Communications to the editor

STUDIES ON THE BIOSYNTHESIS OF PENTALENOLACTONE. III.[1] ISOLATION OF A BIOSYNTHETIC INTERMEDIATE HYDROCARBON, PENTALENENE

Sir:

Pentalenolactone is an antibiotic active against Gram-positive and negative bacteria as well as fungi[2] and produced by several species of Streptomyces such as *Streptomyces chromofuscus**, *S. griseochromogenes*** and *S. baarnensis* (an AA-57 producing organism)[3]***. Recently its mechanism of action was reported[4] to inhibit the enzyme glyceraldehyde-3-phosphate dehydrogenase.

During biosynthetic studies of this antibiotic, we have isolated pentalenolactone G[5], H[1] and pentalenic acid[1] as acidic biosynthetic intermediates of pentalenolactone (Fig. 1) from the filtered fermentation broth of *S. chromofuscus*. More recently, pentalenolactone E was obtained by CANE *et al.*[6] As a next step to shed light on the pathway from unknown precursors to pentalenic acid, a screening was carried out for less oxidized (neutral) metabolites in the mycelia of the above producing organisms. This resulted in the isolation from *S. griseochromogenes* of a sesquiterpene hydrocarbon named pentalenene (**I**)†.

Isolation procedures of **I** were as follows. *S. griseochromogenes* was cultivated for 60 hours in a jar fermentor containing a medium used for preparation of AA-57[3] and the fermentation broth was filtered. The mycelial cake obtained was extracted with 60% aqueous acetone and after removal of the solvent under reduced pressure, the residual solution was extracted with benzene. The benzene layer was concentrated to

Fig. 1.

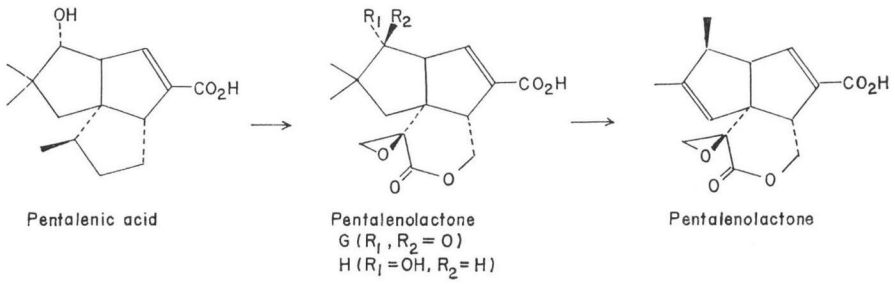
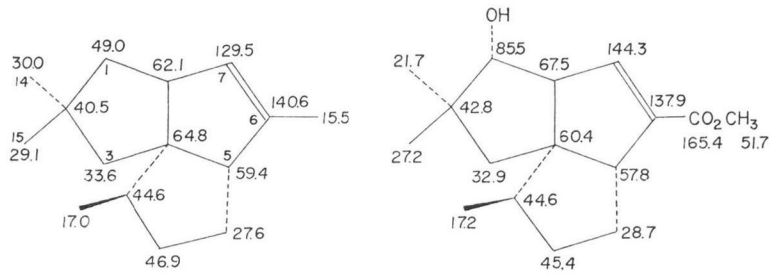


Fig. 2. ^{13}C Chemical shifts of pentalenene and the methyl ester of pentalenic acid.



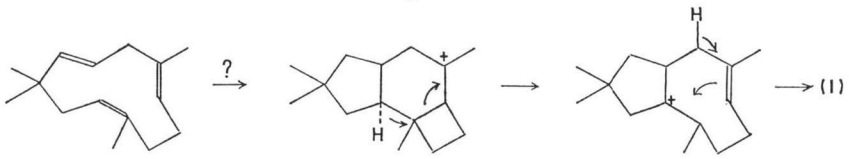
* The organism used in our previous studies[1,5] was classified as described herein.

** This organism, formerly called *Streptomyces* sp. 661[1], was isolated by Dainippon Pharmaceutical Co. (A. TAMURA, unpublished data).

