by suction filtration and was washed with several portions of ether and methanol, and the combined filtrate and washings were evaporated. Molecular distillation of the residue gave 0.76 g (76%) of yellow oil; glpc analysis of this material on column A at 225° showed the β-atlantone (retention time 14.8 min) had been converted to a product with retention time 20.3 min. Purification of the product by preparative glpc on column C at 225° followed by molecular distillation yielded (+)-α-atlantone (**27**) as a yellow oil, bp (bath) <110° (0.05 mm); $[\alpha]^{30}D$ +77° (*c* 0.37, C_2H_5OH); ir (film) 3010 (>C=CH–), 1675 (>C=O), 1625 (conjugated >C=CH–), 1113, 1060, and 878 cm^{-1}; uv_{max} (CH_3OH) 269 mμ (ε 21,000 ± 1100); nmr ($CDCl_3$) τ 4.04 (broadened s, 2 H, >C=C*H*CO-C*H*=C<), 4.69 (m, 1 H, –C*H*=C(CH_3)CH_2–), and 7.7–8.5 ppm [m, 19 H, including τ 7.90 (broadened s, –C(CH_3)=CHCOCH=C(CH_3)$_2$, two methyls cis to carbonyl), 8.17 (broadened s, –CO-CH=C(CH_3)$_2$, methyl trans to carbonyl), and 8.39 ppm (broadened s, –CH=C(CH_3)CH_2–)]; mass spectrum (70 eV) *m/e* (rel intensity) 218 (9), 203 (6), 163 (9), 150 (5), 135 (18), 123 (17), 121 (7), 120 (11), 119 (12), 109 (6), 107 (10), 105 (13), 95 (14), 93 (10), 91 (10), 83 (100), 79 (10), 69 (19), 55 (31).

Anal. Calcd for $C_{15}H_{22}O$: C, 82.51; H, 10.16. Found: C, 82.19; H, 10.25.

A sample of naturally occurring α-atlantone was isolated from the oil of *Cedrus atlantica*. Glpc analysis of the oil on column A at 225° showed that the component with longest retention time could be separated from other components, and had a retention time identical with that of synthetic α-atlantone; the area of this peak constituted *ca.* 8% of the sum of peak areas for the entire oil. The oil was separated by distillation into a low boiling fraction, bp 63–65° (0.10–0.05 mm) and a high boiling fraction, bp (bath) 100–130° (0.05 mm). The α-atlantone in the latter fraction was obtained as a yellow oil after purification by preparative glpc on column C at 230° followed by molecular distillation: bp (bath) 100–110° (0.05 mm) [lit. bp 121–123° (1 mm)[44b]; 142–145° (1 mm)[46]]; $[\alpha]^{30}D$ +3° (*c* 0.38, C_2H_5OH) (lit. $[\alpha]D$ +2° 48′[44b]; $[\alpha]D$ +1.2° [46]); uv_{max} (CH_3OH) 269 mμ (ε 19,600 ± 1200). The ir, nmr, and mass spectra were identical in all respects with the corresponding spectra described above for synthetic α-atlantone (*cf.* lit.[46] ir uv, nmr, and mass spectrum).

Anal. Calcd for $C_{15}H_{22}O$: C, 82.51; H, 10.16. Found: C, 82.56; H, 10.15.

Acknowledgment. The technical assistance of Mr. F. W. Vonderahe is gratefully acknowledged.

Polyene Antibiotics. IV. Structure of Chainin[1,2]

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Abstract: The antifungal pentaene antibiotic chainin has been shown to have the structure 2-(*n*-butyl)-16-methyl-3,5,7,9,11,13,15,26,27-nonahydroxyoctacosa-16,18,20,22,24-pentaenoic acid, 27-lactone (**1**). Mass spectra of the octaacetate of **1**, of the octaacetate of its decahydro derivative, and of its hydrocarbon and methyl ester reduction products were instrumental in the structural investigation. Structural studies on norchainin, homochainin, and filipins II and IV are also described.

Recently, Thirumalachar reported the isolation of a new antibiotic from a *Chainia* species.[3] The new antibiotic, which was named chainin, belongs to the pentaene subgroup of the polyene antibiotics,[4] and like most of the polyenes it has antifungal rather than antibacterial activity. In the present report we assign structure **1** to chainin (Figure 1).

Molecular Formula

Previous studies of pentaene antibiotics have employed trimethylsilyl derivatives for volatilization and mass spectral studies.[5] We have developed an alternative procedure, involving exhaustive acetylation with acetic anhydride in pyridine.[6] We regard this method as somewhat superior, since the acetate derivatives are stable and can be isolated; they give good molecular ions at low electron energy. In the present study chainin (**1**) and its decahydro derivative **3**, obtained upon hydrogenation of chainin in acetic acid over platinum oxide, were acetylated to give polyacetates lacking hydroxyl absorption in their infrared spectra. Mass spectra of both polyacetates **2** and **4** gave molecular ions (Table I). The derivative of chainin (**2**) gave a parent ion at *m/e* 946; decahydrochainin octaacetate (**4**) gave a parent ion at *m/e* 956. The high-resolution mass spectrum of **4** indicates its molecular formula to be $C_{49}H_{80}O_{18}$. Both spectra showed ions for losses of one–eight acetic acid units. These correspond to a molecular weight for chainin of 610 (946 − 8 × 42) and a molecular formula $C_{33}H_{54}O_{10}$.[7] A second series of ions 18 amu higher is also found, corresponding to the loss of 1 mol of ketene (to give ion k, Table I), plus the loss of 1–7 mol of acetic acid.

