STUDIES ON BIOSYNTHESIS OF PENTALENOLACTONE. V*

ISOLATION OF DEOXYPENTALENYLGLUCURON

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Deoxypentalenylglucuron was isolated from the culture broths of three different strains, such as *Streptomyces omiyaensis*, *S. albofaciens* and *S. viridifaciens*. The structure of deoxypentalenylglucuron has been determined by ^{1}H and ^{13}C NMR, mass spectroscopy and by chemical correlation to be an oxidation product of pentalenene **1**. Deoxypentalenylglucuron (**1**) has some antitumor activity against Sarcoma 180 in mice.

Pentalenolactone is one of the sesquiterpenoid antibiotics produced by several species of *Streptomyces* such as *S. chromofuscus*, *S. griseochromogenes*, *S. baarnensis*, *S. arenae* and *S. roseogriseus*. During biosynthetic studies of this antibiotic, SETO *et al.* have isolated pentalenolactones G[1] and H[2] and pentalenic acid[2] from *S. chromofuscus* and the sesquiterpene hydrocarbon, pentalenene,[3] from *S. griseochromogenes* and pentalenolactones O and P[4] from *Streptomyces* sp. CANE *et al.* have found pentalenolactone E in the fermentation broth of *Streptomyces* sp. UC539[5]. In the present paper, we wish to report the structure determination of a novel pentalenolactone derivative named deoxypentalenylglucuron.

Deoxypentalenylglucuron (**1**) was isolated from the culture broths of three different species, such as *S. omiyaensis*, *S. albofaciens*, and *S. viridifaciens*, and was produced together with pentalenolactone[6] and pentalenolactones O and P. Pentalenolactone O (arenaemycin D)[7] is assumed to be an artifact produced from pentalenolactone under acidic conditions in a similar manner to AA-57 (arenaemycin C)[7,8]. Pentalenolactone P may be a key intermediate in the methyl migration reaction which gives pentalenolactone.

From the ethyl acetate extract of the acidified fermentation broth of *S. omiyaensis*, the title compound (**1**) was isolated by column chromatography using Sephadex LH-20. Compound **1**, (mp 186~187°C, $C_{21}H_{30}O_8$, IR ν cm^{-1}: 3450, 1780, 1720 and 1625) gave a monomethyl ester (**2**) (mp 163~165°C) by treatment with diazomethane. The high resolution mass spectrum of **2** showed the molecular ion peak at *m/z* 424.2041, (calcd. for $C_{22}H_{32}O_8$, *m/z* 424.2097) and the base ion peak at *m/z* 217.1592 (calcd. for $C_{15}H_{21}O$, *m/z* 217.1592) corresponding to the sesquiterpenoid part formed by the loss of a highly oxidized moiety, *i.e.* a glucuronic acid residue (*vide infra*).

Acetylation of **2** with acetic anhydride in pyridine gave a triacetate [**3**, oil, $C_{28}H_{38}O_{11}$, IR ν cm^{-1}, 1760, 1730 (sh), 1620 and 1220, *m/z* 490 (M−60)]. The detailed analysis of the 400 MHz ^{1}H NMR and 100 MHz ^{13}C NMR spectra of **2** (in $CDCl_3$ with a drop of CD_3OD) and **3** (in $CDCl_3$) indicated the as-

* For part III, see reference 3.

signed structure. It is noticeable that the ^{13}C NMR spectrum of the terpene moiety of **2** is similar to that of pentalenic acid except for carbons at around C-1. Removal of the hydroxyl substituent from C-1 of pentalenic acid accounts for the upfield shift of the β-carbons (C-2 and C-8) and the downfield shift of the γ-carbons (C-14, C-7 and C-4) in **1**. Treatment of the ethyl acetate extract of an alkaline hydrolysate of **2** with diazomethane afforded a methyl ester (**4**, $C_{16}H_{24}O_2$ *m/z* 248). This result indicated that a sesquiterpenoid carboxylic acid is involved in the formation of an ester bond with the sugar moiety as mentioned above. Further structure evidences for sesquiterpene part were obtained as follows. Reduction of **2** with $LiAlH_4$ in THF gave a terpene alcohol (**5**, $C_{15}H_{24}O$, *m/z* 220). In the ^1H NMR spectrum of **5**, a newly appeared methylene signal due to an allyl alcohol was detected at 4.12 ppm and the olefinic proton at 6.82 ppm of **2** shifted to 5.5 ppm reflecting the reduction of ester carbonyl in an α,β-unsaturated carbonyl system. On the other hand, oxidation of pentalenene with selenium dioxide in ethanol gave an allyl alcohol, which was identified with the terpene alcohol (**5**) by comparison of their ^1H NMR, EI-mass spectra and gas chromatographic analysis (Rt: 3.50, 2% OV-1, 130°C). Therefore, the structure of the terpene part of **1** was established as deoxypentalenic acid. The sugar moiety containing $C_6H_9O_5$ for residual formula includes three hydroxyl groups and one carboxylic acid group. The large coupling constants between H-1 ~ H-5 in **2** apparently show the structure of the carbohydrate to be glucuronic acid. In agreement with this assignment, alkaline hydrolysis of **1** gave D-glucuronic acid, possessing $[\alpha]_D^{22}$ +14.0° (*c* 0.235, H_2O). The ester bond formation involving the oxygen atom at C-1 of the glucuronic acid moiety was established by the above-mentioned ^1H NMR chemical shift of the glucuronic acid moiety. Therefore, the structure of the title compound has been determined as **1**. The structural characteristics of **1** suggest that the oxidation of the allylic methyl in pentalenene takes place prior to the

