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STRUCTURES OF CLEOMISCOSINS, COUMARINO-LIGNOIDS OF *CLEOME VISCOSA* SEEDS†

ANIL B. RAY,* SUNIL K. CHATTOPADHYAY and SANDEEP KUMAR
Department of Medicinal Chemistry, I.M.S., Banaras Hindu University, Varanasi 221005, India

and

CHOHACHI KONNO, YOSHINOBU KISO and HIROSHI HIKINO
Pharmaceutical Institute, Tohoku University, Aoba-Yama, Sendai, Japan

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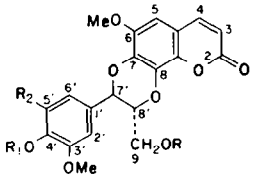
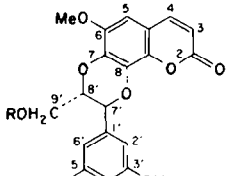
Abstract—Structures of cleomiscosins A, B and C, three new coumarino-lignoids isolated from the seeds of *Cleome viscosa* Linné, have been established as **1a**, **2a** and **1f** respectively, on the basis of spectral and chemical evidence. The compounds exhibited liver-protective properties.

Cleome viscosa Linné (syn. *C. icosandra* Linné), a common weed of the family Capparidaceae, is found throughout the tropical regions of the world. The plant finds use in the traditional systems of Indian medicine[1] and considerable phytochemical work on different parts of this plant has been reported.[2–8] In two preliminary communications,[9,10] we reported the structure determination of cleomiscosins A and B, the first pair of coumarino-lignoids isolated from the seeds of *Cleome viscosa*. Our continued search has now culminated in the isolation of yet another coumarino-lignoid, cleomiscosin C, and the present paper describes the isolation and structure determination of all the three compounds in full details.

Cleomiscosin A (**1a**), $C_{20}H_{18}O_8$ (M^+, 386.0992), m.p. 247°, $[\alpha]_D \pm 0°$, responded to tests for phenols and it was recognized to be a coumarin from its UV (λ_{max} 288 sh, 327 nm; ε 5661, 10550) and IR spectral data (ν_{max} 3500, 1715, 1620 and 1580 cm^{-1}). In conformity with its phenolic nature, cleomiscosin A gave a monomethyl ether (**1b**), $C_{21}H_{20}O_8$ (M^+, 400), m.p. 214°, on treatment with ethereal diazomethane, and a monoethyl ether (**1c**), $C_{22}H_{22}O_8$ (M^+, 414), m.p. 212°, ν_{max} 3450 cm^{-1}, with diethyl sulfate in the presence of anhydrous potassium carbonate. The non-phenolic nature of these monoalkyl ethers proved the presence of a solitary phenolic OH group in cleomiscosin A. On acetylation, cleomiscosin A gave a diacetate (**1d**), $C_{24}H_{22}O_{10}$ (M^+, 470), m.p. 175°, and its monoethyl ether (**1c**) under similar conditions furnished a monoacetate (**1e**), $C_{24}H_{24}O_9$ (M^+, 456), m.p. 160°. The diacetate (**1d**) showed in its IR spectrum, bands for phenol acetate (1765 cm^{-1}), alcohol acetate (1730 cm^{-1}) and coumarin system (1718, 1610, 1575 cm^{-1}) but no band in the OH region. Cleomiscosin A was thus proved to bear a phenolic and an alcoholic OH group in its molecule. The presence of a coumarin moiety in cleomiscosin A was further revealed from the diagnostic coumarin H-signals at δ 6.30 and 7.62 (1H, *d* each, J = 10 Hz) in the ^{1}H-NMR spectrum of its ethyl ether (**1c**) which also showed signals for two aromatic OMe's and four aromatic H's in addition to signals for an OEt grouping (Table 1). Chemical evidence in support of the existence of a coumarin moiety in cleomiscosin A was secured by transformation of **1c** to an *ortho*-methoxy-*trans*-cinnamic acid derivative (**3**) by prolonged reflux of the compound in methanolic alkali and subsequent methylation of the reaction product *in situ* with dimethyl sulfate and alkali. The ^{1}H-NMR spectrum of this acid (**3**), $C_{24}H_{28}O_9$ (M^+, 460), m.p. 199°, showed signals for two mutually coupled *trans* olefinic hydrogens at δ 6.48 and 8.18 (J = 16 Hz) instead of the characteristic coumarin hydrogens, in addition to signals for an aliphatic OMe (δ 3.41, 3H, *s*) and an extra aromatic OMe (δ 3.95, 3H, *s*). The existence of a coumarin moiety, two OMe's, one phenolic OH and an alcoholic OH group in cleomiscosin A accounts for six of the eight O atoms present in the molecule and the remaining two inert O atoms are considered to constitute oxide linkages. The foregoing data thus permit advancement of the part structure, **4**, for cleomiscosin A. Thus the problem that remains to be solved is to ascertain the nature of the C_9-unit and its mode of linkage with the coumarin nucleus. The C_9-unit was recognized as a substituted phenylpropane moiety from its ^{13}C-NMR data which showed signals for six aromatic carbons and three aliphatic carbons (—CH—O— × 2, —CH_2—O— × 1) in addition to the carbon signals associated with the coumarin nucleus. This observation permits extension of the part structure **4** to **5**. The resemblance of the UV spectrum of cleomiscosin A with that of 6,7,8-trioxygenated coumarin[11] and the occurrence of the common





