119.70, 79.33, 50.65, 50.24, 46.63, 39.86, 35.63, 34.65, 34.60, 34.37, 30.95, 29.51, 27.08, 24.99, 24.70, 23.75, 23.52, 17.59, 14.81. Anal. Calcd for $C_{22}H_{34}O_2$: C, 79.95; H, 10.37. Found: C, 80.06; H, 10.32.

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Registry No. (±)-1, 84551-60-0; (±)-2, 75476-24-3; (±)-3, 75476-25-4; (±)-4, 75476-26-5; (±)-5, 98717-57-8; (±)-6, 98653-35-1; (±)-7, 98717-58-9; (±)-8, 98717-59-0; (±)-9, 98653-36-2; (±)-9 (mesylate), 98653-40-8; (±)-10, 98653-37-3; (±)-10 (1,17α-diol), 98677-73-7; (±)-10 (1,17β-diol), 98653-48-6; (±)-11α, 98677-48-6; (±)-11α (MEM deriv), 98653-42-0; (±)-11β, 98653-41-9; (±)-11β (MEM deriv), 98677-72-6; (±)-12α, 98653-38-4; (±)-12α (alcohol), 98653-44-2; (±)-12β, 98653-43-1; (±)-12β (alcohol), 98653-45-3; (±)-12 (3,17-dione), 98653-46-4; (±)-13, 98653-39-5; (±)-13 (dione), 98653-47-5; (±)-5-*epi*-euphane, 98717-60-3; 2-methoxy-5-methyl-1,4-benzoquinone, 614-13-1.

Supplementary Material Available: Crystallographic data for 13: atom coordinates, temperature factors, bond lengths, bond angles, and hydrogen coordinates (20 pages). Ordering information is given on any masthead page.

Herbasterol, an Ichthyotoxic 9,11-Secosterol from the Sponge *Dysidea herbacea*

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A specimen of the marine sponge *Dysidea herbacea* contained the known metabolite dysidin (1) and a new ichthyotoxic 9,11-secosterol, herbasterol (2). The structure of herbasterol was elucidated by interpretation of spectral data. Treatment of herbasterol (2) with either acid or base caused a retro-aldol reaction resulting in the formation of 19-norherbasterol (4).

An unusually diverse array of metabolites has been isolated from various specimens of *Dysidea herbacea*.[1] A reason for the unusual diversity could be that some of the metabolites are produced by the symbiotic microorganisms known to coexist with *D. herbacea*. Alternatively, the species, as presently defined, may not be homogeneous. The metabolites reported to date include polybrominated biphenyl ethers,[2] chlorinated nitrogenous metabolites[3] that are reminiscent of certain blue-green algal metabolites, and sesquiterpenes[4] that are almost certainly true sponge metabolites.

We have examined three samples of *D. herbacea* from different depths and locations on Bowl Reef near Townsville, Australia. We had hoped to find qualitative or quantitative differences in the secondary metabolites from each sample but the composition of the three crude extracts was identical within experimental error. The dichloromethane extracts all contained dysidin (1), as previously reported by Hofheinz and Oberhänsli,[3a] suggesting that this variety of *D. herbacea* had already been studied. We were, therefore, surprised to find that the methanolic extracts of each sample contained a single ichthyotoxic and antimicrobial metabolite. In this paper we describe the structural elucidation of herbasterol (2), a polyhydroxylated 9,11-secosterol responsible for the observed biological activities.

Chromatography of the methanol-soluble material on Sephadex LH-20 with 1:1 dichloromethane/methanol as eluant gave herbasterol (2, 8.6% dry wt) as an off-white solid, mp 113–5 °C. The molecular formula, $C_{27}H_{48}O_6$, was established from a combination of measurements: the highest peak in the mass spectrum gave a molecular formula of $C_{26}H_{46}O_5$ (M – CH_2O) but the ^{13}C NMR spectrum required 27 carbon atoms, including one ketone carbonyl (δ 215.2), and acetylation produced a pentaacetate 3. The ^{13}C NMR spectrum also contained two hydroxymethylene signals at δ 58.9 and 71.4 and three hydroxymethine signals at δ 71.4, 72.6, and 75.3. The infrared spectrum contained bands at 3400 cm^{-1} (broad) and 1700 cm^{-1} due to the hydroxyl and ketone functionalities. The ^1H NMR spectrum gave the first data that indicated a sterol structure. The methyl signals at δ 0.77 (s, 3 H), 1.00 (d, 3 H, J = 6.5 Hz), and 0.88 (d, 6 H, J = 6.5 Hz), could be assigned to carbons 18, 21, 26 and 27 of a "cholestane" skeleton. The absence of a C-19 methyl signal and the presence of two hydroxymethylene proton signals at δ 3.52 (d, 1 H, J = 11.2 Hz) and 4.64 (d, 1 H, J = 11.2 Hz) allowed a hydroxymethylene group to be placed at C-10. The facile loss of a one carbon unit in the mass spectrum and under both acidic or basic conditions can readily be explained by a retro-aldol reaction if the ketone group is at C-9 of a 9,11-secosterol. The remaining hydroxymethylene group that gives rise to a ^1H NMR signal at δ 3.58 (t, 2 H, J = 8.0 Hz) can be assigned to C-11 of a 9,11-secosterol. The mutually coupled (J = 13.4 Hz) signals at δ 3.28 (m, 1 H, J = 13.4, 9.4, 4.7 Hz)

