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CHEMICAL CONSTITUENTS OF *PIPER SCHMIDTII*:[1] STRUCTURE OF A NEW NEOLIGNAN SCHMIDITIN

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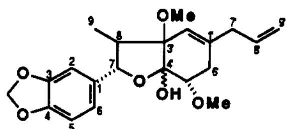
ABSTRACT.—A new neolignan, designated schmiditin [**1**], has been isolated from *Piper schmidtii*. The known lignan galgravin, as well as friedelin, 1-triacontanol, octacosanoic acid, β-sitosterol, and its β-*O*-glucoside were also isolated from the hexane-soluble fraction of the EtOH extract which showed antiamoebic activity (in vitro) at 1000 μg/ml. The structure of schmiditin [**1**] was established by ^{1}H- and ^{13}C-nmr spectroscopy. None of the isolated compounds showed antiamoebic activity.

The genus *Piper* is distributed throughout the tropical and subtropical regions of the world. In India there are about thirty species (1). *Piper* species are rich sources of lignans and neolignans (2). Many of these exhibit biological activities (3–7).

The EtOH extract of the aerial parts of *Piper schmidtii* Hook. f. (Piperaceae) exhibited antiamoebic activity (8) in vitro against *Entamoeba histolytica*, Strain G.S. We report the isolation and structure elucidation of a new neolignan named schmiditin [**1**], along with several other known compounds. The structure of schmiditin [**1**], belonging to the $\Delta^{8'}$-3′,4′,5′,6′-tetrahydro-7.O.4′,8.3′ type of lignans (9), was determined by ^{1}H- and ^{13}C-nmr analysis employing decoupling experiments.

The crude EtOH extractive of the shed-dried aerial parts was fractionated into hexane-, $CHCl_3$-, and MeOH-soluble fractions. Of the three fractions tested against *E. histolytica* (in tissue culture) in vitro for antiamoebic activity, the hexane-soluble fraction exhibited



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[1]CDRI Communication No. 4026.

90% protection at a dose of 1000 μg/ml. On cc over Si gel G, the hexane-soluble fraction yielded a mixture of fatty hydrocarbons (not investigated further), friedelin, 1-triacontanol, β-sitosterol, octacosanoic acid, and an oil which on repeated cc finally yielded schmiditin [**1**] as a colorless crystalline needles (100 mg), mp 98–100°, [α]D −21° (*c* = 1.0, MeOH), another lignan characterized as galgravin (10), and the very common β-D-glucoside of β-sitosterol.

Schmiditin [**1**] analyzed for $C_{21}H_{26}O_6$ (elemental analysis and fdms $[M+H]^+$ *m/z* 375). The ir spectrum showed the presence of a hydroxy group (3500 cm^{-1}), and the uv spectrum in MeOH showed λ max 235 and 285 nm. ^{1}H nmr of schmiditin [**1**], recorded at 400 MHz and aided with decoupling experiments, was very informative. The presence of a methylenedioxy group at δ 5.9 (2H, s) and two methoxy methyls at δ 3.3 (3H, s) and 3.6 (3H, s) was evident. The three aromatic protons resonated between δ 6.72 and 6.84. The H-2 appeared as a narrow doublet at δ 6.7 (d, *J* = 1 Hz) overlapping the high field doublet signal of the two ortho coupled protons H-5 and H-6, which was centered at δ 6.8 (2H, dd, *J* = 10 and 1 Hz), indicating a 1,3,4 substitution pattern of the aromatic ring. The presence of a CH_3-CH-CH-O grouping was indicated by a secondary methyl doublet at δ 0.96 (3H, d, *J* = 7 Hz) coupled to a proton at δ 2.77 (m), which was overlapped by one of the

