Aerothionin and Homoaerothionin: Two Tetrabromo Spirocyclohexadienylisoxazoles from *Verongia* Sponges

By **K. Moody** and **R. H. Thomson,*** Department of Chemistry, University of Aberdeen, Old Aberdeen AB9 2UE, Scotland
E. Fattorusso, L. Minale, and **G. Sodano,** Laboratorio per la Chimica e Fisica di Molecole di Interesse Biologico, C.N.R., Arco Felice, Naples, Italy

Aerothionin and homoaerothionin, tetrabromo-compounds from the sponges *V. thiona* and *V. aerophoba* are shown to be the homologous spirocyclohexadienylisoxazoles (XIII) and (XIV), respectively.

Most natural organobromo-compounds are of marine origin, found especially in algae [1] and sponges.[2-6] Several relatively simple dibromo-compounds have been isolated from *Verongia* spp.[2-4] and we describe here two more complex tetrabromo-metabolites [5] from *V. thiona* and *V. aerophoba* (=*Aplysina aerophoba*).

The major component in both species (10% in *V. aerophoba*), aerothionin, $C_{24}H_{26}Br_4N_4O_8$, is optically active, and shows $\lambda_{max.}$ 284 nm (cisoid diene) and $\nu_{max.}$ 3335, 1660, and 1550 cm^{-1} (secondary amide). Doublets (J 8 Hz) at δ 4·18 and 5·37 p.p.m. in the n.m.r. spectrum indicate the presence of an isolated secondary alcohol function and this was confirmed by D_2O exchange when the hydroxy-signal at δ 5·37 p.p.m. disappeared and the doublet at δ 4·18 p.p.m. collapsed to a singlet, and also by the downfield shift of the methine proton signal to δ 5·83 p.p.m. on acetylation (diacetate, ν_{CO} 1744 cm^{-1}). The symmetrical structure of aerothionin is reflected in the simplicity of its n.m.r. spectrum, which shows also singlets for olefinic and methoxy-protons at δ 6·50 and 3·72 p.p.m., respectively, and the amide proton as a broad triplet at δ 7·58 p.p.m. An isolated methylene group in an asymmetric environment is indicated by an AB quartet with line positions at δ 3·84 and 3·14 p.p.m., while the large geminal coupling constant (J 18 Hz) suggests that the group is adjacent to a π-electron system.[7] A multiplet at δ 3·34 p.p.m., assigned to a methylene group attached to nitrogen, is coupled to another methylene multiplet at δ 1·60 p.p.m. which, from its chemical shift, must be linked only to saturated carbon. This suggests that a C_4 saturated chain is present and hints at the dimeric nature of aerothionin. In confirmation addition of deuterium chloride eliminated the N–H signal at δ 7·58 p.p.m. and simplified the *N*-methylene resonance at δ 3·34 p.p.m., while coupling between the signals at δ 3·34 and 1·60 p.p.m. was demonstrated by double irradiation.

The observation from t.l.c. that treatment of aerothionin with an excess of trimethylsilyl chloride or dihydropyran gives initially both mono- and di-derivatives suggests that two alcoholic functions are present, and that the number of protons is twice the simplest ratio indicated by the n.m.r. spectrum. This was established by mass spectrometry of a rearrangement product (see below) but the mass spectra of aerothionin and its di-tetrahydropyranyl, di-trimethylsilyl and di-acetyl derivatives were not particularly useful as molecular ions were absent. However many of the fragment ions occurred as multiplets, the relative intensity of which was diagnostic for the presence of bromine. Chemical evidence indicated that bromine was attached in either aromatic or vinylic form as the C–Br linkage in aerothionin was stable to attempted oxidation by dimethyl sulphoxide at 70 °C, and was unaffected by boiling aqueous methanolic potassium hydroxide.

Thus the combined evidence shows that aerothionin

[1] J. H. Hodgkin, J. S. Craigie, and A. G. McInnes, *Canad. J. Chem.*, 1966, **44**, 74; T. Irie, M. Suzuki, E. Kurosawa, and T. Masamune, *Tetrahedron*, 1970, **26**, 3277, and previous papers.
[2] G. M. Sharma and P. R. Burkholder, *Tetrahedron Letters*, 1967, 4147.
[3] G. M. Sharma, B. Vig, and P. R. Burkholder, *J. Org. Chem.*, 1970, **35**, 2823.
[4] E. Fattorusso, L. Minale, and G. Sodano, *Chem. Comm.*, 1970, 751.
[5] E. Fattorusso, L. Minale, G. Sodano, K. Moody, and R. H. Thomson, *Chem. Comm.*, 1970, 752.
[6] G. M. Sharma and P. R. Burkholder, *Chem. Comm.*, 1971, 151.
[7] M. Barfield and D. M. Grant, *J. Amer. Chem. Soc.*, 1963, **85**, 1899.

