CHEMISTRY OF *SAURURUS CERNUUS*. I. SAUCERNETIN, A NEW NEOLIGNAN

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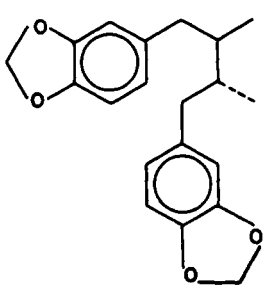
ABSTRACT.—From the lipophilic fraction of the alcoholic extract of an aquatic weed *Saururus cernuus* L., four neolignan components were isolated. One of these, named saucernetin (**3**), was found to be a new member of the 2,5-diaryl-3,4-dimethyltetrahydrofuranoid type neolignans with a 2,3-cis/3,4-trans/4,5-cis stereochemistry. The remaining three compounds were identified as the known neolignans, austrobailignan-5 (**1**), veraguensin (**2**) and guaiacin (**9**).

An aquatic weed known as lizard's tail (*Saururus cernuus* L., N.O. Saururaceae) is a native of North America and has been used in folk medicine as a sedative and as a poultice for tumors (1,2). The leaves and twigs have a pleasant aromatic odor, and the volatile oil has been studied by Tutupalli *et al.* (3) who isolated a series of terpenoid compounds from it. During a search for constituents with possible biological activity, the presence of lignoid compounds became apparent in the extract. In this paper we describe the isolation of a new neolignan, saucernetin, and three other neolignans previously isolated from other plant sources.

The lignoids to be described here were isolated from the dried, above-ground parts of the plant by extraction with ethanol, concentration of the extract and partition between water and chloroform. Partition of the chloroform layer between methanol, water, benzene, and hexane (4:1:1:4) was followed by separation of the benzene-hexane layer into neutral and phenolic fractions. Each was subjected to chromatography on silica gel. Three neutral lignoids (fractions 1, 2 and 3) and one phenolic lignoid (fraction 4) were isolated, all of which were homogeneous as indicated by tlc, gc and hplc.

Lignoid no. 1 is a colorless oil, $C_{20}H_{22}O_4$, $[\alpha]_D -27°$ with uv maxima at 230 and 288 nm and nmr signals to indicate six aromatic protons, two methylenedioxy groups, four protons of the type Ar-C*H*$_2$ and two C*H*–C*H*$_3$ groups. The mass spectrum (M+326) showed a base peak at *m/e* 135 (methylenedioxybenzyl or its equivalent) with little or no other significant fragmentation. These analytical and spectral data were consistent with a 2,3-dimethyl-1,4-diarylbutane type neolignan structure and agreed with the values described for the known neolignan, austrobailignan-5 (**1**) isolated by Murphy *et al.*, (4), although an authentic sample was unavailable for direct comparison.



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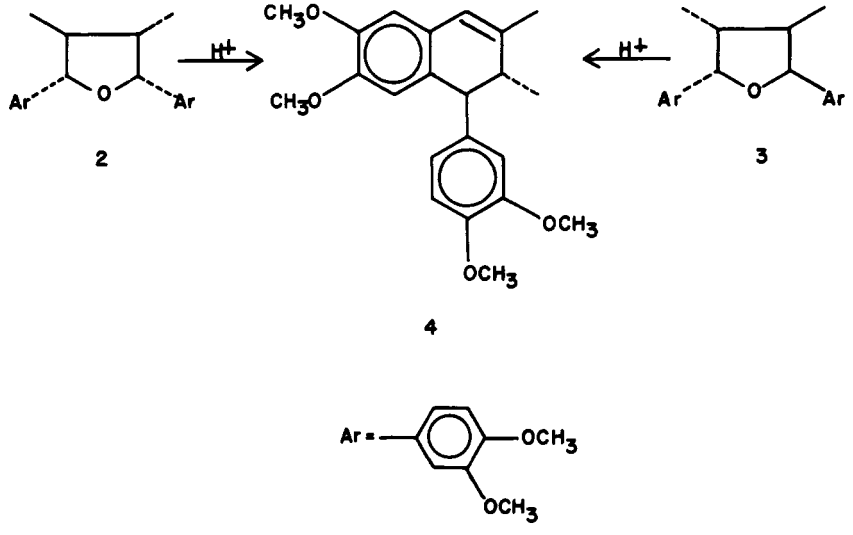
Lignoid no. 2 is a colorless crystalline solid, $C_{22}H_{28}O_5$, $[\alpha]_D +32°$ with uv maxima at 235 and 280 nm and nmr spectrum to indicate six aromatic protons, two nonequivalent protons of the type Ar-C*H*-O, four methoxyls, and two nonequivalent