1a R = R_1 = R_2 = H
1b R = R_2 = H, R_1 = Me
1c R = R_2 = H, R_1 = Et
1d R = R_1 = Ac, R_2 = H
1e R = Ac, R_1 = Et, R_2 = H
1f R = R_1 = H, R_2 = OMe

2a R = R_1 = R_2 = H
2b R = R_2 = H, R_1 = Me
2c R = R_2 = H, R_1 = Et
2d R = R_1 = Ac, R_2 = H
2e R = R_1 = H, R_2 = OH
2f R = R_1 = H, R_2 = OMe

† Liver-protective drugs, Part 21 of Tohoku University group.

Table 1. ^{1}H-NMR data*

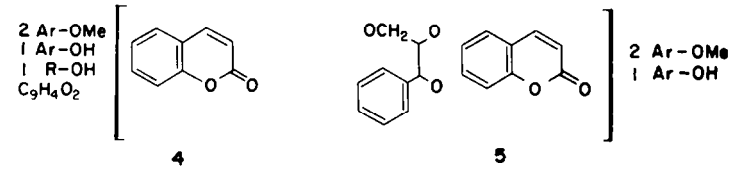
	1c ($CDCl_3$)†	2c ($CDCl_3$)†	1d ($CDCl_3$)‡	1f (DMSO-d_6)‡	6§ ($CDCl_3$)‡
3-H	6.30 *d* (10)	6.30 *d* (9)	6.33 *d* (9.5)	6.35 *d* (9)	2.92 *t* (8)
4-H	7.62 *d* (10)	7.63 *d* (9)	7.60 *d* (9.5)	7.98 *d* (9)	2.61 *t* (8)
5-H	6.56 *s*	6.51 *s*	6.55 *s*	6.93 *s*	6.34 *s*
2'-H			6.99 *d* (2)	6.76 *s*	6.95 *d* (2)
5'-H	7.00 br *s*	6.96 br *s*	7.08 *d* (8.5)	—	6.89 *d* (8)
6'-H			6.99 *dd* (8.5, 2)	6.76 *s*	7.01 *dd* (8, 2)
7'-H	5.10 *d* (7)	5.13 *d* (9)	5.03 *d* (7)	4.98 *d* (8)	4.94 *d* (8)
8'-H	3.8–4.3	3.8–4.3	4.40 *m*	4.38 *m*	4.08 *ddd* (8, 4, 2.5)
9'-CH_2	3.8–4.3	3.8–4.3	4.4 *m*	3.68 *dd* (12.5, 7)	3.85 *m*
			4.12 *dd* (13, 5.5)	3.41 *dd* (12.5, 3.5)	3.58 *ddd* (12.5, 7.5, 4)
OMe	3.90 *s*	3.90 *s*	3.85 *s*	3.79 *s*	3.89 *s*
			3.88 *s*	3.77 *s*	3.84 *s*
					3.81 *s*
					3.69 *s*
OEt	1.45 *t*	1.46 *t*			
	4.13 *q* (7)	4.16 *q* (7)			
OAc			2.06 *s*		
			2.31 *s*		

* Values in δ (ppm), coupling constants (Hz) in parentheses.
† Recorded on 60 MHz instruments.
‡ Recorded at 270 MHz on a Bruker WH-270.
§ The numbering system is after the coumarin from which it is derived.

fragment ion (**a**) at *m/z* 208 in the mass spectra of cleomiscosin A and its O-alkyl ethers (**1b** and **1c**) indicate that a dioxane bridge links the C_9-unit with the coumarin residue and that one of the two OMe's is in the coumarin part of the molecule. This OMe group was found to be at C-6 of the coumarin nucleus from the recognition of the lone aromatic hydrogen appearing as a singlet at δ 6.55 in the ^{1}H-NMR spectrum of the diacetate (**1d**) as coumarin C-5 hydrogen in view of the nOe observed between the C-4 H-signal at δ 7.60 and this H-signal (21%) and that discerned between this H-signal and the OMe hydrogen signal at δ 3.88 (31%). Consequently the oxide linkages were settled to be at C-7 and C-8.

The substitution pattern of the aromatic nucleus of the phenylpropane residue was clarified from the isolation of veratric acid by permanganate oxidation of cleomiscosin A methyl ether (**1b**). Further, the ^{1}H-NMR spectrum of cleomiscosin A diacetate (**1d**) showed an ABX pattern for three aromatic hydrogens in which the low-field hydrogen at δ 7.08 appeared as a doublet (J = 8.5 Hz) and the other two near-equivalent hydrogens appeared at δ 6.99 as a double doublet (J = 8.5 and 2 Hz) and a *meta*-coupled doublet (J = 2 Hz). This observation suggests that the phenol acetate group is symmetrically disposed relative to the near-equivalent hydrogens, or in other words, the acetoxy group is at the 4'-position. The 4-hydroxy-3-methoxy substitution pattern is also consistent with the facts that (1) the calculated ^{13}C resonances for the six aromatic carbons of a 4-acetoxy-3-methoxyphenyl analogue indicated a fair identity with the observed resonances of the diacetate (**1d**) and (2) the observed chemical shifts for the aromatic carbons of the non-coumarin part of cleomiscosin A are in accord with the corresponding carbons in 4-hydroxy-3-methoxyphenyl derivatives.[9]

