ISOLATION AND IDENTIFICATION OF PICRONOLIDE, METHYNOLIDE AND NEOMETHYNOLIDE PRODUCED BY *STREPTOMYCES VENEZUELAE* MCRL-0376

Sir:

In the previous paper,[1] we reported the isolation of the aglycone of narbomycin from the fermentation beer of *Streptomyces venezuelae* MCRL-0376, the producer of picromycin, narbomycin, methymycin and/or neomethymycin, cultured in media containing sodium salt of organic acids such as acetic, succinic, malonic, pyruvic or citric acid.

Expecting accumulation of the aglycones of other macrolide antibiotics by strain MCRL-0376, we attempted to fractionate the metabolites in the culture filtrate of this organism using a medium containing sodium acetate, and isolated three additional macrolide aglycones produced as minor components.

In this paper, we describe the isolation and characterization of these compounds named picronolide (**I**), methynolide (**II**) and neomethynolide (**III**), the aglycones of picromycin, methymycin and neomethymycin, respectively.

Strain MCRL-0376 was cultured in the glucose-peptone medium[1] containing 0.04 M sodium acetate on a rotary shaker at 27°C for 6 days. Then, **I**, **II** and **III** were extracted from culture filtrate with ethyl acetate at pH 4.5. The extract was chromatographed on a silica-gel column using benzene-ethyl acetate as a developing solvent. A mixture of **I** and **II** was eluted with benzene-ethyl acetate (2:3, v/v), and then **III** with benzene-ethyl acetate (1:4, v/v). They were recrystallized from ethyl acetate-*n*-hexane as colorless needles.

The mixture of **I** and **II** was fractionated by neutral alumina column chromatography (benzene-ethylacetate). The elution with benzene-ethyl acetate (4:1, v/v) gave **I**, and the elution with (7:3, v/v) gave **II**. They were recrystallized from ethyl acetate-*n*-hexane to afford colorless needles.

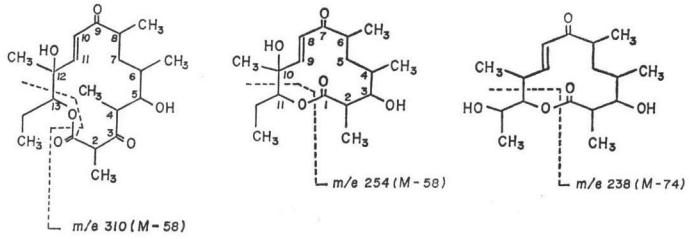
I has m.p. 139°C, $[\alpha]_D + 70°$(MeOH) $\lambda_{max.}^{EtOH}$ nm(log ε) 225(4.06) $\lambda_{max}^{EtOH+NaOH}$ nm(log ε) 280(4.08),[4] and analyzed for $C_{20}H_{32}O_6$. IR spectrum indicated hydroxyl (3500, 3380), lactone (1735), conjugated ketone (1698) and double bond (1635 cm^{-1}) functions. NMR (100 MHz, $CDCl_3$) of **I** exhibited signals at δ 0.8~2.05 (18H, C-CH_3), 3.79(1H, q, J=6.7, H-2), 5.02(1H, d, d, J=3.5, 10, H-13), 6.30 (1H, d, J=16, H-10) and 6.74 (1H, d, J=16, H-11). MS showed a molecular ion peak at *m/e* 368 and other diagnostic fragment peaks at *m/e* 350(M-18), *m/e* 339(M-29) and *m/e* 310(M-58). The acetyl derivative showed a molecular ion peak at *m/e* 410.

These results established the structure of **I** as picromycin aglycone. Moreover, **I** was easily converted to picromycin by the washed cell suspension of *Streptomyces narbonensis* ISP-5016 (narbomycin-producing strain). **I** was also obtained from narbonolide by the cell suspension of strain MCRL-0376. These transformations also support the structure of **I**.

II has m.p. 168~169°C, $[\alpha]_D$+67°, (MeOH), $\lambda_{max.}^{EtOH}$ nm(log ε) 225(3.99), and analyzed for $C_{17}H_{28}O_5$. IR spectrum indicated hydroxyl (3400), lactone (1725), conjugated ketone (1685) and double bond (1625 cm^{-1}). MS showed a molecular ion peak at *m/e* 312 and other diagnostic peaks at *m/e* 294(M-18) and *m/e* 254(M-58). The acetyl derivative showed the molecular ion peak at *m/e* 354. NMR(100 MHz, $CDCl_3$) of **II** exhibited signals at δ 0.8~2.2(15H, C-CH_3), 4.79(1H, d, d, J=2, 10, H-11), 6.33(1H, d, J=16, H-8) and 6.83(1H, d, J=16, H-9). In view of a similarity of the physicochemical properties of **II** with methymycin aglycone, the direct comparison of **II** with the authentic methymycin aglycone (methynolide) prepared according to C. DJERASSI[2] was carried out. IR, UV, MS and m.p. were completely identical with each other.

III has m.p. 102~104°C, $\lambda_{max.}^{EtOH}$ nm(log ε) 226(3.95), and analyzed for $C_{17}H_{28}O_5 \cdot H_2O$. IR spectrum indicated hydroxyl(3450), lactone (1730), conjugated ketone(1680) and double bond(1625 cm^{-1}). MS showed a molecular ion peak at *m/e* 312, and other diagnostic peaks at *m/e* 294(M-18) and *m/e* 238(M-74). The molecular ion peak of the acetyl derivative showed *m/e* 396 corresponding to the diacetate of which IR showed no hydroxyl absorption. These results established the structure of **III** as neomethymycin aglycone (neomethynolide). Further verification was carried out by the comparison of **III** with the authentic neomethynolide prepared by degradation of neomethymycin.[3]

Fig. 1.



I Picronolide II Methynolide III Neomethynolide

The authentic neomethynolide showed no depression of the melting point on mixing with **III**, and the IR, UV and MS were completely identical with each other.

Thus we could separate various macrolide aglycones from the fermentation beer of strain MCRL-0376 cultured in a medium containing sodium acetate. However, the accumulation of these aglycones in the medium containing sodium acetate was limited only to strain MCRL-0376 and those phenomena could not be observed in the culture of *S. narbonensis* ISP-5016 and *Streptomyces zaomyceticus* MCRL-0405 (narbomycin, picromycin and methymycin producer).

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ISAO MAEZAWA
AKIO KINUMAKI
MAKOTO SUZUKI

Microbial Chemistry Research Laboratory, Tanabe Seiyaku Co., Ltd., Toda, Saitama, Japan

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References

1) HORI, T.; I. MAEZAWA, N. NAGAHAMA & M. SUZUKI: Isolation and structure of narbonolide, narbomycin aglycone, from *Streptomyces venezuelae* and its biological transformation into picromycin *via* narbomycin. J. Chem. Soc. Chem. Commun. 1971: 304~305, 1971
2) DJERASSI, C. & J. A. ZDERIC: The structure of the antibiotic methymycin. J. Amer. Chem. Soc. 78: 6390~6395, 1956
3) DJERASSI, C. & O. HALPERN: Macrolide antibiotics. VII. The structure of neomethymycin. Tetrahedron 3: 255~268, 1958
4) RICKARDS, R. W.; R. M. SMITH & J. MAJER: The structure of the macrolide antibiotic picromycin. Chem. Commun. 1968: 1049, 1968