2. It has been established that in the treatment of extracts of saponin-containing plants it is desirable to use IA-1 resin in combinations with KU-2 resin for the preliminary desalting of the extract.

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GLYCOSIDES OF MARINE INVERTEBRATES.

XVI. CUCUMARIOGENIN FROM GLYCOSIDES OF THE HOLOTHURIAN *Cucumaria fraudatrix*

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A new genin of the holostane series has been isolated by the acid hydrolysis of the glycosidic fraction of the holothurian *Cucumaria fraudatrix*. It has been shown that this genin is the native alycone of glycoside G_1 from the same holothurian. The structure of the genin has been established as 16β-acetoxyholosta-7,24-dien-3β-ol.

Continuing an investigation of the glycosides of marine invertebrates, we have studied the structure of the native genin from the glycosides of the Far Eastern holothurian *Cucumaria fraudatrix*. The structure of 16β-acetoxyholosta-7,24-dien-3β-ol (Ia) for this compound was proposed on the basis of the following facts. Cucumariogenin was obtained in the form of the acetate (Ib) from the glycosidic fraction of the holothurian *Cucumaria fraudatrix* after acid hydrolysis of this fraction and acetylation of the hydrolysis products. Cucumariogenin acetate (Ib) had seven methyl groups, since its ^{1}H NMR spectrum had seven 3H singlets in the 0.9-1.68 ppm region. The IR spectrum of (Ib) showed an acetate and a lactone function ($\nu_{C=O}$ 1727 cm^{-1}, ν_{C-O-C} 1253 cm^{-1}, $\nu_{C=O}$ 1755 cm^{-1}). There was no absorption of free hydroxy groups in the spectrum.

The ^{13}C NMR spectrum of cucumariogenin acetate contained three signals of carbonyl carbons (168.9, 170.9, and 179.0 ppm) corresponding to two acetate groups and a γ-lactone, and also four signals of carbon atoms for two tri-substituted double bonds (120.3 d, 144.8 s,

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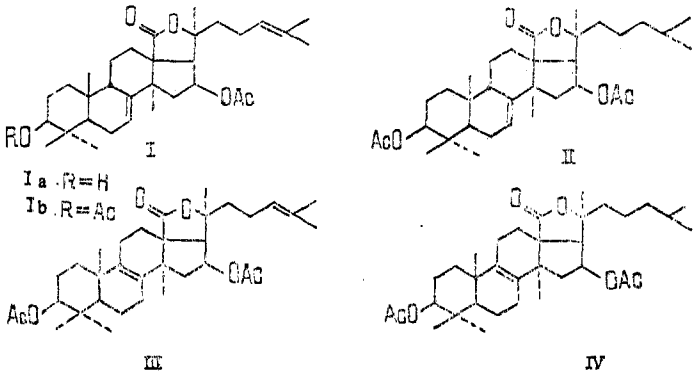
123.4 d, 132.2 s). In cucumariogenin acetate, the lactone group occupies the 18 → 20 position, since the signal of one of the methyl groups (C-21) in the ^{1}H NMR spectrum was shifted to 1.49 ppm because of the influence of the neighboring —O—CO— fragment. These facts permitted the assumption that compound (1b) is a new holostane derivative with two acetate groups and two double bonds.

The mass and ^{1}H and ^{13}C NMR spectra of (Ib) showed the 3β position of one of the acetate groups. Thus, the molecular ion lost a fragment with 129 m/z on the cleavage of ring A, which is characteristic for the mass spectra of 3β-acetoxyholothurinogenins [1]. The ^{1}H NMR spectrum contained a signal at 4.47 ppm, which is characteristic for a 3α-H when there is an acetate group in the 3β position.



To establish the positions of the second acetate group and of the double bonds, several chemical transformations were performed. Thus, the presence of 24(25) unsaturation could be assumed from the ^{1}H NMR spectrum, which contained the signals of the protons of two methyl groups on a double bond (1.67 and 1.58 ppm). This hypothesis was confirmed by the catalytic hydrogenation of (Ib).

The ^{1}H NMR spectrum of the 24,25-dihydro derivative obtained (II) lacked the signals of methyl groups at a double bond and had a doublet (6 H) at 0.87 ppm, which is characteristic for a saturated side chain. In the ^{13}C NMR spectrum of (II), the signals of carbon atoms at a double bond (123.4 and 132.2 ppm) had disappeared and signals corresponding to the carbon atoms of a saturated side chain [2] had appeared (Table 1).

