Structure of Scopafungin, a Potent Nonpolyene Antifungal Antibiotic[1]

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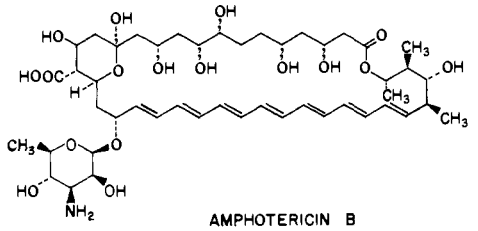
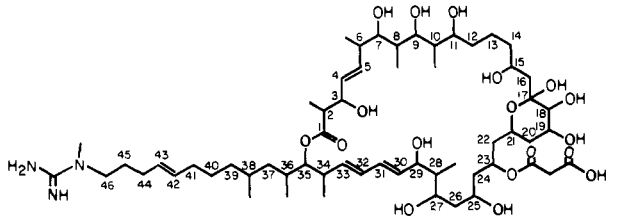
Abstract: The structure of the potent antifungal antibiotic scopafungin has been assigned as **1**, featuring a 36-membered macrolide ring, an unsymmetrically disubstituted guanidine, and a monoester of malonic acid. The molecular formula assigned, $C_{59}H_{103}N_3O_{18}$, agrees with high-resolution fast atom bombardment and field desorption mass spectrometric measurements. The structure of scopafungin was derived by combining structures assigned to key ozonolysis and periodate products, based on ^1H (including two-dimensional FT) and ^{13}C NMR spectroscopy and on high-resolution field desorption and electron ionization mass spectrometry. All alkene linkages were assigned as trans from coupling constants and examination of the two-dimensional ^1H NMR spectrum, while relative stereochemistry at C-6 through C-9 and C-25 through C-28 was assigned from coupling constants. Extensive use of reversed-phase high-performance liquid chromatography was made in separating the complex mixtures of scopafungin components and degradation products.

Scopafungin, first reported in 1971 from *Streptomyces hygroscopicus* var. *enhygrus* var. nova,[3] is only modestly antibacterial but is a potentially useful antifungal antibiotic, its activity (Table I) comparing favorably to that of amphotericin B, the drug of



AMPHOTERICIN B

choice[4] for the treatment of systemic fungal diseases of man. However, scopafungin, unlike amphotericin B, is not a polyene antibiotic, a class noted for its high toxicity. We assign here the structure of scopafungin as **1**, characterized by a 36-membered ring, a guanidine, and a monoester of malonic acid.



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Crude scopafungin was analyzed by reversed-phase high-performance liquid chromatography (HPLC). Since a neutral mobile phase (80:20 methanol-water) did not provide good resolution, the broad shape of the peaks suggesting the presence of a basic functionality, the mobile phase was adjusted to pH 7.5, giving a dramatic sharpening of the peak and excellent resolution. The different constituents of crude scopafungin were isolated on an analytical scale by HPLC and tested against *Bacillus subtilis* and *Saccharomyces cerevisiae*; scopafungin was identified as the major component ($k' = 8$).[5]

Table I. Antifungal Spectra[a]

organism	MIC, μg/mL scopafungin	ampho B	primycin
Geotrichum sp.	2.0	1.0	62.5
Hormodendrum compactum	2.0	2.0	3.9
Phialophora verrucosa	2.0	2.0	2.0
Cryptococcus neoformans 4869	2.0	1.0	15.6
C. neoformans 1139	2.0	≤0.5	3.9
Sporotrichum schenckii	7.8	7.8	15.6
Monosporium apiospermum	3.9	≤0.5	3.9
Candida albicans 7163	15.6	1.0	500
C. albicans 7164	15.6	1.0	500
Microsporum canis	7.8	250	7.8
Trichophyton rubrum	2.0	≤0.5	31.2
T. interdigitale	7.8	250	15.6
T. violaceum	2.0	1.0	125
T. asteroides	1.0	1.0	2.0
T. mentagrophytes 4797	1.0	≤0.5	7.8
T. mentagrophytes 4860	3.9	1.0	125

[a] Determined by J. H. Coats and G. E. Zurenko, The Upjohn Co.

Crude scopafungin[6] was then purified by preparative HPLC, with the same solvent, followed by semipreparative HPLC to yield a material giving a single peak on analytical HPLC (both using the same solvent system). This material could be crystallized from acetone-water, but the tiny white needles were, unfortunately, not suitable for X-ray analysis.

The thus purified scopafungin gave an M + H ion at m/z 1142.7283 by high-resolution field desorption mass spectrometry (HRFDMS), in agreement with the formula $C_{59}H_{104}N_3O_{18}$ (calcd 1142.7314), an M + H ion at m/z 1142.7341 by HR fast atom bombardment (FAB) MS,[7] and microanalyses (C, H, and N) agreeing with the molecular formula $C_{59}H_{103}N_3O_{18}\cdot H_2O$.

The ^{13}C NMR spectrum of scopafungin (Table II) contains peaks for 53 carbons (59 required), including one guanidino carbon,[8,9] three carboxylate carbons, and eight olefinic carbons, which account for eight degrees of unsaturation vs. the ten required

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(3) (a) Johnson, L. E.; Dietz, A. *Appl. Microbiol.* **1971**, *22*, 303-308. (b) Bergy, M. E.; Hoeksema, H. *J. Antibiot.* **1972**, *25*, 39-43.
(4) *Medical Letter* **1980**, *22*, 5-12.
(5) Snyder, L. R.; Kirkland, J. J. "Introduction to Modern Liquid Chromatography"; Wiley: New York, 1974; p 27.
(6) Provided by Dr. T. E. Eble, Infectious Diseases Research, The Upjohn Co., Kalamazoo, Mi 49001.
(7) (a) Surman, D. J.; Vickerman, J. C. *J. Chem. Soc., Chem. Commun.* **1981**, 324-325. (b) Barber, M.; Bordoli, R. S.; Sedgwick, R. D.; Tyler, A. N. *Ibid.* **1981**, 325-327.
(8) Cheng, M. T.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1978**, *100*, 7409-7411.
(9) Carter, G. T.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1978**, *100*, 4302-4304.

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Scheme I

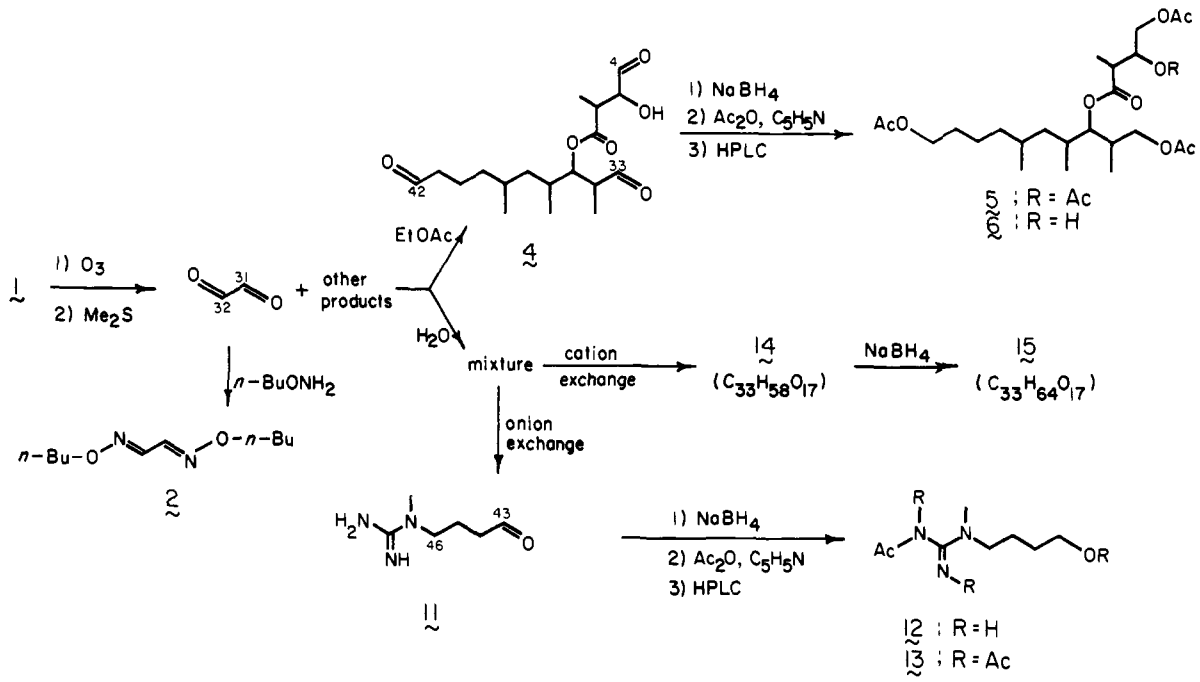


Table II. ^{13}C NMR Absorptions of Scopafungin[a]

carbon type	δ,[b] mult	carbon type	δ, mult
O ‖ -C-O-	177.0, s 174.1, s 171.7, s	-CH-	45.2, d 43.9, d 42.8, d 40.2, d 32.6, d 30.5, d 28.4, d
NH ‖ -N-C-NH$_2$	157.9, s		41.2[c] 40.7[c]
-CH=CH-	136.6, d 136.2, d 134.8, d 132.8,d 131.9, d 131.8, d 131.7, d 129.8, d	-CH$_2$-	44.2, t 42.1, t 41.9, t 39.2, t 37.0, t 33.6, t 33.0, t 30.3, t 29.5, t 27.3, t
-O-C-O	99.8, s		
-CH-O-	80.6, d 77.1, d 75.6, d 75.4, d 75.1, d 74.5, d 72.3, d 72.1, d 71.3, d 69.5, d 69.1, d 66.0, d 65.6, d	-CH$_3$	20.5, q 17.8, q 16.8, q 15.3, q 14.8, q 14.6, q 11.0, q 10.5, q

[a] 360 MHz, CD_3OD solvent, Me_4Si used as marker. [b] Multiplicity in off-resonance spectrum: s = singlet, d = doublet, t = triplet, q = quartet. [c] Signals at 41.2 and 40.7 ppm were of unidentifiable multiplicity. The signals for the NCH_3 and five other carbons may be under the solvent peak.

by its formula (leaving two rings). A carbon signal at 99.8 ppm suggests a cyclic hemiketal as one ring, and a macrolide ring would complete the unsaturation elements. Two major oxidative tools, ozonolysis and periodate oxidation, were employed in elucidating the structure of scopafungin.

Table III. ^1H NMR Signals and Decoupling for Scopafungin[a]

assignment	δ, mult (intensity)	J, Hz	change on irradn
H-32	6.18, dd (1)	15, 10	
H-31	6.05, dd (1)	15, 10	
H-5	5.72, dd (1)	16, 8	
H-30	5.66, dd (1)	15, 7	H-31 → d (15 Hz), H-29 → m
H-33	5.52, dd (1)	15, 9	H-32 → d (15 Hz)
H-42[b]	5.50, dt (1)	16, 6	
H-4	5.46, dd (1)	16, 6	H-3 → d, H-5 → d
H-43[b]	5.42, dt (1)	16, 3.5	
H-35 or H-23[c]	5.20, m (1)		
H-29, H-3, others	4.08, m (4)		H-30 → d (15 Hz), H-4 → d (16 Hz)
	3.85, m (4)		
	3.75, m (3)		
H-46	3.15, t (2)	7	
NCH_3	2.84, s (3)		
H-34	2.53, m (1)		H-33 → d (15 Hz), 34-CH_3 → s, 2-CH_3 → s
H-2	2.44, m (1)		34-CH_3 → s, 2-CH_3 → s, H-3 → m
H-6	2.31, m (1)		H-5 → d (16 Hz), 6-CH_3 → s
H-41 or H-44	1.98, m		H-42 and H-43 → m
6-CH_3	1.08, d (3)	7	H-6 → m
34-CH_3 and 2-CH_3	1.00, d (6)	7	H-2 → d (7 Hz), H-34 → m
	0.90, m (9)		
	0.87, d (6)	7	

[a] 360 MHz, CD_3OD. [b] These assignments may be exchanged. [c] The other proton is presumably masked by the water peak.

Ozonolysis Products

Identification of Units a and b. Ozonolysis was carried out in methanol at −78 °C and was followed by reduction of the ozonide by dimethyl sulfide, as shown in Scheme I. The simplest ozonolysis product, glyoxal, was identified by gas chromatography (GC)/MS of its bis(*O*-*n*-butyloxime) (**2**) and by coinjection of an authentic sample of **2**. The conjugated diene unit a in scopafungin was confirmed by the antibiotic's ultraviolet maximum at 231 nm, which disappeared upon hydrogenation.

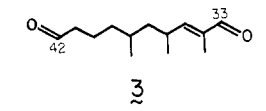
a

Table IV. ^{1}H NMR Signals and Decoupling for Ethyl Acetate Soluble Ozonolysis Products

	3[d]			8[e]		
proton[a]	δ,[b] mult[c]	*J*, Hz	change on irradn	δ, mult	*J*, Hz	change on irradn
H-33	9.38*, s			3.70, m	7	H-34 → quintuplet (8 Hz)
H-42	9.36*, s			4.06, t	7	
H-34				1.95, m		H-35 → s, H-33 → q, 34-CH_3 → s
H-35	5.73, d	10	H-36 → m	3.45, dd	9, 2	H-34 → m, H-36 → q
H-36	2.50, m		H-35 → s, 36-CH_3 → s	1.80, m		H-35 → d (9 Hz), 36-CH_3 → s
H-38				1.40, m		38-CH_3 → s
41-CH_2	1.79, t	7				
34-CH_3	1.71, s			0.84, d	7	
36-CH_3	0.73, d	7	H-36 → m	0.86, d	7	H-34, H-36, H-38 → simplified
38-CH_3	0.63, d	7		0.87, d	7	
CO-CH_3				2.05, s		

[a] For numbering; see formula in text. [b] Assignments with same superscripts may be interchanged. [c] Multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. [d] 360 MHz, C_6D_6. [e] 220 MHz, $CDCl_3$.