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and 3.48 (m, 1 H, J = 13.4, 9.4, 4.7 Hz) were assigned to two axial hydroxymethine protons of a 2,3-dihydroxysterol. The remaining hydroxymethine proton signal at δ 3.81 (br s, 1 H) was initially ascribed to an equatorial proton adjacent to an axial hydroxyl group at either C-6 or C-7, but subsequent analysis of the spectral data of the tetraacetate **5** (vide infra) indicated that the hydroxyl group was at C-6.

Treatment of herbasterol (**2**) with either 6 N hydrochloric acid at 55 °C for 2 h or 1 N potassium hydroxide solution at reflux for 2 h resulted in formation of 19-norherbasterol (**4**) in yields of ~26% and 91%, respectively. The loss of a hydroxymethylene group was immediately apparent from the ^{1}H and ^{13}C NMR data. Acetylation of 19-norherbasterol (**4**) with acetic anhydride in pyridine gave a tetraacetate **5** in almost quantitative yield. Analysis of the ^{1}H NMR data of **5** revealed that the stereochemistry of ring B had changed during the retro-aldol reaction since all three secondary acetoxy groups were equatorial. The relevant ^{1}H NMR signals at δ 4.88 (m, 1 H, J = 11.1, 11.1, 5 Hz, H-2), 4.76 (m, 1 H, J = 15.1, 11.1, 4.7 Hz, H-3), and 5.01 (m, 1 H, J = 10.8. 10.8, 4.0 Hz, H-6) were all assigned to axial protons. Although accurate measurement of the coupling constants of some signals was not possible due to overlap, one decoupling experiment yielded data that allowed the stereochemistry of ring B to be determined. Irradiation at δ 1.74 caused the C-6 signal to collapse to a broad singlet, indicating that the axial protons at C-5 and C-7 were both being irradiated. As expected, the C-7 equatorial proton signal at δ 2.26 (br d, 1 H, J = 13, 3, 2 Hz) collapsed to a broad singlet, indicating that the proton at C-8 must be equatorial. The overlapping C-8 and C-10 proton singals at δ 2.53 (br dd, 1 H, J = 12, 3 Hz) and 2.54 (br t, 1 H, J = ~12 Hz) collapsed to a doublet (J = ~12 Hz) and a broad doublet (J = ~12 Hz), respectively, indicating that the H-5 and H-10 protons must both be axial. A single-crystal X-ray analysis of 19-norherbasterol tetraacetate (**5**) was attempted. While it clearly showed the structure and stereochemistry about the three rings, disorder in the side chain and in the C-2 acetate group prevented successful refinement.[5]

Having defined the stereochemistry of 19-norherbasterol (**4**), the stereochemistry of herbasterol (**2**) can readily be determined. During the retroaldol reaction, the stereochemistry at C-6 appears to undergo inversion. The phenomenon can be explained if herbasterol (**2**) has an A/B cis ring junction and inversion occurs at C-10 during the retro-aldol reaction, forcing ring B to adopt the alternative chair conformation (Figure 1). The circular dichroism (CD) spectrum of herbasterol (**2**) showed a negative Cotton effect at 295 nm, similar to that of 9,11-secosterols with an equatorial substituent at C-8.[6c]

While studying the base-catalyzed reaction of herbasterol (**2**) we observed that treatment of herbasterol (**2**) with sodium deuteroxide in deuterium oxide gave a dideuterio product that was acetylated to obtain a tetraacetate **6**. The same product was obtained from 19-norherbasterol (**4**) under identical reaction conditions. In the ^{1}H NMR spectrum of **6** the C-11 proton signals appeared as an AB quartet at δ 4.04 (d, 1 H, J = 11 Hz) and 4.16 (d, 1 H, J = 11 Hz), indicating that the protons at C-12 had exchanged. The mechanism of deuterium incorporation remains to be established.

