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HERPETETRONE, ANOTHER TETRAMERIC LIGNOID FROM *HERPETOSPERMUM CAUDIGERUM* SEEDS

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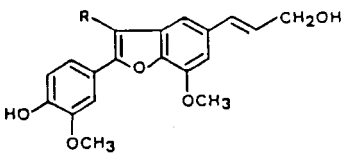
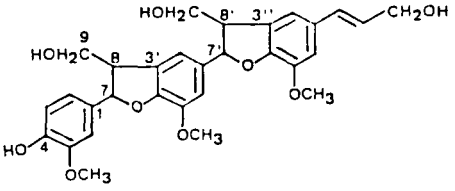
Seeds of *Herpetospermum caudigerum* Wall. (Cucurbitaceae) contain lignoids with different polymerization stages, probably derived by oxidative coupling of coniferyl alcohol units. Present in the MeOH extract of the defatted seeds, these compounds have been identified as structures **1-8** on the basis of their spectroscopic data (1-7). Continuing the investigation of the MeOH extract, we have isolated another tetramer of coniferyl alcohol generated like tetrameric lignoids **5** and **8** by 8.3′- and 8.8′-couplings of the C_6-C_3 units (3,6,8). We now wish to report the identification of this new minor constituent named herpetetrone [**9**].

During fractionation of the MeOH extract of *H. caudigerum* seeds by polyamide cc followed by centrifugal circular tlc on Si gel, compound **9** was eluted just before tetramers **5** and **8**, and its purification was carried out by repeated filtration through a Sephadex LH 20 column. Amorphous herpetetrone [**9**] did not show a molecular ion in eims, and the highest fragment ion observed at *m/z* 536 $(M-194)^{+\cdot}$ corresponded to a trimeric structure such as compound **3** ($M^{+\cdot}$ *m/z* 536). The trimethylsilyl and the acetylated derivatives exhibited, respectively, their molecular ions at *m/z* 1090 and 940, indicating five free hydroxyls in the natural product. These results allowed assignment of the molecular formula $C_{40}H_{42}O_{13}$ to **9**, consistent with a tetramer of coniferyl alcohol. Examination of the ^{1}H-nmr spectra of both the natural product (Table 1) and the acetylated derivative (Table 2) shows four aromatic rings and four methoxy groups identified as guaiacyl residues with only three hydroxy groups appearing at δ 2.31 (6H) and 2.43 (3H) after acetylation. The proton spectra show, furthermore, three partial structures: (a) a 1,2-disubstituted propanonol (4,6), (b) a 2,6-disubstituted 3,7-dioxabicyclo[3.3.0] octane (4,6), and (c) a 2,3-disubstituted dihydrobenzofuran (2,3). The two singlets at δ 1.99 and 2.05 (Table 2) show the acetylated derivatives of two non-aromatic alcohol functions.

In compound **9**, a 1,3,4-trisubstituted aromatic ring was deduced from the following data: an ir band at 1660 cm^{-1}, a uv λ max (MeOH) of 307 nm, which moved to 347 nm in NaOAc, and ^{1}H-nmr peaks at δ 8.34, 8.20, and 7.11 (Table 1, H-2, H-6, and H-3). The multiplicities of these peaks indicated a carbonyl group between *meta* protons and *para* to a hydroxyl group, which was consistent with the bathochromic shift obtained in NaOAc. The fragment ions **10-12** at *m/z* 151, 165, and 223 given, respectively, by the natural product, the permethylated compound, and the trimethylsilyl derivative finally indicated that the third substituent was a methoxyl group. Peaks at δ 6.29, 5.14, and 4.46 in the ^{1}H nmr (Table 1, H-8 and H-9) for **9**, as for **7** and **8**, show



1 R=CHO
2 R=CH_2OH



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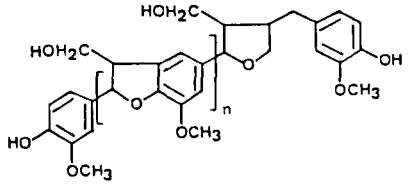
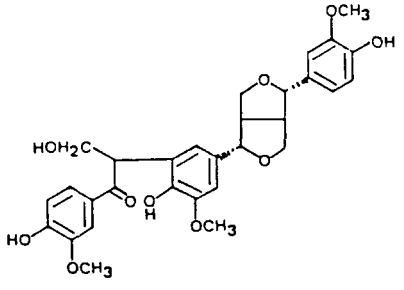


