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STRUCTURES OF APLYSIN AND APLYSINOL, NATURALLY OCCURRING BROMO-COMPOUNDS

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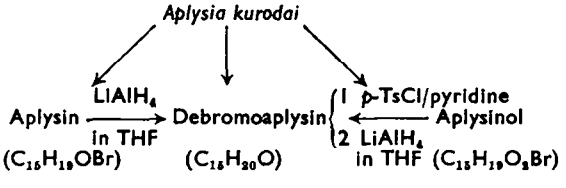
Abstract—The structures of aplysin and aplysinol, new sesquiterpenoid aromatic bromo-compounds isolated from *Aplysia kurodai*, are elucidated and biogenesis of aplysin, debromoaplysin and aplysinol is discussed.

ONLY a few naturally occurring bromo-compounds have so far been reported in the literature. Recently Tanaka and Toyama have reported that *Aplysia kurodai*, which is a kind of mollusca, contains several bromo compounds[1] but it is our experience that the kind and content of bromo-compounds in *Aplysia kurodai* depends on when and where they were gathered. We have isolated three new compounds, named aplysin, aplysinol and debromoaplysin, respectively.[2] It is the purpose of this paper to report the structures of aplysin, debromoaplysin and aplysinol, which may indicate the role of these substances in the metabolism of bromine.

Aplysin, debromoaplysin and aplysinol were isolated from *Aplysia kurodai*. As is shown by the scheme A, both aplysin and aplysinol are related to debromoaplysin. Reduction of aplysin with lithium aluminium hydride gives an oily product which was identified as debromoaplysin (II) obtained from *Aplysia kurodai*. The product showed a negative Beilstein test and the molecular ion peak at m/e 216 in its mass spectrum indicates the removal of a bromine atom from aplysin (m/e = 294) during reduction. On the other hand, aplysinol has a molecular formula, $C_{15}H_{19}O_2Br$, which involves one more oxygen atom than aplysin, $C_{15}H_{19}OBr$. Acetylation of aplysinol with acetic anhydride in pyridine yields aplysinol acetate, which shows no hydroxyl absorption, but a carbonyl band at 1732 cm^{-1} in its I.R. spectrum. Hydrolysis of aplysinol acetate yields aplysinol in a quantitative yield. The results of acetylation and hydrolysis indicate that aplysinol has one primary or secondary hydroxyl group. Aplysinol is resistant to tosylation with *p*-toluenesulfonyl chloride in pyridine at room temperature, but on the reaction at 55°, it yields a tosylate which affords debromoaply sin (II) by reduction with lithium aluminium hydride.

The I.R. and U.V. spectra of aplysin [$\nu_{max}^{CCl_4}$ 1620, 1586, 1486, 1277 and 1240 cm^{-1} (no hydroxyl band); λ_{max} 294 and 234 mμ (log ε 3·66 and 3·95)] suggest that aplysin

Scheme A

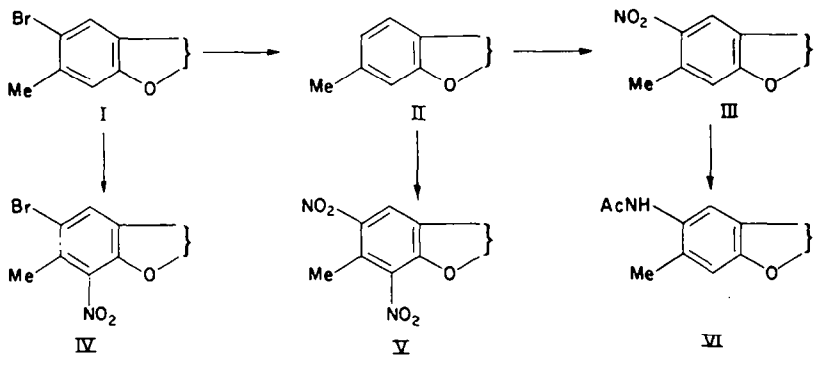


[1] T. Tanaka and Y. Toyama, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*) **80**, 1329 (1959).

[2] *Aplysia kurodai* were gathered at Hokkaido, Japan in September, 1962.

contains a phenolic ether group. The N.M.R. spectrum of the compound shows the presence of two isolated protons attached to an aromatic ring 3·48 (1H s) and 2·95 τ (1H s) and of four methyl groups, which are discussed later. The relative positions of substituents on the aromatic ring were determined by the following reactions. Aplysin was nitrated to mononitroaplysin (IV), the U.V. spectrum of which [λ_{max} 310, 255 (sh), 249 and 225–220 mμ (log ε 3·78, 3·90, 3·92 and 4·08–4·14, respectively)] indicates the presence of an *ortho-* or *meta*-nitroanisole-type chromophore.[3] The ease of nitration indicates that the nitro-compound (IV) has the former type of chromophore. Therefore, at least one of the two *ortho* positions to the ethereal oxygen of aplysin is unsubstituted. Nitration of debromoaplysin (II) under mild conditions yields the mononitro derivative (III). The U.V. spectrum of III [λ_{max} 322 and 239 mμ (log ε 4·01 and 3·90, respectively)] strongly indicates the presence of a typical *para*-nitroanisole-type chromophore.[3] Catalytic hydrogenation of III followed by acetylation yields N-acetylaminodebromoaplysin (VI), $C_{17}H_{23}O_2N$, [ν_{max} 1657 cm^{-1}; λ_{max} 290 and 236 mμ (log ε 3·59 and 3·91, respectively)]. Its U.V. spectrum is analogous to that of a aplysin.[4] The N.M.R. spectrum of III shows two isolated singlet peaks at 2·13 and 3·42 τ which are attributable to the aromatic proton vicinal to the nitro-group and an aromatic proton distant from the nitro-group, respectively[5,6] and also a singlet peak at 7·43 τ, which is attributable to a methyl group on the aromatic ring. The appearance of the methyl proton signal in considerably low magnetic field demands the presence of nitro

Scheme B



The U.V. spectrum of the *ortho-* or *meta*-compound, which has both electron-attracting group (—NO_2,—NO,—COR) and electron releasing groups (—OR, —OH, —NH_2, —NR_2) on the aromatic ring, shows three absorption maxima, the relative intensity of which decreases as the wave length increases. On the other hand, the *para*-isomer shows two absorption maxima. The intensity of the absorption maximum at longer wave length is stronger. Alkyl groups have no effect on the shape of U.V. spectrum except for the case of steric hindrance. These data were mainly gathered from M. J. Kamlet Ed., *Organic Electronic Spectral Data* Vol. I and II. Interscience, New York (1960).