Carbon Skeleton

A modification of the procedure of Cope, *et al.*,[8] was employed to reduce the antibiotic to a saturated hydrocarbon, **5**, as shown in Figure 2. The hydrocarbon had an infrared spectrum characteristic of a saturated hy-

(1) Presented in part at the 7th International Symposium on the Chemistry of Natural Products, IUPAC, Riga, USSR, June 1970, paper E-157.

(2) Paper III in this series: K. L. Rinehart, Jr., W. P. Tucker, and R. C. Pandey, *J. Amer. Chem. Soc.*, **93**, 3747 (1971).

(3) K. S. Gopalkrishnan, N. Narasimhachari, V. B. Joshi, and M. J. Thirumalachar, *Nature (London)*, **218**, 597 (1968).

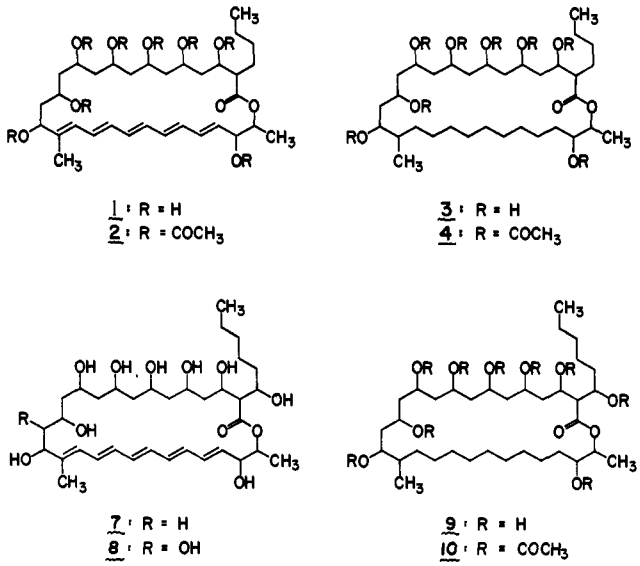
(4) A review of polyene antibiotics: W. Oroshnik and A. D. Mebane, *Fortsch. Chem. Org. Naturst.*, **21**, 17 (1963).

(5) B. T. Golding, R. W. Rickards, and M. Barker, *Tetrahedron Lett.*, 2615 (1964).

(6) R. C. Pandey and K. L. Rinehart, Jr., *J. Antibiot.*, **23**, 414 (1970).

(7) Microanalyses reported[3] for chainin do not agree well with the molecular formula assigned, but would for a hydrate. *Anal.* Calcd for $C_{33}H_{54}O_{10}$: C, 64.89; H, 8.91. Calcd for $C_{33}H_{54}O_{10}\cdot H_2O$: C, 63.03; H, 8.97. Found:[3] C, 63.25; H, 8.62.

(8) 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).



1: R = H
2: R = COCH3
3: R = H
4: R = COCH3
7: R = H
8: R = OH
9: R = H
10: R = COCH3

Figure 1. Structures of pentaene antibiotics and their derivatives: **1**, chainin; **7**, filipin III; **8**, lagosin.

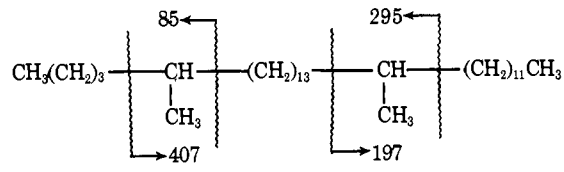
drocarbon and the mass spectrum showed a molecular ion at *m/e* 464.5319 ($C_{33}H_{68}$). The fragmentation pattern shown here indicated the position of the methyl

Table I. Major Mass Spectral Fragments of Polyacetates

Assignments	Chainin octa-acetate (**2**)	Decahydro-chainin octaacetate (**4**)	Decahydro-filipin III nonaacetate
P	946	956.5317[a]	1042.5721
k (P − C_2H_2O)		914.514[b]	
P − HOAc	886	896.513[c]	982.5453
k − HOAc	844	854	
P − 2HOAc	826	836.4880[d]	922
k − 2HOAc	784	794	880
P − 3HOAc	766	776.4719[e]	862
k − 3HOAc	724	734	820
P − 4HOAc	706	716.450[f]	802
k − 4HOAc	664	674.436[g]	760
P − 5HOAc	646	656.427[h]	742
k − 5HOAc	604	614.420[i]	700
P − 6HOAc	586	596.407[j]	682
k − 6HOAc	544	554.397[k]	640
P − 7HOAc	526	536.384[l]	622
k − 7HOAc	484	494.375[m]	580
P − 8HOAc	466	476	562
k − 8HOAc			520
P − 9HOAc			502

