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HIPPURISTANOLS, CYTOTOXIC POLYOXYGENATED STEROIDS FROM THE GORGONIAN *ISIS HIPPURIS*

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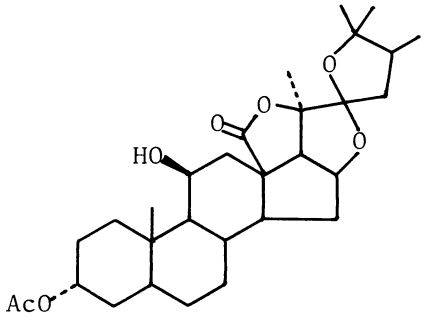
Four new polyoxygenated steroids (4-7) containing a spiroketal function have been isolated from the gorgonian *Isis hippuris*. Their structures were determined by spectral correlation and by interconversion. Previously assigned configuration at the C-22 of hippurin-1 (3) was reversed by spectroscopic evidence. Two (4, 7) of the steroids exhibited potent *in vitro* anticancer activities.

In a recent communication[1] we described the structures of two polyoxygenated steroids (1, 2) isolated from the gorgonian *Isis hippuris* collected in Okinawa Island. They were closely related to hippurin-1 (3)[2] isolated from the same species collected on the Great Barrier Reef. Our subsequent work on the same specimen resulted in the isolation of several new polyoxyganated steroids including hippuristanol (4)[3], 22-epihippuristanol (5), and 22-epihippurin-1 (6). On the other hand, the bioassay-guided separation of the gorgonian extract led the Fujisawa group to the isolation of two potent anticancer constituents, 4 and 2α-hydroxyhippuristanol (7) in addition to 3, 5, and 6. We now wish to report the isolation and structures of these marine steroids. We also present evidence for altering the configuration at the C-22 of hippurin-1 (3) whose structure including the relative stereochemistry has been established by an X-ray analysis.[2]

The gorgonian (5 Kg), collected at Kohama Island, Okinawa, was extracted with methanol at room temperature. The extract was concentrated and partitioned between ethyl acetate and water. The ethyl acetate soluble portion (37 g) was chromatographed on silica gel using a hexane-acetone gradient to afford six fractions. Repeated chromatography of the fraction 4 (9.1 g) over polystyrene gel (MeOH), silica gel (hexane-acetone), and LiChroprep RP-8 (Merck, 80-90% aq. MeOH) furnished, after recrystallization from methanol, pure samples of 3[4] (47 mg), 4 (540 mg), 5 (153 mg), and 6 (145 mg). Similar treatment of the fraction 6 gave rise to 7 (240 mg) as an amorphous solid, mp <140°. An attempted recrystallization was unsuccessful, but it was shown to be homogeneous by TLC in several solvent systems.

The structures of 6 and 7 were established by direct correlation with 3. The steroid 6, $C_{30}H_{48}O_7$ (M^+ m/z 520), mp 243-245°, was proved to be a 22*S*-epimer of 3 which is now shown to have a 22*R* configuration (*vide infra*). When 3 was allowed to stand with a catalytic amount of *p*-toluenesulfonic acid (*p*-TsOH) in THF at room



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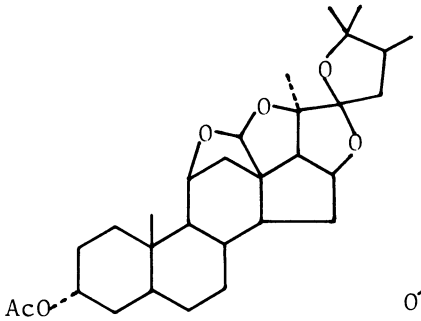

