Table X

Tube	[1], M	Added [VCl], M	Peak area VCl(obsd)
A-1	0.0618	0	175
A-2	0.0618	0	171
B-1	0.0309	0.0183	254[a]
B-2	0.0309	0.0183	251[a]
C-1	0	0.0366	328
C-2	0	0.0366	338
C-3	0	0.0366	324

[a] Predicted peak area VCl = 252.

chloroolefin concentration due to added chloroolefin was determined from C and assumed to apply to chloroolefin added to B, The sum of these predicted chloroolefin peak areas for B gives a calculated peak area for B, which is compared to the observed chloroolefin peak area for B. The agreement between calculated and observed values demonstrates the stability of chloroolefin to the reaction conditions.

Decomposition of 3-Methyl-2-butenoyl Peroxide in the Presence of 3-Methyl-2-butenoic Acid. A degassed carbon tetrachloride solution, 0.500 M in 3-methyl-2-butenoyl peroxide and 0.033 M in 3-methyl-2-butenoic acid, was heated at 78.0 ± 0.2° for 12 hr. The solution was then analyzed for 3-methyl-2-butenoic acid by vpc. Equivolume (50 μl) injections of reaction solution and a standard solution of 0.0331 M in 3-methyl-2-butenoic acid were injected on an SE-30 methyl column (10 ft × 0.25 in., 10% on 45–60 acid-washed Chromosorb W, F & M 720 instrument) at 100° with an injector temperature of 240°, a detector temperature of 310°, and a helium flow of 50 cc/min: 3-methyl-2-butenoic acid, 10.4 min. The average peak area for the solution containing acid was 280.8. Since the yield of acid produced in the thermal decomposition of 3-methyl-2-butenoyl peroxide is immeasurably small at this peroxide concentration, the added acid was therefore recovered unreacted. 2-Methylpropene was not detected; analysis of standard solutions showed that 0.25% yield was readily observable.

3-Methyl-1,1,1,3-tetrachlorobutane. Authentic material was prepared by the benzoyl peroxide catalyzed addition of carbon tetrachloride to 2-methylpropene: bp 79–83° (21 mm); $n^{17.5}$D 1.4855 (lit.[38] bp 64–75° (7 mm); n^{20}D 1.4850); nmr 3.408 (s, 1 H), 1.85 (s, 3 H); ir 3040, 2990, 765, 705 cm^{-1}. 3-Methyl-1,1,1,3-tetrachlorobutane was identified as a reaction product in k_H/k_{Cl} runs by vpc retention time. Approximately 0.05 M 3-methyl-2-butenoyl peroxide in 17:1 toluene:carbon tetrachloride solvent mixtures was decomposed at both 110.0 ± 0.5° for 4 hr and 78.0 ± 0.2° for 12 hr. Analysis on an SE-30 methyl column (10 ft × 0.25 in., 10%, temperature programmed at 2°/min from 70 to 90° and held at 90°, He flow 100 cc/min, F & M 720 instrument, injector 250°, detector 310°) gave a peak which was enhanced in size, but not altered in shape, by the coinjection of authentic 3-methyl-1,1,1,3-tetrachlorobutane. Decomposition of 0.1034 M 3-methyl-2-butenoyl peroxide in a 10.14:1.00 p-chlorotoluene–carbon tetrachloride solvent mixture at 78.0 ± 0.2° for 24 hr (100% conversion) also gave a peak which was enhanced in size, but not altered in shape, by the coinjection of authentic adduct. Decomposition for 10 min (17% conversion) produced no detectable peak in this region, and the presence of a peak in this region was questionable for decomposition for 30 min (37% conversion). Analyses of standard solutions showed that reaction of ~5% of the isobutene to form adduct would have been detected in the lowest conversion run (17%).

Acknowledgment. This work is described in detail in the Ph.D. Thesis of P. G. W., University of Rochester, 1970, and was supported, in part, by the National Science Foundation, GP 13475.

(38) A. V. Topchiev, N. F. Bogomolova, and Yu. Ya. Gol'dfarb, *Dokl. Akad. Nauk USSR*, **107**, 420 (1956).

Polyene Antibiotics. II. The Structure of Tetrin A[1,2]

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Abstract: Structure **1** ($C_{34}H_{51}NO_{13}$) has been assigned to tetrin A, from consideration of the structures of certain degradation products, including 3,15-dimethylhexacosane (**7**) and 12-methyl-13-hydroxy-2,4,6,8,10-tetradecapentaenal (**15**), and from nmr and mass spectral properties of a number of derivatives, especially N-acetyltetrin A and decahydrotetrin A.

The antibiotic tetrin, which inhibits the growth of yeasts and fungi, was first reported in 1960[4] and immediately recognized as a member of the family of antibiotics containing an isolated tetraene chromophore.[5] Somewhat later we reported our initial chemical studies on tetrin,[1] which revealed that the antibiotic consists of two closely related components, tetrins A and B. Tetrin A is about three times as active against *Penicillium oxalicum* and the separated components were also characterized by their chemical and physical properties. From microanalyses of the antibiotics and derivatives, tetrins A and B were assigned the tentative formulas $C_{35}H_{53-55}NO_{13}$ and $C_{34-35}H_{53-55}NO_{14}$, respectively. Both compounds contain the tetraene chromophore; both gave mycosamine on acidic hydrolysis. Since tetrins A and B represent the only reported isolation of two tetraene antibiotics from the same microorganism, their structures, and especially the differences between them, are of special interest. In the present report we assign structure **1** (Figure 1) to tetrin A. Our studies with tetrin B are described separately.[6]

(1) Paper I in this series: K. L. Rinehart, Jr., V. F. German, W. P. Tucker, and D. Gottlieb, *Justus Liebigs Ann. Chem.*, **668**, 77 (1963).
(2) Partial reports of the present work: (a) K. L. Rinehart, Jr., V. F. German, W. P. Tucker, D. Krauss, and Y. Nishikawa, 3rd International Symposium on the Chemistry of Natural Products, Kyoto, April 12–18, 1964; Abstracts, p 148. (b) R. C. Pandey and K. L. Rinehart, Jr., 157th National Meeting of the American Chemical Society, Minneapolis, Minn., April 13–18, 1969, Abstract No. ORGN 155; (c) R. C. Pandey, K. L. Rinehart, Jr., and N. Narasimhachari, 7th International Symposium on the Chemistry of Natural Products, IUPAC, Riga, USSR, June 1970, Paper E 157.
(3) Alfred P. Sloan Foundation Fellow, 1959–1963.
(4) D. Gottlieb and H. L. Pote, *Phytopathology*, **50**, 817 (1960).
(5) A review of polyene antibiotics: W. Oroshnik and A. D. Mebane, *Fortschr. Chem. Org. Naturst.*, **21**, 17 (1963).

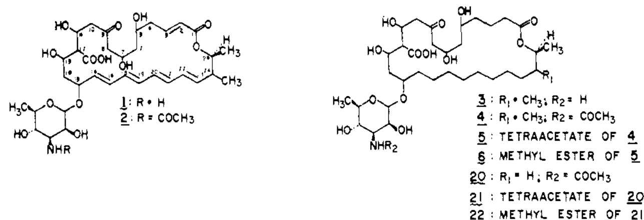


Figure 1. Structures of tetrin A and its derivatives.

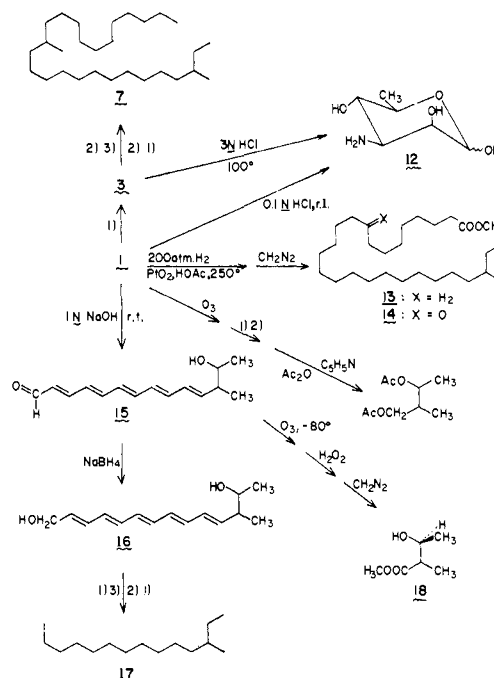
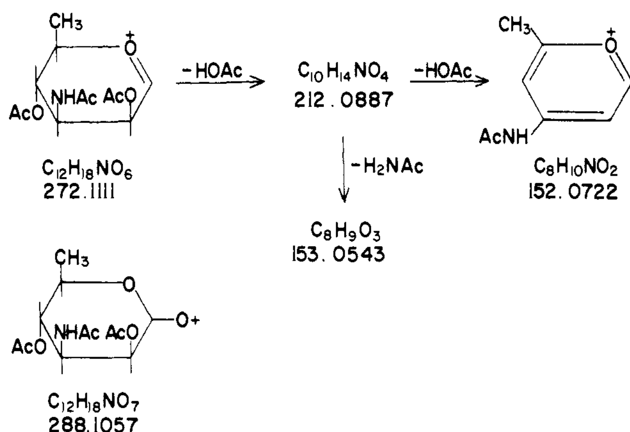


Figure 2. Degradation products of tetrin A. Reagents abbreviated: (1) H_2, PtO_2; (2) $LiAlH_4$; (3) P, 48% HI.

Molecular Formula. In our earlier report[1] we wrote, "Bei Molekülen dieser Grösse und Kompliziertheit können Bruttoformeln jedoch nicht als sicher betrachtet werden, bevor die Struktur aufgeklärt ist." With the general availability of mass spectral techniques,[7] that statement is no longer necessarily true.

In the present study key compounds for mass spectral investigation include the trimethylsilyl derivatives of tetrin A (**1**) and *N*-acetyltetrin A (**2**); underivatized decahydrotetrin A (**3**) and *N*-acetyldecahydrotetrin A (**4**); and, especially, pentaacetyldecahydrotetrin A (**5**), the tetraacetate of **4**, and the corresponding methyl ester (**6**).