[a] $C_{49}H_{80}O_{18}$. [b] $C_{47}H_{78}O_{17}$. [c] $C_{47}H_{76}O_{16}$. [d] $C_{45}H_{72}O_{14}$. [e] $C_{43}H_{68}O_{12}$. [f] $C_{41}H_{64}O_{10}$. [g] $C_{39}H_{62}O_9$. [h] $C_{39}H_{60}O_8$. [i] $C_{37}H_{58}O_7$. [j] $C_{57}H_{56}O_6$. [k] $C_{35}H_{54}O_5$. [l] $C_{55}H_{52}O_4$. [m] $C_{33}H_{50}O_3$.

substituents and suggested the structure to be 5,19-dimethylhentriacontane (**5**).



5

Further information on the carbon skeleton was obtained by Ceder reduction.[9] Hydrogenolysis of chainin

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

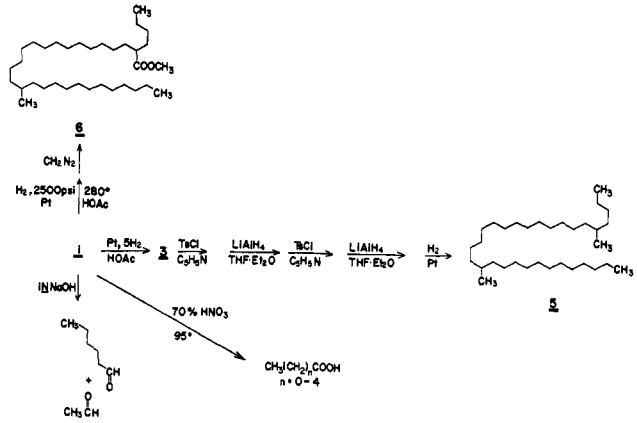
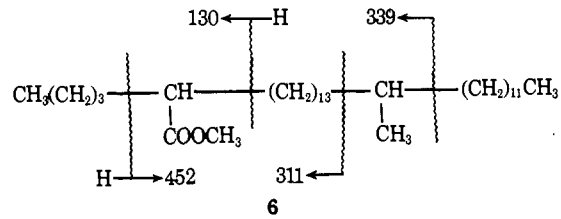


Figure 2. Degradative reactions carried out on chainin (**1**).

at 280° and 2500 psi over platinum oxide in glacial acetic acid and esterification of the product with diazomethane gave a methyl ester (**6**) whose mass spectrum showed a



6

molecular ion at *m/e* 508.524 ($C_{34}H_{68}O_2$). The mass spectral fragments attributed to β cleavage with rearrangement[10] occurred at *m/e* 452.461 ($C_{30}H_{60}O_2$) and 130.100 ($C_7H_{14}O_2$), indicating the position of the carbomethoxyl group. Other prominent ions occurred at *m/e* 339.327 ($C_{22}H_{43}O_2$) and 311.288 ($C_{20}H_{39}O_2$), but not at *m/e* 325, indicating the position of the branching methyl group.[10]

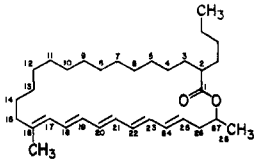
Functional Groups and Structure

The ultraviolet maxima of chainin—at 308 (inflection), 324 (ϵ 51,200), 342 (80,300), and 358 nm (77,800)[3,4]—show that its pentaene unit is all-trans and methyl substituted but not conjugated to a carbonyl group. The location of the pentaene in the carbon skeleton is assigned conclusively from the observation that the methyl group at C-16 is found as a singlet at δ 1.85 (C_5D_5N), a position characteristic of a terminal methyl in a polyene chain but at too high field for an internal methyl in a polyene unit.[11]

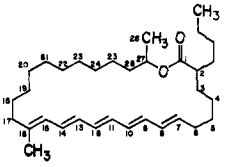
The infrared spectra of chainin (**1**) and its decahydro derivative **3** contain carbonyl bands at 1710 and 1720 cm^{-1} (Nujol), respectively; the Ceder reduction described above indicates these are for an ester group.[12] The ester group must be a lactone since no carbon atoms are lost in the formation of **5** and **6**, and the lactone cannot be conjugated with an alkene linkage since it is not conjugated with the pentaene unit and no additional unsaturation is allowed by the molecular formula. The alkyl attachment of the lactone unit can be fixed at C-27 of the carbon chain by the demonstration that spin decoupling of the methyl doublet at δ

(10) R. Ryhage and E. Stenhagen, *Ark. Kemi*, **15**, 333 (1960).
(11) M. S. Barker, J. B. Davis, L. M. Jackman, and B. C. L. Weedon, *J. Chem. Soc.*, 2870 (1960).
(12) Since chainin is neutral a carboxyl group is eliminated from consideration.