Fig. 1.

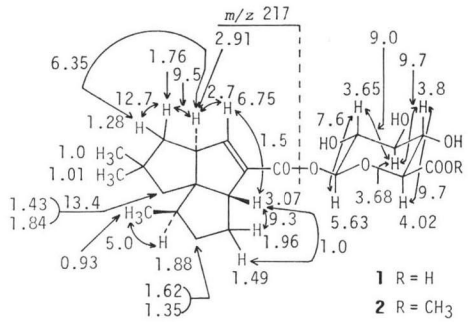


Fig. 2.

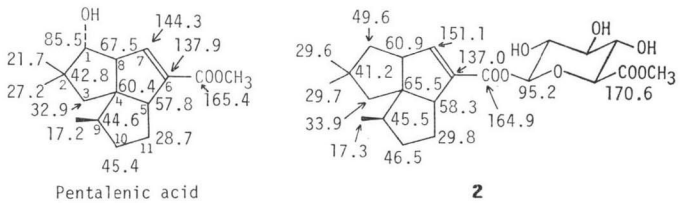


Fig. 3.

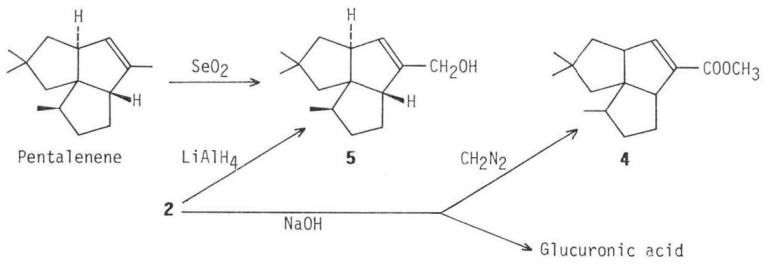
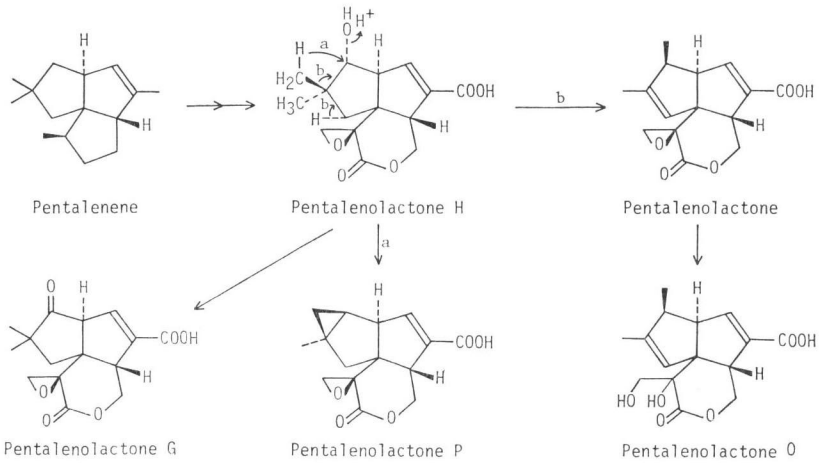


Fig. 4.



introduction of a hydroxyl function at C-1 in the biosynthesis of pentalenolactone. Deoxypentalenylglucuron exhibited no antibacterial activity against Gram-positive and Gram-negative bacteria. On the other hand, **1** showed some antitumor activity against Sarcoma 180 in mice. For example, when **1** in acacia suspension was injected intraperitoneally at daily doses of 0.2 and 1 mg per kg for 7 days to mice implanted with 5×10^6 cells of Sarcoma 180 per mouse 24 hours before initiation of the treatment, **1** improved the survival period (treated/control) by 1.39 and 1.84 times, respectively.

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