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filtered reaction mixture of the bromide **21b**, prepared from **21a** (0.7 mmol) as described above. The mixture was stirred at room temperature for 2 h; then it was concentrated under reduced pressure. The residual oil was chromatographed on silica and crystallized from dichloromethane/light petroleum to give **21d** in 70% yield: mp 145–146 °C; ^{1}H NMR 1.73 (3 H, d, J = 7.5 Hz), 3.73 (3 H, s), 4.05 (1.1 H, d, J = 5 Hz), 4.98 (1 H, q, J = 7.5 Hz), 6.71 (1 H, br d, J = 5 Hz), 7.70–7.85 (4 H, m); MS m/e 291 (90% $^2H_1$), 232 (90% $^2H_1$), 202 (0% $^2H_1$).

***N*-Phthaloylglycyl-α-deuterioglycyl-α-deuterioglycine Methyl Ester (22d).** Addition of tributyltin deuteride (530 mg, 1.8 mmol) to a crude, filtered reaction mixture of **22b**, prepared from **22a** (200 mg, 0.6 mmol) and NBS (430 mg, 2.4 mmol) in dichloromethane (200 mL), as described above for the preparation of **21b**, gave **22d** (0.11 g, 55%): mp 230–231 °C; ^{1}H NMR 3.69 (3 H, s), 3.86 (1.1 H, d, J = 5 Hz), 3.90 (1.05 H, d, J = 6 Hz), 4.37 (2 H, s), 7.70–7.90 (4 H, m), 8.14 (1 H, br d, J = 6 Hz), 8.51 (1 H, br d, J = 5 Hz); MS m/e 335 (90% $^2H_2$, 5% $^2H_1$), 246 (95% $^2H_1$), 218 (95% $^2H_1$), 188 (0% $^2H_1$).

***N*-Phthaloylglycyl-(*Z*)-α,β-dehydroaspartic Acid Dimethyl Diester (24).** Treatment of **23** (400 mg, 1.15 mmol) with NBS (205 mg, 1.15 mmol), as described above for the preparation of **21b**, gave **24** (330 mg, 82%): mp 175–176 °C; ^{1}H NMR 3.73 (3 H, s), 3.79 (3 H, s), 4.50 (2 H, s), 5.57 (1 H, s), 7.70–7.90 (4 H, m), 10.50 (1 H, br s); MS m/e 346, 345, 314, 287, 188, 161, 160. Anal. Calcd for $C_{16}H_{14}N_2O_7$: C, 55.5; H, 4.1; N, 8.1. Found: C, 55.5; H, 4.0; N, 8.1.

Acknowledgment. This work was supported by a grant from the Australian Research Council.

Registry No. **9a**, 1205-08-9; **9b**, 101649-82-5; **10a**, 23244-58-8; **10b**, 135395-15-2; **11a**, 39739-01-0; **11b**, 135395-26-5; **12**, 89928-06-3; **13**, 129309-14-4; **14**, 129309-13-3; **19a**, 132785-19-4; **19b**, 132785-25-2; (*S*)-**20a**, 135395-13-0; (*RS*)-**20a**, 135501-56-3; (2*S*,3*R*)-**20b**, 135395-16-3; (2*S*,3*S*)-**20b**, 135395-17-4; (±)-(*R**,*R**)-**20b**, 135501-57-4; (±)-(*R**,*S**)-**20b**, 135501-58-5; **21a**, 63267-72-1; **21b** (diastereomer 1), 135395-18-5; **21b** (diastereomer 2), 135395-19-6; **21c** (diastereomer 1), 135395-20-9; **21c** (diastereomer 2), 135395-21-0; **21d** (diastereomer 1), 135395-22-1; **21d** (diastereomer 2), 135395-23-2; **22a**, 63199-92-8; **22b**, 135395-24-3; **22d**, 135395-25-4; **23**, 135395-14-1; **24**, 87358-90-5.

Variculanol: Structure and Absolute Stereochemistry of a Novel 5/12/5 Tricyclic Sesterterpenoid from *Aspergillus variecolor*

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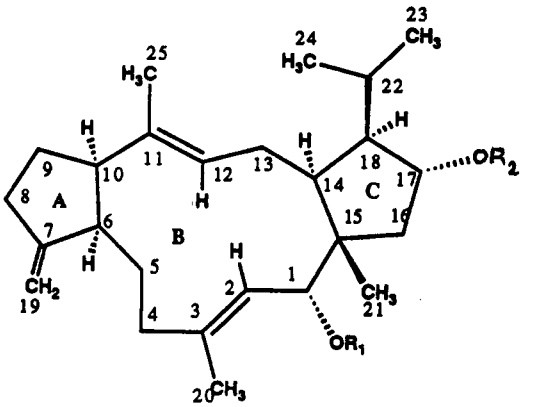
The structure, absolute stereochemistry, and conformation of variculanol, a sesterterpenoid with novel skeleton isolated from *Aspergillus variecolor*, has been described. The molecular structure was deduced from extensive application of 2D NMR methods, in particular HMBC, which established the unambiguous assignment of the novel 5/12/5 ring system. NOEDS measurements were very useful in establishing the relative stereochemistry. The NMR-mandelate method and CD spectral analysis of the 1,17-bis(4-bromobenzoate) were used to determine the absolute stereochemistry. A unique solution-phase conformation was determined from both extensive NOE measurements and MM2 calculations and optimization.

In our search for novel antiparasitic compounds effective against coccidia,[1] we discovered variculanol (**1a**), a sesterterpenoid having a novel 5/12/5 ring system from *Aspergillus variecolor*. We report herein the isolation, structure, absolute stereochemistry, and solution conformation of variculanol (**1a**).