The remaining ozonolysis products were partitioned between ethyl acetate and water. Preparative thin-layer chromatography of the ethyl acetate soluble fraction on silica gel gave **3**, an oil.



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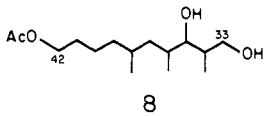

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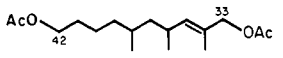

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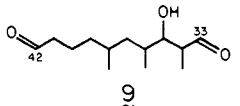

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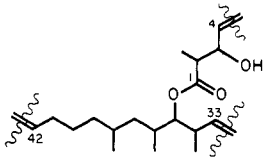
Since there are no olefinic methyls in the ^{1}H NMR spectrum of scopafungin (Table III), **3** was clearly an α,β-elimination product of a β-oxyaldehyde. To prevent the elimination that gave **3**, the organic-soluble ozonolysis product was reduced with sodium borohydride and acetylated. A mixture was obtained that was separated by HPLC to give **5** (Scheme I) as the major component and **6**, **7**, and **8** as minor components. Compound **7** was the reduction product of **3**, while **5** and **6** must have come from **4**, and **8** from **9**. Compound **5** was shown to be derived from **4**, a trialdehyde, by sodium borodeuteride replacement of borohydride. The trialdehyde **4** must have come, in turn, from the unit b. Assignments of structures of **3**, **5**, **6**, **7**, and **8** are discussed in the next section.



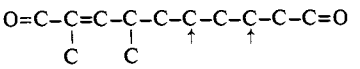
b

Structures of Compounds 3, 5, 6, 7, 8, and 10. 3. The molecular formula of **3** was established by HRMS (electron impact, EI) as $C_{13}H_{22}O_2$, indicating three degrees of unsaturation. The ^{13}C NMR spectrum showed that the unsaturation came from two aldehydes, with signals at 200 and 194 ppm (both d), and from one double bond, with signals at 158 (d) and 138 ppm (s). The UV (λ_{max} 227 nm, ϵ 15 000) and IR (1685 cm^{-1}) as well as the ^{13}C NMR spectra argued for a conjugated aldehyde and an olefinic methyl and a one-proton doublet at 5.7 ppm in the ^{1}H NMR spectrum (Table IV) suggested the unit

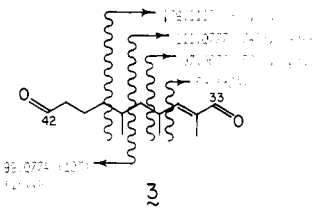
1.71 5.73 2.50 0.73

O=CH–C(CH_3)=CH–CH–(CH_3)–

confirmed by spin decoupling (Table IV). An additional $CHCH_3$ (d, 0.63 ppm) unit observed in the ^{1}H NMR spectrum suggested, on biosynthetic grounds, the skeleton



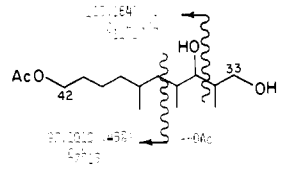
with the third methyl on one of the carbons indicated by the arrows. A two-proton triplet at 1.79 ppm (Table IV, $CH_2C{=}O$) eliminates the right-hand position, assigning the structure **3**, which was confirmed by HREIMS data, as shown.



3

7. The molecular formula of **7** was established as $C_{17}H_{30}O_4$ by HREIMS, allowing for three degrees of unsaturation. The ^{1}H NMR spectrum had sharp singlets at 1.69 and 1.68 ppm and doublets at 0.89 and 0.79 ppm, which are attributed to two acetates and two $CHCH_3$ groups, respectively. A one-proton doublet at 5.14 ppm (=CH, J = 10 Hz) and a narrow doublet at 1.58 ppm (=CCH_3, J = 1 Hz) indicate a double bond substituted by a methyl. All these data indicate that **7** was derived from **3** by borohydride reduction and acetylation.

8. The molecular formula of **8** was established as $C_{15}H_{30}O_4$ by HREIMS, allowing for one degree of unsaturation. An OH band at 3490 cm^{-1} in the IR spectrum indicated one or more hydroxyls in the molecule. The ^{1}H NMR spectrum had a sharp singlet at 2.05 ppm and doublets at 0.87, 0.86, and 0.84 ppm, which are attributed to one acetate (accounting for the unsaturation) and three $CHCH_3$ groups, suggesting that **8** was related to **3** and **7**. A two-proton multiplet at 3.70 ppm and a one-proton doublet of doublets at 3.45 ppm in the ^{1}H NMR spectrum (Table IV) argued for primary and secondary hydroxyls and a two-proton triplet at 4.06 ppm for the group $AcOCH_2CH_2$. Only one structure with these features can be derived for **8** from **3**, and this structure was confirmed by spin decoupling of the ^{1}H NMR spectrum (Table IV) and HREIMS data, as shown.



8

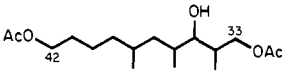
10. Treatment of **8** with acetic anhydride–pyridine gave one major component, **10**, whose ^{1}H NMR spectrum had sharp singlets at 1.70 and 1.65 ppm attributed to two acetates. Four methylene

Table V. ^{1}H NMR Signals and Decoupling for 5[a]

proton	δ, mult	J, Hz	change on irradn
H-3	5.42, m		H-4α → d (J = 12 Hz), H-4β → m, H-2 → q (J = 7 Hz)
H-35	5.0, dd	9, 3	
H-4α[b]	4.54, dd	12, 2.5	H-4β, H-3 → m
H-4β[b] / H-33, H-42[b]	4.0, m		H-3 → m, H-4α → d
H-2	2.83, m		H-3 → m, 2-CH_3 → s
$COCH_3$	1.83, s		
	1.72, s		
	1.70, s		
	1.63, s		
2-CH_3	1.06, d	7	H-2 → d (J = 7 Hz)
CH_3	0.84, d	7	
	0.82, d	7	
	0.78, d	7	

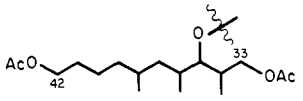
[a] 360 MHz, C_6D_6. [b] The intensity of these signals decreased in the ^{1}H NMR spectrum of deuterated 5.

protons at 4.6 (1 H), 4.2 (1 H), and 4.0 (2 H) indicate that the two acetates are primary and thus establish the structure of **10**.



10

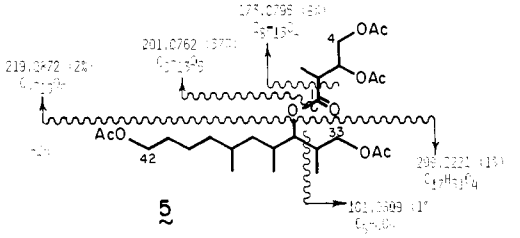
5. The molecular formula of **5** (Scheme I) was established by HREIMS as $C_{26}H_{44}O_{10}$. The ^{1}H NMR spectrum (Table V) had four sharp three-proton singlets attributed to four acetates. The ^{13}C NMR spectrum, however, indicated five carboxylic carbons (172.5, 170.1, 170.0, 169.8, and 169.5, all s) and five carbons singly bonded to oxygen (76.7 (d), 72.7 (d), 65.7 (t), 64.3 (t), and 62.9 (t) ppm). These results indicate an ester functionality in addition to the four acetates. The ^{1}H NMR spectrum also had four three-proton doublets attributed to $CHCH_3$ groups. The number of methyls suggested that **5** included the skeleton of **8** (and **10**) in unit c, and this was confirmed by the HREIMS data shown



c

as well as by hydrolysis of **5** (1 N KOH) and acetylation to give **10**.

Unit c ($C_{17}H_{31}O_5$) accounts then for two acetates and three methyls in **5**, leaving two acetates, one methyl, and the ester carbonyl for the remainder ($C_9H_{13}O_5$) of **5**. Spin decoupling of the ^{1}H NMR spectrum (Table V) assigned the structure of the remaining part of **5**.

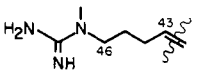


5

Replacement of sodium borohydride by sodium borodeuteride between **4** and **5** in Scheme I increased the molecular weight of **5** by 3 amu, showing that three carbonyls of **4** had been reduced. The ^{1}H NMR spectrum of the deuterated **5** showed a decrease in the intensity of the signals of the carbinyl acetate protons (4.54 and 4.00 ppm, CH_2OAc), establishing the structure of the trialdehyde **4**.

6. The ^{1}H NMR spectrum ($CDCl_3$) of **6** had singlets at 2.09 and 2.04 ppm, containing nine protons attributed to three acetates. The spectrum also had doublets at 1.24, 0.99, 0.88, and 0.85 ppm attributed to four $CHCH_3$ groups. The presence of the four methyls suggested that **6**, like **5**, was derived from unit b. Furthermore, the molecular weight (474, compared to 516 for **5**), the presence of three acetates (vs. four for **5**), and an OH band at 3520 cm^{-1} in the IR spectrum of **6** indicated that **6** must be a monoalcohol parent of **5**. Comparison of ^{1}H NMR spectra of **5** and **6** showed that the signal at 5.42 ppm in **5** assigned to H-3 is missing and that a new signal appears at 3.84 ppm in **6**; otherwise, the two spectra are almost identical. This establishes a hydroxyl group at C-3 and the structure of **6**.

Identification of Unit d. Chromatography of the water-soluble



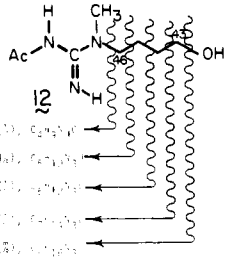
d

ozonolysis products on an anion exchange column using a neutral mobile phase followed by sodium borohydride reduction, acetylation, and HPLC gave **12** and **13** (Scheme I). Replacement of borohydride by borodeuteride increased the molecular weight of **13** by 1 amu, demonstrating the aldehyde (**11**) in the precursor. Unit d in scopafungin, which gives **11**, accounts for all three nitrogens and for the antibiotic's basicity.

Structures of Compounds 12 and 13. 13. The molecular formula of **13** was established as $C_{14}H_{23}N_3O_5$ by HREIMS, with five degrees of unsaturation. Its structure is assigned by inspection of the ^{1}H NMR spectrum, which had sharp singlets at 2.05, 2.20, 2.23, 2.25, and 3.11 ppm attributed to four acetyl groups and one *N*-methyl, respectively. A band at 1735 cm^{-1} in the IR spectrum indicated an acetate and, with five oxygens, **13** must contain one *O*-acetyl and three *N*-acetyl groups. The ^{1}H NMR spectrum also showed two downfield triplets, each integrating for two protons; that at 4.08 ppm is attributed to a carbinyl acetate (CH_2OAc) and that at 3.57 ppm to a methylene group α to a nitrogen ($CH_2N<$). The remaining four protons can be found in two methylene groups near 1.68 ppm, not attached to nitrogen or oxygen, thus defining the unit $>N(CH_2)_4OAc$.

The three *N*-acetyl groups and the remaining carbon require a guanidine group. As noted above, the guanidine accounts for the peak at 157.9 (s) ppm in the ^{13}C NMR spectrum of scopafungin; it also accounts for three protons (which are exchangeable) at 7.5 ppm in the ^{1}H NMR spectrum (Me_2SO) of scopafungin. The fact that only one NH peak is observed indicates that the three protons are equivalent, i.e., that the guanidine is unsymmetrically disubstituted, and assigns the position of the *N*-methyl as in **13**.[10]

12. The molecular formula of **12** was established as $C_8H_{17}N_3O_2$ by HREIMS, with two degrees of unsaturation. The ^{1}H NMR spectrum had sharp singlets at 2.86 and 2.09 ppm, which are attributed to an *N*-methyl and an *N*-acetyl, respectively. A band at 1620 cm^{-1} in the IR spectrum indicated an amide and with the molecular weight (187, FDMS) identified **12** as the mono-*N*-acetyl parent of **13**. HREIMS data established the fragmentation pattern shown for **12**, leaving a $C_4H_9N_3O$ unit that must contain the *N*-acetyl and *N*-methyl groups and one additional unsaturated carbon, i.e., a guanidine group.

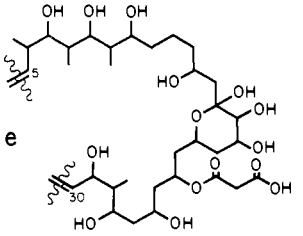


12

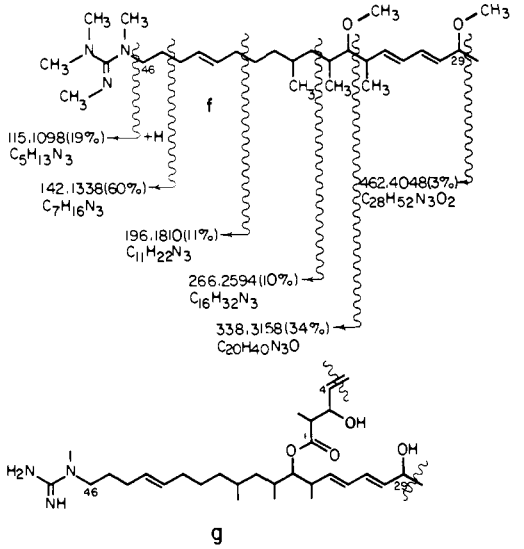
(10) Corral, R. A.; Orazi, O. O.; Gonzalez, M. E. *Rev. Latinoam. Quim.* **1978**, *9*, 184–189.

