solution of 0.39 g (0.001 mol) of **10a/11a** in 0.4 mL of dry toluene at 50 °C was added, in one portion, 0.21 g (0.0015 mol) of Dibal-H in 1 mL of toluene. Isobutene was evolved as the temperature rose to 80 °C. After 5 min the mixture was cooled, and to it were carefully added 0.1 mL of ethanol, 0.45 mL of water, and 0.225 mL of concentrated hydrochloric acid. The organic layer was decanted, and the residue was extracted with diethyl ether. The combined organic layers were washed with water, 5 N sodium hydroxide solution, and water until neutral, dried, and evaporated to leave 0.25 g of crude material. Filtration through silica with hexane gave 0.19 g of **16** (81%). The compound was pure: ^{1}H NMR, TLC, and HPLC pure: ^{1}H NMR ($CDCl_3$) δ 0.87, 0.91, and 1.15 (3 s, 9, 3 CH_3), 1.06–2.26 (m, 9, 4 CH_2 and CH), 2.61–3.00 (m, 2, $ThCH_2$), 6.56–7.02 (AB, 2, Ar H).

Registry No. **7a**, 20895-79-8; **7b**, 73838-25-2; **7c**, 82112-37-6; (±)-(*E*)-**8a**, 82112-38-7; (±)-(*E*)-**8b**, 82112-39-8; (±)-(*E*)-**8c**, 82112-40-1; (±)-(*Z*)-**9a**, 82112-41-2; (±)-(*Z*)-**9b**, 82112-42-3; (±)-**10a**, 82112-43-4; (±)-**10b**, 82112-44-5; (±)-**10c**, 82112-45-6; (±)-**11a**, 82166-43-6; (±)-**11b**, 82166-44-7; (±)-**11c**, 82112-46-7; (±)-**12a**, 82116-45-8; (±)-**12b**, 82166-46-9; (±)-**13a**, 82166-47-0; (±)-**13b**, 82166-48-1; **14a**, 82112-47-8; (±)-14b, 82112-48-9; (±)-**15**, 82112-49-0; (±)-**16**, 82112-50-3; (±)-**17**, 82112-51-4; (±)-**18**, 82112-52-5; (±)-**19**, 82112-53-6; 1-chloro-3,7-dimethyl-2(*Z*),6-octadiene, 20536-36-1; geranyl chloride, 5389-87-7.

Supplementary Material Available: Table of recrystallization solvents, formulas, and C and H elemental analyses for **8b,c**, **9a,b**, **10c**, **13a,b**, and **17** (1 page). Ordering information is given on any current masthead page.

Microbial Transformations of Natural Antitumor Agents. 21. Conversions of Aphidicolin

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Microbial transformations have been employed as a means of preparing analogues of the diterpene aphidicolin. Microbial transformation products were initially identified by thin-layer chromatography of fermentation extracts and then were prepared by larger scale incubations. Each microbial metabolite was subjected to structure elucidation employing carbon-13 and proton NMR, high-resolution mass spectrometry, and infrared analysis. Metabolites were identified as 3α-acetoxy-16,17,18-trihydroxyaphidicolane, 18-acetoxy-3α,16,17-trihydroxyaphidicolane, 3α,16,17-trihydroxyaphidicolan-18-oate, 16,17,18-trihydroxyaphidicolan-3-one, 3β,16,17,18-tetrahydroxyaphidicolane, and 3α,6β,16,17,18-pentahydroxyaphidicolane. The availability of the microbial metabolites enabled the near complete elucidation of the carbon-13 NMR spectrum of aphidicolin. Biological evaluations of these compounds were made by using in vivo and in vitro techniques. None of the metabolites were active in an in vivo antitumor test system, while all of the compounds inhibited the uptake of thymidine to P-388 leukemic cells in vitro.

Introduction

3α,16,17,18-Tetrahydroxyaphidicolane (aphidicolin, 1, Figure 1) is a novel diterpene produced by species of *Cephalosporium aphidicola*[1] and *Nigrosporum sphaerica*.[2] This compound possesses antiviral[1,3] and antitumor activities[4] while demonstrating a lack of mutagenic activity.[5] Aphidicolin is very specific in inhibiting DNA polymerase α.[6,7] These interesting biological properties have prompted the preparation of various derivatives of aphidicolin[8] as well as partial[9] and total synthetic efforts.[10,11]

The structural complexity of aphidicolin renders the preparation of unusual analogues a difficult task. Aphidicolin analogues would be of interest to exploit the known biological activities and to establish structure activity relationships for the unusual diterpene. Microbial transformations have been widely employed in the preparation of difficult-to-synthesize analogues of steroids and structurally complicated antitumor compounds.[12] This report describes the preparation of six analogues of aphidicolin using microbial transformation technology, the elucidation of most of the carbon-13 NMR spectrum of aphidicolin, and a description of the biological activities of the aphidicolin analogues.

