# PLANT ANTICANCER AGENTS XXIX. CLEOMISCOSIN A FROM *SIMABA MULTIFLORA, SOULAMEA SOULAMEOIDES,* AND *MATAYBA ARBORESCENS*[1,2]

MUNEHISA ARISAWA,[3] SUKHDEV S. HANDA,[4] DAVID D. MCPHERSON, DAVID C. LANKIN, GEOFFREY A. CORDELL,* HARRY H.S. FONG, and NORMAN R. FARNSWORTH

Department of Pharmacognosy and Pharmacology, College of Pharmacy,
University of Illinois at the Medical Center, Chicago, IL 60612

ABSTRACT.—Cleomiscosin A (**1**) has been isolated from *Simaba multiflora* and *Soulamea soulameoides* in the Simaroubaceae through bioactivity-directed fractionation and from *Matayba arborescens* in the Sapindaceae. The structure of cleomiscosin A (**1**) was established through spectroscopic studies and chemical reactions.

Recent publications by Hikino and co-workers (2) and by Gottlieb and co-workers (3) on the isolation of coumarinolignan derivatives prompt us to report on our studies of a compound coincidentally obtained from three plants in our laboratory. During the course of our studies, the structure of one of the isolates was revised (4).[5] Our data, obtained independently, are in agreement with this structure revision for cleomiscosin A.

A methanol extract of the stem wood and stem bark of *Soulamea soulameoides* (Gray) Nooteboom (syn. *Amaroria soulameoides* Gray) (Simaroubaceae) displayed good *in vivo* activity in the P-388 lymphocytic leukemia system (T/C 189% at 50 mg/kg) and was also cytotoxic in the Eagles' carcinoma of the nasopharynx (KB) test system in cell culture.[6] The compounds principally responsible for this activity have been disclosed previously (6), but here we report the isolation of cleomiscosin A (**1**) from a P-388 *in vitro* active fraction.

	R_1	R_2
1	H	H
2	$COCH_3$	$COCH_3$
3	CH_3	H
4	CH_3	$COCH_3$

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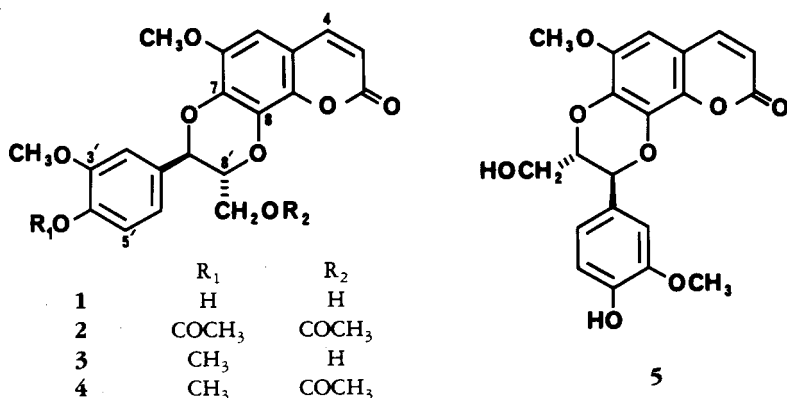
[1]For Paper XXVII in this series see reference (1).

[2]Part of this work was presented at the Joint Meeting of the American Society of Pharmacognosy and the Society for Economic Botany, Boston, Mass., July, 1981.

[3]Present address: Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, Sugitani 2630, Toyama 930-01, Japan.

[4]Present address: Pharmacognosy Section, Department of Pharmaceutical Sciences, Panjab University, Chandigarh, 160014, India.

[5]We thank Professor Hikino for a reprint of this manuscript and for keeping us informed of the progress of his work.

[6]All extracts and fractions were evaluated according to protocols established by the National Cancer Institute (5).