C*H*-C*H*$_3$ groups. These characteristics suggested a 2,5-diaryltetrahydrofuranoid type neolignan structure, and this was confirmed by mass spectrum (M+372) with the base peak at *m/e* 206 and characteristic peaks (5) at *m/e* 191, 175, 165, 151 and 138. The physical and spectral properties agreed with those given for the neolignan veraguensin **2** described by Crossley and Djerassi (6), and a direct comparison with a sample kindly supplied by Professor Robert Stevenson confirmed the identity.

Lignoid no. 3 is a colorless crystalline solid, $C_{22}H_{28}O_5$, isomeric with veraguensin with $[\alpha]_D$+48° and uv maxima at 235 and 280 nm. Its nmr spectrum: δ 6.83, s, six aromatic H; δ 5.45, d, 2 Ar-C*H*-O, δ 3.86, s, 4OCH$_3$; δ 2.08–2.47, m, 2 C*H*-CH$_3$ and δ 1.08, d, CH-C*H*$_3$, also suggested a 2,5-diaryltetrahydrofuranoid type neolignan structure, although the spectrum was significantly different from that of veraguensin. The mass spectrum (M+372), however, was identical with that of veraguensin, as was expected due to the insensitivity of the fragmentation pattern to stereochemical differences in the group of tetrahydrofuranoid neolignans (5). The physical and spectral properties of lignoid no. 3 were distinguishable from the other known members of the 2,5-bisdimethoxyphenyl-3,4-dimethyltetrahydrofuranoid neolignans and the compound was named saucernetin (**3**).

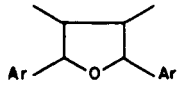
Treatment of saucernetin with trifluoroacetic acid in the cold yielded a crystalline, optically active product $[\alpha]_D$+130°, $C_{22}H_{26}O_4$ with uv maxima at 225 and 285 nm and nmr signals at δ 6.50–6.63, m, 5 aromatic H; δ 6.10, s, 1 olefinic H; δ 3.75, 3.85, s, 4 OCH$_3$; δ 2.2, m, C*H*-CH$_3$, δ 1.80, s, =C-C*H*$_3$ and δ 1.08, d, CH-C*H*$_3$. In comparison with saucernetin, the acid-rearranged product showed one less aromatic H, loss of the low field doublet attributed to Ar-C*H*-O functions, and the presence of an olefinic H and CH$_3$. These differences indicated conversion of saucernetin to a 1-phenyl-1,2-dihydronaphthalene system such as **4** and specifically to cyclogalbelgin, obtained under similar conditions from galbelgin (7) and veraguensin (6). The identity of the physical and spectral properties including the specific rotation of **4** with those of cyclogalbelgin established a trans orientation of the methyl and dimethoxyphenyl groups in **4**.



2 3 4

With regard to the stereochemistry of saucernetin **3**, there are six possible orientations for the symmetrically substituted 2,5-diaryl-3,4-dimethyltetrahydrofuran system as shown in **2**, **3**, **5**, **6**, **7** and **8** and, of these, four have been isolated so far. Two are meso isomers: di-O-methyltetrahydrofuroguaiacin **5** (8) and gal-

gravin **6** (7), and the other two, galbelgin **7** (7) and veraguensin **2**, are optically active. Saucernetin, which is optically active, is therefore different from the two meso isomers, and its spectral and physical properties distinguish it from galbelgin and veraguensin. Hence, it must have the structure of either **3** or **8**.



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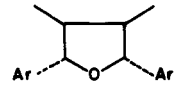

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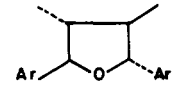

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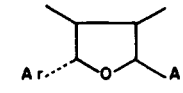

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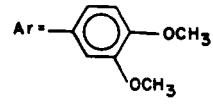


The nmr spectra of the four known members belong to one or the other of two types: one with an element of symmetry as indicated by magnetic equivalency of pairs of groups such as Ar-C*H*-O, C*H*-CH_3, etc., and the other which indicates magnetic nonequivalence for these pairs. Compounds **5**, **6** and **7** belong to the first category because they all possess an element of symmetry; whereas veraguensin **2**, which lacks such symmetry, belongs to the second category. Of the two remaining structures, **3** must belong to the first and **8** to the second category. Saucernetin shows a spectrum which clearly shows an element of symmetry and thus must have the structure **3**. It is significant to note that Crossley and Djerassi (6), during the elucidation of the structure of veraguensin, had the choice between the structures **2** and **3** and selected **2** for veraguensin because of the asymmetry as reflected in the nmr spectrum as opposed to **3**, which is expected to provide a much simpler spectrum.

