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

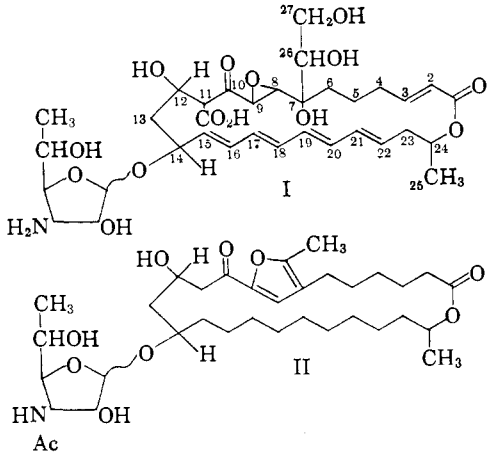
PIMARICIN. II. THE STRUCTURE OF PIMARICIN

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).

enzyme preparations from kidney and that this process is stimulated by any of a number of cytidine nucleotides, including CDP-choline.[3] The amounts of labeled inositol incorporated ranged from 1–7 millimicromoles.

We have observed a much more extensive incorporation (75–150 millimicromoles) of free inositol catalyzed by well-washed, dialyzed microsomes from guinea pig liver in the presence of 0.01 *M* $MnCl_2$ in TRIS buffer of *p*H 7.4. Under these conditions, there is no requirement for cytidine nucleotides nor for an added source of metabolic energy. At low concentrations of $MnCl_2$, however, or in phosphate buffer, the reaction is stimulated by the same range of cytidine nucleotides as reported by Agranoff. It appears that this reaction, and probably the reaction observed by Agranoff, *et al.*,[2] is an enzymatic exchange of free inositol with inositol monophosphatide present in the enzyme particles and that the cytidine nucleotide effect is related to the binding of manganese by the enzyme. These experiments therefore provide little information about the pathways involved in the *de novo* synthesis of inositol monophosphatide.

TABLE I

CONVERSION OF L-α-GLYCEROPHOSPHATE-P^{32} TO INOSITOL MONOPHOSPHATIDE

Each tube contained 0.5 ml. of a dialyzed 20% homogenate of guinea pig liver in 0.05 *M* phosphate buffer of *p*H 7.4, 0.5 μmole of L-α-glycerophosphate-P^{32} (1.7×10^6 counts/μmole), 1.0 μmole of CoA, 0.1 μmole of oleic acid, 5.0 μmoles of ATP (added in portions), 3 μmoles of $MnCl_2$ and 3 μmoles of $MgCl_2$ in a total volume of 1.42 ml. The system was incubated at 37° for 2 hours. The lipides were extracted, washed and then hydrolyzed in methanol–chloroform containing 10% aqueous 10 *N* H_2SO_4. The inositol monophosphate fraction was isolated by chromatography on Dowex-1 formate and counted.

	Additions	L-α-Glycerophosphate-P^{32} converted to inositol monophosphatide (mμmoles)
1	None	0.1
2	1 μmole inositol	0.2
3	1 μmole CTP	4.8
4	1 μmole inositol + 1 μmole CTP	40.5
5	1 μmole inositol + 1 μmole GTP	0.2
6	1 μmole inositol + 1 μmole ITP	0.8
7	1 μmole inositol + 1 μmole UTP	0.0
8	1 μmole inositol + 1 μmole CDP-choline	1.3

It has now been found that the phosphorus moiety of inositol monophosphatide is derived from L-α-glycerophosphate and that the conversion of L-α-glycerophosphate-P^{32} to inositol monophosphatide P^{32} specifically requires CTP and free inositol (Table I). In contrast to the exchange reaction, which is stimulated by CDP-choline,[2] the conversion of L-α-glycerophosphate-P^{32} to inositol monophosphatide is not stimulated by CDP-choline.

