mass determination, calcd for $C_{18}H_{25}NO_4$ 319.1783, found 319.1780.

***cis*-1-(4-Methoxyphenyl)-3(e),4(e)-diacetoxyquinolizidine (16). Method A.** Monoacetate **15** (13 mg, 0.04 mmol) was dissolved in pyridine (0.5 mL) and acetic anhydride (0.5 mL) was added. The reaction mixture was stirred at room temperature for 10 h and concentrated in vacuo. Dichloromethane (25 mL) was added and the mixture washed with saturated aqueous sodium bicarbonate and water. The organic extract was dried and concentrated in vacuo to provide **16** as an oil (13 mg, 88%).

Method B. A mixture of *cis*-3,4-epoxy-1-(4-methoxyphenyl)quinolizidine (**14**) (225 mg, 0.869 mmol), acetic acid (2.5 mL), and acetic anhydride (2.5 mL) was stirred, at 23 °C, for 3 days. The solution was concentrated in vacuo, dichloromethane (50 mL) and saturated aqueous sodium bicarbonate (50 mL) were added, and the mixture was stirred for 2 h at 23 °C. The organic layer was separated and washed consecutively with saturated aqueous sodium bicarbonate and water, dried, and concentrated to provide **16** as an oil (240 mg, 77%). The product was further purified by flash chromatography to provide **16** (154 mg, 49%): IR (Neat) 1735 (C=O); NMR (270 MHz, $CDCl_3$) δ 1.25–2.90 (multiplets, 11 H), 2.040 and 2.048 (two s, 6 H, $COCH_3$), 3.81 (s, 3 H, OCH_3), 4.00 (t, 1 H, 1-H, J = 5.1 Hz), 5.09–5.19 (m, 2 H, 3-H, 4-H), 6.88 and 7.38 (A_2B_2 q, 4 H, Ar H, J = 8.8 Hz); MS, (relative intensity) m/z 361 (5), 302 (20), 301 (13), 243 (11), 242 (70), 159 (96); accurate mass determination, calcd for $C_{20}H_{27}NO_5$ 361.1889, found 361.1894.

***trans*-1-(4-Methoxyphenyl)-3(a),4(a)-diacetoxyquinolizidine (17).** Trifluoroacetic anhydride (0.9 mL, 6.4 mmol) was added dropwise to a solution of hydrogen peroxide (50% aqueous, 0.1 g) in dichloromethane (20 mL) at 0 °C. The reaction mixture was stirred for 3 h at 23 °C, and a solution of *trans*-1-(4-methoxyphenyl)-3,4-didehydroquinolizidine (**8**, 0.48 g, 2.00 mmol) in trifluoroacetic acid (1 mL) was added at ambient temperature. The mixture was stirred for 6 days at ambient temperature. Saturated aqueous sodium bisulfite was added, and the separated organic layer was washed consecutively with aqueous sodium bicarbonate and water and then dried. The residue obtained after concentration in vacuo was dissolved in acetic acid (2.5 mL) and acetic anhydride (2.5 mL) and stirred at 23 °C for 3 days. After concentration in vacuo, the residue was dissolved in dichloromethane (25 mL) and washed consecutively with saturated aqueous sodium bicarbonate and water. The organic extract was dried, filtered, and concentrated in vacuo to provide a thick oil (0.41 g, 57%), which on flash chromatography gave a product (**17**, 122 mg, 17%): IR (neat) 2700–2900 (Bohlmann bands), 1730 (C=O) cm^{-1}; NMR (80 MHz, $CDCl_3$) δ 1.0–2.9 (m, 11 H), 2.05 and 2.10 (two s, 6 H, $COCH_3$), 3.10 (d of d, 1 H, 1-H, J_1 = 4 Hz, J_2 = 11 Hz), 3.72 (s, 3 H, OCH_3), 4.75 (m, 2 H, 3-H, 4-H), 6.75 and 7.15 (A_2B_2 q, 4 H, Ar H, J = 8 Hz); MS, m/z (relative intensity) 361 (5), 302 (20), 301 (12), 243 (10), 242 (55), 159 (80); accurate mass determination, calcd for $C_{20}H_{27}NO_5$ 361.1889, found 361.1861.