Remainder of Scopafungin

Identification of Unit e. The alkene units a, b, and c (C_2H_2, $C_{18}H_{30}O_3$, and $C_6H_{13}N_3$, respectively) required for the formation of glyoxal, **4**, and **11** (Scheme I), account for $C_{26}H_{45}N_3O_3$ (447 amu) of the molecular formula ($C_{59}H_{103}N_3O_{18}$) of scopafungin, leaving $C_{33}H_{58}O_{15}$ (694 amu) for the remainder of the molecule. This remainder was found in **14** (whose structure will be assigned below but whose molecular formula is given in Scheme I) among the water-soluble ozonolysis products by chromatography on a cation exchange column (which retained **11**) using a neutral mobile phase and identified as an acid since it had been retained by anion exchange. Reduction of **14** with sodium borohydride (Scheme I) gave **15** ($C_{33}H_{64}O_{17}$, HRFDMS), which must contain six hydrogens more than **14** from reduction of two ozonolysis termini (hemiacetal carbons at 101.9 and 97.3 ppm in the ^{13}C NMR spectrum of **14**) and the hemiketal carbon (a singlet at 99.8 ppm). Thus, the formula for **14** must be $C_{33}H_{58}O_{17}$, in agreement with the required formula $C_{33}H_{58}O_{15}$ (based on the molecular formula $C_{59}H_{103}N_3O_{18}$ for scopafungin) for the alkene unit e, from which **14** is derived. Structure e will also be developed later.



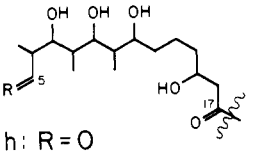
Identification of Unit g. Units a, b, and d can, of course, be linked to one another in a variety of ways. Their juxtaposition was established by HREIMS data on the product derived from room-temperature basic hydrolysis (0.1 N NaOH) of scopafungin and methylation with dimsylsodium–methyl iodide; these data established (with the help of the N_3 marker) the fragmentation pattern shown for f, which in scopafungin must be present in g.



g

Periodate Oxidation Products

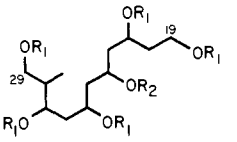
Identification of Units i and j. A first insight into the structure of **14**, and thus of unit e of scopafungin, was provided as follows. Borohydride reduction of **14** (Scheme II; cf. also Scheme I), followed by periodate oxidation, a second borohydride reduction, acetylation, a bicarbonate wash (which removed acidic products), and HPLC gave compound **18** (derived from the intermediates **16** and **17**), with four acetates, as the major component and tetraacetate, triacetate, and diacetate analogues of **18** (**19**, **20**, and **21**, respectively; structures unassigned) as minor products. If the first reductant was replaced by borodeuteride, the product was **18**-d_2, with a single deuterium at the right-hand terminus (C-17). These results define the unit h as part of **14** and thus i as part of e in scopafungin. Although the acidic products **22** and **23** were



h: R = O

i: R = remainder of **14**

not identified in this experiment, the latter was identified subsequently and played a role in locating the malonic acid unit (cf. below).



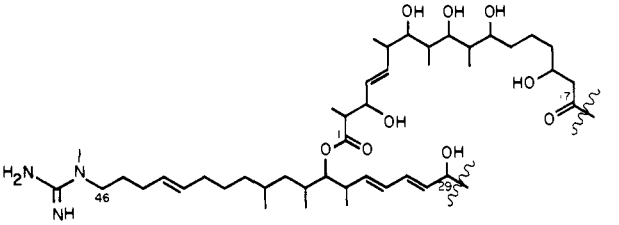
23: $R_1 = H$, $R_2 = COCH_2COOH$

31: $R_1 = R_2 = Ac$

32: $R_1 = R_2 = H$

37: $R_1 = H$, $R_2 = COCH_2COOCH_3$

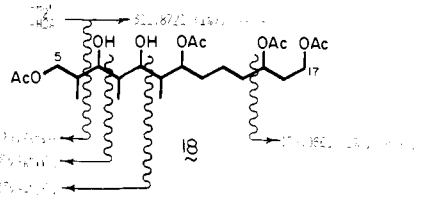
Since units i and g contain one terminal alkene each, it can be assumed that they are joined in scopafungin, giving unit j. This



j

assumption was confirmed by oxidation of scopafungin (**1**) by sodium periodate (Scheme III), followed by reduction with lithium aluminum hydride and acetylation to give **25**, which was isolated by HPLC and must have come from the intermediate **24**. Unit j accounts for $C_{44}H_{77}N_3O_{10}$ in scopafungin, leaving $C_{15}H_{26}O_9$. If hydrogenation of **1** preceded the periodate oxidation, **26**, the dihydro derivative of **25** (cf. Scheme III), was obtained.

Structures of 18, 18-d_2, 18-d, 19, 20, 21, and 25. 18. The molecular formula of **18** was established as $C_{24}H_{42}O_{10}$ by HREIMS. The ^1H NMR spectrum (Table VI) had sharp singlets at 1.72, 1.69, and 1.67 which account together for 12 protons and are attributed to four acetates. Two of these acetates are secondary, as indicated by two methine protons at 5.25 and 5.02 ppm. The other two are primary, as indicated by four methylene protons with signals at 4.38, 4.25, 4.11, and 4.02 ppm marked by geminal coupling. Two methine protons at 3.78 and 3.40 ppm and an absorption at 3450 cm^{-1} in the IR spectrum indicated that the two remaining oxygens come from secondary alcohols. The ^1H NMR spectrum also has doublets at 1.00, 0.72, and 0.71 ppm attributed to three $CHCH_3$ groups. Exhaustive spin decoupling of the spectrum (Table VI) led then to the complete structure of **18** and the assignment of most protons, as shown. In addition,



18

the fragmentations indicated by HREI mass spectrometry agreed with the structure assigned.

Scheme II

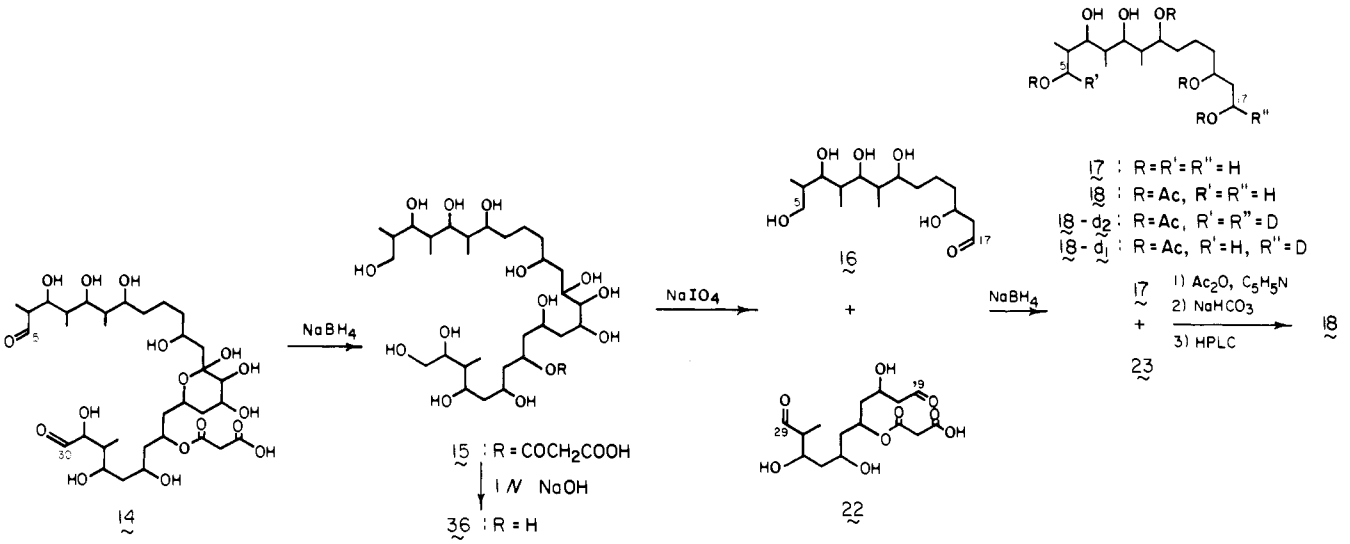


Table VI. ^{1}H NMR Signals and Decoupling for Periodate Oxidation Products of Scopafungin

	18[a]				28[d]		
proton	δ, mult (intensity)	J, Hz	change on irradn	proton	δ, mult (intensity)	J, Hz	change on irradn
H-15	5.25, ddd (1)	12, 10, 2	H-14 → m, H-12 → m, H-16 → m	H-15	5.34, tt	5, 2.5	
H-11	5.02, ddd (1)	9, 5, 4	H-12 → m, H-16 → m	H-11	5.04, dt	10, 4	
H-5				H-5	5.96, d	10	
H-5α	4.38, dd (1)[b]	11, 5.5	H-5β → t, H-6 → q				
H-5β	4.25, dd (1)[b]	11, 6.5	H-5α → d, H-6 → m				
H-17α	4.11, ddd (1)[b,c]	11, 7.5, 6	H-17β → dd, H-12 → m, H-16 → m	H-17α	4.09, m		
H-17β	4.02, ddd (1)[b,c]	11, 6, 5	H-17α → dd, H-12 → m, H-16 → m	H-17β	3.88, m		
H-7	3.78, ddd (1)	10, 6, 2	H-6 → q, H-8 → m, H-10 → m	H-7	5.12, dd	5, 2	
H-9	3.40, ddd (1)	10, 9, 3	H-8 → m, H-10 → m	H-9	3.81, ddd	11, 7, 2	
C(O)CH$_3$	1.72, s (3)			COCH$_3$	1.87, s		
					1.71, s		
					1.70, s		
	1.69, s (6)				1.69, s		
	1.67, s (3)				1.65, s		
H-6	1.92, dddt (1)	6.5, 5.5, 6, 7	6-CH$_3$ → s, H-7 → d (9 Hz), H-5α → d (11 Hz), H-5β → d (11 Hz)	H-6	1.75, m		H-5 → s, 6-CH$_3$ → s, H-7 → s
H-16	1.75, m		H-17α → d (11 Hz), H-17β → d (11 Hz), H-15 → m	H-6, H-8	1.70, m		H-5 → s, H-7 → s, H-9 → d (J = 11 Hz), 6-CH$_3$ → s, 8-CH$_3$ → s
H-12	1.6, m		H-17α and H-17β → dd, H-15 → s, H-11 → s	H-10, H-16	1.6, m		H-9 → m, H-15, H-17α, H-17β, H-11, 10-CH$_3$ → s
H-8 or H-10	1.52 m		H-9 → d (5 Hz), H-7 → d (5.5 Hz), 10- or 8-CH$_3$ → s	H-14	1.3, m		H-19 → m, H-13 → m
				H-13	1.1, m		H-14 → m
H-10 or H-8	1.42, m		H-9 → s, H-7 → d (5.5 Hz), H-11 → m, 10- or 8-CH$_3$ → s	8-CH$_3$	0.86		
H-14α and H-14β	1.20, m		H-15 → d (10 Hz)	6-CH$_3$	0.81		
6-CH$_3$	1.00, d (3)	7	H-6 → q	10-CH$_3$	0.71		
H-13α	0.92, m		H-13 → m, H-16 → m, H-14 → m				
H-13β	0.84, m						
8-CH$_3$	0.72, d (3)	7	H-8 → m, H-10 → m				
10-CH$_3$	0.71, d (3)	7	H-8 → m, H-10 → m				

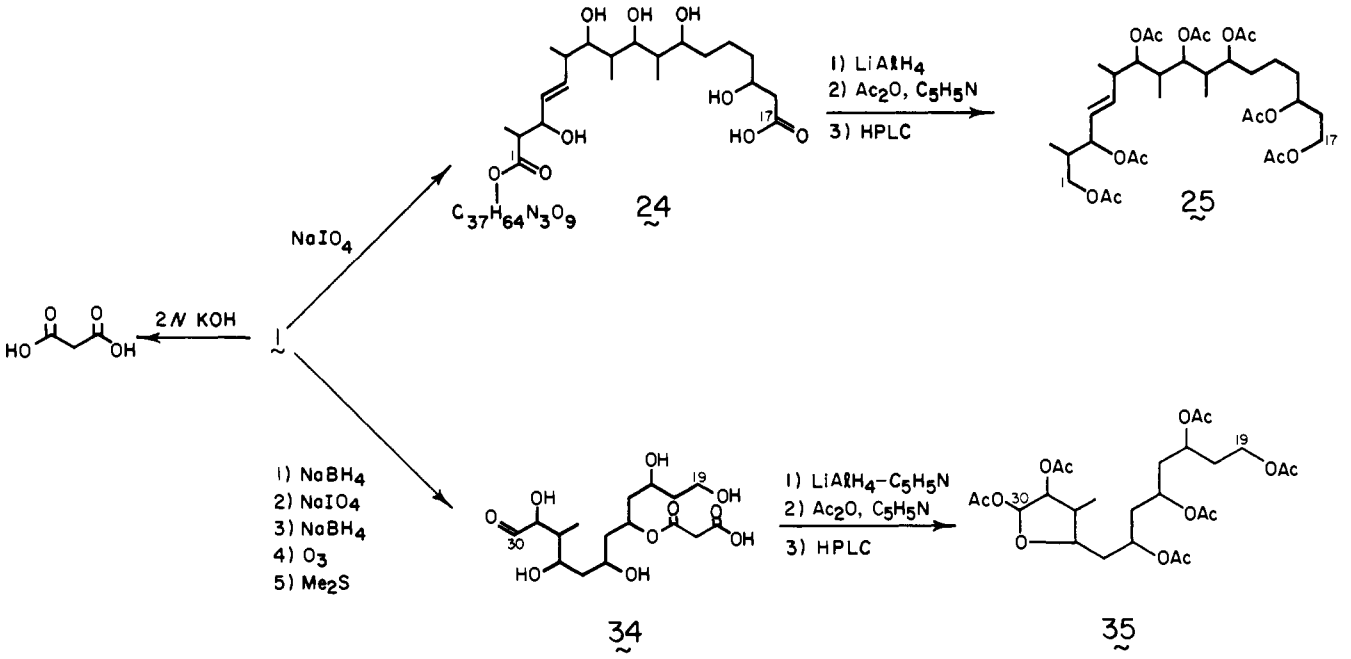
[a] 200 MHz, C_6D_6. [b] Signal intensity decreased in the ^{1}H NMR spectrum of **18**-d_2. [c] Signal intensity decreased in the ^{1}H NMR spectrum of **18**-d. [d] 360 MHz, C_6D_6.