1.46 (J = 7 Hz) is achieved by irradiation at δ 5.16, a position characteristic of an acyloxycarbinyl proton, *i.e.*, –COOCH–.[13] Fitting the lactone and pentaene units into the carbon skeletons of **5** and **6** leads to partial structure a or b for the antibiotic.

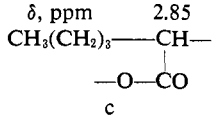


a



b

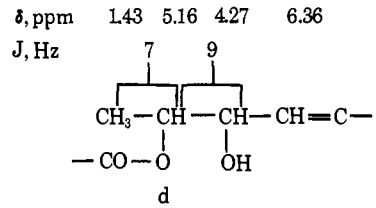
The following experiments decide in favor of a. First, chainin on treatment with alkali afforded 1-hexanal and acetaldehyde as the steam volatile products, which were isolated as their 2,4-dinitrophenylhydrazone derivatives. Hexanal can be formed from this carbon skeleton on base treatment only if the butyl group is unsubstituted and C-2 does not bear a hydroxyl, as shown in c. Further evidence that chainin lacks hydroxyl



c

substitution on the butyl group or C-2 was provided by nitric acid oxidation of the antibiotic, which gave acetic, propionic, *n*-butyric, *n*-valeric, and *n*-caproic acids (Figure 2), identified by gas chromatographic retention times. The proton α to the ester carbonyl (H-2) appears in the nmr spectrum (C_5D_5N, 100 MHz) of decahydrochainin (**3**) as a complex multiplet at δ 2.85, in a region lacking absorption by other protons.

Since no hydroxyl groups are found in the butyl side chain or on C-2, the eight hydroxyl groups of chainin must be located on C-3–C-15 and on C-26 in a, or on C-3–C-6 and on C-17–C-26 in b. The decision can be made between these possibilities from the lack of reaction of **1** with periodate, which rules out any structures containing a vicinal glycol. Since it is impossible to fit eight hydroxyl groups into C-3–C-6 and C-17–C-26 of b without recourse to a vicinal glycol, b is eliminated. For that matter, only one arrangement of eight hydroxyls in a fits the requirements—that shown in **1**, with hydroxyls on C-3, C-5, C-7, C-9, C-11, C-13, C-15, and C-26. Spin decoupling results are in agreement with the placing of a hydroxyl at C-26, since the acyloxy carbinyl proton multiplet at C-27 (δ 5.16) can be converted to a quartet by irradiation at δ 4.27. The proton at δ 4.27 is, moreover, partially decoupled by irradiation in the olefinic region, at δ 6.36. Thus, the unit d is confirmed by spin decoupling.



d

(13) Attachment at C-3′ in the butyl group is prohibited by the isolation of hexanal and hexanoic acid, as described below.

Norchainin and Homochainin

Antibiotics are often isolated as mixtures of closely related compounds. Thus, it is not surprising that chainin, as isolated, is accompanied by two other homologous antibiotics present in small amounts. Since the two homologs are quite minor constituents they would not have been apparent except for the extensive mass spectral studies carried out. Thus, in addition to the ions listed in Table I, there are other much weaker ions in the mass spectra of **2** and **4** attributable to the homologs of chainin. Molecular ions for the decahydro octaacetates of these homologs (norchainin and homochainin) occur at *m/e* 942.515 and 970.539, respectively, corresponding to $C_{48}H_{78}O_{18}$ and $C_{50}H_{82}O_{18}$. The intensity of the homologous ions in the spectrum of **2** indicates crude chainin is a mixture of about 93% chainin, 4% norchainin, and 3% homochainin. The structures of these homologs cannot be completely assigned, since the quantities available did not allow the extensive and difficult separations required for the antibiotics themselves. However, combined gas chromatography–mass spectrometry, with its miniscule sample requirements, did allow study of the carbon skeletons of the homologs. In particular, a gas chromatogram of the crude saturated hydrocarbon **5** indicated three components in the approximate ratio 2:97:1. During combined gas chromatography–mass spectrometry, the middle, major component gave the same fragmentation pattern as that shown above for the crude hydrocarbon, but satisfactory mass spectra could not be obtained for the other components owing to their low concentrations. A mass spectrum of the crude hydrocarbon showed additional, weak molecular ions at *m/e* 450 and 478, in keeping with the assigned natures of norchainin and homochainin.

In a similar fashion the crude methyl ester **6** was subjected to combined gas chromatography–mass spectrometry. The major component's fragmentation pattern was that shown above. Mass spectra of the two homologous methyl esters derived from norchainin and homochainin (which preceded and followed the major component on glc; ratio 2:95:3) showed molecular ions at *m/e* 494 and 522, respectively. Prominent odd-electron ions appear at *m/e* 130 and at M − 56 (*m/e* 438 and 466, respectively) in each spectrum, showing the homologous differences between the chainins do not occur in the side chain attached at C-2.