[4] The N-acetylamino group has no influence on the chromophore of an aromatic ring.

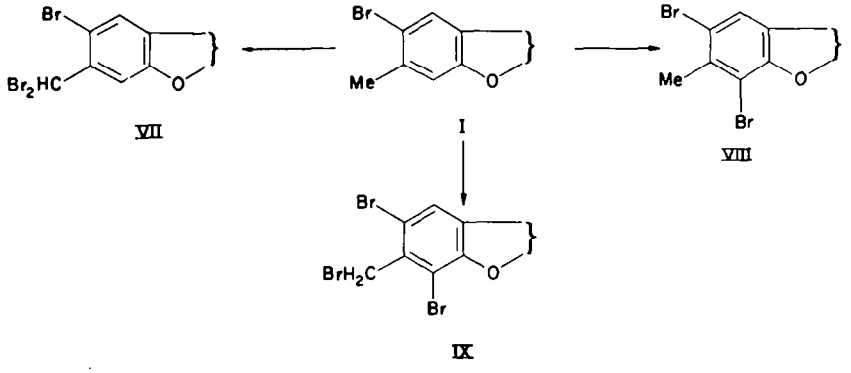
[5] L. M. Jackman, *Application of Magnetic Resonance Spectroscopy* p. 58 Pergamon Press, London (1959).

[6] Georg Van Dyke Tiers, *Characteristic Nuclear Magnetic Resonance* (N.M.R.) Schielding Values' (Spectral Positions) *For Hydrogen in Organic Structures* Table II. (1958); N. S. Bhacca, L. F. Johnson and J. N. Shoolery, "*N.M.R. Spectra Catalog*", Varian Associates, Palo Alto, California (1962).

group vicinal to the methyl group.[5] These nitration reactions indicate that the bromine atom and the methyl group on the aromatic ring should be in *para*- and *meta*-position to the ethereal phenolic oxygen, respectively, and that an *ortho*-position to the oxygen should be unsubstituted. This assumption is supported by the fact that nitration of II under vigorous conditions yields dinitrodebromoaplysin(V) ($C_{15}H_{18}N_2O_5$, ν_{max} 1513 and 1340 cm^{-1}). The N.M.R. spectrum of aplysin has a singlet peak at 7·68 τ (3H) indicative of a methyl group on the aromatic ring, which shifts to a lower magnetic field by 0·2 τ on the nitration.[5] This shift of the methyl proton signal indicates that the nitro group is located in the *ortho*-position to the methyl group.

Bromination of aplysin with N-bromosuccinimide in carbon tetrachloride yields dibromoaplysin (VII), $C_{15}H_{17}OBr_3$. Its N.M.R. spectrum has no peak in 7·6–5·0 τ region, while there are three peaks (3H) in 4·0–2·7 τ region, one of which is attributed to $ArCHBr_2$.[6] Bromination reactions with bromine in acetic acid and in chloroform yield monobromoaplysin (VIII) and isodibromoaplysin (IX), respectively. The N.M.R. spectrum of the former shows only a singlet peak (1H) in the 4·0–2·7 τ region. On the other hand, the latter has a singlet peak (1H) at 5·35 τ, which is attributed to $ArCH_2Br$, and also a singlet peak (1H) at 3·00 τ.[6,7] These bromination experiments indicate the absence of benzylic hydrogens except for a methyl group on the aromatic ring in aplysin. as shown by Scheme C. The only possibility for the relative positions of substituents on the aromatic ring in aplysin can be expressed by the partial structure I as shown in Scheme B.