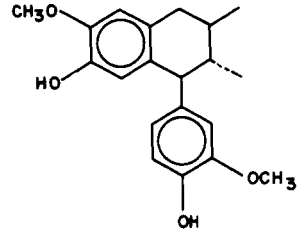
In contrast to all the other known members of the diaryltetrahydrofuran group which show multiplets for the six aromatic protons, saucernetin shows a singlet for these. Another significant difference between **3** and the other members of this group **2**, **5**, **6** and **7** is the chemical shift of the doublet due to the two Ar-C*H*-O functions which is found at a considerably lower field than that of all the others. Neolignans of the diaryltetrahydrofuran group with *trans* orientation of the vicinal methyl and aryl groups (e.g., **6** and **7**) show the signal at δ 4.5–4.7, while veraguensin, which has one *trans* and one *cis* pair, has two doublets, one at δ 4.5 and the other at δ 5.10. In saucernetin, the value for the chemical shift is δ 5.45 which clearly shows a *cis* relationship between each of the methyl and aryl groups. Thus, of the two possible structures **3** and **8**, the symmetrical nature of the spectrum and the low field signal for the Ar-C*H*-O strongly support the structure **3** for saucernetin with a 2,3-*cis*/3,4-*trans*/4,5-*cis* stereochemistry.

Birch *et al.* (7) in their stereochemical studies on galgravin **6** and galbelgin **7** employed acid-catalyzed isomerization as a means of studying the relative steric compressions in the isomers. In the presence of an acid such as perchloric acid/-acetic acid, the oxonium ion first formed changes to the carbonium ion which then undergoes transformation to the more stable *trans* configuration with respect to the methyl and aryl groups. Such an acyclic form exists in equilibrium with the more stable tetrahydrofuran system, although competing with this isomerization, there is also the irreversible rearrangement of the carbonium ion to the aryldihydronaphthalene species such as **4**. A similar reasoning was employed by Blears and Haworth (8) as well as King and Wilson (9) in transforming **5** to **6** by treatment with acid. We observed that when saucernetin was heated with 5% Pd/C in oxydiethanol in the presence of acetic acid (1%), it likewise underwent transformation into two other compounds which were shown by gas chromatography to be veraguensin **2** and galbelgin **7**. This reaction clearly showed that the

2,3-*cis*/4,5-*cis* arrangement in saucernetin was unstable and was transformed to veraguensin by inversion at carbon 2 and then to galbelgin by inversion at both carbons 2 and 5.

After this work was completed, a report appeared recently on the presence of a somewhat related lignan named neoolivil (10). Although it was not actually isolated in pure form, its structure, 2,5-bis(4-hydroxy-3-methoxyphenyl)3,4-bis-hydroxymethyltetrahydrofuran, was derived on the basis of spectral data of the tetraacetate and a similar cis/trans/cis stereochemistry was assigned to it. The influence of the acetoxymethyl and/or acetoxyaryl functions is apparently significant because the doublet due to the Ar-C*H*-O groups was found at δ 5.0.

The fourth lignoid was obtained as a colorless crystalline sold, $C_{20}H_{24}O_4$, $[\alpha]_D+45°$ with uv maxima at 240 and 285 nm. A base-induced shift of the uv maximum from 280 to 295 nm and the manner in which the compound was isolated showed that it was a phenolic compound. Its nmr spectrum, which gave signals to indicate five aromatic protons, two methoxyls, one Ar-CH-Ar, one Ar-CH_2 and two C*H*-CH_3 groups, suggested that the compound might be an aryl tetralin type neolignan. Mass spectral fragmentation showed M$^+$ (328) as the base peak with major fragment ions at *m/e* 272, 241, 204, 189, 164 and 137, all in conformity with the fragmentation pattern of neolignans of this type (11). Acetylation gave a diacetate: M$^+$412; ir 1760 cm^{-1} and nmr: δ 2.30 and 2.20, and methylation, a dimethyl ether. The physical and spectral properties agreed with those described for guaiacin **9** (12); this was confirmed by comparison of the dimethyl ether with an authentic sample of guaiacin dimethyl ether isolated from guaiacum resin (9).



