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18-OXYGENATED POLYFUNCTIONAL STEROIDS FROM THE GORGONIAN ISIS HIPPURIS[1]

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Abstract: Two new 18-oxygenated, polyfunctional steroids (2, 3) related to hippurin-1 have been isolated from the gorgonian Isis hippuris collected off Okinawa and their structures were determined.

A number of polyoxygenated steroids have recently been discovered from octocorals (alcyonaceans, gorgonians) and other marine sources.[2] Of these, hippurin-1 (1),[3] isolated from the gorgonian Isis hippuris collected on the Great Barrier Reef, is particularly interesting for its uncommon structural features, a unique oxygenation pattern and a spiroketal ring structure on the side chain of a 24-methylcholestane skeleton. We now report that the same gorgonian species collected off Okinawa contains two related steroids of further complex structural features, 3α-acetoxy-11β-hydroxy-24-methyl-22,25-epoxy-5α-furostan-18,20β-lactone (2) and 3α-acetoxy-24-methyl-11β,18; 18,20β; 22,25-triepoxy-5α-furostane (3).

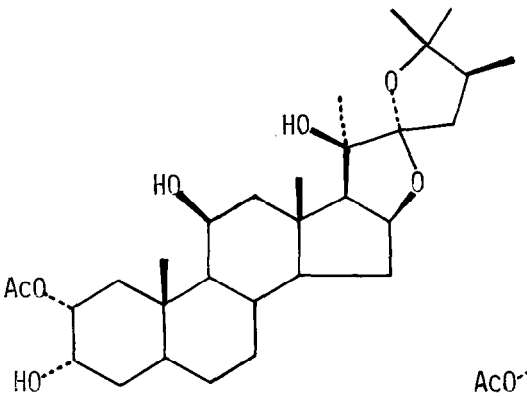
The fresh animal (2.5 Kg) was extracted by steeping in 95% ethanol for several days, and two more extractions were made by further steeping of the material in the fresh solvent. The combined extracts were concentrated and partitioned into the hexane, ethyl acetate, and methanol soluble portions. The hexane soluble portion (15.2 g) was chromatographed on silica gel using hexane and ethyl acetate gradient. The steroids 2 and 3 were contained in the fractions eluted with 1:1 hexane-ethyl acetate. Each of these fractions was subjected to further separation on Sephadex LH-20 followed by TLC on silica gel to furnish 2 (78 mg), mp 260-262°, $[\alpha]_D$ -31° ($CHCl_3$) and 3 (51 mg), mp 245.5-247°.

High resolution mass spectrometry established the molecular formula of 2 $C_{30}H_{44}O_7$ (observed 516.3069, calcd. 516.3084). The ^{13}C NMR spectrum[4] showed resonances for all 30 carbons comprising one γ-lactone carbonyl (δ182.4), an acetoxy carbonyl (δ170.5), a carbon bearing two oxygen atoms (δ116.7s), five carbons singly bonded to oxygen (δ90.1s, 85.6s, 80.2d, 70.0d, 66.8d), two quaternary carbons (δ53.0, 36.2), and six methine, eight methylene, and six methyl carbons. Therefore, the compound must be heptacyclic. These resonances also suggested that 2 has a C_{28} steroidal skeleton containing a spiroketal moiety and thus is closely related to hippurin-1 (1).[3]

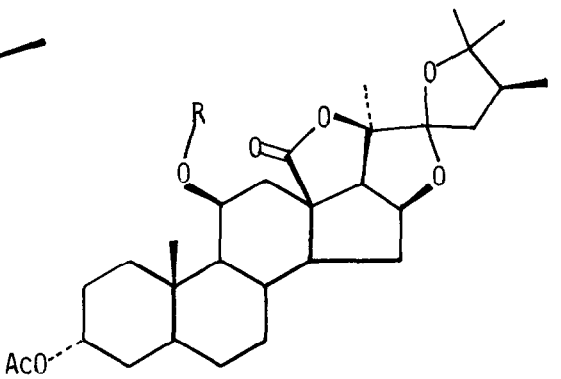
The infrared spectrum (KBr) of 2 showed absorptions at 3472 (OH) and 1733 cm^{-1} (OAc) in addition to several strong absorptions in the region 1300-1000 cm^{-1} due to various C-O stretching vibrations. A shoulder (1743 cm^{-1}) observed when the carbonyl region was expanded was assigned to the carbonyl of an intramolecularly hydrogen-bonded γ-lactone. The presence of this functionality was clearly demonstrated by breaking the hydrogen-bond through the formation of the acetate 4[5] which now exhibited an absorption (1774 cm^{-1}) typical to a γ-lactone. The shift of the carbon signal (δ182.4) to slightly low field comparing to that of γ-lactone (δ178)[6] is also an evidence of the internal hydrogen-bonding. Furthermore, as a result of the hydrogen-bonding conformation of the hydroxyl group is frozen, and thereby the ^1H NMR spectrum[4] revealed the hydroxyl proton as a doublet of an unusually large coupling constant at δ3.94 (J = 13Hz) which disappeared upon addition of D_2O. The signal is coupled to a doublet of quartets at δ4.19 (J = 13, 3Hz). The latter signal is in turn coupled to signals at δ2.37 (dd, J = 14, 3Hz, 12β-H), 1.71 (dd, J = 14, 4Hz, 12α-H), and 0.90 (dd, J = 11, 4Hz, 9α-H) as determined by decoupling experiments. A quartet of doublets at δ2.52 (J = 11, 4.5Hz) which coupled to the signal at δ0.90 was assigned to the 8β-H. These data pointed that the hydroxy group must be located at the C-11β position. A quintet at δ5.02 (J = 3Hz) suggested that an acetoxy group (δ2.04, s, 3H) is axially substituted either at the C-2 or C-3. The assignment of this group to the 3α position was made by comparing ^{13}C NMR resonances for the ring A and B portion of 2 with those of 2β- and 3α-acetoxycholestanes[7] and with estimated chemical shifts of 3α-acetoxy-11β-hydroxycholestane using additivity relationship[8] of substituents.

