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BUTIROSINS A AND B, AMINOGLYCOSIDE ANTIBIOTICS. III. STRUCTURES

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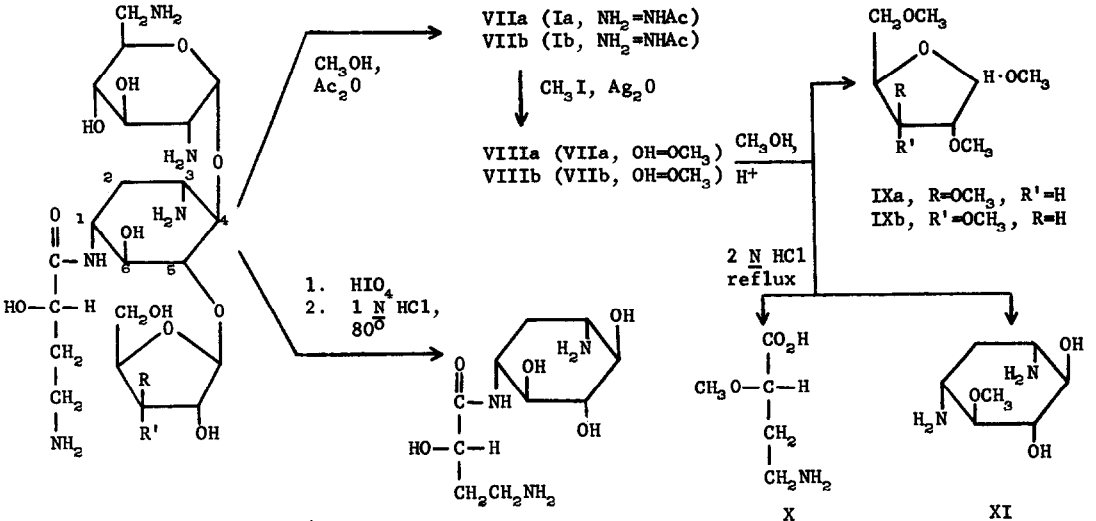
Previous letters[1] have established the molecular formula of butirosins A (Ia) and B (Ib) as $C_{21}H_{41}N_5O_{12}$ and have shown that three of the structural units, (S)-(-)-4-amino-2-hydroxybutyric acid (II), neosamine C (2,6-diamino-2,6-dideoxy-D-glucose) (III), and a pentose (D-xylose (IVa) in Ia, D-ribose (IVb) in Ib), are individually attached, as amide and O-glycosides, to the fourth unit, deoxystreptamine (V). Evidence presented in this communication will confirm this mode of attachments and establish the structures of Ia and Ib.

As reported,[1a] strong acid hydrolysis of Ia and Ib yielded neamine (VI, neomycin A), the structure of which, including absolute configuration, has been established as 4-O-(α-2,6-diamino-2,6-dideoxy-D-glucosyl)-2-deoxystreptamine.[2] The linkage of neosamine C to deoxystreptamine is therefore as indicated in Ia and Ib.

The linkage position and ring size of D-xylose in Ia were established by methylation studies. Treatment of N,N',N'',N'''-tetraacetylbutirosin A (VIIa) with methyl iodide and silver oxide in dimethylformamide[3] gave the corresponding O-methylated derivative (VIIIa). Methanolysis of VIIIa in 0.4 N methanolic hydrogen chloride (9 hr at reflux) yielded methyl α(and β)-2,3,5-tri-O-methylxylofuranoside (IXa), which was identified and distinguished from the respective pyranosides by tlc[4a] and glpc.[4b] Thus, butirosin A is a D-xylofuranoside, as indicated in Ia.

The remaining methanolysis products from VIIIa were subjected to vigorous acid hydrolysis (2 N HCl at reflux, 5.5 hr). Chromatography of the hydrolysate on a cellulose column, using 0.1 N HCl-ethanol (1:7) as eluent, yielded, among other products, 4-amino-2-methoxybutyric acid (X), $C_5H_{11}NO_3$, mp 242°, $[\alpha]_D^{25}$ -67° (c 0.71, water). The structure of X was established by



elemental analyses, Van Slyke determination (0.86 primary amino group), and nmr data[5] (OCH_3, singlet at δ 3.78; C-2 H, quartet at δ 4.25, $J_{2,3}$ 6.5 and 5.5 cps; two C-3 H's, multiplet at δ 2.17 to 2.67; two C-4 H's, triplet at δ 3.60, $J_{3,4}$ 7.0 cps). The isolation of X showed that the C-2 hydroxyl group in 4-amino-2-hydroxybutyric acid is free from substituent in butirosin A, as indicated in Ia, and consequently precluded as a possible site of linkage for D-xylose (or other structural units).