Scheme C

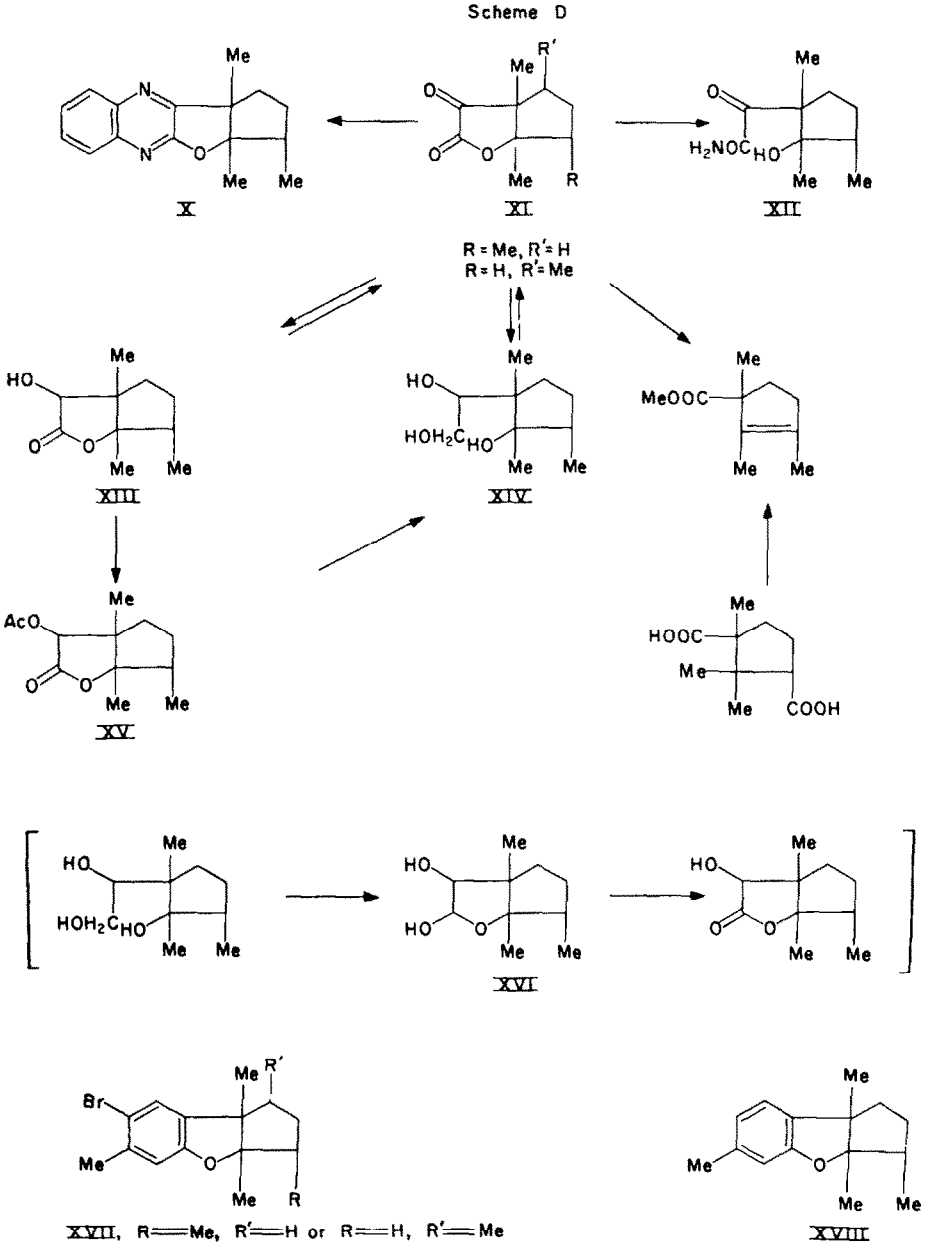


In the reaction with chromic trioxide in acetic acid, aplysin and debromoaplysin do not yield a benzoic acid derivative, but a lactone, apoaplysin (XI; $C_{10}H_{14}O_3$). On the other hand, mononitroaplysin is unaffected by the same reagent. These facts indicate that the aromatic ring in aplysin or debromoaplysin undergoes oxidative degradation, whereas the aromatic ring in mononitroaplysin is stabilized by the nitro group, and that both aplysin and debromoaplysin lack the benzylic hydrogen as suggested by the results of bromination. Apoaplysin (XI) shows two carbonyl bands at 1776 and 1765 cm^{-1}, but no absorption band in the region of 3000–5000 cm^{-1} in its I.R. spectrum. Condensation of apoaplysin (XI) with *o*-phenylenediamine affords a quinoxaline derivative (X). Treatment of apoaplysin (XI) with concentrated ammonia gives a α-keto acid amide (XII), which shows I.R. absorption at 1712 ($\nu_{C=O}$), 1647, 1617 (sh)

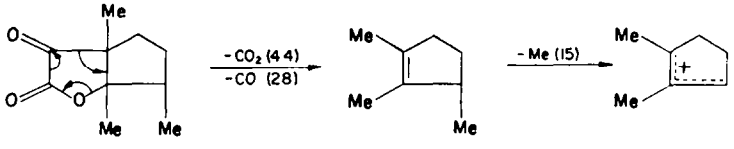
[7] M. Ohnishi, K. Nukada and A. Suzuki, *Absr. of Papers.* 1st symposium on *High Resolution Nuclear Magnetic Resonance* p. 62. Tokyo (1961).

and 1520 cm^{-1} (ν_{CONH_2}). These facts indicate the presence of α-keto-γ-lactone group in apoaplysin, which is further supported by the following reactions. Sodium borohydride reduces apoaplysin (XI) to an α-hydroxy lactone (XIII) (ν_{max} 1752 and 3400 (br) cm^{-1}), which is reversibly oxidized with chromic trioxide in acetic acid to the original apoaplysin in nearly quantitative yield. Furthermore, treatment of the α-hydroxy lactone (XIII) with acetic anhydride and pyridine affords an α-acetoxy

Scheme D

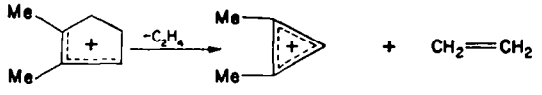


lactone (XV) which shows two carbonyl absorption bands in the I.R. spectrum at 1780 and 1745 cm^{-1} assigned to the γ-lactone and acetoxyl groups, respectively. Reduction of apoaplysin (XI) or α-acetoxy lactone (XV) with lithium aluminium hydride affords a triol (XIV), which may be oxidized to the original apoaplysin (XI) through an intermediate (XVI) under the same conditions as that of XIII. Formation of biacetyl beside apoaplysin in the oxidation of aplysin with potassium permanganate in acetic acid indicates the presence of vicinal methyl groups. Oxidation of debromoaplysin with concentrated nitric acid in a sealed tube at 170° gave succinic acid indicative of the presence of (—CH_2CH_2—) group. Therefore, apoaplysin should have the structural formula XI (R = Me, R′ = H or R = H, R′ = Me) based on the above experiments and its N.M.R. spectrum. The latter spectrum shows the presence of two tertiary and one secondary methyl group and the absence of (—CH—O—) group [τ-value 8·87 (3H d), 8·73 (3H s) and 8·56 (3H s); no proton signal in a lower magnetic field than 7·7 τ].[6] The behavior of aplysin on nitration, bromination and oxidation may be explained by postulating the structural formula XVII (R = Me, R′ = H or R = H, R′ = Me). On oxidation with hydrogen peroxide in alkaline alcoholic solution, and esterification with diazomethane followed by dehydration with thionyl chloride and pyridine, apoaplysin yields a small amount of an oily product, which was identified as 1,2,3-trimethyl-cyclopent-1-ene-3-carboxylic acid methylester by comparison of its I.R. spectrum with that of an authentic sample obtained from camphoric acid according to Ashan's method.[8,9] This fact shows that apoaplysin should be expressed as XI (R = Me and R′ = H). In the conversion of apoaplysin into 1,2,3-trimethyl-cyclopent-1-ene-3-carboxylic acid, isomerization of the double bond may be considered, but both rearrangement of the methyl group and a skeletal change in cyclopentane ring is unlikely. This is supported by its mass spectrum, which has no parent peak, but several remarkable peaks at m/e 110 (50), 95 (100), 67 (52), 44 (82) and 28 (83), which are assigned as follows.