In the course of isolating additional quantities of the quassinoid 6α-senecioyloxy-chaparrinone from *Simaba multiflora* A. Juss (Simaroubaceae) for extensive biological testing, a number of other compounds were isolated through bioactivity-directed fractionation (7). From one of these fractions, cleomiscosin A (**1**) was isolated as an active constituent.

Cleomiscosin A (**1**) was also isolated from the Peruvian plant *Matayba arborescens* Radlk. (Sapindaceae), together with the coumarin scopoletin.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Kofler hot plate and are uncorrected. The uv spectra were obtained with a Beckman model DB-G spectrophotometer. The ir spectra were obtained with a Beckman model 18-A spectrophotometer with polystyrene calibration at 1601 cm^{-1}; absorption bands are recorded in wave numbers (cm^{-1}). Pmr spectra were recorded in $CDCl_3$, C_5D_5N or $(CD_3)_2SO$ with Varian T-60A instrument operating at 60 MHz with a Nicolet Model TT-7 Fourier Transform attachment. Cmr spectra were recorded on a JEOL 100 instrument operating at 25.05 MHz using $CDCl_3$ as a solvent. TMS was used as an internal standard and chemical shifts are recorded in δ (ppm). Mass spectra were obtained with a Varian MAT-112S double focusing mass spectrometer operating at 70 eV.

PLANT MATERIALS.—Stem wood and stem bark of *S. soulameoides* were collected in Fiji in 1979, and the stem wood, stem bark, and twigs of *M. arborescens* were collected in Peru in December, 1978. The wood of *S. multiflora* used for extraction was collected in Peru in 1976. Herbarium specimens documenting these collections have been deposited in the Herbarium of the National Arboretum, Agricultural Research Service, U.S. Department of Agriculture, Washington, DC.

EXTRACTION, FRACTIONATION, AND ISOLATION.—*S. multiflora*. An ethanolic extract of the wood (318 kg) of *S. multiflora* was supplied by Polysciences Inc. Concentration *in vacuo* at 50° afforded 3.18 kg of residue, of which a portion (1.9 kg) was partitioned between H_2O-MeOH (9:1, 3.5 liters) and $CHCl_3$ (2×3.5 liters). The $CHCl_3$-soluble fraction was concentrated *in vacuo* at 50° to yield a viscous residue (950 g) that was partitioned between petroleum ether (2.5 liters) and H_2O-MeOH (1:1, 2.5 liters). Evaporation of solvent from the aqueous MeOH phase afforded a dark brown residue (390 g).

Chromatography of the residue (390 g) on silica gel PF$_{254}$[7] (4.5 kg) eluting successively, by gradient elution, with $CHCl_3$ and $CHCl_3$-MeOH mixtures afforded 75 fractions (2 liters each). Fraction 26 from the column, eluted with $CHCl_3$-MeOH (49:1), crystallized from MeOH to afford colorless needles of cleomiscosin A (**1**, 70 mg, $3.2\times10^{-7}\%$).

Soulamea soulameoides. The dried and milled plant material (35 kg) was thoroughly extracted successively with petroleum ether, $CHCl_3$, and MeOH. Partition of the MeOH extract between $CHCl_3$ (2×2.5 liters) and H_2O (2.5 liters) followed by evaporation afforded 120 g of a residue (KB, ED$_{50}$ 0.043 μg/ml).

A portion (70 g) of the $CHCl_3$-soluble fraction was chromatographed on a column of silica gel PF 254 (800 g) eluting successively with $CHCl_3$ and $CHCl_3$-MeOH mixtures afforded 15 fractions. Fractions 10-15 (1.5 g, KB, ED$_{50}$ 0.01 μg/ml), eluted with $CHCl_3$-MeOH (99:1), were rechromatographed over silica gel (40 g) eluting with C_6H_6-$CHCl_3$ (1:1) and $CHCl_3$. The first fractions eluted with $CHCl_3$ afforded a residue (30 mg) that was washed with Et_2O and crystallized from $CHCl_3$-MeOH to afford cleomiscosin A (**1**, 16 mg, $6.5\times10^{-5}\%$).