Discussion

The outstanding successes realized in microbial transformations of steroids and of other terpenes[12] indicated that a similar approach with aphidicolin could provide interesting new analogues. A broad program of screening microorganisms for their abilities to achieve useful chemical transformations of aphidicolin was undertaken. Some 220 cultures were examined, and numerous of these pro-

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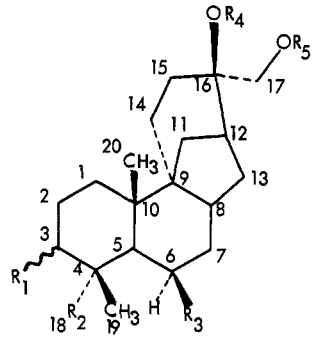

	R_1	R_2	R_3	R_4	R_5
1	α-OH	CH_2OH	H	H	H
2a	α-OH	CH_2OH	H	H	Ac
2b	α-OH	CH_2OAc	H	H	Ac
2c	α-OAc	CH_2OAc	H	H	Ac
2d	α-OH	CH_2OAc	H	H	H
2e	α-OAc	CH_2OH	H	H	H
3	α-OH	COOH	H	H	H
4	=O	CH_2OH	H	H	H
5	β-OH	CH_2OH	H	H	H
6	α-OH	CH_2OH	OH	H	H
7a	α-O-$C(CH_3)_2$-OCH_2-		H	—$C(CH_3)_2$—	
7b	β-O-$C(CH_3)_2$-OCH_2-		H	—$C(CH_3)_2$—	

Figure 1. Structures of aphidicolin (1), aphidicolin derivatives, and microbial metabolites.

vided 6 different metabolites of aphidicolin, some in estimated quantitative yield (TLC). Several of the best metabolite-accumulating cultures were selected for preparative scale work.

Scopulariopsis constantini (UI 1860) consistently provided excellent yields of two major metabolites identified as aphidicolin derivatives **2d** and **2e**. Both compounds exhibited IR (C=O stretching), ^1H NMR (methyl group absorption at 2.1 ppm), ^{13}C NMR (absorbances at 171.5 and 21.3 ppm), and mass spectral properties consistent with the presence of an acetate residue in their structures. Both **2d** and **2e** were interconvertible by heating or by chromatography (two-dimensional TLC). Comparison of ^1H NMR spectra of the metabolite **2d** and **2e** with authentic aphidicolin acetates **2a**, **2b**, and **2c** enabled the assignment of the specific location of acetate functional groups. Acetylation of aphidicolin at positions 3 or 18 results in a downfield shift of carbinol protons at these positions of 1.2 and 0.6 ppm, respectively. Signals for the proton at the 3-position were very similar in **2c** and **2e**, indicating that this metabolite was a 3-acetoxy derivative of aphidicolin, and signals for protons at the 18-position were very similar in **2b** and **2d**, showing that the metabolite was an 18-acetoxy analogue of aphidicolin.

Trichothecium roseum (UI 320) was used to produce 3α,16,17-trihydroxyaphidicolan-18-oate (aphidicolin-18-carboxylic acid, **3**) in 30% yield. The IR and mass spectra of **3** and its solubility characteristics supported the presence of a carboxylic acid functional group. Signals for protons at the 18-position were absent in the ^1H NMR spectrum of the metabolite; the ^{13}C NMR spectrum indicated the loss of the triplet signal at 71.1 ppm for C-18 and displayed a new singlet at 171.9 ppm for a carboxyl carbon atom. Additional evidence supporting the presence of a carboxyl group at the 18-position was obtained by ^1H NMR, where protons at positions 3 and 19 were shifted downfield by 0.27 and 0.69 ppm, respectively.