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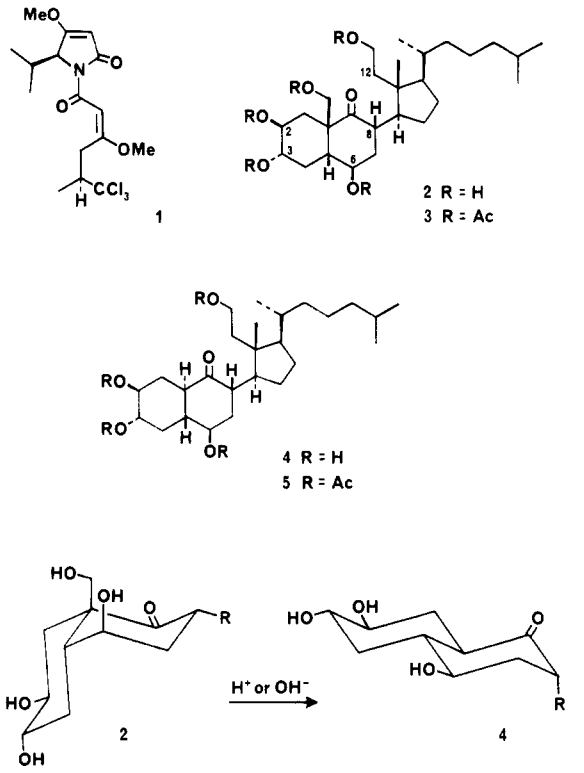

Figure 1.

Both herbasterol (**2**) and 19-norherbasterol (**4**) were toxic to goldfish (*Carassius auratus*) at 10 and 25 μg/mL, respectively, but the corresponding acetates were inactive. The purified herbasterol (**2**) proved to be only mildly active against the bacteria *B. subtilis* and *S. aureus*, although the activity was sufficient to permit the bioassay-directed isolation. Several 9,11-secosterols have been isolated from marine organisms[6] but no biological activity has been reported for these metabolites.

Experimental Section

Three freeze-dried specimens of *Dysides herbacea* (82–113, 82–114, 82–115, 36 g dry wt) were collected at different depths and locations on Bowl Reef near Townsville, Australia. The specimens were extracted separately with 1:1 chloroform–methanol and the extracts were examined by thin-layer chromatography, ^{1}H NMR spectroscopy, and antimicrobial assays. Since the extracts were essentially identical, they were combined, and the solvent was evaporated to obtain a residue that was triturated first with dichloromethane and then with methanol.

Chromatography of the dichloromethane-soluble material on silica using solvents of increasing polarity from hexane through dichloromethane to ethyl acetate gave fractions that contained dysidin (**1**). The combined fractions were decolorized by using activated charcoal and crystallized from ether/hexane to obtain dysidin (**1**, 460 mg, 1.3% dry wt) as white needles, mp 127.5–128.5 °C (lit.[3a] mp 127–129 °C).

Routine screening of the extracts revealed that the methanol-soluble material inhibited the growth of *Bacillus subtilis* and *Staphylococcus aureus*. Chromatography of the methanol-soluble material on Sephadex LH-20 with 1:1 dichloromethane–methanol as eluant gave active fractions that contained herbasterol (**2**, 3.1 g, 8.6% dry wt), a white hygroscopic solid: mp 113–5 °C; $[\alpha]_D$ +1.4° (c 8.4, MeOH); CD (MeOH) $(\theta)_{295}$ −8200°; IR (Nujol) 3400 (br), 1700 cm^{-1}; ^{1}H NMR (CD_3OD), 360 MHz) see Table I; ^{13}C NMR (CD_3OD, 50 MHz) see Table II; HRMS, obsd m/z 438.3340 ($C_{26}H_{46}O_5$ (M − CH_2O) requires 438.3346), 432.3240 ($C_{27}H_{44}O_4$ (M − H_2O − H_2O) requires 432.3240), 420.3240 ($C_{26}H_{44}O_4$ (M − CH_2O − H_2O) requires 420.3240), 402.3145 ($C_{26}H_{42}O_3$ requires 402.3134).

