Hyosmin, a new lignan from *Hyoscyamus niger* L.

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Hyosmin (1) a new lignan has been isolated from the seeds of *Hyoscyamus niger* L. (Solanaceae), and its structure shown to be the 3-{[(2*R*)-2-carbomethoxy-2-hydroxy]ethyl}benzoate ester of {(2*R*,3*S*,4*S*)-2-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-4-[(4-hydroxy-3-methoxyphenyl)methyl]tetrahydrofuran

Keywords: *Hyoscyamus niger*, Solanaceae, hyosmin, lignan, 3',5-dihydroxy-3,4',5',6,7-pentamethoxyflavone

In view of our interest in the plants of Solanaceae family, we undertook a chemical investigation of *Hyoscyamus niger* L., which is commonly known as Henbane and is widely distributed in Europe and Asia. In India, it is found from Kashmir to the Garhwal Himalayas from 8000 to 11,000 feet.[1] The plant is said to possess anti-spasmodic, sedative and analgesic properties.[2] *H. niger* L., is a source of alkaloids,[3] and a tyramine derivative.[4] Recently the presence of some non-alkaloidal constituents including withanolides,[4] lignanamides[5] and flavonoids[6] have also been reported. We now report the isolation and characterisation of five compounds from the methanolic extract of seeds of *H. niger*. These include a novel lignan, hyosmin (**1**), 3',5-dihydroxy-3,4',5',6,7-pentamethoxyflavone, (±)-pinoresinol, vanillin and 5-hydroxymethylfurfural. This is the first report of the isolation of these compounds from *Hyoscyamus*.

Hyosmin (**1**), a colourless amorphous powder, was analysed for $C_{31}H_{34}O_{10}$ by HR–EI–MS (M^+ at *m/z* 566.2161, calcd. 566.2152) and ^{13}C NMR data (Table 1). The UV absorption at λ_{max} 232, 281nm was indicative of phenolic moiety and its

Table 1 ^1H and ^{13}C NMR (500/125 MHz, $CDCl_3$) data of **1** and **1a**

Position	Hyosmin (**1**)		Hyosmin dimethyl ether (**1a**)
	δ_C[a]	δ_H[a]	δ_H
1	131.8 (C)		
2	111.2 (CH)	6.67 (brs)	6.70 (d, 1.7)
3	146.5 (C)		
4	144.0 (C)		
5	114.4 (CH)	6.83 (d, 7.9)	6.80 (d, 8.0)
6	121.1 (CH)	6.69 (brd, 7.9)	6.74 (dd, 8.0, 1.7)
7	33.5 (CH_2)	2.61 (dd, 13.6, 10.6)	2.63 (dd, 13.6,10.6)
	2.91 (dd, 13.6, 5.3)	2.94 (dd, 13.6, 4.9)	
8	42.8 (CH)	2.82 (m)	2.83 (m)
9	72.8 (CH_2)	3.80 (dd, 11.2, 7.7)	3.80 (dd, 11.2, 7.7)
	4.10 (dd, 6.5, 8.6)	4.13 (dd, 9.0, 6.6)	
1'	134.2 (C)		
2'	108.6 (CH)	6.86 (brs)	6.88 (brs)
3'	146.6 (C)		
4'	145.1 (C)		
5'	114.3 (CH)	6.85 (d, 7.5)	6.82 (d, 8.1)
6'	119.2 (CH)	6.88 (brd, 7.5)	6.90 (dd, 8.1, 1.7)
7'	83.6 (CH)	4.85 (d, 6.9)	4.89 (d, 6.6)
8'	49.1 (CH)	2.72 (m)	2.73 (m)
9'	63.4 (CH_2)	4.41 (dd, 11.2, 7.6)	4.44 (dd, 11.2, 7.6)
	4.61 (dd, 11.2, 6.4)	4.62 (dd, 11.2, 6.6)	
1"	129.1 (C)		
2"	130.5 (CH)	7.71 (brs)	7.80 (brs)
3"	136.9 (C)		
4"	134.3 (CH)	7.42 (brd, 7.8)	7.43 (brd, 7.8)
5"	128.4 (CH)	7.33 (t, 7.8)	7.34 (t, 7.8)
6"	128.1 (CH)	7.77 (brd, 7.8)	7.79 (brd, 7.8)
7"	166.3 (C)		
8"	40.1 (CH_2)	2.96 (dd, 13.9, 7.1)	2.98 (dd, 13.9, 7.1)
	3.15 (dd, 13.9, 4.2)	3.17 (dd, 13.9, 4.2)	
9"	70.9 (CH)	4.46 (m)	4.46 (dd, 7.1, 4.2)
10"	174.5 (C)		
3-OMe	55.9 (CH_3)	3.86 (s)	3.85 (s)[b]
4-OMe	3.85 (s)[b]		
3"-OMe	55.9 (CH_3)	3.84 (s)	3.84 (s)[b]
4"-OMe	3.85 (s)[b]		
10"-OMe	52.6 (CH_3)	3.79 (s)	3.78 (s)

[a]Assignments were made with the aid of H-H COSY, HMQC and HMBC spectra.
[b]Assignment may be interchanged.