Streptomyces griseus (ATCC 13273) and *Chaetomium funiculum* (UI 141) both formed a metabolite identified as 16,17,18-trihydroxyaphidicolan-3-one (3-ketoaphidicolin, **4**). The IR spectrum (C=O stretching), chemical behavior (2,4-dinitrophenylhydrazine on TLC), and mass spectral (*m/e* 305, 2 mass units less than aphidicolin) behavior all supported the structure as **4**. The loss of the proton signal at the 3-position was evident in the ^1H NMR spectrum, and the ^{13}C NMR spectrum of **4** contained a new singlet signal at 217.1 ppm, consistent with the presence of a carbonyl carbon atom. At the same time, this spectrum also revealed the loss of a doublet signal at 76.1 ppm for the carbon at the 3-position of aphidicolin. *Streptomyces griseus* provided a mixture of oxidation products of aphidicolin giving **3** and **4** in 40% and 26% yields, respectively, while *C. funiculum* gave **4** in 53% yield.

C. funiculum also formed 3β,16,17,18-tetrahydroxyaphidicolane (3-epiaphidicolin, **5**) in the same incubation mixture. The mass spectrum of **5** and its IR spectrum were nearly identical with those of aphidicolin. When incubated with the 3-keto derivative of aphidicolin (**4**), a 3:2 (by TLC) mixture of **5** and **1** was produced. The ^1H NMR spectrum of **5** indicated an upfield shift of the 3-position proton relative to aphidicolin, consistent with the change of a proton from an equatorial to an axial orientation.[13] This is supported by the upfield shift observed for the methyl carbon atom at the 19-position in the ^{13}C NMR spectrum of **5** as expected through a γ interaction.[14] The upfield shift of the proton at the 3-position was also evident in the spectrum of bis-acetonides **7b** vs. **7a** prepared from **1** and **5**, respectively.

Unambiguous chemical shift assignments for aphidicolin were made earlier[15] for carbons at positions 3, 9, 12, 15, 16, 17, 18, 19, and 20, and these were nearly identical with our measurements using pyridine-d_5 (Table I). Analysis of the ^{13}C spectra of the foregoing metabolites enabled the clear-cut assignment of resonances for carbons at positions 1, 2, 4, 5, 6, 8, and 10. For example, of the unassigned quaternary resonances,[15] only that for C-4 should have been strongly influenced by the structural changes described for our metabolites. Thus, the signal at 40.8 ppm belongs to C-4 and that at 40.0 ppm is assigned to C-10. Of the two unassigned methyne carbons C-5 and C-8, only C-5 could have been strongly affected by the reported structural changes. Thus, the signal at 33.9 ppm belongs to C-5 and that at 40.3 ppm is assigned to C-8. The downfield shift of 5 ppm for C-5 in compounds **4** and **5** is most likely due to the relief of the γ interaction[14] present in **1** between the 3-α-hydroxyl group and C-5.

Among the methylene carbons, a similar downfield shift would be expected for C-1 in compounds **4** and **5** due to relief of its γ interaction with the 3-α-hydroxyl group of aphidicolin. The appearance of new signals at 31.4 and 32.5 ppm in **4** and **5**, respectively, allows the assignment of one signal at 27.2 ppm in **1** to C-1. For C-2, downfield shifts of about 9 ppm in **4** and 2 ppm in **5** were expected.[16] Appropriate new signals were present at 37.1 and 28.5 ppm in **4** and **5**, respectively. Thus, another signal at 27.2 ppm in **1** could be assigned to C-2. In accordance with Adams

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Table I. Carbon-13 NMR Spectral Data for Aphidicolin and Aphidicolin Metabolites