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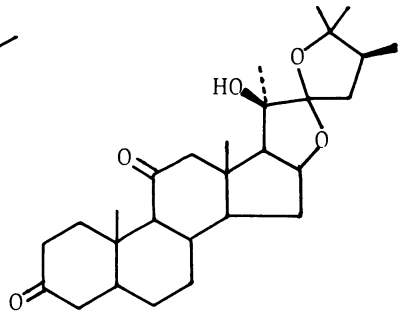
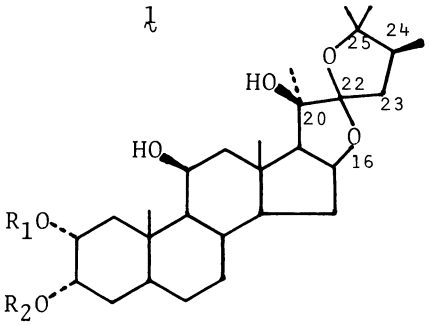
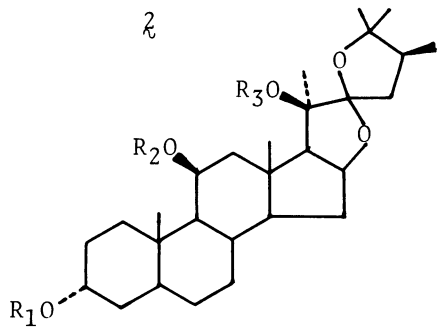


14, 22*R*
15, 22*S*



3, R_1=Ac, R_2=H, 22*R*
6, R_1=Ac, R_2=H, 22*S*
7, R_1=R_2=H, 22*R*
8, R_1=R_2=Ac, 22*R*



4, R_1=R_2=R_3=H, 22*R*
5, R_1=R_2=R_3=H, 22*S*
9, R_1=Ac, R_2=R_3=H, 22*R*
10, R_1=R_2=Ac, R_3=H, 22*R*

11, R_1=Ac, R_2=R_3=H, 22*S*
12, R_1=R_2=Ac, R_3=H, 22*S*
13, R_1=R_2=R_3=Ac, 22*S*

temperature over a period of a week, a major product isolated in 49% yield was indistinguishable with 6 by mp, mmp, TLC, and IR. The epimers 3 and 6 could clearly be differentiated by TLC and IR. In general, in the 22-epimeric pairs of these compounds the *S*-epimers have lower *R*f values than the *R*-epimers. IR absorption bands in the range 1050-900 cm^{-1} are also useful to differentiate the epimeric pairs in this series. Examination of eight each of 22*R*- and 22*S*-epimers including 3 to 15 showed that the *R*-epimers have three bands at 1005-1010 (s), 975-980 (s), 930-932 cm^{-1} (w), while the *S*-epimers have the bands at 1020-1028 (s), 968-972 (s), and 915-923 cm^{-1} (w or m).[5]

The infrared spectrum of 2α-hydroxyhippuristanol (7), $C_{28}H_{46}O_6$ (M+1, m/z 479.3347) contained absorptions at 1007 (s), 980 (s), and 932 (w) in addition to strong bands at 3450, 2910, and 1035 cm^{-1}. The ^{13}C NMR spectrum (Table 1) was virtually identical with that of 3, except minor differences in the chemical shifts attributed to the C-1 to C-3. The signals for the ring A carbons could be assigned through a model compound, 5α-spirostane-2α,3α-diol.[6] The ^1H NMR spectrum ($CDCl_3$) contained five methyl singlets (δ1.03, 1.17, 1.20, 1.29, 1.35), a methyl doublet (δ0.96, J=7 Hz), and four signals [δ3.19 (s, 1H, 20-OH), 3.78 (br d, J=12 Hz, 2β-H), 3.90 (br s, 3β-H), 4.26 (m, 2H, 11α-H and 16α-H)] assignable as indicated by comparing with those of 1-3. These data suggested that 7 is desacetylhippurin-1. It was confirmed by saponification of 3 to form 7 in a quantitative yield. Conversely, transformation of 7 to 3 could also be achieved by a controlled acetylation. Thus, treatment of 7 with acetic anhydride and pyridine at room temperature for 3 hr furnished 3 and the diacetate 8 (glass) in 63 and 32% yield, respectively, while the same reaction for 17 hr gave 8 as a sole isolatable product in 87% yield.