A methyl ethyl ketone extract of a solid-state fermentation of *A. variecolor* was partitioned between hexane and methanol–water. Silica gel chromatography of the hexane extract followed by crystallization from CH_3CN afforded granules of variculanol[2] (**1a**).

Structure Elucidation. High-resolution EI mass spectral analysis of variculanol (**1a**) gave the molecular formula $C_{25}H_{40}O_2$ with six double-bond equivalents (DBE), which was corroborated by ^{13}C NMR spectral data (Table I). The IR spectrum of **1a** showed hydroxy absorption, which was confirmed by formation of a diacetate (**1b**, M^+, 456; IR 1733 cm^{-1}) and a bis(trimethylsilyl ether) (M^+, 516.3809). Because of the presence of only three double bonds, this molecule must have a tricyclic skeleton.

The 400-MHz ^{1}H NMR spectrum of **1a** in $CDCl_3$ and DMSO-d_6 (Table I) exhibited some readily assignable signals such as two vinylic methyls, an angular methyl and a set of methyl doublets, two oxymethines, two exocyclic methylene protons, and a pair of olefinic protons. The



1a: $R_1 = R_2 =$ H, variculanol
b: $R_1 = R_2 =$ acetyl
c: $R_1 =$ (*R*)-*O*-methylmandeloyl, $R_2 =$ H
d: $R_1 =$ H, $R_2 =$ (*R*)-*O*-methylmandeloyl
e: $R_1 =$ (*S*)-*O*-methylmandeloyl, $R_2 =$ H
f: $R_1 =$ H, $R_2 =$ (*S*)-*O*-methylmandeloyl
g: $R_1 =$ 4-bromobenzoyl, $R_2 =$ H
h: $R_1 =$ H, $R_2 =$ 4-bromobenzoyl
i: $R_1 = R_2 =$ 4-bromobenzoyl

(1) Schmatz, D. M.; Crane, M. J. S.; Murray, P. K. *J. Protozool.* **1986**, *33*, 109.
(2) Repeated efforts toward crystallization of variculanol and its derivatives failed to produce crystals suitable for X-ray diffraction.

0022-3263/91/1956-5618$02.50/0

Table I. ^1H and ^{13}C NMR Assignments of Variculanol (1a) in Deuteriochloroform and DMSO-d_6 and HMBC Correlations

position	^{13}C ($CDCl_3$)	^1H ($CDCl_3$)[a]	^1H (DMSO-d_6)	HMBC ($CDCl_3$)
1 H	75.71	4.04, d, 9.9	3.77, d, 9.7	C-1 → H-16α, H-16β, H-14, H-21
2 H	126.78	5.26, dd, 9.9, 1.5	5.15, br d, 9.9	C-2 → H-1, H-4α,β, H-20
3	138.97	-		C-3 → H-1, H-4α,β, H-5α,β, H-20
4 Hα	38.05	2.06, m	2.00, m	C-4 → H-2, H-5α,β, H-20
4 Hβ	-	2.20, m	2.16, m	-
5 Hα	27.70	2.10, m	2.08, m	C-5 → H-4α,β, H-10
5 Hβ	-	0.73, m	0.68, m	-
6 H	49.91	2.48, m	2.42, m	C-6 → H-19 (both), H-10, H-4α,β, H-8, H-5α,β, H-9α,β
7	156.83	-	-	C-7 → H-6, H-8, H-9β, H-10
8 Hα	32.60	2.50, m	2.45, m	C-8 → H-9α,β, H-10, H-19
8 Hβ	-	2.50, m	2.45, m	-
9 Hα	27.98	1.94, ddd, 14, 9, 4	1.87, m	C-9 → H-6, H-8, H-10
9 Hβ	-	1.64, ddd, 14, 7, 2	1.56, m	-
10 H	51.22	3.08, dt, 7.9, 2	3.07, br t, 7.7	C-10 → H-5α,β, H-6, H-12, H-25
11	135.22	-	-	C-11 → H-9α,β, H-10, H-13α,β, H-25
12 H	130.30	5.15, ddd, 11.1, 3.3, 1.2	5.09, br d, 9.2	C-12 → H-10, H-13α,β, H-14, H-25
				-
13 Hα	26.26	2.00, ddd, 14.7, 9.0, 3.5	1.86, m	C-13 → H-12, H-14
13 Hβ	-	2.18, ddd, 14.7, 11.6, 3.0	2.00, dd*, 17.5, 9.1	-
14 H	40.05	2.69, ddd, 11.6, 9.3, 2.1	2.40	C-14 → H-1, H-13α,β, H-16α, H-17, H-21
15	50.09	-	-	C-15 → H-1, H-2, H-13α,β, H-14, H-16α,β, H-17, H-18, H-21
16 Hα	49.43	1.96, br d, 14.7	1.90, dd*, 12.6, 5.9	C-16 → H-1, H-21
16 Hβ	-	1.80, dd, 14.7, 5.2	1.44, dd*, 12.7, 5.6	-
17 H	72.54	3.99, br d, 5.2	3.74, br dd, 13.3, 5.9	C-17 → H-16β, H-18, H-22
18 H	61.62	1.98, dd, 9.5, 5.0	1.63, dd, 12.0, 6.0	C-18 → H-13α, H-14, H-16α, H-22, H-23, H-24
19 H	102.65	4.80, d, 2.2	4.77, br s	C-19 → H-6, H-8
19 H	-	4.72, d, 2.2	4.71, br s	-
20 H	16.13	1.63, d, 1.3	1.54, br s	C-20 → H-2, H-4α,β
21 H	23.00	0.86, s	0.73, s	C-21 → H-1, H-14, H-16β
22 H	25.60	1.85, dhept, 6.8, 4.4	1.68, octet, 6.3	C-22 → H-14, H-18, H-23, H-24
23 H	20.07	0.84, d, 6.6	0.89, d, 6.5	C-23 → H-18, H-22, H-24
24 H	24.39	1.03, d, 6.6	0.93, d, 6.4	C-24 → H-18, H-22, H-23
25 H	13.50	1.40, t, 1.4	1.32, br s	C-25 → H-10, H-12

[a] Scalar coupling constants were determined from HOMO2DJ spectroscopy and *NOE difference spectra. Because of either severe overlap or complex spin systems, some of the coupling constants could not be determined and are presented as m.