XI (m/e 110) (m/e 95)

A peak at m/e 67 can be attributed to formation of a stable cyclopropenyl cation.[10,11] This mechanism is supported by the appearance of a metastable peak at m/e 47·3 (calc. 47·25).[12]



(m/e 95) (m/e 67)

[8] O. Ashan, *Ber. Dtsch. chem. Ges.* **27**, 3504 (1894).

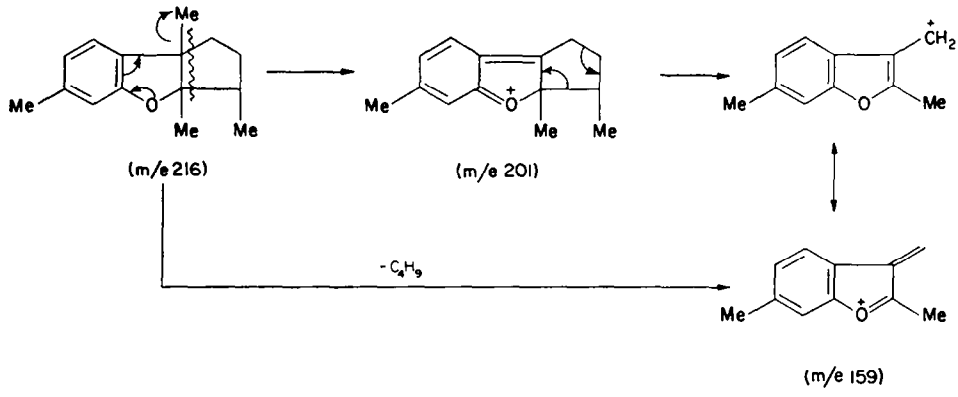
[9] G. S. Skinner, *J. Amer. Chem. Soc.* **45**, 1498 (1923).

[10] According to Huckel's (4n + 2) π-electron rule, this is the corresponding ion with only two π-electrons (n = 0).

[11] *Determination of Organic Structures by Physical Methods* (Edited by F. W. McLafferty in F. C. Nachod and W. D. Phillips) Vol. II, p. 137 Academic Press, New York (1962).

[12] K. Biemann, J. Seibl and F. Gapp, *J. Amer. Chem. Soc.* **83**, 3795 (1961).

The mass spectrum of debromoaplysin has no remarkable peak except for a few peaks at m/e 216 (34), 201 (100) and 159 (50), being consistent with the structure (XVIII).[13] Structures of aplysin and debromoaplysin must, therefore, be XVII (R = Me, R′ = H) and XVIII, respectively.



The proposed structure of aplysinol is based on the comparison of its spectral data [ν_{max} 1615, 1577, 1482, 1277 and 1240 cm^{-1}; λ_{max} 292 and 233 mμ (log ε 3·66 and 3·89, respectively); τ-value 8·93 (3H d), 8·57 (3H s), 7·70 (3H s), 6·22 (2H s), 3·49 (1H s) and 2·99 (1H s)] with that of aplysin. The I.R. and U.V. spectra suggest that aplysinol has the same carbon skeleton as aplysin. On the other hand, the distinct difference between the N.M.R. spectra of aplysinol and aplysin is observed in the higher magnetic field region. As described above, aplysin has four methyl groups, one attached to the aromatic ring (7·68 s), one secondary methyl group (8·91 d) and two tertiary methyl groups (8·75 s, 8·70 s). On the other hand, aplysinol has a singlet peak at 6·22 τ attributed to (—CH_2O—) group, instead of one tertiary methyl group.[6] Furthermore, a singlet peak at 8·57 τ attributed to a tertiary methyl group in aplysinol appears in considerably lower magnetic field region than the corresponding peak in aplysin, suggesting that the tertiary methyl group and —CH_2OH group are in a *cis* configuration to each other.[14] Aplysinol must therefore have the structure XIX or XX.

