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OXIDATIVE DEGRADATION OF RISTOCETIN A

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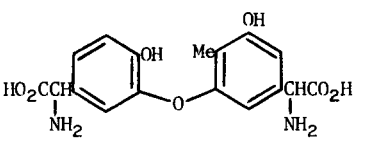
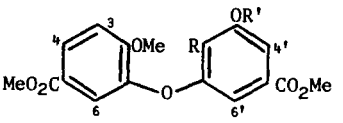
Ristocetin A and B are glycopeptide antibiotics of unknown constitution elaborated by *Nocardia lurida*.[1] The peptide portion of these compounds comprises novel and complex aromatic amino acids. A previous report from this laboratory described the isolation and partial characterization of one of the amino acids obtained by hydrolysis of ristocetin A.[2] This compound was shown to be a 2,2'-(oxydiphenylene)diglycine having a hydroxyl group on one ring and a methyl and a hydroxyl group on the other. Nmr spectra suggested that one ring was substituted 1,2,4 and the other 1,2,3,5, but the substituents could not be placed with certainty. The structure of this amino acid has now been established as 1 by means of oxidative degradation.



1

A mixture of the diastereoisomers[3] of 1 was methylated (Me_2SO_4, aq KOH, 25°, 1 hr) after protection of the amino groups by acetylation. The acetyl groups were then removed (aq KOH, 95°, 60 hr) and the methylated amino acid was oxidized with NaOCl (25°, 24 hr) followed by $KMnO_4$ (25°, 1.5 hr).[4] The resulting carboxylic acids were esterified (CH_2N_2) and separated by hplc (*Corasil II*, 16 ft, 1:1 ether-pentane) to give mainly diester 2: mp 132-135°; *m/e* 360 ($C_{19}H_{20}O_7$ established by exact mass measurement); nmr ($CDCl_3$) δ 2.22 (2'-Me), 3.87 (2 x OMe), 3.95 (2 x OMe), 7.02 (d, J = 8 Hz, 3-H), 7.08 (br, 4'- or 6'-H), 7.32 (br, 6'- or 4'-H), 7.44 (d, J = 2 Hz, 6-H), 7.85 (d x d, J = 8 + 2 Hz, 4-H). The nmr spectrum, when compared with those of methyl dimethoxybenzoates,[6] suggests that one ring is a protocatechuate and the other an α-resorcylate, with the methyl group being on the latter, probably between the oxy substituents. These postulates receive indirect support from the observation that BCl_3, which is known to demethylate *o*-methoxybenzoate esters,[7] had no effect on 2. The oxidation of 1 also gave several minor products which may have arisen from contaminants; one, however, *m/e* 404, appeared

to be the corresponding triester (3) but was obtained in quantities too small for characterization. More of this material was subsequently prepared by direct degradation of ristocetin A.



2, R = Me, R' = Me

3, R = CO_2Me, R' = Me

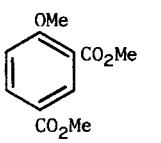
4, R = CO_2Me, R' = H

The aglycone, prepared by hydrolysis (0.5 M H_2SO_4, reflux, 2 hr) of ristocetin A, was methylated (Me_2SO_4, aq NaOH), and then saponified (0.5 M NaOH, reflux, 15 hr). The hydrolysate was treated with excess $KMnO_4$ (84°, 1 hr), the resulting acids were esterified with CH_2N_2, and the esters were separated by hplc to give as a major product triester 3: mp 129°; *m/e* 404 ($C_{20}H_{20}O_9$ established by exact mass measurement); nmr $CDCl_3$) δ 3.81 (OMe), 3.82 (OMe), 3.84 (OMe), 3.88 (OMe), 3.89 (OMe), 6.90 (d, J = 2 Hz, 4'- or 6'-H), 6.95 (d, J = 9 Hz, 3-H), 7.27 (d, J = 2 Hz, 6'- or 4'-H), 7.66 (d, J = 2 Hz, 6-H), 7.86 (d x d, J = 9 + 2 Hz, 4-H). The nmr spectrum corresponds closely to that of 2 with the exception that the C-Me signal has been replaced by an OMe. The fact that the aromatic portions of the spectra of 2 and 3 are very similar argues that the C-Me group of 2 and the new CO_2Me group of 3 are flanked by *ortho* substituents. Demethylation of 3 by BCl_3 (11 hr at 0° and 3 hr at 20° in CH_2Cl_2) gave phenolic ester 4: *m/e* 390; nmr ($CDCl_3$) δ 3.83 (OMe), 3.85 (OMe), 3.88 (OMe), 3.89 (OMe), 6.82 (d, J = 2 Hz, 4'- or 6'-H), 7.00 (d, J = 8 Hz, 3-H), 7.34 (d, J = 2 Hz, 6'- or 4'-H), 7.51 (d, J = 2 Hz, 6-H), 7.85 (d x d, J = 8 + 2 Hz, 4-H), 11.29 (OH). The low field location of the OH signal indicates that the group is hydrogen-bonded to the adjacent CO_2Me group.

