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ECDYSONE-LIKE METABOLITES, 14α-HYDROXYPINNASTEROLS, FROM THE RED ALGA *LAURENCIA PINNATA*

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Key Word Index—*Laurencia pinnata*; Rhodomelaceae; hane-sozo; structure and biological activity; ecdysone-like steroids.

Abstract—Four steroids with moulting hormone activity were isolated from *Laurencia pinnata*. These steroids are related structurally to β-ecdysone.

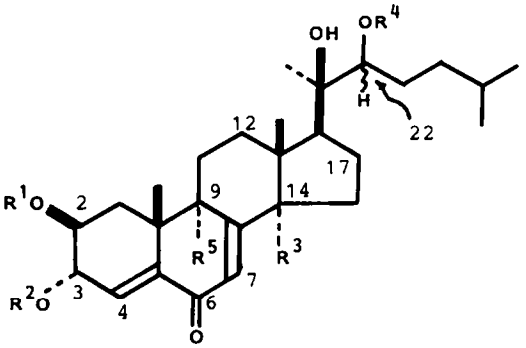
INTRODUCTION

We recently reported [1]* the isolation of two sterols, acetylpinnasterol (**1**) and pinnasterol (**2**), from the title alga, which are the first marine phytosteroids with moulting hormone activity. These sterols are structurally related to β-ecdysone (20-hydroxyecdysone, **3**) [2], but differ from **3** in the presence of a double bond at C-4/C-5 and a *trans*-oriented 2,3-dihydroxy moiety as well as the absence of 14α- and 25-hydroxyl groups. In a continuing study on components of the alga, four phytoecdysones (**4**–**7**) with an α-hydroxyl group at C-14 were isolated. The present paper describes the structure and biological activity of these sterols.

RESULTS AND DISCUSSION

The steroids were isolated from the neutral ether-soluble oil obtained from the methanol extracts of the title alga after repeated chromatography. The phytosteroid **4**, mp 174–176°, $[\alpha]_D$ +91°, had molecular formula $C_{29}H_{44}O_7$ and gave a diacetate **8**. The ^{1}H NMR spectrum of **4** [δ6.28 (1H, *d*, *J* = 2.5 Hz, H-7) and 3.57 (1H, *ddd*, *J* = 12, 7 and 2.5 Hz, H-9α)], compared with that of **1** [δ6.03 (1H, *t*, *J* = 2 Hz, H-7) and 2.54 (1H, *ddd*, *J* = 12, 7 and 2 Hz, H-9α)] and combined with the UV and IR spectra of **4**, indicated that **4** would be 14α-hydroxyacetylpinnasterol. Oxidation of **1** with SeO_2 gave, after chromatography, the corresponding 14α-hydroxy (20%) and 9α,14α-dihydroxy derivatives (**9**) (10%). The former was identified as **4** (IR, UV, ^{1}H NMR, MS, TLC and $[\alpha]_D$), confirming the structure.

The phytosteroid **5**, mp 144–145°, $[\alpha]_D$ +65°, had the same molecular formula $C_{29}H_{44}O_7$ as **4** and revealed almost the same FD-mass, UV and IR spectra as those of **4**. The chemical shifts [except the following two protons, δ2.26 (1H, *dt*, *J* = 13 and 5 Hz, H-12α) and 2.70 (1H, *t*, *J*



1 R^1=Ac, R^2=R^3=R^4=R^5=H, 22β-H(22R)

2 R^1=R^2=R^3=R^4=R^5=H, 22β-H(22R)

4 R^1=Ac, R^2=R^4=R^5=H, R^3=OH, 22β-H(22R)

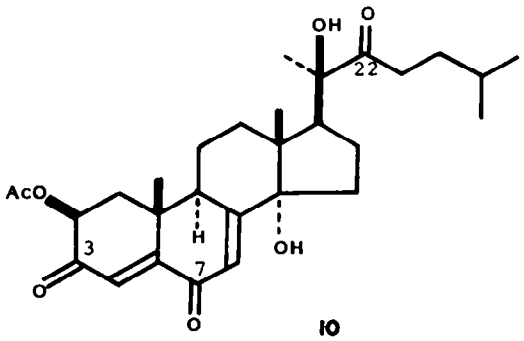
5 R^1=Ac, R^2=R^4=R^5=H, R^3=OH, 22α-H(22S)

6 R^1=R^4=R^5=H, R^2=Ac, R^3=OH, 22β-H(22R)

7 R^1=R^2=R^4=R^5=H, R^3=OH, 22β-H(22R)

8 R^1=R^2=R^4=Ac, R^3=OH, R^5=H, 22β-H(22R)

9 R^1=Ac, R^2=R^4=H, R^3=R^5=OH, 22β-H(22R)



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*The structures of **1** and **2** (H-22α) (22S) given in ref. [1] are incorrect (because of the authors' misunderstanding of the X-ray structure given in ref. [1]), and should be revised to (H-22β) (22R) as shown in this paper.

