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PHYTOCHEMICAL INVESTIGATIONS OF PLANTS OF THE GENUS *ARISTOLOCHIA*, 1. ISOLATION AND NMR SPECTRAL CHARACTERIZATION OF EUPOMATENOID DERIVATIVES

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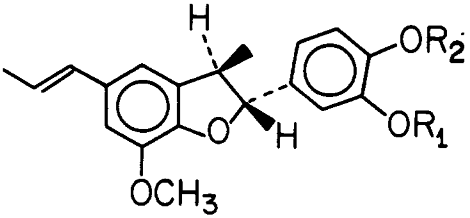
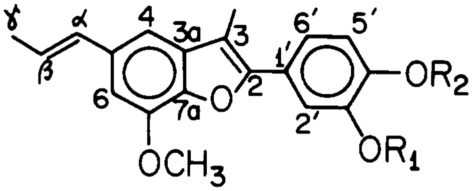
In the course of an investigation of medicinal plants that are, according to Mexican folk medicine, of use in the treatment of dermatological affections, it was found that ground roots and stems of plants of the *Aristolochia* genus, popularly known as *guaco* or *raiz de guaco*, were purported to be effective for this purpose (1).[1] Consequently, we have undertaken a phytochemical investigation of *guaco* in the hope of isolating possible active components. To achieve this isolation, petroleum ether extract of the bark was submitted to column chromatography; four closely related compounds of the lignan type (eupomatenoids) were isolated: (±)-licarin-A [or (+)-*trans*-dehydrodiisoeugenol] (**1a**), (−)-licarin-B (or eupomatenoid-8) (**1b**), eupomatenoid-7 (**2a**), and eupomatenoid-1 (or eupomatene) (**2b**). The characterization of these compounds and the possible significance of their presence in *guaco* are discussed below.



1a $R_1=CH_3, R_2=H$
1b $R_1R_2=-CH_2-$

Eupomatenoids are 3-methyl-2-phenyl-5(E)-propenylbenzofuran derivatives that fall within the general chemical class of lignans and more specifically have been proposed as neolignans (3). They owe their name to the family of Eupomatiaceae, which is a rich source of these compounds (4-7). However, with the exception of **1a**, which has been previously isolated from *Aristolochia taliscana* (8), eupomatenoids have not been found before in species of *Aristolochia*.

Characterization of **1** and **2** was made on the basis of melting points, optical activity, mass spectral parent ions, and particularly pmr and cmr spectroscopy. ^{1}H-Spectral data in $CDCl_3$ were in agreement with those previously reported (4-10). However, the use of higher field (200 MHz) spectra, the use of selective decoupling of benzylic protons [H(2) and H(3)] to remove long-range coupling to the aromatic protons, and the recording of additional spectra in Me_2CO-d_6 allowed assignment of many previously unassigned aromatic proton peaks.



2a $R_1=CH_3, R_2=H$
2b $R_1R_2=-CH_2-$

A combination of normal ^{13}C-spectra and edited ^{13}C-spectra using the pulse sequence DEPT (11) confirmed the presence of the correct numbers and types of carbons. Cmr chemical shifts for

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[1] At least 20 species of *Aristolochia* share these popular names (1), and *guaco* has a number of other claimed medicinal uses in Mexican folk medicine (1,2) (see Discussion section).

TABLE 1. Cmr Chemical Shift Data for **1a, 1b, 2a,** and **2b** in CDCl$_3$, Relative to (CH$_3$)$_4$Si (S=O)

Atom	Carbon[a]			
	1a	**1b**	**2a**	**2b**
2	93.79	93.40	151.46	151.13
3	45.62	45.78	110.19	110.50
3a	133.27	133.07	133.07	132.97
4	113.31	133.34	109.16	109.16
5	132.19[b]	132.22	133.64	133.66
6	109.23	109.26	104.47	104.67
7	144.15	144.11	144.82	144.86
7a	146.56[c]	146.49	142.10	142.12
1′	132.08[b]	134.31	123.69	125.33
2′	108.92	106.79	109.44	107.33
3′	146.67[c]	147.89	146.62	147.85
4′	145.77	147.58	145.74	147.38
5′	114.08	108.05	114.47	108.48
6′	119.96	120.20	120.59	121.05
α	130.92	130.91	131.51	131.45
β	123.48	123.47	124.32	124.39
γ	18.38	18.38	18.42	18.44
CH$_3$	17.57	17.89	9.58	9.61
OCH$_3$	55.94	55.94	56.05	56.15
OCH$_3$	55.98	——	56.08	——
OCH$_2$O	——	101.08	——	101.23

[a] For carbon labeling, see **2**.
[b,c] Pairs of carbons could be interchanged.

