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NEW POLYHYDROXY STEROLS, DYSIDAMIDES, AND A DIDEOXYHEXOSE FROM THE SPONGE *DYSIDEA HERBACEA*

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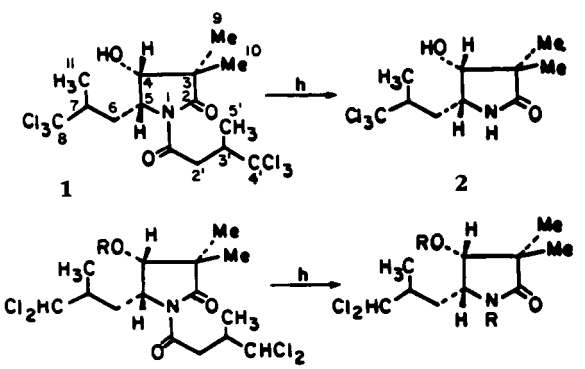
ABSTRACT.—Six novel marine natural products, dysidamides B [**3**] and C [**5**], three highly oxygenated sterols **9**, **11**, and **13**, and a 1,4-dideoxyhexose **15** were isolated from the Red Sea sponge *Dysidea herbacea*, along with the previously known 24-methylene-5α-cholest-7-ene-3β,5,6β-triol [**7**], furodysinin lactone, and α-D-xylopyranose. Structures of all new compounds were determined from spectroscopic data, especially extensive 1D and 2D nmr experiments.

Many interesting metabolites have been isolated from *Dysidea* species (1–7). Recently we have reported the isolation of a chlorinated nitrogenous metabolite, dysidamide [**1**], from a *Dysidea* sp. collected near Massawa, Ethiopia (8), a sponge which has now been fully identified as *Dysidea herbacea* Keller (family Dysideidae, order Dictyoceratida). An unusually diverse array of metabolites has been isolated from various specimens of *D. herbacea* (1,4). Herewith we wish to report several minor metabolites from the same sponge: two new dysidamides, B [**3**] and C [**5**], four polyoxygenated sterols **7**, **9**, **11**, and **13**, and a dideoxyhexose **15**, which were isolated with D-xylose and furodysinin lactone (7).

The EtOH extract of the sponge was separated into four fractions by chromotography on a Sephadex LH-20 column prepared and eluted with petroleum ether-CH_2Cl_2-MeOH (2:1:1); the fractions contained (a) glycerides and non-polar sterols, (b) dysidamides, (c) polar sterols, and (d) monosaccharides. The three latter fractions were further purified by repeated chromatography on Sephadex LH-20 and RP-18 columns and also, in some cases, on Si gel after acetylation with Ac_2O/pyridine (it was verified that there are no natural acetates of the new compounds). Together with compound **1**, which was the major metabolite (2% dry wt of the sponge), we have now isolated small



3 R=H
4 R=Ac

5 R=H
6 R=Ac

h = 0.1% K_2CO_3, MeOH

SCHEME 1

amounts of dysidamides B [**3**] and C [**5**] (0.003%, and 0.015%, respectively). Dysidamide B [**3**], $C_{15}H_{23}Cl_4NO_3$, hrcims found *m/z* 406.0523 (required 406.0510) with a characteristic cluster of four chlorine atoms, ν max 1738 and 1694 cm^{-1} (see Table 1). Dysidamide B gave upon acetylation a monoacetate **4**, *m/z* 447, 449, 451 $[M]^+$ (13:3:3) confirming the secondary alcohol. Comparison of the ^1H- and ^{13}C-nmr data of **4** with those of dysidamide [**1**] showed great similarity. However, there were evidences in **3** for the replacement of the two CCl_3 by two $CHCl_2$ groups, δ_c 78.3 d and 77.2 d with characteristic $J_{CCl_2\text{-}H}$ couplings of 174 Hz (9), and δ_H values of 5.94 brs and 5.96 d (J = 3.5 Hz) for **3**, instead of the two CCl_3 lines, at 107.0 s and 105.9 s, of **1**. The complete structure of **3** was achieved following thorough nmr assignments by COSY (10), HMQC (11), and HMBC (12) experiments. Among others, the most characteristic for the structure elucidation were the following CH-correlations between: C-2/H-4, H-5, Me-9, and Me-10; C-1′/H-4′ (^4J); C-8/H_2-6, and Me-11; and C-4′/H_2-2′ and Me-5′. The proposed relative stereochemistry for C-4 and C-5 is based on comparisons of coupling constants and an nOe between the two protons.

TABLE 1. ^1H-nmr Data of Compounds **3** and **5**.

