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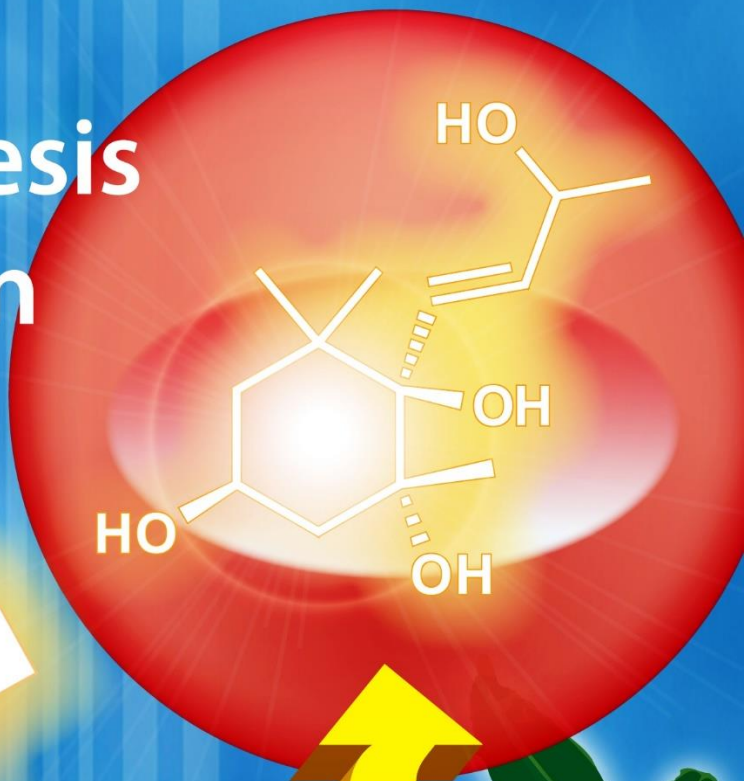

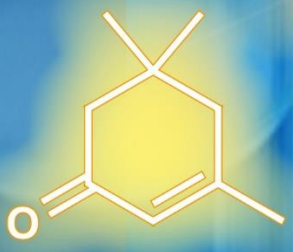

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Full Paper

A Theoretical investigation of furan-AlX_3, pyrrole-AlX_3 and thiophene-AlX_3 (X = H, F, Cl, Br) interactions

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ABSTRACT: *X_3Al-YC_4H_4 (X = H, F, Cl and Br; Y = O in furan, Y=NH in pyrrole, and Y = S in thiophen) have been investigated as donor–acceptor complex types using the DFT level of theory. Both staggered and eclipsed conformations have been examined. For all complexes, the first one is found to be favored. The DFT results including the BSSE contribution show that fluoro complexes are more stable than the others. The interaction diagrams prove that the evolution of complexation energy depends on the coordination mode. In fact, this is a simple "HOMO–LUMO" interaction for X_3Al-YC_4H_4 complexes. This quantum chemistry analysis of the X_3Al-YC_4H_4 donor–acceptor complexes shows no correlation with the charge transfer.*

Keywords: *donor-acceptor; aluminum; furan; pyrrole; thiophen; density functional theory*

Introduction

The binding interactions between an electron pair donor (Lewis base) and an electron pair acceptor (Lewis acid) play an important role in many chemical processes. The reaction course of numerous reactions takes place with formation of donor–acceptor adducts as intermediates. The geometrical parameters are sensitive to intra– and intermolecular interactions; hence they can indicate and characterize these processes. The strength of the adduct bond is generally lower than that of a typical covalent bond.

Complexes formed by Lewis acids and bases are widely known and of great importance in modern chemistry. The types of reactions in which trivalent aluminum

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plays a catalytic role are many and varied. The Friedel-Crafts alkylation and acylation of aromatic rings, removal of tert-butyl groups from phenols, and the well-known Ziegler-Natta polymerization reactions are some examples where the aluminum trichloride acts as catalyst. Many of these important compounds have been experimentally and theoretically studied [1-6]. The complexing behavior of aluminum trihalides AlX_3 has also been the subject of experimental and theoretical works [7-26]. The points that have been more developed are conformational structure, complexation energy and charge transfer. One of the major characteristics of these adducts is the donor-acceptor complexation energy. Mulliken proposed that donor-acceptor complex formation depends on the degree of charge transfer between the HOMO of the donor and the LUMO of the acceptor [27]. According to this point of view, the total charge-transfer Q_T from donor (D) to acceptor (A) should determine the energy of the donor-acceptor bond and, as a result, the complexation energy. However, in recent computational studies it has been shown that in some systems such a correlation is not valid [28]. This can be attributed to the importance of the terminal atoms in the complex formation.

In this work, we report our investigation on the alane-trihalide (AlX_3, X = F, Cl, and Br) donor-acceptor complexes X_3Al-YC_4H_4 (Y = O in furan, Y = NH in pyrrole, and Y = S in thiophen) compared to the alane H_3Al- YC_4H_4 ones. Despite many theoretical works, no comparative ab initio studies of these complexes have been carried out. The electronic structure of these complexes has been analyzed and the relative stabilities are examined.

Material and Methods

Computational details

The geometry optimizations have been carried out at the B3LYP/6–311G(d,p) level. The nature of all stationary point structures were determined by analytical frequency analysis, which also provided zero–point vibrational energies (ZPEs). ZPEs were scaled by the factor 0.9153 [29]. All structures reported here are minima on the potential energy surface (only positive eigenvalues of the Hessian matrix). Final energies were calculated at the B3LYP/6–311G (d,p) + ZPEs level. The basis set superposition error (BSSE) correction was evaluated using the counterpoise method [30]. The electronic structure has been done using the natural bond orbital (NBO) partitioning analysis [31] .The calculations were performed using the GAUSSIAN03 suite of programs [32].

Results and Discussion

Association of AlX_3 (D_{3h} symmetry; X = H, F, Cl and Br), which act as electron pair acceptors, with YC_4H_4 (Y = O in furan, Y = NH in pyrrole, and Y´= S in thiophen),

wish act as electron pair donors, leads to $X_3Al\text{-}YC_4H_4$. For all complexes C_s symmetry is found to be favored. The geometry and electronic structures of these complexes have been analyzed and the relative stability is examined. Table 1 lists relevant optimized bond lengths and bond angles for all the complexes studied in this work. The depicted geometrical parameters are reported in figures 1, 2 and 3.

One can see from Table 1 that upon coordination, there are a number of intramolecular distortions that accompany the formation of the complex. In these C_s complexes there are two different Al–X bond length (there are two chemically different X atoms, Xa and Xb — see figures 1, 2 and 3), two different ∠XAlX angles (namely ∠XaAlXb and ∠XbAlXb), and two different ∠XAlY angles (namely ∠XaAlY and ∠XbAlY). The calculated Al–X bond lengths in $X3Al\text{-}YC_4H_4$ complexes are little longer than that in isolated moieties AlX3 (X = H, F, Cl and Br). This increase does not exceed 1.5%.

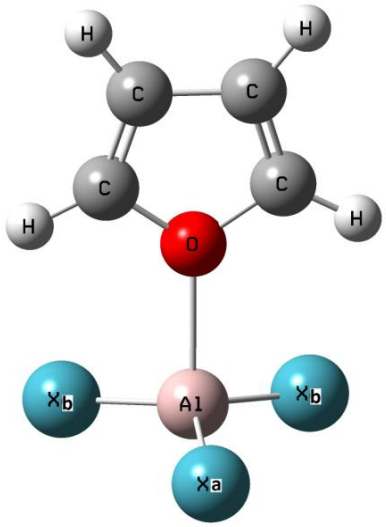

Figure 1. Geometrical parameters of the X_3Al-furan complexes (X = H, F, Cl and Br).

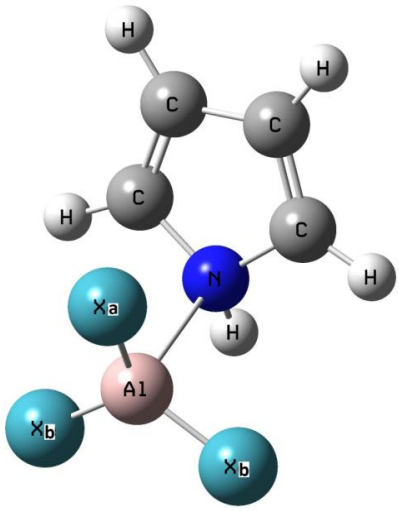

Figure 2. Geometrical parameters of the X_3Al-pyrrole complexes (X = H, F, Cl and Br).

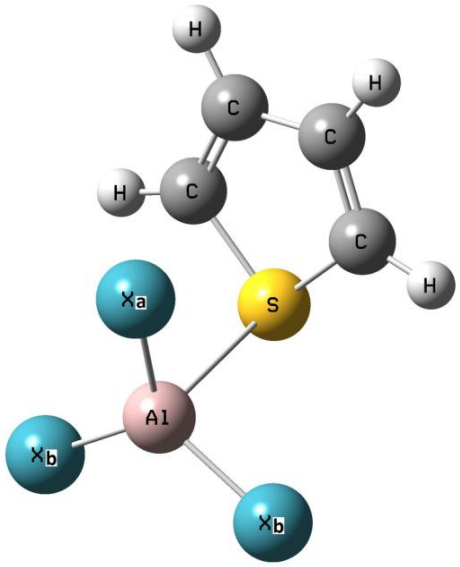

Figure 3. Geometrical parameters of the X_3Al-Thiophen complexes (X=H, F, Cl and Br).

Table 1. B3LYP/6–311G (d, p) calculated geometries (Bond length in Å and Bond angle in degree)

Complex	Al-Xa(Xb)	Al-Y	Y-C	C_1-C_2	C_2-C_3	∠XaAlXb (∠XbAlXb)	∠XaAlY (∠XbAlY)	∠CYC
AlH_3	1.584					120.0		
AlF_3	1.651					120.0		
$AlCl_3$	2.081					120.0		
$AlBr_3$	2.245					120.0		
Furan			1.363	1.358	1.435			106.8
Pyrrole			1.374	1.376	1.424			109.8
Thiophen			1.734	1.364	1.427			91.4
H_3Al-Furan	1.592(1.591)	2.119	1.384	1.349	1.439	118.0(119.7)	99.7(95.6)	107.4
F_3Al- Furan	1.668(1.673)	1.978	1.393	1.346	1.442	116.3(120.5)	103.7(96.5)	107.8
Cl_3Al-Furan	2.112(2.116)	2.023	1.399	1.345	1.441	116.3(117.5)	101.5(100.2)	107.2
Br_3Al-Furan	2.278(2.282)	2.045	1.398	1.345	1.441	116.4(117.0)	101.2(100.5)	107.2
H_3Al-Pyrrole	1.587(1.598)	2.238	1.420	1.354	1.443	119.4(116.2)	101.6(95.3)	106.8
F_3Al-Pyrrole	1.669(1.679)	2.074	1.434	1.349	1.448	117.6(116.0)	106.2(96.7)	106.3
Cl_3Al-Pyrrole	2.112(2.129)	2.102	1.441	1.347	1.449	116.9(114.2)	106.0(99.5)	105.8
Br_3Al-Pyrrole	2.276(2.297)	2.120	1.440	1.347	1.449	117.0(113.9)	106.3(99.3)	105.7
H_3Al-Thiophen	1.588(1.592)	2.656	1.749	1.356	1.436	119.2(118.7)	98.6(94.1)	91.5
F_3Al-Thiophen	1.669(1.673)	2.485	1.756	1.353	1.440	117.2(118.5)	103.3(95.3)	91.6
Cl_3Al-Thiophen	2.119(2.117)	2.532	1.757	1.352	1.441	116.6(117.4)	104.6(98.2)	91.7
Br_3Al-Thiophen	2.276(2.284)	2.551	1.756	1.353	1.441	116.9(117.4)	104.8(97.7)	91.7

Upon complexation, the lengthening of the Al–X bond increases when going from

AlH_3 to $AlBr_3$. This is because in the isolated AlX_3 strong π- donation from the halogen lone pairs into the formally empty p (π) orbital at aluminum stabilizes the molecule, yielding shorter Al–X bonds. Of particular interest is the Y–C (Y = O in furan, Y = NH in pyrrole, and Y = S in thiophen) bond distance. Upon complexation the calculated geometrical parameters show a lengthening of the Y–C (Y = O in furan, Y = NH in pyrrole, and Y = S in thiophen) bonds. Indeed, the NBO calculations show that in isolated furan donor fragment, the pairs on Y (Y = O in furan) atom have lower "s" character than that in X_3Al-furan complexes. On the other hand one notices the reverse in the case of the other complexes X_3Al-pyrrole and X_3Al-thiopnene.

Taking into account that greater "s" character in the complex favors a shorter and stronger bond, we can deduce that this change alone would imply a shortening of the Y–C bond lengths because it increases upon coordination. Moreover, Table 2 shows that the 2s atomic orbital (AO) contribution of Y = O, in the O–C bond is more important in X_3Al-furan (X = H, F, Cl and Br) complexes than that in isolated furan moiety. In the even feel, the calculated Wiberg bond index, from the NBO analysis, of the Y–C bond decreases upon coordination for Y = O, NH and S atoms (Table 2).

Table 2. The Optimized Y–C bond length of the YC_4H_4 moiety and their complexes with AlX_3, Wiberg bond index, and the n_s NBO contribution of Y Atom in the Y–C bond

	d (Y-C) (A°)	Wiberg bond index	n_s(%)
Furan	1.363	1.0475	31.33
AlH_3-Furan	1.384	0.9593	32.21
AlF_3-Furan	1.393	0.9266	32.70
$AlCl_3$-Furan	1.399	0.9189	32.56
$AlBr_3$-Furan	1.398	0.9216	32.49
Pyrrole	1.374	1.1854	36.02
AlH_3-Pyrrole	1.420	1.0465	32.05
AlF_3-Pyrrole	1.434	1.0134	31.92
$AlCl_3$-Pyrrole	1.441	0.9966	30.26
$AlBr_3$-Pyrrole	1.440	0.9985	30.13
Thiophen	1.734	1.2148	19.75
AlH_3-Thiophen	1.749	1.1395	18.97
AlF_3-Thiophen	1.756	1.1163	19.12
$AlCl_3$-Thiophen	1.757	1.1092	18.90
$AlBr_3$-Thiophen	1.756	1.1104	18.88

On the other hand, the bond angle $\angle$X–Al–Y (Y = O, NH, and S) varies slightly in going from AlX_3 free moiety (90°) to $X_3AlYC_4H_4$ complex adducts. It increases on average only by about 10°. This has a consequence for the Al geometrical environment, which passes from D_{3h} (flat) in free AlX_3 to pseudo–pyramidal in the complex. For the bond

angles ∠X–Al–X and ∠C–Y–C we note that no notable deviation in going from isolated AlX_3 to $X_3AlYC_4H_4$ complex. One can see that ∠X–Al–X bond angle decreases by about 4° in going from the isolated AlX_3 (X = H, F, Cl and Br) ligand to the complex adduct. The ∠C–Y–C bond angle increases by about ~1° in going from the isolated YC_4H_4 ligand to the complex adduct. This trend is consistent with the observed Y–C bond lengths which are affected very little by coordination.

Table 3 lists the computed complexation energies for the $X_3AlYC_4H_4$ (X = H, F, Cl and Br; Y = O in furan, Y = NH in pyrrole, and Y = S in thiophen), donor–acceptor complexes and the charge transfer from YC_4H_4 Lewis bases to AlX_3 Lewis acids (Q_t). The complexation energies are calculated as the difference between the energies of the complexes and the respective donor–acceptor moieties. The estimation of the basis set superposition error (BSSE) for all the structures presented here was performed by the full counterpoise method at the B3LYP/6–311G (d,p) level. These results are also presented in Table 3. The BSSE goes from 0.89 kcal/mol for the H_3Al-thiophen complex to 5.52 kcal/mol for the F_3Al-pyrrole complex. Table 3 shows that BSSE has slightly significant values and must be taken into account. The calculated complexation energies E_{comp} of the halogen alane Lewis acids with YC_4H_4 (Y = O in furan, Y = NH in pyrrole, and Y = S in thiophen) Lewis bases show the trend furan > pyrrole > thiophen at the B3LYP/6–311G (d,p) + BSSE corrections level of theory.

Table 3. E_{comp}(B3LYP) (Complexation energies), BSSE, $E_{comp+BSSE}$ (kcal/mol), and charge transfer Q_t (electron)

Complex	E_{comp} [a]	BSSE [b]	$E_{comp+BSSE}$	Q_t
AlH_3-Furan	-11.18	1.64	-9.54	0.099
AlH_3-Pyrrole	-7.42	1.56	-5.86	0.120
AlH_3-Thiophen	-5.82	0.89	-4.93	0.192
AlF_3-Furan	-24.54	5.38	-19.17	0.081
AlF_3-Pyrrole	-21.11	5.52	-15.59	0.101
AlF_3-Thiophen	-15.36	4.40	-10.96	0.191
$AlCl_3$-Furan	-17.34	2.57	-14.77	0.110
$AlCl_3$-Pyrrole	-14.85	2.62	-12.23	0.146
$AlCl_3$-Thiophen	-10.09	1.57	-8.52	0.261
$AlBr_3$-Furan	-14.42	2.57	-11.85	0.109
$AlBr_3$-Pyrrole	-12.03	2.54	-9.49	0.148
$AlBr_3$-Thiophen	-8.01	1.69	-6.32	0.262

[a] $E_{comp} = E(X_3Al\text{-}YC_4H_4)-[E(AlX_3)+E(YC_4H_4)]$ (X = H, F, Cl and Br; Y = O, S and NH)
[b] Calculated using the counterpoise method.

The furan complexes with AlX_3 (X = H, F, Cl and Br) Lewis acids are calculated to be more strongly bound than the respective pyrrole and thiophen complexes. In addition, the energetic results show that the stability decreases when going from Y = O in furan to Y = S in thiophen for all complexes. Indeed, the complexation energies of

H_3Al-furan, H_3Al-pyrrole, and H_3Al-thiophen complexes are –9.54, –5.86, and –4.93 kcal/mol, respectively, while the complexation energies of F_3Al-Furan, F_3Al-Pyrrole, and F_3Al-Thiophen are –19.17, –15.59, and –10.96 kcal/mol, respectively, the complexation energies of Cl_3Al-furan, Cl_3Al-pyrrole, and Cl_3Al-thiophen are –14.77, –12.23, and –8.52 kcal/mol, respectively, and the complexation energies of Br_3Al-furan, Br_3Al-pyrrole, and Br_3Al-thiophen are -11.85, -9.49, and -6.32 kcal/mol, respectively. On the other hand, one can observe that the complexation energy of the alane complexes show the trend AlF_3 > $AlCl_3$ > $AlBr_3$ > AlH_3 at the both levels of calculation B3LYP/6–311G (d,p) and B3LYP/6–311G(d,p) + BSSE. This confirms that the values of the BSSE correction have no influence on the established trend. Indeed, Figure 4 shows nicely that furan leads always to the more stable complex among the Lewis bases.

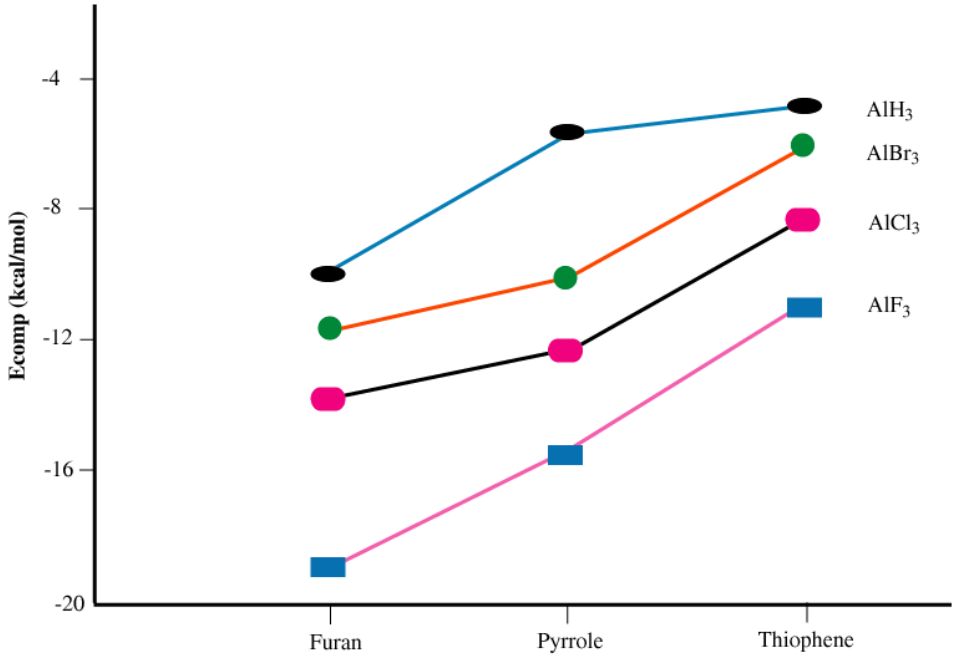

Figure 4. Trend of the calculated complexation energies (including BSSE correction) of the $X_3AlYC_4H_4$ (X = H, F, Cl and Br; Y = O in furan, Y = NH in pyrrole, and Y = S in thiophene) complexes.

The charge transfer from furan to AlX_3 (X = H, F, Cl and Br) is lower than that from pyrrole and thiophene, while the complexation energies of X_3Al-furan complexes are higher than that for X_3Al-pyrrole and X_3Al-thiophene complexes (see Table 3). Moreover, the F_3Al-furan complex is the most stable and it show only a lower charge transfer (0.081 *e*), whereas the less stable complex is H_3Al-thiophene and is shows a charge transfer of 0.192 *e*.

Hence, one can see that from the NBO results it follows that there is no correlation between charge transfer and the calculated complexation energy of $X_3AlYC_4H_4$ (X = H, F, Cl and Br; Y = O in furan, Y = NH in pyrrole, and Y = S in thiophene) donor–acceptor complexes. However and as that is shown on Table 3, for a same base YC_4H_4,

the stability of the complxes increases with the electronegativity of halogen X ($\chi_F > \chi_{Cl} > \chi_{Br} > \chi_H$). This result can also be confirmed by another descriptor. It is about the gap between the HOMO of the donor and the LUMO of the acceptor. It is known that more this gap is narrow more coordination between the donor and the acceptor is strong and more the complex formed donor-acceptor is stable.

The results of the calculations carried out on B3LYP/6–311G (d,p) level shows the opposite as it is shown by Table 4. Indeed, the weakest gap (346, 39 kcal/mol) and the highest gap (491, 98 kcal/mol) correspond to the AlH_3-thiophene complexes the least stable and most stable AlF_3-furan, respectively. It is significant to note within this frame-work which it is known the frontier molecular orbital theory proposed by Fukui takes into account only the interactions between a LUMO and a HOMO. It is obvious that the chem-ical reactivity is not reduced simply to this type of interaction. One must also consider repulsive terms which will intervene between the occupied OM. Consequently, the donor orbital of the C_4H_4Y species is not really the HOMO. So, one must be very careful during the use of the concept of the HOMO in a DFT calculation.

Table 4. B3LYP/6-311G(d,p) level $Gap_{(HOMO-LUMO)}$ (kcal/mol), $E_{comp+BSSE}$ (kcal/mol), and charge transfer (electron)

Complex	$Gap_{(HOMO-LUMO)}$	$E_{comp+BSSE}$	Q_t
AlH_3-Furan	368.35	-9.54	0.099
AlH_3-Pyrrole	364.59	-5.86	0.120
AlH_3-Thiophen	346.39	-4.93	0.192
AlF_3-Furan	491.98	-19.16	0.081
AlF_3-Pyrrole	488.21	-15.59	0.101
AlF_3-Thiophen	470.01	-10.96	0.191
$AlCl_3$-Furan	392.20	-14.77	0.110
$AlCl_3$-Pyrrole	389.06	-12.23	0.146
$AlCl_3$-Thiophen	370.24	-8.52	0.261
$AlBr_3$-Furan	363.96	-11.85	0.109
$AlBr_3$-Pyrrole	360.20	-9.49	0.148
$AlBr_3$-Thiophen	344.51	-6.32	0.262

Conclusion

DFT calculations have been carried out to study the interaction in $X_3Al\text{-}YC_4H_4$ (X = H, F, Cl and Br; Y = O in furan, Y = NH in pyrrole, and Y = S in thiophen) donor–acceptor complexes. We have shown that the formation of the donor-acceptor complexes is accompanied by a certain number of geometrical distortions such as the increase length of Al-X bond while passing by AlH_3 to $AlBr_3$. The same remark is valid for Y-C bond. One can also note significant changes such as the growth of $\angle$XAlY angle because of the passage of the aluminum atom of symmetry D_{3h} (in isolated species AlX_3) to a pseudo-pyramidal geometry in the studied complexes.

We have also shown that the stability of the complexes $X_3Al\text{-}YC_4H_4$ donor-acceptor decreases in the order furane, pyrrole and thiophen. It is significant here not to announce the need for taking into account the values of the BSSE in the evaluation of energies of the donor-acceptor complexes studied in this work. The analysis of the electronic structure based on natural bond orbitals (NBO) partitioning indicates that there is no correlation between the charge transfer and the stability of the complex.

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PEG-400 as an efficient and recyclable reaction medium for the synthesis of polyhydroquinolines *via* Hantzsch reaction

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ABSTRACT: *Polyhydroquinoline derivatives have been prepared efficiently in a one-pot synthesis via Hantzsch condensation using PEG-400 as reaction medium. The present method does not involve any hazardous organic solvents or toxic catalysts. The present methodology offers several advantages such as simple procedure, excellent yields with shorter reaction times and purification of products by non-chromatographic methods.*

Keywords: *polyhydroquinoline derivatives; Hantzsch condensation; green chemistry; PEG-400*

Introduction

Polyhydroquinoline nucleus is a fertile source of biologically important molecules possessing various important pharmacological properties such as vasodilator, anti-hypertensive, bronchodilator, anti-therosclerotic, hepto-protective, anti-tumor, anti-mutagenic, geroprotective and anti-diabetic agents [1]. Polyhydroquinolines have found commercial utility as calcium channel blockers as exemplified by therapeutic agents such as Nifedine, Nitrendipine and Nimodipine [2]. These examples clearly demonstrate the remarkable potential of polyhydroquinoline derivatives as a source of valuable drugs. Owing to the wide range of pharmacological and biological activities, the synthesis of imidazoles has become an important target in current years. There are several methods reported in literature for the synthesis of polyhydroquinoline derivatives in the synthesis

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of various drug sources, reported in many classical methods such as conventional heating [3, 4], the progress in this field is remarkable for microwave irradiation and ultrasound [5, 6], various catalysts such as trimethylsilyl chloride (TMSCl) [7], molecular iodine [8], L-proline [9], $Yb(OTf)_3$ [10], cerric ammonium nitrate [11], iron (III) trifluoroacetate [12], heteropoly acid [13], $Sc(OTf)_3$ [14], microwave irradiation [15], Bakers' yeast [16] *p*-TSA [17], grinding [18], by refluxing in water[19] and nanosized nickel [20]. However, most of the reported methodologies still have certain limitations such as expensive catalysts, toxicity of solvents, restrictions for large scale applications, critical product isolation procedures, difficulty in recovery of high boiling solvents, excessive amounts of catalysts and generation of large amounts of toxic wastes in scaling up for industrial applications leading to environmental issues. Thus, the development of a simple and efficient method under catalyst free conditions for constructing these polyhydroquinoline has been advocated.

