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PAVONININS, SHARK-REPELLING AND ICHTHYOTOXIC STEROID N-ACETYLGLUCOSAMINIDES FROM THE DEFENSE SECRETION OF THE SOLE *PARDACHIRUS PAVONINUS* (SOLEIDAE)

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Abstract—Six steroid N-acetylglucosaminides, pavoninins-1 to -6, have been isolated from the defense secretion of the sole *P. pavoninus* guided by ichthyotoxicity and hemolytic activity, and their structures determined to be **1–6** by spectroscopic studies and chemical correlations. The pavoninins are considered to be the factors responsible for the repellent property of the sole against predatory fishes.

INTRODUCTION

Certain species of fish are known to kill other fishes confined in the same aquarium despite lack of any traumagenic devices. These fishes have special secretory cells which emit ichthyotoxic substances upon extraneous disturbance, and are termed ichthyocrinotoxic fishes.[1] Among common characteristics of these fish are their poor swimming ability, lack of prominent scales, and habitats on tropical reefs, where the struggle for survival is harsh. These ichthyotoxic substances, i.e. ichthyocrinotoxins, are thus presumed to function as repellents against predatory fishes.[2] Similar repellent properties are known for starfishes and sea cucumbers, and a number of steroid and triterpenoid glycosides have been isolated and characterized therefrom.[3] With regard to chemistry of ichthyocrinotoxins, most of them have been found peptidic or proteinaceous,[4] though none have been fully characterized to-date, with the exception of hexadecanoyl cholines from the boxfish *Ostracion lentiginosus*[5] and the smooth trunkfish *Lactophrys triqueter*,[6] both belonging to the trunkfish family. Among the ichthyocrinotoxic fishes, the Red Sea "Moses sole" *Pardachirus marmoratus* is reported to repel sharks at the last moment of their attack.[7] Isolation of an ichthyotoxic and hemolytic protein, pardaxin, from its secretion has been reported by an Israeli group,[8] and it has been suspected to be responsible for the shark-repellent property of the sole.[9] The sole *P. pavoninus*, a close kin of Moses sole in the tropical region of the western Pacific and eastern Indian Oceans, is also known to be ichthyocrinotoxic, possessing characteristic toxin cells lining the base of its dorsal and anal fin spines.[10] In this article, we report isolation and full structural elucidation of six ichthyotoxic, hemolytic and shark-repelling steroid N-acetylglucosaminides, named pavoninins-1 to -6, from the secretion of *P. pavoninus*.[11]

Isolation. Seven individuals of *P. pavoninus*, 20–30 cm long, caught at Ishigaki Island, Ryukyu Archipelago, Japan, were disturbed by taking them out of the water twice, one day apart, to obtain mucous secretion from their toxin cells onto an enameled steel tray. The milky secretion was washed with sea water into a sample bottle to give a soapy suspension, which yielded a white powder upon lyophilization. The powder was resuspended in 0.1 M aqueous ammonia and diluted tenfold with acetone to result in a white precipitate, which comprises mainly proteinaceous material, on the basis of its solubilities and chromatographic behaviors in preliminary experiments. Approximately 60% of total ichthyotoxic and 20% of hemolytic activity was found in the precipitate, which is suspected to contain pardaxin or related materials. The remaining activity was found in a lipophilic fraction of the filtrate, and was concentrated as follows. The filtrate residue was partitioned between ethyl acetate and water, the former layer giving a yellow oil, 1.3 g upon solvent removal, whereas the latter layer giving mostly inorganic salts and possessing no activity. The oil was chromatographed on a silica gel column to yield a mixture of ichthyotoxins (992 mg; i.e. 71 mg per average discharge) eluted with 10–25% methanol/chloroform. The mixture exhibits lethality to the Japanese killifish *Oryzias latipes* of 1 hr LC_{50} 8.5 μg/mL and comparable hemolytic activity to commercial saponin on rabbit erythrocytes. Further separation of the mixture brought about no substantial changes of these bioactivities.

Repeated silica gel column chromatography separated the ichthyotoxin mixture into two fractions, whose respective silica gel tlc gives single spots. Reverse phase column chromatography of the less polar fraction (145 mg) eluted with 80% aqueous methanol yielded predominantly a major component, pavoninin-1 (**1**; 111 mg). The same treatment of the more polar fraction (617 mg) gave pavoninin-2 (**2**; 34 mg), -3 (**3**; 143 mg), and -4 (**4**; 49 mg) in order of elution. A further mixture of two compounds was eluted between **2** and **3**, and finally separated on a column of silica gel impregnated with 10% silver nitrate and eluted with 15–20% MeOH/EtOAc into pavoninin-5 (**5**; 210 mg) and -6 (**6**; 44 mg).

Pavoninin-1. The 360 MHz ^{1}H-NMR spectrum of **1** in methanol-d_4 with double resonance measurements

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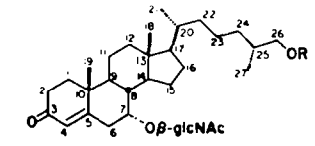
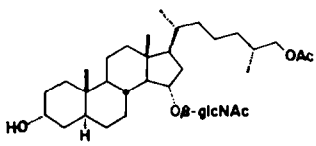
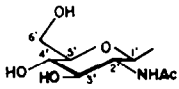


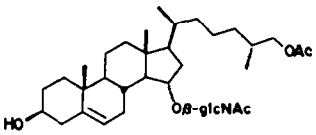
pavoninin-1 1 : R = Ac
" -2 2 : R = H



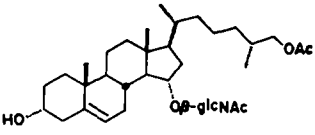
pavoninin-4 4

β-glcNAc =

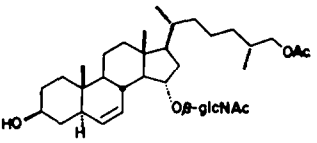




pavoninin-5 5



pavoninin-3 3



pavoninin-6 6

shows five methine protons at 4.42, 3.59, 3.45, 3.32 and 3.26 ppm, which couple to their neighbors in this order, the last further coupling to methylene protons at 3.69/3.88 ppm (Fig. 1). The coupling constants of 9–10 Hz among these methine signals indicate that the molecule has a moiety equivalent to a β-glucopyranoside. In addition to the sugar moiety, readily recognized from the NMR and other spectroscopic data are a secondary acetamide [ν_{max} 1650, 1550 cm^{-1}; δ 1.80 ppm], an acetate [ν_{max} 1725 cm^{-1}; δ 2.03 ppm], two tertiary and two secondary methyls [δ 1.22 (s), 0.73 (s), 0.94 (d, 7 Hz) and 0.93 ppm (d, 7 Hz)], and structural moieties **A** (C-3 to -14) [λ_{max} 244 nm (MeOH); ν_{max} 1660 cm^{-1}] and **B** (C-25, 26) in Fig. 2. The molecular ion peak at *m/z* 662, $(M + H)^+$, in the secondary-ion mass spectrum[12] of **1**, together with the ^{13}C-NMR spectrum and consideration of the functional groups referred to above, led to a molecular formula of $C_{37}H_{59}NO_9$.

Conventional acetylation of **1** introduced three more acetyls to furnish pavoninin-1 peracetate. Its ^{1}H-NMR spectrum in chloroform-*d* shows an exchangeable proton signal at 5.43 ppm coupling to the 2′-H signal at 3.48 ppm by 9 Hz, thus establishing location of the acetamide [ν_{max} 3350 cm^{-1}] at C-2′. Downfield shifts of 3′-H to 5.23 ppm, 4′-H to 5.06 ppm, and 6′-H_2 to 4.14/4.19 ppm upon acetylation indicate that free hydroxyls are attached to these sites in **1**. The remaining oxygenated sites, i.e. C-7, -26, -1′ and -5′, must therefore be linked to ether or ester oxygens.

Acid methanolysis of **1** yielded a ninhydrin-positive

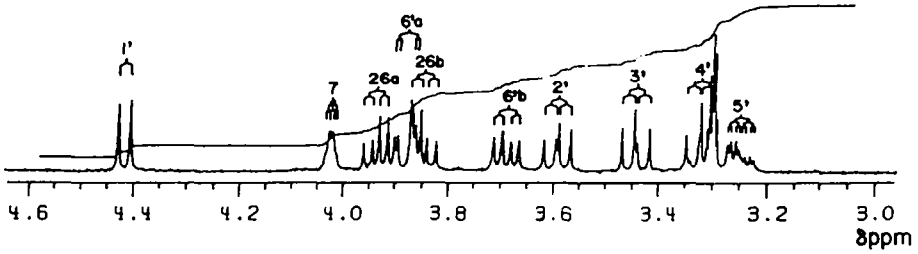


Fig. 1. 360 MHz ^{1}H-NMR spectrum of pavoninin-1 (**1**) in methanol-d_4 (part).

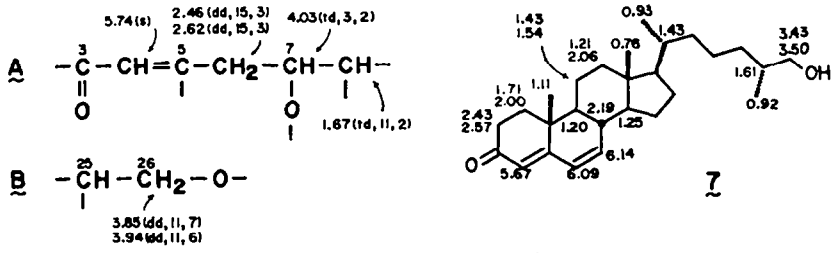


Fig. 2. Structural moieties **A** and **B** of **1**, and its methanolysate **7**. ^{1}H-NMR data in methanol-d_4 (**1**) and in chloroform-*d* (**7**).

substance in the hydrophilic portion. N-Acetylation of the amine with acetic anhydride in pyridine/methanol and subsequent *p*-bromobenzoylation of hydroxyls furnished a tri-*p*-bromobenzoate, which gave an isotopic quartet at *m/z* 782, 784, 786 and 788, $(M + H)^+$, in its FD-MS. The tri-*p*-bromobenzoate shows a typical exciton-split pattern in its CD spectrum, $\Delta\varepsilon_{232/252} + 9/-20$, whose signs and magnitudes are characteristic to D-glucopyranose 3,4,6-tri-*p*-bromobenzoates and are predicted by the additivity rule in the exciton chirality method.[13] Together with the acetylation shifts of the ^{1}H-NMR signals and their coupling constants discussed above, the CD datum established the sugar moiety of **1** as β-N-acetyl-D-glucosamine (β-glcNAc).