= 9.3 Hz, H-17α)] and splitting patterns of all observed protons in the ^{1}H NMR spectrum of **5** resembled closely those of **4** [δ2.56 (1H, *dt*, *J* = 13 and 5 Hz, H-12α) and 2.92 (1H, *t*, *J* = 9.1 Hz, H-17α)]. These spectra suggested that the sterol would differ from **4** only in the configuration of C-22. Oxidation of **5** with acetic anhydride and DMSO afforded 3,6,22-triketone (**10**) (5%), which was identical with the triketone obtained by the same treatment of **4**, indicating **5** to be a 22-epimer of **4**.

The phytosteroids **6**, amorphous, $[\alpha]_D$ +92°, and **7**, mp 210–212°, $[\alpha]_D$ +39°, had molecular formulae $C_{29}H_{44}O_7$ and $C_{27}H_{42}O_6$, and gave the corresponding diacetate and triacetate, respectively. These two acetates were found to be identical with **8**. The ^{1}H NMR of **6** [δ4.23 (1H, *ddd*, *J* = 10, 7 and 3.5 Hz, H-2α) and 5.76 (1H, *dd*, *J* = 7 and 2 Hz, H-3β)] and **7** [δ4.21 (1H, *ddd*, *J* = 10, 7 and 3.5 Hz, H-2α) and 4.64 (1H, *dd*, *J* = 7 and 2 Hz, H-3β)] indicate that the steroids **6** and **7** are formulated as 3-*O*-acetyl-14α-hydroxypinnasterol and 14α-hydroxypinnasterol, respectively.

The biological activities (ED_{50}) of these compounds as moulting hormones, determined by a *Sarcophaga* method [3], were as follows: **1**, 0.54 μg; **3**, 0.018 μg; **4**, 6 μg; **5**, 2 μg; **7**, 0.25 μg.

EXPERIMENTAL

Extraction and isolation. The plant material was collected at Onahama in Fukushima Prefecture, Japan, early in July, 1982. The wet material (12 kg) was digested with MeOH. The MeOH extracts were concd and extracted with Et_2O. The Et_2O soln was washed with 1 M aq. NaOH and 1 M HCl, dried and evaporated to leave a neutral oil (30.8 g), which was fractionated by chromatography over silica gel (Merck, 70–230 mesh, 1 kg, 7 × 58 cm) with C_6H_6, EtOAc and MeOH as eluents. Fractions eluted with EtOAc (Fr. 11, 1.3 g) and with EtOAc–MeOH (1:1) (Fr. 12, 0.24 g) were combined and separated by chromatography over silica gel (50 g) with $CHCl_3$–MeOH (100:3) to yield **1** (400 mg) and a mixture of steroids. The mixture was further separated by prep. HPLC over μ-Porasil with CH_2Cl_2–$CHCl_3$–MeOH (20:20:1), yielding three new phytosteroids, **4** (18 mg), **5** (16 mg) and **6** (8 mg). On the other hand, fractions eluted later with EtOAc–MeOH (1:1) (Fr. 13, 3.8 g) were likewise submitted to rechromatography with $CHCl_3$–MeOH (100:3) to give **2** (170 mg) and a mixture of steroids. The latter was further purified by prep. HPLC over Hitachi-gel #3011 (a copolymer of styrene and divinylbenzene) with MeOH–H_2O (4:1) to give phytosteroid **7** (9 mg).

14α-Hydroxyacetylpinnasterol (**4**). Mp 174–176° ($CHCl_3$–Et_2O); $[\alpha]_D^{20}$ +91° (MeOH; *c* 0.55); FD-MS *m/z*: 504 $[M]^+$, 486, 444, 403, 359, 342 and 145; UV λ_{max}^{EtOH} nm (log ε): 257 (3.99); IR ν_{max}^{KBr} cm^{-1}: 3480, 1730, 1675, 1645 and 1260; ^{1}H NMR (500 MHz, C_5D_5N): δ0.88 and 0.89 (each 3H, *d*, *J* = 6 Hz, H-26 and H-27), 1.21, 1.25 and 1.58 (each 3H, *s*, H-18, H-19 and H-21), 1.64 (1H, *dd*, *J* = 14 and 10.3 Hz, H-1β), 2.00 (3H, *s*, OAc), 2.44 (1H, *dd*, *J* = 14 and 3.5 Hz, H-1α), 2.56 (1H, *dt*, *J* = 13 and 5 Hz, H-12α), 2.92 (1H, *t*, *J* = 9.1 Hz, H-17α), 3.57 (1H, *ddd*, *J* = 12, 7 and 2.5 Hz, H-9α), 3.83 (1H, *d*, *J* = 10 Hz, H-22β), 4.69 (1H, *dd*, *J* = 7 and 2.5 Hz, H-3β), 5.31 (1H, *ddd*, *J* = 10.3, 7 and 3.5 Hz, H-2α), 6.28 (1H, *d*, *J* = 2.5 Hz, H-7) and 6.72 (1H, *d*, *J* = 2.5 Hz, H-4).

