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STRUCTURAL INVESTIGATION OF THE ANTIBIOTIC ACTINOIDIN: IDENTIFICATION OF THE TRIS(AMINO ACID)

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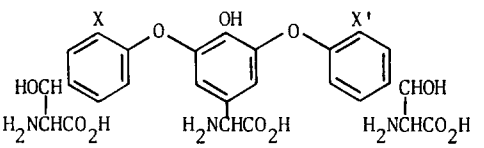
The fugitive triamino tricarboxylic acid, the last remaining unknown constituent of the glycopeptide antibiotic actinoidin, has been identified as monodechlorovancomycinic acid by investigation of the products of base hydrolysis of the aglycone.

Actinoidin, a metabolite of Proactinomyces actinoides, is a member of the vancomycin group of antibiotics.[1] Two forms of this glycopeptide (A and B) have been identified, both of which contain D-glucose, D-mannose, two 3-amino-2,3,6-trideoxyhexoses (L-acosamine and L-actinosamine), L-phenylalanine, and the bis(aryl amino acid) actinoidinic acid[2] but differ in that A contains 4-hydroxyphenylglycine whereas B contains 3-chloro-4-hydroxyphenylglycine.[3] An additional constituent of A and B is the tris(amino acid) Y which has been detected in two peptides resulting from partial acid hydrolysis of the antibiotics.[4] The free tris(amino acid) has not been detected in hydrolysates, presumably because of acid-catalyzed degradation. We wish to propose structure 1 for the Y constituent on the basis of degradative studies described below.



1, X = H, X' = Cl
7, X = X' = Cl
8, X = X' = H

Methylation of actinoidin sulfate with excess CH_2N_2 in 50% aqueous methanol (20°, 24 hr) followed by treatment with 3N anhydrous methanolic HCl (sealed ampoule, 105°, 6 hr) gave partially protected aglycone which was hydrolyzed by treatment with 4N KOH in the presence of 2M $NaBH_4$ (100°, 24 hr) under N_2. The products were acylated with Ac_2O at pH 7.5-8.0 (20°, 2 hr) and methylated with CH_2N_2 (20°, 12 hr) in methanol. Chromatography [silica gel, Merck 60F-254, column developed with $CHCl_3$-MeOH (98:2) followed by CH_2Cl_2-MeOH (95:5)] yielded, in addition to the O-methylated N-acetylated methyl esters of previously identified amino acids, a triaryl constituent identified as 2; m.p. 53-55° (Kofler); ms m/e 517, 515 (M^+); nmr ($CDCl_3$) δ 1.94 (CH_3CO), 3.69 and 3.80 (MeO's), 4.63 (2 x CH_2OH), 5.39 (d, J = 7 Hz, α-CH), 6.61 (broad, NH), 6.68 (d, J = 2 Hz, H_C or H_D), 6.77 (d, J = 2 Hz, H_D or H_C), 6.87 (d, J = 8 Hz, H_G), 6.95 (d, J = 9 Hz, H_B and $H_{B'}$), 7.17 (d x d, J = 2 and 8 Hz, H_F), 7.32 (d, J = 9 Hz, H_A and $H_{A'}$), 7.47 (d, J = 2 Hz, H_E). The relationship of H_E, H_F and H_G was confirmed by decoupling. The presence of chlorine, not recognized in the previous studies[4] of constituent Y, was indicated by the ^{35}Cl: ^{37}Cl isotope peaks in the mass spectrum and confirmed by elemental analysis. The empirical formula, $C_{26}H_{26}NO_8Cl$, and structure 2 were assigned on the basis of the nmr and mass spectra.

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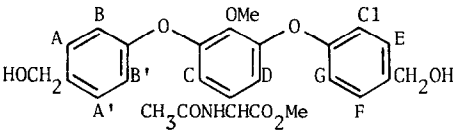
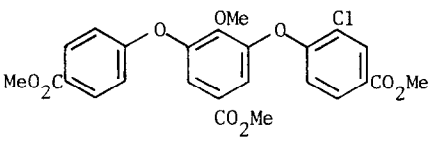


2

Chemical support for the structure of 2 was obtained by conversion to tris(benzoate ester) 3. Treatment of 2 with 2N KOH in 50% methanol (reflux, 3 hr), followed by NaOCl oxidation (20°, 2.5 hr), further base hydrolysis (4N KOH, reflux, 12 hr), oxidation with excess 5% $KMnO_4$ (reflux, 3 hr), and esterification with CH_2N_2 gave 3 in 46% yield; ms m/e 502, 500 (M^+); nmr (d_6-acetone) δ 3.82, 3.85, 3.87, 3.90 (MeO's), 7.10 (d, J = 8 Hz, H_G), 7.15 (d, J = 9 Hz, H_B and $H_{B'}$), 7.63 and 7.64 (2 x d, J's = 2 Hz, H_C and H_D), 7.95 (d x d, J = 2 and 8 Hz, H_F), 8.05 (d, J = 9 Hz, H_A and $H_{A'}$), 8.13 (d, J = 2 Hz, H_E). The empirical formula of 3, $C_{25}H_{21}O_9Cl$, was established by exact mass measurement of a high resolution mass spectrum (Calculated: 500.0874; found: 500.0868). Interpretation of the nmr spectrum of 3 was more definitive than that of 2; deshielding of protons ortho to the carbomethoxy groups reduced the number of overlapping signals in the aromatic region. The arrangement of substituents on the three rings of triester 3 was confirmed by comparison of the nmr spectrum with those of unchlorinated and dichlorinated triesters 4 and 5. Compound 4 has been obtained by oxidative degradation of ristocetin and ristomycin,[5] 5 by a similar degradation of vancomycin.[6]



