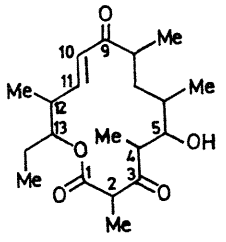
Isolation and Structure of Narbonolide, Narbomycin Aglycone, from *Streptomyces venezuelae* and its Biological Transformation into Picromycin *via* Narbomycin[1]

By T. HORI, I. MAEZAWA, N. NAGAHAMA, and M. SUZUKI*
(*Microbial Chemistry Research Laboratory, Tanabe Seiyaku Co., Ltd., Toda, Saitama, Japan*)

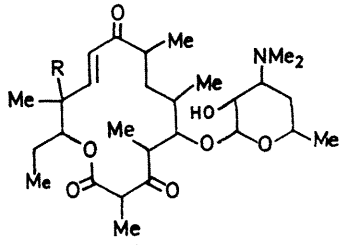
Summary A new crystalline compound, narbonolide (**1**), has been isolated from *Streptomyces venezuelae* MCRL 0376 and shown to be an aglycone of narbomycin (**2**) biologically convertible into picromycin (**3**) *via* narbomycin.

To isolate narbonolide, sodium acetate was added to a culture medium†. The production of macrolide antibiotics, narbomycin (**2**)[2] and picromycin (**3**),[3] and the growth of *Streptomyces venezuelae* MCRL 0376 was retarded for two days, but after the third day, a rate of production of macrolide antibiotics three and a half times that of control‡ was obtained.

From 1 l of the broth incubated for two days, 15 mg of a crystalline compound, named narbonolide (**1**), $C_{20}H_{32}O_5$;§ m.p. 125—126°; $[\alpha]_D^{23}$ + 89° (*c* 1·0 in MeOH); o.r.d. (*c* 0·0485 in MeOH) $[\phi]_{312}$ + 7690°, $[\phi]_{264}$ − 5070°; c.d. (*c* 0·0485 in MeOH) $[\theta]_{286}$ + 8050; ν_{max} (KBr) 3520 (OH), 1741 (lactone and ketone), 1700 (conjugated ketone), and 1635 cm^{-1} (double bond); λ_{max} (MeOH) 228·5 nm (ϵ 8200) was isolated. The n.m.r. spectrum (100 MHz; $CDCl_3$ with Me_4Si) shows two olefinic protons at δ 6·94 p.p.m. (dd, J_1 6·0, J_2 15·5 Hz, 11-H) and 6·13 (dd, J_1 1·0 J_2 15·5 Hz, 10-H), and a methine proton signal due to 13-H at 5·14 (dt, J_1 2·5, J_2 6·3 Hz). On irradiation at 2·70 (12-H), the signal due to the two olefinic protons and 13-H became an AB quartet (J 15·5 Hz) and triplet (J 6·3 Hz), respectively. A carbinyl proton signal due to 5-H appears at 3·91 [shifts to 5·45 (dd, J_1 1·0, J_2 9·0 Hz) on acetylation], and a methine proton signal due to 2-H at 3·75 (q, J 6·7 Hz). Comparison of these data with data for narbomycin, suggested the structure (**1**) for narbonolide. This structure was confirmed by the identity of narbonolide with an aglycone derived from narbomycin by hydrolysis with conc. HCl at room temperature. Neutral reaction products were removed continuously by benzene extraction. Crystals, which were identical with narbonolide (m.p., t.l.c., i.r., and mass spectrum, o.r.d., and c.d.) were obtained in 12% yield from the benzene extract.



(1)



(2) R=H
(3) R=OH

[^{2}H]Narbonolide (69% [$^2H_1$], 25% [$^2H_2$]¶) was transformed into [^{2}H]narbomycin (23% [$^2H_1$], 6% [$^2H_2$]) by the washed mycelium of *S. narbonensis* ISP 5016. Furthermore, [^{2}H]narbomycin (41% [$^2H_1$], 14% [$^2H_2$]¶) was also converted into [^{2}H]picromycin (13% [$^2H_1$], 4% [$^2H_2$]) by the washed mycelium of a picromycin-producing strain, *S. sp.* MCRL 0405. Narbonolide is therefore an intermediate which immediately precedes narbomycin in the biogenetic pathway, and narbomycin is in turn a biogenetic precursor of picromycin.

† The basal medium consists of the following components: glucose, 10·0 g; glycerol, 10·0 g; polypeptone, 10·0 g; meat extracts, 5·0 g; NaCl, 5·0 g; $CaCl_2,2H_2O$, 2·0 g; tap water to 1 l; pH 7·3 adjusted before autoclaving.

‡ Addition of 0·06M of sodium acetate gave the maximum increase in the macrolide antibiotics produced. Biochemical results will be published elsewhere.

§ Elemental analyses for narbonolide and its monoacetate are in good agreement (±0·1%) with the calculated values.

¶ [^{2}H]Narbonolide and [^{2}H]narbomycin were prepared by deuterium exchange with 50% K_2CO_3–D_2O in benzene and basic alumina–deuterium oxide,[4] respectively. Although the deuterium content of [^{2}H]narbonolide was reduced by the prolonged treatment with buffer solution of pH 8·4, all the deuterium atoms were not lost during incubation or isolation of the product.

Although erythromycin biogenetic intermediates[5,6] have been isolated mainly from blocked mutants of *S. erythreus*, the addition of acetic acid to the basal medium caused production** of the narbomycin aglycone, a direct precursor of narbomycin.

The use of deuteriated acetic acid led to the incorporation of CD_3COOH (99% [^2H]) in the narbonolide molecule. The mass spectrum showed 16 atom % excess of deuterium and the most abundant molecular ion was [$^2H_5$]narbonolide. This result indicated that more than one molecule of acetic acid was incorporated into the molecule of narbonolide and was consistent with a suggested biogenesis in which the lactone portions of both narbomycin and picromycin are formed from six propionate units and one acetate unit[7] (assuming biogenetic conversion of acetic acid into propionate units[8,9] as in the erythromycin-producing strain, *S. erythreus*[9,10] and the methymycin-producing strain, *S. venezuelae*.[8])

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** A spot with the same R_F value as narbonolide appeared on t.l.c. [R_F = 0·4—0·5, silica gel with ethyl acetate–hexane (1 : 2) as eluant] of ethyl acetate extracts of the fermentation broth on the addition of the sodium salt of citric, succinic, 2-oxoglutaric, fumaric, malic, pyruvic, malonic, and acetoacetic acid.

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