Notes

STUDIES ON THE BIOSYNTHESIS OF PENTALENOLACTONE. VII[1]. ISOLATION OF PENTALENOLACTONES P AND O

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In previous papers, we reported the structures of biosynthetic intermediates of a sesquiterpene antibiotic pentalenolactone[2], *i.e.* pentalenolactones G[3] and H[4], pentalenic acid[4], a hydrocarbon pentalenene[5] and more recently deoxypentalenylglucuron[6]. Independently CANE *et al.* obtained pentalenolactones E[7] and F[3]. Very recently we have confirmed the structure of pentalenolactone G by X-ray analysis and revised the structure of pentalenolactone F[1] (see Fig. 2).

We also reported the isolation of pentalenolactones P[8] (possessing a cyclopropane ring) and O[8] (containing additional oxygen) from *Streptomyces omiyaensis*[10]. In this paper the structure elucidation of these two metabolites will be reported in detail. Pentalenolactone O seems to be identical with arenaemycin D reported by ZÄHNER *et al.*[11].

Pentalenolactones P (**1**) and O (**2**) were isolated as their free acids from the fermentation broth of *S. chromofuscus* by adsorption on activated carbon, solvent extraction followed by silica gel column chromatography (benzene - ethyl acetate, 9:1). **1** and **2** were obtained from fractions 171~196 and 221~250, respectively, while fractions 51~170 and 197~220 gave known metabolites, pentalenolactone and pentalenolactone G, respectively.

On treatment with diazomethane, **1**, oil, $C_{15}H_{16}O_5$; EI-MS M^+ (*m/z*) 276; IR ($CHCl_3$) 1770 (strained lactone), 1700 (carboxylic acid) and 1630 cm^{-1} (double bond), gave a monomethyl ester (**3**), $C_{16}H_{18}O_5$, EI-MS M^+ (*m/z*) found 290.1136, calcd 290.1154. ^1H NMR spin decoupling experiments together with ^{13}C NMR spectral data revealed the partial structure of **1** as shown in Fig. 1.

The characteristic chemical shifts of C-14 methylene protons at 0.09 and −0.11 imply a cyclopropane structure comprising C_1, C_2 and C_{14}. The high field shift[12] of these carbon signals and large ^{13}C-^1H coupling constants[13] corroborate this conclusion (C-1: 28.9 ppm, J_{C-H}=168 Hz, C-2: 28.0 ppm, C-14: 16.2 ppm, J_{C-H}=160 Hz). The NMR spectral data of the remaining moiety of **1** were very close to those of pentalenolactone H[4] (**5**) and proved the presence of the isolated epoxide methylene (2.20 and 2.68 ppm, J_{gem}=5.0 Hz), an ABX spin system ascribed to $-C(=O)-O-CH_AH_B-CH_X-$ (3.84, 4.56 and *ca.* 2.8 ppm, $J_{A,B}$=12.0, $J_{A,X}$=8.5 and $J_{B,X}$=6.5 Hz) and a *tert*-methyl (0.95 ppm, singlet). These experimental results and struc-

Fig. 1. Partial structures of pentalenolactone P as revealed by NMR spectral analysis.

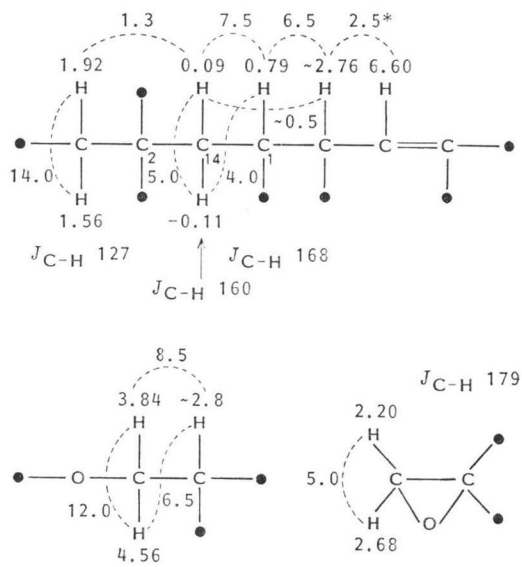
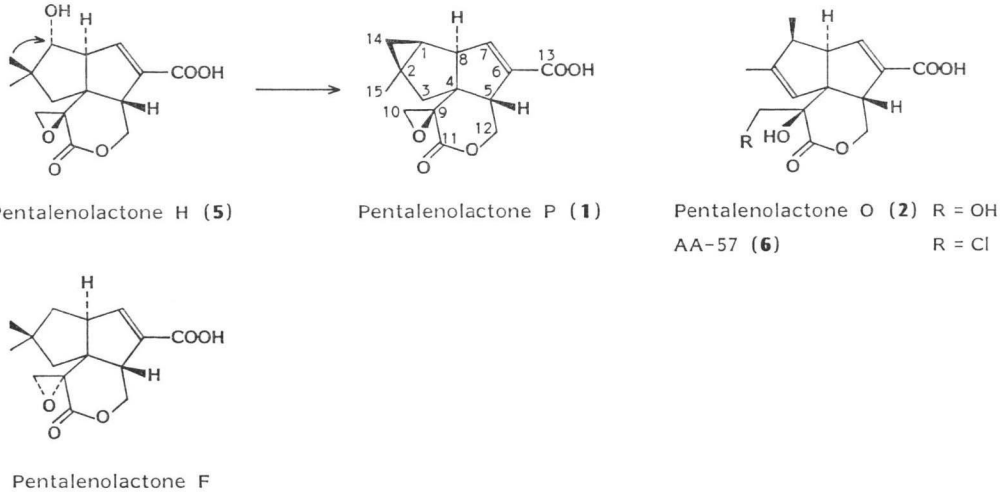


Fig. 2. The structures of pentalenolactones P, O, H and F.



