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Investigación

New Bioactive Derivatives of Xanthorrhizol

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Abstract. The chemical preparation and the antifungic and cytotoxic evaluations of several new derivatives of xanthorrhizol, a bioactive natural product isolated from certain plants used in traditional medicine, are described. Acylation of the phenol, bromination of the benzene ring, as well as reduction and oxidation of the olefin of the natural sesquiterpene, allowed obtaining a series of derivatives which displayed mild antifungic activities and did not show cytotoxic activities toward certain human tumor cell lines.
Key words: Xanthorrhizol, *Iostephane heterophylla,* antifungic agents, bisabolene derivatives.

Resumen. Se decribe la preparación química y las evaluaciones antifúngicas y citotóxicas de algunos derivados novedosos del xantorrhizol, un producto natural bioactivo aislado de ciertas plantas usadas en la medicina tradicional. La acilación del fenol, la bromación del anillo bencénico, y la oxidación y reducción de la olefina del sesquiterpeno natural permitieron la obtención de una serie de derivados, los cuales mostraron actividades fúngicas moderadas y no mostraron actividad citotóxica en ciertas líneas celulares.
Palabras clave: Xantorrhizol, *Iostephane heterophylla,* agentes antifúngicos, derivados del bisaboleno.

Introduction

Xanthorrhizol (**1**), a sesquiterpene of the bisabolene type, has been isolated from Zingiberaceae and Asteraceae families of higher plants [1,2]. This compound constitutes the major secondary metabolite in the chloroform extract of the roots of *Iostephane heterophylla* (Asteraceae), a plant that has been medicinally used in Central Mexico to treat skin illnesses such as wounds and sores, among other diseases [3]. Previous evaluations on this compound, as antifungic and cytotoxic agent [3], for prolonging pentobarbital induced sleeping time [4], and as inhibitor of the tonic contraction of rat uterus [5], have shown positive results. Xanthorrhizol is a sesquiterpenic phenol, with an unsaturated prenylic chain at C-5. The antimicrobial activity of phenol derivatives is well documented in the literature, and phenol itself is employed as a standard of bactericidal activity in the USP Pharmacopoeia, reporting its antimicrobial action as the "phenol coefficient" [6]. Introduction of chlorine or bromine atoms in phenolic ring enhances the antiseptic activities, as well as the presence of alkyl chains, being more potent the lineal than the ramified ones. Acetylation of the phenolic group produces an ester that slowly liberates the phenol when it contacts the tissues. This property diminishes its irritant action and allows it to be employed as an antiinfective agent in nose, throat and ears [6].

Since chemical modifications of a bioactive natural product result in the modification of its biological properties [7-9], some new derivatives of xanthorrhizol (**1**) were prepared, in which the phenol was modified to acetate and benzoate esters. Other modifications of **1** were the bromination of the aromatic ring and the saturation and oxidation of the $\Delta^{12,13}$ double bond. In this paper, we report the preparation of the above mentioned derivatives and their evaluation in the antidermatophytic and cytotoxic bioassays.

Results and discussion

6-Bromo- (**2**), 4-bromo- (**3**), 12,13-dihydro- (**4**), 1-*O*-acetyl- (**7**), and 1-*O*-benzoyl- (**8**), 12, 13-epoxy- (**5**), and 12, 13-dihydro-dihydroxy-xanthorrhizol (**6**) were prepared from xanthorrhizol (**1**), obtained from the $CHCl_3$ extract of the roots of *I. heterophylla* [3]. Halogenation of **1** with NBS afforded the sterically hindered 6-bromo-xanthorrhizol (**2**) as the main product, together with the bromination product at C-4 (**3**). Both compounds possessed similar spectroscopic characteristics and according to their MS, they showed to be isomers. The molecular ion appeared at *m/z* 298 [M^++2], 297 [M^++1] and 296 [M^+], and other fragments were at *m/z* 217 M^+-Br and 135 M^+-$C_6H_{11}O$ (base peak). The ^{1}H NMR spectrum of **2**, showed signals for two *ortho* benzenic hydrogens at δ 6.71 (H-5) and δ 7.03 (H-4), as a pair of doublets (J = 7.8), while in compound **3**, the benzenic hydrogens appeared *para* to each other, as a pair of singlets at δ 6.65 (H-6) and δ 7.26 (H-3).

