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# ISOLATION AND STRUCTURES OF APLYKURODINS A AND B, TWO NEW ISOPRENOIDS FROM THE MARINE MOLLUSK APLYSIA KURODAI

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Abstract: Two new isoprenoids, aplykurodins A and B, were isolated from the marine mollusk, Aplysia kurodai(Aplysiidae), and their structures were determined by chemical, spectral and X-ray crystallographic analyses.

Marine opisthobranchs of the family Aplysiidae have been reported to contain aplysiatoxin[1], halogenated terpenoids[2], biliverdine derivatives[3], a glyceryl ether[4], and others[5]. In this communication we wish to report isolation and structures of two new isoprenoids from the body[6] of the sea hare, Aplysia kurodai.

The $CHCl_3$ soluble part of MeOH extract obtained from the bodies of A. kurodai(10 kg) was fractionated with normal phase($SiO_2$, n-hexane-AcOEt(4:1→1:4) followed by reverse phase(RP-8, 80% MeOH) column chromatography to give aplykurodin A(1)(52 mg), colorless needles, mp 138°, $[\alpha]_D$-44°(c=0.96, $CHCl_3$), HREIMS m/z: 322.2527($M^+$, calcd for $C_{20}H_{34}O_3$: 322.2529) and aplykurodin B(2) (16 mg), colorless plates, mp 130-131°, $[\alpha]_D$-36°(c=0.90, $CHCl_3$), HREIMS m/z: 320.2350($M^+$, calcd for $C_{20}H_{32}O_3$: 320.2350).

Aplykurodin A(1) showed the IR absorptions due to hydroxyl(3400 $cm^{-1}$) and carbonyl(1720 $cm^{-1}$) groups, while the $^1H$[δ0.86, 0.87, 0.93(each 3H, d), 0.95 (3H, s)] and $^{13}C$ NMR(Table 1) spectra suggested the presence of 3 secondary methyls, one tertiary methyl, 7 methylenes, 5 methines, one quarternary carbon, 2 oxygen-bearing methines and one ester. Compound 1 was acetylated to afford the monoacetate(3), colorless plates, mp 74-75°. Since 1 contained 4 degrees of unsaturation, these data suggested 1 to be a bicyclic compound possessing a hydroxyl and a lactone functionalities. The $^{13}C$ NMR signals indicated the existence of the same side chain in 1 as that of cholesterol.