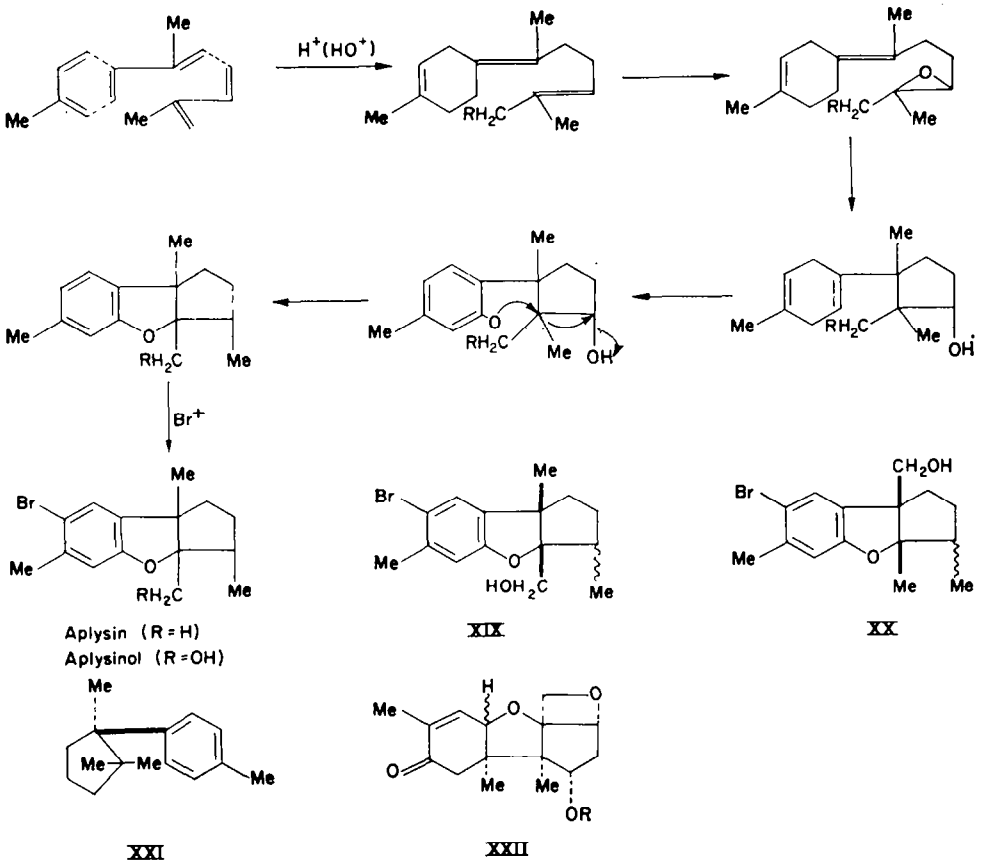
These natural products isolated from *Aplysia kurodai* have a novel type of sesquiterpene carbon skeleton. Their biogenesis is considered to be similar to that of cuparene (XXI) and trichothecin (XXII).[15,16] On the basis of biogenetic considerations the structure XIX seems to be favored, as shown by Scheme E. An alternative structure (XX) has, however, not been excluded, but if an attack of OH^+ occurs as the first step, XIX would be produced. The investigation on the stereochemistry of these natural bromo compounds and their synthesis is in progress and will be reported later.

[13] Any remarkable peak below m/e 159 could not be observed, because of the formation of a stable aromatic benzofuran.

[14] Y. Kawazoe, M. Natume, T. Okamoto, Y. Sato, H. Hasegawa and K. Tuda, *Abstr. of Papers*, 1st symposium on *High Resolution Nuclear Magnetic Resonance* p. 92. Tokyo (1961).

[15] C. Enzel and H. Erdtman, *Tetrahedron* **4**, 361 (1958).

[16] E. R. H. Jones and G. Lowe, *J. Chem. Soc.* 3959 (1960).



EXPERIMENTAL

M.ps. are uncorrected. U.V. spectra were obtained with a Beckman DK-2 spectrophotometer with an automatic recorder in absolute ethanol solution. I.R. spectra were taken with a Hilger H 800 double beam spectrophotometer with a rock salt prism as potassium bromide disk, unless stated otherwise. Optical rotations were measured in chloroform with a Rudolph and San polarimeter. N.M.R. spectra were measured with Nihondensi JNM-3 (40 Mc) and a Varian A-60 analytical N.M.R. spectrometer (60 Mc). The values are given in p.p.m. relative to tetramethylsilane as an internal reference (s: singlet; d: doublet; m: multiplet). Mass spectra were measured by Atlas Werke Co., Ltd, Germany.

Isolation of aplysin and debromoaplysin

Dried *Aplysia kurodai* (2 Kg) were repeatedly extracted with ether, and the extracts saponified with methanolic potassium hydroxide in the usual manner. After addition of water (2 l), unsaponified materials were extracted with ether and after evaporation of the solvent under red. press., the residue was dissolved in n-hexane (500 ml) and refluxed according to the method described by Tanaka and Toyama.[1] After separation of insoluble materials, the n-hexane solution was concentrated to about 100 ml and chromatographed on silica gel (350 g) (Wakojunyaku Co., Ltd.), and the fraction eluted with n-hexane was rechromatographed on alumina (20 g). The first fraction eluted with n-hexane gave 1.5 g debromoaplysin as an oil which was converted to the mononitro compound, mononitrodebromoaplysin, as pale yellow crystals, m.p. 107–108°. The second fraction eluted with benzene gave colorless crystals. Recrystallization from methanol gave 2·3 g aplysin, m.p. 85–86°,

$[\alpha]_D^{27}$ −85·4°, λ_{max} 294 and 234 mμ (log ε 3·66 and 3·95, respectively), τ-value 8·91 (3H d), 8·75 (3H s), 8·70 (3H s), 7·68 (3H s), 3·48 (1H s) and 2·95 (1H s) (in CCl_4), (Found: C, 61·17; H, 6·51; Br, 27·15; O, 5·88. $C_{15}H_{19}OBr$ requires: C, 61·02; H, 6·49; Br, 27·07; O, 5·42%).

Mass spectra of aplysin and debromoaplysin

Aplysin: 296 (47), 294 (47), 282 (14), 281 (100), 280 (14), 279 (99), 240 (19), 239 (39), 238 (19), 237 (34), 202 (19), 201 (51), 185 (13), 160 (24), 159 (16), 128 (10), 115 (16), 109 (10), 91 (10), 77 (10), 55 (10), 44 (10), 43 (13), and 41 (17) m/e.

Debromoaplysin: 216 (34), 202 (15), 201 (100), 187 (13), 173 (19), 160 (26), 159 (50), 145 (15), 91 (14), 55 (10), 43 (15), and 41 (15) m/e.

Isolation of aplysinol

As described above, the n-hexane solution which was concentrated to 100 ml, was chromatographed on silica gel (350 g). After the mixture of aplysin and debromoaplysin was eluted, elution with benzene–n-hexane (1:1) gave an oil, which was rechromatographed on alumina (15 g). Elution with ether-methanol (5:1) gave colorless crystals. Recrystallization from carbon tetrachloride gave 0·06 g aplysinol, m.p. 158–160°, $[\alpha]_D^{19}$ −55·6°, λ_{max} 292 and 233 mμ (log ε 3·60 and 3·89, respectively), τ-value 8·93 (3H d), 8·57 (3H s), 7·70 (3H s), 6·22 (2H s), 3·49 (1H s) and 2·99 (1H s) (in CS_2), (Found: C, 57·60; H, 6·17. $C_{15}H_{19}O_3Br$ requires: C, 57·89; H, 6·15%).

