Amphotericin B. I. Carbon Skeleton, Ring Size, and Partial Structure[1]

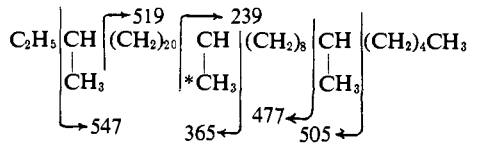
Arthur C. Cope, Udo Axen, Elizabeth P. Burrows, and Jürgen Weinlich

Contribution from the Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139. *Received June 13, 1966*

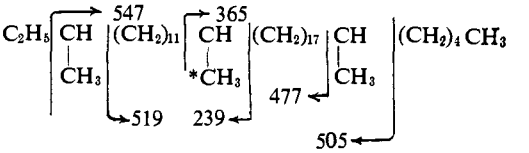
Abstract: Amphotericin B aglycone is shown to be C_{41} by conversion of the antibiotic to the "parent" hydrocarbon, 3,5,23-trimethyloctatriacontane (**1c**). High-pressure hydrogenation of the antibiotic at 290° gave two derived hydrocarbons, 3,5-dimethyloctatriacontane (**2**) and 3,5-dimethylheptatriacontane (**3**). This and other evidence proves the presence of a free carboxyl group at C-16 in addition to the lactone carbonyl at C-1. The location of the heptaene system and the complete structure of carbon atoms 33 through 38 have been established by the isolation of 1,3,5-trihydroxy-2,4-dimethylhexane (**8a**) as a major product of ozonolysis followed by lithium aluminum hydride reduction. This C_8 triol has been found to differ in absolute configuration at at least one center from a similar triol obtained from oleandomycin. The terminus of the lactone ring in amphotericin B is shown to be at C-37. Evidence for further features of a partial structure is presented.

The isolation of the antifungal antibiotics amphotericin A and B, so named because of their amphoteric properties, was reported in 1956.[2] The earliest chemical investigators of the latter antibiotic recognized its macrolide nature and assigned a tentative empirical formula $C_{46}H_{73}NO_{20}$ on the basis of microanalytical and neutralization equivalent determinations.[3] The location of the single basis nitrogen atom was shown by isolation of mycosamine,[4] a frequent glycosidic constituent of macrolides, on acid treatment.[3] The ultraviolet spectrum of amphotericin B[2] indicated a conjugated heptaene system and the hydrogenated antibiotic (7 molar equiv of hydrogen absorbed) showed no specific ultraviolet bands.[3] Recently the isolation of optically active 2-methylheptadecanedioic acid from nitric acid oxidation of perhydroamphotericin B was reported.[5]

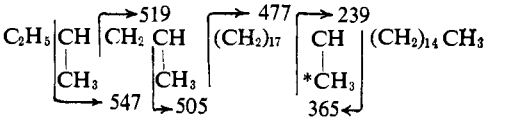
When we undertook the structural determination of amphotericin B in 1964 the first series of experiments was conversion of the antibiotic to a hydrocarbon by the phosphorus–hydriodic acid method, now a well-established tool for determination of the carbon skeleton of a macrolide.[6–8] Analysis of the mass spectrum of the hydrocarbon obtained (Figure 1) proved it was C_{41} (molecular ion at *m/e* 576) and contained three methyl groups, but alone was not sufficient proof for a single structure. Four possible trimethyloctatriacontanes (**1a–1d**) would each expectedly give rise to all of the observed principal fragments on electron impact.



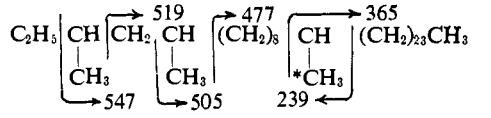
1a



1b



1c



1d

The results of high-pressure hydrogenation, a second method of reductive degradation first applied to the structure determination of macrolides by Ceder,[9] permitted a decision among the four possible "parent" hydrocarbons. In the cases of pimaricin[9] and rimocidin[10] the principal products of high-pressure hydrogenation in glacial acetic acid at elevated temperatures (250–300°) were carboxylic acids. When amphotericin B was hydrogenated under these conditions no acids were found; the only products isolated were two hydrocarbons in a relative ratio 2:1, separated by gas chromatography (vpc). An unambiguous determination of their structures was possible by mass spectrometry. The low-field regions of both spectra were identical, with the same major peaks (at *m/e* 57, 71, 85, 98, and 99) that were present in the spectrum of the C_{41} hydrocarbon. There were no significant peaks in

(1) Support was provided in part by the National Institutes of Health through Public Health Research Grant AI-02241-08. We are grateful to Dr. James D. Dutcher of the Squibb Institute for Medical Research for a generous gift of amphotericin B.

(2) J. Vandeputte, J. L. Wachtel, and E. T. Stiller, *Antibiot. Ann.*, 587 (1955–1956).

(3) J. D. Dutcher, M. B. Young, J. H. Sherman, W. Hibbits, and D. R. Walters, *ibid.*, 866 (1956–1957).

(4) J. D. Dutcher, D. R. Walters, and O. Wintersteiner, *J. Org. Chem.*, **28**, 995 (1963); M. von Saltza, J. D. Dutcher, J. Reid, and O. Wintersteiner, *ibid.*, **28**, 999 (1963).

(5) E. Borowski, W. Mechlinski, L. Falkowski, T. Zimínski, and J. D. Dutcher, *Tetrahedron Letters*, 473 (1965). We thank Dr. Dutcher for informing us of this result prior to publication.

(6) 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. Am. Chem. Soc.*, 84, 2170 (1962).

(7) O. Ceder, *Acta Chem. Scand.*, **18**, 77 (1964).

(8) A. C. Cope, E. P. Burrows, M. E. Derieg, S. Moon, and W. Wirth, *J. Am. Chem. Soc.*, 87, 5452 (1965).

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

(10) A. C. Cope, U. Axen, and E. P. Burrows, *J. Am. Chem. Soc.*, 88, 4221 (1966).

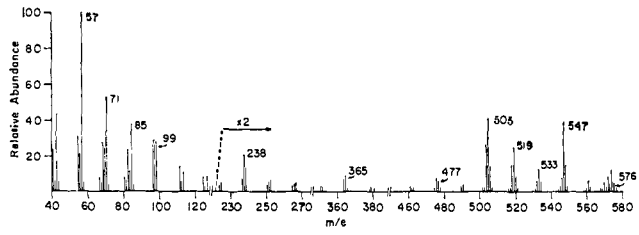
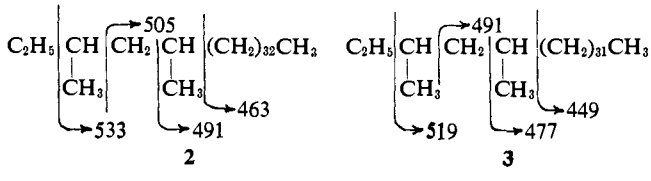


Figure 1. Mass spectrum of 3,5,23-trimethyloctatriacontane.

