Antibiotics from *Polyangium cellulosum* var. *fulvum*. 2.[1] 5-*epi*-5,6-Dihydroxypolyangioic Acid

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An antifungal antibiotic, 5-*epi*-5,6-dihydroxypolyangioic acid (**1**), has been isolated from *Polyangium celluosum* var. *fulvum* and its structure elucidated by a comparison of the ^1H NMR spectra of diacetates **3** and **6**, and by conversion of 5,6-dihydroxypolyangioic acid (**4**) into **1**.

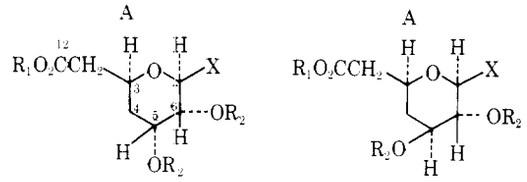
In a recent publication[1] we reported the structure elucidation of 5,6-dihydroxypolyangioic acid[3] (**4**), the major antifungal antibiotic present in the fermentation medium of *Polyangium cellulosum* var. *fulvum*. This antibiotic is highly active against systemic pathogenic fungi such as *Histoplasma capsulatum* and *Coccidioides immitis*. In the present paper we describe the isolation and characterization of a second antifungal antibiotic **1** from the same source. Antibiotic **1** shows a very similar antimicrobial spectrum to **4** *in vitro*.[4]

Bioautography[4] of thin-layer chromatograms of ethyl acetate extracts of the fermentation broth indicated three spots showing antifungal activity. The spot at origin consists of a mixture of components. The other two spots represent pure compounds [**4** (most polar) and **1**]. Antibiotic **1** was usually present in very small amounts compared to **4**, but the ratio varied from fermentation to fermentation.

The most convenient method of separating **1** and **4** was to methylate the mixture with diazomethane, separate the esters (**2** and **5**) by preparative TLC, and hydrolyze the pure esters to the corresponding acids (**1** and **4**). Acid **4** obtained by hydrolysis of ester **5** showed equivalent spectral and biological properties to acid **4** obtained directly from the fermentation indicating no undesirable changes had taken place during methylation and hydrolysis.

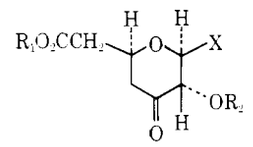
The IR spectra of acids **1** and **4** were almost identical as were those of the corresponding esters (**2** and **5**). The mass spectrum of ester **2** indicated a molecular formula $C_{29}H_{44}O_6$ (488)

Scheme I

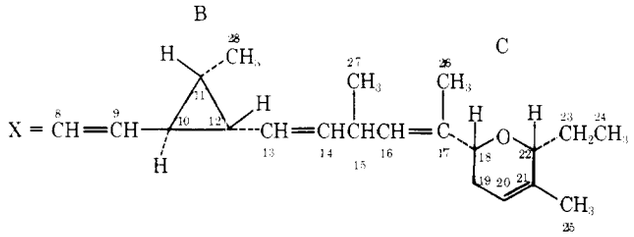


1, $R_1 = H$; $R_2 = H$
2, $R_1 = CH_3$; $R_2 = H$
3, $R_1 = H$; $R_2 = COCH_3$

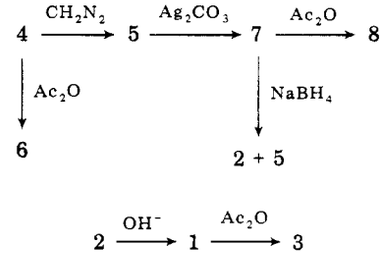
4, $R_1 = H$; $R_2 = H$
5, $R_1 = CH_3$; $R_2 = H$
6, $R_1 = H$; $R_2 = COCH_3$



7, $R_1 = CH_3$; $R_2 = H$
8, $R_1 = CH_3$; $R_2 = COCH_3$
9, $R_1 = H$; $R_2 = H$



Scheme II



and was practically identical to the mass spectrum of **5**. Acid **1** was converted to diacetate **3**. The mass spectrum of **3** displays a molecular ion at 558 ($C_{32}H_{46}O_8$) and a fragmentation pattern which was almost identical to that of **6**. The IR spectra of **3** and **6** were also very similar. Thus **1** has the formula $C_{28}H_{42}O_6$ and is an isomer of **4**. The significant differences in the ^1H NMR spectra of **3** and **6** are shown in Table I. The differences in the chemical shifts of the protons on carbons 3, 5, 6, and 7 in diacetate **3** with respect to the corresponding protons in diacetate **6** indicates **3** and **6** are structurally different in ring A. The multiplet due to H-3 has the same shape in both spectra and the doublet of doublets due to H-7 also has the same splitting pattern in both spectra. Thus the relationship between H-3 and H-4 and between H-6 and H-7 is the same in both molecules. The coupling between H-5 and H-6 is 3.0 Hz in **3** and 9.5 Hz in **6**. Thus diacetates **3** and **6** are epimeric at C_5.