With the present information nothing further can be said about the structures of norchainin and homochainin. We assume their structures, especially their oxygenation patterns, are nearly identical with those of chainin.

Relationship to Filipins II–IV

A striking feature of the structure (**1**) assigned to chainin is that it is nearly identical with that assigned earlier[5,6,14,15] to filipin (**7**, Figure 1). The difference between the two antibiotics lies in the 2-alkyl groups: an *n*-butyl group in chainin, an α-hydroxyl-*n*-hexyl group in filipin. This would be in good agreement with the obvious polyacetate origin[4] of both antibiotics, and

(14) O. J. Ceder and R. Ryhage, *Acta Chem. Scand.*, **18**, 558 (1964).

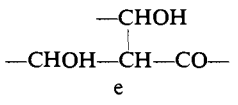
(15) (a) M. L. Dhar, V. Thaller, and M. C. Whiting, *Proc. Chem. Soc.*, 310 (1960); (b) M. L. Dhar, V. Thaller, and M. C. Whiting, *J. Chem. Soc.*, 842 (1964).

with that of the closely related lagosin (**8**),[5,15] which is apparently identical with fungichromin[8] except for possible stereochemical differences.

Filipin has recently been shown to be a mixture of at least four components, filipins I, II, III, and IV.[16] According to mass spectral studies,[6] the most abundant component (53% of the mixture) apparently has the structure **7** assigned earlier to the unrecognized mixture. The mass spectral studies also indicated filipin IV (18%) to be isomeric with filipin III, and filipins II (25%) and I (4%) to lack one and two hydroxyl groups of filipin III, respectively.

In order to compare chainin with filipin, decahydrofilipin III (**9**) was acetylated to afford decahydrofilipin III nonaacetate (**10**). The infrared and nmr spectra of decahydrochainin octaacetate and **10** were almost superimposable and their mass spectra (Table I) were nearly identical except for the shift of most peaks by 86 amu (-CH_2CHOAc-). Though not conclusive of themselves, these comparisons supply confirmatory evidence of the close structural similarity of filipin and chainin.

The antibiotics were further compared by examining the nmr spectra (220 MHz, C_5D_5N) of decahydrofilipins II, III, and IV in the region near δ 3.0 (-CHC(=O) protons).[17] The absorption for decahydrofilipin III (**9**) in this region occurs as a multiplet at δ 3.18 which was demonstrated by the indor method[18] to be coupled with resonances centered at δ 4.89 and 4.53. Irradiation at each of these positions caused the multiplet at δ 3.18 to collapse to a doublet, thus confirming the environment of the corresponding proton as in e.



e

Similarly, the H-2 proton of decahydrofilipin IV, which occurs at δ 3.10, is coupled to two protons centered at δ 4.83 and irradiation at this position caused the H-2 multiplet to collapse to a singlet. In decahydrofilipin II the C-2 proton appears as a complex multiplet at δ 2.84.

These results are in agreement with the proposed structure **7** for filipin III and indicate that filipin IV is probably stereoisomeric at C-3 or C-1′ with filipin III, since the chemical shift of the H-2 proton of its decahydro derivative is shifted slightly from that of **9**. They also suggest that filipin II probably lacks a hydroxyl on C-1′ (or possibly on C-3) since the chemical shift of H-2 in the nmr spectrum of decahydrofilipin II is considerably upfield from those of **9** and decahydrofilipin IV and is like that in the spectrum of decahydrochainin (**3**) which lacks a C-1′ hydroxyl.

To further test these structural conclusions regarding filipins II and IV *vis-à-vis* filipin III, the three antibiotics were oxidized with 70% nitric acid and the acidic products were gas chromatographed. The acids from filipins III and IV were identified as acetic through caproic acids (C_2–C_6 acids) derived from their α-hydroxyhexyl side chains. By contrast, filipin II yielded acetic through caprylic acids (C_2–C_8 acids), in agreement with its lack of an α-hydroxyl group in the side chain. The formation of caprylic acid corresponds to the formation of caproic acid from chainin, whose side chain is two carbon atoms shorter.

In a final comparison of filipin with chainin, filipins II, III, and IV were subjected to basic hydrolysis. As reported elsewhere[19] for the crude filipin mixture, its major component, filipin III, gave hexanal, as did the isomeric filipin IV. However, filipin II did not give any trace of hexanal.

Experimental Section[20]

Chainin. The sample used in the present investigation had mp 222–224° dec, $[\alpha]^{25}D$ −112.2° (*c* 0.16, MeOH). Its ir spectrum (Nujol) had bands at 3390 (OH), 1749 (C=O), and 1615 [(C=C)$_n$] cm^{-1}. Its nmr spectrum (C_5D_5N) contains peaks at 6.8–6.0 (m, 9 H), 5.3–5.0 (m, 1 H), 4.6–4.1 (m, 7 H), 3.8 (m, 1 H), 2.6 (m, 1 H), 2.3–1.55 (m, 14 H), 1.83 (s, 3 H), 1.43 (d, 3 H), 1.27 (m, 4 H), 0.76 (t, 3 H).

