Hyosgerin, a New Optically Active Coumarinolignan, from the Seeds of *Hyoscyamus niger*

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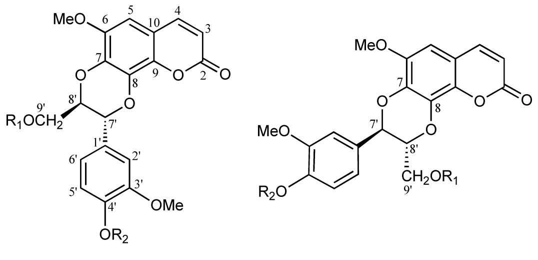
Hyosgerin, a new optically active coumarinolignan, has been isolated and characterized along with three other coumarinolignans, venkatasin, cleomiscosin A and cleomiscosin B, from the seeds of *Hyoscyamus niger* L. The structure was determined on the basis of spectroscopic analysis and chemical conversion. The optical properties and absolute stereochemistry of these coumarinolignans have also been studied and discussed.

Key words hyosgerin; *Hyoscyamus niger*; Solanaceae; coumarinolignan; venkatasin; cleomiscosin

Hyoscyamus niger L, commonly known as Henbane, is widely distributed in Europe and Asia. In India, it is found from Kashmir to Garhwal Himalayas, from 8000 to 11000 feet.[1)] The plant is said to possess anti-spasmodic, sedative and analgesic properties.[2)] *H. niger*, one of the known sources of alkaloids,[3)] has also shown the presence of tyramine derivative,[4)] withanolides,[4)] lignanamides[5)] and flavonoids.[6)] Our continued interest in the chemical constituents elaborated by plants of Solanaceae family prompted us to take up the phytochemical investigation of the seeds of *H. niger* and report the isolation of hyosmin, 3′,5-dihydroxy-3,4′,5′,6,7-pentamethoxyflavone, epoxyconiferyl alcohol, vanillin and 5-hydroxymethylfurfural from its methanolic extract.[7)] Further study of the extract led to the isolation of a new coumarinolignan named hyosgerin (**1**), along with the known coumarinolignans, venkatasin (**2**), and cleomiscosins A and B. In this paper we report the isolation and structural elucidation of hyosgerin (**1**) (Fig. 1).

Results and Discussion

Chromatographic separation of the MeOH extract of the seeds of *H. niger* furnished compounds **1** and **2** as yellow crystals. Compound **1** was found to be more mobile than compound **2** in reversed-phase HPLC. The molecular formulae of **1** and **2** were deduced as $C_{22}H_{20}O_9$ on the basis of high-resolution (HR)-EI-MS data. The two compounds showed essentially identical UV absorption maxima near 232, 287 and 322 nm. The ^1H- and ^{13}C-NMR data (Table 1) for **1** and **2** were closely similar to each other, and resembled cleomiscosins A[8,9)] and B,[8)] except for the signals of an acetyl group, not discernible in the spectra of cleomiscosins. Careful comparison of the ^1H- and ^{13}C-NMR data of **2** with the published data for venkatasin[10)] (durantin-A[11)]) led to the assignment of **2** as venkatasin, while hyosgerin (**1**) was suggested to be a structural isomer of venkatasin.



1: R_1=Ac, R_2=H
1a: R_1, R_2=H
1b: R_1, R_2=(*S*)-MTPA

2: R_1=Ac, R_2=H
2a: R_1, R_2=H
2b: R_1, R_2=(*S*)-MTPA

Fig. 1. Structures of Coumarinolignans

The ^1H-NMR spectrum of **1** showed signals of two methoxy groups (δ 3.92, 3.93), three downfield protons assignable to H-3, H-4 and H-5 of a coumarin nuclei, three aromatic protons due to a 1,3,4-trisubstituted benzene ring, four oxymethine/oxymethylene protons and an acetyl methyl (δ 2.08) assignable to a $-\underline{C}\underline{H}(OR)-\underline{C}\underline{H}(OR)-\underline{C}\underline{H}_2-OAc$ moiety. A large coupling constant (7.3 Hz) between the two adjacent oxymethine protons (δ 4.98, 4.38), which is consistent with the $J_{H\text{-}7,H\text{-}8'}$ value (7.5 Hz) of venkatasin,[10)] indicated the