The ^{1}H and ^{13}C NMR spectra of compound (II) show the presence of only one trisubstituted double bond: 1 H (m) at 5.54 ppm, and C=C signals at 120.3 (d) and 144.8 (s) ppm. This bond was assigned to the C-7(8) position on the basis of a comparison of the spectral characteristics of (II) with the spectra of holost-7-enol derivatives [2] and chemical transformations.

Treatment of the acetate (Ib) with chloroform saturated with hydrogen chloride gave the $\Delta^{8(9),24}$ derivative (III). Its ^{13}C NMR spectrum contained the signals of one trisubstituted double bond (123.7 and 132.2 ppm, d and s, respectively) and one tetrasubstituted double bond (135.5 and 130.2 ppm, both s). Such a migration of a double bond has been described previously for holost-7-enols [3]. The similar treatment of product (II) gave the $\Delta^{8(9)}$ isomer (IV) with one tetrasubstituted double bond. In the holostane skeleton, this double bond can occupy only the C-8(9) position.

The second acetate group was placed at C-16 on the basis of NMR characteristics for compound (IV). From the difference spectrum between the double resonance spectrum with irradiation of CH-OAc (5.67 ppm) and the monoresonance spectrum the protonic environment of the CH—OAc fragments was determined as $-CH_2-CH(OAc)-CH<$. The chemical shifts and spin—spin coupling constants of the protons are given in Table 2. Thus, in the holostane nucleus this acetoxy group can occupy either the C-16 or the C-6 position. The C-6 position does not agree with the ^{13}C NMR spectra of the genins (Ib)-(IV). Thus, the presence of an acetoxy group at C-6 would cause a downfield shift of about 5 ppm in the spectra of the genins (Ib) and (II) as compared with the value of 145.6 ppm for 3β,23(S)-diacetoxyholost-7(8)-ene [2]. Moreover, each of the spectra of the genins (III) and (IV) contains a characteristic signal in the strong field at 18.0 ppm (t) which, as in the case of lanost-8(9)-enols can belong only to C-6 [5]. Consequently, the acetoxy group is located at C-16. Its β configurations follows from an analysis of the spin—spin coupling constants of the H-16, H-17, and

TABLE 1. ^{13}C NMR Spectra of Cucumariogenin Acetate and its Derivatives ($CDCl_3$, ppm, TMS – 0)

Atom	Compound				Atom	Compound			
	Ib	II	III	IV		Ib	II	III	IV
C-1	35,5	35,5	35,35	35,35	C-18	179,2	179,4	176,9	176,9
C-2	24,25	24,25	24,1	24,1	C-19	23,5	23,7	18,7	18,7
C-3	81,2	81,2	81,0	80,9	C-20	84,8	85,1	84,5	84,7
C-4	37,7	37,7	37,8	37,8	C-21	28,2	28,2	28,7	28,6
C-5	47,7	47,6	50,6	50,6	C-22	38,3	38,9	38,4	38,9
C-6	23,0[a]	23,0[a]	18,0	18,1	C-23	23,5	22,7	23,5	22,5
C-7	120,3	120,3	26,8	27,0	C-24	123,4	39,6	123,7	39,6
C-8	144,8	144,8	135,4	135,4	C-25	132,2	28,1	132,2	28,1
C-9	46,7	46,7	130,2	130,2	C-26	25,5	22,7[a]	25,6	22,55
C-10	35,5	35,5	37,1	37,1	C-27	17,7	22,5[a]	17,8	22,55
C-11	22,4[a]	22,5[a]	20,9	21,0	C-30	17,4	17,5	16,6	16,6
C-12	31,65	31,6	28,7	28,6	C-31	28,8	28,8	28,0	28,1
C-13	59,15	59,1	59,6	59,6	C-32	32,3	32,4	28,6	27,0
C-14	47,3	47,2	44,6	44,6	OAc	21,25	21,25	21,25	21,2
									21,4
C-15	43,3	43,3	41,0	41,0		170,9	170,8	170,1	170,9
C-16	74,75	74,9	75,0	75,2		168,9	168,9	170,3	170,2
C-17	54,85	54,9	52,1	52,3					

[a] – assignment of the signals ambiguous.