The line of argument with respect to **5** and **6** is as follows. The number of acetyl groups in **5** was established by its nmr spectrum, while mass spectral analysis of **5** and **6** gave peaks as high as *m/e* 883 and 897, respectively (Table I). Allowing for the five acetyl groups,[8]



which must have introduced 210 mass units (5 × 42 amu), the molecular weight of decahydrotetrin A would be assigned as 673 and that of tetrin A as 663. Thus, the molecular formula indicated for the antibiotic by the mass spectra of **5** and **6** should probably be $C_{34}H_{49}NO_{12}$ or $C_{35}H_{53}NO_{11}$. We shall see in the next section that the carbon skeleton of the macrolide portion of the antibiotic contains 28 carbon atoms. Since mycosamine contains six carbon atoms the molecular formula $C_{34}H_{49}NO_{12}$ would be indicated by the mass spectrum. However, that formula is not in accord with the microanalytical data (see Experimental Section and ref 1) for tetrin A (**1**) or its *N*-acetyl derivative (**2**), which require 13 oxygen atoms. We conclude, therefore, that the highest mass peaks in the mass spectra of **5** and **6** result from the loss of water from the parent peaks and that the molecular formula of tetrin A is $C_{34}H_{51}NO_{13}$, the molecular weight 681.

Other mass spectral data in accord with the molecular formula assigned to tetrin A are found in the intense peaks in the mass spectra of decahydrotetrin A (**3**) and its *N*-acetyl derivative (**4**) at *m/e* 510 [P − sugar (mycosamine or *N*-acetylmycosamine) − H_2O], 492 (510 − H_2O), 474 (510 − $2H_2O$), 456 (510 − $3H_2O$), 412 (510 − $3H_2O$ − CO_2), and 394 (412 − H_2O). Finally, the molecular formula of the aglycone ($C_{28}H_{40}O_{10}$), recovered after mild hydrolytic removal of mycosamine and isolated as its triethylamine salt, agrees with that assigned to tetrin A.

Carbon Skeleton. The Cope procedure for determining the carbon skeleton of polyene antibiotics was first applied to fungichromin[9] and has since then been used in structural studies of many other antibiotics.[7a,c] In the present study (Figure 2), tetrin A was first reduced catalytically over platinum oxide in acetic acid, then with lithium aluminum hydride in refluxing tetrahydrofuran. The resulting polyol was heated at reflux with 48% hydriodic acid and red phosphorus. Lithium aluminum hydride and catalytic reductions of the iodide, followed by chromatography on alumina, afforded an oil with infrared and ultraviolet spectra characteristic of a saturated hydrocarbon which gas–liquid chromatography indicated to be a single compound. The high-resolution mass spectrum of the hydrocarbon obtained by field ionization contained a parent ion at $C_{28}H_{58}$ (*m/e* 394.456), with no ions above *m/e* 394. Strong peaks in the high-resolution mass spectrum obtained by electron impact were found at $C_{17}H_{35}$ (*m/e* 239.273) and $C_{13}H_{27}$ (183.211), in-

(6) K. L. Rinehart, Jr., W. P. Tucker, and R. C. Pandey, *J. Amer. Chem. Soc.*, **93**, 3647 (1971).

(7) Recent pertinent reviews: (a) K. L. Rinehart, Jr., and T. H. Kinstle, *Annu. Rev. Phys. Chem.*, **19**, 301 (1968); (b) G. E. Van Lear and F. W. McLafferty, *Annu. Rev. Biochem.*, **38**, 289 (1969); (c) G. E. Van Lear and K. L. Rinehart, Jr., in "Biochemical Applications of Mass Spectrometry," G. R. Waller, Ed., Interscience, New York, N. Y., in press, Chapter 17.

(8) Three of the acetyl groups in **5** and **6** are in the mycosamine portion of the molecule, as confirmed by the fragmentation scheme shown. The remaining two acetyl groups are on unspecified hydroxyls.

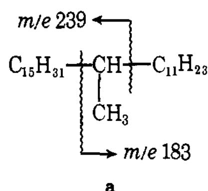
(9) A. C. Cope, R. K. Bly, E. P. Burrows, O. J. Ceder, E. Ciganek, B. T. Gillis, R. F. Porter, and H. E. Johnson, *J. Amer. Chem. Soc.*, **84**, 2170 (1962).

Table I. Corresponding Mass Spectral Peaks for Hydrogenated Tetrin and Pimaricin Derivatives

Origin of peak	Compound: Pentaacetyl-decahydrotetrin A (**5**)	Pentaacetyl-dodecahydro-pimaricin (**21**)	Pentaacetyldeca-hydrotetrin A, methyl ester (**6**)	Pentaacetyldodeca-hydropimaricin, methyl ester (**22**)
$P - H_2O$	883	869	897 (*898*)[b]	883
$P - H_2O - HOAc$	823	809	837 (*838*)[b]	823 (*824*)[b]
$P - H_2O - 2HOAc$	763	749	777 (*778*)[b]	763 (*764*)[b]
$P - H_2O - 2HOAc - CO_2$	719	705	*733* (734)[b]	719 (*720*)[b]
$P - H_2O - 3HOAc$	703	689	717 (*718*)[b]	703 (704)[b]
$P - 2H_2O - 2HOAc - CO_2$	701	687		
$P - 2H_2O - 3HOAc - CO_2$	641	627		
$P - 2H_2O - 4HOAc - CO_2$	581	567		
$P - H_2O - HOAc - C_{12}H_{18}NO_7$[a]	535	521	549	535
$P - H_2O - 2HOAc - C_{12}H_{18}NO_7$[a]	475	461	489	475
$P - 2H_2O - 2HOAc - C_{12}H_{18}NO_7$[a]	457	443	471	457
$C_{12}H_{18}NO_7$[a]	288[c]	288	(289)[b]	(289)[b]
$C_{12}H_{18}NO_6$[a]	272[c]	272	272	272

[a] $C_{12}H_{18}NO_7$ = triacetylmycosaminyloxy; $C_{12}H_{18}NO_6$ = triacetylmycosaminyl.[8] [b] Peaks with one hydrogen more or fewer are in parentheses, with the italicized peak being the strongest in the cluster. [c] Confirmed in a high-resolution spectrum.

dicating partial structure **a** for the hydrocarbon, whose total structure was presumed to be **7**, based on additional evidence developed below.



a

To prove the identity of this hydrocarbon it was synthesized according to the route shown in Figure 3, starting from 11-bromoundecanoic acid (**8**) and pro-

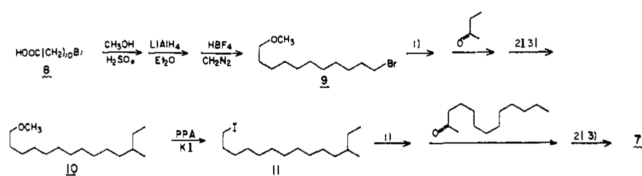
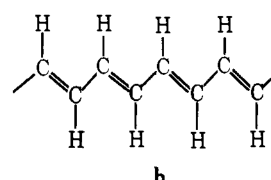


Figure 3. Preparation of 3,15-dimethylhexacosane. Reagents abbreviated: (1) Mg, Et_2O; (2) *p*-TsOH, C_6H_6, reflux; (3) H_2, PtO_2, EtOAc.

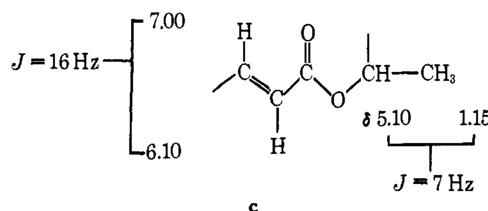
ceeding through 1-methoxy-11-bromoundecane (**9**), 1-methoxy-12-methyltetradecane (**10**), and 1-iodo-12-methyltetradecane (**11**). The Grignard reagent from **11** was then treated with 2-tridecanone, dehydrated, and hydrogenated to afford pure 3,15-dimethylhexacosane (**7**). The mass spectrum of the synthetic sample of **7** was identical with that of the hydrocarbon obtained from tetrin A.

Functional Groups. From the outset tetrin has been recognized to contain an isolated tetraene chromophore whose maxima are of positions (λ_{max} 278, 290, 303, and 318 nm) and extinction coefficients (ϵ_{max} 30,100; 55,300; 78,300; 75,500; respectively) characteristic of an all-trans configuration, like that in lagosin.[10] The nmr spectrum of *N*-acetyltetrin A contains no olefinic methyl absorption, indicating an unsubstituted tetraene, as in **b**.



b

The additional unsaturation required by the 5 *M* hydrogen uptake to give decahydrotetrin A is located in the olefinic group of an α,β-unsaturated ester. That conclusion is derived by comparison of the infrared spectrum of tetrin A, which contains only a conjugated ester carbonyl, at 1710 cm^{-1}, with the corresponding spectrum of decahydrotetrin A, with a saturated ester carbonyl absorption at 1730 cm^{-1}. Additional evidence is found in the characteristic ultraviolet maximum at 210 nm (ϵ_{max} 14,500), at approximately the same location as the maxima in the spectra of other macrolide antibiotics containing α,β-unsaturated lactone units.[11] This maximum, as well as the tetraene chromophore, disappears on hydrogenation to **3**. The conjugated olefinic group is unsubstituted and the α and β protons can be located at δ 6.10 and 7.00 (J = 16 Hz), respectively, in the spectrum of **2**. Since no carbon atoms (except those in mycosamine) are lost in the conversion of tetrin A (**1**) to the saturated hydrocarbon **7**, the ester must be in the form of a lactone. The alkyl portion of the lactone is elaborated by a spin decoupling experiment. A methyl group doublet at δ 1.15 in the nmr spectrum of tetrin A collapses to a singlet on irradiation at δ 5.10; thus, the carbinyl proton splitting the methyl group occurs at a position appropriate for an acyloxycarbinyl (lactone) proton but not for a hydroxycarbinyl proton. From this the partial structure **c** emerges.



c

We earlier reported the isolation of mycosamine (**12**, Figure 2) by hydrolysis of tetrin A.[1] Thus, an amino

(10) P. Nayler and M. C. Whiting, *J. Chem. Soc.*, 3037 (1955).