The lipophilic portion of the methanolysate of **1** yielded a steroid **7**, whose structure was suggested by its ^{1}H-NMR spectrum and by intensive double resonance measurements to be as in Fig. 2. The hydroxymethyl, which is originated from moiety **B** of **1**, was determined to be at C-25 based on simultaneous decoupling of its methylene signals at 3.43/3.50 ppm and a *sec*-Me signal at 0.92 ppm by irradiation at 1.61 ppm. A broader doublet signal for the other *sec*-Me at 0.93 ppm, presumably attached to C-20, was decoupled by irradiation at 1.43 ppm. In order to substantiate attribution of the common cholestane skeleton to **7**, it was hydrogenated in alkaline ethanol to the 5β-3-one **8** (Scheme 1), which was dehydroxylated at C-26 by tosylation/LAH reduction to give an epimeric mixture of the 3-ols. Jones oxidation of the mixture regenerated a ketone, which is identical in all respects with 5β-cholestan-3-one prepared from authentic cholesterol by means of Jones oxidation immediately followed by treatment with hydrochloric acid in methanol,[14] and subsequent hydrogenation. This conversion fully established the skeletal structure of **7**, and thus of **1**, including absolute stereochemistry at C-8, -9, -10, -13, -14, -17 and -20.

Upfield shifts of 26-H's from 3.85/3.94 ppm in **1** to 3.43/3.50 ppm in **7** clarify that C-26 bears the acetate in **1** and the sugar is attached at C-7. The axial orientation of the C-7 oxygen, i.e. 7α-sugar configuration, is based on *J*-values of the 7-H signal in the ^{1}H-NMR spectrum of **1** (**A** in Fig. 2). It also explains the facile dehydration accompanying methanolysis to give the dienone system in **7** as shown by a red shift of the UV absorption maximum to 285 nm.

In order to determine the configuration at C-25, the sole chiral center remaining to be determined, a diastereomeric pair of (+)- and (−)-methoxytrifluoromethylphenylacetates (MTPA esters) were prepared from **7** respectively. It has been empirically shown that the lanthanide-induced shift for the OMe ^{1}H-NMR signal of a primary carbinol (+)-MTPA ester with *R* chirality at the α-carbon, or of an (α-*S*)-carbinol (−)-MTPA ester, is larger than that of its diastereomer.[15] Addition of an equimolar $Eu(fod)_3$ to an uneven mixture of (+)- and (−)-MTPA esters (*ca* 10:7) of **7** in chloroform-*d* shifted the two OMe signals by 0.68 and 0.66 ppm, and addition of 2.8 molar equivalents $Eu(fod)_3$ shifted them by 2.05 and 2.00 ppm, respectively. The larger downfield shift observed for the more intense OMe signal of the (+)-MTPA ester determines the C-25 configuration of **7**, and therefore of **1**, to be *R*.

Pavoninin-2. Lack of the O-acetyl as compared to **1** is the only difference recognizable in spectroscopic data of **2**. Thus **2** was suggested to be de-O-acetyl-**1**. This was supported by the molecular ion peak at *m/z* 620, $(M + H)^+$, in FD-MS of **2** and established by formation of identical peracetates from **1** and **2**. Appearance of methylene signals at 3.33/3.40 ppm in the ^{1}H-NMR spectrum of **2** as expected in place of those of 3.85/3.94 ppm of **1** also confirms regiochemical assignments of the sugar and the acetate groups in **1**.

Pavoninin-3. Double resonance ^{1}H-NMR measurements of **3** in methanol-d_4 showed presence of the β-hexopyranoside, two acetyls which the IR spectrum indicates to be an acetate [ν_{max} 1730 cm^{-1}; δ 2.03 ppm] and a secondary acetamide [ν_{max} 1655, 1560 cm^{-1}; δ 1.98 ppm], two tertiary and two secondary methyls [δ 1.03 (s), 0.73 (s), and 0.93 ppm (6H, d, 7 Hz)], and the moiety **B** (C-25, 26) [δ 3.94 (dd, 11, 6 Hz) and 3.85 ppm (dd, 11, 7 Hz)], all of which correspond to those in **1**. On the other hand, the NMR data show moieties **C** (C-3 to -6) and **D** (C-14 to -17) in Fig. 3 instead of the moiety **A** in **1**. This difference agrees with absence of an absorption maximum in the UV spectrum of **3** and with its molecular formula of $C_{37}H_{61}NO_9$, which has two more hydrogens than **1**; the formula is based on the molecular ion peak at *m/z* 664, $(M + H)^+$, in the FD-MS and on the ^{13}C-NMR spectrum of **3**.

Acetylation of **3** introduced four more acetyls to furnish pavoninin-3 peracetate, whose ^{1}H-NMR spectrum in chloroform-*d*, besides characterizing the sugar to be β-glcNAc as in the case of **1**, indicates that C-15 (in **D**) bears the sugar whereas C-3 (in **C**) bears an OH in **3**. Namely, 3-H shifted from 3.95 ppm to 4.98 ppm upon acetylation, whereas 15-H did not show a downfield shift. The axial orientation of the C-3 oxygen, i.e. 3α-OH configuration, is again indicated by small half-band widths of the 3-H signals in ^{1}H-NMR spectra of **3** and its tetraacetate, and is supported by moderate resistance of the OH to conventional acetylation.

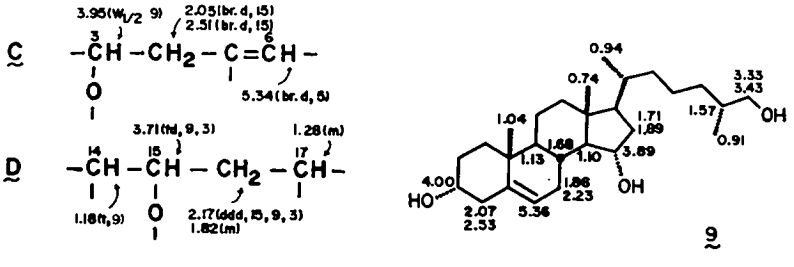


Fig. 3. Structural moeities **C** and **D** of **3**, and its methanolysate **9**. ^{1}H-NMR data in methanol-d_4 (**3**) and in chloroform-*d* (**9**).

Acid methanolysis of **3** yielded a steroid **9** as well as methyl glucosaminide. Intensive double resonance ^{1}H-NMR measurements of **9** enabled one to unite moieties C and D by connecting C-3 through C-16, thus suggesting its structure to be as shown in Fig. 3. The steroidal skeleton of **9**, hence of **3**, including all configurations except at C-15, was established by chemical correlation between **7** and **3**, which was accomplished by conversion of **3** to **8** in the following manner.[16] Careful Jones oxidation of **3** immediately followed by acid methanolysis[14] yielded dihydroxyenone **10**, since C-15 was protected from oxidation by the sugar group. The 26-OH of **10** was then selectively protected as silyl ether by t-butyldimethylsilylation[17] in presence of excess neopentyl alcohol, which scavenges the silyl chloride prior to silylation of the 15-OH, but after the 26-OH. After hydrogenation of the monosilyl ether **11** in alkaline ethanol, the 5β-3-one was dehydroxylated at C-15 by O-phenyl thiocarbonylation and subsequent reduction with tri-n-butyltin hydride.[18] Deprotection of the dehydroxylated product at the 26-oxygen yielded hydroxyketone **8**, which was obtained by hydrogenation of **7** (*vide supra*). The correlations among pavoninins-1 and -3 and authentic cholesterol are summarized in Scheme 1.

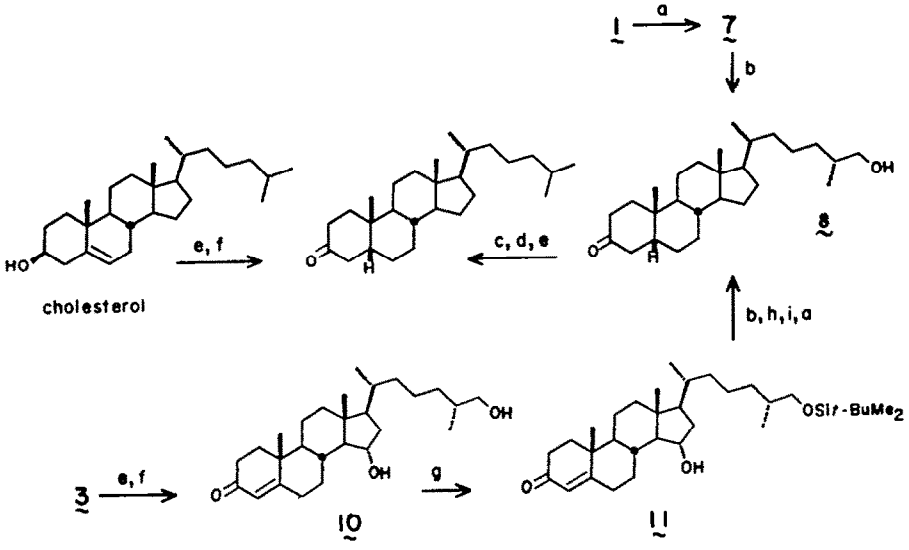
Configuration at C-15 in **3** was determined by the exciton chirality method on enone *p*-bromobenzoate **12** which was readily prepared from the monosilyl ether **11**. The two chromophores in **12** which have their UV absorption maxima in close proximity constitute a coupled oscillator. According to the exciton chirality method,[19] the chromophores in the 15α-benzoate are placed in negative chirality and hence predicted to give rise to a split CD spectrum with a negative curve at longer wavelength, whereas those in the 15β-benzoate in positive chirality would give rise to opposite CD curves. The signs of the split CD spectrum of **12**, $\Delta\varepsilon_{228/247}$ +8/−12, establish the configuration at C-15 as α-OH in **11**, or 15*S* in **3** (Fig. 4).

Pavoninin-4. Lack of an olefin is the only difference readily recognizable in ^{1}H- and ^{13}C-NMR spectra of **4** as compared to **3**. Spectroscopic similarity between **4** and **3** otherwise, identification of the sugar as β-glcNAc by peracetylation and the ^{1}H-NMR studies, and the molecular ion peak at *m/z* 666, $(M + H)^+$, in FD-MS suggested **4** to be a dihydro analogue of **3**. ^{1}H- and ^{13}C-NMR spectra of **4** in methanol-d_4 show its 10-Me signals at 0.81 and 11.9 ppm, respectively, being typical for 5α-steroids, whereas a 5β-steroid would show them at lower-field for both the nuclei.[20] A multiplet proton signal at 3.94 ppm with $W_{1/2}$ 8 Hz, which was assigned to 3-H, indicates orientation of the 3-OH to be axial, thus α, as in **3**.

An attempt to convert **3** to **4** directly by hydrogenation over Pt in AcOH resulted in exclusive formation of the A/B-*cis* isomer, or 5-epi-**4**, probably due to participation of the sugar moiety which conceals the α-side of the olefin. The *cis*-fusion of A/B rings in the hydrogenation product was determined based on a broad multiplet at 3.50 ppm for 3-ax-H in the NMR spectrum, indicating inversion of A ring by hydrogenation at the β-side. Alternatively, validity of the structure, which was suggested for **4** by the spectroscopic analogy, e.g. identical oxygenated sites to **3** in the skeleton, i.e. C-3, -15, and -26, was substantiated by chemical correlations via pavoninin-5 (**5**) (*vide infra*).

