REFERENCES

1. Shimomura, H., Sashida, Y. and Oohara, M. (1987) *Phytochemistry* **26**, 1513.
2. Murphy, S. T., Ritchie, E. and Taylar, W. C. (1975) *Aust. J. Chem.* **28**, 81.
3. Liu, J-S., Huang, M-F. and Gao, Y-L. (1981) *Can. J. Chem.* **59**, 1680.
4. Crossley, N. S. and Djerassi, C. (1962) *J. Chem. Soc.* 1459.
5. King, F. E. and Wilson, J. G. (1964) *J. Chem. Soc.* 4011.
6. Riggs, N. V. and Stevens, J. D. (1962) *Aust. J. Chem.* **15**, 305.
7. Yasuda, I. Uehara, S., Hamano, T., Takano, I., Seto, T., Akiyama, K., Takeya, K. and Itokawa, H. (1985) Abstract papers of the 32th Annual Meeting of the Pharmaceutical Society of Japan p. 30.
8. McAlpine, J. B., Riggs, N. V. and Gordon, P. G. (1968) *Aust. J. Chem.* **21**, 2095.
9. Le Quesne, P. W., Larrahando, J. E. and Raffauf, R. F. (1980) *J. Nat. Prod.* **43**, 353.

Phytochemistry, Vol. 27, No. 2, pp. 636–638, 1988.
Printed in Great Britain.

0031–9422/88 $3.00 + 0.00

CLEOMISCOSIN D, A COUMARINO-LIGNAN FROM SEEDS OF *CLEOME VISCOSA*

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(*Received* 17 *June* 1987)

Key Word Index—*Cleome viscosa*; Capparidaceae; cleomiscosin D; coumarino-lignan.

Abstract—Cleomiscosin D, a minor coumarino-lignan of the seeds of *Cleome viscosa*, has been proved to be regioisomer of cleomiscosin C. A method of degradation of coumarino-lignans for the identification of the coumarin moiety has been developed.

INTRODUCTION

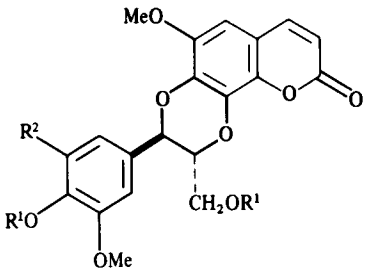
Cleomiscosins A (**1**) and B (**2**), isolated from the seeds of *Cleome viscosa* Linné and fully characterized from comprehensive spectral analysis and some chemical reactions, constitute the first regioisomeric pair of coumarino-lignans in which a coumarin moiety is linked with a phenylpropanoid unit through a dioxane bridge [1–3]. The correctness of these structures were later verified by Merlini and co-workers who achieved their synthesis by oxidative coupling of fraxetin with coniferyl alcohol [4]. We subsequently reported the isolation of a third coumarino-lignan, cleomiscosin C (**3**) from this source [3] and showed it to be identical with aquillochin [5] for which two alternative structures were proposed. Further investigation on this plant material led us to isolate a known coumarin and a new coumarinolignan, cleomiscosin D. The characterization of these two compounds and a method of cleaving the dioxane bridge of cleomiscosins for identification of coumarin moieties will be discussed in the present paper.

RESULTS AND DISCUSSION

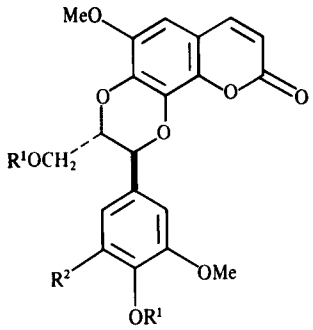
A coumarin, $C_{10}H_8O_5$ (MS *m/z* 208, M^+), was recognized to be oxygenated at its 6, 7 and 8-positions from its UV, IR spectra together with its ^1H NMR signals for two coumarin hydrogens [δ6.17 and 7.74 (1H each, *d*, $J = 9.5$ Hz)], a lone aromatic hydrogen (δ6.61) and a methoxyl grouping (δ3.88). Irradiation of the methoxyl signal at δ3.88 showed an observable NOE of the isolated signal at δ6.61 which in turn showed long range coupling with the coumarin H-4 signal at δ7.74. The observation clearly revealed it to be fraxetin [4].