Also isolated from the cellulose chromatography of the acid hydrolysate (cf. the preceding paragraph) was 6-O-methyl-2-deoxystreptamine (XI), identified as its crystalline N,N'-diacetyl derivative (XII) by comparison of mp (287.5-288°) and ir spectra with an authentic sample. The optical rotation of XII, $[\alpha]_D^{26}$ + 2.4° (c 0.21, water) is similar to those reported for the same compound obtained from zygomycin A_1 and A_2 (+ 4°) and from neomycin B (+ 5°).[6] The isolation of 6-O-methyl-2-deoxystreptamine shows that xylose is attached to the C-5 hydroxyl, but not the C-6 hydroxyl, of the deoxystreptamine moiety, as indicated in Ia.

Through an identical series of reactions, butirosin B was shown to be a ribofuranoside in which ribose is attached to the C-5 hydroxyl of deoxystreptamine, as indicated in Ib.

In the nmr spectrum of tetraacetylbutirosin A (VIIa), the anomeric hydrogen of the D-xylofuranoside moiety appears at δ 5.71, $J_{1,2} \sim 0.6$ cps.[5,7] The small splitting is indicative

of a trans relationship between the two protons at C-1 and C-2[8] and consequently a β-D-xylofuranosyl glycosidic linkage, as in VIIa or Ia. Similarly, the nmr signal of the C-1 proton of the D-ribose moiety in tetraacetylbutirosin B (VIIb), δ 5.71, $J_{1,2} \sim 1$ cps, indicates a β-D-ribofuranosyl linkage, as indicated in VIIb or Ib.

The linkage of the C_4 acid (II) in the form of an amide (instead of ester, ether, or amine) to the rest of the molecule in Ia and Ib has been deduced from mass spectral and infrared data[1b] and is also supported, partially, by chemical data from the methylation studies above (conditions for hydrolytic cleavage, isolation of compound X). The position of linkage of acid II was established by periodate oxidation studies. Since xylose or ribose is attached to the C-5 hydroxyl of deoxystreptamine, the attachment of acid II to any amino group in Ia or Ib, except the C-1 amino of deoxystreptamine, would result in the presence of a vicinal amino-hydroxy grouping at C-1 and C-6 of the deoxystreptamine moiety, which would then be susceptible to periodate cleavage. Accordingly, Ia and Ib, along with paromomycin[9] as a control which contains a vicinal amino-hydroxy grouping at C-1 and C-6 of its deoxystreptamine moiety, were treated with 0.05 M sodium periodate at pH ca. 4.6 (ca. 9.6 mol of periodate per mol of reactant) at 4° for 23 hours. Strong acid hydrolysis of the resulting oxidation product (4 N HCl, 12 hr under reflux) yielded deoxystreptamine from Ia and Ib, but not from paromomycin. Thus acid II is attached to the C-1 amino group of deoxystreptamine, as in Ia and Ib.

Attachment of acid II to deoxystreptamine through an amide linkage in Ia was also confirmed by the isolation of compound XIII, a degradation fragment consisting of II and deoxystreptamine. Butirosin (I; mostly A) was oxidized with 0.014 M periodic acid (5.6 mol oxidant per mol of I) at 5° for nine days (4.7 mol uptake after 7 days), and the product was hydrolyzed under mild conditions (1 N HCl at 80° for 30 min) to give XIII. The composition of XIII was indicated by more vigorous acid hydrolysis (6 N HCl, 7.5 hr, 106°), which yielded deoxystreptamine and acid II. The N,N'-diacetyl derivative of XIII (mp 211-213°, no ir carbonyl absorption above 1700 cm^{-1}) was O-trimethylsilylated[10] to give a product which shows the expected $(M - CH_3)$ mass spectral peak[10] at m/e 620 (direct inlet, 70 ev), thereby confirming the structure of XIII as N^1-((S)-4-amino-2-hydroxybutyryl)-2-deoxystreptamine.

Thus, the structures of butirosins A and B, including absolute configuration, have been established as Ia and Ib.[11]

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References and Footnotes

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4. (a) M. Gee, Anal. Chem., 35, 350 (1963). For detection, the plate was sprayed with 2 N hydrochloric acid, kept at 105° for 15 min., then sprayed with p-anisidine phthalate. (b) Vapor phase chromatography was performed by Dr. Horst G. Schneider using a column of Carbowax 50 M (5% KOH) at 203°.

5. The spectrum was determined at 60 mc using deuterium oxide as solvent and tetramethylsilane as external reference.

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7. A doublet (J = 3.3 cps) at δ 6.07 in the spectrum of VIIa and a doublet (J = 3.5 cps) at δ 5.99 in the spectrum of VIIb were assigned to the C-1 proton of the neosamine C moiety. The corresponding proton in N,N',N'',N'''-tetraacetylneamine appears as a doublet (J = 3.7 cps) at δ 5.82.

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11. A recently-reported antibiotic, ribostamycin (SF-733) [E. Akita, T. Tsuruoka, N. Ezaki, and T. Niida, J. Antibiotics, 23, 173 (1970)] differs from Ib only in the lack of the acid II moiety.