has the following functional groups; 2 OMe, 2 >CHOH, 2 CH=C, 2 $>CH_2$, 4 Br, and a CONH·$[CH_2]_4$·NHCO unit. Two CNO groups remain unidentified. Mild basic treatment of aerothionin converts it quantitatively into an isomeric optically inactive, dihydric phenol ($\lambda_{max.}$ 292 nm). The n.m.r. spectrum of this product is very similar to that of aerothionin except that the >CHOH resonances are absent, olefinic proton absorption is replaced by an aromatic proton singlet at lower field (δ 7·59 p.p.m.), and the isolated methylene group is now benzylic and appears as a singlet at δ 3·82 p.p.m. The CONH·$[CH_2]_4$·NHCO unit is still present so that two CHNO moieties are not yet accounted for; as the compound forms a tetra-acetate (ν_{CO} 1797 and 1760 cm^{-1}) CHNO must contain OH and is therefore an oxime, >C=NOH. The phenol was also characterised by its tetramethyl and tetrabenzyl ethers. Unlike the other derivatives of aerothionin, the mass spectrum of the tetramethyl derivative showed a molecular ion, a quintet centred at *m/e* 874 with relative intensities, 1 : 4 : 6 : 4 : 1, indicative of a tetrabromo-compound. Accurate mass measurement established the formula $C_{28}H_{34}Br_4N_4O_8$, and the structure of the phenol may now be represented by (I). The n.m.r. spectra of these methyl and benzyl ethers were in agreement with those of the model compounds (II; R = Me and $PhCH_2$).

The relative positions of the aromatic substituents in (I) were established by degradative experiments. Oxidation of the tetramethyl derivative of (I) with potassium permanganate in aqueous acetone gave an acid, which, on the basis of its n.m.r. spectrum and i.r. bands at 1675 and 1705 cm^{-1} appeared to be the glyoxylic acid (III); synthesis of an authentic sample, however, from ethyl 2,4-dihydroxyphenylglyoxylate showed that this was not the structure. The acid was subsequently found to be the benzoic acid (IV) identical with material prepared by oxidative decarboxylation of (III) with alkaline hydrogen peroxide. The presence of two carbonyl bands in the i.r. spectrum of the acid (IV) in Nujol mull is probably associated with two different orientations of the carboxy-group in the crystal structure.