(11) Pimaricin, 222 nm (ϵ 22,400);[14] lucensomycin, 218 nm (ϵ 21,210) [G. Gaudiano, P. Bravo, and A. Quilico, *Tetrahedron Lett.*, 3559 (1966)]; chalcomycin, 218 nm (ϵ 22,770) [P. W. K. Woo, H. W. Dion, and Q. R. Bartz, *J. Amer. Chem. Soc.*, 86, 2724 (1964)].

group and two hydroxyl groups of mycosamine are available for acylation. The isolation of pentaacetyldecahydroterin A (5)[8] requires then that at least two additional hydroxyl groups be available for acetylation in the macrolide portion of the antibiotic.

Two additional oxygen atoms are located in tetrin A in a free carboxyl group. The antibiotic itself is essentially neutral; since an amino group is known to be present in mycosamine, overall neutrality requires a carboxyl group. Also, the infrared spectrum of tetrin A contains carboxylate (COO^-) bands (KBr)[1] at 1560 and 1390 cm^{-1},[12] which are absent in the spectra of tetrin A hydrochloride and *N*-acetyltetrin A, where they are replaced by a carboxyl (COOH) band at 1700 cm^{-1}, and of *N*-acetyltetrin A methyl ester, where it is replaced by an ester (COOR) band at 1720 cm^{-1}. Finally, the shift of mass spectral peaks for **6** by 14 amu from those for **5**, due to the formation of a methyl ester, confirms the carboxyl group. While the carboxyl group might in principle have given rise to any of the four methyl groups of **7**, the two terminal methyls are eliminated from consideration by structural unit **c**. The carboxyl is ultimately assigned as shown in **1** by consideration of the results from the Ceder reduction to be described in the next section.

The final demonstrable functional group is a keto group. An infrared band at 1700 cm^{-1} in the spectrum of **1** (at 1710 in that of **3**) is in accord with this assignment and a keto group is also indicated to be present by the results of a retro-aldol cleavage to be discussed in detail in the next section. For purposes of identification of the keto group, however, suffice it to note here that a base-catalyzed retro-aldol cleavage is triggered by the keto group and that sodium borohydride treatment of tetrin A gives a product which no longer undergoes retro-aldol cleavage.

Juxtaposition of Functional Groups. High-pressure hydrogenation (Ceder reduction)[13] of polyene antibiotics followed by esterification usually leads to mixtures of long-chain methyl esters, useful in the characterization of the macrolide protion of the molecule.[7c] Hydrogenolysis of tetrin A over platinum oxide in acetic acid at 245° and 2500 psi afforded 24-methylhexacosanoic acid and 9-oxo-24-methylhexacosanoic acid, which were isolated as their methyl esters (**13** and **14**, respectively) by column chromatography and preparative thin layer chromatography and identified by their infrared and mass spectra. Both infrared spectra showed ester absorption near 1730 cm^{-1}, that of **14** a ketone shoulder at 1710 cm^{-1} as well. Both mass spectra showed parent peaks, at *m/e* 424.428 ($C_{28}H_{56}O_2$) for **13**, at *m/e* 438.408 ($C_{28}H_{54}O_3$) for **14**. In addition, the typical α- and β-cleavages of ketones were apparent in the spectrum of **14** (Figure 4), giving rise to the strong peaks at *m/e* 185.119 ($C_{10}H_{17}O_3$) and 281.285 ($C_{19}H_{37}O$) (α-cleavage) and *m/e* 200.143 ($C_{11}H_{20}O_3$) and 296.309 ($C_{20}H_{40}O$) (β-cleavage with hydrogen transfer from C-12 and C-6, respectively), as shown in Figure 4. These fragmentations locate the ketone function at C-9 of the macrolide portion of the molecule. Isolation of **13** and **14**, lacking a substituent at C-12, also serves to indicate that the methyl in the middle of the chain in **7** must arise

(12) L. J. Bellamy, "The Infra-Red Spectra of Complex Molecules," 2nd ed, Methuen, London, 1958, p 162.

(13) O. Ceder, J. M. Waisvisz, M. G. van der Hoeven, and R. Ryhage, *Acta Chem. Scand.*, 18, 83 (1964).

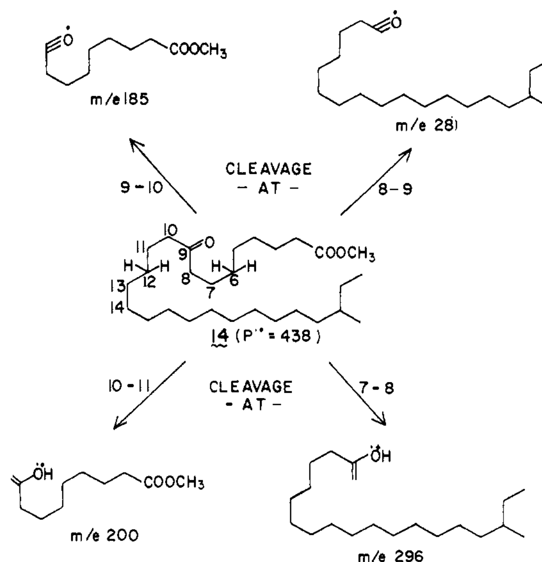
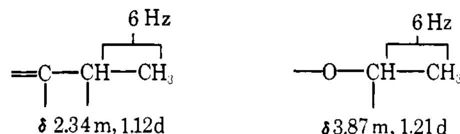


Figure 4. Mass spectral fragmentations of methyl 9-oxo-24-methylhexacosanoate (**14**).

from a carboxyl at C-14 in **1**. The carboxyl is presumably lost by decarboxylation under the vigorous conditions of the Ceder reduction.

Additional structural evidence was provided by treatment of tetrin A with 1 *N* sodium hydroxide at room temperature. Continuous extraction with ether under these conditions gave an ether-soluble solid, which was shown to be homogeneous by tlc and whose structure was assigned as **15**. Its mass spectrum contained a parent ion peak at *m/e* 232 (P, $C_{15}H_{20}O_2$), and other characteristic peaks at *m/e* 214 (P − H_2O) and 187 (P − $CHOHCH_3$). Its ultraviolet spectrum contained a broad maximum at 377 nm (ϵ 50,000), comparable with that of a pentaenal isolated from similar treatment of pimaricin,[14] while its infrared spectrum contains a highly conjugated aldehyde carbonyl band at 1675 cm^{-1} and a polyene band at 1570 cm^{-1} (corresponding absorptions for 2,4,6,8,10-dodecapentaenal are at 1677 and 1573 cm^{-1}).[15] The remainder of structure **15** was assigned from the nmr spectrum, which showed signals for an aldehyde proton at δ 9.60 (d, J = 8 Hz), for ten olefinic protons in a multiplet near δ 6.4, and for the additional groupings shown.



Structure **15** was confirmed by its reduction with sodium borohydride to a crystalline pentaenediol (**16**, $C_{15}H_{22}O_2$),[16] whose ultraviolet spectrum showed the presence of an isolated pentaene chromophore [$\lambda\lambda_{max}$ 304 nm (ϵ 40,800), 316 (69,400), 331 (103,500), and 348 (105,500)], quite comparable with that observed for the pentaenediol isolated from fungichromin.[17] The in-

(14) J. B. Patrick, R. P. Williams, C. F. Wolf, and J. S. Webb, *J. Amer. Chem. Soc.*, **80**, 6688 (1958).

(15) E. R. Blout, M. Fields, and R. Karplus, *ibid.*, 70, 194 (1948).

(16) Owing to the instability of the pentaenal (**15**), the pentaenediol (**16**) was prepared more conveniently without isolation of **15** (*cf.* Experimental Section).

(17) A. C. Cope and H. E. Johnson, *J. Amer. Chem. Soc.*, **80**, 1504 (1958).

frared spectrum did not show any carbonyl band and the nmr spectra of the diol and its diacetate were in complete agreement with the proposed structure. In particular, a two-proton doublet at δ 4.43 (J = 5 Hz) was observed for the new hydroxymethyl group.

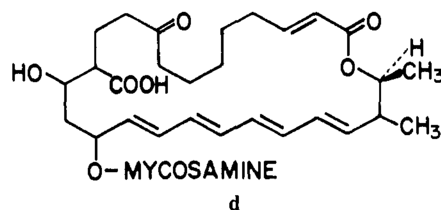
The diol (**16**) was hydrogenated over platinum in ethyl acetate–ethanol (1:1) and the product was heated with 48% hydriodic acid. Lithium aluminum hydride reduction of the resulting diiodide, followed by catalytic hydrogenation over platinum oxide, gave 3-methyltetradecane (**17**), whose structure was confirmed by synthesis. Lithium aluminum hydride reduction of **11**, an intermediate used in the preparation of **7**, followed by catalytic hydrogenation over platinum oxide, gave **17**.

Horeau's method[18] was used to determine the absolute configuration at the carbinol carbon of **15** (C-25 of tetrin A). Ozonolysis of the pentaenal (**15**) in methanol at −80° and oxidative work-up followed by esterification with diazomethane gave methyl 2-methyl-3-hydroxybutanoate (**18**), $[\alpha]^{23}D$ +22.8° ($CHCl_3$), whose identity was confirmed by synthesis of the racemate in the Reformatsky reaction between acetaldehyde and ethyl α-bromopropionate, followed by hydrolysis and esterification with diazomethane. The methyl ester (**18**) was then treated with (±)-α-phenylbutyric anhydride in benzene–pyridine. The recovered α-phenylbutyric acid was levorotatory (αD −0.26°), indicating that the absolute configuration at the carbinol carbon is S,[18] as shown in Figure 1.

With the structure of **15** in hand, attention turns to its formation from tetrin A. The nmr spectrum of N-acetyltetrin A contains a methyl doublet at δ 0.95 (in addition to those at δ 1.15 and 1.41) for the C-24 methyl group. Irradiation at δ 2.65 collapses the doublet, indicating the attached methine to be of the C=CCH– type. Thus, the tetraene system of tetrin A consists of C-16 through C-23. The presence of a C-22, C-23 double bond is also indicated by the results of ozonolysis of tetrin A and reductive work-up, followed successively by lithium aluminum hydride treatment and acetylation. This treatment yielded 2-methyl-1,3-butanediol diacetate, whose nmr spectrum and glc behavior were identical with those of a synthetic sample.