Proton	Compound	
	3	**5**
	δ_H [m, J (Hz)] in $CDCl_3$	δ_H [m, J (Hz)] in DMSO-d_6
H-4	4.11 (brd, J = 7)	3.97 (d, J = 5)
H-5	4.27 (ddd, J = 4.5, 6, 7)	3.71 (dt, J = 9, 5)
H-6	2.02 (ddd, J = 6, 7, 14)	1.91 (ddd, J = 9, 5, 14)
H-6′	1.85 (dt, J = 14, 4.5)	1.70 (ddd, J = 9, 5, 14)
H-7	2.48 (m)	2.47 (m)
H-8	5.94 (brs)	5.82 (d, J = 3)
Me-9	1.24 (s)	1.13 (s)
Me-10	1.24 (s)	1.11 (s)
Me-11	1.20 (d, J = 7)	1.15 (d, J = 7)
H-2′	3.20 (dd, J = 6, 18)	
H-2′	2.98 (dd, J = 7, 18)	
H-3′	2.77 (m)	
H-4′	5.96 (d, J = 3.5)	
Me-5′	1.22 (d, J = 7)	
NH		7.29 (brs)
OH		6.28 (brs)

The second nitrogenous metabolite, dysidamide C [**5**], $C_{10}H_{17}Cl_2NO_2$, eims *m/z* 253, 255, 257 (9:6:1) (for the hrcims see Experimental), ν max 3520 and 1700 cm^{-1}, was very similar in its structure to compound **2**, the basic degradation product of **1** (8). Upon acetylation **5** afforded a diacetate **6** (δ 2.11, OAc; 2.49, NAc) (see Table 2).

From the mass spectrum and the nmr data of dysidamide C (Tables 1 and 2), it was clear that it possesses the same $Me(CHCl_2)CHCH_2$-side chain on C-5 of the γ-lactam, as **3**. Cleavage of the latter side chain in the mass spectrometer resulted in two prominent peaks: *m/z* 128 (13%, $\underline{CH(OH)CH=N^+HCOCMe_2}$) and *m/z* 142 (17%, $\underline{CH(OH)CH(CH_2{}^+)NHCOCMe_2}$). Mild basic hydrolysis of **3** (0.1% K_2CO_3, MeOH, 4 h, room temperature), as in case of the degradation of **1** to **2** (8), afforded compound **5**.

In addition to the more common trichloromethyl compounds, the isolation of dichloromethyl derivatives has already been reported in the case of isodysidenin isolated

from *D. herbacea* (13). This may suggest that the dichloro compounds are intermediate products of the chlorination process undergone by one of the methyls, resulting in the trichloro group.

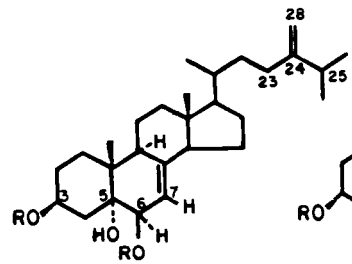
The second group of compounds isolated from this sponge consisted of polar sterols **7**, **9**, **11**, and **13**.

Earlier publications by Gunasekera and Schmitz (3) and Capon and Faulkner (4) and more recent disclosures by West and Cardellina (5,6) have shown that highly functionalized sterols are widespread in the sponge genus *Dysidea*.

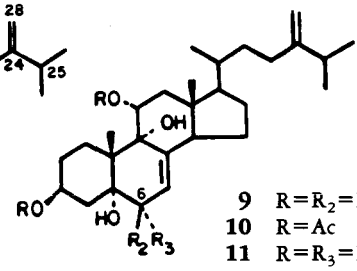
Common to all four sterols was a 24-methylene side chain confirmed by its nmr data (COSY, CH correlations, and chemical shift comparisons with known sterols) (14,15).

Compound **7**, the least polar of the four, was characterized as 24-methylene-5α-cholest-7-ene-3β,5,6β-triol, a sterol earlier isolated from *Spongionella gracilis* (16). Sterols with similar functionalized AB rings (3β, 5α, 6β, 9α tetraols) were also revealed from a *Dysidea* sp. (5).

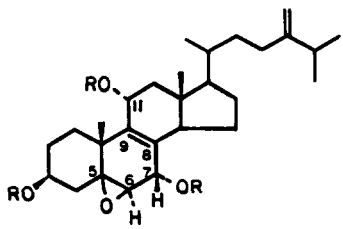
The second sterol, **9**, the major component in the crude sterol mixture, was purified as triacetate **10**, $C_{34}H_{52}O_8$, *m/z* 528 (100%) $[M - HOAc]^+$. The ^{1}H-nmr data (Table 3) suggested three secondary acetates and one trisubstituted double bond in the steroid nucleus. The chemical shift values of all the carbon atoms in **10** are reported in Table 4. These data and a DEPT experiment revealed the presence of two tertiary hydroxyl carbons. The 24-methylene side chain of **10** was confirmed by CH correlations between H-28, -28′/C-23, -25 and between Me's-26, -27/C-25. The comprehensive proton-line assignment (Table 3) was achieved from one-bond CH correlations, determining the 8 geminal pairs of protons, and COSY measurements. The latter experiments together with long-range CH correlations established the complete substitution pattern of **10**. Connectivities between H-1/Me-19; H-1, -1′/H-2, -2′; H-2, -2′/H-3; H-3/H-4, -4′; H-6/H-7 and a homoallylic coupling, of H-6, with H-14; H-7/H-14; H-11 and Me-18/H-12, -12′; H-14/H-15, -15′ as well as correlations of the two hydroxyl protons, 5-O*H*/H-6 and 9-O*H*/H-11, could be seen in the COSY experiment. Hetero-correlations between Me-19/C-5; H-3, -4, -4′/C-5; H-7 and Me-19/C-9; H-7/C-5; Me-18/C-12 and C-14 were observed in the HETCOR experiment (17).