carbon	1[a]	1[b]	2d[b]	3[b]	4[b]	5[b]	6[b]	8[c]
1	27.4[d] t	27.2 t	27.2 t	26.8[d] t	31.4 t	32.5 t	29.4 t	26.4[d] t
2	27.6[e] t	27.2 t	27.2 t	27.6[d] t	37.1 t	28.5[d] t	27.3 t	26.7[e] t
3	76.9 d	76.1 d	70.2 d	73.0 d	217.1	73.4 d	78.1 d	76.9 d
4	41.2	40.8	41.0	51.8	53.3	43.3	41.8	40.6
5	41.7 d	33.9 d	37.6 d	35.9 d	38.6 d	38.7 d	37.3 d	41.3 d
6	23.9[e] t	23.4 t	24.1 t	26.6[d] t	25.2[d] t	23.7 t	68.4 d	22.6[e] t
7	27.1[d] t	27.2 t	27.2 t	26.8[d] t	26.4 t	27.0 t	37.6 t	26.0[d] t
8	34.6 d	40.3 d	40.4 d	40.8 d	40.5 d	40.3 d	34.2 d	32.8 d
9	50.0	49.5	49.8	49.8	48.7	49.3	49.6	49.1
10	40.6	40.0	40.5	40.0	39.2	40.0	41.1	39.6
11[d]	33.5[e] t	33.2 t	33.5 t	33.2 t	33.3 t	33.7 t	33.7 t	34.0[e] t
12	41.0 d	41.9 d	42.2 d	42.0 d	41.8 d	42.0 d	42.2 d	48.3 d
13[d]	32.1[e] t	31.6 t	32.0 t	31.6 t	31.4 t	31.6 t	31.7 t	31.7[e] t
14	25.3[e] t	25.4 t	25.5 t	25.6 t	25.6[d] t	25.3 t	25.4 t	21.6[e] t
15	28.3 t	28.9 t	29.1 t	29.0 t	28.7 t	28.9[d] t	28.8 t	34.5 t
16	77.3	74.2	74.6	74.2	74.1	74.2	74.3	207.2
17	68.1 t	68.1 t	68.6 t	68.2 t	68.3[d] t	68.3 t	68.3 t	
18	17.8[f] q	71.1 t	72.5 t	179.9	68.7[d] t	67.8 t	72.4 t	17.6[f] q
19	71.5[f] t	17.9 q	17.5 q	18.0 q	18.6 q	13.2 q	20.7 q	71.5[f] t
20	15.5 q	15.3 q	15.5 q	15.6 q	14.9 q	15.6 q	18.5 q	15.7 q
misc			171.5, 21.3 (OAc)					

[a] Reference 15, CD_3CO_2D. [b] Pyridine-d_5 at 22.635 MHz. [c] Reference 15, $CDCl_3$. [d] Values within any vertical column may be interchanged. [e] Resonances not assigned by Adams and Bulock, ref 15. [f] Assignments should be interchanged.

and Bu'Lock,[15] the remaining signal at 27.2 ppm in 1 was assigned to C-7. Of the remaining unassigned methylene carbon signals, only that at 23.4 ppm in 1 was affected by the structural changes observed in the metabolites. This signal could thus be assigned to C-6. Assignments given for the three remaining methylene carbons at 11, 13, and 14 are based on structural considerations and the ^{13}C NMR spectral differences between aphidicolin and the 17-*nor*-16-keto derivative (8) (Table I).

Streptomyces punipalus (NRRL 3529) gave 54% yield of 3α,6β,16,17,18-pentahydroxyaphidicolane (6β-hydroxyaphidicolin, 6). The presence of an additional oxygen atom in the metabolite was evident from the mass spectrum. Signals in the ^1H NMR spectrum (4.35 ppm, 1 H) and ^{13}C NMR spectrum (doublet at 68.4 ppm) demonstrated that hydroxylation had occurred on one of eight possible methylene carbon atoms. Protons of methyl groups at the 19- and 20-positions experienced downfield shifts of 0.74 and 0.61 ppm, respectively, in the ^1H NMR spectrum, and a similar effect was observed in the ^{13}C NMR spectrum, where signals for carbons 19 and 20 were shifted downfield by 2.8 and 3.2 ppm, respectively. The anisotropic effect[17] observed in the ^1H NMR spectrum and the δ-synaxial effect[18] observed in the ^{13}C NMR spectrum of the metabolite were suggestive of hydroxylation at either 2β or 6β.

6β-Hydroxylation in androstanes results in an upfield shift in the signal for the carbon at the 8-position (6 ppm) and downfield shifting in signals for carbon atoms at positions 1 (2 ppm), 5 (3 ppm), 6 (43 ppm), and 7 (7 ppm).[16] The effect at the 1-position is an indirect one mediated by the 1,3-diaxial interaction between the angular methyl and 6β-hydroxyl groups. In aphidicolin, the 4β-methyl carbon atom would also experience a 1,3-diaxial interaction with a 6β-hydroxyl group, which should indirectly cause a 2-ppm downfield shifting for C-3. Following these arguments, the ^{13}C NMR spectrum of 6 is readily explained by the presence of a 6β-hydroxyl group. Downfield shifts for carbons 1 (2.2 ppm), 3 (2.0 ppm), 5 (3.2 ppm), 6 (45 ppm), and 7 (10.4 ppm) and an upfield shift for carbon 8 (6.1 ppm) are all consistent with the site of hydroxylation as 6β, and these results are different than what would occur with 2β-hydroxylation.[16,19]