Herbasterol Pentaacetate (3). A solution of herbasterol (**2**, 50 mg) and acetic anhydride (0.5 mL) in pyridine (1 mL) was stirred at room temperature overnight. The solvents were

Table I. Selected 360-MHz ^{1}H NMR Data for Herbasterol (2), Herbasterol Pentaacetate (3), and 19-Norherbasterol Tetraacetate (5)

H at C no.	2	3	5
1α	2.60 (dd, 1 H, J = 13.3, 4.7 Hz)	2.64 (dd, 1 H, J = 13.7, 5 Hz)	
2α	3.48 (ddd, 1 H, J = 13.4, 9.4, 4.7 Hz)	5.14 (ddd, 1 H, J = 11, 11, 5 Hz)	4.88 (ddd, 1 H, J = 11.1, 11.1, 5.0 Hz)
3β	3.28 (ddd, 1 H, J = 13.4, 9.4, 4.7 Hz)	4.75 (ddd, 1 H, J = 11, 11, 4.3 Hz)	4.76 (ddd, 1 H, J = 15.1, 11.1, 4.7 Hz)
5β	2.09 (br d, 1 H, J = 12.5 Hz)	2.44 (br d, 1 H, J = 12.2 Hz)	~1.74
6α	3.81 (br s, 1 H, $W_{1/2}$ = 7.4 Hz)	4.86 (br s, 1 H, $W_{1/2}$ = 9 Hz)	5.01 (ddd, 1 H, J = 10.8, 10.8, 4.0 Hz)
8β	2.55 (br t, 1 H, J = 8.8 Hz)	3.07 (ddd, 1 H, J = 12, 6.5, 3.5 Hz)	2.53 (br dd, 1 H, J = ~12, 3 Hz)
10α			2.54 (br t, 1 H, J = ~12 Hz)
11	3.58 (t, 2 H, J = 8 Hz)	4.01 (ddd, 1 H, J = 10, 10, 5.4 Hz)	4.06 (dd, 1 H, J = 11.2, 11.2, 6.8 Hz)
		4.13 (ddd, 1 H, J = 10, 10, 5.4 Hz)	4.16 (ddd, 1 H, J = 11.2, 11.2, 4.3 Hz)
18	0.77 (s, 3 H)	0.67 (s, 3 H)	0.78 (s, 3 H)
19	3.52 (d, 1 H, J = 11.2 Hz)	4.14 (d, 1 H, J = 11.5 Hz)	
	4.64 (d, 1 H, J = 11.2 Hz)	4.94 (d, 1 H, J = 11.5 Hz)	
21	1.00 (d, 3 H, J = 6.5 Hz)	0.92 (d, 3 H, J = 6.5 Hz)	0.97 (d, 3 H, J = 6.5 Hz)
26	0.88 (d, 3 H, J = 6.5 Hz)	0.86 (d, 3 H, J = 6.5 Hz)	0.86 (d, 3 H, J = 6.5 Hz)
27	0.88 (d, 3 H, J = 6.5 Hz)	0.87 (d, 3 H, J = 6.5 Hz)	0.86 (d, 3 H, J = 6.5 Hz)
OAc		2.00 (s, 3 H)	2.01 (s, 3 H)
		2.01 (s, 3 H)	2.03 (s, 3 H)
		2.03 (s, 6 H)	2.07 (s, 3 H)
		2.16 (s, 3 H)	2.09 (s, 3 H)

Table II. ^{13}C NMR Data for Herbasterol (2), Herbasterol Pentaacetate (3), and 19-Norherbasterol Tetraacetate (5)[a]

