NOVEL 19-OXYGENATED STEROLS FROM THE OKINAWAN SOFT CORAL LITOPHYTON VIRIDIS

Kazuo IGUCHI, Shuichi SAITOH and Yasuji YAMADA*
Tokyo College of Pharmacy, Horinouchi, Hachioji, Tokyo 192-03, Japan

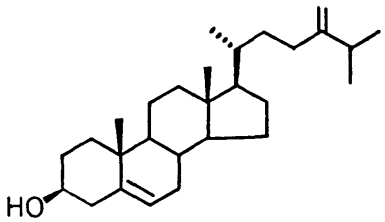
Two new sterols, litosterol (2a) and 5,6-epoxylitosterol (3a), with an oxygen functionality at the C-19 position were isolated from the Okinawan soft coral Litophyton viridis. The structures were elucidated on the basis of spectroscopic analysis.

KEYWORDS soft coral; Litophyton viridis; 19-hydroxylated sterol; ergostane skeleton; litosterol; 5,6-epoxylitosterol; antileukemic activity

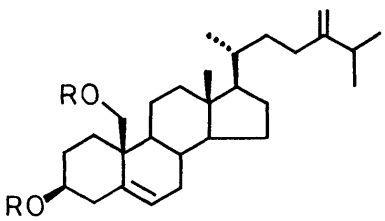
Marine sterols have received considerable attention because of the frequent occurrence of unusual structural features[1] compared with the sterols from terrestrial origins. In the course of our investigations[2] on bioactive substances of Okinawan marine animals, we have isolated two new 19-oxygenated sterols named litosterol (2a) and 5,6-epoxylitosterol (3a) from the soft coral Litophyton viridis. The sterol 3a showed an antileukemic activity (IC_{50} 0.5 μg/ml) against P388 leukemia cells in vitro. This paper describes the isolation and structure elucidation of these sterols on the basis of spectroscopic analysis.

The methanol extract of Litophyton viridis (wet weight 6.5 kg), collected at the coral reef of Ishigaki Island (Okinawa, Japan), was suspended in water and then extracted with ethyl acetate. The ethyl acetate-soluble portion (110 g) was subjected to repeated silica gel column chromatography to give ergosta-5,24(28)-dien-3β-ol (24-methylenecholesterol)[3] (1) (308 mg, mp 134.5-135.5°C), litosterol[4,5] (2a) (18 mg, $C_{28}H_{46}O_2$, mp 147.5-150°C), and 5,6-epoxylitosterol[6] (3a) (80 mg, $C_{28}H_{46}O_3$, mp 179-183°C).

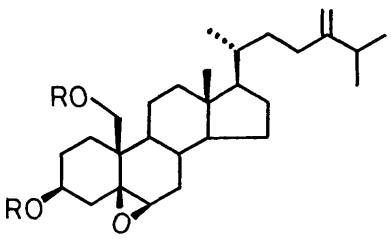
The IR spectrum of litosterol (2a) showed the presence of a hydroxy group (3600 cm^{-1}) and a terminal methylene group (1640, 890 cm^{-1}). The presence of a primary hydroxy group and a secondary hydroxy group was shown by the ^{1}H-NMR (270 MHz, $CDCl_3$) [δ_{ppm} 3.59 (1H, d, J = 11.2 Hz), 3.80 (1H, d, J = 11.2 Hz), 3.56 (1H, m)] and ^{13}C-NMR (67.8 MHz, $CDCl_3$) [δ_{ppm} 62.7 (t), 71.4 (d)] spectra. Acetylation of 2a gave the diacetate 2b[5] [^{1}H-NMR 3.98 (1H, d, J = 12.0 Hz), 4.46 (1H, d, J = 12.0 Hz), 4.64 (1H, m); ^{13}C-NMR 64.5 (t), 73.4 (d)], clearly indicating the presence of primary and secondary hydroxy groups in 2a. The ^{1}H- and ^{13}C-NMR spectra of 2a also showed the signals due to four methyl groups [^{1}H-NMR 0.73 (3H, s), 0.94 (3H, d, J = 6.6 Hz), 1.02 (6H, d, J = 6.6 Hz)], a terminal methylene group [^{1}H-NMR 4.65 (1H, br s), 4.71 (1H, br s); ^{13}C-NMR 105.9 (t), 156.8 (s)], and a trisubstituted olefin [^{1}H-NMR 5.75 (1H, br d, J = 4.0 Hz); ^{13}C-NMR 127.3 (d), 135.6 (s)]. These findings suggested that litosterol (2a) had a structure similar to that of ergosta-5,24(28)-dien-3β-ol (1). The chemical shifts of the ^{13}C signals of 2a are closely related to those of 1[7] as shown in Table I, except for the lack of the 19-methyl signal (19.4 ppm) present in 1, and the appearance of the signal (62.7 ppm) due to the hydroxymethyl group,[8] accompanied with a little changes in the chemical shifts of the signals at C-1, -5, -6, and -10 positions which are neighbors of the C-19 position. From these findings, the structure of litosterol was assigned to be ergosta-5,24(28)-diene-3β,19-diol as depicted in 2a.