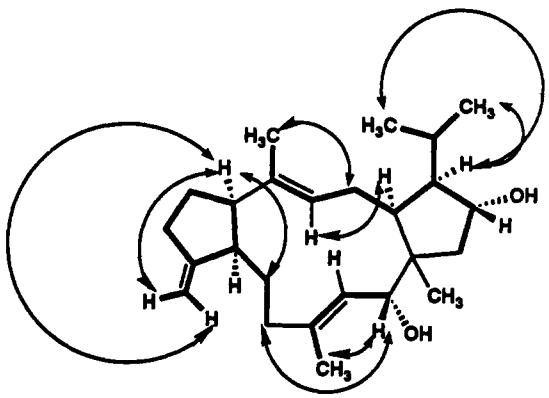


Figure 1. Representation of variculanol showing COSY (bold lines) and relay coherence transfer (arrows) correlations.

^1H,^1H connectivity was determined by 2D ^1H–^1H COSY[3] (Figure 1, bold lines) and relayed ^1H–^1H COSY[4] experiments (Figure 1, arrows). A ^1H–^{13}C COSY[5] experiment was useful not only in assigning the carbon-bearing protons but also in sorting some of the overlapping methylene proton shifts. A highly deshielded (C-18, 61.62 ppm) non-heteroatom-bearing methine was correlated in ^1H–^{13}C COSY to a multiplet at δ 1.98 ppm, and a methylene carbon at δ 27.70 (C-13) was correlated to a proton at δ 2.10 and to a highly shielded counterpart at 0.73 ppm (probably shielded by the olefin at C-11–C-12, Dreiding model). The tricyclic *variculane* ring system was unambiguously ascertained from the ^1H,^{13}C long-range correlations obtained from an HMBC experiment[6] (Table I). The most important correlations were as follows: H-20 (δ 1.63) to C-4 and C-2; H-25 (δ 1.40) and H-12 (δ 5.15) to C-10 (δ 51.22) and the angular methyl (δ 0.86); and H-14 (δ 2.68) and both H-16 protons to C-1.

Stereochemistry and Conformation. ^1H–^1H NOE difference spectroscopy[7] and scalar coupling constants were used to derive the relative stereochemistry which was rationalized with Dreiding models. For example, a cis fusion of ring A was apparent because of a *strong* NOE between H-6 and H-10 and $J_{H6,10}$ = 7.9 Hz (from a decoupling experiment). Stereochemical assignment based on the scalar coupling of a ring junction containing a cyclopentenyl ring is extremely dangerous because of indiscrimination of the vicinal coupling constants between cis and trans protons.[8] However, a *strong* NOE from H-10 to H-6 unambiguously establishes cis A/B-ring fusion in variculanol. Observation of very strong NOEs from H-21 and H-17 to H-16β and H-23 as well as H-24 to H-17 and

(3) Bax, A.; Freeman, R. *J. Magn. Reson.* **1981**, *44*, 542.
(4) (a) Bolton, P. H. *J. Magn. Reson.* **1982**, *48*, 336. (b) Eich, G.; Bodenhausen, G.; Ernst, R. R. *J. Am. Chem. Soc.* **1982**, *104*, 3731. (c) Bax, A.; Drobny, G. *J. Magn. Reson.* **1985**, *61*, 306. (d) Weber, P. L.; Drobny, G.; Reid, B. R. *Biochemistry* **1985**, *24*, 4549.
(5) (a) Bodenhausen, G.; Freeman, R. *J. Magn. Reson.* **1971**, *28*, 471. (b) Bax, A.; Morris, G. A. *J. Magn. Reson.* **1981**, *42*, 501.

(6) (a) Summers, M. F.; Marzilli, L. G.; Bax, A. *J. Am. Chem. Soc.* **1986**, *108*, 4285. (b) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093. (c) Bax, A.; Aszalos, A.; Dinya, Z.; Sudo, K. *J. Am. Chem. Soc.* **1986**, *108*, 8056. Similar but fewer long-range ^1H,^{13}C correlations were obtained from long-range ^1H,^{13}C COSY (delay optimized for J = 10 Hz), COLOC: Kessler, H.; Griesinger, C.; Zerbock, J.; Loosli, H. R. *J. Magn. Reson.* **1984**, *57*, 331.
(7) Most NOE enhancements were obtained in $CDCl_3$ due to sharper lines and less spectral overlap. Several NOEs measured in DMSO-d_6 were identical except that the saturation of H-17 enhance both H-16α and H-16β in $CDCl_3$ but only H-16β in DMSO-d_6. H-17 (J ~ 5 Hz) was coupled to both H-16 protons in DMSO-d_6 but only to H-16β in $CDCl_3$.
(8) (a) Gray, G. R.; Canales, M. W. *Phytochemistry* **1988**, *27*, 1653. (b) Cutler, H. G.; Crumley, F. G.; Cox, R. H.; Springer, J. P.; Arrendale, R. F.; Cole, P. D. *J. Agric. Food Chem.* **1984**, *32*, 778.