In the recent years, PEG emerged as a powerful phase transfer catalyst and performs many useful organic transformations under mild reaction conditions. Moreover, PEG is inexpensive, easy to handle, thermally stable, non-toxic, and recyclable in various organic transformations [21], such as for example, Heck reaction [22], catalytic hydrogenations [23], asymmetric dihydroxylation reaction [24], Baylis-Hillman reaction [25], Biginelli reaction [26], Suzuki-Miyaura reaction, Stille cross-coupling reaction [27], Wacker reaction [28] and asymmetric aldol reaction [29]. This inspired us to focus on the aspect of synthesis of biologically active polyhydroquinolines derivatives under catalyst free conditions by using PEG-400 as an eco-friendly and recyclable media.

Material and Methods

Melting points were determined in an open capillary tube and are uncorrected. IR spectra were recorded in KBr on a Perkin-Elmer spectrometer. ^{1}H-NMR spectra were recorded on a Gemini 300-MHz instrument in $CDCl_3$ as solvent and TMS as an internal standard. The purity of products was checked by thin-layer chromatography (TLC) on silica-gel.

Typical procedure for the synthesis of 2-amino-4H-chromenes 4(a-l)

A mixture of aldehyde **1** (1 mmol), dimedone **2** (1 mmol), ethyl acetoacetate **3** (1 mmol) and ammonium acetate (1 mmol) in PEG-400 (5ml) was stirred at 80 ^{o}C in 25 mL round bottom flask for the appropriate time mentioned in Table 2.The progress of reaction was monitored by TLC. After completion of reaction, the separated solid was filtered and recrystallized from ethyl alcohol.The progress of reaction was monitored by thin layer chromatography (*n*-hexane:EtOAc, 8:2). After completion of reaction the reaction mass was cooled to room temperature and then poured on cold water. The

obtained solid was filtered, washed with water and crude solid was crystallized from ethanol. The aqueous filtrate was distilled at 100 °C to remove water and thus separated PEG-400 was reused.

Spectroscopic data of synthesized some principal compounds

Compound (4a): IR (KBr, cm^{-1}): 3305, 3052, 2967, 1679, 1633, 1351, 762; ^{1}H-NMR ($CDCl_3$ δ, ppm): 1.12 (6H, s, $2CH_3$), 1.23 (3H, t, J=7.1 Hz, CH_3), 1.71 (3H, s, CH_3), 2.31-2.35 (4H, m, $2CH_2$), 4.11 (2H, q, J = 7.1 Hz, CH_2), 5.05 (1H, s, ArCH), 6.41 (1H, br., s, NH), 6.75 (2H, d, J = 8.1 Hz, Ar-H), 7.22 (2H, d, J = 8.1 Hz, Ar-H).

Compound (4b): IR (KBr, cm^{-1}): 3310, 3042, 2967, 1679, 1630, 1498, 1351, 1200, 752; ^{1}H-NMR ($CDCl_3$ δ, ppm): 1.11 (6H, s, $2CH_3$), 1.20 (3H, t, J=7.1 Hz, CH_3), 1.73 (3H, s, CH_3), 2.31-2.35 (4H, m, $2CH_2$), 3.62 (3H,s, OCH_3), 4.11 (2H, q, J = 7.1 Hz, CH_2), 5.05 (1H, s, ArCH), 6.41 (1H, br., s, NH), 6.82 (2H, d, J = 8.1 Hz, Ar-H), 7.22 (2H, d, J = 8.1 Hz, Ar-H).

Compound(4c): IR (KBr, cm^{-1}): 3302, 3274, 3043, 2940, 1656, 1587, 1478, 1344, 1223, 767; ^{1}H-NMR :($CDCl_3$, δ, ppm): 1.18 (6H, s, $2CH_3$), 1.19 (3H, t, J = 7.1Hz, CH_3), 1.83 (3H, s, CH_3), 2.40-2.50 (4H, m, $2CH_2$), 2.45 (3H,s, CH_3), 4.16 (2H,q, J = 7.1 Hz, CH_2), 5.35 (1H, s, ArCH), 5.95 (1H, br., s, NH),7.66 (2H, d, J = 8.7 Hz, ArH), 8.20 (2H, d, J = 8.2 Hz ArH);

Compound (4d): IR (KBr, cm^{-1}): 3305, 3280, 3052, 2967, 1679, 1637, 1460,1351, 1218, 769; ^{1}H-NMR ($CDCl_3$ δ, ppm): 1.11 (6H, s, $2CH_3$), 1.21 (3H, t, J=7.1 Hz, CH_3), 1.74 (3H, s, CH_3), 2.32-2.37 (4H, m, $2CH_2$), 4.62 (1H,s, OH), 4.11 (2H, q, J = 7.1 Hz, CH_2), 5.05 (1H, s, ArCH), 6.41 (1H, br., s, NH), 6.97 (2H, d, J = 8.1 Hz, Ar-H), 7.22 (2H, d, J = 8.1 Hz, Ar-H).

Compound (4e): IR (KBr, cm^{-1}): 3305, 3052, 2967, 1679, 1637,1540, 1460,1351, 1218, 769; ^{1}H-NMR ($CDCl_3$ δ, ppm): 1.13 (6H, s, $2CH_3$), 1.23 (3H, t, J=7.1 Hz, CH_3), 1.71 (3H, s, CH_3), 2.30-2.34 (4H, m, $2CH_2$), 4.12 (2H, q, J = 7.1 Hz, CH_2), 5.02 (1H, s, ArCH), 6.42 (1H, br., s, NH), 7.97 (2H, d, J = 8.1 Hz, Ar-H), 7.20 (2H, d, J = 8.1 Hz, Ar-H).

Compound (4f): IR (KBr, cm^{-1}): 3303, 3054, 2960, 1653, 1632,1524,1460,1351, 1212, 760; ^{1}H-NMR ($CDCl_3$ δ, ppm): 1.10 (6H, s, $2CH_3$), 1.23 (3H, t, J=7.1 Hz, CH_3), 1.71 (3H, s, CH_3), 2.30-2.34 (4H, m, $2CH_2$), 4.12 (2H, q, J = 7.1 Hz, CH_2), 5.02 (1H, s, ArCH), 6.42 (1H, br., s, NH), 8.17 (2H, d, J = 8.1 Hz, Ar-H), 7.30 (2H, d, J = 8.1 Hz, Ar-H).

Compound (4g): IR (KBr, cm^{-1}): 3305, 3052, 2967, 1679, 1637, 1548, 1460,1351, 1218, 710, 776,; ^{1}H-NMR ($CDCl_3$ δ, ppm): 1.13 (6H, s, $2CH_3$), 1.22 (3H, t, J=7.1 Hz, CH_3), 1.73 (3H, s, CH_3), 2.31-2.34 (4H, m, $2CH_2$), 4.10 (2H, q, J = 7.1 Hz, CH_2), 5.14

(1H, s, ArCH), 6.40 (1H, br., s, NH), 7.31 (1H, d, Ar-H), 7.82 (1H, d , Ar-H), 7.72 (1H ,s, Ar-H), 7.52 (1H ,t, Ar-H) .

Compound(4h): IR (KBr, cm^{-1}): 3309, 3056, 2952, 1645, 1621, 1468, 1361, 1232, 761; ^{1}H-NMR ($CDCl_3$ δ ppm): 1.12 (6H, s, $2CH_3$), 1.22 (3H, t, J=7.1Hz, CH_3), 1.79 (3H, s, CH_3), 2.32-2.35 (4H, m, $2CH_2$), 4.15 (2H, q, J = 7.1 Hz, CH_2), 5.10 (1H, s, ArCH), 6.51 (1H, br., s, NH), 6.95 (2H, d, J = 8.1 Hz, Ar-H), 7.22 (2H, d, J = 8.1 Hz, Ar-H).

Compound(4i): IR (KBr, cm^{-1}): 3305, 3056, 2945, 1645, 1621, 1468, 1361, 1242, 742; ^{1}H-NMR ($CDCl_3$ δ ppm): 1.12 (6H, s, $2CH_3$), 1.22 (3H, t, J=7.1Hz, CH_3), 1.79 (3H, s, CH_3), 2.32-2.35 (4H, m, $2CH_2$), 4.15 (2H, q, J = 7.1 Hz, CH_2), 5.10 (1H, s, ArCH), 6.51 (1H, br., s, NH), 7.30-7.26 (m, 1H), 7.22-7.19 (m, 1H), 7.13-7.10 (m, 1H),

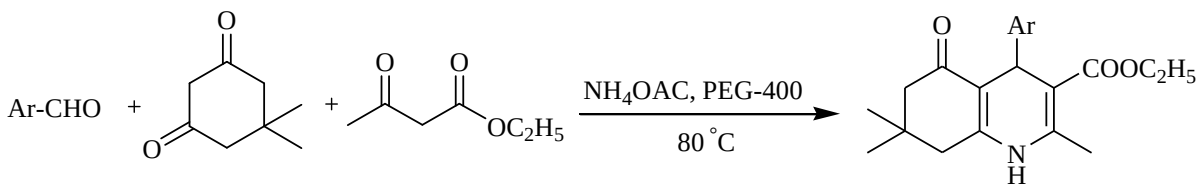
Compound(4j): IR (KBr, cm^{-1}): 3302, 3065, 2942, 1645, 1621, 1468, 1361, 1232, 751; ^{1}H-NMR ($CDCl_3$ δ ppm): 1.12 (6H, s, $2CH_3$), 1.22 (3H, t, J=7.1Hz, CH_3), 1.79 (3H, s, CH_3), 2.32-2.35 (4H, m, $2CH_2$), 4.15 (2H, q, J = 7.1 Hz, CH_2), 5.10 (1H, s, ArCH), 6.51 (1H, br., s, NH), 7.10-7.14 (m, 1H), 7.21-7.19 (m, 1H), 7.13-7.10 (m, 1H),

Compound(4k): IR (KBr, cm^{-1}): 3267, 3020, 2977, 1632, 1515, 1375, 1217, 754; ^{1}H-NMR ($CDCl_3$, δ, ppm):1.09 (6H, s, $2CH_3$), 1.21 (3H,t J = 7.1 Hz, CH_3), 1.81 (3H, s, CH_3), 2.22-2.52 (4H, m, $2CH_2$),4.11 (2H, q, J = 7.1 Hz, CH_2), 5.09 (1H, s, ArCH), 5.98 (1H, br.,s, NH), 7.11 (2H, d, J = 8.2 Hz, ArH), 7.12 (2H, d, J = 8.3 Hz,ArH),2.85 (6H, s, $N[CH_3]_2$]

Compound(4l) : IR (KBr, cm^{-1}) 3312, 3285, 3046, 2910, 1612, 1601, 1458, 1351, 1212, 761; ^{1}H-NMR ($CDCl_3$ δ ppm) : 1.02 (6H, s, $2CH_3$), 1.11 (3H, t, J=7.1Hz, CH_3), 1.75 (3H, s, CH_3),5.12 (1H. S, OH) 2.41-2.45 (4H, m, $2CH_2$), 4.22 (2H, q, J = 7.1 Hz, CH_2), 5.15 (1H, s, ArCH), 6.22 (1H, br., s, NH), 6.95 (1H, d, Ar-H), 7.12 (1H, d , Ar-H), 6.72 (1H ,s, Ar-H), 7.52 (1H ,t, Ar-H).

Results and Discussion

In continuation of our research work on the development of novel synthetic methodologies [30], herein, we have reported a highly efficient route for the synthesis of polyhydroquinolines from one-pot four component coupling of an aromatic aldehydes, dimedone, ethyl acetoacetate and ammonium acetate in PEG-400 (Scheme 1).



1 (a-m) **2** **3** **4 (a-m)**

Scheme 1. Synthesis of polyhydroquinoline derivatives under PEG-400 mediated conditions.

In the initial studies, the reaction of a benzaldehyde **(1a)** as a representative aldehyde, dimedone **(2),** ethyl acetoacetate **(3)** and ammonium acetate was performed in different solvents (5 mL) and at different temperature without any added catalyst to obtain the products. The compound **4a** has been considered as a standard model reaction product for the optimization of reaction condition. It was observed that among the tested solvents (Table 1, entries 4–9). The reaction in PEG-400 was more facile and proceeded to give best yield (93%) when the reaction mixture was stirred at 80 °C for 45 min. Moreover, there are many potential advantages of replacing these volatile or toxic organic solvents with PEG-400. So PEG-400 is the optimal reaction media for the reaction at 80 °C.

The effect of temperature was also studied by carrying out the model reaction of product **4a** in PEG-400 at different temperature. As shown in Table 1 (entries 1–5), the reaction did proceed but the yield is low in entry 5 (Table 1) as compared to entry 4 (Table 1) obtained even after longer reaction time, when the reaction temperature within 25 - 60°C. However, at elevated temperature at 80 °C using PEG-400 gave better results in terms of yield and reaction time. Hence, the conditions of entry 4, shown in Table 1, were the optimized reaction conditions.

Table 1. Effect of solvent and temperature

Entry	Solvent(5ml)	Temperature °C	Time (min)	Yield (%)
1	PEG-400	25	110	75
2	PEG-400	40	80	80
3	PEG-400	60	55	81
4	**PEG-400**	**80**	**45**	**93**
5	PEG-400	100	25	88
6	Dichloromethane	40	60	45
7	Acetonitrile	78	60	55
8	Ethanol	75	60	60
9	Methanol	63	60	55

In order to evaluate the generality of the process, we studied the reaction of various aldehydes **1**, dimedone **2**, ethyl acetoacetate **3**, and ammonium acetate in PEG-400 at 80 °C. Aromatic aldehydes bearing electron withdrawing groups (such as nitro, chloro, bromo) or electron releasing groups (such as methyl, hydroxyl, methoxy, *N,N*-diamine), were smoothly converted to corresponding product (4) in excellent yields, except with 4-nitrobenzaldehyde requires longer reaction time. Results have shown that the substitution groups played a less significant role in governing the reactivity of the substrates. In the present procedure, PEG-400 not only acts as a phase transfer catalyst but also as a clean solvent by significantly enhancing the intramolecular cyclization.

Table 2. Synthesis of polyhydroquinoline (4a–4m) using PEG-400 as reaction medium at 80 °C

Entry	Product	Ar-	Time(min)	Yield (%)	Mp.(°C)	
					Found	Lit.
1	4a	C_6H_5	45	93	201-202	202-205 [9]
2	4b	4-$OCH_3C_6H_4$	30	90	256-258	255-257 [16]
3	4c	4-$CH_3C_6H_4$	35	89	259-261	258-260 [9]
4	4d	4-OHC_6H_4	30	91	232-233	231-233 [16]
5	4e	4-$NO_2C_6H_4$	110	92	242-243	241-243 [9]
6	4f	4-ClC_6H_4	35	89	246-248	245-247 [16]
7	4g	3-$NO_2C_6H_4$	90	94	175-177	174-176 [16]
8	4h	4-BrC_6H_4	40	91	253-254	251-253 [9]
9	4i	2-Furyl	25	89	247-249	246-248 [16]
10	4j	2-Thienyl	20	90	240-241	237-239 [16]
11	4k	4-$N(CH_3)_2C_6H_4$	25	85	231-233	230-232 [16]
12	4l	3-OHC_6H_4	55	93	219-220	218-220 [16]

To check the reusability of medium (PEG-400) we have performed the experiment using same reactants, benzaldehyde **(1a)**, dimedone **(2),** ethyl acetoacetate **(3)** and ammonium acetate using PEG-400 and we found surprising results with this media.

After three successive runs we found the reaction proceed cleanly with good yields were summarized in Table 3, although a little weight loss of PEG-400 was observed from cycle to cycle due to mechanical loss. Further studies to develop the new clean environmentally benign PEG-400 towards the synthesis of biologically active compounds are progress.

Table 3. The recycling of polyethylene glycol for benzaldehyde derivative.

Entry	Time(min.)	Yield(%)
0	45	93
1	45	93
2	45	90
3	45	89

Conclusion

In conclusion, this paper describes a convenient and efficient process for the synthesis of polyhydroquinoline derivatives by use of PEG-400 at 80 ^{o}C as a recyclable medium without the addition of any additive or organic co-solvent. Present methodology offers very attractive features such as simple experimental procedure, reduced reaction times, higher yields and economic viability, when compared with conventional method

as well as with other catalysts, and will have wide scope in organic synthesis.

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Total synthesis of aripuanin, a megastigmane from *Ficus aripuanensis*

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ABSTRACT: *The first total synthesis of the natural product aripuanin, a megastigmane recently isolated, was achieved with moderate yields starting from isophorone.*

Keywords: *aripuanin; synthesis; megastigmane; Ficus aripuanensis*

Introduction

A large number of megastigmanes, which are considered to be substances derived from carotenoids by oxidative cleavage of conjugated double bonds, have been isolated from different species of plants [1]. In 1999, a new natural product denominated aripuanin **1**, the norsesquiterpene (*3S,5R,6R,7E,9ξ*)-megastigmane-7-ene-3,5,6,9-tetrol, was isolated from the leaves of *Ficus aripuanensis* C. C. Berg (Moraceae), which belongs to one of the main families of the Amazonian forest [2]. In spite that no use in traditional medicine has been described particularly for *Ficus Aripuanensis*, some species of the *Ficus* genus are used in folk medicine for their antihelmintic, antirheumatic, antifungal, antimicrobial, antibacterial, antiulcer and anti-inflammatory properties, in leucorrhoea and leprosy [2, 3].

Compound **1** can be considered an aglycone of the natural products kiwiinoside **2** and actinidioionoside **3**, whose structures were already elucidated [4] (Figure 1). To the best of our knowledge, no reports describing the synthesis of aripuanin **1** have yet been reported. In this paper, we describe the first total synthesis of **1** starting from readily available isophorone **4**, as outlined in Scheme 1.

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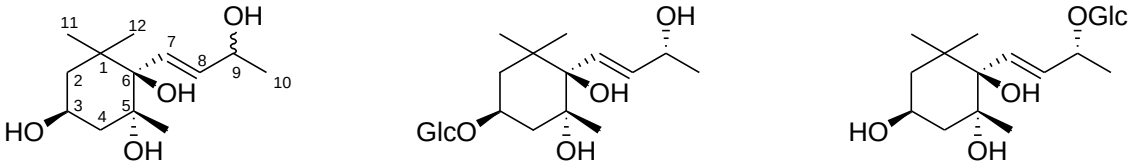


Figure 1. Aripuanin and analogues compounds.

Material and Methods

The common reagents and solvents are commercially available. Column chromatography separation was performed with silica gel 60 (70-230 mesh, Merck). NMR spectra were recorded using a Bruker DRX-400 instrument; chemical shifts are in ppm downfield from a tetramethylsilane internal standard. Infrared spectra were measured with a Perkin Elmer Spectrum RX IFTIR System, and only the most intense or representative bands are reported. HPLC separations were performed on a Shimadzu system with a Shim-pack CLC-CN(M) column. GC-MS analyses were performed by EI ionization at 70 eV on a Shimadzu GC/MS QP-2010 spectrometer. HRMS were recorded on a VG AutoSpec instrument. Elemental analyses were performed with a Carlo Erba instrument EA-1110.

Synthesis of 3,5,5-trimethylcyclohex-3-enol (6)

A solution of compound **5** (5.6911 g, 41.2 mmol), obtained by by the method of Kharasch and Tawney [5], in anhydrous ethyl ether (15 mL), was added to a suspension of $LiAlH_4$ (1.4094 g, 37.1 mmol) in anhydrous ethyl ether (75 mL) at 0 °C. The mixture was heated again to reflux for 4 h, and after the reaction was quenched with cold water (1.5 mL), NaOH 15% (1.5 mL) and finally water (4.5 mL). The solution was filtered through Celite, the filtrate was evaporate and the residue was purified by Kugelrohr distillation under reduced pressure (bp 50 °C, 2 mmHg) to give **6** (5.5375 g, 39.55 mmol, 96%) as a colorless oil.

Analytical data for compound 6: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.10 (br s, 1H), 3.95 (dddd, *J* 11.9, 9.3, 5.6 and 3.5 Hz, 1H), 2.21 (dd, *J* 16.4 and 5.6 Hz, 1H), 1.87 (ddq, *J* 16.4, 9.3 and 1.3 Hz, 1H), 1.72 (ddt, *J* 11.9, 3.5 and 1.3 Hz, 1H), 1.65 (s, 3H), 1.52 (br s, 1H), 1.32 (t, *J* 11.9 Hz, 1H), 0.99 (s, 3H), 0.97 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 131.5 (CH), 128.5 (C), 65.5 (CHOH), 45.9 (CH_2), 39.6 (CH_2), 33.9 (C), 31.4 (CH_3), 29.5 (CH_3), 23.2 (CH_3). IR ν_{max}/cm^{-1} 3338, 1742, 1669, 1394, 1360, 1050, 1014, 835. MS: *m/z* (relative intensity) 140 (12, M^+), 125 (100), 122 (7), 107 (36), 91 (23), 84 (47), 69 (42), 55 (30), 41 (35), 39 (27).

Synthesis of 3,5,5-trimethylcyclohex-3-enyl acetate (7)

To a solution of compound **6** (1.0015 g, 7.15 mmol) in anhydrous CH_2Cl_2 (2 mL), were added Et_3N (0.8640 g, 8.54 mmol), Ac_2O (0.8640 g, 8.46 mmol) and DMAP (0.0872 g, 0.71 mmol). The reaction mixture was stirred at room temperature for 3 h and then extracted with ethyl ether. The combined ethereal extracts were washed with HCl (2*M*), saturated solution of $NaHCO_3$ and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by column chromatography through silica gel using as an eluent a mixture of *n*-hexane and ethyl acetate (9:1) to give **7** (1.1773 g, 6.47 mmol, 90%) as a colorless oil.

Analytical data for compound 7: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.12 (1H, s), 5.07 (1H, dddd, *J* 11.9, 9.1, 5.8 and 3.8 Hz), 2.28 (1H, dd, *J* 16.4 and 5.8 Hz), 2.04 (3H, s), 1.93 (1H, ddq, *J* 16.4, 9.1 and 1.3 Hz), 1.72 (1H, ddt, *J* 11.9, 3.8 and 1.3 Hz), 1.64 (3H, s), 1.44 (1H, t, *J* 11.9 Hz), 1.01 (6H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.8 (C=O), 131.7 (=CH), 128.2 (=C), 69.4 (CH-O), 41.8 (CH_2), 35.8 (CH_2), 33.7 (CH_3), 31.1 (CH_3), 29.4 (C), 23.2 (CH_3), 21.4 (CH_3). IR ν_{max}/cm^{-1} 1733, 1240, 1034.

Synthesis of 3-hydroxy-3,5,5-trimethyl-4-oxocyclohexyl acetate (8)

To a well-stirred mixture of compound **7** (0.2146 g, 1.17 mmol), water (110 mL), and $MgSO_4$ (0.5275 g, 4.38 mmol), previously cooled to 4 °C, was added dropwise a solution of $KMnO_4$ (0.2242 g, 1.42 mmol) in water (70 mL), maintaining the temperature of the reaction mixture below 6 °C. After the mixture was stirred at room temperature for 15 h, enough sodium sulfite was added to decolorize the solution, and the reaction mixture was filtered through Celite. The clear solution was extracted with ethyl ether in a liquid-liquid extractor. The resultant ethereal solution was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by preparative HPLC eluting with a mixture of *n*-hexane and 2-propanol (95:5) to give **8** (0.2096 g, 0.98 mmol, 83%) as a 1:1 mixture of diastereomers *(3R)*-**8a** (white cristalline solid, mp 52-53 °C) and *(3S)*-**8b** (colorless oil).

***Analytical data for compound (3R)-8a*:** ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.26 (1H, ddt, *J* 7.8, 6.6 and 4.8 Hz), 2.22 (1H, ddd, *J* 14.4, 4.8 and 1.5 Hz), 2.11-2.05 (2H, m), 2.01 (3H, s), 1.86 (1H, dd, *J* 13.9 and 7.8 Hz), 1.38 (3H, s), 1.21 (3H, s), 1.17 (3H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 215.8 (C=O), 170.3 (C=O), 74.5 (C-OH), 66.7 (CH-O), 43.1 (CH_2), 43.1 (CH_2), 43.1 (C), 27.6 (CH_3), 27.5 (CH_3), 27.4 (CH_3), 21.3 (CH_3). IR ν_{max}/cm^{-1} 3447, 1745,1718, 1368, 1247, 1167, 1030.

Analytical data for compound (3S)-8b: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.27 (1H, tt, *J* 11.6 and 4.2 Hz), 2.43 (1H, ddd, *J* 12.3, 4.2 and 3.3 Hz), 2.12 (1H, ddd, *J* 13.1, 4.2 and 3.3 Hz), 2.07 (3H, s), 1.89 (1H, dd, *J* 12.3 and 11.6 Hz), 1.74 (1H, dd, *J* 13.1 and 11.6 Hz), 1.47 (3H, s), 1.29 (3H, s), 1.19 (3H, s). ^{13}C-RMN ($CDCl_3$, 100 MHz) δ 217.2 (C=O), 170.8 (C=O), 75.1 (C-OH), 66.5 (CH-O), 45.0 (CH_2), 44.9 (CH_2), 43.3 (C), 28.7 (CH_3),

27.8 (CH_3), 26.8 (CH_3), 21.6 (CH_3). IR ν_{max}/cm^{-1} 3488, 1740,1703, 1368, 1250, 1163, 1031.

Synthesis of 2,4-dihydroxy-2,6,6-trimethylcyclohexanone (9)

A mixture of the diastereomers **8a**/**8b** (0.6046 g, 2.83 mmol), methanol (4 mL), and an aqueous solution of K_2CO_3 10% (4 mL), was stirred at room temperature for 30 min. The methanol was removed under reduced pressure and the residue was extracted with ethyl ether. The organic layer was washed with water, saturated brine, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography through silica gel using as an eluent a mixture of *n*-hexane and ethyl acetate (1:1) to give **9** (0.4373 g, 2.54 mmol, 90%) as a 1:1 mixture of diastereomers *(2R)*-**9a** (white cristalline solid, mp 86-87 °C) and *(2S)*-**9b** (white cristalline solid, mp 63-64 °C).

***Analytical data for compound (2R)-9a*:** ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.38 (1H, ddt, *J* 9.1, 7.8 and 4.7 Hz), 2.28 (1H, ddd, *J* 14.0, 4.7 and 2.3 Hz), 2.01 (1H, ddd, *J* 13.6, 4.7 and 2.3 Hz), 1.96 (1H, dd, *J* 14.0 and 7.8 Hz), 1.84 (1H, dd, *J* 13.6 and 9.1 Hz), 1.64 (br s, 1H), 1.44 (3H, s), 1.27 (3H, s), 1.24 (3H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 216.5 (C=O), 75.1 (C-OH), 63.8 (CH-OH), 47.5 (CH_2), 47.4 (CH_2), 43.6 (C), 28.2 (CH_3), 28.1 (CH_3), 27.8 (CH_3). IR ν_{max}/cm^{-1} 3393, 1708, 1374, 1044. Anal. Calcd. for $C_9H_{16}O_3$: C, 62.77; H, 9.36; O, 27.87. Found: C, 63.01; H, 9.52.