Table 1. ^1H- and ^{13}C-NMR Data (500/125 MHz, $CDCl_3$) for **1** and **2**

No.	**1**		**2**	
	^{13}C	^1H	^{13}C	^1H
2	160.58		160.63	
3	114.41	6.30 (d, 9.5)	114.41	6.33 (d, 9.5)
4	143.57	7.60 (d, 9.5)	143.60	7.61 (d, 9.5)
5	100.62	6.55 (s)	100.41	6.54 (s)
6	145.77		145.89	
7	136.59		137.27	
8	132.59		131.90	
9	138.97		138.91	
10	111.79		111.82	
1′	126.51		126.56	
2′	109.63	6.89 (d, 1.7)	109.65	6.87 (d, 1.9)
3′	146.98		147.01	
4′	146.79		146.88	
5′	114.70	6.95 (d, 8.1)	114.88	6.94 (d, 8.7)
6′	121.05		121.15	
7′	76.30	4.98 (d, 7.3)	76.43	4.99 (d, 7.5)
8′	76.00	4.38 (ddd, 7.3, 5.5, 3.1)	75.48	4.38 (ddd, 7.5, 4.4, 3.0)
9′	62.77	4.07 (dd, 13.2, 5.5)	62.80	4.06 (dd, 12.1, 4.4)
		4.38 (dd, 13.2, 3.1)		4.35 (dd, 12.1, 3.0)
MeO	56.46	3.92 (s)	56.36	3.89 (s)
MeO	56.08	3.93 (s)	56.05	3.91 (s)
Ac	20.71	2.08 (s)	20.67	2.08 (s)
Ac	170.35		170.39	
OH		5.70 (s)		5.72 (s)

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trans-orientation of the two hydrogens (H-7′, H-8′). A nuclear Overhauser effect (NOE) correlation between H-7′ and H_2-9′ further supported the *trans* relationship of H-7′ and H-8′. The ^{13}C signals of **1** (Table 1) were completely assigned with the aid of ^{1}H-detected heteronuclear multiple-bond correlations (HMBC), shown in Fig. 2. These findings suggested that hyosgerin (**1**) should be cleomiscosin B 9′-acetate, a structural isomer of venkatasin in which the positions of the CH_2OAc and phenyl groups are reversed.

In the reported ^{13}C-NMR data of cleomiscosins B and A (**1a**, **2a**), the C-8′ signal of **1a** appeared by δ 0.3 ppm at a field lower than that of **2a**.[8] Consistent with this observation, C-8′ of hyosgerin (**1**) appeared at a lower field than that of venkatasin (**2**) by about 0.5 ppm indicating that hyosgerin is related to venkatasin in the same manner as cleomiscosin B is to cleomiscosin A.

Definitive proof of structure **1** was established by the chemical correlation with the known cleomiscosin B (**1a**). Thus, treatment of hyosgerin with K_2CO_3 in MeOH/THF furnished a compound in a good yield that was identified with cleomiscosin B by direct comparison with the authentic sample (TLC, HPLC). Hence, the structure of hyosgerin (**1**) was established to be cleomiscosin B 9′-acetate.

We also obtained **1a** and **b** from the seeds of *H. niger*, both of which showed $[\alpha]_D$ 0°. An earlier paper reported that cleomiscosin B, isolated from the seeds of *Cleome viscose* (Capparaceae), did not show any optical activity and were thus racemic.[8] The sample of **1** in the present study had $[\alpha]_D^{25}$ $-68.3°$. This discrepancy prompted us to study the optical nature of **1** and cleomiscosin B **1a**. 4′,9′-Bis-(*S*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) ester derived from **1a** showed two sets of ^{1}H-NMR signals (Table 2) in a 1 : 1 ratio, confirming the racemic nature of **1a**. The sample of **1a** ($[\alpha]_D^{25}$ $-50°$) obtained upon deacetylation of **1** was also converted to bis-(*S*)-MTPA ester **1b**. This ester showed only one set of ^{1}H signals, thus establishing the optically active nature of **1**.

The absolute stereochemistry of **1** was determined by analysis of the CD spectrum of **1** (Fig. 3). The UV absorptions at 310—340 and 275—290 nm are due to the coumarin and substituted benzene chromophores, respectively. The CD spectrum of **1** exhibited exiton coupling ($\Delta\varepsilon$ -2.6 at 305 nm and $\Delta\varepsilon$ $+0.4$ at 275 nm) between the two chromophores, although the intensity is weak and the pattern is deformed.[12] The observed negative CD couplet correlated with the