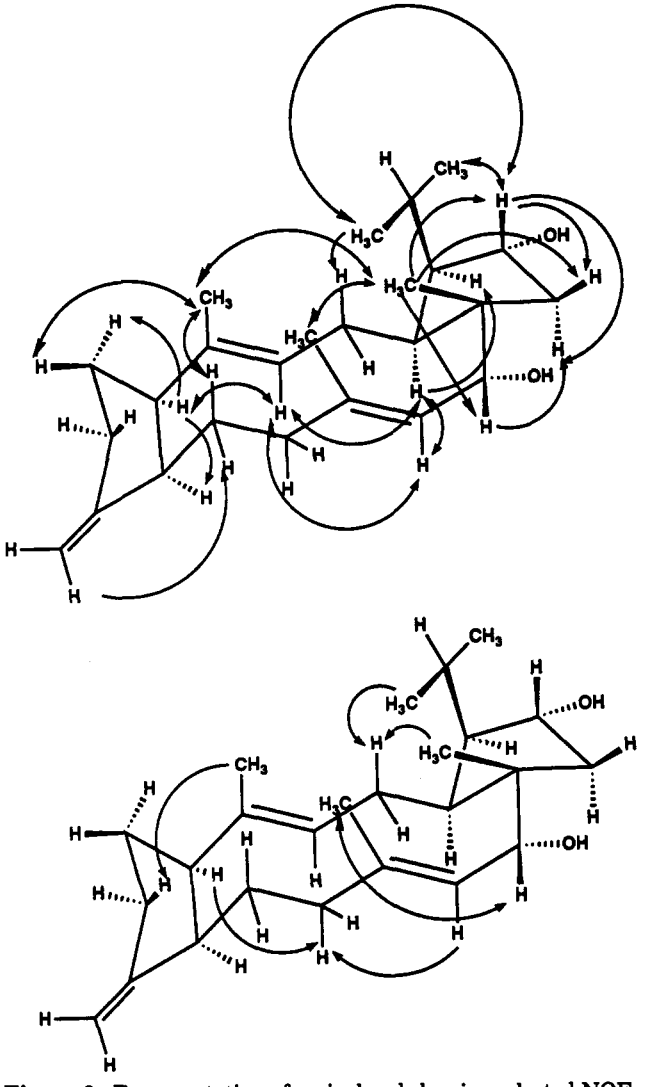


Figure 2. Representation of variculanol showing selected NOEs (arrows) and solution conformation.

H-13β established these protons (groups) on one face of the molecule whereas NOE from H-14 to H-18 ($J_{H14,18}$ = 9.3 Hz) established these on the other face, thus strongly suggesting a trans fusion of ring C. Strong NOE from H-12 to H-10 and H-14 indicated that these three protons are on one face of the molecule, thus transmitting the relative stereochemistry from across the molecule. Similarly H-1 was placed on the same face as H-21 and H-20. Analysis of all the NOE data gave the solution-state conformation of variculanol as depicted in Figure 2, where H-2, H-6, H-10, H-12, and H-14 are on one face and all the methyl groups are on the other. A minimum-energy conformation generated by using a mm2x force field is similar to that obtained from NOE measurements (Figure 3, see supplementary material).

Trost's[9] modification of Dale and Mosher's mandelate method[10] was successfully applied for determination of the absolute stereochemistry of variculanol. Thus both sets of diastereomeric monomethylmandelates (**1c–f**) were prepared by allowing variculanol to react with 2 equiv of both (*R*)- and (*S*)-*O*-methylmandelic acid using oxalyl chloride and DMF in methylene chloride.[9] Comparison

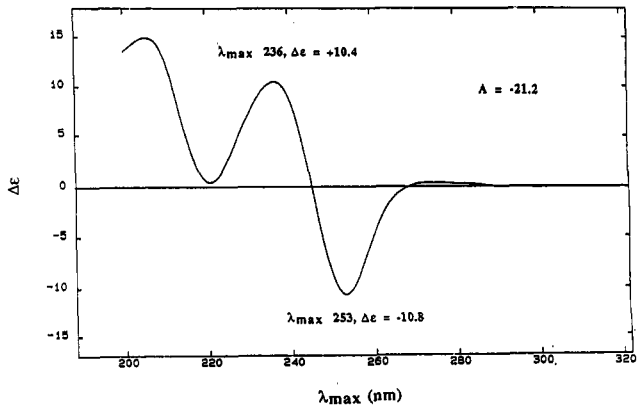


Figure 4. CD spectrum of variculanol 1,17-bis(4-bromobenzoate) (**1i**).

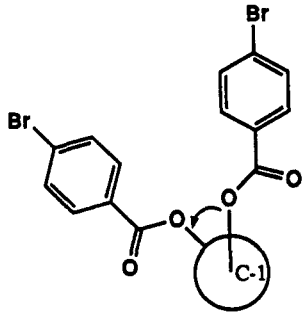


Figure 5.

of ^{1}H NMR spectra (Table II) of (*R*)- and (*S*)-mandelate derivatives **1d** and **1f** revealed significant shielding of H-22, H-23, an H-24 in **1d** and a moderate upfield shift of H-16 (both α and β) and H-21 in **1f**, thus establishing *R* configuration at C-17. The relative stereochemistry derived from NOE experiments requires that C-1 should also have the *R* configuration, and this was found to be consistent with the stereochemistry derived from comparison of the chemical shifts of other mandelate pairs **1c** and **1e**, i.e., an upfield shift of H-21 and H-16 in **1c** and shielding of H-2 and H-5β in **1e**. In order to eliminate the possibility of any unexpected influence of the aromatic shielding effect because of any conformational changes in the molecule, shifts were also compared (Table II) with diacetate **1b** and especially monobenzoates **1g** and **1h**, and $\Delta\delta$ values were consistent with the assignments. Therefore, the stereochemical assignments of all the centers in **1a** are 1*R*, 6*R*, 10*R*, 14*S*, 15*S*, 17*R*, and 18*R*.