***Analytical data for compound (2S)-9b*:** ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.26 (1H, tt, *J* 10.6 and 4.0 Hz), 2.62 (br s, 1H), 2.40 (1H, ddd, *J* 12.6, 4.0 and 3.3 Hz), 2.08 (1H, ddd, *J* 13.1, 4.0 and 3.3 Hz), 1.87 (1H, dd, *J* 12.6 and 10.6 Hz), 1.72 (1H, dd, *J* 13.1 and 10.6 Hz), 1.42 (3H, s), 1.25 (3H, s), 1.20 (3H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 217.3 (C=O), 74.8 (C-OH), 63.9 (CH-OH), 48.6 (CH_2), 48.1 (CH_2), 42.9 (C), 28.3 (CH_3), 27.6 (CH_3), 26.8 (CH_3). IR ν_{max}/cm^{-1} 3391, 1705, 1367, 1049. Anal. Calcd. for $C_9H_{16}O_3$: C, 62.77; H, 9.36; O, 27.87. Found: C, 62.59; H, 9.13.

Synthesis of (2R,4S)-2,4-bis(methoxymethoxy)-2,6,6-trimethylcyclohexanone (10a)

To a solution of pure compound **9a** (0.1247 g, 0.72 mmol) in anhydrous CH_2Cl_2 (2 mL), were added DPEA (0.2792 g, 2.16 mmol) and methoxymethyl chloride (0.2318 g, 2.88 mmol). The reaction mixture was stirred at room temperature for 4 h and then a saturated solution of $NaHCO_3$ was added. The product was extracted with CH_2Cl_2 and the organic layer was washed with water, saturated brine, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography through silica gel using as an eluent a mixture of *n*-hexane and ethyl acetate (8:2) to give **10a** (0.1414 g, 0.54 mmol, 75%) as a colorless oil.

***Analytical data for compound 10a*:** ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.72 (1H, d, *J* 7.1 Hz), 4.70 (1H, d, *J* 7.1 Hz), 4.59 (1H, d, *J* 7.1 Hz), 4.45 (1H, d, *J* 7.1 Hz), 4.33 (1H, tt, *J* 11,1 and 4.3 Hz), 3.40 (3H, s), 3.36 (3H, s), 2.55 (1H, ddd, *J* 13.9, 4.3 Hz and 3.5 Hz), 2.10 (1H, ddd, *J* 13.1, 4.3 and 3.5 Hz), 1.63 (1H, dd, *J* 13.1 and 11.1 Hz), 1.62 (1H, dd, *J* 13.9 and 11.1 Hz), 1.31 (3H, s), 1.29 (3H, s), 1.11 (3H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 211.4 (C=O), 95.2 (CH_2-O), 92.0 (CH_2-O), 79.6 (C-O), 68,3 (CH-O), 55.7 (CH_3-O), 55.3 (CH_3-O), 46.1 (CH_2), 46.0 (CH_2), 44.0 (C), 27.5 (CH_3), 27.2 (CH_3), 21.6 (CH_3). IR ν_{max}/cm^{-1} 1708, 1149, 1102, 1044.

Synthesis of tert-butyl(but-3-yn-2-yloxy)dimethylsilane (11)

To a solution of commercial racemic 3-butyn-2-ol (0.4254 g, 6.0 mmol) in anhydrous THF (7 mL), were added a solution of imidazole (0.4085 g, 6.0 mmol) in anhydrous THF (7 mL). The reaction mixture was stirred at room temperature for 30 min, cooled to 0 °C and then a solution of TBDMSCl (1.0551 g, 7.0 mmol) in anhydrous THF (7 mL) was added. The reaction mixture was stirred overnight at room temperature and then water was added and the product was extracted with ethyl ether. The organic layer was washed with water, saturated brine, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by Kugelrohr distillation (bp 50 °C, 30 mm Hg) to yield **11** (1.0212 g, 5.5 mmol, 92%) as a colorless oil.

***Analytical data for compound 11*:** ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.50 (1H, qd, *J* 6.6 and 2.0 Hz), 2.36 (1H, d, *J* 2.0 Hz), 1.41 (3H, d, *J* 6.6 Hz), 0.89 (9H, s), 0.12 (3H, s), 0.10 (3H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 86.4 (C≡), 71.1 (≡C-H), 58.7 (CH-O), 25.7 (3 CH_3), 25.3 (CH_3), 18.2 (C), -5.0 (CH_3-Si), -4.7 (CH_3-Si). IR ν_{max}/cm^{-1} 3313, 1258, 1104, 1052, 778.

Synthesis of (2R,4S)-1-[3-(tert-butyldimethylsilanoxy)-but-1-ynyl]-2,4-bis-(methoxymethoxy)-2,6,6-trimethylcyclohexanol (12)

To a solution of compound **11** (0.3864 g, 2.10 mmol) in THF (4 mL) at -78 °C was added a solution of *n*-butyllithium in hexane (1.8 mL, 2.34 mmol) and stirred for 40 min. A solution of compound **10a** (0.2188 g, 0.84 mmol) in THF (1 mL) was added and stirred at -78 °C for 5 h. The reaction mixture was quenched by addition of a saturated aqueous solution of ammonium chloride and the product was extracted with ethyl ether. The ethereal layer was washed with water, saturated brine, dried over anhydrous $MgSO_4$ and evaporated. The residue was purified by column chromatography through silica gel using as an eluent a mixture of *n*-hexane and ethyl acetate (8:2) to give compound **12** (0.3183 g, 0.72 mmol, 85%) as a mixture of diastereomers that were used in the next step without further separation.

***Analytical data for compound 12*:** ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4,81 (2H, d, *J* 7.3 Hz),

4.69 (2H, d, *J* 7.3 Hz), 4.65 (2H, d, *J* 6.8 Hz), 4.62 (2H, d, *J* 6.8 Hz), 4.54 (1H, q, *J* 6.6 Hz), 4.53 (1H, q, *J* 6.6 Hz), 3.91 (2H, tt, *J* 11.1 and 4.3 Hz), 3.39 (6H, s), 3.34 (6H, s), 2.20 (2H, ddd, *J* 13.9, 4.3 and 2.3 Hz), 1.82-1.63 (6H, m), 1.47 (6H, s), 1.40 (3H, d, *J* 6.5 Hz), 1.39 (3H, d, *J* 6,5 Hz), 1.14 (6H, s), 1.12 (6H, s), 0.86 (18H, s), 0.08 (6H, s), 0.07 (6H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 94.9 (2 CH_2), 91.6 (2 CH_2), 89.3 (2 C-OH), 83.7 (2 C≡), 82.9 (2 C≡), 77.8 (2 C-O), 69.6 (2 CH-O), 58.9 (2 CH-OSi), 56.3 (2 CH_3), 55.1 (2 CH_3), 43.7 (CH_2), 43.6 (CH_2), 41.2 (CH_2), 41.1 (CH_2), 40.3 (2 C), 29.3 (2 CH_3), 25.7 (6 CH_3), 25.3 (CH_3), 25.2 (CH_3), 23.1 (2 CH_3), 21.7 (2 CH_3), 18.1 (2 C), -5.1 (2 CH_3-Si), -4.7 (2 CH_3-Si). IR ν_{max}/cm^{-1} 3442, 1252, 1145, 1101, 1048, 778.

Synthesis of (2R,4S)-1-[(E)-3-(tert-butyldimethylsilanoxy)-but-1-enyl]-2,4-bis(methoxy-methoxy)-2,6,6-trimethylcyclohexanol (13)

To a solution of compound **12** (0.0411 g, 0.092 mmol) in THF (2 mL), maintained at 0 °C under an nitrogen atmosphere, was added dropwise a solution of Red-Al® 70% in toluene (0.15 mL, 0.54 mmol). The reaction mixture was stirred at room temperature for 6 h. Cold water was added, and the produced white solid was filtered and washed several times with ethyl ether. The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography through silica gel using as an eluent a mixture of *n*-hexane and ethyl acetate (8:2) to yield compound **13** (0.0301 g, 0.067 mmol, 73%) as a mixture of diastereomers that could not be separated.

***Analytical data for compound 13*:** ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.83 (1H, dd, *J* 15.2 and 4.5 Hz), 5.82 (1H, dd, *J* 15,2 and 4.5 Hz), 5.71 (1H, dd, *J* 15.2 and 3.0 Hz), 5.70 (1H, dd, *J* 15.2 and 3.0 Hz), 4.80 (2H, d, *J* 7.3 Hz), 4.73 (2H, d, *J* 7.3 Hz), 4.68 (2H, d, *J* 6.8 Hz), 4.65 (2H, d, *J* 6.8 Hz), 4.30 (1H, qdd, *J* 6.5, 4.5 and 3.0 Hz), 4.29 (1H, qdd, *J* 6.5, 4.5 and 3.0 Hz), 3.99 (2H, tt, *J* 11.6 and 4.5 Hz), 3.42 (6H, s), 3.36 (6H, s), 2,26 (2H, ddd, *J* 13.9, 4.5 and 2.8 Hz), 1.77 (1H, ddd, *J* 13.4, 4.5 and 2.8 Hz),1.76 (1H, ddd, *J* 13.4, 4.5 and 2.8 Hz), 1.49-1.37 (4H, m), 1.16 (6H, s), 1.14 (6H, d, *J* 6.5 Hz), 1.10 (3H, s), 1.05 (3H, s), 0.89 (18H, s), 0.83 (3H, s), 0.79 (3H, s), 0.05 (6H, s), 0.04 (3H, s), 0,03 (3H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 133.4 (2 HC=), 126.8 (=CH), 126.6 (=CH), 94.3 (2 CH_2), 90.6 (2 CH_2), 82.1 (2 C-OH), 77.8 (2 C-O), 69.2 (2 C-O), 68.2 (CH-OSi), 68.1 (CH-OSi), 55.8 (2 CH_3), 54.5 (2 CH_3), 43.7 (2 CH_2), 40.7 (CH_2), 40.6 (CH_2), 38.8 (2 C), 28.3 (2 CH_3), 28.2 (CH_3), 25.7 (6 CH_3), 24.2 (2 CH_3), 24.0 (2 CH_3), 20.8 (CH_3), 20.7 (CH_3), 17.6 (2 C), -5.4 (2 CH_3-Si), -5.3 (2 CH_3-Si). IR ν_{max}/cm^{-1} 3561, 1250, 1147, 1099, 1051, 835, 775.

Synthesis of (3S,5R,6R/6S,7E,9R/9S)-megastigmane-7-ene-3,5,6,9-tetrol (1a and 1b)

Compound **13** (0.0241 g, 0.054 mmol) was dissolved in 2-propanol (1 mL) and

treated with PPTS (0.0272 g, 0.11 mmol). The reaction mixture was stirred at 50 °C for 48 h and then the solvent was removed under reduced pressure. The residue was purified by column chromatography through silica gel using as an eluent a mixture of *n*-hexane and ethyl acetate (1:1) to give **1a** and **1b** (0.0069 g, 0.028 mmol, 53%) in a 1:1 ratio, together another compound (0.0042 g, 0.015 mmol, 27%) still containing only one protective methoxymethoxy group. For analytical purposes, the diastereomers **1a** and **1b** were separated as colorless oils by column chromatography through silica gel using as an eluent a mixture of *n*-hexane and ethyl acetate (2:8).

Spectral data of isomer 1a (3S,5R,6S,9R/9S): ^{1}H NMR (400 MHz, CD_3OD) δ 5.75 (1H, d, *J* 15.5 Hz, H-7), 5.68 (1H, dd, *J* 15.5 and 6.5 Hz, H-8), 4.20 (1H, m, *J* 6.5 Hz, H-9), 4.02 (1H, tt, *J* 11.6 and 4.6 Hz, H-3), 1.88 (1H, ddd, *J* 13.5, 4.6 and 2.6 Hz, H-4eq), 1.55 (1H, ddd, *J* 12.5, 4.6 and 2.6 Hz, H-2eq), 1.43 (1H, dd, *J* 13.5 and 11.6 Hz, H-4ax), 1.39 (1H, dd, *J* 12.5 and 11.6 Hz, H-2ax), 1.19 (OH, br s), 1.14 (3H, s, H-13), 1.12 (3H, d, *J* 6.5 Hz, H-10), 0.94 (3H, s, H-11 or H-12), 0.72 (3H, s, H-12 or H-11). ^{13}C NMR (100 MHz, CD_3OD) δ 134.1 (C-8), 131.4 (C-7), 79.9 (C-6), 77.1 (C-5), 69.3 (C-9), 65.1 (C-3), 48.1 (C-2), 46.9 (C-4), 40.3 (C-1), 29.4 (C-11), 27.6 (C-12), 25.4 (C-13), 24.0 (C-10).

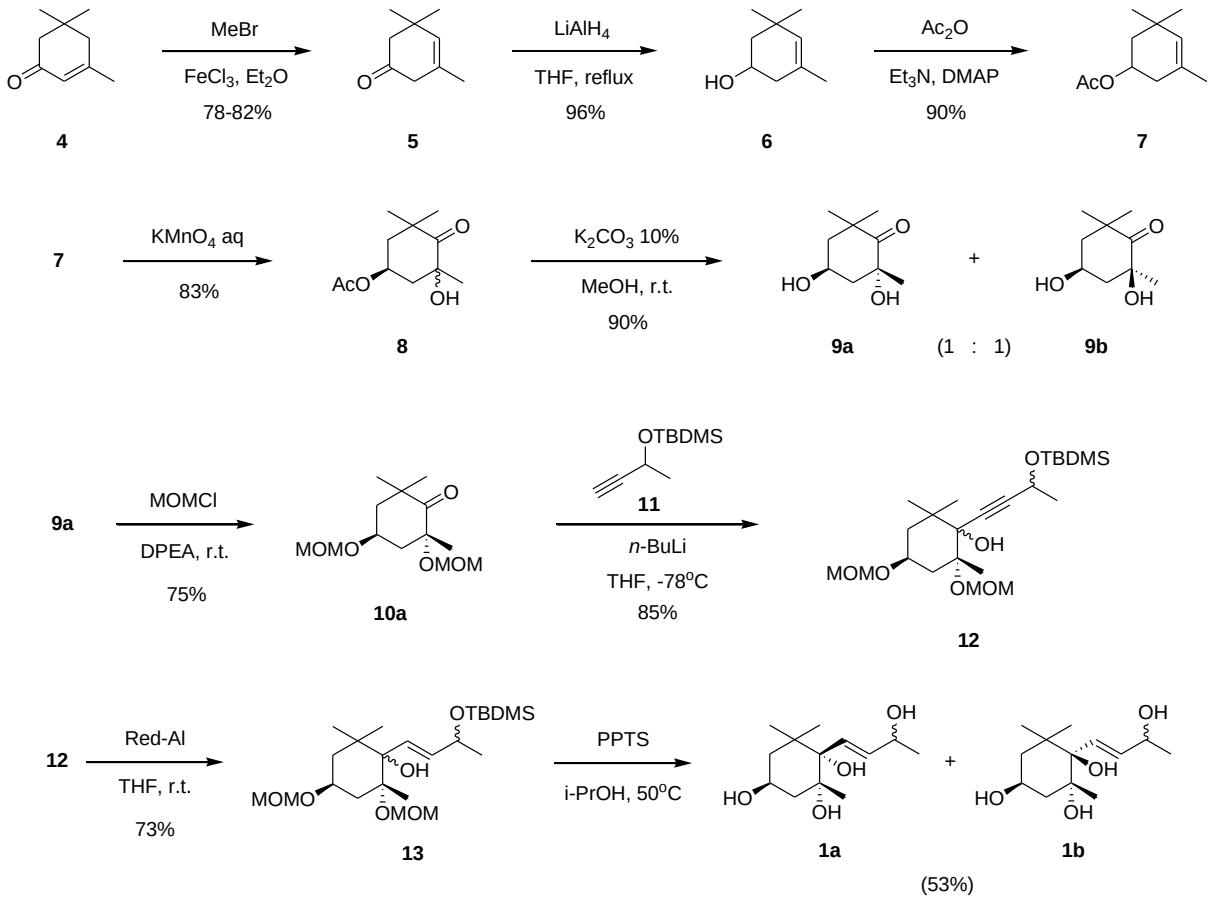
Spectral data of isomer 1b (3S,5R,6R,9R/9S): ^{1}H NMR (400 MHz, CD_3OD) δ 5.75 (1H, d, *J* 15.5 Hz, H-7), 5.67 (1H, dd, *J* 15.5 and 6.5 Hz, H-8), 4.20 (1H, m, *J* 6.5 Hz, H-9), 4.01 (1H, tt, *J* 11.4 and 4.5 Hz, H-3), 1.88 (1H, ddd, *J* 13.4, 4.5 and 2.7 Hz, H-4eq), 1.56 (1H, ddd, *J* 12.5, 4.4 and 2.7 Hz, H-2eq), 1.44 (1H, dd, *J* 13.4 and 11.6 Hz, H-4ax), 1.37 (1H, dd, *J* 12.5 and 11.6 Hz, H-2ax), 1.14 (3H, s, H-13), 1.12 (3H, d, *J* 6.5 Hz, H-10), 0.98 (3H, s, H-11 or H-12), 0.69 (3H, s, H-12 or H-11). ^{13}C NMR (100 MHz, CD_3OD) δ 134.9 (C-8), 129.9 (C-7), 79.9 (C-6), 77.7 (C-5), 68.3 (C-9), 65.0 (C-3), 45.0 (C-2), 44.6 (C-4), 39.5 (C-1), 26.5 (C-11), 26.3 (C-12), 25.3 (C-13), 23.5 (C-10).

Espectral data for mixture of 1a and 1b: IR ν_{max}/cm^{-1} 3395, 1699, 1368, 1229, 981. HRMS *m/z* Calcd for $C_{13}H_{24}O_4$: 244,1675. Found: 244,1642.

Results and Discussion

Several synthetic methods for the megastigmane skeleton have been reported [1, 6]. Our synthesis started from isophorone **4**, that by application of the classic method of Kharasch and Tawney [5] afforded the 3-cyclohexenone **5** in 78-82% yield after purification (Scheme 1). Racemic alcohol **6** was obtained by reduction of **5** with $LiAlH_4$ in 96% yield [7]. Treatment of alcohol **6** with acetic anhydride in triethylamine and 4-*N,N*-dimethylaminopyridine (DMAP) afforded the acetate **7** in 90% yield [8]. Oxidation of **7** with potassium permanganate in neutral aqueous medium gave the keto-alcohol **8** in 83% yield, as a mixture of diastereomers that were separated by preparative HPLC for

analytical purposes. Hydrolysis of the acetate group of **8** was accomplished with a K_2CO_3 solution (10%) in methanol [9] to afford a 1:1 mixture of the diastereomeric keto-diols **9a/9b** in 90% yield, which were separated by column chromatography through silica gel using a 1:1 mixture of *n*-hexane and ethyl acetate as eluent. The protection of the two hydroxyl groups of **9a** by reaction with methoxymethyl chloride and *N,N*-diisopropylethylamine (DPEA) [10] furnished compound **10a** in 75% yield. Reaction of the lithium derivative of compound **11** (obtained by protection of the commercial 3-butyn-2-ol with *tert*-butyldimethylsilyl chloride) [11] with ketone **10a** at -78°C afforded the alkyne **12** in 85% yield, as a 1:1 mixture of diastereomers that could not be separated. Subsequent reduction of **12** with Red-Al® furnished the *7-E* isomer of compound **13** in 73% yield [12]. Treatment of **13** under acidic conditions (HCl/MeOH) cleave the protective groups [10,13] and resulte in a complex mixture of products. On the other hand, the reaction of **13** with 2 equivalents of pyridinium *p*-toluenesulfonate (PPTS) [14] in 2-propanol at 50 °C for 48 h afforded a mixture of compounds **1a**/**1b** (53% yield) in a 1:1 ratio, together another compound (27%) still containing only one protective methoxymethoxy group.



Scheme 1. Synthesis of aripuanin **1** from isophorone **4**.

For analytical purposes, the four diastereomers of **1** were separated in two pairs

of epimers (**1a** and **1b**) by column chromatography through silica gel. The synthetic material **1b** exhibited spectral data in agreement with those reported earlier in literature for aripuanin [2], particularly with regard to the relative stereochemistry at carbons *3S*, *5R* and *6R* that actually corresponds to that of the natural product. However, the absolute configuration of the 9-position remained to be determined.

Conclusion

We have thus accomplished the first total synthesis of the natural product aripuanin **1**, in nine steps starting from commercial isophorone, in an overall yield of 6.5%.

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Full Paper

Análisis físico-químico del aceite de filete de tambaqui (*Colossoma macropomum*) cultivado en el Estado de Roraima, Brasil

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ABSTRACT: *The objective this work was to realize chemometrics analysis and to determine the physicochemical characteristics of tambaqui fish oil (Colossoma macropomum), grown in four localities in the state of Roraima, Alto Alegre (A), Bonfim (B), Uraricoera (U) and Passarão (P). Obtaining oil tambaqui was through his ground meat, dried in oven air circulation following agitation for one hour. Some physicochemical characteristics were analyzed: acidity index (AI), in oleic acid acidity (OAA), of iodine (II), peroxide (PI), saponification (SI), and refractive index (RI), humidity contents and ashes, melting point, density, viscosity, totals lipids and organics substances soluble in ether. For chemometrics analysis was applied to the multivariate analysis, PCA and HCA. The samples different of the localities were discriminated geographically. The samples U1, U2 and U3 showed highest values for II and RI, B1, B2 and B3 showed the highest values for AI, OAA, SI and PI, P1, P2 and P3 showed lowest values for AI, RI, SI, OAA and PI, the samples A1, A2 and A3 had the highest humidity content, density and viscosity.*

Keywords: *Colossoma macropomum; chemometry; fatty acids; HCA; PCA; tambaqui*

Introducción

Según Araujo-Lima y Goulding [1] el *Colossoma macropomum* (tambaqui) es nativo de la bacía Amazónica, Orinoco y sus afluentes. Es la especie que más despierta

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interés a la piscicultura, principalmente por la alta preferencia del consumidor y el alto precio de mercado. Pez bien adaptado a la piscicultura, posee gran capacidad de aprovechar varios tipos de alimentos, se presenta como excelente filtrador de plancton y posee rápido crecimiento, alcanza de 700 a 900 g en el primer año de cultivo y presenta buena resistencia al manoseo. El hábito alimentar de los adultos es muy amplio y predominantemente herbívoro, pero se puede alimentar de insectos, caracol y raramente de otros peces.

El tambaqui ha sido creado intensivamente en cautiverio en Brasil y en algunos países de Latino América, por presentar gran potencial para esa actividad y posee buenas calidades zootécnicas. El cultivo intensivo de peces requiere la utilización de una alimentación equilibrada, a la base de raciones que son formuladas con los más diversos ingredientes y procesos de elaboración, para un mejor aprovechamiento por los peces. En ese modelo de cultivo, el costo con alimentación podrá representar 60 a 80 % de los costos de producción de una piscigranja (o piscifactoría). Una de las alternativas para diminución de esos costos seria el uso de ingredientes regionales introducidos en las formulaciones de las raciones. En estudios de nutrición no bastan los conocimientos de los elementos que el animal consume, ni de los contenidos de nutrientes y energía, pero es necesario tener la idea de los niveles de consumo por la especie, para que se pueda subsidiar con informaciones más precisas para la elaboración de dietas que efectivamente se aproveche lo máximo [2].

El estado de Roraima exporta todos los anos para el estado del Amazonas 40% de la producción anual de pez en cautiverio. Esto significa que de las 2.492 toneladas comercializadas, 996 van para la mesa de los amazonenses [3]. Por otro lado, desde 2004 hay aumento de la producción de pescado en cautiverio en Roraima, con alto investimento de la iniciativa privada. Algunos creadores llegan a retirar de los tanques 500 toneladas en un año. Actualmente existen grandes intereses de las industrias alimenticias y farmacéuticas en la cuestión del aceite de peces, con vista a la tendencia del desarrollo de suplementos alimentares, implicando en el consumo de carne y aceite de peces [4].

El objetivo de este trabajo es de realizar análisis físico-químicas del aceite del pez de tambaqui: índice de acidez, acidez en ácido oleico, índice de yodo, índice de peróxido, índice de saponificación, índice de refracción, contenido de humedad, contenido de cenizas, punto de fusión, densidad, viscosidad, lípido totales y substancias orgánicas solubles en éter. Como también lo de realizar análisis exploratorio de estos dados a través de análisis multivariada, PCA (análisis de componentes principales) y HCA (análisis de agrupamientos jerárquicos), debido a la cantidad de variables analizadas.

Materiales y Métodos

Obtención de las muestras

Fueron colectados tres ejemplares de peces de forma aleatoria de un lote con cerca de dos mil tambaqui en lo cual habían sido capturado de cuatro lagunas de cultivo semi-intensivo. Este procedimiento fue realizado en cuatro localidades del estado de Roraima, a saber: Alto Alegre, Bonfim e Boa Vista (región de Passarão y Uraricoera). Las muestras fueron identificadas y analizadas separadamente. Los pescados poseían cerca de ocho meses de vida.

Secado, extracción y rendimiento del aceite

El método utilizado para la extracción del aceite fue a partir de modificaciones descrito por Duarte [5]:

Después de abatido los peces se retiraron sus filetes de carne y en seguida este fue triturado en una licuadora. Se pesó 100 g de filetes triturados, estos fueron llevados a la estufa de circulación de aire, en temperatura de 100 ^{o}C, por cerca de 8 hora hasta la masa constante. La muestra seca fue transferida para un erlenmeyer de 1000 mL donde fue añadido 20 g de sulfato de sodio anhidro y 400 mL de hexano. Luego este fue llevado a un mezclador, con agitación constante por una hora. Después de la agitación del material fueron hechas filtración y evaporación del solvente en rotavapor. El aceite extraído fue acondicionado en frasco ámbar en atmosfera de nitrógeno gaseoso para posterior análisis.

Rendimiento del aceite del filete de tambaqui

El cálculo de rendimiento fue obtenido por la relación entre la masa del aceite en 100 g de filete y el resultado expreso en masa de aceite / 100 g de filete.

Caracterización fisicoquímica

Las determinaciones hechas en los análisis de aceites y grasas son, generalmente, llamados índices, que son expresiones de propiedades físicas o químicas de los mismos y no los porcentajes de los constituyentes. Estos índices sirven para identificar y evaluar la mayoría de los aceites y grasas, siendo el resultado del análisis basado en este conjunto de dados.

Para los análisis de las constantes físicas de este aceite fueron realizados los análisis de punto de fusión, densidad, viscosidad, insolubles orgánicos en éter, índice de acidez, acidez en ácido oleico, índice de saponificación, índice de yodo, índice de peróxido, índice de refracción, contenido de humedad, contenido de cenizas y lípidos totales de acuerdo con la metodología del Instituto Adolfo Lutz [6]. Todos los análisis fueron hechos en triplicado y los resultados obtenidos con sus desvíos padrones.

Análisis estadística

Los resultados fueron sometidos al teste de variancia, ANOVA, y por lo teste de comparaciones múltiples de Tukey, en nivel de 5% de probabilidad utilizando el software Statistica 6.0. Los dados fueron sometidos al análisis exploratorio de dados: análisis de componentes principales – PCA (*Principal Components Analysis*) y análisis de agrupamientos jerárquicos – HCA (*Hierarchical Cluster Analysis*).

