PENTALENOLACTONE F, A NEW METABOLITE ISOLATED FROM *STREPTOMYCES* ISOLATION AND STRUCTURE ELUCIDATION

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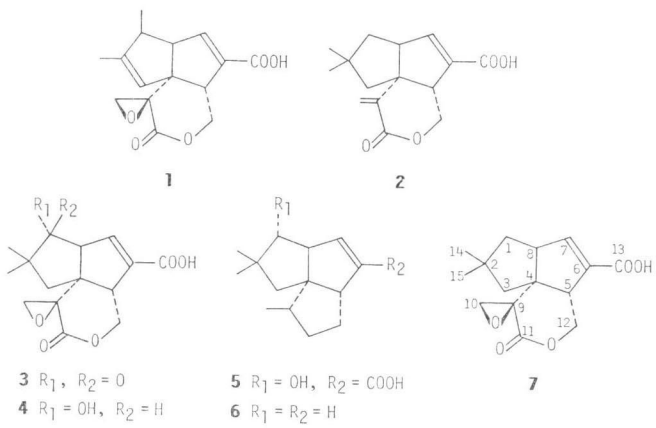
Since the isolation and identification of the sesquiterpene antibiotic pentalenolactone (**1**),[1~7] several other related metabolites have been recognized in strains of *Streptomyces*, notably pentalenolactones E (**2**),[8,9] G (**3**),[10] and H (**4**),[11] pentalenic acid (**5**),[11,12] and the parent hydrocarbon, pentalenene (**6**).[13~15]* Besides their novel structural features, these compounds are all potential intermediates or shunt metabolites in the biosynthesis of pentalenolactone, whose mevalonoid origin we have recently established.[16] We wish to report here a new metabolite, pentalenolactone F (**7**), isolated as the methyl ester, whose structure has been established based on the spectroscopic data reported below.

Treatment of the acidic extract from 2.4 liters of *Streptomyces* UC5319 with ethereal diazomethane gave a mixture of crude methyl esters which were purified by flash column chromatography using benzene - ethyl acetate (5: 1). The fractions containing pentalenic acid (**5**) methyl ester were further purified by preparative layer chromatography (silica gel, benzene - ethyl acetate, 5: 1) to give a mixture of the methyl esters of **5** and a new compound (**7**) which appeared together as an unresolved spot by TLC analysis. The final separation of these two substances was performed by HPLC using a hexanes - ethyl acetate, (1: 1) mixture as eluant to give pure **5** and **7** methyl esters.

The high resolution mass spectrum of **7** methyl ester established the elemental composition as $C_{16}H_{20}O_5$ and suggested that the new compound was a sesquiterpene. The infrared spectrum exhibited bands characteristic of a lactone (1765 cm^{-1}), an ester (1710 cm^{-1}), and a double bond (1630 cm^{-1}) but no hydroxyl absorbtion, thereby accounting for four of the five oxygen atoms and suggesting the presence of four rings and an ether function. Structure **7** was assigned based on analysis of the proton and ^{13}C NMR spectra. The 250 MHz ^1H NMR spectrum of **7** methyl ester showed a broad triplet at δ 6.88, characteristic of the conjugated olefinic (H-7) proton present in the majority of the known pentalenane metabolites. The 62.9 MHz ^{13}C NMR supported the presence of a trisubstituted double bond, C-6 (s, 132.2 ppm) and C-7 (d, 151.1 ppm). The high-field ^1H NMR revealed an AB quartet centered at δ 2.99 and 3.03 (J=5.2 Hz, 2H), indicative of a terminal epoxide residue (C-9, 58.2 ppm, s and C-10, 49.3 ppm, t). Further examination



1 2

3 R_1, R_2 = O
4 R_1 = OH, R_2 = H

5 R_1 = OH, R_2 = COOH
6 R_1 = R_2 = H

7

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* We have recently demonstrated the enzymatic conversion of farnesyl pyrophosphate to pentalenene (**6**) using a cell free extract of *Streptomyces* UC5319 and have established the conversion of pentalenene to **1**, **2**, **5**, and **7** by intact cells (D. E. CANE and A. M. TILLMAN, J. Am. Chem. Soc., in press).

