Aeroplysinin-1, an Antibacterial Bromo-compound from the Sponge *Verongia aerophoba*

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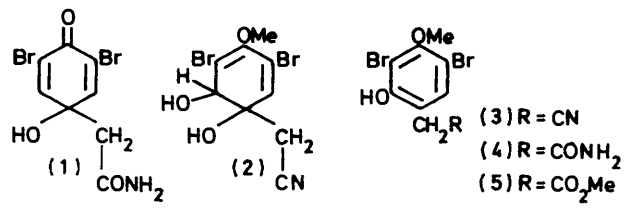
Aeroplysinin-1, an antibacterial bromo-compound from the sponge *Verongia aerophoba*, is the first example of a naturally occurring 1,2-dihydroarene-1,2-diol.

Verongia (= *Aplysina*) *aerophoba*, a marine sponge common in the Mediterranean sea, has not been chemically investigated until now apart from some observations on its yellow pigments.[1]

From methanolic extracts of fresh tissues we have now isolated four bromo-compounds; one (yield *ca.* 3%) proved to be the amide (1) previously isolated from *Verongia cauliformis*,[2] the other three being new compounds which we describe in this and the following paper.

The dibromo-compound, aeroplysinin-1, $C_9H_9Br_2NO_3$, m.p. 120°, $[\alpha]_D$ +186°, shows antibacterial activity against *Staphylococcus albus*, *Bacillus cereus*, and *B. subtilis*.* It shows $\lambda_{max.}$ (MeOH) 284 nm and contains hydroxy (3380 cm^{-1}) and nitrile (2265 cm^{-1}) groups. The n.m.r. spectrum indicates the presence of a methylene group (δ 2·74 p.p.m., s, 2H), a methoxy-group (δ 3·74 p.p.m., s, 3H), a >C=CH– system (δ 6·34 p.p.m., s, 1H), and a



>CHOH group attached to two tertiary carbon atoms (δ 4·16 p.p.m., ill-defined multiplet, 2H; the signal quenches to a sharp one-proton singlet at δ 4·10 p.p.m. on deuterium exchange), the remaining signal at δ 2·28 p.p.m. (s, 1H), which is eliminated on deuterium exchange, must be assigned to a tertiary hydroxy-group. The presence of a secondary and a tertiary hydroxy-group is further supported by conversion of aeroplysinin-1 into a diacetate, $C_{13}H_{13}Br_2NO_5$, whose n.m.r. spectrum shows a downfield shift (2·09 p.p.m.) of the >C*H*OAc signal relative to that of the parent compound.

On treatment of the diacetate in methanol with cold aqueous potassium hydroxide, or aeroplysinin-1 with hot alkali, a crystalline compound, $C_9H_7Br_2NO_2$, m.p. 158° (decomp.), was obtained. The following spectral data [$\lambda_{max.}$ (MeOH) 252, 292, and 312 nm; $\lambda_{max.}$ (MeOH–NaOH) 252 and 312 nm; $\nu_{max.}$ (Nujol) 3365 (OH) and 2260 (CN) cm^{-1}; δ ($CDCl_3$) singlets at 3·70 (2H, CH_2), 3·88 (3H, OMe), 5·87 (1H, exchangeable with D_2O, OH), and 7·53 p.p.m. (1H, ArH)] suggest that this compound may be a dibromo-hydroxy-methoxyphenylacetonitrile. This was established by treatment with concentrated sulphuric acid which gave an amide $C_9H_9Br_2NO_3$, m.p. 166°, converted by hydrolysis with boiling 6N-hydrochloric acid into 3,5-dibromo-2-hydroxy-4-methoxyphenylacetic acid identified as the methyl ester (5), m.p. 71°, by comparison with an authentic sample synthesised by bromination of methyl 2-hydroxy-4-methoxyphenylacetate.

The structure of the phenolic nitrile is, therefore, (3) and, as this is derived from aeroplysinin-1 by loss of a molecule of water, the natural compound must be (2) in agreement with all the evidence.

Six months after this work was published as a preliminary communication,[3] Fulmor *et al.*[4a] reported the

* Assays carried out at the laboratories of Gruppo Lepetit (Torre Annunziata, Naples).

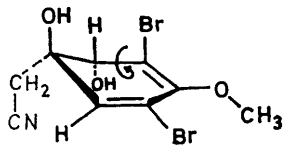
[1] C. F. W. Krukenberg, *Vergl. Physical. Studien. Inst. Heidelberg*, 1882, **2**; A. A. Christomanos, *Prakt. Akad. Athenon*, 1957, **32**, 433.

[2] G. M. Sharma and P. R. Burkholder, *J. Antibiotics, Ser. A (Japan)*, 1967, **20**, 200; *Tetrahedron Letters*, 1967, 4147.