Los dados fueron previamente auto-escalados, antes de someterlos a los análisis de componentes principales y análisis de agrupamiento jerárquico, una vez que hay una gran variación de respuestas de las diversas variables, o sea, difieren en orden de grandeza, se atribuye así un mismo peso para todas las variables. Para la obtención de dendrograma del HCA fueron utilizados la distancia euclidiana y el método de conexión incremental, utilizando el software Einsight.

Resultados y Discusión

Humedad y lípidos totales en filete de pez

La Tabla 1 presenta los resultados de los contenidos de humedad y lípidos totales en el filete de tambaqui. Los resultados muestran las medias finales obtenidas de 100 g de filete.

Tabla 1. Dados del filete de tambaqui (*C. macropomum*)

Localidad	Masa húmeda, g	Masa seca, g	Humedad, %	Lípido total, %
Alto Alegre	100	22,92	77,08±0,54^{a}	3,05±0,19^{c}
Bonfim	100	32,57	67,43±0,32^{b}	8,16±0,13^{a}
Uraricoera	100	34,36	65,64±1,81^{bc}	6,97±0,08^{b}
Passarão	100	35,94	64,06±0,10^{c}	8,11±0,52^{a}
Media		31,45±5,85	68,55±5,36	6,57±2,19

Las medias seguidas de una misma letra, en la columna, no difieren estadísticamente, por el teste de Tukey en nivel de 5% de probabilidad.

Los resultados de los análisis de humedad entre los filetes de pez, indican estadísticamente al nivel de 5%, por el teste de Tukey, que el filete de tambaqui de Alto Alegre posee valores de humedad más elevados que los resultados encontrados de las humedades de filetes de peces de otras localidades estudiadas. Los filetes de peces de las localidades de Bonfim y Uraricoera, Uraricoera y Passarão no poseen diferencias significativas ($p < 0,05$) entre ellos. Los porcentajes de lípidos totales en el filete indican que solo los filetes de pez de Bonfim y Passarão no presentan diferencias significativas ($p < 0,05$). Los contenidos de lípidos en el filete de tambaqui varían de 3,05% a 8,16% media de 6,57 ± 2,41% así, no presenta diferencia significativa al nivel de 5% de probabilidad con los resultados obtenidos por Almeida [4], 7,6%, y Maia y Rodriguez-Amaya [7], 6,0%.

De acuerdo con los valores presentados en la Tabla 2, el tambaqui de la región de Alto Alegre puede ser clasificado como pez de bajo contenido de grasa, el pez de Uraricoera posee medio contenido y de Bonfim y Passarão con alto contenido de grasa según la clasificación de Ackman [8].

Para Castro [9] las diferencias existentes entre los lípidos totales en los filetes de esos peces, pueden variar en una misma especie de acuerdo con la edad, maturación sexual, variación sazonal, dieta alimentar entre otros factores.

En la Tabla 2, se encuentran los resultados de rendimiento de aceite del filete (%), punto de fusión (ºC), densidad (g/cm^3) y las medias de los resultados encontrados.

Tabla 2. Dados del aceite de filete de tambaqui

Localidad	Rendimiento, %	Punto de fusion, ºC	Densidad, g/cm^3	Viscosidad, ºC
Alto Alegre	3,28±0,14^c	30^a	0,910±0,003^a	44,14
Bonfim	8,74±0,19^a	29^b	0,908±0,001^{ab}	42,69
Urariquera	7,58±0,21^b	29^b	0,905±0,001^b	39,20
Passarão	8,69±0,29^a	29^b	0,906±0,001^{ab}	40,73
Media	7,07±2,09	29,00±0,82	0,907±0,002	41,69±2,17

Las medias seguidas de una misma letra, en la columna, no difieren estadísticamente, por el teste de Tukey en nivel de 5% de probabilidad.

Comparando los resultados de los rendimientos de aceite del filete de pez de las localidades estudiadas, se verificó que el aceite de pez de la localidad de Alto Alegre posee significativamente ($p < 0,05$) un valor menor cuando comparados al aceite de pez de las localidades de Bonfim, Uraricoera y Passarão. Los mayores rendimientos fueron encontrados en Bonfim y Passarão. En el análisis de resultados de los puntos de fusión, solo en Alto Alegre posee diferencia significativa ($p < 0,05$), esto, posiblemente, se debe a la presencia de una gran concentración de ácidos grasos saturados comparados con los aceites de pez de otras localidades.

Las densidades de los aceites de peces en las localidades estudiadas presentaron valores muy próximos, pero existe una diferencia significativa al nivel de 5% de probabilidad entre los cultivados en Alto Alegre y Uraricoera. Esas pequeñas variaciones de densidades pueden haber ocurrido debido a una diferencia de ácidos grasos saturados entre los aceite, pues cuanto mayor el porcentual de ácidos grasos insaturados, menor será la densidad [10].

En la Tabla 3, se encuentran los resultados de las características físico-químicas del aceite de filete de tambaqui: índices de acidez, IA, (mgKOH/g muestra), acidez en

ácido oleico, AAO, (%), índice de yodo, IY, (gI_2/100g muestra), índice de saponificación, IS, (mgKOH/g muestra), índice de refracción, (40 ºC), IR, índice de peróxido, IP, (número de mols/1000g de la muestra) y las medias de esos resultados.

Tabla 3. Características físico-química del aceite de *C. macropomum*

	Alto Alegre	Bonfim	Uraricoera	Passarão	Morais *et al.* [11]	Hartman y Esteves [12]
IA	4,27±0,26[a]	4,37±0,29[a]	1,93±0,08[b]	1,42±0,17[b]	6,80±0,70	7,50±5,50
AAO	2,14±0,04[a]	2,34±0,36[a]	0,97±0,04[b]	0,91±0,04[b]	-	-
IY	143,17±0,16[b]	148,63±0,54[a]	149,91±0,82[a]	148,89±0,54[a]	130±8,0	155±15
IS	195,45±0,43[a]	196,37±0,59[a]	196,12±0,69[a]	194,98±0,09[a]	191±1,0	179,3±15
IR	1,459±0,001[a]	1,459±0,001[a]	1,461±0,001[a]	1,460±0,003[a]	-	-
IP	2,65±0,18[a]	2,72±0,29[a]	2,66±0,30[a]	2,31±0,15[a]	2,5±0,3	-

Las medias seguidas de una misma letra, en la columna, no difieren estadísticamente, por el teste de Tukey en nivel de 5% de probabilidad.

Observando los resultados de índice de acidez de ese trabajo se puede decir que existe una diferencia significativa al nivel de 5% de probabilidad entre los resultados encontrados en aceite de peces de las localidades de Alto Alegre y Bonfim con los resultados de Uraricoera y Passarão. Según Santos *et al.* [13] el aceite que presenta AAO inferior a 1% son clasificados como del tipo 1, presentando en lo máximo 2,5% de acidez es considerado del tipo 2 y encima de ese valor es considerado tipo 3. Los resultados confirman que según esa clasificación, los aceites de peces de Alto Alegre y Bonfim son del tipo 2 mientras los aceites de peces de Uraricoera y Passarão son del tipo 1. Esta variable está íntimamente relacionada con la naturaleza y calidad de la materia prima, grado de pureza de la grasa, como también depende del procesamiento y las condiciones de conservación del aceite [10].

Comparando los resultados de este trabajo con los de Morais *et al.* [11] y Hartman y Esteves [12] para la acidez de los aceites, demuestran un indicativo que el procesamiento en la extracción del aceite y su conservación resultaron con un buen desempeño.

El IY es un indicativo cualitativo en los aceites, o sea, cuanto mayor la insaturación de un ácido graso, mayor será su capacidad de absorción de yodo y, consecuentemente, mayor será ese índice [10]. Los aceites de peces de la región de Alto

Alegre poseen diferencias significativas al nivel de 5% de probabilidad con los aceites de las demás regiones, ese hecho posibilita informar que ese aceite posee una menor cantidad de ácidos grasos insaturados que los aceites de peces de otras regiones implicando en un mayor grado de saturación. Comparando a los resultados de Morais y colaboradores [11] y Hartman y Esteves [12] se puede decir que los aceites de peces analizados poseen valores intermediarios de insaturación.

Los resultados demuestran que no existen diferencias significativas al nivel de 5% de probabilidad en los resultados de los IS, eso muestra que los aceites de peces de las regiones estudiadas poseen semejanzas en termos de pesos moleculares de los ácidos grasos, pues ese índice é un indicativo en la calidad de los aceites, como puede establecer el grado de deterioro y la estabilidad del aceite. Comparando los resultados en este trabajo con la literatura, los valores obtenidos por Morais *et al.* [11], poseen masas moleculares próximas, mientras que los valores apreciados por Hartman y Esteves [12] indican que las masas moleculares de los ácidos grasos poseen valores inferiores a los del presente trabajo.

Los IP en este trabajo se llevaron a cabo simplemente para acompañar el estado de oxidación de los aceites, una variación de este índice indica un avanzo en la oxidación del aceite. Según Malacrida [14], los aceites no deben superar el valor de 10 meq/1000g de muestra, pues indica una baja posibilidad de deterioro oxidativo. Los resultados presentados en este trabajo indican valores bajos, evidenciando avanzos no significativos en la oxidación, pues durante los procedimientos no hubo cambios expresivos.

Análisis de componentes principales (PCA)

Realizando análisis de componentes principales (PCA) se verificó que los componentes PC1 e PC2 describen 93,21% de la variación total de los dados y fornecen informaciones discriminatorias de las muestras. Siendo que el primer componente principal (PC1) describe 50,15% de la variación total, 43,06% de la variación total de los dados es descrita por el según componente principal (PC2). A través del gráfico de scores (Figura 1) se observa la formación de cuatro agrupamientos.

Analizando PC1 *versus* PC2 es posible observar la discriminación de los cuatro diferentes municipios del estado de Roraima: Uraricoera (U1, U2 y U3), Passarão (P1, P2 y P3), Alto Alegre (A1, A2 y A3) y Bonfim (B1, B2 y B3). Analizando apenas PC1 es posible observar la formación de dos grandes grupos del lado derecho de las muestras de los municipios de Bonfim y Alto Alegre que de acuerdo con el AAO, los aceites de pez de estos municipios son del tipo 2 mientras que el agrupamiento del lado izquierdo del PC1, están localizados las muestras provenientes de las localidades de Uraricoera y Passarão los aceites de los peces son del tipo 1.

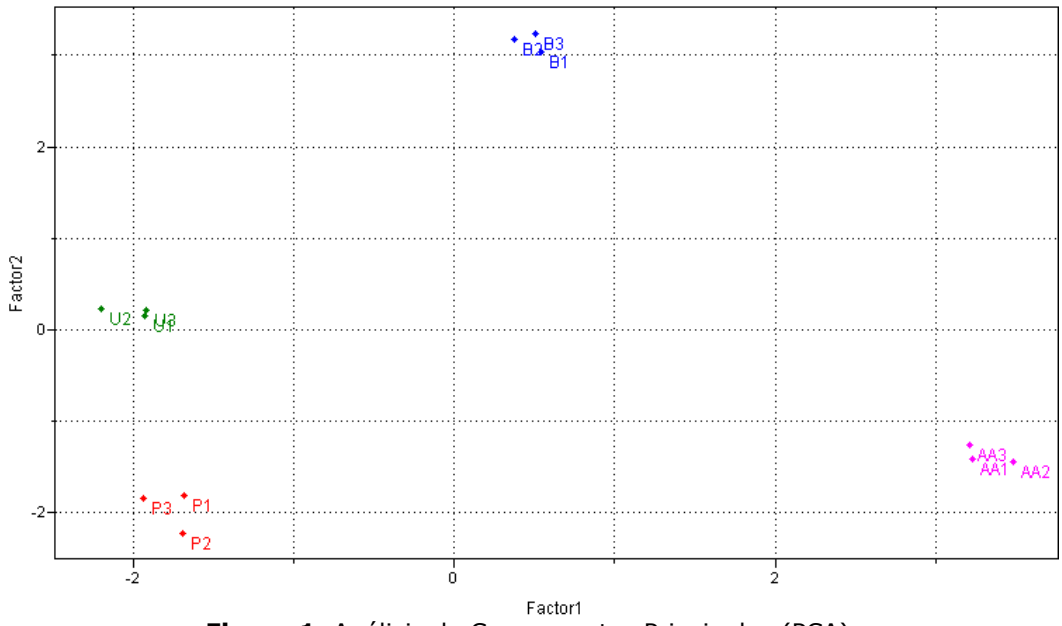

Figura 1. Análisis de Componentes Principales (PCA).

A través del gráfico de los *loadings* (Figura 2) es posible observar las influencias de las variables sobre las muestras, gráfico de los scores. Las diferentes muestras proveniente de diferentes localidades de Roraima fueron discriminadas geográficamente porque, visualizando el gráfico de *loadings*, las muestras U1, U2 y U3 presentaron los mayores valores de IY y IR, las muestras B1, B2 y B3 presentan los mayores contenidos de IA, AAO, IS y IP, las muestras de P1, P2 y P3 presentan los menores valores de IA, IR, IS, AAO y los menores contenidos de IP, las muestras A1, A2 y A3 presentan los mayores contenidos de humedad, densidad y viscosidad, Bonfim y Passarão presentaron los mayores porcentajes de rendimiento.

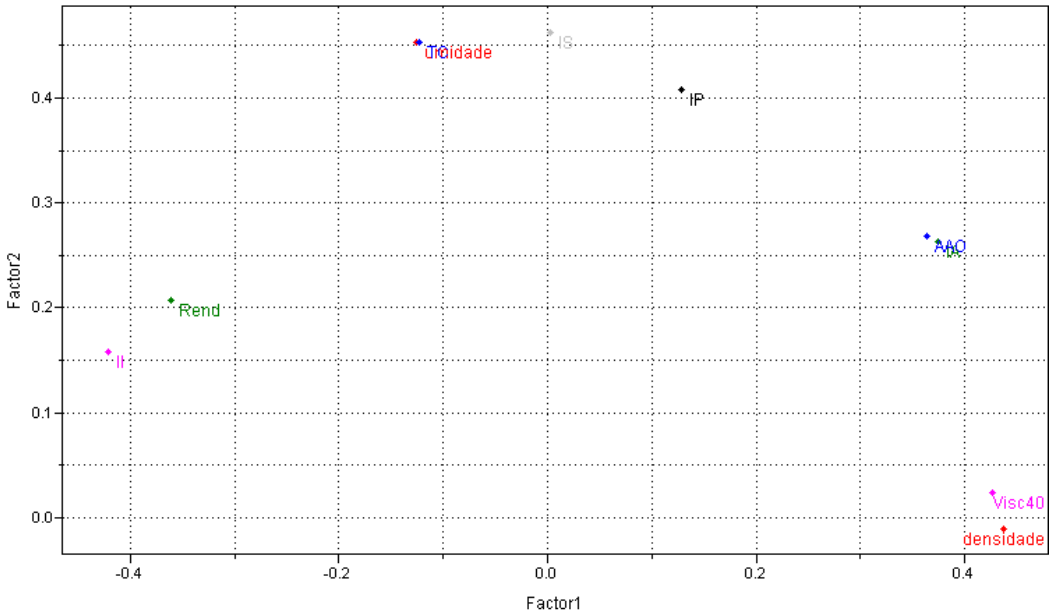

Figura 2. Análisis de Componentes Principales (PCA).

Análisis de agrupamientos jerárquicos (HCA)

Las tendencias observadas a través del PCA fueron confirmadas a través del dendrograma obtenido por el HCA (Figura 3), o sea, es posible observar la formación de cuatro agrupamientos, la discriminación de las cuatro diferentes localidades donde fueron colectadas las muestras de tambaqui, y la formación de dos grandes agrupamientos, las muestras de Bonfim y Alto Alegre (aceite de pez tipo 2) y Passarão y Uraricoera (aceite de pez tipo1).

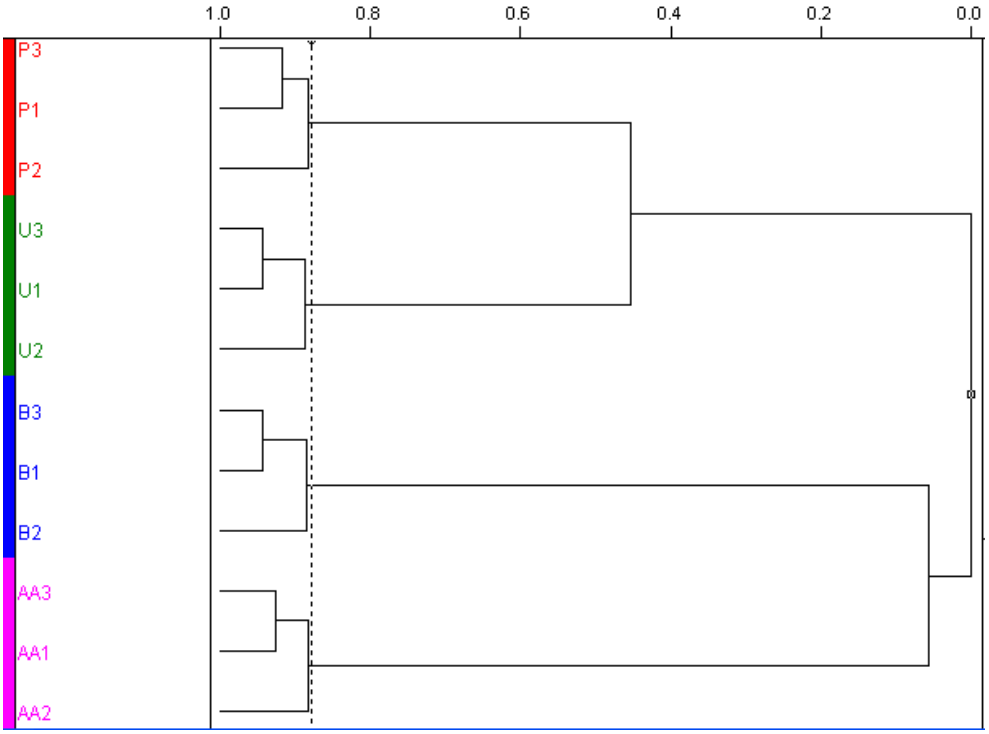

Figura 3. Análisis de Agrupamientos Jerárquicos (HCA).

Conclusiones

Los resultados físico-químicos obtenidos para el aceite de filete de tambaqui de Alto Alegre, Bonfim, Uraricoera e Passarão, muestran la importancia de estas caracterizaciones, pues a través de ellas es posible evaluar la calidad del aceite en cada localidad. La masa corporal del pez es un factor importante para los piscicultores, en vista del factor económico. A través de la aplicación del HCA y PCA fue posible obtener informaciones rápidas y eficientes sobre la igualdad entre las muestras a través de la visualización gráfica. Siendo así, es posible clasificar las muestras de acuerdo con su local de origen y el tipo de aceite de pez.

Agradecimientos

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Full Paper

Evaluation of biological activity of dioxouranium complexes of some Schiff base and dithiocarbamate ligands

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ABSTRACT: *A number of dioxouranium (VI), UO_2^{2+} complexes of Schiff base and monodithiocarbamate ligands have been synthesized and characterized. Antimicrobial activities of these complexes have been studied. The results of inhibition zones of the selected microorganisms showed that all complexes exhibited mild to prominent activities against the pathogens tested herein.*

Keywords: *Schiff base and dithiocarbamate ligands; uranium complexes; anti-microbial activity*

Introduction

Schiff bases are versatile ligands which are synthesized from the condensation of an amino compound with carbonyl compounds. These compounds and their metal complexes are very important as catalysts in various biological systems, polymers, dyes and medicinal and pharmaceutical fields. Their use in birth control, food packages and as an O_2 detector is also outlined. Transition metal complexes with Schiff base as ligand have been amongst the widely studied coordination compounds in the past few years, since they are found to be widely applicable in many fields such as biochemical and analytical fields. They have actively associated with antibacterial, antifungal, antitubercular and anticancer activities.

Antibacterial activity of complexes of Cu (II), Ni (II), Zn (II), Fe (II), Co (II), Mn

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(II), Vo (IV), Hg (II) and Cd (II) metal ions with Schiff base derived from salicylaldehyde and *o*-amino benzoic acid have been studied [1-3]. Antimicrobial activity of dithiocarbamate and their titanium complexes have also been studied [4].

Antibacterial activity of complexes of Cu (II), Ni (II), Zn (II), Fe (II), Co (II), Mn (II), Vo (IV), Hg (II) and Cd (II) metal ions with Schiff base derived from salicylaldehyde and *o*-amino benzoic acid have been studied [1-3]. Antimicrobial activity of dithiocarbamate and their titanium complexes have also been studied [4].

Dioxouranium (VI), UO_2^{2+}, is one of the most studied oxocations for which a large number of complexes with varying geometries ranging the coordination numbers from 7 to 12 are possible [5]. Dioxouranium (VI), UO_2^{2+} complexes of Schiff bases or dithiocarbamates are few. Patil and Shaikh [6] reported the synthesis, characterization and antibacterial studies of mixed ligand dioxouranium(VI) complexes prepared with 8-hydroxyquinoline as a primary ligand and amino acids. Chowdhury et al. prepared several new dioxouranium (VI) complexes of Schiff bases from salicylaldehyde, substituted salicylaldehydes, 2-hydroxy-1-naphthaldehyde with 2-aminothiophenol [7] and aniline [8]. Complexes with mono-dithiocarbamate $(dtcH)_2$ also ligands themselves have practical application in agriculture and in medicine as fungicide and pesticides [9]. Chowdhury et al. also studied synthesis and characterization of dioxouranium (VI), UO_2^{2+} complexes of bidentate monodithiocarbamate ligands [10]. Antimicrobial activities of uranium complexes have not been widely studied. The aim of this work is to study the possible biological antifungal and antibacterial activity of dioxouranium (VI), UO_2^{2+} complexes of the bidentate and tridentate Schiff bases and bidentate monodithiocarbamate ligands.

Material and Methods

Uranylnitrate hexahydrate, $UO_2(NO_3)_2.6H_2O$, was obtained from BDH Chemicals Ltd. Ethylenediamine, *N,N*-dimethylethylenediamine, *N,N*-diethylethylenediamine, 1,3-propanediamine, *N,N*-dibutyl-tri-methylenediamine, 1,6–hexanediamine, carbondisulphide, methanol, ethanol, chloroform, *N,N*-dimethylformamide were obtained from M/S. E . Merck, west Germany. Salicylaldehyde (Sal)/substituted salicylaldehyde, 5-chloro-salicylaldehyde (^{5}Cl-Sal), 5-bromo-salicylaldehyde (^{5}Br-Sal), 2-hydroxyacetphenone (HAP), 2-hydroxypropiophenone (HPP), 2-hydroxy-1-napthaldehyde (HNP), 2-aminothiophenol (OATP), aniline (Ani) and other chemicals used were obtained from the M/S Aldrich Chemicals Co. Ltd.

Preparation of monobasic bidentate Schiff base ligands, (LH)

Salicylaldehyde or substituted salicylaldehyde (Sal), 2-hydroxyacetphenone (HAP), 2-hydroxypropiophenone (HPP), 2-hydroxy-1-napthaldehyde (HNP) (10 mmol

each) were reacted with aniline (Ani) in a round bottom flask, containing 50 mL ethanol, fitted with a reflux condenser and a silica gel guard tube. Each mixture was refluxed for 30 min. with continuous stirring, using magnetic stirrer. The mixture was then cooled in ice-bath and kept over night where upon crystalline precipitate of respective ligands separated out. The product was filtered off, washed with ethanol and dried in vacuo over calcium chloride. Colour, yield and melting points of prepared Schiff base ligands are given in Table 1.

Table 1. Physical state, colour and melting point of the prepared monobasic bidentate Schiff bases.

Ligand	Physical state	Colour	Yield (%)	m.p.(°C)
Sal-AnilineH	Solid	Pale green	80	48
^{5}BrSal-AnilineH	Solid	Orange	85	112
^{5}ClSal-AnilineH	Solid	Orange	80	100
HNP-AnilineH	Solid	Deep orange	80	62
HAP-AnilineH	Liquid	Light yellow	-	-
HPP-AnilineH	Liquid	Light orange	-	-

Preparation of dibasic tridentate Schiff base ligand, L´H$_2$

10 mmol of salicylaldehyde, substituted salicylaldehydes (Sal), 2-hydroxyacetphenone (HAP), 2-hydroxypropiophenone (HPP) or 2-hydroxy-1-napthaldehyde (HNP) with 10 mmol of 2-aminothiophenol (OATP) were taken in a round bottomed flask, containing 50 mL of ethanol, fitted with a reflux condenser and a silica gel guard tube. The mixture was refluxed for 30 min. It was then cooled in an ice-bath and kept over night whereupon crystalline precipitate of the respective ligand was formed. The product was filtered off and washed with ethanol and dried in vacuo over calcium chloride. Colour, yield and melting points of the prepared Schiff base ligands are given in Table 2.

Table 2. Colour, yield and melting point of the prepared dibasic tridentate Schiff bases.

Ligand	Colour	Yield (%)	m.p. (°C)
Sal – OATPH$_2$	Yellow	80	139
^{5}BrSal – OATPH$_2$	Yellow	80	137
^{5}ClSal – OATPH$_2$	Yellow	80	144
HNP – OATPH$_2$	Pale yellow	90	136
HAP – OATPH$_2$	Light green	60	90
HPP – OATPH$_2$	Green	60	76

Preparation of some diamine-monodithiocarbamates, (dtcH)

Diamine or their substituted diamine(150 mmol) in 50 mL methanol was introduced in a 250 mL conical flask and the mixture was allowed to cool in a freezing mixture of ice and salt (0 ^{o}C). To this carbondisulphide (140 mmol; a bit less to protect formation of bis-derivative) was added drop-wise over a period of about half an hour with constant stirring, immediately white oily precipitate was formed. The precipitate was allowed to stand for about 5 hours in the ice-salt bath and thereafter filtered off and

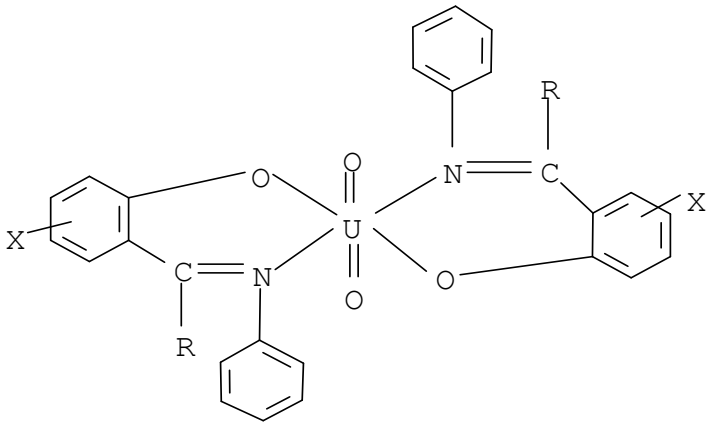
washed with methanol and dried over calcium chloride in a vacuum desiccator. Colour, yield and melting points of the prepared Schiff base ligands are given in Table 3.