The aromatic substitution pattern of the phenylpropane residue being settled, the only problem that remains to be solved is to decide which of the three aliphatic oxycarbons of this residue participate in the dioxane bridge formation. This was clarified from the observation that the signals for the methylene grouping exhibited downfield shifts by 0.2 and 1.3 ppm in the ^{1}H- and ^{13}C-NMR spectra, respectively, when the methyl ether (**1b**) was acetylated, demonstrating that the methylene bears an OH and consequently the two



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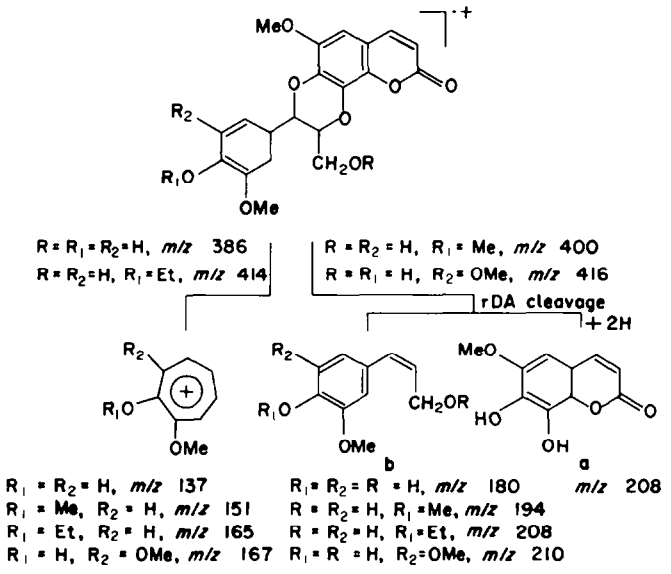
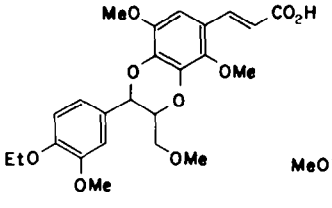


methines carry oxide linkages. Based on these findings, the structure of cleomiscosin A was restricted to two alternative formulations **1a** or **2a**.

In order to make a choice between the two structures, cleomiscosin A methyl ether (**1b**) was hydrogenated with Adams catalyst in acetic acid medium with the idea of opening the benzyl ether linkage of the dioxane bridge by hydrogenolysis. Though a similar dioxane system in the xanthono-lignoid, kielcorin[12] has been reported to be resistant to hydrogenolysis, the reaction product from **1b** responded to tests for phenols after work up and the derived product was methylated with diazomethane *in situ*. The methylated product, $C_{23}H_{28}O_9$, m.p. 122–123°, was revealed to be a substituted phenylpropionate (**6**) instead of the expected hydrogenolysis product from ^{1}H-NMR analysis of the compound. The spectrum showed the diagnostic doublet (J = 8 Hz) at δ 4.94 and doublet of double doublets (J = 8, 4, 2.5 Hz) at δ 4.08 for the two carbinyl hydrogens associated with the dioxane bridge, in addition to signals for four aromatic methoxyls, two vicinal methylenes of the phenylpropionate unit, an isolated aromatic hydrogen and three aromatic protons of a 1,3,4-trisubstituted phenyl nucleus (Table 1). Base-catalysed opening of the dioxane bridge, as reported in the case of kielcorin,[12] was also unsuccessful in the case of cleomiscosin A and as a result the problem had to be solved by hetero-decoupling experiments. In cleomiscosin A diacetate (**1d**), when C-7′ and C-8′ H-signals (δ 5.03 and 4.4 respectively) were irradiated separately, the C-signals for C-7 (δ 136.9) and C-8 (δ 131.7) respectively, showed significant sharpening. Cleomiscosin A was thus framed as **1a**. The coupling constants between H-7′ and H-8′ in the derivatives (**1c**, **1d** and **1e**) of cleomiscosin A were observed to be 8 Hz, demonstrating that the two hydrogens are *trans*-oriented. However, in view of the optical inactivity of the compound it was concluded to



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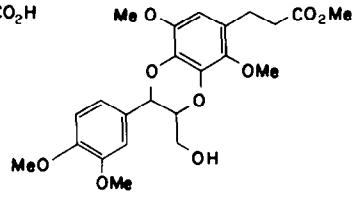

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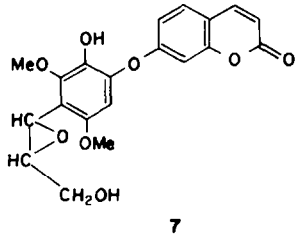

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Table 2. Carbon-13 shieldings in cleomiscosins and their derivatives (δ)

