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NEW SECO- AND HYDROXY-LIGNANS FROM *PHYLLANTHUS NIRURI*[1]

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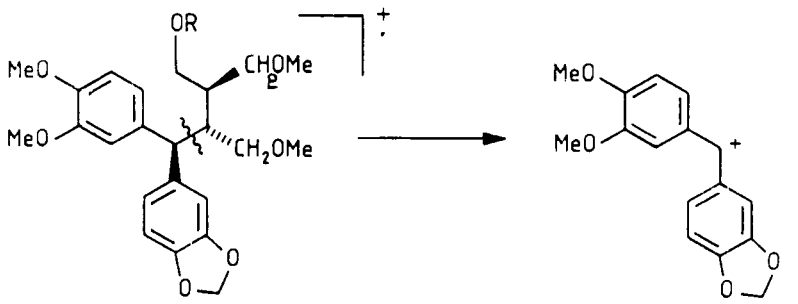
ABSTRACT.—The structures of an unusual seco-lignan [**1a**] and two new hydroxy-lignans [**2a**, **3a**] isolated from *Phyllanthus niruri* have been determined. The conversion of [**2a**] into phyllanthin [**2c**] and [**3a**] into nirtetralin [**4**] is reported. In addition, a known dibenzylbutyrolactone [**5**], reported to exhibit antitumor activity, is reported, and full details of lintetralin [**6**] are included.

Previous extraction of the bitter leaves of *Phyllanthus niruri* L. (Euphorbiaceae), a well-known medicinal plant (1,2), has yielded six lignans (3–5) of which two (phyllanthin and niranthin) are members of the diarylbutane class and four (hypophyllanthin, nirtetralin, phyltetralin, and lintetralin) belong to the aryltetralin group. The present paper describes the isolation of four additional compounds, of which one is an unusual seco-lignan [**1a**] and two, seco-isolariciresinol trimethyl ether [**2a**] and hydroxyniranthin [**3a**], are new members of the diarylbutane class. The fourth compound is a dibenzylbutyrolactone **5** previously isolated from *Bursera schlechtendalii* and reported to exhibit antitumor activity (6).

RESULTS AND DISCUSSION

Seco-4-hydroxylintetralin [**1a**], $C_{23}H_{30}O_7$ (M^+ 418), exhibited uv maxima at 230 and 286 nm (log ϵ 3.84 and 3.62), and showed a strong absorption at 3450 cm^{-1} in its ir spectrum, forming a monoacetate **1b** (M^+ 460) with Ac_2O and pyridine. In its ^1H- and ^{13}C-nmr and mass spectra, it gave clear evidence for the presence of both 3,4-methylenedioxyphenyl and 3,4-dimethoxyphenyl groups and two methyl ethers. The two-proton signal at δ 3.65 in the ^1H-nmr spectrum (Table 1) was shifted downfield to δ 4.20 in the spectrum of acetate **1b**, which was consistent with the presence of a primary CH_2OH group. This conclusion was further supported by the ^{13}C-nmr spectrum



1a R=H (M^+ 418)
1b R=Ac (M^+ 460)

m/z 271 (100)

SCHEME 1

[1]Preliminary communication presented at the 14th IUPAC International Symposium on the Chemistry of Natural Products, 9–14 July 1984, Poznan, Poland.

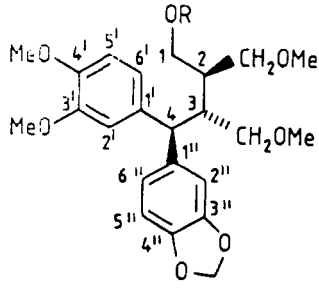
TABLE 1. ^{1}H-Nmr Spectra.[a]

