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A NEW LIGNAN AND A NEW NEOLIGNAN FROM *PHYLLANTHUS NIRURI*[1]

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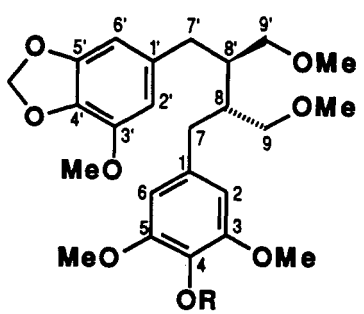
ABSTRACT.—A new lignan, nirphyllin, and a new neolignan, phyllnirurin, isolated from the aerial parts of *Phyllanthus niruri* were established as 3,3′,5,9,9′-pentamethoxy-4-hydroxy-4′,5′-methylenedioxylignan [**1**] and 3,4-methylenedioxy-5′-methoxy-9′-hydroxy-4′,7-epoxy-8,3′-neolignan [**2**] on the basis of spectral evidence.

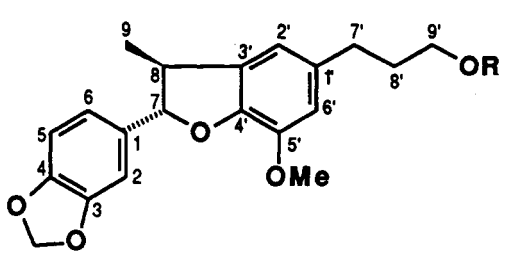
Phyllanthus niruri L. (Euphorbiaceae) has a well-known reputation as a liver-protecting drug in the indigenous system of medicine (1). Earlier, we reported the isolation of several chemical constituents from the hexane extract of the aerial parts (2). The antihepatotoxic activity was found to be due to lignans and acyclic compounds (3). Further investigation has now led to the isolation of two constituents, designated as nirphyllin [**1**] and phyllnirurin [**3**].

The powdered aerial parts of *P. niruri*, mixed with $Ca(OH)_2$ in the ratio 10:3, were extracted with *n*-hexane. The crude *n*-hexane extract was subjected to cc and preparative tlc, leading to the isolation of two polar lignans. Nirphyllin [**1**], $C_{24}H_{32}O_8$ $[M]^+$ 448, exhibited ir absorption bands at 3400 cm^{-1} (hydroxyl), 1595 cm^{-1} and 1575 cm^{-1} (aromatic rings) and a band at 930 cm^{-1} (methylenedioxy). Its ^1H nmr displayed a multiplet at δ 1.98 for a pair of aliphatic methines, a broad doublet ($J = 6$ Hz) at δ 2.68 corresponding to a pair of benzylic methylenes, two methoxy methyl groups at δ 3.34 (6H), three aryloxymethyls at δ 3.82 (6H) and 3.86 (3H), and a methylenedioxy group at δ 5.93 (2H). The appearance of the aryl methine signals at δ 6.53 (1H) and 6.70 (3H) represented the meta protons of the C-2, C-6, C-2′, and C-6′ positions of the 3,4,5-trioxysubstituted aromatic rings (4).

These ^1H-nmr spectral features were consistent with the 8,8′-type of lignans (5) possessing methoxyl groups in 9,9′ positions (6,7). The ms showed peaks at *m/z* $[M]^+$ 448, 223, and 225, the latter of which arose due to the cleavage of the C-8 and C-8′ bond (5–7). The fragment ions at *m/z* 165 and 167 were characteristic of 3,5-dimethoxy-4-hydroxybenzyl and 3-methoxy-4,5-methylenedioxybenzyl ions that could further be rearranged to tropylium ions (8), thus revealing the presence of the above-mentioned substitution pattern in the aromatic rings.



1 R=H
2 R=Ac



3 R=H
4 R=Ac

[1]Part 7 in the series "Studies on Lignan and Neolignans". For part 6 see P.K. Agrawal and R.S. Thakur (15). CIMAP Communication No. 664.

Compound **1** formed a monoacetyl derivative **2** which displayed bands at 1750 cm^{-1} and 1270 cm^{-1} for an acetoxyl group in its ir and displayed an acetoxymethyl resonance at δ 2.25 in its ^{1}H-nmr spectrum. The chemical shifts of the aryl methine protons (see Experimental) remained almost unaffected, revealing the presence of the substituents in ortho and para positions with respect to the acetoxyl group and hence implying C-4 as the site of hydroxyl substitution.

