

SUPPLEMENTAL METHODS

Isolation of Steroids **6**, **8**, **10**, **15**, **17**, and **19**.

The crude EtOAc extraction obtained from the MeOH concentrate from *I. hippuris* was partitioned between hexane and 50% aqueous MeOH. The hexane layer was concentrated to give 18.3 g. The aqueous MeOH layer was partitioned with CH₂Cl₂ and the CH₂Cl₂ layer concentrated to yield 4.0 g of an oil. Both hexane and CH₂Cl₂ extracts were used to separate hippuristanol congeners.

The hexane extract was first separated by VFC (vacuum flash chromatography) on silica gel. The third fraction (2.4 g) eluted with hexane/EtOAc (5:1, v/v) was further separated on a silica gel column to give six fractions. The fourth fraction (89.7 mg) eluted with CH₂Cl₂/EtOAc (1:1, v/v) gave precipitates that were found to be compound **19** (73.5 mg). The fourth fraction (1.1 g) eluted with hexane/EtOAc (1:1, v/v) from VFC was successively separated on a Sephadex LH20 column (CH₂Cl₂/MeOH, 1:1, v/v), a silica gel column (hexane/CH₂Cl₂/EtOAc/MeOH, 1:1:0:0, 5:1:0:0, 1:1:0:0, 1:5:0:0, 0:1:0:0, 0:5:1:0, 0:1:1:0, 0:1:5:0, 0:0:1:0, 0:0:10:1, 0:1:1:0, v/v), a C18 column (MeOH/H₂O, 9:1, v/v), and finally subjected to repeated reversed phase HPLC (MeOH/H₂O, 8:2, v/v) to yield hippuristanol (**1**, 23.0 mg), epihippuristanol (**10**, 15.1 mg), hippurin-1 (**6**, 6.7 mg), and epihippurin-1 (**15**, 1.5 mg).

The CH₂Cl₂ extract was separated on a silica VFC column (hexane//EtOAc, 1:0, 10:1, 5:1, 2:1, 1:5, 0:1, v/v), followed by a Sephadex LH20 column (CH₂Cl₂/MeOH, 1:1, v/v). A fraction (400 mg) eluted with EtOAc was further separated on a silica gel column (/EtOAc) twice and finally subjected to reversed phase HPLC (MeOH/H₂O, 19:1, v/v) to give 2-desacetyl-hippurin-1 (**8**, 3.5 mg) and 2-desacetyl-epihippurin-1 (**17**, 20.4 mg).

Additional amount of steroids **1**, **6**, **8**, **10**, **15**, and **17** were obtained from other fractions.

Preparation of Steroids 7 and 16. A dried Indonesian specimen of the gorgonian *I. hippuris*. (3.2 kg) collected off Flores Island on Aug. 2001 was extracted with acetone, and its EtOAc soluble portion (45.3 g) obtained. The extract was separated on a silica gel column twice followed by HPLC separation (silica, hexane/EtOAc, 1:5, v/v) to give 2-desacetyl-hippurin-1 3-acetate (**7**, 17.5 mg). Additional amounts of **7** were obtained by purification of other fractions.

Compound **7** (24.6 mg) was treated with one drop of 1M hydrochloric solution in THF (1 mL) at RT for 3 hr. The mixture was partitioned between EtOAc and water, and the organic layer was dried over Na₂SO₄ and concentrated. The crude product was purified by HPLC (C18, MeOH/H₂O, 8:2, v/v) to give 22.1 mg of epimeric compound **16** (89%).

Acetylation of hippuristanol (1) to give hippuristanol 3-acetate (2) and hippuristanol 3, 11-diacetate (3). A mixture of hippuristanol (**1**, 10.0 mg), acetic anhydride (0.2 mL), and dry pyridine (0.3 mL) was allowed to stand at room temperature for 13 days. The mixture was concentrated to remove excess acetic anhydride and pyridine. The crude product was separated by silica gel thin layer chromatography (TLC) (CHCl₃/EtOAc, 3:1, v/v) to give 8.5 mg of hippuristanol 3-acetate (**2**, 8.5 mg, 75%) and 1.7 mg of hippuristanol 3,11-diacetate (**3**, 14%).

