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°).

Acknowledgment. This work was supported in part by Grants AI 01278 and AI 04768 from the National Institute of Allergy and Infectious Diseases. We also thank the Upjohn Co. and Lederle Laboratories for samples of crude tetrin, the latter for a sample of pimaricin, and Dr. Olof Ceder for helpful discussions.

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

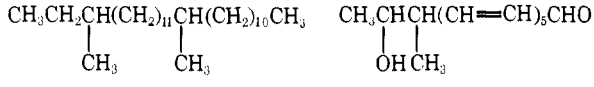
Kenneth L. Rinehart, Jr.,*[3] William P. Tucker,[4] and Ramesh C. Pandey

Contribution from the Department of Chemistry, University of Illinois, Urbana, Illinois 61801. *Received June 20, 1970*

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.



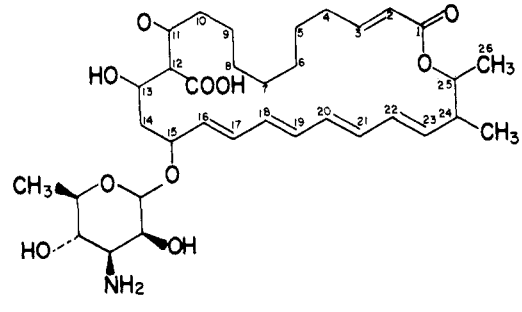
3 **4**

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

(1) Paper II in this series: R. C. Pandey, V. F. German, Y. Nishikawa, and K. L. Rinehart, Jr., *J. Amer. Chem. Soc.*, **93**, 3738 (1971).

(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, 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) National Institutes of Health Postdoctoral Fellow, 1962-1963.

(5) D. Gottlieb and H. L. Pote, *Phytopathology*, **50**, 817 (1960).

(6) K. L. Rinehart, Jr., V. F. German, W. P. Tucker, and D. Gottlieb, *Justus Liebigs Ann. Chem.*, **668**, 77 (1963).

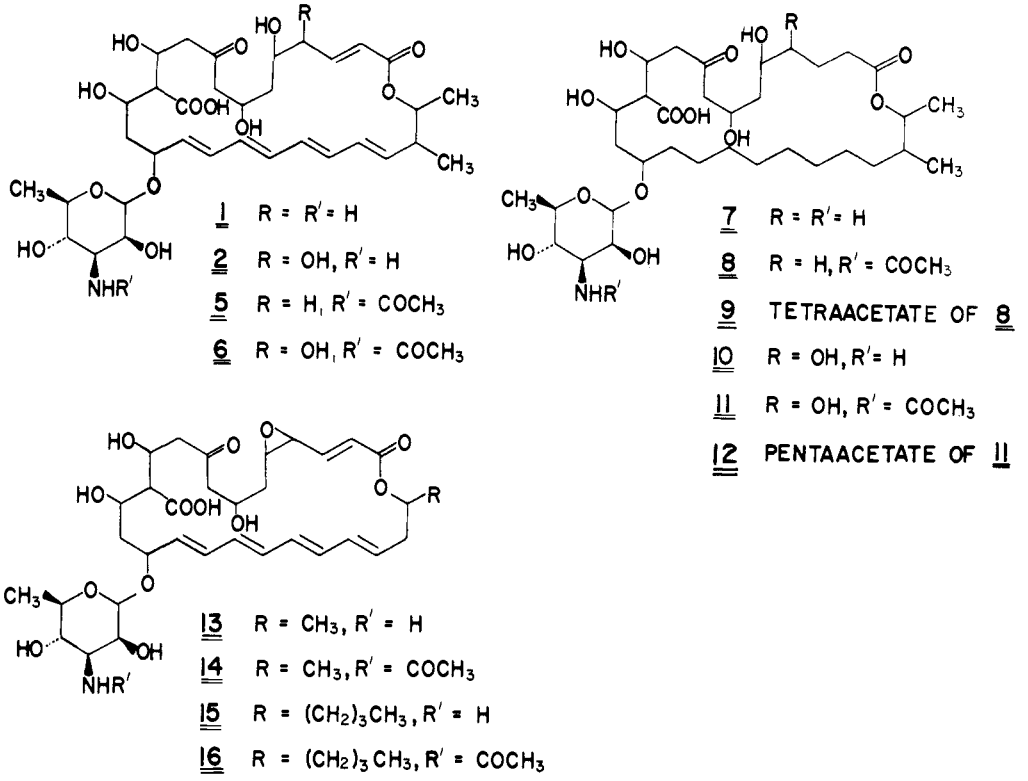
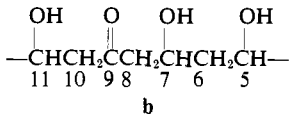