Pentalenolactone H (**5**)

Pentalenolactone P (**1**)

Pentalenolactone O (**2**) R = OH

AA-57 (**6**) R = Cl

Pentalenolactone F

Table 1. ^{13}C Chemical shifts of pentalenolactones P (**1**, in $CDCl_3$) and O (**2**, in $CDCl_3$ - CD_3OD, 3: 1).

Carbon	1	2
1	28.9	44.8
2	28.0	145.8
3	43.9	124.6
4	58.0	62.9
5	53.0*	49.5
6	132.4	134.5
7	149.5	146.2
8	53.7*	55.4
9	56.4	77.1
10	49.5	62.7
11	170.2	173.6
12	67.9	69.3
13	168.4	166.6
14	16.2	15.4
15	21.9	14.4

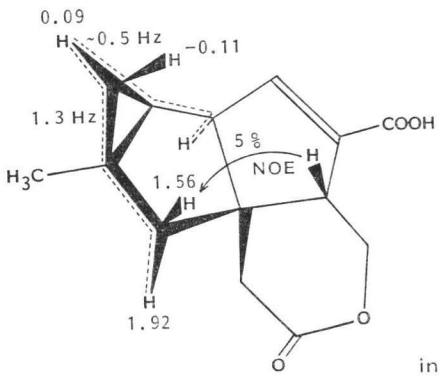
* Assignments may be exchanged.

tural comparison with **5** established the structure of **1** as shown in Fig. 2. The ^{13}C NMR spectral data of **1** (Table 1) are in good agreement with this structure.

The configuration of C-1 was deduced to be (*S*) based on the W-form type long range coupling between H-14 and H-8 (^4J=*ca.* 0.5 Hz), and H-14 and H-3α (^4J=1.3 Hz) (see Fig. 3). H-3α was distinguished from H-3β which showed 5% NOE enhancement upon irradiation of H-5.

This stereochemistry is consistent with biosynthetic consideration that, following the elimination of the OH group, the β-methyl carbon

Fig. 3.



at C-2 of pentalenolactone H would participate in the cyclopropane ring formation (Fig. 2).

2, mp 203~205°C; $[\alpha]_D^{25}$ −160.1° (*c* 3.0, $CHCl_3$); $C_{15}H_{18}O_6$: found C 61.19, H 6.21, calcd C 61.21, H 6.17; IR (KBr) 3400, 1720 (lactone), 1700 (ester) and 1640 cm^{-1} (double bond), gave a monomethyl ester (**4**), mp 169~171°C; $C_{16}H_{20}O_6$; EI-MS M^+ (*m/z*) found 308.1284, calcd 308.1260, IR (KBr) 3600, 1730 and 1720 cm^{-1}, upon treatment with diazomethane. The ^1H NMR spectrum of **4** showed the presence of two olefinic protons at 6.73 and 5.41 ppm, an allylic methyl at 1.68 ppm and a *sec*-methyl at 1.04 ppm. These spectral features are reminiscent of those of pentalenolactone itself. However, the epoxide methylene protons in pentalenolactone at 2.60 and 3.10 ppm were replaced by an oxymethylene (3.39 and 4.06 ppm,

J=12 Hz) in **4** indicating the cleavage of the epoxide ring which was also supported by the shift of the lactone absorption band (1765 cm^{-1} in pentalenolactone and 1720 cm^{-1} in **3**). In agreement with this structure, the ^{13}C NMR spectral data of **2** (Table 1) is very close to those of AA-57[14] (**6**), which had been obtained by treatment of pentalenolactone with hydrochloric acid, excepting for the downfield shift of C-10 from 46.8 ppm in **6** to 62.7 ppm in **2**. Treatment with 35% $HClO_4$ of **2** in THF/H_2O at room temp for two days gave a product which was completely identical with a natural product in every respects. Therefore, the structure of **2** is concluded to be as shown in Fig. 2.

Experimental

Proton and ^{13}C NMR spectra were obtained on a Jeol GX-400 spectrometer operating at 400 MHz and 100 MHz, respectively. Routine and high resolution EI mass spectra were taken on a Jeol JMS-01SG.

Isolation of **1** and **2**

Acidified filtered broth of a 72-hour growth of *S. chromofuscus* was treated with activated carbon. After washing with water, the carbon was eluted with 50% aqueous acetone and the eluate was concentrated *in vacuo* to give an aqueous residue which was extracted with ethyl acetate at pH 2. Concentration of the solvent fraction gave a crude powder which was subjected to silica gel column chromatography (benzene - ethyl acetate, 9:1, 2×50 cm). Eluates were collected in 15 ml each and monitored by TLC for UV absorbing compounds.

Pentalenolactone related metabolites were separated as follows; fractions 51~170, pentalenolactone; fractions 171~196, **1**; fractions 197~220, pentalenolactone G; fractions 221~250, **2**. Each component was further purified by Sephadex LH-20 column chromatography (MeOH).

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