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NAPHTHOQUINOIDS FROM *LIPPIA SIDOIDES*

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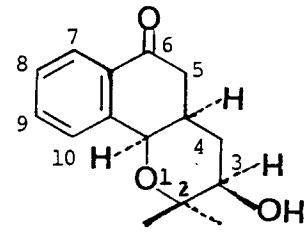
Lippia sidoides Cham. (Verbenaceae) is an aromatic shrub, widespread in occurrence in the deciduous vegetation "caatinga" of Northeastern Brazil. Our specimen was collected near Mossoró in the state of Rio Grande do Norte. Observations of the use of an aqueous extract of leaves by rural populations verified the presence of healing and antiseptic effects. Some chemical and pharmacological studies of this plant were made previously, including chemical analysis of the essential oils of the leaves, which contain thymol and carvacrol as major constituents (2). The essential oil shows bactericidal, bacteriostatic, fungicidal, and fungistatic activities against *Staphylococcus aureus* and other microorganisms (3). The residual water resulting from the steam distillation process presented several pharmacological effects on isolated organs and promoted arterial hypotension on rats (4).

Interesting naphthoquinoids have been reported in plants of the genus *Lippia* (5,6), but no mention is made of their occurrence in *L. sidoides*. These observations and demonstration of several biological activities in preparations of this plant stimulated us to reexamine the chemical constituents of the species, especially the nature of the nonvolatile components.

The following substances were identified in the methanolic extract of leaves and branches: palmitic acid, stearic acid, behenic acid, arachidic acid, lignoceric acid, thymol, carvacrol, β-sitosterol, lapachenol, isocatalponol, and a new substance, 6-oxo-3,4,4a,5-tetrahydro-3-hydroxy-2,2-dimethylnaphtho-1,2-pirane (**1**). All substances but the last were reported previously in plants of the genus *Lippia* but not in this species.



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The ir spectrum of the new substance (**1**) presented an absorption at 3530 cm^{-1} assignable to a nonassociated OH group as well as absorptions for an aromatic ketone (1670 cm^{-1}), an aromatic double bond (1580 cm^{-1}), for four adjacent hydrogens in an aromatic ring (775 cm^{-1}), and an indication of a cyclic ether (1280 cm^{-1}) (7). Absorption at 256 nm in the uv spectrum confirmed the presence of a carbonyl group conjugated with the aromatic ring (8). The presence of this carbonyl group is also supported by an absorption at δ-196.7 in the ^{13}C-nmr spectrum (9). The ms of **1** presented a significant peak at 246 daltons assignable to a molecular ion of $C_{15}H_{18}O_3$ as a molecular formula. The ^1H-nmr spectrum of **1** taken at 60 MHz in $CDCl_3$ contained the following signals: (a) a multiplet from 7.3 to 8.0 δ, corresponding to four aromatic protons; (b) a one hydrogen doublet at 4.5 δ (J=9.0 Hz) in agreement with absorp-

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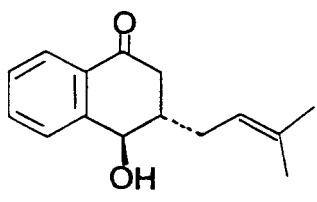
tion due to a proton on a benzylic carbon bonded to oxygen; (c) a one hydrogen quartet centered at 3.6 δ(J=5.0 and 12.0 Hz) attributed to a carbinolic proton; (d) a broad absorption at 2.5 δ corresponding to one hydrogen assignable to an axial methynic proton; (e) a two hydrogen multiplet from 2.4 to 2.7 δ corresponding to the methylene protons of carbon five; (f) a multiplet from 1.9 to 2.2 δ attributed to two aliphatic protons of carbon four; (g) two 3-hydrogen singlets at 1.3 and 1.4 corresponding to the two *gem* methyl groups.