2H-15 protons. These constants were calculated for the 16β- and 16α-acetoxy isomers using the results of x-ray structural analysis for 22,25-epoxyholosta-7,9(11)-dien-3β-ol (V) [6] and assuming that the conformations of rings D in compounds (IV) and (V) did not appreciably differ. A comparison of the observed and calculated constants (see Table 2) indicates the β configuration of the acetoxy group.

The 9β-H configuration in the acetates (Ib) and (II) followed from a comparison of their ^{13}C NMR spectra with that of 23(S)-acetoxyholosta-7,25-dien-3β-ol, the native genin of the glycoside from the holothurian *Astichopus multifidus*, the configurations of the asymmetric centers of which have been determined by x-ray structural analysis [7]. The values of 23.5 and 23.6 ppm for the chemical shifts of C-19 in (Ib) and (II) respectively, show the 9β-H configuration in these genins [8].

A comparison of the signals of the aglycone part of the ^{13}C NMR spectra of cucumarioside G_1, mp 216-218°C, $[\alpha]_D^{20} - 21.8°$ (c 3; ethanol–water (4 : 1)) isolated from *Cucumaria fraudatrix* (Table 3) and the spectrum of genin (Ib) (Table 1) showed that the carbohydrate moiety was attached to C-3, and compound (II) was the acetate of the native genin of this glycoside (the signal for C-3 of the glycoside was shifted to 88.7 ppm, while the other signals of the aglycone moiety of the glycoside and of genin (Ib) did not differ so greatly). On the basis of these facts, the structure of the native cucumariogenin can be represented by formula (Ia).

EXPERIMENTAL

Melting points were determined on a Boëtius stage and optical rotations on a Perkin-Elmer 141 polarimeter in chloroform solutions at room temperature. IR spectra were recorded on a Specord IR-75 spectrometer in chloroform solutions, mass spectra on a LKB-9000 S instrument, and ^1H and ^{13}C NMR spectra on a Bruker HX-90E spectrometer in deuterochloroform. The chemical shifts were measured relative to tetramethylsilane.

The holothurian *Cucumaria fraudatrix* was collected in Posyet Bay, Sea of Japan, in August, 1979, at a depth of 1 m.

The glycosidic fraction of the holothurian *Cucumaria fraudatrix* was isolated as the result of extraction with methanol in a Soxhlet apparatus of the musculocutaneous sacs of the holothurians that had previously been defatted with methylene chloride.

Acid Hydrolysis. The glycosidic fraction was hydrolyzed with 2 N H_2SO_4 at 90°C for 3 h. The mixture of aglycones was obtained by the usual method.

Acetylation was performed with acetic anhydride in pyridine at room temperature for 16 h. The mixture of acetates of aglycones was separated on a column containing SiO_2 in the hexane–ethyl acetate (7 : 1) system. Column 80 × 1.5 cm; support, silica gel L (Chemapol, 40/100 μ).

TABLE 2. Comparison of the Observed and Calculated Spin–Spin Coupling Constants of H_{16} in Compound (IV)

	Dihedral angle*	$J^{\dagger}_{H,H'}$, Hz	$J_{exp\ H,H'}$, Hz
α-OAc	116°($H_{16}H_{17}$)	3.6	
	21°18′ ($H_{16}H^{I}_{15}$)	8.3	
	98°30′ ($H_{16}H_{15}$)	1.8	
β-OAc	4°18′ ($H_{16}H_{17}$)	8.9	9.0
	142°($H_{16}H^{I}_{15}$)	7.5	7.1 10.3
	21°18′ ($H_{16}H_{15}$)	8.4	
	109°28′ ($H^{I}_{15}H^{II}_{15}$)		−12.0

*The angles between the substituents in positions 15, 16, and 17 were taken as equal to the tetrahedral angles.
†$J_{H,H} = (7.8 - 1.0\cos\varphi + 5.6\cos 2\varphi)\cdot(1 - 0.1\,\Sigma\Delta X_i)$ [9]; $X_H = 2.1$, $X_C = 2.5$, $X_{OAc} = 3.7$.