1a and **1b** were in close agreement with those previously reported by Wenkert (12) *et al.*, and the carbon multiplicities were consistent with the earlier spectral assignments (12). The spectra for **2a** and **2b** differed from **1a** and **1b** mainly in the expected replacement of two aliphatic CH signals by nonprotonated olefinic carbon signals. Spectral assignments for **2a** and **2b** were based on previously reported chemical shift assignments for **1a** and **1b** (12) and for benzofuran (13), 3-methylbenzofuran (13), 2-phenylbenzofuran (14), and 2-(4-methoxyphenyl)benzofuran (14), plus chemical shift increments for OH, OCH$_3$, and (E)-1-propenyl substituents in benzene derivatives (15). Results are summarized in Table 1.

The existence of **1a** as a racemic mixture was confirmed by both the lack of optical activity and by the coincidence of its melting point with that of synthetic (±)-*trans*-dehydrodiisoeugenol (16), while **1b** showed optical activity consistent with that reported for (−)-licarin-B (17). The absolute stereochemistry shown in **1b** is the mirror image of that determined for (+)-licarin-B by X-ray diffraction methods (18).

In addition to treatment of dermatological affections, the *Aristolochia* genus has various other uses and attributed properties in Mexican folk medicine, including healing of wounds and treatment of intestinal disorders (1,2).[2] In this regard, it is intriguing to note that **1a** has been shown to have definite antimicrobial activity, strongly inhibiting the growth of a variety of molds, yeasts, and bacteria (19). These observations suggest a possible scientific basis for some of the claimed medicinal properties of *guaco*. Compounds **1b, 2a,** and **2b** apparently have not been tested

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[2] These are the major uses of *guaco* in the Mexico City area (1). Other species of *Aristolochia*, also known as *quaco*, are used throughout Central America for treatment of snake bites (2).

TABLE 2. Pmr Chemical Shifts (δ scale) and Coupling Constants (J, Hz) for **1a**, **1b**, **2a** and **2b** in $CDCl_3$[a]

Compound	Proton						
	α^b	β^b	γ^b	4^e	6^c	$2'^d$	$5'^d$
1a	6.35	6.11	1.87	6.79 J_{46}= 1.3	6.77	6.97 $J_{2'5'}$= 1.9	6.91 $J_{5'6'}$= 8.0
1b	6.38	6.11	1.86	6.77 J_{46}= 1.5	6.75	6.92 $J_{2'5'}$= 1.7	6.77 $J_{5'6'}$= 8.2
2a	6.47	6.20	1.89	7.01 J_{46}= 1.3	6.80	7.29 $J_{2'5'}$= 2.0	6.98 $J_{5'6'}$= 8.2
2b	6.46	6.23	1.90	7.03 J_{46}= 1.4	6.82	7.28 $J_{2'5'}$= 1.7	6.89 $J_{5'6'}$= 8.5

[a] Labeling as shown in **2**.

[b] All four compounds had $J_{\alpha\beta}$= 15.7 Hz, $J_{\alpha\gamma}$= 1.6 Hz and $J_{\beta\gamma}$=6.5 Hz.

[c] Assignments of H_4 and H_6 could be interchanged for **1a** and **1b**.

[d] Coupling constants for **1a** and **1b** estimated from spectral measured in $(CD_3)_2CO$.

[e] Assignments of pairs of OCH_3 signals are uncertain for **1a** and **2a**.

previously for pharmacological activity. A series of pharmacological tests are being carried out for **1a**, **1b**, **2a**, and **2b**, both singly and in combination.[3] In addition, further phytochemical investigations of this and other types of *guaco* are being carried out.

