TOTAL SYNTHESIS OF CLEOMISCOSIN B, A COUMARINOLIGNOID

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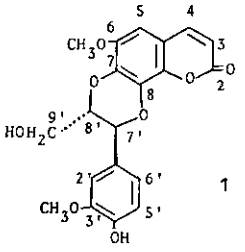
Abstract——Direct alkylation of fraxetin (3) with ethyl 2-bromo-3-(4-benzyoxy-3-methoxyphenyl)-3-oxopropionate (4) by utilizing about 1 equivalent of sodium hydride afforded the desired compound (6) as the major product, which was reduced with lithium borohydride in tetrahydrofuran to give the threo and erythro isomers (7a,b). The isomers underwent cyclization reaction with 5% sulfuric acid and subsequent hydrolysis of the cyclization product (10) furnished cleomiscosin B (1).

Coumarinolignoids are a relatively new class of natural product and to date five compounds (propacin,[1] cleomiscosin A (2),[2-5] cleomiscosin B,[3] aquillochin,[6] and daphneticin,[7]) have been isolated from the plant kingdom. These materials possess a novel skeleton which consists of a phenylpropane unit and a coumarin nucleus. From interest in the biological activity of these substances, particularly their cytotoxity, we have recently reported[8] the effective synthesis of cleomiscosin A (2). Cleomiscosin B (1), a regioisomer of cleomiscosin A, has been isolated[3] from the seeds of Cleome viscosa (Capparidaceae) and was recently found[5] in the bark of Aesculus turbinata (Hippocastanaceae). Cordell[9] and Merlini et al.[10] have achieved the synthesis of cleomiscosin B by oxidative coupling reactions of fraxetin (3) and coniferyl alcohol, and obtained cleomiscosin B as the minor product together with the main product, cleomiscosin A.

Herein, we wish to report a convenient synthesis of Cleomiscosin B from the readily available materials (fraxetin and ethyl 2-bromo-3-(4-benzyoxy-3-methoxyphenyl)-3-oxopropionate (4)).

In monoalkylation reaction of two hydroxyl groups of coumarins (fraxetin and 4-methylesculetin), it was demonstrated[8,11] that the monoanion is predominantly formed at C_7-OH position when treated with about 1 equivalent of sodium hydride

and then alkylation occurs regioselectively at C_7-OH. Reaction of fraxetin with 4 prepared by the method described in the literature,[12] in the presence of ca 1 equivalent of sodium hydride in anhydrous N,N-dimethylformamide was carried out



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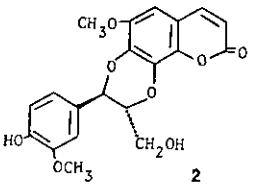

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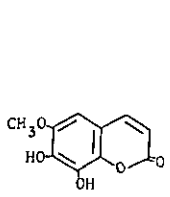

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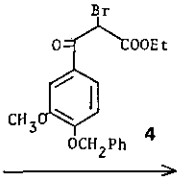

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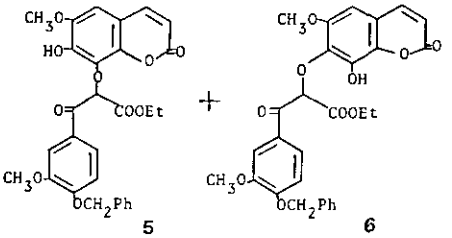
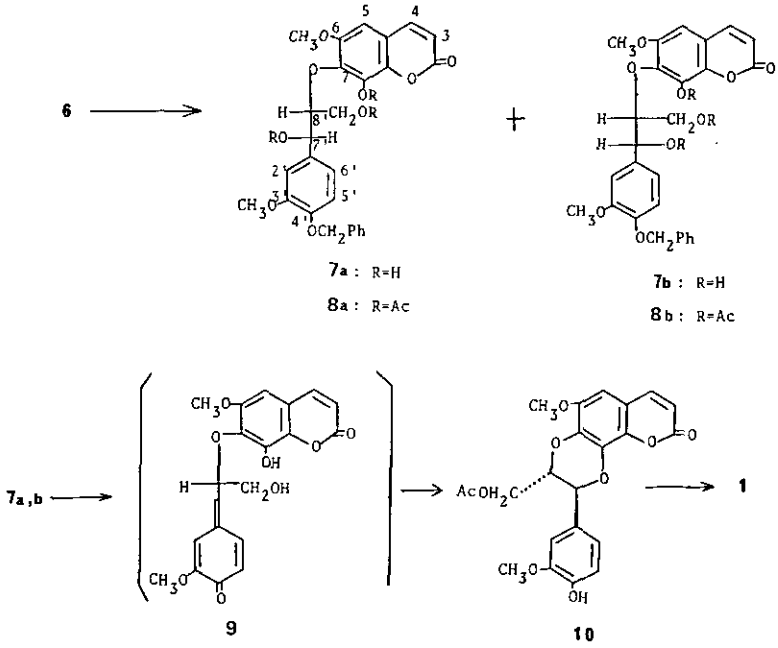