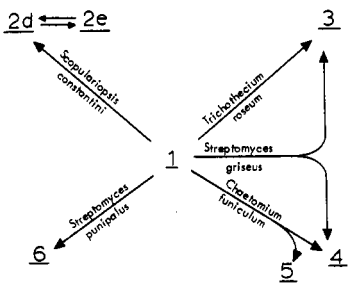

Figure 2. Summary of microbial transformation reactions observed with aphidicolin.

Microbial transformation reactions observed with aphidicolin reveal the richness with which microorganisms achieve chemical transformations of structurally complicated substrates. Examples of regioselective oxidation and acylation are evident in this work. Within some microorganisms such as *C. funiculum*, multiple transformations occur including initial C-3 alcohol oxidation and subsequent reduction of the ketone to a mixture of epimeric alcohol products. The transformations observed with aphidicolin are summarized in Figure 2.

Biological Activity. In vivo evaluation of the various aphidicolin analogues in the 06C631 colon test system in the rat led to uniformly disappointing results. None of the analogues were active at the following dosage levels: 18-acetoxyaphidicolin (NSC 339660, 200 mg/kg), 6β-hydroxyaphidicolin (NSC 340292, 200 mg/kg), aphidicolin-18-carboxylic acid (NSC 342436, 80 mg/kg), and 3-ketoaphidicolin (NSC 346198, 200 mg/kg). The 3-epiaphidicolin analogue has been submitted, but test results have not yet been obtained. It is noteworthy that aphidicolin demonstrates clear-cut activity vs. the 06C631 colon system at the dosage levels indicated.

An in vitro P-388 antileukemia test system described by Fuska et al.[20] was also utilized. This sensitive system

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Table II. Effect of Aphidicolin and Its Metabolites on the Incorporation of ^{14}C-Labeled Precursors into P-388 Cells in Vitro

compd	concn, μg/mL	% inhibition of incorporation of adenine	thymidine	uridine
1	200	35.2	62.0	18.7
	100	10.4	61.4	6.6
2d	200	50.5	72.9	47.7
	100	26.8	67.8	19.9
3	200	8.8	56.2	0
	100	(−)20.0[a]	51.6	(−)7.8[a]
4	200	48.1	62.9	28.2
	100	23.0	58.9	21.4
5	200	19.3	60.1	12.8
	100	6.9	53.9	5.9
6	200	10.3	58.8	2.5
	100	(−)4.5[a]	49.7	(−)8.2[a]

[a] Compounds causing a stimulation of uptake of radiolabeled precursor.

measures the abilities of compounds to inhibit the uptake of radiolabeled adenine, valine, thymidine, and uridine by in vitro grown P-388 leukemic cells. Evaluations were conducted by using levels of 100 and 200 μg/mL of each compound, including aphidicolin, in two separate tests. All incubations were compared to controls containing no antitumor drug. The results of these tests are shown in Table II. Aphidicolin and its analogues had no effect on the uptake of L-valine by tumor cells. Since the recognized mode of action of aphidicolin is through specific inhibition of DNA polymerase α, an inhibition in the uptake of nucleotide bases would be expected. The greatest effect for all compounds was measured on thymidine uptake by the P-388 cells. All of the aphidicolin analogues were similar in their inhibition of thymidine uptake at both levels tested. The slightly enhanced activity of **2d** may be explained by enhanced passive transport of the more lipophilic ester derivative of aphidicolin into the P-388 cells. None of the compounds caused a similar inhibition in adenine uptake and utilization by the cells. These compounds are apparently the first to exhibit this type of activity. Certain 3-benzazepines inhibit [^{14}C]uridine utilization without affecting adenine uptake in this system.[21]

Experimental Section

General. Melting points were obtained in open-ended capillaries and are uncorrected. Infrared spectra were obtained in KBr discs in a Beckman Model 4240 spectrophotometer. NMR spectra were taken in either pyridine-d_5 or in $CDCl_3$ with Me_4Si as an internal standard. Proton magnetic resonance spectra were taken with a Varian EM360 spectrometer, while ^{13}C NMR spectra were taken on a Bruker HX-90E instrument. Most low-resolution mass spectra were obtained with a Finnigan Model 3200 spectrometer using a direct inlet probe. Low-resolution mass spectra for **7a** and **7b** were obtained on a Hewlett-Packard 5985 GC/MS system using a direct inlet probe. High-resolution mass spectra were obtained through the Midwest Center for Mass Spectrometry, Chemistry Department of the University of Nebraska. Elemental analyses were provided by Integral Micro-analytical Laboratories, Inc., Raleigh, NC.