Comparison of the ^{13}C NMR data with those of various steroids[7] suggested an A/B trans junction and no substituents other than the acetoxy group on these rings. A base peak at m/e 388 (observed 388.2249, calcd. for $C_{23}H_{32}O_5$ 388.2249) in the electron impact mass spectrum[4] indicated a C20-C22 bond cleavage of the side chain with two oxygen atoms ($M^+ - C_7H_{12}O_2$).[9] This suggested that the acetoxy, hydroxy, and lactone functions are located in the nucleus of the steroid. Therefore, the most plausible location of the lactone carbonyl is at the C-18 which is cyclized by the 20β-hydroxyl group as shown in the structure 2. This structure can best explain the observed coupling between the hydrogen-bonded hydroxyl proton and the 11α-H. The two protons , as a model shows, can nearly be aligned in the opposite direction in the same plane. The same stereochemistry with 1 is realized at the C-3, C-11, and C-20. Relative configurations at the C-22 and C-24 are possibly the same with 1, but we have no conclusive evidences at present.

Compound 3 also showed a cleavage of the $C_7H_{12}O_2$ unit from the molecular ion (m/e 500, 12 rel.%) to give a base peak at m/e 372. The molecular formula $C_{30}H_{44}O_6$ of 3 was established by a high resolution mass measurement on the deacetyl derivative 5 (observed 458.3017, calcd. for $C_{28}H_{42}O_5$ 458.3029) which was obtained by saponification of 3. The ^1H and ^{13}C NMR spectra[10] revealed the presence of a cyclic acetal function (δ5.33s, 107.5d) in addition to the spiroketal moiety and an acetoxy group on a C_{28} steroidal skeleton. Since the formula requires 9 sites of unsaturation, the absence of olefinic carbons suggested that 3 must be an octacyclic compound. Therefore, the structure 3 was proposed and confirmed by a synthesis from 2. Reduction of 2 with an excess of $LiAlH_4$ in refluxing THF afforded, after usual work up, a mixture consisting mainly of 5 and an unidentified minor product, presumably the 18-epimer of 5.

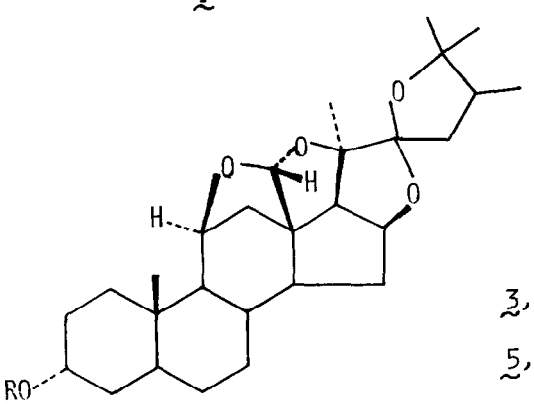


1



2, R = H

4, R = Ac



3, R = Ac

5, R = H

Acetylation of the mixture with acetic anhydride and pyridine furnished 3 (52% overall yield) which was identical with the natural product in all respects (mp, TLC, IR, ^{1}H NMR, and MS).

Relative configuration at the C-18 was proposed to be (R*) as shown in the structure 3. This configuration requires less strained cis junction of the two five-membered rings composed of the bicyclic acetal, while 18S configuration requires the trans ring junction which is highly strained. Treatment of 3 with p-toluenesulfonic acid in THF at room temperature for 30 hr showed no signs of epimerization, suggesting that 3 is the most stable epimer. The chemical shifts of the 11α-H (δ4.81, d, J = 5.5Hz) and 18-H (δ5.33, s) are comparable to those (δ4.72, d, J = 6Hz; 5.37, s) reported[11] for a synthetic steroid (18R) containing the same bicyclic acetal moiety.

To our knowledge naturally occurring 18-substituted steroids are uncommon except the aglycones of sea cucumber constituents, holothurinogenins[12] which have 18→20 lactone functionality on the lanostane skeleton. The most notable exception, however, is the highly active hormone aldosterone and its metabolic products,[13] to one of which the nucleus of 3 is reminiscent

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References and Notes

1. Presented in a part at 3rd International Symposium on Marine Natural Products, Brussels, 1980. We erroneously reported the species as Euplexaura robusta. It has now been correctly identified as Isis hippuris Linnaeus by Dr. Katherine Muzik, Museum of Comparative Zoology, Harvard University. A specimen has been deposited at the museum.