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EXPERIMENTAL[1]

PLANT SOURCE.—The plant material was collected in Gainesville, Florida, and was authenticated at the Herbarium, University of Florida.

EXTRACTION AND FRACTIONATION.—Dried, coarsely ground leaves and stems (5 kg) were macerated with ethanol at room temperature for two days. Three such extracts were combined and concentrated to a thick syrup which was partitioned between water (2 liters) and chloroform (4 liters). The solvent layer was concentrated to a syrup and partitioned in a countercurrent fashion (three 4 liter-aspirator bottles were used) with one liter portions of each layer of the sytem: methanol, water, benzene, hexane (4:1:1:4). The combined benzene hexane layer was concentrated partially to 1 liter, and the concentrate was washed twice with 0.1 N aqueous sodium hydroxide (500 ml each time). The solvent phase containing the "neutral fraction" was dried over sodium sulfate and concentrated to a thick syrup (250 g). The alkaline layer was acidified and extracted twice with ether; the extract, when concentrated, yielded the "phenolic fraction" as an oil (15 g).

[1]Melting points were determined on a Fisher-Johns apparatus and were uncorrected. Thin-layer chromatography was performed on microslides (1 x 2 or 2 x 2 inches) coated with silica gel Merck HF 254+366 without a binder. The samples were visualized by uv light and by spraying with 1% sulfuric acid in acetic acid, followed by gentle heating, whereby violet brown to crimson red colors developed. Column chromatography was carried out on silica gel Merck 200–400 mesh mixed with an equal weight of thin-layer grade cellulose powder (Brown & Co., Berlin, N.H.). Instrumentation used in this paper is as follows: uv, Beckman 25; ir, Beckman, Acculab 3; nmr, Varian T60 spectrometer with tetramethylsilane as internal standard; mass spectra, DuPont 49 chemical ionization spectrometer; gas-chromatography, Varian 2100 with a flame-ionization detector; hplc, Spectra Physics model SP 3500 B with a Partisil 5/25 (Whatman) column with a flow rate of 1.6–2.5 ml/min.; and optical rotations, Perkin Elmer polarimeter 141 with all solutions in chloroform at 1% concentration.

CHROMATOGRAPHY OF NEUTRAL FRACTION.—The "neutral fraction" (25 g) was subjected to chromatography on silica gel cellulose (500 g) with 1:3 benzene-hexane and eluted successively with 1:1 benzene-hexane, benzene and 2% acetone in benzene. The fractions were monitored by absorbance at 285 nm and tlc, combined and concentrated to recover the appropriate components.

Compound **1** (austrobailignan-5) was obtained as a colorless oil by elution with 1:1 benzene-hexane; yield, 0.2%; $[\alpha]_D -27°$; max 230, 288, log ϵ, 3.91, 3.85 respectively, *m/e* 326 (M^+) 191, 190, 163, 149, 136, 135, 105 and 77; nmr: (δ) 6.43–6.67, m, 6H; 5.85, s, 4H; 2.42, t, 4H; 1.7, q, 2H; 0.78, d, 6H.

Compounds **2** and **3** appeared together in the benzene eluate and were separated by preparative tlc with the system: 5% acetone in benzene. Compound **2** was obtained as a colorless crystalline solid: yield, 0.02%; mp 122–123°; λ max 235, 280 nm; log ϵ 4.16, 3.66; (lit. 231, 278 nm (6)); $[\alpha]_D +34°$; hplc-R_T, 7 minutes; *m/e* 372 (M^+), 287, 206, 191, 178, 176, 175, 165, 151, 138; nmr: (δ) 6.87–7.11, m, 6H; 5.13, d, *J*8, 1H; 4.44, d, *J*8, 1H; 3.85, 3.87, 3.88, 3.90, s, 12H; 1.6–2.5, m, 2H; 1.07, d, *J*7, 3H; 0.67, d, *J*7, 3H.