Table 3. Colour, yield and melting points of the prepared Dicarbamate ligands

Sl. No.	Ligands	Physical state	Color	Yield (%)	m.p. °C
1	en-mono-dtc	Solid	White	85	178
2	dme-en-mono-dtc	Solid	White	85	135
3	det-en-mono-dtc	Solid	Light yellow	80	140
4	1,3-pn-mono-dtc	Solid	White	80	192
5	dbtt-tda-mono-dtc	Solid	White	75	98
6	Had-mono-dtc	Solid	White	80	128

Preparation of complexes, UO_2L_2

The ligand (2 mmol) was taken in a round flux. Ethanol (15 mL) and distilled water (10 mL) were added to this. The mixture was refluxed at 60°C in a paraffin bath and was stirred for a few minutes by a magnetic stirrer. Then uranylnitrate hexahydrate, $UO_2(NO_3)_2.6H_2O$ (1 mmol) was added to this solution. The colour of the solution was changed into orange–red. The mixture was refluxed at 60 °C for half an hour with constant stirring when yellow precipitate separated out. The precipitate was filtered and washed with ethanol and finally dried under vacuum over $CaCl_2$. Colour, yield and melting points of the prepared complexes are given in Table 4.



$[UO_2L_2]$

X= Br, Cl,4,5-fused phenyl

R= H, CH_3, CH_3CH_2,

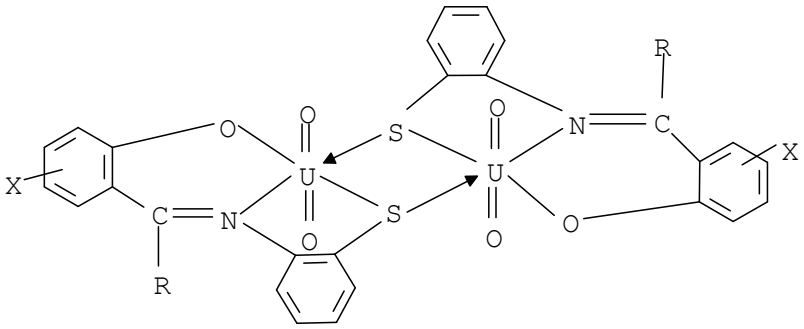
Figure 1. Proposed structure of complexes of monobasic bidentate Schiff base Ligands, (LH).

Table 4. Analytical and some physical data for the of the prepared dioxouranium (VI) complexes with monobasic bidentate Schiff bases

Sl. No	Complexes	Colour	Yield (%)	m.p. (°C)	% M*
1	UO_2(Sal- Aniline)$_2$	Orange	85	250	34.49 (35.93)
2	UO_2(^{5}BrSal- Aniline)$_2$	Orange	70	247	27.92 (29.02)
3	UO_2(^{5}ClSal-Aniline)$_2$	Deep yellow	75	250	31.83 (32.55)
4	UO_2(HNP- Aniline)$_2$	Orange	85	270	30.86 (31.21)
5	UO_2(HAP- Aniline)$_2$	Deep orange	75	208	33.02 (34.47)
6	UO_2(HPP- Aniline)$_2$	Yellow	40	150	32.45 (33.13)

Preparation of complexes, $[UO_2L´]_2$

4 mmol of respective ligand ($L´H_2$) was taken in a round bottomed flask containing 20 mL of a 10:1 mixture of dichloromethane and ethanol. When hot $UO_2(NO_3)_2.6H_2O$ (4 mmol) was added to this with continuous stirring, colour changed immediately. The mixture was refluxed for one and half hour on water bath when coloured precipitate came out. The precipitate was filtered in a sintered funnel, washed with the same solvent and was dried in vacuo over calcium chloride. Colour, yield and melting points of the prepared complexes are given in Table 5.



$[UO_2L]_2$

X= Br, Cl,4,5-fused phenyl

R= H, CH_3, CH_3CH_2,

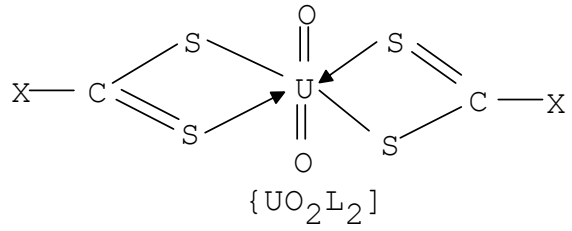
Figure 2. Proposed structure of complexes of dibasic tridentate Schiff base Ligand, $L´H_2$.

Table 5. Analytical and some physical data of the prepared dioxouranium (VI) complexes with dibasic tridentate Schiff bases

Sl. No	Complexes	Colour	Yield (%)	m.p. °C	% M*
1	$[UO_2(Sal\text{-}OATP)]_2$	Dark brown	50	>250	46.27 (47.89)
2	$[UO_2(^5BrSal\text{-}OATP)]_2$	Deep yellow	70	166	40.01 (41.31)
3	$[UO_2(^5ClSal\text{-}OATP)]_2$	Green	60	162	44.04 (44.76)
4	$[UO_2(HNP\text{-}OATP)]_2$	Deep orange	80	208	43.27 (43.49)
5	$[UO_2(HAP\text{-}OATP)]_2$	Dark violet	50	>250	45.91 (46.55)
6	$[UO_2(HPP\text{-}OATP)]_2$	Black	50	>250	44.21 (45.31)

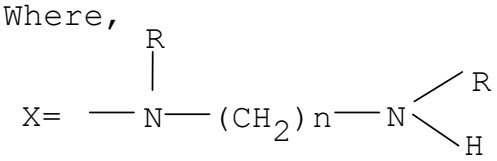
Preparation of complexes, UO_2dtc_2

4 mmol of respective ligand (LH) was taken in a round bottom flask containing 20 mL of mixed solvent of dichloromethane and ethanol by 10:1 (v/v). At reflux $UO_2(NO_3)_2.6H_2O$ (2 mmol) was added to this with continuous stirring when colour changed immediately. The mixture was refluxed for one and half an hour on water bath when coloured precipitate came out. Mixture was then kept overnight. The precipitate was filtered in sintered funnel, washed with same solvent, and dried in vacuo over calcium chloride. Colour, yield and melting points of the prepared complexes are given in Table 6.



$\{UO_2L_2\}$

Where,



n= 2,3,6

R= H, CH_3-, CH_3-CH_2-, -$(CH_2)_3$-CH_3

Figure 3. Proposed structure of the complexes of diamine-monodithiocarbamate ligands, (dtcH).

Table 6. Analytical and some physical data of the complexes of diamine-monodithiocarbamate ligands

Sl. No.	Complexes	Color	Yield (%)	m.p. °C	M (%)*
1	UO_2(en-mono-dtc)$_2$	yellow	70	>250	43.20 (44.04)
2	UO_2(dme-en-mono-dtc)$_2$	yellow	75	>250	38.13 (39.90)
3	UO_2(det-en-mono-dtc)$_2$	yellow	75	>250	35.45 (36.47)
4	UO_2(1,3-pn-mono-dtc)$_2$	yellow	75	>250	40.60 (41.88)
5	UO_2(dbtt-tda-mono-dtc)$_2$	yellow	65	>250	29.30 (30.02)
6	UO_2(Had-mono-dtc)$_2$	yellow	65	>250	35.60 (36.49)

*Values in parentheses indicate calculated values.

Characterization of the complexes

All the prepared complexes have been synthesized and characterized by some physico-chemical studies; elemental, spectral (IR, UV, NMR), magnetic and conductance analyses [7, 8, 10].

Evaluation of the prepared complexes against bacteria

For the detection of antibacterial activities and sensitivity spectrum analysis, the disc diffusion method by Bauer et al. [4, 11] was followed. Nutrient Agar (NA) was used as basal medium for culture of test bacteria and N, N-dimethylformamide (DMF) was used as the solvent to prepare the desired solution (1%) of the compounds initially.

Nutrient Agar (NA) medium was prepared using the composition Beef extract, Peptone, NaCl, Agar and Distilled water. 1000 mL of distilled water was taken in a beaker and 15 g of agar powder, 3 g of beef extract, 5 g of peptone and 0.5 g of NaCl were added slowly in and they were mixed thoroughly with a glass rod and then heated to boiling for 10 min. After 10 min. of boiling, the medium was transferred in 250 mL conical flasks at the rate of 200 mL per flask. The conical flasks were closed with the cotton plugs and autoclaved at 121 °C and 15 psi pressure for 15 min. and then culturing of different micro-organisms was performed.

Sensitivity spectrum analysis: Paper discs of 5 mm diameter were soaked with 10 μL of 2% solution of the test complexes. 0.2 mL of the suspension of test organism was taken in sterilized glass Petri plates of 100 cm diameter and then the molted and cooled (45 ^{o}C) NA medium was poured at the rate of 10 mL per Petri plate and shaked gently. Then the discs with test complexes were placed on the seeded agar plate. A control plate was also maintained in each case with solvent. The plates were kept firstly for 24 hours at low temperature (4 ^{o}C) and the test complex diffused from the disc to the surrounding medium by this time. The plates were then incubated at 35±2 ^{o}C for growth of test organisms and were observed at 24 hours interval for two days. The activity was determined by measuring the diameter of the zone of inhibition in mm.

Evaluation of chemicals against fungi

The antifungal activities of the test complexes were studied against four plant pathogenic fungi. Potato Dextrose Agar (PDA) was used as basal medium for culture of test fungi. Dimethyl formamide (DMF), was used as solvents to prepare the desired solution (2%) of the test complexes. Proper control was maintained with DMF in each case of fungi. The materials and methods of the present work is described below in detail.

Preparation of the PDA (Potato Dextrose Agar) medium: PDA (Potato Dextrose Agar) medium was used throughout the study. The compositions of PDA medium are as Potato 200 g, dextrose 20 g, Agar 15 g, Distilled water 1000 mL. 200 g of sliced potato was boiled in 500 mL distilled water and the extract was decanted after proper boiling. The extract was taken in a 1000 mL beaker and the solution was made up to the mark with distilled water and 20 g dextrose was added in the solution, then 15 g of agar powder was added in the solution and they were mixed thoroughly with a glass rod. After 10 min. of boiling, the medium was transferred to a 250 mL conical flask. Then the medium was autoclaved at 121 °C and 15 psi pressure for 15 minutes.

Preparation and Maintenance of cultures: Glass petri plates were sterilized and the molted sterilized PDA medium was poured at the rate of 10 mL per petri plate. After solidification of the medium, the small portions of mycelium of each fungus were placed carefully at the centre of each PDA plate with the help of sterilized needles. Thus each fungus was transferred to a number of PDA plates. Test tube slants of PDA medium were prepared for the maintenance of cultures. Small portions of mycelia of the collected pathogens were transferred to the test tubes separately from old cultures with the help of sterilized needles. A number of test tubes were freshly prepared for each fungal pathogen. The inoculated slants were incubated at room temperature under laboratory conditions.

Efficacy of the Chemicals: Efficacy of the chemicals was tested by "poison food

technique" [12]. The petri plates of 70-90 mm in diameter were used throughout this experiment. 0.01 mL of test complexes with definite concentration (2%) was taken in sterilized glass petri plates and then molted sterilized PDA medium was poured at the rate of 10 mL per petri plate. Proper control was maintained separately with sterilized PDA medium without complex but in the presence of solvent. After solidification of the medium, the fungal inoculums (5 mm mycelia block) were placed at the center of each petri plate. All the plates were inoculated at room temperature on the laboratory desk for five days. The linear growth of fungal colony was measured in two directions at right angle to each other after five days of incubation. The percentage inhibition of mycelia growth of test fungi was calculated as follows:

$$I = [C\text{-}TIC] \times 100$$

Where, I =Percentage of inhibition; C = Diameter of fungal colony in control; T = Diameter of fungal colony in treatment.

The test tube cultures of the fungal pathogens were prepared in the pathology laboratory of the Department of Microbiology, University of Chittagong. Table represents the identification no. and name of the synthetic compounds used and microrganisms.

Table 7. Identification no. and name of the synthetic compounds used and microganisms used for the evaluation microbial activity

Sl No.	ID no.	Name of the complex	*Selected bacteria*	*Fungal pathogens*
1	M_1	$[UO_2(Sal\text{-}OATP)]_2$	*Bacillus cereus* *Bacillus subtilis* *Bacillus megaterium* *Salmonella paratyphi* *Salmonella typhi* *Shigella dysenteriae* *Staphylococcus aureus* *Pseudomonas aeruginosa* *INABA ET (Vibrio)* *Escherichia coli*	*Fusarium equiseti (Corda) Sacc.* *Curvularia lunata (Wakker) Boedijn* *Macrophomina phaseolina (Maubl) Ashby.* *Botrydiplodia theobromae Pat.*
2	M_2	$[UO_2(^5BrSal\text{-}OATP)]_2$		
3	M_3	$[UO_2(^5ClSal\text{-}OATP)]_2$		
4	M_4	$[UO_2(HNP\text{-}OATP)]_2$		
5	M_5	$[UO_2(HPP\text{-}OATP)]_2$		
6	M_6	$[UO_2(HAP\text{-}OATP)]_2$		
7	M_7	$UO_2(Sal\text{-}Aniline)_2$		
8	M_8	$UO_2(HAP\text{-}Aniline)_2$		
9	M_9	$UO_2(HPP\text{-}Aniline)_2$		
10	M_{10}	$UO_2(HNP\text{-}Aniline)_2$		
11	M_{11}	$UO_2(^5BrSal\text{-}Aniline)_2$		
12	M_{12}	$UO_2(^5ClSal\text{-}Aniline)_2$		
13	T_1	$UO_2(en\text{-}mono\text{-}dtc)_2$	*Bacillus megaterium* *Shigella sonnei* *Shigella dysenteriae* *Salmonella typhi* *Bacillus cereus*	*Collectorichum corchori* *Curvularia lunata* *Fusarium equiseti* *A. niger* *A.funiculosus*
14	T_2	$UO_2(dme\text{-}en\text{-}mono\text{-}dtc)_2$		
15	T_3	$UO_2(det\text{-}en\text{-}mono\text{-}dtc)_2$		
16	T_4	$UO_2(1,3\text{-}pn\text{-}mono\text{-}dtc)_2$		
17	T_5	$UO_2(dbu^t\text{-}tda\text{-}mono\text{-}dtc)_2$		
18	T_6	$UO_2(Had\text{-}mono\text{-}dtc)_2$		

Results and Discussion

Effects of the tested complexes the selected bacteria

The results of inhibition zones of the selected bacteria due to the prepared complexes T_1-T_6 showed that all of these complexes exhibited mild to prominent antibacterial activities against the pathogenic bacteria tested herein.

The results of inhibition zones of the selected bacteria due to the prepared complexes in figures 4 and 5 showed that all complexes exhibit mild to prominent antibacterial activities against the pathogenic bacteria tested herein. The complexes M_5, M_6, M_{11} and M_{12} were found to be more effective than the others. So, the prepared dioxouranium(VI) complexes specially M_5, M_6, M_{11} and M_{12} can be used as antibacterial agents.

From Figure 4 it is appeared that the complexes M_5 and M_6 were found to be very active against all of the selected bacteria except *Bacillus megaterium* which was found less sensitive to the compound M_6. The largest zone of inhibition (30 mm in diameter) was recorded against *Pseudomonas aeruginosa* to the compound M_5 and the largest zone of inhibition (30 mm in diameter) was recorded against *Escherichia coli* to the compound M_6.

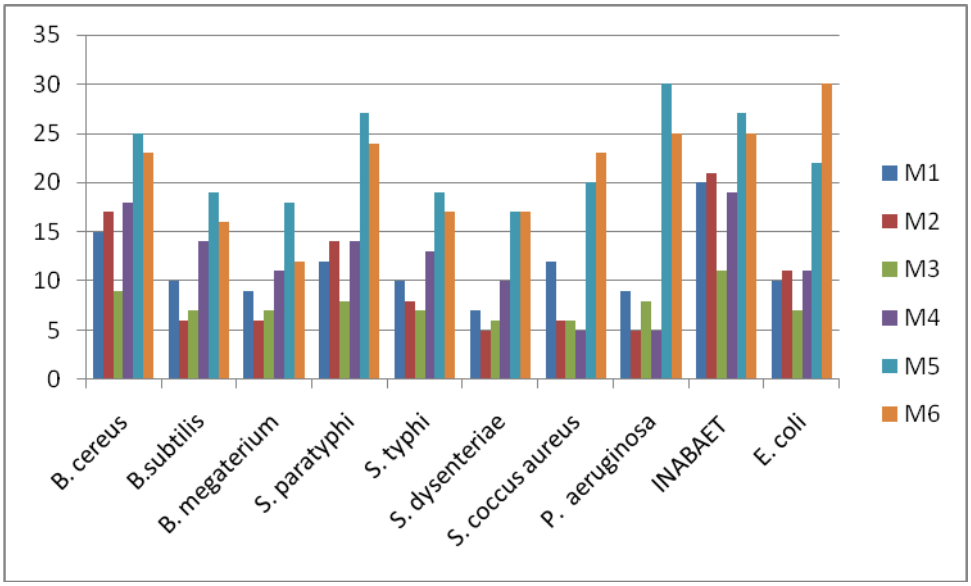


Figure 4. Zone of inhibition produced by the tested complexes M_1-M_6 against the selected organism (bacteria).

Complexes M_7, M_9, M_{11} and M_{12} as shown in Figure 5 were found to be more active against the bacteria *Bacillus cereus*, *Salmonella paratyphi*, *Pseudomonas aeruginosa* and INABA ET (vibrio); M_8 and M_{10} were found to be more active against the bacteria *Bacillus cereus*, *Pseudomonas aeruginosa* and INABA ET (vibrio); M_1, M_2 and M_4 were found to be more active against *Bacillus cereus* and INABA ET (vibrio) than the other selected

bacteria.

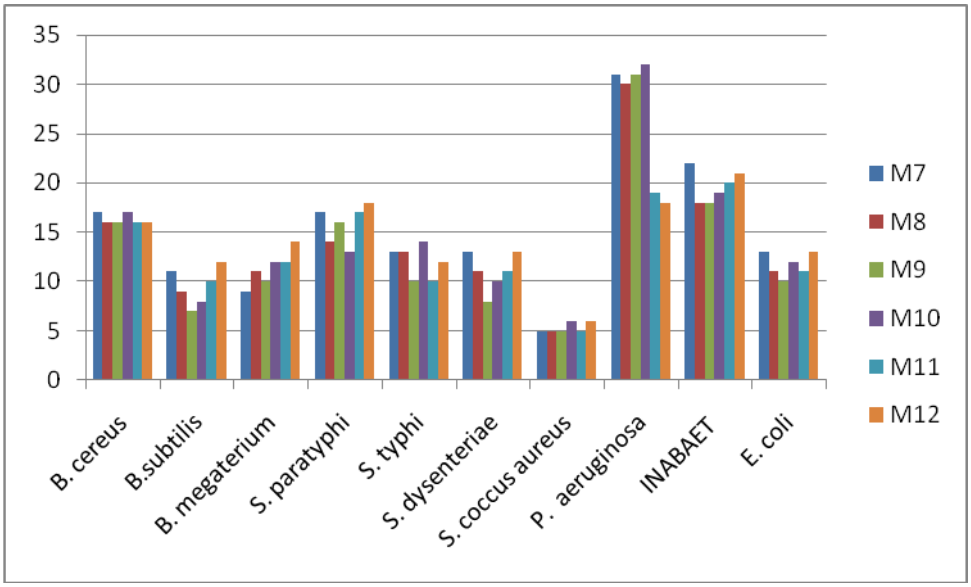


Figure 5. Zone of inhibition produced by the tested complexes M_7-M_9 against the selected organism (bacteria).

The largest zones of inhibition were recorded to the compounds M_1 (20 mm in diameter), M_2 (21 mm in diameter), M_3 (11 mm in diameter) and M_4 (19 mm in diameter) against the bacteria INABA ET (vibrio); compounds M_7 (31 mm in diameter), M_8 (30 mm in diameter), M_9 (31 mm in diameter) and M_{10} (32 mm in diameter) against the bacteria *Pseudomonas aeruginosa*; and compounds M_{11} (20 mm in diameter) and M_{10} (21 mm in diameter) against the bacteria INABA ET (vibrio).

Determination of minimum inhibitory concentration (MIC) studies

Among the twelve prepared complexes, M_5, M_6, M_{11} and M_{12} showed promising antibacterial activity against some selected bacterial strains tested herein. So, an attempt was made to determine the minimum inhibitory concentration (MIC) values of the highly active compounds (M_5, M_6, M_{11} and M_{12}) against the highly sensitive bacterial strains. The active samples M_5, M_6, M_{11} and M_{12} were subjected for testing using different two fold concentrations by disc diffusion technique. The MIC values of the complex M_5 were found 12.5, 12.5, 12.5, 6.25, 6.25 and 25 (in μg/disc) against *Bacillus cereus*, *Salmonella paratyphi*, *Staphylococcus aureus, Pseudomonas aeruginosa*, INABA ET (Vibrio) and *Escherichia coli* respectively. The lowest MIC 6.25 μg/disc was recorded against *Pseudomonas aeruginosa* and INABA ET (Vibrio). The MIC values of the complex M_6 were found 6.25, 6.25, 6.25, 12.5, 6.25 and 6.25 (in μg/disc) against Bacillus cereus, *Salmonella paratyphi*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, INABA ET (Vibrio) and *Escherichia coli* respectively. The lowest MIC 6.25 μg/disc was recorded against *Bacillus cereus, Salmonella paratyphi, Staphylococcus aureus, Escherichia coli* and INABA ET (Vibrio). The MIC values of the complexes M_{11} and M_{12} were found 6.25

μg/disc against INABA ET (Vibrio).

Effects of chemicals on mycelial growth of the selected fungi:

i. *Fusarium equiseti:* The radial mycelial growth inhibition of *Fusarium equiseti* due to the prepared complexes were found to be very effective (figures 6, 7). So we can use all of these complexes except T_4 as antifungal agents. All the complexes were found to be more active than the standard antifungal antibiotic Nystatin.

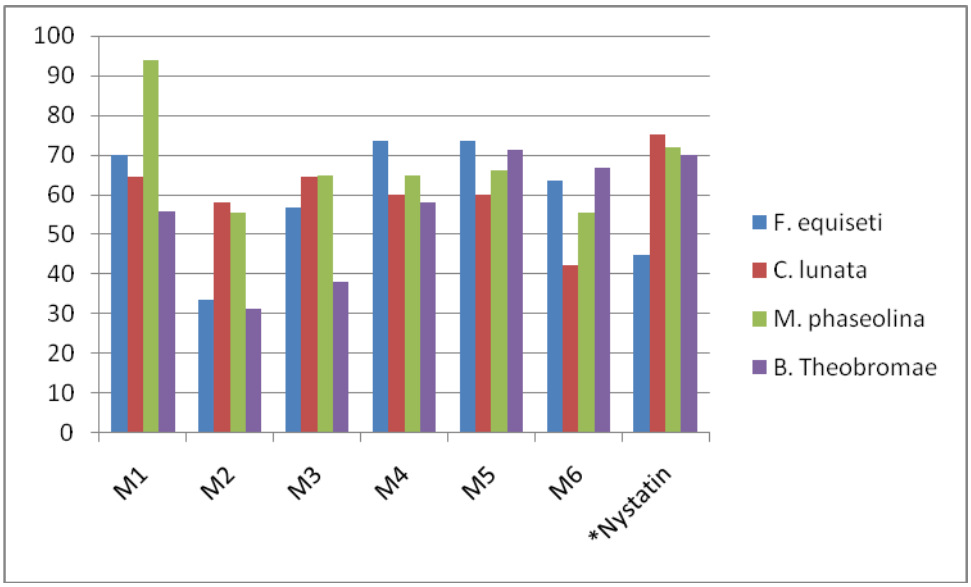


Figure 6. Antifungal activities of the synthesized test samples M_1-M_6 and standard antifungal antibiotic Nystatin against selected fungi.

ii. *Curvularia lunata:* From the (figures 6-8), the inhibition radial mycelial growth of *Curvularia lunata* for all of these complexes were found to be very effective. Complex M_6, T_1, T_4 were found to be less effective than the others. All of these complexes can be used as novel antifungal agents against this organism. Antifungal antibiotic Nystatin was found to be active against the organism compared to the complexes M_7 (77.78%), M_{11} (98.09%), M_{12} (75.56%), T_2 (44.33%) and T_3 (52.43%).

iii. *Macrophomina phaseolina:* From the figures 6-7, the complexes were found to be very effective against *Macrophomina phaseolina.* So, all of these complexes also can be used as antifungal agents. From the inhibitions of mycelial growths of these complexes, M_8 and M_9 were found to be less effective than the others. M_1 (93.85%) and M_7 (81.54%) were found to be more active than the standard antifungal antibiotic Nystatin.

iv. *Botrydiplodia theobromae:* The complexes (except M_8) in figures 6-7 were found to be very effective against *Botrydiplodia theobromae*. These complexes also can

be used as antifungal agents. Complex M_5 (71.11%) was found to be more active than the standard antifungal antibiotic Nystatin.

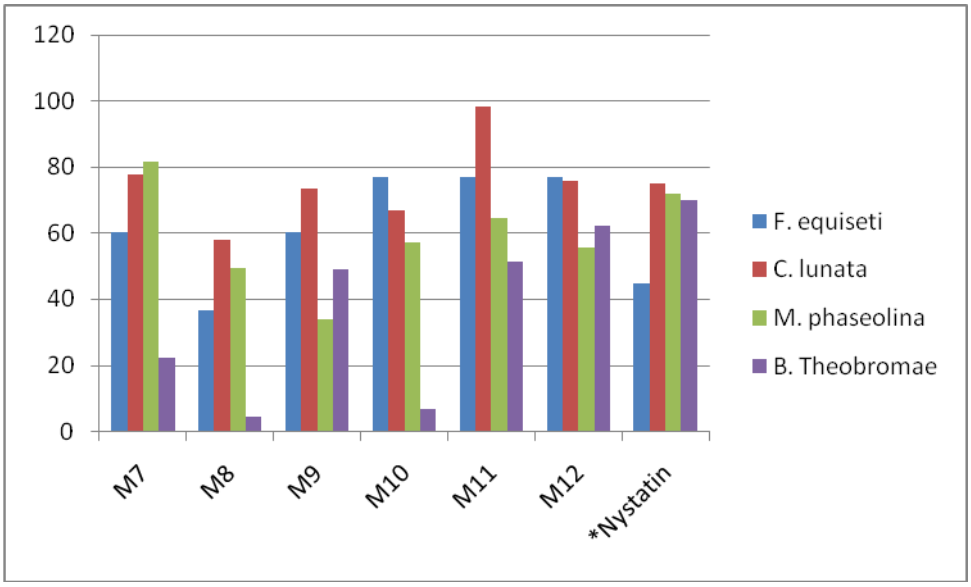


Figure 7. Antifungal activities of the synthesized test samples M_7-M_9 and standard antifungal antibiotic Nystatin against selected fungi.

v. *Collectorichum corchori:* As shown in Figure 8 complexes T_2, T_5 are mild effective whereas T_4 highly effective against the organism. The remaining two shows less activity than standard.

vi. *A. niger:* As shown in Figure 8 complexes T_2, $T_{4\text{-}6}$ more whereas remaining two showed less activity against the tested fungi.

vii. *A. funiculosus:* As shown in Figure 8 complexes $T_{1\text{-}3}$,T_6 are effective and remaining less effective against *A. funiculosus.*

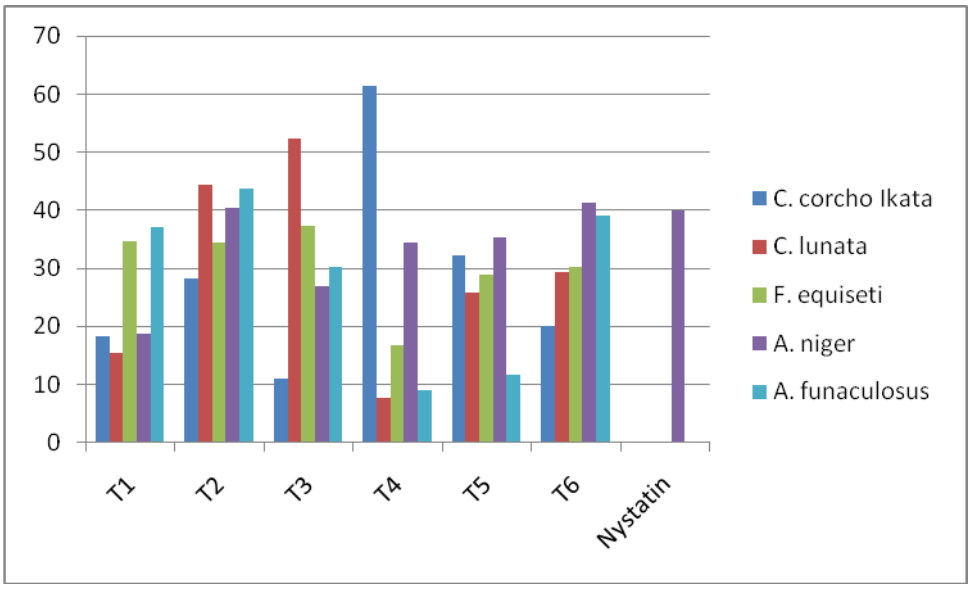


Figure 8. Antifungal activities of the synthesized test samples T_1-T_6 and standard antifungal antibiotic Nystatin against selected fungi.