Acetylation of epihippuristanol (10) to give epihippuristanol 3-acetate (11) and epihippuristanol 3,11-diacetate (12). Epihippuristanol (**10**, 10.0 mg) was acetylated in the same manner as described above for hippuristanol (**1**). The crude product was separated by silica gel TLC (CHCl₃/EtOAc, 3:1, v/v) to give 8.8 mg of epihippuristanol 3-acetate (**11**, 81%) and 1.7 mg of epihippuristanol 3,11-diacetate (**12**, 14%).

Oxidation of hippuristanol (1) to yield hippuristanol 11-one (4) and hippuristanol 3, 11-dione (5). To an ice-cooled solution of hippuristanol (**1**, 31.7 mg) in pyridine (0.5 mL), Cornforth reagent (0.7 mL) was added dropwise. The mixture was kept stirring for 30 min. in an ice bath, then 3 hr at RT. The mixture was taken up in ether and the suspension was filtered. The filtrate was washed with dilute hydrochloric acid, dried over Na₂SO₄, and concentrated. The resulting product was separated on silica TLC (CHCl₃/EtOAc, 3:1, v/v) to give 8.8 mg of hippuristanol 11-one (**4**, 28%) and 17.7 mg of hippuristanol 3,11-dione (**5**, 56%).

Oxidation of epihippuristanol (10) to give epihippuristanol 11-one (13) and epihippuristanol 3,11-dione (14). Epihippuristanol (**10**, 31.4 mg) was oxidized in the same fashion as described above for hippuristanol (**1**). The crude product was separated by TLC (CHCl₃/EtOAc, 1:1, v/v) to give 12.6 mg of epihippuristanol 11-one (**13**, 40%) and 14.0 mg of epihippuristanol 3, 11-dione (**14**, 45%).

Preparation of 2-Desacetyl-hippurin-1 2-glutarate (9) and 2-Desacetyl-epihippurin-1 2-glutarate (18). A mixture of 2-desacetyl-hippurin-1 (**8**, 9.6 mg), glutaric anhydride (14 mg), and pyridine (0.1 mL) was allowed to stand at 70 °C for two days. After removal of pyridine, the crude product was separated by silica TLC (EtOAc), then by HPLC (C18, MeOH/H₂O, 9:1, v/v) to give 2.9 mg of glutarate (**9**, 24%).

Compound **18** was prepared by treating compound **17** (23.6 mg) with glutaric anhydride and pyridine in the same way as described above for 2-desacetyl-hippurin-1 (**8**). The product was separated by silica TLC (EtOAc) followed by silica HPLC (hexane/EtOAc, 1:4, v/v) to give 3.4 mg of glutarate (**18**, 11%).

Steroids 1, 6, 8, 10, 15, and 17. Known steroids **1, 6, 8, 10, 15,** and **17** were identified by comparing their ^1H and ^{13}C NMR data with those previously isolated by Higa et al.

Hippuristanol 3-acetate (2). amorphous solid, ^1H NMR (CDCl_3) δ 5.00 (brs, 1H), 4.29 (2H, m), 3.19 (s, 1H), 2.37 (dd, $J = 13.5, 7.5$ Hz, 1H), 2.20 (dd, $J = 14.0, 2.5$ Hz, 1H), 2.04 (s, 3H), 1.39 (s, 3H), 1.31 (s, 3H), 1.22 (s, 3H), 1.19 (s, 3H), 1.05 (s, 3H), 0.98 (d, $J = 7.0$ Hz, 3H); IR (KBr) 3510, 1715, 1240 cm^{-1} ; EIMS m/z 505 ($[\text{M}+1]^+$), 489 (2), 283 (100%).