The acidic hydrolysis of tetrin A (**1**) to give mycosamine (**12**) proceeds under quite mild conditions (0.1 N aqueous hydrochloric acid at room temperature[1]), but a drastic change in the environment of the glycosidic bond must be effected by hydrogenation since under identical conditions only starting material was recovered from decahydrotetrin A (**3**). Hydrolysis of the latter compound was effected only with acid concentration in excess of 3 N and at 100°. The comparative ease of formation of **12** from **1** and **3** indicates that the lability of the glycosidic bond toward acidic hydrolysis is much lessened by hydrogenation and suggests that the glycosidic bond is at an allylic position, *i.e.*, α to the tetraene unit at C-15. Mycosamine at that position would be lost then by β-elimination from an aldehyde formed during the retro-aldol cleavage which gives **15**. That mycosamine is indeed formed by base-catalyzed elimination is supported by the observation that tetrin A on mild alkaline treatment (insufficient to cause rupture of a glycosidic unit)[19] gives a volatile basic material, presumably ammonia, which could come from a free 3-amino sugar but not from a glycoside of a 3-amino sugar. The aldehyde group itself does not exist in tetrin A and must be formed from a secondary alcohol during the retro-aldol cleavage.

From all the above considerations the nearly complete structure **d** emerges for tetrin A. There remain only two points (outside of stereochemistry)—the location of three additional oxygen atoms and the ring form of mycosamine.



d

The partial formula **e** corresponds to $C_{34}H_{51}NO_{10}$, *vs.* $C_{34}H_{51}NO_{13}$ for tetrin A. Thus the free remaining oxygen atoms must be in hydroxyl groups.[20] As potential locations for these three hydroxyls C-13 through C-26 are eliminated from consideration by the structure of **15**, as are C-1, C-2, C-3, and C-9 by the nature of their substituents. N-Acetyltetrin A does not consume any sodium metaperiodate, indicating the absence of a 1,2-glycol system in the macrolide unit; this eliminates C-12.

Steam distillation of a suspension of tetrin A in dilute alkali afforded acetone and acetaldehyde, isolated as their 2,4-dinitrophenylhydrazones. The aqueous residue on extraction with ether followed by acidification and further steam distillation gave more acetaldehyde, again isolated as its 2,4-dinitrophenylhydrazone. The isolation of acetone requires that the carbon atoms adjacent to the ketone at C-9 be unsubstituted[21] and that hydroxyl groups be on C-11 and C-7 to effect the retro-aldol cleavage releasing acetone (Figure 5). By a similar argument a hydroxyl group must be on C-5 to release acetaldehyde (from C-6 and C-7) before acidification and steam distillation (when it can come from C-11 and C-12).[22] Location of two of the three hydroxyls at C-7 and C-11 is further substantiated by the failure of sodium borohydride reduced tetrin A to give **15**, acetone, or acetaldehyde on alkali treatment. This indicates that the retro-aldol reaction is triggered by the ketone function alone rather than by a lactone or carboxyl function. Thus, a C-11 hydroxyl is required to sustain the successive retro-aldol cleavages to give **15**, as summarized in Figure 5, and a C-7 hydroxyl is required to give acetaldehyde.[22]

Relationship to Pimaricin. At this juncture the relationship of tetrin A to the tetraene antibiotic pimaricin is obvious. Although it has undergone numerous revisions over 10 years,[14,23] the structure of pimaricin

(18) (a) A. Horeau, *Tetrahedron Lett.*, 506 (1961); (b) *ibid.*, 965 (1962).

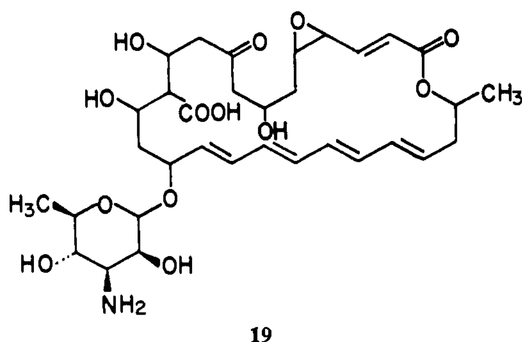
(19) F. A. Hochstein and P. P. Regna, *J. Amer. Chem. Soc.*, 77, 3353 (1955).

(20) It is unclear why decahydrotetrin A forms the pentaacetyl derivative rather than a heptaacetyl; obvious possible explanations are hydrogen bonding and steric hindrance.

(21) The failure of N-acetyltetrin A to consume periodate also argues against a hydroxyl at C-8 or C-10.

(22) The additional acetaldehyde isolated after acidification and steam distillation presumably comes from decarboxylation of formylacetic acid (C-11, C-12, and C-12 carboxyl).

(23) (a) J. B. Patrick, R. P. Williams, and J. S. Webb, *J. Amer. Chem. Soc.*, 80, 6689 (1958); (b) O. Ceder, J. M. Waisvisz, M. G. van der



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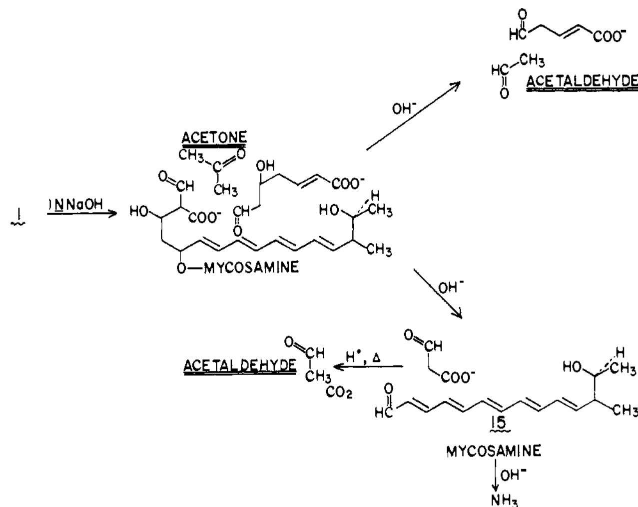

Figure 5. Retroaldol cleavage of tetrin A (1).

now seems settled as **19**. This structure (**19**) lacks the C-24 methyl group of tetrin A and has a 4,5-epoxy unit instead of the 5-hydroxyl group of **1**. In view of the great similarity of the two antibiotics the physical and chemical properties of several derivatives of the two antibiotics were compared. Spectral data for pentaacetyldodecahydropimaricin (**21**) and its methyl ester (**22**) were especially valuable. The mass spectra were essentially identical (Table I) except that all peaks for pentaacetyldecahydrotetrin A (**5**) and its methyl ester (**6**) appear 14 mass units higher than those for **21** and **22**, respectively. The only ions appearing at the same positions were those due to mycosamine fragments (Table I and ref 8). The nmr spectra of **5** and **21** were identical except for the extra methyl doublet in the spectrum of **5**. For that matter the nmr spectra of **2** and the *N*-acetyl derivative of **19** were nearly identical, one obvious difference being the extra methyl doublet, at δ 0.95, in the spectrum of **2**.

Finally, the identity of the nmr spectra of **2** and **19** and of **5** and **21** in those regions due to the mycosamine unit, *e.g.*, the $NCOCH_3$ singlet and the mycosamine methyl doublet (at δ 1.41 in the nmr spectra of **2**, at δ 1.28 in that of **5**), and the near identity in the low mass region of the mass spectra, also due to mycosamine, point to an identity of ring form for mycosamine in the two antibiotics. That has been assigned as pyranose in pimaricin on the basis of periodate oxidation studies.[23] Hence, we assign mycosamine a pyranose form in tetrin A as well and write the antibiotic's structure as **1**.

Experimental Section[24]

Isolation of Tetrins A and B. Details of the fermentation and assay procedures have been described by Gottlieb and Pote.[4] The crude material for the present investigations was supplied by the Upjohn Co., Kalamazoo, Mich., from which tetrin A and tetrin B were isolated by countercurrent distribution in the solvent system *sec*-butyl alcohol–ethyl acetate–buffer (4:1:5).[1]

Separation of 40 g of crude tetrin gave 1.2 g of dark crystalline tetrin A. The antibiotic was recrystallized from methanol or aqueous *n*-butyl alcohol to give fine colorless needles, mp >350° dec, $[\alpha]^{21}D$ +8.3° (*c* 0.72, pyridine). Thin layer chromatography (BAW 415) showed a single spot. The infrared spectrum (Nujol) contained bands at 3500, 3300 (OH, NH), 2700 (-COOH), 1710 (C=O), 1625 (C=C), and 1545 cm^{-1} (COO^-). Ultraviolet, circular dichroism, and optical rotatory dispersion spectra are shown in Figure 6.

Anal. Calcd for $C_{34}H_{51}NO_{13}$: C, 59.89; H, 7.54; N, 2.06; $(CCH_3)_3$, 6.16. Calcd for $C_{34}H_{49}NO_{12}$: C, 61.52; H, 7.44; N, 2.11; $(CCH_3)_3$, 6.80. Calcd for $C_{35}H_{53}NO_{13}$: C, 60.48; H, 7.69; N, 2.02; $(CCH_3)_3$, 6.49. Calcd for $C_{35}H_{55}NO_{13}$: C, 60.24; H, 7.94; N, 2.01; $(CCH_3)_3$, 6.46. Found: C, 60.28, 60.36; H, 7.71, 7.80; N, 2.05, 2.26; CCH_3, 6.20.

Evaporation of the contents of tubes containing tetrin B gave no crystals; hence, the residue was dissolved in ethanol, precipitated with diethyl ether, filtered, washed with ether, and dried *in vacuo* to give 3.3 g of brown amorphous tetrin B, mp 130°, $[\alpha]^{28}D$ +45° (pyridine).