The antimicrobial activity of complexes was tested in vitro against selected bacteria and fungi. The metal chelates have higher antibacterial activity than the control against the same microorganism under identical experimental conditions. This different effect of the compounds against microorganism may be because of the structure of the compound, types of ligands and also the nature of solvent. The diffusion capacity of the compounds varies with the employed solvent, which may be because of the polarity of the solvent.

Such enhanced activity of metal chelates is due to increased lipophilic nature of the metal ions in complexes. The increase in activity with concentration is due to the effect of metal ions on the normal process. The antibacterial activity of these complexes varies with the nature of ligands and it follows the order L´> L >dtc. This high antibacterial activity of complexes can be attributed due to the chelation trends to make a liquid more potential bacterial agent. The increased activity depends upon chelation is attributed to the positive charge of the metal partially shared with donor atom present on the Ligand and possible ɳ-electron delocalization over the whole chelate ring. This, in turn, increases the lipophilic character of the metal chelate and favours its permeation through the lipid layer of the bacterial membranes. Inhibition was found to increase with increasing the concentration of metal complex.

The Figure 9 indicates the zone of inhibition produced by the tested complexes M_1-M_6 against the selected organism (bacteria) and figures 10 and 11 shows the antifungal activities of the synthesized test samples M_7, M_9 and T_4, T_5, respectively against selected fungi.

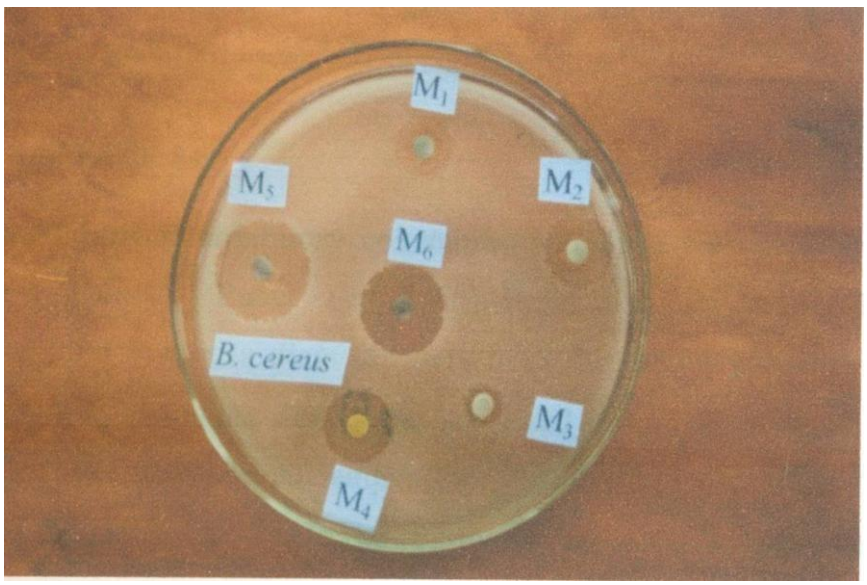


Figure 9. Zone of inhibition produced by the tested complexes M_1-M_6 against the selected organism (bacteria).

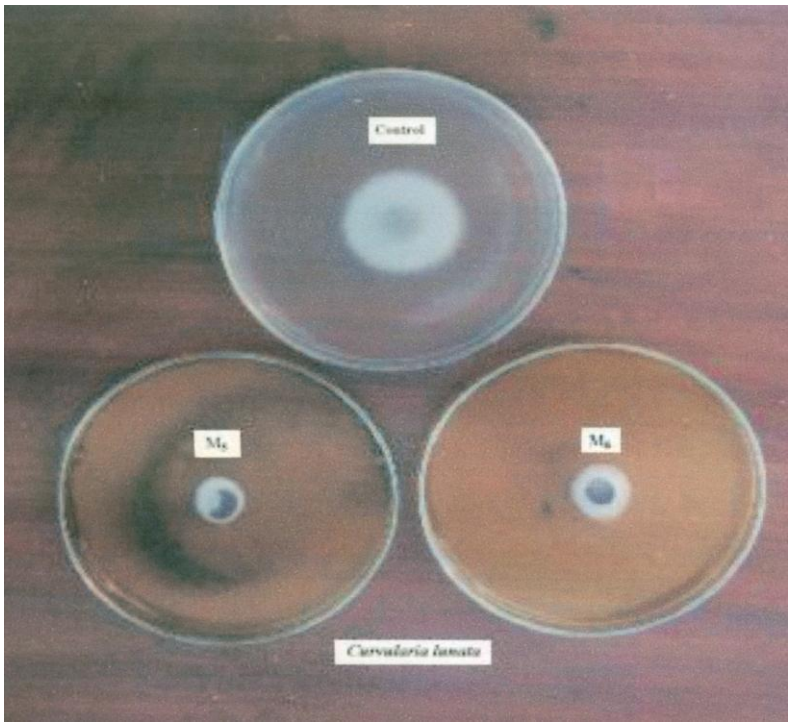


Figure 10. Antifungal activities of the synthesized test samples M_7, M_9 and standard antifungal antibiotic Nystatin against *Curvularia lunata*.

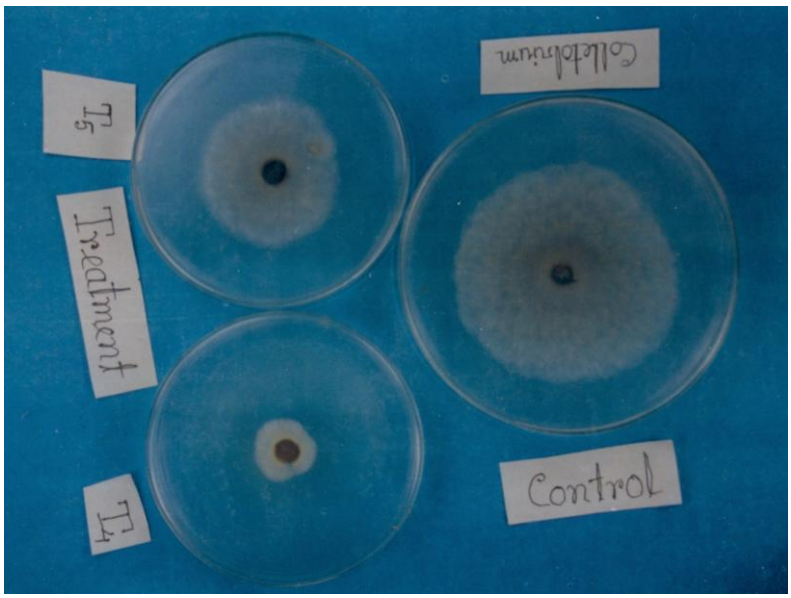


Figure 11. Antifungal activities of the synthesized test samples T_4, T_5 and standard antifungal antibiotic Nystatin against *Curvularia lunata.*

Conclusion

The chemistry of Schiff bases and carbamates is a field that is being noticed. These ligands are considered privileged ligands because they are easily prepared. Their metal complexes had a variety of applications including clinical, analytical, industrial they also play important roles in catalysts. The biological activities of uranium complexes have been summarized. It can be deduced from these results that the different response of the

synthesized ligands arises because of their structural differences.

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Full Paper

2-Methoxyethanol: A remarkably efficient and alternative reaction medium for iodination of reactive aromatics using iodine and iodic acid

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ABSTRACT: Remarkably effective iodination of reactive aromatics carried out using iodine and iodic acid in 2-methoxyethanol as an efficient and alternative reaction medium. The comparison has been made by carrying out iodination reaction in acetic acid and ethanol. The 2-methoxyethanol is found to be excellent reaction solvent in terms of clean reaction conditions, short reaction time giving quantitative yields of product and no need of further purification.

Keywords: iodoaromatics; 2-methoxyethanol; iodine; iodic acid

Introduction

Aromatic iodo compounds are valuable and versatile synthetic intermediates in organic chemistry [1]. They react with nucleophiles such as amines or alkoxides to give the corresponding substituted products and can be lithiated to introduce electrophiles via halogen lithium exchange reaction [2]. They are also important and most reactive intermediate for various cross-coupling reactions and especially useful for formation of carbon-carbon and carbon-heteroatom bonds [3].

The moderate reactivity of iodine with aromatic substrates requires the addition of activating agents for its utilization. Generally, aromatic compounds are iodinated using iodine in presence of Lewis acid or an oxidizing agent [4].

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Synthesis of iodoaromatics using various reaction medium and iodinating reagent involves I_2/petroleum ether [5], KI/H_2SO_4 in H_2SO_4 [6], NaI/Chloramine-T in methanol [7], NaOCl/NaI in aqueous alcohol medium [8], KI/H_2O_2 in CH_3COOH [9], $KClO_3$ /KI/HCl in aqueous medium [10], and I_2/$NaBO_3.H_2O$ in ionic liquid [11]. The utility of alternative reaction solvents such as water [12], ionic liquid [13], flourous [14], supercritical media [15] and polyethylene glycol [PEG] [16] is rapidly growing. These solvents have attracted the attention of organic chemists due to their solvating ability and aptitude to act as a phase transfer catalyst, negligible vapour pressure, non-hazardous, easy work- up and economical cost. However many of these reported procedures have one or more disadvantages such as use of expensive catalysts, long reaction time, low selectivity, requirements of special apparatus and side reaction.

In continuation of earlier research program on iodination of reactive aromatics using iodine and iodic acid [17], herein we wish to report the use of 2-methoxyethanol as a reaction solvent.

Material and Methods

Melting points were determined in an open capillary tube and are uncorrected. IR spectra were recorded in KBr on a Perkin-Elmer spectrometer. ^1H NMR spectra were recorded on a Gemini 300-MH_Z instrument in $CDCl_3$ as solvent and TMS as an internal standard. The mass spectra were recorded on EI-Shimadzu-GC-MS spectrometer. Elemental analyses were performed on a Carlo Erba 106 Perkin-Elmer model 240 analyzer.

General procedures for iodination of hydroxy aromatic aldehydes, hydroxy acetophenones, substituted anilines and phenols in ethanol, acetic acid and 2-methoxyethanol:

Mixture of different aromatic compounds (50 mmol), iodine (20 mmol) dissolved in 5 mL of 2-methoxyethanol and iodic acid (10 mmol) dissolved in water (1 mL) was added with shaking and refluxed for 3-8 minutes (tables 1-4). On cooling reaction mixture, crystalline solid product separated out (reaction monitored on TLC).

Obtained solid product was filtered through Buchner funnel. Physical data is given in Tables 1-4. For synthesis of di-iodo product 40 mmol of iodine and 20 mmol of iodic acid with 50 mmol of substrate was used.

Similar procedure was carried out by using 25 mL of ethanol or 20 mL of acetic acid. Results obtained by these procedures are shown in tables 1-4.

Spectral data of some selected compounds

4-Hydroxy-3,5-diiodo-benzaldehyde: IR (cm^{-1}): 2852 (C-H *stretch of* CHO),

Table 1. Physical data of iodo hydroxy benzaldehydes

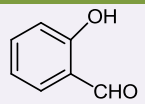
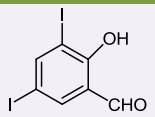
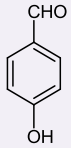
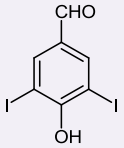
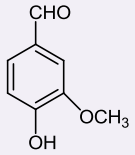
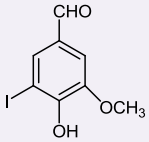
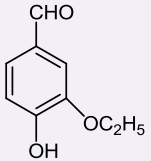
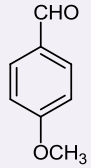
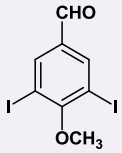
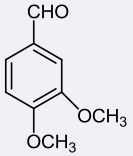
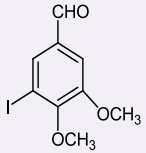
Entry No.	Substrate	Product	M.P. (^{0}C) Found (Reported)	Effect of solvent on iodination of hydroxy aldehydes					
				Ethanol		Acetic acid		2-Methoxyethanol	
				Time (min)	Yield (%)	Time (min)	Yield (%)	Time (min)	Yield (%)
1			110 (110) [19]	05	82	18	58	03	96
2			194 (195) [18]	07	80	20	62	05	88
3			184 (185) [18]	09	85	22	50	05	90
4			185 (185) [19]	05	80	17	63	04	86
5			105 (107) [19]	08	80	21	55	05	92
6			70 (69-72) [19]	10	80	23	68	05	85

Table 2. Physical data of iodo hydroxy acetophenones

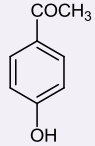
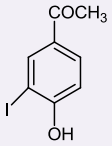
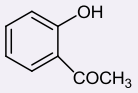
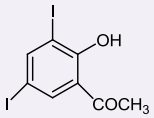
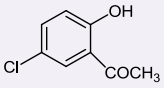
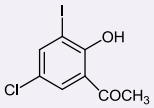
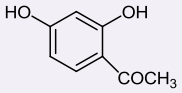
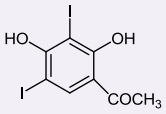
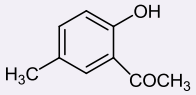
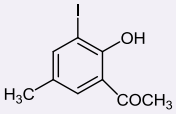
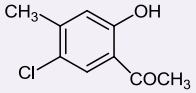
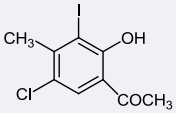
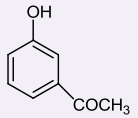
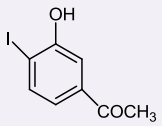
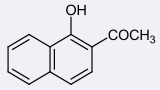
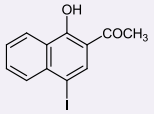
Entry No.	Substrate	Product	M.P.(^{0}C) Found (Reported)	Effect of solvent on iodination of hydroxy acetophenones					
				Ethanol		Acetic acid		2-Methoxyethanol	
				Time (min)	Yield (%)	Time (min)	Yield (%)	Time (min)	Yield (%)
1			164 (162) [19]	05	80	18	58	05	87
2			132 (132) [18]	08	85	22	64	06	92
3			89 (90) [19]	05	84	17	60	04	95
4			177 (178) [18	09	85	24	62	05	90
5			90 (90) [19]	05	82	18	50	03	85
6			78 (76) [19]	06	85	25	70	05	88
7			156 (155) [19]	07	82	19	62	04	90
8			156 (156) [19]	05	79	16	55	05	96

Table 3. Physical data of iodo anilines

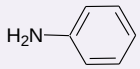
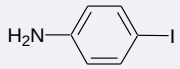
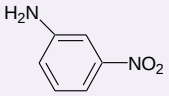
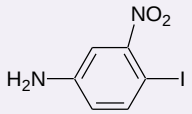
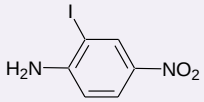
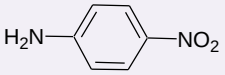
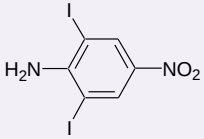
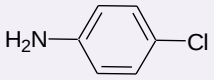
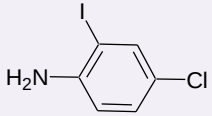
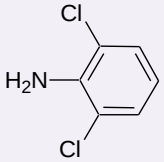
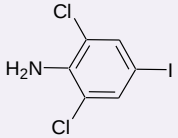
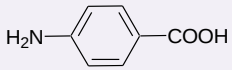
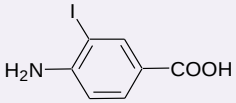
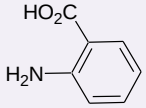
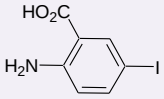
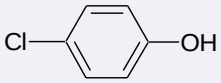
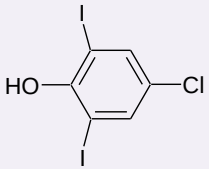
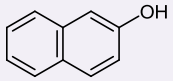
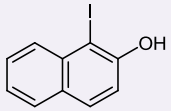
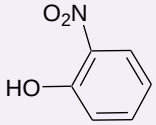
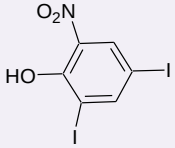
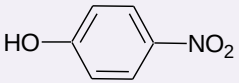
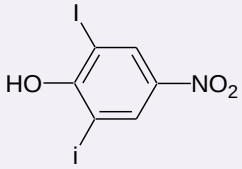
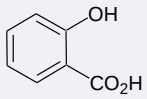
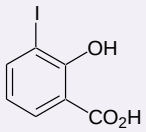
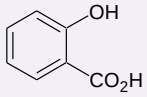
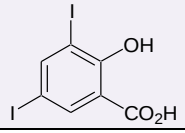
Entry No.	Substrate	Product	M.P.(^{0}C) Found (Reported)	Effect of solvent on iodination of anilines					
				Ethanol		Acetic acid		2-Methoxyethanol	
				Time (min)	Yield (%)	Time (min)	Yield (%)	Time (min)	Yield (%)
1			62 (62-63) [20]	10	80	25	62	05	88
2			120 (122) [20]	05	75	17	54	03	84
3			115 (116) [19]	06	80	19	60	07	82
4			252 (251-253) [19]	09	75	23	50	08	90
5			40 (39-41) [19]	07	75	21	58	05	94
6			85 (80) [19]	05	80	17	60	08	86
7			255 (258) [19]	10	70	28	48	06	82
8			222 (220-225) [19]	06	60	25	42	08	85

Table 4. Physical data of iodo phenols

Entry No.	Substrate	Product	M.P (^{0}C) Found (Reported)	Effect of solvent on iodination of Phenols					
				Ethanol		Acetic acid		2-Methoxyethanol	
				Time (min)	Yield (%)	Time (min)	Yield (%)	Time (min)	Yield (%)
1			105 (107) [21]	10	82	27	52	08	88
2			108 (108-110) [19]	06	80	22	60	05	92
3			95 (93) [22]	12	85	30	66	04	90
4			150 (152) [19]	13	80	28	52	07	82
5			185 (190) [19]	09	80	25	60	05	85
6			220 (220) [19]	12	80	27	52	08	86

1660 (C=O), 1558 (C=C stretch). ^{1}H NMR ($CDCl_3$): δ 7.72 (s, 2H, Ar-H), 8.42 (s, 1H, OH), 10.02 (s, 1H, CHO). ^{13}C NMR (75 MHz, DMSOd_6, δ,ppm):: 91.12 (C *of two* Ar-I), 131.64 (C of Ar-C), 139.42 (C *of two* Ar-H), 182.21 (C *of* Ar-OH), 191.37 (C *of* CHO). MS m/z: 374 (M^+). Anal. calcd. for: $C_7H_4O_2I_2$: C, 22.45; H, 1.06; I, 67.91. Found: C, 22.49; H, 1.09; I, 67.94.

3,5-Diiodo-4-methoxy-benzaldehyde: IR (cm^{-1}): 2845 (C-H *stretch of* CHO), 1652 (C=O), 1548 (C=C stretch). ^{1}H NMR ($CDCl_3$): δ 3.27 (s, 3H, OCH_3), 7.85 (s, 2H, Ar-H), 9.97 (s, 1H, CHO). ^{13}C NMR (75 MHz, DMSOd_6, δ,ppm): 190.78 (C=O), 137.60, 88.23 (*C of Aromatic ring*), 60.92 (OCH_3). MS m/z: 388 (M^+). Anal.calcd. for: $C_8H_6O_2I_2$: C, 24.74; H, 1.54; I, 65.46. Found: C, 24.68; H, 1.51; I, 65.42.

2,4-Dihydroxy-3,5-diiodoacetophenone: IR (cm^{-1}): 3428 (OH), 1660 (C=O), 1555 (C=C stretch). ^{1}H NMR ($CDCl_3$): δ 2.37 (s, 3H, CH_3), 7.90 (s, 1H, Ar-H), 12.62 (s, 1H, OH), 8.92 (s, 1H, OH). ^{13}C NMR (75 MHz, DMSOd_6, δ,ppm): 24.17 (C *of methyl group*) 76.14 (C *of two* Ar-I), 141.29 (C of Ar-H), 178.24 (C *of two* Ar-OH), 197.68 (C *of carbonyl group*). MS m/z: 404 (M^+). Anal.calcd. for: $C_8H_6O_3I_2$: 23.76; H, 1.48; I, 62.87. Found: C, 23.80; H, 1.51; I, 62.93.

1-(1-Hydroxy-4-iodo-naphthalen-2-yl)-ethanone: IR (cm^{-1}): 3438 (OH), 1664 (C=O), 1560 (C=C stretch). ^{1}H NMR ($CDCl_3$): δ 2.35 (s, 3H, CH_3), 6.25-7.91 (m, 5H, Ar-H), 14.12 (s, 1H, OH). ^{13}C NMR (75 MHz, DMSOd_6, δ, ppm): 200.37 (C=O), 164.95, 135.29, 133.20, 130.73, 127.28, 124.62, 122.47, 91.82(*C of Aromatic ring*), 26.62 (CH_3). MS m/z: 312 (M^+). Anal.calcd for: $C_{12}H_9O_2I$: C, 46.15; H, 2.88; I, 40.70. Found: C, 46.10; H, 2.86; I, 40.67.

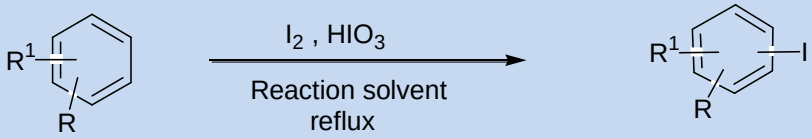
2,6-diiodo-4-nitroaniline: IR (cm^{-1}): 3348 (NH), 1553 (C=C), 1368 (NO_2). ^{1}H NMR ($CDCl_3$): δ 4.08 (s, 2H, NH_2), 7.89 (s, 2H, Ar-H). ^{13}C NMR (75 MHz, DMSOd_6, δ, ppm): 156.20, 145.89, 135.33, 86.90 (*C of Aromatic ring*). MS m/z: 390 (M^+). Anal.calcd. for: $C_6H_4N_2O_2I_2$: C, 18.46; H, 1.02; I, 65.12. Found: C, 18.52; H, 1.05; I, 65.17.

2,6-diiodo-4-nitrophenol: IR (cm^{-1}): 3442 (OH), 1557 (C=C), 1352 (NO_2). ^{1}H NMR ($CDCl_3$): δ 7.82 (s, 2H, Ar-H). ^{13}C NMR (75 MHz, DMSOd_6, δ, ppm): 161.86, 140.18, 134.70, 90.85. MS m/z: 391 (M^+). Anal.calcd. for: $C_6H_3O_3I_2$: C, 18.41; H, 0.76; I, 64.96. Found: C, 18.38; H, 0.75; I, 64.92.

Results and Discussion

2-methoxyethanol is non-halogenated, inexpensive and water soluble which facilitate its removal from reaction product. In view of this observation it was thought worthwhile to carry out iodination of reactive aromatics using iodine and iodic acid as

iodinating agent in 2-methoxyethanol as an efficient and alternative reaction solvent (Scheme-**1**).



Reaction solvent: **A**: ethanol; **B**: acetic acid; **C**: 2-methoxyethanol
R = CHO, $COCH_3$, NH_2, OH;
R_1 = OH, OCH_3, OC_2H_5, Cl, CH_3, NO_2, COOH

Scheme 1. Iodination of reactive aromatics

In order to optimize the reaction conditions in terms of solubility, clean reaction conditions, isolation of product, time required for completion of reaction, yield and purity of product, we carried out the above reaction in ethanol and acetic acid and our results summarized in Table:**1-4**. We found that 2-methoxyethanol is remarkably effective reaction solvent consuming shorter reaction time besides increasing yield of product (Table.**1-4**). Encouraged by the results, we turned our attention towards variety substituted reactive aromatics. In all cases, the reaction proceeded smoothly in high yields at 120°C using 2-methoxyethanol as an attractive reaction solvent for iodination reaction.

Conclusion

We reported remarkably efficient reaction medium for modified practical procedure for iodination of aromatics using iodine-iodic acid in 2-methoxyethanol. Present method, offer additional advantages such as comparatively least requirement of amount of solvent, simple reaction conditions, no need of catalyst, economical process with easier setup and workup procedure giving high yields of desired product.

Acknowledgements

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Full Paper

Gas chromatographic–mass spectrometric validated method for the determination of Bisphenol A in public-supply water: An investigation in Campo Grande, MS, Brazil

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ABSTRACT: *A new validated method is described for determining bisphenol A (BPA) in surface and public-supply water samples. Recovery rates of 91% to 113%, with coefficients of variation below 8.9%, were obtained within the limit of detection (2.4 ng L^{-1}). Concentrations of 13 to 113 ng L^{-1} were found in tap water. An analysis of BPA stability in solid-phase extraction cartridges revealed that the analyte can be stored in such containers for at least 90 days. An evaluation of BPA leached from polyvinyl chloride (PVC) piping showed that BPA can contaminate water distribution systems.*

Keywords: *Bisphenol A; drinking water; PVC; SPE; GC/MS*

Introduction

The primary objects of investigation of endocrine toxicology are xenobiotic compounds (exogen chemicals such as synthetic products and environmental pollutants, among others) that interfere with the production, release, transport, metabolism, binding, or elimination of natural hormones responsible for maintaining homeostasis and

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for regulation of developmental processes. Xenobiotics include estrogen-mimicking compounds, antiandrogens, and molecules that interact with components of the endocrine system in the thyroid, pituitary gland, and hypothalamus, among other organs [1-4].

