BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 44, 2560—2562 (1971)

A Naturally-occurring Bromo-compound, Aplysin-20 from *Aplysia kurodai*

Shosuke YAMAMURA* and Yoshimasa HIRATA
Chemical Institute, Nagoya University, Chikusa, Nagoya

(Received April 6, 1971)

Two bromo-compounds, aplysin and aplysinol, were isolated from *Aplysia kurodai*, whose structures have already been established.[1] We could further isolate a small amount of a third bromo-compound, aplysin-20, from the same materials, which were gathered at Hokkaido (in September.)[1] However, no bromo-compound has been found in the *Aplysia kurodai* which were gathered at Sugashima, Mie-ken (in June).[2] The above different results seem to result from the different kinds of marine plants which were eaten by *Aplysia kurodai*.[3] Especially, the biogenesis of aplysin-20 strongly suggests that its origin must be attributable to the marine plants, as will be discussed later.

Aplysin-20, mp 146—147°C, is regarded as a diterpenoid bromo-compound on the basis of its molecular formula ($C_{20}H_{35}O_2Br$) and spectral data, as will be shown below. In addition to an end absorption in the UV spectrum of aplysin-20, the IR absorption band at 1675 cm^{-1} and a triplet at δ 5.41 ppm (1H, J=7.0 Hz) in the NMR spectrum (see Table 1) indicate the presence of an isolated double bond.

Aplysin-20 was treated with acetic anhydride-pyridine to give a monoacetate (mp 59—62°C; *m/e* 430 and 428 (M^+): ν_{max} 3520, 1730 sh., 1713, 1675 and 1260 cm^{-1}), which could be converted into the starting material with methanolic potassium hydroxide. The acetylation study suggests that aplysin-20 has a primary or secondary hydroxyl group as well as a tertiary one (ν_{max} 3520 cm^{-1}). Furthermore, when the NMR spectra of aplysin-20 and its monoacetate were compared, the doublet at δ 4.16 ppm (2H, J=7.0 Hz) and the triplet at δ 5.41 ppm (1H, J=7.0 Hz) were shifted to the doublet at δ 4.60 ppm (2H, J=7.0 Hz) and the triplet at δ 5.60 ppm (1H, J=7.0 Hz) respectively. The remaining signals are nearly identical in both compounds except for the appearance of the singlet at δ 2.02 ppm (3H, CH_3COO–) in the latter. The above fact, coupled with the observation of the methyl broad

TABLE 1. NMR SIGNALS OF APLYSIN-20

δ-Value	Number of proton and coupling const.	Assignment
0.96	3H (s)	Me+
1.00	3H (s)	Me+
1.08	3H (s)	Me+
1.16	3H (s)	Me+
1.30—2.20	16H (br. m)	
1.70	3H (br. s)	Me–C(=)
3.85	1H (q, J=3.1, 8.5 Hz)	$-CH_2-CHBr-$ ($-CH-CHBr-CH-$)
4.16	2H (d, J=7.0 Hz)	$-C=CH-CH_2OH$
5.41	1H (br. t, J=7.0 Hz)	$-C=CH-CH_2OH$

* Present address: Faculty of Pharmacy, Meijo University, Showa-ku, Nagoya.

1) S. Yamamura and Y. Hirata, *Tetrahedron*, **19**, 1485 (1963).

2) We can catch them in Sugashima only during the rainy season, and the attempted isolation of bromo-compounds has failed.

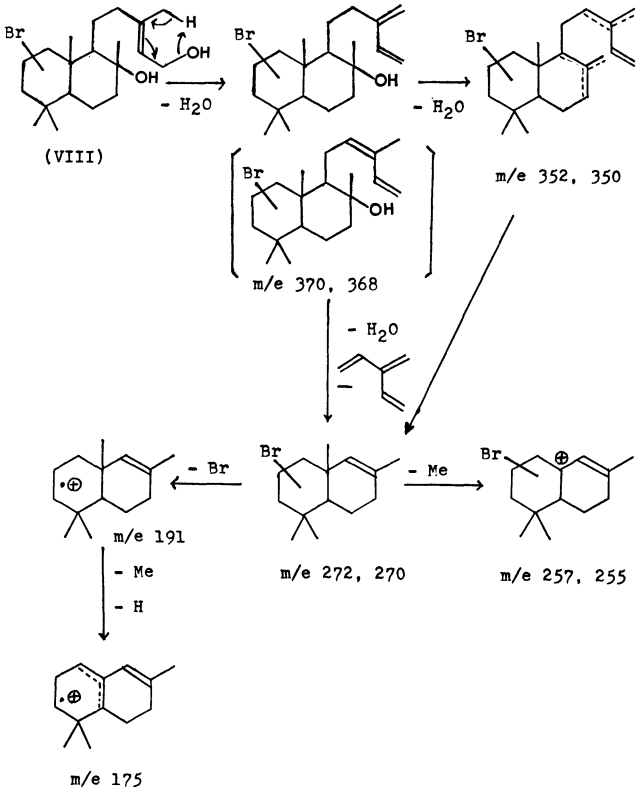
singlet at δ 1.70 ppm, indicates the presence of the partial structure (I). Furthermore, when considered in the light of the partial structure (I) and the presence