Matayba arborescens. The coarsely ground stem wood, stem bark, and twigs (14.9 kg) of *M. arborescens* were extracted thoroughly with MeOH and the extract concentrated *in vacuo* to 5 liters. On standing, a precipitate (228 g) formed and was removed through filtration. After evaporation of the filtrate, the residue (271 g) was redissolved in H_2O-MeOH (2:1) and successively partitioned with petroleum ether and $CHCl_3$. Chromatography of the $CHCl_3$-soluble fraction (25.3 g) on silica gel 60[6] (500 g) eluting with $CHCl_3$-EtOAc-MeOH (9:1:1) afforded 140 fractions (25 ml each). Fractions 62-80 (1.09 g) were redissolved in MeOH and, on standing, a white precipitate formed, which was recrystallized from EtOH to afford cleomiscosin A (**1**, 34 mg, $2.3\times10^{-4}\%$). The mother liquor was evaporated and crystallized from MeOH to afford scopoletin (27.4 mg, $1.8\times10^{-4}\%$), identified by co-tlc, mmp, and spectroscopic properties with an authentic sample.

CLEOMISCOSIN A.—The isolate displayed mp 250-252°, $[\alpha]^{25}D \pm 0°$ (c 0.5, MeOH); ir, ν max (KBr) 3460, 1710, 1610, 1575, 1520, 1500, 1440, 1420, 1370, 1300, 1280, 1230, 1195, 1155, 1130, 1100, 1080, 1050, 1030, 990, 940, 850, 820, and 750 cm^{-1}; uv, λ max (MeOH) (log ε) 325 (4.25), 287

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[7]E. Merck, Darmstadt, West Germany.

(4.10), 232 (4.40), and 211 nm (4.47); pmr (60 MHz, pyridine-d_5) δ 3.70 (3H, s, OCH$_3$), 3.78 (3H, s, OCH$_3$), 3.91-4.50 (3H, m, 8'-H, 9'H$_2$), 5.53 (1H, d, J=8.0 Hz, 7'-H), 6.39 (1H, d, J=9.5 Hz, 3-H), 6.70 (1H, s, 5-H), 7.19 (1H, m, OH, exchanged with D$_2$O), 7.27 (3H, m, 2', 5', 6'-H), 7.70 (1H, d, J=9.5 Hz, 4-H), and 11.18 (1H, m, OH, exchanged with D$_2$O); (60 MHz, DMSO-d_6) δ 3.12-3.66 (2H, m, 9'-H$_2$), 3.78 (6H, s, 2×-OCH$_3$), 4.24-4.41 (1H, m, 8'-H), 4.99 (2H, m, J=7.6 Hz, 7'-H and OH, exchanged with D$_2$O), 6.30 (1H, d, J=9.5 Hz, 3-H), 6.86 (3H, bs, 2', 5', 6'-H), 7.03 (1H, d, J=9.5 Hz, 4-H), and 9.14 (1H, s, OH, exchanged with D$_2$O); ms m/z 386 (M$^+$, 41%), 368 (15), 356 (7), 249 (13), 219 (22), 208 (6), 181 (10), 180 (84), 162 (25), 161 (15), 152 (10), 151 (11), 150 (22), 138 (13), 137 (100), 124 (67), 120 (10), 119 (19), 91 (21), 79 (30), 72 (14), 65 (12), 54 (20), and 51 (25). Identity was established by direct comparison with an authentic sample of cleomiscosin A.