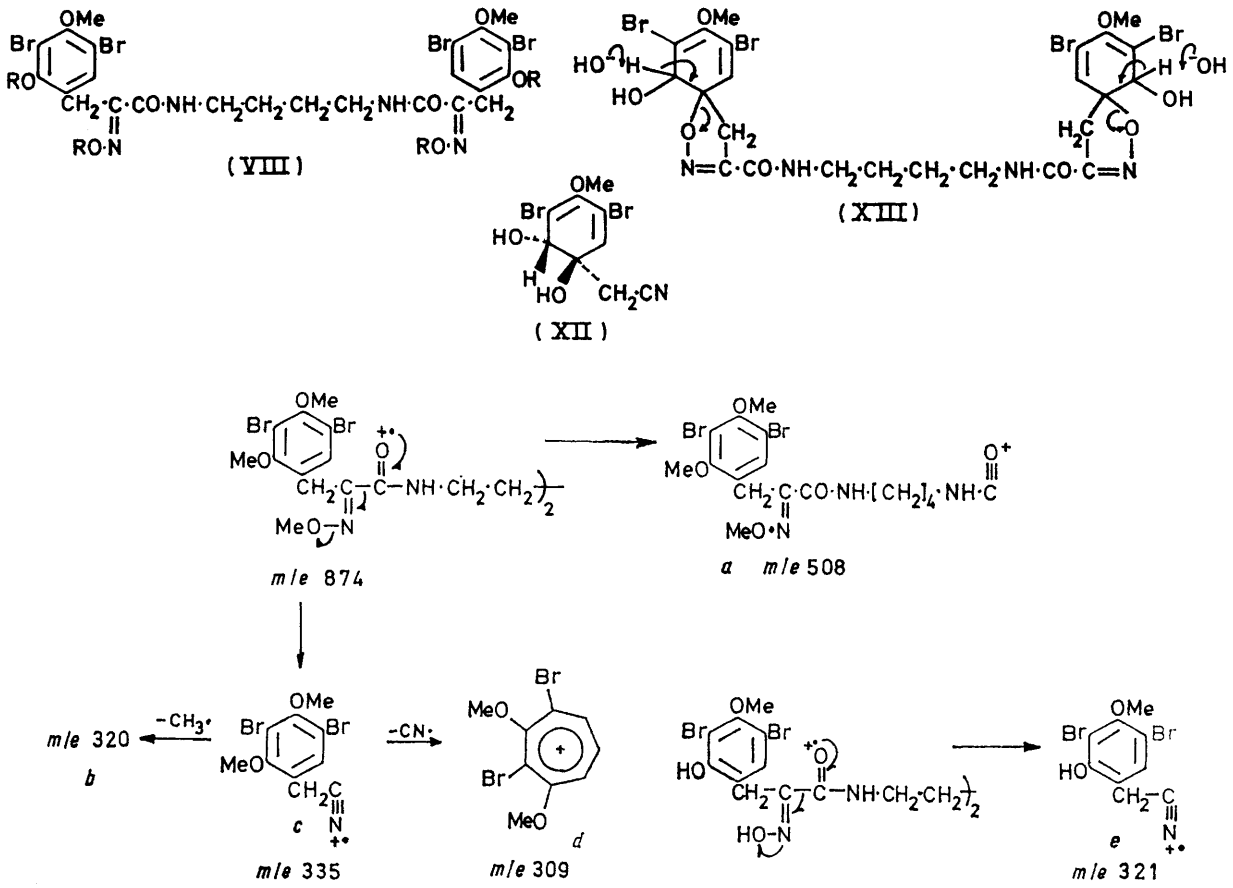
Hydrolysis of the tetramethyl ether of (I) with 25% aqueous methanolic potassium hydroxide yielded the oximinopyruvic acid (V; R = Me). This structure agrees with the spectroscopic properties of the acid and its methyl ester, and the acid was synthesised from methoxyamine and the arylpyruvic acid (VI; R = Me), derived [8] from 3,5-dibromo-2,4-dimethoxybenzaldehyde and *N*-acetylglycine by way of the acetamidocinnamic acid (VII; R = Me). The structure of the tetramethyl ether of (I) can now be defined as (VIII; R = Me). Supporting evidence comes from the mass spectrum which shows prominent ions at *m/e* 508, 335, 320, and 309 (all triplets) suggesting the fragmentation pattern below [the ions (*b*), (*c*), and (*d*) also give significant peaks in the mass spectrum of (V; R = Me)]. The structure (VIII; R = Me) was finally confirmed by synthesis from the acid chloride of (V; R = Me) and 1,4-diaminobutane.

The position of the phenolic groups in (I) was established by hydrolysis with 6M-hydrochloric acid, which gave the arylacetic acid (IX) (characterised as its methyl ester by comparison with a synthetic sample), and also the coumarin (X; R = OMe) (after methylation with diazomethane). These products establish that the structure of (I) is (VIII; R = H) with the phenolic groups *ortho* to the side-chain. One attempt to synthesise (VIII; R = H) from (V; R = CH_2Ph), on a very small scale, was unsuccessful. In another approach, condensation of the appropriate benzaldehyde with the amide (XI) could not be achieved (some experiments on the benzylation of 3,5-dibromo-2-hydroxy-4-methoxybenzaldehyde are described in the Experimental section), and in the reaction of 2-acetoxy-3,5-dibromo-4-methoxybenzaldehyde with *N*-acetylglycine, the coumarin (X; R = NHAc) was obtained instead of the expected acetamidocinnamic acid (VII; R = Ac).

It will be recalled that the transformation of aerothionin into (VIII; R = H) is accompanied by aromatisation, the formation of benzylic methylene groups, and the conversion of two secondary alcohol functions into

[8] *Org. Synth.*, Coll. Vol. II, pp. 1 and 519.

phenolic groups. Bearing in mind the structure and properties of aeroplysinin-1 (XII),[4] a co-metabolite in *V. aerophoba*, which has very similar u.v. and (where relevant) n.m.r. spectra, it is clear that aerothionin must have structure (XIII). This is in complete agreement with all the evidence and its rearrangement to the phenol (VIII; R = H) is straightforward (XIII, arrows). The mass spectrum of aerothionin shows an intense peak at *m/e* 321, also present in the spectrum of the phenol



(VIII; R = H), which may be attributed to the ion (*e*). By analogy with aeroplysinin-1 (XII) [4] and its enantiomer,[9] the hydroxy-groups are probably *trans* to the ring oxygens, and as aerothionin is optically active the asymmetric end units must be identical and not in a mirror-image relationship.