Chainin Octaacetate. Chainin (109.8 mg) was acetylated with acetic anhydride (5 ml) and pyridine (2 ml) at room temperature, under anhydrous conditions, until tlc showed no starting material (20 hr). Ice and water were added and the mixture was extracted with chloroform. The chloroform extract was dried and evaporated. The residue was redissolved in ether and precipitated by addition of pentane to give 98.2 mg (58%) of chainin octaacetate, mp 80–83°, $[\alpha]^{25}D$ −37.4° (*c* 1.31, $CHCl_3$). Its uv spectrum ($\lambda\lambda_{max}$ 312, 325, 342, 360 nm) was like that of the starting material, its ir spectrum contained strong bands at 1740 and 1210 cm^{-1} but no hydroxyl bands, its nmr spectrum ($CDCl_3$) was dominated by the acetyl singlet at δ 2.10 (24 H), other protons being found at 6.5–4.8 (m, 18 H), 2.7 (m, 1 H), 1.9 (m, 15 H), 1.3 (m, 9 H), and 0.9 (m, 3 H). Peaks from the mass spectrum are given in Table I.

Anal. Calcd for $C_{49}H_{70}O_{18}$: mol wt, 946. Found: 946 (mass spectrum).

Decahydrochainin. A solution of 508.9 mg of chainin in 50 ml of glacial acetic acid was hydrogenated at atmospheric pressure and room temperature over 115 mg of prereduced platinum oxide until absorption of hydrogen (*ca.* 5 mol) ceased after 8 hr. The catalyst was filtered and washed well with fresh solvent, and the solvent was removed to afford 523.3 mg of residue, which showed a single spot on tlc. The residue was dissolved in methanol and precipitated from ether–hexane to afford 487 mg (92%) of a white precipitate, mp 130° dec, $[\alpha]^{26}D$ −3.7° (*c* 1.07, MeOH). The uv spectrum showed only very weak end absorption (ϵ_{210} *ca.* 109), the ir spectrum contained bands at 3400 (−OH) and 1740 cm^{-1} (C=O), and the nmr spectrum [C_5D_5N; 220 MHz; δ 5.35 (m, 1 H), 5.12–3.75 (m, 8 H), 2.85 (m, 1 H), 2.45–1.63 (m, 22 H), 1.53 (d, 3 H), 1.28 (sb, 20 H), 0.85 (t, 3 H)] did not show any olefinic protons.

Anal. Calcd for $C_{33}H_{64}O_{10}\cdot 0.5H_2O$: C, 62.94; H, 10.40. Found: C, 62.93; H, 10.09.

(16) M. E. Bergy and T. E. Eble, *Biochemistry*, 7, 653 (1968).

(17) Crude decahydrofilipin, prepared from a sample of filipin containing filipins I, II, III, and IV (expected ratio 4:25:53:18), gave three peaks in the δ 3.0 region whose areas were in the ratio 29:56:15.

(18) V. J. Kowalewski, D. G. Kowalewski, and E. C. Ferra, *J. Mol. Spectrom.*, 20, 203 (1966).

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(20) Melting points, determined on a Kofler micro hot stage apparatus or a Hoover Uni-Melt apparatus, and boiling points are uncorrected. Infrared spectra were recorded on Perkin-Elmer spectrophotometers, Models 221, 237, and 137. Ultraviolet spectra were taken on samples dissolved in methanol or 95% ethanol, employing Beckman, Model DB, and Cary, Model 1115, recording spectrophotometers. Nuclear magnetic resonance (nmr) spectra were obtained by Mr. R. Thrift and Associates on Varian HA-100 or HR-220 spectrometers, employing TMS or DSS as the internal standard. Optical rotations were taken on a Zeiss polarimeter. Thin-layer chromatography was carried out on plates prepared with silica gel G, developing with chloroform containing a suitable proportion of methanol. The spots were revealed by spraying with 50% sulfuric acid and subsequent heating at 160°. Low-resolution mass spectra were obtained with a Varian MAT spectrometer, Model CH4, employing a molecular beam inlet and E4B ion source, by Mr. J. Wrona. High-resolution measurements were made by Mr. R. J. Wnuk, The Upjohn Co., on a CEC 21-110B mass spectrometer, and by Mr. J. C. Cook, Jr., on a Varian MAT SM1B mass spectrometer. Combined gas chromatography–mass spectrometry was carried out with a Varian gas chromatograph, Series 1700, in tandem with a Varian MAT CH7 mass spectrometer, equipped with a two-step Watson-Biemann separator. The column used was a 6-ft helical glass tube (2 mm i.d.) packed with 3% OV 17 on Gaschrom Q (100–120 mesh), operating under a flow rate of 20 ml per min.