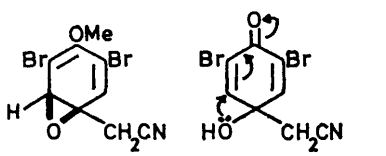
[3] E. Fattorusso, L. Minale, and G. Sodano, *Chem. Comm.*, 1970, 751.

[4] (*a*) W. Fulmor, G. E. Van Lear, G. O. Morton, and R. D. Mills, *Tetrahedron Letters*, 1970, 4551; (*b*) D. B. Cosulich and F. M. Lovell, *Chem. Comm.*, 1971, 397.

occurrence of the enantiomorphic (—)-isomer of aeroplysinin-1 in the Caribbean sponge *Ianthella ardis*, to which they assigned the absolute configuration (6) on the basis of the c.d. curve. This was later confirmed by *X*-ray crystallographic analysis.[4b]



(6)



(7) (8) (9) * = · OR +

The glycol (2) is the first example of a naturally occurring 1,2-dihydroarene-1,2-diol and it could be formed by hydrolysis of an arene oxide [5] in agreement with the stereochemistry (6). On this hypothesis the precursor of (2) would be (7), the epoxide oxygen being derived from the atmosphere. It has been suggested that this may be a general path for the epoxidation and hydroxylation of aromatic compounds *in vivo*.[5] However, the co-existence of (2) and (1) suggests that an alternative biogenetic route may be utilised by *V. aerophoba* in which the epoxide ring is derived from a tertiary hydroxy-group by nucleophilic attack on an enone system. On this basis the precursor of (2) would be (8) and the epoxide oxygen may originate from water. This could be a general path for the epoxidation and hydroxylation of phenols (in contrast to hydrocarbons), oxygen being introduced initially by reaction of a radical or, more probably, a cation (9) with water.

EXPERIMENTAL

Sponges [*Verongia* (= *Aplysina*) *aerophoba*], collected in the bay of Naples, were obtained from the supply department of the Zoological Station, Naples.

Isolation of Aeroplysinin-1 (2).—Fresh sponge (120 g, dry weight after extraction) was extracted four times with acetone at room temperature for 3 days; after concentration the aqueous residue was extracted with ether (4 × 375 ml). The combined ethereal extracts were taken to dryness and the gummy mass (25 g) was treated successively with light petroleum (b.p. 40—70°) and ether. The ether-soluble material (3·5 g) was chromatographed on a column of silica gel (250 g; Merck) to give, by elution with chloroform–ether (1 : 1), a crude product (1·8 g) which later crystallised. Recrystallisation from chloroform yielded *aeroplysinin-1* (2), m.p. 120—121° (1·4 g), $[\alpha]_D$ +186° (MeOH); $\lambda_{max.}$ (MeOH) 231 and 284 nm (ε 3220 and 4915); $\nu_{max.}$ (Nujol) 3380, 2265, 1635, and 1585 cm^{-1}; δ (CD_3CN) 2·28 (1H, s, t-OH), 2·74 (2H, s, CH_2), 3·70 (3H, s, OMe), 4·10 (2H, bm, >CHOH), and 6·34 p.p.m. (1H, s, CH=C); *m/e* 341, 339, 337 (M^+); 323, 321, 319 ($M^+ - H_2O$); 240, 242 ($M^+ - H_2O - Br$; base peak) (Found: C, 31·65; H, 2·5; N, 4·1. Calc. for $C_9H_9Br_2NO_3$: C, 31·85; H, 2·65; N, 4·15%). Treatment with acetic anhydride in cold pyridine gave the *diacetate*, which crystallised from benzene–light petroleum (b.p. 80—100°), m.p. 114°, $[\alpha]_D$ +218° ($CHCl_3$); $\lambda_{max.}$ (MeOH) 230 and 284 nm (ε 3905 and 5094); $\nu_{max.}$ ($CHCl_3$) 2250, 1750, 1630, 1585, and 1220 cm^{-1}; δ ($CDCl_3$) 2·10 and 2·23 (each 3H, s, CH_3CO_2), 3·10 (2H, s, CH_2), 3·78 (3H, s, OMe), 6·25 (1H, s, >CH–OAc), and 6·58 p.p.m. (1H, s, CH=C); *m/e* 425, 423, 421 (M^+); 365, 363, 361 ($M^+ - CH_3CO_2H$), 323, 321, 319 ($M^+ - CH_3CO_2H - CH_2=C=O$; base peak) (Found: C, 36·5; H, 3·05; N, 3·1. Calc. for $C_{13}H_{13}Br_2NO_5$: C, 36·9; H, 3·05; N, 3·3%).