Pavoninin-5. A signal at 3.39 ppm (tt, 10, 4 Hz) in the ^{1}H-NMR spectrum of **5** in methanol-d_4 suggests an equatorial OH at C-3 instead of the axial one as in **3**; this appeared to be the only difference between the two in their NMR and other spectroscopic data. Aside from the sugar moiety confirmed by acetylation shifts in the ^{1}H-NMR spectra of **5** and its tetraacetate, identity of the rest of the structure was established by oxidation of **5** immediately followed by methanolysis to yield **10**, which was obtained from **3** by the same treatment (*vide supra*). Reflecting the closest structural resemblance to cholesterol, **5** is the most abundant of the six pavoninins.

Pavoninin-6. The spectroscopic data of **5** and **6** sug-



Scheme 1. Chemical correlations among pavoninins-1 (**1**) and -3 (**3**) and cholesterol. Reagents: (a) 5% HCl/MeOH, (b) H_2/Pd–C, 10 mM KOH/EtOH, (c) *p*-TsCl/Py, (d) $LiAlH_4/Et_2O$, (e) Jones oxidation, (f) 30% conc. HCl/MeOH, (g) *t*-$BuMe_2SiCl$, imidazole, *t*-$BuCH_2OH$/DMF, (h) PhOCSCl/Py, (i) *n*-Bu_3SnH, AIBN/PhMe.

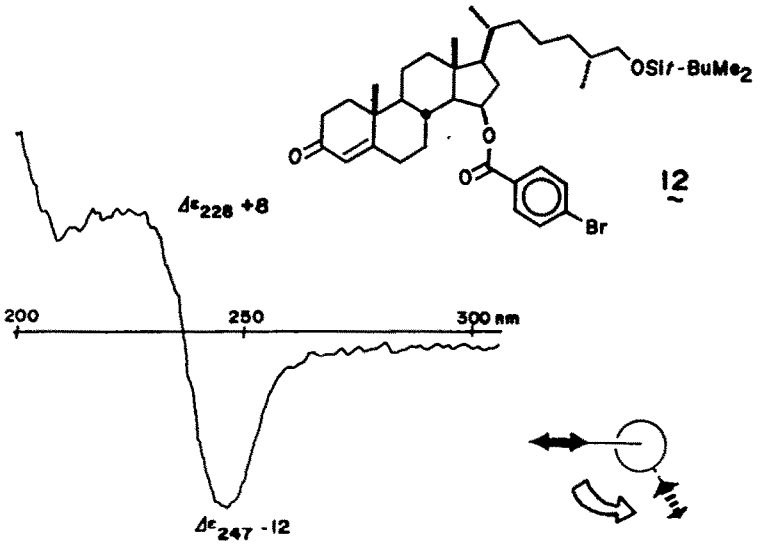


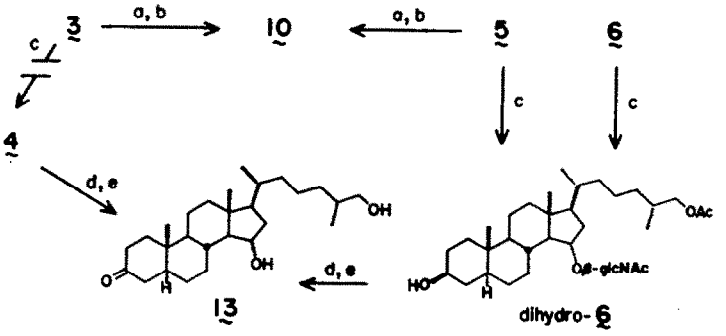
Fig. 4. The CD spectrum of 12, whose two chromophores constitute a coupled oscillator of negative chirality.

gested their structural similarity which had been inferred by their chromatographic behaviors. Existence of a disubstituted *cis*-olefin in **6**, instead of the trisubstituted olefin in **5**, is indicated by its ^{1}H-NMR spectrum in methanol-d_4 with signals at 5.69 (ddd, 10, 3, 2 Hz) and 5.25 ppm (br d, 10 Hz). Location of the *cis*-olefin was determined to be at C-6, 7 by sequential decoupling from 6-H through 15-H; 2.11 (br dd, 12, 10 Hz; 8-H), 1.32 (dd, 12, 9 Hz; 14-H), and 3.86 ppm (td, 9, 3 Hz; 15-H). Configuration at C-5 was determined to be 5α-H, as in **4**, by high-field chemical shifts of 10-Me; 0.79 ppm for ^{1}H's and 11.7 ppm for ^{13}C. *J*-values of the 3-H signal at 3.55 ppm (tt, 10, 4 Hz) indicated the 3-OH to be equatorial, thus β, as in **5**.

Hydrogenation of **6** over Pt in AcOH yielded dihydro-**6**. Corresponding hydrogenation of **5** yielded an epimeric mixture at C-5, which was not readily separable by either normal or reverse phase HPLC, in a ratio of *ca* 4:1 based on intensity of 10-Me signals at 0.97 (5β) vs 0.83 ppm (5α) in its ^{1}H-NMR spectrum. Furthermore, the more abundant 5β-isomer gives rise to a narrow 3-eq-H signal at 4.03 ppm, thus indicating inversion of ring A upon hydrogenation of **5** at the β-side. All signals attributed to the minor 5α-isomer in the 360 MHz ^{1}H-NMR spectrum are superimposable upon those in the spectrum of dihydro-**6**, thereby chemically correlating **5** and **6** to each other.

Finally, both **4** and dihydro-**6** were subjected to Jones oxidation followed by acid methanolysis, yielding the identical dihydroxyketone **13**, and thus concluding the chemical correlations among the six pavoninins **1** to **6**. The correlations among **3** to **6** are summarized in Scheme 2. The ^{13}C-NMR data of the six pavoninins (Table 1) corroborate the presence of the common cholestane skeleton.

Shark-repellent activity. In order to examine whether the pavoninins, which were isolated on the basis of their ichthyotoxicity, function as repellents against predatory fishes, qualitative assays were carried out with the pavoninins and the dog shark *Mustelus griseus*. When the baited shark was exposed to pavoninins ejected around the bait from a stainless-steel pipe dipped in the water, it tended to turn around immediately and swim away, though clear concentration dependency could not be obtained. Repellency of the ejection appeared to depend more on whether it hit the shark's nostrils. No escape behavior was brought about by the proteinaceous precipitate. The shark, however, spat out the bait which had been smeared with the precipitate. These observations hinted that the



Scheme 2. Chemical correlations among pavoninins-3 to -6 (3–6). Reagents: (a) Jones oxidation, N_2, (b) 30% conc. HCl/MeOH, (c) H_2/Pt, AcOH, (d) Jones oxidation, (e) 5% HCl/MeOH.

Table 1. ^{13}C-NMR chemical shifts (δ in ppm) of pavoninins-1 to -6 (1 to 6) in methanol-d_4. Carbons were assigned based on INEPTR measurements, mutual comparison, and comparison of published data[20]

^{13}C	1	2	3	4	5	6
1	36.5	36.5	33.9	33.8	38.6	35.8
2	34.8	34.8	29.6	30.1	32.3	32.0
3	201.9	201.9	67.8	67.1	72.4	71.9
4	126.7	126.6	40.3	36.8	43.0	37.0
5	173.3	173.3	139.6	40.0	141.8	45.9
6	37.3	37.3	123.7	29.6	122.8	130.7
7	73.9	74.0	33.6	33.4	33.5	130.8
8	40.7	40.7	32.9	36.5	33.0	38.9
9	46.2	46.2	51.2	55.7	51.5	53.9
10	39.7	39.7	38.2	37.2	37.7	35.2
11	21.9	21.9	21.6	21.7	21.9	22.0
12	40.4	40.4	40.0	39.8	40.0	39.5
13	43.4	43.4	43.5	43.8	43.5	44.5
14	50.6	50.5	61.8	62.2	61.9	60.4
15	24.2	24.2	85.7	85.2	85.7	84.4
16	29.3	29.3	41.2	41.4	41.2	41.5
17	57.2	57.2	55.0	55.1	55.0	55.2
18	12.3	12.2	13.5	13.8	13.5	13.9
19	17.3	17.4	19.4	11.9	20.0	11.7
20	37.0	37.0	36.5	36.5	36.5	36.6
21	19.2	19.2	19.1	19.1	19.1	19.0
22	37.2	37.3	37.0	37.0	37.0	37.0
23	24.2	24.4	24.3	24.3	24.3	24.4
24	34.8	34.8	34.7	34.7	34.7	34.7
25	33.8	36.9	33.7	33.7	33.7	33.7
26	70.6	68.5	70.6	70.6	70.6	70.6
27	17.1	17.1	17.1	17.1	17.1	17.1
1'	99.1	99.0	103.8	103.8	103.9	103.5
2'	57.2	57.2	57.8	57.7	57.7	57.9
3'	75.9	75.9	76.1	76.0	76.2	76.3
4'	72.3	72.3	72.2	72.2	72.2	72.2
5'	77.7	77.6	77.5	77.5	77.6	77.7
6'	63.2	63.1	63.0	63.0	63.0	63.0
OAc;CO	173.3	—	173.1	173.0	173.1	173.1
" ;Me	20.8	—	20.8	20.9	20.9	20.9
NAc;CO	173.3	173.3	173.4	173.3	173.4	173.6
" :Me	23.6	23.6	23.4	23.3	23.4	23.5

pavoninins are repellents which act on the shark's olfactory sense, whereas the proteinaceous toxin is an antifeedant which acts on its gustatory sense.

Recently, a shark-repellent assay based on termination of tonic immobility of the shark (TI test) has been developed by Gruber for the purpose of screening potential shark repellents, and has been used successfully for screening a series of surfactants, including the ichthyocrinotoxic secretion from the Moses sole.[9] According to preliminary TI tests on the lemon shark *Negaprion brevirostris* at Miami, the pavoninins have been shown to be relatively strong repellents by acting reproducibly on buccal receptors as well as on the olfactory rosette at a concentration of 5 mg/mL.[21] The sole can discharge more than 70 mg of pavoninins at once based on the isolation yield (*vide supra*), and the discharge takes place at the last moment of the attack by a predator[7] so that the toxin may reach the senses of the attacker before much dilution. If one considers these facts, the pavoninins may well achieve their function as repellents against predators.

EXPERIMENTAL

IR spectra were recorded of sample films on a Hitachi EPI-G2 spectrophotometer, or on a Nicolet 7199A FT-IR spectrometer. IR data represent ν_{max} in cm^{-1}. EI- and SI-MS's were recorded on a Hitachi M-80 mass spectrometer. FD-MS's were recorded on the Hitachi instrument or on a Jeol JMS-01SG-2 mass spectrometer. MS data represent *m/z* (an interpreted positive ion, or the site of fission with positive charge/the site of fission with neutral charge, relative intensity). ^{1}H-NMR spectra were recorded on a Nicolet NT-360 spectrometer at 360 MHz. TMS was used as the standard at 0.00 ppm for measurements in $CDCl_3$ or in $CDCl_3/CD_3OD$. A signal at 3.30 ppm for C^1HD_2OD was used as the standard for measurements in CD_3OD. The difference spectrum technique was frequently used in double resonance measurements to observe decoupling of overlapping signals. ^{1}H-NMR data represent δ in ppm (multiplicity, *J*-value(s) in Hz; assignments). ^{13}C-NMR spectra were recorded on a Jeol FX100 spectrometer at 25 MHz. A signal for $^{13}CD_3OD$ was used as the standard at 49.0 ppm. UV spectra were recorded on a Shimadzu UV-210A double-beam spectrophotometer. UV data represent λ_{max} in nm. CD spectra were recorded on a Jasco J-20C spectropolarimeter. Optical rotations were measured with a Perkin-Elmer 161 polarimeter.