4 n=1
5 n=2
6 n=3



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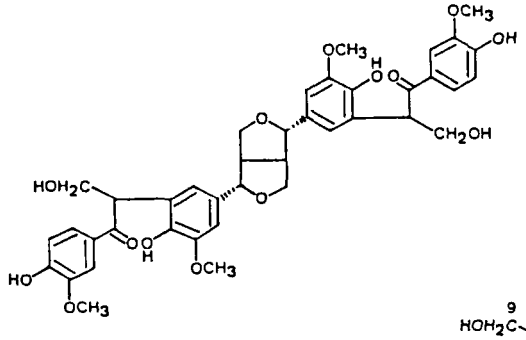
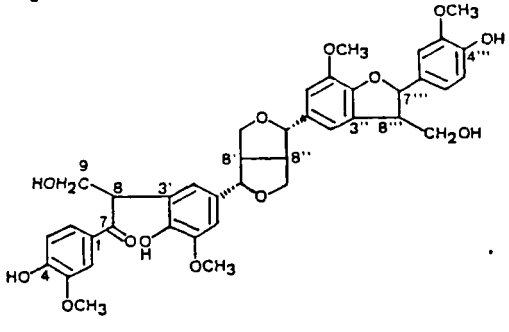
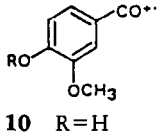


8



9



10 R=H
11 R=Me
12 R=TMSi

the partial structure >CH-CH_2OH of a 7-oxidized coniferyl alcohol unit common to these three compounds.

The next two coniferyl alcohol units are combined into a 2,6-diguaiacyl-3,7-dioxabicyclo[3.3.0]octane moiety as shown by the ^{1}H-nmr peaks at δ 4.85, 4.83, 3.13, 4.22, and 3.92 for **9**, like similar peaks for **7** and **8** (Table 1, H-7′, H-7″, H-8′, H-8″, H-9′, and H-9″). The assignment of the relative configuration 7′*S*, 8′*S*, 7″*S*, 8″*S* to **9**, as in **7** and **8** (4,6,10), resulted from the ^{1}H-nmr data (Tables 1 and 2) indicating (a) the equatorial configuration of the aromatic rings according to deshielding of the equivalent methylene-oxy H-9′ and H-9″, and, consequently, the axial configuration of the equivalent benzylic protons H-7′ and H-7″, (b) the equatorial configuration of

TABLE 1. ^{1}H-nmr Data of Lignoids **7** (250 MHz), **8**, and **9** (350 MHz) in C_5D_5N (δ ppm/TMS)

Proton	Compound		
	7	**8**	**9**
H-2	8.33, dd, J=8, 2 Hz	8.31, dd, J=8.4, 2.1 Hz	8.34, dd, J=8.4, 2 Hz
H-3	7.11, d, J=8 Hz	7.11, d, J=8.4 Hz	7.11, d, J=8.4 Hz
H-6	8.22, d, J=2 Hz	8.20, d, J=2.1 Hz	8.20, d, J=2 Hz
H-8	6.29, dd, J=9, 4.5 Hz	6.26, dd, J=9.1, 4.6 Hz	6.29, dd, J=9, 4.5 Hz
H-9	5.14, dd, J=10, 9 Hz 4.46, dd, J=10, 4.5 Hz	5.13, dd, J=10.2, 9.1 Hz 4.44, dd, J=10.2, 4.6 Hz	5.14, dd, J=10.5, 9 Hz 4.46, dd, J=10.5, 4.5 Hz
H-2′	7.51, d, J=1.5 Hz[a]	7.47, d, J=1.5 Hz[b]	7.35, br s[d]
H-6′	7.14, d, J=1.5 Hz[a]	7.05, d, J=1.5 Hz[b]	7.08, br s[d]
H-7′	4.86, d, J=4.5 Hz	4.80, d, J=4.2 Hz	4.85, d, J=4.2 Hz
H-8′	3.16, m	3.06, m	3.13, m
H-9′	4.20, dd, J=8.5, 4.5 Hz 3.94, m	4.11, dd, J=9.1, 3.5 Hz 3.83, m	4.22, dd, J=9, 3.7 Hz 3.92, m
H-2″	7.28, dd, J=8, 2 Hz	7.43, d, J=1.5 Hz[c]	7.35, br s[d]
H-3″	7.03, d, J=8 Hz		
H-6″	7.12, d, J=2 Hz	7.03, d, J=1.5 Hz[c]	7.14, br s[d]
H-7″	4.90, d, J=4.5 Hz	4.78, d, J=4.2 Hz	4.83, d, J=4.2 Hz
H-8″	3.16, m	3.06, m	3.13, m
H-9″	4.20, dd, J=8.5, 4.5 Hz 3.94, m	4.11, J=9.1, 3.5 Hz 3.83, m	4.22, dd, J=9, 3.7 Hz 3.92, m
H-2‴		8.29, dd, J=8.4, 2.1 Hz	7.25, br d, J=8.4 Hz
H-3‴		7.09, d, J=8.4 Hz	7.18, d, J=8.4 Hz
H-6‴		8.18, d, J=2.1 Hz	7.14, br s
H-7‴			6.13, d, J=6.7 Hz
H-8‴		6.26, dd, J=9.1, 4.6 Hz	4.02, m
H-9‴		5.13, dd, J=10.2, 9.1 Hz 4.44, dd, J=10.2, 4.6 Hz	4.29, m
OMe	3.78, 3.74, 3.70	3.73, 3.73, 3.70, 3.68	3.86, 3.73, 3.69, 3.64

