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THE STRUCTURE OF THE MACROLIDE ANTIBIOTIC PIMARICIN

B. T. Golding and R. W. Rickards
Department of Chemistry, University of Manchester
W. E. Meyer and J. B. Patrick
Lederle Laboratories, Pearl River, New York
and M. Barber
Associated Electrical Industries Ltd., Manchester

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Pimaricin, an antifungal antibiotic of the polyene macrolide group (1, 2) first isolated (3) in 1957 from Streptomyces natalensis, was originally suggested to have the structure (I) by Patrick and co-workers (4, 5). After extensive re-investigation, Ceder (6-12) proposed the revised formula (II: R = OH). That these structures correspond respectively to molecular formulae $C_{34}H_{49}NO_{14}$ and $C_{33}H_{47}NO_{14}$, neither of which is correct, is a measure of the difficulties involved in working with such compounds. We present here evidence that pimaricin has the molecular formula $C_{33}H_{47}NO_{13}$ and the structure (II: R = H). Tennecetin (13) from S. chattanoogensis is believed (14) to be identical with pimaricin.

N-acetylpimaricin itself was, as expected, too involatile and thermally unstable to afford a useful mass spectrum even by direct-inlet insertion. We therefore applied the trimethylsilylation procedure which we have developed (15) for the determination of molecular formulae of high molecular weight polyols and used successfully (15) in the case of the less-complex macrolides lagosin and filipin. Ceder's structure (II: R = OH), $C_{33}H_{47}NO_{14}$, should give an N-acetyl TMS-ester

hexa-TMS-ether* $C_{56}H_{105}NO_{15}Si_7$, of molecular weight 1227. However, N-acetylpimaricin afforded a TMS derivative, the mass spectrum† of which showed a clear molecular ion at m/e 1139. This corresponds to the molecular formula $C_{53}H_{97}NO_{14}Si_6$, i.e. to the TMS-ester penta-TMS-ether of an N-acetyl compound $C_{35}H_{49}NO_{14}$, and necessitates the formula $C_{33}H_{47}NO_{13}$ for pimaricin itself. Mass spectra obtained after trimethylsilylation of three other pimaricin derivatives confirmed this conclusion (cf. Table). In each case the determined molecular weight was 88 mass units less than that expected from Ceder's structure (II; R = OH). This difference corresponds formally to the replacement of $-OSi(CH_3)_3$ by $-H$, and necessitates that pimaricin‡ contains one less hydroxyl group than proposed by Ceder (12).

TABLE

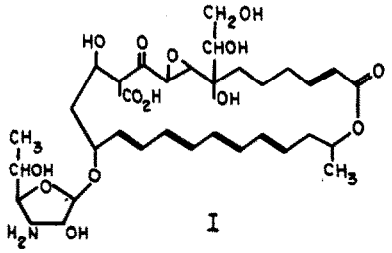
Molecular Ions of TMS Derivatives of Pimaricin

Trimethylsilylated Pimaricin Derivative	(II: R = OH) requires M^+	Formula	Found M^+	Formula
N-Acetyl	1227	$C_{56}H_{105}NO_{15}Si_7$	1139	$C_{53}H_{97}NO_{14}Si_6$
N-Acetyl methyl ester	1169	$C_{54}H_{99}NO_{15}Si_6$	1081	$C_{51}H_{91}NO_{14}Si_5$
N-Acetyl p-iodophenacyl ester	1399	$C_{61}H_{102}INO_{16}Si_6$	1311	$C_{58}H_{94}INO_{15}Si_5$
N-Acetyl dodecahydro	1311	$C_{59}H_{125}NO_{15}Si_8$	1223	$C_{56}H_{117}NO_{14}Si_7$

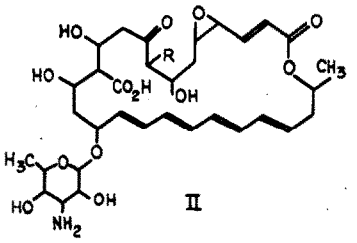
* The abbreviation TMS will be used for trimethylsilyl.

