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BUTIROSINS A AND B, AMINOGLYCOSIDE ANTIBIOTICS. I. STRUCTURAL UNITS

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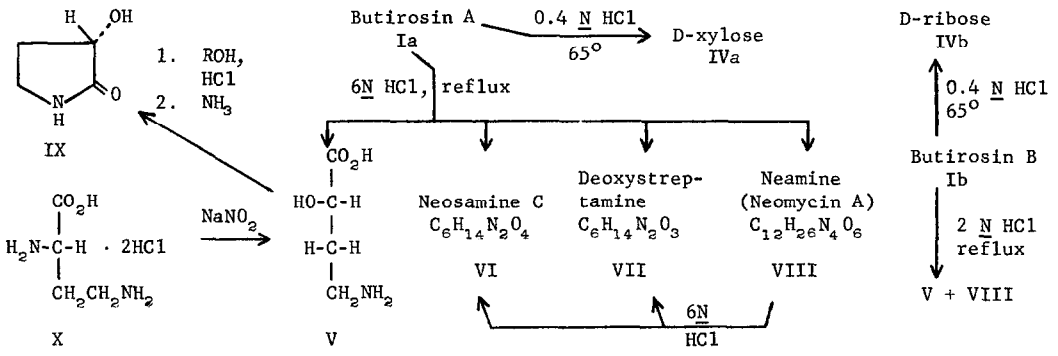
Butirosin,[1] an aminoglycosidic antibiotic complex active against many clinically important bacteria, consists of a major component, butirosin A (Ia), and a minor component, butirosin B (Ib).

The mixture of Ia and Ib was resolved by chromatography on Dowex 1 x 1 or Dowex 1 x 2 in the borate form.[2] Component Ia was eluted with water, whereas Ib was eluted with 5% boric acid solution. They were then isolated from the appropriate eluate fractions by adsorption on IRC 50[2] (NH_4^+) followed by elution with aqueous ammonia. The purified products thus obtained, Ia, $[\alpha]_D^{25}$ + 26.0° (c 1.46, water), and Ib, $[\alpha]_D^{25}$ + 33° (c 1.5, water), were white, amorphous solids (no mp, dec over a wide range above ca. 146°).

Elemental analyses of Ia are in fair agreement with the formulation $C_{21}H_{41}N_5O_{12}$; those of Ib agree with $C_{21}H_{41}N_5O_{12}\cdot 2H_2O$. The analyses of poly-N-acetylbutirosin A (IIa),[3] mp ca. 163-196°, $[\alpha]_D^{25}$ + 25° (c 2.11, water), prepared by treatment of Ia with acetic anhydride in methanol, and of poly-N-acetylbutirosin B (IIb),[3] similarly prepared, mp ca. 168-186°, $[\alpha]_D^{25}$ + 33° (c 1.34, water), agree with $C_{21}H_{41}N_5O_{12}\cdot(C_2H_2O)_4\cdot H_2O$. Also, the analyses of butirosin sulfate (III) (mixture of A and B) agree with $C_{21}H_{41}N_5O_{12}\cdot 2H_2SO_4\cdot 2H_2O$. Thus, according to elemental analyses, $C_{21}H_{41}N_5O_{12}$ is a possible empirical formula for Ia and Ib.

Mild acid hydrolysis of Ia and Ib (0.4 N HCl, 65°) slowly liberated D-xylose (IVa) and D-ribose (IVb), respectively; the pentoses were identified by paper chromatography[4] and as the crystalline (p-tolylsulfonyl)hydrazones.[5]

Strong acid hydrolysis of Ia (6 N HCl, 6 hr at reflux) followed by cellulose chromatography using tert-butyl alcohol-acetic acid-water (BAW)[6] mixtures as eluent, yielded fractions A, B, and C below.



Fraction A, BAW (2:1:1) as eluent, yielded the hydrochloride of V, crystals from absolute ethanol, mp 114-115.5°.

Fraction B, BAW (2:1:1) as eluent, gave a mixture of neosamine C[7,8] (VI, 2,6-diamino-2,6-dideoxy-D-glucose, $C_6H_{14}N_2O_4$) and deoxystreptamine[7,9] (VII, 1,3-diamino-1,2,3-trideoxy-scyllo-inositol, $C_6H_{14}N_2O_3$). They were separated by further chromatography on a cellulose column using ethanol-methanol-acetic acid-water (4:1:1:1) as eluent. Neosamine C was eluted first and identified as its crystalline N,N'-diacetyl derivative.[8b] Deoxystreptamine was characterized as the crystalline dihydrobromide.[9b]

Fraction C, BAW (2:2:1) as eluent, gave neamine[7,10] (VIII, $C_{12}H_{26}N_4O_6$, neomycin A), identified as its crystalline N,N',N'',N'''-tetraacetyl derivative. Neamine reportedly is hydrolyzed by 6 N hydrochloric acid heated under reflux (ca. 50% hydrolysis after 18 hr[9b]) to VI and VII, the same as those found in fraction B.

