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

Jie-fei Cheng,^{1a} Yasushi Ohizumi,^{1a} Markus R. Wälichli,^{1b} Hideshi Nakamura,^{1a} Yoshimasa Hirata,^{1c} Takuma Sasaki,^{1d} and Jun'ichi Kobayashi^{*1a}

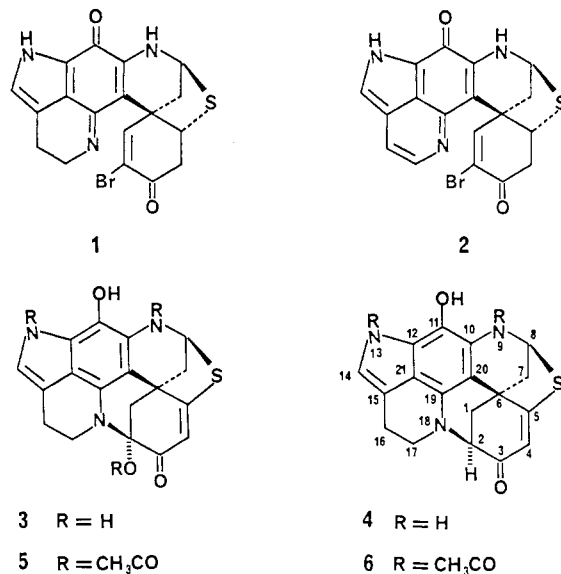
Mitsubishi Kasei Institute of Life Sciences, Minamiooya, Machida, Tokyo 194, Japan, Bruker Japan Company, Tsukuba, Ibaragi 305, Japan, Faculty of Pharmacy, Meijo University, Nagoya 468, Japan, and Cancer Research Institute, Kanazawa University, Kanazawa 920, Japan