Figure 1. Structures of tetraene antibiotics and their derivatives: **1**, tetrin A; **2**, tetrin B; **13**, pimaricin; **15**, lucensomycin.

from the decarboxylation of formylacetic acid.[1] Thus, the structural unit **b** is indicated, as in tetrin A. Moreover, this unit must be fit into C-4 through C-11 of unit **a**. Biosynthetic considerations, based on an assumed polyacetate origin, argue for oxygenation at C-5, C-7, C-9, and C-11. Although these could in principle be arranged in the reverse order from that shown in **b**, to place the keto group at C-7, analogy to tetrin A strongly suggests the order shown.



b

Functionality and Structure of Tetrin B. In an earlier report on tetrin B elemental analyses indicated that tetrin B contains one oxygen atom more than tetrin A.[6] Since tetrin B moves considerably slower on paper chromatography and also in countercurrent distribution than tetrin A,[6] a reasonable assumption would be that the additional oxygen is present in a hydroxyl group. Another argument pointing to an additional hydroxyl group in tetrin B is provided by the results of analytical acetylation, in which tetrin B consumed 1 more mol of acetic anhydride than tetrin A.

From the structural features already established for tetrin B, an additional hydroxyl over tetrin A would require the molecular formula $C_{34}H_{51}NO_{14}$. Elemental analyses reported earlier[7] are in agreement with this formula but are insufficiently precise to define it. On the other hand, mass spectrometry establishes the formula with certainty. In particular, the mass spectrum of hexaacetyldecahydrotetrin B (**12**) contains peaks at *m/e* 821 (P − H_2O − 2HOAc), 777 (P − H_2O − 2HOAc − CO_2), 761 (P − H_2O − 3HOAc), 759 (P − $2H_2O$ − 2HOAc − CO_2), 743 (P − $2H_2O$ − 3HOAc), 699 (P − $2H_2O$ − 3HOAc − CO_2), and 639 (P − $2H_2O$ − 4HOAc − CO_2), all corresponding to peaks in the spectrum of pentaacetyldecahydrotetrin A (**9**),[1] but shifted 58 amu higher, in accord with an extra acetoxyl group.

The additional hydroxyl group in tetrin B is indicated to be adjacent to another hydroxyl by periodate oxidation. Tetrin B itself consumed 2 mol of periodate very rapidly (Figure 2), while tetrin A reacted with 1 mol rapidly. Similar results were obtained for hydrogenated derivatives of tetrins A and B (**7** and **10**, repectively). In all these reactions the initial periodate consumption was followed by slow additional uptake in the mycosamine fragment (Figure 2). The extra mole of rapid periodate uptake by tetrin B and decahydrotetrin B reflects a *vic*-glycol group in tetrin B, in addition to the *vic*-amino alcohol grouping found in both antibiotics. A more straightforward argument is provided by *N*-acetyltetrin B (**6**), which consumes 1 mol of periodate, whereas *N*-acetyltetrin A (**5**) is inert to periodate.[1]

The location of an extra hydroxyl is effectively limited to C-4 or C-12 by the requirements of units **a** and **b**. Of the two, C-12 is tentatively eliminated by the presumed origin of acetaldehyde on acidification and steam distillation of the mild base hydrolysate from tetrin B—formed from decarboxylation of formylacetic acid arising from C-11, C-12, and the C-12 carboxyl group.

To locate positively a hydroxyl at C-4 the nmr spectrum of *N*-acetyltetrin B (**6**) is decisive. Although the region containing the C-4 proton (>CHO–) is com-

(7) Microanalyses reported earlier[6] for tetrin B and decahydrotetrin B agree with values calculated for their presently assigned molecular formulas. *Anal.* Calcd for $C_{34}H_{51}NO_{14}$: C, 58.52; H, 7.37; N, 2.00. Found (tetrin B):[6] C, 58.27; H, 7.48; N, 1.95. Calcd for $C_{34}H_{61}NO_{14}$: C, 57.69; H, 8.68; N, 1.97. Found (decahydrotetrin B):[6] C, 57.64; H, 8.82; N, 1.89.

