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THE BIPHENYL CONSTITUENT OF RISTOCETIN A

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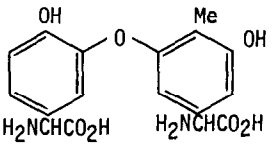
Ristocetins A and B, elaborated by Nocardia lurida,[1] and related antibiotics are glycopeptides which inhibit cell wall biosynthesis in Gram positive bacteria by a mechanism involving complexation with peptide intermediates.[2] The site of activity in the ristocetins has been shown to reside in the peptide portion which comprises complex and unusual amino acids.[3] A previous report from this laboratory described the structure of one of the constituents (1) of ristocetin A which has two α-aminoarylacetic acid fragments joined via an ether linkage between the aromatic rings.[4] We now wish to report the isolation and identification of a second constituent (2), in this case containing two α-aminoarylacetic acids joined through a biphenyl linkage.



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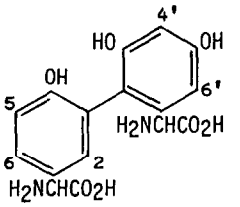

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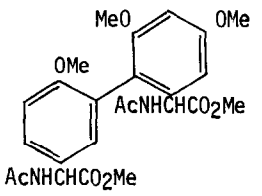
Tarbell and coworkers have reported obtaining by ion-exchange chromatography of hydrolysates of ristocetin A a bis(amino acid) for which the empirical formula $C_{16}H_{16}N_2O_7$ was assigned by mass spectrometry using diethyl esters of bis(t-BOC) and bis(thio-t-BOC) derivatives.[5] The similarity of this bis(amino acid) to 1 led them to suggest that the compound might be a nor-methyl homolog but further structural assignments were not made.

In the present study the nor-methyl bis(amino acid) was isolated from ristocetin A as derivative 3 by hydrolysis (aq 6M HCl/HOAc 2:1, reflux, 48 hr or aq 4M KOH, 1.8M $NaBH_4$, reflux, 24 hr) of N-acetylated O-methylated aglycoristocetin followed by derivatization as the N-acetyl methyl esters. Careful separation by hplc (Waters μ-Porasil, MeOH/CH_2Cl_2 3:97) resolved two diastereoisomers of 3 from each other and from other constituents of the mixture including the diastereoisomers 4 derived from bis(amino acid) 1.[6] Compounds 3 and 4 have the same empirical formula but the stereoisomers of 3 were readily distinguished from those of 4 by nmr on the basis of the number of O-methyl groups and the lack of an aryl C-methyl group. Samples of the nor-methyl amino acid[8] were derivatized by acetylation (Ac_2O in MeOH, 0° warming to 20°) and

methylated (CH_2N_2 followed by Me_2SO_4, K_2CO_3, refluxing acetone) to give diastereoisomers of 3 which were compared by tlc, nmr, and ms with the samples prepared above, thus establishing that the bis(amino acid) which we have now isolated as derivative 3 is the same one that Tarbell and coworkers had previously isolated and studied. It should be pointed out that only single stereoisomers of 1 and 2 are thought to be present in the native antibiotic; epimerization of α-aminophenylacetic acids occurs under the conditions employed for hydrolysis of the peptide.



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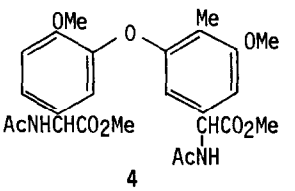
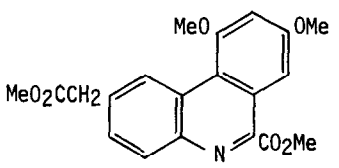


4

Bis(amino acid) 2 was recently identified by Williams and coworkers as a constituent of the peptide portion of the closely related antibiotic vancomycin.[9] The structure was established on spectroscopic grounds using derivatives 3 and 5. From hydrolysis and derivatization of methylated aglycovancomycin following the procedure used with ristocetin A two stereoisomers of 3 were obtained; these were shown by tlc, nmr, ms, ir and uv to be identical with the corresponding species from ristocetin A. As further evidence for the identity of these pairs of samples of 3 from the two antibiotics and for the epimeric relationship between the two isomers obtained from each source, the four samples of 3 were hydrolyzed (aq 1M HCl, reflux, 18 hr) to the free bis(amino acids) and degraded (excess NaOCl, 1M KOH, 25°, 1 hr) to give bis(nitrile) 6 in all cases.[10] In addition to showing the structural relationship among the four samples, the formation of benzonitriles establishes that the amino acid precursors are α-aminophenylacetic acids rather than homologous species.



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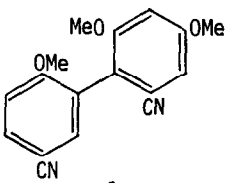
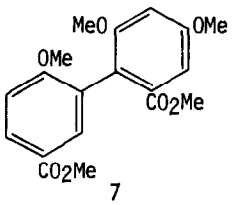