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Registry No. **1b**, 40179-98-4; **3**, 86933-78-0; **4**, 86933-77-9; **5** (isomer 1), 87781-81-5; **5** (isomer 2), 87781-91-7; **6** (isomer 1), 87781-82-6; **6** (isomer 2), 87781-92-8; **7**, 87781-83-7; **8**, 87781-85-9; **9**, 87781-84-8; **10**, 87781-86-0; **11a**, 87781-79-1; **11b**, 86880-50-4; **12a**, 87781-80-4; **12b**, 86880-49-1; **14**, 87781-87-1; **15**, 87781-88-2; **16**, 87781-89-3; **17**, 87781-90-6; 2-piperidylpropanone, 4396-01-4; *p*-anisaldehyde, 123-11-5; (*p*-toluenesulfonyl)hydrazide, 1576-35-8.

Molecular Structures of the Briantheins, New Insecticidal Diterpenes from *Briareum polyanthes*[1]

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Extracts of the soft coral *Briareum polyanthes*, recently found for the first time in Bermudian waters, have yielded three related diterpenes, briantheins X, Y, and Z. Detailed analysis of the spectral data and chemical correlation of briantheins X and Z established the gross structures of these highly oxidized, chlorinated diterpenes; X-ray diffraction studies confirmed the placement of the butyrate ester in briantheins Y and defined the absolute configuration for the trio. The chemotaxonomic significance and the insecticidal activity of the briantheins is discussed.

The soft coral *Briareum asbestinum* is widely distributed and fairly abundant in Caribbean waters and has been the subject of chemical investigation by several groups. These studies have revealed a rich diterpene chemistry and have resulted in the identification of briarein A (**1**)[2] and the asbestinins.[3] Briarein A possesses a novel carbon skeleton which has since been observed only in compounds from sea pens (distantly related coelenterates): stylatulide (**2**)[4] from *Stylatula sp.*, ptilosarcone (**3**)[5] from *Ptilosarcus gurneyi*, and **4**–**6** from *Scytalium tentaculatum*[6] (Chart I).

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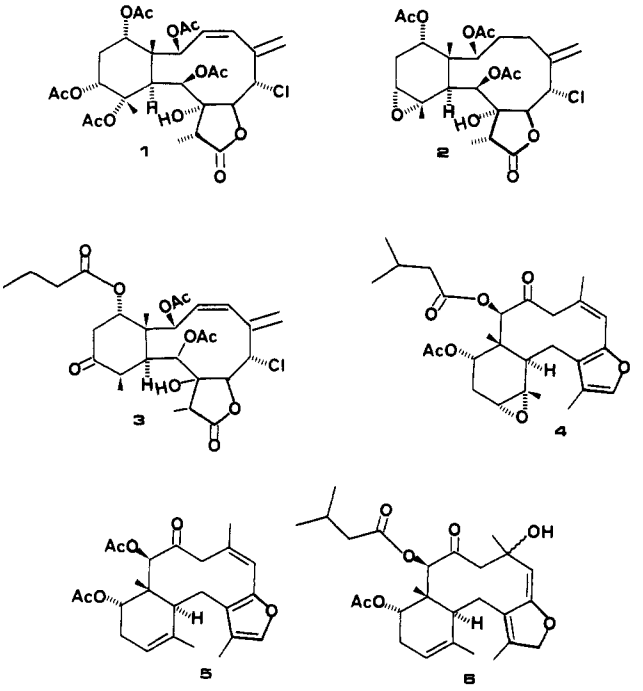
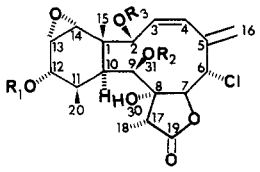
Chart I



We recently proposed structure 7 for brianthein Z, a novel diterpene[7] isolated from *Briareum polyanthes*, a species of confused taxonomy, variously described as a morphological variant of *B. asbestinum*, a different species of *Briareum*, or a member of an altogether distinct genus.[8] We can now confirm the structure and stereochemistry of 7 and fully describe two related compounds, 8 and 9.