Compound **3** was obtained as a colorless crystalline solid, mp 80–81°; yield, 0.05%; $[\alpha]_D +48°$; hplc-R_T, 4 minutes; λ max 235, 280 nm; log ϵ, 4.10, 3.69; ν 1590, 1510, 1450, 1410, 1375, 1355, 1340, 1255, 1230, 1160, 1135, 1025, 960, 850, 810, 760 cm^{-1}; *m/e* 372 (M^+), 287, 206, 191, 178, 176, 175, 165, 151, 138.

Anal. calc. for $C_{22}H_{28}O_6$: C, 70.94; H, 7.58. Found: C, 71.12; H, 7.63.

ACID-CATALYZED REARRANGEMENT OF **2** AND **3**.—A solution of **2** or **3** (0.2 g) in benzene (5 ml) was boiled under reflux with a saturated solution of *p*-toluenesulfonic acid in benzene (5 ml) for 30 minutes. The cooled mixture was washed with aqueous sodium bicarbonate, and the solvent layer was concentrated to dryness. The solid was crystallized from ether; yield 0.15 g; mp 101–102°, $[\alpha]_D +130°$ (lit., mp 100–101°, $[\alpha]_D +135.5°$ (6)).

Alternatively, the neolignan (0.2 g) was dissolved in trifluoroacetic acid (2 ml) at 5° and, after 30 min at this temperature, was diluted with water. The mixture was neutralized with sodium bicarbonate and filtered, and the solid was crystallized from ether; mp 101–102°.

ISOMERIZATION OF **3**.—Compound **3** (0.2 g) was boiled under reflux in oxydiethanol (10 ml) with 5% Pd/C (0.1 g) and acetic acid (1 ml) for 4 hours. The cooled mixture was diluted with water and extracted with benzene, and the benzene layer was concentrated to dryness. The glassy solid was dissolved in benzene and analyzed by gas-chromatography on the column OV 225, temperature 265°; R_T **2**, 4.3 min, **3**, 4.9 min; 7, 4.6 min. In each case, the identity of the respective peaks was confirmed by the addition of the particular reference sample and by observing the enhancement of the peak. The original sample gave a single peak, R_T 4.9 min.

COMPOUND **9**.—The "phenolic fraction" (5 g) from the initial solvent fractionation was subjected to chromatography on silica gel-cellulose (100 g) with benzene. The major component (**9**) was eluted with 2% acetone in benzene. It was obtained as a colorless crystalline solid; yield, 0.05%; mp 204–206°; $[\alpha]_D +42°$; λ max 230, 280 nm; log ϵ 4.12, 3.80; ν: 3540, 3420, 1610, 1510, 1450 cm^{+1}; *m/e* 328 (M^+), 272, 241, 204, 189, 164, 137; nmr; (δ) 7.65, broad, 2H; 6.2–6.9, m, 5H; 3.8, s, H6; 3.33, m, H; 2.4, d, *J*8, 2H; 1.55, m, 2H; 1.05, d, *J*8, 3H; 0.83; d, *J*5, 3H.

Anal. calc. for $C_{20}H_{24}O_4$: C, 73.14; H, 7.36. Found: C, 72.92; H, 7.40.

METHYLATION OF **9**.—A mixture of **9** (0.2 g), dimethyl sulfate (0.3 ml) and anhydrous potassium carbonate (1 g) in acetone (20 ml) was boiled under reflux for 6 hours. After filtration of the reaction mixture, the filtrate was concentrated to dryness and the solid crystallied from ether; yield 0.19 g; mp 130–132° (lit. 130° (12)).

Anal. calc. for $C_{22}H_{28}O_4$: C, 74.13; H, 7.91. Found: C, 73.91; H, 7.86.

ACETYLATION OF **9**.—A solution of **9** (0.1 g) in acetic anhydride (2 ml) and pyridine (0.2 ml) was heated at 100° for 15 minutes, cooled, and diluted with water and the solid was removed by filtration. It was crystallized from aqueous methanol; mp 116–117°; *m/e* 412 (M^+); nmr: δ 6.3–7.0, m, 5H; 3.7, s, 6H; 3.5, d, 1H; 2.25, d, 2H; 2.30, 2.20, s, 6H; 1.6, m, 2H; 1.05, 0.83, d, 6H.

Anal. calc. for $C_{24}H_{28}O_6$: C, 69.88; H, 6.84. Found: C, 70.22; H, 7.01.

ACKNOWLEDGMENT

The authors express their appreciation to Professor Robert Stevenson of Brandeis University for the sample of veraguensin.

Received 6 August 1981

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