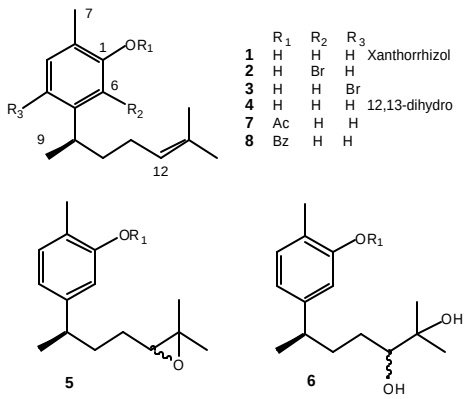
Table 1. ^{13}C NMR Data ($CDCl_3$, 75 MHz) for compounds **1-8**.

C	1	2	3	4	5[a]	6[a]	7	8
1	153.3	150.03	155.25	153.64	153.96	153.91, 154.02	149.28	155.25
2	121.0	113.29	131.02	120.60	121.37	121.45	127.17	127.37
3	130.8	144.33	134.46	130.77	130.77	130.69, 130.78	130.83	134.46
4	119.5	118.80	118.46	119.37	118.89	118.95, 119.22	124.36	124.35
5	147.2	129.67	144.63	147.56	146.18	146.16	146.93	144.63
6	113.7	122.71	113.9	113.46	113.34, 113.52	113.25, 113.38	120.32	120.42
7	15.3	16.39	15.05	15.25	15.44	15.48	15.75	15.84
8	39.1	38.09	37.66	39.42	39.18, 39.52	39.28, 39.54	38.82	38.85
9	22.3	21.28	21.22	22.25	18.62	22.26	22.12	22.16
10	38.5	37.32	37.66	36.52	34.69, 35.12	35.29	38.29	38.31
11	26.2	25.98	26.18	25.38	26.84, 27.16	29.61, 29.78	26.03	26.04
12	124.6	124.24	124.74	39.00	64.63, 64.91	78.63, 78.82	124.71	124.71
13	131.4	131.67	131.63	27.75	58.75, 58.83	73.41	131.52	131.63
14	17.7	17.61	17.22	22.49	22.35, 22.5	23.07, 23.17	17.63	17.64
15	25.7	25.70	25.42	22.59	24.81	26.41	25.67	25.67
1’							20.80	129.73
3’, 5’								128.53
C=O							169.24	

[a] Epimeric mixture at C-12.

The remaining signals corresponded to a benzylic hydrogen, and four methyls (^1H NMR data in the Experimental). Compound **4** was obtained quantitatively by catalytic hydrogenation of xanthorrhizol (10 % Pd / C), and its spectroscopic constants were similar to those of xanthorrhizol, being observed a difference of two additional units in the MS of **4**. **4** was previously characterized [1]. Compounds **5** and **6** were obtained previously as minor natural products [3] and they were prepared as a C-12 epimeric mixture from **1**: epoxidation with *m*-chloro-perbenzoic acid, afforded **5**, and diol **6** was obtained via *trans*-hydroxylation with performic acid [3]. Acetylation and benzoylation of xanthorrhizol (**1**) led to compounds **7** and **8**. ^1H (se Experimental) and ^{13}C NMR (Table 1) data for compounds **2-8** were in agreement with the proposed structures, and the assignments were supported by comparison with the data in the literature [10].

Compounds **1-8** were tested for their antimicrobial activities toward *Trichophyton mentagrophytes* and *Microsporum gypseum* growing *in vitro*, and the results (Table 2) showed that the majority of the structural modifications to xanthorrhizol (derivatives **2**, **3**, **5-8**) led to reduced but clear activities (MIC < 400 µg / mL), while the 12,13-dihydro derivative (**4**) displayed the same bioactivities of the standard antifungic reference (nystatin) and xanthorrhizol (**1**). These preliminary results suggested the structural requirements of xanthorrhizol (**1**) for the activity toward the tested microorganisms. Regarding the antiploriferative activities, although xanthorrhizol (**1**) showed activity against sarcoma 180 ascites in mice [11], the evaluation of compounds **1-8** in KB, UISO-SQC-1 and HCT-15 human tumor cell lines indicated that they did not display significant cytotoxicity (ED_{50} > 4 µg / ml).