Assignment	Seco-4-hydroxy-lintetralin [**1a**]	Seco-4-hydroxy-lintetralin acetate [**b**]	Seco-isolariciresinol trimethyl ether [**2a**]	Seco-lariciresinol trimethyl ether acetate [**2b**]	Hydroxy-niranthin [**3a**]	Hydroxy-niranthin acetate [**3b**]
H-1	3.65 d (5)	4.20 m	2.6 m	2.6 m	4.75 br d (6)	5.80 d (6)
H-4	3.85 d (6)	4.05 br s			2.6 d (9)	2.05–2.30 m
H-2	2.10 m	2.2 m	1.9 m	1.7 m	1.95 m	
H-3	2.60 m	2.6 m				
	3.20 m					
CH_2's		3.20–3.60 m	3.25–3.68 m	3.45 m	3.30 m	3.20 m
	3.45 d (7)			4.2 m	3.45 d (6)	3.53 d (6)
R-OCH_3	3.13 s, 3.23 s	3.10 s, 3.15 s	3.32 s	3.3 s	3.37 s	3.25 s, 3.80 s
Ar-OCH_3	3.77 s, 3.80 s	3.85 s, 3.90 s	3.80 s, 3.85 s	3.82 s, 3.84 s	3.87 s, 3.90 s	3.80 s, 3.85 s
OCH_2O	5.81 s	5.92 s	—	—	5.85 s	5.90 s
ArH	6.65–6.73 m	6.7–6.9 m	6.5–6.8 m	6.5–6.8 m	6.19	6.2–6.80
					6.50–6.70	6.5–6.80
OAc	—	2.05 s	—	2.03 s	—	1.95 s
OH	3.5 br m	—	3.4 br m	—	3.3 br m	—

[a]All spectra recorded in $CDCl_3$ solution (coupling constants in parentheses).

(Table 2), which contained a low-field triplet at δ 65.67. Further analysis of the ^{1}H- and ^{13}C-nmr spectra led to structure **1a**, which was supported by the presence of a base peak at *m/z* 271 in the mass spectrum corresponding to formation of a doubly benzylic carbocation (Scheme 1).

Seco-isolariciresinol trimethyl ether [**2a**], $C_{23}H_{32}O_7$ (M^+404), exhibited uv maxima at 230 and 278 nm (log ε 4.20 and 3.15) and gave a prominent peak at 3400 cm^{-1} in its ir spectrum, forming a monoacetate **2b** (M^+446) on acetylation. The ^{1}H- and ^{13}C-nmr spectra (Tables 1 and 2) gave clear evidence for the presence of both a hydroxymethyl group and a methoxymethyl group. These observations led to the formulation of structure **2a**, which was also supported by the mass spectrum. A partial synthesis of **2a** was achieved treating (±)-2,3-bis(3,4-dimethoxybenzyl)-butane-1,4-diol (7) with one equivalent of MeI/Ag_2O, furnishing the monomethyl ether, mp 83°, which was identical in all respects with seco-isolariciresinol trimethyl ether [**2a**]. In another experiment **2a** was methylated to yield a product (mp 96°) identical with phyllanthin [**2c**].



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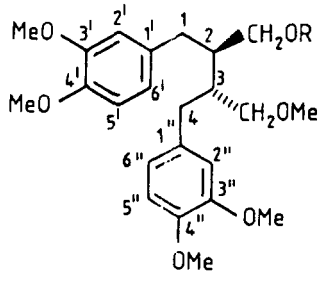
1b R=Ac



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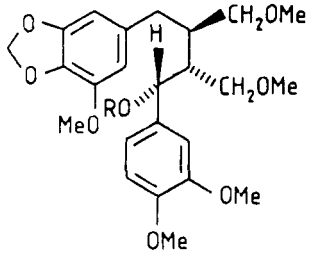
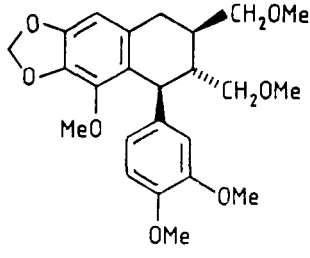
2b R=Ac
2c R=Me