ACETYLATION OF CLEOMISCOSIN A.—Cleomiscosin A (**1**, 30 mg) was treated with Ac$_2$O-pyridine (1:1, 0.5 ml) at 100° for 1 h. Work-up in the usual way afforded a diacetate derivative (**2**, 25 mg) as colorless needles, mp 178-180°; ir ν max (KBr) 1755, 1725, 1715, 1610, 1560, 1505, 1460, 1445, 1420, 1370, 1310, 1290, 1265, 1225, 1190, 1160, 1135, 1090, 1060, 1030, 995, 940, 905, 840, and 780 cm^{-1}; pmr (360 MHz, CDCl$_3$) δ 2.054 (3H, s, 9'-OAc), 2.316 (3H, s, 4'-OAc), 3.839 (3H, s, 3'-OCH$_3$), 3.886 (3H, s, 6-OCH$_3$), 4.103 (1H, dt, J=5.7, 7.2 Hz, 8'-H), 4.383 (2H, m, J=5.61, 10.82 Hz, 9'-H$_2$), 5.044 (1H, d, J=7.13 Hz, 7'-H), 6.321 (1H, d, J=9.5 Hz, 3-H), 6.541 (1H, s, 5-H), 6.96-6.99 (2H, m, 2', 6'-H), 7.072 (1H, d, J=8.5 Hz, 5'-H) and 7.603 (1H, d, J=9.5 Hz, 4-H); cmr (25.05 MHz, CDCl$_3$) 170.2 (ArOCOCH$_3$), 168.5 (t, J=7.0 Hz, -CH$_2$OCOCH$_3$), 160.4 (dd, J=4.3, 11.0 Hz, C-2), 151.7 (m, C-3'), 145.9 (m, C-6), 143.5 (dd, J=163.0, 5.5 Hz, C-4), 140.9 (m, C-4'), 138.8 (m, C-8a), 137.0 (br d, J=9.2 Hz, C-7), 133.6 (bd dd, J=5.2, 7.6 Hz, C-1'), 131.7 (br s, C-8), 123.3 (d, J=163.6 Hz, C-5'), 119.9 (ddd, J=4.3, 7.3, 163.0 Hz, C-6'), 114.4 (d, J=172.7 Hz, C-3), 111.9 (dt, J=1.8, 7.9 Hz, C-4a), 111.5 (ddd, J=4.6, 6.4, 157.7 Hz, C-2'), 100.5 (dd, J=162.0, 4.0 Hz, C-5), 76.7 (bd d, J=154.4 Hz, C-7'), 75.1 (bd d, J=3.0, 149.2, C-8'), 62.4 (bd t, J=150.1 Hz, C-9'), 56.3 (q, J=144.8 Hz, Ar-OCH$_3$), 56.0 (q, J=144.6 Hz, Ar-OCH$_3$), and 20.6 (q, J=130.0 Hz, -COCH$_3$); ms m/z 470 (M$^+$, 16%), 428 (8), 368 (40), 291 (9), 223 (11), 222 (80), 219 (9), 180 (14), 179 (29), 162 (18), 161 (10), 147 (10), 131 (16), 119 (14), 91 (12), 79 (12), and 43 (100).

METHYLATION OF CLEOMISCOSIN A.—Cleomiscosin A (**1**, 30 mg) was treated with ethereal methanolic CH$_2$N$_2$[8] at room temperature overnight. Evaporation afforded a crude solid which was crystallized from MeOH to afford colorless needles of methyl cleomiscosin A (**3**, 20 mg), mp 210-212°; ir ν max (KBr) 3440, 3070, 2940, 2840, 1700, 1610, 1580, 1520, 1450, 1425, 1380, 1355, 1305, 1270, 1235, 1200, 1165, 1135, 1105, 1090, 1065, 1030, 950, 855, and 750 cm^{-1}; pmr (60 MHz, CDCl$_3$) δ 2.68 (1H, m, OH exchanged with D$_2$O), 3.62 (2H, m, 9'-H$_2$), 3.88 (9H, s, 3×-OCH$_3$), 4.13 (1H, m, 8'-H), 5.08 (1H, d, J=8.0 Hz, 7'-H), 6.28 (1H, d, J=9.5 Hz, 3-H), 6.51 (1H, s, 5-H), 6.96 (3H, m, 2', 5', 6'-H), and 7.59 (1H, d, J=9.5 Hz, 4-H); ms m/z 400 (M$^+$, 46%), 382 (12), 370 (7), 367 (8), 342 (4), 341 (4), 322 (10), 321 (43), 307 (17), 306 (82), 292 (18), 291 (5), 276 (7), 263 (5), 262 (21), 249 (13). 219 (16), 195 (14). 194 (100), 176 (16), 175 (11), 166 (13), 165 (17), 164 (18), 163 (14), 152 (15), 151 (94), 139 (14), 138 (76), 131 (10), 130 (12), 123 (15), 119 (12), 116 (16), 107 (11), 102 (11), 91 (23), 79 (31), 77 (28), 64 (11), 62 (12), 54 (23), and 51 (27).