Scheme 1

Experimental

IR spectra were taken on an 80A Nicolet FT. MS were measured on a 5985-B Hewlett Packard instrument. Optical rotation was determined on a 241 Perkin Elmer polarimeter. NMR spectra were recorded on a Varian VXR 300 spectrometer, operating at 300 MHz (^1H) and 75 MHz (^{13}C). $CDCl_3$ was used as solvent. Chemical shifts are reported in parts per million (δ) relative to TMS, and coupling constants (*J*) are in Hertz. Carbon substitution degrees were established by DEPT multipulse sequence. Xanthorrhizol (**1**) was isolated from the roots of *I. heterophylla*, as described previously [2,3].

6-Bromo-xanthorrhizol (2) and 4-Bromo-xanthorrhizol (3). Xanthorrhizol (**1**) (225 mg, 1.02 mmol) and *N*-bromo-succinimide (178 mg, 1 mmol) were dissolved in CCl_4 (2.5 mL). The reaction was initiated adding catalytic quantities of dibenzoyl peroxide, and refluxed for 2 h. The starting material was

Table 2. Data of the Antimicrobial activities for compounds **1-8**.[a]

	Compounds								
Microorganisms	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	Nystatin
T. m.[b]	10	200	400	10	200	400	100	400	10
M. g.[c]	10	400	400	10	200	400	100	400	10

[a] MIC values, μg / mL, calculated as previously reported [12].
[b] *T. mentagrophytes*. [C] *M. gypseum.*

consumed, and the formation of two reaction products less polar than xanthorrhizol was observed by TLC. Evaporation of the solvent afforded a residue which was loaded to a Si gel column chromatography (70-230 mesh) packed with petroleum ether. The chromatography was developed using a gradient of petroleum ether-EtOAc. Two oily compounds were separated, and characterized as **2** (205 mg, 66 %), and **3** (66 mg, 21.6 %). **2**: IR $\nu_{máx}$ (film): 3507, 2961, 2856, 1607, 1489, 1450, 1120, 811 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.19 (3H, d, J 7.0, H-9), 1.51 (3H, s, H-15), 1.58 (2H, m, H-11), 1.67 (3H, s, H-14), 1.93 (2H, m, H-10), 2.27 (3H, s, H-7), 3.12 (1H, q, J=7.0. H-8), 5.09 (1H, t, J=7.1, H-12), 6.71 (1H, d, J=7.8, H-5), 7.03 (1H, d, J=7.8, H-4); ^{13}C NMR (75 MHz, $CDCl_3$) in Table 1; EIMS *m/z* (rel. int.): 298 [M^++2], 297 [M^++1], 296 [M^+, 2.0], 217 (M^+-Br, 32.6), 214 (22.1), 201 (12.4), 135 (100), 134 (39.3), 115 (17.4), 105 (17.7), 91 (31.9), 79 (22.4), 77 (31.4). **4-Bromo-xanthorrhizol (3):** IR ν_{max} (film): 3500, 2950, 2860, 1610, 1450, 1115, 990, 815 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.14 (3H, d, J=6.8, H-9), 1.53 (3H, s, H-15), 1.67 (3H, s, H-14), 1.4-1.7 (2H, m, H-11), 1.93 (2H, m, H-10), 2.18 (3H, s, H-7), 3.10 (1H, m, H-8), 4.72 (1H, br s, OH), 5.09 (1H, m, H-12), 6.65 (1H, s, H-6), 7.26 (1H, s, H-3); ^{13}C NMR ($CDCl_3$, 75 MHz) in Table 1.