† Spectra, details of which will be published elsewhere, were obtained with an A.E.I. Ltd. MS9 spectrometer.

‡ Pimaricin used throughout this work was Myprozine^R produced by the American Cyanamid Company; authentic Dutch pimaricin (3) and Myprozine^R gave similar mass spectra (as their N-acetyl TMS derivatives), optical rotations, and microbiological assays, further confirming the identity established previously (4).



I



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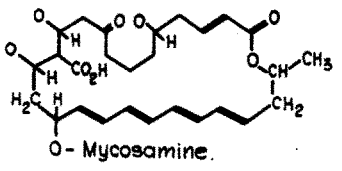

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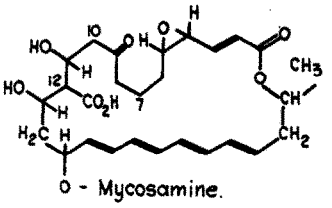

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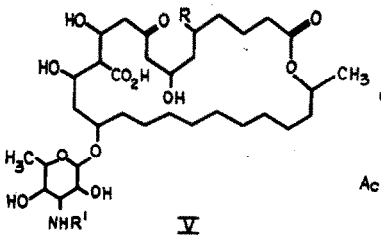

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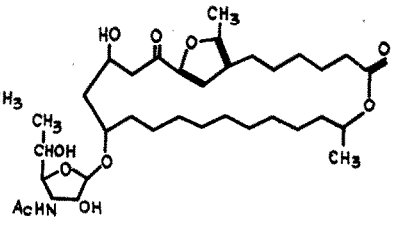

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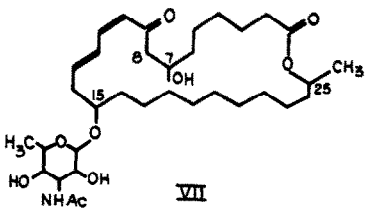

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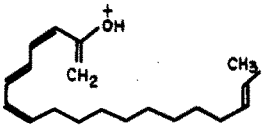

VIII

Of the published evidence (4-12), we can accept that leading to the partial structure (III). Placement of the epoxide function adjacent to the αβ-unsaturated lactone (as in II: R = OH) relied (12) primarily on a spectroscopic adaptation of the Bodforss test (16) for a conjugated epoxide (involving reduction to the corresponding olefin with warm acetic acid and potassium iodide), which may well be misleading when applied to a molecule of this complexity. If we tentatively accept Ceder's positioning of the epoxide, for which we will provide confirmation later, we can expand (III) to (IV), to which only one hydroxyl group must now be added. The published evidence beyond this point is conflicting. On the one hand, vigorous oxidation (10) of pimaricin itself gave no succinic or higher dibasic acid, indicating that the remaining hydroxyl should be located at $C_{(7)}$ (as in II: R = H). In contrast, both N-acetylpimaricin (5, 12) and N-acetyldodecahydropimaricin (12) (in which the epoxide has been opened by hydrogenolysis) were each found to consume two moles of periodate, consistent with the presence of a 1,2,3-trioxygenated system and requiring the remaining hydroxyl to be at $C_{(10)}$ or possibly $C_{(12)}$ in (IV). However, these titrations on N-acetyl derivatives seemed to us incompatible with the report (12), presented without comment, that dodecahydropimaricin itself consumes only two moles of periodate, whereas the mycosaminide moiety here would itself be expected to utilise either one or two moles of reagent according to its furanoid* or pyranoid ring size.

Direct evidence that the remaining hydroxyl is correctly located at $C_{(7)}$ in (IV), and for the $C_{(7)}$ - $C_{(11)}$ environment of the carbonyl function in (II: R = H), comes from our finding that acetone is obtained on retroaldolisation of pimaricin with hot aqueous alkali, in addition to the acetaldehyde previously reported (12). N-acetylpimaricin affords the same result.

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* Cf., however, Weiss et al. (18) who have observed uptake of two moles of periodate by some 3-aminofuranosides.