either spectrum between *m/e* 100 and 440. The high-field regions of the two spectra are reproduced in Figure 2; relative abundances were determined with respect to the base peak above *m/e* 99 in each case. The spectrum of the major hydrocarbon (Figure 2a) showed most prominent peaks at *m/e* 533, 505, and 491, significant peaks at *m/e* 547, 519, 477, and 462, and a molecular ion peak at *m/e* 562. The spectrum of the other hydrocarbon (Figure 2b) showed the same pattern with each peak 14 *m/e* units lower. The respective molecular formulas are thus $C_{40}H_{82}$ and $C_{39}H_{80}$. Comparison of these mass spectra with that of the "parent" C_{41} hydrocarbon (Figure 1) shows that the structures of these hydrocarbons are related to that of the C_{41} hydrocarbon by a loss of one and two methyl groups, respectively. Loss of one and two carboxyl groups under the conditions of high-pressure hydrogenation would explain the formation of the two hydrocarbons. Loss of the secondary carboxyl group which gives rise to the methyl group designated by an asterisk in structures **1a–d** would give the C_{40} hydrocarbon. Since the mass spectrum of the C_{39} hydrocarbon shows two methyl branches in the same positions from the ends of the chain as the C_{40} hydrocarbon, both methyl branches must be on the same end (in the 3 and 5 positions), and the primary carboxyl group that is lost must be on the opposite end. This fact establishes 3,5-dimethyloctatriacontane (**2**) and 3,5-dimethylheptatriacontane (**3**) as the only possi-



ble structures for the two hydrocarbons from high-pressure hydrogenation and eliminates **1a** and **1b** from consideration as structures for the C_{41} hydrocarbon. The isolation of 2-methylheptadecanedioic acid[5,11] from nitric acid oxidation of perhydroamphotericin B eliminates **1d** and establishes 3,5,23-trimethyloctatriacontane (**1c**) as the structure of the "parent" hydrocarbon from amphotericin B. Hydrocarbon **1c** was synthesized as outlined in Scheme I, and mass spectra of the two samples were identical except for differences of relative intensity in the region of the molecular ion (*m/e* 570–576).[12]

(11) We too isolated the acid (as the dimethyl ester) and identified it by comparison of its mass spectrum with that of a sample obtained from nystatin (A. J. Birch, C. W. Holzapfel, R. W. Rickards, C. Djerassi, P. C. Seidel, M. Suzuki, J. W. Westley, and J. D. Dutcher, *Tetrahedron Letters*, 1491 (1964)), for which we thank Professor Djerassi.

(12) Synthetic **1c** displayed a molecular ion peak (*m/e* 576) nearly twice as intense as the M − 2 peak, while the M − 2 peak in the spectrum of **1c** from amphotericin B was more than twice as intense as the molecular ion. We have sometimes observed large M − 2 peaks in the spectra of hydrocarbons obtained by the phosphorus–hydriodic acid method (7,21-dimethyltritriacontane from fungichromin and 3,5-dimethylhentriacontane from amphotericin; see later in this paper), but not always (3-methylhentriacontane from rimocidin and 3,5-dimethyldocosane from amphotericin; see later). All hydrocarbons were hydrogenated before collection by vpc and the apparent persistence of unsaturation in some instances but not in others is not understood.

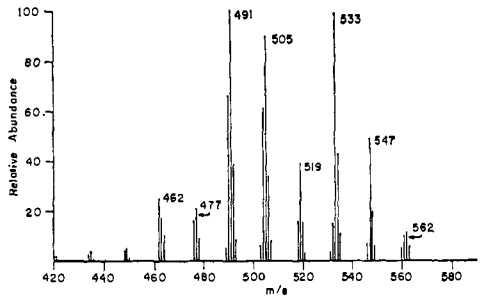


Figure 2a. Mass spectrum of 3,5-dimethyloctatriacontane.

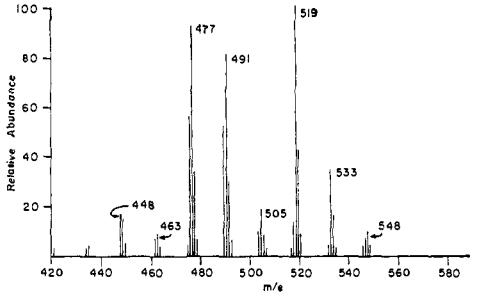
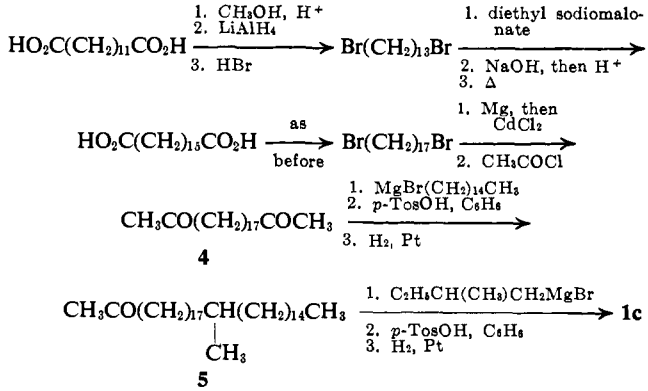


Figure 2b. Mass spectrum of 3,5-dimethylheptatriacontane.

The isolation of 2-methylheptadecanedioic acid[5] established the location of the heptaene system as most probably between the carbon atoms constituting C-6 through C-19 of **1c**. Consequently we sought to isolate a C_8 fragment from ozonolysis of amphotericin

Scheme I



B followed by hydrogenation and lithium aluminum hydride reduction to a mixture of polyols. The method of choice for identification of the expected polyol was conversion of the mixture to methyl ethers in order to facilitate separation by vpc and analysis by mass spectrometry. The structure of the major low molecular weight product from this sequence of reactions was deduced from its mass spectrum alone (Figure 3) and was later confirmed by other experiments. No molecular ion was found; the significant peaks were at *m/e* 188, 175, 172, 158, 117, 103, 59 (base peak), and 45. It was known from mass spectra of model compounds that methyl ethers with the structural feature CH_3-

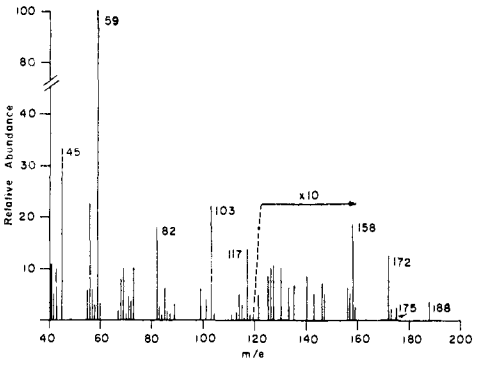
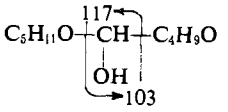


Figure 3. Mass spectrum of 1,5-dimethoxy-3-hydroxy-2,4-dimethylhexane.

$CHOCH_3$ give large peaks at M − 15 and at *m/e* 59 (oxonium cations). Thus the primary working hypothesis was the presence of such a secondary methoxyl group and a molecular weight of 175 + 15 = 190. The presence of a hydroxyl group was indicated by the relatively large peaks at *m/e* 172 (M − 18) and *m/e* 188 (M − 2).[13] The peak at *m/e* 45 was typical of a compound having the expected CH_2OCH_3 group. It was noted that the *m/e* values of the two most intense peaks in the spectrum above *m/e* 82 (103 and 117) added up to 220, or 190 + 30, which suggested cleavage on either side of a secondary hydroxyl group as shown in partial formula **6**.