	1a (C_5D_5N)	**1d** ($CDCl_3$)	**2a** (C_5D_5N)	**2d** ($CDCl_3$)	**1f** (C_5D_5N)
C-2	160.8 *s*	160.4 *s*	160.7 *s*	160.4 *s*	160.8 *s*
C-3	113.6 *d*	114.4 *d*	113.8 *d*	114.3 *d*	113.7 *d*
C-4	144.5 *d*	143.5 *d*	144.4 *d*	143.5 *d*	144.5 *d*
C-5	101.1 *d*	100.5 *d*	101.2 *d*	100.8 *d*	101.0 *d*
C-6	146.3 *s*	145.8 *s*	146.2 *s*	145.7 *s*	146.3 *s*
C-7	138.4 *s*	136.9 *s*	138.1 *s*	136.3 *s*	138.1 *s*
C-8	133.0 *s*	131.7 *s*	133.2 *s*	132.1 *s*	132.5 *s*
C-9	139.3 *s*	140.8 *s*	139.4 *s*	140.7 *s*	139.2 *s*
C-10	111.9 *s*	111.9 *s*	111.8 *s*	111.8 *s*	111.9 *s*
C-1′	127.5 *s*	133.5 *s*	127.5 *s*	133.5 *s*	126.3 *s*
C-2′	112.3 *d*	111.5 *d*	112.3 *d*	111.3 *d*	106.1 *d*
C-3′	150.0 *s*	151.7 *s*	150.1 *s*	151.6 *s*	149.1 *s*
C-4′	149.0 *s*	138.8 *s*	149.1 *s*	138.8 *s*	135.9 *s*
C-5′	116.6 *d*	123.3 *d*	116.5 *d*	123.2 *d*	149.1 *s*
C-6′	121.7 *d*	119.9 *d*	121.7 *d*	119.8 *d*	106.1 *d*
C-7′	77.5 *d*	76.7 *d*	77.1 *d*	76.0 *d*	77.7 *d*
C-8′	79.9 *d*	75.1 *d*	80.2 *d*	75.6 *d*	79.7 *d*
C-9′	60.7 *t*	62.4 *t*	61.1 *t*	62.4 *t*	60.7 *t*
OCH_3	55.8 *q*	56.0 *q*	55.9 *q*	56.0 *q*	56.2 *q*
	56.2 *q*	56.3 *q*	56.1 *q*	56.4 *q*	56.4 *q*
					56.4 *q*
		20.6 *q*		20.6 *q*	
		20.6 *q*		20.6 *q*	
OAc		168.5 *s*		168.6 *s*	
		170.2 *s*		170.2 *s*	

be racemic. The expression **1a** thus shows the relative stereostructure of cleomiscosin A which has been confirmed by its recent synthesis.[13]

The isomeric compound cleomiscosin B (**2a**), $C_{20}H_{18}O_8$ (M^+, 386.1014), m.p. 274°, $[\alpha]_D \pm 0°$, showed striking resemblance with cleomiscosin A in all its spectral properties, indicating a close structural similarity between the two molecules. Like cleomiscosin A, it also furnished a monomethyl ether (**2b**), $C_{21}H_{20}O_8$ (M^+, 400), m.p. 212–213°, a monoethyl ether (**2c**), m.p. 192°, and a diacetate (**2d**), $C_{24}H_{22}O_{10}$ (M^+, 470), m.p. 174°. Structural similarity of the two compounds became fully disclosed from ^{1}H- and ^{13}C-NMR spectral comparisons of common derivatives of the two compounds (Tables 1 and 2). Thus, in the ^{1}H-NMR spectrum of the diacetate (**2d**), a 1H singlet at δ 6.53 for an isolated aromatic hydrogen exhibited a long range coupling with the C-4 H-signal and on nOe (10%) with the OMe hydrogens at δ 3.92. Hence the two oxide linkages in cleomiscosin B are at C-7 and C-8 as in cleomiscosin A.

As for the phenylpropanoid portion, examination of the ^{1}H-NMR spectrum of the diacetate (**2d**) which disclosed signals at δ 6.98 (1H, *d*, J = 8 Hz), 7.00 (1H, *s*) and 7.05 (1H, *d*, J = 8 Hz) in an ABC pattern, indicated the presence of a 3,4-dioxygenated phenyl group. The signals for the aliphatic portion appeared at δ 5.04, 4.4, 4.12 and 4.4 in the ^{1}H and at δ 76.0, 75.6 and 62.4 in the ^{13}C-NMR spectra of the diacetate (**2d**), which were in agreement with those of cleomiscosin A diacetate (**1d**). In view of these similarities, cleomiscosin B (**2a**) was logically assumed to be the position isomer of cleomiscosin A (**1a**) and this was verified by the observation of the ^{13}C—^{1}H spin couplings between the C-8 signal at δ 132.1 and H-7′ signal at δ 5.04, and between the C-7 signal at δ 136.3 and H-8′ signal at δ 4.40 in the diacetate (**2d**). Although cleomiscosin B is a racemate, the coupling constant between the C-7′ and C-8′ H-signals was found to be 8 Hz, showing that these two hydrogens are situated in *trans*.