Kieselgel 60, E. Merck, was used for silica gel column chromatography (230–400 mesh), and for tlc unless otherwise mentioned. Preparative silica-gel HPLC was performed with a Radial Pack B column, Waters Assoc.

Ichthyotoxicity. A lipophilic sample of known weight was dissolved in 0.1 mL of EtOH and diluted with water, or a hydrophilic sample in 0.05 M phosphate buffer (pH 7.2), to a volume of 10 mL. An individual of the Japanese killifish *Oryzias latipes* was put in the soln, and death time was measured. The reciprocal of death time was plotted vs a series of concentrations, and 1 hr lethal concentration (LC_{50}) was obtained.

Hemolytic activity. A lipophilic sample of known weight was dissolved in 20 μL of EtOH and diluted with 0.15 M choline chloride in Tris-HCl buffer (pH 7.4), or a hydrophilic sample directly in the isotonic buffer, to a volume of 2 mL. 1 mL rabbit erythrocyte suspension (6×10^7 cell) in the same isotonic buffer was added to the sample soln, and the whole suspension was incubated at 37° for 20 min. It was then centrifuged at 2000 rpm for 10 min, and absorbance at 560 nm was recorded for the supernatant. Absorbance for the supernatant from the incubated control suspension (i.e. 2×10^7 cell) was taken as 0% hemolysis, and absorbance for the supernatant from the erythrocyte suspension diluted with distilled water threefold was taken as 100% hemolysis. A 50% hemolysis concentration was obtained by the probit method with a series of sample dilutions. Thus obtained hemolytic activity is expressed as relative activity to that of standard

saponin (E. Merck) on the erythrocyte suspension from the same lot. 1 saponin unit (SU) stands for a hemolytic activity equivalent to 1 mg standard saponin.

Isolation of pavoninin-1 to -6 (**1–6**). Lyophilized secretion of *P. pavoninus*, 14 discharges from 7 specimens (30 g; LC_{50} *ca* 100 μg/mL), was dissolved in 350 mL 0.1 M aqueous ammonia, then diluted tenfold by cold acetone with agitation. The resulting suspension was allowed to stand at 4° overnight, then a white ppt was filtered off. The procedure was repeated with the ppt, and bioactivities of the first ppt were recovered in the second ppt (10.66 g; LC_{50} *ca* 60 μg/mL, 0.018 SU/mg).

The combined filtrate was evaporated to dryness and extracted with 3 portions of 400 mL EtOAc from 400 mL water. Solvent was evaporated from the organic extract to give a yellow oil (1.256 g), which was applied to a silica gel (40 g) column and eluted consecutively with 250 mL each of $CHCl_3$, and 5, 10, 25 and 60% MeOH in it. More than 90% of both bioactivities were recovered in the eluates with 10 and 25% MeOH, which were combined to give a mixture of pavoninins (992 mg; LC_{50} 8.5 μg/mL, 0.92 SU/mg), whose silica gel tlc (25% MeOH/$CHCl_3$) showed two spots at R_f 0.6 and 0.5. The eluates with $CHCl_3$ (65 mg; LC_{50} *ca* 50 μg/mg, < 0.05 SU/mg), with 5% MeOH (42 mg ; LC_{50} *ca* 50 μg/mL, 0.15 SU/mg), and with 60% MeOH (136 mg; LC_{50} *ca* 20 μg/mL, 0.38 SU/mg) retained only a minor part of activities. The pavoninins in the shark assays are composed of this pavoninins mixture.

The mixture (884 mg) was separated by repeated silica gel column chromatography (10–25% MeOH/$CHCl_3$) into two fractions corresponding to the tlc spots. Reverse-phase column chromatography (LiChroprep RP-8, E. Merck; 80% aq MeOH) of the less polar fraction (145 mg) yielded a single major component, pavoninin-1 (**1**; 111 mg), $[\alpha]_D^{20}$ +19° (*c* 1.1, $CHCl_3$); IR: 3330, 1740, 1660, 1650 (sh), 1550, 1235 and 1080; SI-MS (glycerol matrix): 684 ((M + Na)$^+$, 1.0%), 662 ((M + H)$^+$, 2.0%), 441 ((M + H)$^+$ – HOglcNAc, 6.5%) and 204 (glcNAc$^+$, 100%); FD-MS: 440 (M$^+$ – HOglcNAc, 100%) and 221 (HOglcNAc$^+$, 21%); ^{1}H-NMR(CD_3OD): 4.42 (d, 9; 1′-H), 3.88 (dd, 12, 2; 6′-H_a), 3.59 (dd, 12, 6; 6′-H_b), 3.59 (dd, 10, 9; 2′-H), 3.45 (dd, 10, 9; 3′-H), 3.32 (dd, 10, 9; 4′-H), 3.26 (ddd, 10, 6, 2; 5′-H), 2.48 (ddd, 17, 15, 5; 2β-H), 2.28 (br d, 17; 2α-H), 2.07 (m; 1β-H), 2.03 (3H, s; OAc), 1.80 (3H, s; N-Ac), 1.68 (m; 1α-H), 1.22 (3H, s; 10-Me), 0.94 (3H, d, 7; 20-Me), 0.93 (3H, d, 7; 25-Me), 0.73 (3H, s; 13-Me), and others in Fig. 2; ^{13}C-NMR(CD_3OD): see Table 1; UV(MeOH): 244 (log ε 4.1).

The eluate from the same chromatography of the more polar fraction (617 mg) was further fractionated into four. The earliest eluate yielded pavoninin-2 (**2**; 34 mg), $[\alpha]_D^{29}$ +31° (*c* 1.6, EtOH); IR: 3300, 1650, 1550 and 1070; FD-MS: 642 ((M + Na)$^+$, 22%), 620 ((M + H)$^+$, 40%), 398 (M$^+$ – HOglcNAc, 100%) and 381 (M$^+$ – HOglcNAc – H_2O, 10%); ^{1}H-NMR(CD_3OD): 5.74 (br s; 4-H), 4.42 (d, 9; 1′-H), 4.03 (td, 3, 2; 7-H), 3.88 (dd, 12, 2; 6′-H_a), 3.69 (dd, 12, 6; 6′-H_b), 3.59 (dd, 10, 9; 2′-H), 3.45 (dd, 10, 9; 3′-H), 3.40 (dd, 11, 6; 26-H_a), 3.33 (dd, 11, 4; 26-H_b), 3.32 (dd, 10, 9; 4′-H), 3.26 (ddd, 10, 6, 2; 5′-H), 2.62 (dd, 15, 3; 6α-H), 2.48 (ddd, 17, 15, 3; 2β-H), 2.46 (dd, 15, 3; 6β-H), 2.28 (br d, 17; 2α-H), 1.79 (3H, s; N-Ac), 1.67 (td, 11, 2; 8-H), 1.22 (3H, s; 10-Me), 0.94 (3H, d, 7; 20-Me), 0.90 (3H, d, 7; 25-Me) and 0.73 (3H, s; 13-Me); ^{13}C-NMR(CD_3OD): see Table 1; UV(EtOH): 244 (log ε 4.0).

The next eluate yielded pavoninin-3 (**3**; 143 mg), $[\alpha]_D^{29}$ +15° (*c* 0.7, EtOH); IR: 3330, 1730, 1655, 1560, 1240 and 1075; FD-MS: 664 ((M + H)$^+$, 100%), 663 (M$^+$, 79%), 646 ((M + H)$^+$ – H_2O, 12%), 621 (M$^+$ – H_2C–C–O, 52%) and 204 (glcNAc$^+$, 12%); ^{1}H-NMR(CD_3OD): 4.40 (d, 8; 1′-H), 3.94 (dd, 11, 6; 26-H_a), 3.85 (2H, dd, 12, 3; 6′-H_a and dd, 11, 7; 26-H_b), 3.69 (dd, 12, 5; 6-H_b), 3.61 (dd, 10, 8; 2′-H), 3.44 (dd, 10, 9; 3′-H), 3.33 (dd, 10, 9; 4′-H), 3.23 (ddd, 10, 5, 3; 5′-H), 2.03 (3H, s; OAc), 1.98 (3H, s; N-Ac), 1.76 (m; 25-H), 1.35 (m; 20-H), 1.03 (3H, s; 10-Me), 0.93 (6H, d, 7; 20- and 25-Me's), 0.73 (3H, s; 13-Me), and others in Fig. 3; ^{13}C-NMR(CD_3OD): see Table 1.

The last eluate yielded pavoninin-4 (**4**; 49 mg), $[\alpha]_D^{30}$ +36° (*c* 0.8, EtOH); IR: 3320, 1735, 1655, 1560, 1240 and 1075; FD-MS: 688 ((M + Na)$^+$, 37%), 666 ((M + H)$^+$, 100%) and 624 ((M + H)$^+$ – H_2C–C–O, 23%); ^{1}H-NMR(CD_3OD): 4.39 (d, 8; 1′-H), 3.94 (2H, m, $W_{1/2}$ 8; 3-H and dd, 11, 6; 26-H_a), 3.85 (2H, dd, 12, 2; 6′-H_a and dd, 11, 7; 26-H_b), 3.69 (dd, 12, 6; 6′-H_b), 3.67 (td, 9, 3; 15-H), 3.59 (dd, 10, 8; 2′-H), 3.43 (dd, 10, 9; 3′-H), 3.33 (t, 9; 4′-H), 3.23 (ddd, 9, 5, 2; 5′-H), 2.18 (ddd, 15, 8, 3; 16α-H), 2.03 (3H, s; OAc), 1.93 (3H, s; N-Ac), 1.79 (ddd, 15, 10, 9; 16β-H), 1.27 (td, 10, 8; 17-H), 1.15 (dd, 11, 9; 15-H), 0.92 (3H, d, 7; 25-Me), 0.91 (3H, d, 6; 20-Me), 0.81 (3H, s; 10-Me) and 0.70 (3H, s; 13-Me); ^{13}C-NMR(CD_3OD): see Table 1.