Hippuristanol 3,11-diacetate (3). glass, $[\alpha]_{\text{D}}^{22} +45.7^\circ$ (c 0.44, CHCl_3); ^1H NMR (CDCl_3) δ 5.32 (brq, $J = 2.8$ Hz, 1H), 4.99 (brt, $J = 2.6$ Hz, 1H), 4.30 (dt, $J = 7.0, 7.6$ Hz, 1H), 3.11 (s, 1H), 2.37 (dd, $J = 13.4, 7.6$ Hz, 1H), 2.28 (dd, $J = 14.4, 2.5$ Hz, 1H), 2.04 (s, 3H), 2.02 (s, 3H), 1.29 (s, 3H), 1.26 (s, 3H), 1.23 (s, 3H), 1.20 (s, 3H), 0.98 (d, $J = 7.0$ Hz, 3H), 0.88 (s, 3H); IR (KBr) 3500, 1720, 1235 cm^{-1} ; EIMS m/z 546 (M^+ , 0.4), 531 (3), 488 (19), 418 (13), 358 (84), 298 (100 %).

Hippuristanol 11-one (4). white crystals. mp 205.5-208°C, ^1H NMR (CDCl_3) δ 4.42 (brdt, $J = 7.0, 7.5$ Hz, 1H), 4.03 (brs, 1H), 3.01 (s, 1H), 2.57 (d, $J = 11.9$ Hz, 1H), 2.36 (dd, $J = 13.7, 8.0$ Hz, 1H), 2.26 (dt, $J = 13.4, 3.0$ Hz, 1H), 2.19 (d, $J = 11.9$ Hz, 1H), 2.09 (m, 1H), 2.03 (d, $J = 8.8$ Hz, 1H), 1.31 (s, 3H), 1.21 (s, 3H), 1.19 (s, 3H), 1.08 (s, 3H), 1.01 (s, 3H), 0.98 (d, $J = 7.0$ Hz, 3H); ^{13}C NMR (CDCl_3) δ 210.6, 115.0, 84.8, 80.5, 79.1, 66.3, 64.5, 63.4, 59.0, 54.9, 46.4, 41.8, 40.5, 38.9, 35.8, 35.6, 35.3, 33.6, 32.8, 30.9, 28.9, 28.7, 28.5, 27.9, 23.0, 17.2, 14.8, 10.8; IR (KBr) 3470, 1705 cm^{-1} ; EIMS m/z 460 (M^+ , 1), 402 (8), 317 (99), 129 (100%).

Hippuristanol 3,11-dione (5). white crystals. mp 181-183.5°C; ^1H NMR (CDCl_3) δ 4.42 (m, 2H), 3.02 (s, 1H), 2.82 (ddd, $J = 13.0, 6.5, 2.0$ Hz, 1H), 2.63 (d, $J = 12.0$ Hz, 1H),

2.45 (dt, $J = 6.5, 14.5$ Hz, 1H), 2.37 (dd, $J = 13.0, 8.0$ Hz, 1H), 1.31 (s, 3H), 1.22 (s, 6H), 1.20 (s, 3H), 1.11 (s, 3H), 0.99 (d, $J = 7.0$ Hz, 3H); ^{13}C NMR (CDCl_3) δ 211.6, 210.0, 115.0, 84.9, 80.4, 79.1, 63.9, 63.4, 58.8, 54.5, 46.9, 46.3, 44.3, 41.8, 40.5, 38.0, 37.0, 35.5, 35.2, 33.7, 32.4, 28.7, 28.5, 28.2, 23.0, 17.3, 14.8, 11.0; IR (KBr) 3510, 1695 cm^{-1} ; EIMS m/z 458 (M^+ , 5), 316 (92), 84 (100%).

