Tetrahedron Letters Vol. 21, pp 4477 - 4480 0040-4039/80/1108-4477$02.00/0

STRUCTURE OF CLEOMISCOSIN A, A COUMARINO-LIGNOID OF *CLEOME VISCOSA* SEEDS

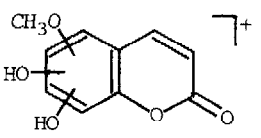
Anil B. Ray and Sunil K Chattopadhyay
Department of Medicinal Chemistry, I. M. S., Banaras Hindu University, Varanasi, India
and
Chohachi Konno and Hiroshi Hikino
Pharmaceutical Institute, Tohoku University, Aoba-yama, Sendai, Japan

Abstract — Cleomiscosin A, isolated from the seeds of *Cleome viscosa*, has been shown to be a coumarino-lignoid and its structure has been advanced as I on the basis of chemical and physical evidence.

Cleome viscosa Linné (syn. *C. icosandra* Linné) (Capparidaceae) is a common weed found throughout the tropical regions of the world. In India, its seeds are utilized as a remedy for infantile convulsions, as an anthelmintic and as a counterirritant in chronic painful joints. Systematic fractionation and chromatographic resolution of the defatted seeds of *C. viscosa* furnished a pale yellow crystalline substance which has been named as cleomiscosin A.

Cleomiscosin A (C-A), m.p. 247-249°, was shown to have the composition $C_{20}H_{18}O_8$ (MS *m/e* 386.0992, M^+). In agreement with this, the ^{13}C NMR spectrum indicated the presence of twenty carbons which were further classified as follows: seven aliphatic carbons (CH_3-O × 2, $-CH_2$-O × 1, >CH-O × 2, -CH=CH- × 1), twelve aromatic carbons (CH × 4, C × 2, C-O × 6) and one carbonyl carbon. Since C-A has a number of aromatic carbons of the C-O type, exhibited a strong IR band[1] at 3500 cm^{-1} and gave a positive phosphomolybdic acid test for phenols, it was treated with diethyl sulfate in the presence of potassium carbonate to yield the monoethyl ether (II), m.p. 208-210°, $C_{22}H_{22}O_8$ (C, 63.48; H, 5.11%, MS *m/e* 414, M^+) which did not respond to tests for phenols. The ether (II) still disclosed a hydroxyl band at 3450 cm^{-1} in the IR spectrum and, therefore, it was acetylated with acetic anhydride in triethylamine to give the monoethyl ether monoacetate (III), m.p. 162-164°, $C_{24}H_{24}O_9$ (C, 63.10, H, 5.85%, MS *m/e* 456, M^+). C-A also gave on acetylation with acetic anhydride in triethylamine the diacetate (IV), m.p. 174-177°, $C_{24}H_{22}O_{10}$ (MS *m/e* 470, M^+). The IR spectra of the derivatives (III and IV) displayed no more hydroxyl band, indicating that C-A contains one phenolic and one alcoholic hydroxyl group.

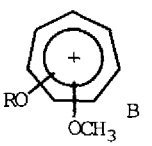
The presence of a coumarin moiety was revealed from the UV (maximum at 325 nm with humps at 288 and 232 nm in EtOH), IR (bands at 1720 and 1620 cm^{-1}) and ^1H NMR (two 1H doublets at δ 6.30 and 7.62 (*J* 10 Hz) for H-3 and H-4) spectra of the ether (II). The existence of a coumarin moiety, two methoxyls, a phenolic and an alcoholic hydroxyl thus accounted for six of the eight oxygen atoms present in the molecule and the remaining two oxygens were thus considered to constitute oxide linkages. Additional evidence in support of the presence of the coumarin moiety was secured from the mass spectra of C-A and its derivative (III) which showed a common fragment peak at *m/e* 208 due to the cation $C_{10}H_8O_5^+$ (A). These data further showed that the coumarin nucleus bears one methoxyl and two O-substituted groups in the aromatic ring. In conformity with this,