Acetylation of aplysinol and hydrolysis of aplysinol acetate

Aplysinol (15 mg) was added to a solution of acetic anhydride and pyridine (2 ml; 1:1). The mixture was allowed to stand at room temp. overnight, and the solvent removed under red. press. leaving an oily product (ν_{max} 1735 cm^{-1} no hydroxyl band in the region of 3000–5000 cm^{-1}), which was saponified with 2 N methanolic potassium hydroxide (2 ml) for 2 hr. under reflux on a water bath. After addition of a large amount water, the precipitate was collected. Recrystallization from carbon tetrachloride gave original aplysin (7 mg; mixed m.p. and I.R. spectrum).

Mononitrodebromoaplysin from debromoaplysin

A mixture of debromoaplysin (150 mg) and conc. nitric acid (2 ml) in acetic acid (2 ml) was refluxed on a water bath for 15 min and then cooled. Dilution with a large amount of water gave pale yellow crystals (95 mg), which were recrystallized from methanol to give mononitrodebromoaplysin, m.p. 107–108°. $[\alpha]_D^{26}$ −122·5°, ν_{max} 1516 and 1320 cm^{-1}, λ_{max} 322 and 239 mμ (log ε 4·01 and 3·90, respectively), τ-value 7·43 (3H s), 3·42 (1H s) and 2·13 (1H s) (in CCl_4), (Found: C, 69·08; H, 7·51; N, 5·75. $C_{15}H_{19}NO_3$ requires: C, 68·94; H, 7·33; N, 5·36%).

Mononitrodebromoaplysin from aplysin

Aplysin (150 mg) in dry tetrahydrofuran (10 ml) was added to lithium aluminum hydride (400 mg) in dry tetrahydrofuran (20 ml). The mixture was refluxed for 40 hr on a water bath. The unreacted hydride was decomposed with ethyl acetate. After acidification with dil. HCl the product was extracted with ether. The ethereal solution was evaporated under red. press. The residue was dissolved in benzene and chromatographed on alumina. Elution with benzene gave an oil, the I.R. spectrum of which was identical with that of debromoaplysin. The oil in acetic acid (2 ml) was treated with conc. nitric acid (2 ml) under the same condition as that for nitration of debromoaplysin. Recrystallization of the product from methanol gave 40 mg of mononitrodebromoaplysin (mixed m.p. and I.R. spectrum).

Conversion of aplysinol into mononitrodebromoaplysin

A suspension fo 25 mg of *p*-toluenesulfonyl chloride in 3 ml pyridine was added slowly to a stirred solution of aplysinol (30 mg) in 4 ml pyridine under cooling. After being stirred for 2 hr, the solution was allowed to stand at 55° overnight. The mixture was treated with ice-water, and the aqueous solution repeatedly extracted with ether. The ethereal solution was washed with water several times and evaporated. The crude product was dried completely and then dissolved in tetrahydrofuran (2 ml). An excess of lithium aluminum hydride (10 mg) was added to the solution, and the mixture was refluxed for 15 hr, and then poured into dil. hydrochloric acid. The product was extracted

with three 20 ml-portions of ether. The solvent was removed under red. press. to give debromoaplysin, which was nitrated with conc. nitric acid in acetic acid to mononitrodebromoaplysin (3 mg; mixed m.p. and I.R. spectrum).

Mononitroaplysin (IV)

A mixture of aplysin (100 mg) and conc. nitric acid (1·5 ml) in acetic acid (2 ml) was refluxed, addition of water (20 ml) gave pale yellow crystals (70 mg). Recrystallization from methanol gave mononitroaplysin (IV), m.p. 109–110°, $[\alpha]_D^{25}$ −60·2, ν_{max} 1515 and 1340 cm^{-1}, λ_{max} 310, 255 (sh), 249 and 225–220 mμ (log ε 3·78, 3·90, 3·92 and 4·10–4·14, respectively), τ-value 7·47 (3H s) and 2·99 (1H s) (in CCl_4), (Found: C, 53·08; H, 5·44; N, 4·41. $C_{15}H_{16}NO_3Br$ requires: C, 52·96; H, 5·33; N, 4·12%).

N-*Acetyl-aminodebromoaplysin* (VI)

A solution of mononitrodebromoaplysin (50 mg) in ethanol (20 ml) was hydrogenated at room temp under the atm. press. of hydrogen in the presence of Adams' catalyst (5 mg). After complete absorption of hydrogen the solution was filtered and the filtrate evaporated under red. press. Treatment of the residue with acetic anhydride (3 ml) and pyridine (3 ml) gave colorless crystals (20 mg). Recrystallization from methanol gave N-acetyl-aminodebromoaplysin (VI), m.p. 157–158°, $[\alpha]_D^{12}$ −72·6°, ν_{max} 1657 cm^{-1}, λ_{max} 290 and 236 mμ (log ε 3·59 and 3·91, respectively), (Found: C, 74·52; H, 8·66. $C_{17}H_{23}NO_3$ requires: C, 74·69; H, 8·48%).

Dinitrodebromoaplysin (V)

A suspension of debromoaplysin (100 mg) in conc. nitric acid (3 ml) was heated in a sealed tube at 140–145° for 6 hr, in the meantime the suspension disappeared, and the solution was poured into excess water to give a pale yellow precipitate. Crystallization from methanol gave 40 mg of dinitrodebromoaplysin (V), m.p. 123–125°, $\nu_{max}^{CHCl_3}$ 1513 and 1340 cm^{-1}, (Found: C, 58·97; H, 6·13. $C_{15}H_{18}N_2O_6$ requires: C, 58·81; H, 5·92%).