The crystals from fraction A (hydrochloride of V) were repeatedly crystallized from ethanol-water to give the hemihydrochloride of V, $C_4H_9NO_3 \cdot 0.5$ HCl,[11] mp 167-168°. The residue recovered from the mother liquor was adsorbed on a Dowex 1 x 2[2] (OH^-) column and eluted with 5% acetic acid to give V, crystals from methanol-water, $C_4H_9NO_3$,[11] mp 203-206°, $[\alpha]_D^{25}$ -28.2° (c 1.22, water). The formulation of V as 4-amino-2-hydroxybutyric acid was consistent with Van Slyke determination (1.09 primary amino groups), pKa values (2.9 and 9.9, water), and spin-decoupling nmr data (D_2O, tetramethylsilane as external reference) (C-2 H, quartet at δ 4.15, $J_{2,3}$ 7.2 and 4.8 cps; two C-3 H's, multiplet at δ 2.31-2.68; two C-4 H's, triplet at δ 3.63, $J_{3,4}$ 7.4 cps). Esterification of V with 2 N methanolic or ethanolic hydrogen chloride gave the corresponding amine hydrochloride esters, ir ($CHCl_3$) 1740 cm^{-1} (C=O). Treatment of the methyl ester in methanol or the ethyl ester in methylene chloride with ammonia resulted in lactam for-

mation to give, after sublimation (ca. 0.1 mm Hg and 90°), (S)-(-)-3-hydroxy-2-pyrrolidinone (IX), $C_4H_7NO_2$,[11] ir ($CHCl_3$) 1706 cm^{-1} (C=O), mp 103-104.5°, $[\alpha]_D^{25}$ -113° (c 0.77, chloroform). The mass spectrum of IX (70 ev, 130°) shows a molecular-ion peak at m/e 101, thus confirming the presence of four carbon atoms, rather than multiples of four, in IX, and hence in V and its hemihydrochloride.

The absolute configuration of V was established by synthesis through partial deamination of L-(+)-2,4-diaminobutyric acid dihydrochloride (X)[12] with sodium nitrite. The reaction should proceed with retention of configuration,[13] resulting in a (S)[14] configuration at C-2. The product, isolated by chromatography on Dowex 50W x 8[2] (H^+ phase, elution with HCl) and converted to the free base by treatment with Dowex 1 x 2[2] as described above, was identical to V. Thus, V is (S)-(-)-4-amino-2-hydroxybutyric acid.[15]

Acid hydrolysis of butirosin B (Ib) (2 N HCl, 5.5 hr at reflux) also yielded V and VIII.

Thus butirosin A (Ia) contains, as its structural units, deoxystreptamine (VII), neosamine C (VI), (S)-(-)-4-amino-2-hydroxybutyric acid (V), and D-xylose (IVa). Butirosin B (Ib) similarly contains VII, VI, and V, but differs in having D-ribose (IVb).

References and Footnotes

1. (a) Formerly known as ambutyrosin. (b) P. W. K. Woo, H. W. Dion, G. L. Coffey, S. A. Fusari, and G. Senos (Parke, Davis & Co.), Ger. Offen. 1,914,527, 09 Oct 1969 (C. A. 72, 41742y (1970)); U.S. Patent 3,541,078, 17 Nov 1970.

2. Dowex 1 x 1 and Dowex 1 x 2, strongly basic anion exchange resins, and Dowex 50W x 8, a strongly acidic cation exchange resin, are obtained from The Dow Chemical Co. Amberlite IRC 50, a weakly acidic cation exchange resin, is obtained from the Rohm & Haas Co. Dowex 1 x 1 and Dowex 1 x 2, in the hydroxyl phase, were converted to the borate phase by treatment with 5% boric acid.

3. The N-acetates IIa and IIb may be separated by paper chromatography in the solvent system n-butyl alcohol-pyridine-5% boric acid (6:4:3). The R_f values in descending chromatograms were 0.34 - 0.38 for IIa and 0.17 - 0.20 for IIb. The compounds were detected by exposure to chlorine, followed by spraying with ethanol, then with starch-potassium iodide solution (cf. S. C. Pan and J. D. Dutcher, Anal. Chem., 28, 836,(1956)).