3a R=H
3b R=Ac



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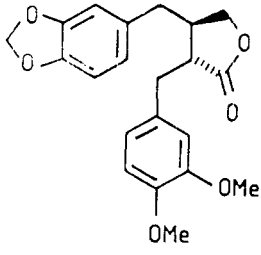

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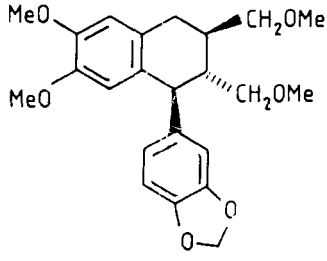

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TABLE 2. ^{13}C Nmr Spectra.

Assignment	Seco-4-Hydroxy-lintetralin [**1a**]	Seco-isolarici-resinol trimethyl ether [**2a**]	Phyllanthin [**2c**]
C-1	65.67	35.79[a]	35.02
C-4	51.75	36.22	
C-2	40.95	42.47	40.77
C-3	43.34	44.63	
CH_2OR	71.74	60.89	72.73
	72.50	71.10	
C-1′	131.73	133.20	133.70
C-1″	132.13	133.45	
C-2′	108.12	111.27	111.27
C-2″	110.48		
C-3′	146.10	147.37	147.37
C-3″	147.18		
C-4′	147.34	148.90	148.83
C-4″	147.89		
C-5′	111.29	112.38	112.36
C-5″	113.26		
C-6′	121.25	121.08	121.25
C-6″	122.75		
Ar*OMe*	55.82	55.84	55.75
	56.12	55.93	55.91
RO*Me*	59.15	58.88	58.72
OCH_2O	100.75	—	—

[a]Values may be interchanged.

Hydroxyniranthin [**3a**], $C_{24}H_{32}O_8$ (M^+448), had uv maxima at 208 and 276 (log ϵ 4.26 and 3.76) and gave an absorption at 3450 cm^{-1} in its ir spectrum. It was readily acetylated to **3b**, $C_{26}H_{34}O_9$ (M^+490). The ^1H-nmr spectrum of **3a** contained a characteristic one-proton signal at δ 4.75 that was shifted downfield to δ 5.80 in the spectrum of the acetate, consistent with the presence of a secondary CHOH group. Further analysis of the ^1H-nmr spectra of **3a** and **3b** (Table 1) and ms led to structure **3a** for hydroxyniranthin. When **3a** was stirred in CH_2Cl_2 with TFA at room temperature, it was converted into nirtetralin [**4**] identical with an authentic sample.

The dibenzylbutyrolactone **5** was isolated as an oil having a lactone carbonyl absorption at 1760 cm^{-1}. It was identified by comparison with an authentic sample obtained by synthesis (8).

Lintetralin [**6**], $C_{23}H_{28}O_6$ (M^+400), was identified by analysis of its ^1H- and ^{13}C-nmr spectra (5). Its structure has been confirmed subsequently by independent synthesis (9).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were recorded on a Shimadzu AR 408 and uv spectra on a Beckman instrument. Mass spectra were recorded on an AEI MS9 spectrometer and ^1H- and ^{13}C-nmr spectra on Varian HA 100 and XL 100 instruments.

ISOLATION OF LIGNANS.—The leaves of *P. niruri* were collected from the Simachalam hills, and the identification certificate was submitted to the Botany Department of this campus. The leaves (10 kg) were powdered and successively extracted with hexane following the procedure reported by Row *et al.* (3). The only operational modification was that the leaf powder was not mixed with lime. The oil (200 g) obtained from the hexane extract was adsorbed on Si gel (400 g BDH [British Drug House], 200 mesh) and chromatographed using a column of Si gel (1500 g) eluting successively with hexane (85 liters), hexane-C_6H_6 (75:25, 75 liters; 50:50, 25 liters; 25:75, 25 liters), C_6H_6 (30 liters), C_6H_6-EtOAc (90:10, 30 lit-

ers). The elution was monitored by tlc (Si gel G), and 1-liter fractions were collected. The compounds isolated are listed in Table 3.

