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STUDIES ON THE BIOSYNTHESIS OF PENTALENOLACTONE, PART II[1)]
ISOLATION OF PENTALENIC ACID AND PENTALENOLACTONE H

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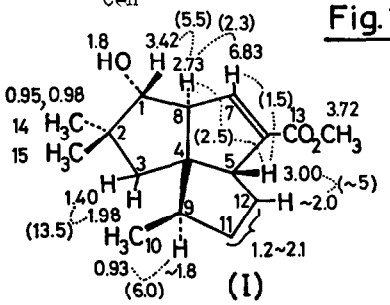
In the previous paper[1)] we reported the structure of pentalenolactone G, a shunt pathway product of pentalenolactone biosynthesis. As a result of further screening for biosynthetic intermediates, we have isolated the less oxidized metabolites named pentalenic acid and pentalenolactone H from the fermentation broth of Streptomyces sp.

From the acidic fraction containing pentalenolactone G[1)], was isolated pentalenic acid as its methyl ester (I) by preparative tlc (benzene/ethyl acetate = 3:1, Rf 0.48).[2)]

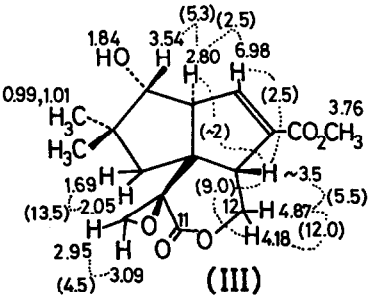
I, $C_{16}H_{24}O_3$ (M^+ m/e found 264.1705, calcd. 264.1725), oil, $\nu_{max}^{CHCl_3}$ 3450 cm^{-1}(OH), 1710 and 1630(α,β-unsaturated ester), λ_{max}^{MeOH} 227 nm (ε 5400) gave upon treatment with acetic anhydride/pyridine a monoacetate, $C_{18}H_{26}O_4$ (M^+ m/e 306, $M^+-CH_3CO_2H$ found 246.1609, calcd. 246.1620), m.p. 67-70°C, δ_H 1.67 (CH_3CO_2-) and 4.63 (d, J=5.0 Hz, $CH_3CO_2\underline{CH}$-).

^1H- and ^{13}C-nmr spectral data[3)] of I and comparison with those of a dihydro derivative (III) of pentalenolactone G (vide infra) revealed the partial structure from C_1 to C_8 shown in Fig.1. (The values show δ_H and those in parentheses are coupling constants in Hz). $^{13}C-\{^1H\}$ Long range selective proton decoupling (LSPD), a technique extensively exploited in the structural elucidation of pentalenolactone G (II)[1)], proved the relationship between gem-dimethyl and C_1. Thus, irradiation at methyls (δ_H 0.96) collapsed the C_1 methin to a sharper signal and eliminated long range coupling ($^3J_{C-H}$) from methyl resonances[1)] at 21.7 and 27.2 ppm.

Fig.1



(I)



(III)

C-1:85.5, C-2:42.8, C-3:32.9, C-4:60.4
C-5:57.8[+], C-6:137.9, C-7:144.3, C-8:67.5[+]
C-9:44.6, C-10:17.2, C-11:45.4*, C-12:28.7
C-13:165.4, C-14:21.7, C-15:27.2, OCH_3:51.7

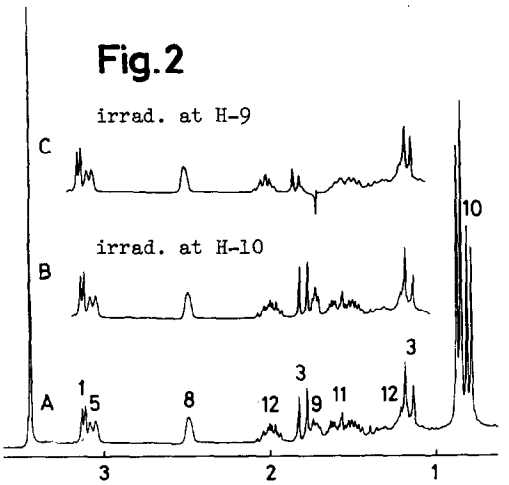
C-1:84.3, C-2:43.3, C-3:48.8, C-4:51.7
C-5:55.7, C-6:135.0, C-7:146.5, C-8:55.7
C-9:60.0, C-10:49.5, C-11:170.8, C-12:67.6
C-13:163.0, C-14:22.9, C-15:27.2, OCH_3:51.8

*See footnote 4. [+]Assignments of these carbons were confirmed by selective decoupling in $CDCl_3$.

