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1-ARYL TETRALIN LIGNANS FROM *LARREA TRIDENTATA*[1]

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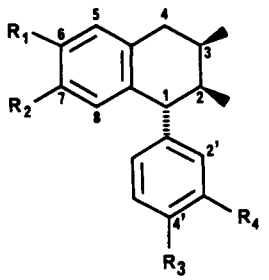
ABSTRACT.—Continuing investigation of the constituents of *Larrea tridentata* has afforded two new 1-aryl tetralin lignan derivatives, **1** and **2**, whose structures were determined through interpretation of their physical and spectroscopic data. Of particular importance was the use of 2D nmr experiments for structure elucidation.

As part of our continuing studies on the isolation and structure elucidation of fertility regulating agents from plants, we have investigated the creosote bush, *Larrea tridentata* (DC.) Coville (Zygophyllaceae), which has been utilized as a contraceptive agent in Mexico (1). [In the recent literature relating to the botanical aspects of this plant, there is a tendency towards the recognition of *L. tridentata*, native to the southwestern U.S. and northern Mexico, as a separate species from *Larrea divaricata* Cavanilles, native to northwestern Argentina (2,3). Because the material used in this study was collected in Arizona, we have used the name *L. tridentata*. Extracts of this plant have also been reported to display uterine relaxation activity in vitro (4). Previous phytochemical work has indicated the presence of flavonoids, lignans, volatile oils, and saponins (5–14), and we have recently reported on the isolation and structure elucidation of two new triterpenes from the stems (15) and on the bioassay-directed isolation of the active anti-implantation agent, 3′-demethoxy-6-*O*-demethylisoguaiacin [**3**], from the leaf and twig parts (16) of this plant. [Compound **3** was referred to as "nor-3′-demethoxyisoguaiacin" by Fernandez *et al.* (14) and in our previous publication (16). We now feel that the "nor" series should be designated as "6-*O*-demethyl-" derivatives. The isolation and biological evaluation of a number of other 1-aryl tetralin and perhydrofurano lignans from the leaf and twig and stem parts have also been summarized (16). A recent



1 $R_1=R_2=R_3=R_4=OH$
3 $R_1=R_2=R_3=OH$, $R_4=H$
5 $R_1=R_2=R_3=R_4=OMe$
6 $R_1=R_2=R_3=OH$, $R_4=OMe$

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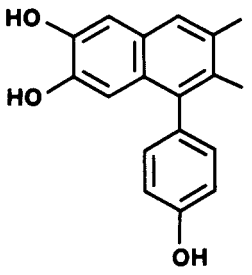

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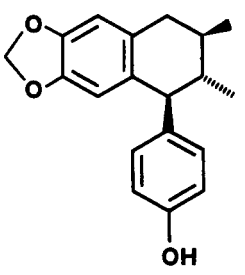

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paper describes the X-ray crystallographic analysis of the triacetate of **3** also from *L. tridentata*, but without any spectroscopic studies (17).

Dried leaves and twigs of *L. tridentata* were extracted with MeOH and the extract treated in the usual way to afford a phenolic fraction, which was subjected to extensive chromatography on Si gel. Several new lignans were isolated during the course of this work, and their structures were determined. In this paper we report on two of these which are members of the 1-aryl tetralin group of lignans. In a subsequent paper[6] we will present the evidence for several new members of the perhydrofurano class of lignan.

6,3′-Di-*O*-demethylisoguaiacin [**1**] [designated as "3′-hydroxynorisoguaiacin" in our previous publication (16)], mp 85–87°, displayed a molecular ion at *m/z* 300.1370 (calcd 300.1361), analyzing for $C_{18}H_{20}O_4$ by hrms. The ir spectrum revealed a hydroxyl group absorption (ν max 3380 cm^{-1}), and its ^{1}H-nmr spectrum indicated the presence of two methyl doublets at δ 0.87, two methine multiplets at δ 1.88 and 2.02, two magnetically non-equivalent methylene protons at δ 2.37 and 2.80, a dibenzylic methine doublet at δ 3.51, two aromatic protons at δ 6.26 and 6.56, and three coupled aromatic protons at δ 6.43, 6.44, and 6.71. These signals were very similar to those of 3′-demethoxy-6-*O*-demethylisoguaiacin [**3**], which we had also isolated, except for the ABC system of a 3,4-dihydroxyphenyl moiety in **1**. Similarity of the ^{13}C-nmr data with those of epicatechin (18) confirmed the presence of the 3,4-dihydroxyphenyl unit, which was also supported by a mass spectral fragment ion at *m/z* 123.