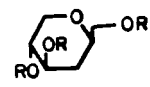
7 R=H
8 R=Ac



9 R=R$_2$=H, R$_3$=OAc
10 R=Ac
11 R=R$_3$=H, R$_2$=OAc
12 R=Ac



13 R=H
14 R=Ac



15 R=H
16 R=Ac

TABLE 2. ^{13}C-nmr Data of Compounds **1**, **4**, and **6** in $CDCl_3$.

Carbon	Compound		
	1[a]	**4**	**6**
	δ_c (m)	δ_c (m, $^1J_{CH}$)[b]	δ_c (m)
C-2	177.4 (s)	177.2 (s)	177.9 (s)
C-3	45.2 (s)	45.5 (s)	45.5 (s)
C-4	73.4 (d)	73.6 (d, 156)	73.6 (d)
C-5	54.9 (d)	55.1 (d, 148)	54.9 (d)
C-6	33.6 (t)	32.1 (t, 130, 126)	32.2 (t)
C-7	52.7 (d)	42.2 (d, 126)	42.2 (d)
C-8	105.4 (s)	78.2 (d, 174)	78.2 (d)
C-9	23.9 (q)	24.2 (q, 126)	24.2 (q)
C-10	19.7 (q)	19.7 (q, 130)	19.7 (q)
C-11	16.3 (q)	15.1 (q, 126)	15.1 (q)
C-1′	171.8 (s)	172.2 (s)	
C-2′	41.6 (t)	40.7 (t, 130, 126)	
C-3′	50.3 (d)	39.9 (d, 139)	
C-4′	104.7 (s)	77.6 (d, 174)	
C-5′	16.9 (q)	15.4 (q, 126)	
4-OAc	169.6 (s), 20.5 (q)	169.9 (s), 20.7 (q)	169.9 (s), 20.6 (q)
N-Ac			171.7 (s), 25.9 (q)

[a]Data are for the more soluble acetate.
[b]$^1J_{CH}$ data were obtained from an HMQC experiment.

The observable coupling constants (Table 3) ($J_{3,4ax} = J_{3,2ax} = 11$ Hz, $J_{3,4eq} = J_{3,2eq} = 5$ Hz, $J_{6,7} < 1$ Hz, $J_{11,12ax} = 11$ Hz, $J_{11,12eq} = 4$ Hz) are also in full agreement with the 3β,6α,11α-triacetoxy-5α-cholest-7-ene-5,9α-diol stereochemistry. Further support of the suggested configurations was achieved from nOe enhancements between: Me-19/H-2ax, -4ax, -6ax, -11ax (28%, together, with the latter two) (all β); 5ax-O*H*/H-3ax (α); 9ax-O*H*/H-1eq, 14ax (α); and Me-18/H-11ax (β).

The nOe between Me-19 and H-6, together with the small coupling constant between H-6 and H-7 (<1 Hz, agreeable with a dihedral angle $\phi_{6\beta,7} \approx 85°$), determined the configuration of C-6, which was not self-evident because of the twisted conformation of ring B. Sterol **9** is close in its structure to the 5α-cholest-7-ene-2α,3β,5,6β,9α,11α,19-heptaol isolated from *Dysidea etheria* (5).

TABLE 3. Selected 360 MHz ^{1}H-nmr Data for Compounds **10**, **12**, and **14** in $CDCl_3$.

Proton	Compound			
	10	**12**	**14**	**7**[a]
H-3	5.00 (tt, J = 11, 5)	5.09 (tt, J = 11, 5)	4.89 (tt, J = 11, 5)	4.08 (m)
H-6	5.16 (brs)	4.88 (dd, J = 5.5, 2)	3.31 (d, J = 2.5)	3.63 (brs)
H-7	4.95 (brs)	5.42 (dd, J = 5.5, 2)	5.53 (brs)	5.36 (brd, J = 4.9)
H-11	5.20 (dd, J = 11, 4)	5.32 (dd, J = 12, 5)	5.40 (dd, J = 4, 8)	
Me-18	0.62 (s)	0.62 (s)	0.52 (s)	0.59 (s)
Me-19	1.10 (s)	1.10 (s)	1.13 (s)	1.09 (s)
Me-21	0.89 (brd, J = 5)	0.89 (brd, J = 5.5)	0.90 (d, J = 6)	0.96 (d, J = 6.9)
Me-26	0.99 (d, J = 7)	0.98 (d, J = 7)	0.98 (d, J = 7)	1.03 (d, J = 6.9)
Me-27	0.99 (d, J = 7)	0.98 (d, J = 7)	0.98 (d, J = 7)	1.03 (d, J = 6.9)
H-28a	4.71 (brs)	4.68 (brs)	4.68 (brs)	4.72 (brs)
H-28b	4.60 (brs)	4.60 (brs)	4.58 (brs)	4.66 (brs)

[a]Data are from Piccialli and Sica (16).