Glutarate 9. amorphous solid, ^1H NMR (CDCl_3) δ 5.00 (ddd, $J = 11.8, 4.5, 3.0$ Hz, 1H), 4.30 (brq, $J = 6.5$ Hz, 1H), 4.25 (brs, 1H), 4.05 (brs, 1H), 3.21 (brs, 1H), 2.46 (m, 3H), 2.37 (dd, $J = 7.6, 13.4$ Hz, 1H), 2.17 (brd, $J = 14.0$ Hz, 1H), 2.00 (m, 1H), 1.37 (s, 3H), 1.31 (s, 3H), 1.21 (s, 3H), 1.19 (s, 3H), 1.10 (s, 3H), 0.97 (d, $J = 7$ Hz, 3H); ^{13}C NMR (CDCl_3) δ 175.9, 172.1, 118.6, 84.2, 82.7, 79.0, 72.9, 68.0, 67.4, 66.3, 60.4, 58.2, 57.9, 48.8, 42.1, 41.0, 39.9, 39.0, 37.4, 36.7, 33.5, 32.7, 32.1, 31.6, 29.6, 29.1, 27.1, 26.9, 23.0, 20.0, 19.4, 15.1, 13.9.

Epihippuristanol 3-acetate (11). white solid, mp 193.5-195°C, ^1H NMR (CDCl_3) δ 5.01 (brs, 1H), 4.44 (dt, $J = 5.0, 7.5$ Hz, 1H), 4.30 (brs, 1H), 2.26 (m, 1H), 2.16 (dd, $J = 2.5, 14.0$ Hz, 1H), 2.04 (s, 3H), 1.34 (s, 3H), 1.30 (s, 3H), 1.28 (s, 3H), 1.04 (s, 3H), 0.99 (s, 3H), 0.94 (d, $J = 7.0$ Hz, 3H); IR (KBr) 3490, 1710, 1245 cm^{-1} ; EIMS m/z 504 (M^+ , 2), 489 (3), 446 (16), 376 (60), 358 (100%).

Epihippuristanol 3,11-diacetate (12, hippurin-2). white solid, mp 253-256.5°C, ^1H NMR (CDCl_3) δ 5.30 (m, 1H), 5.00 (brs, 1H), 4.44 (dt, $J = 5.5, 7.5$ Hz, 1H), 2.25 (m, 2H), 2.04 (s, 3H), 2.01 (s, 3H), 1.29 (s, 3H), 1.27 (s, 3H), 1.22 (s, 3H), 0.98 (s, 3H), 0.94 (d, $J = 7.0$ Hz, 3H), 0.89 (s, 3H); IR (KBr) 3520, 1725, 1250 cm^{-1} .

Epihippuristanol 11-one (13). white crystals, mp 254-257°C; ^1H NMR (CDCl_3) δ 4.49 (q, $J = 7.1$ Hz, 1H), 4.04 (brs, 1H), 2.51 (d, $J = 11.9$ Hz, 1H), 2.25 (d, $J = 11.9$ Hz, 1H),

1.29 (s, 3H), 1.28 (s, 3H), 1.05 (s, 3H), 1.01 (s, 3H), 0.98 (s, 3H), 0.94 (d, $J = 7.0$ Hz, 3H); ^{13}C NMR (CDCl_3) δ 210.5, 118.6, 84.4, 81.9, 79.1, 66.3, 64.6, 63.1, 58.4, 56.1, 45.9, 41.0, 39.6, 39.0, 35.8, 35.3, 32.7, 31.4, 30.9, 29.0, 28.9, 27.9, 25.8, 23.0, 17.6, 14.0, 10.9; IR (KBr) 3545, 3480, 1690 cm^{-1} ; EIMS m/z 460 (M^+ , 3), 445 (0.7), 402 (6), 332 (41), 317 (100 %).

Epihippuristanol 3,11-dione (14). white crystals, mp 226-229°C; ^1H NMR (CDCl_3) δ 4.48 (brq, $J = 7.0$ Hz, 1H), 2.82 (ddd, $J = 13, 6.5, 2$ Hz, 1H), 2.57 (d, $J = 12$ Hz, 1H), 2.45 (dt, $J = 6.5, 14$ Hz, 1H), 1.30 (s, 3H), 1.28 (s, 3H), 1.22 (s, 3H), 1.08 (s, 3H), 0.98 (s, 3H), 0.94 (d, $J = 7$ Hz, 3H); ^{13}C NMR (CDCl_3) δ 211.4, 209.9, 118.6, 84.5, 81.9, 79.0, 64.1, 63.2, 58.2, 55.8, 47.0, 45.9, 44.3, 41.0, 39.6, 38.0, 37.1, 35.8 (2C), 35.3, 32.3, 31.5, 29.0, 28.2, 26.0, 23.0, 17.6, 14.0, 11.1; IR (KBr) 3450, 1700 cm^{-1} ; EIMS m/z 458 (M^+ , 6), 443 (16), 400 (7), 330 (32), 315 (100 %).