7 $R_1=R_2=R_3=Ac$

8 $R_1=R_2=Ac$
$R_3=COCH_2CH_2CH_3$

9 $R_1=R_2=Ac$
$R_3=H$

Results and Discussion

Although it had long been believed that no *Briareum* specimens existed in Bermudian waters, one of us (J.H.C.) discovered a fairly extensive community of *Briareum polyanthes* growing at the eastern end of the archipelago in 1979. The collected specimens were extracted successively with acetone and dichloromethane, and the combined, reduced extracts were distributed between water and dichloromethane. The organic-soluble extracts were then bulk separated by means of a solvent partitioning scheme popularized by Kupchan.[9] ^1H NMR analysis of the carbon tetrachloride and chloroform soluble fractions indicated the presence of significant quantities of terpenoid constituents.

Step gradient gel permeation chromatography[10] of the chloroform-soluble extracts was utilized as a first separation; the first elution phase, employing hexane–dichloromethane (1:4), gave five fractions. ^1H NMR analysis suggested that fractions 2 and 4 were comprised largely of terpenoids. Separation and purification of the individual components was achieved by additional gel permeation chromatography, followed by HPLC on a nitrile-bonded phase column (Ultrasphere-Cyano, eluted with hexane–2-propanol, 2:1). Briantheins Z and Y (7 and 8) were obtained from fraction 2 and brianthein X (9) was isolated from fraction 4 (vide supra).

Among the salient features of the structure elucidation of brianthein Z (7) were a positive Beilstein test and the indication of a γ-lactone in the infrared spectrum. Partial structures developed from ^1H NMR decoupling experiments were reminiscent of the briaran skeleton, but those NMR data also indicated some distinct differences in the cyclohexane ring portion of the molecule. The functional array on the cyclohexane ring was revealed by consideration of ^1H and ^{13}C NMR chemical shifts and coupling constants together with the molecular formula, deduced from nominal mass and ^{13}C NMR spectral data. The only way to accommodate all the sp^3 carbons bearing heteroatoms was to incorporate an epoxide into the structure; correct placement was secured by SFORD experiments and ^1H NMR chemical shifts.

The most polar of the three compounds, brianthein X (9) differed from 7 by the absence of one acetate group, the shift of a one-proton signal from δ 6.22 in 7 to δ 5.23 in 9, and a corresponding shift of an sp^3 methine from δ 75.66 to 72.16. These data indicated that the acetate at C(2)[11] in 7 was replaced by a hydroxyl group in 9. This conclusion and the relationship between the two compounds was confirmed by acetylation of 9 to give a product identical with 7 (by ^1H NMR and melting point).

Brainthein Y (8), the most abundant diterpene, differed from 7 only in the high-field regions of their respective ^1H and ^{13}C NMR spectra. While four ester-type carbonyls persisted in the ^{13}C NMR, only two acetate methyl signals were observed near δ 2.0 in the ^1H NMR spectrum of 8; instead, a methyl triplet at δ 0.92, a two-proton multiplet at δ 1.64, and additional integral strength in the complex pattern of overlapping signals around δ 2.2 suggested a butyrate ester. The presence of two additional sp^3 methylenes in the ^{13}C NMR of 8 and its increased molecular weight (568/570) substantiated this finding. Tables I and II offer a tabulated comparison of the ^1H and ^{13}C NMR data of the briantheins X, Y, and Z.

Mass spectral analyses of the three compounds yielded no molecular ions in either the electron-impact or chemical-ionization mode. Fast atom bombardment mass spectrometry, using a glycerol–potassium iodide matrix, did give very weak, but discernible, $[M + K]^+$ ions.