5 6

and afforded in good yield the desired condensation product (6) along with the minor regioisomer (5), synthesized[8] previously in this laboratory. Its IR spectrum of 6 exhibited the presence of the β-ketoester group at 1675 and 1745 cm^{-1}, and the ^{1}H-NMR spectrum and the mass spectrum were very similar to those of 5. The IR spectrum and mass spectral fragmentation suggested the structure (6) and all of the signals in the ^{1}H-NMR spectrum was also in agreement with the structure (6). Next, the compound (6) was reduced with lithium borohydride in terahydrofuran at room temperature to give an inseparable mixture of diols (7a,b) in 70% yield. The structure of the diols was determined on the basis of spectroscopic data. The high resolution mass spectrum of the diols displayed the molecular formula $C_{27}H_{26}O_9$. The ^{1}H-NMR spectrum revealed the signals due to two aromatic methoxyl groups at δ 3.83 and 3.85, one methylene protons of benzyl group at δ 5.07, and one aromatic proton (C_5-H) at δ 6.64. Further, the proton at C-7' indicated signals as two doublets at δ 5.24 and 5.34. The coupling constant of the doublet at δ 5.24 was 8 Hz and that for the doublet at δ 5.34 was 4 Hz. It is known[13] that the vicinal coupling constant (J_{AB}) of the threo form is larger than that of the erythro form. Hence, the signal observed at δ 5.24 was ascribed to the threo isomer (7a) and consequently the signal appeared at δ 5.34 was attributed to the erythro isomer

(7b). The ratio of the threo and erythro isomers was about 1:2 by the ^{1}H-NMR spectral analysis. The diols were transformed into a mixture of triacetate



derivatives (8a,b) by usual acetylation , which was separated by a preparative TLC and then characterized by spectroscopic data.

Finally, the diols were treated with 5% sulfuric acid in acetic acid at 60°C for 30 min to provide the desired cleomiscosin B monoacetate (10) in 63% yield. We postulate the following mechanism of the cyclization reaction via such quinone methide intermediate as that proposed in the synthesis of cleomiscosin A[8] and silybin.[14] The diols were first subjected to debenzylation and removal of the hydroxyl group (C_7,-OH) by sulfuric acid affording the quinone methide intermediate (9) and subsequently cyclized to cleomiscosin B monoacetate (10). The mass spectrum of 10 displayed a molecular ion peak at m/z 428 and further revealed the characteristic peak at m/z 222 which was formed from the retro Diels-Alder reaction[3] of the dibenzodioxane unit. In the ^{1}H-NMR spectrum, the proton signal of C-7' was observed as doublet at δ 4.94 whose coupling constant was typical[3] for trans-orientation of the benzodioxane moiety. Alkaline hydrolysis of 10 gave in quantitative yield cleomiscosin B (1).