The absolute configuration and conformation of variculanol were independently supported by CD measurements of 1,17-bis(4-bromobenzoate) (**1i**) and application of the well-established 1,4-dibenzoate distant chirality method.[11] The CD spectrum (Figure 4) of **1i** exhibited a negative exciton chirality (A = −21.2) with a negative bisignate Cotton effect [λ_{max} 253 ($\Delta\epsilon$ = −10.8); λ_{max} 236 ($\Delta\epsilon$ = +10.4)]. Strong NOEs between H-1 and H-16α and also between H-1 and the H-20 and H-21 methyl groups suggest that these groups are close in space. Examination of the Dreiding model and twisting it to account for the NOEs forces the C-1 benzoate toward the 12-membered ring. Although the 12-membered ring of variculanol has some flexibility, the C-17 benzoate group must be disposed to the left-hand side of the C-1 benzoate group at all times in order to have left-handed screwness (Figure 5), thus

(9) (a) Trost, B. M.; Belletire, J. L.; Godleski, S.; McDougal, P. G.; Balkovec, J. M.; Baldwin, J. J.; Christy, M. E.; Ponticello, G. S.; Varga, S. L.; Springer, J. P. *J. Org. Chem.* **1986**, *51*, 2370. (b) Trost, B. M.; Curran, D. P. *Tetrahedron Lett.* **1981**, *22*, 4929.

(10) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512.

(11) Harada, N.; Nakanishi, K. *Circular Dichroic Spectroscopy: Exciton Coupling in Organic Stereochemistry*; University Science Books: Mill Valley, CA, 1983.

Table II. ^{1}H NMR Assignments of Variculanol Derivatives 1b–i in $CDCl_3$ Solutions[a]

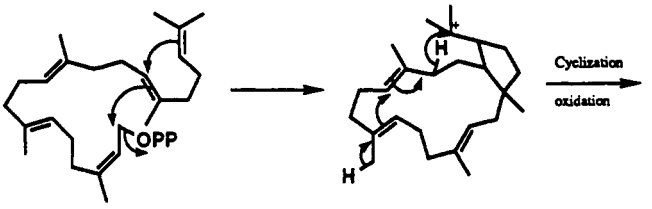
assign.	1b	1c	1e	1g	1d	1f	1h	1i
1	5.10, d, 9.0	5.16, d, 10.1	5.06, d, 9.9	5.33, d, 10.0	4.01, d, 9.6	3.94, d, 9.8	4.03, d, 9.9	5.35, d, 10.2
2	*5.14, d, 9.0*	*5.12, d, 10.1*	*4.95, d, 9.9*	*5.23, br d, 10.3*	5.19, d, 9.6	5.18, d, 9.8	5.23, d, 9.9	5.26, d, 10.2
4α	2.02, m	2.00, m	1.98, m	2.02, m	2.05, m	2.05, m	2.05, m	2.03, m
4β	2.20, m	2.15, m	2.18, m	2.20, m	2.20, m	2.20, m	2.20, m	2.22, m
5α	2.10, m	2.07, m	2.10, m	2.10, m	2.10, m	2.10, m	2.10, m	2.10, m
5β	*0.75, m*	*0.73, m*	*0.65, m*	*0.78, m*	0.75, m	0.74, m	0.77, m	0.79, m
6	2.45, m	2.45, m	2.41, m	2.45, m	2.42, m	2.46, m	2.45, m	2.48, m
8	2.50, m	2.50, m	2.50, m	2.53, m	2.50, m	2.50, m	2.56, m	2.53, m
9α	1.93, m	1.92, m	1.90, m	1.93, m	1.92, m	1.93, m	1.93, m	1.93, m
9β	1.60, m	1.60, m	1.60, m	1.65, m	1.62, m	1.63, m	1.60, m	1.65, m
10	3.05, d, 6.3	3.02, t, 7.9	3.00, t, 7.2	3.05, t, 7.7	3.04, br t, 7.5	3.04, br t, 8.4	3.06, t, 7.6	3.06, t, 7.8
12	5.05, m	5.00, ddd, 8.3, 7, 1.2	4.93, m	5.10, br t, 6.3	5.02, m	5.02, m	5.07, br dd, 7.6, 2.7	5.10, m
13α	1.96, m	1.83, m	1.90, m	1.98, m	1.85, m	1.95, m	2.00, m	2.05, m
13β	2.00, m	1.95, m	2.00, m	2.10, m	2.05, m	2.15, m	2.15, m	2.18, m
14	2.52	2.30	2.52	2.67, m	2.52	2.53	2.60	2.72, mt, 9.9
16α	*1.90*	0.85, d, 12.5	1.59, d, 12.9	1.69, br d, 12.2	*2.05*	*1.82*	*2.18*	1.92, m
16β	*1.69*	1.39, dd, 12.1, 6.1	1.45, dd, 12.9, 4.5	1.62, dd, 12.2, 5.8	*1.82*	*1.65, dd, 12.6, 6.6*	*1.87*	1.83, m
17	4.93, dt, 8.5, 5.5	3.61, m	3.85, m	3.98, dt, 9.6, 6.1	4.95, m	5.06, m	5.22	5.17
18	1.62, m	1.95, m	1.75, m	1.70, m	1.90, m	1.95, m	2.15, m	2.16, m
19	4.80, d, 2.0	4.78, d, 2.1	4.79, br s	4.80, d, 2.0	4.80, br s	4.78, br s	4.80, d, 1.8	4.81, br s
	4.71 d, 2.0	4.69, d, 2.1	4.69, br s	4.71, d, 2.0	4.71, br s	4.69, br s	4.71, d, 1.8	4.73, br s
20	1.70, br s	1.68, br s	1.66, br s	1.76, br s	1.60, br s	1.58, br s	1.62, br s	1.78, br s
21	*0.89, s*	*0.75, s*	*0.81, s*	*0.92, s*	0.83, s	0.80, s	0.88, s	1.02, s
22	*1.70, m*	1.62, m	1.78, m	1.81, m	*1.58, m*	*1.78, m*	*1.85, m*	2.04, m
23	*0.90, d, 6.3*	0.91, d, 6.7	0.99, d, 6.6	1.03, d, 6.6	*0.48, d, 6.6*	*0.84, d, 6.6*	*0.91, d, 6.6*	0.95, d, 6.6
24	*0.96, d, 6.3*	0.93, d, 6.7	1.00, d, 6.6	1.06, d, 6.6	*0.75, d, 6.6*	*0.94, d, 6.6*	*1.00, d, 6.6*	1.04, d, 6.6
25	1.37, br s	1.32, br s	1.34, br s	1.39, br s	1.35, br s	1.35, br s	1.39, br s	1.42, br s
1-OH	–	–	–	–	1.86	1.56	1.90	–
17-OH	–	0.37, br d, 10	3.42, d, 4.4	2.00	–	–	–	–