The PND ^{13}C-nmr spectrum showed the presence of 15 spectral lines in agreement with the proposed molecular formula. In addition to the carbonyl (196.7 δ), six aromatic carbons are clearly present (131.0, 144.6, 125.0, 127.4, 126.8, and 134.0 δ). Three carbons are bonded to oxygen (76.5, 73.4, and 70.8 δ), one carbon is in the vicinity of a carbonyl (43.1 δ), and four aliphatic carbons (16.2, 28.1, 39.8, and 35.0 δ) are observed. Specific assignments are given in the Experimental section. The placement of the hydroxyl group at carbon 3 instead of carbon 4 was possible from analysis of ^{13}C-nmr spectrum where the difference in absorptions of the *gem* methyl groups (28.1 and 16.2 δ) can be attributed to the γ-effect of the hydroxyl in the vicinity. Furthermore, it is reasonable to propose isocatalponol (**2**) as the biosynthetic precursor which, by cyclization, produces **1**. In fact, treatment of isocaltalponol (**2**) with *m*-chloroperbenzoic acid in $CHCl_3$ followed by reaction with BF_3. Et_2O gave **1**, identical, by tlc in three systems of solvent and ^{1}H-nmr spectrum, with the natural product. The same argument also favors positioning the hydroxyl at position 3. Acetylation of **1** gave a monoacetyl derivative, confirming the presence of a secondary aliphatic hydroxyl.



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The proposed structure (**1**) for this naphthoquinoid has no parallel in the literature, as far as we know.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Mettler FP-5/52 and are uncorrected. Spectral data were obtained on the following instruments: ir, Perkin-Elmer Model 283-B; ^{1}H nmr, Varian EM-360; ^{13}C nmr; Varian XL-100; ms, Hewlett, Packard HP 5995A; gc/ms, HP-5933 equipped with data system; uv, Varian Model 634-S. Adsorbents for cc and tlc, silica gel 60, 230-400 mesh and silica gel 60 tlc plates 250 mesh were purchased from Merck and Co.

PLANT MATERIAL.—Leaves and branches of *L. sidoides* were collected around Mossoró, Rio Grande do Norte, in the semiarid area of the Brazilian Northeast in June 1981. Voucher specimens are deposited in the "Herbario Prisco Bezerra" of the Universidade Federal do Ceará, Fortaleza-Ceará, Brazil.

EXTRACTION AND PURIFICATION.—Dried parts (23 g) were extracted with MeOH in a Sohxlet apparatus, giving 760 g of a resinous extract. This material (250 g) was adsorbed on silica gel (500 g) and eluted with $CHCl_3$, Me_2CO, and MeOH successively to give fractions of 41.9 g, 107.6 g, and 80.6 g. These fractions were submitted to fractionation by repetitive cc to give a fixed oil, an aromatic volatile oil, four known solid substances, and the new naphthoquinone (**1**).

The fixed oil was treated with BF_3-MeOH complex, and the product was extracted with hexane. Gc/ms analysis of the hexane extractives gave five peaks which were compared with ms spectra and retention times of standard methyl esters of fatty acids and identified as the methyl esters of palmitic, stearic, arachidic, behenic, and lignoceric acids.

The volatile oil was obtained by further cc of the Me_2CO eluate and is showed by gc/ms analysis two major components that were identified as thymol and carvacrol by comparison of their ms and Kovat's indices with standards.

6,7-Dimethoxy-5,4′-dihydroxyflavone was isolated by silica gel cc of the Me_2CO eluate and recrystallized from $CHCl_3$-Me_2CO to give an amorphous yellow solid, 50 mg, with mp 285-288° (10,11). β-Sitosterol was isolated (970 mg) from the $CHCl_3$ eluate by further cc and purified

by repetitive precipitations with MeOH until pure, mp 136-139°. Lapachenol was isolated (100.0 mg) from the $CHCl_3$ eluate by repetitive cc. Recrystallization of the main fraction gave a yellow solid, mp 57-60°. Its ir, ^1H nmr, and mass spectra were identical to those obtained from an authentic sample (5). Isocatalponol was isolated by cc of the $CHCl_3$ eluate followed by decolorizing with charcoal and a new cc to give needles (150.0 mg), mp 78-79°. Its mp, ir, ^1H nmr, and mass spectra were in agreement with those of isocatalponol (6,12).