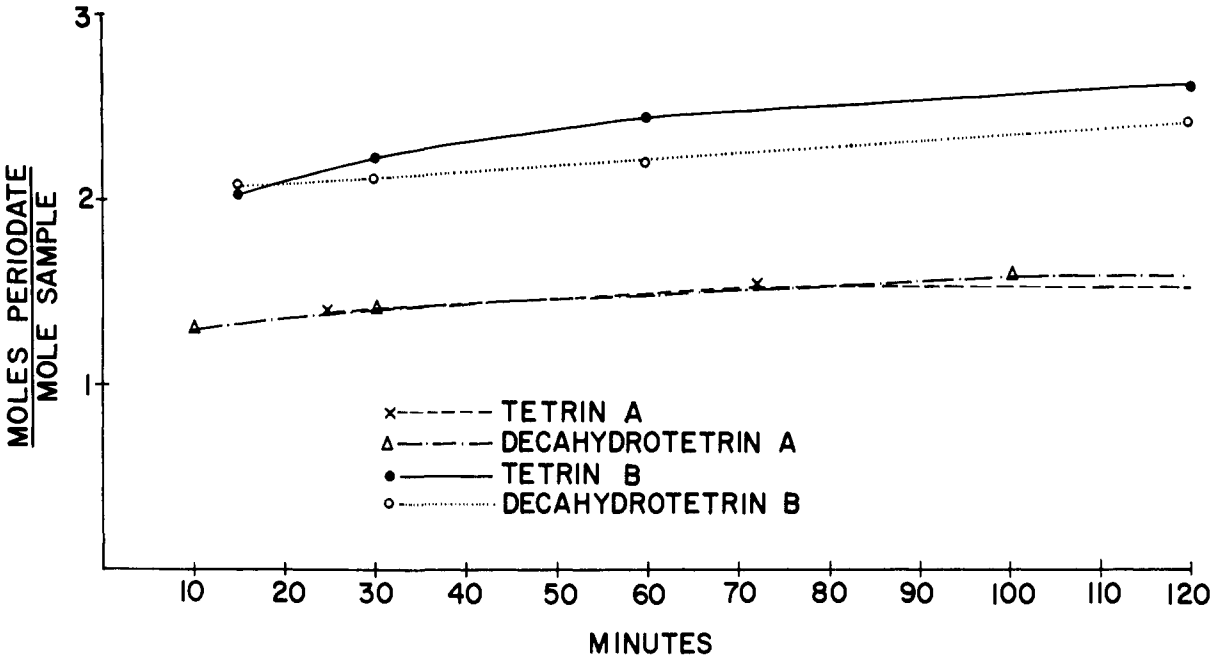
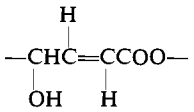


Figure 2. Periodate uptake of tetrins A and B (**1** and **2**) and of decahydrotetrins A and B (**7** and **10**).

plicated by the presence of similar protons from mycosamine, the region containing the conjugated olefinic protons is simpler. In particular, H-3 is very strongly deshielded by the lactone carbonyl. That proton (H-3) appears at δ 7.00 as a doublet of doublets (J_{23} = 16 Hz, J_{31} = 4 Hz), with coupling constants appropriate for a *trans*-olefin and allylic coupling, respectively. The lack of further multiplicity indicates only a single proton is on C-4. Moreover, the C-4 proton can be located at δ 4.66 by irradiation of H-3; thus, it is of the allylic alcohol type and the C-4 through C-1 unit is



In the nmr spectrum of *N*-acetyltetrin A (**5**), the character of the C-3 proton is different, as predicted by its structure (Figure 1). The C-3 proton of **5** appears, at δ 6.96, as a more complex multiplet (doublet of quartets; J_{23} = 16 Hz, J_{34} = 8 Hz, $J_{34'}$ = 6 Hz).