Endocrine disruptors are a class of compounds—including synthetic and natural hormones, natural substances, and a large number of synthetic chemicals—that interfere with endocrine functions. In the environment, concentrations in the order of milligrams or nanograms per liter are sufficient to adversely affect human and animal health [5], with outcomes including, but not limited to, increased incidence of breast cancer [6], declining sperm counts, decreased fertility [7,8], and birth defects secondary to fetal exposure [9-11].

One widely debated endocrine disruptor is bisphenol A (BPA), or 2,2-bis(4-hydroxyphenyl)propane, a monomer formed by two phenolic rings. BPA estrogenic activity was unexpectedly detected [12]. Its minimum effective concentration range is reported as 2280-4580 ng L^{-1} [13].

In the plastics industry, BPA is an intermediate compound employed in the production of epoxy resins and polycarbonates, which are widely used, for instance, in food can coating and food and beverage packaging. Polymers employed in dental treatment may also contain BPA [14,15]. Akin to other synthetic estrogens, this compound has been evaluated in various matrices, in an effort to elucidate its behavior in different environments [16].

Studies conducted in the United States [17], Germany [16, 18, 19], Japan [20], and the Netherlands [21] have revealed BPA concentrations of less than 8 ng mL^{-1} in river waters, with the exception of a river in Germany (21 ng mL^{-1}) where the compound was possibly released from a wastewater treatment station located in the vicinity of the sample collection plots [21].

In river waters, BPA can be degraded under aerobic [22, 23], but not anaerobic conditions [24]. Ten out of 11 bacterial species isolated from three rivers have been shown to biodegrade BPA, albeit with differences in removal rates (18-91%). Of these species, only *Pseudomonas putida* and an unidentified species of the same genus were capable of degrading the compound at high rates (around 90%) [22].

Despite bacterial degradation of BPA in river waters, its calculated half-life of 3-6 days may be sufficiently long to affect aquatic organisms [24].

A wide range of analytical methods have been developed to quantify BPA in aqueous samples (surface water [25–29], drinking water [30–32], mineral water [33, 34], seawater [27–36], residual waters [37] and sewage effluents [38, 39]). Published

analytical methods are often based on solid phase extraction (SPE) and derivatization and detection by gas chromatography coupled with mass spectrometry (GC-MS), as well as liquid chromatography coupled with mass spectrometry (LC-MS) or with fluorescence (LC-FL).

In Brazil, published studies on BPA remain scarce and the reports available focus on surface waters, drinking water, and sewage effluents. Values from 25 to 64200 ng L^{-1} for surface water and from 160 to 7300 ng L^{-1} for drinking water have been found in samples from Campinas and Araraquara (in São Paulo State), respectively [29, 38, 40, 41]. In other countries, concentrations of 5 to 26100 and 5 to 540 ng L^{-1} have been reported for surface water [21, 25, 26, 37, 42–47] and drinking water samples [31, 44–49], respectively — thus at times exceeding the minimum effective concentration range (2280-4580 ng L^{-1}) [13].

To date, BPA has not been included in American or European lists of priority pollutants or among the pollutants listed in ordinances 357/2005 of Brazil's National Council for the Environment (Conama) [50] or ordinance 2.914 of the Health Ministry [51]. Furthermore, no standard analytical method for the compound is available from Standard Methods for the Examination of Water and Wastewater [52].

The present study evaluated the presence of BPA in the water supply system of Campo Grande, the capital city of Mato Grosso do Sul State, in Brazil, using SPE, GC-MS, and derivatization with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA). The method proposed was validated in this work and can be applied to evaluate samples of surface and public-supply water. BPA stability in SPE cartridges was evaluated. The BPA leached from polyvinyl chloride (PVC) water pipes was qualitatively assessed.

Material and Methods

Study area

With 786,797 residents occupying an area of 8,096 km^2, 2010 data, Campo Grande has the highest population density (97.2 inhabitants km^{-2}) in Mato Grosso do Sul [53].

Located in the state's central region (between latitudes 20°13′N and 20°26′34″S and longitudes 53°36′E and 54°38′47″W), the county lies predominantly within the Paraná river basin, with a small northwestern portion extending into the Paraguay river basin [54].

The county's water system supplies approximately 98% of households. Water is extracted from surface sources and underground aquifers. Major surface sources include the Guariroba, Lajeado, and Desbarrancado river catchment areas, which jointly account

for 65% of the total supply. The remaining 35% is drawn from wells [54]. Table 1 shows the amounts supplied by each type of source.

Table 1. Water output of the public supply system in Campo Grande, MS, Brazil [54]

Type of source	Catchment area	Input to the system (%)	Total input (%)	Output (m^3 $month^{-1}$)
Surface	Guariroba	51.86		3 285 321
	Lajeado	12.71	64.87	804 729
	Desbarrancado	0.30		18 697
Ground	Wells	14.97		947 999
	Special wells	20.16	35.13	1 276 857
	Total	**100**		**6 333 603.97**

Sample collection

Surface and supply water samples were collected in 4 L amber glass bottles previously cleaned and rinsed with water from the sample source to prevent contamination. All samples were collected during a single sampling period—namely, 13-20 January 2010, when local precipitation is high. Collection took place from 8 to 10 a.m.

The samples were transported, stored at room temperature, and pre-concentrated in SPE cartridges within 5 h of collection.

Surface waters

Samples were taken from the Lajeado and Guariroba rivers, both of which supply the city. Sampling plots were located in private land, approximately 100 m upstream of the points of entry into the water treatment plant (WTP). At the sampling point, the Guariroba river is about 6 m wide and 2.5 m deep, whereas the width and depth of creek-sized Lajeado is 1.5 m and 0.6 m, respectively. River measurements were made with a measuring tape and depth gauge.

Public-supply water

Supply water samples were collected from nine residential water meters (Figure 1) located in the county's urban area. The samples were identified as to their source (wells or WTP).

Glassware cleaning

All glassware was soaked in 5% Extran solution (MA-02 neutral, Merck) for 24 h and rinsed in running water, followed by a final rinse with distilled water. After being dried in an oven at 40 °C, the material was rinsed in methanol (Dinâmica, HPLC grade) to remove possible traces of BPA and dried again in an oven at 40 °C. Volumetric ware was cleaned in the same manner, but dried at room temperature.

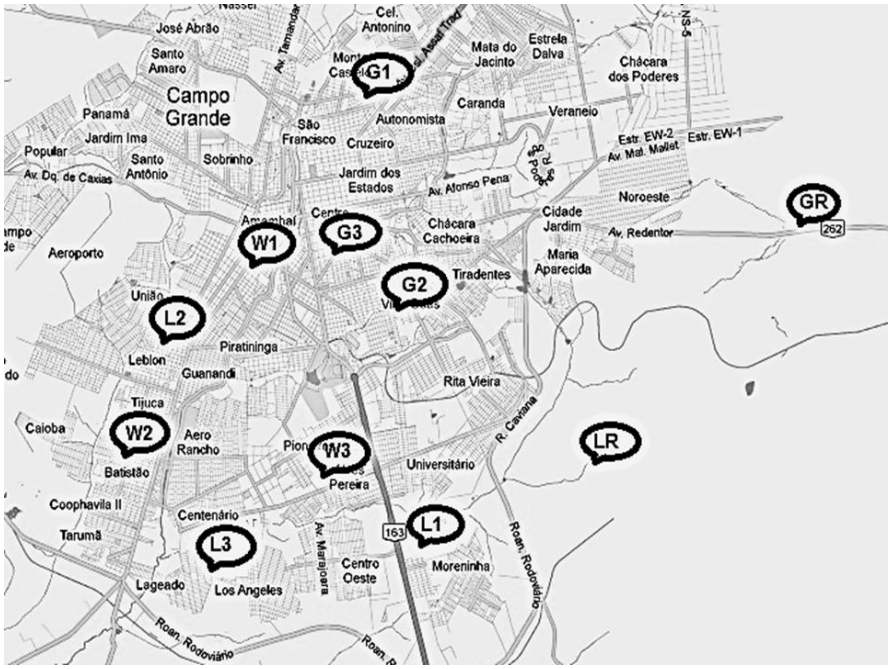

Figure 1. Water sampling points in Campo Grande country, in Mato Grosso do Sul state, mid-west Brazil.

Standards, solutions, and materials

A BPA (Aldrich, 95%) stock solution (100 mg L^{-1}) and working solutions (1.0 and 10.0 mg L^{-1}) were prepared in ethanol (Vetec, 95%). An anthracene-d10 (Supelco, neat) solution (200 μg L^{-1}) was prepared in HPLC-grade dichloromethane (Mallinckrodt) and employed as the internal standard (IS).

BPA derivatization was performed with BSTFA containing 10% trimethylchlorosilane (Fluka, 99%).

Ethyl acetate (J.T. Baker) and methanol (Dinâmica), both HPLC grade, were used for SPE. In the pre-concentration step, 200 mg/3 mL Strata-X cartridges (Phenomenex, USA) were employed in a 12-position vacuum extraction box (Supelco) coupled to a vacuum pump (Tecnal).

Witness sample

Witness sample testing was performed to find a BPA-free water sample for use in the recovery assays. Tap water samples collected from Campo Grande and the adjoining county of Nova Alvorada do Sul were tested, as were samples of distilled, bidistilled, ultrapure (Milli-Q), and well water. Traces of BPA were found in all samples except that from Nova Alvorada do Sul (pH 7.9)—which was therefore selected as the witness sample. Traces of BPA detected in distilled water were probably released from plastic and metal parts of the distillation equipment.

Sample preparation

Pre-concentration and purification

The SPE cartridges were conditioned by serial rinsing (at a constant 3 mL min^{-1} flow) with 50 mL of ethyl acetate, 5.0 mL of methanol (followed by a 5 min rest), and 15.0 mL of BPA-free water. Subsequently, 1.0 L of sample previously filtered through qualitative filter paper (to trap particulates) was transferred to a SPE column at an approximate flow rate of 3 mL min^{-1}. After sample pre-concentration, the cartridges were washed with 9:1 (v:v) water : methanol solution and vacuum-dried for 90 min [36].

Elution was performed with 5.0 mL of methanol at a flow rate of 2 mL min^{-1} and the eluate was allowed to dry under a nitrogen flow. The resulting extract was redissolved in methanol and transferred to a 2 mL vial [36].

Derivatization with BSTFA

The extract was dried again under a gentle stream of nitrogen, and derivatization was performed by adding 100.0 μL of anthracene-d10 solution (internal standard) and 10.0 μL of BSTFA. The flask was shaken for 30 s and kept in an oven at 40 °C for 10 min. The derivatized sample was then dried under a gentle stream of nitrogen, completed to 1.0 mL with dichloromethane, and analyzed by GC-MS. Figure 2 depicts the derivatization reaction of BPA with BSTFA [55].

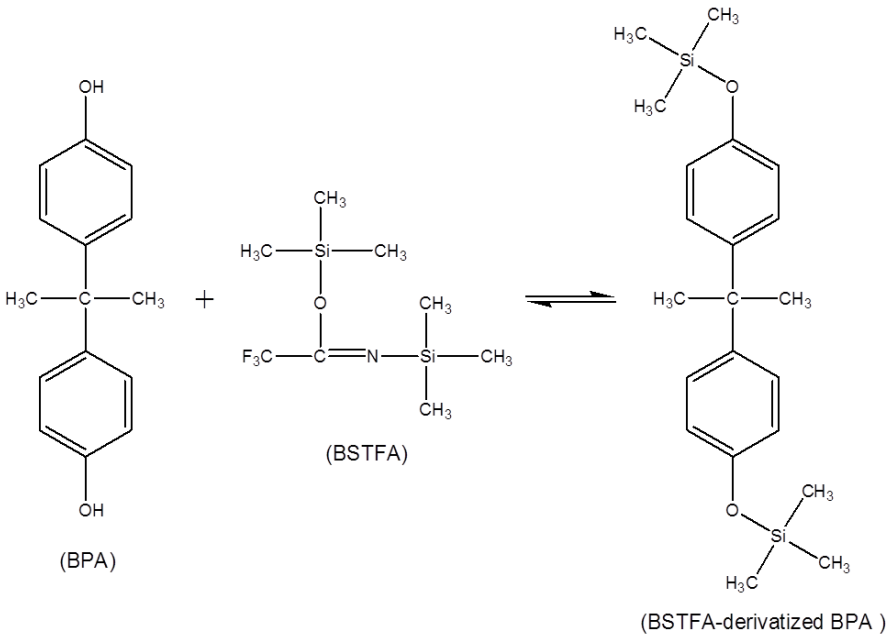

Figure 2. BPA derivatization with BSTFA.

Stability of BPA in SPE cartridges

Samples spiked with BPA (50 ng L^{-1}) were pre-concentrated in SPE cartridges.

After vacuum drying for 90 min, the cartridges were wrapped in aluminum foil and stored under refrigeration at –10 °C for subsequent elution and analysis.

Analyses were performed in triplicate 1, 2, 3, 5, 7, 15, 30, 45, 60, and 90 days after analyte pre-concentration. The analysis was performed immediately after pre-concentration furnished the first point of the curve depicting BPA in-cartridge stability.

BPA leaching from PVC piping

BPA leaching from PVC pipes was evaluated using BPA-free tap water from Nova Alvorada do Sul and a segment of residential-purpose PVC 6.3 pipe for cold water (750 kPa nominal pressure, 50 mm inner diameter, compliant with Brazilian technical norm NBR 5648)—the predominant type of piping employed in the water supply network of Campo Grande.

A roughly 1 m-long pipe segment was cut into 10 cm-long sections, which were kept in 40 L of BPA-free water (pH 7.9) at room temperature in a covered aluminum container. Aliquots of 1 L were withdrawn in triplicate over the course of 60 days, at varying intervals. The volume was manually stirred for 30 s before each aliquot was taken. The samples were analyzed to monitor changes in the concentration of BPA leaching from the pipe material.

Analyses

The analyses were performed at the Department of Chemistry of the Universidade Federal de Mato Grosso do Sul in a GC 2010 high-resolution gas chromatograph coupled to a QP-2010 Plus mass spectrometer (Shimadzu) equipped with an AOC-20i autosampler. An Rtx-5MS chromatographic column (30 m × 0.25 mm × 0.25 μm, from Restek) was employed. The injection volume was 1 μL in splitless mode.

The injector and detector temperatures were 270 and 280 °C, respectively. The column temperature program began with a 2 min period at 120 °C, followed by heating to 210 °C (at 15 °C min^{-1}) and finally to 270 °C (at 10 °C min^{-1}). The final temperature was maintained for 4.7 min. The carrier gas (helium 5.0, White Martins, analytical grade) was flowed at 1.2 mL min^{-1}. Total analysis time was 19 min.

Ion monitoring in scan mode covered the interval from *m/z* 60 to 500. The ions detected in SIM mode were at *m/z* 357 and 372. The spectra were obtained using the electron impact technique, with an impact energy of 70 eV. Chromatograms, mass spectra, and analyte quantification were obtained using GCMS Solutions software (Shimadzu).

Method validation

Validation was based on the following recommended criteria: selectivity, working

range, linearity, sensitivity, accuracy, precision, limit of detection (LD), and limit of quantification (LQ) [56, 57].

Results and Discussion

Method validation

Selectivity

The method's selectivity was evaluated by comparing a sample spiked with 30 ng L^{-1} BPA with the witness sample and the blank. No chromatographic peaks were found with retention times equal to or close to that of BPA. No analyte was detected in the witness sample. These results demonstrated that samples collected from Nova Alvorada do Sul were suitable for the recovery assays run during the validation step, allowing BPA to be unequivocally detected and quantified.

Limit of detection (LD) and limit of quantification (LQ)

Calculation of the LD and LQ of the equipment was based on baseline signal-to-noise ratio, using solutions of known concentrations [56, 57]. These limits were 3.5 and 10.0 μg L^{-1}, respectively.

The method's LD and LQ were calculated as proposed by Their and Zeumer [58], yielding values of 2.4 ng L^{-1} and 10 ng L^{-1}, respectively, with m and n = 5 and $m + n - 2$ degrees of freedom.

These results are considered very good, revealing the method's ability to detect and quantify BPA at concentrations similar to those reported in the literature [16, 21, 25, 26, 31, 37, 41–49].

Working range, linearity, and sensitivity

The method's working range was 10 to 100 μg μL^{-1}.

Linearity was evaluated by injecting BSTFA-derivatized BPA standards. The equation of the curve obtained using anthracene-d10 as the internal standard was y = 0.0170x – 0.0760, with R = 0.9964, revealing a strong correlation between the concentrations studied and the signal generated by the equipment.

Huber's test [59] with a linear coefficient different from zero was employed for consistent rejection of outliers. As shown in Figure 3, none of the values of $(A - a)/C$ was off-limits, thus confirming the linearity of the analytical curve. Additionally, a value of R = 0.9964 indicates excellent linearity.

Curve slope shows that the method is sufficiently sensitive to distinguish between concentration values in close proximity.

Recovery assays

Recovery assays were performed to investigate the method's precision and accuracy. Table 2 shows the results obtained for spiked samples.

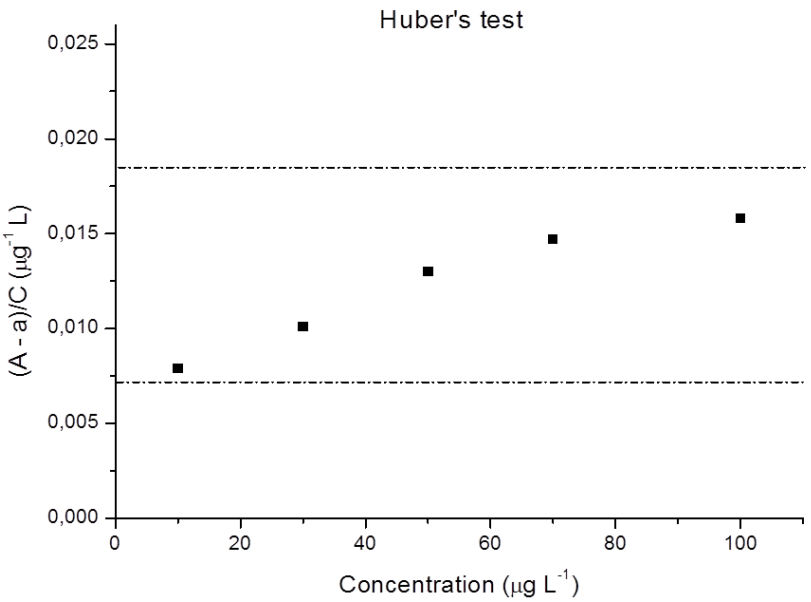

Figure 3. Linearity curve.

Table 2. Results of BPA recovery assays performed on water samples

Level of fortification (ng L^{-1})[a]	CV(%)[b]	Rec (%)[c]	$t_{calculated}$
10	7,0	113	$-7{,}8 \times 10^{-1}$
30	8,9	91	$4{,}2 \times 10^{-2}$
50	6,4	94	$-3{,}1 \times 10^{-1}$

[a]Concentration of BFA in the matrix; [b]Coefficient of variation; [c]Average recovery.
$t_{tabulated}$ (95%) 2,776; $t_{tabelated}$ (99%) 4,604; n = 5.

Precision and accuracy

Coefficients of variation (CV) from 6.4% to 8.9% demonstrated the method's precision, considering the 20% maximum limit recommended in the literature [56, 57].

Recovery rates from 70% to 120% showed the method to be accurate, according to published parameters for the analysis of pesticide residues [56, 57].

The significance of mean recovery rates for each spiking level was assessed using Student's *t* test [60], adopting Rec = 100% as the null hypothesis (H_0) and Rec ≠ 100% as the alternative hypothesis (H_1).

All calculated *t* values were found to fall within the acceptance region of H_0. At 95% and 99% confidence levels, no differences were detected between measured recovery values and those accepted as true—a finding that validates the method's

accuracy for the purposes of the present investigation.

Stability of BPA in SPE cartridges

Data concerning the conservation of samples in the SPE cartridges are shown in Figure 4, revealing that pre-concentrated BPA can remain unaltered in the cartridges under refrigeration for at least 90 days as shown by recovery rates exceeding 81% and CVs below 3.8%. Although variable, analyte recovery lay within recommended levels (above 80%), confirming the method's accuracy and precision even when the analyte is stored for 90 days. A further advantage of in-cartridge storage is the smaller storage space required, compared to the much larger volumes of water required in other methods. In addition, BPA is not promptly degraded or absorbed by cartridge walls.

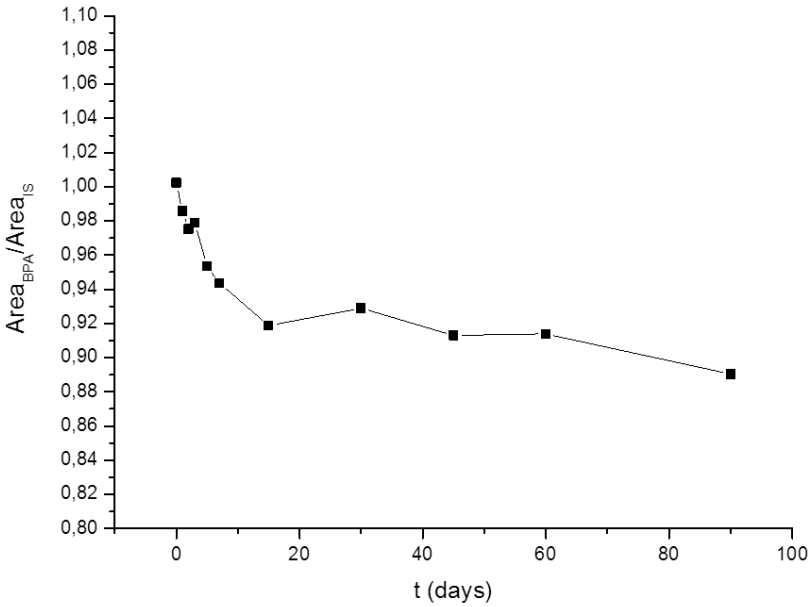

Figure 4. Stability curve for BPA in SPE cartridges kept under refrigeration (−10 °C).

BPA leached from PVC piping

See Figure 5.

BPA concentration increased significantly over the first 5 days, showing that BPA leaches from PVC piping in Campo Grande's water distribution network, with potential contamination risks. After 5 days, however, the concentration of leached BPA decreased, possibly owing to degradation, since the compound has a reported half-life of 3-6 days in surface waters [24].

These results explain why BPA-free water suitable for use as a witness sample in the recovery assays was found in neighboring Nova Alvorada do Sul but not in Campo Grande: in Nova Alvorada do Sul all public-supply water is extracted from artesian wells

and distributed through metal tubing. Water treatment in this county is limited to chlorination.

Levels of BPA leached from PVC piping were similar to those found by Yamamoto and Yasuhara [61] (4.0-1730 μg L^{-1}) for PVC hoses soaked in BPA-free water at room temperature for variable periods (0-24 h).

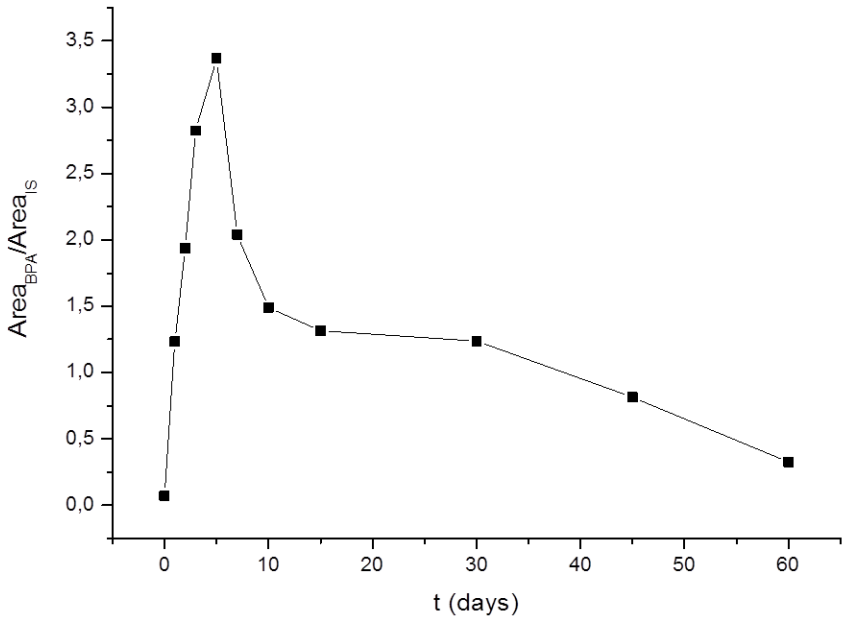

Figure 5. Curve for BPA leaching from PVC piping (water, room temperature).

Public-supply water samples

The analytical results (Table 3) lay within the calibration curve bracket, with the exception of sample G3, whose final extract was subsequently diluted to 2.0 mL with dichloromethane and retested.

Table 3. Results obtained for public-supply water samples

Sample	Geographic Coordinates	BFA (ng L^{-1})
Guariroba River (GR)	20°30'10.32"S 54°14'56.19"O	26 ± 1,5
Lageado River (LR)	20°32'21.75"S 54°30'41.11"O	13 ± 1,9
Lageado 1 (L1)	20°31'53.61"S 54°35'36.87"O	24 ± 0,56
Lageado 2 (L2)	20°29'55.34"S 54°39'24.21"O	21 ± 0,90
Lageado 3 (L3)	20°33'12.99"S 54°38'49.95"O	23 ± 0,93
Guariroba 1 (G1)	20°26'15.25"S 54°36'36.58"O	26 ± 2,3
Guariroba 2 (G2)	20°29'6.93"S 54°36'12.74"O	35 ± 3,2
Guariroba 3* (G3)	20°28'19.62"S 54°37'5.78"O	114 ± 3,0
Well 1 (W1)	20°28'54.97"S 54°38'5.11"O	ND
Well 2 (W2)	20°31'32.12"S 54°40'11.70"O	<LQ
Well 3 (W3)	20°31'38.43"S 54°36'28.50"O	<LQ

LD = 2.4 ng L^{-1}; LQ = 10 ng L^{-1}; n = 3. <LQ = lower than LQ but higher than LD.
*Sample diluted to 2.0 mL with dichloromethane; ND = non detected (concentration below LD).

With the exception of sample W1, BPA was detected in all samples.

The compound was also detected in water from both rivers that feed Campo Grande's supply network—a worrying finding, since these waterbodies account for roughly 65% of the county's water supply.

Worldwide, chemical industries and landfills are usually the main culprits for BPA contamination, but since no industrial plants are present in the study area, contamination can probably be attributed to improper installation and maintenance of landfills.

Given a lack of permits for sample collection immediately upstream and downstream of the WTP's intake and discharge points, it was not possible to ascertain whether or not BPA is efficiently removed at the treatment plant.

However, BPA levels in samples of WTP water collected from household was even higher than in river waters, suggesting the existence of an additional source of BPA. Our data suggest that this potential source of contamination is PVC pipes, which account for 78% of the tubing used in the water distribution network of Campo Grande.

While in the USA, Canada, Japan, and many European countries the environmental impact of endocrine disruptors, especially BPA, has been the focus of debate, few studies have addressed methods for the detection and quantification of these potentially hazardous compounds—hence the timeliness of the present study.

To our knowledge, official regulations on maximum permitted BPA levels in water for human consumption are lacking worldwide. Broader monitoring would ideally provide more comprehensive data to support public policies designed to limit water contamination by BPA and other endocrine disruptors.