The mutual relationship between the C-9,9′ substituents in **1** was established as *trans* in view of resemblance of ^{1}H-nmr chemical shifts and splitting pattern for H-7, -7′,-8,-8′,-9, and -9′ to the reported values for phyllanthin and niranthin (6,7) and was, moreover, supported by the positive value of the optical rotation (+15°). Based on the above evidence, the structure of nirphyllin could be confirmed as 3,3′,5,9,9′-pentamethoxy-4-hydroxy-4′,5′-methylenedioxylignan [**1**].

Phyllnirurin [**3**], $C_{20}H_{22}O_5$ $[M]^+$, 342, showed ir absorption bands at 3400 cm^{-1} and 1035 cm^{-1} (hydroxyl), 1650, 1600, and 1560 cm^{-1} (aromatic rings), and 930 cm^{-1} (methylenedioxy). The uv maxima at 286 nm, in conjunction with the presence of an AMX_3 type of splitting pattern [an oxybenzylic methine doublet (J = 9 Hz), at δ 5.05, another benzylic methine as double quartet (J = 9,7 Hz) at δ 3.35 and a doublet (J = 7 Hz) at δ 1.41 for a secondary methyl group] in its ^{1}H-nmr spectrum suggested a 7.0.4′, 8.3′ (benzofuran) type of skeleton (5,9). Other ^{1}H-nmr signals at δ 2.68, 1.71, and 3.68, each integrating for two protons, appeared as a triplet (J = 7 Hz), multiplet, and triplet (J = 7 Hz), respectively, suggesting the existence of an *n*-propanol side chain substituent on the benzofuran ring system (5,10). The appearance of signals for an aryl methoxyl at δ 3.85 (3H) and a methylenedioxy group at δ 5.90 (2H), in addition to the five aromatic protons in the region δ 6.55–7.00, led to a prediction of the presence of a piperonyl substituent (11) and a methoxyl group in the benzofuran moiety.

Structure **3** was further supported by its ms fragmentation pattern, as it showed a prominent fragment at *m/z* 149 due to the benzylic cleavage of the piperonyl moiety (8). The detection of a fragment ion at *m/z* 297 could be rationalized in view of the benzylic cleavage of the *n*-propanol side chain. Remaining fragments were characteristic of 4′,7-epoxy-8,3′-type neolignans (5,9,10).

Compound **3** formed a monoacetyl derivative **4**, which exhibited a multiplet at δ 4.10 and a singlet at δ 2.07 in its ^{1}H-nmr spectrum due to acetoxymethylene and acetoxymethyl groups. Hence, the existence of the CH_2OH group was confirmed.

The stereochemistry between the piperonyl substituent and methyl group of **3** was established as *trans* due to the characteristic coupling constant value (J = 9 Hz) exhibited by H-7 (12). Thus, on the basis of the foregoing evidence, the structure of phyllnirurin could be established as 3,4-methylenedioxy-5′-methoxy-9′-hydroxy-4′,7-epoxy-8,3′-neolignan [**3**].

It is worthwhile to mention here that the lignans so far characterized from *P. niruri* (6,7,13) have possessed veratryl (3,4-dimethoxyphenyl), piperonyl (3,4-methylenedioxyphenyl), and methoxypiperonyl (3,4-methylenedioxy-5-methoxyphenyl) as the aryl substituent and methoxyl groups, respectively, at C-9 and C-9′ positions. These were either lignan (8,8′-linked) or 2,4′-cyclolignan (8.8′,2.7′-linked lignans) (14–16) which were earlier regarded as the 1,4-diaryl butane and 1-aryl tetralin types of carbon framework. Phyllanthin and niranthin were representatives of the former, whereas hypophyllanthin, nirtetralin, lintetralin, and phylltetralin belonged to the later type. The 3,5-dimethoxy-4-hydroxyphenyl substitution pattern present in nirphyllin had not been earlier recognized in *Phyllanthus* lignans but has been found frequently in lignans from other plant sources (14,15). Phyllnirurin belongs to the 4′,7-epoxy-8,3′-type of neolignan skeleton, which is recognized for the first time in this genus.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURE.—Ms were recorded on JEOL-JMS 0300 at 70 ev; ir in $CHCl_3$ on Perkin-Elmer model 399B; ^{1}H-nmr spectra were measured on an 80 MHz (CFT-20) spectrometer in $CDCl_3$ with TMS as internal standard. The adsorbents for cc and tlc were from BDH.