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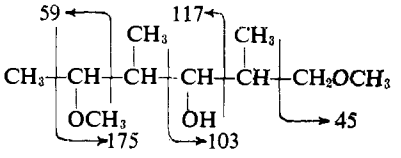
Only one structure can be written incorporating all these features and retaining the skeleton $C_2H_5CH(CH_3)CH_2CH(CH_3)$– required from the structures of hydrocarbons **1c**, **2**, and **3** from amphotericin B: 1,5-dimethoxy-3-hydroxy-2,4-dimethylhexane (**7**).



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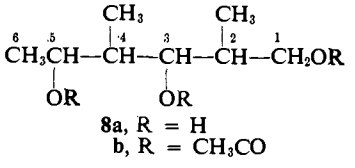


8a, R = H
b, R = CH_3CO

The parent triol **8a** was next isolated by vpc from the products of ozonolysis followed by hydrogenation and lithium aluminum hydride reduction. As anticipated, its identification could not have been made solely on the basis of its mass spectrum, which displayed no peaks above *m/e* 129 (loss of water plus methyl from the molecular ion). Its nuclear magnetic resonance (nmr) spectrum, in combination with microanalytical and infrared data, constituted proof for the assigned structure, 1,3,5-trihydroxy-2,4-dimethylhexane (**8a**). The two methyl groups attached to C-2 and C-4 appeared as two partially overlapping doublets centered at δ 0.72 (J = 7 cps) and 0.88 (J = 7 cps). The C-6 methyl group was a doublet (J = 6 cps) at δ 1.14. The two methine protons on C-2 and C-4 appeared as a multiplet centered at δ 1.65 and the four protons attached to hydroxyl-bearing carbon atoms as an irregular multiplet between δ 3.33 and 3.93. A broad three-proton singlet at δ 4.33, further broadened and shifted downfield to *ca.* δ 4.65 by the addition of pyridine, was due to the hydroxyl protons.

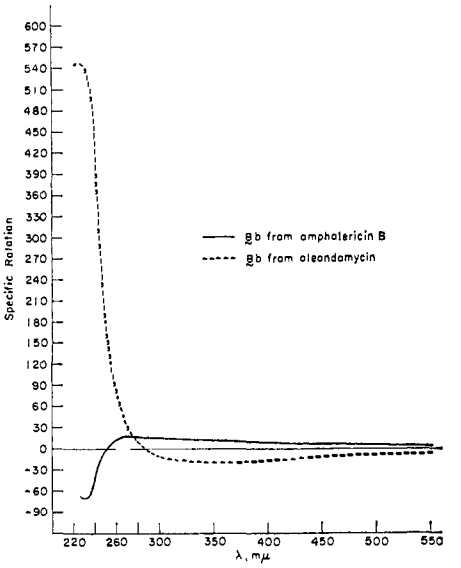


Figure 4. Rotatory dispersion curves of isomeric 1,3,5-triacetyl-2,4-dimethylhexanes (**8b**).

Triol **8a** was compared with a triol of the same gross structure, of known absolute configuration at all four centers, obtained from oleandomycin.[14] The mass spectra of the triols were identical, further substantiating our assigned structure. The specific rotations were not significantly different ($[\alpha]^t_D$ +23.4 and +17.5°). Hence it seemed desirable to prepare derivatives which might display a Cotton effect in order to determine whether our triol had the same configuration at all centers as the triol from oleandomycin. The triacetates proved ideally suited for this purpose; the optical rotatory dispersion curves are reproduced in Figure 4. It is seen that as the wavelength decreases toward the region of absorption maximum the rotation of triacetate **8b** from oleandomycin becomes increasingly highly positive, and that of triacetate **8b** from amphotericin becomes increasingly negative (to a lesser degree) and actually passes through a negative maximum at 230 mμ. At present, therefore, we may state with certainty only that triol **8a** from amphotericin B differs from the triol from oleandomycin in absolute configuration at at least one center.

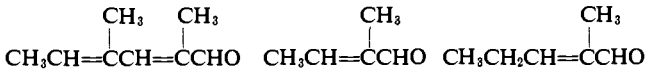
The communication outlining the structural determination of oleandomycin[15] mentioned the isolation

(13) M − 2 peaks are not infrequently found as genuine fragments in the mass spectra of saturated alcohols: H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Interpretation of Mass Spectra of Organic Compounds," Holden-Day Inc., San Francisco, Calif., 1964, p 32.

(14) W. D. Celmer, *J. Am. Chem. Soc.*, 87, 1797 (1965), in his determination of the total absolute configuration of oleandomycin, described compounds closely related to the triol. Dr. Celmer very kindly furnished us a sample of the triol itself.

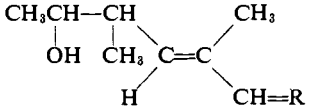
(15) F. A. Hochstein, H. Els, W. D. Celmer, B. L. Shapiro, and R. B. Woodward, *ibid.*, 82, 3225 (1960).

of 2,4-dimethyl-2,4-hexadienal (**9**) from a degradative sequence utilizing alkaline treatment. Because this compound possesses the skeleton of triol **8a** with all centers of asymmetry removed, we attempted to isolate it from amphotericin by alkaline treatment of the mixture of products after ozonolysis and hydrogenation. The only aldehyde isolated,[16] however, had an ultraviolet spectrum identical with that of tiglic aldehyde (**10**) and its mass spectrometric molecular weight was indicative of the next higher homolog. Its identity as 2-methyl-2-pentenal (**11**) was substantiated by comparison with a sample synthesized from propionaldehyde. Thus alkaline treatment occasioned retroaldol cleavage instead of the desired γ,δ elimination.[17]



9 **10** **11**

Acid treatment of the products after ozonolysis and hydrogenation also failed to give any trace of dienal **9**. A product isolated in very low yield had an ultraviolet spectrum identical with that of tiglic aldehyde (**10**) 2,4-dinitrophenylhydrazone. Its infrared spectrum established the presence of a hydroxyl group ($\nu_{max}^{CHCl_3}$ 3600 cm^{-1}). Microanalyses substantiated the formula $C_{14}H_{18}N_4O_5$ assigned on the basis of a mass spectrometric molecular weight of 322. The structure of the aldehyde consequently was formulated as *trans*-2,4-dimethyl-5-hydroxy-2-hexenal (**12a**). The 2,4-dinitrophenylhydrazone **12b** was compared with a sample of



12a, R = O
b, R = $NNHC_6H_3(NO_2)_2$

the same gross structure and of established configuration obtained from oleandomycin.[18] Mass spectra of the two 2,4-dinitrophenylhydrazones were identical and their melting points were very nearly the same. However, a mixture melting point was depressed by 20°, proving a difference in stereochemistry. Both 2,4-dinitrophenylhydrazones had the same sign of optical rotation; consequently the configurations at only one of the two centers are opposite.