[a] All of the spectra were assigned on the basis of ^{1}H–^{1}H COSY, and blank multiplicities could not be assigned because of severe overlap. Additional assignments are as follows. **1b**: 2.01, 2.10 (CH_3CO). Mandelate H's: **1c**, 3.38 (3 H, s, OCH_3), 4.72 (1 H, s), 4.72 (1 H, s, α-H), 7.36–7.50 (5 H, m); **1d**, 3.38 (3 H, s, OCH_3), 4.68 (1 H, s, α-H), 7.33–7.43 (5 H, m); **1e**, 3.44 (3 H, s, OCH_3), 4.82 (1 H, s, α-H), 7.32–7.48 (5 H, m); **1f**, 3.38 (3 H, s, OCH_3), 4.69 (1 H, s, α-H), 7.31–7.43 (5 H, m). Benzoates: **1g**, 7.56, 7.90 (2 H each, d, J = 8.6 Hz); **1h**, 7.56, 7.88 (2 H each, d, 8.6 Hz); **1i**, 7.63, 7.93 (2 H each, d, J = 8.4 Hz) and 7.53, 7.82 (2 H each, d, J = 7.8 Hz).

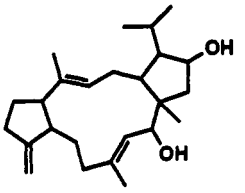
producing a negative chirality. The dihedral angle between the C-1 benzoate and the C-17 benzoate is in the range of 20–30°.

Biogenetically, variculanol may be produced from geranylfarnesyl pyrophosphate after a requisite folding and cyclizations followed by a 1,5-hydride shift to the carbocation (Scheme I).

Scheme I. Proposed Biogenesis of Variculanol



Geranyl-farnesyl pyrophosphate



1a

Experimental Section

All the reagents and deuterated solvents were obtained from Aldrich Chemical Company and were used without any purification. E. Merck (Darmstadt) silica gel plates (0.25 mm) were used for TLC and developed either with 3% ceric sulfate in 3 N H_2SO_4 spray or iodine vapors or both. Stationary phases used for column chromatography were E. Merck silica gel (70–230 or 40–63 mesh). Melting points were uncorrected.

Spectral Measurements. The IR absorption spectra were obtained with a multiple internal reflectance cell (MIR, ZnSe) on neat 10–20-mg samples. The UV absorption spectra were measured in MeOH solution. Mass spectral data were obtained either by electron impact at 70 eV or by FAB. Trimethylsilyl derivatives were prepared with a 1:1 mixture of BSTFA–pyridine at room temperature. Exact mass measurements were made on the same instrument at high resolution by the peak matching method using perfluorokerosene (PFK) as internal standard.

^{1}H NMR chemical shifts in $CDCl_3$ and DMSO-d_6 are given relative to the solvent peaks at 7.256 and 2.49 ppm, respectively. ^{13}C NMR chemical shifts in $CDCl_3$ are given relative to the solvent peak at 77.05 ppm.

^{1}H–^{1}H COSY spectra were recorded by using the standard pulse sequence of Bax et al.[3] ^{1}H–^{1}H relay coherence transfer correlation spectra (single step relayed COSY)[4] were recorded with mix times of 0.026 s and a delay of 1.2 s. The 1K–2K data set was accumulated in 512 increments with 32 transients respectively for each value of t_1 for full phase cycling. Homonuclear (^{1}H) 2D-J resolved spectra (HOM2DJ)[12] were obtained by using a delay of 2.0 s, 2K points in the chemical shift axis (f_2), and 256 increments to define the J-coupling axis (f_1). Zero filling to 512 (f_1) was followed by 2D transformation, the data tilted by 45° and symmetrized.

NOEs were measured by using NOE difference or NOE mult microprograms in $CDCl_3$ and DMSO-d_6, and the sample was not degassed. In the NOE difference method, a relaxation delay of 2.0 s, irradiation time of 0.75–1.0 s, and decoupler power of 40 L was used. In the NOE mult method, multiplets were irradiated for 4.8 s (12 sequential irradiations of 0.4 s each) with a 5-ms delay

(12) (a) Aue, W. P.; Karhan, J.; Ernst, R. R. *J. Chem. Phys.* **1976**, *64*, 4226. (b) Nagayama, K.; Bachmann, P.; Wuthrich, K.; Ernst, R. R. *J. Magn. Reson.* **1978**, *31*, 133.

