THE TERMINAL AMINOGUANIDINE CHAIN OF PHLEOMYCIN G

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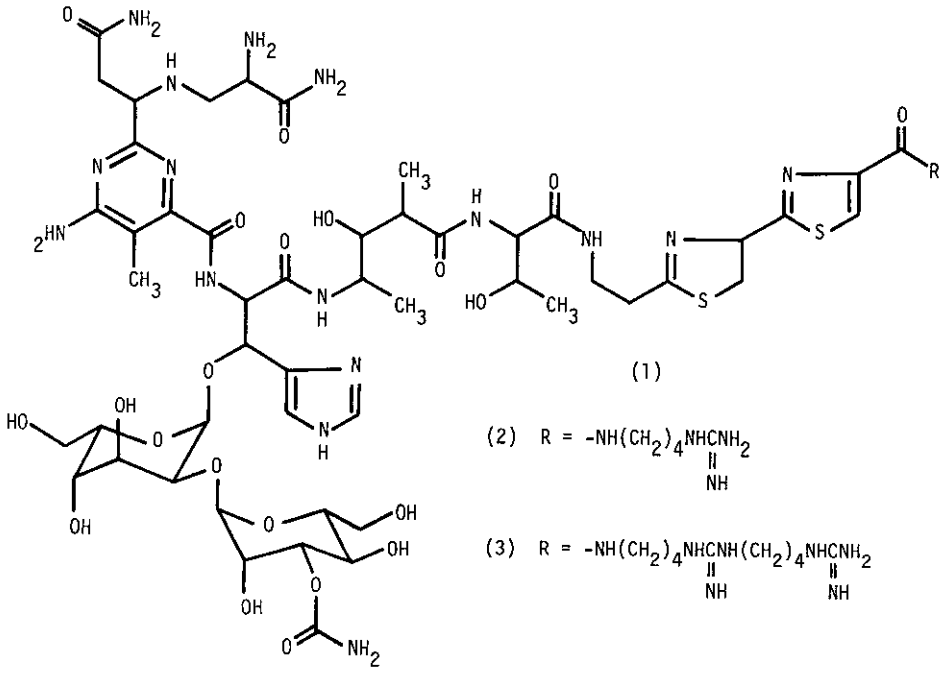
The terminal aminotriguanidine obtained by hydrolysis of phleomycin G is identical with synthetic 1-[[4-((3-[4-{3-(4-aminobutyl)guanidino}butyl]guanidino)butyl]]guanidine (4). The branched isomer 1-(4-aminobutyl)-2,3-bis(4-guanidinobutyl)guanidine (5) has also been prepared for comparison with the hydrolysis base.

Extensive studies of the phleomycins[1,2,3,4] in recent years have established that these peptide antibiotics, which, like the closely related bleomycins, are isolated in the form of copper complexes from cultures of Streptomyces verticillus, have the common structure (1). The terminal amine group R in this structure varies according to the availability of suitable amine precursors in the culture medium. Although the structures of the terminal amines have been determined for a number of phleomycins,[1,2,3] e.g. phleomycin D, R=(2), phleomycin E, R=(3), the structure of the terminal amine from phleomycin G remained incompletely determined. This terminal amine has been stated to contain two or more guanidine groups,[5] and bleomycin B6 has been shown to have the same terminal amine as phleomycin G.[6]

Our interest in phleomycin G arose from studies by Grigg and co-workers who established that the activity of individual phleomycins against E. coli can be markedly enhanced by means of certain compounds such as caffeine and Crystal Violet.[7] Accordingly a sample of pure phleomycin G isolated from a phleomycin mixture (Bristol Co, Batch 648) was subjected to the hydrolysis procedure previously described.[3] After isolation as a picrate, the hydrolysis base was recovered on a column of Dowex 1 (OH^- form) and obtained as a colourless gum. Mass spectroscopic examination of the hydrolysis base under chemical ionization conditions, using isobutane as reagent gas, showed an MH^+ peak at m/z 356 consistent with an aminotriguanidine structure. The base has now been identified as 1-[[4-((3-[4-{3-(4-aminobutyl)guanidino}butyl]guanidino)butyl]]guanidine (4) by comparison with a synthetic sample which had an identical mass spectrum. The branched isomer, 1-(4-aminobutyl)-2,3-bis(4-guanidinobutyl)guanidine (5), was also synthesized for comparison and it can be distinguished from the linear triguanidine (4) by marked differences in mass spectra