of the spectroscopic data showed the presence of geminal methyls (H-14, 15, δ 1.01, s and 1.03, s, 6H; C-14, 15, 28.5, q* and 31.0, q*), a methoxyl (H-13′, 3.78, s, 3H; C-13′, 51.7 ppm, q), an ester (C-13, 164.1 ppm, s), and a lactone (C-11, 169.9 ppm, s). A pair of double doublets centered at δ 4.44 (J=11.9, 2.6 Hz, 1H) and 4.77 (J=11.9, 2.2 Hz, 1H) corresponded to the methylene protons at C-12 (66.4 ppm, t). Two methines were apparent at δ 3.44 (m, 2H) (C-5, C-8, 56.2, d* and 55.4, d*), as were two methylenes (H-1, δ 1.46, AB portion of ABX, J=13.5, 12.9 and less than 1 Hz and H-3, δ 1.45, m, combined area 4H; C-1, C-3, 47.8, t and 44.0, t). Irradiation at δ 3.44 collapsed the H-1 signals to a simple AB pattern, J=13.5 Hz. Two quaternary carbon signals corresponded to C-2 (40.9 ppm, s) and C-4 (54.1, s). The assigned structure **7** follows unambiguously from the above data and is supported by comparison with spectra of **1**[1,3,16] **2**,[8,16] **3**,[10] and **4**.[11] The epoxide stereochemistry has been assigned based on that established for pentalenolactone, and on the close agreement with the ^{13}C NMR signals for C-10 in pentalenolactone H(**4**) and pentalenolactone (**1**) itself, which appear at 49.5 ppm[11] and 47.1 ppm,[3,16] respectively.

Pentalenolactone F may be derived biogenetically by epoxidation of pentalenolactone E (**2**) and conceivably is a direct precursor of pentalenolactone H (**4**). Alternatively, **7** may be a shunt metabolite of a pathway from pentalenene (**6**) to pentalenolactone *via* pentalenic acid (**5**). Experiments to test the intermediacy of compounds **2**~**7** in pentalenolactone biosynthesis are underway.

Experimental

Proton and ^{13}C NMR spectra were obtained on a Bruker WM-250 spectrometer. Chemical shifts are reported in parts per million downfield of Me_4Si (δ 0). Multiplicities are as follows: s=singlet, d=doublet, t=triplet, q=quartet, b=broad, m=multiplet. Infrared spectra were recorded on a Perkin-Elmer Model 681 spectrophotometer. Routine mass spectra were obtained on an Hitachi-Perkin Elmer RMU-6D mass spectrometer at 70 eV. High resolution mass spectra were obtained on a VG Micromass 7070H at the University of Pennsylvania. High pressure liquid chromatography separations were carried out using a Waters M6000A solvent delivery system and RI 401 refractive index detector. Fermentations were carried out in a New Brunswick G25 gyrotory shaker.

Isolation of Pentalenolactone F (7) Methyl Ester

Streptomyces UC5319,* was maintained and grown as previously described.[16] Extraction with chloroform of the acidified culture filtrate of a 60-hour growth gave a brown oil which was treated with benzylamine in ether to precipitate the carboxylate salts. Regeneration of the acids and reaction with diazomethane gave a mixture of methyl esters which was purified by flash column chromatography (silica gel, benzene - ethyl acetate, 5: 1) followed by preparative layer chromatography (silica gel, benzene - ethyl acetate, 5: 1) to give a mixture (2: 1, one spot by TLC) of **5** and **7** methyl esters. Separation of these metabolites was accomplished by HPLC on μ Porasil (7.8 mm × 30 cm, hexanes - ethyl acetate, 1: 1, 3.0 ml/minute) and provided pentalenic acid methyl ester (**5**) (*ca.* 8 mg/liter culture; k′ 0.32) and pentalenolactone F methyl ester (*ca.* 5 mg/liter culture; k′ 0.74). **7** Methyl ester: ^{1}H NMR ($CDCl_3$): δ 6.88 (bt, 1H), 4.77 (dd, J=11.9, 2.2 Hz, 1H), 4.44 (dd, J=11.9, 2.6 Hz, 1H), 3.77 (s, 3H), 3.44 (m, 2H), 2.99, 3.03 (ABq, J=5.2 Hz, 2H), 1.46 (AB portion of ABX, J=13.5, 12.9, $\leq$1 Hz) and 1.45 (m), (combined area 4H), 1.03 (s, 3H), 1.01 (s, 3H); ^{13}C NMR ($CDCl_3$) 169.9 (s), 164.1 (s), 151.1 (d), 132.2 (s), 66.4 (t), 58.2 (s), 56.2 (d), 55.4 (d), 54.1 (s), 51.7 (q), 49.3 (t), 47.8 (t), 44.0 (t), 40.9 (s), 31.0 (q), 28.5 (q); IR $\lambda_{max}^{CHCl_3}$ 1765 cm^{-1} (lactone), 1710 (ester), 1630 (double bond). $C_{16}H_{20}O_5$: m/z, M^+ calcd. 292.1311, found 292.1326.

Note Added in Proof

We have recently confirmed the structure of pentalenolactone F by total synthesis of the racemic methyl ester (D. E. CANA and P. J. THOMAS, to be submitted.),

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* These assignments may be reversed.

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