In order to confirm that this was the only difference between the two molecules, acid **4** was converted to acid **1** via the route shown in Scheme II. Ester **5** was oxidized to keto ester **7**[1] with silver carbonate on celite. Attempts to convert acid **4** to the corresponding keto acid **9** under the same conditions gave tars. The ^1H NMR of keto acetate **8** indicated that **7** and **8** have the structure shown in Scheme I. Ketone **7** was reduced to epimeric alcohols **2** and **5**. The alcohols were separated by preparative TLC, and **5** was identical (TLC mobility and IR) to ester **5** obtained by methylation of the naturally occurring acid **4**. Ester **2** was hydrolyzed to acid **1**, which was acetylated to give diacetate **3**. Acid **1** obtained by the route shown in Scheme II was identical (TLC mobility and IR) to **1** obtained directly from the fermentation broth. The ^1H NMR of **3** (obtained via the route shown in Scheme II) was identical in all respects to that of **3** (obtained by acetylation of the naturally occurring acid **1**). Thus the less polar antibiotic isolated from the fermentation broth of *Polyangium cellulosum* var. *fulvum* has the structure of 5-*epi*-5,6-dihydroxypolyangioic acid (**1**).

Experimental Section[6]

^1H NMR spectra were run in $CDCl_3$ on a Varian HR-220[7] spectrometer with Me_4Si used as internal standard. Mass spectra were obtained with an AEI MS-902 instrument. TLC was performed on silica gel plates (Quantum) using iodine vapor for visualization.

Isolation of Methyl 5-*epi*-5,6-Dihydroxypolyangioate (2). The

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Table I. Ring A protons from ^{1}H NMR Spectra of Acetates 3, 6, and 8

compd	registry no.	H-3	H-4 (2 H)	H-5	H-6	H-7	CH_3CO
3	66966-11-8	4.21 (m)		between 5.45 and 5.32	4.66 (dd, 9.5, 3.0)	4.15 (dd, 9.5, 6.0)	2.13, 1.98
6	63511-83-1	3.95 (m)		4.95 m	4.77 (t, 9.5)	3.71 (dd, 9.5, 6.0)	2.00, 1.98
8	63511-89-7	4.11 (m)	2.65 (dd, 15.0, 3.0) and 2.54 (d^5, 15.0)		4.99 (d, 10.0)	4.00 (dd, 10.0, 6.0)	2.15

crude mixture of antibiotics, which was extracted from the fermentation broth with ethyl acetate, was dissolved in absolute ethanol and methylated with diazomethane in ether. After 10 min at room temperature a few drops of acetic acid were added to decompose the excess diazomethane. The solvents were removed at reduced pressure to give a brown gum. The gum was fractionated by preparative TLC with the solvent system ethyl acetate–cyclohexane (4:1) into two fractions. Methyl 5,6-dihydroxypolyangioate **5** (97 mg) (most polar compound) and methyl 5-*epi*-5,6-dihydroxypolangioate **2** (73 mg), a colorless oil (homogeneous by TLC): IR (film) 3600–3200 (br, OH), 1730 cm^{-1} (CO); mass spectrum *m/e* (rel intensity) 488 (24), 470 (10), 459 (57), 393 (33), 195 (85), 193 (100). Found: M^+ 488.3178. $C_{29}H_{44}O_6$ requires 488.3138.

5-*epi*-5,6-Dihydroxypolyangioic Acid (1). A solution of **2** (46 mg) in methanol (2 mL) and 5% aqueous sodium hydroxide (5 mL) was refluxed under nitrogen for 30 min, cooled, acidified with 1 N hydrochloric acid, and extracted with chloroform. The extracts were dried ($MgSO_4$) and evaporated to give **1** as a colorless oil (homogeneous by TLC) (40 mg 89%): IR (film) 3600–3200 (br, OH), 2800–2400 (OH), 1720 cm^{-1} (CO).

