Stereochemical Studies of Isoprenoid Biosynthesis. Biosynthesis of Fomannosin from [1,2-^{13}C$_2$]Acetate

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Abstract: Feeding of [1,2-^{13}C$_2$]acetate to cultures of *Fomes annosus* gives labeled fomannosin (**1**). The observed pattern of enhancements and couplings in the ^{13}C NMR spectrum of **1** supports a pathway in which mevalonate is converted to humulene via intramolecular cyclization of *trans,trans*-farnesyl pyrophosphate. Further cyclization to a protoilludyl cation or its equivalent followed by oxidative cleavage of the appropriate bond would yield fomannosin. Detailed stereochemical analysis of the course of fomannosin biosynthesis supports the conclusion that, in the key cyclization step, it is the *si* face of the distal double bond of farnesyl pyrophosphate which is attacked by the developing carbonium ion at C-1. The extension of the stereochemical analysis developed here to biogenetically related metabolites is discussed.

The exploitation of ^{13}C NMR techniques has had a significant impact on the study of terpenoid biosynthesis.[1] Most investigations have utilized relatively simple precursors such as [1-^{13}C]-, [2-^{13}C]-, or [1,2-^{13}C$_2$]acetate or [2-^{13}C]-,[2] [5-^{13}C]-,[3] [3,4-^{13}C$_2$]-,[4] [3',4-^{13}C$_2$]-,[3] or [4,5-^{13}C$_2$]-[5] mevalonate. The use of multiply labeled substrates has been particularly effective in monitoring bond-forming and bond-breaking processes which define the often subtle and complex rearrangements which occur during terpenoid biosynthesis. Nonetheless, the majority of studies have concentrated on rather straightforward precursor–product relationships and there have been few reports of deliberate stereochemical investigations.[6] Arigoni has recently cited the need for a "simple and unifying stereochemical concept of sesquiterpene biosynthesis".[7] As a first step in this direction and based on exhaustive biosynthetic studies of a group of cadalane and closely related sesquiterpenes he has put forward a stereochemical analysis of the farnesyl pyrophosphate cyclizations and concomitant 1,3-hydride shifts which are involved in the biogenesis of these substances. In connection with our own interest in the application of stable isotopes to biosynthetic studies[8,9] we recently reported an investigation of the biosynthesis of the phytotoxic, fungal metabolite fomannosin (**1**) from [1,2-^{13}C$_2$]acetate.[10] It was apparent to us at the time that a great deal of stereochemical information was in fact implicit in the biosynthetic results which had been obtained. In this paper, after a recapitulation of our previous incorporation studies, we describe a set of spectroscopic experiments which allow an unambiguous assignment of the ^{13}C NMR signals corresponding to the geminal methyl carbons of fomannosin. Based on these assignments we then present a detailed analysis of the stereochemical course of fomannosin biosynthesis. Finally, using the stereochemical concepts put forward, we show how a similar analysis may be applied to a group of biogenetically related fungal metabolites.

In 1967, Bassett et al. reported the isolation of an antibiotic substance, fomannosin (**1**), from still cultures of the wood-rot Basidiomycete *Fomes annosus*.[11] Fomannosin showed phytotoxic activity in assays with *Chlorella pyrenoidosa* and against *Pinus taeda* seedlings. Although no fomannosin could be detected in specimens of naturally infected sapwood it was suggested that this phytotoxin might play a role in the pathogenic activity of *F. annosus*. Recently, Donnelly has reported the isolation from *F. annosus* of a dihydrobenzofuran, fomannoxin (**2**), which is 100 times more toxic than **1** against *C. pyrenoidosa*.[12] Fomannosin has also been isolated from cultures of the related fungus *Fomitopsis insularis*.[11,13]