Cleomiscosin C (**1f**), a minor congener of cleomiscosins A and B, analysed for the molecular formula $C_{21}H_{20}O_9$ (M^+, 416), m.p. 255°, $[\alpha]_D \pm 0°$. While the UV and IR spectral data of cleomiscosin C were virtually identical with those of its congeners, its ^{1}H-NMR spectrum showed signals for coumarin C-3 and C-4 hydrogens at δ 6.35 and 7.98 (1H, *d* each, J = 9 Hz), an isolated aromatic hydrogen at δ 6.93 (1H, *s*), two equivalent aromatic hydrogens at δ 6.76 (2H, *s*) and three OMe's at δ 3.79 (3H, *s*) and 3.77 (6H, *s*) instead of two OMe groups observed in the molecule of its companions. The presence of an extra OMe group in cleomiscosin C was further verified from its mass spectral data which showed a molecular ion at *m/z* 416 which is 30 mass units higher than that for cleomiscosin A or B. Further, the spectrum showed the coumarin cation at *m/z* 208 like its congeners but phenylpropenol ion (**b**) at *m/z* 210 instead of *m/z* 180. Thus the excess molecular weight of cleomiscosin C was reflected in its ion peak at *m/z* 210 which suggests that the extra OMe group is in the phenylpropanoid unit. The appearance of the two aromatic hydrogens as a singlet further suggests a symmetrical substitution pattern of this unit as shown in formulation **1f** or **2f**. In order to distinguish these two possibilities, the ^{13}C-NMR spectra of cleomiscosin C and its diacetate (aquillochin diacetate[14]) were compared with those of cleomiscosins A and B, and their diacetates. As a result, it was found that the resonances for cleomiscosin C were in good agreement with those for cleomiscosin A rather than those for cleomiscosin B, especially in the chemical shifts for C-7′, C-8′ and C-9′, which were most affected by the structural difference between **1f** and **2f** (Table 2). Again, the coupling constant (8 Hz) between the C-7′

and C-8′ H-signals in the ^{1}H-NMR spectrum revealed these two hydrogens to be *trans*-oriented, though cleomiscosin C is a racemic compound.

Thus, cleomiscosin C was proved to have the same structural framework (**1f**) as cleomiscosin A.

In a recent publication[14] Rastogi *et al.* reported the isolation of a coumarino-lignan, aquillochin, from *Aquilaria agallocha* Roxb. and advanced two alternative formulations for the molecule. Aquillochin which has been identified with cleomiscosin C by direct comparison, has, therefore, the structure **1f**.

Attachment of a phenylpropane unit with a polyphenolic compound through a dioxan bridge was earlier witnessed in the flavono-lignoid, silybin, and xanthono-lignoid, kielcorin. Cleomiscosins are the first members of coumarino-lignoid. In fact, as coumarin itself is a C_6—C_3 unit, cleomiscosins represent a new class of lignans which may be termed as coumarino-lignans.

After the publication of our first paper on cleomiscosin A,[9] it came to our knowledge that a similar compound (**2e**) and an optically active mixture of two related compounds were isolated from the mineral-stained wood of sugar maple (*Acer saccharum* Marsh.) The compounds constituting the mixture were alleged to have the structures **2a** and **2f** on the basis of some spectral evidence and biogenetic consideration.[15] However, the isolation of the position isomers, cleomiscosins A and B, from the same source reveals that biogenetic speculation is not enough to determine the mode of fusion of the coumarin moiety with the phenylpropanoid unit.

Cleosandrin (**7**), isolated from the seeds of *Cleome icosandra*[16] and formulated as a novel 7-phenoxy-coumarin, has been found to be identical with cleomiscosin A by direct comparison of the methyl ethers of the two compounds.[10]

The reported *in vitro* cytotoxicity[17] of cleomiscosin A could not be substantiated from the screening data of our sample provided by the National Cancer Institute, Bethesda, U.S.A. All the cleomiscosins were subjected to screening for their probable antihepatotoxic activity according to the method of Kiso *et al.*[18,19] and the data (Table 3) reveal that while all the cleomiscosins are significantly active against D-galactosamine-induced cytotoxicity in primary cultured rat hepatocytes, cleomiscosin B is the most potent amongst them.

Table 3. Effect of cleomiscosins A, B and C on carbon tetrachloride- and galactosamine-induced cytotoxicity in primary cultured rat hepatocytes

Substance	Dose (mg/ml)	GPT (%)	
		CCl_4	GalN
Control	—	100±2	100±2
Cleomiscosin A	0.01	103±1	92±2
	0.1	100±0	68±1**
	1.0	87±3	86±2
Cleomiscosin B	0.01	96±3	79±2*
	0.1	95±3	19±0**
	1.0	82±0**	49±1**
Cleomiscosin C	0.01	97±1	93±3
	0.1	97±2	86±1
	1.0	92±1	55±1**

$n = 3$ (dishes). Significantly different from the control, $p < 0.01$* or $p < 0.001$**.

EXPERIMENTAL

M.ps were taken on a Toshniwal m.p. apparatus and are uncorrected. Column chromatography was carried out over B.D.H. silica gel (60–120 mesh) and TLC over silica gel G (Centron). All the analytical samples were routinely dried over P_2O_5 at 80–144° (depending upon the m.p. of the compounds) for 24 hr *in vacuo* and were tested for purity by TLC and mass spectrometry. Optical rotations were measured in MeOH.