In the COSY spectrum of **1**, H-1 (δ 3.51) was coupled to a multiplet at δ 1.88, which was assigned to H-2. Because the coupling constant between these protons was 6.3 Hz, similar to that in **3**, it was concluded that the phenyl group and the methyl group were trans to each other. From the chemical shift of the two methyl groups (δ 0.87), which was comparable to that of **3**, but not to that of attenuol [**4**] which shows doublets at δ 0.85 and 1.05 (19), the relative stereostructure was deduced to be the same as that in **3**. Indeed, the remaining signals in the ^{13}C-nmr spectrum also corresponded well with those of **3**. The assignment of carbon resonances of **1** was made on the basis of those reported for similar lignan derivatives (20–22), with the aid of APT experiments. From the negative Cotton effects at 217 and 270 nm and the positive Cotton effect at 290 nm, it was deduced that the absolute structure of 6,3′-di-*O*-demethylisoguaiacin is represented by **1**. Methylation (Me_2SO_4, K_2CO_3) afforded isoguaiacin dimethyl ether [**5**], which could also be prepared from 6-*O*-demethylisoguaiacin (norisoguaiacin) [**6**].

Didehydro-3′-demethoxy-6-*O*-demethylguaiacin [**2**], mp 127–128°, displayed an $[M]^+$ at *m/z* 280.1027 (calcd 280.1099), analyzing for $C_{18}H_{16}O_3$ in the high resolution mass spectrum. Its ir spectrum revealed a hydroxyl group absorption at 3280

[6]C. Konno, Z.-Z. Lu, H.-Z. Xue, C.A.J. Erdelmeier, D. Meksuriyen, G.A. Cordell, D.D. Soejarto, D.P. Waller, and H.H.S. Fong, manuscript in preparation.

cm^{-1}, and its ^{1}H-nmr spectrum demonstrated the presence of two aromatic methyl groups at δ 2.05 and 2.36, three singlet aromatic protons at δ 6.44, 7.12, and 7.38, and a pair of doublets at δ 6.98 and 7.00, suggesting that this lignan contained a 4-hydroxyphenyl moiety. Confirmation of this was achieved through examination of the ^{13}C-nmr spectrum, which showed pertinent signals at δ 131.0, 131.9 (2C), 116.0 (2C) and 157.1, comparing well with the corresponding data for this moiety in ephedradine D (23).

In the ^{1}H-nmr spectrum of **2**, the aliphatic protons present in the spectrum of **1** were absent. In their place were two aromatic methyl groups and a singlet aromatic proton. All other aspects of the spectrum were identical with those of **3**. This observation and the molecular formula, which was four hydrogens less than that of **3**, suggested that **2** was the didehydro derivative of **3**.

In order to establish the structure of this lignan, a delayed homonuclear COSY spectrum was obtained in which the long range couplings were emphasized. In this way we were able to establish long-range coupling between the methyl groups, between the methyl group at δ 2.36 (3-Me) and the methine signal at δ 7.38 (H-4), between the signal at δ 7.38 (H-4) and the protons at δ 7.12 (H-5) and 6.64 (H-8), and between the protons at δ 7.12 (H-5) and 6.64 (H-8). The upfield shift of this latter proton suggested that it was both adjacent to one of the hydroxyl groups and shielded by the 4-hydroxyphenyl moiety. One of the aromatic methyl groups is also somewhat shielded (to δ 2.05) indicating that it too is under the influence of this moiety and can therefore be assigned to C-2. The available evidence therefore suggests that didehydro-3′-demethoxy-6-*O*-demethylguaiacin has the structure **2**. When the 2D nOe spectrum was examined, enhancement was observed between the methyl groups, and between the methyl group at δ 2.36 (3-Me) and the proton at δ 7.38 (H-4), which should therefore be proximate, as expected for **2**. The ^{13}C-nmr spectrum of **2** was assigned from an APT experiment and by comparison with the reported chemical shift values for similar lignans (24).

The biological properties of two of these isolates have been described elsewhere (16). In summary, 3′-demethoxy-6-*O*-demethylisoguaiacin [**3**] was demonstrated to be the orally active anti-implantation agent in rats, whereas 6,3′-di-*O*-demethylisoguaiacin [**1**] was inactive. Didehydro-3′-demethoxy-6-*O*-demethylguaiacin [**2**] has not been evaluated thus far.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Kofler-type hot-stage apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer Model 241 polarimeter. Cd spectra were recorded on a JASCO J-40A automatic recording spectropolarimeter using a quartz cell of 20 mm length and 3.5 ml volume. Uv spectra were recorded with a Beckman model DU-7 spectrophotometer, and ir spectra were obtained with a Nicolet MX-1 interferometer. Mass spectra were determined with a Varian MAT 112S double focusing mass spectrometer at 80 eV. The ^{1}H-nmr spectra were obtained with either a Nicolet NMC 360 instrument operating at 360 MHz or a Varian XL-300 instrument operating at 300 MHz. TMS was used as the internal standard, and chemical shifts are reported in δ ppm downfield from TMS.