6

In our earlier study of ristocetin A, degradation of the crude mixture of amino acids obtained from alkaline hydrolysis of N-acetylated O-methylated aglycoristocetin by a procedure involving oxidation with NaOCl followed by methylation with CH_2N_2 gave dimethyl 4-methoxyisophthalate in addition to the bis(benzoates) derived from 1 and a tris(benzoate) derived from an as yet unidentified tris(aryl amino acid).[4] New oxidation studies under milder conditions have now given additional products which are biphenyl compounds. Treatment of the crude mixture of O-methylated amino acids with NaOCl (pH 8, 25°, 24 hr) followed by neutral $KMnO_4$ (25°, 1 hr) gave a mixture of acids which were esterified with CH_2N_2 and separated by hplc (Waters μ-Porasil, EtOAc/pentane 1:9) to give bis(benzoate) 7, mp 147°,[11] in addition to the esters seen

in the previous oxidation. Compound 7 was also prepared from 6 by alkaline hydrolysis (30% NaOH, 6 hr, reflux) followed by esterification (CH_2N_2). The nmr spectrum of 7 was more informative than that of protected bis(amino acid) 3 or bis(nitrile) 6 because of deshielding of protons ortho to the carbomethoxy groups. The assignment of substituents in the more heavily substituted ring could be made with certainty but those of the other ring were less secure. The structure of 7 was confirmed, however, by an independent synthesis which involved Ullmann-type cross-coupling of methyl 4-methoxy-3-iodobenzoate[12] with methyl 2-bromo-3,5-dimethoxybenzoate[13] (3:1 mole ratio, Cu powder, 220°, 30 min) to give 7, mp 145°, in 70% yield along with self-condensation products of the two halobenzoates. Compound 7 was isolated from the mixture by hplc (Waters μ-Porasil, EtOAc/pentane 1:5).



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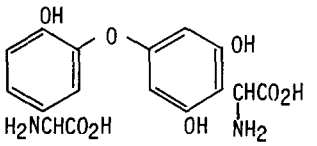

8

Lomakina and coworkers have asserted that the peptide portions of actinoidin, ristomycin, ristocetin, and vancomycin have in common a bis(amino acid) which they term actinoidinic amino acid.[14] They have proposed structure 8 for this compound. Williams finds no evidence for the presence of 8 in vancomycin and has suggested that actinoidinic amino acid may actually be 2, which differs not only in the arrangement of substituents but also in empirical formula. Our present identification of 2 in ristocetin A (with no indication of 8 being present) supports Williams' proposal. Further studies of actinoidin and ristomycin, including degradations of the type described herein and mass spectroscopic determination of empirical formulas will be needed to establish this point.[15]

ACKNOWLEDGMENT. We wish to thank Professors D. S. Tarbell and R. Bognár for their interest in these investigations, Abbott Laboratories and Eli Lilly & Co. for samples of ristocetin A and vancomycin, and the U. S. Public Health Service for generous support of this research (GM-23593).

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(6) The diastereoisomers of 3 had retention times intermediate between those of the two forms of 4. $C_{25}H_{30}N_2O_9$ requires 502.1950; found: (3a) 502.1979; (3b) 502.1973; (4a) 502.1944; (4b) 502.1958.[7]

(7) Exact mass measurements were carried out in the Mass Spectroscopy Laboratories at Columbia and Florida State Universities.

(8) We are grateful to Professor Tarbell for providing two samples of bis(amino acid) 2 for use in this study. One of the samples was, by the terminology of his paper,[5] amino acid I, the other was mainly II although it contained a substantial amount of I. The order of elution of the two diastereoisomers of 3 from silica gel is the same as that of the corresponding diastereoisomers (I and II) of 2 during ion exchange chromatography on Aminex Q-150S.

(9) G. A. Smith, K. A. Smith, and D. H. Williams, J. Chem. Soc. Perkin I, 2108 (1975). Williams has prepared 3 from vancomycin but employed a preparative tlc system which did not separate the stereoisomers.

(10) 6: $C_{17}H_{14}N_2O_3$ requires 294.1003; found 294.0999.[7] NMR (CD_3COCD_3) δ 3.81, 3.88, 3.93 (3 x s, 3MeO's), 6.96 (s, 4'- and 6'-H's), 7.30 (d, J = 8 Hz, 5-H), 7.59 (d, J = 2 Hz, 2-H), 7.82 (d x d, J = 8 + 2 Hz, 6-H). In $CDCl_3$ the 4' and 6' protons were no longer equivalent (δ 6.77 and 6.85, d's, J = 2 Hz).

(11) 7: $C_{19}H_{20}O_7$ requires 360.1209; found: 360.1211.[7] NMR ($CDCl_3$) δ 3.56, 3.70, 3.76, 3.85, 3.86 (5 x s, 5 MeO's), 6.70 (d, J = 2 Hz, 4'-H), 6.96 (d, J = 8 Hz, 5-H), 7.06 (d, J = 2 Hz, 6'-H), 7.82 (d, J = 2 Hz, 2-H), 8.04 (d x d, J = 8 + 2 Hz, 6-H).

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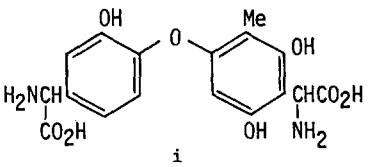
(13) C. T. Calam and A. E. Oxford, ibid., 280 (1939).

(14) N. N. Lomakina, M. S. Yurina, Y. N. Sheinker, and K. F. Turchin, Antibiotiki, 17, 488 (1972).

(15) A similar error in the assignment of empirical formula and structure of ristomycinic amino acid (i) may also have occurred.[16] Lomakina has reported the substance to be present in both ristomycin and ristocetin; we are readily able to isolate bis(amino acid) 1 from ristocetin but can find no evidence for i.



i

(16) N. N. Lomakina, V. A. Zenkova, R. Bognár, F. Sztaricskai, Y. N. Sheinker, and K. F. Turchin, ibid., 13, 675 (1968); N. N. Lomakina and N. L. Tokareva, ibid., 17, 874 (1972).