Related Antibiotics. In connection with the study of tetrins A and B we have also investigated the nmr spectra of related tetraene antibiotics (Figure 1). The C-3 protons of the *N*-acetyl derivatives (**14** and **16**, respectively) of pimaricin (**13**) and lucensomycin (**15**) also appear as doublets of doublets, at δ 6.65 and δ 6.76, respectively (J_{23} = 16 Hz, J_{31} = 7 Hz). The C-4 protons of **14** and **16** are found at δ 3.36 (q, J = 7, 2 Hz) and δ 3.40 (q, J = 6, 1 Hz), respectively, located in *trans*-epoxide groups in confirmation of the structures assigned earlier.[8,9]

At least one other antibiotic—PA 166[10]—is clearly closely related to the four previously cited, though structural parameters of PA 166 remain unreported. The nmr spectrum of *N*-acetyl-PA 166 contains H-3 absorption in the form of a quartet (J_{23} = 16 Hz, J_{31} = 6 Hz) at δ 6.64. Moreover, it has two epoxide protons near δ 3.2. Thus, PA 166 is of the pimaricin–lucensomycin type. Finally, the alkyl region of the nmr spectrum of PA 166 is nearly identical with that of lucensomycin, suggesting the butyl group of **15** is reproduced in PA 166. Comparison of the reported properties (Table I) and infrared spectra of the two antibiotics indicates they are either identical or closely related.

Table I. Physical and Chemical Properties of Lucensomycin and PA 166[a,b]

Property	Lucensomycin	PA 166
Molecular formula	$C_{36}H_{53}NO_{13}$	$(C_{35}H_{53}NO_{14})^c$
Lit. C, %	59.70	59.59
Lit. H, %	8.05	7.66
Lit. N, %	2.04	2.00
Hydrogen uptake	6 mol	6 mol
λ_{max} ($E_{1cm}^{1\%}$)	319 (1180), 305 (1390), 290 (840), 220 (450)	319 (990), 304 (1098), 291 (715), 223 (287)
$[\alpha]^{25}D$, deg	+296 (C_5H_5N)	+275 (C_5H_5N)
Mp, °C	>150	>260

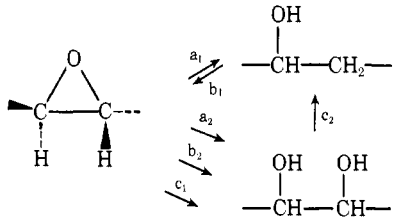
[a] See ref 9. [b] See ref 10. [c] Tentative assignment.

Biosynthetic Considerations. The structures of tetrins A and B (**1** and **2**) are clearly in accord with the general aspects of a polyacetate (malonate) biosynthetic sequence, as are the structures of other tetraene antibiotics (and also pentaene, hexaene, and heptaene antibiotics) such as pimaricin (**13**), lucensomycin (**15**), and nystatin.[11] At the present time confirming biosynthetic studies have been reported only for nystatin[12] and lucensomycin.[13] A study of the biosynthesis of

(8) B. T. Golding, R. W. Rickards, W. E. Meyer, J. B. Patrick, and M. Barker, *Tetrahedron Lett.*, 3551 (1966).
(9) G. Gaudiano, P. Bravo, A. Quilico, B. T. Golding, and R. W. Rickards, *ibid.*, 3567 (1966).
(10) B. K. Koe, F. W. Tanner, Jr., K. V. Rao, B. A. Sobin, and W. D. Celmer, *Antibiot. Annu.*, 897 (1957–1958).
(11) W. Oroshnik and A. D. Mebane, *Fortschr. Chem. Org. Naturst.*, **21**, 17 (1963).
(12) A. J. Birch in "Antibiotics. II. Biosynthesis," D. Gottlieb and P. D. Shaw, Ed., Springer-Verlag, Berlin, 1967, p 228.

mycosamine indicates that this amino sugar component of many polyene antibiotics, including the tetrins, is formed from glucose without rearrangement of the carbon skeleton.[14]

Points of particular interest with regard to the tetrins are the presence of the methyl group at C-24 and the differing states of oxidation at C-4 in **1** and **2**. The C-24 methyl could arise either from propionate incorporation (more likely) or from methylation by methionine. The C-12 carboxyl could arise from malonate or from oxidation of a methyl derived either from propionate or methionine. The propionate origin seems most likely from results on the carboxyl groups of nystatin[12] and lucensomycin.[13] With regard to the second point, the differing states of oxidation at C-4 for tetrins A and B, it seems reasonable that a C-4, C-5 epoxide might be an intermediate, like the epoxide at that position in both pimaricin and lucensomycin. The epoxide could then be opened reductively at C-4 to give tetrin A (step a_1) or hydrolytically to give tetrin B (a_2). Alternative explanations would involve formation of tetrin A as an intermediate, its conversion (b_1) to an epoxide (not isolated) and the epoxide's conversion to tetrin B by hydrolysis (b_2), or the opening of the epoxide to give tetrin B (c_1), followed by reductive removal of the C-4 hydroxyl (c_2).



