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Isolation of Aplysin, Debromoaplysin, and Aplysinol from *Laurencia Okamurai* Yamada[1]

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As part of a continuing search for constituents from *Laurencia* species,[2] we have examined *L. Okamurai* Yamada (Japanese name, Mitsudesozo; Rhodomelaceae). In this paper we will report the isolation of aplysin (I),[3] debromoaplysin (II),[3] aplysinol (III),[3] laurinterol (IV),[2c] and debromolaurinterol (V)[2c] and also a new, unidentified dibromo-compound (VI) from the seaweed.

Methanol extracts of the dried seaweed were evaporated *in vacuo*, and the residue was percolated with ether. The ethereal solution was washed successively with a dilute potassium hydroxide solution, dilute hydrochloric acid, and water, and evaporated. The oily neutral fraction thus obtained was chromatographed on silica gel.

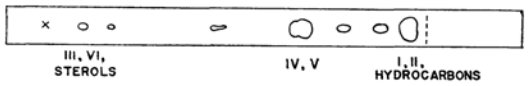
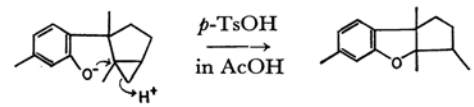


Fig. 1. TLC of the neutral fraction (silica gel, benzene).

From fractions eluted with 9 : 1 *n*-hexane - benzene, there was obtained a crystalline bromo-compound (I), $C_{15}H_{19}OBr$; mp 85—86°C, $[\alpha]_D$ −85°. The UV, IR, and NMR spectra of I were superimposable upon those of aplysin, which was isolated by Yamamura and Hirata[3] from *Aplysia kurodai* (sea rat) and which was also obtained by treatment with *p*-toluenesulfonic acid of laurinterol.[2c] The identity was further confirmed by a comparison of the R_f value on TLC and by a mixed-melting-point determination with an authentic sample.

Fractions eluted with 7 : 3 *n*-hexane - benzene left a colorless oil upon the removal of the solvent; this oil was purified by rechromatography on silica gel to give a pure, oily substance (II), $C_{15}H_{20}O$, $[\alpha]_D$ −63°. The IR and NMR spectra suggest that II is debromoaplysin;[3] this is confirmed by the synthesis from debromolaurinterol (V) shown below.



V II

Benzene eluates gave a mixture of alcohols, from which cholesterol, a monobromo-alcohol (III), $C_{15}H_{19}O_2Br$, mp 157—159°C, and a dibromo-compound (VI), $C_{14}H_{20}O_5Br_2$ (M^+ 428), mp 148.5—149°C, were isolated. The compounds III and VI could be purified *via* their acetates, VII, $C_{17}H_{21}O_3Br$, $[\alpha]_D$ −26°, and VIII, $C_{16}H_{22}O_6Br_2$, mp 154—156°C, $[\alpha]_D$ −44°, respectively. Compound III was identified as aplysinol[3] by a comparison of its UV, IR, and NMR spectra with those of an authentic sample. In the IR spectrum, the new dibromo-compound (VI) showed alcoholic group(s) at ν_{max} 3500 cm^{-1}, but no carbonyl function. The IR and NMR spectra of its acetate (VIII) exhibited the presence of an acetoxyl group [ν_{max} 1755 cm^{-1}; τ 7.86 (3H, s)], four tertiary methyl groups (3H, s's at τ 8.82, 8.70, 8.54 and 8.27) (two of which could be assumed to be olefinic and/or attached to epoxide-ring carbon, judging from their chemical shifts), two protons on carbons bearing bromine atoms (τ 5.2—5.8), and a proton on a carbon bearing an acetoxyl group (τ 4.71). The structure elucidation of VI is now in progress.

The principal part of the neutral fraction was eluted with 1 : 1 *n*-hexane - benzene; it was found to consist of laurinterol (IV) and debromolaurinterol (V), which were isolated from *L. intermedia* Yamada.[2c] It is noteworthy that these bromo-compounds have been found in only one seaweed.

Experimental

Isolation. Air-dried seaweed (1.4 kg), collected at Hakata-shima, the Inland Sea of Japan, was extracted with methanol, and then the methanolic solution was concentrated *in vacuo*. The residue was percolated

1) Part XII of "Constituents from Marine Plants." Part XI: T. Irie, T. Suzuki, Y. Yasunari, E. Kurosawa and T. Masamune, *Tetrahedron*, in press.

2) a) T. Irie, M. Suzuki and T. Masamune, *Tetrahedron Letters*, **1965**, 1091; b) T. Irie, Y. Yasunari, T. Suzuki, N. Imai, E. Kurosawa and T. Masamune, *ibid.*, **1965**, 3619; c) T. Irie, M. Suzuki, E. Kurosawa and T. Masamune, *ibid.*, **1966**, 1837; d) T. Irie, M. Izawa and E. Kurosawa, *ibid.*, **1968**, 2901, 2735; e) T. Irie, M. Suzuki and T. Masamune, *Tetrahedron*, **24**, 4193 (1968).

3) S. Yamamura and Y. Hirata, *ibid.*, **19**, 1485 (1963).

with ether, and the ethereal solution was shaken with a 5% potassium hydroxide solution and then with 1N hydrochloric acid to remove the acidic and basic components. After the removal of the solvent, a neutral, brown-colored oil (30 g) was obtained; it was subsequently chromatographed on silica gel.