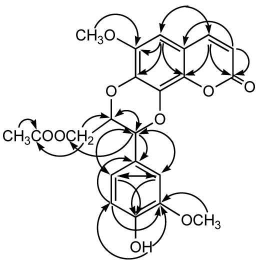


Fig. 2. HMBC Correlations (Recorded at the Set of $J_{C-H}=5$ Hz) from H to C of Hyosgerin (**1**)

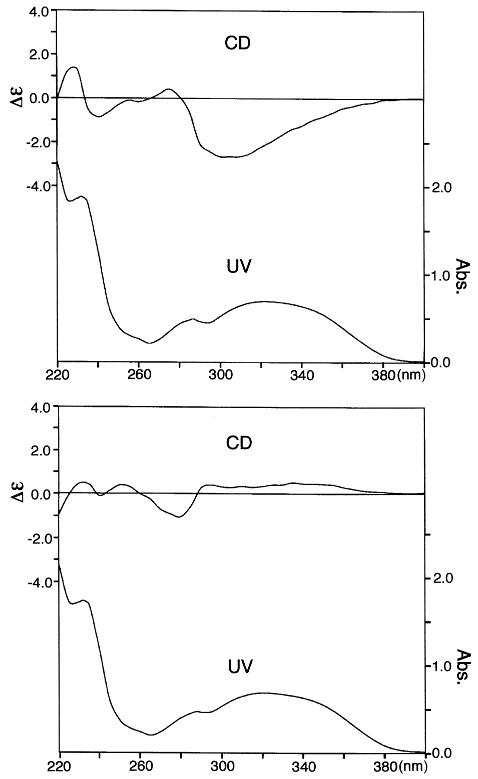
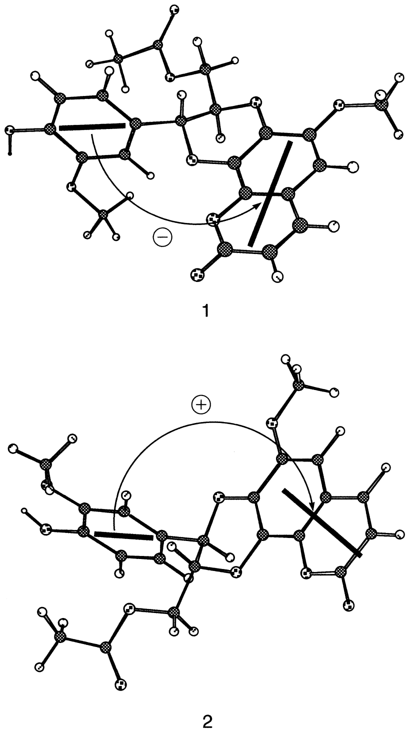


Fig. 3. CD and UV Spectra of Hyosgerin (**1**) (Top) and Venkatasin (**2**) (Bottom)

Table 2. ^{1}H-NMR Data (500 MHz, $CDCl_3$) for Bis-(*S*)-MTPA Esters **1b** and **2b**

No.	Cleomiscosin B bis-(*S*)-MTPA ester		Cleomiscosin A bis-(*S*)-MTPA ester	
	1b	**1b** isomer[a]	**2b** (major)	**2b** (minor)
3	6.32 (d, 9.6)	6.32 (d, 9.6)	6.34 (d, 9.5)	6.37 (d, 9.5)
4	7.62 (d, 9.6)	7.60 (d, 9.6)	7.62 (d, 9.5)	7.64 (d, 9.5)
5	6.59 (s)	6.55 (s)	6.55 (s)	6.56 (s)
2′	6.99 (d, 1.7)	7.01 (d, 1.7)	7.02 (d, 1.8)	7.00 (d, 1.7)
5′	7.00 (d, 8.0)	7.04 (d, 8.1)	7.07 (d, 8.1)	7.00 (d, 8.1)
6′	6.82 (dd, 8.0, 1.7)	6.93 (dd, 8.1, 1.7)	6.98 (dd, 8.1, 1.8)	6.84 (dd, 8.1, 1.7)
7′	4.94 (d, 7.8)	4.80 (d, 7.8)	4.97 (d, 7.6)	5.00 (d, 8.0)
8′	4.38 (dt, 7.8, 2.4)	4.39 (ddd, 7.8, 4.2, 2.4)	4.41 (ddd, 7.6, 4.5, 2.4)	4.38 (dt, 8.0, 2.5)
9′	4.00 (dd, 12.4, 2.4)	4.17 (dd, 12.3, 4.2)	4.24 (dd, 12.5, 4.5)	3.98 (dd, 12.6, 2.5)
	5.03 (dd, 12.4, 2.4)	4.90 (dd, 12.3, 2.4)	4.75 (dd, 12.5, 2.4)	4.99 (dd, 12.6, 2.5)
MeO	3.78 (s)	3.81 (s)	3.81 (s)	3.80 (s)
MeO	3.94 (s)	3.90 (s)	3.89 (s)	3.88 (s)

[a] Bis-(*S*)-MTPA ester of (+)-cleomiscosin B.