The structure and relative configuration of fomannosin were determined by x-ray crystallographic analysis of the *p*-bromobenzoylurethane of dihydrofomannosin (**5**).[14] More re-

cently we have prepared the camphanate ester of dihydrofomannosin (**6**) and determined the structure by x ray.[15] Since the configuration of camphanic acid is known unambiguously
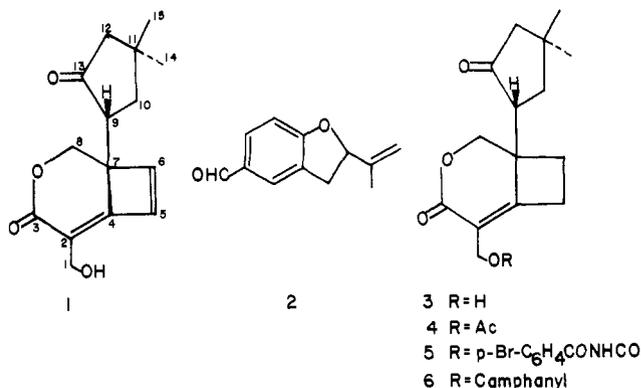
by its synthesis from (+)-camphor,[16,17] the absolute configuration of fomannosin is established as 7*S*,9*R*.[15]

The proton–noise-decoupled carbon magnetic resonance spectrum of fomannosin exhibits 15 signals which are readily assigned with the aid of off-resonance decoupling (SFORD) and by comparison with known chemical-shift data and shift parameters.[18] The assignments are listed in Table I. The geminal methyl signals were not distinguishable on the basis of simple shift rules since 1,3 interactions in cyclopentyl systems have only small (ca. 1–2 ppm) effects on chemical shifts.[18b] The methods used to assign these latter signals are described below.

Sodium [1,2-^{13}C$_2$]acetate (90% enriched), diluted with three parts of unlabeled acetate and mixed with 1.76 × 10^6 dpm of sodium [2-^{14}C]acetate, was administered under sterile conditions to 42-day-old surface cultures of *F. annosus*, FSLD 63, and again on the 46th day. After a total of 8 days the cultures were harvested, and the culture filtrate was extracted with chloroform. The fomannosin was isolated by column chromatography on silica gel and repurified by preparative layer chromatography.

The ^{13}C NMR spectrum of enriched **1** exhibited six pairs of spin-coupled doublets appearing as satellites about the (enhanced) natural abundance singlets, as well as three enhanced singlets. The observed ^{13}C enrichment was ca. 1.4% at each carbon, consistent with that calculated from measurement of the ^{14}C content of **1**, assuming incorporation of nine acetates per mol of fomannosin.[19] The results are presented in Table I and illustrated in Scheme I. The observed labeling pattern supports a pathway in which mevalonate is converted to humulene via intramolecular cyclization of *trans,trans*-farnesyl pyrophosphate. Further cyclization to a

Table I. ^{13}C NMR of Fomannosin[a]

Carbon	δ_C[b]	J_{CC}, Hz[c]	Carbon	δ_C	J_{CC}, Hz
1	58.4 t[d]	52	9	46.5 d	35
2	114.2 s	51	10	38.3 t	36
3	166.1 s	s	11	33.7 s	35
4	155.1 s	44	15	28.1 q	35
5	140.2 d	44	12	53.4 t	38
6	146.5 d	s	13	219.3 s	37
7	52.7 s	37	14	29.7 q	s
8	73.7 t	38			

[a] Bruker WP-60, 15.08 MHz; spectral width 3906 Hz, acquisition time 1.048 s, pulse delay 2.0 s, pulse width 35 μs, 26104 transients, 0.92 M solution in $CDCl_3$, 10-mm sample tube. [b] Me_4Si = 0.00 ppm. [c] Observed coupling of satellite doublets for ^{13}C-enriched **1**. [d] Multiplicity in SFORD spectrum: s = singlet, d = doublet, t = triplet, q = quartet.

Scheme I
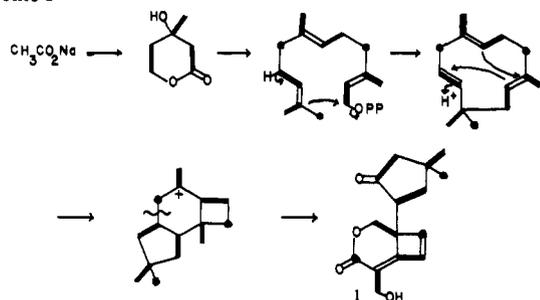
protoilludyl cation or its equivalent followed by oxidative cleavage of the appropriate bond would yield fomannosin.