4. P. Colombo, D. Corbetta, A. Pirotta, G. Ruffini, and A. Satori, J. Chromatogr., 3, 343 (1960).

5. D. G. Easterby, L. Hough, J. K. N. Jones, J. Chem. Soc., 3416 (1951).

6. In an ascending chromatogram using BAW (2:2:1) the various degradation products, applied as the hydrochlorides, appeared as ninhydrin-positive spots having R_f values as follows: V, 0.34 or 0.50, or both; VI, 0.14; VII, 0.13; VIII, 0.05. In cellulose tlc using n-propyl alcohol-pyridine-acetic acid-water (15:10:3:10), R_f values were 0.43 for VI and 0.35 for VII.

7. Kenneth L. Rinehart, Jr., "The Neomycins and Related Antibiotics," John Wiley & Sons, Inc., New York, N. Y., 1964; K. L. Rinehart, Jr., J. Infec. Dis., 119, 345 (1969); S. Hanessian and T. H. Haskell in "The Carbohydrates," Vol. IIA, 2nd ed, W. Pigman and D. Horton, Ed., Academic Press, Inc., New York, N. Y., 1970, Chapter 31.

8. (a) K. L. Rinehart, Jr., M. Hichens, K. Striegler, K. R. Rover, T. P. Culbertson, S. Tatsuoka, S. Horii, T. Yamaguchi, H. Hitomi, and A. Miyake, J. Am. Chem. Soc., 83, 2964 (1961); (b) H. Weidmann and H. K. Zimmerman, Jr., Ann. Chem., 644, 127 (1961).

9. (a) F. A. Kuehl, Jr., M. N. Bishop, and K. Folkers, J. Am. Chem. Soc., 73, 881 (1951); R. U. Lemieux and R. J. Cushley, Can. J. Chem., 41, 858 (1963); (b) J. R. Dyer, Ph.D. Thesis, University of Illinois, Urbana, Illinois, 1954.

10. H. E. Carter, J. R. Dyer, P. D. Shaw, K. L. Rinehart, Jr., and M. Hichens, J. Am. Chem. Soc., 83, 3723 (1961); M. Hichens and K. L. Rinehart, Jr., ibid., 85, 1547 (1963); S. Tatsuoka and S. Horii, Proc. Japan Acad., 39, 314 (1963).

11. Elemental analyses (C, H, and N, also Cl if applicable) showing good agreement with the indicated formula were obtained.

12. The absolute configuration of L-(+)-2,4-diaminobutyric acid has been shown by enzymatic method to be identical to that of other α-amino acids in the L-series [J. P. Greenstein, S. M. Birnbaum, and M. C. Otey, J. Biol. Chem., 204, 307 (1953)]. It has also been prepared by treatment of L-(+)-glutamic acid with hydrazoic acid [H. Paulus and E. Gray, ibid., 239, 865 (1964); D. W. Adamson, J. Chem. Soc., 1564 (1939)] during which the asymmetric center was not affected.

13. It has been generally accepted that nitrous acid deamination of aliphatic α-amino acids proceeds with retention of configuration. The neighboring carboxylate ion group was considered responsible for this stereospecificity [P. Brewster, F. Hiron, E. D. Hughes, C. K. Ingold, and P. A. D. S. Rao, Nature, 166, 179 (1950)]. An extensively studied example is the deamination of L-(+)-glutamic acid, the configuration of which relative to L-(+)-isoleucine has been determined by enzymatic method [J. P. Greenstein, et al. (12)]. The configuration of the product, (S)-(-)-2-hydroxyglutaric acid, relative to R-(+)-glyceraldehyde and hence to (+) tartaric acid, has been determined through chemical reactions [O. Červinka and L. Hub, Collect. Czech. Chem. Commun., 33, 2927 (1968); R. Kuhn and R. Brossmer, Angew. Chem., 74, 252 (1962)]. Since the absolute configurations of both (-)-isoleucine and (+)-tartaric acid have been established by X-ray technique [E. Eliel, "Stereochemistry of Carbon Compounds," McGraw Hill, 1962, p. 96], it is possible to deduce that the deamination of L-glutamic acid proceeded with retention of configuration. It may be safely assumed, therefore, that the similar deamination of 2,4-diaminobutyric acid (X) also proceeded with retention of configuration.

14. R. S. Cahn, C. K. Ingold, V. Prelog, Experientia, 12, 81 (1956).

15. Although 4-amino-2-hydroxybutyric acid is reportedly involved in biochemical reactions and has been the subject of much investigation [A. D. Homola and E. E. Dekker, Biochemistry, 6, 2626 (1967); L. P. Bouthillier, J. J. Pushpathadam, and Y. Binette, Can. J. Biochem., 44, 171 (1966); P. M. Dunnill and L. Fowden, Phytochemistry, 4, 445 (1965); D. R. Curtis and J. C. Watkins, Pharmacol. Rev., 17, 347 (1965)], the physical constants of either enantiomer of this compound apparently have not yet been reported.