Experimental Section[15]

Isolation of tetrin B (2) has been reported elsewhere.[1,6] Samples from those studies, isolated by countercurrent distribution, always showed some impurity in the nmr spectrum. This was later identified as the triethylamine salt of tetrin B. Further purification of tetrin B was effected by trituration with water, centrifugation, and decantation. The procedure was repeated eight to ten times until the aqueous layer had only a very faint yellow color. The residue, after decanting the water, was dried in a desiccator containing sulfuric acid. The dried brown amorphous powder had the following constants: mp >360° (after darkening at 160–165°, blackening at 250–295°), $[\alpha]^{24}D$ +43.5° (*c* 0.14, MeOH).[15] The ir spectrum showed characteristic bands at 3400 (OH), 1725 (C=O), 1650 (C=C), and 1575 cm^{-1} (COO^-). The uv spectrum was as reported earlier: $\lambda\lambda_{max}$ 212, 282 (sh), 293, 306, 322 nm.

***N*-Acetyltetrin B (6).** A mixture of 181 mg of tetrin B and 0.1 ml of acetic anhydride in 20 ml of methanol was stirred at 0° for 2 hr. Solvent was removed at room temperature and the residue was precipitated from methanol with ether, then dried under vacuum to afford 155 mg (81%) of *N*-acetyltetrin B; mp 190° (with decomposition, after darkening at 140°), single spot on tlc (BAW 415). The uv spectrum had $\lambda\lambda_{max}$ 212, 282 (inflection), 294, 306, and 322 nm; the ir spectrum contained characteristic bands at 3400 (OH), 2700 (COOH), 1720 (C=O), 1650 (C=C), and 1550 cm^{-1} (amide).

Anal. Calcd for $C_{36}H_{53}NO_{15}\cdot H_2O$: C, 57.05; H, 7.31, N, 1.85. Found: C, 56.59; H, 6.92; N, 2.25.

***N*-Acetylpimaricin,[16] *N*-acetyllucensomycin,[17] and the *N*-acetyl derivative of PA 166** were prepared by the method described in the preceding section for *N*-acetyltetrin B, a modification of the procedure employed by Gaudiano, *et al.*,[17] for the earlier preparation of *N*-acetyllucensomycin. The *N*-acetyl derivatives were purified by trituration with ethyl acetate, giving the *N*-acetyl derivatives in yields of 56, 78, and 88% from pimaricin, lucensomycin, and PA 166, respectively. Each *N*-acetyl derivative gave a single spot on tlc in the solvent system BAW 415.

Decahydrotetrin B (10) was prepared by the procedure reported earlier.[6] A mixture of 201 mg of tetrin B and 150 mg of prereduced platinum oxide in 30 ml of glacial acetic acid was stirred at room temperature under hydrogen until the absorption of hydrogen ceased, after 12 hr. The catalyst was filtered and washed with fresh acetic acid and solvent was removed under vacuum. The residue on precipitation from ether gave 131 mg (64%) of decahydrotetrin B, mp 200–204° (after sintering at 180°), single spot on tlc (BAW 415).[15] The ir spectrum showed characteristic bands at 3400 (OH), 1740 (C=O), and 1575 cm^{-1} (COO^-). The uv spectrum showed only very weak end absorption (ϵ_{212} 1201). Crystalline decahydrotetrin B could be isolated as fine white needles by covering its solution in butanol-saturated water with heptane and allowing it to stand at room temperature, mp 190° dec, R_f 0.60 (BAW 415).