Cleomiscosin D (**4**), $C_{21}H_{20}O_9$ (MS, *m/z* 416, M^+), responded to tests for phenol. The UV and IR data of cleomiscosin D were essentially identical with those of cleomiscosin C (**3**), an isomer of cleomiscosin D. The ^{13}C NMR spectrum of cleomiscosin D disclosed the presence of eight aliphatic carbons (Me–O × 3, $-CH_2-O$ × 1, > CH–O × 2, –CH=CH– × 1), 12 aromatic carbons (CH × 3, C × 2, C–O × 7) and one carbonyl-like cleomiscosin C. Cleomiscosin D gave a diacetate (**4a**), $C_{25}H_{24}O_{11}$ (MS, *m/z* 500, M^+).

The ^1H NMR spectrum of cleomiscosin D in pyridine-d_5 showed signals for three methoxyl groupings (δ3.77, 3.77 and 3.82), two coumarin hydrogens (δ6.41 and 7.73), an isolated aromatic hydrogen (δ6.74) and four aliphatic hydrogens on carbons carrying oxygen functions (δ3.96, 4.30, 4.57 and 5.55). Significant amounts of NOE were observed between the isolated signal at δ6.74 and a methoxyl signal at 3.82, between the isolated signal at 6.74



1 $R^1 = R^2 = H$
1a R^1 = Ac, $R^2 = H$
3 $R^1 = H$, R^2 = OMe



2 $R^1 = R^2 = H$
2a R^1 = Ac, $R^2 = H$
4 $R^1 = H$, R^2 = OMe
4a R^1 = Ac, R^2 = OMe

and a doublet at 7.73, and between the two doublets at 7.73 and 6.41. These spectral data clearly indicated the presence of a fraxetin moiety in cleomiscosin D which was further corroborated from the fragment ion peak at *m/z* 208 in the mass spectrum of cleomiscosin D (**4**) and from the comparison of ^{13}C NMR signals due to the coumarin part of cleomiscosin D diacetate (**4a**) with the corresponding signals of the diacetates of cleomiscosins A and B (Table 1). The chemical shift and splitting pattern of ^{1}H NMR signals of cleomiscosin D diacetate also resembled those of the diacetates of cleomiscosins A (**1**) and B (**2**) with the exception that the former showed an extra aromatic methoxyl signal and consequently two, instead of three, aromatic hydrogen signals for the non-coumarin part of the molecule. The chemical equivalence of these two aromatic hydrogens, as revealed from their appearance as 2H singlet at δ6.63, suggested a symmetrical substitution pattern of the aromatic ring of the phenylpropanoid moiety. The extra methoxyl group was, therefore, placed at the 5′-position with the aromatic acetoxyl function at the 4′-position, flanked on either side by a methoxyl group. Such a substitution pattern received support also from the presence of a fragment ion at *m/z* 209 in the mass spectrum and ^{13}C NMR signals at δ132.9 (1C), 104.0 (2C), 152.5 (2C) and 129.5 (1C). The ^{1}H NMR signals due to the propane part of the phenylpropane moiety in cleomiscosin D diacetate (**4a**) were very similar to those of diacetates of cleomiscosins A and B, indicating that oxygens on C-7′ and C-8′ were involved in ether linkages and that the oxygen at C-9′ was acetylated. The coupling constant ($J = 7$ Hz) of H-7′ indicated that the phenyl group at C-7′ and the acetoxymethyl group at C-8′ are *trans*. Based on these findings and the fact that cleomiscosins C and D showed virtually identical ^{1}H NMR and mass spectra, cleomiscosin D (**4**) was formulated as a regioisomer of cleomiscosin C (**3**). The assumption was verified from the observation that the chemical shifts of C-7, C-7′ and C-8′ in ^{13}C NMR spectrum of cleomiscosin D diacetate (**4a**) closely resembled those of cleomiscosin B diacetate (**2a**) rather than cleomiscosin A diacetate (**1a**). This is the first report of the natural occurrence of cleomiscosin D, though its synthesis was reported earlier [7]. The physical and spectral properties of our natural product agreed fairly well with those of the synthetic compound.

The occurrence of fraxetin and two regioisomeric pairs of coumarino-lignans in the same plant is a strong circumstantial evidence to infer that in the biosynthesis of these coumarino-lignans, the union of two C_6–C_3 units follows the same mechanism as in their synthesis.

While our earlier attempt to open the dioxane bridge linking the two C_6–C_3 units in cleomiscosins met with failure [3], we have now been successful in providing unambiguous chemical evidence in support of the presence of the fraxetin moiety in cleomiscosins A and B [8]. Thus, when cleomiscosin A (**1**) or its regioisomer (**2**) was heated with pyridine hydrochloride at 210° for 5 min, two crystaline phenolic coumpounds were obtained and identified as fraxetin and its demethylated derivative.