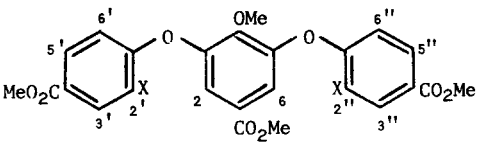
The structures of 1-4 were established[8] by an independent synthesis of 3. An Ullmann condensation of the sodium salt of dimethyl 2-hydroxy-6-methoxyterephthalate[9] with methyl 3-bromo-4-methoxybenzoate (190°, 12 hr with Cu_2Br_2) gave a low yield of 3, which was identical (nmr, ms) with material obtained from ristocetin A.

Two other esters were obtained in substantial quantities from the oxidation of ristocetin A. For one of these, dimethyl 4-methoxyisophthalate (5): mp 94-94.2° (lit.[10] mp 94°); *m/e* 224; nmr (CCl_4) δ 3.79 (OMe), 3.81 (OMe), 3.89 (OMe), 6.84 (d, J = 9 Hz, 5-H), 7.97 (d x d, J = 9 + 2 Hz, 6-H), 8.26 (d, J = 2 Hz, 2-H), the structure was readily established by comparison with an authentic sample. The other ester (6): mp 67°; *m/e* 466; nmr (CCl_4) δ 3.71 (OMe), 3.83 (OMe), 3.85 (2 x OMe), 6.89 (d, J = 8 Hz, 2'-, 2''-, 6'-, 6''-H), 7.55 (s, 2- and 6-H), 7.94 (d, J = 8 Hz, 3'-, 3''-, 5'-, and 5''-H), was recognized to contain three aromatic rings arranged ArOArOAr and having one OMe and three CO_2Me substituents. Structure 6, which was

assigned after study of model compounds[6] and accounts for the equivalence of two protons at 7.52 and four each at 6.88 and 7.92, was established by an independent synthesis involving an Ullmann condensation of the potassium salt of methyl 4-hydroxybenzoate with methyl 3,5-dibromo-4-methoxybenzoate in the presence of Cu_2Cl_2 (180°, 8 hr). The product (*Anal.* Calcd for $C_{25}H_{22}O_9$: C, 64.37; H, 4.75. Found: C, 64.20; H, 4.71), which was obtained in low yield, was shown (mp, nmr, ms) to be identical with 6 derived from ristocetin A.



5



6, X = H

7 X = Cl

Esters 5 and 6 are of particular interest, because 5 and 7, the latter being a dichloro derivative of 6, have been found by Williams and coworkers among the products of a similar oxidation of vancomycin.[11] Vancomycin is also a glycopeptide antibiotic for which the full structure is not known. The identification of 5 and 6 as oxidation products of ristocetin A points to a close structural relationship between the two. Although it seems likely that 5, 6, and 7, like 2 and 3, are oxidation products of complex amino acids, the corresponding amino acids have not yet been isolated from either of the antibiotics. Williams has, however, obtained evidence which suggests that with vancomycin 5 may actually be the oxidation product of a biphenyl-type dimer of a phenylglycine.[11]

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(2) J. R. Fehlner, R. E. J. Hutchinson, D. S. Tarbell, and J. R. Schenck, *Proc. Nat. Acad. Sci.*, 69, 2420 (1972).
(3) The diastereoisomers were designated IV and V in ref. 2.
(4) The conditions were chosen so as to avoid oxidation of the C-Me group. Treatment of the amino acid with NaOCl brought about an oxidative decarboxylation;[5] the resulting aldehyde was oxidized readily to the acid by $KMnO_4$ at 25°.
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(8) These assignments are supported by the observed acid-catalyzed H/D exchange reaction of the aromatic protons on the more highly substituted ring of 1,[2] which reflects the additional activation provided by the *meta* arrangement of the oxy substituents as compared with the *ortho* arrangement on the other ring.

(9) Treatment of dimethyl 2,6-dimethoxyterephthalate with BCl_3 in CH_2Cl_2 (0° for 2 hr and 25° for 2 hr) gave a good yield of dimethyl 2-hydroxy-6-methoxyterephthalate: mp 96-96.5° (*Anal.* Calcd for $C_{11}H_{12}O_6$: C, 55.00; H, 5.04. Found: C, 55.32; H, 5.12).

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