Table III. Composition of Seed and Production Media

KF Seed Medium			
component	g/L	component	mg/L
corn steep liquor	5.0	$FeSO_4 \cdot 7H_2O$	10.0
tomato paste	40.0	$MnSO_4 \cdot H_2O$	10.0
oat flour	10.0	$CuCl_2 \cdot 2H_2O$	0.25
dextrose	10.0	$CaCl_2 \cdot 2H_2O$	1.0
		H_3BO_3	0.56
		$(NH_4)_2Mo_7O_{24} \cdot 4H_2O$	0.19
		$ZnSO_4 \cdot 7H_2O$	2.0

NPF-2 Production Medium			
component	g/L	component	g/L
dextrose	150.0	$MgSO_4 \cdot 7H_2O$	0.25
urea	4.0	KCl	0.25
NZ amine A	4.0	$ZnSO_4 \cdot 7H_2O$	0.9
K_2HPO_4	0.5	$CaCO_3$	16.5

for frequency switching. The observe pulse width was 90°, and relaxation delay was used. Decoupler power was between 40 and 50 L.

^1H–^{13}C chemical shift correlation spectra (COSY) were recorded in $CDCl_3$ by using the standard pulse sequence of Bax and Morris.[5] The 512 × 4K data set was accumulated in 128 increments with 768 transients for each value of t_1. The delay time between transients was 0.5 s, and the experiment was optimized for $^1J_{CH}$ = 150 Hz. The corresponding long-range experiment was optimized for a multiple-bond carbon–proton coupling constant of 10 Hz. Inverse-mode HMBC experiments were performed by using the pulse sequence of Bax et al.[6] The 512 × 2K data set was accumulated in 200 increments and 256 transients for each value of t_1. The delay between transients was 2 s, and the experiment was optimized for a coupling constant of 10 Hz (0.05 s).

Fermentation of Variculanol (1a). The fungus *A. variecolor* was obtained from the Merck Culture Collection, No. MF 138. The composition of seed and production media is shown in Table III. The seed medium was prepared in distilled water and the pH adjusted to 6.8 prior to sterilization. The medium was dispensed at 54 mL/250 mL plain Erlenmeyer flask, closed with cotton, and sterilized at 121 °C for 20 min. Seed cultures were inoculated with a source of *A. variecolor* and grown on a gyratory shaker (220 rpm, 5.1-cm throw) for 72 h at 25 °C. A portion of the seed culture (12 mL) was used to inoculate each production flask, which consisted of 70 g of vermiculite (this portion of media was sterilized separately in 2-L flasks at 121 °C for 60 min) and 250 mL of production medium which was previously sterilized in 500-mL Erlenmeyer flasks at 121 °C for 15 min. Production flasks were incubated statically at 25 °C for 17–19 days.

Isolation of Variculanol (1a). The solid-state production fermentation (16 flasks, each with 70 g of vermiculite-based medium) of *A. variecolor* was extracted with methyl ethyl ketone (500 mL each, 8 L total) by shaking for 4 h. The extract was concentrated to a volume of 500 mL (mainly aqueous), methanol (600 mL) was added, and the mixture was filtered to remove any solid material. The filtrate was extracted with hexane (3 × 600 mL). The hexane extract was evaporated (5.5 g) under vacuum and chromatographed on a silica gel column (volume of packed column 800 mL). Elution of the column with hexane–EtOAc (7:3) and evaporation of the solvents followed by crystallization from hot CH_3CN afforded colorless granules of variculanol (1a, 400 mg): mp 188–189 °C; $[\alpha]^{25}_D$ +23.2° (c, 0.56, $CHCl_3$); IR (ZnSe) ν_{max} 3245, 2930, 1646, 1542, 1451, 1434, 1230, 1196, 1016, 926, 901, 872, 854, 820, 736 cm^{-1}; HREIMS (*m/z*) 372.3021 (M^+, calcd for $C_{25}H_{40}O_2$ 372.3028), 293.2261 (calcd for $C_{22}H_{29}$ 293.2269), 201.1622 (calcd for $C_{15}H_{21}$ 201.1643). For NMR spectral data, see Table I.

Variculanol 1,17-Diacetate (1b). To a solution of variculanol (2.9 mg) in anhydrous pyridine (0.5 mL) was added acetic anhydride (0.5 mL), and the mixture was stirred at room temperature overnight. Methanol was added to consume excess reagent, and volatiles were evaporated under a stream of nitrogen, followed by filtration through a pipet filled with silica gel. Elution with CH_2Cl_2–MeOH (99.5:0.5) gave pure diacetate **1b** (3.0 mg) as a glass from methanol: mp 82–84 °C; IR (ZnSe) ν_{max} 2960, 1733, 1436, 1370, 1240, 1023, 974, 815, 760, 735 cm^{-1}; HREIMS (*m/z*) 456.3236 (M^+, calcd for $C_{29}H_{44}O_4$ 456.3240). For ^1H NMR spectral data, see Table II.