Hippuristanol (4), $C_{28}H_{46}O_5$ (M+1, m/z 463.3417), mp 188-190°, exhibited similar spectral properties with those of 3 and 7. The IR [3460 (s), 2920 (s), 1009 (s), 979 (s), 932 cm^{-1} (w)] resembled that of 7 and was indicative of a 22*R* configuration. The ^1H NMR spectrum ($CDCl_3$) contained six methyl signals [δ0.98 (d, J=7 Hz), 1.03 (s), 1.20 (s), 1.22 (s), 1.32 (s), 1.38 (s)] and three resonances [δ3.19 (s, 1H, 20-OH), 4.04 (br s, 3β-H), 4.30 (m, 2H, 11α-H and 16α-H)] which could be assigned as indicated. The ^{13}C NMR data (Table 1) of 4 were virtually identical with those of 3 and 7 except the signals attributed to the ring A carbons. The chemical shifts of the ring A and portions of the ring B and C carbons were also consistent with those predicted by using the values[7] of 3α-cholestanol and 11β-androstanol. Acetylation (Ac_2O/Py, room temp., 13 days) of 4 gave the monoacetate 9 (amorphous) and the diacetate 10 (amorphous) in 78 and 14% yield, respectively. These data permitted us to assign the structure of 4 as indicated.

Epimeric nature of 22-epihippuristanol (5), $C_{28}H_{46}O_5$ (M^+ m/e 462), mp 248-249°, with 4 was suggested by IR [3640 (m), 3480 (s), 2900 (s), 1023 (s), 968 (s), 916 cm^{-1} (m)], ^1H NMR [10% $CD_3OD/CDCl_3$, δ0.94 (d, 3H, J=7 Hz), 0.98 (s, 3H), 1.03 (s, 3H), 1.27 (s, 3H), 1.30 (s, 3H), 1.34 (s, 3H), 4.01 (br s, 3β-H), 4.28 (m, 11α-H), 4.43 (ddd, J=7,7,5 Hz, 16α-H)], and by ^{13}C NMR data (Table 1). Indeed 5 was obtained in 60% yield when 4 was treated with a catalytic amount of *p*-TsOH in THF at room temperature overnight. Acetylation of 5 with Ac_2O/Py (room temp., 13 days) afforded the monoacetate 11, mp 193.5-195°, and the diacetate 12, 252-253°, in 81 and 14% yield, respectively, while the reaction with Ac_2O/*p*-TsOH (room temp., 3 days) gave 12 (53%) and the triacetate 13 (amorphous, 24%). The diacetate 12 was identical with the compound previously isolated[8] from the gorgonian. The ^{13}C NMR data of 12 are shown in Table 1.

As discussed above 22-epimeric pairs of these compounds can easily be differenciated by TLC, IR, ^1H and ^{13}C NMR data. All of these techniques allowed to place 3, 4, and 7 in the same configurational group and 5 and 6 in the other. Small but sub-

Table 1. ^{13}C NMR Data (ppm) of Hippuristanols in $CDCl_3$

Carbon	4	5	7	12[a]	Carbon	4	5	7	12
1	32.6	32.5	40.3	32.7[e]	15	34.0	31.9	33.6	32.2[e]
2	28.7	28.8	68.8[d]	25.7	16	80.1	79.0	80.1	78.9
3	66.4	66.4[b]	69.0[d]	69.6[f]	17	66.1	66.7[b]	66.1	66.4
4	35.3	35.5	34.1	32.2[e]	18	28.4	27.2	28.4	27.3
5	40.0	40.1	39.1	40.9[g]	19	14.0	14.1[h]	15,2	14.1[h]
6	27.9	28.0	27.1	27.6	20	79.2	82.7	79.3	82.4
7	31.7	31.7	32.5	31.5	21	18.6	19.5	18.7	18.8
8	30.2	30.4	30.0	30.8	22	115.2	118.5	115.2	118.6
9	58.3	58.5[c]	58.1	58.0	23	40.9	40.1	40.9	39.9
10	36.3	36.4	37.2	35.7	24	41.9	41.1	41.9	41.0[g]
11	68.1	68.2	68.1	69.5[f]	25	84.5	84.1	84.5	84.1
12	49.8	49.0	49.7	44.6	26	22.9	23.0	23.0	23.0
13	42.2	42.3	42.2	42.0	27	29.0	29.2	29.0	29.1
14	57.2	58.3[c]	57.0	56.6	28	14.7	14.0[h]	14.7	14.0[h]

