8.28), which on selective oxidation with N-bromoacetamide in aqueous acetone yields 21-acetoxy-11α,17α - dihydroxy - 16β - methylpregnan-3,20-dione, m.p. 187.5–190.5°, $[\alpha]_D$ +77.9°; *anal.* Found: C, 68.43; H, 8.61. The 1,4-dien-3-one system, is introduced in the usual manner (dibromination at C-2 and C-4 followed by dehydrobromination with dimethylformamide) to give 21-acetoxy - 11α,17α - dihydroxy - 16β -methyl-$\Delta^{1,4}$-pregnadiene-3,20-dione, m.p. 225–228°, $[\alpha]_D$ +100.0, λ_{max}^{MeOH} 247 mμ (ϵ 16,700). *Anal.* Found: C, 69.35; H. 7.79.

Introduction of the 9α-fluoro-11β-hydroxy group is performed in the usual fashion[2]: the 11α-tosylate is dehydrated to 21-acetoxy-17α-hydroxy-16β-methyl-$\Delta^{1,4,9(11)}$-pregnatriene-3,20-dione, m.p. 205–207°, $[\alpha]_D$ +140.3°, λ_{max}^{MeOH}, 239 mμ (ϵ 19,300). Addition of HOBr with N-bromosuccinimide followed by closure with potassium acetate to the epoxide gives 9β,11-epoxy-21-acetoxy-17α-hydroxy-$\Delta^{1,4}$-pregnadiene-3,20-dione (m.p. 210–216°, λ_{max}^{MeOH} 249 mμ (ϵ 15,600)) which on treatment with hydrogen fluoride produces 9α-fluoro-16β-methylprednisolone acetate (I), m.p. 196–201°, $[\alpha]_D$ +107.5°, λ_{max}^{MeOH} 239 mμ (ϵ 25,200); *anal.* Found: C, 66.27; H, 7.51.

The physiological properties of 16β-methyl-9α-fluoroprednisolone acetate in man appear to be quantitatively similar to those of the α-isomer.[1d] The reduction in the number of circulating eosinophiles, the effect on an oral glucose load, the elevation of the fasting blood sugar and the excretion of sodium are responses readily induced by both the 16α- and 16β-methyl isomers.

(2) *Cf.* J. Fried and E. Sabo, THIS JOURNAL, **79**, 1130 (1957).

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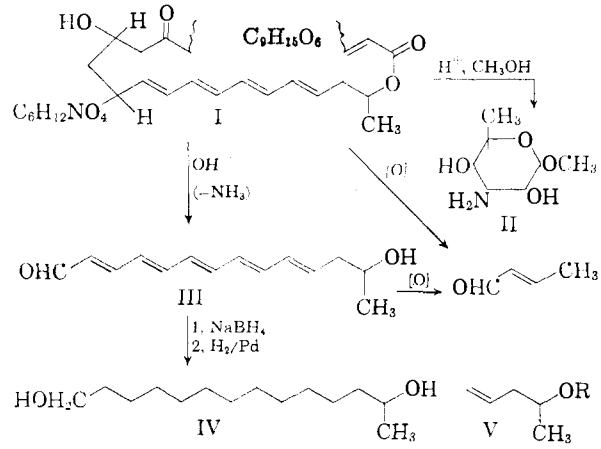
PIMARICIN. I. OXIDATION AND HYDROLYSIS PRODUCTS

Sir:

During investigations on the tetraene antifungal antibiotic pimaricin[1,2,3] we have accumulated evidence for partial structure I for this antibiotic. Pimaricin (calcd. for $C_{34}H_{49}NO_{14}$ (695.74): C, 58.69; H, 7.10; N, 2.01. Found:[4] C, 58.53 ± 0.32; H, 7.32 ± 0.17; N, 2.12 ± 0.14; C-methyl, 1.43, 1.71 methyl groups) gives the usual tests for a primary amino group (Van Slyke), a carboxyl group, and a somewhat hindered keto group. An α,β-unsaturated lactone is present (saponification equivalent: 1 mole of alkali without fission of the molecule, infrared peak at 5.84 μ, shifting to 5.77 μ on hydrogenation of the antibiotic; ultraviolet maximum at 222 mμ, ϵ = 22,400, which disappears on hydrogenation).



Refluxing pimaricin with methanolic hydrogen chloride produced the methyl glycoside of the amino sugar mycosamine,[5] identified by mixed melting point and X-ray powder diffraction pattern comparison of the triacetate with authentic methyl mycosaminide triacetate from nystatin.