As mentioned above, [1,2-[13]C_2]acetate is a particularly useful precursor of isoprenoid metabolites in biosynthetic investigations.[20] All carbons in any subsequent metabolite which are derived from paired atoms C-3 and C-3′, and C-4 and C-5 of mevalonate, respectively, give rise to coupled doublets in the ^{13}C NMR spectrum, provided that the paired atoms remain connected. Adjacent carbon atoms derived from distinct molecules of acetate will not show spin-spin coupling as long as there is a significant pool of unlabeled acetate, either administered externally or endogenously generated. Furthermore, since C-1 of mevalonate is lost as carbon dioxide in the formation of isopentenyl pyrophosphate, all carbons in any subsequent metabolite which are derived from C-2 of mevalonate give rise to enhanced singlets. The ^{13}C NMR spectrum of labeled **1** indicates that C-3 and -6 as well as the low-field methyl group of fomannosin are derived from C-2 of mevalonate.[21]

The geminal methyl signals were assigned by analysis of a series of derivatives of fomannosin. Fomannosin was reduced catalytically to dihydrofomannosin (**3**), and the latter was acetylated to dihydrofomannosin acetate (**4**). Comparison of the ^{1}H NMR spectra of each of these substances ($CDCl_3$, 60 and 270 MHz) revealed that in going from **1** to **3** to **4**, the chemical shift of one of the two methyls remains essentially unchanged while that of the second methyl moves downfield from δ 1.16 to 1.24 and then to 1.25, respectively. Since it is the bulky substituent of the cyclopentanone which is being altered in this series of derivatives, it seems clear that it is the magnetic environment of the methyl cis to this substituent which will be affected while the environment of the trans methyl is not seriously perturbed. (This argument does not depend on the *sign* of the anisotropy of the cyclobutene double bond but only its relative *magnitude*.) The ^{13}C NMR spectra of **1**, **3**, and **4** do not show a corresponding regularity in the positions of the respective methyl signals since carbon chemical shifts are sensitive to conformational as well as electronic effects. The proton and carbon spectra could be correlated, however, using the method of Birdsall.[22] Thus a series of
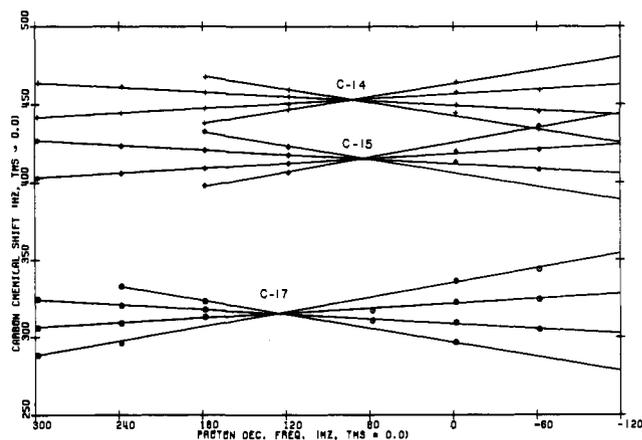
Figure 1. Birdsall plot of SFORD data for C-14, C-15, and C-17 in dihydrofomannosin acetate (**4**).

Table II. Birdsall Plot Correlation for Dihydrofomannosin Acetate (**4**) in $CDCl_3$

| C	[13]C, ppm	[1]H correlation (δ, ±0.05)	[1]H obsd (δ)
14	30.2	1.21	1.25
15	27.6	1.06	1.11
17	20.8	2.08	2.07

SFORD ^{13}C NMR spectra of **4** was recorded with the ^{1}H decoupling frequency (H_2) set at different points in the ^{1}H NMR region, starting at 2 ppm upfield of Me_4Si and moving downfield in increments of 60 Hz. Twelve such spectra were recorded. As H_2 approaches a proton resonance frequency, the residual [13]C-^{1}H coupling goes to zero. Plotting the ^{13}C multiplet frequencies as a function of H_2 yields a series of straight lines at whose intersection the abscissa corresponds to the mean resonance frequency of the ^{1}H atoms bound to that particular carbon atom. Dihydrofomannosin acetate (**4**) was chosen for this analysis because its geminal methyls show a maximum separation in both the [1]H (0.14 ppm) and [13]C (2.6 ppm) spectra. A typical plot of the geminal methyls as well as the acetate methyl is shown in Figure 1 and the correlations are summarized in Table II. From the above data we are able to correlate the downfield (cis) methyl in the proton spectrum of **4** with the downfield methyl in the carbon spectrum of **4**. A Birdsall analysis was also carried out on fomannosin itself in C_6D_6. In the latter solvent the ^{1}H signals for the geminal methyls are shifted upfield to δ 0.86 and 0.72 while the corresponding carbon signals are not significantly affected. (A control spectrum, in 1:1 $CDCl_3$-C_6D_6, established that no crossover of the methyl proton signals occurs in C_6D_6.) Once again the downfield carbon resonance, C-14, is correlated with the downfield proton signal (calcd δ 0.89) while the upfield carbon, C-15, corresponds to a calculated δ 0.78 in the proton spectrum. Finally, in order to relate the carbon spectra of **1**, **3**, and **4**, biosynthetically labeled **1** was converted as above to **4**. In the ^{13}C spectra of enriched **1**, **3**, and **4**, the downfield methyl signals each appear as an enhanced singlet while the corresponding upfield methyl exhibits [13]C-^{13}C satellites, J = 34–35 Hz. This last experiment rules out the possibility of crossover in the various carbon spectra. It was therefore concluded that the enhanced singlet at 29.7 in the ^{13}C spectrum of enriched fomannosin corresponds to the cis methyl (C-14) and furthermore that this methyl is biosynthetically derived from C-2 of mevalonate.