TABLE 3. ^{13}C NMR Spectrum of the Aglycone Moiety of Cucumarioside G_1, $(CD_3)_2SO$, ppm, TMS – 0)

Atom	Aglycone of glycoside G_1	Atom	Aglycone of glycoside G_1	Atom	Aglycone of glycoside G_1
C-1	35.6	C-11	22.05	C-21	28.5
C-2	26.6	C-12	30.96	C-22	38.6
C-3	88.7	C-13	58.9	C-23	23.1
C-4	39.1	C-14	47.1	C-24	123.9
C-5	47.7	C-15	43.4	C-25	131.7
C-6	23.1	C-16	74.6	C-26	25.6
C-7	119.7	C-17	53.9	C-27	17.7
C-8	145.5	C-18	179.40	C-30	17.7
C-9	47.1	C-19	23.7	C-31	28.5
C-10	35.2	C-20	85.2	C-32	32.2
				—OAc	21.1
					169.8

Holosta-7,24-diene-3β,16β-diol Diacetate (Ib). $C_{34}H_{50}O_6$, mp 213–215°C (MeOH), $[\alpha]_D^{20}$ + 8° (c 0.56; $CHCl_3$), R_f 0.65 (hexane–ethyl acetate (3 : 1); TLC on silica gel). Mass spectrum, m/z: 554 (M^+), 539, 512, 494, 479, 449, 435, 425, 419, 389, 375, 339.

PMR ($CDCl_3$) (δ): 0.96 (30-CH_3, 3 H, s); 0.90 (31-CH_3, 3 H, s); 1.06 (19-CH_3, 3 H, s); 1.15 (32-CH_3, 3 H, s); 1.49 (21-CH_3, 3 H, s); 1.58 (26-CH_3, 3 H, s); 1.67 (27-CH_3, 3 H, s); 4.47 (H3, 1 H, m); 5.54 (H7, 1 H, m); 3.21 (H9, 1 H, m); 5.67 (H16, 1 H, m); 2.49 (H17, 1 H, d, J = 8.8 Hz); 5.01 (H24, 1 H, m); 2.05 (2 Ac, 6 H, s).

Holosta-7-ene-3β,16β-diol Diacetate (II). The diacetate (Ib) (20 mg) was hydrogenated in ethyl acetate over PtO_2 at room temperature for 4 h. The catalyst was filtered off, the ethyl acetate was evaporated off, and the residue was chromatographed on silica gel. This gave 18 mg of the hydrogenated product (II), $C_{34}H_{52}O_6$, mp 229–231°C (MeOH), $[\alpha]_D^{20}$ + 4° (c 0.50; $CHCl_3$). Mass spectrum, m/z: 556 (M^+) PMR ($CDCl_3$, δ): 0.87 (26,27-CH_3, 6 H, d, J = 6.1 Hz).

Holosta-8,24-diene-3β,16β-diol Diacetate (III) and Holosta-8-ene-3β,16β-diol Diacetate (IV). The acetate (Ib) or (II) (20 mg) was treated with (hydrogen chloride)-saturated chloroform for 1 h. Then the chloroform was evaporated off and the residue was chromatographed on silica gel. In this way, (Ib) yielded 12 mg of (III), $C_{34}H_{50}O_6$, mp 228–230°C (MeOH),

$[\alpha]_D^{20}$ + 34° (c 0.45; $CHCl_3$), PMR ($CDCl_3$, δ): 0.91 (30,31-CH_3, 6 H, s); 1.06 (19-CH_3, 3 H, s); 1.11 (32-CH_3, 3 H, s); 1.50 (21-CH_3, 3 H, s); 1.59 (26-CH_3, 3 H, s); 1.69 (27-CH_3, 3 H, s); 4.53 (H3, 1 H, m); 5.67 (H16, 1 H, m); 2.49 (H17, 1 H, d, J = 9.1 Hz); 4.97 (H24, 1 H, m); 2.02, 2.05 (Ac 6 H, s); and (II) yielded 13 mg of (IV), mp 246-248°C (MeOH), $[\alpha]_D^{20}$ + 31° (c 0.31, $CHCl_3$). Mass spectrum, m/z: 556 (M^+). PMR ($CDCl_3$, δ): 0.89 (30,31-CH_3, 6 H, s); 1.06 (19-CH_3, 3 H, s); 1.10 (32-CH_3, 3 H, s); 1.46 (21-CH_3, 3 H, s); 0.87 (26,27-CH_3, 6 H, d, J = 6.0 Hz); 4.50 (H3, 1 H, m); 5.64 (H16, 1 H, m); 2.49 (H17, 1 H, d, J = 9.1 Hz); 2.04 (Ac, 6 H, s).

SUMMARY

It has been shown that cucumariogenin from the holothurian *Cucumaria fraudatrix* has the structure of 16β-acetoxyholosta-7,24-dien-3β-ol.

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