CONSTITUENTS OF *PIPER SYLVATICUM*: STRUCTURE OF SYLVATESMIN

AVIJIT BANERJI and SUDHIR PAL

Department of Pure Chemistry, University College of Science, 92, Acharya Prafulla Chandra Road, Calcutta—700 009, India

ABSTRACT.—Two constituents of the seeds of *Piper sylvaticum* Roxb. have been fully characterized by chemical and spectroscopical investigation. These are the new epieudesmin-type lignan sylvatesmin (**1**), and 3',5-dihydroxy-4',7-dimethoxy flavone (**2**).

We have been working on the constituents of Indian *Piper* species for a number of years (1–5) with the aim of isolating and characterizing biologically active compounds. In the present communication, we report the isolation and characterization of two or more compounds from the seeds of *Piper sylvaticum* Roxb.

RESULTS AND DISCUSSION

In the course of the present work, the crushed dried seeds of *Piper sylvaticum* Roxb. were extracted with petroleum ether (bp 60–80°) in a Soxhlet apparatus. The concentrated extract on chromatography over silica gel gave, in addition to the known constituents, four compounds which had not been isolated previously from this plant. These were an amide, the lignans PS–VI and sylvatesmin, and a flavone. The characterization of the latter two compounds are discussed in the present communication.

Sylvatesmin, $C_{21}H_{24}O_6$ (M^+ 372), mp 123° $[\alpha]^{21}D$+158° (chloroform), was obtained in the chloroform eluates. Its ir spectrum showed the presence of a hydroxyl group (3475 cm^{-1}) and a 1,2,4-trisubstituted phenyl nuclei (850, 825, 765, 740, 725 cm^{-1}) and the absence of carbonyl groups. The uv absorption spectrum (γ max (EtOH) 231, 280 and 343 nm; log ϵ; 4.16, 375 and 3.58 respectively) indicated its aromatic nature. A marked bathochromic shift of the maxima to 252 and 281 nm (log ϵ: 4.09 and 3.85) in the presence of alkali indicated that a phenolic hydroxyl was present.

A careful study of the 270 MHz ^1H nmr (table 1) and 20 MHz ^{13}C nmr (table 2) spectra as well as the mass spectrum of the compound suggested that sylvatesmin was a lignan having the furofuranoid skeleton. Its ^1H nmr spectrum ($CDCl_3$) exhibited signals for three aromatic methoxyls (δ 3.89, 3.90 and 3.91; 3H, s each),

TABLE 1. Assignment of the 270 MHz ^1H nmr ($CDCl_3$) signals of the aliphatic protons of sylvatesmin (**1**).

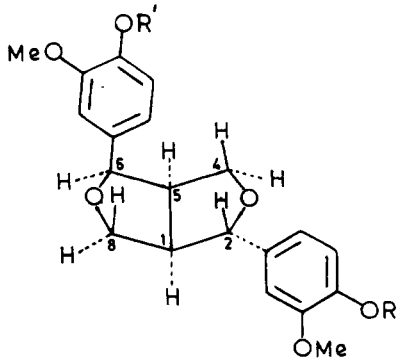
Chemical shift (δ in ppm)	No. of protons	Multiplicity (J in Hz)	Additional data	Assignment
4.88	1	d(5.4)	collapsed to s on irr. at δ 3.85	C_6–*H*
4.43	1	d(7.3)	collapsed to s on irr. at δ 2.90	C_2–*H*
4.13	1	d(9.8)	—	C_8–H_e
ca. 3.85	2	m	The shape of the signal was influenced upon successive irr. at (i) δ 4.13, (ii) δ 3.35 or (iii) δ 2.90	C_8–H_a & C_4–H_e
3.26–3.40	2	m	Splitting pattern was changed on irr. at (i) δ 2.90 or (ii) δ 4.88	C_4–H_a & C_5–*H*
2.85–2.98	1	m	Multiplicity changed on irr. at (i) δ 3.36 or (ii) δ 4.43	C_1–*H*

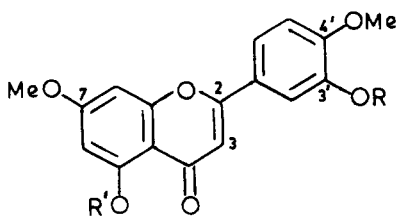
TABLE 2. 20 MHz ^{13}C nmr ($CDCl_3$) data of sylvatesmin (1) and its acetate (4).