Isolation of cleomiscosins. Air-dried and pulverised seeds of *Cleome viscosa* (5 kg) were first defatted with petroleum ether and then extracted with EtOAc in a Soxhlet apparatus (24 hr). The EtOAc extract was chromatographed over a column of silica gel (240 g). The column was eluted initially with benzene and then with EtOAc. EtOAc eluates furnished a mixture of cleomiscosins (4 g) which was rechromatographed over silica gel when pure cleomiscosin A (2 g) eluted out of the column from the early fractions of benzene–EtOAc (1:1) mixture. Continued elution with the same solvent furnished a mixture of cleomiscosins A and C, and finally cleomiscosin B (0.2 g). The separation of cleomiscosin C from A was effected by repeated silica gel chromatography of the mixture to yield cleomiscosin C (20 mg).

Cleomiscosin A (**1a**) crystallised from MeOH–EtOAc as clusters of colourless needles, $C_{20}H_{18}O_8$ (M^+, *m/z* 386.0992), m.p. 247°, $[\alpha]_D \pm 0°$ (*c* 0.1); TLC: R_f 0.55 in benzene–EtOAc (1:1); UV (EtOH): λ_{max} 288 sh (ε 5661) and 327 nm (ε 10 550); IR (nujol): ν_{max} 3500, 1720, 1620 and 1580 cm^{-1}; ^{13}C-NMR: shown in Table 2; MS *m/z* (rel. int. %): 386 (M^+, 52), 368 (18), 354 (14), 249 (18), 208 (48), 180 (94), 162 (20), 151 (10), 137 (100), 124 (54). (Found: C, 62.50; H, 4.55. Calc for $C_{20}H_{18}O_8$: C, 62.18; H, 4.66%.)

Cleomiscosin A methyl ether (**1b**). Treatment of **1a** (0.15 g) with an excess of ethereal soln of CH_2N_2 for 48 hr afforded **1b** which crystallised from MeOH as colourless needles (0.14 g), m.p. 214°. (Found: C, 62.92; H, 4.90. Calc for $C_{21}H_{20}O_8$: C, 63.00; H, 5.10%.)

Cleomiscosin A ethyl ether (**1c**). A mixture of **1a** (0.6 g), K_2CO_3 (6 g) and Et_2SO_4 (1 ml) was refluxed in dry acetone (200 ml) under anhydrous conditions for 8 hr. The product was chromatographed over silica gel (8 g). Elution with benzene–EtOAc (3:1) gave **1c** as a homogeneous solid which crystallised from MeOH as colourless needles (0.59 g), m.p. 210°; IR (nujol): ν_{max} 3450, 1720 cm^{-1}; ^{1}H-NMR: shown in Table 1; ^{13}C-NMR (C_5D_5N): δ 14.9, 55.7, 56.1 (*q* each); 60.6, 64.4 (*t* each); 77.3, 79.7, 101.0, 111.6, 113.3, 113.8, 121.0, 144.4 (*d* each); 112.2, 129.1, 132.0, 138.2, 139.2, 146.3, 149.6, 150.1, 160.7 (*s* each). (Found: C, 63.48; H, 5.11. Calc for $C_{22}H_{22}O_8$: C, 63.77; H, 5.31%.)

Cleomiscosin A diacetate (**1d**). A mixture of **1a** (0.3 g), Ac_2O (5 ml) and Et_3N (2 ml) was kept at room temp for 48 hr under anhydrous conditions. Usual work up followed by purification through silica gel column and crystallisation from MeOH yielded **1d** as colourless needles (0.26 g), m.p. 175°; IR (nujol): ν_{max} 1765, 1730, 1718, 1610 and 1575 cm^{-1}; ^{1}H- and ^{13}C-NMR: shown in Tables 1 and 2; MS *m/z* (rel. int. %): 470 (M^+, 76), 428 (36), 368 (100), 369 (25), 222 (87), 179 (31), 180 (16), 162 (33), 151 (9), 149 (15), 131 (16). (Found: C, 61.30; H, 4.80. Calc for $C_{24}H_{22}O_{10}$: C, 61.28; H, 4.68%.)

Cleomiscosin A ethyl ether monoacetate (**1e**). Acetylation of **1c** (0.2 g) with Ac_2O and Et_3N at room temp by usual procedure afforded **1e** as a homogeneous solid (0.18 g). Crystallised from MeOH as colourless needles, m.p. 160°, $[\alpha]_D \pm 0°$ (*c* 0.07), IR (nujol): ν_{max} 1755, 1725, 1630 and 1590 cm^{-1}; ^{1}H-NMR (90 MHz, $CDCl_3$): δ 1.47 (3H, *t*, J = 7 Hz), 4.12 (2H, *q*, J = 7 Hz), 2.08 (3H, *s*) 3.86, 3.88 (3H, *s* each), 4.36 (2H, *m*), 4.01 (1H overlapped with the signal at δ 4.12), 5.02 (1H, *d*, J = 7 Hz), 6.33, 7.64 (1H, *d* each, J = 8 Hz), 6.56 (1H, *s*), 6.92 (3H, *s*); MS *m/z* (rel. int. %): 456 (M^+, 50), 396 (48), 367 (27), 250 (100), 208 (18), 207 (29), 191 (31), 190 (15), 179 (29), 161 (15), 151 (13), 137

(12), 119 (20). (Found: C, 63.10; H, 5.85. Calc for $C_{24}H_{24}O_9$: C, 63.15; H, 5.26%.)