EXPERIMENTAL

Isolation of cleomiscosin D and fraxetin. General directions and work-up of the plant material have been given before [3]. Continued elution of the column resolving the mixture of cleomiscosins with C_6H_6–EtOAc (1:1) furnished a mixture of cleomiscosins B and D, and then the fraxetin fraction. Recryst. of the fraxetin with MeOH–EtOAc afforded fraxetin (yield, 0.002%) as pale yellow needles, mp 225–226°, EIMS *m/z* (rel. int. %): 208 (M^+, 100), 194 (50), 180 (22), 166 (22), 137 (24), 109 (22), 81 (28); UV λ_{max}^{EtOH} nm (log ε): 232 (4.09), 255 (3.72), 342 (4.08); IR ν_{max}^{KBr} cm^{-1}: 3425, 1683, 1610; ^{1}H NMR (100 MHz, $CDCl_3$): δ3.88 (3H, *s*), 6.17 (1H, *d*, $J = 9.5$ Hz), 6.61 (1H, *s*), 7.74 (1H, *d*, 9.5 Hz).

Repeated chromatography of the mixture of cleomiscosins B and D over silica gel and elution with C_6H_6–EtOAc (3:1) yielded cleomiscosin D (yield, 0.0002%) as colourless needles from MeOH–EtOAc, mp 243–246°, EIMS *m/z* (rel. int. %): 416 (M^+, 19), 398 (14), 384 (19), 210 (100), 208 (27), 192 (13), 182 (31), 167 (68), 154 (25), 149 (32), 121 (16), 91 (15), 79 (19); UV λ_{max}^{EtOH} nm (log ε): 214 (4.38), 232 (3.97), 325 (3.58); IR ν_{max}^{KBr} cm^{-1}: 3420, 1710, 1690; ^{1}H NMR (100 MHz, C_5D_5N): δ3.77 (6H, *s*), 3.82 (3H, *s*), 3.96 (1H, *m*), 4.30 (1H, *m*), 4.57 (1H, *m*), 5.55 (1H, *d*, $J = 8.1$ Hz), 6.41 (1H, *d*, $J = 10.3$ Hz), 6.74 (1H, *s*), 7.73 (1H, *d*, $J = 10.3$ Hz).

Acetylation of cleomiscosin D. Cleomiscosin D (10 mg) was acetylated with Ac_2O (1 ml) and pyridine (0.2 ml) at 140° for 2 hr. After work-up in the usual manner and recryst. from MeOH, cleomiscosin D diacetate (7 mg) was obtained as colourless needles, mp 203–205°, EIMS *m/z* (rel. int. %): 500 (M^+, 100), 458 (15), 440 (29), 428 (22), 398 (10), 252 (28), 250 (20), 210 (10), 209 (10), 150 (10), 149 (10); IR ν_{max}^{KBr} cm^{-1}: 1760, 1732, 1132; ^{1}H NMR

Table 1. ^{13}C NMR data of cleomiscosins and their derivatives

C	**4** (C_5D_5N)	**4a** ($CDCl_3$)	**1a** ($CDCl_3$)	**2a** ($CDCl_3$)	**3** (C_5D_5N)
2	160.7 *s*	160.4 *s*	160.4 *s*	160.4 *s*	160.8 *s*
3	113.9 *d*	114.4 *d*	114.4 *d*	114.3 *d*	113.7 *d*
4	144.4 *d*	143.5 *d*	143.5 *d*	143.5 *d*	144.5 *d*
5	101.2 *d*	100.8 *d*	100.5 *d*	100.8 *d*	101.0 *d*
6	146.3 *s*	145.7 *s*	145.8 *s*	145.7 *s*	146.3 *s*
7	138.7 *s*	136.3 *s*	136.9 *s*	136.3 *s*	138.1 *s*
8	133.8 *s*	132.0 *s*	131.7 *s*	132.1 *s*	132.5 *s*
9	139.5 *s*	138.8 *s*	138.8 *s*	138.8 *s*	139.2 *s*
10	111.8 *s*	111.8 *s*	111.9 *s*	111.8 *s*	111.9 *s*
1′	126.5 *s*	132.9 *s*	133.5 *s*	133.5 *s*	126.3 *s*
2′	106.6 *d*	104.0 *d*	111.5 *d*	111.3 *d*	106.1 *d*
3′	150.2 *s*	152.5 *s*	151.7 *s*	151.6 *s*	149.1 *s*
4′	135.1 *s*	129.5 *s*	140.8 *s*	140.7 *s*	135.9 *s*
5′	150.2 *s*	152.5 *s*	123.3 *d*	123.2 *d*	149.1 *s*
6′	106.6 *d*	104.0 *d*	119.9 *d*	119.8 *d*	106.1 *d*
7′	77.5 *d*	76.3 *d*	76.7 *d*	76.0 *d*	77.7 *d*
8′	80.2 *d*	75.6 *d*	75.1 *d*	75.6 *d*	79.7 *d*
9′	61.1 *t*	62.5 *t*	62.4 *t*	62.4 *t*	60.7 *d*
OMe	56.1 *q*	56.2 *q*	56.0 *q*	56.0 *q*	56.2 *q*
OMe	56.4 *q*	56.4 *q*	56.3 *q*	56.4 *q*	56.4 *q*
OMe	56.4 *q*	56.4 *q*			56.4 *q*
OAc		20.4 *q*	20.6 *q*	20.6 *q*	
		20.7 *q*	20.6 *q*	20.6 *q*	
		168.3 *s*	168.5 *s*	168.6 *s*	
		170.2 *s*	170.2 *s*	170.2 *s*	