Chemical shift (δ in ppm) of		SFORD multiplicity	Assignments
1	4		
49.70	49.79	d	C-5
54.01	54.25	d	C-1
55.51	55.61	q	methoxyl carbons
69.12	69.45	t	C-8
70.64	70.89	t	C-4
81.63	81.68	d	C-6
87.30	86.97	d	C-2
108.43	109.70	d	C-2'
108.91	108.65	d	C-2"
110.93*	110.98*	d	C-5"
114.05*	117.74*	d	C-5'
117.41*	117.50*	d	C-6"
118.66*	122.35*	d	C-6'
130.71	130.75	s	C-1"
132.64	138.93	s	C-1'
145.06	140.07	s	C-4'
146.51	150.92	s	C-3'
147.71	147.83	s	C-3" or C-4"
148.56	148.63	s	C-4" or C-3"
—	168.83	s	C-OCH_3
—	20.44	q	-$COCH_3$

*Displayed virtual coupling in SFORD spectrum.

one phenolic hydroxyl (δ 5.55, 1H, broad s, disappearing on deuteration), six aromatic protons (δ 6.8–7.0, 6H, m) and eight aliphatic protons. The assignments of the aliphatic protons are shown in table 1 and are based on extensive decoupling experiments. From the unsymmetrical ^1H nmr spectrum of the aliphatic protons, it appeared that the compound belonged to the epi-series rather than the symmetrical normal and dia-series. Its specific rotation (+158°) was also characteristic of the epi-series, the specific rotations of the normal series of compounds being usually below 100°, while those of the dia-series are usually above 300° (6). Thus sylvatesmin seemed to have the carbon skeleton and stereochemistry of





1: R = H, R' = Me
3: R = R' = Me
4: R = Ac, R' = Me
5: R = Me, R' = H
6: R = R' = H

2: R = R' = H
7: R = Me, R' = H
8: R = R' = Ac

epieudesmin. Its molecular formula and spectral properties showed that the compound contained three methoxyls and a phenolic hydroxyl instead of the four methoxyl groups in epieudesmin. Methylation of sylvatesmin with methyl iodide/sodium hydride in THF yielded (+)epieudesmin (**3**), mp 125°, $[\alpha]^{25}D$ +129.7° (acetone). This settled its structural features, the absolute stereochemistry and the oxygenation pattern of the aromatic rings. The mass spectral fragmentation (cf. experimental) pattern which was characteristic of furofuranoid lignans (6) further reinforced the structural proposals.

The ^{13}C nmr spectrum (noise-decoupled and SFORD) showed the presence of all three methoxyls at δ 55.51 (q in SFORD spectrum), four oxygenated sp^3 carbons (two methylenes and two methines), two other aliphatic methines and twelve signals for aromatic carbons (six methines and six quaternary carbons). The assignments are tabulated in table 2. In order to determine the position of the hydroxyl group, we undertook the study of the ^{13}C nmr spectrum of sylvatesmin monoacetate (**4**), $C_{23}H_{26}O_7$, mp 116–17°. The latter was obtained by acetylation of sylvatesmin with acetic anhydride-pyridine at room temperature. It showed the expected spectroscopical properties [ir: νmax (KBr) 1765 cm^{-1}; 80 MHz ^1H nmr: 3H,s at δ 2.26; 20 MHz ^{13}C nmr: δ 168.8 & 20.4, $-COCH_3$]. A comparison of the carbon chemical shifts (table 2) of the two compounds showed that a 4-hydroxy-3-methoxyphenyl ring was present. The non-oxygenated quaternary aromatic carbon at δ 132.64 suffered a down-field shift of ~6.3 ppm in the acetate, indicating its *para*-orientation with the phenolic hydroxyl.

It then remained to be decided which of the two aryl groups was axial. Of the two non-oxygenated quaternary carbons at δ 130.71 and δ 132.64, the upfield one clearly belonged to the axially substituted aryl group. This upfield shift is due to a steric compression effect and has been noted earlier (6) in similar systems. Since this did not appreciably shift in the acetate, it followed that the 3,4-dimethoxyphenyl group was axially oriented. Hence, the 4-hydroxy-3-methoxyphenyl group was equatorial. It could be mentioned that the chemical shift of the quaternary carbons, C-1′ and C-1″, is not particularly dependent on the substitution of a hydroxyl by a methoxy group in the *para* position. For example, in epipinoresinol (**6**) these carbons (δ 133.09 and δ 130.36) appeared within 0.5 ppm of those (δ 133.51 and δ 130.81) of epieudesmin (**3**) (7). The stereochemical assignment of the two aryl rings is thus unequivocal. Sylvatesmin, therefore, has the structure and absolute configuration as shown in **1**. Thus it is the mono-methyl ether of (+)-epipinoresinol. (+)-Phillygenol (forsythigenol), a monomethyl ether of (+)-epipinoresinol, has been earlier reported to occur as the β-D-glycoside, phillyrin (forsythin) in various *Phillyrea* and *Forsythia* species (6,8). In these earlier papers, the stereochemistry of the two aryl groups were not confirmed and two alternative structures (**1** or **5**) were given. Sylvatesmin corresponds to **1**, but it is not certain whether it is identical with phillygenol as an authentic sample of the latter could not be obtained.