TABLE 4. ^{13}C-nmr Data (90 MHz, $CDCl_3$) of Compounds **8**, **10**, **12**, and **14**.[a]

Carbon	Compound				
	8	**10**	**12**	**14**	24-Methylene-5α-cholest-7-ene-3β,6α-diol[b]
C-1	27.7 (t)	27.5 (t)	27.7 (t)	28.3 (t)	37.3
C-2	32.2 (t)	27.8 (t)	28.0 (t)	28.8 (t)	31.2
C-3	70.7 (d)	69.4 (d)	70.0 (d)	70.6 (d)	70.9
C-4	35.6 (t)	36.4 (t)	35.9 (t)	35.8 (t)	34.0
C-5	76.0 (s)	77.2 (s)	76.3 (s)	63.5 (s)	49.1
C-6	73.6 (d)	72.8 (d)	72.8 (d)	59.1 (d)	70.1
C-7	114.0 (d)	117.9 (d)	119.2 (d)	69.4 (d)	122.0
C-8	145.7 (s)	142.6 (s)	142.4 (s)	132.2 (s)	141.6
C-9	43.2 (d)	75.4 (s)	75.9 (s)	134.6 (s)	49.4
C-10	43.3 (s)	42.3 (s)	42.4 (s)	46.5 (s)	35.4
C-11	22.8 (t)	72.6 (d)	73.0 (d)	68.6 (d)	21.5
C-12	39.2 (t)	41.3 (t)	41.5 (t)	45.7 (t)	39.5
C-13	43.9 (s)	42.7 (s)	41.8 (s)	38.8 (s)	43.8
C-14	54.8 (d)	50.6 (d)	50.5 (d)	48.1 (d)	54.9
C-15	22.8 (t)	22.4 (t)	22.8 (t)	23.1 (t)	22.9
C-16	26.9 (t)	26.5 (t)	26.9 (t)	26.8 (t)	27.9
C-17	56.1 (d)	55.1 (d)	55.8 (d)	55.1 (d)	56.2
C-18	12.1 (q)	12.2 (q)	12.4 (q)	13.0 (q)	11.9
C-19	18.1 (q)	18.6 (q)	20.3 (q)	21.9 (q)	13.9
C-20	36.1 (d)	35.7 (d)	35.8 (d)	35.1 (d)	36.1
C-21	18.7 (q)	19.0 (q)	18.8 (q)	18.4 (q)	18.9
C-22	34.6 (t)	34.3 (t)	34.9 (t)	34.2 (t)	34.8
C-23	31.1 (t)	30.9 (t)	30.9 (t)	30.9 (t)	31.3
C-24	156.8 (s)	156.5 (s)	156.5 (s)	156.5 (s)	156.8
C-25	33.9 (d)	33.7 (d)	33.9 (d)	33.8 (d)	36.1
C-26	21.3 (q)	21.0 (q)	21.3 (q)	21.3 (q)	21.9
C-27	21.3 (q)	21.2 (q)	21.3 (q)	21.3 (q)	22.0
C-28	106.2 (t)	106.0 (t)	106.1 (t)	106.0 (t)	106.1
OAc's	170.8, 169.9 (all s) 21.9(q)(3×C)	169.2, 170.2 170.5 (all s) 21.9(q)(3×C)	169.4, 170.2 173.7 (all s) 21.8(q)(3×C)	170.1, 170.8 (×2) (all s) 21.7(q)(3×C)	

[a]Another good model for comparison of the chemical shifts of the side chain is the steroidal glycoside isolated from *Sinularia crispa* (15): 29.7 (C-20), 18.3 (C-21), 35.0 (C-22), 31.3 (C-23), 157.0 (C-24), 34.0 (C-25), 21.8 (C-26) and 21.9 (C-27) (these may be interchanged), 106.0 (C-28).

[b]Data are from Piccialli and Sica (14).

The third sterol **11** was identified as the C-6 epimer of sterol **9**. The complete ^{1}H and carbon atom assignments of the triacetate **12**, eims *m/z* 528 (49%) $[M - HOAc]^+$, (Tables 3 and 4) are based on HMQC, HMBC, and H-H COSY measurements. A coupling constant of 5.5 Hz between H-6 and H-7 (both possessing homoallylic couplings with H-14) agrees with a dihedral angle of ca. 30° between the two. Change of the stereochemistry of C-6 influences, as expected, the Me-19 group, $\delta_c = 20.3$ q for **12** against 18.6 q for **10** (Table 4).