***N*-Acetyltetrin A (2).** A stirred solution of 442.5 mg (0.64 mol) of tetrin A and 0.2 ml (2 mmol) of acetic anhydride in 40 ml of absolute methanol was maintained at 0°. Thin layer chromatography indicated the reaction was complete after 2 hr. Solvent was removed under vacuum at room temperature and the residue was crystallized from methanol–ethyl acetate to furnish 177.5 mg (37%) of fine white needles, mp 160–162°. Recrystallization from absolute ethanol gave the analytical sample: mp 167–171°, $[\alpha]^{21}D$ +54° (*c* 0.5, MeOH). The derivative had $\lambda\lambda_{max}$ (95% EtOH) 214, 278, 290, 303, and 319 nm; its infrared spectrum (Nujol) contained bands at 3415 (OH, NH), 2700 and 1720 (COOH), 1695 (conjugated C=O), 1630 and 1545 cm^{-1} (amide, bands I and II). The compound consumed no periodate at 0°.

Anal. Calcd for $C_{36}H_{53}NO_{14}$: C, 59.97; H, 7.34; N, 1.94. Calcd for $C_{35}H_{53}NO_{14} \cdot H_2O$: C, 58.38; H, 7.10; N, 1.80. Found: C, 58.86; H, 7.52; N, 1.90.

Decahydrotetrin A (3). Tetrin A (1.8 g) was hydrogenated over prereduced platinum oxide in 50 ml of glacial acetic acid at atmospheric pressure. Hydrogen uptake ceased after 1 hr, but stirring was continued for 1 hr more, when 320 ml of hydrogen (at 25° (736 mm), representing 96% of 5 molar equiv) had been absorbed. Filtration, concentration, and addition of ether gave a white pre-

Hoeven, and R. Ryhage, *Chimia*, 17, 352 (1963); (c) O. Ceder, *Acta Chem. Scand.*, 18, 126 (1964); (d) B. T. Golding, R. W. Rickards, W. E. Meyer, J. B. Patrick, and M. Barker, *Tetrahedron Lett.*, 3551 (1966).

(24) Melting points were determined on a Kofler hot stage and are uncorrected. Infrared spectra were determined on Perkin-Elmer infrared spectrophotometers, Models 21B, 237, and 137B, ultraviolet spectra on Cary Model 14M, Bausch and Lomb Model 505, or Beckman Model DB spectrophotometers. Optical rotations were measured on a Zeiss polarimeter. Optical rotatory dispersion and circular dichroism spectra were determined on a modified Jasco spectropolarimeter, Model E. Proton magnetic resonance spectra were determined by Mr. R. Thrift and associates on Varian A-60 and HA-100 spectrometers. Chemical shifts are reported on the δ scale from TMS as internal standard (δ 0). Low-resolution mass spectra were obtained by Mr. J. Wrona on an Atlas CH4B spectrometer, employing direct sample introduction techniques. High-resolution mass spectra were determined at the Purdue Mass Spectrometry Center by Dr. G. E. Van Lear and Dr. W. L. Budde on a CEC 21-110 mass spectrometer. Microanalyses were determined by Mr. J. Nemeth and associates. Gas–liquid chromatographic analyses were performed on F & M Model 500 or Aerograph Model A-90-P instruments, using helium as carrier gas. Analytical and preparative thin layer chromatography was carried out on silica gel G (Merck). The spots were visualized by ninhydrin spray reagent, iodine vapor, or sulfuric acid–nitric acid mixture (2:1). Florisil (Florisil Co.), Woelm neutral alumina, activities I and II, and silica gel G were used as adsorbents for both tlc and column chromatography. For ascending paper chromatography Whatman 3MM paper was used, for descending paper chromatography, Whatman No. 1. The solvent system BAW 415 was frequently employed. This consisted of the upper phase of *n*-butyl alcohol–acetic acid–water (4:1:5). Countercurrent distribution experiments were carried out as described earlier,[1] except that a 400-tube (10 ml/phase) automatic Craig instrument (H. O. Post Co.) was employed.

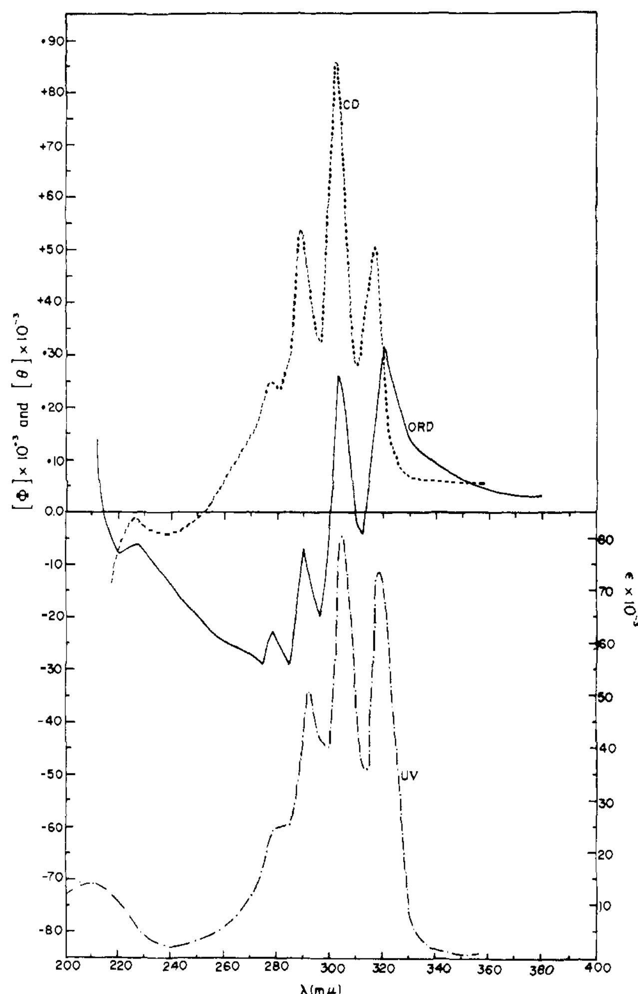

Figure 6. Ultraviolet (UV), circular dichroism (CD), and optical rotatory dispersion (ORD) spectra of tetrin A (1).

cipitate, which was filtered, washed with ether, and dried, giving 1.35 g (75%) of decahydrotetrin A: mp 206–208° dec, $[\alpha]^{26}D$ −44.1° (*c* 2.06, MeOH), $[\alpha]^{26}D$ ~60° (*c* 0.5, pyridine). A thin layer chromatogram (BAW 415) showed a single spot. Optical rotatory dispersion (*c* 0.085, methanol, 25°) gave a negative plain curve and the ultraviolet spectrum showed only a weak maximum (95% EtOH) at 208 nm (ε 676). The infrared spectrum (Nujol) showed bands at 3360 (OH, NH), 1730 (C=O), and 1080 cm^{-1} (OH). The nmr spectrum (HA-100, C_5D_5N) did not show any olefinic protons between δ 10.0 and 5.4.

Anal. Calcd for $C_{34}H_{61}NO_{13}$: C, 59.04; H, 8.82; N, 2.02. Found: C, 58.38; H, 9.08; N, 2.15.

***N*-Acetyldecahydrotetrin A (4).** A mixture of 240 mg of decahydrotetrin A, 0.1 ml of acetic anhydride, and 20 ml of absolute methanol was stirred at 0° for 2 hr while the antibiotic derivative gradually dissolved. The resulting solution was concentrated at room temperature and the residue was precipitated from ether-hexane (1:1) to give 174 mg (72%) of *N*-acetyldecahydrotetrin A, sintering *ca.* 125° and melting 134–136°, $[\alpha]^{21}D$ −62.2° (*c* 1.13, MeOH). Analytical tlc indicated a single component, R_f 0.63 (BAW 415). The infrared spectrum (Nujol) contained bands at 3400, 1740, 1650, and 1550 cm^{-1}.

Anal. Calcd for $C_{38}H_{63}NO_{14} \cdot H_2O$: C, 57.49; H, 8.72; N, 1.86. Found: C, 56.84; H, 8.43; N, 1.99.

Pentaacetyldecahydrotetrin A (5). A mixture of 142.7 mg of decahydrotetrin A, 3 ml of pyridine, and 2 ml of acetic anhydride stood at room temperature for 24 hr while the course of the reaction was followed by tlc (BAW 415). The mixture was then cooled, diluted with cold water, and extracted with chloroform. Work-up afforded a foamy residue (0.235 g), which was dissolved in ether and precipitated with hexane. The white precipitate was filtered, washed with fresh hexane, and dried at 56° (1 mm) for 6 hr to yield 132.0 mg (70%) of pentaacetyldecahydrotetrin A, mp 118–120°. The infrared spectrum ($CHCl_3$) contained bands at 3600 and 3440 (OH), at 1737 and 1200 (acetate), and at 1670 cm^{-1} (amide). The nmr spectrum ($CDCl_3$) clearly indicated the presence of five acetyl groups, with peaks at δ 2.14 (s, 3), 2.06 (s, 6), 2.02 (s, 3), and 1.91 (s, 3). Esterification with diazomethane in ether gave the methyl ester (6) of pentaacetyldecahydrotetrin A. Mass spectral peaks for both compounds are found in Table I.

Pentaacetyldodecahydropimaricin (21) was prepared from pimaricin *via* dodecahydropimaricin,[14] following exactly the same conditions as employed in the preparation of pentaacetyldecahydrotetrin A: sintered 106°, mp ~120°; $[\alpha]^{26}D$ −50.6 (*c* 0.79, $CHCl_3$). The infrared spectrum ($CHCl_3$) had bands at 3600 and 3440 (OH), 1735 and 1200 (acetate), and 1675 cm^{-1} (amide). The nmr spectrum ($CDCl_3$) indicated the presence of five acetyl groups: δ 2.20 (s, 3), 2.12 (s, 6), 2.08 (s, 3), 1.98 (s, 3). Esterification with diazomethane in ether gave the methyl ester, **22**. Mass spectral peaks of both compounds are listed in Table I.