The compound **2**, $C_{17}H_{14}O_6$, mp 237°, appeared to be a flavone on the basis of its spectral data. It was identified as the 4′,7-dimethyl ether of luteolin, previously designated as pilloin (9), on the basis of the mp, uv, ir, and ^1H-nmr data of the compound as well as those of its monomethyl ether, **7** and its diacetate **8**.

EXPERIMENTAL[1]

PLANT MATERIAL.—Seeds of *Piper sylvaticum* Roxb. were collected in West Bengal. The herbarium sample no. PS-S is preserved in our laboratory.

[1]Mp's were taken on a Köfler block and are uncorrected. R_f (tlc) data given were recorded in benzene-ethylacetate (1:1). Uv spectra were recorded in aldehyde-free ethanol with a Varian 634 S instrument. Ir spectra were taken in KBr pellets with a Beckman IR-20 and Pye-Unicam 1025 spectrometers. ^1H and ^{13}C nmr data were recorded with TMS as internal standard with a Varian CFT-20 instrument.

ISOLATION OF SYLVATESMIN AND FLAVONE.—Air-dried seeds (2 kg) of *Piper sylvaticum* (Roxb) were powdered and extracted with petroleum ether (bp 60–80°) (8 liters) in a Soxhlet apparatus for 3 days. The extract was concentrated and chromatographed over silica gel (60–100 mesh) with solvents of increasing polarity.

Sylvatesmin was obtained [yield 120 mg; 0.006%] as a white solid, R_f (tlc) 0.50, mp 122–23°, $[\alpha]^{21}D$ (chloroform) +158° and $[\alpha]^{21}D$ (95% EtOH) +147° from chloroform eluates. For uv and significant ir absorptions see discussions. For ^1H nmr data see discussions and table 1. It gave a ms (70 ev): 372 (M^+, 25%), 341 (M^+-OMe, 3%); 194 ($ArCH=\overset{+}{O}-CH_2$, 9%), 180 ($ArCH=\overset{+}{O}-CH_2$, 11%), 178 ($ArCH=CHMe^{\cdot+}$, 15%); 177 ($Ar\overset{+}{C}H-CH=CH_2$, 60%); 166 ($ArCHO^{\cdot+}$, 55%), 165 ($ArC\equiv\overset{+}{O}$, 75%); 164 ($Ar\overset{+}{C}H=CHMe^{\cdot+}$, 16%); 163 ($Ar\overset{+}{C}H-CH=CH_2$, 16%); 152 ($ArCHO^{\cdot+}$, 60%); 151 ($ArC\equiv\overset{+}{O}$ and $ArCH_2$, 100%); 138 ($ArH^{\cdot+}$, 45%); 137 ($Ar\overset{+}{C}H_2$) and (Ar^+, 90%); 124 ($ArH^{\cdot+}$, 35%); 123 Ar^+, 10%) [Ar≡3,4-dimethoxyphenyl and Ar≡3-methoxy-4-hydroxyphenyl].

The flavone was obtained as yellow needle-shaped crystals, R_f (tlc) 0.60, mp 237°, from benzene-chloroform eluates [yield 20 mg, 0.001%]; uv: λmax (EtOH) 253–54, 268, 292–97, 345–46 nm [log ε: 4.28, 4.23, 4.01 and 4.31 respectively]; λmax (EtOH/$AlCl_3$) 263–64, 278, 354 and 382–84 nm [log ε: 4.31, 4.53, 4.32 and 4.48 respectively]; λmax (EtOH/OH^-) 270 and 374–76 nm [log ε: 4.48 and 4.17]; ir: νmax (KBr) 3240 (broad), 1660, 1595, 1500, 1445, 1200, 1145, 1042, 870, 840, 820, 775 and 645 cm^{-1}. *Anal*: Calcd. for $C_{17}H_{14}O_6$: C, 65.05; H, 4.40%. Found: C, 64.96; H, 4.46%.