18-d_2 **and 18**-d. If the first reductant was replaced by sodium borodeuteride, two deuteriums were incorporated, as shown by an increase of 2 amu in the molecular ion of **18**-d_2. The ^{1}H NMR spectrum indicated a decrease in intensity of the signals at 4.38, 4.25, 4.11, and 4.02 ppm, locating one deuterium at each terminus of **18**-d_2. If the second reductant was replaced by sodium borodeuteride, a single deuterium was incorporated, as demonstrated by an increase of 1 amu in the molecular ion of **18**-d. The ^{1}H NMR spectrum indicated a decrease in the intensity of the signals at 4.11 and 4.02 ppm, which can be seen in Table VI to be the carbinyl acetate protons at the C-17 terminus of **18**-d_2.

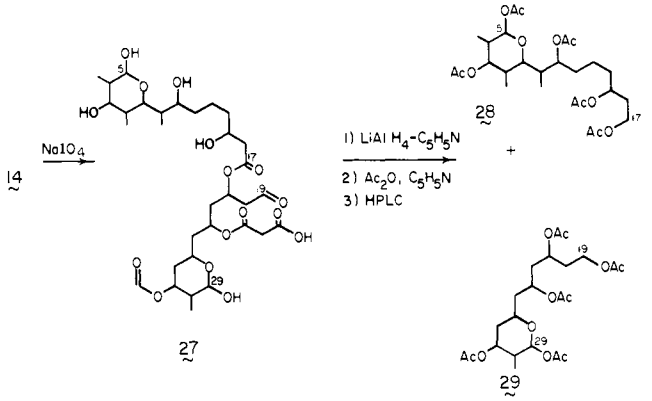
19, 20, and 21. The other components, **19**, **20**, and **21**, isolated at the same time as **18** were all, according to their ^{1}H NMR and mass spectra, analogues of **18** involving acetylation of different hydroxyls. Their complete structures were not assigned.

25. The FD mass spectrum of **25** had an M + H ion at m/z 701 and the HREI mass spectrum an ion for $C_{31}H_{50}O_{11}$ (M − HOAc − ketene); its ^{1}H NMR spectrum ($CDCl_3$) had sharp

Scheme III



Scheme IV



singlets at 1.94, 1.82, 1.80, 1.73, 1.72, 1.71, and 1.70 ppm attributed to acetates and doublets at 0.97, 0.95, 0.85, and 0.75 attributed to four $CHCH_3$ groups. Isolation of the dihydro derivative of **25**, following hydrogenation of **1** and periodate oxidation, demonstrated a double bond in **25**. This indicated that **25** must include C-1 through C-4 of g, since the other alkene-containing portions are largely oxygen free. Carbons 1 through 4 account for two acetates and one *C*-methyl of **25**, and the remainder must then contain three *C*-methyls and five acetates, as expected for a product from unit i. The structures of units i and C-1 to C-4 of g define the structure of **25**.

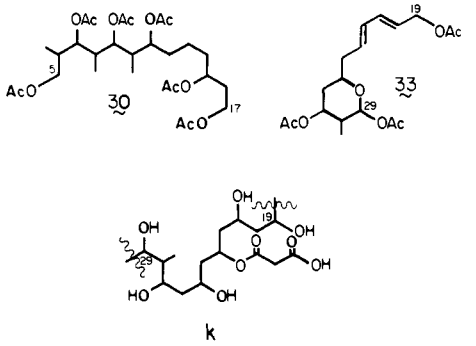
Identification of Unit k. The remainder ($C_{15}H_{26}O_9$) of scopafungin was assigned as follows. The other major piece of **14** was obtained by treatment of **14** with sodium periodate for 12 h (Scheme IV), followed by lithium aluminum hydride–pyridine and acetic anhydride–pyridine, and isolated by HPLC. The two major products isolated were acetates of hemiacetals—**28**, related to **18**, and **29**, from the other half of **14**, both derived from the intermediate **27**. In addition, open-chain analogues, **30** (the hexaacetate of **17**) and **31** (the hexaacetate of **32**, the lithium aluminum hydride cleavage product of **23**), were isolated. Similarly, treatment of **14** with periodate for 24 h followed by the same sequence gave **33** as the major component. The off-resonance ^{13}C NMR spectrum of **14** (cf. Experimental Section) also shows that the two terminal aldehydes of **14** underwent ring closures, producing two hemiacetal carbons (101.9 and 97.3 ppm, both d). The survival of the hemiacetals through lithium aluminum hydride reduction may be due to the unusual reactivity

Table VII. ^1H NMR Signals and Decoupling for **33**[a]

proton	δ, mult	*J*, Hz	change on irradn
H-21	6.05, dd	15, 10	H-20 + H-23 → m
H-22	5.90, dd	15, 10	H-20 + H-23 → m
H-20 + H-23	5.55, m		H-21, H-22 → d, H-19, H-24 → m
H-29	5.47, d	10	H-28 → m
H-27	4.68, td	11, 2.5	H-28 → m, H-26 → m
19-CH_2	4.45, d	7	H-20 + H-23 → m
H-25	3.2, dtd	11, 6, 2.5	
H-24α	2.15, m		H-25 → m, H-20 + H-23 → m
H-24β	2.05, m		
H-28	1.82, m		H-27 → d (11 Hz), H-29 → s, 28-CH_3 → s
$COCH_3$	1.66, s		
	1.65, s		
	1.62, s		
26-CH_2	1.2, m		H-25 → m, H-27 → m
28-CH_3	0.86, d	6.5	

[a] 470 MHz, C_6D_6.

of the lithium aluminum hydride–pyridine complex.[11] These results define compound **22** and unit k in scopafungin, except for



the nature and position of the acid, which will be discussed in a separate section below. Units j and k account for all eight $CHCH_3$ groups of scopafungin indicated by its ^{13}C NMR spectrum (Table II).

(11) Lansbury, P. T.; Peterson, J. *O. J. Am. Chem. Soc.* **1963**, *85*, 2236–2242.

Structures of 33, 29, 31, 28, and 30. 33. Mass spectral data (HREIMS, FDMS) established the molecular formula of **33** as $C_{18}H_{26}O_7$, allowing for six degrees of unsaturation. Its ^1H NMR spectrum (Table VII) had three sharp 3-proton singlets attributed to three acetates and 26 protons over the entire spectrum. An *E,E* conjugated diene was clearly indicated by signals at 6.05 (dd, 1 H, J = 15, 10 Hz), 5.90 (dd, 1 H, J = 15, 10 Hz), and 5.55 ppm (m, 2 H) in the ^1H NMR spectrum, by a UV_{max} at 230 nm, and by an absorption at 990 cm^{-1} in the IR spectrum. The three acetates and the two double bonds account for five degrees of unsaturation, leaving one. An acetal ring accounts for the last degree of unsaturation, with a one-proton doublet at 5.98 ppm attributed to the anomeric proton, and for the absence of OH bands in the IR spectrum. Exhaustive spin decoupling of the ^1H NMR spectrum (Table VII) established the structure, as shown.

29. Mass spectra (FDMS, HREIMS) of **29** were in agreement with the molecular formula $C_{22}H_{34}O_{11}$, requiring six degrees of unsaturation. The ^1H NMR spectrum of **29** (cf. Experimental Section) had five sharp singlets attributed to acetates, a doublet at 5.3 ppm attributed to the anomeric proton of an acetal, and one doublet attributed to a $CHCH_3$ group. The ^1H NMR spectrum does not, however, contain any olefinic protons. The presence of only one *C*-methyl indicated that **29** and **33** are derived from the same unit. This, along with the number of acetates (five vs. three), argued that a precursor of **29** gave rise to a precursor of **33** by elimination on standing with periodate for a longer time (24 vs. 12 h). The two extra acetates must occupy nonadjacent positions to avoid reaction with periodate, assigning structure **29**.

31. The molecular formula of **31** was assigned as $C_{24}H_{38}O_{12}$ (six degrees of unsaturation) by FDMS and HREIMS; the ^1H NMR spectrum of **31** (cf. Experimental Section) had four sharp singlets (accounting for 18 protons) attributed to six acetates. The spectrum also had a single three-proton doublet attributed to a $CHCH_3$ group, indicating that **31** was related to **29** and **33** and also derived from **27**. The six acetates and the molecular formula assign the structure of **31**.

28. The molecular formula of **28** was assigned as $C_{26}H_{42}O_{11}$ by FDMS and HREIMS. The ^1H NMR spectrum of **28** (Table VI) had five sharp three-proton singlets attributed to five acetates and three three-proton doublets attributed to $CHCH_3$ groups. The three *C*-methyls indicated that **28** was derived from the precursor of **18**, but the molecular formula requires one degree of unsaturation (which must be a ring) beyond the five acetates. The only possibility for ring closure is between the aldehyde formed by ozonolysis on C-5 and the hydroxyl on C-9 and a doublet at 5.96 ppm in **28** is assigned to the anomeric proton. Further evidence is found by comparison of the ^1H NMR spectra of **28** and **18** (Table VI).

The signals corresponding to the H-5 methylene protons of **18** (4.38 and 4.25 ppm) are no longer present in **28**, and the H-9 methine proton has moved downfield from 3.40 ppm in **18** to 3.81 ppm in **28**. All these data require an acetal ring between C-5 and C-9, which also accounts for the absence of an OH band in the IR spectrum of **28**. Spin decoupling of the ^1H NMR spectrum of **28** (Table VI) confirmed the structure.

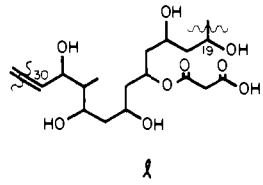
30. The molecular formula of **30** was assigned as $C_{28}H_{46}O_{12}$ by FDMS and HREIMS, and the ^1H NMR spectrum (cf. Experimental Section) had five sharp singlets (accounting for 18 protons) attributed to six acetates. There were three three-proton doublets assigned to $CHCH_3$ groups, indicating that **30** must be related to **18**, and the number of acetates (six) and the molecular formula indicated that **30** is the fully acetylated derivative of **18**.

Identification of Unit h. The twin questions of (1) whether j and k contain a common carbinol carbon or are linked by a vicinal diol and (2) which end of k is linked to which end of j were answered by the following sequence (Scheme III). Scopafungin was treated with borohydride, periodate for 12 h, borohydride, and ozone, the ozonolysis products were partitioned between ethyl acetate and water, and water-soluble products were reduced by lithium aluminum hydride–pyridine. Acetylation and HPLC gave **35**, not previously encountered, as well as the expected **28** and **30** (derived from the unacetylated analogue of **28**). Neither **29** nor **31** was found, indicating that units j and k are not linked by a vicinal diol; periodate cleavage would then have given rise to **22** (and thus to **29** or **31**). The structure of **35**, which must have come from **34** (Scheme III), with an aldehyde derived from the alkene carbon, defines the linkage of j and k as involving unit l. Combining units j and l (overlapping =CHCHOH—) gives $C_{55}H_{99}N_3O_{14}$, leaving $C_4H_4O_4$ from scopafungin still unassigned.

Table VIII. ^1H NMR Signals and Decoupling for 35[a]

proton	δ, mult	J, Hz	change on irradn
H-30	6.48, d	11	H-29 → d
H-29	5.25, dd	6.5, 1	H-30 → s, H-28 → m
2 H H-25 H-23	5.25, m		
1 H H-22	5.16, m		
H-27	4.26, ddd	8, 8, 3.5	
H-19α	4.04, dt	11, 6	
H-19β	3.97, dt	11, 6	
H-28	2.37, m		H-27 → dd, H-29 → s, 28-CH_3 → s
$COCH_3$	1.80, s		
	1.79, s		
	1.78, s		
	1.70, s		
	1.66, s		
	1.57, s		
28-CH_3	0.69, d	7	H-28 → m

[a] 360 MHz, C_6D_6.



l

Sturcture of Compound 35. The molecular formula of **35** was assigned as $C_{25}H_{38}O_{13}$ by FDMS and HREIMS, and its ^1H NMR spectrum (Table VIII) had six sharp three-proton singlets attributed to six acetates and one three-proton doublet for a $CHCH_3$ group, relating it to **29** but with an extra CHOAc unit. Spin decoupling of the ^1H NMR spectrum (Table VIII) in the furanoside region assigned the proposed structure, with the remainder of **35** (C-19 to C-24) being identical with **29**.