Variculanol 1- and 17-[(*R*)-*O*-Methylmandelate] (1c and 1d). To a cooled (0 °C) solution of DMF (0.063 mL, 0.081 mmol) in CH_3CN (1 mL) were added oxalyl chloride (0.027 mL of 2 M CH_2Cl_2 solution, 0.053 mmol) and (*R*)-*O*-methylmandelic acid (9 mg, 0.054 mmol). The solution was stirred at 0 °C for 10 min, and then a solution of variculanol (10 mg, 0.027 mmol) in CH_2Cl_2 (1 mL) followed by pyridine (0.009 mL) was added. After 2 h, the mixture was poured onto CH_2Cl_2 (60 mL), the organic solution was washed with 10% citric acid and water and dried (Na_2SO_4), the solvent was evaporated, and the product was chromatographed on a silica gel plate (20 × 20 cm, 500 μm), which was developed in hexane–EtOAc (4:1). Both bands were eluted with EtOAc and then separately filtered through a pipet filled with silica gel. Elution with hexane–EtOAc (9:1 to 7:3) gave pure mandelates **1d** (4 mg) and **1c** (6 mg). **1c**: amorphous solid from CH_2Cl_2–hexane, mp 75–76 °C; IR (ZnSe) ν_{max} 3527, 2930, 1742, 1455, 1369, 1180, 1111, 1016, 875, 738 cm^{-1}; HREIMS (*m/z*) 520.3546 (M^+, calcd for $C_{34}H_{48}O_4$ 520.3553). For ^1H NMR spectra data, see Table II. **1d**: powder from acetone–hexane, mp 65–67 °C; IR (ZnSe) ν_{max} 3567, 2937, 1744, 1650, 1493, 1455, 1388, 1369, 1253, 1177, 1112, 1076, 1046, 997, 953, 924, 872, 846, 825, 735 cm^{-1}; HREIMS (*m/z*) 520.3546 (M^+, calcd for $C_{34}H_{48}O_4$ 520.3553). For ^1H NMR spectra data, see Table II.

Variculanol 1- and 17-[(*S*)-*O*-Methylmandelate] (1e and 1f). Via the procedure just described, **1e** and **1f** were prepared. **1e**: solid from EtOAc–hexane, mp 73–74 °C; IR (ZnSe) ν_{max} 3516, 2931, 1743, 1650, 1496, 1455, 1369, 1316, 1260, 1200, 1179, 1106, 1076, 1017, 939, 914, 874, 847, 826, 732 cm^{-1}; HREIMS (*m/z*) 520.3546 (M^+, calcd for $C_{34}H_{48}O_4$ 520.3553). For ^1H NMR spectra data, see Table II. **1f**: powder from EtOAc–hexane, mp 70–71 °C; IR (ZnSe) ν_{max} 3498, 2932, 1745, 1650, 1495, 1455, 1388, 1369, 1253, 1199, 1178, 1116, 1076, 1049, 1030, 1000, 971, 924, 876, 846, 823, 734 cm^{-1}; HREIMS (*m/z*) 520.3541 (M^+, calcd for $C_{34}H_{48}O_4$ 520.3553). For ^1H NMR spectra data, see Table II.

Variculanol 1- and 17-(4-Bromobenzoate) (1g and 1h) and 1,17-Bis(4-bromobenzoate) (1i). Pyridine (0.1 mL) followed by 4-bromobenzoyl chloride (12 mg, 0.054 mmol) was added to a solution of variculanol (10 mg, 0.027 mmol) in 1 mL of CH_2Cl_2, and the solution was stirred under nitrogen for 16 h. The mixture was concentrated to dryness and then chromatographed over a silica gel plate (20 × 20 cm, 500 μm), which was developed in CH_2Cl_2–MeOH (99:1), and the bands were eluted with CH_2Cl_2–MeOH (95:5) to give pure monobenzoates **1g** (4.6 mg) and **1h** (9.1 mg) (**1g** has a lower R_f than does **1h** on TLC). **1g**: amorphous powder from MeOH, mp 144–145 °C; IR (ZnSe) ν_{max} 3520, 2932, 1717, 1650, 1591, 1485, 1451, 1397, 1369, 1314, 1289, 1271, 1173, 1119, 1105, 1070, 1013, 990, 939, 914, 874, 848, 806, 759, 733 cm^{-1}; HREIMS (*m/z*) 554.2377 (M^+, calcd for $C_{32}H_{43}O_3Br$ 554.2396). For ^1H NMR spectra data, see Table II. **1h**: amorphous solid from acetone–hexane, mp 87–88 °C; IR (ZnSe) ν_{max} 3433, 2936, 1717, 1650, 1590, 1484, 1452, 1434, 1397, 1369, 1330, 1269, 1234, 1173, 1118, 1102, 1070, 1049, 1012, 958, 927, 873, 847, 824, 757 cm^{-1}; HREIMS (*m/z*) 554.2377 (M^+, calcd for $C_{32}H_{43}O_3Br$ 554.2396). For ^1H spectral data, see Table II.

This reaction also produced 0.5 mg of 1,17-bis(4-bromobenzoate) (**1i**), which was quantitatively prepared from a similar reaction but using 100 mg of 4-bromobenzyl chloride. The bis(benzoate) was obtained as an amorphous solid from ethyl acetate–hexane: mp 80–82 °C; IR (ZnSe) ν_{max} 2934, 1718, 1651, 1590, 1484, 1455, 1435, 1397, 1317, 1267, 1173, 1116, 1102, 1070, 1012, 959, 930, 914, 873, 847, 824, 756 cm^{-1}; HREIMS (*m/z*) 736.1755 (M^+, calcd for $C_{39}H_{46}O_4Br_2$ 736.1763); UV λ_{max} (MeOH) 206 (ε 49 126); 244 (ε 36 710); CD λ_{max} (MeOH) 253 ($\Delta\epsilon$ = −23.8), 245 ($\Delta\epsilon$ = 0.0), 236 ($\Delta\epsilon$ = +22.8), 206 ($\Delta\epsilon$ = +34.0). For ^1H NMR spectra data, see Table II.

Acknowledgment. We acknowledge with thanks the help of Mr. Ralph Mosely in collecting MM2 data and Dr. G. Sanyal of West Point for providing CD data.

Supplementary Material Available: ^1H and ^{13}C NMR, ^1H–^1H COSY, ^1H–^{13}C COSY, relayed ^1H–^1H COSY, HMBC, and NOEDS spectra in $CDCl_3$ and Figure 3 (23 pages). Ordering information is given on any current masthead page.