Decahydrochainin Octaacetate. Decahydrochainin (103 mg) was acetylated with acetic anhydride (5 ml) and pyridine (2 ml) during 22 hr; then the reaction mixture was cooled and diluted with ice and water. The acetate was extracted with chloroform; work-up by the procedure employed for chainin octaacetate yielded a viscous gum (142 mg, 90%), $[\alpha]^{25}D$ −37.4° (*c* 0.565, $CHCl_3$), which was pure by tlc (10% CH_3OH in $CHCl_3$, H_2SO_4–HNO_3 (1:1) as spray reagent) but could not be crystallized. Its ir spectrum contained strong bands at 1740, 1240, and 1220 cm^{-1} but no hydroxyl bands, while its uv spectrum showed only very weak end absorption (ϵ_{210} *ca.* 756). Its nmr spectrum ($CDCl_3$) contained peaks at δ 5.3–4.8 (m, 9 H), 2.7 (m, 1 H), 2.12 (s, 24 H), 1.92 (m, 15 H), 1.35 (m, 22 H), 1.25 (d, 3 H), 0.92 (m, 6 H). Peaks from the mass spectrum are found in Table I.

Anal. Calcd for $C_{49}H_{80}O_{18}$: mol wt, 956.5317. Found: 956.5317 (HRMS).

Conversion of Chainin to 5,19-Dimethylhentriacontane. A solution of 628 mg of decahydrochainin and 3.2 g of *p*-toluenesulfonyl chloride (16 mmol) in 10 ml of pyridine was kept under anhydrous conditions for 36 hr at 4° while the reaction was followed by the change of color from yellow to brown to pink to purple, and by the separation of pyridine hydrochloride as long needles. The mixture was poured over ice and extracted with chloroform. Work-up yielded a residue which was dried in a desiccator overnight to afford 1.598 g of a foamy residue, whose ir spectrum indicated a very weak band for hydroxyl groups at 3550 cm^{-1}. A solution of the tosylate in tetrahydrofuran–ether (1:1) was added to a cold suspension of 3.0 g of lithium aluminum hydride in 200 ml of ether during 15 min. The mixture was stirred at room temperature for 48 hr and at reflux for 48 hr, then cooled and treated with saturated aqueous sodium sulfate. The white precipitate was filtered and the filtrate was extracted with ether. Work-up of the ether extract afforded 475 mg of an alcohol (ir spectrum, 3370 and 1060 cm^{-1} for hydroxyl, but no carbonyl bands).

The alcohol (400 mg) was treated with 1.0 g of *p*-toluenesulfonyl chloride and 10 ml of dry pyridine, precisely as in the preceding paragraph, to give 707 mg of crude tosylate whose ir spectrum contained no hydroxyl bands. The tosylate was then reduced with lithium aluminum hydride (1.5 g), as above, to give 325 mg of crude hydrocarbon, which was chromatographed over a column of silica gel (20 g; 10 cm × 2.2 cm). Elution with pentane gave 74 mg of saturated and unsaturated hydrocarbon whose mass spectrum showed molecular ions at 464 and 462. The hydrocarbon was then catalytically reduced over a suspension of 25 mg of pre-reduced platinum oxide in *n*-hexane. After 4 hr the catalyst was filtered and the filtrate was passed through a small column of silica gel and eluted with hexane. Solvent removal afforded 69 mg (14% from decahydrochainin) of a colorless residue, $n^{25}D$ 1.4643, whose ir spectrum (liquid film) was characteristic of a saturated hydrocarbon (2920, 2850, 1455, 1380 cm^{-1}).

Anal. Calcd for $C_{33}H_{68}$: mol wt, 464.5320. Found: mol wt, 464.5319 (HRMS).

Gas chromatographic analysis of the material showed one major component having a retention time of 6.0 min under isothermal column conditions at 270°. Minor components appeared at 5.7 (2%) and 6.9 min (1%). Low-resolution mass spectral data given in the Discussion were recorded for the major component during combined gas chromatography–mass spectrometry.

Hydrogenolysis of Chainin to Methyl 2-*n*-Butyl-16-methyloctacosanoate. A suspension of 1.2 g of chainin plus 500 mg of platinum oxide in 25 ml of glacial acetic acid was hydrogenated in a bomb at 2500 psi and 280° for 5 hr, then cooled, filtered, and washed with hexane–ethanol. Removal of solvent gave a white foamy acid (950 mg), which was esterified with diazomethane and purified by chromatography on silica gel to afford 186 mg (20%) of hydrocarbon (ir 2930, 2870, 1470, 1380, cm^{-1}), 166 mg (17%) of ester (ir 1745 and 1170 cm^{-1}), and 488 mg (52%) of hydroxy ester (ir 3400, 1745, and 1245 cm^{-1}), identified by their ir spectra. The ester fraction was rechromatographed and the purified fraction was distilled: 72 mg (7%); $n^{22.5}D$ 1.4620; nmr ($CDCl_3$) δ 3.73 (s, 3 H), 2.17 (m, 1 H), 1.26 (m, 54 H), 0.89 (m, 9 H).