a: Acetate carbons at δ170.5, 170.0, 21.8, and 21.5. b-h: Assignments may be reversed.

stantial low field shifts of the 16α-H (δ4.43) and 24α-H [δ2.26 (ddq, J=13, 6, 7 Hz)] of 5 relative to those [δ4.30, 1.88 (ddq, J=9.5, 8, 7 Hz)][9] of 4 indicated that these protons of 5 must be subject to deshielding from the 22,25- and 16,22-epoxy oxygens, requiring 5 to have the 22*S* configuration which is contradicted by the reported[2] stereochemistry (22*S*) of 3. The 1H sharp singlet at δ3.19 observed with only 3, 4, 7 and their derivatives is indicative of the presence of the intramolecular H-bond between the 20-OH and the 22,25-epoxy oxygen, and that requires these compounds to have the 22*R* configuration. In order to gain further evidence, the diketones 14 and 15 were prepared[10] from 4 and 5, respectively, and subjected to infrared studies to see dilution effect on hydroxyl absorption bands. Infrared spectra were recorded with 7.5, 5, and 2.5% each of the ketones in $CHCl_3$. The ketone 15 showed two hydroxyl bands at 3610 (sharp, free OH) and 3470 cm^{-1} (broad, bonded OH). Upon dilution relative intensity of the former band gradually increased at the expense of the latter. To the contrary 14 exhibited a single broad band at 3515 cm^{-1} which was not affected by dilution. These results clearly demonstrated the presence of the intramolecular H-bond in 14 and not in 15. We therefore came to the conclusion that hippurin-1 (3) must have the *R* configuration at the C-22.[11] The 22*R* epimers could easily be converted to the *S*-epimers by acid treatment, while the reverse could not be effected. Thus, the *S*-epimers 5 and 6 may partly be artefacts of the isolation procedure.

Hippuristanol (4) and 2α-hydroxyhippuristanol (7) showed 50% inhibition (*in vitro*) of DBA/MC fibrosarcoma at 0.8 and 0.1 μg/ml, respectively. Compound 4 was also active against lymphocytic leukemia P-388 in mice, while 7 was slightly active.

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References

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2) R. Kazlauskas, P. T. Murphy, R. J. Quinn, R. J. Wells, and P. Schönholzer, Tetrahedron Lett., 1977, 4439.

3) The trivial name hippuristanol is given to the simplest member (4) of this series. Other new members except 6 are named as derivatives of 4.

4) Mp 182-185° (lit.,[2] mp 183-185°). ^1H NMR spectrum was identical with that of hippurin-1.

5) All spectra were recorded as KBr disks on a Hitachi IR 260-10 instrument. Accuracy of the instrument is ±3 cm^{-1} in the range 2000-650 cm^{-1}.

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8) T. Higa and J. Tanaka, unpublished result. It was also found to be identical with hippurin-2[12]

9) The spectra were recorded by Dr. K. Tachibana on a 360 MHz instrument. Assignments are based on decoupling.

10) Ketones 14, mp 181-183.5°, and 15, mp 229.5-232°, were prepared by Cornforth oxidation[13] of 4 and by Jones oxidation of 5, respectively.

11) It appears that the hippurin-1 monoacetate used for the x-ray determination had been epimerized in the acetylation process of 3. The ^{13}C NMR data sent by the authors contain signals [δ118.5 (C-22), 82.5 (C-20), 79.0 (C-16)] identical to those of 5, but not to 3 (115.2s, 79.3s, 80.1d).

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