Stereochemical Considerations. Fomannosin. A detailed picture of the stereochemistry of fomannosin biosynthesis can now be projected. To do so one must first examine the various stereochemical possibilities.

Table III. Stereochemical Combinations

re, re, cis:trans, *R*	*si, re,* cis:cis, *R*
re, re, trans:cis, *S*	*si, re,* trans:trans, *S*
re, si, cis:cis, *S*	*si, si,* cis:trans, *S*
re, si, trans:trans, *R*	*si, si,* trans:cis, *R*

Scheme II

Scheme III

7   R = CH$_2$OH

8   R = CH$_3$

9

Scheme IV

10   11
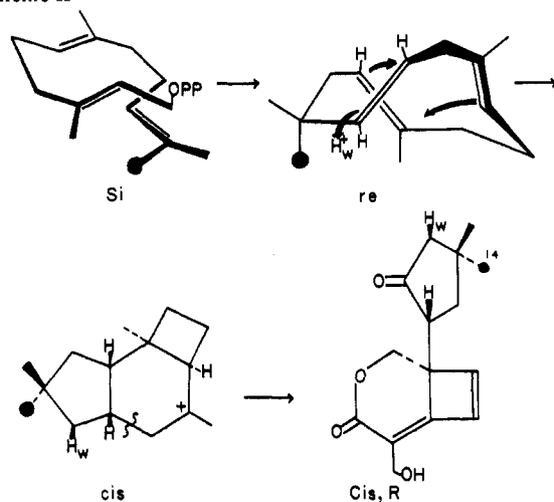
When *trans,trans*-farnesyl pyrophosphate cyclizes, electrophilic attack by the C-1 carbinyl carbon at C-11 may occur on the *re* or *si* face of the distal double bond. If attack occurs on the *re* face, the methyl group derived from C-2 of mevalonate becomes the *pro-S* methyl of humulene. Conversely, attack on the *si* face of the distal double bond generates humulene in which the *pro-R* methyl is derived from C-2 of mevalonate. The 9,10 double bond of humulene is now protonated at position 10 from either the *si* or the *re* face and the concomitant intramolecular cyclization can generate either a cis- or a trans-fused cyclopentane ring in the resultant protoilludane cation or its equivalent. For a sequence of cyclization to the *re* face of the distal double bond of FPP, *re* attack on the humulene double bond, and cis ring fusion, the fomannosin which is ultimately generated would have the following measurable characteristics: (1) the relationship of the methyl derived from C-2 of mevalonate to the cyclobutyl substituent would be trans with respect to the cyclopentane ring; (2) the configuration at C-9 of fomannosin would be *R*. The entire sequence may be abbreviated *re, re,* cis:trans, *R*. For attack on the *si* face of the FPP double bond, followed by *re* protonation of the humulene and generation of a cis-fused cyclopentane, the observable results are different: (1) a cis relationship between the methyl derived from C-2 of mevalonate and the cyclobutyl substituent; and (2) *R* configuration at C-9 of fomannosin. This sequence, which is abbreviated *si, re,* cis:cis, *R*, is illustrated in Scheme II. Altogether there are eight possibilities and these are summarized in Table III.

