Prianosins B, C, and D, Novel Sulfur-Containing Alkaloids with Potent Antineoplastic Activity from the Okinawan Marine Sponge *Prianos melanos*

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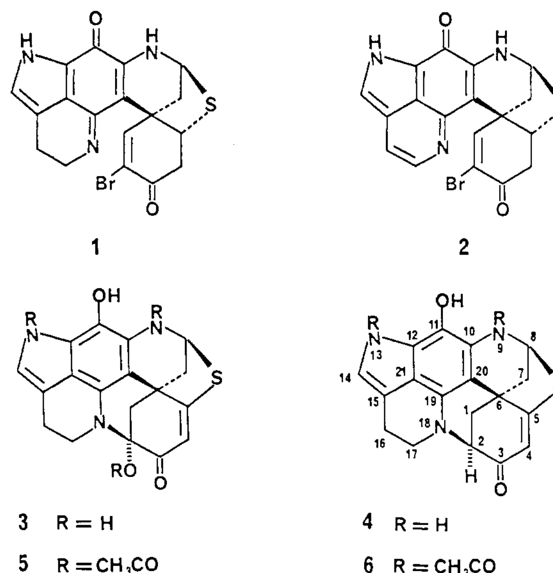

Although a variety of alkaloids have been isolated from terrestrial or marine sources, relatively few sulfur-containing alkaloids have been reported.[2] During our survey of bioactive metabolites from marine organisms,[3] we have recently isolated prianosin A (1),[4] a novel sulfur-containing alkaloid with potent antineoplastic activity from the Okinawan marine sponge *Prianos melanos*. Continuing studies on bioactive compounds from this sponge resulted in the isolation and structure determinations of prianosins B (2), C (3), and D (4), three more novel antineoplastic alkaloids possessing the same tetrahydrothiophene ring as 1. Prianosins C (3) and D (4) showed extremely unfavorable solubility properties, like calliactine,[5] making structure elucidations of these alkaloids challenging. Furthermore, extensive $^{1}H-^{13}C$ long-range correlations were essential for assignments of the structures, owing to the abundance of nonprotonated carbons and heteroatoms in the molecules. ^{1}H-detected heteronuclear multiple-bond $^{1}H-^{13}C$ correlation (HMBC) experiments[6] were very useful for these intractable samples, due to the higher sensitivity.

The green-colored sponge *P. melanos* (900 g, wet weight) was collected at Motobu Peninsula, Okinawa (−2 to −3 m), and kept frozen until used. The methanol/toluene (3:1) extract was partitioned with toluene and water. The chloroform extract of the aqueous layer, exhibiting potent cytotoxicity against L1210 murine leukemia cells, was subjected to a silica gel column chromatography ($CHCl_3$/MeOH, 98:2 to 80:20) followed by a Sephadex LH-20 column ($CHCl_3$/MeOH, 1:1) to afford prianosins C (3) and D (4) in the yields of 0.008% and 0.007% of wet weight, respectively, and a mixture of prianosins A (1) and B (2), which was further separated by a silica gel column (petroleum ether/$CHCl_3$/MeOH, 20:5:1) to give 1 and 2 in the yields of 0.02% and 0.001% of wet weight, respectively.