EXPERIMENTAL

All melting points are uncorrected. Column chromatography was run on Merck silica gel 60 (70~230 mesh) and TLC was performed on glass plates precoated with

Kieselgel 60 F_{254} (Merck). Mass spectrum (MS) were recorded on a Hitachi M-52 spectrometer, and high resolution mass spectra and secondary ion mass spectrometry (SIMS) on a Hitachi M-80 spectrometer. IR spectra were obtained on a JASCO IRA-3 spectrophotometer. ^{1}H-NMR spectra were recorded on a JEOL JNM-PS-100 nuclear magnetic resonance spectrometer and ^{13}C-NMR spectra on a JEOL JNM-FX-100 Fourier transform spectrometer operating at 25.00 MHz, with tetramethylsilane as an internal standard. Chemical shifts are quoted in parts per million (s=singlet, d=doublet, dd=doublet of doublets, t=triplet, q=quartet, br=broad, m=multiplet).

Condensation of 3 with 4 (Formation of 5 and 6)——NaH (60% in mineral oil) (175 mg), washed twice with dry ether (5 ml), was suspended in dry DMF (10 ml). The slurry was cooled to 0°C under a stream of nitrogen and a solution of fraxetin (1.5 g) in dry DMF (10 ml) was slowly added. After the addition, the solution was stirred at room temperature for 1 h, cooled to 0°C, and a solution of 4[12] (2.91 g) in dry DMF (5 ml) was added dropwise. The mixture was stirred at room temperature for 8 h and then quenched by adding water (10 ml). The resulting mixture was extracted with AcOEt. The organic layer was washed with water, dried over Na_2SO_4, and evaporated to dryness. The residue was chromatographed on a silica gel column with a mixture of $CHCl_3$ and acetone (10:1) giving colorless needles (5) (380 mg, 10% based on consumed starting material) and colorless needles (6) (1.54 g, 80%) and fraxetin (750 mg) was recovered.[15]

Compound (5): The compound was recrystallized from AcOEt. Colorless needles. mp 157-158°C (lit.,[8] mp 157-158°C). TLC (silica gel/$CHCl_3$-acetone (10:1), Rf=0.47). SIMS m/z: 535 (M^++1). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3630, 1745, 1730, 1675, 1580. ^{1}H-NMR ($CDCl_3$) δ: 1.24 (3H, t, J=7 Hz, $OCH_2\underline{CH_3}$), 3.90, 3.95 (6H, 2 x s, 2 x OCH_3), 4.30 (2H, q, J=7 Hz, OCH_2CH_3), 5.25 (2H, s, CH_2Ph), 6.20 (1H, s, C_8-H), 6.25 (1H, d, J=10 Hz, C_3-H), 6.71 (1H, s, C_5-H), 7.00 (1H, d, J=8 Hz, C_5-H), 7.39 (5H, br s, 5 x aromatic protons), 7.58 (1H, d, J=10 Hz, C_4-H), 7.68 (1H, d, J=3 Hz, C_2-H), 7.95 (1H, dd, J=8, 3 Hz, C_6-H). The compound (5) was shown to be identical by direct comparison of physical and spectral properties with an authentic sample.[8]

Compound (6): The substance was recrystallized from AcOEt. Colorless needles. mp 160-161°C. TLC (silica gel/$CHCl_3$-acetone (10:1), Rf=0.46). Anal. Calcd for $C_{29}H_{26}O_{10}$: C, 65.16; H, 4.90. Found: C, 64.90; H, 4.82. SIMS m/z: 535 (M^++1). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 1745, 1730, 1675, 1580. ^{1}H-NMR ($CDCl_3$) δ: 1.18 (3H, t, J=7 Hz, $OCH_2\underline{CH_3}$), 3.76, 3.94 (6H, 2 x s, 2 x OCH_3), 4.26 (2H, q, J=7 Hz, OCH_2CH_3), 5.21 (2H, s, CH_2Ph), 5.76 (1H, s, C_8-H), 6.28 (1H, d, J=10 Hz, C_3-H), 6.40 (1H, s, C_5-H), 6.95 (1H, d, J=8 Hz, C_5-H), 7.36 (5H, br s, 5 x aromatic protons), 7.52

(1H, d, J=10 Hz, C_4-H), 7.63 (1H, d, J=3 Hz, $C_{2'}$-H), 7.72 (1H, dd, J=8, 3 Hz, $C_{6'}$-H).