Re-examination* of the conflicting periodate oxidation experiments has removed any argument against the oxygenation pattern of structure (II: R = H). In contrast to the previous results (5, 12), N-acetyldodecahydropimaricin showed no uptake of oxidant even overnight, as measured by arsenite titration or ultraviolet spectrometry (17). Thus the dodecahydro-antibiotic contains no vicinal oxygen functions, in agreement with the revised structure (V: R = OH; R' = H) which would result from allylic† hydrogenation of the epoxide. Furthermore, both methyl mycosaminide (3-amino-3-deoxy-α-D-mannopyranoside) and free dodecahydropimaricin consumed two moles of periodate within an hour, oxidation then effectively ceasing. Thus, the mycosamine ring in pimaricin is probably pyranose‡ (as in II: R = H), a conclusion also presented by Ceder (12) without full substantiation. The iodoform test given by pimaricin in aqueous bicarbonate solution (5), upon which the earlier suggestion (5) of a furanoid mycosaminide ring was based, probably results after hydrolysis of the lactone.

It remains to consider the crystalline "N-acetyldecarboxytrianhydrododecahydropimaricin" prepared in low yield (~8%) by Patrick et al. (5) from N-acetyldodecahydropimaricin on brief warming with acid, and suggested by them to have the key furyl ketone structure (VI). Ceder (12) revised this to an alternative furyl ketone (which is, in fact, at the wrong oxidation level to arise by dehydration from his formula for N-acetyldodecahydropimaricin), but pointed out that the ultraviolet chromophore ($\lambda_{max.}$ 280 mμ, $\epsilon_{max.}$ 21, 500) could also be that of a conjugated dienone. We believe this compound to be the dienone (VII), arising from acidic dehydration and decarboxylation not of N-acetyldodecahydropimaricin itself

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* Compounds were dissolved in 0.1 M aqueous potassium bicarbonate and treated with four equivalents of sodium periodate at 27° in darkness.

† That this is the preferred, if not the sole, direction of ring opening is indicated by the chromic acid oxidation of dodecahydropimaricin to glutaric acid in good yield.

‡ Further work on this point is in progress; see footnote, previous page.

(V: R = OH; R' = Ac), but rather from an impurity, the desoxy-analogue (V: R = H; R' = Ac). This latter would be formed by complete hydrogenolysis of the oxygen function of the conjugated epoxide, a reaction for which there is ample precedent (19). Consequently, the structure (VII) of this dienone proves the location of the epoxide in pimaricin independently of the Bodforss test (12), and also confirms the point of glycosidic linkage to mycosamine.

High resolution mass spectrometry of the dienone (VII) indicates the molecular formula $C_{34}H_{57}NO_9$ (found: M^+, 623.4046; $C_{34}H_{57}NO_9$ requires M^+, 623.4033) in agreement with the earlier elemental analyses (5). Alkaline hydration and retroaldolisation afforded acetone and acetaldehyde, whilst chromic acid oxidation occurred with preferential cleavage between $C_{(7)}$ and $C_{(8)}$ to give a good yield of pimelic acid (together with the expected higher dibasic acids from the $C_{(15)}$ - $C_{(25)}$ region). Major mass spectral fragmentations of the molecular ion (VII) occur by elimination of N-acetylmycosamine, both alone to give m/e 418 (found: m/e 418.3077; $C_{26}H_{42}O_4$ requires m/e 418.3083) and together with water to give m/e 400. A metastable peak at m/e 179.5 relates m/e 418 to the other intense ion in the spectrum at m/e 274. This daughter ion is formulated as (VIII) (found: m/e 274.2301; $C_{19}H_{30}O$ requires m/e 274.2297) arising by a double McLafferty rearrangement (20), and taken in conjunction with the chemical evidence confirms the dienone structure (VII).

We believe (II: R = H) to be the first accurate structure (without regard to stereochemistry) to be presented for a macrolide antibiotic of the carbohydrate-containing polyene group (1, 2).

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