The location of the butyrate ester, however, remained to be assigned. The structure of ptilosarcone (3) suggested a position of attachment on the cyclohexane ring, in this case C(12), but direct evidence for such an assignment did not appear to be at hand. In order to determine both the position of the butyrate and the stereochemical configuration of these new compounds, X-ray diffraction analysis of 8 was undertaken.

The X-ray experiment showed the diterpenoid molecule to have a molecular constitution similar to that of briarein A, with the butyrate ester located at C(2) (Figure 1). This

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(11) Numbering system of ref 4 is used in this report.

Table I. ^{1}H NMR Assignments for Briantheins X, Y, and Z

	chemical shifts, δ				coupling constant, Hz		
proton	X (9)	Y (8)	Z (7)	mult	X (9)	Y (8)	Z (7)
C_8-OH	3.47	3.18	3.20	s			
H_2	5.23	6.22	6.22	d[a]		9.1	8.8
H_3	5.21	5.60	5.61	dd[a]		11.8, 9.1	11.9, 8.8
H_4	5.83	5.91	5.93	br d	8, 1	11.8, 1	11.9, 1
H_6	5.25	5.25	5.25	ddd	3.3, 3, 2.7	3.3, 2.7, 2.3	3, 2.4, 2
H_7	4.93	4.90	4.90	d	3	3.3	3
H_9	5.19	5.22	5.22	br d	8.2	8.5	8.5
H_{10}	2.18-2.27	2.15-2.26	2.15-2.26	m			
H_{11}	2.18-2.27	2.15-2.26	2.15-2.26	m			
H_{12}	4.73	4.68	4.68	dd	6.1, 2.7	5.5, 3.2	5.8, 3
H_{13}	3.61	3.55	3.55	dd	6.1, 3.1	5.5, 3.3	5.8, 3.2
H_{14}	3.14	2.92	2.95	d	3.1	3.3	3.2
H_{15}	1.04	1.07	1.09	s			
H_{16a}	5.59	5.99	5.99	br d	2.4	2.3	2
H_{16b}	5.90	6.32	6.30	br d	3.0	2.7	2.4
H_{17}	2.32	2.29	2.29	q	7.7	7.0	6.5
H_{18}	1.15	1.15	1.15	d	7.7	7.0	6.5
H_{20}	1.04	1.04	1.05	d	7.2	7.2	7.6
R_1 (3 H)	2.15[b]	2.14[b]	2.15[b]	s			
R_2 (3 H)	2.07[b]	2.05[b]	2.05[b]	s			
R	2.18 (1 H, s)[c]	2.29 (2 H, t)	2.05 (3 H, s)			6.7	
		1.64 (2 H)		sextet		6.7	
		0.92 (3 H)		t		6.7	

[a] Overlapping multiplets in X. [b] May be interchanged within each column. [c] Hydroxyl proton.

Table II. ^{13}C Assignments for Briantheins X, Y, and Z

	chemical shifts, δ			
carbon	9	8	7	mult
1	41.54	40.78	40.81	s
2	72.16	75.34[j]	75.66	d
3	135.63	131.09	131.07	d
4	126.12	127.86	127.92	d
5	138.00	136.75	136.80	s
6	62.50	62.38	62.42	d
7	69.39	68.99[j]	69.18	d
8	84.61	84.58	84.64	s
9	77.66	77.41	77.10	d
10	33.24[h]	32.69[b]	32.86[e]	d
11	36.45[h]	36.71[b]	36.78[e]	d
12	69.84	69.38[j]	69.45	d
13	53.24	52.64[j]	52.70	d
14	62.36	61.80[j]	61.85	d
15	6.22	6.01	6.04	q
16	117.91	118.88	118.94	t
17	44.63	44.75	44.78	d
18	15.28	15.81	15.81	q
19	174.42	174.40	174.27	s
20	12.55	12.35	12.41	q
R_2	20.35[i]	20.30[c]	20.29[f]	q
R_1	21.70[i]	21.82[c]	21.78[f]	q
R_3		36.13		t
R_3		18.26		t
R_3		13.45		q
R_3 (CO)		172.48	169.75	s
R_2 (CO)	170.13[g]	170.18[a]	170.07[d]	s
R_1 (CO)	169.98[g]	170.16[a]	169.98[d]	s