Analogy with the known structures of other macrolides strongly suggested that the terminus of the lactone ring was at one of the two hydroxyl groups in the C_8 moiety. This expectation was confirmed by the observation that mild alkaline treatment either before or after acid treatment of the hydrogenated ozonolysis products resulted in a tenfold increase in yield of hydroxy aldehyde **12a**. That the double bond α,β to the aldehyde group was actually present after acid treatment and before alkaline hydrolysis was shown by the isolation of tiglic aldehyde (**10**) from the acid-treated ozonolysis products after a second ozonolysis followed by hydrogenation and steam distillation. These facts constitute proof for closure of the lactone ring at C-37[19] of the partial structure **13** that we may now formulate for amphotericin B.

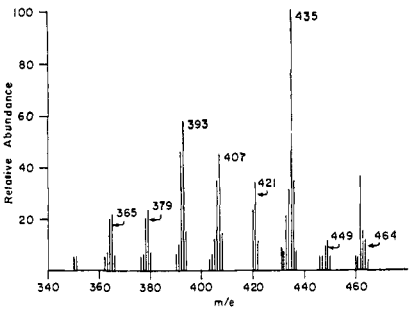
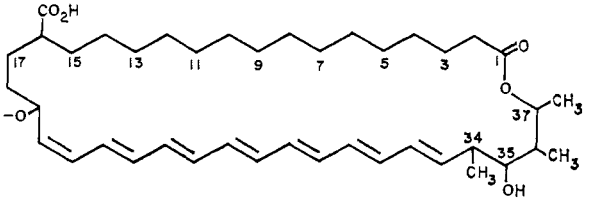


Figure 5a. Mass spectrum of 3,5-dimethylhentriacontane.

The possibility that the carboxyl group at C-16 rather than the primary carboxyl group formed the lactone was eliminated in the following way. Decarboxylation of perhydroamphotericin B proceeded smoothly in water at 100°. Titrations before and after heating showed that one acidic group had been lost but that the lactone function was still present after heating. The hydrocarbon prepared from decarboxyperhydroamphotericin B by the phosphorus–hydriodic acid method had a mass spectrum identical with that of hydrocarbon **2** from high-pressure hydrogenation. This proved that the primary carboxyl group forms the lactone as shown in partial structure **13**.



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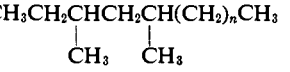
Some further information concerning the oxygenation pattern of amphotericin B was obtained through treatment of perhydroamphotericin with a variety of reagents prior to conversion to hydrocarbons by the phosphorus–hydriodic acid method. Treatment of perhydroamphotericin **B** (or amphotericin B itself) with either periodic acid for 5 min or sodium metaperiodate overnight followed by lithium aluminum hydride reduction and conversion to hydrocarbons gave a single hydrocarbon, identified from its mass spectrum (Figure 5a) as 3,5-dimethylhentriacontane (**14a**).[20] As a conse-



14a, n = 25
b, n = 16
c, n = 14

(16) In this and all subsequent experiments involving isolation of aldehydes the products were separated and characterized as the 2,4-dinitrophenylhydrazones.

(17) A similar isolation of aldehyde **11** was reported by E. Borowski, W. Mechlinski, L. Falkowski, T. Ziminski, and J. D. Dutcher, *Roczniki Chem.*, **39** (12), 1933 (1965). We thank Dr. Dutcher for sending us a preprint of this communication, in which the authors proposed the correct structure of the C_8 moiety without isolating any more complex degradation products than aldehyde **11**.

(18) W. D. Celmer, *J. Am. Chem. Soc.*, 87, 1787 (1965). It is a pleasure to acknowledge the contribution of Dr. Celmer not only in providing samples for comparison but in making available to us a stimulating discussion of correlations in macrolide biogenesis and stereochemistry in advance of publication: "Antimicrobial Agents and Chemotherapy—1965," G. L. Hobby, Ed., American Society for Microbiology, Ann Arbor, Mich.

(19) Borowski and co-workers (see footnote 17) also proposed closure of the lactone ring at this position but presented no experimental evidence.

(20) Treatment of perhydroamphotericin with acid for a longer time followed by periodate oxidation and conversion to hydrocarbons also gave **14a** and no significant amounts of any lower hydrocarbons.

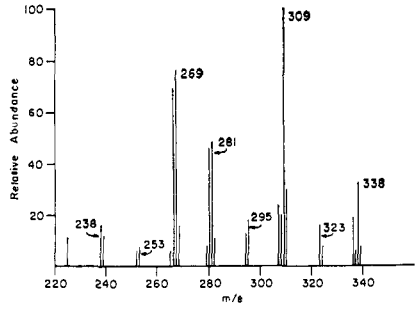


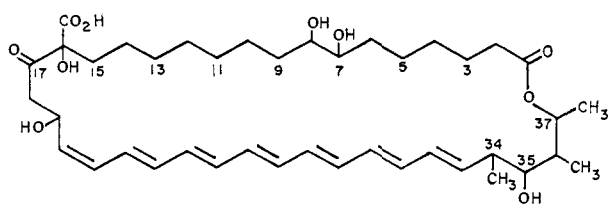
Figure 5b. Mass spectrum of 3,5-dimethyldocosane.

quence, hydroxyl groups must be placed on C-7 and C-8. Loss of the secondary carboxyl group at C-16 (**13**) on periodate treatment was attributed to an oxidative process since it occurred with metaperiodate as well as with periodic acid.

The facile decarboxylation described earlier required the presence of a keto group at one of the two positions β to the carboxyl group (C-15 or C-17). Treatment of perhydroamphotericin B with alkali followed by lithium aluminum hydride reduction and conversion to hydrocarbons gave a complex mixture containing two major products in a relative ratio 2:1. The mass spectrum of the more predominant one (Figure 5b) proved its identity as 3,5-dimethyldocosane (**14b**); the other was similarly identified on the basis of a strictly analogous mass spectrum (not shown) as 3,5-dimethyleicosane (**14c**). Cleavage between carbon atoms 16 and 17 yielding hydrocarbon **14b** was attributed to the acid cleavage of a β-keto acid with alkali; cleavage between carbon atoms 18 and 19 yielding hydrocarbon **14c** was attributed to retroaldol fission of a 1,3-ketol. Thus the results of alkaline treatment not only prove the presence of a keto group at C-17 but also the presence of a (potential) hydroxyl at C-19, which had been inferred from the earlier nitric acid oxidation experiments.[5,11]

Reduction of perhydroamphotericin B with lithium aluminum hydride and work-up with care to avoid strongly acidic conditions, followed by a brief treatment with periodic acid and then by lithium aluminum hydride reduction and conversion to hydrocarbons, gave as a major product (*ca.* 60%) the same hydrocarbon obtained from alkaline treatment, 3,5-dimethyldocosane (**14b**). A minor product (*ca.* 20%) was 3,5-dimethylhentriacontane (**14a**). These facts necessitate placement of a hydroxyl group at C-16, α to the keto group. The α-hydroxy ketone remained intact on brief treatment with periodic acid (as shown by the isolation of **14a** described previously), but after lithium aluminum hydride reduction the resulting 1,2-diol was more readily oxidized and the major hydrocarbon product was **14b**.

With the incorporation of these features the partial structure for amphotericin B aglycone is formulated as **15**, with the additional requirement that none of the remaining oxygen functions to be placed between C-8 and C-16 be vicinal.



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Further work is in progress to determine the complete oxygenation pattern of amphotericin B and the position of attachment of mycosamine to the aglycone.

