ANTIBIOTIC GLYCOSIDES. VII 10,11-DIHYDROPICROMYCIN: ANOTHER METABOLITE OF *STREPTOMYCES VENEZUELAE*

Sir:

10,11-Dihydropicromycin (**I**) has been found as a metabolite of *Streptomyces venezuelae* ATCC 15068. Its structure was deduced from spectral data and confirmed by comparison with an authentic semisynthetic sample. The biogenetic relationship between picromycin (**II**) and **I** has also been investigated.

The new metabolite has been isolated from the culture filtrates of *Streptomyces venezuelae* ATCC 15068. The medium used was that of DEVOE *et al.*[1], consisting of corn starch (3 %), corn-steep liquor (2.5 %), calcium carbonate (0.9 %), and ammonium sulfate (0.33 %); pH after sterilization: 6.9. The cultures were grown in Fernbach flasks (2,800-ml capacity, containing 350 ml of the medium) on a rotary shaker (300 r.p.m., 2.5 cm turn radius) for 72 hours at 28°C. The beer was centrifuged (30 minutes at 1,300×*g*) and the resulting supernatant was adjusted to pH 9.5 with sodium hydroxide (10 % w/v, aq.) after the addition of an equal volume of zinc sulfate solution (10 % w/v, aq.). The filtrate was extracted four times with chloroform (one fifth of the volume of the filtrate). After a volume reduction to one third *in vacuo* the combined chloroform extracts were shaken twice with a half volume of citric acid solution (1 % w/v, aq.) and the bases from the alkalinized aqueous layer (pH 9.5) were again taken into chloroform as above. The combined chloroform extracts were dried over anhydrous sodium sulfate, filtered and evaporated to dryness. Picromycin[2,3] was obtained from the oily residue upon crystallization from ethanol. The mother liquors were subjected to column chromatography on Sephadex LH-20 in chloroform-hexane mixture (1:1, v/v). Narbomycin[4] and picromycin eluted first at relative elution volumes (r.e.v.) of 0.58 and 0.70 respectively. A new compound followed at an r.e.v. of 0.76; it yielded rhomboid platelets (m.p. 137°C) upon crystallization from acetone-water.

The assignment of a structure to the unknown compound is based on the following evidence: The mass spectrum displayed a molecular ion peak at *m/e* 527 (7.3 %) which is consistent with $C_{28}H_{49}NO_8$. Other prominent peaks at *m/e* 509 (4.8 %), 353 (14.3 %), 335 (41.7 %), 174 (100 %), 158 (88.1 %), 116 (27.4 %) were indicative of the presence of the aglycone and the basic sugar respectively.*

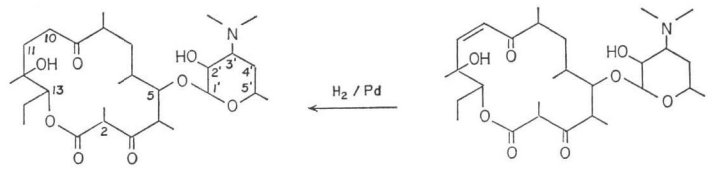
Further evidence for the proposed structure has been obtained from the 100 MHz p.m.r. spectra (Varian HA 100) in $CDCl_3$. Extensive spin-decoupling experiments allowed for a thorough analysis of the methine and methyl proton signals in the 10,11-dihydropicromycin molecule (Table 1). Diagnostically important were the quartet at 3.96 p.p.m. consistent with a proton at a carbon between two carbonyls and a methyl group (*cf.* references 2 *vs* 3 and 5) and a double doublet centered at 4.88 p.p.m. characteristic for a proton coupled with two diastereotopic protons present in macrolides possessing a tertiary hydroxyl group on the C_{12} (erythromycin A,C, picromycin). Also notable in comparison with picromycin was the absence of any signals downfield of 5.0 p.p.m. from T.M.S.

The I.R. spectrum (neat film) indicated maxima at 3460 (hydroxyl), 1740 (lactone) and 1710 cm^{-1} (ketone); in the U.V. spectrum

Table 1. Proton chemical shifts (ppm from TMS).

	Me
H−2 3.96	C−2 1.40
H−4 3.08	C−4 1.34
H−5 4.10	C−6 1.02
H−6 1.92	C−8 1.05
H−8 2.60	C−12 1.17
H−13 4.88	C−14 0.88
H−1′ 4.29	C−5′ 1.23
H−2′ 3.21	N 2.27
H−3′ 2.35	
H−5′ 3.52	

* The mass spectrum of picromycin (**II**), molecular weight 525 contained prominent fragments at *m/e* 174, 158, and 116 indicative of the desosamine portion common both to **I** and **II**. Other fragments including the M+ from **II**, *i.e.*, *m/e* 525, 507, 351, 333 were two units less as compared to those derived from **I**, *i.e.*, 527, 509, 353 and 335 respectively.



I II

(EtOH) only end absorption by the unknown was observed, whereas the addition of NaOH produced a maximum at 294 nm (log ε 4.3) due to the enolate ion formation of the keto group in a β-position to the lactonic carbonyl[2]. Catalytic hydrogenation[6] of picromycin with Pd on charcoal in MeOH afforded a product identical with **I** based on chromatographic, spectral and physical criteria. Raney nickel reduction of picromycin yielded 10,11-dihydropicromycin as the only product.*

The washed mycelium of the producer organism converted ^{3}H, ^{14}C labeled picromycin into **I** in a 2.2% yield, thus suggesting a possible precursor-product relationship between picromycin and 10,11-dihydropicromycin. The reduction of the 10,11-double bond is a step reminiscent of saturated fatty acid biosynthesis.

10,11-Dihydropicromycin exhibits antimicrobial activity when tested against both the erythromycin "sensitive" and "resistant" strains of *Bacillus subtilis* 168,[8] ID_{50} values being 1 and 6.5 μg/ml respectively.[9] With the two strains the ID_{50} values were 0.58 and 1.5 μg/ml for picromycin and 0.025 and 3.2 μg/ml for erythromycin.

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* With the same reducing agent, 4″-oxo-erythromycin B has been shown to yield a stereospecific reduction of the 4″-oxofunction in the presence of the C_9 ketone. See ref. 7.

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