ACETYLATION OF METHYL CLEOMISCOSIN A.—Methyl cleomiscosin A (**3**, 10 mg) was treated with Ac$_2$O-pyridine (1:1, 0.5 ml) at 100° for 1 h. Work-up in the usual way afforded a mono-acetate (**4**, 8 mg) as colorless needles, mp 200-202°; ir ν max (KBr) 2960, 2930, 2870, 2855, 1725, 1710, 1610, 1575, 1520, 1460, 1445, 1420, 1380, 1310, 1280, 1265, 1230, 1190, 1160, 1140, 1130, 1060, 1020, 990, 950, 930, 875, 860, 835, 805, 775, 760, and 720 cm^{-1}; pmr (60 MHz, CDCl$_3$) δ 2.08 (3H, s, 9'-OAc), 3.89 (9H, s, 3×-OCH$_3$), 4.24 (3H, m, 8'-H, 9'-H$_2$), 5.03 (1H, d, J=7.0 Hz, 7'-H), 6.31 (1H, d, J=9.4 Hz, 3-H), 6.90 (3H, m, 2', 5', 6'-H), and 7.60 (1H, d, J=9.4 Hz, 4-H); ms m/z 442 (M$^+$, 60%), 414 (8), 383 (15), 382 (63), 367 (22), 291 (8), 237 (13), 236 (100), 219 (10), 208 (11), 194 (31), 193 (48), 178 (10), 177 (60), 176 (52), 175 (29), 165 (26), 164 (10), 163 (12), 161 (22), 151 (20), 146 (21), 138 (17), 133 (13), 131 (12), 119 (15), 118 (12), 115 (10), 105 (10), 103 (12), 91 (20), 79 (28), 77 (16), 54 (11), 51 (17), and 43 (87).

BIOLOGICAL ACTIVITY.—Cleomiscosin A (**1**, NSC-346197) was inactive in the KB test system in cell culture (ED$_{50}$ 4.9 μg/ml), but was weakly active (ED$_{50}$ 3.8 μg/ml) in the P-388 lymphocytic leukemia test system in cell culture (5).

RESULTS AND DISCUSSION

The isolate displayed a molecular ion at m/z 386, in agreement with a molecular for-