Experimental Section[21]

Conversion of Amphotericin B to 3,5,23-Trimethyloctatriacontane (1c). A suspension of amphotericin B (2.01 g) in methanol (100 ml) and acetic acid (10 ml) was hydrogenated over platinum as catalyst (150 mg of platinum oxide) for 16 hr at room temperature and atmospheric pressure. The cstalyst was removed by filtration, the solvents were evaporated, and the residue was dried at 0.5 mm overnight. The resulting amorphous white solid (2.27 g, hereafter referred to as perhydroamphotericin B) was suspended in 100 ml of tetrahydrofuran (THF) and added to 4 g of lithium aluminum hydride in 70 ml of THF. The mixture was stirred under reflux for 2 days. Water (20 ml) was added slowly with cooling, the mixture was filtered, and the filtrate was evaporated to dryness yielding 1.46 g of polyol. The filter cake was dissolved in 10% sulfuric acid (300 ml) and the solution was extracted with two 150-ml portions of 1-butanol. The butanol extracts were washed with 10% sodium carbonate solution (100 ml) and water (two 100-ml portions) and evaporated to give an additional 0.91 g of polyol. The polyol (2.37 g) was heated under reflux for 24 hr with hydriodic acid (80 ml) and red phosphorus (2.4 g). The cooled mixture was diluted with water (100 ml) and extracted with two 150-ml portions of chloroform. The chloroform solution was filtered, washed with water, 2% sodium thiosulfate solution, and water, dried, and evaporated. The residue (2.47 g) was dissolved in THF (65 ml) and added slowly to 2.5 g of lithium aluminum hydride in 50 ml of THF. The mixture was stirred under reflux for 16 hr and cooled, and the excess lithium aluminum hydride was destroyed with ethyl acetate. The mixture was filtered, the filter cake was washed with hexane, and the filtrate and washings were evaporated to dryness. The residual oil (631 mg) was hydrogenated for 20 hr over 150 mg of platinum oxide in hexane (80 ml) and acetic acid (5 ml). The catalyst was removed by filtration and the filtrate was evaporated to dryness. The residue (435 mg) was dissolved in hexane and filtered through a column of activity I alumina (4 g).[22] The resulting hydrocarbon (108 mg, >90% pure by vpc) was later identified as 3,5,23-trimethyloctatriacontane (**1c**). Samples were collected by vpc for mass spectrometric and other analyses.

Anal. Calcd for $C_{41}H_{84}$: C, 85.33; H, 14.67. Found: C, 85.55; H, 14.30.

Synthesis of 3,5,23-Trimethyloctatriacontane (1c). A. 2,20-Heneicosanedione (4). 1,11-Undecanedicarboxylic acid (Aldrich Chemical Co.) was esterified with methanol-sulfuric acid and converted in 52% over-all yield to 1,13-dibromotridecane, bp 162–165° (2 mm) [lit.[23] bp 185–187° (9 mm)], by the procedure described previously.[6] The dibromide (16.8 g) was added in portions to a solution of ethyl sodiomalonate prepared from 3.3 g of sodium and 24.5 g of diethyl malonate in 42 ml of ethanol, and the mixture was heated under reflux for 18 hr. After saponification of the products followed by acidification, heating of the crude tetracarboxylic acid to 200°, and recrystallization of the residue from benzene, 12.0 g of 1,15-pentadecanedicarboxylic acid (78% yield), mp 115–118° (lit. mp 118°), was obtained. Repetition of

(21) A Hitachi Perkin-Elmer RMU-6D mass spectrometer was used throughout. Nuclear magnetic resonance (nmr) spectra were determined in deuteriochloroform on a Varian Associates A-60 instrument. Optical rotatory disperison curves were determined using a Cary 60 recording spectropolarimeter. Melting points were taken on a Kofler hot stage and are not corrected. Analyses were performed by the Scandinavian Microanalytical Laboratory, Herlev, Denmark. Gas chromatographic (vpc) analyses and collections (100–325°) were made using an F and M Model 720 instrument equipped with 2 ft × 0.25 in. and 8 ft × 0.25 in. columns packed with 3, 10, or 20% silicone rubber (SE-30) on 60–80 mesh Chromosorb W, and an F and M Model 810 (with thermal conductivity detector only) equipped with a 4 ft × 0.25 in. 5% silicone rubber column (on 60–80 mesh Diatoport S). Solvents were removed under reduced pressure using a rotary evaporator, and magnesium sulfate or sodium sulfate were used as drying agents.

(22) In subsequent conversions to hydrocarbons the products of the second lithium aluminum hydride reduction were filtered through alumina before hydrogenation, but the persistent unsaturated impurities present in some cases after hydrogenation still remained.[12]

(23) P. Chuit, *Helv. Chim. Acta*, **9**, 264 (1926).

the first three steps of the synthesis gave 1,17-dibromoheptadecane, bp 196–202° (1.5 mm) [lit.[24] bp 208–210° (3 mm)], in 50% over-all yield. Treatment of the Grignard reagent prepared from 7.7 g of the dibromide and 1.02 g of magnesium in ether with cadmium chloride (5 g, in two portions), followed by replacement of the ether with benzene and addition of acetyl chloride (5 g) in benzene in the manner previously described in detail for the synthesis of 2,16-heptadecanedione,[25] afforded the homolog 2,20-heneicosanedione (**4**). Pure **4**, mp 90–93° (lit.[26] mp 95.5°), homogeneous to vpc, was obtained in 60% yield (3.8 g) after several recrystallizations from ethanol.

B. **2-Methyl-2-pentatriacontanone (5).** 1-Bromopentadecane, bp 141–143° (2 mm), >99% pure by vpc, was prepared (76% yield) by treatment of 1-pentadecanol (Aldrich Chemical Co.) with anhydrous hydrogen bromide at 135–145° for 1 hr. A solution of the Grignard reagent prepared from this bromide (1.46 g) and magnesium (0.26 g) in ether (15 ml) was added slowly to a boiling solution of diketone **4** (1.6 g) in 1:1 ether–benzene (40 ml). Heating was continued for 19 hr. The work-up employed was that described for the reaction of *n*-dodecylmagnesium bromide with 2,16-heptadecanedione[25] except that benzene was used to extract the organic products (2.9 g). Vpc analysis revealed a mixture of three components in an approximate ratio 2:1:1.5 in order of increasing retention times. The first two were identified as **4** and *n*-dotriacontane, respectively. The hydrocarbon was removed by chromatography of the mixture on activity I alumina. Repeated chromatography of mixtures of the other two components, eluted with benzene–chloroform mixtures, resulted in enrichment of the desired product to the extent of 80%, but no further separation was attained. This mixture (1.2 g) was treated for 16 hr with *p*-toluenesulfonic acid (0.4 g) in refluxing benzene (120 ml). The solvent was removed by distillation and the residue was dissolved in ether (200 ml), washed with 5% sodium hydroxide solution and with water, and dried. After removal of the solvent the oily residue (1.0 g) was hydrogenated in *n*-hexane (150 ml) using platinum as catalyst. Vpc analysis of the product (0.95 g) revealed a 1:4 mixture of diketone **4** and a major component of longer retention time. Its identity as **5** was confirmed by the mass spectrum of a collected sample (molecular ion at *m/e* 520). The mixture was not separable by chromatography on alumina or by recrystallization and was used directly in the subsequent step. A collected sample of **5** was analyzed.