Lintetralin [**6**] was obtained as an oil, R_f 0.43 (C_6H_6-EtOAc, 8:2) $[\alpha]^{28}D$ +2.6° ($CHCl_3$), and gave a positive Labat test for the OCH_2O group. Found: C 68.45%, H 6.91%; $C_{23}H_{28}O_6$ requires C 68.95%, H 7.05%; uv (MeOH) 225 and 281 nm; ir ($CHCl_3$) 3000, 2900, 1610, 1584, 1510 cm^{-1}; eims *m/z* $[M]^+$ 400 (52), 368 (7), 324 (14), 323 (68), 296 (21), 165 (20), 151 (55), 137 (23), 135 (68), 111 (100), 109 (60). For ^1H- and ^{13}C-nmr spectra, see Satyanarayana *et al.* (5).

Trans-2-(3,4-dimethoxybenzyl)-3-(3,4-methylenedioxybenzyl)butyrolactone [**5**] was obtained as an oil, R_f 0.50 (hexane-$CHCl_3$, 1:1), $[\alpha]^{28}D$ −151° ($CHCl_3$). It gave a positive Labat test for the OCH_2O group and dissolved in concentrated H_2SO_4, giving a pink color which changed to red. Found: C 68.15%, H 6.07%; $C_{21}H_{20}O_6$ requires C 68.10%, H 5.94%; uv (MeOH) 232 and 283 nm (log ε 4.15 and 3.98); ir ($CHCl_3$) 2960, 2930, 1760, 1610, 1590, 1500 cm^{-1}; eims *m/z* $[M]^+$ 370 (47), 235 (3), 208 (6), 200 (5), 152 (14), 151 (100), 135 (22). For ^1H- and ^{13}C-nmr spectra, see Pelter *et al.* (8). Identity confirmed by direct comparison with an authentic sample.

Seco-4-hydroxylintetralin [**1a**] was isolated as an oil, R_f 0.35 (C_6H_6-EtOAc, 8:2), $[\alpha]^{28}D$ +11.5° ($CHCl_3$), and gave a positive Labat test for the OCH_2O group. Found: C 66.15%, H 7.4%, $C_{23}H_{30}O_7$ requires C 66.03%, H 7.1%; uv (MeOH) 230 and 286 nm (log ε 3.84 and 3.62); ir ($CHCl_3$) 3400, 3000, 2960, 2940, 1600 cm^{-1}; eims *m/z* $[M]^+$ 418 (2), 386 (1), 362 (2), 297 (2), 272 (18), 271 (100). For ^1H- and ^{13}C-nmr spectra, see Tables 1 and 2.

Acetate of seco-4-hydroxylintetralin [**1b**]: seco-4-hydroxylintetralin (20 mg) was dissolved in dry pyridine (0.5 ml), and dry Ac_2O (1 ml) was added. The reaction mixture was kept at room temperature for 24 h. After the usual work-up, a colorless oil (20 mg) was obtained, R_f 0.68 ($CHCl_3$-EtOAc, 95:5). Found:

TABLE 3. Isolation Data.

Eluent	Fractions	Compounds Isolated
Hexane	1–85	oil } (110 mg)
Hexane-C_6H_6 (75:25)	86–125	oil }
Hexane-C_6H_6 (75:25)	126–165	lintetralin (150 mg) + nirtetralin + niranthin + hypophyllanthin (9.5 g)
Hexane-C_6H_6 (50:50)	166–190	nirtetralin + niranthin + hypophyllanthin (10.0 g) + phyltetralin + dibenzylbutyrolactone [**5**] (150 mg)
Hexane-C_6H_6 (25:75)	191–215	hypophyllanthin + phyltetralin + phyllanthin (17.0 g)
C_6H_6	216–230	hypophyllanthin + phyltetralin + phyllanthin (15.0 g)
C_6H_6	231–245	phyllanthin (3 g)
C_6H_6-EtOAc (90:10)	246–255	phyllanthin
C_6H_6-EtOAc (90:10)	256–275	phyllanthin + seco-4-hydroxylintetralin (200 mg) + seco-isolariciresinol trimethyl ether (150 mg) + hydroxyniranthin (40 mg)