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mula of $C_{20}H_{18}O_8$. A coumarin nucleus was evident from the ir (ν max 1710 and 1610 cm^{-1}) and pmr (doublets, $J=9.5$ Hz at 6.39 and 7.70 ppm) spectra, together with two aromatic methoxy (3.70 and 3.78 ppm) and two hydroxy groups (7.19 and 11.18 ppm). Three protons on a second aromatic nucleus were observed as well as one singlet aromatic proton (6.70 ppm). The coumarin nucleus must, therefore, be trisubstituted and, from a consideration of the number of oxygens atoms to be placed, trioxygenated (either 5,6,7- or 6,7,8-). Subtraction of the coumarin nucleus (C-9) from the C-10 skeleton left another C-9 unit to be accounted for. Six of these carbon atoms are aromatic, and, on biogenetic grounds, it was considered that the second unit was a C-6-C-3, phenylpropane moiety. Cleomiscosin A formed a diacetate derivative (**2**), and a monomethyl ether (**3**), which could be acetylated. In the diacetate at 60 MHz, singlets were observed at 2.04 and 2.30 ppm, typical for aliphatic and aromatic acetate groups, respectively. Two protons in the complex region 3.12-3.66 ppm of **1** shifted downfield slightly (~0.75 ppm) on acetylation, indicating that the isolate contained a primary hydroxy group. With the observation of a deshielded (5.53 ppm) doublet ($J=8.0$ Hz) typical for a benzylic methine substituted by oxygen, and its typical *trans*-coupling, it became apparent that the final degree of unsaturation to be accounted for was in the form of a *trans*-substituted 1,4-dioxane ring. In support of this, a prominent (84% of base peak) ion at m/z 180 could be rationalized in terms of a retro-Diels Alder reaction in the dioxane ring (9,10). Thus, with all the carbons, hydrogens, and oxygens and their functional groups accounted for, the problem of regiosubstitution could be subdivided into substitution on (a) the phenyl ring, (b) the coumarin nucleus, and (c) the dioxane ring.

The ion at m/z 180, which shifted to m/z 194 in **3** and m/z 236 in **4**, clarified that one phenolic group and one methoxy group were present on the aromatic nucleus. From biogenetic considerations and comparison with alternative substitution patterns, the multiplicity and chemical shift of the three remaining aromatic protons indicated that these groups should be at C-3' and C-4'. A distinction between a 3-hydroxy-4-methoxy- or a 4-hydroxy-3-methoxy-substituted phenyl ring was made (2) on the basis of the carbon-13 assignments observed and calculated for the diacetate derivative. These data suggested that a 4-hydroxy-3-methoxy phenyl moiety was present.

From the broad-band decoupled and off-resonance decoupled cmr spectra of the diacetate derivative, and by comparison with established data for the coumarin nucleus (11-13) and for related flavonolignan (14) and neolignan (15,16) derivatives, assignments for most of the carbons in the nucleus and the functional groups could be made.

The absence of homonuclear coupling between H-4 and H-8 (17) suggested that the substitution pattern was 6,7,8-. However, the relative orientations of the methoxy and dioxane units could place the methoxy group at C-6 (C-7, C-8 dioxane) or C-8 (C-6, C-7-dioxane). This distinction was made, and the substitution pattern confirmed, through nOe experiments. Irradiation of the aromatic methoxy group at 3.886 ppm caused a positive nOe (25%) in the adjacent proton (H-5), and a negative nOe (8%) in the doublet at 7.603 ppm (18). Irradiation of the singlet at 6.541 ppm caused a positive nOe for both the aromatic methoxy group at δ 3.886 (16%) and the *peri*-proton at H-4 (15%). The substitution pattern is, therefore, established to be 6,7,8- on the coumarin nucleus.

At 360 MHz, H-2' and H-6' appeared in the region δ 6.96-6.99, and H-5' as a sharp doublet ($J=8.5$ Hz) at δ 7.072, and this chemical shift difference permitted the distinction to be made between a 4-hydroxy-3-methoxy phenyl and a 3-hydroxy-4-methoxy phenyl moiety. Thus, irradiation of the methoxy group at δ 3.839 caused a 10% nOe at about δ 6.98, indicating that this methoxy group was at the 3'-position and the hydroxy in cleomiscosin A at C-4'.

Some resolution of the aliphatic protons was achieved through irradiation at δ 5.044, which, through observation of the difference spectrum, indicated collapse of the signal at δ 4.383 to a doublet of doublets (J=5.61, 10.82 Hz) and that at δ 4.103 to a triplet (J=5.7 Hz). The long-range coupling of H-7' to the two 9'-protons, although observed, was not measured.

Substitution on the dioxane nucleus by the hydroxymethyl and 4-hydroxy-3-methoxy-phenyl units led to the proposition of two alternative structures **1** and **5**. This functional group placement problem parallels the situation observed previously in the flavonolignan series of compounds (9).