Anal. Calcd for $C_{36}H_{72}O$: C, 83.00; H, 13.93. Found: C, 82.38; H, 13.22.[27]

C. **3,5,23-Trimethyloctatriacontane (1c).** To a Grignard reagent prepared from 0.9 g of 1-bromo-2-methylbutane (bp 119–121°, >99.5% pure by vpc, prepared by treatment of 2-methyl-1-butanol with phosphorus tribromide) and 0.24 g of magnesium in ether (20 ml) was added a solution of 75 mg of the mixture of **4** and **5** in benzene (20 ml). The mixture was stirred under reflux for 20 hr, then cooled and treated with aqueous ammonium chloride. The aqueous layer was extracted with hexane and the residue (89 mg) obtained on evaporation of the combined, dried organic solutions was treated with *p*-toluenesulfonic acid (0.1 g) and hydrogenated as described above. Vpc analysis of the mixture of hydrocarbons (32 mg) obtained after filtration through alumina in hexane revealed a major component (*ca.* 50%) with the same retention time as **1c** from amphotericin B. Mass spectra of the two hydrocarbons were identical except for differences in relative intensity of the peaks in the region of the molecular ion (*m/e* 570–577).

High-Pressure Hydrogenation of Amphotericin B. Isolation of 3,5-Dimethyloctatriacontane (2) and 3,5-Dimethylheptatriacontane (3). Amphotericin B (600 mg) was hydrogenated in glacial acetic acid (8 ml) at 290–295° and 3300 psi for 5 hr using 5% palladium on alumina (330 mg) as catalyst. The mixture was cooled and filtered, and the filter cake was extracted with three 50-ml portions of boiling ethanol. The combined filtrate and extracts on evaporation to dryness yielded a semisolid (179 mg) which was shown by vpc to contain only two major components (>90% of the total mixture). The infrared spectrum of the mixture had no absorption in the carbonyl region and was typical of a saturated hydrocarbon. Analysis of the mass spectra of collected samples of the two products was sufficient to prove their identities as **2** and **3** (see Discussion secion).

Ozonolysis of Amphotericin B. A. Isolation of 1,5-Dimethoxy-3-hydroxy-2,4-dimethylhexane (7). A suspension of amphotericin B (2.5 g) in methanol (1.2 l.) was ozonized just to completion (15 min) at −78°. Nitrogen was then bubbled through the clear colorless solution for 30 min at −78°. Platinum oxide (300 mg) was added and the mixture was allowed to warm to room temperature during hydrogenation at atmospheric pressure for 2 hr. The catalyst was removed by filtration and the solvent was evaporated to a residue of 2.5 g, which was suspended in THF (150 ml) and added to a stirred suspension of lithium aluminum hydride (6 g) in THF (100 ml). The mixture was stirred under reflux for 18 hr, then cooled. To it was slowly added methanol (65 ml) and water (12 ml); after standing several hours the resulting mixture was filtered and the filtrate was evaporated to dryness. The residue was dissolved in 1:1 chloroform–methanol and filtered through activity III alumina yielding 3.1 g of a mixture of polyols. A portion of the mixture (1.38 g) was dissolved in dimethylformamide (10 ml) and methyl iodide (10 ml) and was added dropwise to a stirred mixture of barium oxide (4 g), barium hydroxide octahydrate (150 mg), methyl iodide (4 ml), and dimethylformamide (5 ml).[28] The mixture was stirred at 60° for 30 min and at 40° for 16 hr. It was diluted with chloroform (500 ml) and filtered, and the chloroform solution was washed with sodium thiosulfate solution and repeatedly with water, dried, and evaporated. Vpc analysis of the material obtained (224 mg) by ether extraction of the residue revealed a complex mixture. A major product was collected and on the basis of its mass spectrum (Figure 3) was identified as 1,5-dimethoxy-3-hydroxy-2,4-dimethylhexane (**7**).

B. **Isolation of 1,3,5-Trihydroxy-2,4-dimethylhexane (8a).** When the mixture of polyols obtained above was subjected directly to vpc analysis a single major low molecular weight compound (except for 1,4-butanediol, believed to result from attack by lithium aluminum hydride on THF under prolonged heating) was found. Pure samples of the triol, identified as **8a** on the basis of microanalysis and spectral data, were obtained by preparative vpc. Its optical rotation was measured in methanol: $[\alpha]^{27}D$ +23.4 ± 2° (*c* 1.02). Triol **8a** from oleandomycin[14] had $[\alpha]^{25}D$ +17.5° (*c* 2, methanol). Mass spectra of the two triols were identical.

Anal. Calcd for $C_8H_{18}O_3$: C, 59.23; H, 11.18. Found: C, 59.53; H, 11.01.

C. **1,3,5-Triacetyl-2,4-dimethylhexane (8b).** Treatment of the mixture of polyols with excess acetic anhydride in pyridine at room temperature gave (in addition to 1,4-butanediol diacetate) triacetate **8b** as the major volatile product. A collected sample had $\nu_{max}^{CCl_4}$ 1735 cm^{-1} (no hydroxyl absorption), $[\alpha]^{25}D$ +4.6 ± 0.5° (*c* 0.51, ether). Triacetate **8b** prepared in the same manner from the triol from oleandomycin[14] had $[\alpha]^{25}D$ −8.4° (*c* 0.50, ether). Optical rotatory dispersion curves of the two samples, measured in ether, are reproduced in Figure 4. Mass spectra of the two triacetates were nearly identical except for differences in the relative intensities of the peaks at *m/e* 187 and 173 and at *m/e* 145 and 142.

Anal. Calcd for $C_{14}H_{24}O_6$: C, 58.32; H, 8.39; $3COCH_3$, 44.78. Found: C, 58.79; H, 8.36; $COCH_3$, 45.41.

Ozonolysis of Amphotericin B. A. Isolation of 2-Methyl-2-pentenal (11). A suspension of amphotericin B (1 g) in methanol (500 ml) was ozonized at −78° and then hydrogenated as described in the previous section. The catalyst was removed by filtration, the solvent was evaporated, and the residue was heated to 100° with a solution of potassium hydroxide (1.5 g) in water (50 ml). Forty milliliters of distillate was collected and extracted with three 50-ml portions of ether. The ether solution was concentrated and added to a solution of 0.6 g of 2,4-dinitrophenylhydrazine in 25 ml of 30% perchloric acid. The ether was removed by evaporation and the precipitate was collected by filtration and washed with water. Purification by preparative thin layer chromatography[29] followed by recrystallization from ethyl acetate yielded **11** as the 2,4-dinitrophenylhydrazone, mp 164–165° (lit.[30]

(24) P. Chuit and J. Hausser, *Helv. Chim. Acta*, **12**, 854 (1929).

(25) See ref 6, p 2175.

(26) L. Canonica and T. Bacchetti, *Atti Accad. Nazl. Lincei, Rend. Classe Sci. Fis., Mat. Nat.*, **15**, 278 (1953); *Chem. Abstr.*, **49**, 8121 (1955).