[a-i] Assignments are interchangeable. [j] Assignments verified by SFORD.

would be consistent with the supposition that brianthein X (**9**) serves as precursor to both **7** and **8**. The absolute configuration of brianthein Y is 1*S*,2*S*,6*S*,7*R*,8*R*,9*S*,10*S*,11*R*,12*S*,13*R*,14*R*,17*R*.

The substitution patterns of **8** and **1** are very similar, and yet analogous torsion angles in the 10-membered rings differ substantially, the mean difference in magnitude being 23.0°. The largest difference, 45.1°, is about the C(9)-C(10) bond; the smallest, 2.4°, is about the C(3)–C(4) double bond. The result is that **8** adopts a much more open 10-membered-ring conformation.

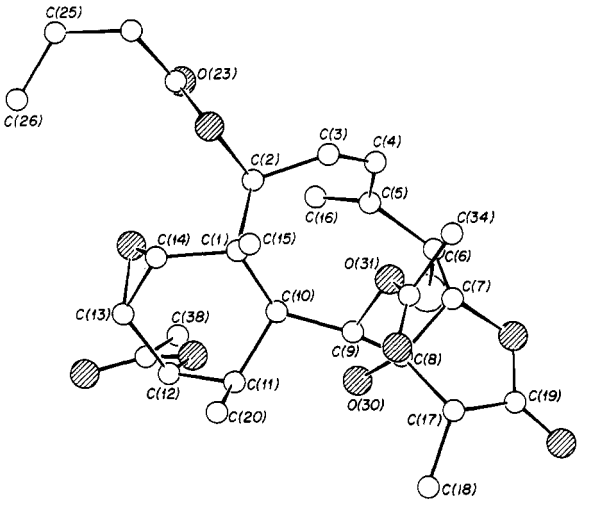

Figure 1. View of brianthein Y with oxygen atoms indicated by crosshatching. Hydrogen atoms have been omitted for clarity.

The differences arise from the fact that in briarein A C(11) bears both a β-methyl group and an α-acetoxy group while in **8** C(11) bears only a β-methyl group. In **1** the α-side transannular interactions between the α substituent on C(11) and O(30) and between O(30) and C(16) are mitigated by the β-side transannular interactions among C(15), C(20), and O(31). In **8** there is no C(11) α substituent so the α-side interactions are much diminished with a concomitant diminution of β-side transannular interactions. This point is emphasized by observation of the C(16)···O(30) and C(20)···O(31) separations; in briarein A they are, respectively, 3.35 and 3.35 Å, while in **8** they are 3.82 and 4.2 Å. This relaxation of strain diminishes the C(3)–C(4)–C(5)–C(16) torsion angle from about 68.9° in **1** to 48.7° in **8**. The torsion angle about the C(3)–C(4) double bond also diminishes from −5.4° in **1** to −3.0° in **8**.

These conformational differences explain the variation in the H-9,H-10 coupling constants in the briantheins (J = 8.2 Hz) and briarein A (J < 0.5 Hz). The conformational analyses of **1** and **8** also suggest that the stereochemical assignments for ptilosarcone (**3**) should be reconsidered. The configuration of C(9) in **3** was proposed to be opposite

that in briarein A because of the substantially different coupling constant (J = 5.5 Hz) of H-9 in **3** compared to that in **1**. Since ptilosarcone, like the briantheins, lacks a C(11) oxygen substituent, a similar conformation of the cyclodecene ring should be expected. It would seem, then, that the stereochemistry of the cyclodecene ring substituents is the same throughout this series of diterpenes.