A

the ^{1}H NMR spectrum of the diacetate (IV) displayed a 1H singlet at δ 6.54 attributable to an isolated hydrogen. The finding of NOE's between the H-4 signal at δ 7.58 and the above singlet at δ 6.54 (21%) and between the same singlet at δ 6.54 and the methoxyl hydrogen signal at δ 3.88 (31%) established the location of the hydrogen in question at C-5, the methoxyl at C-6 and consequently the remaining oxide linkages at C-7 and C-8. This was further verified by comparison of the observed chemical shifts of the ^{13}C NMR signals for C-2--C-10 in C-A and its derivatives (III and IV) with the predicted shifts of C-2—C-10 in a 6,7,8-tri-O-alkylated coumarin described below. Assignments of the observed shifts were first confirmed by the presence of the ^{13}C-^{1}H spin couplings between the C-7 signal and the H-5 signal, between the C-9 signal and the H-5 signal and between the C-6 signal and the methoxyl hydrogen signal. The predicted shifts of a 6,7,8-trihydroxycoumarin were calculated from the shieldings of 6,7-dihydroxycoumarin and the additive substituent parameters for the additional hydroxyl at C-8. Since in coumarins, conversion of hydroxyls into O-alkyls is known to cause no significant changes in the chemical shifts,[2] the predicted shifts thus deduced were compared with the observed shifts, showing that both the sets of values were coincident (Table I).

As was revealed by the previous ^{13}C NMR data, there was another phenyl group in the molecule. In the ^{1}H NMR spectra of the derivatives (III and IV), signals originating from three hydrogens in the phenyl group in question were visible, indicating that the phenyl was disubstituted. Although the three hydrogens appeared as a singlet at the same position δ 6.90 in the spectrum of the ether acetate (III), they occurred separately at δ 6.99 (doublet, *J* 8 Hz), 7.07 (doublet, *J* 8 Hz) and 7.02 (singlet) in an ABC pattern in that of the diacetate (IV). This fact pointed to the three possibilities, 2,4-, 2,5- and 3,4-dioxygenated features, for the substitution pattern of the phenyl group. That one of the two oxygen substituents was a methoxyl and the other a hydroxyl was confirmed by the finding that the fragment peak at *m/e* 137 due to the cation B (R=H) in the mass spectrum of C-A shifted to *m/e* 165 (B, R=C_2H_5) in that of the ether (II). It was reported that the three ^{1}H NMR signals appeared at δ 6.3 (2H) and 6.9 (1H) in 2,4-dihydroxy-1-propylbenzene, at δ 6.50 (3H) in 2,5-dihydroxy-1-methylbenzene (DMSO-d_6+$CDCl_3$) and at δ 6.65 (3H) in 3,4-dihydroxy-1-methylbenzene.[3] Thus the observed resonances for the three hydrogens at δ 6.90 in the ether acetate (III) suggested the 3,4-dioxygenated pattern for the phenyl group.



B

In the further examination of the substitution pattern of the phenyl group, the observed chemical shifts of the six ^{13}C NMR signals for C-1'—C-6' in the phenyl group were found to be consistent with the calculated shifts of C-1—C-6 in 2,5- and 3,4-dioxygenated-1-methylbenzenes but not with those of 2,4-dioxygenated-1-methylbenzenes (Table I and II). The predicated resonances for C-1'—C-6' in the diacetate (IV) were then calculated by adding the acetylation effects on the carbon resonances in C-A.[4] Comparison of the predicted resonances with the observed ones for C-1'—C-6' in the acetate (IV) indicated a fair identity for the two sets of data if C-A were a 4-hydroxy-3-methoxy derivative and not if it were a 3-hydroxy-4-methoxy, 2-hydroxy-5-methoxy or 5-hydroxy-2-methoxy derivative (Table III). A discrepancy still existed for the resonances of the two carbons bearing oxygen functions and this may be attributed to the vicinal position of the two carbons in question. The 4-hydroxy-3-methoxy arrangement was further verified by the following fact: 1) an NOE was found between the singlet for the methoxyl hydrogens at δ 3.84 and the singlet for the