methylene protons at C-6′. The latter proton in turn was coupled to an oxybenzylic methine proton at δ 4.2 (1H, d, $J=10$ Hz), as confirmed by decoupling experiments. The presence of an allyl side chain attached to an olefinic carbon was evidenced by the presence of the C-7′ methylene protons centered at δ 3.1 (2H, d, d, $J_{7'a-7'b}=14$ Hz, $J_{7'-8'}=3$ Hz), while H-8′ appeared as a multiplet at δ 5.8 (m). The exocyclic methylene protons resonated at δ 5.14 (1H, d, d, $J_{8'-9'a}=8$ Hz and $J_{9'a-9'b}=1.5$ Hz) and δ 5.16 (d, d, $J_{8'-9'b}=16$ Hz and $J_{9'a-9'b}=1.5$ Hz). A singlet at δ 5.38 indicated the presence of an isolated trisubstituted double bond and was assigned to H-2′. The presence of a carbinol proton H-5′ coupled to methylene protons was indicated by a double doublet at δ 4.1 (1H, dd, $J_{5'-6'a}=4$ and $J_{5'-6'b}=3$ Hz). The two methylene protons at C-6′, in turn, appeared at δ 1.98 (1H, dd, $J_{6'a-6'b}=14$ and $J_{6'a-5'}=4$ Hz) and δ 2.77 (m), suggesting their presence in a rigid framework. The signal at δ 2.77 (2H, m) accounted for H-6′b and H-8 protons overlapping each other, leading to its multiplicity. The above findings were fully in agreement with structure **1** for schmiditin.

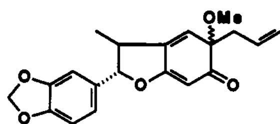
Schmiditin [**1**] on acetylation (Ac_2O/pyridine) yielded a monoacetate as a viscous oil consistent with the formula $C_{23}H_{28}O_7$ ($[M]^+$ 416 *m/z*). The ^1H nmr of the monoacetate showed the presence of an acetoxy methyl singlet at δ 2.0, and there was no shift of any carbinol proton. This was in agreement with the tertiary nature of the hydroxyl group in **1**.

^{13}C nmr aided with DEPT further confirmed the placement of the functional groups in schmiditin [**1**]. Comparison of ^{13}C-nmr data (Table 1) of **1** with those reported for neolignans (11) such as $\Delta^{8'}$-1′,6′-dihydro-6′-oxo-7.O.4′,8.3′ lignan [**2**] (12), mirandin A [**3**] (11) and mirandin B [**4**] (11) fully supported the linkage of the two C_6–C_3

TABLE 1. ^{13}C Chemical Shifts of Compounds **1**, **2**, **3**, and **4** in ppm.

Carbon	Compound			
	1	2[a]	3[a]	4[a]
C-1	134.2	131.4	135.5	132.7
C-2	107.5	106.1	102.6	103.5
C-3	148.1	148.1	152.8	153.3
C-4	147.6	148.1	137.2	138.4
C-5	107.8	108.2	152.8	153.5
C-6	120.5	120.0	102.6	103.5
C-7	84.9	93.7	94.3	91.2
C-8	48.9	42.6	46.9	49.8
C-9	9.2	16.1	16.1	6.9
C-1′	146.1	80.8	142.5	142.8
C-2′	121.1	134.1	131.6	130.9
C-3′	81.3	140.2	80.9	77.6
C-4′	104.6	172.0	172.6	174.3
C-5′	65.9	99.5	104.6	102.7
C-6′	34.2	199.3	186.8	186.8
C-7′	39.1	45.0	33.2	33.5
C-8′	135.4	130.7	134.8	134.8
C-9′	117.1	119.0	116.9	117.1
-O-CH_2-O-	101.1	101.3		
O-Me	48.9, 51.8	53.5	50.3, 56.1, 60.7	51.5, 56.1, 60.7

[a]The values for compounds **2**, **3**, and **4** are from Wenkert *et al.* (11).