The six-membered ring adopts a C_s form with the mirror plane through C(11) and C(14). While the epoxide portion of this ring in **8** is flatter than that in stylatulide (**2**), the overall conformations are similar.

The trans-fused, saturated γ-lactone in **8** adopts the expected envelope conformation with C(8) as the "flap" atom. The endocyclic torsion angles about the C(8)–C(17) and C(17)–C(19) bonds are slightly enlarged due to the C(18)···O(30) interaction (2.89 Å).

Most bond distances and angles are normal; however, two distances involving the quaternary carbon, C(1), are quite long [C(1)–C(10), 1.584 Å; C(1)–C(2), 1.567 Å]. There are also four other C–C bond distances greater than 1.550 Å, all of which occur between carbons bearing three or four nonhydrogen substituents [C(7)–C(8), C(8)–C(9), C(9)–C(10), and C(10)–C(11)]. The mean valency angle in the ten-membered ring, which incorporates three sp^2 carbons, is 117.9°, very similar to the accepted mean for cyclodecane, 116.5°.[12]

Brianthein Y (**8**) forms a strong hydrogen bond (O···O separation 2.77 Å) between O(30), the hydroxyl oxygen atom, and O(23), the butyrate carbonyl oxygen atom, of a molecule related by $^1/_2 - x$, $1 - y$, $^{-1}/_2 + z$. All other intermolecular separations conform to standard van der Waals contacts.

Subsequent analysis of the carbon tetrachloride soluble extracts has led to the isolation of a substantial quantity (~1 g) of a mixture of **7** and **8**. The chemotaxonomic significance of these findings[7] centers on the discovery of these three related compounds as major constituents of the organic extracts and the complete absence of briarein A. These facts lend credence to the chemotaxonomic argument that the producing organism is indeed a species of *Briareum* different from *B. asbestinum*.

Insecticidal Activity

We have begun testing isolates from marine organisms for insecticidal and insect repellant activity as part of a program to develop new models for agriculturally useful chemicals. Brianthein Y, the most abundant of the briantheins, was employed in tests to determine its effects on the feeding behavior of the grasshopper *Melanoplus bivittatus*, a major agricultural nuisance in the plain and plateau states.

An ethanol–water solution of **8** was applied to freshly cut squares (2 × 2 cm) from leaves of sunflower plants; 3 mg of **8** was coated on each leaf square. Test grasshoppers were offered these treated squares and water. One set of controls was offered water and similar squares treated only with ethanol and water, while a second set of controls was maintained on untreated sunflower leaves and water. Brianthein Y proved to be toxic to grasshoppers; all the insects exposed to **8** expired within 3 days, while all but one (of eight) controls survived.

Brianthein Y exhibited no mutagenicity in *Salmonella* strains TA 98 and TA 100 over a range of concentrations. Concentrations approaching 7 μg/mL initiated toxicity to the bacteria but still showed no sign of mutagenicity. Similarly, **8** proved negative in tests for selective toxicity to bacteria deficient in DNA repair capacity.[13]

Experimental Section

NMR spectra were recorded with a Bruker WM-250 Fourier transform spectrometer; chemical shifts are reported in δ units relative to tetramethylsilane (δ 0) with $CDCl_3$ as the solvent and internal standard. Mass spectra were obtained with a VG 7070HE mass spectrometer operating in the fast atom bombardment mode. IR spectra were determined on a Beckman IR-20 spectrophotometer and UV spectra on a Varian G34 spectrophotometer. Melting points were determined on a Fisher-Johns apparatus and are uncorrected.

Collection and Extraction. *Briareum polyanthes* was collected in October 1979 and September 1981 at depths of 4–7 m in the channel between Governor's Island and Smith's Island. The specimens were chopped and stored in acetone at −5 °C prior to extraction. The acetone was decanted, and the gorgonian pieces were macerated in a Waring blender with fresh acetone; the combined filtered extracts were reduced to an aqueous suspension. The soft coral marc was then extracted twice with CH_2Cl_2 after which the CH_2Cl_2 extracts and the aqueous suspension were equilibrated. Evaporation of the CH_2Cl_2 phase in vacuo gave 25.4 g of a thick brown oil (from 482.3 g dry weight).