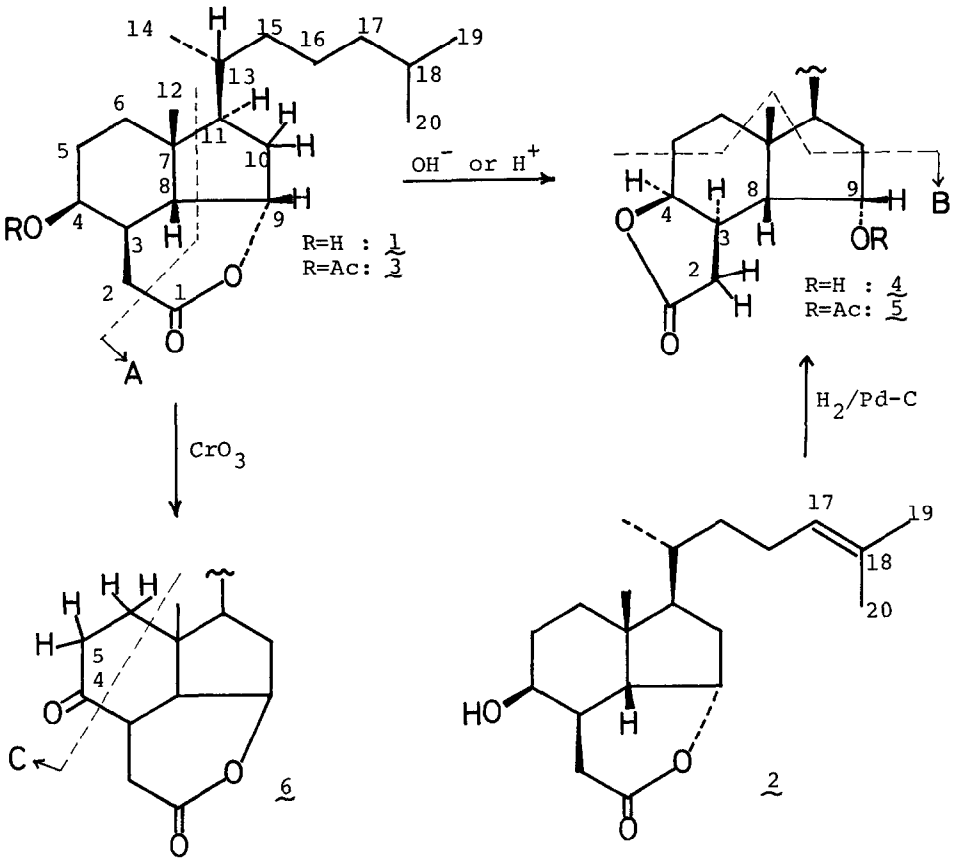
Detailed analysis of the $^1$H NMR spectrum(400 MHz) of 1 including spin-decoupling experiments implied the presence of the partial structure A in 1 [irradiation of 13-H at δ1.41 collapsed a br.dd at δ1.98(11-H) to br.d, irradiation of 11-H a multiplet at δ2.10(10-$H_2$) to br.dd, and irradiation of 10-$H_2$ a ddd at δ4.97(9-H) to d]. Upon treatment with alkali or acid, 1 gave a γ-lactone isomer(4)[oil, $[\alpha]_D$+14°(c=0.2, $CHCl_3$), EIMS m/z: 322($M^+$, $C_{20}H_{34}O_3$), IR $cm^{-1}$: 3400(OH), 1764(C=O), $^{13}$C NMR(Table 1)] which yielded the monoacetate (5). This compound was suggested to possess the partial structure B by $^1$H NMR double resonance studies [irradiation of 4-H at δ4.62 collapsed a dddd at δ2.51(3-H) to ddd, irradiation of 3-H dds at δ2.34 and 2.75(2-$H_2$)to ds and a dd at δ1.71(8-H) to d, and irradiation of 9-H at δ5.30 a dd at δ1.71 to d]. Irradiation of 8-H affected only 3-H and 9-H, which indicated C-8 was adjacent to a quarternary carbon. Oxidation of 1 afforded a ketone(6), $C_{20}H_{32}O_3$, which showed signals at δ2.38(ddd) and δ2.43(ddd) in the $^1$H NMR spectrum due to a methylene group(5-$H_2$) linked to a ketone group. This secured that 6 has a partial structure C. Thus the gross structure 1 was assigned for aplykurodin A.

The relative stereostructure of 1 was deduced by X-ray crystallography. A single crystal of 3(acetate of 1), $C_{22}H_{36}O_4$, suitable for X-ray diffraction study was obtained by crystallization from aq. MeOH. It is monoclinic, space group $P2_1$ with unit cell dimention a=14.241(2), b=7.278(1), c=10.370(2) Å, V=1074.7(3) Å$^3$, β=90.02(1), d(calcd.)=1.126 g/cm$^3$(for z=2, mol.wt. 324), d(obsd.)=1.095 g/cm$^3$(in KI solution). The intensities of all reflections with θ <60° were taken with Mo-Kα(0.70926 Å) radiation on a Rigaku AFC 5R diffractometer + RASA System(2θ<20°, ω-scan; 2θ>20°, 2θ-ω scan). The structure was solved by the direct method(MULTAN 84[7]) using 1824 independent structure factors($I_o \geq 3\sigma(I_o)$). The parameters were refined by the block-diagonal least square method to an R-factor of 0.04. A view of the molecule of 3(or its mirror image) is given in Fig.1.

The absolute configulation of 1 was determined by application of octant rule[8] to the CD spectrum of 6, which showed a negative Cotton effect($[\theta]_{283}$ -7900) due to the six-membered ring ketone.