As discussed above, the cis methyl of fomannosin is in fact derived from C-2 of mevalonate and the configuration at C-9 has been established as *R*. We therefore infer that in the cyclization of *trans,trans*-farnesyl pyrophosphate the substrate is folded at the enzyme active site so as to promote *si* attack on the distal double bond by the developing carbonium ion at C-1. From Table III, it is clear that this conclusion ultimately involves no assumptions as to the stereochemistry of the steps leading from humulene to fomannosin. If, however, one makes the further working assumption, based on analogy to other known dimethylcyclopentane metabolites (see below), that the cyclopentane intermediate generated has a cis ring fusion, then protonation of the humulene double bond is seen to occur from the *re* face. It is therefore predicted that the 12β trans H of
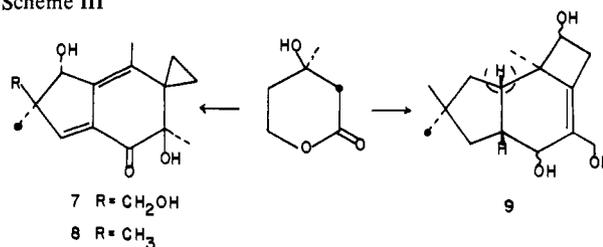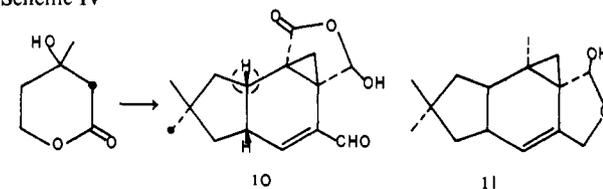
fomannosin is derived from the medium while the H-12α originates from the 4-*pro-R* position of mevalonate. This prediction is currently being tested using deuterated substances and analysis by ^{2}H NMR.[23] Finally, the relative configurations of C-7 and C-9 in fomannosin are consistent only with an overall cis addition to the 2,3 double bond of *all-trans*-humulene.

Extension to Biogenetically Related Metabolites. A number of fungal metabolites biogenetically related to fomannosin are known. Biosynthetic studies have been reported for illudins,[24,25] marasmic acid,[26] coriolins,[27,28] and hirsutic acid.[29] While remembering that one is dealing with a heterogeneous and somewhat incomplete set of results, it is nonetheless useful to reexamine some of the literature data using the stereochemical analysis developed above.

Illudin S (7) and illudin M (8) (see Scheme III) are produced by the fungus *Clitocybe illudens*.[30] The former metabolite exhibits both antitumor and antibiotic activity. The absolute configuration of 7 has been assigned by the dibenzoate chirality rule.[31] Feeding of [2-^{14}C]mevalonate and Kuhn–Roth degradation of 7 have suggested that the methyl attached to C-2' is labeled while the geminal hydroxymethyl is essentially devoid of label.[25] Accompanying these metabolites is illudol (9) (a potential precursor of fomannosin in *F. annosus*).[32] Although no labeling studies have been reported for illudol itself it is reasonable to assume that 7, 8, and 9 are derived from a common protoilludyl cation. By extension, therefore, we assume that the C-2'(α) methyl of illudol is derived from C-2 of mevalonate. The absolute configuration of a derivative of 9 has been determined and illudol is seen to belong to the same absolute configurational series as fomannosin.[33] Using the terminology developed above, illudol can be described as a *cis,* "*R*" metabolite. (Here "*R*" refers to the configuration with reference to C-9 of fomannosin. The configuration at C-1 of illudol is of course *S*, and the complication derives from application of the Cahn–Ingold–Prelog sequence rule. "*R*" is used only to be consistent with Table III. To be perfectly correct one needs only construct a separate table for each metabolite; the results of the analysis are the same.) The complete stereochemical description of the biosynthesis of illudol, and presumably illudins S and M, is therefore *si, re,* cis:cis, "*R*", the same as that for fomannosin.

Marasmic acid (10), of the absolute configuration shown (see Scheme IV),[33] is produced by *Marasmius conigenus*. A closely related substance, 11, is a co-metabolite of fomannosin in *F. insularis*.[34] Preliminary degradation experiments on 10 indicated that the geminal methyls are derived from C-2 of mevalonate but no distinction could be made as to which of the two methyls was labeled.[26]

On the basis of the close parallel to fomannosin and illudin structure and biogenesis as well as the cooccurrence of **1** and **11** we predict that it is the cis (α) methyl of marasmic acid which is labeled by C-2 of mevalonate.