***N*-Acetyldecahydrotetrin B.** A mixture of 175 mg of decahydrotetrin B, 0.13 ml of acetic anhydride, and 20 ml of dry methanol was shaken mechanically for 12 hr at room temperature. The pale yellow solution was treated with charcoal, filtered, and concentrated *in vacuo* to approximately one-fifth its original volume. The product was precipitated by addition of diethyl ether and petroleum ether (bp 30–60°), collected, washed well with ether–petroleum ether, and dried, affording 145 mg (80%) of an amorphous white solid, 123–127°. This material was partially dissolved in hot ethyl acetate, and the solution was filtered and concentrated to give 85 mg of a still amorphous solid, mp 127–128°. Addition of petroleum ether to the mother liquor afforded an additional 33 mg, mp 125–127°.

Anal. Calcd for $C_{36}H_{53}NO_{15}$: C, 57.66; H, 8.47; N, 1.87. Found: C, 58.62; H, 8.37; N, 1.79.

Hexaacetyldecahydrotetrin B (12). A mixture of 120 mg of decahydrotetrin B, 2 ml of acetic anhydride, and 2 ml of pyridine stood at room temperature for 20 hr under anhydrous conditions, then was decomposed by ice. The hexaacetyl derivative was extracted into chloroform and the chloroform extract was worked up to give 97.2 mg (60%) of the hexaacetyl derivative (**12**), mp 130–133°. The ir spectrum (Nujol) contained characteristic peaks at 3500 (OH), 2700 (COOH), 1740, and 1240 cm^{-1} (acetate). The nmr spectrum ($CDCl_3$) showed a strong peak (broad) at δ 1.24 ($-CCH_2C-$), the C-24 methyl doublet (J = 7 Hz) at δ 1.08, and acetate methyl singlets at δ 1.84, 1.96, 2.02 (6 H), 2.04, and 2.10.

Anal. Calcd for $C_{46}H_{73}NO_{20}$: C, 57.55; H, 7.67; N, 1.46. Found: C, 57.89; H, 7.45; N, 1.63.

Analytical Acetylation. The procedure used was essentially that described by Fritz and Hammond.[18] Acetylations employed a solution of acetic anhydride–pyridine (1:3, volume) at room temperature for 72 hr and the resulting solutions were potentiometri-

(13) D. G. Manwaring, R. W. Rickards, G. Gaudiano, and V. Nicolella, Abstracts, 7th International Symposium on the Chemistry of Natural Products, IUPAC, Riga, USSR, June 1970, p 623.

(14) C. P. Schaffner, C. H. Li, and K. Mosbach, paper in preparation.

(15) 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. Proton magnetic resonance spectra were determined by Mr. R. Thrift and associates on Varian A-60, HA-100, and HR-220 spectrometers. Chemical shifts are reported on the δ scale from TMS as internal standard (δ 0). Spectra of the *N*-acetyl derivatives of the antibiotics were determined on deuteriopyridine solutions. Mass spectra were obtained by Mr. J. Wrona on an Atlas CH4B spectrometer, employing direct sample introduction techniques. 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 (Floridin 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).

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

(17) G. Gaudiano, P. Bravo, and A. Quilico, *Gazz. Chim. Ital.*, **96**, 1322 (1966).

(18) J. S. Fritz and G. S. Hammond, "Quantitative Organic Analysis," Wiley, New York, N. Y., 1957, p 261.

cally titrated with 0.498 *N* methanolic sodium hydroxide solution to pH 9.8.

A. Tetrin A. Titration of the reaction product from 83.0 mg (0.119 mmol) of tetrin A (**1**)[1] indicated 1.02 ml (0.523 mequiv) or 4.47 mol of acetic anhydride/mol of sample had reacted.

B. Tetrin B. Titration of the product from 85.4 mg (0.122 mmol) of tetrin B indicated 1.26 ml (0.627 mequiv) or 5.14 mol of acetic anhydride/mol of sample had reacted. Two additional samples of tetrin B had reacted with 6.02 and 4.79 mol of acetic anhydride/mol of sample. Thus, for three determinations an average of 5.32 mol of acetic anhydride was consumed per mole of sample.

Periodate oxidations were carried out at 0° except as noted, in flasks wrapped with aluminum foil, employing standard solutions of sodium *m*-periodate, sodium arsenite, iodine, and starch prepared with reagent grade chemicals according to standard procedures.[19] *tert*-Butyl alcohol was purified by distillation.

A. Tetrin A. Run I. A mixture of 23.8 mg (0.0343 mmol) of tetrin A (**1**),[1] 10 ml of 50% aqueous *tert*-butyl alcohol, and 10 ml of 0.1 *M* sodium *m*-periodate had consumed 1.22 mol of periodate per mol of sample after 2 min, 1.26 mol after 10 min.