Synthesis of 3,15-Dimethylhexacosane (7). A. From Tetrin A. Tetrin A (0.4357 g) was hydrogenated over 100 mg of platinum oxide in glacial acetic acid at room temperature to afford 0.4064 g of decahydrotetrin A (3), mp 206–208° dec. A mixture of this material, 1.0 g of lithium aluminum hydride, and 100 ml of dry tetrahydrofuran was then heated under reflux for 3 days. Excess hydride was destroyed by addition of ethyl acetate, and the mixture was evaporated nearly to dryness under reduced pressure. The inorganic material was dissolved in cold 15% aqueous sulfuric acid and the polyol was extracted into *n*-butyl alcohol. Work-up of the butanol extract afforded 0.1951 g of a noncrystalline material whose infrared spectrum (smear) contained a strong band at 3350 cm^{-1} (OH) and a very weak band at 1700 cm^{-1} (C=O).

A mixture of 0.1865 g of the crude polyol, 5 ml of glacial acetic acid, 400 mg of red phosphorus, and 40 ml of 48% hydriodic acid was heated under reflux for 48 hr and then cooled. Water was added and the reaction mixture was extracted with ether. The ether extract was washed with water and aqueous 2% sodium thiosulfate solution, then dried. Removal of the solvent yielded a yellow viscous residue (0.1864 g). A mixture of the polyiodide and 0.5 g of lithium aluminium hydride in dry ether was heated under reflux for 48 hr. Excess hydride was destroyed by addition of ethyl acetate, the complex was decomposed by aqueous sulfuric acid, and the organic material was extracted into ether. Work-up afforded 0.1232 g of a residue, which was hydrogenated over 20 mg of prereduced platinum oxide. Filtration and concentration furnished 0.1236 g of residue, which was chromatographed over a small column of grade I alumina. Elution with 25 ml of pentane afforded 21 mg (8% overall from tetrin A) of hydrocarbon: bp (bath) 180–200° (0.2 mm); ir (smear) 2905, 1455, 1375, and 725 cm^{-1}. Gas-liquid chromatography (206°, 6-ft column, 10% SE-30 on Chromosorb P) showed a single peak.

Anal. Calcd for $C_{28}H_{58}$: mol wt, 394.4539. Found: mol wt, 394.4556 (HRMS).

B. Authentic Sample. 11-Bromoundecanoic acid (**8**, 25 g, Aldrich Chemical Co.) was esterified with methanol (20 ml) in benzene (200 ml) containing 6 ml of concentrated sulfuric acid. The methyl ester (25 g, 95%) after the usual work-up had bp 151–152° (2.1 mm), $n^{20}D$ 1.4641 [lit.[25] bp 131–132° (5 mm), $n^{20}D$ 1.4652].

Anal. Calcd for $C_{12}H_{23}BrO_2$: mol wt, 278. Found: mol wt, 278 (mass spectrum).

A solution of 25 g of the methyl ester and 6.0 g of lithium aluminum hydride in 200 ml of ether was stirred under reflux for 10 hr. The usual work-up and crystallization of the residue from pentane afforded 18.2 g (81%) of white crystalline 11-bromo-1-undecanol, mp 40–41° [lit.[26] mp 44–46°], ir (Nujol) 3400 cm^{-1} (OH).

Anal. Calcd for $C_{11}H_{23}BrO$: mol wt, 250. Found: mol wt, 250 (mass spectrum).

A solution of diazomethane plus 8.6 g of fluoroboric acid[27] in methylene chloride was added to 15.1 g of 11-bromoundecanol in methylene chloride until the yellow color persisted. The reaction mixture stood for 1 hr, then excess diazomethane was destroyed with a few drops of glacial acetic acid. Work-up gave 17.37 g of a colorless mobile liquid which was chromatographed over silica gel, using benzene–ethyl acetate as eluent, to afford 9.4 g (58%) of pure 1-methoxy-11-bromoundecane (**9**), bp 109–

(25) R. E. Bowman and R. G. Mason, *J. Chem. Soc.*, 4151 (1952).
(26) T. D. Perrine, *J. Org. Chem.*, 18, 1356 (1953).
(27) M. Neeman, M. C. Caserio, J. D. Roberts, and W. S. Johnson, *Tetrahedron*, 6, 36 (1959).

110° (0.3 mm), $n^{25.5}D$ 1.4575 [lit.[25] bp 114–115° (0.25 mm), $n^{20}D$ 1.4624], ir (smear) 1135 cm^{-1} (-OCH_3).

Anal. Calcd for $C_{12}H_{25}BrO$: C, 54.33; H, 9.43; mol wt, 264. Found: C, 54.48; H, 9.44; mol wt, 264 (mass spectrum).

A mixture of 3.0 g of methyl ethyl ketone and the Grignard reagent prepared from 7.95 g of 1-methoxy-11-bromoundecane (**9**) and 0.73 g of magnesium ribbon, in 100 ml of ether, was stirred at room temperature for 16 hr. Work-up afforded 9.58 g of a semicrystalline residue, which gave infrared bands at 3400 (OH) and 1625 cm^{-1} (C=C). A solution of the residue plus 3.0 g of *p*-toluenesulfonic acid monohydrate in 50 ml of benzene was then heated for 2 hr under reflux using a Dean–Stark trap. Work-up afforded a liquid residue (8.79 g), which was eluted from a column of alumina (grade I, 60 g) with pentane to give 7.03 g (97%) of the unsaturated methyl ether; ir 1625 (C=C) and 1130 cm^{-1} (-COC-). The unsaturated methyl ether (4.7 g) was then hydrogenated at atmospheric pressure over platinum oxide in ethyl acetate and the hydrogenated product was purified on a silica gel column. Elution with benzene gave 3.7 g (78%) of pure (tlc, glc) 1-methoxy-12-methyltetradecane (**10**): bp 121–122° (0.9 mm), $n^{24}D$ 1.4352, ir (smear) 1125 cm^{-1} (methyl ether). The nmr spectrum (CCl_4) contained peaks at δ 3.24 (t, 2, J = 6 Hz, CH_2O–), 3.20 (s, 3, CH_3–O), 1.27 (sb, 21), 0.87 (overlapping doublet and triplet, 6, J = 6 Hz).

Anal. Calcd for $C_{16}H_{34}O$: C, 79.37; H, 14.14; mol wt, 242. Found: C, 78.75; H, 14.34; mol wt, 242 (mass spectrum).

A mixture of 2.5 g of 1-methoxy-12-methyltetradecane (**10**), 2.5 g of phosphorus pentoxide, 6 ml of 85% phosphoric acid, and 9.96 g of potassium iodide was stirred at 135–140° (bath temperature) for 5.5 hr, cooled, diluted with water, and extracted with ether. The ether extract was washed with water, 2% sodium thiosulfate, water, and brine, then dried and evaporated to give 3.3 g of the colorless iodide (positive Beilstein test), which was purified over a column (2 × 18 cm) of silica gel, eluting with 100 ml of 5% ethyl acetate in benzene. The product, 1-iodo-12-methyltetradecane (**11**), was purified by distillation: 2.5 g (73%), bp 135–136° (0.3 mm), $n^{24}D$ 1.4834. The nmr spectrum (CCl_4) contained peaks at δ 3.13 (t, 2, J = 7 Hz), 1.81 (d, 2, J = 7 Hz), 1.26 (s, 19), 0.84 (t, 3, J = 7 Hz), and 0.83 (d, 3, J = 6 Hz).

Anal. Calcd for $C_{15}H_{31}I$: C, 53.25; H, 9.23; mol wt, 338. Found: C, 52.51; H, 9.05; mol wt, 338 (mass spectrum).

2-Tridecanone was prepared in 76% yield by oxidation of 2-tridecanol (the product of the Grignard reaction of dodecanal with methylmagnesium iodide) with chromic oxide in acetone, bp 128° (6.0 mm) (lit.[28] bp 145° (10 mm)). A mixture of 1.4 g of 2-tridecanone and the Grignard reagent prepared from 2.2 g of 1-iodo-12-methyltetradecane (**11**) and 170 mg of magnesium, in 45 ml of ether, was then heated at reflux for 24 hr. Work-up yielded the crude tertiary alcohol, which was heated at reflux with 1.0 g of *p*-toluenesulfonic acid in 20 ml of benzene for 12 hr. Work-up yielded 3.07 g of the crude product, which was passed over an alumina–silica column. Elution with pentane gave 1.79 g of the oily olefin; ir (smear) 1645, 970, and 914 cm^{-1} (C=C).

Anal. Calcd for $C_{28}H_{56}$: mol wt, 392. Found: mol wt, 392 (mass spectrum), the main peak.

The olefin was hydrogenated at atmospheric pressure over 250 mg of prereduced platinum oxide in ethyl acetate. Filtration of the catalyst and removal of solvent yielded 1.5 g of an oil; glc showed one major (85%) and two minor components. The major component (7) was collected by preparative glc, employing a 6-ft column of 10% SE-30 on Chromosorb P: bp (bath) 120–140° (0.1 mm); $n^{20}D$ 1.4315; ir (smear) 2950, 1466, 1375, and 720 cm^{-1}.

Anal. Calcd for $C_{28}H_{58}$: C, 85.19; H, 14.81; mol wt, 394. Found: C, 85.01; H, 14.81; mol wt, 394 (mass spectrum).

High-Pressure Hydrogenolysis of Tetrin A. A mixture of 1.00 g of tetrin A, 30 ml of glacial acetic acid, and 500 mg of platinum oxide was shaken for 5 hr in a hydrogenation bomb at 245° and a pressure of 2500 psi of hydrogen. After the mixture was cooled and vented, the catalyst was filtered and washed and the solvent was removed under suction to give 1.0175 g of the crude acid, which was treated with diazomethane in ether to yield 0.8834 g of methyl ester. The crude ester, which showed a number of spots on tlc, was purified by silica gel column chromatography and preparative thin layer chromatography to afford two pure esters (tlc, mass spec.). The less polar ester was identified as methyl 24-methylhexacosanoate (**13**), ir ($CHCl_3$) 1730, 1125 cm^{-1} (ester).

(28) C. W. Hoerr, R. A. Reck, G. B. Corcoran, and H. J. Harwood, *J. Phys. Chem.*, **59**, 457 (1955).

Anal. Calcd for $C_{28}H_{56}O_2$: mol wt, 424.4280. Found: mol wt, 424.4277 (HRMS).