Formic Acid from C-18. Three lines of reasoning argue for C-18 as an additional >CHOH group between the termini of j (C-17) and l (C-19). First, the ^{13}C NMR spectrum of scopafungin (Table II) contains 13 >CHO carbons (80.6, 77.1, 75.6, 75.4, 75.1, 74.5, 72.3, 72.1, 71.3, 69.5, 69.1, 66.0, and 65.6 ppm, all d, whereas only 12 have been assigned thus far. Second, the polypropionate–acetate biosynthetic origin of scopafungin requires one (or three) extra carbon(s) to preserve the β-polyol sequence. Third, scopafungin was shown by titration to react with 2 equiv of periodate,[12] indicating two vicinal diols. Formic acid, the required periodate cleavage product from a *vic*-triol unit at C-17 through C-19, was identified as follows. Desalted scopafungin was oxidized with sodium periodate in distilled water. Distillation of the acidified solution (H_2SO_4) after the reaction gave a dilute aqueous solution of formic and acetic acids, identified by FDMS of their sodium salts. With all the carbons of the macrolide ring assigned, it is clear from the structure of **18**-*d* that the hemiketal noted at the outset (99.8 ppm) must be formed between the C-17 carbonyl and the C-21 hydroxyl group, since no five- or six-membered rings are allowed by other hydroxyls.

Malonic Acid and Its Location. A $C_3H_2O_3$ unit remains to be assigned in scopafungin. Several lines of evidence argue for this as a monoester of malonic acid, especially the two unassigned carboxylate carbons in scopafungin's ^{13}C NMR spectrum (Table II) and the identification of sodium acetate (together with sodium

(12) Quantitative periodate oxidations were carried out by Dr. W. C. Christopfel, University of Illinois.

formate) in the basified distillate of the preceding section. Indeed, basic hydrolysis of scopafungin (2 N KOH, room temperature) followed by chromatography on LH20, acidification of the salt fraction, and extraction with ether gave a white solid, identified as malonic acid by its FD mass spectrum [m/z 105 (M + H), 60 (M − CO_2)].

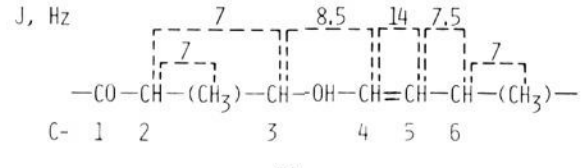
The malonate unit must be present in the portion of scopafungin that gives **15** (C-5 to C-30), from the molecular formula of **15**. This location would account for the acidity of **14**, for the two carboxylate carbons in the ^{13}C NMR spectrum of **14**, and for a sharp two-proton singlet at 3.2 ppm in the ^1H NMR spectrum (Me_2SO-d_6) of **14** (dimethyl malonate, 3.35 ppm). To confirm this location, **15** was treated overnight with 1 N sodium hydroxide; the product (**36**, Scheme II) gave an M + H ion at m/z 647 (FDMS) due to the replacement of $COCH_2COOH$ by H (86 amu).

The malonate may be located more precisely by the following arguments. Periodate oxidation of **15** followed by borohydride reduction should give a mixture of **17** and **32** (molecular weights 322 and 266, respectively), with whichever of these two units bears the malonate having its molecular weight increased by 86 amu ($COCH_2COOH$ − H). The FD mass spectrum of the mixture experimentally obtained showed ions at m/z 323 (322 + H), 345 (322 + Na), and 375 (266 + 86 + Na), but none at m/z 409 (322 + 86 + H) or 431 (322 + 86 + Na). Additional evidence came from the treatment of the previous mixture with diazomethane. The FD mass spectrum of the resulting products indicated an ion at m/z 367 (266 + 86 + 14 + H), but no ion at m/z 423 (322 + 86 + 14 + H). These results define the malonate as being on **32** (specifically in **23** and **37**, the methyl ester of **23**; cf. below) and in unit k. The presence of the malonate in unit k accounts also for the isolation of **18** and not **29** after the bicarbonate wash and for the isolation of **29** only after lithium aluminum hydride reduction.

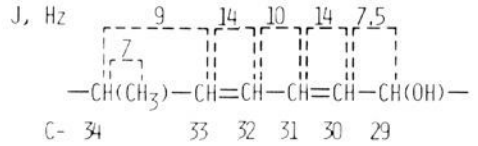
The hydroxyls on C-19 and C-29 of scopafungin (**1**) are eliminated by the periodate cleavage that gives **29**, that on C-21 by its involvement in hemiketal formation with the C-17 ketone, that on C-25 by the pyranose formation in **29**, and that on C-27 by the furanose formation in **35**. Thus, the malonate must be (by elimination) on C-23. This position may account for the facile elimination to give **33**.

Stereochemical Assignments in Scopafungin

Alkene Bonds. C-4, C-5, C-30 to C-33, C-42, and C-43. Due to the presence of similar structural features, the ^1H NMR spectrum of scopafungin itself is poorly resolved even at 360 MHz (in methanol-d_4 and other solvents), and in the ranges 1–2 and 3.5–5 ppm only a few resonances could be assigned (Table III). However, spin decoupling locates all of the olefinic and most of the allylic protons to assign E (trans) stereochemistry to all of the alkene linkages of scopafungin, including the units m and n.



m



n

Thus, the E,E geometry of the diene is confirmed by the H-30 to H-31 and H-32 to H-33 coupling constants (J = 15 Hz), and the H-4 to H-5 and H-42 to H-43 coupling constants (J = 16 Hz) establish an E geometry for those two double bonds. Two-dimensional FT ^1H NMR analysis[13] of the olefinic region (Figure 1) confirmed for H-32, H-31, H-5, and H-30 (6.18, 6.05, 5.72, and 5.66 ppm, respectively) the results obtained by decoupling (Table III) and proved all those protons to be doublets of doublets. The two-dimensional FT data are interpreted for the remaining four olefinic protons in the 5.4–5.65-region as follows: 5.52 (dd, H-33 by decoupling, J = 15, 9 Hz), 5.50 (dd, interpreted as dt with the two inner peaks obscured, ,H-42 or H-43, J = 16, 6 Hz), 5.46 (d, interprted as dd with the two outer peaks obscured, H-4, J = 16, 6 Hz), 5.42 (dt, H-43 or H-42, J = 16, 3.5 Hz).

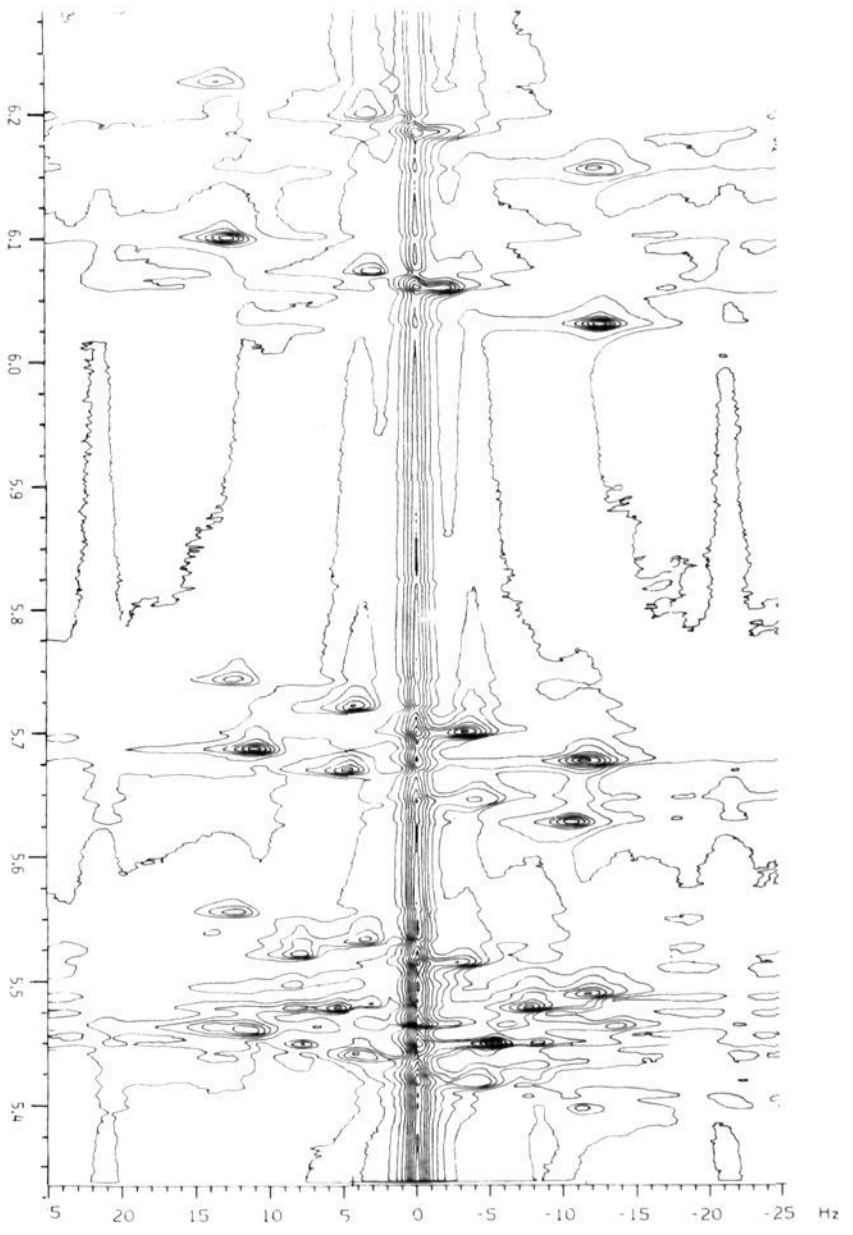
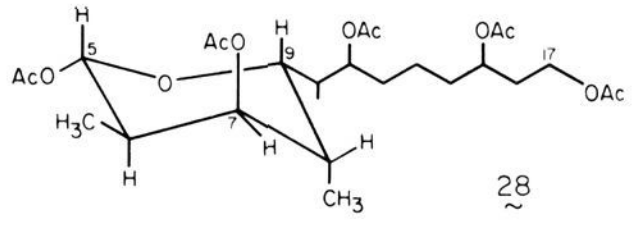


Figure 1. Two-dimensional FT ^1H NMR spectrum of the olefinic region of scopafungin (**1**).

C-6 to C-9. The relative stereochemistry at C-6, C-7, C-8, and C-9 was deduced from the ^1H NMR spectrum of **28**. The



28

chemical shifts and multiplicities of the signals allowed assignment of the downfield protons (Table VI): H-5 and H-6 must be diaxial (J = 10 Hz) and H-7 (J = 5, 2 Hz) equatorial. The difference between the two coupling constants, however, suggests that H-7 has both equatorial–axial (5 Hz) and equatorial–equatorial (2 Hz) coupling. Since H-6 is axial, H-8 must therefore be equatorial. For H-9, J = 11, 7, 2 Hz; spin decoupling of the spectrum showed $J_{9,10}$ = 11 Hz, with J = 7 Hz then attributed to H-9 to H-8 coupling and J = 2 Hz to long-range coupling. $J_{9,8}$ = 7 Hz indicates axial–equatorial coupling and thus H-9 as axial. These results argue the relative configurations shown for **28** and at C-6, C-7, C-8, and C-9 in unit o of scopafungin.

C-25 to C-29. The relative stereochemistry at C-28, C-27, and C-25 was deduced from the ^1H NMR spectrum of **33** (Table VII), which shows H-27, H-28, and H-29 all to be axial ($J_{27,28}$ = 11 Hz, $J_{28,29}$ = 10 Hz). In addition, $J_{27,26a}$ = 11 Hz and $J_{27,26e}$ =

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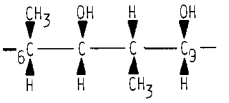
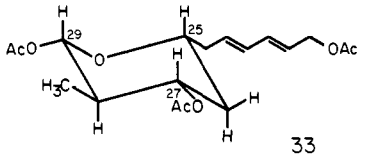
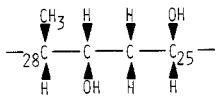


o

2.5 Hz. For H-25, J = 11, 6, 6, 2.5 Hz, indicating it to be axial. These results define the relative configurations shown for **33** (as well as for **29**) and at C-28, C-27, and C-25 in unit p of scopafungin.

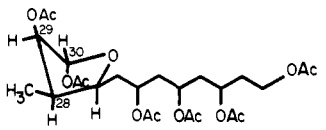


33



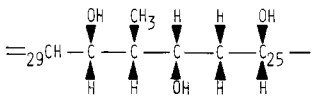
p

Spin decoupling of the ^{1}H NMR spectrum of **35** (Table VIII)



35

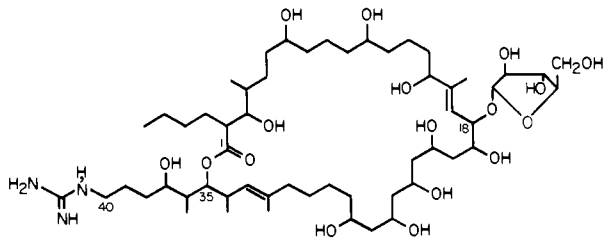
showed $J_{29,30}$ = 1 Hz, $J_{28,29}$ = 6 Hz, and $J_{27,28}$ = 8 Hz. In the furanose system we can then conclude that H-29 and H-30 are trans; in addition, the relative stereochemistry at C-27 and C-28 is known to be cis from **33**. Inspection of molecular models with an approximately 90° dihedral angle between H-29 and H-30 shows that the relative stereochemistry at C-28 and C-29 must be cis to account for the H-28 to H-29 coupling constant of 6 Hz. Thus, the relative configurations must be as shown for **35** and at C-29, C-28, C-27, and C-25 in unit q of scopafungin.



q

Related Compounds

Scopafungin is related to the polyene antifungal antibiotics mainly in that it contains a macrolide ring; for example, the hexaene antibiotics dermostatins A and B[14] also contain a 36-membered macrolide ring. However, scopafungin is much more closely related to primycin,[15] which contains both a 36-membered macrolide ring (the largest ring found outside the polyenes) and a guanidine group. Scopafungin is much larger than primycin, and it also contains a malonate group, which primycin lacks; on the other hand, primycin contains a sugar, which scopafungin lacks. In view of the differences between (and similarities of) scopafungin and primycin, it is of interest to compare the antimicrobial spectra of the two antibiotics (Tables I and IX). Primycin is generally more active against bacteria, and scopafungin more active against fungi. Activity against anaerobes is mixed, with scopafungin more active against *B. fragilis* and primycin more active against *C. perfringens*. The origins of these rather sharp reversals of relative activity in related molecules are of some interest.