The residue insoluble in ether (20 g) was chromatographed on silica gel (800 g; Merck) in chloroform–methanol yielding homoaerothionin and aerothionin (see the following paper) and (1) (3·5 g) which was crystallised from acetone, m.p. 192—194° (lit.,[2] 193—195°); M^+ at *m/e* 327, 325, and 323; u.v., i.r., and n.m.r. spectra were identical to those reported by Sharma and Burkholder.[2]

Conversion of Aeroplysinin-1 (2) *and its Diacetate into 3,5-Dibromo-2-hydroxy-4-methoxyphenylacetonitrile* (3).—To a solution of the diacetate of (2) (200 mg) in methanol (15 ml), 0·15N-potassium hydroxide (15 ml) was added and the solution was stirred for 3 h at room temperature. After acidification with 2N-hydrochloric acid, the solution was extracted with ether (3 × 100 ml). The combined ether extracts were evaporated to dryness and the residue was crystallised from light petroleum (b.p. 80—100°) to give the *nitrile* (3), m.p. 158° (decomp.) (130 mg), $\lambda_{max.}$ (MeOH) 252, 292, and 312 nm (ε 4984, 2300, and 1790); $\lambda_{max.}$ (MeOH–NaOH) 252 and 312 nm (ε 8460 and 3610); $\nu_{max.}$ (Nujol) 3365 and 2260 cm^{-1}; δ ($CDCl_3$) 3·70 (2H, s, CH_2), 3·88 (3H, s, OMe), 5·87 (1H, s, exchangeable with D_2O, OH), and 7·53 p.p.m. (1H, s, ArH) (Found: C, 33·45; H, 2·15; N, 4·25. Calc. for $C_9H_7Br_2NO_2$: C, 33·65; H, 2·2; N, 4·35%).

To aeroplysinin-1 (200 mg) in methanol (15 ml), 0·15N-potassium hydroxide (15 ml) was added and the solution was refluxed for 2 h. Work-up as above afforded (3) (125 mg).

3,5-Dibromo-2-hydroxy-4-methoxyphenylacetamide (4).—A solution of (3) (200 mg) in concentrated sulphuric acid (1·5 ml) was heated for 6 min at 100°. The mixture was poured onto ice–water and the precipitate was crystallised from chloroform to give the *amide* (4), m.p. 166—167° (160 mg), $\lambda_{max.}$ (MeOH) 251, 292, and 312 nm (ε 1596, 2474, and 478); $\nu_{max.}$ (Nujol) 3405, 3350, 3180, and 1670 cm^{-1}; δ [$(CD_3)_2CO$] 2·81 (2H, b, NH_2), 3·66 (2H, s, CH_2), 3·82 (3H, s, OMe), 7·27 (1H, s, ArH), and 7·70 p.p.m. (1H, b, exchangeable with D_2O, OH); *m/e* 341, 339, 337 (M^+), 324, 322, 320 (base peak, $M^+ - NH_3$), 297, 295, 293 ($M^+ - CONH_2$) (Found: C, 31·5; H, 2·55; N, 4·1. Calc. for $C_9H_9Br_2NO_3$: C, 31·85; H, 2·65; N, 4·1%).

Methyl 3,5-Dibromo-2-hydroxy-4-methoxyphenylacetate (5).—The amide (120 mg) in 6N-hydrochloric acid (5 ml) was refluxed for 1 h, cooled, diluted with water, and extracted with ether. After removal of the solvent the residual acid was treated with methanolic hydrogen chloride (20 ml) for 12 h at room temperature to give the *methyl ester* (5), which was crystallised from light petroleum (b.p. 40—70°), m.p. 70—71° (80 mg), identical (mixed m.p., i.r., u.v., n.m.r.) with an authentic sample.

[5] D. M. Jerina, J. W. Daly, B. Witkop, P. Zaltzman-Nirenberg, and S. Undenfriend, *Arch. Biochem. Biophys.*, 1968, **128**, 176; *J. Amer. Chem. Soc.*, 1968, **90**, 6523, 6525; D. M. Jerina, H. Ziffer, and J. W. Daly, *J. Amer. Chem. Soc.*, 1970, **92**, 1056.

The ester (5) was synthesised by adding bromine (250 mg in 5 ml of acetic acid) slowly (1·5 h), with stirring, at room temperature to a solution of methyl 2-hydroxy-4-methoxyphenylacetate [6] (196 mg) in acetic acid (5 ml). The solution was taken to dryness to give (5), which was crystallised from light petroleum (b.p. 40—70°), m.p. 70—71° (180 mg), $\lambda_{max.}$ (MeOH) 252, 292, and 313 nm (ε 1430, 2324, and 178); $\nu_{max.}$ (Nujol) 3450, 1730, and 1600 cm^{-1}; δ ($CDCl_3$) 361 (2H, s, CH_2), 3·71 (3H, s, CO_2Me), 3·86 (3H, s, OMe), 6·33 (1H, b, exchangeable with D_2O, OH), and 7·33 p.p.m. (1H, s, ArH) (Found: C, 33·9; H, 2·8. Calc. for $C_{10}H_{10}Br_2O_4$: C, 34·0; H, 2·8%).

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[6] S. Gripenberg and B. Juselius, *Acta Chem. Scand.*, 1954, **8**, 734.