(1)

(2) R = $-NH(CH_2)_4NHCNH_2$ (NH)

(3) R = $-NH(CH_2)_4NHCNH(CH_2)_4NHCNH_2$ (NH, NH)

(4) $NH_2(CH_2)_4NHCNH(CH_2)_4NHCNH(CH_2)_4NHCNH_2$ (NH, NH, NH)

(5) $NH_2(CH_2)_4NHCNH(CH_2)_4NHCNH_2$ (NH, NH) with N substituted by $(CH_2)_4NHCNH_2$ (NH)

$PhCONH(CH_2)_4NHC(SMe)=NH_2]^+I^- \xrightarrow{H_2N(CH_2)_4NH_2} PhCONH(CH_2)_4NHCNH(CH_2)_4NH_2$ (NH)

(6) (7)

$[NH_2-C(SMe)=NH_2]_2^+SO_4^= \longrightarrow PhCONH(CH_2)_4NHCNH(CH_2)_4NHCNH_2$ (NH, NH)

(8)

$\xrightarrow{HCl} NH_2(CH_2)_4NHCNH(CH_2)_4NHCNH_2$ (NH, NH)

(9)

$PhCONH(CH_2)_4NHC(SMe)=NH_2]^+I^- \longrightarrow PhCONH(CH_2)_4NHCNH(CH_2)_4NHCNH(CH_2)_4NHCNH_2$ (NH, NH, NH) $\xrightarrow{HCl}$ (4)

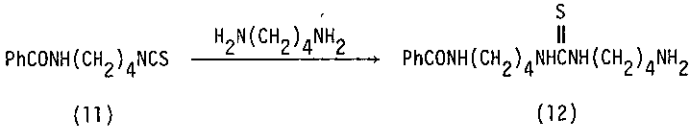
(10)

[linear isomer (4), MH^+, m/z 357 (0.2), 315 (3), 244 (7), 227 (5), 202 (21), 185 (5), 170 (2), 156 (5), 131 (18), 114 (100), 89 (23), 72 (22); branched isomer (5), MH^+, m/z 357 (0.2), 315 (0.3), 244 (0.4), 227 (10), 202 (2), 185 (20), 170 (1), 156 (1), 131 (100), 114 (92), 89 (38), 72 (22)]. Because of the difficulty of preparing crystalline derivatives of these compounds the hydrolysis base from phleomycin G and the linear (4) and branched (5) aminotriguanidines were compared by chromatographic techniques. On a column of CM Sepharose CL-6B (cation exchanger) the linear and branched isomers showed respective elution times of 725 and 710 minutes (linear gradient over 24 hr. from 0.1 M pyridine adjusted to pH 4.6 with formic acid to 1.0 M pyridine adjusted to pH 4.4 with formic acid; post-column derivatization with o-phthalaldehyde[8]).

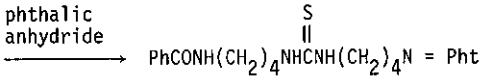
The linear triguanidine (4) was prepared as follows: reaction of 4-benzamidobutyl-S-methyl-isothiouronium iodide (6) with an excess of 1,4-diaminobutane afforded 1-(4-aminobutyl)-3-(4-benzamidobutyl)guanidinium iodide (7), m.p. 115.5-117°, 93% yield, which in turn reacted with S-methylisothiouronium sulphate to give 1-[4-{3-(4-benzamidobutyl)guanidino}butyl]guanidine (8). The product (8) was added to a column of IRA400 (OH^-) to remove $SO_4^=$ and I^-, and eluted with methanol/water (9:1). It was purified by gradient elution in ammonium formate solution from a column of CM Sephadex C25 and fractions containing (8) were freed of ammonium formate on a column of XAD-2 resin. Elution with methanol/water (1:1) gave (8) which was then passed through a column of IRA400 (OH^-) to remove formate ions. The product (8) was finally obtained as a glassy gum which partly crystallized on standing (MH^+, m/z 348). The benzoyl compound (8) was hydrolyzed by HCl (25% w/v) at reflux temperature for 15 hr. After removal of Cl^- ions on a column of IRA400 (OH^-), elution from the column with water gave 1-[4-{3-(4-aminobutyl)guanidino}butyl]guanidine (9) as a colourless gum (MH^+, m/z 244).