The third eluate yielded a mixture of two components (272 mg), which was separated through a column of silica gel (50–100 mesh) impregnated with 10% $AgNO_3$ (15–20% MeOH/EtOAc). The component in the earlier eluate was extracted by the lower layer of a two-phase mixture, $CHCl_3$–MeOH–water (7:18:8), from the upper layer which removed $AgNO_3$. Purification of the extract through a silica gel column (18% MeOH/$CHCl_3$) yielded pavoninin-5 (**5**; 210 mg), $[\alpha]_D^{29}$ +21° (*c* 0.7, EtOH); IR: 3300, 1725, 1650, 1555, 1240 and 1080; FD-MS: 664 ((M + H)$^+$, 84%), 663 (M$^+$, 100%), 646 ((M + H)$^+$ – H_2O, 24%), 645 (M$^+$ – H_2O, 25%) and 621 (M$^+$ – H_2C–C–O, 70%); ^{1}H-NMR(CD_3OD): 5.37 (br d, 4; 6-H), 4.38 (d, 8; 1′-H), 3.94 (dd, 11, 6; 26-H_a), 3.85 (2H, dd, 11, 2; 6′-H_a and dd, 11, 7; 26-H_b), 3.71 (td, 9, 3; 15-H), 3.69 (dd, 11, 5; 6′-H_b), 3.62 (dd, 10, 8; 2′-H), 3.42 (dd, 10, 9; 3′-H), 3.39 (tt, 10, 4; 3-H), 3.33 (dd, 10, 9; 4′-H), 3.23 (ddd, 9, 5, 2; 5′-H), 2.22 (2H, m; 4-H_2), 2.17 (ddd, 15, 9, 3; 16α-H), 2.03 (3H, s; OAc), 1.96 (3H, s; N-Ac), 1.02 (3H, s; 10-Me), 0.92 (6H, d, 7; 20- and 25-Me's) and 0.73 (3H, s; 13-Me); ^{13}C-NMR(CD_3OD): see Table 1.

The component in the later eluate was extracted by 10% MeOH in $CHCl_3$ from water. Solvent removal and purification through a silica gel column (18% MeOH/$CHCl_3$) yielded pavoninin-6 (**6**; 44 mg), $[\alpha]_D^{30}$ –25° (*c* 2.4, EtOH); IR: 3300, 1720, 1640, 1540, 1240, 1070 and 720; FD-MS: 686 ((M – Na)$^+$, 65%), 664 ((M + H)$^+$, 100%) and 663 (M$^+$, 33%); ^{1}H-NMR(CD_3OD; assignments partially aided by a 2D ^{1}H-^{1}H correlation measurement): 5.69 (ddd, 10, 3, 2; 7-H), 5.25 (br d, 10; 6-H), 4.48 (d, 8; 1′-H), 3.94 (dd, 11, 6; 26-H_a), 3.86 (td, 9, 3; 15-H), 3.85 (2H, dd, 12, 2; 6′-H_a, and dd, 11, 7; 26-H_b), 3.69 (dd, 12, 6; 6-H_b), 3.61 (dd, 10, 8; 2′-H), 3.55 (tt, 10, 4; 3-H), 3.43 (dd, 10, 9; 3′-H), 3.33 (t, 9; 4′-H), 3.25 (ddd, 9, 6, 2; 5′-H), 2.22 (ddd, 15, 8, 3; 16α-H), 2.11 (br dd, 12, 10; 8-H), 2.03 (3H, s; OAc), 1.97 (3H, s; N-Ac), 1.89 (m; 5-H), 1.82 (ddd, 15, 10, 9; 16β-H), 1.78 (m; 25-H), 1.36 (m; 20-H), 1.32 (dd, 12, 9; 14-H), 1.02 (ddd, 12, 10, 3; 9-H), 0.92 (3H, d, 7; 25-Me), 0.91 (3H, d, 6; 20-Me), 0.79 (3H, s; 10-Me) and 0.74 (3H, s; 13-Me); ^{13}C-NMR(CD_3OD): see Table 1.

Acetylation of **1**. Pavoninin-1 (**1**; 5.5 mg) was treated with 0.1 mL Ac_2O in 0.5 mL pyridine at room temp overnight. After addition of MeOH and solvent removal as the benzene azeotrope, the product was purified through a silica gel column (3% MeOH/$CHCl_3$) to yield **1** triacetate (5.8 mg); IR: 3350, 1740, 1665, 1655 (sh), 1540, 1235 and 1040; EI-MS: 787 (M$^+$, 0.3%), 727 (M$^+$ – AcOH, 0.2%), 440 (M$^+$ – HOglcNAc·Ac_3, 100%), 330 (glcNAc·Ac$_2^+$, 8%) and 269 (C-17$^+$/20 – HOglcNAc·Ac_3, 25%); ^{1}H-NMR($CDCl_3$): 5.65 (br s; 4-H), 5.43 (br d, 9, D_2O exchangeable; NH), 5.23 (dd, 11, 9; 3′-H), 5.06 (dd, 10, 9; 4′-H), 4.65 (d, 8; 1′-H), 4.19 (dd, 12, 5; 6′-H_a), 4.14 (dd, 12, 3; 6′-H_b), 3.95 (m; 7-H), 3.94 (dd, 11, 6; 26-H_a), 3.85 (dd, 11, 7; 26-H_b), 3.84 (ddd, 11, 9, 8; 2′-H), 3.66 (ddd, 10, 5, 3; 5′-H), 2.50 (dm, 15; 6-H_a), 2.42 (ddd, 17, 15, 5; 2β-H), 2.39 (dm, 15; 6-H_b), 2.34 (dm, 17; 2α-H), 2.08 (3H, s; OAc), 2.06 (3H, s; OAc), 2.03 (3H, s; OAc), 2.02 (3H, s; OAc), 1.80 (3H, s; N-Ac), 1.18 (3H, s; 10-Me), 0.93 (3H, d, 7; 25-Me), 0.91 (3H, d, 7; 20-Me) and 0.67 (3H, s; 13-Me); UV(MeOH): 242 (log ε 4.1).

Methanolysis of **1**. Pavoninin-1 (**1**; 10 mg) was treated with 2 mL 5% HCl in MeOH at 64° for 2 hr. After solvent removal, the residue was partitioned between water and EtOAc. Sol-

vent removal from the aqueous layer as the 2-propanol azeotrope and elution through a reverse-phase column (Sep-Pak C_{18}, Waters Assoc.; H_2O) yielded an anomeric mixture of methyl D-glucosaminidium salts (1.5 mg), which gave a ninhydrin-positive spot at R_f 3.8 on silica gel tlc (n-BuOH–AcOH–H_2O (4:1:1)). The mixture was treated with 0.1 mL pyridine and 0.2 mL Ac_2O in 1 mL MeOH at room temp for 2 hr. After solvent removal as the benzene azeotrope, the residue was treated with *p*-bromobenzoyl chloride (6 mg) and N,N-dimethyl-4-aminopyridine (3 mg) in 1 mL pyridine at 60° overnight. After addition of 1 mL MeOH and solvent removal as the benzene azeotrope, the major product was eluted through a silica gel column (67% EtOAc/hexane) and purified by silica gel HPLC (33% EtOAc/hexane) to yield methyl α-N-acetyl-D-glucosaminide tri-*p*-bromobenzoate; FD-MS: 788, 786, 784 and 782 ($(M + H)^+$, 8, 15, 15 and 9%), and 43 (Ac^+, 100%); ^{1}H-NMR($CDCl_3$): identical to that of the tri-*p*-bromobenzoate correspondingly prepared from authentic N-acetyl-D-glucosamine (methanolysis, N-acetylation, and O-*p*-bromobenzoylation); 7.88 (2H, d, 9; Bz_a-*m*-H's), 7.76 (2H, d, 8; Bz_b-*m*-H's), 7.73 (2H, d, 8; Bz_c-*m*-H's), 7.58 (2H, d, 9; Bz_a-*o*-H's), 7.50 (2H, d, 8; Bz_b-*o*-H's), 7.49 (2H, d, 8; Bz_c-*o*-H's), 5.82 (d, 9, D_2O exchangeable; NH), 5.63 (t, 10; 3-H), 5.60 (dd, 10, 9; 4-H), 4.85 (d, 3; 1-H), 4.59 (ddd, 10, 9, 3; 2-H), 4.56 (dd, 13, 3; 6-H_a), 4.42 (dd, 13, 5; H_b), 4.28 (ddd, 9, 5, 3; 5-H), 3.50 (3H, s; OMe) and 1.88 (3H, s; N-Ac); UV(MeOH): 246, CD(MeOH, based on absorbance at λ_{max} of UV, where ε was assumed to be 57,200[13]): $\Delta\varepsilon_{232}$ +9, $\Delta\varepsilon_{252}$ −20.

After solvent evaporation from the organic layer of the methanolysate, the major component was purified by silica gel HPLC (3% EtOAc/CH_2Cl_2) to yield **7** (4.6 mg); IR: 3440, 1660, 1610 and 1030; EI-MS: 398 (M^+, 100%), 269 (C-17$^+$/20, 19%) and 136 (C-7;10$^+$/8;9, 30%); ^{1}H-NMR($CDCl_3$; assignments in Fig. 2): 6.14 (ddd, 10, 2, 1), 6.09 (dd, 10, 2), 5.67 (br s), 3.50 (dd, 10, 6), 3.43 (dd, 10, 7), 2.57 (ddd, 18, 14, 5), 2.43 (dddd, 18, 5, 2, 1), 2.19 (br t, 10), 2.06 (dt, 13, 3), 2.00 (ddd, 13, 5, 2), 1.71 (ddd, 14, 13, 5), 1.61 (m), 1.54 (dtd, 13, 4, 3), 1.43 (2H, tdd, 13, 12, 3; 11β-H, and m; 20-H), 1.21 (td, 13, 4), 1.20 (ddd, 12, 10, 4), 1.11 (3H, s), 0.93 (3H, d, 7), 0.92 (3H, d, 7) and 0.76 (3H, s); UV(MeOH): 286.

Hydrogenation of **7**. Hydroxydienone **7** (6 mg) was stirred with 5% Pd–C (5 mg) in 5 mL 10 mM ethanolic KOH under H_2 at room temp for 50 min. After removal of Pd–C by filtration, solvent evaporation, and elution through a silica gel column (3% MeOH/$CHCl_3$), the major product was purified by silica gel HPLC (10% EtOAc/CH_2Cl_2) to yield **8** (2.9 mg); IR: 3400, 1710 and 1035; EI-MS: 402 (M^+, 100%), 332 (C-5;10$^+$/4;1, 71%) and 231 (C-13;14$^+$/17;15 − H, 73%); ^{1}H-NMR($CDCl_3$): 3.50 (m; 26-H_a), 3.43 (m; 26-H_b), 2.70 (dd, 15, 14; 4α-H), 2.34 (td, 15, 6; 2α-H), 2.16 (dm, 15; 2β-H), 1.02 (3H, s; 10-Me), 0.92 (6H, d, 7; 20- and 25-Me's) and 0.68 (3H, s; 13-Me).