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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 ¹H-¹³C long-range correlations were essential for assignments of the structures, owing to the abundance of nonprotonated carbons and heteroatoms in the molecules. ¹H-detected heteronuclear multiple-bond ¹H-¹³C correlation (HMBC) experiments⁶ 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 (CHCl₃/MeOH, 98:2 to 80:20) followed by a Sephadex LH-20 column (CHCl₃/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/CHCl₃/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, [α]_D²⁵ +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, C₂₂H₁₉N₃O₄S (HREIMS, m/z 421.1094, $\Delta + 0.2$ mmu), [α]_D²⁵ +341° (c 0.03, CHCl₃), 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 ¹H NMR spectrum showed totally 19 protons (Table I). The proton at δ 10.7, suggested not to be attached to a carbon by the ¹H-¹³C COSY,⁷ was assigned to a strongly hydrogen-bonded hydroxy group⁸ (11-OH) of phenol. The ¹³C NMR data (Table I) including DEPT⁹ experiments disclosed the presence of two methyls (δ 23.4 and 23.5), four methylenes (δ 22.3–48.0), two sp³ methines (δ 60.3 and 64.6), two sp² methines (δ 116.4 and 117.6), and one sp³ (δ 45.0), eight sp² (δ 110.7–176.3) quaternary carbons other than two amide carbonyls (δ 171.0 and δ 173.3), and an α,β -unsaturated ketone (δ 187.9), thus accounting for all the carbons of 6. A combination of the COSY¹⁰ and HOHAHA¹¹ data allowed a complete assignment of all proton resonances as shown in Table I. Two CH₂-CH spin systems (C-1 to C-2 and C-7 to C-8) were deduced by the presence of the vicinal couplings. The ¹H-¹³C COSY data implied the presence of a sp³ 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 H₂-17 (δ 2.95 and 3.37) and allylic couplings to H₂-16, suggesting the presence of a partial structure CH=CCH₂CH₂ (C-14~C-17). The molecular framework of 6 was elucidated by extensive long-range ¹H-¹³C correlations obtained by HMBC experiments (Figure 1). The sp³ 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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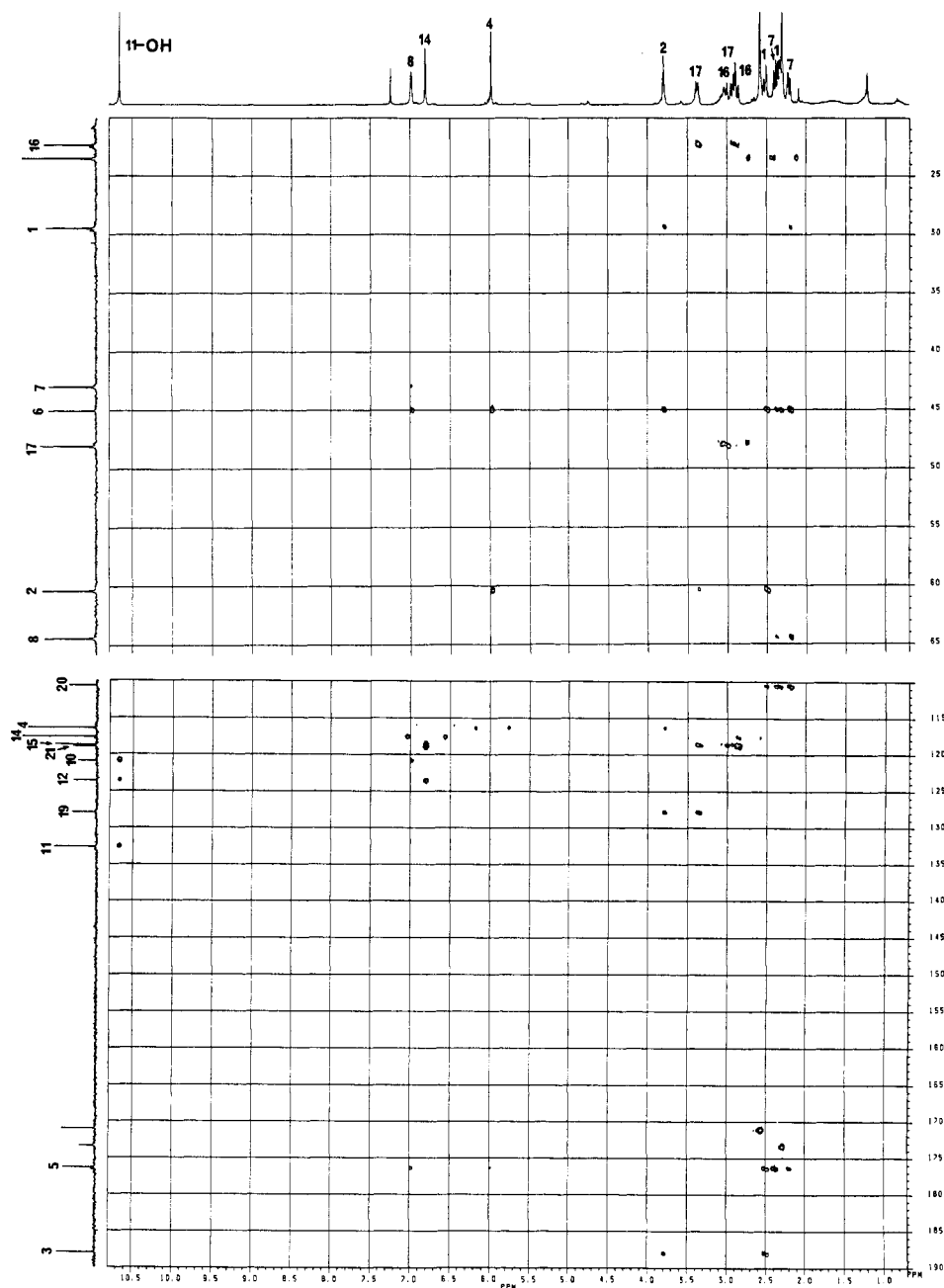


Figure 1. The HMBC spectrum of prianosin D acetate (6).

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-8¹² 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 peaks¹⁴ 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 $\text{CH}=\text{CCH}_2\text{CH}_2$ (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. ^1H 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	^1H	J_{HH} , Hz	HMBC (^1H)
1	29.5	2.34 (dd)	3.0, 12.9	H-2, H-7
		2.52 (dd)	3.0, 12.9	
2		3.86 (t)	3.0	H-1, H-4, H-17
3	60.3			H-1, H-2
4	187.9			H-2
5	116.4	5.98 (s)		H-1, H-4, H-7, H-8
	176.3			H-1, H-2, H-4, H-7, H-8
6	45.0			H-8
7	43.0	2.21 (dd)	3.8, 11.5	H-7
		2.45 (d)	11.5	
8	64.4	6.99 (d)	3.8	H-7
9-NCOCH ₃	23.4	2.30 (s)		9-NCOCH ₃
9-NCOCH ₃	173.3			H-8, 11-OH
10	120.9			11-OH
11	132.6			
11-OH		10.7 (br s)		
12	123.6			H-14, 11-OH
13-NCOCH ₃	23.5	2.53 (s)		
13-NCOCH 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 hydroxy group at C-11.¹⁵