12, 13-Dihydro-xanthorrhizol (4). A solution of xanthorrhizol (**1**) (80 mg) dissolved in ethyl acetate (10 mL) was hydrogenated using 10 % Pd / C (20 mg), as catalyst. The reaction was monitored by TLC (petroleum ether / ethyl acetate 85:15), and the reaction product was purified by preparative chromatography as a yellowish oil (100 % yield). $[\alpha]_D$-5.61° (*c* 1.3, MeOH); UV λ_{max} (MeOH): 215, 273 nm (ε 17,982, 2324); IR ν_{max} (film): 3400, 3372, 2955, 2867, 1620, 1587, 1458, 1365, 1160, 1116, 993, 811 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 0.62 (3H, d, J=6.6, H-14), 0.63 (3H, d, J=6.6, H-15), 1.11-1.19 (2H, m, H-12, 13), 1.18 (3H, br d, J=7.2, H-9), 1.44-1.52 (2H, m, H-10), 2.21 (3H, s, H-7), 2.57 (1H, m, H-8), 4.85 (1H, br s, -O<u>H</u>), 6.61 (1H, d, J=1.6, H-6), 6.68 (1H, dd, J=7.8, 1.6, H-4), 7.03 (1H, d, J=7.8, H-3); ^{13}C NMR ($CDCl_3$, 75 MHz) in Table 2; EIMS *m/z* (rel. int.): 220 [M^+, 8], 136 (27), 135 (100), 121 (15), 91 (15), 77 (5), 79 (5), 43 (7).

1-*O*-Acetyl-xanthorrhizol (7). Xanthorrhizol (**1**) (322 mg) was acetylated with acetic anhydride (3.3 mL) and pyridine (1 ml) overnight at room temperature. Usual work-up afforded **7** which was purified by column chromatography to obtain 280 mg as an oil (68.7 %). ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.19 (3H, d, J=6.8, H-9), 1.50-1.63 (2H, m, H-10), 1.52 (3H, s, H-15), 1.67 (3H, s, H-14), 1.94-1.83 (2H, m, H-11), 2.13 (3H, s, H-7), 2.31 (3H, s, H-1'), 2.66 (1H, qt, H-8), 5.08 (1H, m, H-12), 6.82 (1H, d, J=1.8, H-6), 6.99 (1H, dd, J=7.7, 1.8, H-4), 7.14 (1H, d, J=7.7, H-3); ^{13}C NMR ($CDCl_3$, 75 MHz) in Table 1.

1-*O*-Benzoyl-xanthorrhizol (8). Xanthorrhizol (**1**) (250 mg), benzoyl chloride (0.5 mL) and pyridine (0.5 mL) were allowed to react overnight at room temperature. Usual work-up and purification of the product by column chromatography allowed to obtain 240 mg of **6** (62 %). IR ν_{max} (film): 3061, 2960, 2865, 1601, 1579, 1451, 1176, 1120, 862, 821, 708 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.24 (2H, d, J 7.0, H-9), 1.51-1.69 (2H, m, H-10), 1.67 (3H, s, H-15), 1.54 (3H, s, H-14), 1.98-1.86 (2H, m, H-11), 2.19 (3H, s, H-7), 2.78-2.63 (1H, m, H-8), 5.09 (1H, m, H-12), 6.96 (1H, d, J=1.6, H-6), 7.01 (1H, dd, J=7.70, 1.6, H-4), 7.19 (1H, d, J=7.7, H-3), 7.69-7.48 (3H, m, H-3', 4', 5'), 8.23 (2H, m, H-2', 6'); ^{13}C NMR ($CDCl_3$, 75 MHz) in Table 1; EIMS *m/z* (rel. int.): 322 [M^+, 6], 254 (35), 217 (20), 105 (100), 91 (18), 68 (5), 43 (7).

Biological Assays. Antimicrobial activity. Studies were performed with cultures of *Trichophyton mentagrophytes* (ATCC 10742) and *Microsporum gypseum* (ATCC 10435). The dermatophytes were maintained on Saboureaud's dextrose agar [12]. **Cytotoxic activity.** Cell cultures were maintained in Basal Medium Eagle (BME) with 10 % fetal bovine serum (FBS), and the assays were performed using standard protocols [12].

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