Dehydroxylation of **8**. Hydroxyketone **8** (5 mg) was treated with *p*-tosyl chloride (10 mg) in 0.5 mL pyridine at 4° overnight. After solvent removal as the toluene azeotrope, the product was purified through a silica gel column (CH_2Cl_2) to yield ketone tosylate (5.5 mg). The tosylate was stirred with LAH (10 mg) in 3 mL dry diethyl ether at room temp for 1 hr, then under reflux for 10 min. EtOAc (0.1 mL) and $MgSO_4$ (500 mg) was added sequentially to the mixture, which was then filtered. Solvent removal from the filtrate yielded an epimeric mixture of 5β-cholestan-3-ols (4.0 mg), which was treated with 5 μL Jones reagent (2 M chromium(VI) oxide in 32% aqueous H_2SO_4) in 1 mL acetone at room temp for 20 min. After addition of 0.1 mL 2-propanol, solvent evaporation, and extraction by diethyl ether from water, the product was purified through a silica gel column (CH_2Cl_2) to yield 5β-cholestan-3-one (3.6 mg), whose spectroscopic data are identical to those of a sample prepared from authentic cholesterol; IR: 1715; EI-MS: 386 (M^+, 100%), 316 (C-5;10$^+$/4;1, 40%) and 231 (C-13;14$^+$/17;15 − H, 74%); ^{1}H-NMR($CDCl_3$): 2.70 (dd, 15, 14; 4α-H), 2.34 (td, 15, 6; 2α-H), 2.16 (dm, 15; 2β-H), 1.02 (3H, 3; 10-Me), 0.92 (3H, d, 7; 20-Me), 0.88 (3H, d, 7; 25-Me_a), 0.87 (3H, d, 7; 25-Me_b) and 0.68 (3H, s; 13-Me); CD(MeOH): $\Delta\varepsilon_{288}$ −0.5.

(+)- and (−)-MTPA'ation of **7**. Hydroxydienone **7** (2.0 mg) was treated with freshly distilled (+)-methoxytrifluoromethylphenylacetyl chloride (large excess) in 1 mL pyridine–CCl_4 (1:1) at 60° overnight. After solvent removal as the benzene azeotrope and extraction by diethyl ether from water, the product was eluted through a silica gel column ($CHCl_3$) and purified by silica gel HPLC (17% EtOAc/hexane) to yield **7** (+)-MTPA ester (2.0 mg); IR: 3060, 1750, 1665, 1620, 1270, 1170, 1025, 875 and 710; EI-MS: 615 (M^+, 100%), 269 (C-17$^+$/20, 18%) and 189 ($(MeO)(CF_3)PhC^+$, 56%); ^{1}H-NMR($CDCl_3$): 7.52 (2H, m; Ph-*o*-H's), 7.40 (3H, m; Ph-*m*- and -*p*-H's), 6.14 (br d; 10, 7-H), 6.10 (dd, 10, 2; 6-H), 5.67 (s; 4-H), 4.24 (dd, 11, 6; 26-H_a), 4.08 (dd, 11, 7; 26-H_b), 3.55 (3H, q, < 1; OMe), 2.57 (ddd, 18, 14, 5; 2β-H), 2.43 (br dd, 15, 5; 2α-H), 2.19 (br t, 10; 8-H), 2.05 (dt, 12, 3; 12β-H), 2.00 (ddd, 13, 5, 2; 1β-H), 1.71 (ddd, 14, 13, 5; 1α-H), 1.11 (3H, s; 10-Me), 0.91 (3H × 2, d, 7; 25-Me and d, 6; 20-Me) and 0.75 (3H, s; 13-Me).

Treatment of **7** with (−)-methoxytrifluoromethylphenylacetyl chloride with the same procedure as above yielded **7** (−)-MTPA ester; IR: 3060, 1750, 1665, 1620, 1265, 1170, 1025, 875, 720 and 670; EI-MS: 615 (M^+, 100%), 269 (C-17$^+$/20, 13%), and 189 ($(MeO)(CF_3)PhC^+$, 38%); ^{1}H-NMR($CDCl_3$): 4.16 (2H, d, 6; 26-H_2), 0.92 (3H, d, 7; 25-Me), 0.90 (3H, d, 6; 20-Me), and others indistinguishable from signals in the spectrum of the (+)-MTPA ester.

A 10:7 mixture of the (+)- and (−)-MTPA esters; ^{1}H-NMR (with 1 mol equiv Eu(fod)$_3$ in $CDCl_3$): 10.19 (4-H), 7.90 (Ph-*o*-H's), 7.52 (Ph-*m*- and -*p*-H's), 7.49 (2-H_2), 7.02 (6-H), 6.53 (7-H), 4.45 (26-H_a of (+)-MTPA), 4.36 (26-H_2 of (−)-MTPA), 4.29 (26-H_b of (+)-MTPA), 4.18 (OMe of (+)-MTPA), 4.16 (OMe of (−)-MTPA), 3.78 (1α-H), 3.25 (1β-H), 3.04 (8-H), 2.38 (12β-H), 2.27 (10-Me), 1.07 (20-Me), 1.02 (13-Me); 1.03 (25-Me of (−)-MTPA) and 1.02 (25-Me of (+)-MTPA), ^{1}H-NMR (with 2.8 mol equiv Eu(fod)$_3$ in $CDCl_3$): 14.86 (4-H), 11.40 (2-H_2), 9.01 (Ph-*o*-H's of (+)-MTPA), 8.98 (Ph-*o*-H's of (−)-MTPA), 7.81 (6-H), 7.78 (Ph-*m*- and -*p*-H's), 6.77 (7-H), 5.60 (OMe of (+)-MTPA), 5.55 (OMe of (−)-MTPA), 5.43 (1α-H), 4.95 (26-H_a of (+)-MTPA), 4.85 (26-H_2 of (−)-MTPA), 4.78 (26-H_b of (+)-MTPA), 4.25 (1β-H), 3.70 (8-H), 3.20 (10-Me), 2.98 (9-H), 2.79 (11α-H), 2.63 (12β-H), 2.50 (11β-H), 1.25 (13-Me), 1.21 (20-Me) and 1.19 (25-Me).

Acetylation of **2**. Pavoninin-2 (**2**; 2.7 mg) was treated with 0.2 mL Ac_2O in 1 mL pyridine at room temp overnight. After the same work-up as acetylation of **1**, the product was purified through a silica gel column (50% EtOAc/$CHCl_3$) to yield **2** tetraacetate (2.9 mg); IR, EI-MS, ^{1}H-NMR($CDCl_3$) and UV(MeOH): all identical to those of **1** triacetate.

Acetylation of **3**. Pavoninin-3 (**3**; 3.3 mg) was treated with 30 μL Ac_2O in 0.3 mL pyridine at room temp overnight. After the same work-up as acetylation of **1**, the residue was applied on a silica gel column. Elution with 50% EtOAc in benzene yielded **3** tetraacetate (2.4 mg); IR: 3280, 1730, 1650, 1540, 1230 and 1040; EI-MS: 831 (M^+, 7%), 771 (M^+ − AcOH, 1.1%), 424 (M^+ − HOglcNAc·Ac_3 − AcOH, 100%), 330 (glcNAc·Ac‡, 61%), and 158 (C-8;11$^+$/14;12 − 2H − AcOH, 48%); ^{1}H-NMR($CDCl_3$): 5.45 (d, 9, D_2O exchangeable; NH), 5.20 (ddm, 11, 10; 3'-H), 5.17 (m; 6-H), 5.03 (t, 10; 4'-H), 4.98 (m, $W_{1/2}$ 8; 3-H), 4.57 (d, 8; 1'-H), 4.19 (2H, d, 4; 6'-H_2), 3.93 (dd, 11, 6; 26-H_a), 3.89 (ddd, 11, 9, 8; 2'-H), 3.85 (dd, 11, 7; 26-H_b), 3.65 (m; 15-H), 3.64 (dt, 10, 4; 5'-H), 2.47 (dm, 15; 4α-H), 2.19 (dm, 15; 4β-H), 2.07 (3H, s; OAc), 2.06 (3H, s; OAc), 2.03 (3H × 2, s × 2; OAc × 2), 2.01 (3H, s; OAc), 1.93 (3H, s; N–Ac), 1.27 (t, 10; 14-H), 1.00 (3H, s; 10-Me), 0.92 (3H, d, 7; 25-Me), 0.90 (3H, d, 6; 20-Me) and 0.68 (3H, s; 13-Me).

Further elution with EtOAC yielded **3** 3'O,4'O,6'O-triacetate (1.5 mg); IR: 3450, 3300, 1740, 1655, 1540, 1235 and 1040; EI-MS: 789 (M^+, 2%), 771 (M^+ − H_2O, 3%), 729 (M^+ − AcOH, 4%), 424 (M^+ − HOglcNAc·Ac_3 − H_2O, 100%), 330 (glcNAc·Ac‡, 36%), 288 (glcNAc·Ac‡ − H_2C–C–O, 40%), and 158 (C-8;11$^+$/14;12 − 2H − H_2O, 57%); ^{1}H-NMR($CDCl_3$): 5.45 (d, 9; NH), 5.32 (m; 6-H),

5.30 (dd, 11, 10; 3'-H), 5.03 (t, 10; 4'-H), 4.67 (d, 8; 1'-H), 4.19 (2H, d, 4; 6'-H_2), 4.00 (m, $W_{1/2}$ 15; 3-H), 3.93 (dd, 11, 6; 26-H_a), 3.84 (dd, 11, 7; 26-H_b), 3.77 (ddd, 11, 9, 8; 2'-H), 3.66 (2H, m; 15-H and dt, 10, 4; 5'-H), 2.57 (dm, 15; 4α-H), 2.07 (3H, s; OAc), 2.06 (3H, s; OAc), 2.03 (6H, s; OAc × 2), 1.94 (3H, s; N-Ac), 1.46 (d, 7; OH), 1.25 (t, 4; 14-H), 1.01 (3H, s; 10-Me), 0.92 (3H, d, 7; 25-Me), 0.90 (3H, d, 6; 20-Me) and 0.68 (3H, s; 13-Me).

Methanolysis of **3**. Pavoninin-3 (**3**; 14.4 mg) was treated with 2 mL 5% HCl in MeOH at 64° for 6 hr. After solvent evaporation, the residue was partitioned between EtOAc and water. Solvent removal from the aqueous layer as the 2-propanol azeotrope and elution through a polystyrene (SM-2, BioRad Lab.) column (H_2O) yielded an anomeric mixture of methyl-D-glucosaminidium salts, which gave a ninhydrin-positive spot at the same R_f on the tlc as the methanolysate of **1**. After solvent evaporation from the organic layer, the major component was purified through a silica gel column (2–10% MeOH/$CHCl_3$) to yield enetriol **9** (6.6 mg); EI-MS: 400 ($M^+ - H_2O$, 77%), 367 ($M^+ - 2H_2O$ – Me, 94%), and 158 (C-8;11$^+$/14;12 – 2H – H_2O, 100%); FD-MS: 419 ((M + H)$^+$, 30%), 418 (M^+, 26%), 400 ($M^+ - H_2O$, 100%) and 383 ((M + H)$^+ - 2H_2O$, 23%); ^{1}H-NMR($CDCl_3$–CD_3OD (1:1 v/v); assignments in Fig. 3): 5.36 (m, $W_{1/2}$ 10), 4.00 (m, $W_{1/2}$ 8), 3.89 (td, 9, 4), 3.43 (dd, 10, 6), 3.33 (dd, 10, 7), 2.53 (br d, 15, $W_{1/2}$ 8), 2.23 (dtd, 18, 5, 2), 2.07 (br d, 15, $W_{1/2}$ 6), 2.00 (ddd, 12, 4, 3; 1β- or 12β-H), 1.89 (ddd, 14, 9, 7), 1.86 (br dd, 18, 10), 1.71 (ddd, 14, 8, 4), 1.68 (tdd, 11, 10, 5), 1.57 (m), 1.13 (td, 11, 5), 1.10 (dd, 11, 9), 1.04 (3H, s), 0.94 (3H, d, 6), 0.91 (3H, d, 7) and 0.74 (3H, s).