$$-CMe=CH-CH_2OR$$
(I) R=H; (II) R=Ac

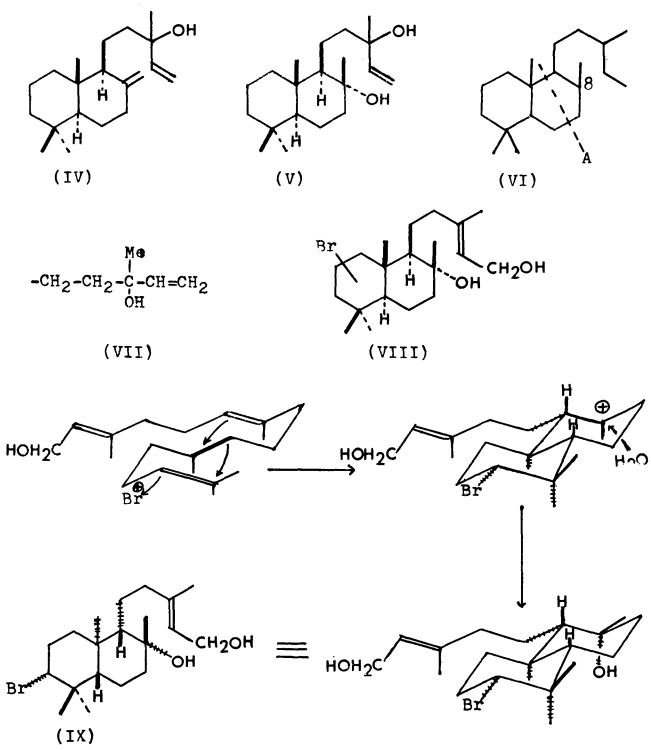
of two hydroxyl groups in aplysin-20 (or the partial structure (II) in a monoacetate with a tertiary hydroxyl group), the appearance of a pair of strong peaks (*m/e* 272 and 270), coupled with the strongest peak in their mass spectra at *m/e* 191, suggests the presence of the partial structure (III) in aplysin-20, as was discussed

$$-CH_2CH_2-CMe=CH-CH_2OH \quad (III)$$

in connection with Scheme I. Accordingly, aplysin-20, which has four tertiary methyl groups (see Table 1) and the partial structure (III),[4] may be regarded as a bicyclic diterpene with a side chain (III); thus, it is analogous to many bicyclic diterpenes with the same number of methyl or potential methyl groups and the same type of side chain as aplysin-20.[5] Manool (IV) and sclareol (V) are typical bicyclic diterpenes; both have the carbon skeleton (VI) with the side chain (VII).[6] Therefore, the structure (VIII) has been proposed for aplysin-20 except for the position of the



Scheme I. Fragment peaks in the mass spectrum of Aplysin-20.



Scheme II. Biogenesis of Aplysin-20.

bromine atom.[7] The tentative structure (VIII) can be satisfactorily explained by means of the mass spectra of both aplysin-20 and its monoacetate (in Scheme I) except for the following point: there are no typical peaks corresponding to the fragmentation, A, which can be found in the spectra of manool, sclareol, and other diterpenes with the exocyclic double bond or equatorial hydroxyl group at the C_8-position (in VI).[8] Accordingly, it seems that aplysin-20 has a configuration different from that of sclareol at the C_8-position or a carbon skeleton different from those of usual bicyclic diterpenes. The final structure was deduced by X-ray analysis,[9] indicating that aplysin-20 has the absolute configuration (IX), which is different from those of manool, sclareol, and streoids. The biogenesis of aplysin-20 is shown in Scheme II. The most interesting point in this study is that aplysin-20 has an axial hydroxyl group at the C_8-position and an equatorial bromine atom located at the C_3-position, a position usually substituted in diterpenes and steroids. From a biogenetic point of view particularly, aplysin-20 is the first bicyclic diterpene with an axial hydroxyl group at the C_8-position

Experimental

The mps are uncorrected. The IR spectra were taken with a JASCO IR-S spectrophotometer on a potassium bromide disk. The optical rotation was measured in methanol

3) Aplysin-type sesquiterpenes have been isolated from marine plants by T. Irie and his co-workers. (*Cf.* T. Irie, *Nippon Kagaku Zasshi*, **90**, 1179 (1969) and the references cited therein.)

4) Aplysin-20 has two rings which consist of less than ten carbon atoms.

5) D. H. R. Barton, "Chemistry of Carbon Compounds," Vol. II, Elsevier Publishing Co. (1953), p. 696; P. deMayo, "The Higher Triterpenoids," Interscience Publishers, N. Y. (1959), p. 1.

6) The transformation of the side chain (VII) to II has been known (G. Ohloff, *Ann.*, **617**, 134 (1958).