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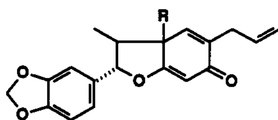
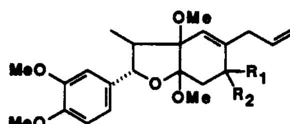
units in **1** to be of the $\Delta^{8'}$,3′,4′,5′,6′-tetrahydro-7.O.4′,8.3′ type with the stereochemistry as shown in **1**. The significant features of ^{13}C-nmr signals of schmiditin [**1**] were the presence of four carbons bearing oxygen functions at δ 65.9, 84.9, 81.3, and 104.6 in addition to those carrying a methylenedioxy group in a benzene ring (δ 147.6 and 148.1). Of these, the secondary carbon at δ 84.9 was assigned to C-7, and the other at δ 65.9 accounted for a secondary carbon carrying the methoxy group at C-5′ (adjacent to a methylene group as evidenced by ^{1}H nmr). The presence of two tertiary carbinol carbons was evident by an anomeric carbon at C-4′ resonating at δ 104.6, and the other tertiary carbon at δ 81.3 could best be assigned to the carbon carrying the other methoxyl group at C-3′. The assignments of other carbons (Table 1) were fully in agreement with structure **1** for schmiditin.

Piperenone [**5**], reported from *Piper futokadzura* Sieb. et Zucc. (13), carries an oxo group at C-6′, and its reduction with $LiAlH_4$ yielded the epimeric alcohols **6** and **7** which were found to be different from schmiditin [**1**] and as such the methoxy group in **1** was placed at C-5′, which was more feasible on biogenetic considerations.

The mass spectrum of **1** showed a weak molecular ion ([M]$^+$ *m/z* 374) and the parent ion appeared at *m/z* 342 [P]$^+$, showing easy loss of MeOH from the molecular ion. A weak peak at *m/z* 356



3 R=β-OMe
4 R=α-OMe



5 R_1,R_2=O
6 R_1=OH, R_2=H
7 R_1=H, R_2=OH

accounted for the loss of H_2O from the molecular ion. The other significant features in the ms of **1** were the presence of an ion at *m/z* 301 [P − allyl group]$^+$, which subsequently showed the loss of methoxyl to give a peak at *m/z* 271. The characteristic peaks arising from the aromatic part of the molecule appeared at *m/z* 121, 135 and 150. A significant peak at *m/z* 211 could best be assigned to an ion arising by the cleavage of the C_9 aromatic unit of the neolignan. None of the isolated compounds showed antiamoebic activity.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's are uncorrected and were taken in open capillaries; ir spectra were determined on a Perkin-Elmer model 331 grating recording spectrometer in KBr pellets. The uv spectra in MeOH were recorded on a Perkin-Elmer Model 202 recording spectrometer. ^{1}H-nmr spectra were recorded in $CDCl_3$ unless otherwise stated, on a 400 MHz (Brucker WM) spectrometer, and ^{13}C-nmr spectra were recorded on the Brucker WM spectrometer at 100 mHz. The mass spectra were recorded on a JMSD-300 mass spectrometer fitted with a direct inlet system.

PLANT MATERIAL.—The aerial parts of *P. schmidtii* were collected from Dodolabetta Reserve Forest, Tamil Nadu, India, in July 1984, by the Botany Division of CDRI, and a voucher specimen (no. 4526) of the plant is preserved in the herbarium of the Institute.

EXTRACTION AND ISOLATION.—The whole plant was air-dried under shade (6 kg dry wt), powdered, and extracted with 95% EtOH 5 × 15 liters at room temperature. The total EtOH extract was concentrated under reduced pressure, and the crude extract was successively fractionated into hexane-soluble and $CHCl_3$-soluble fractions. The anti-amoebic activity was localized in the hexane fraction. The individual fractions were tested in vitro (tissue culture method) against *E. histolytica* at different dilutions. The hexane-soluble fraction at 1000 μg/ml dilution killed 98% of

the trophozoites, while other fractions had only marginal or no effects.