The coupled carbon spectrum of cleomiscosin A diacetate revealed most of the $^1J_{CH}$, $^2J_{CH}$ and $^3J_{CH}$ coupling constants, and these are given in the experimental section. Observed values were quite typical of those observed previously for coumarins (12). The multiplicities and coupling constants of the signals at 137.0, 133.6, and 131.7 ppm indicated C-7 to be a broadened doublet (J=9.2 Hz) at 137.0 ppm, C-1' to be a broadened doublet of doublets (J=5.2, 7.6 Hz) at 133.6 ppm, and C-8 to be a broadened singlet at 131.7 ppm. These assignments are in agreement with the revised data of Hikino and co-workers (2). The multiplicity of C-4a (111.9 ppm) as a doublet ($^3J_{CH}$=7.9 Hz) of triplets ($^2J_{CH}$=1.8 Hz) confirms C-5 to be unsubstituted. In the aromatic nucleus, C-2' and C-6' are observed as doublets of doublets of doublets in which the $^2J_{CH}$ is quite substantial (4.6 and 4.2 Hz, respectively) (12).

However, these data do not distinguish between the two alternative structures **1** and **5** for cleomiscosin A. Hikino (2) resolved this problem through the selective irradiation of the C-7' and C-8' protons, observing the changes in multiplicity for C-7 and C-8, as well as several other carbons. But this technique relies on the unambiguous assignment of C-7 and C-8.

Although we were convinced of the assignment of C-7 and C-8 based on their multiplicities, we suggest an alternative method of deducing the structure, which is independent of the assignment of C-7 and C-8. In the technique we have developed, reliance is placed on (a) an aromatic proton β to one of the dioxane ring aromatic carbons, and (b) an observable $^3J_{CH}$ coupling across the dioxane ring oxygen between an aliphatic H and an aromatic C. The premise is that irradiation of the aromatic H simplifies the β carbon while irradiation of the benzylic H simplifies either the same (isomer A) or a different carbon (isomer B) (Figure 1).
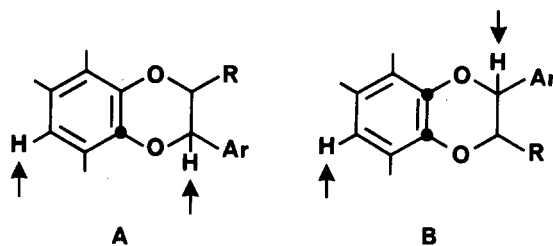

FIGURE 1.   Low-power single frequency selective decoupling
technique to distinguish isomers A and B.

In the case of cleomiscosin A diacetate, irradiation of the C-5 proton (6.54 ppm) simplified the signals at 100.5 (C-5), 137.0, 138.8 (c-8a), 140.9 (C-4'), and 143.5 ppm (C-4) but did not affect the signal at 131.7 ppm.[9] Irradiation at 5.04 ppm (H-7')

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[9]In practice, the close chemical shift of H-3 and H-5 also means that in this irradiation a number of carbons which couple with H-3 are also simplified.

now simplified the signals at 62.4 (C-9′), 76.7 (C-7′), 111.6 (C-2′), and 119.9 (C-6′), and markedly sharpened the signal at 137.0 (Figure 2).[10] Because this latter signal was affected from both irradiation experiments, isomer A is indicated to be correct, and cleomiscosin A has the structure **1**.
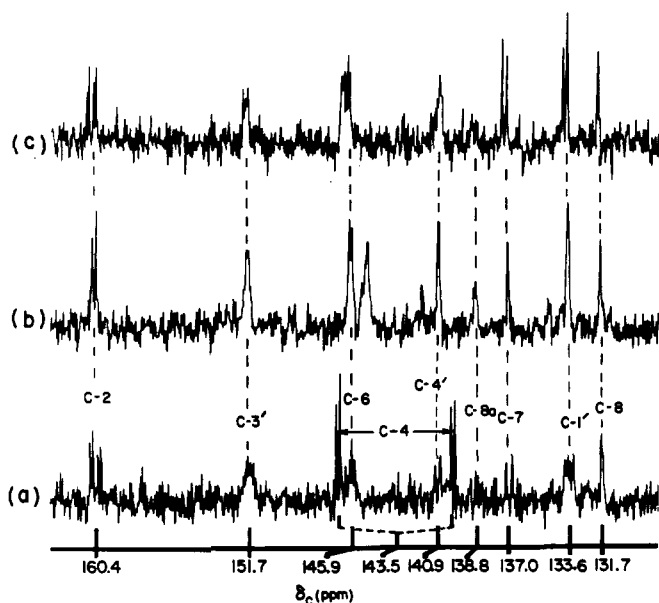