[a-d]Assignments with the same letter designation may warrant changing.

TABLE 2. ^{1}H-nmr Data of the Acetates **7a** (250 MHz), **8a**, and **9a** (350 MHz) in $CDCl_3$ (δ ppm/TMS)

Proton	Compound		
	7a	**8a**	**9a**
H-2	7.48, dd, J=8, 2 Hz	7.54, dd, J=8, 2 Hz	7.53, dd, J=8, 2 Hz
H-3	7.00, d, J=8 Hz	7.05, d, J=8 Hz	7.04, d, J=8 Hz
H-6	7.55, d, J=2 Hz	7.60, d, J=2 Hz	7.60, d, J=2 Hz
H-8	5.07, dd, J=9, 5 Hz	5.08, dd, J=9, 4.5 Hz	5.11, dd, J=8.5, 4.5 Hz
H-9	4.63, dd, J=11, 9 Hz ca. 4.28, m	4.64, dd, J=11, 9 Hz ca. 4.28, m	4.70, dd, J=10, 8.5 Hz ca. 4.30, m
H-2′	6.88, d, J=2 Hz[a]	6.92, m	6.84, br s[b]
H-6′	6.95, d, J=2 Hz[a]	6.92, m	6.99 br s[b]
H-7′	4.72, d, J=4.5 Hz	4.68, d, J=4.5 Hz	4.68, d, J=4.5 Hz
H-8′	3.00, m	3.02, m	3.03, m
H-9′	4.28, dd, J=10, 5.5 Hz 3.85, m	4.28, dd, J=10.5, 5 Hz ca. 3.85, m	4.30, dd, J=10.5, 5.5 Hz ca. 3.90, m
H-2″	6.82, dd, J=8, 2 Hz	6.92, m	6.84, br s[b]
H-3″	6.97, d, J=8 Hz		
H-6″	6.63, d, J=2 Hz	6.92, m	6.99, br s[b]
H-7″	4.69, d, J=4.5 Hz	4.68, d, J=4.5 Hz	4.68, d, J=4.5 Hz
H-8″	3.00, m	3.02, m	3.03, m
H-9″	4.28, dd, J=10, 5.5 Hz 3.85, m	4.28, dd, J=10.5, 5 Hz ca. 3.85, m	4.30, dd, J=10.5, 5.5 Hz 3.90, m
H-2‴		7.54, dd, J=8, 2 Hz	6.95, br d, J=8 Hz
H-3‴		7.05, d, J=8 Hz	7.03, d, J=8 Hz
H-6‴		7.60, d, J=2 Hz	6.71, br s
H-7‴			5.55, br d, J=7 Hz
H-8‴		5.08, dd, J=9, 4.5 Hz	3.73, ddd, J=8, 7, 5.5 Hz
H-9‴		4.64, dd, J=11, 9 Hz 4.28, m	4.46, dd, J=11.5, 5.5 Hz ca. 4.30, m
OMe	3.86, 3.85, 3.83	3.84, 3.84, 3.81, 3.79	3.92, 3.86, 3.85, 3.82
OAc	2.42, 2.35, 2.32, 1.92	2.42, 2.38, 2.31, 2.30, 1.98, 1.97	2.43, 2.31, 2.31, 2.05, 1.99

[a-b]Assignments with the same letter designation may warrant changing.

the two equivalent methines H-8′ and H-8″ according to the coupling values $J_{7',8'}=J_{7'',8''}=4.2$ Hz in the natural product and 4.5 Hz in the acetylated derivative.