Hydrogenation of *N-acetyl pimaricin* (m.p. 200°; $[\alpha]^{25}_D$ + 230°, calcd. for $C_{36}H_{51}NO_{15}$ (737.38): C, 58.60; H, 6.97; N, 1.90. Found: C, 58.86; H, 7.00; N, 1.93) produced *N-acetyldodecahydropimaricin* (m.p. 155–156°; $[\alpha]^{25}_D$ −67.5°; calcd. for $C_{36}H_{63}NO_{15}$ (748.48): C, 57.66; H, 8.47. Found: C, 57.69: H, 8.64: N, 1.91) which was oxidized by sodium dichromate-sulfuric acid to *sebacic acid*, demonstrating that the tetraene system carried no alkyl substituents.

Acid dichromate oxidation of pimaricin itself yielded crotonaldehyde, suggesting the presence of the grouping V.[6]

Treatment of pimaricin with alkali caused liberation of ammonia, indicating labilization of the glycoside linkage, possibly by beta-elimination.[7] The other isolated product was *13-hydroxy-2,4,6,-8,10-tetradecapentaeneal* (III) [orange crystals, m.p. 124–128°; λ_{max} 375 mμ (MeOH)] whose structure follows from its positive Fehling reaction and sodium borohydride reduction to *1,13-dihydroxy-2,4,6,8,10-tetradecapentaene* (yellow crystals, m.p. 187–188°; $\lambda\lambda_{max}$ 313, 328, 346 mμ). This diol was hydrogenated to 1,13-tetradecanediol (IV) (m.p. 49.0–50.0°; $[\alpha]^{25}_D$ −6.5°; calcd. for $C_{14}H_{30}O_2$ (230.4): C, 72.98; H, 13.12; C-methyl, (1) 6.5; active hydrogen, 2. Found: C, 72.77; H, 13.15; C-methyl, 5.26; active hydrogen, 1.76) which after hypobromite oxidation, yielded tridecanedioic (brassylic) acid.

(1) A. P. Struyk, *et al.*; *Antibiotics Annual* (1957–1958), 878 (Medical Encyclopedia, Inc., New York, 1958).

(2) Our antibiotic was isolated and identified as pimaricin by comparison of infrared and ultaviolet spectra and paper chromatographic mobilities of the antibiotics (M. Dann, E. J. Backus, R. W. Sharpe, J. H. Mowat, and N. Bohonos, unpublished data) and their N-acetyl derivatives. X-Ray powder diffraction patterns of the antibiotics and their N-acetyl derivatives were compared also. Pimaricin was kindly provided by Dr. J. C. Hoogerheide, Royal Dutch Yeast and Fermentation Industries, Delft.

(3) The registered trademark of the American Cyanamid Company for pimaricin is Myprozine.

(4) Average and average deviation of seventeen analyses.

(5) D. Walters, J. D. Dutcher and O. Wintersteiner, THIS JOURNAL, **79**, 5076 (1957).

(6) Compare D. E. Ames and R. E. Bowman, *J. Chem. Soc.*, 4264 (1955).

(7) F. A. Hochstein and P. P. Regna, THIS JOURNAL, **77**, 3353 (1955).

We postulate that III is formed by dealdolization of a β-hydroxy ketone system, and then elimination of the mycosamine moiety, which is *beta* to the newly formed aldehyde group. Since III, which contains the system V (R = H), gives a much poorer yield of crotonaldehyde than pimaricin itself does on dichromate oxidation, we believe that pimaricin has a protecting group, probably the lactone, serving as R in formula V.

The partial structure I embodies all of the above features. Evidence to substantiate and extend this partial structure is presented in the following communication.

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RECEIVED SEPTEMBER 26, 1958

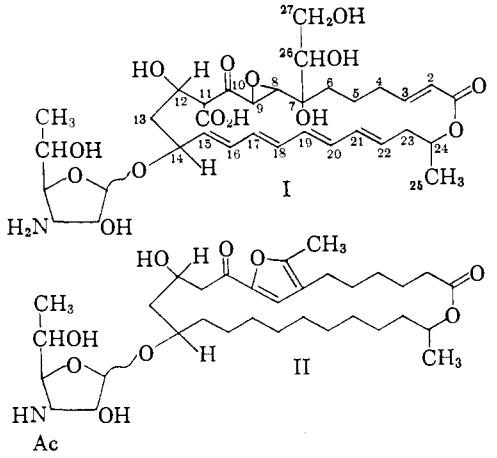
PIMARICIN. II. THE STRUCTURE OF PIMARICIN

Sir:

A partial structure for the tetraene antifungal antibiotic pimaricin[1] has been proposed.[2] We now present evidence that this antibiotic has the total structure I.