PRIMYCIN

Table IX. Antimicrobial Spectra[a]

organism	zone of inhibition,[b] mm scopafungin	primycin	ampho B
Bacillus subtilis	20	29	0
B. subtilis ME	23	30	0
B. subtilis 6033	21	30	0
Staphylococcus aureus	18	22	0
S. aureus 3665	15	21	0
S. aureus 6029	15	21	0
Sarcina lutea	16	23	0
S. lutea 3383	17	25	0
S. lutea sens	21	30	0
Streptococcus pyogenes	16	23	0
Mycobacterium avium	16	18	0
Klebsiella pneumoniae	0	14	0
Clostridium perfringens	14	20	0
Bacteroides fragilis	21	14	0
Penicillium oxalicum	30	19	25
Saccharomyces pastorianus	16	0	16

[a] Determined by J. H. Coats and G. E. Zurenko, The Upjohn Co. [b] *Escherichia coli, Salmonella schottmuelleri, Proteus vulgaris, Pseudomonas aeruginosa,* and *Rhodopseudomonas spheroides*: all 0 mm.

In addition to being related to primycin, whose structure is known, scopafungin has recently been reported to be related to the antibiotics niphithricins A and B,[16] niphimycin,[17] melanosporin,[18] E-79,[19] and B-15565 A and B.[20] The structures of the latter seven antibiotics have not been assigned.

Experimental Section

General Data. Infrared (IR) and ultraviolet (UV) spectra were determined on Beckman spectrophotometers, Models IR-12 and DB, respectively, the IR spectra by G. Elliott. Most ^{1}H NMR and all two-dimensional FT NMR spectra were determined by Dr. S. E. Ulrich and associates on Varian HR 220 and Nicolet NTC 360 spectrometers; some were determined by Drs. D. L. Delaware and R. E. Schwartz at the Purdue University Biochemical Magnetic Resonance Laboratory on Nicolet NTC 360 and NTC 470 spectrometers and by S. A. Mizsak at The Upjohn Company on a Varian XL-200 spectrometer. ^{13}C NMR spectra were determined by Dr. Ulrich on Varian XL-100, JEOL FX 60 and Nicolet NTC 360 spectrometers; chemical shifts (δ) are reported as parts per million (ppm) downfield from internal Me_4Si. Low-resolution electron impact mass spectrometry (EIMS) was carried out on a Varian MAT CH-5 DF mass spectrometer by K. M. Broga, gas chromatography (GC)/MS was carried out on a VG 7070 mass spectrometer, high-resolution (HR) MS and field desorption (FD) MS were performed on a Varian MAT 731 mass spectrometer equipped with a multichannel analyzer,[21] and fast atom bombardment (FAB) MS was carried out on a

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VG Analytical MM ZAB 1F high-resolution mass spectrometer by Drs. M. Barber and R. D. Sedgwick, University of Manchester Institute of Science and Technology, and Dr. B. N. Green, VG Analytical.

Preparative thin-layer chromatography (TLC) was carried out on commercial plates (Analtech, 20 cm × 20 cm × 1 mm). All analytical and semipreparative high-performance liquid chromatography (HPLC) was carried out on a reversed-phase Altex column (Ultrasphere ODS 5 μm, 25 cm, 10-mm i.d.) with a Waters M 6000A pump, employing a Waters differential refractometer R401 as detector. Preparative HPLC was carried out in a Waters Associates 500 instrument, employing a Prep/Pak 500 C_{18} column.

Purification of Scopafungin. Crude scopafungin (3 g, U-29479, 9089BBC-35A) was chromatographed on a Prep/Pak 500 C_{18} column eluted with methanol–water (80:20, adjusted to pH 7.5); 350-mL fractions were collected, freeze-dried, and analyzed by HPLC, with the same solvent. Fraction **7** afforded 377 mg of 70% pure scopafungin, while fractions **8** and **9** produced, respectively, 210 and 110 mg of material that gave a single peak by using HPLC but was still colored. A portion of combined fractions **8** and **9** (200 mg) was further purified by HPLC, with the same solvent system. Fractions corresponding to the scopafungin peak were collected, combined, and freeze-dried to give 120 mg of a white powder, pure by HPLC. This white powder (10 mg) was dissolved in the minimum amount of acetone–water (1:1) and allowed to crystallize overnight at room temperature. Filtration gave small white needles of scopafungin (5 mg): mp 133–135 °C; IR (KBr) 3450 (OH), 1715 (C=O), 1645 (C=C), 990 (C=CC=C, *E,E*) cm^{-1}; UV (EtOH) λ_{max} 231 nm (ϵ 5000). The ^1H NMR spectrum is shown in Table III and the ^{13}C NMR signals are listed in Table II.

Anal. Calcd for $C_{59}H_{103}N_3O_{18}\cdot H_2O$: C, 61.08; H, 9.05; H, 3.62; mol wt, 1142.7314 (M + H). Found: C, 60.70; H, 9.21; N, 3.70; mol wt, 1142.7283 (M + H, HRFDMS), 1142.7341 (HRFABMS).

Ozonolysis of Scopafungin. A solution of purified scopafungin (1 g) in methanol (50 mL) was cooled to −78 °C in dry ice–acetone and treated with ozone until the solution turned blue. Excess ozone was then removed by using a stream of nitrogen, and dimethyl sulfide (4 mL) was added. The solution was allowed to warm slowly to room temperature and was concentrated first with a stream of nitrogen, and then at reduced pressure, to give 1.1 g of a crude ozonolysis mixture.

Glyoxal Bis(*O*-*n*-butyloxime) (2). *O*-*n*-Butylhydroxylamine (20 mg) was added to a solution of the crude ozonolysis mixture (5 mg) in methanol (80 μL) and pyridine (20 μL). After 4 h at room temperature, the solution was analyzed by GC (3% OV1 on GCQ, 3 ft, 70 °C) and GC/MS; a peak having a retention time of 6 min gave *m/z* (rel intensity) 200 (8, M), 199 (8), 128 (14), 113 (22), 100 (49), 97 (96), 88 (54), 83 (52), 57 (97), 41 (87). An authentic sample was prepared from glyoxal (2 mg) and *O*-*n*-butylhydroxylamine (10 mg) in the same way as above. Coinjection of this sample with **2** gave a single peak by GC.

Partition of the Crude Ozonolysis Mixture. A suspension of crude ozonolysis mixture (1 g) in 50 mL of water was extracted with ethyl acetate (3 × 15 mL). The ethyl acetate layers were combined and concentrated at reduced pressure to give 280 mg of an organic-soluble fraction. The aqueous layer was freeze-dried to afford 700 mg of a water-soluble fraction.

Thin-Layer Chromatography of the Organic-Soluble Fraction To Give 3. Preparative TLC of 50 mg of the organic-soluble fractin on silica gel (3:97 MeOH–$CHCl_3$) and methanol extraction of the UV-active band (R_f 0.6), followed by evaporation of solvent, gave 5 mg of oily **3**: UV (EtOH) λ_{max} 227 nm (ϵ 15 000); IR ($CHCl_3$) 2730 (CHO), 1730 (C=O), 1685 and 1650 (C=C—C=O) cm^{-1}; ^1H NMR (see Table IV); ^{13}C NMR (15 MHz, C_6D_6) δ 200.3 (d), 194.0 (d), 158.5 (d), 138.0 (s), 44.1 (t), 37.1 (t), 31.0 (d), 30.8 (d), 30.1 (t), 20.3 (q), 19.4 (q), 9.4 (q); MS (70 eV) *m/z* (rel intensity) 139 (9), 135 (22.1), 111 (74), 99 (10), 97 (50), 69 (82), 41 (100).

Anal. Calcd for $C_{13}H_{22}O_{20}$: mol wt, 210.1634. Found: mol wt, 210.1620 (HREIMS).

Sodium Borohydride Reduction and Acetylation of the Organic-Soluble Fraction To Give 5, 6, 7, and 8. Sodium borohydride (50 mg) was added to a solution of the organic-soluble fraction (150 mg) in ethanol (5 mL). The solution was stirred at room temperature for 2 h, neutralized with dilute hydrochloric acid, and evaporated to dryness. The residue was treated with acetic anhydride–pyridine (5 mL, 1:9) for 12 h at room temperature, hydrolyzed with ice, and extracted with chloroform (3 × 5 mL). The chloroform extracts were washed (2×) with saturated sodium bicarbonate followed by brine to neutrality. Solvent was evaporated at reduced pressure to give 110 mg of a crude reaction mixture, which was subjected to HPLC (80:20 MeOH–H_2O) to give, in order of elution, **8** (10 mg), **6** (7 mg), **5** (20 mg), and **7** (10 mg).

5: IR ($CHCl_3$) 1745 (OC=O) cm^{-1}; ^1H NMR (see Table V); ^{13}C NMR (25.2 MHz, C_6D_6) δ 172.5 (s), 170.15 (s), 170.06 (s), 169.8 (s), 169.5 (s), 76.7 (d), 72.8 (d), 65.7 (t), 64.3 (t), 62.9 (t), 41.6 (dd), 34.9 (d), 31.5 (d), 29.8 (t), 29.3, 23.5 (t), 20.7 (q), 20.5 (q), 20.2 (q), 20.1 (q), 20.0 (q), 14.6 (q), 14.2 (q), 13.7 (q); MS (70 eV) *m/z* (rel intensity) 457 (<1), 415 (<1), 331 (1), 299 (11), 257 (4), 239 (6), 201 (37), 159 (100); FDMS 517 (M + H).

Anal. Calcd for $C_{26}H_{45}O_{10}$: mol wt (M + H), 517.3010. Found: mol wt, 517.3010 (M + H, HREIMS).

6: IR ($CHCl_3$) 3490 (OH), 1735 (OC=O) cm^{-1}; ^1H NMR (220 MHz, $CDCl_3$) δ 4.85 (m, 1 H), 4.54 (m, 1 H), 4.05 (m, 5 H), 3.84 (m, 1 H), 2.65 (m, 1 H), 2.09 (s, 3 H), 2.04 (s, 6 H), 1.24 (d, 3 H, *J* = 7 Hz), 0.99 (d, 3 H, *J* = 7 Hz), 0.885 (d, 3 H, *J* = 7 Hz), 0.85 (d, 3 H, *J* = 7 Hz); MS (70 eV) *m/z* (rel intensity) 159 (2), 137 (3), 123 (11), 95 (13), 69 (28), 43 (58), 27 (100); FDMS 475.

7: IR ($CHCl_3$) 1740 (OC=O) cm^{-1}; ^1H NMR (360 MHz, C_6D_6) δ 5.14 (d, 1 H, *J* = 10 Hz), 4.45 (s, 2 H), 3.97 (t, 2 H, *J* = 7 Hz), 2.42 (m, 1 H), 1.69 (s, 3 H), 1.68 (s, 3 H), 1.58 (d, 3 H, *J* = 1 Hz), 1.41 (m), 1.17 (m), 0.98 (m), 0.89 (d, 3 H, *J* = 6.5 Hz), 0.79 (d, 3 H, *J* = 6.5 Hz); MS (70 eV) *m/z* (rel intensity) 298 (1, M), 256 (10), 124 (34), 82 (23), 43 (100).

Anal. Calcd for $C_{17}H_{30}O_4$: mol wt, 298.2142. Found: mol wt, 298.2147 (HREIMS).

8: IR ($CHCl_3$) 3520 (OH), 1740 (OC=O) cm^{-1}; ^1H NMR (see Table IV); MS (70 ev) *m/z* (rel intensity) 215 (15), 155 (12), 137 (46), 97 (45), 84 (88), 69 (73), 55 (97), 43 (100).

Anal. Calcd for $C_{15}H_{30}O_4$: mol wt, 274.2160. Found: mol wt, 274.2139 (HREIMS).

10 from 8. Compound **8** (3 mg) was treated with acetic anhydride–pyridine (50 mL, 1:9) and left at room temperature for 1 h. The solution was hydrolyzed with ice and extracted with chloroform (3 × 1 mL). The combined chloroform extracts were treated with bicarbonate followed by brine to neutrality. Evaporation of the solvent at reduced pressure afforded 2 mg of **10**: ^1H NMR (360 MHz, $CDCl_3$), δ 4.31 (dd, 1 H, *J* = 11, 5.5 Hz), 4.09 (dd, 1 H, *J* = 11, 4 Hz), 4.00 (t, 2 H, *J* = 6.5 Hz), 3.16 (dd, 1 H, *J* = 9, 2.5 Hz), 1.69 (s, 3 H), 1.65 (s, 3 H), 0.82 (d, 3 H, *J* = 6.5 Hz), 0.81 (d, 3 H, *J* = 6 Hz), 0.76 (d, 3 H, *J* = 7 Hz); MS (70 eV) *m/z* (rel intensity) 317 (M + H) (<1), 299 (<1), 257 (<1), 215 (18), 137 (33), 131 (38), 97 (22), 83 (24), 71 (76), 55 (44), 43 (100).

Anal. Calcd for $C_{15}H_{19}O_3$ (M − OAc): 257.2116. Found: 257.2061 (HREIMS).