Run II. A mixture of 25.1 mg (0.0386 mmol) of tetrin A (**1**), 10 ml of *tert*-butyl alcohol, and 10 ml of 0.1 *M* sodium *m*-periodate had consumed 1.40 mol of periodate/mol of sample after 25 min, 1.60 mol after 260 min. Results are plotted in Figure 2.

B. *N*-Acetyltetrin A. A mixture of 29.6 mg of *N*-acetyltetrin A (**5**),[1] 10 ml of *tert*-butyl alcohol, and 10 ml of 0.1 *M* sodium *m*-periodate consumed no periodate.

C. Decahydrotetrin A. Run I. A solution containing 23.2 mg (0.0331 mmol) of decahydrotetrin A (**7**),[1] 10.0 ml of 50% aqueous *tert*-butyl alcohol, and 10.0 ml of 0.1 *M* sodium *m*-periodate consumed 0.98 mol of periodate/mol of sample during 2 min, 1.35 mol during 10 min.

Run II. A mixture of 22.2 mg (0.032 mmol) of crystalline decahydrotetrin A (**7**), 10 ml of *tert*-butyl alcohol, and 10 ml of 0.1 *M* sodium *m*-periodate had consumed 1.30 mol of periodate/mol of sample after 10 min, 2.07 mol after 1445 min. Results are plotted in Figure 2.

D. *N*-Acetyldecahydrotetrin A (6). A mixture of 29.6 mg (0.045 mmol) of *N*-acetyldecahydrotetrin A (**8**),[1] 10 ml of 50% aqueous *tert*-butyl alcohol, and 10 ml of 0.1 *M* sodium *m*-periodate consumed no periodate.

E. Tetrin B. Run I. A solution containing 32.7 mg (0.0467 mmol) of tetrin B (**2**), 10.0 ml of 50% aqueous *tert*-butyl alcohol, and 10.0 ml of 0.1 *M* sodium *m*-periodate consumed 1.96 mol of periodate/mol of sample during 15 min at 4°, 3.44 mol during 22 hr.

Run II. A solution containing 36.13 mg (0.0502 mmol) of tetrin B (**2**), 10.0 ml of 50% aqueous *tert*-butyl alcohol, and 10.0 ml of 0.1 *M* sodium *m*-periodate consumed 2.03 mol of periodate/mol of sample during 15 min at 4°, 3.90 mol during 22 hr. Periodate consumption is plotted in Figure 2.

F. Decahydrotetrin B. Run I. A solution of 30.4 mg (0.0428 mmol) of decahydrotetrin B (**10**), 10.0 ml of 50% aqueous *tert*-butyl alcohol, and 10.0 ml of 0.1 *M* sodium *m*-periodate stood at 4°. The consumption of periodate was 2.08 mol/mole of sample after 15 min, 3.87 mol after 34 hr. The results are plotted in Figure 2.

Run II. A solution of 31.9 mg (0.0450 mmol) of decahydrotetrin B (**10**), 10.0 ml of 50% aqueous *tert*-butyl alcohol, and 10.0 ml of 0.1 *M* sodium *m*-periodate at 4° had consumed 1.86 mol of periodate/mol of sample after 15 min, 2.86 mol after 34 hr.

G. *N*-Acetyldecahydrotetrin B. A solution of 23.9 mg (0.032 mmol) of *N*-acetyldecahydrotetrin B (**11**) in 10.0 ml of 50% aqueous *tert*-butyl alcohol and 10.0 ml of 0.1 *M* sodium *m*-periodate had consumed 0.98 mol of periodate/mol of sample after 15 min. No addition consumption of periodate was noted during 1 hr.

Preparation of 3,15-dimethylhexacosane (3) followed the procedure described for its preparation from tetrin A.[1] Tetrin B was hydrogenated over reduced platinum oxide in acetic acid as described above and the decahydro derivative (**10**) (1.0 g) was reduced by lithium aluminum hydride (2 g) in refluxing tetrahydrofuran (100 ml). The usual work-up afforded a viscous material (971 mg) whose ir spectrum indicated the presence of hydroxyl bands (3350, 1060 cm^{-1}) and a very weak carbonyl band (1700 cm^{-1}). The polyol was heated at reflux with glacial acetic acid (15 ml), hydriodic acid (50 ml), and 1.2 g of red phosphorus for 36 hr, cooled, and diluted with water. The organic material was extracted with ether and ethyl acetate and the combined organic extracts were washed with water, 2% aqueous sodium thiosulfate solution, water, and brine, then dried over anhydrous sodium sulfate. Solvent removal afforded 753 mg of a gummy residue, which gave a positive Beilstein test.