Chromatography. Analytical thin-layer chromatography (TLC) was performed on activated 0.25-mm layers of silica gel GF_{254} (Merck). Plates were developed in a mobile phase of $CHCl_3$–EtOH (5:1), and chromatograms were visualized by using a *p*-anisaldehyde spray reagent (0.5:60:1 *p*-anisaldehyde–HOAc–concentrated H_2SO_4) followed by warming with a heat gun.

Open-column chromatography was performed on silica gel (Baker 3404, 60-200 mesh) slurry packed in chloroform into glass tubes. Samples were generally applied to columns by preadsorption from solutions in $CHCl_3$ or $CHCl_3$–EtOH (9:1).

High-performance liquid chromatography was performed with a Waters ALC/GPC 202 instrument equipped with an M6000 solvent delivery system, a U6K Universal Injector, and an R401 differential refractometer detector. Preparative separations of aphidicolin (1) and 3-epiaphidicolin (5) were obtained on a 0.94 × 50 cm Whatman Partisil M9 ODS column linked in series to a 0.21 × 7 cm guard column packed with Co:Pell ODS with a solvent of MeOH–H_2O (7:3) at a flow rate of 2 mL/min and an operating pressure of 1200 psi. Retention volumes of 5 and 1 were 41.4 and 51 mL, respectively, with this system.

Aphidicolin. Aphidicolin (1) was provided by Imperial Chemical Industries, Ltd., and possessed physical properties identical with those reported in the literature,[1] including ^{1}H NMR (pyridine-d_5) [δ 0.78 (s, 3 H, 19-CH_3), 1.02 (s, 3 H, 20-CH_3), 3.54 and 3.88 (dd, *J* 10 Hz, 2 H, 18-CH_2OH), 3.76, (s, 2 H, 17-CH_2OH), 3.96 (br, s, 1 H, H-3)]. Authentic aphidicolin acetates[1] (**2a–c**) were obtained from Imperial Chemical Industries.

Fermentation Methods. Incubations were conducted according to a standard two-stage fermentation procedure.[22] All cultures used in this work are maintained and deposited in the University of Iowa, College of Pharmacy Culture Collection. Aphidicolin was added to a final medium concentration of 0.5 mg/mL in a 5% solution in dimethylformamide, and cultures were incubated for varying periods of time. Samples of substrate-contaning cultures (4 mL) were withdrawn at various time intervals and extracted with 1.0 mL of ethyl acetate–1-butanol (7:3), and 10 μL of the extracts were examined by TLC. All preparative scale incubations were conducted with 3 g of aphidicolin and with protocols established on smaller scale screening experiments.

Production of 2d and 2e from Aphidicolin by *Scopulariopsis constantini* (UI 1860). After substrate addition to stage II cultures, fermentation was continued for 2 days when cells were separated from the fermentation beer and were exhaustively extracted with methanol. The filtrate was extracted with ethyl acetate. Chromatography of the cell and culture filtrate extracts on separate silica gel columns (4 × 46 cm, 210 g) using $CHCl_3$–EtOH (15:1) gave a combined weight of 3.5 g of 90% pure metabolites, neither of which were completely free from the other. Two-dimensional TLC indicated that the two metabolites were interconverting during chromatography, and simple warming in solution also resulted in their interconversion. Purest preparations were obtained by rapid column chromatography over several small silica gel columns.