Homonuclear COSY spectra were recorded at 1K with a Varian XL 300 spectrometer. Standard Varian pulse sequences were used. NOe difference spectra were measured on a Nicolet NMC 360 spectrometer. The samples were degassed by using a repeated freeze-pump-thaw cycle and then closed under N_2. Data sets of 16K covering a spectral width of 2 MHz were acquired. A 2.0 Hz line broadening was applied to the data prior to Fourier transformation.

PLANT MATERIAL.—The leaves and twigs of *L. tridentata* were collected by Mr. V.M. Gass and Ms. Wendy Hodgson, Desert Botanical Garden, Phoenix, Arizona in the vicinity of Phoenix, Arizona in June 1983. The identity of the sample was confirmed by one of us (D.D.S.). Herbarium specimens representing the collection are deposited in the herbarium of the Desert Botanical Garden and in the John G. Searle Herbarium, Field Museum of Natural History, Chicago, Illinois.

ISOLATION OF LIGNANS.—The leaves and twigs of *L. tridentata* (23.4 kg) were exhaustively, extracted with MeOH at room temperature concentrated in vacuo, and partitioned in petroleum ether-MeOH-H_2O (10:1:9). The resulting MeOH-H_2O fraction was further partitioned between H_2O and $CHCl_3$ to afford a $CHCl_3$-soluble fraction (2.32 kg) on evaporation. This fraction was then partitioned against 5% NaOH solution, and the aqueous alkaline phase was acidified with 1 N HCl and extracted with $CHCl_3$. Evaporation of the $CHCl_3$ fraction, after drying with Na_2SO_4, afforded a phenolic fraction (1.93 kg).

The phenolic fraction was chromatographed over Si gel (6 kg) eluting successively with $CHCl_3$, $CHCl_3$-MeOH (98:2, 95:5, 90:10, 80:20), and pure MeOH. Fractions eluted with $CHCl_3$-MeOH (95:5) and $CHCl_3$-MeOH (90:10) were combined (F-009), evaporated, and rechromatographed on Si gel (3.3 kg) eluting with $CHCl_3$ and mixtures of $CHCl_3$-MeOH of increasing polarity (98:2, 95:5, 93:7, 90:10, and 80:20).

Elution of F-009 with $CHCl_3$-MeOH (93:7) afforded a fraction (F-037) which was subjected to bioassay-directed chromatographic separation in a series of Si gel columns. Fractional crystallization of the active fraction (F-073) from C_6H_6/MeOH afforded 3′-demethoxy-6-*O*-demethylisoguaiacin [**3**] (82 mg, 0.00035%) and didehydro-3′-demethoxy-6-*O*-demethylguaiacin [**2**] (66 mg, 0.00028%). Elution of F-009 with $CHCl_3$-MeOH (9:1) afforded a fraction (F-038), which on concentration in vacuo gave the known linear lignan, nordihydroguaiaretic acid (NDGA, 106.3 g). Repeated Si gel cc of the post-NDGA mother liquor led to the isolation of 6,3′-di-*O*-demethylisoguaiacin [**1**] (763 mg, 0.0033%). The new isolates had the following physical and spectroscopic properties.

6,3′-Di-O-demethylisoguaiacin [**1**].—Mp 85–87°; cd (c=0.017, MeOH) $[\theta]_{207}$ +2700, $[\theta]_{217}$ −6000, $[\theta]_{270}$ −2300, $[\theta]_{290}$ +2100; ir ν max (KBr) 3380, 3120, 3095, 1700, 1615, 1619, 1446, 1355, 1280, 1190, 1111, 772 cm^{-1}; uv λ max (MeOH) (log ϵ) 227 (4.14) and 285 (3.78) nm, (MeOH + NaOH) 231 (4.31) and 286 (3.88) nm; ^{1}H-nmr (360 MHz, Me_2CO-d_6) δ 0.87 (6H, d, J = 6.8 Hz, 2- and 3-Me), 1.88 (1H, m, H-2), 2.02 (1H, m, H-3), 2.37 (1H, dd, J = 16.5 and 7.7 Hz, H-4a), 2.80 (1H, dd, J = 16.5 and 5.4 Hz, H-4b), 3.51 (1H, d, J = 6.3 Hz, H-1), 6.26 (1H, s, H-8), 6.43 (1H, dd, J = 8.7 and 2.1 Hz, H-6′), 6.44 (1H, br s, H-2′), 6.56 (1H, s, H-5), and 6.71 (1H, d, J = 8.7 Hz, H-5′); ^{13}C-nmr (90.54 MHz, Me_2CO-d_6) δ 16.0 (Me), 16.2 (Me), 29.8 (C-3), 35.4 (C-4), 41.5 (C-2), 50.8 (C-1), 115.5 (C-5 or C-5′), 115.7 (C-5′ or C-5), 116.8 (C-2′), 117.6 (C-8), 121.2 (C-6′), 128.0 (C-4a), 130.3 (C-1′), 140.1 (C-8a), 143.7 (C-6 and C-7), 144.0 (C-4′), 145.2 (C-3′); ms *m/z* (rel. int.) $[M]^+$ 300 (100%), 244 (27), 243 (46), 227 (97), 226 (32), 190 (10), 175 (15), 123 (23).