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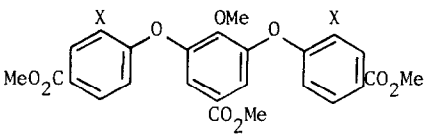
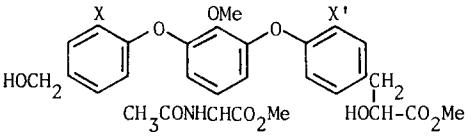


4, X = H

5, X = Cl

Base hydrolysis of actinoidin A,B (4N KOH, reflux, 24 hr, N_2) yielded glycine which was isolated as the N-(2,4-dinitrophenyl) methyl ester by treatment with 2,4-dinitrofluorobenzene followed by CH_2N_2; the derivative was identified by m.p. and tlc comparison with authentic material and by nmr and mass spectroscopy. Glycine is not present among the products of acid hydrolysis of actinoidin.

Two additional triaryl compounds were obtained from base hydrolysis of protected actinoidin aglycone in the presence of $NaBH_4$. The compounds, m.p. 51-53° and 59-62°, were tentatively assigned as 6a or 6b on the basis of the mass spectra [589, 587 (M^+) for both compounds] and the close relationship of their nmr spectra to the spectrum of 2. Low yields of the compounds precluded further investigation.



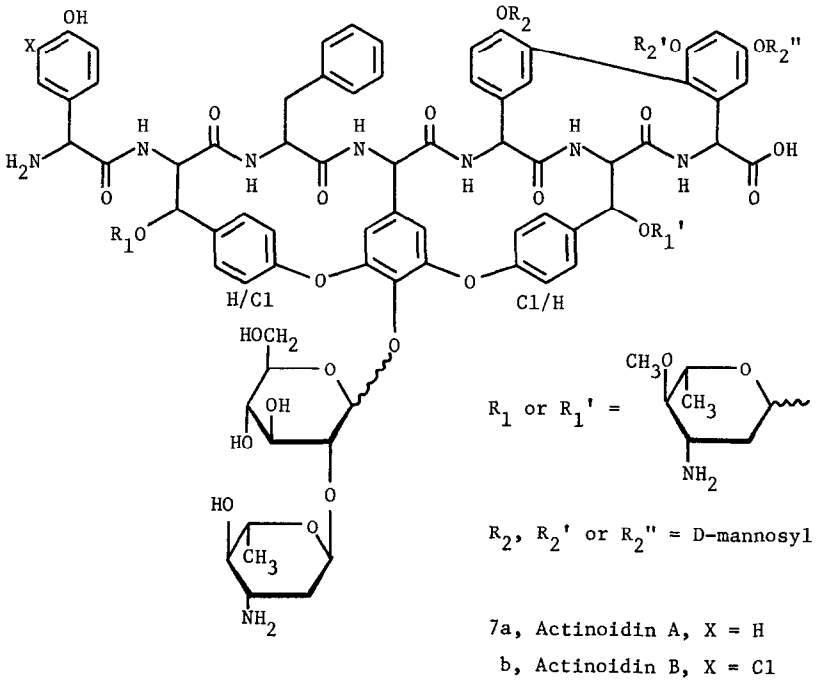
6a, X = H, X' = Cl

b, X = Cl, X' = H

These degradation products are consistent with 1 being the structure of component Y. Under basic conditions, 1 undergoes retro-aldol cleavage of the β-hydroxy-α-aminopropionate groups contained in the phenylserine moieties to give glycine plus arylaldehydes; the latter undergo

reduction by $NaBH_4$ to give benzyl alcohols. The lactate side chains of 6a-b arise by dehydration of the β-hydroxy-α-aminopropionate, hydrolysis to an arylpyruvate and then reduction. The apparent destruction of the Y constituent during acid hydrolysis of actinoidin can be accounted for by the sensitivity of the benzylic alcohols of the phenylserines to acid-catalyzed electrophilic reactions with activated aromatic nuclei.

Tris(amino acids) have been identified in other antibiotics in the vancomycin class. Dichloro compound 7, vancomycinic acid, is present in vancomycin,[6] while bisdechlorovancomycinic acid (8) is present in ristocetin and ristomycin.[5] The structure of a derivative of vancomycin has been established by X-ray crystallography.[7] Antibiotics in the vancomycin class must have similar peptide sequences because their mechanism of action involves a highly specific coordination between the antibiotic peptide moieties and bacterial cell-wall constituents containing the peptide fragment acyl-D-ala-D-ala.[8] Taking this observation into account along with previous work,[1-3] particularly that in which the N-terminal amino acids of actinoidins A and B were deduced[4] and certain of the glycosidic linkages were established,[9] structures 7a and b can be proposed for actinoidins A and B. Further studies are in progress to confirm the peptide sequence and to establish remaining structural and stereochemical details.



7a, Actinoidin A, X = H
b, Actinoidin B, X = Cl

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