(II):1-keto derivative of **(III)**

The established partial structure leaves two methylenes and a methin to form a five membered ring substituted by a methyl, the position of which was determined by the FT ^{1}H-nmr spectra (270 MHz, 0.367 Hz/data point) of I in C_6D_6 (Fig. 2).

The irradiation of a complex multiplet at 2.0 ppm collapsed a broad doublet (H_5) at 3.06 ppm to a broad singlet with a methyl doublet at 0.80 ppm unchanged (not shown in Fig. 2). Therefore, the methyl should be located either at C_9 or C_{11}. Were the methyl at C_{11}, the perturbation of the methin resonance (H_9) at 1.73 ppm which was coupled to the methyl signal (Fig. 2B) would collapse the very complicated methylene at 1.4-1.7 ppm to an AB quartet. This turned out not to be the case (Fig. 2C). It follows therefore that the methyl under consideration is located at C_9.



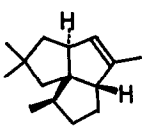
The stereochemistry at C-1 and C-9 in I

Reduction of II with $NaBH_4$ followed by separation by tlc (benzene/ethyl acetate =3:1) gave two epimeric dihydro derivatives in the ratio of ~3:1 (major[5]:minor). Therefore, the configuration at C-1 of the minor product (III) was deduced to be R.

The ^{1}H- and ^{13}C-nmr spectra of III, $C_{16}H_{20}O_6$ (M^+ m/e 308, M^+-H_2O found 290.1153, calcd. 290.1154), oil $\nu_{max}^{CHCl_3}$ 3680, 1765 and 1710 cm^{-1}, proved the same configuration at C-1 of I (Fig. 1). On the other hand, the following spectral data of the major product (IV) were apparently different from those of I; H_1 3.75, H_8 3.1 ($J_{1,8}$=7.3 Hz), H_3 1.85 ppm (singlet), C-1 80.8 and C-8 55.8 ppm.

It should be noted that C-8 was suffered marked downfield shift on going from III or IV to I (55.7 and 55.8 → 67.5 ppm). This is evidently caused by the lack in I of the γ-effect by C-10 which was operating in III and IV. Accordingly, the configuration of C-9 in I must be S. The significant upfield shift of C-3 was also due to the γ-effect by C-10. Thus, the structure of I has been unambiguously determined as shown in Fig. 1.

It is interesting to note that a compound shown which was obtained by formolysis of protoilludyl cation equivalents[6] possesses the same stereochemistry as I.



Isolation of pentalenolactone H Based on biosynthetic considerations, we tried to isolate III from the same fraction containing I and II. Preparative tlc (benzene/ethyl acetate =3:1) followed by HPLC (n-hexane/ethyl acetate = 3:1, μporasil) gave a UV absorbing compound which was completely identical with III by GC/MS analysis. Thus, III which we named pentalenolactone H has been shown to be a pivotal intermediate to pentalenolactone and pentalenolactone G.

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References and Footnotes

1) For part I see H. Seto et al. Tetrahedron Lett. 1978, 923. 2) This compound was also isolated from Streptomyces sp. 661 together with pentalenolactone by A. Tamura et al, Dainippon Pharmaceutical Co. 3) Unless otherwise stated, ^{1}H- and ^{13}C-nmr spectra were taken in $CDCl_3$ at 100 MHz and 25.05 MHz, respectively. Chemical shifts are expressed in ppm from internal TMS. 4) The assignment of this carbon was made by selective decoupling based on 270 MHz spectral data in C_6D_6. 5) this compound was reported in ref.1. 6) Y. Ohfune et al. Tetrahedron Lett. 1976, 2869

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