The most polar component, prianosin D (4), a green solid, mp >300 °C, $[\alpha]^{26}_{D}$ +344° (*c* 0.01, MeOH), showed a quasimolecular ion peak at m/z 338 (M^+ + H) in the FABMS, while it gave ion peaks at m/z 337 (M^+), 304 (M^+ − HS), and 249 ($M^+ - C_3H_4OS$) in the EIMS. The EIMS fragmentation pattern was very similar to that of prianosin A (1), indicating their structures closely related to each other. Structure elucidation was carried out initially with prianosin D acetate (6), a yellow crystal, mp 256–259 °C dec, $C_{22}H_{19}N_3O_4S$ (HREIMS, m/z 421.1094, Δ + 0.2 mmu), $[\alpha]^{25}_{D}$ +341° (*c* 0.03, $CHCl_3$), since 4 precipitated from solution and was not suitable for NMR measurements. The IR spectrum of 6 revealed absorptions at 3500–3200, 1660, and 1640 cm^{-1}, which were attributed to hydroxy, α,β-unsaturated ketone, and amide carbonyl groups, respectively. The ^{1}H NMR spectrum showed totally 19 protons (Table I). The proton at δ 10.7, suggested not to be attached to a carbon by the $^{1}H-^{13}C$ COSY,[7] was assigned to a strongly hydrogen-bonded hydroxy group[8] (11-OH) of phenol. The ^{13}C NMR data (Table I) including DEPT[9] experiments disclosed the presence of two methyls (δ 23.4 and 23.5), four methylenes (δ 22.3–48.0), two sp^3 methines (δ 60.3 and 64.6), two sp^2 methines (δ 116.4 and 117.6), and one sp^3 (δ 45.0), eight sp^2 (δ 110.7–176.3) quaternary carbons other than two amide carbonyls (δ 171.0 and δ173.3), and an α,β-unsaturated ketone (δ 187.9), thus accounting for all the carbons of 6. A combination of the COSY[10] and HOHAHA[11] data allowed a complete assignment of all proton resonances as shown in Table I. Two CH_2–CH spin systems (C-1 to C-2 and C-7 to C-8) were deduced by the presence of the vicinal couplings. The $^{1}H-^{13}C$ COSY data implied the presencce of a sp^3 methine proton (H-8), which was shifted to very low field (δ 6.99). In the HOHAHA spectrum of 6, H-14 (δ 6.81) revealed relayed cross peaks to H_2-17 (δ 2.95 and 3.37) and allyic couplings to H_2-16, suggesting the presence of a partial structure CH=CCH_2CH_2 (C-14∼C-17). The molecular framework of 6 was elucidated by extensive long-range $^{1}H-^{13}C$ correlations obtained by HMBC experiments (Figure 1). The sp^3 quaternary carbon (C-6) at δ 45.0 correlated with H-1, H-2, H-4, H-7, and H-8 in the HMBC spectrum, indicating that C-6 was a conjunct point of two

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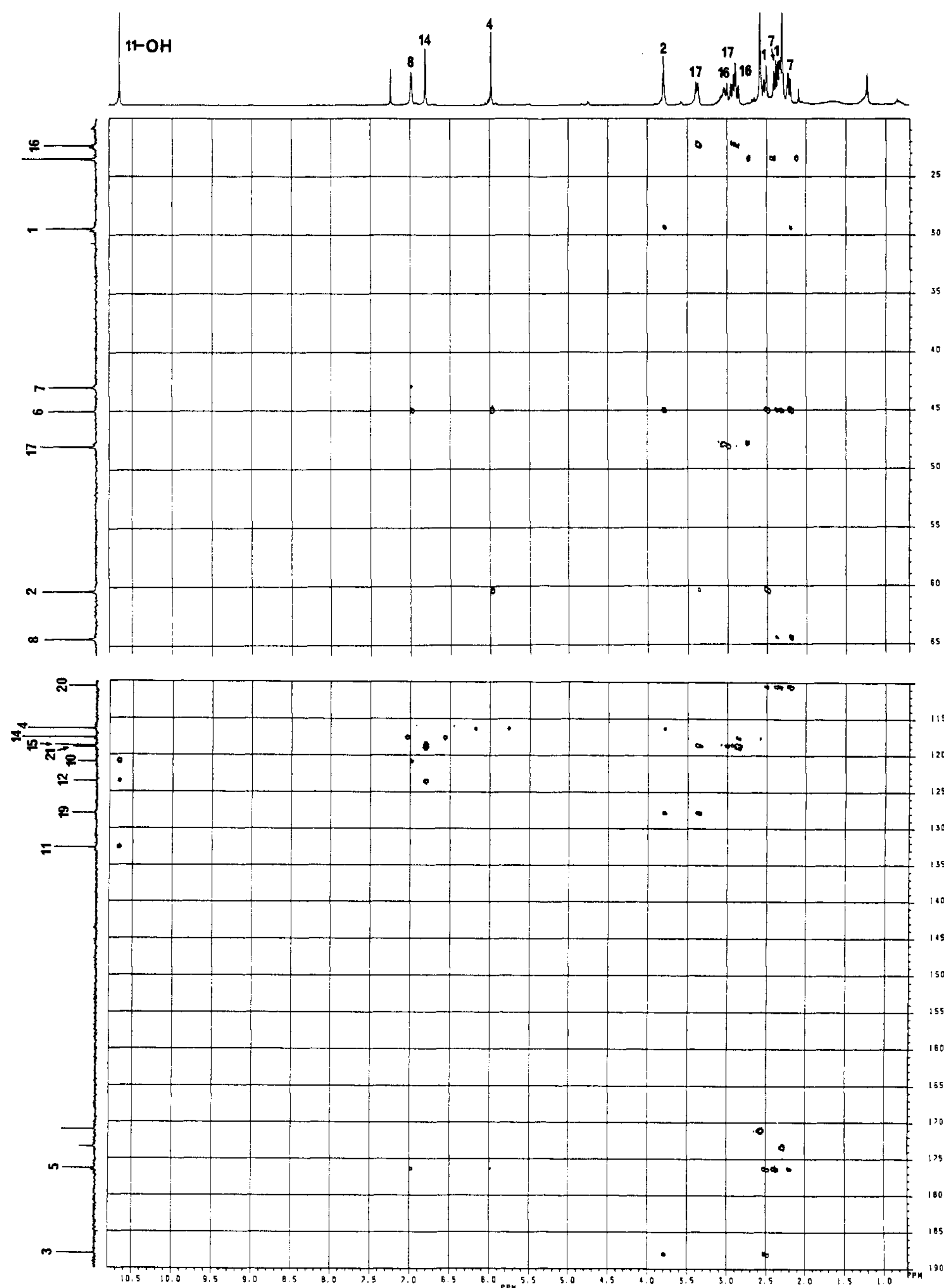