The fourth sterol, **13**, (0.01% dry wt) was purified as the triacetate derivative **14**, *m/z* 510 (18%) $[M - HOAc]^+$ and *m/z* 450 (100%) $[M - 2HOAc]^+$.

From the nmr spectra (Tables 3 and 4) it was clear that compound **13** has the same 24-methylene side chain as compounds **7–12**. The nmr data also pointed to three secondary acetoxy groups, a tetrasubstituted double bond (δ_c 132.4 and 134.6), and a trisubstituted epoxide, δ_H 3.31 d ($J = 2.4$ Hz), δ_C 59.1 d and 63.5 s. The proton and

carbon-line assignments of **14** were based on COSY, one bond, and multiple-bond CH-correlations (HMQC, and HMBC experiments). The following homo- and hetero-correlations established the locations of the three acetates at C-3, -7, and -11: i.e., connectivities between H-3/H-2, -2′, -4, -4′; H-6/H-7; H-7/H-14; H-14/H-15, -15′; H-18/H-12, -12′; C-8/H-6, -7; and C-9/H-7; C-11/H-12, -12′. The correlation between H-14 and the geminal pair H-15,H-15′ established unequivocally the position of the double bond at $\Delta^{8(9)}$. Further support for the proposed structure came from the hetero-correlations between H-18/C-17, -14, -13, -12 and between H-19/C-10, -9, -5, -1.

The coexistence of the 8(9) double bond and the 5,6-epoxide in the same ring causes ring B to adopt one of two possible conformations, that is, either a conformation in which C-7 and -10 are above the plane of C-5, -6, -8, and -9 ("C-7, -10-up") or below the plane ("C-7, -10-down"). This holds for both the 5,6α and the 5,6β epoxides. The special conformation of ring B brings about a unique twisting of both ring A and C (observable on a Dreiding model). Furthermore, for each conformer of B more than a single conformer of ring A has to be taken into consideration. Therefore it was incorrect to compare the chemical shifts and coupling constants of rings A–C of **14** with those of the known sterols, which possess only a single functionality on ring B.

On the basis of difference nOe measurements summarized in Table 5, the 5,6β-epoxy-3β,11α,7α-trihydroxy stereochemistry was suggested for **14**. The extent of the enhancement between Me-19 and H-11 indicated the β-epoxide was preferred over the α one. Only in the "C-7,-10-down" conformation of the β-epoxide is Me-19 close enough to H-11β for an nOe. Observation of Dreiding models has shown that the "C-7,-10-down" conformation is geometrically impossible in case of an α epoxide. The above-mentioned nOe between Me-19 and H-11, which is only possible for H-11β, suggests the 11α-acetoxy configuration. The latter stereochemistry is also in agreement with the vicinal couplings of H-11 with H-12. Furthermore, the nOe experiment also establishes the configuration of C-7. An enhancement between H-7 and Me-18 and Me-19 (in addition to H-6) determined the 7α-acetoxy configuration. The observed nOe's, and more particularly the simultaneous enhancement of both H-11 and H-7 while irradiating Me-19, require the existence of ring B in two conformations. On the grounds of the 3β-acetoxy stereochemistry of sterols **8**, **10**, and **12**, we assume also for **14** the same configuration of C-3. Because of the twisted structure of **14** the configuration of the latter carbon atom was difficult to determine from the coupling constants of H-3. The upfield shift of Me-18 (from 0.62 in case of **10** and **12** to 0.52) can be explained by the expected diamagnetic shift from the 8(9) double bond. Sterols **8**, **10**, and **12** were found to possess antitumor activity (against P388) with IC_{50} values of 1.46, 4.78, and 6.5 μg/ml, respectively.

Two more compounds were purified in the final stage and identified as the peracetates of α-D-xylopyranose tetraacetate (18) and a new natural product, derivative **16**.

Compound **16** was found to be a triacetate, $C_{12}H_{18}O_7$, hrcims *m/z* 275.1142

TABLE 5. Summary of nOe's of Compound **14**.

Irradiation of:	nOe effect on:	Irradiation of:	nOe effect on:
H-6	H-7, H-4eq[a]	Me-18	H-7, H-11, Me-19, H-12β, H-15eq
H-7	H-6 (11%)	Me-19	H-7, H-11, H-4ax[a], Me-18
H-11	Me-19	Me-21	H-12β[a]
H-14, H-12β	H-11, H-12α[a]		

[a]The following coupling constants could be measured from the enhanced signals observed during this nOe experiment: H (m, *J*), H-4ax (t, *J* = 12), H-4eq (dd, *J* = 12, 4). H-12β (dd, 8, 14), H-12α (dd, 4, 14).

([MH]$^+$ required *m/z* 275.1131). The mass, together with the ^{13}C-nmr spectra (one methylene, two methyleneoxy, and three methinoxy groups), was in full agreement with a structure of a dideoxyhexose.

The complete structure of **16** was deduced from the ^{1}H-nmr spectrum (Table 6).