Partitioning and Fractionation of Crude Extract. The crude extract was distributed between hexane and 10% aqueous MeOH. The polar phase was increased to 25% water and then extracted with CCl_4. Finally, the upper phase was increased to 35% water and extracted with $CHCl_3$. The MeOH was then evaporated from the aqueous phase; the remaining aqueous solution was then extracted with EtOAc. Evaporation of the hexane phase gave 18.62 g of extract, the CCl_4 phase gave 3.75 g, the $CHCl_3$ phase gave 2.27 g, and the EtOAc phase gave 0.13 g.

The $CHCl_3$-soluble extract was applied to a 2.2 × 195 cm column of Sephadex LH-20 and eluted with hexane–CH_2Cl_2 (1:4); five fractions were collected. Subsequent elution with acetone–CH_2Cl_2 (2:3 and then 4:1), yielded three fractions with each solvent combination.

Isolation of Briantheins Z and Y. Fraction 2 from the step gradient gel permeation chromatography (565 mg) was permeated through a Sephadex LH-60 (column 2 × 168 cm) with CH_2Cl_2–CH_3CN (3:2). Two fractions were obtained; the first (492 mg) was permeated through Bio-Beads (S-X8; column 1.7 × 126 cm) with cyclohexane–CH_2Cl_2 (2:3) to give five fractions. The third fraction was subjected to HPLC on an Ultrasphere-Cyano column (0.9 × 25 cm); elution with hexane–*i*-PrOH (2:1) gave seven fractions. The fourth was brianthein Y (**8**, 129 mg), and the fifth was brianthein Z (**7**, 30 mg).

Isolation of Brianthein X. Fraction 4 from the step gradient gel permeation chromatography (528 mg) was permeated through Sephadex LH-20 (column 2 × 122 cm) with CH_2Cl_2–MeOH (1:1). The second of three fractions (310 mg) was permeated next through Sephadex LH-60 with CH_2Cl_2–CH_3CN (3:2). The second of three fractions (241 mg) was submitted to HPLC on an Ultrasphere-Cyano column (0.9 × 25 cm); elution with hexane–*i*-PrOH (2:1) gave brianthein X (**9**, 105 mg) as the second of three fractions.

Characterization of Briantheins X, Y, and Z. Brianthein X (9): $C_{24}H_{31}ClO_9$, mp 230–232°(dec); UV (CH_2Cl_2) λ_{max} 231 nm (ε 6900); IR ($CHCl_3$) ν_{max} 3566, 2963, 1788, 1739, 1363, cm^{-1}; MS, m/z (relative intensity) 539 (M + 2 + K, 0.3), 537 (M + K, 1).

Brianthein Y (8): $C_{28}H_{37}ClO_{10}$, mp 233–235 °C dec; UV (CH_2Cl_2) λ_{max} 229 nm (ε 6500); IR ($CHCl_3$) ν_{max} 3575, 2964, 1788, 1739, 1364, cm^{-1}; MS, m/z (relative intensity) 609 (M + 2 + K, 1), 607 (M + K, 2.7).

Brianthein Z (7): $C_{26}H_{33}ClO_{10}$, mp 240–242 °C dec; UV (CH_2Cl_2) λ_{max} 230 nm (ε 6700); IR ($CHCl_3$) ν_{max} 3540, 2930, 1790, 1739, 1365 cm^{-1}; MS, m/z (relative intensity) 581 (M + 2 + K, 0.2), 579 (M + K, 0.4).

The ^1H and ^{13}C NMR data are tabulated and presented in Tables I and II.

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(13) We thank Dr. Samuel Rogers and Dr. Guylyn Warren for these tests; full details of these bioassay results will be reported elsewhere. The assay work was funded by NIEHS Grant ES 02995-02.