The biosynthesis of inositol monophosphatide is thus like that of lecithin and phosphatidylethanolamine in that it requires CTP but is strikingly different in that the phosphorus of lecithin and phosphatidylethanolamine is not derived from glycerophosphate.[4] The present evidence is consistent with a route of formation involving either the hypothetical compound cytidine diphosphate diglyceride, which has been suggested by Agranoff, *et al.*,[1] as a possible intermediate, or cytidine diphosphate glycerol.[5]

(3) Abbreviations: ATP, CTP, GTP, ITP and UTP are the 5′-triphosphates of adenosine, cytidine, guanosine, inosine and uridine, respectively; CDP-choline = cytidine diphosphate choline; CMP = cytidine-5′-phosphate; CoA = coenzyme A; Tris = tris-(hydroxymethyl)-aminomethane.

(4) E. P. Kennedy, *Ann. Rev. Biochem.*, **26**, 119 (1957).

(5) J. Baddiley, J. G. Buchanan, B. Carss, A. P. Mathias and A. R. Sanderson, *Biochem. J.*, **64**, 599 (1956).

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OXIDATION OF SECONDARY PHOSPHINES TO SECONDARY PHOSPHINE OXIDES

Sir:

Secondary phosphines, like most derivatives of trivalent phosphorus, are easily oxidized. Indeed the lower alkyl secondary phosphines inflame spontaneously when exposed to the air.[1] Air oxidation of diphenylphosphine proceeds more gently, and the product has been reported to be diphenylphosphinic acid.[2] Phosphinic acids also result from the oxidation of secondary alkyl phosphines with strong oxidizing agents such as nitric acid.[3]

$$R_2PH \longrightarrow \left[R_2\overset{\overset{O}{\|}}{P}H \right] \longrightarrow R_2\overset{\overset{O}{\|}}{P}OH$$

Although secondary phosphine oxides have been considered as possible intermediates, their isolation from such reactions has heretofore been thought impossible.[4] Moreover, secondary phosphine oxides, prepared by treating dialkyl phosphites with aliphatic[5,6,7] and aromatic[8] Grignard reagents or by reducing phosphinyl chlorides with lithium aluminum hydride,[6] have been found to be relatively stable toward oxidation.[5] Therefore, it seemed reasonable that if secondary phosphine oxides occurred as intermediates during the oxidation of secondary phosphines, they could be isolated by careful control of conditions.

We found this to be the case. Thus, crystalline secondary phosphine oxides were obtained by treating solutions of di-*n*-butylphosphine, di-*n*-octylphosphine, and bis-(2-cyanoethyl)-phosphine in isopropyl alcohol with air at 70°. Di-*n*-butylphosphine oxide, m.p. 66° (calcd. for $C_8H_{19}OP$: P, 19.08. Found: P, 18.84) was characterized by its infrared spectrum and by its reaction with chloral hydrate in refluxing isopropyl alcohol to give 1-hydroxy-2,2,2-trichloroethyldi-*n*-butylphosphine oxide, m.p. 132–133° (calcd. for $C_{10}H_{20}Cl_3O_2P$:

(1) A. W. Hofmann, *Ber.*, **4**, 605 (1871); **6**, 292 (1873).

(2) C. Dorken, *ibid.*, **21**, 1505 (1888).

(3) A. W. Hofmann, *ibid.*, **5**, 104 (1872); **6**, 303 (1873); A. R. Stiles, F. F. Rust and W. E. Vaughan, THIS JOURNAL, **74**, 3282 (1952).

(4) G. M. Kosolapoff, "Organophosphorus Compounds," John Wiley and Sons, Inc., New York, N. Y., 1950, p. 137.

(5) R. H. Williams and L. A. Hamilton, THIS JOURNAL, **74**, 5418 (1952).

(6) R. H. Williams and L. A. Hamilton, *ibid.*, **77**, 3411 (1955).

(7) R. C. Miller, J. S. Bradley and L. A. Hamilton *ibid.*, **78**, 5299 (1956).

(8) B. B. Hunt and B. C. Saunders, *J. Chem. Soc.*, 2413 (1957).