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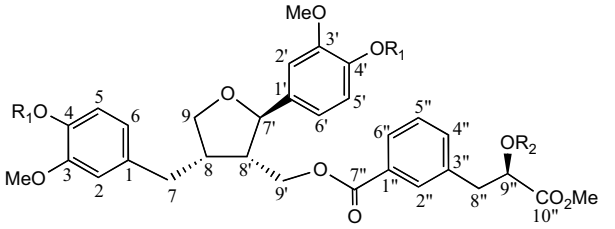
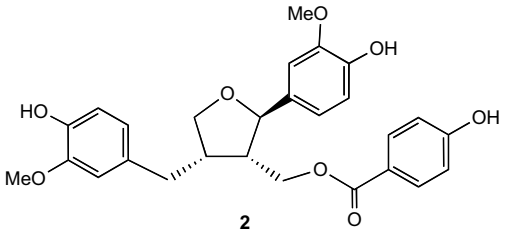
IR spectrum showed strong absorption bands at ν_{max} 3530 cm^{-1} (hydroxyl group) 1735, 1720 cm^{-1} (ester carbonyls) and 1590 cm^{-1} (aromatic functionalities). The ^{13}C NMR and DEPT spectra indicated the presence of three methoxy carbons (δ 52.6, 55.8 and 55.8 ppm), four methylene carbons (δ 33.4, 40.1, 63.3 and 72.8 ppm), four methine carbons (δ 42.7, 49.0, 70.9 and 83.6 ppm) and 18 carbons of three aromatic rings attached to 10 protons, four oxygen atoms and four carbon atoms.

The ^1H NMR spectrum of **1** revealed that the 10 aromatic hydrogens (δ 6.67, 6.69, 6.83, 6.85, 6.86, 6.88, 7.33, 7.41, 7.72 and 7.77 ppm) comprised two 1, 3, 4-trisubstituted aromatic rings and one 1, 3-disubstituted aromatic ring. These assignments and the linkage of the side chain were determined using a combination of 2D NMR methods including H-H COSY, HMQC and HMBC (Fig. 1) data, which allowed all the protons and carbons to be assigned. The ^1H and ^{13}C NMR spectral data (Table 1) of **1** resembled those of a monofuranoid lignan, agastinol[7] (**2**), except that the structure of one of the substituents and the position of the substituent in the disubstituted benzene ring were different. The C-3" substituent consists of a methoxy carbon (δ 52.6), a methylenic carbon (δ 40.1), a methine carbon (δ 70.9) and an ester carbonyl carbon (δ 174.5), and was formulated as $–CH_2–CH(OH)–CO_2CH_3$ on the basis of H-H COSY and HMBC data. The relative stereochemistry at C-7', C-8' and C-8 (the same numbering system as that of **2** is used except for systematic naming) of the THF ring in hyosmin (**1**) and agastinol (**2**) were identified by comparison of the ^1H NMR data for both the compounds (Table 1). Like agastinol, **1** also exhibited a correlation between H-8 and H-8' but no correlation was observed between H-7' and H-8' in the NOESY spectrum.



1: R_1, R_2=H
1a: R_1=Me, R_2=H
1b: R_1=Me, R_2=(*R*)-MTPA
1c: R_1=Me, R_2=(*S*)-MTPA