The relationships between the various protons were determined both by a COSY and double irradiation experiments. The proposed 3α,4β-diacetoxy-6β-methylene-acetoxypyrane is a new marine natural product. A stereoisomer of **15**, hexahydrokojic acid, was synthetically prepared by hydrogenation of kojic acid (19).

The last compound that was identified from the sponge was furodysinin lactone, earlier isolated from *D. etheria* (7).

TABLE 6. ^{1}H nmr Data of Compound **16**.

Proton	δ_H, m, *J* (Hz)	Proton	δ_H, m, *J* (Hz)
H-2ax	2.80 dd (10, 11)	H-5eq	1.64 ddd (2, 5, 11)
H-2eq	3.90 dd (5, 11)	H-6ax	3.00 dddd (2, 4, 6, 11)
H-3ax	4.95 ddd (5, 10, 10)	H-7	3.82 dd (6, 11)
H-4ax	4.90 ddd (5, 10, 11)	H-7′	3.75 dd (4, 11)
H-5ax	1.13 dt (2, 11)	3×OAc	2.02 s, 2.03 s, 2.08 s

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Lrms and hrms were taken on a Du Pont B491 instrument and a VG-70, VSEQ instrument, respectively. Optical rotations were measured on a Perkin-Elmer 241 polarimeter with a 10-cm microcell. Ir spectra were recorded on a Perkin-Elmer model 177 spectrophotometer. ^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker AM 360 spectrometer equipped with an Aspect 3000 computer and operating at 360.1 and 90.5 MHz for ^{1}H and ^{13}C, respectively. All chemical shifts are reported with respect to TMS (δ = 0).

EXTRACTION AND ISOLATION.—*D. herbacea* was collected during the spring of 1989 near Masawa, Ethiopia. Voucher specimens are available from the senior author. Freshly collected specimens were extracted with EtOH. After removal of the solvent in vacuo, the residue was separated into four fractions by chromatography on a Sephadex LH-20 column which was eluted with petroleum ether-CH_2Cl_2-MeOH (2:1:1). The second fraction was further chromatographed on an RP-18 column eluted with MeOH-H_2O (1:1) and a Sephadex LH-20 column eluted with MeOH-CH_2Cl_2 (1:1) to give dysidamides **1** (2%), **3** (0.003%), and **5** (0.015%). The third fraction, which contained the polar sterols, was acetylated with Ac_2O/pyridine (room temperature, 24 h) and then chromatographed on a Si gel column eluted with petroleum ether/EtOAc mixtures to give compounds **8** (0.014%), **10** (0.07%), **12** (0.03%), and **14** (0.01%).

The fourth fraction was acetylated as described above and further purified by chromatography on a Si gel column eluted with petroleum ether/EtOAc mixtures to give α-D-xylopyranose tretraacetate (0.05%) and **16** (0.02%).

COMPOUND **3**.—[α]D +6° (*c* = 0.16, $CHCl_3$); hrcims (isobutane) [MH]$^+$ *m/z* found 406.0523, required 406.0510; ir ($CHCl_3$) 1738, 1694 cm^{-1}; ^{13}C nmr (90.1 MHz, $CDCl_3$) δ 177.8 (s, C-2), 46.6 (s, C-3), 72.7 (d, C-4), 56.6 (d, C-5), 31.8 (t, C-6), 42.7 (d, C-7), 78.3 (d, C-8), 24.0 (q, C-9), 19.3 (q, C-10), 15.1 (q, C-11), 170.0 (s, C-1′), 40.8 (t, C-2′), 39.9 (d, C-3′), 77.2 (d, C-4′), 15.3 (q, C-5′).

COMPOUND **4**.—[α]D +8° (*c* = 1.1, $CHCl_3$); ir ($CHCl_3$) 1750, 1710 cm^{-1}; ^{1}H nmr (360 MHz, $CDCl_3$) δ 5.17 (1H, d, *J* = 7 Hz, H-4), 4.44 (1H, ddd, *J* = 7, 6, 5 Hz, H-5), 1.75 (1H, ddd, *J* = 6, 7, 14 Hz, H-6), 1.95 (1H, dt, *J* = 14, 5 Hz, H-6′), 2.36 (1H, m, H-7), 5.88 (1H, d, *J* = 2.5 Hz, H-8), 1.24 (3H, s, Me-9), 1.20 (3H, s, Me-10), 1.10 (3H, d, *J* = 6.5 Hz, Me-11), 2.89 (1H, dd, *J* = 7, 18 Hz, H-2), 3.21 (1H, dd, *J* = 6, 18 Hz, H-2′), 2.79 (1H, m, H-3′), 5.95 (1H, d, *J* = 3 Hz, H-4′), 1.18 (3H, d, *J* = 6.5 Hz, Me-5′), 2.16 (3H, s, OAc); eims (rel. int. %) *m/z* [M]$^+$ 447 (13), 449 (3), 451 (3), 415 (18), 414 (91), 413 (23), 412 (100), 378 (10), 376 (13), 366 (45), 364 (64), 341 (24), 324 (14), 322 (26), 304 (27), 302 (83), 279 (12), 273 (12), 260 (18), 256 (13), 236 (10), 192 (23), 170 (61), 150 (10), 149 (10), 137 (19), 136 (83), 125 (12), 123 (18), 94 (18), 82 (12), 60 (12).