FIGURE 2.   Partial cmr spectrum (25.04 MHz) of cleomiscosin A
             diacetate (a) proton-coupled spectrum; (b) low-power
             single frequency selective decoupling of H-5 (δ 6.54); (c)
             low-power single frequency selective decoupling of H-7′
             (δ 5.04).

Coumarino-lignans are a relatively new, though not unexpected, group of natural products. Hikino and co-workers were the first to describe the structure determination of a member of this series (2), cleomiscosin A from *Cleome viscosa* (Capparidaceae). Prior to this, Gottlieb and co-workers had reported on the structure of propacin from *Protium opacum* in the family Burseraceae (3), but they were unable to distinguish between the two alternative structural isomers. Even earlier, the wood of *Acer saccharum* (Aceraceae) had yielded a compound in this series (19); once again the isomers could not be distinguished, although on biogenetic grounds, structure **5** was preferred. Recently, Rastogi and co-workers isolated aquillochin from *Aquilaria agallocha* (Thymelaeaceae) (20), but in spite of having complete pmr and cmr data, the two alternative structures could not be distinguished. On the other hand, Wagner and co-workers have demonstrated a structure for the corresponding 6-demethoxy derivative, daphneticin (21,22).

Further work by Hikino and co-workers afforded a second coumarinolignan cleomiscosin B (4). Cmr analysis indicated an identical structure with that proposed initially for cleomiscosin A. Additional irradiation experiments resulted in a reassignment of two signals in the cmr spectrum of the diacetate of cleomiscosin A and a revision in the structure from **5** to **1**. Comparison of the methyl ether of cleosandrion (8) established an identity with the methyl ester of **1** (4).

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[10]Based on their multiplicity and intensity the assignments of C-4′ and C-8a have been reversed (2,4).

Journal of Natural Products

This paper has described the isolation of cleomiscosin A (**1**) from three new sources, *Simaba multiflora, Soulamea soulameoides,* and *Matayba arborescens,* and two new plant families, the Simaroubaceae and Sapindaceae. Our structural conclusions, obtained independently using a combination of nOe and selective decoupling experiments, are in agreement with the revised structure (**1**) for cleomiscosin A, and all spectral data are comparable to those published. Direct comparison of our isolate, as its diacetate, with samples of the diacetates of cleomiscosins A and B, kindly provided by Professor Hikino, indicated our isolate to be identical with cleomiscosin A. The regio-isomer cleomiscosin B (**5**) was detected, but not isolated, in the extracts of each of these plants.

Biogenetically, cleomiscosin A and B may be derived from the coumarin fraxetin (**6**) and coniferyl alcohol (**7**) through a radical coupling process, as postulated for the formation of silybin and isosilybin (14). A biomimetic synthesis along these lines has been achieved (14). One may also predict that these derivatives will be formed from other *ortho*-dihydroxycoumarins (*e.g.,* aesculetin and daphnetin) and a *para*-hydroxy cinnamyl alcohol (*e.g.,* coniferyl alcohol, syringenin, and *p*-coumaroyl alcohol), and successful synthetic studies in this area have been reported recently (23).

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