10 from 5. Compound **5** (4 mg) was heated at reflux for 2 h with 5% potassium hydroxide in methanol (1 mL). The solution was allowed to cool to room temperature, neutralized with dilute hydrochloric acid, and evaporated at reduced pressure. The residue was treated with acetic anhydride–pyridine (100 mL, 1:9) for 1 h at room temperature, hydrolyzed, extracted with chloroform, and worked up as usual. Evaporation of the solvent at reduced pressure afforded 2 mg of a material that coeluted with **10** (prepared from **8**) by HPLC (80:20 MeOH–H_2O) and whose ^1H NMR spectrum was identical with that of **10** from **8**.

Sodium Borodeuteride Reduction and Acetylation of the Organic-Soluble Fraction. A solution of the organic-soluble fraction (50 mg) in ethanol (2 mL) was treated with sodium borodeuteride (20 mg) with stirring at room temperature for 2 h and then worked up as described above. The residue was treated with acetic anhydride–pyridine (2 mL, 1:9) in the usual manner and the crude acetylated material was separated by HPLC (80:20 MeOH–H_2O). The material eluted with the same retention time as **5** was collected to give 3 mg of deuterated **5**: ^1H NMR (See Table V); FDMS 520 (M + H).

Isolation of 12 and 13 from the Water-Soluble Fraction. The water-soluble fraction from ozonolysis (300 mg) was chromatographed on an anion exchange column (Dowex 1X8). The materials eluted with ethanol were combined, and sodium borohydride (100 mg) was added to the solution. After 2 h at room temperature the solution was neutralized with dilute hydrochloric acid and evaporated to dryness, and the residue was treated with acetic anhydride–pyridine (5 mL, 1:9). After 12 h, the solution was hydrolyzed with ice and extracted with chloroform (3 × 15 mL). The combined chloroform extracts were washed with sodium bicarbonate followed by brine to neutrality. Evaporation of the solvent at reduced pressure afforded 60 mg of crude material that was separated by HPLC (45:55 MeOH–H_2O) to give **12** (10 mg) and **13** (15 mg), in order of elution.

12: IR ($CHCl_3$) 1610 (NC=O) cm^{-1}; ^1H NMR (220 MHz, $CDCl_3$) δ 3.73 (t, 2 H, *J* = 7 Hz), 3.39 (m 2 H), 2.86 (s, 3 H), 2.09 (s, 3 H), 1.66 (m, 4 H); MS (70 eV) *m/z* (rel intensity) 187 (1), 186 (14), 170 (11), 157 (24), 156 (12), 142 (30), 130 (31), 128 (19), 115 (31), 114 (21), 100 (31), 99 (35), 83 (40), 70 (86), 57 (95), 43 (100).

Anal. Calcd for $C_8H_{17}N_3O_2$: mol wt, 187.1319. Found: mol wt, 187.1313 (HREIMS).

13: IR ($CHCl_3$) 1735 (OC=O), 1610 (NC=O) cm^{-1}; ^1H NMR (220 MHz, $CDCl_3$) δ 4.08 (t, 2 H, *J* = 5.5 Hz), 3.57 (t, 2 H, *J* = 7.5 Hz), 3.11 (s, 3 H), 2.25 (s, 3 H), 2.23 (s, 3 H), 2.20 (s, 3 H), 2.05 (s, 3 H), 1.68 (m, 4 H); ^{13}C NMR (25.2 MHz, C_6D_6) δ 63 (t), 46 (t), 34, 26.3,

26.0, 25, 23.4, 22.5, 20.4; MS (70 eV) m/z (rel intensity) 298 (1), 270 (6), 256 (10), 229 (20), 70 (21), 56 (29), 43 (100); FDMS 313.

Anal. Calcd for $C_{14}H_{23}N_3O_5$: mol wt, 313.1636. Found: mol wt, 313.1650 (HREIMS).

Isolation of Deuterated 13. An aliquot (2 mL) of the ethanol solution obtained above by chromatography on the anion exchange column was treated with sodium borodeuteride (10 mg) for 2 h at room temperature and then worked up as described above. The residue was treated with acetic anhydride–pyridine (0.5 mL, 1:9) in the usual manner, and the crude acetylated material was separated by HPLC (45:55 MeOH–H_2O). The material eluted with the same retention time as **13** was collected to give 1 mg of deuterated **13**: FDMS 314.

Isolation of 14 and 15 from the Water-Soluble Fraction. The water-soluble ozonolysis fraction (300 mg) was chromatographed on a cation exchange column (Dowex 50W-X8), and the material eluted with water was collected and freeze-dried to give 200 mg of **14**, a white hygroscopic solid: IR (KBr) 3430 (OH), 1725 (C=O) cm^{-1}; ^{13}C NMR (25.2 MHz, CD_3OD) δ 174.0 (s), 171.3 (s), 101.9 (d), 99.8 (s), 97.3 (d), 83.2 (d), 79.7 (d), 78.3 (d), 73.2 (d), 72.0 (d), 71.6 (d), 70.8, 70.0 (d), 69.08 (d), 65.8 (d), 65.5 (d), 43.09, 42.9 (t), 41.95 (d), 39.4, 39.2 (d), 36.9 (t), 35.0 (t), 32.4 (t), 29.1 (t), 28.8 (d), 22.4 (t), 15.4 (q), 12.8 (q), 9.5 (q), 8.14 (q).

Sodium borohydride (100 mg) was then added to a solution of **14** (200 mg) in ethanol (5 mL). After 2 h of stirring at room temperature, the solution was neutralized with dilute hydrochloric acid and evaporated to dryness, and the residue was chromatographed on a short LH20 column. The first material eluted by methanol was collected, and the solvent was evaporated under reduced pressure to give 150 mg of **15**: IR (KBr) 3440 (OH), 1725 (C=O) cm^{-1}; ^{13}C NMR (90 MHz, CD_3OD) δ 172.5 (s), 170.9 (s), 78.0 (d), 76.25 (d), 76.0 (d), 75.5 (d), 73.5 (d), 72.8 (d), 71.6 (d), 69.6 (d), 68.4 (d), 66.7 (d), 65.9 (d), 65.7, 65.5 (t), 64.4 (t), 44.4 (t), 44.1 (t), 43.3 (t), 42.6 (d), 41.8 (d), 40.6 (d), 40.3 (d), 38.8 (t), 36.9 (t), 33.4 (t), 30.1 (t), 29.7 (t), 15.6 (q), 13.7 (q), 10.5 (2 C, both q); FDMS (18 mA) 755 (M + Na), 733 (M + H), 671, 669, 647, 631.

Anal. Calcd for $C_{33}H_{65}O_{17}$: 733.4220 (M + H). Found: 733.4213 (HRFDMS). Calcd for $C_{33}H_{64}NaO_{17}$: 755.4036 (M + Na). Found: 755.4021 (HRFDMS).

Periodate Oxidation of 15. A. Isolation of 18, 19, 20, and 21. Sodium periodate (150 mg) was added to a solution of **15** (300 mg) in methanol–water (10 mL, 1:1). After standing at room temperature overnight, the solution was diluted with brine (20 mL) and extracted with 1-butanol (3 × 5 mL). The combined 1-butanol extracts were washed with brine and evaporated to dryness at reduced pressure. Sodium borohydride (150 mg) was added to the residue dissolved in ethanol (5 mL) and the solution was left at room temperature for 2 h before being neutralized with dilute hydrochloric acid and evaporated to dryness. A solution of the residue in acetic anhydride–pyridine (10 mL, 1:9) was left overnight at room temperature, hydrolyzed with ice, and extracted with chloroform (3 × 10 mL). The combined chloroform extracts were washed with sodium bicarbonate (2×) followed by brine to give a neutral solution from which solvent was evaporated at reduced pressure. The residue (120 mg) was separated by HPLC (60:40 MeOH–H_2O) to give, in order of elution, compounds **21** (10 mg), **20** (7 mg), **19** (10 mg), and **18** (30 mg).

18: IR ($CHCl_3$) 3450 (OH), 1730 (OC=O) cm^{-1}; ^1H NMR (see Table VI); ^{13}C NMR (25.2 MHz, C_6D_6) δ 172.3 (s), 170.4 (s), 170.1 (s), 169.9 (s), 75.5 (d), 74.1 (d), 73.6 (dd, 2 C), 66.3 (t), 60.9 (t), 44.4 (d), 39.5 (d), 37.7 (t), 36.5 (d), 30.0 (t), 29.67 (t), 28.72 (t), 20.52 (dq), 14.87 (d), 13.91 (d), 9.8 (q); MS (70 eV) m/z (rel intensity) 490 (M), 473, 412, 389, 371, 352, and 329 (all <1), 311 (1), 251 (2.5), 196 (7), 189 (2), 131 (7), 107 (14), 68 (45), 43 (100).

Anal. Calcd for $C_{22}H_{36}O_7$ (M – H_2O – HOAc): 412.2458. Found: 412.2457 (HREIMS).

19: ^1H NMR (220 MHz, $CDCl_3$) δ 2.070 (s, 3 H), 2.068 (s, 3 H), 2.06 (s, 3 H), 2.04 (s, 3 H), 1.00 (d, 3 H, J = 7 Hz), 0.95 (d, 3 H, J = 7 Hz), 0.91 (d, 3 H, J = 7 Hz); FDMS 491 (M + H).

20: ^1H NMR (200 MHz, $CDCl_3$) δ 2.06 (s, 3 H), 2.049 (s, 3 H), 2.042 (s, 3 H), 0.97 (d, 3 H, J = 7 Hz), 0.96 (d, 3 H, J = 6.5 Hz), 0.94 (d, 3 H, J = 6.5 Hz); FDMS 449 (M + H).

21: ^1H NMR (220 MHz, $CDCl_3$) δ 2.06 (s, 3 H), 2.05 (s, 3 H), 0.97 (d, 3 H, J = 7.5 Hz), 0.92 (d, 3 H, J = 7 Hz), 0.91 (d, 3 H, J = 7 Hz); FDMS 407 (M + H).

Isolation of 18-d_2. Compound **14** (30 mg) was treated with sodium borodeuteride (20 mg) in ethanol (2 mL). After 2 h at room temperature, the solution was neutralized with dilute hydrochloric acid and evaporated to dryness. The residue was treated with sodium periodate (15 mg) in methanol–water (1 mL, 1:1). After standing at room temperature overnight, the solution was diluted with brine (2 mL) and extracted with 1-butanol (3 × 1 mL). The combined 1-butanol extracts were evaporated to dryness at reduced pressure. The residue was dissolved in ethanol (2 mL) and treated with sodium borohydride (15 mg). After 2 h at room temperature, the solution was neutralized with dilute hydrochloric acid and evaporated to dryness. A solution of the residue in acetic anhydride–pyridine (1 mL, 1:9) was left overnight at room temperature, hydrolyzed with ice, and extracted with chloroform (3 × 1 mL). The combined chloroform extracts were washed with sodium bicarbonate (2×) followed by brine to give a neutral solution from which solvent was evaporated at reduced pressure. The residue was separated by HPLC (60:40 MeOH–H_2O) and the material with the same retention time as **18** was collected to give 3 mg of **18-d_2**: ^1H NMR (see Table VI); FDMS 493.

Isolation of 18-d. The same experimental procedure using the same amounts as for **18-d_2** was followed, except that sodium borohydride was used for the first reduction of **14** and sodium borodeuteride for the reduction following periodate oxidation. The residue obtained after acetylation was separated by HPLC (60:40 MeOH–H_2O), and the material with the same retention time as **18** was collected to give 3 mg of **18-d**: ^1H NMR (see Table VI); FDMS 492.

B. Identification of 17, 32, 23, and 37. Compound **15** (20 mg) was oxidized with sodium periodate and reduced with sodium borohydride as above. After evaporation of the solvent, the residue was chromatographed on a short LH20 column in methanol. The first material eluted was collected and concentrated to give a mixture (5 mg) of **17** and **23**; FDMS 323 and 345 (M + H, M + Na, **17**), 375 (M + Na, **23**). A solution of this mixture (4 mg) in methanol was treated with fresh diazomethane in ether until a yellow color persisted. After 30 min at room temperature, excess diazomethane was removed with a stream of nitrogen, and the solution was evaporated to dryness to yield a mixture (4 mg) of **17**, **23**, **32**, and **37**; FDMS (20 mA) 367 (M + H, **37**), 323 (M + H, **17**), 309 (M + H – CO_2, **23**), 267 (M + H, **32**).

Periodate Oxidation of 14. A. Isolation of 29 and 28. Sodium periodate (50 mg) was added to a solution of **14** (100 mg) in methanol–water (10 mL, 1:1). After standing for 12 h at room temperature, the solution was diluted with brine (20 mL) and extracted with 1-butanol (3 × 3 mL). The combined 1-butanol extracts were washed with brine and evaporated to dryness. The residue was dissolved in pyridine (5 mL) and added at room temperature to a stirred suspension of lithium aluminum hydride (100 mg) in THF–dioxane–pyridine (20 mL, 1:1:1). The reaction mixture was stirred at room temperature overnight, hydrolyzed with ice, neutralized with dilute hydrochloric acid, and evaporated to dryness at reduced pressure. The residue was treated with acetic anhydride–pyridine (5 mL, 1:9), and the solution was stirred overnight at room temperature and worked up as usual. After evaporation of the solvent, the residue (40 mg) was separated by HPLC (6:35 MeOH–H_2O) to give, in order of elution, compounds **31** (3 mg), **29** (4 mg), **28** (3 mg), and **30** (3 mg).