Reduction of 6 with Lithium Borohydride (Formation of 7a,b)———A solution of (899 mg) in dry THF (8 ml) was added to a suspension of $LiBH_4$ (74 mg) in dry THF (5 ml) at 0°C under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 1.5 h and then carefully quenched by adding ice, and the mixture was extracted with AcOEt. The organic layer was washed with water, dried over Na_2SO_4 and evaporated. The residue was chromatographed on a silica gel column with a mixture of $CHCl_3$ and acetone (5:1) affording a colorless oil (7a,b) (580 mg, 70%). High MS m/z: 494.1575. Calcd for $C_{27}H_{26}O_9$ (M^+). Found: 494.1552. MS m/z: 494 (M^+), 476, 385, 286, 270, 234, 208. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3350, 1720, 1615, 1575. ^{1}H-NMR ($CDCl_3$) δ: 3.56-4.32 (3H, m, $C_{9'}$-H and $C_{8'}$-H), 3.83, 3.85 (6H, 2 x s, 2 x OCH_3), 5.07 (2H, s, CH_2Ph), 5.24 (1/3 H, d, J=8 Hz, $C_{7'}$-H), 5.34 (2/3 H, d, J=4 Hz, $C_{7'}$-H), 6.18 (1/3 H, d, J=10 Hz, C_3-H), 6.19 (2/3 H, d, J=10 Hz, C_3-H), 6.64 (1H, s, C_5-H), 6.72-7.08 (3H, m, $C_{2'}$-H, $C_{5'}$-H, and $C_{6'}$-H), 7.34 (5H, br s, 5 x aromatic protons), 7.54 (1/3 H, d, J=10 Hz, C_4-H), 7.55 (2/3 H, d, J=10 Hz, C_4-H).

Acetylation of 7a,b———A mixture of 7a,b (50 mg), Ac_2O (0.5 ml), and pyridine (0.5 ml) was stirred at room temperature for 2 h, then poured into water and extracted with AcOEt. The organic layer was washed with water, dried over Na_2SO_4, and evaporated. The residue was chromatographed on a preparative TLC with a mixture of benzene and AcOEt (2:1) giving (8a) (8 mg, 13%) and (8b) (32 mg, 51%).

Compound (8a): A colorless oil. TLC (silica gel/benzene-AcOEt (2:1), Rf=0.45). High MS m/z: 620.1891. Calcd for $C_{33}H_{32}O_{12}$ (M^+). Found: 620.1883. MS m/z: 620 (M^+), 518, 458, 427, 367, 335, 312, 293, 269, 251, 243, 208. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770, 1740, 1575. ^{1}H-NMR ($CDCl_3$) δ: 1.98, 2.01, 2.32 (9H, 3 x s, 3 x $COCH_3$), 3.85, 3.89 (6H, 2 x s, 2 x OCH_3), 3.92 (1H, dd, J=11, 4 Hz, $C_{9'}$-H), 4.32 (1H, dd, J=11, 3 Hz, $C_{9'}$-H), 4.98 (1H, m, $C_{8'}$-H), 5.10 (2H, s, CH_2Ph), 6.08 (1H, d, J=8 Hz, $C_{7'}$-H), 6.38 (1H, d, J=10 Hz, C_3-H), 6.64-7.06 (3H, m, 3 x aromatic protons), 6.70 (1H, s, C_5-H), 7.34 (5H, br s, 5 x aromatic protons), 7.59 (1H, d, J=10 Hz, C_4-H).

Compound (8b): A colorless oil. TLC (silica gel/benzene-AcOEt (2:1), Rf=0.44). High MS m/z: 620.1891. Calcd for $C_{33}H_{32}O_{12}$ (M^+). Found: 620.1877. MS m/z: 620 (M^+), 518, 458, 427, 367, 335, 312, 293, 269, 251, 243, 208. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770, 1740, 1575. ^{1}H-NMR ($CDCl_3$) δ: 1.90, 2.12, 2.20 (9H, 3 x s, 3 x $COCH_3$), 3.80, 3.89 (6H, 2 x s, 2 x OCH_3), 4.04 (1H, dd, J=13, 4 Hz, $C_{9'}$-H), 4.41 (1H, dd, J=13, 7 Hz, $C_{9'}$-H), 4.98 (1H, m, $C_{8'}$-H), 5.12 (2H, s, CH_2Ph), 6.00 (1H, d, J=4 Hz, $C_{7'}$-H), 6.33 (1H, d, J=10 Hz, C_3-H), 6.68-7.02 (3H, m, 3 x aromatic protons), 6.76 (1H, s, C_5-H), 7.34 (5H, br s, 5 x aromatic protons), 7.57 (1H, d, J=10 Hz, C_4-H).

