Pimaricin: the Glycosidic Form of Mycosamine

By WALTER E. MEYER

(*Lederle Laboratories, A Division of American Cyanamid Company, Pearl River, New York* 10965)

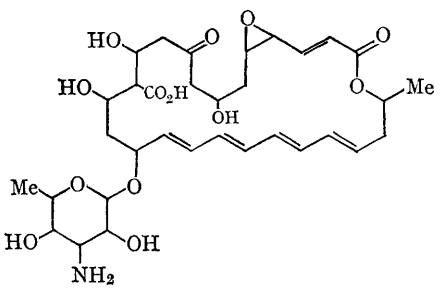
IN 1958 a structure for pimaricin, an antifungal, antibiotic, polyene macrolide, was suggested by Patrick, *et al.*[1,2] Subsequently Ceder, *et al.*,[3,4] utilizing high-pressure reductions and the newly developed general procedure devised by Cope and his co-workers for the determination of the carbon skeleton of complex polyols,[5] revised this structure. The measurement of mass spectra from trimethylsilyl derivatives of similar large molecular weight polyols[6] enabled Golding, *et al.*,[7] to obtain strong evidence for (I) for pimaricin.

Although the 3-amino-3,6-dideoxyhexose mycosamine, isolated from pimaricin as a methyl glycoside,[1] has the D-mannose configuration,[8] there has been general disagreement concerning the ring form of the hexose as it occurs in the antibiotic. Initially Patrick and his co-workers suggested the furanose form, primarily on the basis of a positive haloform test,[2] a result which, as Golding,[7] pointed out, would have been observed if any lactone hydrolysis had occurred in the alkaline test medium. Ceder[4] and, later, Golding,[7] independently postulated a pyranose ring; however, the former claim appeared without substantiation while the latter, based upon periodate oxidations of methyl mycosaminide, dodecahydropimaricin, and *N*-acetyldodecahydropimaricin, required additional verification because of anomalous periodate uptakes reported for some aminofuranosides.[9] To clarify these results a definitive approach was sought to establish the ring form of mycosamine in pimaricin.

If mycosamine is present as a pyranoside, per-*O*-methylation followed by hydrolysis with acid would result in the formation of 3-amino-3,6-dideoxy-2,4-di-*O*-methyl-D-mannose, which would not reduce periodate. On the other hand, similar treatment of the furanoside would lead to the

periodate-sensitive 3-amino-3,6-dideoxy-2,5-di-*O*-methyl-D-mannose. Therefore, the following sequence was undertaken.

N-Acetylpimaricin[1] was exhaustively methylated with methyl iodide in dimethylformamide and sodium hydrogen carbonate, and then hydrolysed with 50% methanolic 6N-hydrochloric acid. An ethereal extract of the hydrolysate was evaporated, distilled at 67° (0·1 mm.), and separated into two fractions (A and B) by preparative v.p.c. on a 6-ft. 3% ECNSS-M column at 128°. The n.m.r. and mass spectra of A, the slower component, were in accord with a methyl glycoside of di-*O*-methylmycosamine. The molecular ion at *m/e* 205 was peak-matched and found to represent a molecular formula of $C_9H_{19}NO_4$. The n.m.r. spectrum ($CDCl_3$) contained, in addition to other peaks, three singlets (3 H) at δ 3·53, 3·47, and 3·37 p.p.m. (3-OMe), a singlet (2 H) at δ 1·86 ($-NH_2$) and a doublet (3 H) centred at δ 1·3 p.p.m. (>CHMe). Similarly, fraction B was shown to be a methyl glycoside of *N*-methyl-di-*O*-methyl mycosamine. The n.m.r. spectrum ($CDCl_3$) contained an additional singlet (3 H) at δ 2·45 (>NMe) and a singlet (1 H) at δ 1·80 p.p.m. (>NH). A molecular formula of $C_{10}H_{21}NO_4$ was determined from an integrated proton count from the n.m.r. spectrum and a molecular-ion peak in the mass spectrum at *m/e* 219. Hydrolysis of fraction A with 6N-hydrochloric acid was complete after 18 hr. at 100° (disappearance of the δ 3·37 p.p.m. peak in the n.m.r. spectrum). Periodate consumption measured polarographically at −0·25 v (saturated calomel electrode)[10] after 3·5 hr. at 25° was 0·11 mole per mole of carbohydrate. Fraction B, after similar treatment, consumed 0·05 mole of periodate per mole of carbohydrate; thus, the mycosamine ring of pimaricin is pyranose, presumably in the chair form.



I thank J. Karliner for mass spectra, W. Muller for v.p.c. separations, H. Ferrari for polarographic assays, and E. Radenberg and G. Morton for n.m.r. spectra. I also thank Dr. J. B. Patrick for his interest and encouragement during this investigation.

(*Received, March 6th,* 1968; *Com.* 280.)

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