COMPOUND **5**.—$[\alpha]_D$ −41° (c = 1, MeOH); hrcims $[MH]^+$ m/z found 254.0743, required 254.0715; ir ($CHCl_3$) 3520, 1701 cm^{-1}; ^{13}C nmr (90.1 MHz, DMSO-d_6) δ 180.1 (s, C-2), 44.5 (s, C-3), 75.6 (d, C-4), 52.3 (d, C-5), 32.6 (t, C-6), 40.0 (d, C-7), 80.6 (d, C-8), 23.3 (q, C-9), 18.7 (q, C-10), 14.2 (q, C-11); eims (rel. int. %) m/z $[M]^+$ 253 (100), 255 (50), 257 (11), 142 (17), 128 (13).

COMPOUND **6**.—$[\alpha]_D$ +21° (c = 0.5, $CHCl_3$); ir 1750, 1720 cm^{-1}; ^1H nmr $CDCl_3$) δ 5.15 (1H, d, J = 7 Hz, H-4), 4.40 (1H, ddd, J = 5, 6, 7 Hz, H-5), 1.95 (1H, dt, J = 14, 5 Hz, H-6), 1.74 (1H, ddd, J = 6, 7, 14 Hz, H-6′), 2.36 (1H, m, H-7), 5.87 (1H, d, J = 2.5 Hz, H-8), 1.22 (3H, s, Me-9), 1.19 (3H, s, Me-10), 1.09 (3H, d, J = 6.5 Hz, Me-11), 2.49 (3H, s, NAc), 2.11 (3H, s, OAc); eims (rel. int. %) m/z $[M]^+$ 337 (3), 304 (17), 303 (14), 302 (60), 301 (19), 266 (12), 254 (38), 213 (12), 212 (100), 170 (49).

HYDROLYSIS OF **3** TO GIVE **5**.—Compound **3** (2 mg) was kept for 4 h in a 0.1% K_2CO_3 in MeOH solution at room temperature. After neutralization and evaporation of the solvent, the residue was purified on a short Si gel column eluted with petroleum ether/EtOAc mixtures to afford **5** (1 mg).

3β,6α,11α-TRIACETOXY-5α-CHOLEST-7-ENE-5,9α-DIOL [**10**].—$[\alpha]_D$ +9° (c = 0.3, $CHCl_3$); ir ($CHCl_3$) 3420, 1730, 1720 cm^{-1}; ^1H nmr (360 MHz, $CDCl_3$) δ 0.62 s (3H, Me-18), 0.89 (3H, brd, J = 5.2 Hz, Me-21), 0.99 (6H, d, J = 6.8 Hz, Me-26, Me-27), 1.08 (1H, H-22), 1.10 (3H, s, Me-19), 1.23 (1H, H-17), 1.32 (1H, H-20), 1.32 (1H, H-15), 1.47 (1H, H-22′), 1.50 (2H, H-16, H-15′), 1.52 (1H, H-2), 1.53 (2H, H-1′, H-4), 1.70 (1H, H-12), 1.85 (1H, H-23), 1.87 (1H, H-4′), 1.89 (1H, H-2′), 1.98 (1H, H-12′), 2.05 (1H, H-23′), 2.15 (1H, H-25), 2.40 (1H, H-1′), 2.52 (1H, H-14), 2.80 (1H, 5-O*H*), 4.00 (1H, 9-O*H*), 4.60 (1H, H-28), 4.71 (1H, H-28′), 4.95 (1H, brs, H-7), 5.00 (1H, tt, J = 11, 5 Hz, H-3), 5.16 (1H, brs, H-6), 5.20 (1H, dd, J = 11, 4 Hz, H-11), 1.98 (3H, s, OAc), 2.05 (3H, s, OAc), 2.10 (3H, s, OAc); eims (rel. int. %) m/z $[M - HOAc]^+$ 528 (100), $[M - 2HOAc]^+$ 468 (26), $[M - 3HOAc]^+$ 408 (14), 134 (60).