2. For review, F. J. Schmitz, in 'Marine Natural Products, Chemical and Biological Perspectives' (P. J. Scheuer, ed.), Vol. I, p 241, Academic Press, New York, 1978.

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

4. Mass spectrum m/e (rel. int. %) 516 (70, M^{+}), 498 (16), 458 (19), 456 (30), 438 (45), 423 (23), 388 (100), 299 (27), 286 (23), 285 (64), 280 (29), and 239 (35). ^{13}C NMR ($CDCl_3$, assignments are tentative) δ 182.4s (C-18), 170.5s (OCOMe), 116.7s (C-22), 90.1s (C-20), 85.6s (C-25), 80.2d (C-16), 70.0d (C-3), 66.8d (C-11 and C-17), 60.0d (C-14), 57.8d (C-9), 56.8d (C-24), 53.0s (C-13), 41.1d (C-5), 40.8t (C-12), 40.6t (C-15), 38.6t (C-23), 36.2s (C-10), 34.9t (C-7), 32.4t (C-1), 32.2t (C-4), 29.7d (C-8), 29.0q (C-26), 27.4t (C-6), 25.7t (C-2), 23.0q (C-27), 21.5q (OCOMe), 18.9q (C-21), and 14.0q (C-19 and C-28). ^{1}H NMR (360 MHz, $CDCl_3$) δ0.90dd (1H, J = 11, 4Hz, H-9), 0.97d (3H, J = 7Hz, H-28), 1.01s (3H, H-19), 1.11s (3H, H-26), 1.31s (3H, H-27), 1.48s (3H, H-21), 1.71dd (1H, J = 14, 4 Hz, H-12α), 1.80t (1H, J = 13Hz, H-23α), 2.04s (3H, OCOMe), 2.13dd (1H, J = 13, 6.5Hz, H-23β), 2.32m (1H, H-24), 2.33dt (1H, J = 11, 6Hz, H-15), 2.37dd (1H, J = 14, 3Hz, H-12β), 2.52qd (1H, J = 11, 4.5Hz, H-8), 2.67d (1H, J = 8Hz, H-17), 3.94d (1H, J = 13Hz, OH), 4.19dq (1H, J = 13, 3Hz, H-11α), 4.68ddd (1H, J = 8, 7, 6Hz, H-16), and 5.02quin. (1H, J = 3Hz, H-3β).

5. Mp 258-259°. Acetylation of 2 was accomplished by reacting with acetic anhydride in the presence of p-toluenesulfonic acid. Ac_2O/Py did not effect the acetylation even under reflux for 3 hr.

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7. H. Eggert, C. L. VanAntwerp, N. S. Bhacca, and C. Djerassi, J. Org. Chem., 41, 71 (1976). J. W. Blunt and J. B. Stothers, Org. Magn. Reson., 9, 439 (1977).

8. C. L. VanAntwerp, H. Eggert, G. D. Meakins, J. O. Miners, and C. Djerassi, J. Org. Chem., 42, 789 (1977).

9. The same bond cleavage was observed with hippurin-1 and derivatives, all of which gave base peaks at m/e 129 ($C_7H_{13}O_2^{+}$)[3].

10. Mass spectrum m/e (rel. Int.%) 500 (M^{+}, 12), 430 (14), 373 (25), 372 (100), 329 (18), 302 (12), and 269 (25). ^{13}C NMR ($CDCl_3$, assignments are tentative) δ170.4s (OCOMe), 117.0s (C-22), 107.5d (C-18), 93.3s (C-20), 84.9s (C-25), 81.5d (C-11), 80.9d (C-16), 69.8d (C-3), 63.7s (C-13), 58.0d (C-14), 57.4d (C-9), 49.7d (C-24), 40.9d (C-5), 40.1, 39.6, 39.0, 36.1s (C-10), 33.0t, 32.3t, 29.2q (C-26), 28.3t (C-6), 25.8t (C-2), 23.0q (C-27), 21.4q (OCOMe), 20.7q (C-21), 14.0q (C-28), and 11.9q (C-19). ^{1}H NMR (360MHz, $CDCl_3$) δ0.85d (1H, J = 10.5 Hz, H-9), 0.92s (3H, H-19), 0.94d (3H, J = 7Hz, H-28), 0.98s (3H, H-26), 1.28s (3H, H-27), 1.38s (3H, H-21), 1.74t (1H, J = 13 Hz, H-23α), 2.04s (3H, OCOMe), 2.25m (1H, H-24), 2.29dd (1H, J = 11.5, 5.5Hz, H-12β), 2.62d (1H, J = 7Hz, H-17), 4.59td (1H, J = 7, 2.5Hz, H-16), 4.81d (1H, J = 5.5Hz, H-11α), 5.01bs (1H, W h/2 = 7.7Hz, H-3β, and 5.33s (1H, H-18).

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