7) S. Yamamura and Y. Hirata, The 16th Annual Meeting of Chemical Society of Japan (Tokyo, April, 1963; Abstract of Papers, p. 84).

8) C. Enzell, *Acta Chem. Scand.*, **15**, 1303 (1961).

9) H. Matsuda, Y. Tomiie, S. Yamamura, and Y. Hirata, *Chem. Commun.*, **1967**, 898.

with a Rudolph and San polarimeter. The NMR spectra were measured in $CDCl_3$ with Nihondenshi JNM-3 and Varian A-60 analytical NMR spectrometers (60 MHz). The values are given in ppm relative to tetramethyl silane as an internal reference (s: singlet; d: doublet; q: quartet; t: triplet: m: multiplet). The mass spectra were measured by the Atlas Werke Co., Ltd., Germany (70 eV).

Isolation of Aplysin-20. Dried *Aplysia kurodai* (2 kg) was treated according to the method described in Ref. 1, and the insoluble materials were removed. The *n*-hexane solution was concentrated to about 100 m*l* and then chromatographed on silica gel (Wakojunyaku Co., Ltd.) (350 g). After elution with *n*-hexane to give debromoaplysin, aplysin, and aplysinol,[1] the second fraction, eluted with *n*-hexane-ether (10:1), was rechromatographed on alumina (Wakojunyaku Co., Ltd.) (15 g). After subsequent elution with *n*-hexane-ether (10:1) to give a yellow liquid, the second fraction, when eluted with *n*-hexane-ether (4:5), afforded a white solid which was then recrystallized from methanol to give colorless needles of aplysin-20 (320 mg),[10] mp 146—147°C; *m/e* 370 and 368 (4) (M^+-18), 352 (19), 350 (19), 289 (2) (M^+-Br), 272 (30), 271 (17), 270 (30), 257 (10), 255 (10), 191 (100, base peak), 175 (13), 149 (16), 147 (10), 135 (29), 134 (32), 133 (11), 123 (19), 121 (18), 119 (12), 109 (22), 107 (21), 105 (10), 98 (39), 97 (12), 96 (11), 95 (41), 94 (11), 93 (22), 91 (10), 84 (15), 83 (20), 82 (18), 81 (62), 79 (14), 71 (46), 69 (64), 68 (17), 65 (20), 57 (14), 55 (43), 43 (39), and 41 (56); ν_{max} 3500—3300 and 1675 cm^{-1} (Found: C, 61.98; H, 9.01%. Calcd for $C_{20}H_{35}O_2Br$: C, 62.00; H, 9.11%).

Acetylation of Aplysin-20. Alpysin-20 (100 mg) was dissolved in a solution of acetic anhydride and pyridine (1:1; 1 m*l*). The reaction solution was allowed to stand at room temperature overnight, and then the unreacted reagent was removed under reduced pressure to give a white solid. Recrystallizaton from methanol afforded colorless crystals of monoacetate (75 mg); mp 59—62°C; *m/e* 430 and 428 (0.4) (M^+), 370 (13), 368 (13), 352 (7), 350 (7), 312 (5), 310 (5), 289 (16) (M^+–Br), 272 (10), 271 (10), 270 (10), 257 (4), 255 (4), 191 (37), 175 (10), 152 (15), 149 (18), 147 (10), 137 (11), 135(23), 134 (16), 133 (10), 123 (19), 121 (20), 119 (15), 118 (17), 109 (26), 107 (23), 105 (11), 99 (13), 98 (15), 97 (10), 95 (41), 94 (10), 93 (23), 84 (15), 83 (12), 82 (12), 81 (50), 79 (14), 71 (37), 69 (68), 68 (12), 67 (20), 57 (17), 55 (39), 43 (100, base peak) and 41 (53); ν_{max} 3520, 1730 sh, 1713, 1675 and 1260 cm^{-1}; δ0.97 (3H, s), 1.00 (3H, s), 1.10 (3H, s), 1.13 (3H, s), 1.30—2.30 (15 H, br.m), 1.66 (3H, br. s), 2.02 (3H, s), 3.75 (1H, br.), 4.60 (2H, d, J=7.0 Hz) and 5.60 ppm (1H, t, J=7.0Hz) (Found: C, 61.20; H, 8.50%. Calcd for $C_{22}H_{37}O_3Br$: C, 61.53; H, 8.68%).

Hydrolysis of the Monoacetate. A solution of the monoacetate (30 mg) in 2N methanolic potassium hydroxide (2 m*l*) was stirred at room temperature for 2hr, poured into a large amount of water, and then extracted with ether. The ethereal solution was washed well with a saturated NaCl solution and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure to give a crystalline solid (20 mg), which was then recrystallized from methanol to afford colorless needles of aplycin-20 (mp and IR spectrum).

The authors wish to express their thanks to Professor Minoru Yamada, Hokkaido University, for providing us with the *Aplysia kurodai*, and also to Professor Yoshiyuki Toyama, formerly of Nagoya University, for his suggestions.

10) Aplysin-20 was used directly for the X-ray analysis.