PLANT MATERIAL AND EXTRACTION.—The plant material was collected from the Institute farm during August 1983 and identified by the Botany Department of our Institute. A specimen No. 439 is also preserved in the herbarium. Dried powdered aerial parts (1 kg) were mixed with $Ca(OH)_2$ in a 10:3 ratio and extracted exhaustively with hexane, and the total hexane extractive was concentrated. The concentrate (30 g) was dissolved in hot hexane (1 liter), and MeOH (3 × 200 ml) was added to obtain a precipitate (5 g). The filtrate was concentrated to yield a residue (25 g). A part (10 g) was chromatographed over Si gel (330 g) and eluted with a hexane/EtOAc gradient with increasing polarity of EtOAc. Fractions 137–140 eluted with hexane-EtOAc (8:2) showed the presence of two compounds which were further purified by plc [Si gel, hexane-EtOAc, (8:2)] affording nirphyllin (7 mg) and phyllnirurin (6 mg) at the R_f values 0.17 and 0.15, respectively.

NIRPHYLLIN [**1**].—Ir ν max 3400 (OH), 2910, 2845, 1595, 1575, 1510, (aromatic), 1460, 1440, 1410, 1370, 1255, 1235, 1140, 1025, 930 (-OCH_2O-), 810 cm^{-1}; ^{1}H nmr δ 1.98 (2H, bs, H-8,8′), 2.68 (4H, bd, J = 6 Hz, H-7,7′), 3.34 (6H, s, 9,9′-OMe), 3.34 (4H, m, H-9,9′), 3.86 (9H, s, 3,3′,5-OMe), 5.93 (2H, s, 4′,5′-OCH_2O), 6.53–6.70 (4H, m, Ar-H); ms *m/z* [M]$^+$ 448, [M − OMe]$^+$ 418, 400, 386, 342, 278, 236, 225, 223, 208, 203, 189, 181, 167, 165, 151, 149, 137, 109, 94, 83, 71, 57, 55, 43, 41. Calcd for $C_{24}H_{32}O_2$, C 64.29, H 7.14; found C 64.15, H 7.16%.

NIRPHYLLIN ACETATE [**2**].—Nirphyllin [**1**] (4 mg) was treated with pyridine and Ac_2O (5 drops each) for 2 h at room temperature, and on usual workup it afforded an acetate **2** (3 ml) R_f 0.38 [hexane-EtOAc (8:2)]; ir ν max 2920, 2830, 1750 (OCO), 1600, 1580, 1520 (aromatic), 1460, 1440, 1415, 1360, 1270, 930, 800 cm^{-1}; ^{1}H nmr δ 1.96 (2H, bs, H-8,8′), 2.25 (3H, s, 4-Ac), 2.64 (4H, d, J = 6 Hz, H-7,7′), 3.30 (6H, s, 9,9′-OMe), 3.31 (4H, m, H-9,9′), 3.35, 3.80 (9H, s, 3,3′,5-OMe), 5.92 (2H, s, 4′,5′-OCH_2O), 6.55–6.70 (4H, m, Ar-H); ms *m/z* [M]$^+$ 490, 460, 448, 428, 384, 267, 266, 231, 225, 223, 209, 193, 167, 165, 151, 109, 94, 71, 55, 43, 42.