The polyiodide was then stirred for 24 hr with 1.5 g of lithium aluminum hydride in 50 ml of refluxing tetrahydrofuran. Work-up in the usual fashion gave 394 mg of a residue which was passed through a column of alumina (grade I). Elution with hexane afforded 70.3 mg of a hydrocarbon, which was first hydrogenated over platinum oxide catalyst in hexane–ethyl acetate, then passed through a small column of alumina (grade I). Elution with hexane gave 31.2 mg (2.5% overall yield) of a saturated hydrocarbon with characteristic ir absorption, which was analyzed by mass spectrometry. Gas chromatography on a 10% SE-30 column indicated it to be essentially a single compound.

Basic Hydrolysis of Tetrin B. A. Isolation of 12-Methyl-2,4,6,8,10-tetradecapentaene-1,13-diol. A solution of 100 mg of tetrin B in 140 ml of 0.4 *N* aqueous sodium hydroxide was extracted continuously with ether while it was stirred at room temperature for 26 hr. The yellow ethereal solution was concentrated at room temperature to a residue of the crude pentaenal (**4**), λ_{max} 380 nm, which gave one major yellow spot on tlc (silica gel, $CHCl_3$–EtOAc 3:2, R_f 0.63). A mixture of the residue and excess sodium borohydride in methanol was stirred at room temperature until the yellow color was discharged, then was diluted with water and extracted with ether. Work-up of the ether extract gave the crude pentaenediol (one major spot on tlc as above; R_f 0.42), which was recrystallized from ethyl acetate to give 15 mg (45%) of pale yellow plates, mp 159–160°; mixture melting point with 12-methyl-2,4,6,8,10-tetradecapentaene-1,13-diol from tetrin A, 159–160°. The infrared and ultraviolet spectra of the two samples were identical.

B. Identification of Acetone and Acetaldehyde. A suspension of tetrin B (0.2 g) in 50 ml of water containing 0.2 g of sodium hydroxide was steam distilled into 2 *N* aqueous hydrochloric acid saturated with 2,4-nitrophenylhydrazine until no more 2,4-dinitrophenylhydrazone formed. The dinitrophenylhydrazone was filtered and dried (41.5 mg). Tlc indicated it to be a mixture of two compounds, a conclusion further supported by mass spectra. The two compounds were separated by preparative tlc and the bands were eluted with ether. Removal of solvent and crystallization from hexane–ether gave the 2,4-dinitrophenylhydrazones of acetone and acetaldehyde, each identified by melting point, mixture melting point with an authentic sample, and mass spectrum.

The aqueous basic residue after steam distillation was cooled and extracted continuously with ether for 24 hr, then acidified with hydrochloric acid and steam distilled again into 2,4-dinitrophenylhydrazine-saturated 2 *N* aqueous hydrochloric acid. The 2,4-dinitrophenylhydrazone derivative isolated was purified by preparative tlc, crystallized from hexane–ether, and identified as acetaldehyde 2,4-dinitrophenylhydrazone by the methods of the preceding paragraph.

Acknowledgment. This work was supported in part by Grants AI 01278 and AI 04769 from the National Institute of Allergy and Infectious Diseases. We also thank Dr. T. E. Eble, The Upjohn Co., and Dr. N. Bohonos, Lederle Laboratories, for samples of crude tetrin; Dr. J. S. Webb, Lederle Laboratories, for a sample of pimaricin; Dr. G. Gaudiano, Politecnico di Milano, for a sample of lucensomycin; Dr. W. D. Celmer, Chas. Pfizer & Co., for a sample of PA 166; and Dr. Olof Ceder, University of Göteborg, for helpful discussions.

(19) I. M. Kolthoff, E. B. Sandell, E. J. Meehan, and S. Bruckenstein, "Quantitative Chemical Analysis," 4th ed, Macmillan, New York, N. Y., 1969.