31: IR ($CHCl_3$) 1730 (OC=O) cm^{-1}; ^1H NMR (220 MHz, $CDCl_3$) δ 5.00 (m, 4 H), 4.08 (t, 2 H, J = 6.5 Hz), 4.00 (dd, 1 H, J = 11, 6 Hz), 3.9 (dd, 1 H, J = 11, 6 Hz), 2.06 (s), 2.05 (s), 2.04 (s), 2.01 (s), (18 H for the 4 singlets above), 1.81 (m, 9 H), 0.96 (d, 3 H, J = 7 Hz); MS (70 eV) m/z (rel intensity) 416 (6), 315 (5), 255 (12), 236 (12), 176 (8), 159 (6), 135 (18), 81 (11), 43 (100); FDMS 519 (M + H).

Anal. Calcd for $C_{22}H_{35}O_{10}$ (M – OAc): 459.2228. Found: 459.2227 (HREIMS).

29: IR ($CDCl_3$) 1730 (OC=O) cm^{-1}; ^1H NMR (220 MHz, $CDCl_3$) δ 5.30 (d, 1 H, J = 9 Hz), 5.04 (m, 2 H), 4.70 (m, 1 H), 4.06 (t, 2 H, J = 6.5 Hz), 3.60 (m, 1 H), 2.13 (s, 3 H), 2.07 (s, 3 H), 2.04 (s, 3 H), 2.03 (m, 3 H), 2.02 (s, 3 H), 1.88 (m), 1.79 (m), 1.36 (m, 2 H), 0.91 (d, 3 H, J = 6.5 Hz); MS (70 eV) m/z (rel intensity) 355 (1), 325 (2), 265 (3), 259 (5), 157 (11), 139 (10), 108 (25), 95 (21), 43 (100); FDMS 475 (M + H).

Anal. Calcd for $C_{18}H_{27}O_7$ (M – HOAc – OAc): 355.1755. Found: 355.1750 (HREIMS).

28: IR ($CHCl_3$) 1730 (OC=O) cm^{-1}; ^1H NMR (see Table VI); MS (70 eV) m/z (rel intensity) 350 (1), 189 (1), 187 (1), 162 (3), 147 (4), 135 (5), 125 (12), 95 (16), 60 (37), 43 (100); FDMS 531 (M + H).

Anal. Calcd for $C_{20}H_{30}O_5$ (M – 3HOAc): 350.2093. Found: 350.2093 (HREIMS).

30: IR ($CHCl_3$) 1730 (OC=O) cm^{-1}; ^1H NMR (360 MHz, C_6D_6) δ 5.31 (m, 1 H), 5.20 (m, 1 H), 5.06 (m, 2 H), 4.1 (m, 1 H), 4.01 (m, 3 H), 1.90 (s, 3 H), 1.82 (s, 3 H), 1.73 (s, 3 H), 1.72 (s, 3 H), 1.710 (s, 3 H), 1.708 (s, 3 H), 0.93 (d, 3 H, J = 7 Hz), 0.83 (d, 3 H, J = 7 Hz), 0.74 (d, 3 H, J = 7 Hz); MS (70 eV) m/z (rel intensity) 515 (<1), 352 (1), 334 (1), 295 (2), 259 (5), 251 (4), 175 (5), 159 (10), 157 (11), 147 (10), 108 (28), 95 (25), 43 (100); FDMS 575 (M + H).

Anal. Calcd for $C_{27}H_{43}O_{10}$ (M – OAc): 515.2853. Found: 515.2847 (HREIMS).

B. Isolation of 33. Sodium periodate (50 mg) was added to a solution of **14** (100 mg) in methanol–water (5 mL, 1:1). After standing at room temperature for 24 h, the solution was diluted with brine and extracted with 1-butanol (3 × 5 mL). The combined 1-butanol extracts were

evaporated to dryness, and the residue was reduced with lithium aluminum hydride (100 mg) in THF–dioxane–pyridine (20 mL, 1:1:1), acetylated, and separated by HPLC as in A above to give as the major component **33** (5 mg): IR ($CHCl_3$) 1730 (OC=O), 990 (diene, *E,E*) 965 (diene, *E,E*) cm^{-1}; ^1H NMR (see Table VII); MS (70 eV) *m/z* (rel intensity) 354 (<1), 294 (2), 234 (5), 155 (14), 113 (38), 95 (26), 80 (62), 43 (100); FDMS 355 (M + H).

Anal. Calcd for $C_{18}H_{26}O_7$: 354.1678. Found: 354.1679 (HREIMS).

Isolation of 25. Scopafungin (100 mg) in methanol–water (5 mL, 1:1) was oxidized overnight with sodium periodate (50 mg). The solution was diluted with brine and extracted with 1-butanol (3 × 5 mL). After evaporation of the solvent, the residue was heated at reflux for 12 h with a suspension of lithium aluminum hydride (50 mg) in tetrahydrofuran (20 mL) and then cooled to room temperature. Ice was added to destroy excess hydride, and the solution was neutralized with dilute hydrochloric acid and evaporated to dryness. The residue was treated overnight at room temperature with acetic anhydride–pyridine (10 mL, 1:9) and then worked up as usual. After evaporation of the solvent, the residue (29 mg) was separated by HPLC (80:20 MeOH–H_2O) to give **25** (5 mg): IR ($CHCl_3$) 1730 (OC=O), 1650 (C=C) cm^{-1}; ^1H NMR (220 MHz, $CDCl_3$) δ 5.70 (m, 1 H), 5.40 (m, 5 H), 5.05 (m, 3 H), 4.04 (m, 4 H), 2.44 (m, 1 H), 1.94 (s, 3 H), 1.82 (s, 3 H), 1.80 (s, 3 H), 1.73 (s, 3 H), 1.72 (s, 3 H), 1.71 (s, 3 H), 1.70 (s, 3 H), 0.97 (d, 3 H, J = 7 Hz), 0.95 (d, 3 H, J = 7 Hz), 0.85 (d, 3 H, J = 7 Hz), 0.75 (d, 3 H, J = 7 Hz); MS (70 eV) *m/z* (rel intensity) 598 (<1), 538 (2), 478 (4), 460 (4), 400 (6), 311 (5), 251 (8), 191 (9), 173 (10), 121 (24), 108 (46), 43 (100); FDMS 701 (M + H).

Anal. Calcd for $C_{31}H_{50}O_{11}$ (M – HOAc – ketene): 598.3353. Found: 598.3337 (HREIMS).

Isolation of 26. A mixture of scopafungin (150 mg) and palladium on carbon (100 mg, 5%) in ethanol (20 mL) was stirred under hydrogen at atmopsherica pressure for 24 h and then filtered. Evaporation of the solvent at reduced pessure gave perhydroscopafungin (140 mg), which was treated sequentially with sodium periodate (70 mg), lithium aluminum hydride (70 mg), and acetic anhydride–pyridine (10 mL, 1:9), as described for scopafungin in the foregoing section. After workup and evaporation of the solvent, the residue (25 mg) was separated by HPLC (80:20 MeOH–H_2O) to give **26** (7 mg): IR ($CHCl_3$) 1730 (OC=O) cm^{-1}; ^1H NMR (220 MHz, $CDCl_3$) δ 5.15 (m, 5 H), 4.06 (m, 4 H), 1.95 (s, 3 H), 1.83 (s, 3 H), 1.80 (s, 3 H), 1.76 (s, 3 H), 1.74 (s, 3 H), 1.73 (s, 3 H), 1.72 (s, 3 H), 0.95 (d, 3 H, J = 7 Hz), 0.83 (d, 3 H, J = 7 Hz), 0.79 (d, 3 H, J = 7 Hz), 0.75 (d, 3 H, J = 7 Hz); FDMS 703 (M + H).

Anal. Calcd for $C_{33}H_{55}O_{12}$ (M – OAc): 643.3696. Found: 643.3696.

Basic Hydrolysis of Scopafungin. A mixture of scopafungin (10 mg) and 0.1 N sodium hydroxide in methanol (1 mL) was left overnight at room temperature, neutralized with dilute hydrochloric acid and evaporated to dryness. The residue was dissolved in dimethyl sulfoxide (Me_2SO, 0.5 mL) and treated at room temperature with 1 mL of a solution of dimsylsodium prepared from dry Me_2SO (4 mL) and sodium hydride (0.25 g).[22] After it was stirred for 2 h, the reddish solution was cooled at 10 °C, iodomethane (1 mL in 2 mL of Me_2SO) was added dropwise until the red color disappeared, and stirring was continued for 1 h at room temperature. Excess iodomethane was removed with a stream of nitrogen, and the solution was diluted with toluene, washed with brine, and concentrated at reduced pressure to give a complex mixture; MS (70 eV) *m/z* (rel intensity) 490 (1), 462 (3), 430 (1), 338 (34), 325 (27), 266 (10), 254 (10), 196 (11), 142 (60), 129 (33), 115 (19), 100 (41), 85 (100).

Isolation of 35. A solution of scopafungin (200 mg) in ethanol (5 mL) was treated with sodium borohydride (50 mg) at room temperature for 2 h, neutralized with dilute hydrochloric acid, diluted with water (5 mL) and treated with sodium periodate (50 mg). After standing at room temperature overnight, the solution was diluted with brine (10 mL) and extracted with 1-butanol (3 × 5 mL). The combined 1-butanol extracts were evaporated to dryness, and the residue was reduced with sodium borohydride (50 mg) in ethanol and worked up as usual. After evaporation of the solvent, the residue was chromatographed on a short LH20 column (MeOH). The first material eluted was collected, cooled at −78 °C and treated with a stream of ozone until the solution turned blue. Excess ozone was removed with a stream of nitrogen, dimethyl sulfide (1 mL) was added, and the solution was allowed to return slowly to room temperature. Excess dimethyl sulfide and the solvent were evaporated under a stream of nitrogen and then at reduced pressure. The residue (100 mg) was suspended in water (10 mL) and extracted with ethyl acetate (3 × 3 mL). The aqueous layer was freeze-dried, and the residue was dissolved in pyridine (3 mL) and then added to a suspension of lithium aluminum hydride (50 mg) in THF–dioxane–pyridine (10 mL, 1:1:1). The mixture was stirred for 12 h at room temperature, hydrolyzed with ice, neutralized with dilute hydrochloric acid, and evaporated to dryness at reduced pressure. The residue was acetylated as usual with acetic anhydride–pyridine (10 mL, 1:9). After workup and evaporation of the solvent, the residue (40 mg) was separated by HPLC (65:35 MeOH–H_2O) to give compounds **35** (5 mg), **28** (4 mg), and **30** (4 mg).

35: IR ($CHCl_3$) 1730 (OC=O) cm^{-1}; ^1H NMR (see Table VIII); MS (70 eV) *m/z* (rel intensity) 487 (2), 417 (2), 331 (3), 271 (5), 255 (5), 211 (5), 141 (6), 135 (9), 109 (7), 99 (9), 43 (100); FDMS 487 (M – OAc).

Anal. Calcd for $C_{23}H_{35}O_{11}$ (M – OAc): 487.2179. Found: 487.2174.

Sodium Formate and Sodium Acetate. Scopafungin (100 mg) was chromatographed on an LH20 column in methanol. The first material eluted was collected and concentrated at reduced pressure. The residue was suspended in distilled water and stirred with sodium periodate (50 mg) overnight at room temperature. The reaction mixture was acidified with dilute sulfuric acid, and the aqueous layer was distilled at atmospheric pressure to give a slightly acidic solution (pH 3) that was neutralized with dilute sodium hydroxide and then freeze-dried to yield a mixture (5 mg) of sodium formate and sodium acetate; FDMS 105 ($CH_3CO_2Na_2$), 91 (HCO_2Na_2), 60 (CH_3COOH), 46 (HCOOH).

Malonic Acid. Scopafungin (150 mg) was treated for 2 h at room temperature with sodium borohydride (50 mg) in ethanol (5 mL) and aqueous potassium hydroxide (4 N, 5 mL) was added. After standing overnight at room temperature, the reaction mixture was neutralized with dilute hydrochloric acid, evaporated to dryness, and chromatographed on an LH20 column (MeOH). The last chromatographic fractions, containing the salts, were combined and concentrated at reduced pressure. The residue was dissolved in 2 N aqueous hydrochloric acid (5 mL) and extracted with ether (3 × 1 mL). The combined ether fractions were washed with 1 N aqueous hydrochloric acid and evaporated to dryness at reduced pressure to give malonic acid (0.1 mg); FDMS 105 (M + H), 60 (M – CO_2).

Basic Hydrolysis of 15. Compound **15** (5 mg) was treated at room temperature with sodium hydroxide (1 mL, 1 N) in methanol–water (1:1). The solution was left overnight, was neutralized with dilute hydrochloric acid, and was evaporated to dryness. The residue was chromatographed on an LH20 column. The first material eluted with methanol was collected and concentrated at reduced pressure to give 3 mg of **36**; FDMS 647.

Note Added in Proof. The nearly complete structures recently assigned to the antibiotics azalomycins F_{3a},[23] F_{4a},[24,25] and F_{5a},[23–25] to copiamycin,[26] and to niphithricins A and B[27] resemble in overall pattern but differ in detail from that of scopafungin.

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Registry No. 1, 11056-18-1; **2**, 81875-38-9; **3**, 81875-39-0; **5**, 81875-40-3; **6**, 81875-41-4; **7**, 81875-42-5; **8**, 81875-43-6; **10**, 81875-44-7; **12**, 81875-45-8; **13**, 81875-46-9; **14**, 81875-47-0; **15**, 81875-48-1; **18**, 81875-49-2; **25**, 81875-50-5; **26**, 81897-64-5; **28**, 81875-51-6; **29**, 81875-52-7; **30**, 81875-53-8; **31**, 81875-54-9; **33**, 81875-55-0; **35**, 81875-56-1; sodium formate, 141-53-7; sodium acetate, 127-09-3; malonic acid, 141-82-2.

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