3β,6β,11α-TRIACETOXY-5α-CHOLEST-7-ENE-5,9α-DIOL [**12**].—$[\alpha]_D$ −80° (c = 0.6, $CHCl_3$); ir ($CHCl_3$) 3420, 1720, 1718, 1715 cm^{-1}; ^1H nmr (360 MHz, $CDCl_3$) δ 0.62 (s, 3H, Me-18), 0.89 (3H, d, J = 5.5 Hz, Me-21), 0.98 (6H, d, J = 6.8 Hz, Me-26, Me-27), 1.08 (1H, H-22), 1.10 (3H, s, Me-19), 1.28 (1H, H-17), 1.33 (1H, H-20), 1.39 (1H, H-15), 1.41 (1H, H-1), 1.50 (2H, H-4, H-22′), 1.52 (1H, H-2), 1.57 (2H, H-15′, H-16), 1.62 (1H, H-12), 1.70 (1H, H-4′), 1.83 (1H, H-23), 1.90 (2H, H-2′, H-16′), 2.06 (2H, H-12′, H-23′), 2.16 (1H, H-25), 2.28 (1H, H-1′), 2.50 (1H, H-14), 3.60 (1H, 9-O*H*), 4.40 (1H, 5-O*H*), 4.60 (1H, H-28), 4.68 (1H, H-28′), 4.88 (1H, dd, J = 5.5, 2 Hz, H-6), 5.09 (1H, tt, J = 11, 5 Hz, H-3), 5.32 (1H, dd, J = 12, 5 Hz, H-11), 5.42 (1H, dd, J = 5.5, 2 Hz, H-7), 2.00 (3H, s, OAc), 2.04 (3H, s, OAc), 2.07 (3H, s, OAc); eims (rel. int. %) m/z $[M - H_2O]^+$ 570 (5), $[M - HOAc]^+$ 528 (49), $[M - HOAc - H_2O]^+$ 510 (24), $[M - 2HOAc - H_2O]^+$ 450 (100), $[M - 3HOAc - H_2O]^+$ 390 (10).

5,6β-EPOXY-3β,6α,11α-TRIACETOXY-5β-CHOLEST-8-ENE [**14**].—$[\alpha]_D$ −15° (c = 0.3, $CHCl_3$); ir ($CHCl_3$) 1730, 1720 cm^{-1}; ^1H nmr (360 MHz, $CDCl_3$) δ 0.52 (3H, s, Me-18), 0.90 (3H, d, J = 5.8 Hz, Me-21), 0.98 (6H, d, J = 6.8 Hz, Me-26, Me-27), 1.09 (1H, H-22), 1.13 (3H, s, Me-19), 1.20 (1H, H-17), 1.23 (1H, H-15), 1.31 (1H, H-20), 1.40 (1H, H-12), 1.42 (1H, H-4), 1.47 (1H, H-22′), 1.51 (2H, H-1, H-2), 1.70 (H-15′), 1.72 (1H, H-16), 1.79 (1H, H-23), 1.80 (1H, H-1′), 1.92 (1H, H-16′), 1.99 (1H, H-23′), 2.00 (1H, H-2′), 2.14 (1H, H-25′), 2.18 (1H, H-4′), 2.52 (1H, H-14), 3.31 (1H, H-6), 4.58 (1H, H-28), 4.60 (1H, H-28′), 4.89 (1H, tt, J = 11, 5 Hz, H-3), 5.40 (1H, dd, J = 4, 8 Hz, H-11), 5.53 (1H, brs, H-7), 2.17 (3H, s, OAc), 2.04 (6H, s, OAc); eims (rel. int. %) m/z $[M - HOAc]^+$ 510 (17), $[M - 2HOAc]^+$ 450 (100), 438 (17), 436 (15), 435 (19), 432 (11), $[M - 3HOAc]^+$ 390 (90), 378 (15), 376 (14), 374 (19), 372 (30), 230 (11).

HYDROLYSIS OF **10** TO OBTAIN **9**.—A solution of **10** (2 mg) in 5% ethanolic KOH was kept at room temperature for 24 h. After acidification and removal of the EtOH in vacuo the solution was extracted with CH_2Cl_2. Compound **9** was obtained after evaporation of the solvent. ^1H [$CDCl_3$-CD_3OD (4:1)] δ 0.58 (3H, s, Me-18), 0.82 (3H, s, Me-21), 0.98 (6H, s, Me-26, Me-27), 1.10 (3H, s, Me-19), 3.82 (1H, H-6), 3.93 (2H, H-3, H-11), 4.58 (1H, H-28), 4.65 (1H, H-28′), 5.06 (1H, H-7).

HYDROLYSIS OF **12** TO OBTAIN **11**.—Compound **11**: ^1H nmr (200 MHz, $CDCl_3 + CD_3OD$) δ 0.58 (3H, s, Me-18), 0.85 (3H, s, Me-21), 0.92 (6H, s, Me-26, Me-27), 1.12 (3H, s, Me-19), 3.58 (1H, H-6), 3.92 (1H, H-3), 4.00 (1H, H-11), 5.39 (1H, H-7).

3α,4β-DIACETOXY-6β-METHYLENEACETOXYPYRAN [**16**].—$[\alpha]_D$ +32° (c = 1, $CHCl_3$); ir ($CHCl_3$) 1720, 1718 cm^{-1}; ^1H nmr ($CDCl_3$) see text; ^{13}C nmr ($CDCl_3$) δ 20.8 (q, 3C), 32.7 (t), 65.7 (t), 67.0 (d), 71.3 (d), 73.8 (d), 170.0 (s), 170.3 (s), 170.6 (s); eims (rel. int. %) m/z $[M - 3HOAc]^+$ 94 (100%).

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