Jones oxidation of **3** *and methanolysis*. Pavoninin-3 (**3**; 10.2 mg) was treated with 20 μL Jones reagent in 5 mL acetone at room temp for 10 min, and 50 μL 2-propanol was added to the mixture. Immediately after solvent evaporation, the residue was treated with 8 mL 30% conc HCl in MeOH under reflux for 5 hr. Up to this stage, all the reagents and solvents were previously bubbled with N_2 stream to expel O_2. After solvent evaporation, extraction with diethyl ether from water, and elution through a silica gel column (5% MeOH/$CHCl_3$), the product was purified by reverse-phase HPLC (μBondapack C18, Waters Assoc.; 75% aq MeOH), then by silica-gel HPLC (5% EtOH/CH_2Cl_2) to yield dihydroxyenone **10** (3.8 mg); IR: 3400, 1660, 1615 and 1045; EI-MS: 416 (M^+, 54%), 398 ($M^+ - H_2O$, 45%), 227 (C-17$^+$/20 – H_2O – H_2C–C–O, 55%) and 124 (C-6;10$^+$/7;9 + 2H, 100%); ^{1}H-NMR($CDCl_3$): 5.73 (br s; 4-H), 3.98 (dd, 9, 3; 15-H), 3.50 (dd, 10, 6; 26-H_a), 3.43 (dd, 10, 7; 26-H_b), 1.20 (3H, s; 10-Me), 1.05 (dd, 12, 9; 14-H), 0.92 (3H, d, 7; 25-Me), 0.91 (3H, d, 6; 20-Me) and 0.74 (3H, s; 13-Me); UV(MeOH): 242.

Selective silylation of **10**. Dihydroxyenone **10** (3.5 mg) was treated with t-butyldimethylsilyl chloride (12 mg), imidazole (8 mg), and neopentyl alcohol (8 mg) in 0.2 mL N,N-dimethylformamide at room temp for 70 min. After addition of MeOH, extraction with EtOAc from water, and solvent removal as the toluene azeotrope, the product was purified through a silica gel column (10–25% EtOAc/$CHCl_3$) to yield hydroxysiloxyenone **11** (3.4 mg); IR: 3430, 1670, 1615, 1250, 840 and 775; EI-MS: 530 (M^+, 0.5%), 529 (M^+ – H, 0.6%), 515 (M^+ – Me, 1.8%), 473 (M^+ – t-Bu, 100%) and 75 (Me_2Si^+OH, 16%); ^{1}H-NMR($CDCl_3$): 5.73 (br.s; 4-H), 3.98 (tdd, 9, 5, 3; 15-H), 3.42 (dd, 10, 6; 26-H_a), 3.36 (dd, 10, 7; 26-H_b), 1.20 (3H, s; 10-Me), 1.05 (dd, 11, 9; 14-H), 0.91 (3H, d, 6; 20-Me), 0.90 (9H, s; t-Bu), 0.86 (3H, d, 7; 25-Me), 0.75 (3H, s; 13-Me), and 0.04 (6H, s; $Si-Me_2$); UV(MeCN): 239.

Hydrogenation of **11**. Hydroxysiloxyenone **11** (2.1 mg) was stirred with 5% Pd–C (5 mg) in 2 mL 10 mM ethanolic KOH under H_2 at room temp for 3 hr. After neutralization by 30 μL 1 M aqueous ammonium chloride, removal of the catalyst by filtration, and solvent evaporation, the product was extracted with EtOAc from water, and purified through a silica gel column (10% EtOAc/$CHCl_3$) to yield 5β-dihydro-**11** (1.8 mg); IR: 3440, 1710, 1250, 1095, 840 and 775; EI-MS: 532 (M^+, 0.7%), 517 (M^+ – Me, 1.4%), 499 (M^+ – Me – H_2O, 1.8%), 475 (M^+ – t-Bu, 53%), 457 (M^+ – t-Bu – H_2O, 40%) and 75 (Me_2Si^+OH, 100%); ^{1}H-NMR($CDCl_3$): 3.98 (m; 15-H), 3.43 (dd, 10, 6; 26-H_a), 3.36 (dd, 10, 7; 26-H_b), 2.72 (dd, 15, 14; 4α-H), 2.34 (td, 14, 5; 2α-H), 2.17 (dm, 14; 2β-H), 1.16 (dd, 11, 9; 14-H), 1.04 (3H, s; 10-Me), 0.91 (3H, d, 6; 20-Me), 0.90 (9H, s; t-Bu), 0.86 (3H, d, 7; 25-Me), 0.72 (3H, s; 13-Me) and 0.04 (6H, s; $Si-Me_2$).

O-Phenyl thiocarbonylation of 5β-*dihydro-***11**. 5β-Dihydro-**11** (1.7 mg) was treated with 25 μL O-phenyl thiocarbonyl chloride in 0.5 mL pyridine at room temp overnight. After addition of MeOH and solvent removal as the toluene azeotrope, the product was extracted with EtOAc from water, and purified through a silica gel column (PhH to CH_2Cl_2) to yield 5β-dihydro-**11** O-phenyl thiocarbonate (1.3 mg); IR: 1710, 1590, 1490, 1290, 1200, 1090, 840 and 770; EI-MS: 514 (M^+ – PhOCSOH, 0.3%), 499 (M^+ – PhOCSOH – Me, 1.9%), 457 (M^+ – PhOCSOH – t-Bu, 100%), and 75 (Me_2Si^+OH, 41%); ^{1}H-NMR($CDCl_3$): 7.41 (2H, t, 8; Ph-*m*-H's), 7.28 (t, 8; Ph-*p*-H), 7.09 (2H, d, 8; Ph-*o*-H's), 5.28 (td, 9, 3; 15-H), 3.43 (dd, 10, 6; 26-H_a), 3.37 (dd, 10, 7; 26-H_b), 2.71 (dd, 15, 14; 4α-H), 2.33 (td, 15, 5; 2α-H), 1.64 (dd, 11, 9; 14-H), 1.05 (3H, s; 10-Me), 0.94 (3H, d, 6; 20-Me), 0.90 (9H, s; t-Bu), 0.84 (3H, d, 7; 25-Me), 0.80 (3H, s; 13-Me) and 0.04 (6H, s; $Si-Me_2$).

Reductive cleavage of 5β-*dihydro-***11** O-*phenyl thiocarbonate*. 5β-Dihydro-**11** O-phenyl thiocarbonate (0.8 mg) was treated with freshly distilled 30 μL tri-n-butyltin hydride and α,α'-azobis-iso-butyronitrile (0.5 mg) in 0.2 mL dry N_2-prebubbled toluene under N_2 at 110° for 12 hr. After addition of $CHCl_3$ and solvent evaporation, the product was purified through silica gel columns (50% PhH/hexane and 50% CH_2Cl_2/PhH) to yield 5β-dihydrodehydroxy-**11** (0.5 mg); IR: 1715, 1255, 1095, 840 and 750; EI-MS: 516 (M^+, 0.7%), 515 (M^+ – H, 1.2%), 501 (M^+ – Me, 3.2%), 459 (M^+ – t-Bu, 100%), 161 (C-22$^+$/20 + H – t-Bu, 43%) and 75 (Me_2Si^+OH, 82%).

Desilylation of 5β-*dihydrodehydroxy-***11** (*O-silyl-***8**). 5β-Dihydrodehydroxy-**11** (0.5 mg) was treated with 0.5 mL 5% HCl in MeOH at room temp overnight. After solvent evaporation, the product was purified through a silica gel column (20% EtOAc/$CHCl_3$) to yield **8** (0.3 mg); IR, EI-MS, and ^{1}H-NMR($CDCl_3$): all identical to those of **8** from **7**.

p-*Bromobenzoylation of* **11**. Hydroxysiloxyenone **11** (3.5 mg) was treated with *p*-bromobenzoyl chloride (11.5 mg) and N,N-dimethyl-4-aminopyridine (12.0 mg) in 0.5 mL pyridine at 65° for 12 hr. After addition of 5 μL MeOH and solvent removal as the toluene azeotrope, the product was extracted by EtOAc from 1 M aqueous ammonium chloride, then purified through silica gel columns ($CHCl_3$ and 5% EtOAc/$CHCl_3$) to yield **11** *p*-bromobenzoate (**12**; 3.6 mg); IR: 1715, 1675, 1620, 1590, 1270, 1115, 1100, 1010, 840 (sh), 835 and 775; EI-MS: 657 and 655 (M^+ – t-Bu, 3.3% and 2.6%), 455 (M^+ – Br $C_4H_6CO_2H$ – t-Bu, 100%), 185 and 183 ($BrC_4H_6CO^+$, 17% and 20%), and 75 (Me_2Si^+OH, 45%); ^{1}H-NMR($CDCl_3$): 7.87 (2H, d, 8; Bz-*o*-H's), 7.59 (2H, d, 8; Bz-*m*-H's), 5.72 (br s; 4-H), 5.05 (td, 9, 3; 15-H), 3.40 (dd, 10, 6; 26-H_a), 3.34 (dd, 10, 7; 26-H_b), 1.53 (dd, 11, 9; 14-H), 1.20 (3H, s; 10-Me), 0.94 (3H, d, 6; 20-Me), 0.87 (9H, s; t-Bu), 0.84 (3H × 2, s; 13-Me and d, 7; 25-Me) and 0.02 (6H, s; $Si-Me_2$); UV(MeCN): 241 (log ε 4.5); CD(MeCN): $\Delta\varepsilon_{228}$ +8, $\Delta\varepsilon_{247}$ −12.

Acetylation of **4**. Pavoninin-4 (**4**; 2.7 mg) was treated with 0.2 mL Ac_2O in 1 mL pyridine at room temp overnight. After the same work-up as acetylation of **1**, the product was purified through a silica gel column (6% EtOH/$CHCl_3$) to yield **4** tetraacetate (3.5 mg); IR: 3280, 1745, 1660, 1555, 1230 and 1040; EI-MS: 833 (M^+, 2.2%), 427 (M^+ – HOglcNAc·Ac_3 – AcOH, 64%), and 241 (C-2';5'$^+$/1';O – AcOH, 100%); ^{1}H-NMR($CDCl_3$): 5.42 (d, 9; NH), 5.22 (dd, 11, 9; 3'-H), 5.03 (dd, 10, 9; 4'-H), 4.99 (m, $W_{1/2}$

7; 3-H), 4.58 (d, 8; 1'-H), 4.19 (2H, d, 4; 6'-H_2), 3.93 (dd, 11, 6; 26-H_a), 3.86 (ddd, 11, 9, 8; 2'-H), 3.85 (dd, 11, 7; 26-H_b), 3.65 (dt, 10, 4; 5'-H), 3.63 (td, 10, 3; 15-H), 2.07 (3H, s; OAc), 2.06 (6H, s; OAc × 2), 2.03 (6H, s; OAc × 2), 1.91 (3H, s; N-Ac), 1.24 (t, 10; 14-H), 0.91 (3H, d, 7; 25-Me), 0.88 (3H, d, 6; 20-Me), 0.78 (3H, s; 10-Me) and 0.65 (3H, s; 13-Me).