Preparation of o-methoxy-trans-cinnamic acid derivative (**3**) *from cleomiscosin A ethyl ether* (**1c**). Compound **1c** (0.2 g) was refluxed with NaOMe in MeOH (1 M, 30 ml) on a water bath for 8 hr. The mixture, after removal of the solvent, was taken in KOH aq and heated (50°) with Me_2SO_4 for 2 hr. The alkaline mixture was cooled, acidified and extracted with EtOAc. The EtOAc extract was chromatographed over silica gel (5 g). Elution with $CHCl_3$—MeOH (99:1) gave **3** that crystallised from MeOH as clusters of colourless needles, m.p. 199°, ^{1}H-NMR (60 MHz, $CDCl_3$): δ 1.50 (3H, *t*, J = 7 Hz), 4.16 (2H, *q*, J = 7 Hz), 3.41 (3H, *s*), 3.91 (6H, *s*), 3.95 (3H, *s*), 3.42–4.2 (3H), 5.12 (1H, *d*, J = 7 Hz), 6.06 (1H, br *s*, lost in D_2O), 6.48, 8.18 (1H, *d* each, J = 16 Hz), 6.75 (1H, *s*), 7.01 (3H, *s*); MS *m/z* (rel. int. %): 460 (M^+, 36), 428 (54), 399 (23), 222 (100), 190 (34), 191 (38), 177 (18), 162 (14), 161 (20), 119 (11). (Found: C, 62.50; H, 6.38. Calc for $C_{24}H_{28}O_9$: C, 62.61; H, 6.08%.)

Potassium permanganate oxidation of cleomiscosin A methyl ether (**1b**). To a soln of **1b** (0.2 g) in aqueous acetone (30 ml), was added a $KMnO_4$ aq (0.8 g) with stirring until the $KMnO_4$ colour was found to persist. The mixture was heated for 30 min, acidified with dil H_2SO_4, cooled at room temp, treated with $Na_2S_2O_5$ until the soln became colourless and then extracted with Et_2O. The Et_2O extract on removal of solvent yielded a residue which crystallised from boiling water into colourless needles (25 mg), m.p. 182°, identical with veratric acid by direct comparison (m.m.p., TLC, IR).

Preparation of substituted phenylpropionate (**6**) *from cleomiscosin A methyl ether* (**1b**). A soln of **1b** (0.2 g) in glacial AcOH (30 ml) was shaken in an atmosphere of H_2 for 3 hr in the presence of PtO_2 catalyst (30 mg). The soln was freed from catalyst by filtration and the filtrate was diluted with cold water and extracted with EtOAc (50 × 3 ml). The EtOAc extract yielded, on removal of solvent, an amorphous solid (0.12 g) which was dissolved in MeOH and methylated with ethereal CH_2N_2. The mixture was freed from solvent and chromatographed over silica gel (5 g). Elution with benzene–EtOAc (19:1) yielded **6** which crystallised from EtOAc–petroleum ether as colourless stout needles (65 mg), m.p. 122–123°; UV (MeOH): λ_{max} 280 nm; ^{1}H-NMR: shown in Table 1; MS *m/z* (rel. int. %): 448 (M^+, 58), 430 (23), 297 (48), 267 (16), 224 (28), 194 (100), 176 (58), 151 (86), 138 (52), 123 (16). (Found: C, 61.80; H, 6.39. Calc for $C_{23}H_{28}O_9$: C, 61.61; H, 6.25%.)

Cleomiscosin B (**2a**) crystallised from MeOH—EtOAc as colourless flaky needles, m.p. 274°, $[\alpha]_D \pm 0°$ (*c* 0.12); TLC: R_f 0.39 in benzene–EtOAc (1:1); UV (EtOH): λ_{max} 285 and 328 nm; IR (nujol): ν_{max} 3510, 1720, 1610, 1560 cm^{-1}; ^{13}C-NMR: shown in Table 2; MS *m/z* (rel. int. %): 386 (M^+, 48), 368 (2), 208 (5), 180 (100), 137 (85), 124 (48). (Found: C, 62.61; H, 4.80. Calc for $C_{20}H_{18}O_8$: C, 62.18; H, 4.66%.)

Cleomiscosin B methyl ether (**2b**). Treatment of **2a** (50 mg) with an ethereal soln of CH_2N_2 at room temp afforded **2b** (40 mg) which crystallised from MeOH as colourless needles, m.p. 212–213°; ^{13}C-NMR (C_5D_5N): δ 55.9, 56.1 (*q* each), 61.2 *t*, 75.8, 79.2, 100.1, 110.4, 111.1, 113.8, 120.2, 143.5 (*d* each), 111.4, 127.7, 132.6, 136.8, 138.8, 145.6, 149.2, 149.6, 160.8 (*s* each). (Found: C, 62.51; H, 4.85. Calc for $C_{21}H_{20}O_8$: C, 63.00; H, 5.00%.)