Figure 1. The HMBC spectrum of prianosin D acetate (**6**).

sets of CH_2CH system (C-1 to C-2 and C-7 to C-8). The couplings of H-1 and H-7 to C-5 (δ 176.3) and C-20 (δ 110.7) implied the connectivities of C-6 to C-5 and C-6 to C-20. Both H-1 and H-2 showed cross peaks to a carbonyl carbon at δ 187.9, which could not be more than three bonds away from H-1 and therefore should be C-3. The cross peaks of H-4 to C-2, C-5, and C-6 (Figure 1) indicated the bonds of C-3~C-6. In the HMBC H-8 revealed couplings to C-5 (δ 176.3) and C-10 (δ 120.9) as well as to C-6 and C-7. The C-5 and C-10 should be just three bonds away from H-8, since no coupling of H-8 to any other carbons, except for C-6, adjacent to C-10 or C-5 was observed. From this consideration and structural analogy to 1, a nitrogen (N-9) atom was indicated between C-8 and C-10, while a sulfur atom was located between C-5 and C-8. The chemical shift (δ 64.6) of C-8[12] and the proton coupling pattern between H-8 and H-7 supported the N and S substitution at C-8 to make a tetrahydrothiophene ring (S and C-5~C-8) like 1.[4]

The presence of a substituted indole chromophore (C-10~C-15, C-21, C-19, and C-20) was suggested by the UV absorptions [247 (ϵ 19 700), 280 (17 600), and 350 (4400) nm].[13] The hydroxy proton at δ 10.7, exhibiting cross peaks[14] to C-10, C-11, and C-12, was connected to C-11, since couplings of C-10 to H-8 and C-12 to H-14 were observed. The partial structure $CH{=}CCH_2CH_2$ (C-14~C-17) deduced from the HOHAHA experiment was confirmed by the HMBC spectrum, in which cross peaks were revealed for C-14/H-16, C-15/H-14, H-16 and H-17, C-16/H-17, and C-17/H-16 as shown in Table I. The connectivity of C-15 to C-21 (δ 119.0) was indicated by cross

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Table I. ^{1}H and ^{13}C NMR Chemical Shifts (ppm) of Prianosin D Acetate (6) and Protons to Which Long-Range Correlations Were Observed in the HMBC Experiments[a]

position	^{13}C	^{1}H	J_{HH}, Hz	HMBC (^{1}H)
1	29.5	2.34 (dd)	3.0, 12.9	H-2, H-7
		2.52 (dd)	3.0, 12.9	
2	60.3	3.86 (t)	3.0	H-1, H-4, H-17
3	187.9			H-1, H-2
4	116.4	5.98 (s)		H-2
5	176.3			H-1, H-4, H-7, H-8
6	45.0			H-1, H-2, H-4, H-7, H-8
7	43.0	2.21 (dd)	3.8, 11.5	H-8
		2.45 (d)	11.5	
8	64.4	6.99 (d)	3.8	H-7
9-NCOCH_3	23.4	2.30 (s)		
9-N$CO$$CH_3$	173.3			9-NCOCH_3
10	120.9			H-8, 11-OH
11	132.6			11-OH
11-OH		10.7 (br s)		
12	123.6			H-14, 11-OH
13-NCOCH_3	23.5	2.53 (s)		
13-N$CO$$CH_3$	171.0			13-NCOCH_3
14	117.6	6.81 (d)	2.0	H-16
15	118.7			H-14, H-16, H-17
16	22.3	2.89 (m)		H-17
		3.06 (m)		
17	48.0	2.95 (m)		H-16
		3.37 (m)		
19	127.9			H-2, H-17
20	110.7			H-1, H-7
21	119.0			H-14, H-16