C 65.92%, H 7.68%; $C_{25}H_{32}O_8$ requires C 65.21%, H 7.39%; uv (MeOH) 206 and 280 nm (log ε 4.30 and 3.26); ir ($CHCl_3$) 2950, 2850, 1730, 1600, 1510 cm^{-1}; eims *m/z* $[M]^+$ 460 (3), 323 (1), 297 (1), 273 (3), 272 (17), 271 (100). For ^1H-nmr spectrum, see Table 1.

Seco-isolariciresinol trimethyl ether [**2a**] was obtained as a colorless oil. R_f 0.35 (C_6H_6-EtOAc, 8:2), $[\alpha]^{28}D$ −2.3° ($CHCl_3$). Found: C 68.15%, H 7.78%; $C_{23}H_{32}O_6$ requires C 68.25%, H 7.70%; uv (MeOH) 230, 278 nm (log ε 4.20 and 3.15); ir ($CHCl_3$) 3400, 3000, 2960, 2900, 2845, 1580 cm^{-1}; eims *m/z* $[M]^+$ 404 (12), 372 (1), 364 (1), 339 (2), 312 (1), 203 (8), 177 (8), 152 (26), 151 (100). For ^1H- and ^{13}C-nmr spectra, see Tables 1 and 2.

Seco-isolariciresinol trimethyl ether acetate [**2b**] was prepared using Ac_2O and pyridine as described above and was obtained as an oil. R_f 0.65 (C_6H_6-EtOAc, 8:2). Found: C 68.35%, H 8.15%; $C_{25}H_{34}O_7$ requires C 67.87%, H 7.62%; uv (MeOH) 203, 222, 275 nm (log ε 4.58, 4.55, 3.88); ir ($CHCl_3$) 3000, 2900, 1735, 1630, 1510 cm^{-1}; eims *m/z* $[M]^+$ 446. For ^1H-nmr spectrum, see Table 1.

Conversion of seco-isolariciresinol trimethyl ether [**2a**] into phyllanthin [**2c**]: Seco-isolariciresinol trimethylether [**2a**] (25 mg) was dissolved in dry DMF (5 ml) and MeI (5 ml), and freshly prepared Ag_2O (300 mg) was added in small portions. The reaction mixture was kept at room temperature for 24 h, after which a colorless oil was obtained that, upon crystallization from petroleum ether, afforded the methyl ether as colorless needles mp 96°, R_f 0.35 (hexane-EtOAc, 3:1). Found: C 68.60%, H 8.27%; $C_{24}H_{34}O_6$ requires C 68.90%, H 8.13%; uv (MeOH) 232 and 279 nm (log ε 4.16 and 3.79); ir (Nujol) 1605, 1600, 1590, 1520 cm^{-1}. The mmp was not depressed by an authentic sample of phyllanthin [**2c**].

Partial synthesis of (±)-seco-isolariciresinol trimethyl ether [**2a**]: (±)-2,3-bis(3,4-dimethoxybenzyl)-butane-1,4-diol (50 mg) was dissolved in dry DMF (5 ml) and MeI (1 ml), and freshly prepared Ag_2O (300 mg) was added in small quantities and worked up as usual to give residual oil that was chromatographed on Si gel. Elution with C_6H_6-EtOAc (95:5 v/v) eluted an oil that crystallized from hexane to yield phyllanthin as colorless needles (20 mg) mp 95°. Further elution with C_6H_6-EtOAc (90:10 v/v) afforded oil, which on crystallization from C_6H_6-light petroleum ether, gave (±)-seco-isolariciresinol trimethyl ether as colorless plates (22 mg) mp 82°, R_f 0.35 (C_6H_6-EtOAc, 8:2). Found: C 68.53%, H, 7.86%; $C_{23}H_{32}O_6$ requires C 68.25%, H, 7.9%; uv (MeOH) 230 and 278 nm (log ε 4.20 and 3.15); ir ($CHCl_3$) 3400, 3000, 2960, 2900, 2845, 1580, 1450, 1320, 1230, 1155, 1140, 1100, 1030 cm^{-1}; ^1H nmr ($CDCl_3$) 1.9 (m, 2H), 2.6 (m, 4H), 3.25–3.65 (m, 4H), 3.80 (s, 6H), 3.85 (s, 6H), 3.32 (s, 3H), 6.5–6.8 (m, 6H). All the physical characteristics and spectral data were found to be identical in all respects with natural **2a**.