1

2

Fig. 4. The Most Stable Conformations of **1** and **2** (Major Enantiomer)

$7'R,8'R$ stereochemistry of the molecule, the most stable conformation (deduced by MM2) of which is depicted in Fig. 4. Thus, the absolute stereochemistry of **1** was determined to be $7'R,8'R$.

We further explored the optical properties of **2a** and **b**. Samples of cleomiscosin A isolated from the seeds of *C. viscosa*[8] and the stem of *Soulamea soulameoides* (Simaroubaceae)[9] were described as racemic because of $[\alpha]_D$ 0°. Our sample of cleomiscosin A was found to be racemic since the ^{1}H-NMR spectrum of the corresponding $4',9'$-bis-(S)-MTPA ester exhibited two sets of ^{1}H signals in a 1 : 1 ratio (Table 2). The sample of **2** isolated in the present study showed $[\alpha]_D^{25}$ $+16.0°$. This is in contrast to the previous report that venkatasin **2** had $[\alpha]_D$ 0°.[10] A sample of **2a** obtained from **2** was converted to bis-(S)-MTPA ester **2b**. The ^{1}H-NMR spectrum of the ester **2b** displayed a major and minor set of signals in 87 : 13 ratio, indicating that compound **2** is 74% ee.

The absolute stereochemistry of the major enantiomer of **2** was proposed as $7'R,8'R$ in the same manner as described for **1**. The CD spectrum of **2** showed a positive exciton couplet ($\Delta\varepsilon$ $+0.4$ at 295 nm and $\Delta\varepsilon$ -1.1 at 280 nm) due to the two chromophores. The most stable conformation of the $7'R,8'R$-enantiomer of **2** is illustrated in Fig. 4.

In conclusion, we have isolated hyosgerin (**1**) from the seeds of *H. niger*, along with venkatasin (**2**), cleomiscosin B (**1a**) and cleomiscosin A (**2a**), and established the structure of **1** by spectroscopic data and chemical conversion. Studies of the optical properties of these samples revealed that **1** is 100% optically active, whereas **2** is 74% ee, in contrast to the racemic nature of **1a** and **2a**. Further, it was proposed that hyosgerin (**1**) has $7'R,8'R$ stereochemistry, and the major enantiomer of **2** has $7'R,8'R$ stereochemistry. It is likely that racemic cleomiscosin B was acetylated by an acylation enzyme, which reacts with $(-)$-($7'R,8'R$)-cleomiscosin B stereospecifically, to give $(-)$-($7'R,8'R$)-**1**. With regard to the formation of **2**, the enzyme action is not completely stereospecific, yielding a mixture of $(+)$-($7'R,8'R$)- and $(-)$-($7'S,8'S$)-venkatasin, with the former being predominant.

Experimental

General Melting points were determined using a Yazawa hot-stage apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-360 polarimeter. IR spectra were recorded on a Perkin-Elmer FT-IR Paragon 500 spectrophotometer, and UV on a Shimadzu UV-1600PC spectrometer. CD spectra were measured with a JASCO J-500C polarimeter at 25 °C in MeOH solution. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker DRX500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometer in $CDCl_3$ solution. The $CDCl_3$ signal was used as a reference (δ 77.0) for ^{13}C-chemical shifts. EI-MS (70 eV) and FAB-MS (NBA as matrix) were obtained on a JEOL JMS-700 spectrometer. Preparative HPLC was performed on a Waters HPLC system composed of a 600-multisolvent delivery unit, model 590 solvent delivery module and model 740 data module coupled with a Lambda max model 481 detector, using a normal phase column (GROM Saphir 110 Si, 25×2 cm i.d.). Analytical HPLC was performed on a Shimadzu LC-6A instrument with a SPD-6A UV detector, using a reversed-phase (ODS) column (Shiseido CAPCEL PAC, 25 cm×4.6 mm i.d.).