C no.	2	3	5
1	38.6 (dd)	34.4 (t)	29.1 (t)
2	72.6 (d)	69.9[a] (d)	70.2[a] (d)
3	75.3 (d)	72.8 (d)	72.3[a] (d)
4	36.6[a] (t)	31.6[b] (t)	33.3[b] (t)
5	42.3[b] (d)	41.0[c] (d)	51.1 (d)
6	71.4 (d)	72.0[a] (d)	72.6[a] (d)
7	36.9[a] (t)	33.0[b] (t)	35.1[b] (t)
8	40.2 (d)	39.6 (d)	35.1 (d)
9	215.2 (s)	209.4 (s)	211.5 (s)
10	58.0 (s)	54.0 (s)	45.4 (d)
11	58.9 (t)	61.2 (t)	60.5 (t)
12	41.2 (t)	35.5 (t)	35.4 (t)
13	46.4 (s)	45.4 (s)	45.5 (s)
14	47.9[b] (d)	43.6[c] (d)	45.4 (d)
15	23.3 (t)	22.8 (t)	24.5 (t)
16	27.0 (t)	25.9 (t)	26.5 (t)
17	50.6 (d)	49.3 (d)	50.2 (d)
18	17.8 (q)	17.4 (q)	15.8 (q)
19	71.4 (t)	69.9 (t)	
20	35.7 (d)	34.6 (d)	33.4 (d)
21	19.7 (q)	19.3 (q)	19.0 (q)
22	36.4 (t)	36.1 (t)	35.4 (t)
23	25.5 (t)	24.6 (t)	24.5 (t)
24	40.5 (t)	39.6 (t)	39.4 (t)
25	29.0 (d)	28.0 (d)	27.9 (d)
26	23.0[c] (q)	22.7[d] (q)	22.5[c] (q)
27	23.1[c] (q)	22.9[d] (q)	22.7[c] (q)
$OCOCH_3$		169.6 (s)	169.8 (s)
		169.8 (s)	170.3 (s)
		170.3 (s)	170.5 (s)
		170.4 (s)	171.3 (s)
		170.9 (s)	
$OCOCH_3$		20.6 (q)	20.9 (q)
		21.0 (q)	21.0 (q)
		21.1 (q)	21.0 (q)
		21.2 (q)	21.0 (q)
		21.4 (q)	

[a] Spectra of 3 and 5 were recorded in $CDCl_3$ (2 in CD_3OD) at 50 MHz. a–d, values with identical superscripts within a column may be interchanged.

evaporated, and the product was eluted through a silica Sep-pak with dichloromethane to obtain herbasterol pentaacetate (3; 72 mg, quantitative) as an oil: $[\alpha]_D$ +2.2° (c 1.7, MeOH); IR (Nujol) 1740, 1710 (sh) cm^{-1}; ^{1}H NMR ($CDCl_3$) see Table I; ^{13}C NMR ($CDCl_3$) see Table II; HRMS, obsd m/z 618.3757 ($C_{35}H_{54}O_9$ (M − AcOH) requires 618.3767).

19-Norherbasterol (4) and 19-Norherbasterol Tetraacetate (5). (a) A solution of herbasterol (2, 100 mg) in 6 N hydrochloric acid was stirred under dry nitrogen at 55 °C for 2 h. The solvent was evaporated in vacuo, and the residue was stirred with acetic anhydride (1 mL) and pyridine (2 mL) at room temperature overnight. The solvents were evaporated, and the residue was eluted through a silica Sep-pak with dichloromethane to obtain an oil that was chromatographed by LC on Partisil M9 using 4:1 dichloromethane–ethyl acetate as eluant. The major product, 19-norherbasterol tetraacetate (5, 33 mg, 26% theoretical), crystallized from ether–pentane as white needles: mp 146–7 °C; $[\alpha]_D$ +14.1° (c 1.3, MeOH); IR (Nujol) 1735, 1705 cm^{-1}; ^{1}H NMR ($CDCl_3$) see Table I, ^{13}C NMR ($CDCl_3$) see Table II; HRMS, obsd m/z 546.3559 ($C_{32}H_{50}O_7$ (M − AcOH) requires 546.3556).

(b) A solution of herbasterol (2, 40 mg) in 1 N potassium hydroxide solution was stirred under nitrogen at 100 °C for 2 h. The cooled reaction mixture was passed through a C-18 reversed-phase Sep-pak which was then washed with water until the washings were neutral, and the product was eluted with methanol. The product appeared to be almost pure 19-norherbasterol (4, 34 mg, 91% theoretical). Acetylation of the product as described above gave 19-norherbasterol tetraacetate (5, 36 mg, 70% overall yield), identical with material prepared by the acid-catalyzed route.

(c) A solution of sodium deuteroxide in deuterium oxide was prepared by dissolving sodium (100 mg) in deuterium oxide (1 mL). A solution of herbasterol (80 mg) in the sodium deuteroxide solution was reacted according to the conditions above to obtain 12,12-dideuterio-19-norherbasterol tetraacetate (70 mg, 68% theoretical): ^{1}H NMR ($CDCl_3$) identical with that of 5 except for δ 4.06 (d, 1 H, J = 11 Hz) and 4.16 (d, 1 H, J = 11 Hz); HRMS, obsd m/z 548.3660 ($C_{32}D_2H_{48}O_7$ (M − AcOH) requires 548.3682).

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