The molecular weight [FABMS, m/z 354 ($\text{M}^+ + \text{H}$)] of prianosin C (3), a green solid, mp $>300^\circ\text{C}$, $[\alpha]_{\text{D}}^{25} +358^\circ$ (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 $200\text{--}201^\circ\text{C}$ dec, $[\alpha]_{\text{D}}^{25} +384^\circ$ (c 0.1, CHCl_3), was used for structure elucidation, since 3 was unstable in solution as was prianosin D (4). The ^1H 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 ($\text{M}^+ + \text{H}$) and 416 ($\text{M}^+ + 2 + \text{H}$)] of prianosin B (2), a red crystal, mp $250\text{--}251^\circ\text{C}$ dec, $[\alpha]_{\text{D}}^{30} +360^\circ$ (c 0.1, CHCl_3), indicated that 2 was a dehydrogenated form of prianosin A (1). Comparison of the ^1H 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 ($\Delta\epsilon -3.7$), 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 cytotoxic¹⁹ 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 $5.0\text{ }\mu\text{g/mL}$) $\mu\text{g/mL}$ for 2, 0.15, 0.024, and $0.57\text{ }\mu\text{g/mL}$ for 3, and 0.18, 0.048, and $0.46\text{ }\mu\text{g/mL}$ 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 \times 2). The extract was partitioned between toluene (500 mL \times 2) and 1 M NaCl (1500 mL). The aqueous layer was extracted with chloroform (500 mL \times 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 \times 600 mm) with $\text{MeOH}/\text{CHCl}_3$ (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 \times 900 mm) with $\text{CHCl}_3/\text{MeOH}$ (1:1) followed by a silica gel column (Wako gel C-300, 15 \times 600 mm) with petroleum ether/ $\text{CHCl}_3/\text{MeOH}$ (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 \times 900 mm) with $\text{CHCl}_3/\text{MeOH}$ (1:1) to give prianosins C (3, 60 mg) and D (4, 71 mg), respectively.

Prianosin A⁴ (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; $[\alpha]_{\text{D}}^{30}$ +360° (c 0.1, CHCl_3); UV (MeOH) λ_{max} 228 (ϵ 17800), 263 (15000), 410 (sh), and 430 (11200) 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 ($\text{CDCl}_3/\text{CD}_3\text{OD}$, 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 \times 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; $[\alpha]_{\text{D}}^{22}$ +358° (c 0.01, MeOH); UV (MeOH) λ_{max} 231 (ϵ 12300), 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^+ - $\text{C}_3\text{H}_5\text{OS}$).

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 \times 100 mm) with $\text{CHCl}_3/\text{MeOH}$ (99:1) to give the triacetate (5, 4.6 mg): a yellow crystal; mp 200-201 °C dec; $[\alpha]_{\text{D}}^{23}$ +384° (c 0.1, CHCl_3); UV (MeOH) λ_{max} 245 (ϵ 21000), 279 (17000), 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 - $\text{C}_3\text{H}_5\text{OS}$), 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; $[\alpha]_{\text{D}}^{26}$ +344° (c 0.01, MeOH); UV (MeOH) λ_{max} 250 (ϵ 18100), 284 (11100), 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^+ - $\text{C}_3\text{H}_4\text{OS}$).

Prianosin D Acetate (6): To 25.0 mg of prianosin D (4) were added pyridine (2 mL) and acetic anhydride (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; $[\alpha]_{\text{D}}^{25}$ +341° (c 0.03, CHCl_3); UV (MeOH) λ_{max} 247 (ϵ 19700), 280 (17600), 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 - $\text{C}_3\text{H}_5\text{OS}$), and 250 (292 - Ac); HREIMS found m/z 421.1094, calcd for $\text{C}_{22}\text{H}_{19}\text{N}_3\text{O}_4\text{S}$ 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 $\mu\text{g}/\text{mL}$ 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 $\mu\text{g}/\text{mL}$ 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-Methylenecyclopentane

Gerald D. Andrews and John E. Baldwin*¹

Department of Chemistry, University of Oregon, Eugene, Oregon 97403

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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

(1) Present address: Department of Chemistry, Syracuse University, Syracuse, NY 13244.