The structure and absolute configuration of hirsutic acid (**12**), a metabolite of *Stereum complicatum*, have been assigned by x-ray analysis.[35] Hirsutic acid, while having the usual cis-fused cyclopentane structure, has stereochemistry antipodal to **1**, **9**, and **10**. A biosynthetic study involving [1-^{13}C]- and [2-^{13}C]acetate did not distinguish whether the carboxyl group or its geminal methyl are derived from C-2 of mevalonate.

12
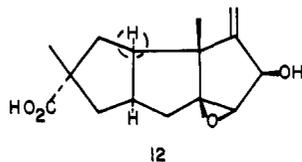
Extracts of *Coriolus consors* produce a set of antitumor antibiotics with structures **13**, **14**, and **15** closely related to that of hirsutic acid.[36] Tanabe has used [1,2-^{13}C$_2$]acetate to deduce the mode of biosynthesis of these compounds, distinguishing among a number of possible rearrangement pathways in the process. While it was determined that the methyl resonating at 26.5 ppm was derived from C-2 of mevalonate (enhanced singlet, methyl at 21.4 has satellites, $J = 34$ Hz) no attempt was made to assign cis or trans stereochemistry to the individual methyls. In order to apply our stereochemical analysis we have made a preliminary assignment based on simple shift parameters.[18] Taking 1,1-dimethylcyclopentane as a reference for the geminal methyls (29.1 ppm)[18] and applying the known upfield shifts induced by vicinal hydroxyl substitution (trans, −1.9; cis, −6.5),[18] the calculated shifts for 2,2-dimethylcyclopentan-1-ol are 22.5 (cis methyl) and 27.5 (trans methyl). Since acylation of the alcohol is expected to perturb the methyl shifts only slightly[18] and 1,3 interactions are known to be small (±1-2 ppm), the signal at 26.5 in **16** may be provisionally assigned to the methyl trans to the α-acetoxy ester and cis to the 2,3 bond. (The effects of the C-12 methyl and the acetoxy at C-8 are unknown.) Furthermore, although the absolute configuration of the coriolins has not been determined directly, we assume for the purpose of argument that the configuration is the same as that of the closely related hirsutic acid, i.e., antipodal to fomannosin. Keeping the above assumptions in mind, coriolin (and, by extension, hirsutic acid) biosynthesis may be described as *re, si*, cis:cis, "*S*". (Again, "*S*" refers to the analogous position, C-9, of fomannosin.) The *re* attack on the distal double bond of farnesyl pyrophosphate is in contrast to the demonstrated *si* attack in the antipodal fomannosin series. This result has interesting implications for the nature of the enzyme reactions involved in cyclizing farnesyl pyrophosphate. Either free humulene is produced in the various organisms by enzymes with either *re* or *si* specificity, or else the *absolute stereochemistry of the ultimate product dictates the folding of farnesyl pyrophosphate at the active site*, humulene being generated in a conformation amenable to further transannular cyclization. In the latter regard it should be noted that the coriolins are not derived from a protoilludyl cation but are formed by way of a tricyclic structure such as hirsutene (**17**) (see Scheme V).[27,28,36]

In summary, analysis of the labeling pattern of biosynthetically derived fomannosin has allowed a detailed analysis of the stereochemical course of fomannosin biosynthesis. The analysis developed here may also be extended to metabolites biogenetically related to fomannosin, with the understanding that the validity depends on the assumptions noted. While these latter conclusions must be regarded as preliminary, they do suggest a number of important experiments and make clear predictions as to the results expected.