The fact that dodecahydropimaricin contains one more acetylatable hydroxyl group than the parent antibiotic, although the keto group remains unreduced, suggests the presence of an epoxide in pimaricin. The liberation of iodine from potassium iodide–acetic acid[3] by the antibiotic confirmed this and indicated that the epoxide probably is adjacent to a carbonyl group.[4]

The carboxyl group in pimaricin is present as a β-keto acid, since pimaricin and dodecahydropimaricin, but not sodium borohydride-reduced dodecahydropimaricin, are readily decarboxylated by warm dilute sulfuric acid.



The previous communication[2] and the arguments above account for all but three of the oxygen atoms in pimaricin. The status of these three is shown by (a) N-acetyl pimaricin consumes two moles of periodate, one immediately and the second in two hours. Formaldehyde is produced. (b) When N-acetyldodecahydropimaricin is heated with *N* sulfuric acid at 90° for 3 minutes, crystalline *N-acetyldecarboxytrianhydrododecahydropimaricin* (II) (m.p. 205–211°; found: C, 64.50; H, 9.08, N, 2.26; N-acetyl, 5.01) is produced. The ultraviolet spectrum of II is that of an alkyl furyl ketone (λ_{max} 280 mμ: ϵ = 21,500).

We consider that the triol structure at positions 7, 26, and 27 accommodates these findings thus: (a) The first mole of periodate obviously cleaves the 26–27 bond, liberating formaldehyde. The splitting of the 7–26 bond is considerably slower, since the hydroxyl group at 7 is tertiary. (b) The reductive opening of the epoxide ring on hydrogenation makes the system 10, 9, 8, 7, 26, 27 the equivalent of a desoxy hexose, the correct oxidation state for acid dehydration to a furyl ketone.

If the above arguments are correct, there remain five carbon atoms at 2, 3, 4, 5 and 6 which should appear as pimelic acid after oxidation of dodecahydropimaricin. Therefore, we reinvestigated this oxidation and succeeded in obtaining pimelic acid (identified by gas chromatography and infrared spectrum of the methyl ester), from chromic acid oxidation. We feel that the isolation of this fragment completes the minimum proof of the structure of the large ring.

Two details remain: (a) The mycosamine moiety is presumed to be furanose because pimaricin gives a positive iodoform test in aqueous bicarbonate solution where the lactone ring is not opened. (b) We prefer to place the carboxyl group at 11 rather than 9 on the basis of a number of indications, none of which, however, amounts to a definitive proof. We shall confine ourselves here to the point that a carboxyl at 9 should be capable of lactone formation with hydroxyl groups at 7, 12 or 26. We have not observed any such lactonization.

This is, we believe, the first complete structure determination on any of the numerous polyene antifungal antibiotics reported in the literature. It seems likely that most of these substances are macrolides of the same general type as pimaricin.[5]

(1) A. P. Struyk, *et al.*, *Antibiotics Annual* (1957–1958), 878 (Medical Encyclopedia, Inc., New York, 1958).

(2) J. B. Patrick, R. P. Williams, C. F. Wolf and J. S. Webb, THIS JOURNAL, **80**, 6688 (1958).

(3) S. Bodforss, *Ber.*, **49**, 2801 (1916).

(4) Nystatin and rimocidin also give this test.

(5) *Cf.* also M. L. Dhar, V. Thaller and M. C. Whiting, *Proc. Chem. Soc.*, 148 (1958).

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THE ENZYMATIC SYNTHESIS OF INOSITOL MONOPHOSPHATIDE[1]

Sir:

Experiments on the enzymatic synthesis of inositol monophosphatide have been described by Agranoff, *et al.*,[2] who have reported that labeled free inositol is incorporated into a phosphatide by

(1) Supported by grants from the Nutrition Foundation, Inc., the Life Insurance Medical Research Fund and the National Institute for Neurological Diseases and Blindness (B-1199). Mr. Henry Paulus is a pre-doctoral fellow of the National Science Foundation.

(2) B. W. Agranoff, R. M. Bradley and R. O. Brady, *J. Biol. Chem.*, **233**, 1072 (1958).