(27) This analysis was the better of two trials. Values lower than the theoretical are attributed to partial decomposition at the high temperature (325°) required for collection by vpc.

(28) R. Kuhn, H. H. Baer, and A. Seeliger, *Ann. Chem.* **611**, 236 (1958); R. Kuhn, H. Egge, R. Brossmer, A. Gauhe, P. Klesse, W. Lochinger, E. Roehm, H. Trischmann, and D. Tschampel, *Angew. Chem.* **72**, 805 (1960).

(29) All separations of 2,4-dinitrophenylhydrazones by preparative thin layer chromatography were made using Merck silica gel G, activated 2 hr at 100°, with benzene–ethyl acetate–methanol, 100:8:1, as the solvent.

(30) D. R. Howton, *J. Org. Chem.*, **14**, 1 (1949).

mp 161–162°). A mixture melting point with authentic material synthesized from propionaldehyde[31] was not depressed.

B. Isolation of 2,4-Dimethyl-5-hydroxy-2-hexenal 2,4-Dinitrophenylhydrazone (12b). A suspension of amphotericin B (1.2 g) in methanol (600 ml) was ozonized and hydrogenated as described previously. The catalyst was filtered, the filtrate was evaporated to dryness, and the residue was dissolved in 30 ml of 0.1 *N* potassium hydroxide in 99% methanol and allowed to stand 11 hr. It was then neutralized with 6 *N* hydrochloric acid and evaporated to dryness. The residue was dissolved in 30 ml of water containing 12 drops of 10% sulfuric acid and refluxed 1 hr on a steam bath. The mixture was cooled and extracted continuously with ether, and the extracts were concentrated and treated with excess (3.0 g) 2,4-dinitrophenylhydrazine in 30% perchloric acid (140 ml) as described above. Purification of the resulting crude 2,4-dinitrophenylhydrazone (180 mg) by preparative thin layer chromatography[29] yielded 67.5 mg of 2,4-dinitrophenylhydrazone **12b** which was recrystallized from ethanol, mp 175–176°.

Anal. Calcd for $C_{14}H_{18}N_4O_5$: C, 52.17; H, 5.63; N, 17.38. Found: C, 52.25; H, 5.80; N, 17.43.

2,4-Dinitrophenylhydrazone **12b** from oleandomycin had mp 173–174°. A mixture melting point of equal amounts of the two samples was depressed by 20°.

Optical rotatory dispersion curves of the two samples, measured in chloroform, each showed positive values increasing in magnitude from *ca.* [α] +80 to +190° as the wavelength was decreased from 589 to 510 mμ.[32]

Proof of Closure of the Lactone. A suspension of amphotericin B (600 mg) in methanol (500 ml) was ozonized and hydrogenated and the products were evaporated to dryness as described above. The residue was refluxed for 1 hr with 40 ml of water containing 10 drops of 10% sulfuric acid, cooled, and neutralized with sodium bicarbonate. The water was evaporated under reduced pressure and the organic residue was dissolved in methanol (60 ml) and filtered. The solution was divided into three equal parts which were treated as follows.

Solution 1. To the solution were added water (10 ml) and 10% sulfuric acid (2 drops) and the mixture was distilled while water was added continuously. The distillate (70 ml) was added to 20 ml of the previously described solution of 2,4-dinitrophenylhydrazine in 30% perchloric acid. The methanol was removed by evaporation and the precipitate was collected. Preparative thin layer chromatography[29] yielded only trace amounts of 2,4-dinitrophenylhydrazone **12b** (<1 mg) and tiglic aldehyde 2,4-dinitrophenylhydrazone (<0.5 mg).

Solution 2. Potassium hydroxide (110 mg) was added and the resulting solution was allowed to stand at room temperature for 4 days. It was neutralized with 10% sulfuric acid and then made slightly acidic and distilled as described for solution 1. Preparative thin layer chromatography[29] of the 2,4-dinitrophenylhydrazones obtained from the distillate afforded **12b** (10 mg)[33] and a trace of tiglic aldehyde 2,4-dinitrophenylhydrazone (<1 mg).

Solution 3. The solution was ozonized at − 78° and hydrogenated as described previously. The catalyst was removed by filtration and to the filtrate were added water (10 ml) and 10% sulfuric acid (2 drops). Distillation was carried out as described above, and the 2,4-dinitrophenylhydrazones obtained from the distillate were separated by preparative thin layer chromatography.[29] Tiglic aldehyde (**10**) 2,4-dinitrophenylhydrazone was the major product (13.5 mg); less than 1.5 mg of 2,4-dinitrophenylhydrazone **12b** was isolated.

Decarboxylation of Perhydroamphotericin B. Conversion to 3,5-Dimethyloctatriacontane (2). A stirred suspension of perhydroamphotericin B (750 mg) in water (5 ml) was heated slowly to 100° under a stream of nitrogen. The effluent gas was passed through a barium hydroxide trap. Evolution of carbon dioxide was observed at 95° and the mixture was kept at 95–100° for 1 hr. It was then evaporated to dryness and the residue (decarboxyperhydroamphotericin B) was reduced with lithium aluminum hydride and converted to hydrocarbons in the manner described previously for the conversion of amphotericin B to 3,5,23-trimethyloctatriacontane (**1c**). The major hydrocarbon product was identified as 3,5-dimethyloctatriacontane by comparison of its mass spectrum with that of **2** obtained from high-pressure hydrogenation of amphotericin B. Hydrocarbons **1c** and **3** were not detected.

Acid–Base Titrations of Perhydroamphotericin and Decarboxyperhydroamphotericin B. Duplicate samples of each material (197 ± 0.5 mg) were dissolved in 10.00 ml of 0.090 *N* potassium hydroxide in 99:1 methanol–water. The samples were titrated *vs.* 0.100 *N* hydrochloric acid (phenolphthalein indicator) immediately and also after standing 4 hr. Blanks were run before and after each determination. Hydrolysis of the lactone was found to proceed extremely rapidly under these conditions and was partially complete even when 0.09 *N* potassium hydroxide (7 ml) was added to a sample of perhydroamphotericin (197 mg) suspended in methanol (7 ml) at 5° and the resulting solution titrated as rapidly as possible. The results (estimated accuracy ±0.05) are summarized in Table I.

Table I. Titrations of Perhydroamphotericin and Decarboxyperhydroamphotericin B

Compound (conditions)	mmoles of KOH/mmole of compound[a] Assume mol wt 900	Assume mol wt 1000
Perhydroamphotericin (5°, immediately)	2.38	2.62
Perhydroamphotericin (room temp, immediately)	2.62	2.89
Perhydroamphotericin (room temp, after 4 hr)	3.00	3.30
Decarboxyperhydroamphotericin (room temp, immediately)	1.48	1.63
Decarboxyperhydroamphotericin (room temp, after 4 hr)	2.03	2.24

[a] In each case 1 mmole of base was consumed in excess of that required for the lactone group (and the free carboxyl group, when present). The "extra" acidic function is attributed to salt formation of the amino group with an acid (acetic or hydrochloric) at some earlier stage.