Scheme V

13 R'= O; R²= H
14 R'= O; R²= COCHOHC$_6$H$_{13}$
15 R'= H,OH; R²= COC$_7$H$_{15}$

17

16 R = COCHOAcC$_6$H$_{13}$
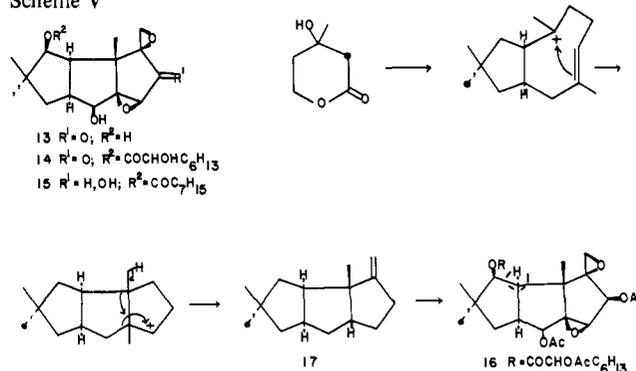
Experimental Section

Instrumentation. Proton NMR spectra were obtained on Varian A60A and Bruker WP60 (60 MHz) and Bruker HX 270 (270 MHz) spectrometers. Carbon spectra were obtained on the Bruker WP 60 at 15.08 MHz. Infrared spectra were recorded on a Perkin-Elmer Model 257 grating spectrophotometer. Radioactivity measurements were performed with a Packard 3330 liquid scintillation counter using 10-mL toluene solutions containing 7.20 g of Bu-PBD and 0.45 g of PBBO per L of toluene. Melting points were taken in unsealed glass capillaries in a Hoover-Thomas melting point apparatus and are uncorrected. Fermentations were carried out in a Freas 816 incubator.

Materials. Sodium [1,2-^{13}C$_2$]acetate (90% enriched) was purchased from Merck & Co. Sodium [2-^{14}C]acetate was obtained from new England Nuclear. *Fomes annosus* (FSLD 63) was a gift from Dr. George Kuhlmann of the Forestry Sciences Laboratory, Durham, N.C. Cultures were maintained as slants on modified malt agar (20 g of Difco malt extract, 20 g of dextrose, 1 g of Difco peptone, and 25 g of Difco dehydrated agar per L of distilled water) and stored at 4 °C.

Incorporation of Sodium [1,2-^{13}C$_2$]Acetate into Fomannosin. A modified Raulin's medium,[11] consisting of 46.6 g of saccharose, 2.66 g of *d*-tartaric acid, 0.40 g of potassium dihydrogen phosphate, 2.70 g of ammonium nitrate, 0.40 g of potassium carbonate, 0.26 g of magnesium carbonate, 0.20 g of calcium sulfate (trihydrate), 0.11 g of zinc sulfate (heptahydrate), 0.11 g of ferrous sulfate (heptahydrate), and 0.1 mg of thiamine hydrochloride in 1 L of distilled water, pH adjusted to 4.50 with 0.1 N sodium hydroxide, was divided between two 800-mL Roux bottles (250 mL of broth per bottle) fitted with Morton closures and autoclaved at 120 °C for 20 min. The flasks were each inoculated with a plug of mycelium from an agar slant of *F. annosus*, FSLD 63, and the surface cultures were incubated for 42 days at 24 °C in the dark. A solution of 62 mg (0.74 mmol) of sodium [1,2-^{13}C$_2$]acetate diluted with 186 mg (2.22 mmol) of unlabeled acetate and mixed with 1.76×10^6 dpm of sodium [2-^{14}C]acetate was divided into two equal portions which were administered on day 42 and again on day 46 via a Swinnex-13 Millipore filter unit to the growing cultures, care being taken not to disturb the mycelial mat. After a total of 50 days the cultures were harvested by filtration, and the filtrate was extracted two times with 150 mL of chloroform, Celite 503 being used to break up emulsions. The chloroform extracts were dried over sodium sulfate and the solvent was evaporated under reduced pressure to give 0.33 g of residue. Chromatography on 20 g of silica gel using 2:1 chloroform–acetone gave crude fomannosin which was further purified by PLC (chloroform–acetone, 1:1), yielding 0.19 g of fomannosin, 3.24×10^5 dpm/mmol: incorporation, 13.1%; ^{13}C enrichment, 1.4%; NMR (CDCl$_3$, 270 MHz) δ 1.10 (s, *H*-15, 3 H), 1.16 (s, *H*-14, 3 H), 1.57 (dd, $J = 12.1$, 12.8 Hz, *H*-10, 1 H), 1.74 (ddd, $J = 2.4$, 9.0, 12.8 Hz, *H*-10, 1 H), 1.95 (d, $J = 18.3$ Hz, *H*-12, 1 H), 2.23 (br d, $J = 18.3$ Hz, *H*-12, 1 H), 2.64 (s, O*H*, 1 H), 3.18 (dd, $J = 9.0$, 12.1 Hz, *H*-9, 1 H), 4.29 (d, $J = 10.1$ Hz, *H*-8, 1 H), 4.35 (d, $J = 13.7$ Hz, *H*-1, 1 H), 4.43 (d, $J = 13.7$ Hz, *H*-1, 1 H), 4.91 (d, $J = 10.1$ Hz, *H*-8, 1 H), 6.70 (d, $J = 2.4$ Hz, *H*-6, 1 H), and 6.90 (d, $J = 2.4$ Hz, *H*-5, 1 H); IR ν_{max} (CHCl$_3$) 3520 (OH), 1729, 1700 cm^{-1} (C=O).