The crude hexane-soluble extract (60 g) was chromatographed over Si gel (1000 g), eluting with hexane, hexane-C_6H_6 (1:1), hexane-C_6H_6 (1:3), C_6H_6, C_6H_6-EtOAc (99:1), C_6H_6-EtOAc (95:5), C_6H_6-EtOAc (9:1), EtOAc, and MeOH mixtures of increasing polarity. About 400 fractions of 150 ml each were collected and yielded the following compounds: friedelin, 1-triacontanol, β-sitosterol, galgravin, and β-sitosterol-β-D-glucoside.

SCHMIDITIN [**1**].—Successive elution with C_6H_6/EtOAc 5% (fractions 224–228) yielded a mixture (800 mg) as oil which was rechromatographed on a Si gel column. On elution with C_6H_6-EtOAc (95:5), a crystalline compound was obtained which on repeated crystallization with MeOH gave white crystalline needles of schmiditin [**1**] (100 mg): mp 98–100°, $[\alpha]D -21°$ (MeOH); uv λ max (MeOH) 235 (log ε, 2.86), 285 (log ε, 2.93) nm; ir ν max 3500, 2995, 1630, 1640, 1500, 1430, 1260, 1120, 1089, 980, 940, 840 cm^{-1}; ^1H nmr δ 0.96 (3H, d, J=7 Hz, Me-9), 1.9 (1H, dd, J= 14, 3.5 Hz, H-6′b), 2.77 (2H, m, H-8, H-6′a), 3.1 (2H, dd, J= 14′, 3 Hz, H-7′), 3.3 (3H, s, OMe), 3.6 (3H, s, -OMe); 4.1 (1H, dd, J=4, 3 Hz, H-5′); 4.2 (1H, d, J= 10 Hz, H-7), 5.16 (2H, dd, J= 10, 2.5 Hz, H-9′a, -9′b), 5.38 (1H, s, H-2), 5.80 (1H, m, H-8′), 5.94 (2H, s, -OCH_2O-), 6.72 (2H, H-2, -6), 6.84 (1H, H-5); ms *m/z* $[M]^+$ 374 (2%), 342 (64%), 327 (42%), 301 (32%), 271 (77%), 236 (50%), 211 (100%), 162 (54%), 135 (74%), 121 (40%), 150 (44%); calcd for $C_{21}H_{26}O_6$, C 67.4, H 6.9, found C 67.25, H 7.35%; ^{13}C nmr ($CDCl_3$) see Table 1.

ACETYLATION OF **1**.—Compound **1** (20 mg) was taken in pyridine (1 ml) treated wth Ac_2O (0.5 ml), refluxed at 120° for 4 h, processed by pouring over H_2O, left overnight, and extracted with Et_2O. The Et_2O-soluble residue on chromatography over Si gel and elution with hexane yielded an oily acetate, $C_{23}H_{28}O_7$ (10 mg): ir ν max (KBr) 2970, 1730, 1570, 1499, 1450, 1380, 1250, 1120, 1089, 980, 840, 840 cm^{-1}; ^1H nmr δ 0.96 (3H, d, J=7 Hz, Me-9), 1.9 (1H, dd, J= 14, 3.5 Hz, H-6′b), 2.0 (s, 3H, OAc-4′), 2.77 (2H, m, H-8, H-6′a), 3.1 (2H, dq, J= 14, 3 Hz, H-7′), 3.3 (3H, s, -OMe), 3.6 (3H, s, -OMe), 4.1 (1H, dd, J=4 Hz, H-5′), 4.2 (1H, d, J= 10 Hz, H-7), 5.16 (2H, dd, J= 10, 4 Hz, =CH_2-9′), 5.37 (1H, s, H-2′), 5.80 (1H, m, H-8′), 5.94 (2H, s, -OCH_2O-1), 6.72 (2H, H-2, -6), 6.84 (1H, H-5); ms *m/z* $[M]^+$ 416 (60%), 400 (15%), 377 (15%), 315 (20%), 271 (100%), 236 (20%), 135 (50%).

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