[a] Spectra recorded on a Bruker AM-400 spectrometer in $CDCl_3$.

peaks of H-14 and H-16 to C-21. The bonds of C-17 to N-18, C-2 to N-18, and N-18 to C-19 were implied by cross peaks of H-17 and H-2 to C-19 (δ 127.9), and H-17 to C-2 (Figure 1). The remaining bonds of C-21 to C-19, C-19 to C-20, and C-20 to C-10 were connected to complete a benzene ring of indole nucleus. The structure of prianosin D acetate was thus established to be **6**. The acetyl group at N-13 was considered to have migrated from the hyroxy group at C-11.[15]

The molecular weight [FABMS, m/z 354 (M^+ + H)] of prianosin C (**3**), a green solid, mp >300 °C, $[\alpha]^{22}_D$ +358° (c 0.01, MeOH), was greater than that of prianosin D (**4**) only by 16 Da, indicating that **3** may be an oxidation product of **4**. Prianosin C acetate (**5**), a yellow crystal, mp 200–201 °C dec, $[\alpha]^{23}_D$ +384° (c 0.1, $CHCl_3$), was used for structure elucidation, since **3** was unstable in solution as was prianosin D (**4**). The ^{1}H and ^{13}C NMR data of **5** were different from those of **6** in the lack of an H-2 signal and in the presence of an O-acetyl group [δH 2.63; δC 169.4 (CO) and 21.3 (CH_3)], corresponding to IR absorption at 1740 cm^{-1}. The lower field resonance of C-2 (δ 88.6) of **5** provided supporting evidence for the presence of a carbinolamine carbon.[16] Thus, the structure of prianosin C acetate (**5**) was concluded to be 2-acetoxy form of **6**.

The FABMS [m/z 414 (M^+ + H) and 416 (M^+ + 2 + H)] of prianosin B (**2**), a red crystal, mp 250–251 °C dec, $[\alpha]^{30}_D$ +360° (c 0.1, $CHCl_3$), indicated that **2** was a dehydrogenated form of prianosin A (**1**). Comparison of the ^{1}H NMR data of **2** with those of **1** demonstrated that only the proton resonances at C-16 and C-17 were clearly different. The vicinal methylene signals at C-16 and C-17 of **1** were replaced by AB resonances at δ 7.51 (d, J = 5.9 Hz) and δ 8.46 (d, J = 5.9 Hz) for **2**, typical ortho-coupling pattern of α,β-protons on a pyridine ring. Accordingly, prianosin B (**2**) was established to be the dehydrogenated form at C-16 and C-17 of **1**.

The absolute stereochemistry for **2** was assigned by comparison of the CD curves with that of **1**, since the stereostructure of **1** has been unambiguously determined by X-ray diffraction analysis.[4] Prianosin A (**1**) exhibited four CD extrema; MeOH λ_{ext} 360 (Δε −3.7), 309 (+2.4), 271 (+2.0), and 233 (−7.1) nm. These Cotton effects, especially in the region of 220–280 nm, were considered to result from interactions between the substituted indole and α,β-unsaturated ketone chromophores and therefore to reflect the chirality at C-6.[17] The CD curve in the region of 220–280 nm of **2** was coincident with that of **1**, indicating the same C-6 configuration (S) of **2** as that of **1**. R configuration at C-5 was deduced by the presence of axial H-5 (δ 4.79, dd, J = 12.5 and 6.7 Hz), NMR parameters of which were almost the same as those of **1**. The configuration at C-8 was governed to be S by the configuration of C-6. The relative configurations at C-6 and C-8 of **3** and **4** were postulated to be the same as those of **1** and **2**, since **3** and **4** were considered to be biogenetically related to **1**. The CD spectra observed for **3** and **4** rationalized well this consideration as follows. The CD curves of **3** and **4** were identical with each other, whereas the signs in the region of 220–280 nm of **3** or **4** were opposite to those of **1**. This would be explained by opposite orientation (R configuration at C-6) of the α,β-unsaturated ketone and the substituted indole chromophores of **3** or **4** to that of **1**. The C-8 of **3** or **4** was assigned as S configuration by the same reason as described for **2**. The assignment for C-2 of **3** or **4** was made by considering each molecular model, in which a β OH for **3** or a β H for **4** was impossible. An evidence supporting this assignment for **4** was provided by a NOE enhancement (6%) of H-2 on irradiation of H-17(α) at δ 3.37 in **6**.