Dihydrofomannosin (3). A solution of 0.116 g (0.44 mmol) of fomannosin in 10.0 mL of absolute ethanol was added via syringe to a stirred suspension of 0.033 g of 5% palladium/carbon in 15.0 mL of ethanol, previously equilibrated with hydrogen. After 10.6 mL (0.435

mmol) of hydrogen gas had been consumed, the reaction mixture was filtered to remove the catalyst and concentrated under vacuum. The crude product was purified by PLC (ethyl acetate–chloroform, 2:1), giving 0.072 g (62%) of dihydrofomannosin (**3**): NMR (CDCl$_3$) 1.10 (s, *H*-15, 3 H), 1.24 (s, *H*-14, 3 H), 2.0 (m, *H*-6, *H*-10, *H*-12, 6 H), 3.0 (m, *H*-5, *H*-9, O*H*, 4 H), 4.28 (d, *J* = 10.5 Hz, *H*-8, 1 H), 4.30 (s, *H*-1, 2 H), and 4.90 (d, *J* = 10.5 Hz, *H*-8, 1 H); ^{13}C NMR (CDCl$_3$) δ 217.6 (s, *C*-13), 165.2 (s, *C*-3), 159.8 (s, *C*-4), 121.3 (s, *C*-2), 75.1 (t, *C*-8), 56.9 (t, C-1), 53.7 (t, *C*-12), 47.3 (d, *C*-9), 46.8 (s, *C*-7), 39.9 (t, *C*-10), 33.4 (s, *C*-11), 30.3 (t, *C*-5), 29.9 (q, *C*-14), 27.4 (q, *C*-15), 25.5 (t, *C*-6); IR ν_{max} (CHCl$_3$) 3550 (OH), 1730, 1710 cm^{-1} (C=O).

Dihydrofomannosin Acetate (4). Pyridine (1.0 mL) was added to a solution of 0.038 g (0.14 mmol) of dihydrofomannosin (**3**) in 1.0 mL of acetic anhydride and the reaction mixture was stirred for 2 h at 0 °C. Water (1.0 mL) was added; the mixture was stirred 0.5 h and then worked up by addition of 10.0 mL of water and extraction with three 10-mL portions of ether. The combined ethereal extracts were washed with 10 mL of 1% hydrochloric acid, twice with 10 mL of half-saturated sodium bicarbonate, and twice with 10 mL of water. After drying over magnesium sulfate, the solvent was evaporated and the crude product was purified by PLC (ethyl acetate–chloroform, 2:1) yielding 0.031 g (72%) of dihydrofomannosin acetate (**4**): NMR (CDCl$_3$) 1.11 (s, *H*-15, 3 H), 1.25 (s, *H*-14, 3 H), 2.0 (m, *H*-6, *H*-10, *H*-12, 6 H), 2.08 (s, C*H*$_3$CO, 3 H), 3.1 (m, *H*-5, *H*-9, 3 H), 4.25 (d, *J* = 11 Hz, *H*-8, 1 H), 4.78 (s, *H*-1, 2 H) and 4.93 (d, *J* = 11 Hz, *H*-8, 1 H); ^{13}C NMR (CDCl$_3$) δ 217.2 (s, *C*-13), 170.6 (s, *C*-16), 163.8 (s, *C*-3), 162.8 (s, *C*-4), 117.6 (s, *C*-2), 75.1 (t, *C*-8), 57.9 (t, *C*-1), 53.8 (t, *C*-12), 47.5 (s, d, *C*-7, *C*-9), 40.1 (t, *C*-10), 33.6 (s, *C*-11), 30.7 (t, *C*-5), 30.2 (q, *C*-14), 27.6 (q, *C*-15), 25.4 (t, *C*-6), 20.8 (q, *C*-17); IR ν_{max} (CHCl$_3$) 1725 cm^{-1} (C=O).

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