Bromination of aplysin with N-*bromosuccinimide*

A solution of aplysin (185 mg), N-bromosuccinimide (430 mg) and benzoyl peroxide (10 mg) in carbon tetrachloride (15 ml) was refluxed for 30 min. After addition of potassium acetate (2 g) in acetic acid (2 ml), the solution was boiled for 30 min and then poured into ice-water (about 50 ml), and extracted with ether. Evaporation of the solvent under red. press. gave an amorphous product, which was chromatographed on alumina. Elution with benzene gave colorless crystals (120 mg). Recrystallization from ethanol gave dibromoaplysin (VII), m.p. 165°, $[\alpha]_D^{27}$ −51·1°, λ_{max} 312 and 218 mμ (log ε 3·69 and 4·41, respectively), τ-value 3·81 (1H s), 3·45 (1H s) and 2·90 (1H s) (in CCl_4), (Found: C, 40·05; H, 3·77. $C_{15}H_{17}OBr_3$ requires: C, 39·77; H, 3·78%).

Monobromoaplysin (VIII)

An excess of bromine (100 mg) in acetic acid (10 ml) was added to a solution of aplysin (50 mg) in acetic acid (2 ml) with stirring, and reaction mixture allowed to stand at room temp overnight. The solvent was removed under red. press. and the residue dissolved in benzene chromatographed on alumina. Elution with benzene gave colorless crystals. Recrystallization from methanol gave 57 mg of monobromoaplysin (VIII), m.p. 65–67°, λ_{max} 302 (log ε 3·70), τ-value 7·67 (3H s) and 2·99 (1H s) (in CCl_4), (Found: C, 48·04; H, 4·99. $C_{15}H_{18}OBr_2$ requires: C, 48·13; H, 4·85%).

Isodibromoaplysin (IX)

A solution of bromine (100 mg) in chloroform (2 ml) was added to a solution of aplysin (100 mg) in chloroform (3 ml) and the mixture was allowed to stand at room temp overnight. On evaporation of the solvent under red. press., the solution gave colorless crystals (76 mg). Recrystallization from methanol gave isodibromoaplysin (IX), m.p. 140–143°, λ_{max} 307 and 219 mμ (log ε 3·65 and 4·51, respectively), τ-value 5·35 (2H s) and 3·00 (1H s) (in CCl_4), (Found: C, 39·86; H, 3·73. $C_{15}H_{17}OBr_3$ requires: C, 39·77; H, 3·78%).

Oxidation of aplysin with chromic trioxide

Aplysin (200 mg) in acetic acid (3 ml) was added dropwise to a solution of chromic trioxide (250 mg) in 85% acetic acid (1 ml), and the mixture was allowed to stand at 40° overnight. After addition of a large amount of water, the solution was extracted with chloroform and the solvent evaporated on a water bath. The residue was chromatographed on silica gel (1 g). Elution with benzene–ether (5:1) gave colorless crystals (25 mg). Recrystallization from methanol gave colorless crystals of *apoaplysin*, m.p. 163–164°, $[\alpha]_D^{26}$ −90°, $\nu_{max}^{CHCl_3}$ 1776 and 1765 cm^{-1}, τ-value 8·87 (3H d), 8·73 (3H s), 8·56 (3H s) and 8·27–7·70 (5H m) (in $CHCl_3$), m/e 111 (21), 110 (50), 95 (100), 79 (14), 77 (14), 69 (15), 67 (52), 55 (45), 53 (23), 51 (10), 47·3 (metastable peak), 44 (82), 43 (61), 41 (69), 39 (47), 29 (23), 28 (83), and 27 (41), (Found: C, 65·81; H, 7·72. $C_{10}H_{14}O_3$ requires: C, 65·91; H, 7·74%).

Ozonolysis of aplysin

Aplysin (250 mg) in acetic acid (15 ml) was ozonized at room temp for 6 hr, and then the solvent removed under red. press. After decomposition of the ozonide with a large amount of water, the product was extracted with chloroform, and the solvent evaporated on a water-bath. Crystallization from methanol gave 20 mg apoaplysin (mixed m.p. and I.R. spectrum).

Oxidation of aplysin with potassium permanganate

To a solution of aplysin (300 mg) in acetic acid (20 ml) was added dropwise a solution of potassium permanganate (1·5 g) in minimum amount of water at room temp over a period of 30 min, and after being heated at 80–85° on a water bath for 1 hr the solution was allowed to stand at room temp overnight. The unreacted potassium permanganate was decomposed with alcohol, and after acidification with 2 N HCl, the solution was subjected to steam distillation, which was stopped in 30 min. The distillate was poured into a saturated solution of 2,4-dinitrophenylhydrazine in 2 N HCl. (A) The first precipitate was collected, and washed well with water. The dried products were chromatographed on silica gel, and elution with benzene–ether (5:1) gave yellow crystals (20 mg). Recrystallization from ethanol gave apoaplysin 2,4-*dinitrophenylhydrazone*, m.p. 210–211°, ν_{max} 1765 cm^{-1}, τ-value 8·87 (3H d), 8·57 (3H s) and 8·41 (3H s) (in $CHCl_3$), (Found: C, 52·91; H, 4·86; N. 15·32. $C_{16}H_{18}N_4O_6$ requires: C, 53·03; H, 5·01; N, 15·46%). (B) The second precipitate was treated in usual manner to give 6 mg of diacetyl-2,4-dinitrophenylhydrazone (m.p. and I.R. spectrum). The residue of the steam distillation was extracted with chloroform, and the solvent was evaporated on a water bath. The residue was chromatographed on a silica gel and elution with benzene–ether (5:1) gave 10 mg of apoaplysin (mixed m.p. and I.R. spectrum).

Quinoxaline derivative (X)

A mixture of apoaplysin (10 mg) and o-phenylenediamine (4 mg) was refluxed for 2 hr under nitrogen. After cooling, chloroform (50 ml) was added and the chloroform solution was washed with 2 N HCl and with water, and dried (Na_2SO_4). Evaporation of the solvent gave an amorphous product, which could not be crystallized, but formation of quinoxaline derivative was confirmed by I.R. and U.V. spectra (ν_{max} 1660, 1640, 1605, 1577 and 1500 cm^{-1}; λ_{max} 346 (sh), 331, 315 (sh), 331, 315 (sh), 251, 247 and 226 mμ).