Table I. Carbon-13 shieldings in cleomiscosin A and related substances (δ)

	cleomiscosin A (C_5D_5N)	ether (II) (C_5D_5N)*	diacetate (IV) ($CDCl_3$)**	6,7,8-OH coumarin[2]	ephedradine B (C_5D_5N)[4]
C-2	160.8 s	160.7 s	160.4 s	161.4	
C-3	113.6 d	113.3 d	114.4 d	112.0	
C-4	144.5 d	144.5 d	143.5 d	144.5	
C-5	101.1 d	101.0 d	100.5 d	103.5	
C-6	146.3 s	146.3 s	145.8 s	143.5	
C-7	138 4 s	138.2 s	136.9 s	137.5	
C-8	133.0 s	132.0 s	133.5 s	132.0	
C-9	139.3 s	139.2 s	140.8 s	139.1	
C-10	111 9 s	112.2 s	111.9 s	112.0	
C-1'	127 5 s	129.1 s	131.7 s		130.8 s
C-2'	112.3 d	111.6 d	111.5 d		111.1 d
C-3'	150.0 s	150.1 s	151.7 s		147.9 s
C-4'	149.0 s	149.6 s	138,8 s		145.9 s
C-5'	116.6 d	113 8 d	123.3 d		115.7 d
C-6'	121.7 d	121.0 d	119.9 d		120.5 d
C-7'	79 9 d	79.7 d	76.7 d		
C-8'	77.5 d	77,3 d	75 1 d		
C-9'	60.7 t	60.6 t	62.4 t		
OCH_3	55.8 q	55.7 q	56.0 q		
OCH_3	56.2 q	56.1 q	56.3 q		

*64.4 t, 14 9 q for OCH_2CH_3 **168.5 s, 170.2 s, 20.6 q, 20.6 q for two $COCH_3$'s

Table II. Carbon-13 shieldings in the hydroxy-methoxy-methylbenzenes (δ)

	C-1	C-2	C-3	C-4	C-5	C-6
2-hydroxy-4-methoxy-1-methylbenzene (1)	117.0	157.1	101.3	158.4	106.7	131.6
4-hydroxy-2-methoxy-1-methylbenzene (2)	115.7	162.0	101.3	153.5	108.0	131.6
2-hydroxy-5-methoxy-1-methylbenzene (3)	125,7	148.4	116.7	112.6	152.5	116.2
5-hydroxy-2-methoxy-1-methylbenzene (4)	124.4	153 3	115.4	113.9	147.9	117.5
3-hydroxy-4-methoxy-1-methylbenzene (5)	131.1	117.5	140.9	144.3	115.4	122.9
4-hydroxy-3-methoxy-1-methylbenzene (6)	131.1	116.2	147 1	138.1	116.7	122.9

Table III. Carbon-13 shieldings of the phenyl side chain in cleomiscosin A and its acetate

	C-1'	C-2'	C-3'	C-4'	C-5'	C-6'
in the case of 2-hydroxy-5-methoxy derivative						
cleomiscosin A (obs)	127,5	149.0	116.6	112.3	150.0	121.7
the diacetate (calc)	137.8	142.7	126,9	110.3	157,9	119.7
the diacetate (obs)	131.7	136.9	123,3	111.5	151.7	119,9
in the case of 5-hydroxy-2-methoxy derivative						
cleomiscosin A (obs)	127.5	150,0	116.6	112.3	149.0	121.7
the diacetate (calc)	125.5	157.9	114.6	122.6	142.7	132.0
the diacetate (obs)	131.7	151.7	111.5	119.9	136.9	123.3
in the case of 3-hydroxy-4-methoxy derivative						
cleomiscosin A (obs)	127.5	116.6	149.0	150.0	112.3	121.7
the diacetate (calc)	125.5	126.9	142.7	160.3	110.3	129,6
the diacetate (obs)	131.7	119.9	136.9	151.7	111.5	123.3
in the case of 4-hydroxy-3-methoxy derivative						
cleomiscosin A (obs)	127.5	112.3	150.0	149.0	116.6	121,7
the diacetate (calc)	135.4	110.3	160.3	142,7	126.9	119,7
the diacetate (obs)	131.7	111.5	151.7	136.9	123,3	119.9