Cleomiscosin B Monoacetate (10)———A solution of 7a,b (70 mg) in 5% H_2SO_4 (0.5 ml) and acetic acid (1 ml) was heated at 60°C for 30 min, then poured into water and extracted with AcOEt. And the organic layer was washed with water, dried over Na_2SO_4, and evaporated. The residue was chromatographed on a silica gel column with a mixture of $CHCl_3$ and acetone (10:1) giving a colorless oil (10) (38 mg, 63%). High MS m/z: 428.1106. Calcd for $C_{22}H_{20}O_9$ (M^+). Found: 428.1102. MS m/z: 428 (M^+), 368, 222, 208, 180, 179, 162. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 1735, 1725, 1615, 1575. ^{1}H-NMR ($CDCl_3$) δ: 2.05 (3H, s, $COCH_3$), 3.87, 3.90 (6H, 2 x s, 2 x OCH_3), 3.88-4.46 (3H, m, C_9,-H and C_8,-H), 4.94 (1H, d, J=8 Hz, C_7,-H), 5.76 (1H, br s, OH), 6.26 (1H, d, J=10 Hz, C_3-H), 6.51 (1H, s, C_5-H), 6.78-7.00 (3H, m, 3 x aromatic ptotons), 7.55 (1H, d, J=10 Hz, C_4-H).

Cleomiscosin B (1)———A mixture of 10 (35 mg), 1% NaOH (0.5 ml), and MeOH (0.5 ml) was stirred at room temperature for 2 h, then poured into water and extracted with AcOEt. And the organic layer was washed with water, dried over Na_2SO_4, and evaporated to afford an essentially pure solid (1). The solid was recrystallized from MeOH. Colorless needles (31 mg, 98%). mp 275-276°C (lit.,[3] mp 274°C; lit.,[5] mp 273-275°C; lit.,[10] mp 275-278°C). Anal. Calcd for $C_{20}H_{18}O_8$: C, 62.17; H, 4.70. Found: C, 61.98; H, 4.77. MS m/z: 386 (M^+), 368, 249, 219, 208, 181, 180, 162, 161, 152, 137, 124. IR ν_{max}^{KBr} cm^{-1}: 3400, 1710, 1610, 1570. ^{1}H-NMR (pyridine-d_5)) δ: 3.70, 3.82 (6H, 2 x s, 2 x OCH_3), 3.92 (1H, dd, J=13, 3 Hz, C_9,-H), 4.30 (1H, dd, J=13, 2 Hz, C_9,-H), 4.54 (1H, m, C_8,-H), 5.18 (1H, br s, OH), 5.54 (1H, d, J=8 Hz, C_7,-H), 6.39 (1H, d, J=10 Hz, C_3-H), 6.72 (1H, s, C_5-H), 7.24-7.56 (3H, m, C_2,-H, C_5,-H, and C_6,-H), 7.71 (1H, d, J=10 Hz, C_4-H). ^{13}C-NMR (pyridine-d_5) δ: 160.8 (s, C-2), 149.9 (s, C-3'), 149.2 (s, C-4'), 146.5 (s, C-6), 144.5 (d, C-4), 139.5 (s, C-9), 138.5 (s, C-7), 133.2 (s, C-8), 127.7 (s, C-1'), 121.7 (d, C-6'), 116.7 (d, C-5'), 113.9 (d, C-3), 112.4 (d, C-2'), 112.0 (s, C-10), 101.3 (d, C-5), 80.0 (d, C-8'), 77.6 (d, C-7'), 60.8 (t, C-9'), 56.3 (q, OCH_3), 55.9 (q, OCH_3). This compound was identical with an authentic sample[5] by mixed melting point and direct comparison of IR spectra (KBr).

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