(100 MHz, $CDCl_3$): δ2.06 (3H, *s*), 2.34 (3H, *s*), 3.82 (6H, *s*) 3.91 (3H, *s*) 4.16 (1H, *dd*, $J = 12.5$, 6 Hz), 4.15 (3H, *m*), 4.98 (1H, *d*, $J = 8$ Hz), 6.27 (1H, *d*, $J = 9.5$ Hz), 6.53 (1H, *s*), 6.63 (2H, *s*), 7.57 (1H, *d*, $J = 9.5$ Hz).

Degradation of cleomiscosin A to fraxetin and 6,7,8-trihydroxy coumarin. A mixture of cleomiscosin A (0.25 g) and pyridine hydrochloride (1 g) was heated at 210° for 5 min. The reaction mixture was poured into H_2O, extracted with EtOAc and the extract chromatographed over silica gel. Elution with C_6H_6–EtOAc (3:1) gave fraxetin as pale yellow needles (30 mg), mp 225–226°, indistinguishable from an authentic sample (mp, co-TLC, ^1H NMR). Continued elution with C_6H_6–EtOAc (1:1) furnished 6,7,8-trihydroxy coumarin (25 mg) as pale yellow needless, mp 258°, ^1H NMR (270 MHz, CD_3OD): δ6.17 (1H, *d*, $J = 9$ Hz), 6.54 (1H, *s*), 7.75 (1H, *d*, $J = 9$ Hz); trimethyl ether (CH_2N_2), mp 103°, identical with dimethyl ether of fraxetin (mmp, Co-TLC); triacetate (Ac_2O, Et_3N), ^1H NMR (270 MHz, $CDCl_3$): δ2.30, 2.34, 2.40 (3H, *s* each), 6.44 (1H, *d*, $J = 9.6$ Hz), 7.30 (1H, *s*), 7.60 (1H, *d*, $J = 9.6$ Hz).

Acknowledgement—Sincere thanks are due to Professor G. A. Cordell, University of Illinois, for supplying a synthetic sample of cleomiscosin. D. S. K. is thankful to Ministry of Health & Family Welfare, Government of India, for the award of fellowship.

REFERENCES

1. Ray, A. B., Chattopadhyay, S. K., Konno, C. and Hikino, H. (1980) *Tetrahedron Letters* **21**, 4477.
2. Ray, A. B., Chattopadhyay, S. K., Konno, C. and Hikino, H. (1982) *Hetrocycles* **19**, 19.
3. Ray, A. B., Chattopadhyay, S. K., Kumar, S., Konno, C., Kiso, Y. and Hikino, H. (1985) *Tetrahedron* **41**, 209.
4. Arnoldi, A., Arnone, A. and Merlini, L. (1984) *Hetrocycles* **22**, 1537.
5. Bhandari, P., Pant, P. and Rastogi, R. P. (1982) *Phytochemistry* **21**, 2147.
6. Wessely, F. and Demmer, E. (1929) *Chem. Ber.* **62**, 120.
7. Lin, L.-J. and Cordell, G. A. (1984) *J. Chem. Soc. Chem. Commun.* 160.
8. Kumar, S. (1986) Ph.D. Thesis, Banaras Hindu University (India).