EXPERIMENTAL

A sample of *guaco* was purchased at the Sonora market in Mexico City in the form of dried aerial parts (primarily bark and stems). A voucher specimen was placed at the IMSS herbarium. The physical appearance of this sample exactly matched the detailed description of *gauco* (as sold in Mexico City) provided by Martinez (2). Unfortunately, this source, while unambiguously assigning *guaco* as obtained from an *Aristolochia* species, provides no more detailed classification. However, microscopic examination of a cross-section of stem and comparison with specimens of plants of the *Aristolochia* genus deposited at the National herbarium (Department of Biology, UNAM) indicated that the sample was most probably either *Aristolochia maxima* L (2) or *Aristolochia taliscana* Hook (2). More precise identification could not be made, because of the absence of leaves to aid in identification. We are endeavoring to obtain a complete plant specimen for more definitive classification.

EXTRACTION PROCEDURE.—Dried, ground aerial parts (bark and stems) of the sample (800 g) were macerated in petroleum ether for periods of 48 h four times. Each time the solvent was removed in vacuo. The collected residues (12.9 g) were chromatographed in a column of silica gel, eluting with a gradient of hexane-EtOAc from 100:0 to a 80:20 ratio, respectively. Elution followed the order **1b**, **2b**, **1a**, and **2a**. Rf values in Si gel tlc (for the elution system hexane-EtOAc, in an 85:15 ratio) were, respectively, 0.54, 0.47, 0.19, and 0.17.

COMPOUND CHARACTERIZATION.—**1a** *m/z* 326, mp 136° [lit. 132-133° (16), 134-136° (7)], $[\alpha]^{25}\mathrm{D}$ 0.0; **1b** *m/z* 324, mp 89-90° [lit. 91-92° (8), 86-88° (17)], $[\alpha]^{25}\mathrm{D}$ −43° [−45.4° (17)]; **2a** *m/z* 324, mp 106-107° [lit. 105-106° (5)]; **2b** *m/z* 322, mp 158-159° [lit. 154-156° (4)]. Cmr and pmr spectral data are reported in Tables 1 and 2, respectively. ^1H-Spectra were obtained at 200.0 MHz, and ^{13}C-spectra at 50.1 MHz on a Varian XL-200 spectrometer operating in pulsed Fourier transform mode. ^1H-Spectra were obtained in both $CDCl_3$ and $(CD_3)_2CO$ with TMS (δ=0.00) as internal reference. For ^1H-spectra, 2000 Hz spectral widths, 16K data points zero-filled to 32K (0.13 Hz data point resolution), 45° pulses and 4-second repetition rates were used. Normal ^{13}C-spectra were obtained in $CDCl_3$

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[3] Preliminary screening tests indicate that the phenolic derivatives (**1a** and **2a**) in particular show antibiotic activity.

TABLE 2. (Continued)

Compound	Proton						
	6'[d]	2	3	3-CH_3	OH	-OCH_2O-	OCH_3[e]
1a	6.91 $J_{2'6'}=$ 0.5	5.09 $J_{23}=$ 9.5	3.42 J_{3CH_3} 6.8	1.37	5.63	—	3.88 3.89
1b	6.87 $J_{2'6'}=$ 0.7	5.09 $J_{23}=$ 9.5	3.40 $J_{3CH_3}=$ 6.7	1.37	—	5.94	3.88
2a	7.26 $J_{2'6'}=$ 0.6	—	—	2.37	5.80	—	3.91 4.01
2b	7.28 $J_{2'6'}=$ 0.5	—	—	2.39	—	6.00	4.03

using 10,000 Hz spectral widths, 32K data points (0.66 Hz data point resolution), 45° pulses and 1.6-second repetition rate. Four separate DEPT spectra were obtained for each sample using θ pulses of 45°, 90°, 90°, and 135° in the standard DEPT pulse sequence (11). These were used to generate "edited" spectra (CH only, CH_2 only, and CH_3 only), using the subroutine ADEPTS incorporated in the standard Varian software package (H-release) for the XL-200. Mass spectra were obtained on a Dupont 21-490 Mass spectrometer.

NOTE ADDED IN PROOF: The sample of *guaco* has now been classified as *Aristolochia taliscana* on the basis of botanical comparisons with an authentic specimen of that plant, plus comparisons of composition of petroleum ether extracts from the two samples.

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