Didehydro-3′-demethoxy-6-O-demethylguaiacin [**2**].—Mp 127–128°; ir ν max (KBr) 3280, 1620, 1526, 1522, 1441, 1437, 1264, 1231, 1185, 1171, 855, 773 cm^{-1}; uv λ max (MeOH) (log ϵ) 231 (4.83), 232 (4.82), 243 (4.75), 316 (3.66) and 332 (3.73) nm, (MeOH + NaOH) 278 (5.17) and 342 (3.78) nm; ^{1}H-nmr (360 MHz, Me_2CO-d_6) δ 2.05 (3H, s, 2-Me), 2.36 (3H, s, 3-Me), 6.64 (1H, s, H-8), 6.98 (2H, d, J = 7.7 Hz, H-3′, H-5′), 7.00 (2H, d, J = 7.7 Hz, H-2′, H-6′), 7.12 (1H, s, H-5), 7.38 (1H, s, H-4); ^{13}C-nmr (90.54 MHz, Me_2CO-d_6) δ 17.5 (Me), 21.0 (Me), 109.5 (C-5 or C-8), 109.8 (C-8 or C-5), 116.0 (C-3′ and C-5′), 126.1 (C-4), 128.9 (C-2 and C-3), 131.0 (C-1′), 131.9 (C-2′ and C-6′), 132.8 (C-8a), 133.1 (C-4a), 137.4 (C-1), 145.9 (C-6 or C-7), 146.2 (C-7 or C-6), 157.1 (C-4′); ms *m/z* (rel. int.) $[M]^+$ 280 (100%), 265 (10), 264 (8), 247 (4), 218 (3), 189 (3), 140 (7), 125 (3), 123 (4), 109 (7), 94 (7).

METHYLATION OF 6,3′-DI-*O*-DEMETHYLISOGUAIACIN [**1**].—To a solution of 6,3′-di-*O*-demethylisoguaiacin [**1**] (20 mg) in dry Me_2CO (10 ml) were added Me_2SO_4 (0.5 ml) and anhydrous K_2CO_3 (1 g), and the mixture was heated under reflux for 3 h. At the end of this time, the reaction mixture was filtered and evaporated in vacuo, H_2O and diluted NH_4OH were added, and the residue after decantation was precipitated from MeOH to afford isoguaiacin dimethyl ether [**5**] (13 mg) as an amorphous solid: $[\alpha]_D$ −20° (c = 1.02, MeOH); ir ν max (KBr) 2820, 1245, 1030 cm^{-1}; ^{1}H-nmr (360 MHz, Me_2CO-d_6) δ 0.89 (3H, d, J = 7.0 Hz, 2-Me), 0.90 (3H, d, J = 7.0 Hz, 3-Me), 1.98 (2H, m, H-2 and H-3), 2.45 (1H, dd, J = 8.1 and 16.2 Hz, H-4a), 2.87 (1H, dd, J = 4.8 and 16.2 Hz, H-4b), 3.58 (3H, s, OMe), 3.72 (3H, s, OMe), 3.72 (1H, m, H-1), 3.76 (3H, s, OMe), 3.78 (3H, s, OMe), 6.38 (1H, s, H-8), 6.47 (1H, dd, J = 1.8 and 8.5 Hz, H-6′), 6.69 (1H, s, H-5), 6.72 (1H, d, J = 1.8 Hz, H-2′) and 6.81 (1H, d, J = 8.5 Hz, H-5′); ms *m/z* (rel. int.) $[M]^+$ 356 (62), 325 (7), 299 (14), 269 (100), 238 (16), 203 (30), 187 (10), 165 (29), 151 (50), 121 (17).

METHYLATION OF 6-*O*-DEMETHYLISOGUAIACIN (NORISOGUAIACIN) [**6**].—A mixture of 6-*O*-demethylisoguaiacin [**6**] (40 mg), Me_2SO_4 (1 ml), anhydrous K_2CO_3 (2 g), and Me_2CO (20 ml) was refluxed for 4 h. After working up in the usual way, crystallization from aqueous MeOH afforded **5** as colorless needles, mp 88–90°, identical (co-tlc, uv, ir, ^{1}H-nmr, ms), except for $[\alpha]_D$ −49° (c = 0.49, MeOH), with the sample obtained previously.

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