Hydrogenation of **3**. Pavoninin-3 (**3**; 3.0 mg) was stirred with Pt catalyst (from 5 mg PtO_2) in 3.5 mL AcOH under H_2 at room temp for 5 hr. After removal of the catalyst by filtration and solvent evaporation as the toluene azeotrope, the product was purified through a silica gel column (20% MeOH/$CHCl_3$) to yield 5β-dihydro-**3** (5-epi-**4**; 1.5 mg); ^{1}H-NMR(CD_3OD): 4.37 (d, 8; 1'-H), 3.94 (dd, 11, 6; 26-H_a), 3.85 (2H, dd, 11, 3; 6'-H_a and dd, 11, 7; 26-H_b), 3.69 (dd, 11, 6; 6'-H_b), 3.66 (m; 15-H), 3.60 (dd, 10, 8; 2'-H), 3.50 (m; 3-H), 3.43 (dd, 10, 9; 3'-H), 3.33 (dd, 10, 9; 4'-H), 3.22 (ddd, 10, 6, 3; 5'-H), 2.17 (m; 16α-H), 2.03 (3H, s; OAc), 1.95 (3H, s; N-Ac), 0.95 (3H, s; 10-Me), 0.93 (3H, d, 7; 25-Me), 0.91 (3H, d, 6; 20-Me) and 0.69 (3H, s; 13-Me).

Acetylation of **5**. Pavoninin-5 (**5**; 3.0 mg) was acetylated and worked up as **4**. The product was purified through a silica gel column (28% EtOAc/$CHCl_3$) to yield **5** tetraacetate (3.3 mg); IR: 3300, 1745, 1660, 1560, 1240 and 1045; EI-MS: 831 (M^+, 2.0%), 771 (M^+ – AcOH, 1.4%), 424 (M^+ – HOglcNAc·Ac_3 – AcOH, 100%), 330 (glcNAc·Ac_3^+, 80%) and 158 (C-18;11$^+$/14;12 – 2H – AcOH, 60%); ^{1}H-NMR($CDCl_3$): 5.48 (d, 9; NH), 5.29 (m; 6-H), 5.28 (dd, 10, 9; 3'-H), 5.02 (t, 10; 4'-H), 4.65 (d, 8; 1'-H), 4.60 (m; 3-H), 4.19 (2H, d, 4 and d, 3; 6'-H_2), 3.93 (dd, 11, 6; 26-H_a), 3.85 (dd, 11, 7; 26-H_b), 3.79 (ddd, 11, 9, 8; 2'-H), 3.65 (ddd, 10, 4, 3; 5'-H), 3.64 (td, 9, 3; 15-H), 2.31 (2H, m; 4-H_2), 2.06 (3H, s; OAc), 2.05 (3H, s; OAc), 2.03 (9H, s; OAc × 3), 1.94 (3H, s; N-Ac), 1.24 (dd, 11, 9; 14-H), 1.02 (3H, s; 10-Me), 0.91 (3H, d, 7; 25-Me), 0.89 (3H, d, 6; 20-Me) and 0.67 (3H, s; 13-Me).

Jones oxidation of **5** *and methanolysis*. With the same care as **3**, pavoninin-5 (**5**; 28.1 mg) was treated with 55 μL Jones reagent in 30 mL acetone at room temp for 10 min, and 1 mL 2-propanol was added. Immediately after solvent evaporation, the residue was treated with 30 mL 30% conc HCl in MeOH under reflux for 3 hr. The same work up and purification procedure as in the treatment of **3** yielded **10** (16.7 mg); IR, EI-MS, ^{1}H-NMR($CDCl_3$) and CD(MeOH): all identical to those of **10** from **3**.

Hydrogenation of **6**. Pavoninin-6 (**6**; 3.0 mg) was hydrogenated as **3** for 2 hr. The same work-up yielded dihydro-**6** (3.0 mg); ^{1}H-NMR(CD_3OD): 4.37 (d, 8; 1'-H), 3.93 (dd, 11, 6; 26-H_a), 3.85 (2H, dd, 12, 2; 6'-H_a and dd, 11, 7; 26-H_b), 3.69 (dd, 12, 6; 6-H_b), 3.67 (td, 9, 3; 15-H), 3.60 (dd, 10, 8; 2'-H), 3.50 (m; 3-H), 3.42 (dd, 10, 9; 3'-H), 3.32 (dd, 10, 9; 4'-H), 3.22 (ddd, 10, 6, 2; 5'-H), 2.19 (ddd, 14, 8, 3; 16α-H), 2.03 (3H, s; OAc), 1.93 (3H, s; N-Ac), 0.93 (3H, d, 7; 25-Me), 0.91 (3H, d, 6; 20-Me), 0.83 (3H, s; 10-Me) and 0.70 (3H, s; 13-Me).

Hydrogenation of **5**. Pavoninin-5 (**5**; 2.0 mg) was hydrogenated as **3** for 3 hr. The same work-up and purification procedure yielded an epimeric mixture of 5β- and 5α-dihydro-**5** (4:1; 1.0 mg); ^{1}H-NMR(5β-epimer; CD_3OD): 4.35 (d, 8; 1'-H), 4.03 (m, $W_{1/2}$ 8; 3-H), 3.66 (td, 9, 3; 15-H), 3.62 (dd, 10, 8; 2'-H), 3.41 (dd, 10, 9; 3'-H), 2.17 (ddd, 14, 8, 3; 16α-H), 1.94 (3H, s, N-Ac), and others indistinguishable from signals in the spectrum of dihydro-**6**. The following signals attributed to the minor 5α-epimer are clearly observed and superimposable to those in the spectrum of dihydro-**6**: 4.37 (1'-H), 3.60 (2'-H), 3.42 (3'-H), 1.93 (N-Ac) and 0.83 (10-Me).

*Jones oxidation of dihydro-***6** *and methanolysis*. Dihydro-**6** (3.0 mg) was treated with 25 μL Jones reagent in 2 mL acetone at room temp for 10 min, and 0.5 mL 2-propanol was added. After solvent evaporation, the residue was treated with 1 mL 5% HCl in MeOH at 60–65° for 4 hr. After solvent evaporation and extraction by EtOAc from water, the extract was washed by 5% aq $NaHCO_3$. After solvent evaporation of the organic extract, the product was eluted through a silica gel column (EtOAc), and purified by silica gel HPLC (40% EtOAc/$CHCl_3$) to yield **13** (0.7 mg); IR: 3340, 1715 and 1050; EI-MS: 418 (M^+, 2.1%), 400 (M^+ – H_2O, 100%), 271 (C-17$^+$/20 – H_2O, 90%), 261 (C-13;15$^+$/17;16 – H, 50%) and 233 (C-13;14$^+$/17;15 + H, 65%); ^{1}H-NMR($CDCl_3$): 3.96 (tdd, 9, 5, 3; 15-H), 3.50 (ddd, 11, 6, 5; 26-H_a), 3.43 (ddd, 11, 7, 5; 26-H_b), 2.38 (ddd, 15, 14, 6; 2β-H), 2.30 (dm, 15; 2α-H), 2.27 (dd, 15, 14; 4β-H), 2.10 (ddd, 15, 4, 2; 4α-H), 1.72 (ddd, 14, 8, 3; 16α-H), 1.07 (dd, 11, 9; 14-H), 1.03 (3H, s; 10-Me), 0.92 (3H, d, 7; 25-Me), 0.91 (3H, d, 6; 20-Me), 0.79 (ddd, 12, 11, 4; 9-H) and 0.72 (3H, s; 13-Me); CD(MeOH): $\Delta\varepsilon_{287}$ +1.1.

Jones oxidation of **4** *and methanolysis*. Pavoninin-4 (**4**; 3.9 mg) was treated with 30 μL Jones reagent in 2 mL acetone as dihydro-**6**. The same work-up and methanolysis procedure, the product was eluted through a silica-gel column (2% MeOH/$CHCl_3$), and purified in the same manner as above to yield **13** (1.4 mg); IR, EI-MS, ^{1}H-NMR($CDCl_3$), and CD(MeOH): all identical to those of **13** from dihydro-**6**.

Shark-repellent assay. The assay was performed on two specimens of the dog shark *Mustelus griseus* (40 cm long) in a plastic basin with continuously replacing 500 L sea water at Okinawa Expo Aquarium, Marine-Expo Memorial Park, Motobu, Okinawa, Japan. The sample ejecting system comprised a stainless-steel HPLC pipe (3 i.d. × 1000 mm) connected to a 10 mL syringe at one end out of water, and bent to hook bait fish (*ca* 10 cm long) at the other in the water near the bottom of the basin. Ejection of 10 mL sample solns was made from the hooked end of the pipe when the shark was in proximity of the bait (within *ca* 10 cm) at the rate of 1–2 mL/sec, and continued until the shark was repelled or the bait was taken. Ejection was resumed when the repelled shark approached back to the bait, repeatedly until the sample soln expired or the bait was taken. The following samples were assayed; sea water and 70% aqueous EtOH as control (several portions each), the proteinaceous ppt solns in sea water at concentrations of 20 and 100 mg/mL, pavoninins solns (*cf isolation*) in 70% aqueous EtOH at concentrations of 0.3, 0.9, 1.1 and 3.5 mg/mL, and commerical saponin (E. Merck) solns in sea water at concentrations of 2, 5, 10 (three portions), and 15 mg/mL. In the course of continual ejection the shark was repelled repeatedly until the 10 mL soln expired by 0.9 mg/mL pavoninins and by a portion of 10 mg/mL saponin. It was repelled a few times at the beginning but the bait was eventually taken before the sample soln expired by 1.1 and 3.5 mg/mL pavoninins and by 5, a potion of 10, and 15 mg/mL saponin. With the 100 mg/mL ppt, the shark appeared discouraged to feed, but stayed around the bait and eventually took it. The bait was taken at the first attack with ejection of any other sample or control solns.

Antifeedant assay. The assay was performed on the same specimens of sharks. Bait fish were smeared with pavoninins (1 mg; with 70% aqueous EtOH) or with the proteinaceous ppt (40 mg; as powder) and put in the water. The bait smeared with pavoninins appeared to turn away approaching sharks several times before being eaten by a single bite. The bait smeared with the ppt was immediately taken, but was spat out. The shark repeated biting and spitting a few times before eventually giving up to feed. The bait was consumed by the next morning.

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