Anal. Calcd for $C_{34}H_{68}O_2$: mol wt, 508.522. Found: mol wt, 508.524 (HRMS).

The ester was homogeneous on tlc in the solvent system methanol–chloroform, 1:20. Gas chromatographic analysis revealed that 97% of the material appeared as one component ($M \cdot^+$ 508) having a retention time of 9.8 min at a constant oven temperature of 280°, with minor peaks appearing at 9.2 min (2%, $M \cdot^+$ 494) and 10.7 min (1%, $M \cdot^+$ 522).

Isolation of 1-Hexanal and Acetaldehyde from Hydrolysis of Chainin. A suspension of 100 mg of chainin in 50 ml of 1 *N* aqueous sodium hydroxide was steam distilled. The steam distillate was collected in 50 ml of a saturated solution of 2,4-dinitrophenylhydrazine in 2 *N* aqueous hydrochloric acid. The 2,4-dinitrophenylhydrazone was filtered, washed with water, and dried. The residue (48.6 mg) was then purified on a 20 × 20 cm preparative tlc plate (silica gel G) using benzene–ethyl acetate (19:1) as solvent. Two bands (R_f 0.607 and 0.413) were removed from the preparative plate and eluted separately with ether. The faster moving component (10.7 mg) was crystallized from hexane and identified as 1-hexanal 2,4-dinitrophenylhydrazone by melting point and mixture melting point (104°) with an authentic sample. Its ir and mass spectra ($M \cdot^+ = m/e$ 280) were identical with those of the authentic sample. The slower moving component (33.1 mg) was crystallized from hexane and identified as acetaldehyde 2,4-dinitrophenylhydrazone by mp (156–160°), mixture melting point with an authentic sample, and ir and mass spectra ($M \cdot^+ = m/e$ 224).

Periodate Oxidation of Decahydrochainin. A solution of 16 mg of decahydrochainin (2.58×10^{-5} mol) in 10 ml of methanol and 10 ml of 0.1 *M* sodium metaperiodate was allowed to stand at room temperature.[21] Essentially no periodate had been consumed after 46 hr.

Nitric acid oxidation of chainin was carried out with 2 mg of the antibiotic and 0.1 ml of 70% nitric acid, which were incubated at 95° for 1 hr and then cooled. The solution was made basic by addition of sodium bicarbonate and extracted with ether. The aqueous solution was acidified with hydrochloric acid and again extracted with ether. The acidic extract was then dried over anhydrous magnesium sulfate and concentrated to about 50 μl. About 2 μl of the solution of acids was injected onto a glass column (2 ft × 2 mm i.d.) containing Porapak Q·S (flow rate 40 ml/min, oven temperature 240°). The retention times of the peaks were compared with those of standards, demonstrating the presence of acetic, propionic, *n*-butyric, valeric, and caproic acids.

Decahydrofilipin Nonaacetate. Decahydrofilipin[22] (150 mg) was acetylated with acetic anhydride (2 ml) and pyridine (2 ml) for 24 hr at room temperature. The usual work-up gave a viscous gum (208 mg, 87%) whose ir spectrum contained intense bands at 1750 and 1220 cm^{-1} but no hydroxyl bands. Its nmr spectrum [$CDCl_3$, δ 5.3–4.8 (m, 10 H), 2.15 (s, 27 H), 1.90 (m, 12 H), 1.36 (sb. 22 H), 1.25 (d, 3 H), 0.90 (t, 6 H)] was nearly superimposable on that of decahydrochainin octaacetate except for the acetyl region. Mass spectral peaks are found in Table I.

Anal. Calcd for $C_{66}H_{86}O_{20}$: mol wt, 1042.5712. Found: 1042.5721 (HRMS).

Hydrogenations of Filipins II, III, and IV. Each antibiotic (50 mg) was hydrogenated over 6 mg of Adams catalyst in 10 ml of ethanol for 4 hr at 20° and atmospheric pressure; then the solution was filtered through Celite to remove the catalyst, and evaporated *in vacuo*. Each residue was dissolved in 0.5 ml of pyridine-d_5 and transferred to an nmr tube.

Nitric acid oxidations of filipins II, III, and IV were carried out precisely as described above for the oxidation of chainin. Gas chromatographic analysis of the acidic products from filipin III indicated the formation of C_2–C_6 acids, by comparison of the retention times with those of acetic through *n*-caproic acids. The same results were obtained with filipin IV. Filipin II, however, produced C_2–C_8 acids (acetic through caprylic).

Basic hydrolyses of filipins II, III, and IV were carried out by heating 5 mg of each antibiotic with 0.1 ml of 1 *N* sodium hydroxide in a sealed tube inside a steam bath for 1.5 hr. Each solution was extracted with ether and the ether extracts were concentrated to about 20 μl each. An aliquot (2 μl) of each solution was injected onto a Porapak Q·S column (2 ft × 2 mm i.d.) at 240°. The peaks emerging were compared with a standard sample of hexanal. Filipins III and IV gave peaks corresponding to hexanal but filipin II gave no hexanal.

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