A very closely-related compound, homoaerothionin,[10] found in smaller quantity in both *V. thiona* and *V. aerophoba* would not crystallise and was purified as its diacetate. (Aerothionin crystallises fairly easily but retains solvents tenaciously.) Like aerothionin, homoaerothionin could be aromatised in base to give a phenol very similar to (VIII; R = H) which, in turn, could be degraded with acid to (IX) and (X; R = OMe) (after methylation). The difference between the two compounds can best be seen in the n.m.r. spectra of the crystalline diacetates which are virtually the same except that the high-field multiplet at δ 1·3—1·9 p.p.m. integrates for *six* protons. Thus the central part of the homoaerothionin molecule (XIV) is a pentamethylene chain, and this was confirmed by synthesis of the tetramethyl ether (XV; R = Me) of the derived phenol by condensing 1,5-diaminopentane with the acid chloride of (V).

It seems very probable that 3,5-dibromotyrosine [2] is a precursor of all the *Verongia* metabolites (XII), (XIII), (XIV), (XVI),[2] and (XVII),[3] and presumably the central C_4N_2 and C_5N_2 chains of aerothionin and homoaerothionin are derived from ornithine and lysine, respectively. (Both dibromotyrosine and lysine have been found in sponge protein.[11]) The spirocyclohexadienylisoxazoline systems in (XIII) and (XIV) could arise in various ways including nucleophilic attack by an oxime function in a modified tyrosinyl unit on an arene oxide [12] (XVIII), or by conversion of the latter into a phenol

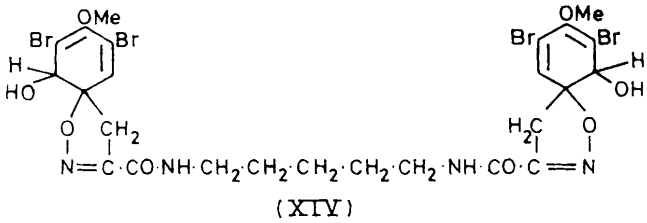
[9] W. Fulmor, G. E. Van Lear, G. O. Morton, and R. D. Mills, *Tetrahedron Letters*, 1970, 4551; D. B. Cosulich and F. M. Lovell, *Chem. Comm.*, 1971, 397.

[10] E. Fattorusso, L. Minale, K. Moody, G. Sodano, and R. H. Thomson, *Gazzetta*, 1971, **101**, 61.

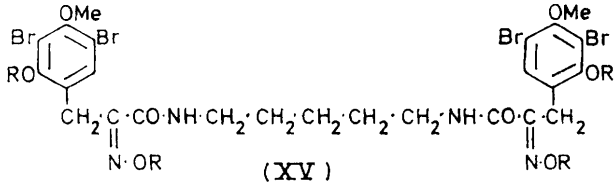
[11] D. Ackermann and E. Müller, *Z. Physiol. Chem.*, 1941, **269**, 146; E. M. Low, *J. Marine Research*, 1951, **10**, 239.

[12] D. M. Jerina, H. Ziffer, and J. W. Daly, *J. Amer. Chem. Soc.*, 1970, **92**, 1056, and references therein; J. E. Baldwin, H. H. Basson, and H. Krauss, *Chem. Comm.*, 1968, 984.

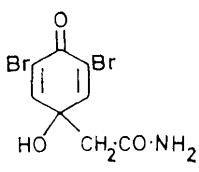
followed by intramolecular phenol-oxime coupling. It has been suggested [13] that nitriles may be derived *in vivo* from α-amino-acids by way of α-keto- and α-oximino-acids, and there is experimental support, both *in*



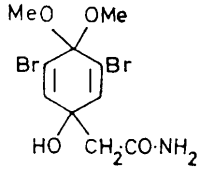
(XIV)



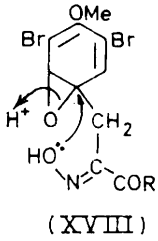
(XV)



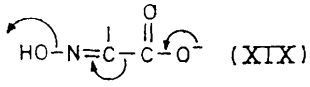
(XVI)



(XVII)



(XVIII)



(XIX)

vitro [13,14] and *in vivo*,[15] for the last step. Thus (XVIII; R = OH) seems a likely precursor of the nitrile (XII) [16] as indicated by (XIX).