2

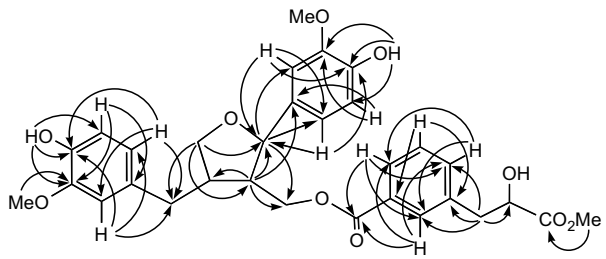


Fig. 1 The key HMBC correlations of **1.**

Additional NOE correlations were observed between H-8' and H_2-7 and between H-8' and H_2-9'. This led to the assignment of *trans*-H-7'/H-8' and *cis*-H-8'/H-8 stereochemistry (C-7'*R**, C-8'*S**, C-8*S**). The optical rotation of **1,** –17.9°, may suggest the C-7'*R*, C-8'*S*, C-8*S* absolute configuration from analogy of the negative rotation of agastinol.[7] The configuration at the oxymethine centre at the C-3" substituent was investigated by the modified Mosher's ester method.[8] Compound **1** was treated with excess diazomethane to give the 3,3'-dimethylated derivative **1a** which was converted to the (*R*)- and (*S*)-MTPA esters (**1b** and **1c**). Comparison of their ^1H NMR data (Table 2) allowed us to assign the C-9" configuration as *R*. The structure of hyosmin (**1**) was, therefore, shown to be the 3-{[(2*R*)-2-carbomethoxy-2-hydroxy]ethyl}benzoate of {(2*R*,3*S*,4*S*)-2-(4-hydroxy-3-methoxyphenyl)–3hydroxymethyl-4-[(4-hydroxy-3-methoxy phenyl)methyl]tetrahydrofuran ester. The EIMS of **1** exhibited fragment ions as depicted in Fig. 2, which corroborated the assigned structure.

The structure of vanillin,[9] (±)-pinoresinol {$[\alpha]_D^{25} = 0.0$ (c 0.4, $CHCl_3$)},[10] 3',5-dihydroxy-3,4',5',6,7-pentamethoxyflavone[11] and 5-(hydroxymethyl)furfural,[12] were established by the comparison of spectral data (UV, IR, ^1H NMR and ^{13}C NMR) with the reported literature data.

Experimental

General

IR spectra were recorded on a JASCO-FT/IR-5300 spectrometer. UV spectra were recorded on a Shimadzu UV-1600PC spectrophotometer using spectroscopic grade methanol. ^1H and ^{13}C NMR spectra were measured using a Bruker DRX-500 spectrometer with TMS as internal reference. Mass spectra (EI, FAB, HREI, HRFAB) were recorded on a JEOL JMS-700 spectrometer using a direct inlet system. Optical rotations were recorded on JASCO DIP-360 polarimeter (cell length 5 cm). Silica gel used for column chromatography refers to Centron Research Laboratories (India) material.

Plant material

The seeds of *Hyoscyamus niger* were purchased from a local market in Varanasi, India and authenticated by Dr V. K. Joshi, Department of Dravya Guna, IMS, Banaras Hindu University, Varanasi, India. A voucher specimen (AS/HN/02) has been deposited in the Department of Medicinal Chemistry of the same Institution.

Extraction and isolation

The dried and milled seeds of *H. niger* (5 kg) were extracted successively with hexane and CH_3OH for 48 h in a Soxhlet extractor. The methanolic extract was concentrated under reduced pressure and partitioned between hexane and water. The aqueous methanolic portion was extracted with $CHCl_3$, EtOAc and *n*-BuOH successively. The residue (109.5 g) obtained from the $CHCl_3$ fraction after concentration under reduced pressure, was chromatographed over silica gel column and eluted using solvents of increasing polarity.

Rechromatography of the mixed C_6H_6 eluates after concentration under reduced pressure, over SiO_2 column and elution with C_6H_6 (fractions of 100 ml each were collected) afforded two compounds. C_6H_6 eluates (fraction 1-3) yielded epoxyconiferyl alcohol (0.12 g). C_6H_6 eluates (fraction 4-8) were mixed purified by prep-TLC, using C_6H_6 –EtOAc (9: 1) as mobile phase, which yielded 3',5-dihydroxy-3,4',5',6,7-pentamethoxyflavone (0.093 g).

Rechromatography of the C_6H_6 –EtOAc (95: 5) eluate over silica gel, followed by prep-TLC using C_6H_6 –EtOAc–$CHCl_3$ (50: 20 : 30) solvent system yielded hyosmin (0.05 g) (**1**).

Rechromatography of C_6H_6 –EtOAc (75: 25) eluate over RP-18 column using MeOH as eluent yielded 5-(hydroxymethyl)furfural (0.08 g). C_6H_6–EtOAc (25: 75) eluates of the column yielded vanillin (0.63 g) on crystallisation.

Hyosmin (**1**): Colourless amorphous solid, $[\alpha]_D^{25}$ –17.9° (c 0.58, MeOH); IR (cm^{-1}) 3530, 1735, 1720, 1590; UV (nm, MeOH) 232, 281; ^1H NMR (500 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 1; EIMS (*m/z*) EIMS *m/z* 566 (M^+, 6%), 534 (1%), 506 (1%), 342 (24%), 207 (49%), 205 (31%), 175 (14%), 150 (60%), 137 (100%), 131 (26%), 123 (16%), 103 (20%), 91 (20%); *m/z* found: M^+ 566.2161, $C_{31}H_{34}O_{10}$ requires M_r 566.2152.