Oxidation of **1** *with* SeO_2. A soln of **1** (50 mg) in dioxane (0.5 ml) was stirred with SeO_2 (100 mg) in 50% aq. dioxane (1 ml) at 60° for 24 hr under N_2. The soln was evaporated and passed through a silica gel column with EtOAc to remove Se and SeO_2, and the eluent was separated by HPLC over μ-Porasil (CH_2Cl_2–MeOH) to give **4** (10 mg), mp 174–175°, $[\alpha]_D^{22}$ +54° (MeOH; *c* 0.27), and **9**, mp 225–227° (CH_2Cl_2–MeOH), $[\alpha]_D^{22}$ +11° (MeOH, *c* 0.10); FD-MS *m/z*: 521 $[MH]^+$, 442 and 401; UV λ_{max}^{EtOH} nm (log ε): 232 (3.82); IR ν_{max}^{KBr} cm^{-1}: 3460, 1730, 1675, 1635 and 1265; ^{1}H NMR (500 MHz, C_5D_5N): δ 0.84 and 0.85 (each 3H, *d*, *J* = 6 Hz, H-26 and H-27), 1.24, 1.30 and 1.56 (each 3H, *s*, H-18, H-19 and H-21), 2.00 (3H, *s*, OAc), 2.85 (1H, *t*, *J* = 9.0 Hz, H-17α), 2.99 (1H, *dd*, *J* = 14 and 4 Hz, H-1α), 3.79 (1H, *d*, *J* = 10 Hz, H-22β), 4.69 (1H, *dd*, *J* = 7 and 2 Hz, H-3β), 6.00 (1H, *ddd*, *J* = 10, 7 and 4 Hz, H-2α), 6.30 (1H, *s*, H-7), 6.95 (1H, *br s*, OH) and 7.06 (1H, *d*, *J* = 2 Hz, H-4).

Acetylation of **4**, **6** *and* **7**. Compound **4** (1 mg) was treated with Ac_2O (0.1 ml) and pyridine (0.1 ml) at room temp. for 12 hr. The reaction mixture was worked up as usual and purified by chromatography over silica gel ($CHCl_3$–MeOH) to give **8** (1 mg), oil; $[\alpha]_D^{22}$ +98° (MeOH; *c* 0.10); EI-MS *m/z*: 588 $[M]^+$, 570, 560, 552, 528, 492 and 450; IR $\nu_{max}^{CHCl_3}$ cm^{-1}; 1740, 1665, 1640, 1255 and 1030; ^{1}H NMR (400 MHz, C_5D_5N): δ0.83 and 0.84 (each 3H, *d*, *J* = 6Hz, H-26 and H-27), 1.15, 1.20 and 1.62 (H-18, H-19 and H-21), 2.03, 2.05, and 2.07 (each 3H, *s*, OAc); 2.87 (1H, *t*, *J* = 8 Hz, H-17α), 5.23 (1H, *ddd*, *J* = 11, 7 and 3 Hz, H-2α), 5.45 (1H, *d*, *J* = 10 Hz, H-22β), 5.71 (1H, *dd*, *J* = 6 and 3 Hz, H-3β), 6.29 (1H, *d*, *J* = 2 Hz, H-7), 6.39 (1H, *d*, *J* = 3 Hz, H-4), and 6.63 (1H, *br s*, OH). The same compound (**8**) was obtained by acetylation of **6** and **7** under the same conditions.

22-epi-14α-Hydroxyacetylpinnasterol (**5**). Mp 144–145° (Me_2CO–hexane); $[\alpha]_D^{20}$ +65° (MeOH; *c* 0.23); FD-MS (*m/z*: 504 $[M]^+$, 486, 403 and 359; UV λ_{max}^{EtOH} nm (log ε): 253 (3.93); IR ν_{max}^{KBr} cm^{-1}: 3490, 1730, 1675, 1638 and 1265; ^{1}H NMR (500 MHz, C_5D_5N): δ0.86 and 0.88 (each 3H, *d*, *J* = 6.5 Hz, H-26 and H-27), 1.25, 1.30 and 1.52 (each 3H, *s*, H-18, H-19 and H-21), 2.01 (3H, *s*, OAc), 2.26 (1H, *dt*, *J* = 13 and 5 Hz, H-12α), 2.70 (1H, *t*, *J* = 9.3 Hz, H-17α), 3.45 (1H, *ddd*, *J* = 12, 7 and 2.5 Hz, H-9α), 3.75 (1H, *d*, *J* = 10 Hz, H-22α), 4.68 (1H, *dd*, *J* = 7 and 2.5 Hz, H-3β), 5.26 (1H, *ddd*, *J* = 10. 7 and 3.5 Hz, H-2α), 6.49 (1H, *d*, *J* = 2.5 Hz, H-7), and 6.86 (1H, *d*, *J* = 2.5 Hz, H-4).