2d: R_f 0.65; no distinct melting point; IR 3430 (OH), 2935, 2860, 1735, 1718 (C=O), 1250, 1040 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.92 (s, 3 H, 19-CH_3), 0.98 (s, 3 H, 20-CH_3), 2.08 (s, 3 H, $COCH_3$), 3.50 (s, 2 H, 17-CH_2OH), 3.63 (br s, 1 H, 3-H), 3.93 and 4.17 (dd, *J* = 10 Hz, 2 H, 18-CH_2OH); ^{13}C NMR (pyridine-d_5), see Table I; high-resolution mass spectrum, *m/e* 349.2376 (M − CH_2OH for $C_{21}H_{34}O_4$, 100%, calculated at 349.2380), 320 (3.6), 302 (20), 289 (8.9), 284 (6.9), 275 (4.6), 272 (14), 271 (35).

2e: R_f 0.80; no distinct melting point; IR 3420 (OH), 2935, 2860, 1717 (C=O), 1260, 1040, 752 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.95 (s, 3 H, 19-CH_3), 1.03 (s, 3 H, 20-CH_3), 2.13 (s, 3 H, $COCH_3$), 3.37 (s, 2 H, 18-CH_2OH), 3.47 (s, 2 H, 17-CH_2OH), 4.85 (br s, 1 H, H-3); low-resolution mass spectrum, *m/e* 349 (M − CH_2OH, 100%), 320 (4.8), 302 (2.8), 290 (9.7), 289 (7.8), 275 (7.8), 272 (4.8), 271 (15). No ^{13}C NMR or high-resolution mass spectrum was obtained on this unstable compound.

Production of 3 from Aphidicolin by *Trichothecium roseum* (UI 320). Fermentation was continued for 4 days when cultures were adjusted to pH 3.0 and cells were separated from the fermentation beer. Cells and culture filtrates were extracted exhaustively with EtOAc. The combined extracts were adsorbed onto silica gel and subjected to silica gel column chromatography (4 × 46 cm, 210 g) eluting with $CHCl_3$–EtOH–HCOOH (90:10:0.1) to give 1.0 g of the clean metabolite (R_f 0.35), which crystalized in prisms from MeOH; mp 232–234 °C; IR 3400 (br extending to 2200), 2955, 2950, 2865, 1702 (C=O), 904 cm^{-1}; ^{1}H NMR

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(pyridine-d_5) δ 1.07 (s, 3 H, 20-CH_3), 1.47 (s, 3 H, 19-CH_3) 3.73 (s, 2 H, 17-CH_2OH), 4.23 (br s, 1 H, H-3); mass spectrum, m/e 321.2062 (M - CH_2OH for $C_{19}H_{29}O_4$, 100%, calculated at 321.2067), 316 (7.8), 307 (6.8), 291 (11), 275 (9.7), 273 (30).

Production of 3 and 4 from Aphidicolin by *Streptomyces griseus* (ATCC 13273). Fermentation was continued for 3 days when whole cultures were adjusted to pH 3.3 with 6 N HCl and cells were separated from the beer by centrifugation (10000*g* for 10 min). Cells and culture supernatants were extracted exhaustively with EtOAc–*n*-BuOH (9:1). Extracts were combined and purified by column chromatography over silica gel (4 × 46 cm, 210 g) by using $CHCl_3$–EtOH–HOAc (10:1:0.1) as the eluting solvent to obtain the major metabolite (*Rf* 0.35, 1.2 g), which was crystalized from MeOH in prisms, mp 233–235 °C. The compound was identical with **3** produced by *T. roseum* (IR, mass spectrum ^1H NMR, mixture melting point). A second metabolite (R_f 0.65) was purified by a second column and by preparative TLC to yield an analytical sample identical wity **4**, the major metabolite formed by *C. funiculum* (see below).

Production of 4 and 5 from Aphidicolin by *Chaetomium funiculum* (UI 141). Fermentations were continued for 4 days when cells were separated from cultures by filtration and exhaustively extracted with MeOH. The filtrate was extracted with EtOAc–*i*-PrOH (9:1), and the combined extracts were purified by chromatography over silica gel (4 × 46 cm, 210 g) by elution with $CHCl_3$–EtOH (10:1) to give 1.6 g of pure metabolite **4** and 0.6 g of **5** (R_f 0.5). The latter fraction was contaminated with approximately 20% aphidicolin.