1



2a R = H
2b R = Ac



3a R = H
3b R = Ac

The structure of 3a was also elucidated on the basis of spectroscopic data. The presence of a primary hydroxy group and a secondary hydroxy group in 3a was indicated by the ^{1}H-NMR spectrum [3.61 (1H, d, J = 11.0 Hz), 4.23 (1H, d, J = 11.0 Hz), 3.80 (1H, m)], and was confirmed by acetylation giving the diacetate 3b[6] [^{1}H-NMR 4.13 (1H, d, J = 12.3 Hz), 4.46 (1H, d, J = 12.3 Hz), 4.87 (1H, m)]. The IR spectrum of 3b showed no absorption due to hydroxy group, indicating that one of the three oxygen atoms in 3a ($C_{28}H_{46}O_3$) forms an ether linkage. The ^{1}H-NMR spectrum of 3a also showed signals of four methyls [0.72 (3H, s), 0.95 (3H, d, J = 6.5 Hz), 1.03 (6H, d, J = 6.5 Hz)] and a terminal methylene [4.69 (1H, s), 4.76 (1H, s)]. However the signal due to an epoxy proton [3.07 (1H, d, J = 2.0 Hz)] was newly observed in 3a instead of the signal due to the olefinic proton at C-6 present in 1 and 2a. This suggests that 3a is the corresponding 5,6-epoxide of litosterol (2a). This was confirmed by comparison of the ^{13}C-NMR spectrum of 3a with that of 2a as shown in Table I: the olefinic carbon signals at C-5 and C-6 shift to the higher field at δ 63.6 (s) and 61.5 (d) ppm in 3a, respectively, and the other signals are very similar to each other. The β-configuration of the epoxide was elucidated by comparison of the ^{1}H-NMR spectrum of 3a with those of 5β,6β-epoxycholesterol acetate and its 5α,6α-isomer, which were prepared by oxidation of cholesterol acetate with m-chloroperbenzoic acid.[9] The chemical shift and coupling constant of the epoxy proton in 3a were in good accordance with those of 5β,6β-epoxycholesterol acetate [3.05 (1H, d, J = 2.0 Hz)] rather than those of the 5α,6α-epoxide [2.87 (1H, d, J = 3.8 Hz)]. Thus the structure of 3a was assigned as 5β,6β-epoxyergost-24(28)-ene-3β,19-diol.

Table I. ^{13}C-NMR Data (δ_{ppm}, 67.8 MHz, $CDCl_3$) of 1, 2a and 3a

Carbon	1[7]	2a	3a
1	37.2	33.3	33.2
2	31.6	31.2	31.2
3	71.7	71.4	68.7
4	42.3	42.3	41.8
5	140.7	135.6	63.6
6	121.5	127.3	61.5
7	31.9	32.0	31.8
8	31.9	33.4	32.0
9	50.1	50.4	50.2
10	36.5	41.5	38.3
11	21.1	21.9	21.6
12	39.8	40.0	40.0
13	42.3	42.6	42.7
14	56.7	57.7	56.9
15	24.3	24.1	24.1
16	28.2	28.2	28.1
17	56.0	56.0	56.0
18	11.9	12.2	11.9
19	19.4	62.7	66.7
20	35.7	35.7	35.7
21	18.7	18.7	18.7
22	34.7	34.7	34.7
23	31.0	31.0	31.0
24	156.7	156.8	156.8
25	33.8	33.8	33.8
26	21.9	21.9	21.9
27	22.0	22.0	22.0
28	105.9	105.9	106.0

The 19-hydroxylated sterols are very rare in nature,[10] and are of interest as a possible intermediate in the biosynthesis of 19-norsterols.[11]

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REFERENCES AND NOTES

1) L. J. Goad, "Marine Natural Products," Vol. II, ed. by P. J. Scheuer, Academic Press, New York, pp 75-172 (1978). Recent examples: G. A. Doss and C. Djerassi, J. Am. Chem. Soc., 110, 8124 (1988); R. Riccio, M. Santaniello, O. S. Greco and L. Minale, J. Chem. Soc. Perkin Trans. 1, 1989, 823.

2) Recent reports: K. Iguchi, S. Kaneta, K. Mori and Y. Yamada, Chem. Pharm. Bull., 35, 4375 (1987); K. Mori, K. Iguchi, N. Yamada, Y. Yamada and Y. Inouye, Chem. Pharm. Bull., 36, 2840 (1988).

3) U. H. M. Fagerlund and D. R. Idler, J. Org. Chem., 21, 372 (1956).

4) All new compounds gave satisfactory high-resolution mass measurements.

5) 2a; $[\alpha]_D$ -25.8° (c 0.24, $CHCl_3$). 2b; $[\alpha]_D$ -51.9° (c 0.32, $CHCl_3$). IR ($CHCl_3$) 1720, 1635, 890 cm^{-1}.

6) 3a; $[\alpha]_D$ +3.8° (c 0.26, $CHCl_3$). IR ($CHCl_3$) 3620, 3520, 1638, 890 cm^{-1}. 3b; $[\alpha]_D$ -13.4° (c 0.7, $CHCl_3$). IR ($CHCl_3$) 1720, 1640, 890 cm^{-1}.

7) Assignments of the ^{13}C signals were done by comparison with the data of cholesterol and campesterol; N. Koizumi, Y. Fujimoto, T. Takeshita and N. Ikekawa, Chem. Pharm. Bull., 27, 38 (1979).

8) H. L. Holland, P. R. P. Diakow and G. J. Taylor, Can. J. Chem., 56, 3121 (1978).

9) J. G. Phillips and V. D. Parker, "Steroid Reactions," ed. by C. Djerassi, Holden-Day, San Francisco, pp 593-657 (1963).

10) M. Bortolotto, J. C. Braekman, D. Daloze, D. Losman and B. Tursch, Steroids, 28, 461 (1976); S. P. Gunasekera and F. J. Schmitz, J. Org. Chem., 48, 885 (1983).

11) S. Popov, R. M. K. Carlson, A.-M. Wegman and C. Djerassi, Tetrahedron Lett., 1976, 3491.

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