Plant Material The seeds of *H. niger* were purchased from a local market in Varanasi, India, and were 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* (5.0 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 aqueous methanol. The aqueous methanolic portion was extracted with $CHCl_3$, EtOAc and n-BuOH, successively. The $CHCl_3$ soluble portion (109.5 g) was chromatographed over silica gel and eluted with solvents of increasing polarity. The fractions eluted with benzene–$CHCl_3$ (2 : 1) were concentrated, and the residue was subjected to preparative HPLC (solvent, a gradient of AcOEt–hexane (1 : 1), reaching 100% AcOEt within 20 min; flow rate, 20 ml/min; UV detection at 254 nm). The peaks eluted at 9.4 and 11.4 min were collected and concentrated to give **2** (9.1 mg) and **1** (12.5 mg), respectively.

Further elution of the column with benzene–$CHCl_3$ (1 : 2) gave fractions containing cleomiscosins A and B. This was rechromatographed over silica gel with benzene–$CHCl_3$ (1 : 1) as an eluant to yield cleomiscosin A (1.2 g) in the earlier fraction and cleomiscosin B (0.51 g) in the later fraction.

Hyosgerin (1) Yellow crystals from MeOH, mp 198—199 °C, $[\alpha]_D^{25}$ $-65.4°$ (c=0.38, $CHCl_3$), UV λ_{max} (MeOH) 232 (4.29), 287 (3.71) and 322 (3.87) nm, IR ν_{max} ($CHCl_3$) 3530, 1720, 1610, 1570 cm^{-1}, ^{1}H and ^{13}C data see Table 1, CD data see Fig. 3, HR-EI-MS m/z: 428.1111 (M^+), $C_{22}H_{20}O_9$ requires 428.1107. EI-MS m/z (relative intensity %): 428 (M^+, 20), 368 (100), 353 (15), 222 (23), 219 (12), 179 (12), 162 (71), 147 (22), 131 (13), 119 (13), 91 (15). In analytical HPLC (conditions: solvent, MeOH–H_2O (1 : 1); flow rate, 0.8 ml/min; UV detection at 282 nm), compounds **1** and **2** were eluted at 17.52 and 16.62 min, respectively.

Conversion of 1 to Cleomiscosin B (1a) Powdered K_2CO_3 (1.1 mg) was added to a solution of compound **1a** (1.0 mg) in MeOH (50 μl) and THF (50 μl), and the mixture was stirred for 10 min. Powdered NH_4Cl was added to the mixture and the solution part was filtered through a cotton plug. The filtrate was concentrated to give a yellow solid (0.9 mg, 95%), $[\alpha]_D^{25}$ $-50°$ (c=0.03, MeOH). In analytical HPLC (conditions: solvent, MeOH–H_2O (1 : 1); flow rate, 0.8 ml/min; UV detection at 282 nm), the deacetylated sample of **1** was eluted at 7.87 min, while authentic samples of cleomiscosin B (**1a**) and cleomiscosin A (**2a**) were eluted at 8.86 and 7.87 min, respectively.

Conversion of 1a to Bis-(*S*)-MTPA Ester (1b) Compound **1a** (0.9 mg) in pyridine (50 μl) was treated with (R)-MTPACl (5 μl) for 1h. MeOH (30 μl) was added to the reaction mixture to destroy the acid chloride, and the solution was applied to p-TLC plate. The plate was developed with $CHCl_3$–MeOH (50 : 1), and the band of the desired product furnished **1b** (1.2 mg, 89%) as a yellow oil. ^{1}H-NMR data, see Table 2. HR-FAB-MS m/z: 819.1880 $[M+H]^+$, $C_{40}H_{33}O_{12}F_6$ requires 819.1876.

Venkatasin (2) Yellow crystals from MeOH, mp 113—115 °C (lit. 120—121 °C[9]), $[\alpha]_D^{25}$ $+16.0°$ (c=0.09, $CHCl_3$), UV λ_{max} (MeOH) 232 (4.24), 288 (3.67) and 319 (3.85) nm, IR ν_{max} ($CHCl_3$) 3530, 1720, 1610, 1570 cm^{-1}, ^{1}H and ^{13}C data see Table 1, CD data see Fig. 3, HR-EI-MS, m/z:

428.1156 (M^+), $C_{22}H_{20}O_9$ requires 428.1107. EI-MS *m*/*z* (relative intensity %): 428 (M^+, 24), 368 (100), 353 (16), 222 (35), 219 (6), 179 (18), 162 (63), 147 (20), 131 (18), 119 (15), 91 (16).

Conversion of **2** to **2a** and further derivatization to bis-(*S*)-MTPA ester **2b** was carried out as described for **1**.

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