Finally, the fourth C_6-C_3 unit of **9**, corresponding to a 2,3-disubstituted benzofuran moiety, had ^{1}H-nmr peaks at δ 6.13, 4.02, and 4.29 (Table 1, H-7‴, H-8‴, and H-9‴) just like compounds **3-6** (see Table 3 for **3** and **5**). Acetylation improved the signals for H-8‴ and H-9‴ of **9** (Table 2) and the corresponding signals of **3** and **5** (Table 4).

TABLE 3. ^{1}H-nmr Data of the Aliphatic 2,3-Disubstituted Dihydrobenzofuran Protons in Compounds **3** and **5** at 250 MHz and **9** at 350 MHz (C_5D_5N; δ ppm/TMS)

Proton	Compound		
	3	**5**	**9**
H-7	6.14, d, J=6.5 Hz	6.09, d, J=6.5 Hz[a]	
H-7′	6.14, d, J=6.5 Hz	6.11, d, J=6.5 Hz[a]	
H-7‴			6.13, d, J=6.7 Hz
H-8	ca. 4.00, m	ca. 4.00, m	
H-8′	ca. 4.00, m	ca. 4.00, m	
H-8‴			ca. 4.02, m
H-9	ca. 4.22, m	ca. 4.26, m	
H-9′	ca. 4.22, m		
H-9‴			4.29, m

[a]Assignments may warrant changing.

From the above results, it was clearly established that the guaiacyl propanonol unit was one end of compound **9**, as indicated by the two *ortho*-coupled protons at δ 8.20 and 7.11 in the natural product and δ 7.53 and 7.04 in the acetylated derivative. To define the other terminal group of **9** as a guaiacyl dioxabicyclooctane (like that of **7**) or as a guaiacyl dihydrobenzofuran (as in **3-6**) was not possible from the nmr of Table 1 because the *ortho*-coupled protons of the involved guaiacyls could not be differentiated from each other. Localization of the guaiacyl dihydrobenzofuran at one end of this molecule, as shown in **9**, was deduced from the long range coupling observed at 500 MHz between H-7‴ (δ 5.55) and two *meta*-related protons (δ 6.95 and 6.71) in the ^{1}H-nmr spectrum of the acetylated derivative. One of these aromatic protons (δ 6.95) being also *ortho*-coupled with one at δ 7.03, it was clearly established that the guaiacyl di-

TABLE 4. ^{1}H-nmr Data of the Aliphatic 2,3-Disubstituted Dihydrobenzofuran Protons in Acetates **3a**, **5a**, and **9a** at 350 MHz ($CDCl_3$; δ ppm/TMS)

Proton	Compound		
	3a	**5a**	**9a**
H-7	5.50, d, J=7 Hz[a]	5.54, d, J=7 Hz	
H-7′	5.54, d, J=7 Hz[a]	5.54, d, J=7 Hz	
H-7‴			5.55, br d, J=7 Hz
H-8	ca. 3.90, m	3.77, ddd, J=7, 7, 3 Hz	
H-8′	ca. 3.90, m	3.77, ddd, J=7, 7, 3 Hz	
H-8‴			3.73, ddd, J=8, 7, 5.5 Hz
H-9	4.46, dd, J=11, 3 Hz[b] 4.32, dd, J=11, 7.5 Hz[c]	4.43, br d, J=11.5 Hz[d] 4.30, dd, J=11.5, 7 Hz	
H-9′	4.44, dd, J=11, 3 Hz[b] 4.24, dd, J=11, 7.5 Hz[c]	4.45, br d, J=11.5 Hz[d] 4.30, dd, J=11.5, 7 Hz	
H-9‴			4.46, dd, J=11.5, 5.5 Hz ca. 4.30, m

[a-d]Assignments with the same letter designation may warrant changing.

hydrobenzofuran is the other terminal unit. Compound **9** is, therefore, considered *rel*-(7′*S*,8′*S*,7″*S*,8″*S*)-4,9,4′,4‴,9‴-pentahydroxy-5,5′,5″,5‴-tetramethoxy-7-oxo-8.3′,7′.0.9″,8′.8″,9′.0.7″,3″.8‴,4″.0.7‴-lignoid, according to the nomenclature used for lignans and neolignans (9).