ACETYLATION OF SYLVATESMIN.—Sylvatesmin (1) (60 mg) was treated with acetic anhydride (5 ml) and two drops of pyridine and was kept at 30° for 15 hrs. About 30 ml water was added and the acid was neutralized with $NaHCO_3$. The mixture was extracted with ether (3 x 20 ml) and the extract was washed successively with dil. HCl and water. The ether solution was dried over anhydrous Na_2SO_4 and the solvent was removed to afford the monoacetyl derivative, mp 116–17°, R_f (tlc) 0.60 (yield 50 mg, 75%); uv: λmax (EtOH) 210, 223–24 and 279–80 nm [log ε: 4.22, 4.18 and 3.77 respectively]; ir: νmax (KBr) 1765 (s), 1600, 1505, 1455, 1412, 1255, 1210, 1150, 1070, 1015, 845, 825, 800, 750, 730 and 710 cm^{-1}; ^1H nmr (80 MHz): δ ($CDCl_3$) 7.00–6.75 (6H, m), 4.82 (1H, d, J=5 Hz), 4.45 (1H, d, J=7 Hz), 4.15 (1H, d, J=10 Hz), 3.83 (3H, s), 3.81 (3H, s), ca. 3.72 (2H, m), ca. 3.26 (2H, m), ca. 2.90 (1H, m), 2.24 (3H, s). *Anal.*: Calcd. for $C_{23}H_{26}O_7$: C, 66.67; H, 6.28%; Found: C, 66.85; H, 6.20%.

METHYLATION OF SYLVATESMIN.—Substrate (10 mg) in dry tetrahydrofuran (20 ml) was treated with MeI (0.2 ml) and NaH (1.5 mg, 50–55% in oil dispersion, washed with dry benzene). The mixture was refluxed for half an hour. After the reaction was completed, the solvent was removed at reduced pressure and 20 ml of water was added and the solution extracted with ether. From the ethereal extract the methyl derivative was isolated as a white solid, mp 125°, $[\alpha]^{21}D$+129.7° (acetone), R_f (tlc) 0.55 (yield 8 mg, 77%); ir: νmax (KBr) 1590, 1450, 1405, 1262, 1230, 1155, 1135, 1072, 845, 800, 750, 730 cm^{-1}; ^1H nmr (80 MHz): δ ($CDCl_3$) 6.96–6.70 (6H, m), 4.78 (1H, d, J=5.5 Hz), 4.37 (1H, d, J=7.5 Hz), 4.07 (1H, d, J=9.5 Hz), 3.85 (3H, s), 3.81 (3H, s), 3.80 (6H, s), ca. 3.75 (2H, m), ca. 3.26 (2H, m), ca. 2.90 (1H, m). *Anal.* Calcd. for $C_{23}H_{26}O_6$: C, 68.29; H, 6.73%; Found: C, 68.20; H, 6.82%.

ACETYLATION OF FLAVONE.—The diacetate of the flavone was obtained as a pale yellow crystalline solid, mp 195°, R_f (tlc) 0.65, by acetylation of the flavone following the procedure used for acetylation of sylvatesmin. *Anal.*: Calcd. for $C_{21}H_{18}O_8$: C, 63.3; H, 4.5%; Found: C, 63.7; H, 4.55%. ^1H nmr (90 MHz): δ ($CDCl_3$) 7.72 (1H, dd, J_1=9 Hz & J_2=3 Hz), 7.57 (1H, d, J=3 Hz), 7.05 (1H, d, J=9 Hz), 6.52 (1H, s), 6.45 (1H, d, J=3 Hz), 6.35 (1H, d, J=3 Hz), 3.89 (3H, s), 3.85 (3H, s), 2.32 (3H, s), 2.13 (3H, s).

METHYLATION OF FLAVONE.—The flavone (10 mg) in ether (100 ml) was treated with excess ethereal solution of diazomethane. After completion of the reaction (20 hrs), the solvent was removed; the yellow crystalline solid, mp 165°, R_f (tlc) 0.65 (yield 8 mg, 75%) was found to be identical with an authentic sample of 5-hydroxy-3',4',7-trimethoxyflavone [mp, mmp, co-tlc, superimposable ir and identical ^1H nmr (80 MHz)].

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