Aplykurodin B exhibited the spectral features quite similar to those of 1 except for signals due to the terminal isobutyl functionality [$^1$H NMR: δ1.60, 1.69(3H each, br s), 5.02(1H, m), $^{13}$C NMR(Table 1[9])] and was hydrogenated to give 4[10](identical with $^{13}$C NMR and $[\alpha]_D$). Thus aplykurodin B was assignable to the 17,18-dehydroderivative of 1.

Though aplykurodins are thought to be novel type diterpenoids or steroids, their biosyntheses are not explainable at present. We treat them new isoprenoids[11] in the present paper. The biological activities of aplykurodins will be examined.



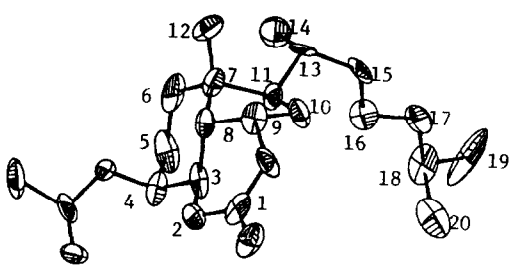



Fig.1 ORTEP drawing of 3

Table 1 $^{13}C$ NMR of 1, 2 and 4 (25 MHz in $CDCl_3$)

| | 1 | 2 | 4 | | 1 | 2 | 4 |
|---|---|---|---|---|---|---|---|
| C-1 | 172.2(s) | 172.4(s) | 177.8(s) | C-11 | 47.5(d) | 47.5(d) | 47.0(d) |
| C-2 | 37.8(t) | 37.7(t) | 37.9(t) | C-12 | 23.0(q) | 23.0(q) | 24.2(q) |
| C-3 | 33.2(d) | 33.2(d) | 32.7(d) | C-13 | 35.5(d) | 35.2(d) | 34.6(d) |
| C-4 | 66.8(d) | 66.7(d) | 79.7(d) | C-14 | 18.6(q) | 18.6(q) | 19.0(q) |
| C-5 | 28.9(t)[a] | 28.9(t) | 23.6(t) | C-15 | 36.6(t) | 36.4(t) | 36.3(t) |
| C-6 | 29.0(t)[a] | 28.9(t) | 30.4(t) | C-16 | 24.0(t) | 24.8(t) | 24.0(t) |
| C-7 | 43.2(s) | 43.2(s) | 42.3(s) | C-17 | 39.3(t) | 124.5(d) | 39.4(t) |
| C-8 | 43.7(d) | 43.6(d) | 53.2(d) | C-18 | 28.0(d) | 131.4(s) | 28.0(d) |
| C-9 | 80.7(d) | 80.8(d) | 71.2(d) | C-19 | 22.5(q) | 25.7(q) | 22.5(q) |
| C-10 | 33.6(t) | 33.6(t) | 39.4(t) | C-20 | 22.8(q) | 17.6(q) | 22.8(q) |

a: may be reversed

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References and Notes

1. Y.Kato and P.J.Scheuer, Pure Appl.Chem., 41, 1 (1975).
2. Y.Hashimoto,"Marine Toxins and Other Bioactive Marine Metabolites", Japan Scientific Societies Press, Tokyo, 1979, p. 295-298.
3. W.Rüdiger, Hoppe-Seyler's Z.Physiol.Chem., 348, 129, 1554 (1967).
4. T.Komori, J.Kitajima, S.Taguchi, H.Yoshi and T.Kawasaki, Nippon Kagaku Kaishi, 1981, 639.
5. D.J.Faulkner, Nat.Prod.Rep., 1, 551 (1984).
6. The body means the part obtained by removal of internal organs from the animal.
7. This computer program was developed by P.Main, G.Germain and M.M.Woolfson of the University of York, England.
8. W.Moffit, R.B.Woodward, A.Moscowitz, W.Klyne and C.Djerassi, J.Am.Chem.Soc., 83, 4013 (1961).
9. The $^{13}C$ NMR signals of 1, 2 and 4 were assigned by comparison with the spectra of cholesterol, linalool, 3, 5 and 6.
10. The lactone ring must be isomerized in the course of reaction.
11. Numbering of carbons of 1 and 2 follows that of steroid, tentatively.

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