5-*epi*-5,6-Diacetoxypolyangioic Acid (3). Acetic anhydride (1 mL) was added to a solution of **1** (101 mg) in pyridine (2 mL). The reaction mixture was allowed to stand at room temperature overnight. A few drops of water were added and the solvents were removed at reduced pressure to give **3** as a pale yellow oil (homogeneous by TLC) (100 mg 84%): IR (film) 3600–3200 (br, OH), 1745 (CO), 1720 cm^{-1} (CO); ^{1}H NMR δ 5.57 (d br, 1, $C_{20}H$), 5.45 (dd, 1, J = 15.5 and 6.2 Hz, $C_{14}H$), 5.45–5.32 (m, 3, C_5H, C_8H, C_9H), 5.25 (d, 1, J = 9.0 Hz, $C_{16}H$), 5.05 (dd, 1, J = 15.5 and 8.5 Hz, $C_{13}H$), 4.66 (dd, 1, J = 9.5 and 3.0 Hz, C_6H), 4.21 (m, 1, C_3H), 4.15 (dd, 1, J = 9.5 and 6.0 Hz, C_7H), 4.11 (br, 1, $C_{22}H$), 3.86 (dd, 1, J = 2.2 and 10.5 Hz, $C_{18}H$), 3.06 (m, 1, $C_{15}H$), 2.65 (dd, 1 J = 5.5 and 16.0 Hz; C_2H), 2.45 (dd, 1, J = 5.5 and 16.0 Hz, C_2H), 2.13 (s, 3, CH_3CO), 1.98 (s, 3, CH_3CO), 1.64 (s, 3, CH_3C=), 1.59 (s, 3, CH_3C=), 1.04 (m, 6, 27-CH_3 and 28-CH_3), 0.89 (t, 3, 24-CH_3); mass spectrum *m/e* (relative intensity) 558 (26), 529 (44), 463 (30), 343 (12), 305 (14), 259 (14), 245 (21), 195 (90), 193 (100). Found: M^+ 558.3249. $C_{32}H_{46}O_8$ requires 558.3271.

Preparation of Esters 2 and 5 by Reduction of Methyl 6-Hydroxy-5-oxopolyangioate (7). Sodium borohydride (230 mg, 0.0058 mol) was added to a solution of **7** (230 mg, 0.00047 mol) in methanol (35 mL). The reaction mixture was stirred under nitrogen for 2 h. The solvent was evaporated under reduced pressure to give a white solid. The solid was dissolved in water, acidified with 1 N hydrochloric acid, and extracted with chloroform. The extracts were dried ($MgSO_4$) and evaporated to give a pale yellow gum (187 mg). The gum was fractionated by preparative TLC with the solvent system ethyl acetate–cyclohexane (4:1) into two fractions, **5** (most polar compound), a colorless oil (86 mg 37%), and **2**, a colorless oil (49 mg 21%): IR (film) 3600–3200 (OH), 1730 cm^{-1} (CO). The thin layer chromatographic mobilities of **2** and **5** were identical to those of **2** and **5** (obtained by methylation of naturally occurring acids).

Conversion of Ester 2 to Acid 1. Ester **2** (obtained by reduction of **7**) (49 mg) was hydrolyzed by the method described above to give **1** as a colorless oil (25 mg 53%) (homogeneous by TLC): IR (film) 3600–3200 (br, OH), 2800–2400 (OH), 1720 cm^{-1} (CO); mass spectrum *m/e* (rel intensity) 474 (14), 456 (9), 445 (29), 379 (19), 361 (8), 279 (64), 245 (19), 235 (21), 195 (75), 193 (100). Found: M^+ 474.3010. $C_{28}H_{42}O_6$ requires 474.2981.

Conversion of Acid 1 to Diacetate 3. Acid **1** (synthesized from **7** via **2**) (25 mg) was acetylated by the method described above to give **3** as a pale yellow gum (28 mg 90%) (homogeneous by TLC): IR (film) 3600–3200 (br, OH), 1745 (CO), 1720 cm^{-1} (CO); mass spectrum *m/e* (rel intensity) 558 (17), 529 (50), 463 (21), 343 (10), 305 (10), 259 (7), 245 (23), 195 (23), 193 (100). Found: M^+ 558.3295. $C_{32}H_{46}O_8$ requires 558.3271.

Registry No.—**1**, 66965-37-5; **2**, 66966-10-7; **5**, 62711-77-7; **7**, 63511-88-6.

References and Notes

(1) Part 1: D. T. Connor, R. C. Greenough, and M. von Strandtmann, *J. Org. Chem.*, **42**, 3664 (1977). There is an error in Figure 1 in this paper. The relative stereochemistry at C-18 and C-22 is the reverse of that shown. A correction has been sent to the editor.
(2) ICI North America, Wilmington, Del 19897.
(3) Ambruticin is the USANC name.
(4) S. M. Ringel et al., *J. Antibiot.*, **30**, 371 (1977).
(5) Although this resonance appears to be a doublet, the actual splitting may be more complex as these lines overlap the lines due to one of the hydrogens on the methylene adjacent to the carbonyl of the ester group.
(6) The compounds described in this paper were either oils or gums. The purity of each compound was checked by TLC with the solvent systems ethyl acetate–cyclohexane (4:1) and ethyl acetate–2-propanol–water (85:10:5).
(7) 220-MHz ^{1}H NMR spectra were run by Morgan-Schaffer Corp., Montreal, Canada.