Hyosmin dimethyl ether derivative (**1a**): To compound **1** (4 mg)

Table 2 Pertinent ^{1}H NMR (500 MHz, $CDCl_3$) data of MTPA esters **1b** and **1c**

Position	**1b**	**1c**	Δδ (**1c**–**1b**)
2"	7.79 (brs)	7.69 (brs)	–0.10
4"	7.41 (brd, 7.8)	7.45 (brd, 7.8)	+ 0.04
5"	7.34 (t, 7.8)	7.33 (t, 7.8)	–0.01
6"	7.82 (brd, 7.8)	7.75 (dt, 7.8, 1.6)	–0.07
8"	3.35 (dd, 14.0, 3.7)	3.25 (dd, 14.5, 3.8)	–0.10
	3.16 (dd, 14.0, 9.8)	3.13 (dd, 14.5, 8.7)	–0.03
9"	5.41 (dd, 9.8, 3.7)	5.41 (dd, 8.7, 3.8)	–
10"-OMe[a]	3.76 (s)	3.85 (s)	+ 0.09

[a]Assigned on the basis of NOE experiments.

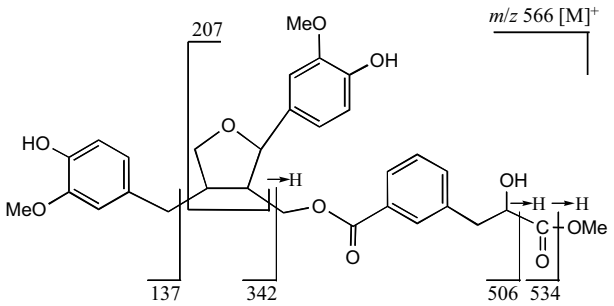


Fig. 2 EIMS fragmentation pattern of **1.**

dissolved in MeOH (0.1 ml) was added excess ethereal diazomethane and the mixture was stirred overnight. Concentration of the mixture and purification of the residue on silica gel chromatography (eluted with hexane–ethyl acetate 3 : 1) yielded 2.5 mg of pure **1a**. Yellow amorphous, $[\alpha]_D^{25}$ –20.0° (c 0.25, MeOH); UV (nm, MeOH) 232 (4.41), 279 (3.81); ^{1}H NMR (500 MHz, $CDCl_3$) see Table 1; EIMS *m/z* 594 (M^+, 66%), 534 (2%), 370 (65%), 339 (17%), 219 (58%), 207 (47%), 165 (63%), 151 (100%); *m/z* found: M^+ 594.2450, $C_{33}H_{38}O_{10}$ requires M 566.2152.

(R)- and (S)-MTPA esters of **1a:** Treatment of **1a** (1 mg) with (*S*)-MTPACl (1 µl) in pyridine (50 µl) and purification of the product on p-TLC after dilution with MeOH gave the (*R*)-MTPA ester **1b** (1 mg). ^{1}H NMR (500 MHz, $CDCl_3$) see Table 2. HRFABMS *m/z* found: MH^+ 811.2908, $C_{43}H_{46}F_3O_{12}$ requires 811.2941.

Similar reaction using (*R*)-MTPACl afforded (*S*)-MTPA ester **1c** (1 mg). ^{1}H NMR (500 MHz, $CDCl_3$) see Table 2. HRFABMS *m/z* found: MH^+ 811.2970, $C_{43}H_{46}F_3O_{12}$ requires 811.2941.

3',5-dihydroxy-3,4',5',6,7-pentamethoxyflavone: Yellow coloured solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 12.54 (1H, s, 5-OH), 7.35 (2H, s, 2', 6'-H, this signal became an AB doublet (J = 2.0 Hz) at δ 7.27 and 7.35 when a few drops of CD_3OD was added), 6.51 (1H, s, 8-H), 5.91 (1H, s, 3'-OH), 3.89 (3H, s, 3-OMe), 3.93 (3H, s, 6-OMe), 3.95 (3H, s, 5'-OMe), 3.97 (3H, s, 7-OMe), 4.01 (3H, s, 4'-OMe); ^{13}C (100 MHz; $CDCl_3$) δ: 179.0 (C-4), 158.9 (C-7), 155.4 (C-2), 152.8 (C-8a), 152.4 (C-5), 152.1 (C-5'), 149.2 (C-3'), 139.4 (C-3), 137.8 (C-4'), 132.3 (C-6), 126.0 (C-1'), 108.5 (C-2'), 106.7 (C-4a), 105.1 (C-6'), 90.4 (C-8), 61.1 (4'-OMe), 60.9 (6-OMe), 60.3 (3-OMe), 56.1 (5'-OMe), 56.4 (7-OMe). EIMS *m/z* 404 (M^+ 100%), 389 (17%), 371 (17%), 361 (11%), 331 (8%), 181 (6%), 83 (9%), 57 (13%), 44 (93%), 18 (24%).

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