In toxicological terms, the results of the present study raise concerns, given the estrogenic activity exhibited by BPA even at concentrations below 1 ng L^{-1}.[1] However, the mere finding of BPA in tap water does not necessarily imply a potential health risk, whose evaluation requires an assessment of estrogenic activity using biological assays, among them *in vitro* and/or *in vivo* immunoassays based on the generation of antibodies and/or receptors capable of binding specifically to estrogenic compounds [10-12].

Given the unfeasibility of replacing PVC with alternative materials in the water supply systems of entire counties, new processes would have to be applied to ensure removal of this endocrine disruptor. Activated carbon filters for drinking water, for instance, are highly efficient (>99%) for the removal of BPA at concentrations of less than 15 mg L^{-1} [62].

Other methods include ozonation [63], advanced oxidation processes [64], nanofiltration membranes, and reverse osmosis [65].

However, because BPA is just one of many endocrine disruptors, the treatment of

water for human consumption should cover other compounds of the same class in a bid to prevent deleterious effects.

Conclusion

In this study, the quality of water from the public distribution network of Campo Grande was evaluated for BPA content. Samples were collected not only from households but also from the waterbodies that feed the county's WTP. The proposed method, also validated here, employed Strata-X polymer-phase SPE cartridges, derivatization with BSTFA, and GC-MS detection. The method exhibited excellent precision and accuracy, with recovery rates from 91% to 113%, CVs of less than 8.9%, and LD and LQ of 2.4 and 10 ng L^{-1}, respectively. Fast, efficient, and simple to operate, the proposed method can be reliably applied to quantify BPA at low concentrations, in the order of nanograms per liter.

The possibility of storing the analyte in SPE cartridges for at least 90 days without analytical impairment is a highly relevant contribution for future studies of BPA based on SPE.

The range of BPA values found in surface water samples (13-26 ng L^{-1}) and samples collected from household water meters (ranging from nondetectable levels to 114 ng L^{-1}) and the finding that BPA actually leaches from PVC pipes strongly suggest that the piping material used in the water supply network is implicated in BPA contamination. Although BPA levels were inferior to the minimum effective concentration range for this compound (2280-4580 ng L^{-1}), BPA's deleterious effects warrant its listing in ordinances 2.914 of the Ministry of Health and 357/2005 of CONAMA [50, 51]. Studies focused on alternative materials safer than PVC and investigations on new techniques for removing contaminants are expected to help mitigate the current risks.

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Review

Ozonized vegetable oils and therapeutic properties: A review

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ABSTRACT: *Ozonized oils represent an interesting pharmaceutical approach to the management of a variety of dermatological pathologies. Ozone reacts with carbon-carbon double bonds of unsaturated fatty acids according to the mechanism described by Criegee, forming ozonides or 1,2,4 trioxolane rings and peroxides as the most important products, responsible for the antimicrobial activity and stimulating tissue repair and regeneration properties. The ozonized vegetable oils can be liquids or semisolids at room temperature and have stability periods that may be adequate for commercial distribuition. Ozonized sunflower oil (Oleozon®), a drug registered nationally and developed in the Ozone Research Center in Cuba has been tested and it was found to have valuable antimicrobial activity against bacteria, fungi and virus. FT-IR and NMR technics are used to confirm the structural changes undergone by oil during the ozonation. For determining the quality of ozonized oils, analytical methods such as peroxide, acidity and iodine values are usually carried out. Products are available for an alternative use of available resources, natural and renewable sources, simple technology, low cost and with extensive biological activity with reduced collateral effects.*

Keywords: *ozonized oil; 1,2,4-trioxolanes; ozonides; ozonetherapy; antimicrobial activity*

Introduction

Although there is no precise data in Brazil, some authors estimate that almost 3%

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of the Brazilian population suffers from any type of skin lesion. Furthermore, approximately four million people present chronic lesions or some form of complication in the cicatricial process [1]. This demands that health professionals have not only a broader knowledge and training to work with the problem, but also implies the need for greater investment in research, both to quantify this population more precisely and to discover new resources and technologies, with lower cost and greater effectiveness, besides being more appropriate and more accessible to the Brazilian population [2].

It has been reported that ozonetherapy and derived products are very useful in treating many pathologies, such as chronic osteomyelitis, pleural emphysema, abscesses with intractable fistulae, infected wounds, bed sores, chronic ulcers and initial gangrene, necrotizing fasciitis, diabetic foot, skin, mouth, vaginal and rectal bacterial, fungus, viral infections and burns [3], especially when in combination with topical therapy by ozonated oils due to permeation and controlled release of active oxygen species into the skin layers.

The skin barrier offers microbiological, physical and biological barrier against external aggressions and has access routes for penetration of cosmetics or active pharmaceuticals [4]. The skin layers are stratum corneum (10-20 μm), viable epidermis (50-100 μm), the dermis (1-2 mm) and subcutaneous tissue. Each layer has specific functions and features [5].

Barata [4] reports that a product will be absorbed by the body following ways of penetration: transdermal (very slow penetration *via*, but of considerable importance); by sudoriparous glands (lines minor penetration), and by pilo-sebaceous apparatus (easier penetration zones). Vegetable oils may alter skin permeation through three different mechanisms namely: increasing occlusion, widening the polar pathway and widening the non-polar pathway [6]. In addition, it has been found that vegetable oils in general produce virtually no skin irritation or sensitization problems [7].

The best absorption and skin penetration enhancers are oils with a high proportion of unsaturated fatty acids such as oleic acid (omega 9), linoleic acid (omega 6) and linolenic acid (omega 3), but mainly oleic and linoleic acids [8]. Absorption and penetration of products into the skin are influenced due to the skin condition and composition of the product to be applied. Gomes [9] described that to improve the absorption and penetration it's important to study the biological, physiological, physicochemical and cosmetologycal features that facilitate or interfere with the application of the product. The effective penetration into the skin aims the best use of the product in the body and the desired effects are the efficiency of the therapeutic properties of active prevention, balance and maintenance of healthy skin [9].

In recent years there has been a growing interest in modificating technology of

oils and fats. It can be attributed to the fact that these materials are obtained from natural sources and used as important raw materials for the chemical industries, pharmaceutical, cosmetic and food [10].

Ozone reacts with the carbon-carbon double bonds of unsaturated fatty acids from vegetable oils giving rise to the formation of chemical species, such as ozonides and peroxides that are responsible for the germicidal action, as well as the properties of stimulating tissue repair and regeneration [11, 12]. The antimicrobial properties of ozonized oils represent an interesting pharmaceutical approach to the management of a variety of dermatological pathologies [12, 13].

This paper presents a bibliographic review on ozonized oils, synthesis of ozonides, physico-chemical characterization, therapeutic properties and antimicrobial activity. Generally, in the literature there is little information about the chemical composition of ozonized oils, due to the complexity of the mixture of compounds formed during the reaction. This aspect is a major constraint to the registration of these products as drugs.

Ozonetherapy

Ozonetherapy is a term of medicine that describes a number of different practices in which oxygen, ozone or hydrogen peroxides are administered *via* gas, water or oil to kill microorganisms, improve cellular function and promote the healing of damage tissues [14].

The history of ozone began in 1839, through the German chemist Christian Friedrich Schonbein, who initially identified its characteristic odor and began to investigate it. Ozone occurs naturally in the atmosphere, 6 to 30 miles above the earth's surface at a concentration of approximately 10 ppm (parts per million). This ozone layer helps protect the earth's surface from harmful ultraviolet radiation and prevents heat loss from the surface [15].

Ozone, the triatomic form of oxygen, is a colorless gas of pungent odor. It is a very strong oxidizer and can oxidize organic substances, destroying microorganisms, such as bacteria, sterilizing the air, and destroying odors [16].

On the 19th century, ozone had been already identified as a potent bactericidal gas and it was used during World War I for treating German soldiers affected by gaseous gangrene due to *Clostridium* anaerobic infections. In two pioneristic studies, Stroke reported the first 21 medical cases successfully treat with ozone at the Queen Alexandria Military Hospital [17, 18].

Ozone has been used therapeutically in several countries such as Cuba, Germany, Italy, Switzerland, Austria, Spain, Russia, Japan, Chile, Peru, Argentina, United States, among others. Cuba is one of the pioneers in the implementation of this therapy in Public

Health Services for over 22 years [19]. OLEOZON® is a therapeutical drug obtained from the reaction of ozone with sunflower oil. The process was developed at the Ozone Research Center – National Center for Scientific Research in Cuba [20]. Topical ozonetherapy have been reported to activate local microcirculation, improve cellular oxygen up-take, stimulate oxidative defensive enzymatic systems and to improve granulation and tissue growth [21].

The ozone gas quickly becomes unstable in the atmosphere so it must be generated practically before use. Ozonization of vegetable oils seems to enhance its stability for clinical uses, keeping activity for a period of up to 2-3 years [22, 23]. Ozonated materials, in which the ozone molecule is stabilized as an ozonide, have the capacity to deliver nascent oxygen deep into the treated area without causing irritation [24].

As a natural preparation, ozonized oil is available in several countries [25]. Ozonized sunflower oil (Oleozon®) has been tested and it found to have valuable antimicrobial activity against bacteria, fungi and virus [26-28].

At the University Hospital pharmacy of Siena, they make their own preparation by bubbling ozone in pure olive oil for at least 30 minutes in a cooled bath. In other countries the pure olive oil is ozonized for two days until it solidifies [29].

There are many other commercially produced ozonated vegetable oils on the market like Cocozone – made from coconut oil in Great Britain, OOO (Ozonized Olive Oil) – made in Canada, O2-Zap – made from olive oil in USA [30].

There are numbers of pharmaceuticals and cosmetics on the market that use ozonized oils as active principles, including Oxaktiv® (Pharmoxid Arznei GmbH&Co.KG, German) [31], Oleoforte® (NaturOzone, Spain) [32] and Ozonia 10® (Innovares, Italy) [33].

Ozonized vegetable oils

The vegetable oils are formed by 97-98% of triglycerides. Depending on their origin and nature they have a variable composition of saturated and unsaturated fatty acids bonded to the glycerol backbone [34].

Industrial exploitation of oils and fats, both for food and oleochemical products, is based on chemical modification of both the carboxyl and alkene groups present in fatty acids, especially *via* oxidation process. The unsaturated triglycerides give the oil many favorable properties. Oxidation of double bonds is used to cleave the alkyl chain or to introduce additional functionality along the chain [35].

An important example described in literature involves the oxidative cleavage of

double bonds using ozonolysis reaction. Ozonolysis is a convenient and highly effective method owing to the complete reaction of ozone with the starting material [36].

The reaction of the ozone with unsaturated fatty acids from vegetable oils generates ozonides, peroxides and aldehydes [37]. The peroxides are the most important products formed. This group includes ozonides, hydroperoxides, polymeric peroxides and other organic peroxides [38, 39] and, probably, is responsible for the wide biological activity of described ozonized vegetable oils [40].

The mechanism of ozonolysis (Figure 1) was described by Criegee in 1975 [41]. First step is a 1,3 dipolar cycloaddition of ozone to the olefin leading to the malozonide (1) (Criegee intermediate), which is very unstable and decomposes to give a zwitterion (2) and a carbonyl compound (3) In the presence of reactive solvent, such as water or alcohol, the zwitterion interact with the solvent to give hydroperoxides (4) in high yield, since the concentration of the solvent far exceeds that of any other substances with which the zwitterion may react. Ozonides (5), dimeric (6) or polymeric peroxides may be by-products. When the solvent is inert, the zwitterion must react either with itself or with carbonyl compound. Reaction with carbonyl compound (3) to form a monomeric ozonide (5) as the major product and polymeric ozonides as minor products usually predominates if (3) is an aldehyde. The zwitterion (2) generally dimerizes to form 6 or polymerizes when 3 is a ketone, less susceptible to nucleophilic attack [11].

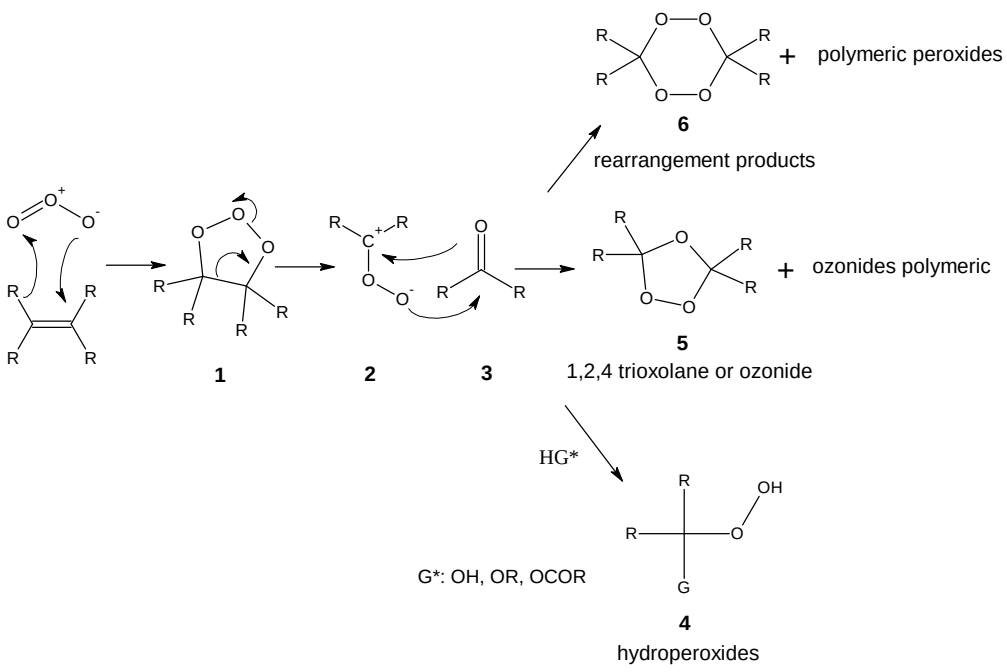

Figure 1: Mechanism of ozonolysis proposed by Criegee [11].

The chemical reactions of ozone when bubbled into oil are very complex. The

analyses of these reactions provide information on the functional group change during ozonation as well as the identification of the products without use of prior separation techniques [41].

The yield of products depends on reaction conditions, such as temperature, time, ozone generator, reactor type and ozone concentration [12]. Referring to ozonolysis of vegetable oils, many oils such as olive [28], canola [37], sunflower [28, 40, 42, 43], sesame [12, 44] and coconuts [45] have been investigated.

Analyses FT-IR, NMR ^{1}H and ^{13}C confirm structural changes undergone by oil during the ozonation. In IR spectra, the bands corresponding to both C=C (1654 cm^{-1}), =C-H (3009 cm^{-1}) stretching and to ozonide C-O stretching (1105 cm^{-1}) are the most important. The intensity of the bands corresponding to the double bonds (C=C) decrease and the band that identify the formation of ozonides increase with respect to the time reaction [12, 46, 47].

The main reaction is the formation of 1,2,4-trioxolane rings, that can be identified by signals observed at ^{1}H NMR spectra with characteristic chemical shifts, like 5.17-5.08 ppm (protons on oxolane ring carbons), at 1,63 (methylene protons α to oxolane ring) and at 1.35 ppm (methylene protons β to ring carbons) [44, 48, 59, 50]. In ^{13}C NMR spectra, oxolane ring carbons have chemical shift at about 103-104 ppm.

Almeida et al. [43] perfomed the ozonolysis reaction in sunflower oil under different conditions and the product is in the process of patent. The IR and ^{1}H and ^{13}C NMR of ozonized oils confirm the formation of 1,2,4-trioxolane ring according to the mechanism proposed by Criegee [43].

Díaz & Gavín [51] studied the products of ozonated methyl linoleate (7) using ^{1}H, ^{13}C and 2DCOSY RMN spectroscopy. Figure 2 shows oxygenated compounds along with their molecular weight that could possibly be obtained in the reaction. All functional groups of the products were well characterized as ozonides (8), hydroperoxides (9) and aldehydes (10) present in ozonized methyl linoleate [51].

In the absence of any participating solvent, the cyclic intermediate (1) called malozonide (Figure 1) leads to the formation of 1,2,4-trioxolanos and peroxide oligomer [47].

Soriano & collaborators [47] performed the decomposition of linoleate and oleoate in neat sunflower oil and in presence of water. In the IR spectrum of ozonated oil in presence of water showed a band at 3471 cm^{-1}, due to the presence of OH groups. According to the Criegee mechanism, water reacts the intermediate carbonyl oxides to give hydroxyl-alkyl-hydroperoxides, thereby preventing the formation of 1,2,4-trioxolane.

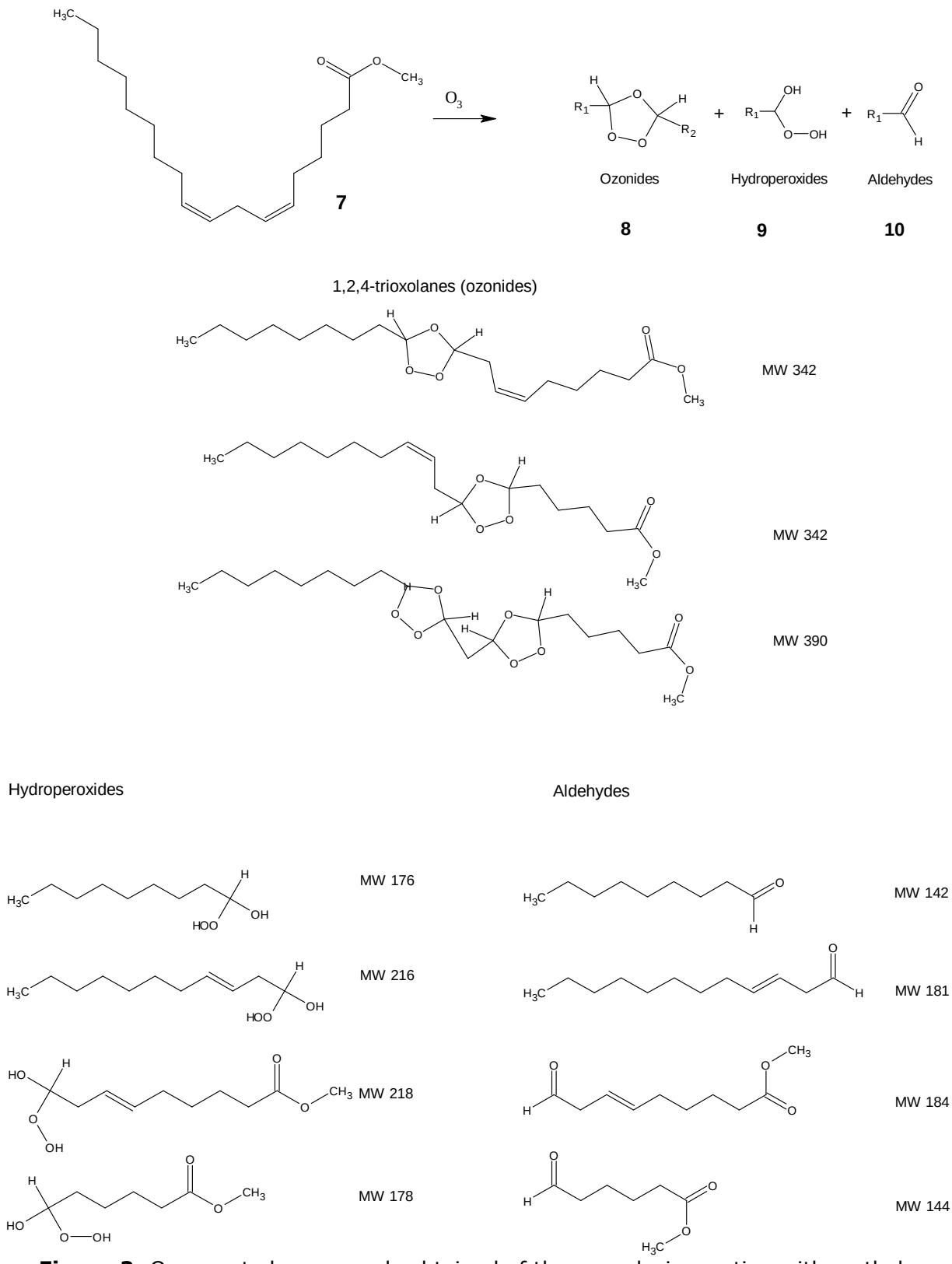

Figure 2: Oxygenated compounds obtained of the ozonolysis reaction with methyl linoleate [51].

The presence of α-hydroxyl-alkyl-hydroperoxides would be evidenced in ^{1}H NMR spectrum, with signals for protons that was expected to resonate between 4.9 and 5.1

ppm [52]. What was not observed by Soriano et al. [46]. The hydroxihydroperoxides may lose hydrogen peroxide to give aldehyde or rearrange to carboxylic acids.

Acording to Diaz et al. [45], the ozonization of coconut oil in presence of water leads to higher ozonides formation. When ethanol is added to the reaction of coconut oil with ozone, higher peroxide decomposition occurs and this favors formation of acids and aldehyde [45].

The study of the physico-chemical properties of ozonated vegetable oils has great importance for their characterization and identification. For determining the quality of ozonized products, analytical methods such as peroxide, acidy and iodine values are usually carried out [12, 38, 53].

The peroxide value (PV) represents the quantity of peroxide in the sample; acid value (AV) represents the present free acids; and iodine value (IV) is a measure of total number of double bonds in the sample. All values are well described according to the European pharmacopoeia [54, 55, 56] and Official Methods of Analysis of the Association of Official Analytical Chemists [57, 58].

The peroxide value represents the quantity of peroxide expressed in milliequivalents of active oxygen contained in a 1000 g sample. In the case of materials characterizated by a high peroxide content, some authors determined the PV introducing changes into the method described in the official monograph due the slow iodide reactivity with diallylperoxides [12, 59]. In accordance with the official methods of analysis, after addition of potassium iodide, the sample is allowed to stand for 1 minute so that the peroxide oxidizes iodide to iodine. During the ozonolysis of sunflower oil, polymeric peroxides and other organic peroxides have been formed [60], and due the high concentration of peroxides a long reaction time is required for these compounds the oxidize iodide to iodine [39]. Some methods include increased reaction time and reflux until 60 ºC. Peroxide content of ozonated sunflower oil using iodometric assay achieved the maximum values at 24 hours of reaction time [39].

Other difficult found in the iodometric assay is susceptible to interference by molecular oxygen as well as the reaction of liberated iodine with other components in the systems [61].

According to a Cuban patent (U.S. Pat No. PI 0309256-1 A), published in 2005, the ozonolysis reaction was continued until obtaining peroxide value between 600-800 units and acid number less than 15 mg/g for the sunflower oil; peroxide value between 1000-1200 units and acid value below 30 mg/g for cacao oil, used in the preparation of therapeutic and cosmetic creams formulations [62].

The acid value of ozonized oils does not directly indicate the oil quality or process

of rancidity [12]. An increase in acid value was observed in several works as increasing the reaction time [12, 44, 63], that may be due to acid formation during the ozonation and due to peroxide decomposition.

For the ozonated oils, the iodine value showed a decrease in relation to applied ozone dose. Ozone reaction with the unsaturated fatty acids led to rapid decrease of iodine values [12, 63].

Antimicrobial property of ozonated oils

Ozone germicidal action was widely proved on a broad group of microorganisms, including Gram-positive and Gram-negative bacteria as well as fungi spores and vegetative cells [64].

Telles Silveira et al. [65] compared antimicrobial activities of ozone and sodium hypochlorite on *Enterococcus sp* (Gram positive bacteria), which are often found in hospital sewage. Ozone was more effective than sodium hypochlorite against these microorganisms, even on Vancomycin-resistant Enterococcus.

Inactivation of bacteria by ozone is a complex process once ozone attacks numerous cellular constituents, including proteins, unsaturated lipids and respiratory enzymes in cell membranes, peptidoglycans in cell wall, enzymes and nucleic acids in the cytoplasm, and proteins and peptidoglycan in spore coats and virus capsids. Some authors concluded that molecular ozone is the main inactivator of microorganisms, while others emphasize the antimicrobial activity of the reactive by-products of ozone decomposition such as OH^-, O_2^-, and $OH^\cdot$ [66-68].

Chlorine acts specifically by diffusion through the cell wall, acting on the vital elements within the cell, such as enzimes, protein, DNA and RNA. Unlike chlorine, the ozone may oxidize various components of cell envelope including polyunsaturated fatty acids, membrane-bound enzymes, glycoproteins and glycolipids, leading to leakage of cell contents and eventually causing lysis [69]. It acts directs on the cell wall, causing rupture and death in a short contact time, preventing the recovery of microorganisms of the attack [70].

Bactericidal, fungicidal and virucidal properties of ozone are attributed to its ability to destroy many of the enzymatic structures. Naturally each microorganism has specific sensitivity to ozone. Bacteria are more sensitive than yeast and fungi. As a result of differences in structure of the cells walls, Gram positive bacteria are more sensitive to ozone than Gram negative ones [20, 64, 71].

Probably, when stable ozonide comes into contact with the warn exudate of the wound, it slowly decomposes in different peroxides, that can explain the prolonged antimicrobial and stimulatory activity of tissue repair [24].

Ozonized sunflower oil has a wide antimicrobial spectrum showing inhibition and lethal activity on gram positive and gram negative bacteria resistant to antibiotics, as *Mycobacterium* species, yeast of the gender Candida and some protozoa like *Giardia lamblia* [25, 27, 72, 73].

The ozonized olive and sunflower oils show activity against *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 10536, *Bacillus subtilis* ATCC 6633 and *Pseudomonas aeruginosa* ATCC 27853. The olive and sunflower ozonized oil with peroxide value of 2439 and 2506 mmol-equiv. Kg^{-1} respectively, showed Minimum Inhibitory Concentrations (MICs) of 0.95 mg Ml^{-1} for all tested bacteria. Diaz et al. [28] research indicates that at higher peroxide value, higher antimicrobial activity of ozonized sunflower oil.

Because of the antimicrobial properties of ozonized vegetable oils, there are many patents describing their use for the treatment of infectious diseases such as dermatitis, acne, ulcers, sores, burns and other skin lesions [74-77], treatment of asthma [78], use as a laxative and for treating intestinal infections, where they act against pathogenic intestine microorganisms [79], therapy for gastroduodenal ulcers [78] and to treat *Giardia lamblia* infections [80]. Recently their use has been described for the treatment of infections caused by pinworms, genital herpes simplex, human papilloma virus (HPV), and fungi, such as microorganisms of the genus *Candida* [38].

Conclusion

Ozonetherapy, ozonized vegetable oils are an interesting alternative for investigation. The availability of vegetable oils and fatty materials in Brazil and the ease of obtaining these products, in addition to low operating costs and high cost-benefit ratio allow the use of ozone and related products in the treatment of various diseases, especially chronic wounds. Ozonized oils, containing 1,2,4-trioxolane rings formed in unsaturated fatty acid chain, can be considered as an active principle and a vehicle at the same time, enhancing absorption and skin penetration. Using and/or synthesizing natural molecules or modified bioinspired products are rational strategies for achieving adhesiveness, biological and positive immunological activities, to control drugs' release and to promote easier penetration into normal skin. The technology for oils and fats modifications considers traditional knowledge for the synthesis of low cost and innovative products, with new applications due to biological activity resulting from generation of radicals and oxidizing species. Possessing extensive biological activity with reduced collateral effects these products are an alternative to use of available resources, natural and renewable sources, using simple low cost technology.

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