Acetylation of Brianthein X. Brianthein X (5 mg) was dissolved in 0.5 mL of dry pyridine; 0.5 mL of Ac_2O was added, and the mixture was allowed to stand, in a sealed flask, at 50 °C for 1 h. Solvent and excess reagent were removed in vacuo, and the residue was permeated through Bio-Beads (S-X8) with CH_2Cl_2–cyclohexane (3:2) to give, in quantitative yield, a crystalline solid (mp 243–246 °C) whose ^1H NMR spectrum was superimposable on that of brianthein Z (7).

Crystallographic Data and X-ray Structure Analysis of 8. Small single crystals of 8 ($C_{28}H_{37}ClO_{10}$, mol wt 569.0) were grown by slow evaporation of an EtOAc solution. These crystals belong to the orthorhombic space group $P2_12_12_1$ with a = 20.307 (3) Å, b = 10.548 (1) Å, c = 13.444 (1) Å, U = 2880 Å^3, Z = 4, P_{calcd} = 1.312 g cm^{-3}, and P_{measd} (flotation) = 1.30 g cm^{-3}. An octant of data to θ = 65° was collected on a Syntex $P2_1$ automated diffractometer using Ni-filtered Cu Kα (λ = 1.5418 Å) radiation (θ–2θ scan mode). Of the 2189 reflections collected, the 1909 reflections with $I > 2\delta(I)$ were used in the structure solution and refinement. The structure was solved by direct phasing methods (MULTAN 80) and refined by full-matrix least-squares[14] methods. After several cycles of refinement of positional and thermal parameters, anomalous scattering corrections for the chlorine atom were introduced into the structure factor calculations to establish the absolute configuration. For coordinates corresponding to the absolute stereochemistry represented in Figure 1, R was 0.070, whereas for the enantiomer R was 0.073. The highly significant[15] difference indicates that 8 correctly represents the absolute stereochemistry. Further refinement led to convergence at R = 0.050. Positions for hydrogen atoms on C(25) were calculated and given a B of 10.0 Å^2 but were not refined. Hydrogen atoms were not placed on C(13), C(14), C(26), C(34), or C(38).

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Supplementary Material Available: Listings of final atomic positional and thermal parameters (anisotropic Cl, O, C; isotropic H) are given in Tables III–V. Bond distances and valency angles are in Table VI, and torsion angles are in Table VII (9 pages). Ordering information is given on any current masthead page.

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Synthesis of (24*R*)- and (24*S*)-5,28-Stigmastadien-3β-ol and Determination of the Stereochemistry of Their 24-Hydroxy Analogues, the Saringosterols

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Both epimers at C-24 of 5,28-stigmastadien-3β-ol (24-vinylcholesterol) were synthesized from 5,24(28)-ergostadien-3β-ol, and their configuration at C-24 was determined by conversion into clionasterol and sitosterol. 24-Vinylcholesterol has not yet been found in nature, but it is a possible intermediate in the biosynthesis of 24-propylcholesterol and 24-propylidenecholesterol. A structurally related compound, 5,28-stigmastadiene-3β,24ξ-diol (saringosterol), first isolated from a brown seaweed, was shown to be a mixture of epimers at C-24. They were separated and their configuration was determined by correlation with fucosterol and isofucosterol 24(28)-epoxides of known stereochemistry.

Analysis of sterol mixtures isolated from marine animals has resulted in the discovery of many sterols with side chain alkylation patterns that have not been found in sterols of terrestrial organisms. Such unusual sterols may result from dietary accumulation by the animal, from de novo synthesis by algal symbionts, or from metabolism of dietary sterols by the animal.[2] An investigation of the mechanisms of the formation of such unusual side chains is clearly of biosynthetic interest.

Work on the biosynthesis of sterols resulting from dietary accumulation had to wait until the primary producers, i.e., species of unicellular marine algae which constitute phytoplankton, had been found. That is the reason why, until now, only one paper on sterol side chain biosynthesis

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