Compound 16. colorless crystals, ^1H NMR (CDCl_3) δ 5.12 (brs, 1H), 4.43 (m, 1H), 4.29 (brs, 1H), 3.89 (m, 1H), 2.24 (m, 1H), 2.12 (s, 3H), 1.34 (s, 3H), 1.28 (s, 6H), 1.06 (s, 3H), 0.98 (s, 3H), 0.94 (d, $J = 6.5$ Hz, 3H), 0.83 (dd, $J = 11.0, 3.5$ Hz, 1H).

Glutarate 18. amorphous solid, ^1H NMR (CDCl_3) δ 5.00 (m, 1H), 4.43 (dt, $J = 7.5, 5.5$ Hz, 1H), 4.26 (brs, 1H), 4.06 (brs, 1H), 2.42 (m, 2H), 2.25 (m, 1H), 2.14 (dd, $J = 14.5, 2.5$ Hz, 1H), 1.33 (s, 3H), 1.30 (s, 3H), 1.27 (s, 3H), 1.10 (s, 3H), 0.98 (s, 3H), 0.94 (d, $J = 7.0$ Hz, 3H).

Compound 19. colorless crystals, mp: 240°C; $[\alpha]_{\text{D}}^{24} -38.0^\circ$ (c 2.41, CHCl_3); ^1H NMR (CDCl_3) δ 5.31 (s, 1H), 5.00 (brs 1H), 4.53 (q, $J = 7$ Hz, 1H), 4.24 (brs 1H), 2.69 (dd, $J = 14.5, 3.5$ Hz, 1H), 2.61 (d, $J = 6.5$ Hz, 1H), 2.28 (m, 1H), 2.12 (m, 1H), 2.06 (m, 1H), 2.04 (s, 3H), 1.87 (m, 1H), 1.82 (m, 2H), 1.74 (m, 1H), 1.71 (m, 2H), 1.64 (m, 1H), 1.61

(dd, $J = 14.5, 4$ Hz, 1H), 1.48 (m, 3H), 1.41 (s, 3H), 1.41 (m, 1H), 1.34 (m, 1H), 1.29 (s, 3H), 1.27 (m, 1H), 1.18 (m, 2H), 1.00 (s, 3H), 0.96 (s, 3H), 0.93 (d, $J = 7$ Hz, 3H), 0.79 (m, 1H); ^{13}C NMR (CDCl_3) δ 170.6 s, 117.9 s, 101.3 d, 90.7 s, 84.7 s, 80.6 d, 70.0 d, 66.0 d, 64.3 d, 58.5 d, 56.1 s, 56.0 d, 41.1 d, 40.5 d, 39.2 t, 38.7 t, 35.9 s, 32.4 t, 33.7 t, 32.1 t, 32.1 t, 31.0 d, 29.0 q, 27.4 t, 25.6 t, 22.8 q, 21.4 q, 19.6 q, 14.4 q, 13.9 q; positive NOEs: H-6 β /H-8, H-8/H-18, H-12 β /H-21, H-12 α /H-16, H-14/H-16, H-15 β /H-18, H-16/H-17, H-17/H-21, and H-21/H-23 α ; IR (KBr) 3470, 3300, 1730, 1240 cm^{-1} ; EIMS m/z 500 (M- H_2O), 485 (4), 442 (4), 372 (100%). The stereochemistry at C-18 was elucidated to be *R* configuration by positive NOEs between the proton pairs: H-18/H-8 and H-18/H-15 β .