The reaction apoaplysin with conc. ammonia

To a solution of apoaplysin (15 mg) in ethanol (2 ml) was added conc. ammonia (1 ml) and the solution was allowed to stand at room temp for 2 days, and then the solvent removed under red. press. Crystallization of the residue from ethanol gave 5 mg of α-keto acid amide, m.p. 173–174°. The elementary analysis was not carried out, but its I.R. spectrum (ν_{max} 1712, 1647, 1617, (sh) and 1520 cm^{-1}) showed the presence of carbonyl and amide groups.

Reduction of apoaplysin with sodium borohydride

A mixture of apoaplysin (15 mg) and sodium borohydride (10 mg) in tetrahydrofuran (3 ml) was refluxed on a water bath for 10 min, and then allowed to stand at room temp for 1 hr. The solution was poured into dil. HCl and extracted with ether. The solvent was evaporated on a water bath to give 9 mg of colorless needles. Recrystallization from carbon tetrachloride gave α-hydroxyapoaplysin, m.p. 84–87°, ν_{max} 1752 and 3400 (br) cm^{-1}. The elementary analysis was not carried out, but the product was used directly for the next experiment.

Conversion of α-hydroxy apoaplysin into apoaplysin

A solution of α-hydroxy apoaplysin (10 mg) in acetic acid (1 ml) was treated with chromic trioxide (15 mg) in 85% acetic acid (1 ml) under the same reaction condition as that of aplysin. Recrystallization of the product from carbon tetrachloride gave 4 mg of apoaplysin (mixed m.p. and I.R. spectrum). The formation of apoaplysin from α-hydroxyapoaplysin suggests that the carbon skeleton has not been changed during the oxidation and reduction.

Acetylation of α-hydroxy apoaplysin

α-Hydroxyapoaplysin (7 mg) was added to a solution of acetic anhydride and pyridine (1:1; 1 ml). The mixture was allowed to stand at room temp overnight, and then the unreacted reagent was removed under red. press. to give colorless crystals. Recrystallization from carbon tetrachloride gave 3 mg of *α-acetoxyapoaplysin*, m.p. 98–99°, ν_{max} 1780 and 1745 cm^{-1}, (Found: C, 63·50; H, 7·70. $C_{18}H_{18}O_4$ requires: C, 63·70; H, 8·02%).

Reduction of α-acetoxyapoaplysin with lithium aluminum hydride

To a solution of α-acetoxyapoaplysin (2 mg) in dry tetrahydrofuran (1 ml) was added an excess of lithium aluminum hydride (10 mg) in dry tetrahydrofuran (1 ml). The mixture was allowed to stand at room temp for 3 hr, and then worked up in the usual manner. Recrystallization of the product from carbon tetrachloride gave colorless crystals of trihydroxyapoaplysin (about 0·5 mg), m.p. 99–101°, the I.R. spectrum (ν_{max} 3460, 3266 and 3100 (br) cm^{-1}, and no carbonyl band in the region of 1800–1600 cm^{-1}) of which shows the presence of more than two hydroxyl groups. This compound was converted into apoaplysin with chromic trioxide, as described below.

Reduction of apoaplysin with lithium aluminum hydride and reconversion of the product into the starting substance with chromic trioxide

(1) Apoaplysin (15 mg) in dry tetrahydrofuran (2 ml) was treated with excess of lithium aluminum hydride (20 ml) in dry tetrahydrofuran (2 ml) under the same reaction condition as that of α-acetoxyapoaplysin. Recrystallization from carbon tetrachloride gave colorless crystals, which were identical with trihydroxyapoaplysin, the derivative from α-acetoxyapoaplysin (mixed m.p. and I.R. spectrum), yield 3 mg

(2) Trihydroxyapoaplysin (5 mg) was treated with chromic trioxide under the same oxidation condition as that of α-hydroxyapoaplysin to give apoaplysin (about 2 mg; mixed m.p. and I.R. spectrum).

Oxidation of debromoaplysin with conc. nitric acid

Debromoaplysin (50 mg) in conc. nitric acid (3 ml) was heated in a sealed tube at 160–170° for 24 hr, and then the solution was extracted with a large amount of ether. The solvent was evaporated on a water-bath. The residue was washed with a small amount of ether 3 times to give 2 mg pure succinic acid (m.p. and I.R. spectrum).

Formation of 1,2,3,-trimethyl-cyclopent-1-ene-3-carboxylic acid

Hydrogen peroxide (30%; 1 ml) was added to a solution of apoaplysin (45 mg) in 10% methanolic potassium hydroxide (2 ml) with stirring and then the solution was allowed to stand at 55° for 48 hr. After acidification with dil. HCl, the solution was repeatedly extracted with ether. The solvent was evaporated on a water bath. The residue was dissolved in 2 ml dry ether, to which an excess of diazomethane in ether was added dropwise. The ethereal solution was allowed to stand at room temp for 1 hr, and then the solvent was evaporated on a water bath. The methyl ester was obtained as a colorless oil (17 mg) which was used directly for the next experiment. Thionyl chloride (1 ml) was added dropwise to a stirred mixture of methyl ester and dry pyridine (1 ml) with cooling. After addition was complete, the mixture was allowed to stand at 55° for 3 hr, and then cooled to room temp. The solution was poured into ice water. The aqueous solution was extracted fully with ether. The ethereal solution was washed with water, and dried (Na_2SO_4). The solvent was evaporated on a water bath to give an oil, which was dissolved in benzene and chromatographed on silica gel (500 mg).

Elution with benzene–ether (10: 1) gave a colorless oil (about 4 mg), ν_{max}^{film} 1728, 1265, 1159 and 1102 cm^{-1}, which was identified as 1,2,3-trimethylcyclopent-1-ene-3-carboxylic acid methyl ester by comparision of its I.R. spectrum with that of an authentic sample obtained from camphoric acid (Wakojunyaku Co., Ltd.) according to Ashan's method.[8,9]

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