Hydroxyniranthin [**3a**] was obtained as a colorless oil, R_f 0.74 (C_6H_6-EtOAc, 8:2), $[\alpha]^{28}D$ −50° ($CHCl_3$), and gave a positive Labat test for the OCH_2O group. Found: C 64.45%, H 7.3%; $C_{24}H_{32}O_8$ requires C 64.45%, H 7.1%; uv (MeOH) 208 and 276 nm (log ε 4.28 and 3.76); ir ($CHCl_3$) 3450, 3000, 2960, 2800, 1610, 1590, 1550, 1500 cm^{-1}; eims *m/z* $[M]^+$ 448 (60), 431 (8), 430 (30), 416 (6), 398 (8), 384 (65), 367 (10), 251 (8), 250 (40), 272 (9), 218 (8), 208 (15), 167 (25), 166 (40), 165 (100), 151 (18), 139 (13). For ^1H-nmr spectrum, see Table 1.

Acetate of hydroxyniranthin [**3a**] was prepared using Ac_2O and pyridine as described above and was obtained as a colorless oil, R_f 0.80 (C_6H_6-EtOAc, 8:2). Found: C 63.39%, H 7.02%; $C_{26}H_{34}O_9$ requires C 63.37%, H 6.93%; uv (MeOH) 221 and 280 nm (log ε 4.10 and 3.61); ir ($CHCl_3$) 2960, 2870, 1730, 1600, 1516 cm^{-1}; eims *m/z* $[M]^+$ 490 (70), 460 (5), 459 (5), 458 (4), 430 (6), 429 (23), 398 (8), 367 (8), 353 (18), 223 (18), 222 (18), 215 (10), 209 (13), 208 (53), 210 (10), 191 (9), 168 (16), 167 (50), 166 (50), 165 (100), 152 (9), 151 (44), 139 (19). For ^1H-nmr spectrum, see Table 1.

CONVERSION OF HYDROXYNIRATHIN [**3a**] TO NIRTETRALIN [**4**].—Hydroxyniranthin [**3a**] (15 mg) was dissolved in dry CH_2Cl_2 (5 ml), and TFA (0.2 ml) was added. The mixture was stirred for 6 h at room temperature. Work-up followed by crystallization from petroleum ether afforded colorless crystals mp 55°, $[\alpha]^{28}D$ +14.4° ($CHCl_3$). Found: C 66.76%, H 7.43%; $C_{24}H_{30}O_7$ requires C 66.96%, H 7.02%; eims *m/z* $[M]^+$ 430 (52); uv (EtOH) 226 and 284 nm; ir (Nujol) 2850, 1635, 1605 cm^{-1}; ^1H nmr ($CDCl_3$), 1.856 (m, 2H), 2.50–2.65 (m, 2H), 3.21 (s, 6H), 3.45 (s, 3H), 3.75 (s, 6H), 3.30 (m, 4H), 4.19 (m, 1H), 5.98 (s, 2H), 6.33 (s, 1H), 6.55–6.60 (m, 3H). Identified by co-tlc, R_f 0.53 (petroleum ether-EtOAc, 3:1), mmp was not depressed by an authentic sample of nirtetralin [**4**].

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