Prianosins are novel sulfur-containing polycyclic alkaloids. A sponge metabolite, discorhabdin C,[18] from the genus of *Latrunculia* is closely related to the structures of prianosins but does not contain sulfur. All four prianosins may have a similar biogenetic basis, although the structure of prianosin C (**3**) or D (**4**) is obviously different from that of prianosin A (**1**) or B (**2**) in the presence of a linkage of C-2 to N-18. A plausible biosynthetic pathway of these compounds could involve tyrosine (C-1∼N-9) and tryptophan (C-10∼C-21) units. Prianosins B (**2**), C (**3**), and D (**4**) were cytotoxic[19] against murine lymphomas L1210 and L5178Y cells and human epidermoid carcinoma KB cells in vitro with the IC_{50} values of 2.0, 1.8, and >5.0 (24% inhibition at 5.0 μg/mL) μg/mL for **2**, 0.15, 0.024, and 0.57 μg/mL for **3**, and 0.18, 0.048, and 0.46 μg/mL for **4**, respectively. In addition, prianosin D (**4**) induced Ca^{2+} release from sarcoplasmic reticulum,[20] 10 times more potent than caffeine in this assay, whereas such activity was not observed for **2** or **3**.

Experimental Section

General Methods. All melting points were measured on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were measured on a Hitachi 260-50 infrared spectrometer. UV spectra were taken on a JASCO 660 UV/VIS

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spectrometer. Optical rotations were obtained on a JASCO DIP-360 polarimeter and CD spectra on a JASCO J-40A spectrometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker AM-400 or AM-500 spectrometer. The 7.27 ppm resonance of residual $CHCl_3$ and 76.9 ppm of $CDCl_3$ were used as internal references for ^1H and ^{13}C NMR, respectively. ^1H Selective probehead for inverse experiments (400 MHz, Bruker Co.) was used in HMBC measurements. Mass spectra were obtained on a Shimadzu GC-MS QP-1000A operating at 70 eV (for LREI) or a JEOL HX-100 spectrometer (for FAB and HREI).

Collection, Extraction, and Separation. *P. melanos*, a green sponge, was collected at Motobu Peninsula (-2 to -3 m) of Okinawa Island in June 1986 by using SCUBA and kept frozen until used. The sponge (900 g, wet weight) was crushed and extracted with methanol/toluene (3:1, 1500 mL × 2). The extract was partitioned between toluene (500 mL × 2) and 1 M NaCl (1500 mL). The aqueous layer was extracted with chloroform (500 mL × 2). After evaporation of the solvent under reduced pressure, the chloroform-soluble material (1.49 g) was chromatographed on a silica gel column (Wako gel C-300, Wako Chemicals, 30 × 600 mm) with $MeOH/CHCl_3$ (2:98 to 20:80) to give three fractions of a (780–990 mL), b (1210–1900 mL), and c (2110–2660 mL). The less polar fraction a was further purified on a Sephadex LH-20 column (Pharmacia Fine Chemicals, 30 × 900 mm) with $CHCl_3/MeOH$ (1:1) followed by a silica gel column (Wako gel C-300, 15 × 600 mm) with petroleum ether/$CHCl_3$/MeOH (20:5:1) to afford prianosins A (**1**, 180 mg) and B (**2**, 14 mg). Each of polar fractions (b and c) was evaporated under reduced pressure and passed through a Sephadex LH-20 column (30 × 900 mm) with $CHCl_3/MeOH$ (1:1) to give prianosins C (**3**, 60 mg) and D (**4**, 71 mg), respectively.

Prianosin A[4] (1): CD (MeOH) λ_{ext} 360 ($\Delta\epsilon$ −3.7), 309 (+2.4), 271 (+2.0), and 233 (−7.1) nm.