The more polar ester, which crystallized after several days at room temperature (mp 40°), was identified as methyl 9-oxo-24-methylhexacosanoate (**14**), ir ($CHCl_3$) 1725, 1130 cm^{-1} (ester).

Anal. Calcd for $C_{28}H_{54}O_3$: mol wt, 438.408. Found: mol wt, 438.407 (HRMS).

Acidic Hydrolyses of Tetrin A and Derivatives. A. Identification of Mycosamine (12). Paper chromatographic analyses of hydrolysates indicated that mycosamine [R_f 0.23 (BAW 415), R_f 0.70 (*tert*-butyl alcohol–acetic acid–water 2:2:1)] was formed from tetrin A under the following mild conditions: (a) 0.1 N hydrochloric acid in 50% aqueous ethanol at 20° for 20 hr; (b) 3 N hydrochloric acid in 50% aqueous methanol at −20° for 40 hr. However, when decahydrotetrin A was treated with hydrochloric acid at different temperatures, it was found that mycosamine was formed only with hydrochloric acid at least 3 N and at temperatures of at least 100°.

B. Isolation of Aglycone. Hydrochloric acid hydrolysis of 316 mg of tetrin A was carried out as described earlier[1] for the isolation of mycosamine. The butanol extract of the hydrolysate was washed with deionized water to pH 5 and concentrated under reduced pressure. The pale orange residue (225 mg, 93%) was purified by countercurrent distribution (200 transfers) in the solvent system *sec*-butyl alcohol–ethyl acetate–triethylamine–water (8:2:1:9). Microanalyses of the material isolated from countercurrent distribution were consistent with those for the triethylammonium salt of the aglycone.

Anal. Calcd for $C_{25}H_{40}O_{10} \cdot C_6H_{15}N$: C, 64.03; H, 8.69. Found: C, 64.28; H, 9.25.

The ultraviolet spectrum showed the characteristic absorption bands for an isolated tetraene chromophore at 290, 304, and 319 mμ. The material consumed 5.05 molar equiv of hydrogen in glacial acetic acid over platinum oxide (assumed mol wt, 572.7) and was inert toward sodium metaperiodate.

Basic Hydrolysis of Tetrin A. A. Detection of Ammonia. Tetrin A (10 mg) was treated with 2 ml of 1 N aqueous sodium hydroxide at *ca.* 50° whereupon a volatile base was given off, as indicated by a color change in pH paper and a faint odor, characteristic of ammonia.

B. Isolation of Acetone and Acetaldehyde. A suspension of 0.2 g of tetrin A in a solution of 0.2 g of sodium hydroxide in 50 ml of water was steam distilled into a saturated solution of 2,4-dinitrophenylhydrazine in 2 N aqeuous hydrochloric acid until precipitation of 2,4-dinitrophenylhydrazones ceased. The precipitate was filtered and dried (55.4 mg, mp 145–148°), then chromatographed on a column (1.8 × 8 cm) of alumina, grade II. Elution with benzene and crystallization from hexane furnished yellow-orange shining plates, mp 162–163°. Recrystallization raised the melting point to 166–168°, undepressed on mixture with authentic acetaldehyde 2,4-dinitrophenylhydrazone, mp 166–168°. The identity was further confirmed by superimposable infrared and mass spectra.

The filtrate from the above crystallizations and later benzene eluates were mixed and purified by preparative thin layer chromatography employing benzene or benzene–ethyl acetate (95:5), then crystallized from hexane to furnish yellow golden crystals, mp 123–124°. The product was identified as acetone 2,4-dinitrophenylhydrazone by mixture melting point with an authentic sample, mp 122–124°, and by superimposable infrared and mass spectra.

After the steam distillation above the aqueous basic residue was continuously extracted with ether for 24 hr, then acidified with hydrochloric acid to pH 2. The acidified aqueous residue was steam distilled into a 2,4-dinitrophenylhydrazine-saturated 2 N aqueous hydrochloric acid solution, until no more hydrazone was formed. The precipitated 2,4-dinitrophenylhydrazone was filtered, washed with water, and dried to yield 2 mg of product, mp 145–148°. Recrystallization from hexane raised the melting point to 166–168°. As before, this compound was identified as acetaldehyde 2,4-dinitrophenylhydrazone by mixture melting point and superimposable infrared and mass spectra. No other products could be isolated.

C. Isolation and Characterization of 12-Methyl-13-hydroxy-2,4,6,8,10-tetradecapentaenal (15). A mixture of 560 mg of tetrin A and 250 ml of 1 N sodium hydroxide was extracted continuously with ether for 24 hr. The ether extract was worked up to give 100 mg of a yellow residue, which was purified by chromatography over Florisil. The major component (**15**) when crystallized from cyclohexane had mp 112–113°, $[\alpha]^{22.3}D$ −52.9° (c

0.34, $CHCl_3$), λ_{max} (95% C_2H_5OH) 377 mμ (ϵ 50,000), infrared bands (KBr) at 3420 (OH), 1675 (highly conjugated C=O), 1570, 1005, and 928 cm^{-1} (C=C).

Anal. Calcd for $C_{15}H_{20}O_2$: mol wt, 232. Found: mol wt, 232 (mass spectrum).

Alkali Treatment of Sodium Borohydride Reduced Tetrin A. A suspension of 200 mg of tetrin A and excess solid sodium borohydride in 25 ml of methanol stood 24 hr at room temperature. The reaction mixture was then treated with Amberlite MB-3 ion exchange resin in 95% aqueous methanol, the resin was removed by filtration, and the filtrate was concentrated under reduced pressure. The partially deionized residue, whose ultraviolet spectrum was identical with that of the starting material, was treated with 50 ml of 0.4 *N* aqueous sodium hydroxide solution and continuously extracted with ether during 24 hr. This yielded no ether-soluble solid (12-methyl-13-hydroxy-2,4,6,8,10-tetradecapentaenal) and the aqueous alkaline solution still retained the tetrin chromophore. Additional studies of the reaction of alkali on tetrin A and sodium borohydride-reduced tetrin A at room temperature indicated that tetrin A loses its chromophore after 24 hr, while sodium borohydride reduced tetrin A remains unaffected even after 10 days or more.

Preparation of 12-Methyl-2,4,6,8,10-tetradecapentaene-1,13-diol (16). A. From the Pentaenal (15). A mixture of 50 mg of 12-methyl-13-hydroxy-2,4,6,8,10-tetradecapentaenal (**15**) and 50 mg of solid sodium borohydride in 25 ml of methanol stood at room temperature for 30 min, then was diluted with deionized water and concentrated under reduced pressure to remove methanol. The aqueous solution was extracted twice with ether and the ether extract was worked up to give a residue which on crystallization from ethyl acetate or an ether-pentane mixture furnished 23 mg (45%) of pale yellow plates: mp 161–163°; $[\alpha]^{28}D$ $-116.5°$ (*c* 0.84, pyridine); λ_{max} (95% C_2H_5OH) 348 (ϵ 105,500), 331 (ϵ 103,500), 316 (ϵ 69,400), and 304 nm (ϵ 40,800); ir (Nujol) 3350 (OH) and 976, 935, 910 cm^{-1} (C=C). The nmr spectrum (C_5D_5N) contained peaks at δ 5.3 [s, 10, (-CH=CH-)$_5$], 4.43 (d, 2, J = 5 Hz, =CCH_2O), 3.88 (m, 1, -CHO-), 2.52 (m, 1, CH_3CHC=), 1.32 (d, 3, J = 6 Hz, CH_3CHO-), and 1.22 (d, 3, J = 6 Hz, CH_3-CHC=).

Anal. Calcd for $C_{15}H_{22}O_2$: C, 76.87; H, 9.47; $(CCH_3)_2$, 12.83; mol wt, 434. Found: C, 76.71; H, 9.49; CCH_3, 11.86; mol wt, 434 (mass spectrum).

B. From Tetrin A. A stirred mixture of 132 mg of tetrin A and 175 ml of 0.4 *N* sodium hydroxide was extracted continuously with ether for 24 hr. The extract was washed with water and concentrated under reduced pressure at a temperature less than 20°. The residual orange solid was then treated with methanolic sodium borohydride and worked up as in A. The product was crystallized repeatedly from ethyl acetate to give 23 mg (54%) of pale yellow plates, mp 158–159°.

Preparation of 3-Methyltetradecane (17). A. From 12-Methyl-2,4,6,8,10-tetradecapentaene-1,13,diol (16). 12-Methyl-2,4,6,8,10-tetradecapentaene-1,13-diol (**16**, 20 mg) was hydrogenated over prereduced platinum oxide in 10 ml of ethyl acetate-methanol (1:1) and the crude hydrogenated product, isolated after the usual work-up, was heated for 12 hr at reflux with 0.5 ml of glacial acetic acid, 40 mg of red phosphorus, and 5 ml of 48% hydriodic acid. The organic material was extracted with ether, and the ether solution was washed with water, 2% aqueous sodium thiosulfate solution, and water, then dried and concentrated. The residue was then heated for 24 hr at reflux with 100 mg of lithium aluminum hydride in dry ether. The usual work-up gave a product which was hydrogenated over prereduced platinum oxide in hexane. Purification of the hydrogenated product by chromatography over a small column of alumina, eluting with pentane, yielded 5 mg (33% overall from **16**) of a colorless liquid, the saturated hydrocarbon **17**, ir 2950, 1465, 1380 cm^{-1}.

Anal. Calcd for $C_{15}H_{32}$: mol wt, 312. Found: mol wt, 312 (mass spectrum).

B. From 1-Iodo-12-methyltetradecane (11). 1-Iodo-12-methyltetradecane (**11**) (an intermediate in the synthesis of 3,15-dimethylhexacosane, **7**, described above) was subjected to lithium aluminum hydride reduction followed by catalytic hydrogenation over prereduced platinum oxide in hexane. The usual work-up and purification yielded a colorless liquid, bp (bath) 145–150° (7.5 mm), ir 2950, 1468, 1380 cm^{-1}.

Anal. Calcd for $C_{15}H_{32}$: C, 84.82; H, 15.18; mol wt, 312. Found: C, 84.84; H, 15.05; mol wt, 312 (mass spectrum).