This is the third tetrameric lignoid isolated from the seeds of *H. caudigerum*. Like all the known lignoids of this species, its structure reflects 8.3′-couplings of coniferyl alcohol units; like trimer **7** and tetramer **8**, the 8.3′-coupling is associated with the 8′.8″-coupling generating a dioxabicyclooctane.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Analytical tlc was carried out on Si gel 60F-254 plates (E. Merck). Si gel 60PF-254 containing gypsum (E. Merck) for preparative layer chromatography was used for centrifugal circular tlc. Polyamide CC6 (Macherey Nagel) and Sephadex LH20 (Pharmacia Fine Chemicals) were used for column chromatography. Uv spectra were measured in MeOH on a Beckman 25 spectrometer. Ir spectra (KBr) were taken on an Unicam SP1100 spectrometer. ^{1}H-nmr spectra (C_5D_5N or $CDCl_3$; δ ppm; TMS) were recorded on WM250 Bruker, Cameca 250, Cameca 350, and Bruker 500 MHz nmr spectrometers. Extensive decoupling was used to verify assignments. Ei mass spectra were taken on an AEI MS902 mass spectrometer (70 eV).

PLANT MATERIAL.—*H. caudigerum* seeds (2 kg) were obtained as a gift from la Mission CNRS-Népal-RCP 253 and identified by Dr. M. Keraudren, Laboratoire du Phanérogame, Museum d'Histoire Naturelle, Paris. A voucher specimen HCS-77 has been deposited at Laboratoire de Pharmacognosie de Grenoble, Domaine de La Merci, F-38700 La Tronche.

ISOLATION OF HERPETETRONE.—*H. caudigerum* seeds, first defatted with *n*-hexane then with $CHCl_3$, were extracted at room temperature by MeOH, to afford, after removal of the solvent, a brown viscous mass. After precipitation with H_2O, a portion (1 g) of the insoluble part was subjected to fractionation through a polyamide cc with a gradient from C_6H_6-MeOH (95:5) to MeOH. Combination of similar eluates provided twenty fractions on the basis of tlc analysis on Si gel in C_6H_6-butanone-MeOH (85:10:5 and 75:15:10). Fractions 7-9 (135 mg), containing tetramer **9**, were treated by repeated cctlc on Si gel with different solvent mixtures to concentrate the related product. This procedure yielded 8 mg of impure herpetetrone; final purification was accomplished by filtration through a Sephadex LH 20 column (MeOH) providing 3 mg of an amorphous powder.

HERPETETRONE [**9**].—Amorphous powder; uv λ MeOH 307 sh, 280, 230; /NaOMe=/NaOAc 347 nm; ir ν KBr 3450, 2950, 1660, 1595, 1520, 1450, 1425, 1270, 1170, 1040, 820 cm^{-1}; ms (70 eV) *m/z* (%) 536 (M-194; 2), 518 (13), 506 (10), 488 (5), 458 (2), 446 (2), 344 (6), 330 (12), 206 (11), 175 (17), 167 (13), 165 (15), 151 (28), 137 (30), 124 (100), 109 (98); ^{1}H nmr, see Table 1.

ACETYLATED DERIVATIVE.—(**9**, C_5H_5N, Ac_2O, room temperature, 2h), viscous mass extracted by C_6H_6 and purified by Si cc with C_6H_6-MeOH (98:2); uv λ MeOH 305 sh, 290 sh, 280 sh, 260 nm; /NaOMe 352, 280 sh, 260 sh; /NaOMe+HCl 305 sh, 280 nm; ir ν KBr 3050, 2990, 2960, 2880, 1770, 1740, 1670, 1615, 1600, 1505, 1470, 1415, 1370, 1260, 1200, 1170, 1040, 900, 800 cm^{-1}; ms (70 eV) *m/z* (%) 940 ($M^{+\cdot}$; 2; 940.316; $C_{50}H_{52}O_{18}$=940.3153), 880 (9), 838 (6), 797 (2), 778 (5), 737 (7), 429 (2), 297 (6), 221 (5), 151 (74), 137 (17), 60 (35), 45 (38), 43 (100); ^{1}H nmr, see Table 2.

PERMETHYLATED DERIVATIVE.—(**9**, Na, DMF, MeI, room temperature, 2 h), viscous mass extracted by C_6H_6 and purified by tlc on Si with several developments in C_6H_6-MeOH (98:2); ms (70 eV) *m/z* (%) 768 (M-MeOH; 6), 766 (10), 754 (11), 734 (9), 722 (4), 209 (12), 179 (13), 165 (100), 153 (23), 151 (21), 135 (24), 121 (30), 109 (25).

TRIMETHYLSILYL DERIVATIVE.—(**9**, BSTFA+1% TMCS, room temperature, 24 h); ms (70 eV) *m/z* (%) 1090 ($M^{+\cdot}$; 2), 1000 (5), 910 (6), 612 (5), 556 (35), 547 (72), 482 (40), 457 (42), 355 (28), 297 (64), 281 (72), 267 (40), 223 (100), 209 (70), 204 (80), 193 (22), 179 (25), 147 (70).

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