Prianosin B (2): a red crystal; mp 250–251 °C dec; $[\alpha]^{30}_D$ +360° (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} 228 (ϵ 17 800), 263 (15 000), 410 (sh), and 430 (11 200) nm; IR (KBr) ν_{max} 3350, 1670, 1640, 1600, 1460, 1300, and 1210 cm^{-1}; CD (MeOH) λ_{ext} 360 ($\Delta\epsilon$ −2.7), 265 (+3.6), and 233 (−8.8) nm; ^1H NMR ($CDCl_3$) δ 2.88 (m, H-7), 2.95 (dd, *J* = 16.9 and 6.7 Hz, H-4), 3.01 (dd, *J* = 16.9 and 12.5 Hz, H-4), 4.79 (dd, *J* = 12.5 and 6.7 Hz, H-5), 5.47 (m, H-8), 7.51 (d, *J* = 5.9 Hz, H-16), 7.78 (s, H-14), 7.96 (s, H-1), and 8.46 (d, *J* = 5.9 Hz, H-17); ^{13}C NMR ($CDCl_3/CD_3OD$, 4:1) δ 40.3 (t, C-7), 45.8 (t, C-4), 51.1 (s, C-6), 56.6 (d, C-5), 61.8 (d, C-8), 113.8 (d, C-16), 117.4 (s), 119.0 (s), 120.2 (s, C-2), 125.3 (d, C-14), 128.4 (s, 2 × C), 142.9 (d, C-17), 143.7 (s), 149.1 (s), 156.8 (d, C-1), 170.5 (s, C-11), and 189.2 (s, C-3); FABMS (glycerol), *m/z* 414 (M^+ + H) and 416 (M^+ + 2 + H).

Prianosin C (3): a green solid; mp >300 °C; $[\alpha]^{22}_D$ +358° (*c* 0.01, MeOH); UV (MeOH) λ_{max} 231 (ϵ 12 300), 263 (3900), 292 (2100), and 370 (1280) nm; IR (KBr) ν_{max} 3400–3100, 2945, 1650, 1630, 1600, 1540, 1500, 1430, 1320, 1220, 1180, 1130, 1090, 1040, 990, 845, 800, and 740 cm^{-1}; CD (MeOH) λ_{ext} 352 ($\Delta\epsilon$ +41.1), 308 (−17.3), and 258 (−43.3) nm; FABMS (glycerol), *m/z* 354 (M^+ + H); EIMS, *m/z* 353 (M^+), 336 (M^+ − HO), and 266 (M^+ − C_3H_3OS).

Prianosin C Acetate (5): To 25.0 mg of prianosin C (**3**) were added pyridine (2 mL) and acetic anhydride (2 mL), standing at room temperature overnight. After evaporation of organic solvents under reduced pressure, the residue was chromatographed on a short silica gel column (Wako gel C-300, 10 × 100 mm) with $CHCl_3/MeOH$ (99:1) to give the triacetate (**5**, 4.6 mg): a yellow crystal; mp 200–201 °C dec; $[\alpha]^{23}_D$ +384° (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} 245 (ϵ 21 000), 279 (17 000), 370 (3200), and 419 (2800) nm; IR (KBr) ν_{max} 3500–3200, 3090, 2925, 2850, 1740, 1650, 1570, 1370, 1310, 1240, 1180, 1150, 1050, 1030, 990, 900, 845, 800, and 740 cm^{-1}; FABMS (glycerol), *m/z* 480 (M^+ + H); EIMS, *m/z* 479 (M^+), 437 (M^+ − 2Ac - H), 352 (M^+ − 3Ac), 308 (M^+ − 2Ac − C_3H_2OS), and 266 (308 − Ac); ^1H NMR ($CDCl_3$) δ 2.24 (s, Me), 2.32 (dd, *J* = 11.7 and 4.0 Hz, H-7), 2.34 (s, Me), 2.44 (d, *J* = 11.7 Hz, H-7), 2.53 (d, *J* = 12.2 Hz, H-1), 2.53 (m, H-17), 2.63 (s, Me), 2.93 (m, *J* = 15.7, 2.0, and 1.5 Hz, H-16), 3.04 (m, *J* = 15.7, 4.8, and 2.0 Hz, H-16), 3.45 (d, *J* = 12.2 Hz, H-1), 3.83 (ddd, *J* = 12.5, 4.8, and 2.0 Hz, H-17), 5.98 (s, H-4), 6.88 (d, *J* = 1.5 Hz, H-14), 7.03 (d, *J* = 4.0 Hz, H-8), and 10.18 (s, 11-OH); ^{13}C NMR ($CDCl_3$) δ 21.3 (q), 22.2 (t, C-16), 23.4 (q), 23.5 (q), 32.7 (t, C-1), 42.7 (s and t, C-6 and C-7), 47.9 (t, C-17), 64.7 (d, C-8), 88.6 (s, C-2), 111.8 (s, C-20), 115.0 (d, C-5), 117.6 (d, C-14), 118.8 (s, C-15), 119.5 (s, C-21), 120.4 (s, C-10). 123.9 (s, C-12), 126.6 (s, C-19), 133.6 (s, C-11), 169.4 (s, 2-OCO), 171.0 (s, 9-NCO), 173.1 (s, 13-NCO), 174.8 (s, C-5), and 181.0 (s, C-3).