The aminodiguanidine (9) was then converted into 1-[[4-((3-[4-{3-(4-benzamidobutyl)guanidino}butyl]guanidino))butyl]]guanidine (10) by reaction with 4-benzamidobutyl-S-methylisothiouronium iodide (6) in ethanol. In order to achieve complete reaction in a reasonable time it was necessary to use a 50% molar excess of (6). After 12 hr at reflux temperature n-octylamine was added to react with excess (6), as n-octylamine and the guanidine derived from it are easily separated from the required product (10). The benzamidotriguanidine (10) was purified in a manner similar to the benzamidodiguanidine (8), and then hydrolyzed with HCl to give 1-[[4-((3-[4-{3-(4-aminobutyl)guanidino}butyl]guanidino))butyl]]guanidine (4), as a colourless gum (MH^+, m/z 357).

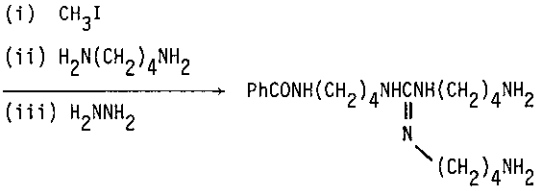
The branched aminotriguanidine (5) was prepared as follows: N-(4-aminobutyl)benzamide was converted into 4-benzamidobutylisothiocyanate (11) by a described method[9], and thence by reaction with an excess of 1,4-diaminobutane into 1-(4-aminobutyl)-2-(4-benzamidobutyl)thio-



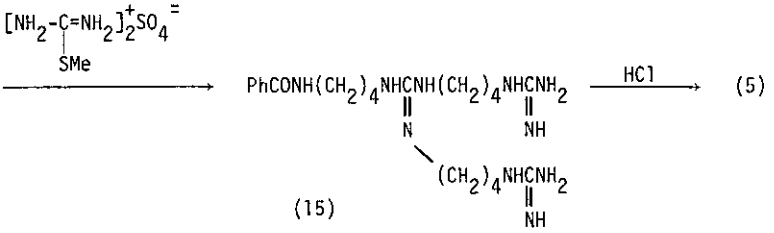
(11) (12)



(13) (Pht = phthaloyl)



(14)



(15) (5)

urea (12) which was heated with phthalic anhydride to give 1-(4-benzamidobutyl)-2-(4-phthalimidobutyl)thiourea (13), colourless crystals, m.p. 152-153°. Reaction of (13) with methyl iodide gave a non-crystalline S-methylisothiouronium iodide which was stirred in ethanolic solution at 40° with an excess of 1,4-diaminobutane for 6 hr. After removal of ethanol and excess 1,4-diaminobutane under vacuum the residue was dissolved in ethanol and refluxed in the presence of hydrazine in order to hydrolyze the phthaloyl groups. From this reaction mixture crude 1-(4-benzamidobutyl)2,3-bis(4-aminobutyl)guanidine (14) was obtained and it was subsequently purified by chromatography on a column of XAD-2 resin, from which it was eluted by water/methanol (1:1), and by chromatography on CM-Sephadex C25 in ammonium formate solution. The benzamide (14) was finally obtained as a highly viscous oil (MH^+, m/z 377) which was heated with S-methylisothiouronium sulphate in ethanolic solution. The resulting 1-(4-benzamidobutyl)-2,3-bis(4-guanidinobutyl)guanidine (15) was isolated in a manner similar to (8) and n-octylamine was used to react with the excess S-methylisothiouronium iodide. Purification of benzoyl compound (15), as in the case of (8), and hydrolysis with HCl gave 1-(4-aminobutyl)-2,3-bis(4-guanidinobutyl)guanidine (5), a colourless gum (MH^+, m/z 357).

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