*** Production of pentalenolactone together with AA-57 by this organism has been confirmed (H. SETO and S. AIZAWA, unpublished data).

† The term pentalenane is proposed for the saturated hydrocarbon.

Fig. 3.



a small volume and *n*-hexane was added. After removal of precipitates by filtration, the filtrate was applied to a silica gel column and developed with *n*-hexane. Fractions giving a single peak by gas chromatographic analysis (retention time 7.2 minutes, column 1.5% OV-1 on Shimalite, 0.4 cm × 1 m, 100°C, flow rate of N_2 gas, 40 ml/minute) were combined and concentrated to give a pure sample of **I**, oil, $C_{15}H_{24}$ (M^+, *m/e* found 204.1946, calcd. 204.1876), $[\alpha]_D^{25}$ +11.8° (*c* 6.8, $CHCl_3$).

The ^{1}H-nmr spectrum of **I** in $CDCl_3$ showed the presence of a sec-methyl (0.88 ppm, 3H, d, J=7.0 Hz), two geminal methyls (0.97, 6H, s), an allylic methyl (1.60, 3H, very broad s), two allylic methines (~2.25, 2H, unresolved multiplets) and an olefinic proton (5.13, 1H, unresolved multiplet).

The ^{13}C-nmr spectrum of **I** revealed the following carbons: $4\times CH_3$, $4\times CH_2$, $3\times CH$, $2\times -\overset{|}{\underset{|}{C}}-$ and –HC=C–. Comparison of the ^{13}C-nmr spectra of **I** and pentalenic acid methyl ester[1] strongly supports the structure of **I** as shown in Fig. 2. The downfield shift of C-14 by 7~8 ppm in **I** was caused by the lack of a hydroxy group present at C-1 in **II**. The upfield shift of C-7 in **I** by 15 ppm reflects the structural change of an α,β-unsaturated carbonyl system in **II** to an isolated double bond system in **I**. Further structural evidences were obtained by direct comparison of natural and synthetic samples[7] which were completely identical in physicochemical properties. MATSUMOTO *et al.*[7] had synthesized **I** by formolysis of protoilludyl cation equivalents such as **III** and proposed the following mechanism for the formation of **I**.

The isolation of **I** from a petalenolactone producing organism may be taken as strong evidence suggesting the intermediacy of humulene in the biosynthesis of pentalenolactone.

Acknowledgment

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References

1) For part II, see SETO, H.; T. SASAKI, J. UZAWA, S. TAKEUCHI & H. YONEHARA: Studies on the biosynthesis of pentalenolactone. II. Isolation of pentalenic acid and pentalenolactone H. Tetrahedron Lett. 1978: 4411~4412, 1978
2) KOE, B. K.; B. A. SOBIN & W. D. CELMER: PA 132, a new antibiotic. I. Isolation and chemical properties. Antibiot. Annual 1956/1957: 672~675, 1957
3) AIZAWA, S.; H. AKUTSU, T. SATOMI, S. KAWABATA & K. SASAKI: AA-57, a new antibiotic related to pentalenolactone. J. Antibiotics 31: 729~731, 1978
4) HARTMAN, S.; J. NEEFF, U. HEER & D. MECKE: Arenaemycin (pentalenolactone): A specific inhibitor of glycolysis. FEBS Letters 93: 339~342, 1978
5) SETO, H.; T. SASAKI, H. YONEHARA & J. UZAWA: Studies on the biosynthesis of pentalenolactone. I. Application of long range selective proton decoupling (LSPD) and selective ^{13}C-{^{1}H} NOE in the structural elucidation of pentalenolactone G. Tetrahedron Lett. 1978: 923~926, 1978
6) CANE, D. E. & T. ROSSI: The isolation and structural elucidation of pentalenolactone E. Tetrahedron Lett. 1979: 2973~2974, 1979
7) OHFUNE, Y.; H. SHIRAHAMA & T. MATSUMOTO: Formolysis of protoilludyl cation equivalents, a new mode of rearrangement of bicyclo [4.2.0]oct-2-yl cation. Tetrahedron Lett. 1976: 2869~2872, 1976