Prianosin D (4): a green solid; mp >300 °C; $[\alpha]^{26}_D$ +344° (*c* 0.01, MeOH); UV (MeOH) λ_{max} 250 (ϵ 18 100), 284 (11 100), 325 (6600), and 392 (6950) nm; IR (KBr) ν_{max} 3400–3100, 2945, 1660, 1630, 1600, 1540, 1500, 1430, 1320, 1300, 1220, 1180, 1135, 1110, 980, and 900 cm^{-1}; CD (MeOH) λ_{ext} 360 ($\Delta\epsilon$ +45.8), 304 (−11.5), and 255 (−34.4) nm; FABMS (glycerol), *m/z* 338 (M^+ + H); EIMS, *m/z* 337 (M^+), 304 (M^+ − HS), and 249 (M^+ − C_3H_4OS).

Prianosin D Acetate (6). To 25.0 mg of prianosin D (**4**) were added pyridine (2 mL) and acetic anhydide (2 mL). The mixture stood overnight at room temperature. The same workup as described for **5** yielded the diacetate (**6**, 14.6 mg): a yellow crystal; mp 256–259 °C dec; $[\alpha]^{25}_D$ +341° (*c* 0.03, $CHCl_3$); UV (MeOH) λ_{max} 247 (ϵ 19 700), 280 (17 600), and 350 (4400) nm; IR (KBr) ν_{max} 3500–3200, 3125, 2925, 2850, 1660, 1640, 1615, 1570, 1480, 1420, 1360, 1300, 1270, 1140, 990, and 740 cm^{-1}; ^1H and ^{13}C NMR (Table I); FABMS (glycerol), *m/z* 422 (M^+ + H); EIMS, *m/z* 421 (M^+), 379 (M^+ − Ac), 346 (M^+ − Ac − HS), 336 (M^+ − 2Ac − H), 292 (M^+ − Ac − C_3H_3OS), and 250 (292 − Ac); HREIMS found *m/z* 421.1094, calcd for $C_{22}H_{19}N_3O_4S$ 421.1092 (M).

Biological Assay. The extravesicular Ca^{2+} concentration in sarcoplasmic reticulum was monitored with a Ca^{2+} electrode prepared by the method of Tsien and Rink with modifications.[20]

Antitumor activity was determined by using murine lymphomas L1210, L5178Y, and human epidermoid carcinoma KB cells. Roswell Park Memorial Institute Medium 1640 supplemented with 10% heat-inactivated fetal bovine serum and 50 μg/mL of kanamycin was used as the cell cultured medium. Tumor cells (5×10^4 cells/mL) were cultured in a CO_2 gas incubator at 37 °C for 48 h in 1 mL of medium containing various concentrations of test compound. Their viability, estimated by use of a variation of a colorimetric [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) assay,[21] was compared to that of control cells incubated in the identical medium without the compound. The antitumor activity evaluated as IC_{50} (the concentration in μg/mL required for 50% inhibition of cell growth). The IC_{50} value was obtained by plotting the logarithm of concentration of test compound vs the growth rate (percentage of control) of the treated cells.

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Supplementary Material Available: Two figures containing the HOHAHA spectrum of prianosin D acetate (**6**) and the ^1H–^{13}C COSY spectrum of prianosin D acetate (**6**) (3 pages). Ordering information is given on any current masthead page.

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Thermal Isomerizations of 2-Methylenebicyclo[2.1.0]pentane

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It has been known since 1966 that 1,2,5-hexatriene (**1**) isomerizes at 340–385 °C in a flow system to both 3- and 4-methylenecyclopentene (**3** and **4**), presumably by way

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