Periodate Oxidations. Isolation of 3,5-Dimethylhentriacontane. A. Sodium Periodate Oxidation of Perhydroamphotericin B. To a solution of perhydroamphotericin B (1 g) in *t*-butyl alcohol (40 ml) and water (80 ml) was added a solution of sodium metaperiodate (0.9 g) in water (10 ml). The mixture was allowed to stand at room temperature for 20 hr and was then diluted with water (100 ml) and extracted with two 250-ml portions of 1-butanol. The butanol extracts were washed with water and evaporated to dryness. The residue was reduced with lithium aluminum hydride and converted to hydrocarbons as described previously. Vpc analysis of the product (42 mg) revealed a single major component (95%) identified as 3,5-dimethylhentriacontane (**14a**) on the basis of the mass spectrum of a collected sample (Figure 5a).

B. Periodic Acid Oxidation of Perhydroamphotericin B. To a suspension of perhydroamphotericin B (1 g) in methanol (75 ml) was added a solution of periodic acid (1.95 g of $HIO_4 \cdot 2H_2O$) in water (26 ml). The mixture became homogeneous at once and was stirred for 5 min. The excess periodic acid was quenched with ethylene glycol (7 ml) and the mixture was diluted with water (300 ml) and extracted with two 200-ml portions of 1-butanol. The butanol extracts were washed with water and evaporated to dryness. The residue was reduced with lithium aluminum hydride and converted to hydrocarbons as described previously. The composition of the product (38 mg) was very similar to that obtained after sodium periodate treatment except that the major component **14a** constituted only 75–80% of the mixture; the remainder was a number of lower molecular weight hydrocarbons in approximately the same relative amounts as before.

C. Periodic Acid Oxidation of Amphotericin B. A suspension of amphotericin B (1 g) in 1-butanol (140 ml) was oxidized with aqueous periodic acid for 5 min as described above. Acetic acid (10 ml) was added to the butanol extracts from the work-up, and the solution was hydrogenated over platinum as catalyst (150 mg of platinum oxide) until hydrogen uptake ceased (6 hr). The com-

(31) O. Doebner and A. Weissenborn, *Ber.*, **35**, 1143 (1902).

(32) Owing to the high ultraviolet absorption the precision of measurement was only ±20%.

(33) The mass spectrum of **12b** obtained by distillation from acid solution displayed an intense peak at *m/e* 125 which was not present when **12b** was obtained *via* ether extraction. This peak is attributed to the presence of the *cis* isomer which could be removed preferentially as the hemiacetal by distillation from an equilibrium mixture in which the *trans* isomer predominated.

position of the product after conversion to hydrocarbons was largely **14a** (60%) with smaller amounts of **1c** (20%) and **2** (10%); the remainder was a complex mixture of low molecular weight hydrocarbons.

Lithium Aluminum Hydride Reduction of Perhydroamphotericin B. Isolation of 3,5-Dimethyldocosane (14b). Perhydroamphotericin B (1 g) was refluxed 24 hr with lithium aluminum hydride (2 g) in THF (160 ml). The excess lithium aluminum hydride was destroyed by slow addition of water (20 ml) diluted with THF (50 ml). The mixture was filtered and the filter cake was dissolved in 5% sulfuric acid (250 ml) at − 5° while 1-butanol (250 ml) was added simultaneously. The layers were separated and the aqueous layer was extracted as rapidly as possible with two 250-ml portions of cold 1-butanol. The combined butanol extracts were washed with sodium bicarbonate solution until neutral and then with water, and evaporated to dryness. A solution of the resulting polyol (1.3 g) in methanol (75 ml) was treated with a solution of periodic acid (1.95 g) in water (26 ml) and allowed to stand for 5 min before work-up as described previously (part B of periodic acid oxidations) and conversion to hydrocarbons. The major product (60%) was identified as 3,5-dimethyldocosane (**14b**) on the basis of its mass spectrum (Figure 5b); a minor product (20%) was **14a**. A small amount of the C_{41} hydrocarbon **1c** was also present.

Alkaline Treatment of Perhydroamphotericin B. A solution of perhydroamphotericin B (1 g) in methanol (20 ml) and water (40 ml) containing 600 mg of sodium hydroxide was heated on a steam bath for 15 min. It was cooled, diluted with water (100 ml), and extracted with two 250-ml portions of 1-butanol. The butanol extracts were washed with water until the washings were neutral and then evaporated to dryness. The residue was reduced with lithium aluminum hydride and converted to hydrocarbons as described previously. Vpc analysis revealed a complex mixture of more than 20 products. Two were clearly predominant, constituting roughly one-quarter of the total and present in a relative ratio 2:1. The major hydrocarbon was **14b**, identical with the major product of lithium aluminum hydride reduction of perhydroamphotericin followed by periodate cleavage described above, and the other on the basis of its mass spectrum was **14c**.

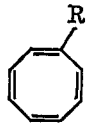
Nuclear Magnetic Resonance Spectroscopy. Ring Inversion in 3,5,7-Cyclooctatrienone[1a]

Camille Ganter, Sanford M. Pokras,[1b] and John D. Roberts

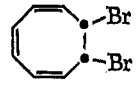
Contribution No. 3358 from the Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena, California. *Received March 31, 1966*

Abstract: The rate of ring inversion in 3,5,7-cyclooctatrienone was determined by the temperature dependence of the nmr spectrum of the methylene protons adjacent to the carbonyl group. Inversion does not occur by rapid reversible formation of hydroxycyclooctatetraene because deuterium exchange of the methylene protons is much too slow for the operation of such a mechanism. It is suggested that a nearly planar transition state is involved. The activation energy for ring inversion was determined to be 11.9 ± 0.5 kcal/mole.

The rates and activation energies of bond shift and/or ring inversion in cyclooctatetraene (I),[2] fluorocyclooctatetraene (II),[3] and *cis*-1,2-dibromo-3,5,7-cyclooctatriene (III)[4] have been determined by nuclear magnetic resonance spectroscopy. We report here a similar investigation of ring inversion of 3,5,7-cyclooctatrienone (IV)[5,6] from the temperature dependence of its proton nmr spectrum.

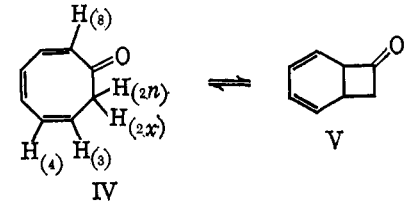


I, R=H
II, R=F



III

A possible complication in the study of ring inversion of IV arises from the fact that infrared[5] and nmr measurements[7] indicate that at equilibrium at 20° IV contains about 5% of the valence tautomer V. However, the proportion of IV decreases rapidly with decreasing temperature[7] and the equilibrium between IV and V is established rather slowly.[7] For this reason,



IV

we have assumed that the equilibrium IV ⇄ V has no appreciable effect on the rate of ring inversion of IV.

The temperature dependence of the proton nmr spectrum of IV was studied with the aid of a Varian Model HR-60 spectrometer equipped with a special low-temperature probe using a 25% by volume solution of IV in carbon disulfide with tetramethylsilane (TMS) as internal standard.

The spectrum of IV at room temperature (Figure 1a) shows a doublet (two protons) centered on δ 2.97, assigned to the protons on C(2); a quartet (one proton), split further, and centered on δ 5.77, assigned to the proton on C(3); and a complex jumble of lines (five

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