insulated hydrogen at δ 7.02, 2) the observed chemical shifts for C-1'—C-6' in C-A fitted with those for the corresponding carbons in 4-hydroxy-3-methoxypnenyl derivatives, *e.g.*, ephedradine B[4] (Table I) and 3) the shifts for C-3' and C-4' in the diacetate (IV) were in good agreement with those for the corresponding carbons in ephedradine B triacetate (δ 151.0 and 139.8[4]).

Inspection of the ^{1}H NMR spectrum of the ether (II) with the aid of double resonance experiments showed the presence of the part structure C. The H-9' signal exhibited a downfield shift by 0.2 ppm when the ether (II) was acetylated, indicating that the C-9' methylene bears a hydroxyl and consequently the C-7' and C-8' methines carry oxide linkages. Hence, the above 4-hydroxy-3-methoxyphenyl system is to be attached to the C-7' methine of the part structure C to build up the C_6-C_3 unit. Such an arrangement is consistent with the fairly deshielded line position of the H-7' signal (δ 5.05, 5.01 and 5.03 for the derivatives (II, III and IV)). The genesis of the fragment ion (D) (*m/e* 180.0794 in C-A (R^1-R^2=H), *m/e* 208.1075 in the ether (II) (R^1=H, R^2=C_2H_5) and *m/e* 250 in the ether acetate (III) (R^1=$COCH_3$, R^2=C_2H_5)) by retro Diels-Alder type fission confirmed this assumption. C-A was thus represented by formula I, though an alternative structure by interchange of substituents at C-7' and C-8' is equally probable. However, the correctness of the structure I was ascertained by the observation of the ^{13}C-^{1}H spin couplings between the C-7 signal at δ 136.9 and the H-8' signal at δ *ca.* 4.1 and between the C-8 signal at δ 133.5 and the H-7' signal at δ 5.03 in the diacetate (IV).

The coupling constant between the H-7' and H-8' signals in the derivatives (II, III and IV) was 8 Hz, demonstrating that the two hydrogens are *trans*-oriented. The relative stereostructure I was thus deduced for C-A. However, in view of the optical inactivity of the derivatives (II and III), C-A was concluded to be racemic.

C-A possesses a novel skeleton in which a C_6-C_3 unit is linked with a coumarin nucleus through a dioxane bridge. It is the first member of a new class of compounds which may be termed as coumarino-lignans.

Acknowledgment We thank Dr. K. Matsushita, JEOL Ltd., for some NMR data.

NOTE AND REFERENCES

1) Unless stated otherwise, IR and ^{1}H NMR spectra were taken in Nujol and $CDCl_3$, respectively.
2) N. J. Cussans and T. N. Huckerby, *Tetrahedron*, 31, 2719 (1975)
3) C. J. Pouchert and J. R. Campbell, *The Aldrich Library of NMR spectra*, Vol. IV, p. 134, 138, 141 (1974)
4) M. Tamada, K. Endo and H. Hikino, *Heterocycles*, 12, 783 (1979)

Addendum A. G. R. Nair (*Ind. J. Chem.*, 17B, 438 (1979)) has quite recently reported the isolation of cleosandrin from the seeds of *C. icosandra* and its structure has been proposed as 7-O-[2-hydroxy-3,5-dimethoxy-4-(1'-*cis*-epoxy-3'-hydroxypropyl)phenyl]coumarin. From the reported physical constants, cleosandrin appears to be identical with our cleomiscosin A but the Nair's structure is untenable with the data we have obtained.

(Received in Japan 29 July 1980)