The analytical sample of **4** was obtained by crystalization from EtOAc–hexanes and further recrystalization from acetone; mp 137–139 °C; IR 3400 (OH, br), 2930, 2860, 1689 (C=O), 1035, 960 cm^{-1}; ^1H NMR (pyridine-d_5) δ 0.95 (s, 3 H, 19-CH_3), 1.02 (s, 3 H, 20-CH_3), 3.78 (s, 2 H, 17-CH_2OH), 4.08 and 3.62 (dd, J = 10 Hz, 2 H, 18-CH_2OH); ^{13}C NMR, see Table I; mass spectrum, m/e 305.2109 (M - CH_2OH for $C_{19}H_{29}O_3$, 77%, calculated at 305.2118), 275.2013 ($C_{18}H_{27}O_2$, 100%).

The analytical sample of **5** was obtained by preparative HPLC and by recrystallization from EtOAc: mp 193.5–195.5 °C; IR 3380 (OH, br), 2940, 2865, 1060, 1037, 962 cm^{-1}; ^1H NMR (pyridine-d_5) δ 1.06 (s, 6 H, 19- and 20-CH_3), 3.58 (br s, 1 H, H-3), 3.77 (s, 2 H, 17-CH_2OH), 4.03 and 4.32 (dd, 2 H, 18-CH_2OH); ^{13}C NMR, see Table I; mass spectrum, m/e 307.2268 (M - CH_2OH for $C_{19}H_{31}O_3$, 100%, calculated at 307.2274), 289 (5.5), 271 (12).

Production of 6 from Aphidicolin by *Streptomyces punipalus* (NRRL 3529). Fermentation was continued for 3–5 days when cells were separated from the beer and extracted once with EtOAc–*n*-BuOH (9:1) and then exhaustively with EtOAc. The culture filtrate was extracted exhaustively with EtOAc. The extracts were combined and subjected to column chromatography on silica gel (4 × 46 cm, 210 g) by eluting with 200 mL of $CHCl_3$ and then mixtures of varying proportions of $CHCl_3$–EtOH to obtain 1.7 g of the metabolite **6**. The analytical sample of **6** was obtained by crystalization from EtOAc; R_f 0.40; mp 185.5–187.5 °C; IR 3410 (OH), 2950, 2907, 2868, 1036 cm^{-1}; ^1H NMR (pyridine-d_5) δ 1.52 (s, 3 H, 19- or 20-CH_3), 1.63 (s, 3 H, 19- or 20-CH_3), 3.82 (s, 2 H, 17-CH_2OH), 3.95 (s, 2 H, 18-CH_2OH), 4.13 (br s, 1 H, H-3), 4.35 (br, 1 H, H-6); ^{13}C NMR (pyridine-d_5), see Table I; mass spectrum, m/e 323.2216 (M - CH_2OH for $C_{19}H_{31}O_4$, 100%, calculated at 323.2223), 318 (7.8), 305 (22), 303 (38), 287 (16), 275 (7.3).

Synthesis of the Bis Acetonides of Aphidicolin (1) and 3-Epiaphidicolin (5). The bis acetonides of **1** and **5** were prepared as previously described.[1] 3α,18;16,17-Bis(isopropylidene)dioxyaphidicolane (**7a**) was obtained as white needles and was identical with the compound reported in the literature: mp 144–145 °C; ^1H NMR ($CDCl_3$) δ 0.72 (s, 3 H, 19-CH_3), 0.98 (s, 3 H, 20-CH_3), 1.38 (s, 4 × CH_3), 3.23, 3.68 (dd, J = 12 Hz, 2 H, 18-CH_2O), 3.69 (br s, 1 H, H-3), 3.56 and 3.81 (dd, J = 8 Hz, 2 H, 17-CH_2O); low-resolution mass spectrum, m/e 403 (M - CH_3, 100%). Anal. Calcd for $C_{26}H_{42}O_4$: C, 74.60; H, 10.11. Found: C 74.13; H, 10.32.

3β,18;16,17-Bis(isopropylidene)dioxyaphidicolane (**7b**) was obtained as white needles and gave the following data: mp 212.5–215 °C; ^1H NMR ($CDCl_3$) δ 0.99 (s, 3 H, 20-CH_3), 1.07 (s, 3 H, 19-CH_3), 1.42 (s, 4 × CH_3), 3.36 (br, 1 H, H-3), 3.50 (s, 2 H, 18-CH_2O), 3.80 and 3.55 (dd, 8 Hz, 2 H, 17-CH_2O); mass spectrum, m/e 403 (M - CH_3, 30%). Anal. Calcd for $C_{26}H_{42}O_4$: C, 74.60; H, 10.11. Found: C, 74.36; H, 10.18.

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