3-O-Acetyl-14α-hydroxypinnasterol (**6**). Oil; $[\alpha]_D^{20}$ +92° ($CHCl_3$; *c* 0.5); FD-MS *m/z*: 505 $[MH]^+$, 487, 486, 145 and 101; UV λ_{max}^{EtOH} nm: 255 (4.00); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3455, 1680, 1643 and 1270; ^{1}H NMR (500 MHz, C_5D_5N): δ0.84 and 0.85 (each 3H, *d*, *J* = 6.4 Hz, H-26 and H-27), 1.20, 1.26 and 1.60 (each 3H, *s*, H-18, H-19 and H-21), 2.94 (1H, *t*, *J* = 9 Hz, H-17α), 3.83 (1H, *d*, *J* = 10 Hz, H-22β), 4.23 (1H, *ddd*, *J* = 10, 7 and 3.5 Hz, H-2α), 5.76 (1H, *dd*, *J* = 7 and 2 Hz, H-3β), 6.31 (1H, *d*, *J* = 2.5 Hz, H-7), 6.41 (1H, *d*, *J* = 2 Hz, H-4) and 6.52 (1H, *br s*, OH).

14α-Hydroxypinnasterol (**7**). Mp 210–212° (MeOH–H_2O); $[\alpha]_D^{22}$ +39° (MeOH; *c* 0.35); FD-MS *m/z*: 462 $[M]^+$, 444, 351, 317 and 145; UV λ_{max}^{EtOH} nm (log ε): 256 (3.88); IR ν_{max}^{KBr} cm^{-1}: 3450, 1685, 1655 and 1075; ^{1}H NMR (500 MHz, C_5D_5N): δ0.84 and 0.85 (each 3H, *d*, *J* = 6.7 Hz, H-26 and H-27), 1.22, 1.26 and 1.59 (each 3H, *s*, H-18, H-19 and H-21), 2.94 (1H, *t*, *J* = 9 Hz, H-17α), 3.54 (1H, *ddd*, *J* = 12, 7 and 2 Hz, H-9α), 3.83 (1H, *d*, *J* = 11 Hz, H-22β), 4.21 (1H, *ddd*, *J* = 10, 7 and 3.5 Hz, H-2α), 4.64 (1H, *dd*, *J* = 7 and 2 Hz, H-3β), 6.28 (1H, *d*, *J* = 2 Hz, H-7) and 6.79 (1H, *d*, *J* = 2 Hz, H-4).

Oxidation of **4** *and* **5** *with* Ac_2O *and DMSO*. A soln of **4** (15 mg) in DMSO (0.5 ml) was treated with Ac_2O (0.5 ml) at 3° for 12 hr under stirring. The mixture was evaporated *in vacuo* to leave an oil, which was separated by prep. TLC (silica gel, EtOAc–C_6H_6, 1:4), giving **10** (0.5 mg) with **4** (12 mg); **10**, oil, $[\alpha]_D^{22}$ −21° ($CHCl_3$; *c* 0.3); FD-MS *m/z*: 501 $[MH]^+$, 143 and 100; UV λ_{max}^{EtOH} nm (log ε): 262 (3.52); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1740, 1720 and 1250; ^{1}H NMR (500 MHz, $CDCl_3$): δ0.91 and 0.92 (each 3H, *d*, *J* = 6.8 Hz, H-26 and H-27), 0.94, 1.21 and 1.28 (each 3H, *s*, H-18, H-19 and H-21), 2.19 (3H, *s*, OAc), 5.47 (1H, *dd*, *J* = 13.2 and

5.4 Hz, H-2α), 6.09 (1H, *d*, *J* = 2.0 Hz, H-7), and 6.26 (1H, *s*, H-4). The same triketone (**10**, 0.4 mg) was obtained by oxidation of **5** (7 mg) under the same conditions as those with **4** (3.5 mg).

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REFERENCES

1. Fukuzawa, A., Kumagai, Y., Masamune, T., Furusaki, A., Katayama, C. and Matsumoto, T. (1981) *Tetrahedron Letters* **22**, 4085.
2. Faux, A., Horn, D. H. S., Midolleton, E. J., Fales, H. M. and Lowe, M. E. (1969) *J. Chem. Soc. Chem. Commun.* 175.
3. Ohtaki, T., Milkman, R. D. and Williams, C. M. (1968) *Biol. Bull.* **135**, 322.