Methyl 2-Methyl-3-hydroxybutanoate (18). A. From Ozonolysis of 12-Methyl-13-hydroxy-2,4,6,8,10-tetradecapentaenal (15). A stream of ozone (*ca.* 0.6 mmol/min) was passed through a solution of 12-methyl-13-hydroxy-2,4,6,8,10-tetradecapentaenal (**15**, 250 mg) in methanol (30 ml) at −80° for 15 min. The ozonide was then transferred to a round-bottomed flask and the solvent was distilled at room temperature. The residue was heated at 60° for 2 hr with hydrogen peroxide (30%, 10 ml), potassium carbonate (500 mg), and water (10 ml), then left at room temperature overnight. The usual work-up gave 33.2 mg of an acid, which was esterified with diazomethane and purified through a small column of silica gel to afford 24 mg (82%) of methyl 2-methyl-3-hydroxybutanoate (**18**): ir (smear) 3375 (OH), 1730 cm^{-1} ($COOCH_3$); $[\alpha]^{23}D$ $+22.8°$ (*c* 0.99, $CHCl_3$).

B. Stereochemical Studies. A solution of 120 mg of ($\pm$)-α-phenylbutyric anhydride, 12 mg of methyl 2-methyl-3-hydroxybutanoate (from the pentaenal **15**), 0.5 ml of pyridine, and 5 ml of benzene was kept at room temperature for 24 hr, then was diluted with 5 ml of water. The mixture was stirred for 30 min, then mixed with 25 ml of dilute hydrochloric acid and 25 ml of ether. The ether layer was washed with water and extracted with sodium bicarbonate. The bicarbonate extract was acidified with dilute hydrochloric acid, extracted with ether, and dried. Removal of solvent afforded a residue (49 mg) which in 1 ml of benzene had $\alpha^{23}D$ -0.26.

C. Preparation of an Authentic Sample. Approximately 10 ml of a mixture of 50 ml of benzene, 45 g of distilled ethyl α-bromopropionate, and 16 g of acetaldehyde was added with stirring to 22.5 g of zinc, cut into small pieces. The mixture was heated for 5 min to start the reaction, then the remainder of the liquid reactants were added at a rate to continue refluxing. The mixture was then heated at reflux for a final 30 min, decanted over ice, decomposed with dilute sulfuric acid, saturated with solid ammonium sulfate, and extracted with ether–benzene (1:1). Work-up of the organic layer and fractionation of the residue gave 8.3 g (24%) of ethyl 2-methyl-3-hydroxybutanoate as a colorless mobile liquid: bp 87–88° (15 mm), $n^{22}D$ 1.4245 [lit.[29] bp 85–87° (15 mm)].

A portion (2.5 g) of the ethyl ester was heated for 2.5 hr at reflux with 50 ml of 5% methanolic potassium hydroxide solution. Methanol was removed under vacuum and the residue was worked up to give the hydroxy acid (ir bands at 3300, 2600, and 1715 cm^{-1}), which was esterified with diazomethane to give methyl 2-methyl-3-hydroxybutanoate as a colorless mobile liquid, bp 85° (15 mm); $n^{22}D$ 1.4256. The ir spectrum (smear) contained bands at 3310 (OH) and 1725 cm^{-1} ($COOCH_3$). The nmr spectrum ($CDCl_3$) contained peaks at δ 1.14 (d, 3, J = 6.5 Hz), 1.16 (d, 3, J = 7 Hz), 2.45 (m, 1), 3.7 (s, 3), and 3.94 (m, 1).

2-Methyl-1,3-butanediol Diacetate. A. From Tetrin A. Ozone was passed through a solution of 500 mg of tetrin A in 80 ml of methanol at 0° until oxidation was complete. The solution was concentrated, 100 mg of platinum oxide was added, and the mixture was hydrogenated under atmospheric pressure, then filtered and concentrated under reduced pressure. The residue was stirred with *ca.* 1 g of lithium aluminum hydride and 100 ml of dry tetrahydrofuran at reflux for 20 hr. Excess hydride was decomposed with glacial acetic acid and solvent was removed under reduced pressure. The residue, plus 80 ml of ether, 50 ml of pyridine, and 20 ml of acetic anhydride, was heated at reflux for 20 hr. Work-up gave a residue, which was distilled, bp 150° (0.1 mm), into a receiver cooled in a Dry Ice–acetone bath. Gas–liquid chromatographic analysis of the distillate (8-ft Apiezon L column, 170°) showed two principal overlapping components, which were collected together *via* preparative glc (8-ft Apiezon L column, 170°).

The major component was shown to be 2-methyl-1,3-butanediol diacetate by comparison of glc retention times (8-ft columns: Apiezon L, 170°; LAC 728, 175°; LAC 446, 168°) and nmr spectra of this material and an authentic sample prepared as described in the next section.

B. Authentic Sample. A solution of 267 ml (3.06 mol) of methyl ethyl ketone and 200 ml (2.46 mol) of 37% aqueous formaldehyde was maintained below 35° and at pH 9–9.5 by slow addition of 2 *N* sodium hydroxide during 5 hr. Excess methyl ethyl ketone and water were removed under reduced pressure and the residue was distilled to yield 60 g of 4-hydroxy-3-methyl-2-butanone, bp 91–94° (17 mm) [lit.[30] bp 86–87° (15 mm)]. An excess of solid sodium borohydride was then added batchwise to a mixture of 15 g of the hydroxybutanone in 50 ml of 50% aqueous methanol. Water was added and the mixture was extracted with *n*-butyl alcohol.

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Butanol was removed by slow distillation through a 2-ft semimicro column and the 2-methyl-1,3-butanediol was distilled, bp 110–112° (15 mm). A solution of 2 ml of the distillate, 20 ml of dry pyridine, and 8 ml of acetic anhydride was heated at 100° for 6 hr, then worked up to give an analytical sample of 2-methyl-1,3-butanediol diacetate, isolated by preparative glc (8-ft Apiezon column, 170°).

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Polyene Antibiotics. III. The Structure of Tetrin B[1,2]

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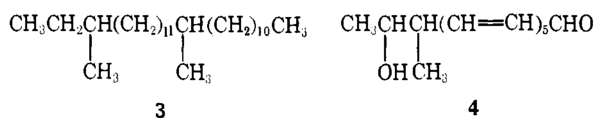
Abstract: The structure of the tetraene antifungal antibiotic tetrin B has been assigned as **2**. Evidence presented includes the reduction of tetrin B to the saturated hydrocarbon 3,15-dimethylhexacosane (**3**) and its basic hydrolysis to 12-methyl-13-hydroxytetradecapentaenal (**4**). Nmr and mass spectra are also fundamental in the structural assignment.

Tetrin, an antifungal antibiotic belonging to the class containing an isolated tetraene chromophore,[5] was earlier shown to consist of two related components,[6] tetrins A and B. Although tetrin B is a somewhat less active antibiotic it is the more abundant component. Recently we assigned structure **1** (Figure 1) to tetrin A.[1] In the present report we assign structure **2** to tetrin B.

Structural Units Like Those in Tetrin A. Many of the degradative reactions which led to the assignment of structure **1** to tetrin A follow an identical course when applied to tetrin B. First, Cope reduction of tetrin B gives 3,15-dimethylhexacosane (**3**), the same hydrocarbon isolated from tetrin A,[1] establishing the carbon skeleton. Second, treatment of tetrin B with mild base gives the same conjugated aldehyde as that from tetrin A, 12-methyl-13-hydroxytetradecapentaenal (**4**), establishing the general location of the tetraene unit.

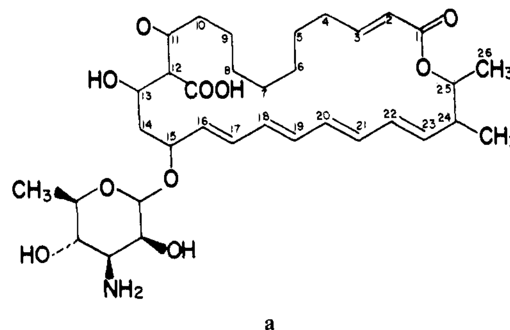


Close similarities in the methyl region of the nmr spectra of the *N*-acetyl derivatives of the two antibiotics (**5** and **6**, respectively) indicate like structural units for their methyl groups—lactone at C-25, pyranose form for mycosamine, allylic methyl at C-24—and this is substantiated by spin decoupling of *N*-acetyltetrin B (**6**), which locates the carbinyl protons as shown: –COOCHCH$_3$ (δ 4.99, 1.05, J = 6.5 Hz); –OCHCH$_3$ (δ 3.57, 1.32, J = 6.5 Hz); =CCHCH$_3$ (δ 2.66, 0.85, J = 7 Hz). These units and the structure of **4** establish the precise location of the tetraene chromophore in the antibiotic (**2**) and the easy hydrolysis of the mycosamine glycoside bond, reported earlier,[6] locates the amino sugar at the position allylic to the tetraene system of **2**. The mode of formation of the pentaenal locates an additional oxygen function (hydroxyl or ketone) in the carbon chain of **2** two carbons beyond the aldehyde carbon of **4**; the latter must have been a carbinol carbon in **2**.

The substituent which becomes a methyl group near the center of the carbon skeleton in the saturated hydrocarbon (at C-15 of **3**) cannot be a methyl in tetrin B since there are only three methyl groups in the nmr spectrum of the antibiotic. It is identified as a carboxyl by the carboxylate bands at 1560 and 1390 cm^{-1} in the infrared spectrum (KBr)[1,6] of tetrin B.

Tetrin B takes up 5 mol of hydrogen,[6] like tetrin A. The fifth olefinic group is located in an unsaturated lactone by the ultraviolet spectrum (λ_{max} 212 nm) of tetrin B, analogous to that of tetrin A.[1]

The results described thus far establish partial structure **a** in tetrin B, the same as in tetrin A.



a

Mild basic hydrolysis of tetrin B gives acetone and acetaldehyde on steam distillation, plus additional acetaldehyde after acidification and further steam distillation; the latter acetaldehyde presumably comes

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