PHYLLNIRURIN [**3**].—Ir ν max 3400 (OH), 2920, 2850, 1650, 1600, 1560 (aromatic), 1490, 1460, 1440, 1370, 1245, 1200, 1140, 1035, 930 (-OCH_2O-), 810 cm^{-1}; uv λ max 252, 286, 324 nm; ^{1}H nmr δ 1.41 (3H, d, J = 7 Hz, H-9), 1.71 (2H, m, H-8′), 2.68 (2H, t, J = 7 Hz, H-7′), 3.35 (1H, dq, J = 9, 7 Hz, H-8), 3.68 (2H, t, J = 7 Hz, H-9′), 3.85 (3H, s, 5′-OMe), 5.05 (1H, d, J = 9 Hz, H-7), 5.90 (2H, s, 3′,4′-OCH_2O), 6.56–7.00 (5H, m, H-2,2′,5,6,6′); ms *m/z* [M]$^+$ 342, 326, 297, 279, 189, 175, 167, 163, 149, 135, 125, 117, 95, 82, 71, 57, 55, 45. Calcd for $C_{20}H_{22}O_5$, C 70.18, H 6.43; found C 70.20, H 6.41%.

PHYLLNIRURIN ACETATE [**4**].—Phyllnirurin [**3**] (3 mg) was treated with pyridine and Ac_2O (5 drops each) for 2 h at room temperature, and on usual workup it afforded an acetate **4** (2.5 mg), R_f 0.34 [hexane-EtOAc (8:2)] which exhibited ir ν max 2918, 2845, 1720 (OAc), 1650, 1600, 1565 (aromatic), 1485, 1460, 1440, 1370, 1265, 1240, 1200, 1140, 1035, 1030 (methylenedioxy), 800 cm^{-1}; ^{1}H nmr δ 1.41 (3H, d, J = 7 Hz, H-9), 1.71 (2H, m, H-8′), 2.07 (3H, s, OAc), 2.68 (2H, t, J = 7 Hz, H-7′), 3.35 (1H, dq, J = 9, 7 Hz, H-8), 4.10 (2H, m, H-9′), 3.85 (3H, s, 5′-OMe), 5.05 (1H, d, J = 9 Hz, H-7), 5.90 (2H, s, 3′,5′-OCH_2O), 6.55–7.00 (5H, m, H-2,2′,5,6,6′); ms *m/z* [M]$^+$ 384, 354, 342, 325, 324, 311, 297, 282, 281, 191, 189, 167, 159, 149, 148, 135, 117, 95, 87, 73, 59, 45, 43, 41.

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LITERATURE CITED

1. R.N. Chopra, S.L. Nayar, and I.C. Chopra, "Glossary of Indian Medicinal Plants," CSIR, New Delhi, 1956, p. 191.
2. B. Singh, P.K. Agrawal, and R.S. Thakur, *Indian J. Chem.*, **25B**, 600 (1986).
3. K.V. Syamasundar, B. Singh, R.S. Thakur, A. Husain, Y. Kiso, and H. Hikino, *J. Ethnopharmacol.*, **14**, 41 (1985).
4. W.D. MacRae and G.H.N. Towers, *Phytochemistry*, **24**, 561 (1985).
5. P.K. Agrawal and R.P. Rastogi, *Phytochemistry*, **21**, 1459 (1982).
6. L.R. Rao, C. Srinivasulu, M. Smith, and G.S.R. Subba Rao, *Tetrahedron Lett.*, 1957 (1964).
7. A.S.R. Anzaneyulu, K.J. Rao, L.R. Row, and G. Subramanyam, *Tetrahedron*, **29**, 1291 (1973).

8. A.M. Duffield, *J. Heterocyclic Chem.*, **4**, 16 (1967).
9. P.W. Lequesne, J.E. Larrahondo, and R.F. Raffauf, *J. Nat. Prod.*, **43**, 353 (1980).
10. P.K. Agrawal, S.K. Agarwal and R.P. Rastogi, *Phytochemistry*, **19**, 1260 (1980).
11. J. Harmatha, M. Budesinsku, and A. Trka, *Collect. Czech. Chem. Commun.*, **47**, 644 (1982).
12. P.K. Agrawal, R.P. Rastogi, and B.G. Oesterdahl, *Org. Magn. Reson.*, **21**, 119 (1983).
13. R.S. Ward, P. Satyanarayana, L.R. Row, and B.V.G. Rao, *Tetrahedron Lett.*, 3034 (1979).
14. O.R. Gottlieb, *Prog. Chem. Org. Nat. Prod.*, **35**, 1 (1978).
15. P.K. Agrawal and R.S. Thakur, *Magn. Reson. Chem.*, **23**, 389 (1985).
16. G.P. Moss, "Lignan and Neolignan Nomenclature," IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN), **25.1**, 1 (1987).

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