Cleomiscosin B ethyl ether (**2c**) was prepared from **2a** (50 mg) by the usual procedure, crystallised from MeOH as colourless needles (30 mg), m.p. 192°; ^{1}H-NMR: shown in Table 1. (Found: C, 63.50; H, 5.55. Calc for $C_{22}H_{22}O_8$: C, 63.70; H, 5.31%.)

Cleomiscosin B diacetate (**2d**). Acetylation of **2a** with Ac_2O and Et_3N at room temp for 48 hr yielded **2d** which crystallised from MeOH as colourless needles, m.p. 174°; IR (nujol): ν_{max} 1762, 1730, 1718, 1612, 1570 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.04 (3H, *s*), 2.32 (3H, *s*), 3.84 (3H, *s*), 3.92 (3H, *s*), 4.12 (1H, *dd*, J = 13, 6 Hz), 4.4 (2H, *m*), 5.04 (1H, *d*, J = 7 Hz), 6.28 (1H, *d*, J = 10 Hz), 6.53 (1H, *s*), 6.98 (1H, *d*, J = 8 Hz), 7.00 (1H, *s*), 7.05 (1H, *d*, J = 8 Hz), 7.58 (1H, *d*, J = 10 Hz); ^{13}C-NMR: shown in Table 2. (Found: C, 61.36; H, 4.96. Calc for $C_{24}H_{22}O_{10}$: C, 61.28; H, 4.68%.)

Cleomiscosin C (**1f**) crystallised from MeOH—EtOAc as colourless needles, m.p. 255°, $[\alpha]_D \pm 0°$ (*c* 0.08), TLC: R_f 0.59 in $CHCl_3$—MeOH (9:1); UV (MeOH): λ_{max} 232 sh (ε 25 600), 280 sh (ε 4160), 322 nm (ε 12 160); UV (MeOH—NaOH): λ_{max} 258, 292 sh, 328 nm; IR (KBr): ν_{max} 3420, 1720, 1685 cm^{-1}; ^{1}H- and ^{13}C-NMR: shown in Tables 1 and 2; MS *m/z* (rel. int. %): 416 (M^+, 7), 249 (5), 210 (56), 208 (45) 193 (21), 180 (34), 167 (100), 149 (50), 137 (76), 109 (50), 91 (49), 79 (78), identical with aquillochin[14] by direct comparison (TLC and ^{1}H-NMR).

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REFERENCES

[1] K. R. Kirtikar and B. D. Basu, *Indian Medicinal Plants* (Edited by E. Blatter, J. F. Caius and K. S. Mhaskar), Vol. 1, p. 183. Singh & Singh (1975).
[2] M. P. Gupta and S. Dutt, *J. Indian Chem. Soc.* **15**, 532 (1938).
[3] R. C. Badami, G. S. Deshpande and M. R. Shanbheg, *J. Oil. Technol. Assoc. India* **7**, 76 (1975).
[4] C. Rukmini, *Ind. J. Chem. Res.* **67**, 604 (1978).
[5] A. H. Chen, *Tai-wan K'o Hsuah* **29**, 40 (1975).
[6] S. B. Mahato, B. C. Pal, T. Kawasaki, K. Miyahara, O. Tanaka and K. Yamasaki, *J. Am. Chem. Soc.* 4720 (1979).
[7] J. S. Chauhan, S. K. Srivastava and S. D. Srivastava, *Indian J. Chem.* **17B**, 300 (1979).
[8] J. S. Chauhan, S. K. Srivastava and S. D. Srivastava, *Phytochemistry* **18**, 691 (1979).
[9] A. B. Ray, S. K. Chattopadhyay, C. Konno and H. Hikino, *Tetrahedron Lett.* **21**, 4477 (1980).
[10] A. B. Ray, S. K. Chattopadhyay, C. Konno and H. Hikino, *Heterocycles* **19**, 19 (1982).
[11] R. H. Goodwin and B. M. Pollock, *Arch. Biochem. Biophys.* **49**, 5 (1954).
[12] H. Nielson and P. Arends, *Phytochemistry* **17**, 2040 (1978).
[13] A. Arnoldi, A. Arnone and L. Merlini, *Heterocycles* **22**, 1537 (1984).
[14] P. Bhandari, P. Pant and R. P. Rastogi, *Phytochemistry* **21**, 2147 (1982).
[15] J. F. Manville and N. Lavintin, *Environ. Can. For. Serv.* **30**, 3 (1974).
[16] A. G. R. Nair, *Indian J. Chem.* **17B**, 438 (1979).
[17] M. Arisawa, A. D. Kinghorn, G. A. Cordell and N. R. Farnsworth, *J. Nat. Prod.* **46**, 222 (1983).
[18] Y. Kiso, M. Tohkin and H. Hikino, *Planta Med.* **49**, 222 (1983).
[19] Y. Kiso, M. Tohkin and H. Hikino, *J. Nat. Prod.* **46**, 841 (1983).