Aplysin (I). Fractions eluted with 9 : 1 *n*-hexane - benzene left a crystalline substance upon the removal of the solvent. Recrystallization from methanol gave colorless crystals, mp 85—86°C; $[\alpha]_D$ −85° (*c*, 1.25; $CHCl_3$); UV, λ_{max}^{EtOH} 293, 232 mμ (ε 4400, 8100); IR, ν_{max}^{Nujol} 1610, 1580, 1474, 1393, 1380, 1263, 1230, 1100, 1005, 880, 860 cm^{-1}; NMR, τ 8.90 (3H, d, J=6 c/s), 8.75 (3H, s), 8.70 (3H, s), 7.68 (3H, s), 3.48 (1H, s), 2.95 (1H, s). (Found: C, 61.16; H, 6.41%).

The IR and NMR spectra were superimposable upon those of aplysin, and the R_f value on TLC and the mixed-melting-point were the same as those of an authentic sample.

Debromoaplysin (II). Fractions eluted with 7 : 3 *n*-hexane - benzene left an oily substance, which was then purified by rechromatography over silica gel to give a pure, colorless oil, $[\alpha]_D$ −63° (*c*, 1.15; $CHCl_3$); IR, ν_{max}^{film} 1620, 1595, 1500, 1280, 1265, 1120, 1010, 950, 800 cm^{-1}; NMR, τ 8.90 (3H, d, J=6 c/s), 8.75 (3H, s), 8.70 (3H, s), 7.72 (3H, s), 3.60 (1H, broad s), 3.50 (1H, broad d, J=7 c/s), 3.20 (1H, d, J=7 c/s). (Found: C, 82.98; H, 9.20%).

The IR and NMR spectra were superimposable upon those of debromoaplysin, to be described below.

Laurinterol (IV) and Debromolaurinterol (V). From fractions eluted with 1 : 1 *n*-hexane - benzene two compounds, IV, $C_{15}H_{19}OBr$, mp 54—55°C, and V, $C_{15}H_{20}O$ (an oil) were obtained and identified as laurinterol and debromolaurinterol respectively.

Aplysinol (III). Fractions eluted with benzene gave a mixture of alcohols. This mixture was then acetylated with acetic anhydride and pyridine at room temperature. The acetylated product was chromatographed on silica gel to give a pure, colorless oil (VII); $[\alpha]_D$ −26° (*c*, 0.85; $CHCl_3$); UV, λ_{max}^{EtOH} 292, 233 mμ (ε 2600, 5100); IR, ν_{max}^{film} 1750, 1615, 1585, 1490, 1230, 1050 cm^{-1}; NMR, τ 8.93 (3H, d, J=6 c/s), 8.63 (3H, s), 8.07 (3H, s), 7.74 (3H, s), 5.83 (2H, AB-q), 3.44 (1H, s), 2.95 (1H, s).

The acetate VII was saponified with 1.5N methanolic potassium hydroxide under reflux to give the original alcohol (III) in a good yield. Recrystallization from carbon tetrachloride gave colorless crystals, mp 157—159°C; $[\alpha]_D$ −54° (*c*, 0.90; $CHCl_3$); UV, λ_{max}^{EtOH} 292, 233 mμ (ε 5400, 10200); IR, ν_{max}^{Nujol} 3240, 1582, 1490, 1230, 1157, 1102, 1056, 998, 880, 857, 840, 790 cm^{-1}; NMR, τ 8.93 (3H, d, J=6 c/s), 8.54 (3H, s), 7.71 (3H, s), 6.28 (2H, m), 3.47 (1H, s), 2.98 (1H, s).

(Found: C, 57.67; H, 6.17%).

The IR and NMR spectra were superimposable upon those of aplysinol. Aplysinol (III) has also been isolated directly from the original alcoholic mixture by careful rechromatography.

Dibromo-compound (VI). The rechromatography of the afore-mentioned alcoholic mixture led to the isolation of crystals melting at 148.5—149°C (VI); IR, ν_{max}^{Nujol} 3500, 1252, 1080, 1050, 1000, 915, 896, 823, 786 cm^{-1}. Acetate (VIII), colorless crystals, mp 154—156°C (from CCl_4), $[\alpha]_D$ −44° (*c*, 0.75; $CHCl_3$); UV, λ_{max}^{EtOH} 290 mμ (ε 180); IR, ν_{max}^{Nujol} 1755, 1403, 1225, 1082, 1050, 1032, 1012, 942, 878, 828, 790 cm^{-1}; NMR, τ 8.82 (3H, s), 8.70 (3H, s), 8.54 (3H, s), 8.27 (3H, s), 7.86 (3H, s), 7.5—8.2 (2H, m), 7.05 (1H, s), 5.2—5.9 (2H, m), 4.71 (1H, s).

Found: C, 40.65; H, 4.67%. Calcd for $C_{16}H_{22}O_6Br_2$: C, 40.86; H, 4.72%.

The saponification of VIII gave VI in a good yield.

Debromoaplysin (II) from Debromolaurinterol (V). A solution of V (51 mg) and *p*-TsOH (25 mg) in glacial acetic acid (2 ml) was heated at 50°C for 25 hr. After being cooled, the reaction mixture was extracted with ether, and the ethereal solution was washed successively with water, a 5% sodium bicarbonate solution, and a saturated sodium chloride solution, and dried over sodium sulfate. The ether was then removed, and the residual oil was chromatographed over silica gel to give a pure, colorless oil (47 mg), $[\alpha]_D$ −61° (*c*, 0.74, $CHCl_3$); mass spectrum, *m/e* (rel. abund.) 216 (M^+), 202 (15), 201 (100), 187 (11), 173 (18), 160 (31). 159 (50), 145 (14), 119 (9), 91 (9), 77 (6), 55 (4), 43 (5), and 41 (8). The IR and NMR spectra were superimposable upon those of II, and the mass spectral data were identical with those of debromoaplysin reported in the literature.[3]

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