6-OXO-3,4,4A-5-TETRAHYDRO-3-HYDROXY-2,2-DIMETHYLNAPHTHO [1,2] PYRAN (**1**).—Obtained by cc of the Me_2CO eluate. Recrystallized from C_6H_6, mp 148-149°, M^+ 246. $C_{15}H_{18}O_3$; ir ν max (KBr) 3530, 3050, 1670, 1580, 1450, 1280, 1250, 1110, 1070, 1050, 1020, 775, 640 cm^{-1}; ms *m/z* (%) 246 (M^+, 1), 228 (1), 188 (10), 157 (3), 145 (26), 144 (100), 129 (4), 116 (23), 115 (28), 105 (2,2), and 91 (3); ^1H nmr (60 MHz, $CDCl_3$) δ 8.02-7.30 (m, 4, ArH), 4.5 (d, J=9.0 Hz, 1, H-1a), 3.63 (dd, J=5.0; 12.0 Hz, 1, H-3), 2.50 (m, 1, H-4a), 2.40-2.70 (m, 2, H-5), 1.99-2.20 (m, 2, H-4), 1.41 (s, 3, CH_3), 1.30 (s, 3, CH_3); ^{13}C nmr (25.2 MHz, $CDCl_3$ δ 196.7 (s, C-6), 144.6 (s, C-10a), 134.0 (d, C-9), 131.0 (s, C-7a), 127.4 (d, C-7), 126.8 (d, C-8), 125.0 (d, C-10), 76.5 (s, C-2), 73.4 (d, C-1a), 70.8 (d, C-3), 43.1 (t, C-5), 39.8 (d, C-4a), 35.0 (t, C-4), 28.1 (q, *CH*$_3$ eq.), and 16.2 (q, *CH*$_3$ ax.); $[\alpha]^{25}D$ +22.46.

6-OXO-3,4,4A,5-TETRAHYDRO-3-HYDROXY-2,2-DIMETHYL-NAPHTHO [1,2] PYRAN ACETATE.—To pyridine (1.0 ml) and Ac_2O (2.0 ml) was added **1** (250 mg). The reaction mixture was kept for 24 h at room temperature. Usual workup gave an oil. ^1H nmr (60 MHz, $CDCl_3$) δ 8.02-7.3 (m, 4, ArH), 4.6 (d, J=10.0 Hz, H-1a), 4.7 (dd, J=5.0 and 12.0 Hz, 1, H-3), 2.5 (m, 3, H-4a and H-5), 2.2-1.8 (m, 2, H-4), 2.08 (s, 3, *CH*$_3$-C=0), 1.43 (s, 3, *CH*$_3$), 1.36 (s, 3, *CH*$_3$); ^{13}C nmr (25.2 MHz, $CDCl_3$) δ 195.9 (s, C-6), 170.2 (s, CH_3-*C*=0), 133.8 (d, C-9), 130.8 (s, C-7a), 127.4 (d, C-7), 126.9 (d, C-8), 124.7 (d, C-10), 74.6 (s, C-2), 74.5 (d, C-1a), 70.7 (d, C-3), 42.7 (t, C-5), 39.0 (d, C-4a), 31.4 (t, C-4), 27.9 (q, *CH*$_3$ ax), 21.1 (q, *CH*$_3$-C=0), 17.6 (q, *CH*$_3$ eq.).

SYNTHESIS OF **1**.—Isocatalponol (0.304 g) in $CHCl_3$ (5.0 ml) was treated with *m*-chloroperbenzoic acid (0.226 g) in $CHCl_3$. Treatment of the reaction mixture with $BF_3 \cdot Et_2O$ (traces) followed by workup produced **1** (0.0235 g) identical with natural naphthoquinoid (**1**). ^1H nmr 7.9-7.1 (m, 4, ArH), 4.5 (d, 1.10 Hz, H-1a), 3.6 (dd, J=5.0 Hz and J=12.O Hz, 1, H-3), 2.3-2.7 (m, 3, H-4 and H-5), 1.7-2.2 (m, 2, H-4), 1.45 (s, 3, CH_3), 1.35 (s, 3, CH_3).

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