EXPERIMENTAL

Extraction of Verongia thiona.—Dried sponge (1160 g), from La Jolla, California, was digested with cold acetone (6 l) for 3 days, filtered, and the extract taken to dryness. The oily residue (35·6 g) was redissolved in a small volume of cold acetone, and the solution was filtered and evaporated. Crystallisation of the crude product from chloroform gave *aerothionin* (XIII) (15·1 g, 1·3% dry wt. of sponge), which was recrystallised from ethyl acetate and then, for analysis, from acetone–benzene to give plates, m.p. 134—137° (decomp.), $[\alpha]_D$ +252° (acetone) [Found: C, 35·4; H, 2·9; Br, 39·2; N, 7·1%; *M* (osmometric), 844. $C_{24}H_{26}Br_4N_4O_8$ requires C, 35·2; H, 3·2; Br, 39·1; N, 6·9%; *M*, 817·6], $\lambda_{max.}$ (EtOH) 234 and 284 nm (log ε 4·16 and 4·13); $\nu_{max.}$ (Nujol) 3335, 3160, 1675, 1660, 1580, and 1550 cm^{-1}; δ [100 MHz, ($^2H_6$-acetone)] 7·58 (2H, bt, N*H*CH_2), 6·50 (2H, s, >C=C*H*), 5·37 (2H, d, *J* 8 Hz, OH), 4·18 (2H, d, *J* 8 Hz, >C*H*OH), 3·72 (6H, s, OCH_3), 3·84 and 3·14 (each 2H, d, *J* 18 Hz, CH_2), 3·34 (4H, m, NHCH_2), and 1·60 p.p.m. (4H, m, NHCH_2CH_2). The *diacetate*, prepared with acetic anhydride in cold pyridine, formed needles, m.p. 206—208° (from acetone), $[\alpha]_D$ +236° ($CHCl_3$) (Found: C, 37·5; H, 3·3; Br, 35·6; N, 6·1. $C_{28}H_{30}Br_4N_4O_{10}$ requires C, 37·3; H, 3·4; Br, 35·4; N, 6·2%), $\nu_{max.}$ (Nujol) 3335, 1744, 1652, and 1540 cm^{-1}; δ (60 MHz, $CDCl_3$) 6·67 (2H, bt, N*H*CH_2), 6·28 (2H, s, >C=C*H*), 5·83 (2H, s, >C*H*OAc), 3·75 (6H, s, OCH_3), 3·41 and 3·06 (each 2H, d, *J* 18·5 Hz, CH_2), 3·35 (4H, m, NHCH_2), 2·13 (6H, s, CH_3CO), and 1·63 p.p.m. (4H, m, NHCH_2CH_2).

The chloroform mother liquor from the initial crystallisation was diluted with light petroleum which precipitated a mixture of bromo-compounds (5·47 g) leaving sterols and fatty acids in solution. The former were separated (p.l.c.) on silica gel in ethyl acetate–benzene (1 : 1) to give more aerothionin, and *homoaerothionin* (XIV) as a gum (0·91 g) (two other products were isolated as gums but were not obtained pure). Homoaerothionin was precipitated from acetone solution with light petroleum as an amorphous solid (0·77 g); δ [$CDCl_3$–($^2H_6$-DMSO)] 7·60 (2H, b, N*H*CH_2), 6·28 (2H, s, >C=C*H*), 4·16 (2H, s, >C*H*OH), 3·73 (6H, s, OCH_3), 3·87 and 3·02 (each 2H, d, *J* 18·5 Hz, CH_2), 3·35 (4H, m, NHCH_2), and 1·60 p.p.m. (6H, m, $CH_2CH_2CH_2CH_2$-CH_2). The *diacetate*, purified by p.l.c. on silica gel in ethyl acetate–benzene (2 : 3), crystallised from ethanol as nodules, m.p. 166—167°, $[\alpha]_D$ +191·5° ($CHCl_3$) (Found: C, 38·4; H, 3·5; N, 5·9. $C_{29}H_{32}Br_4N_4O_{10}$ requires C, 38·0; H, 2·5; N, 6·1%), $\lambda_{max.}$ (MeOH) 289 nm (log ε 4·13); $\nu_{max.}$ (Nujol) 3310, 1740, 1658, and 1540 cm^{-1}; δ ($CDCl_3$) 6·60 (2H, b, N*H*CH_2), 6·30 (2H, s, >C=C*H*), 5·80 (2H, s, >C*H*OAc), 3·75 (6H, s, OCH_3), 3·41 and 3·07 (each 2H, d, *J* 18·5 Hz, CH_2), 3·30 (4H, m, NHCH_2), 2·13 (6H, s, CH_3CO), and 1·63 p.p.m. (6H, m, $CH_2CH_2CH_2CH_2CH_2$).

Extraction of V. aerophoba.—The ether-insoluble fraction (20 g) (see preceding paper) was chromatographed on a silica-gel column in chloroform–methanol, 500-ml fractions being collected. The crude product (12·2 g) from fractions 10—13 was crystallised from acetone–chloroform to give aerothionin, melting range, 130—150° (gas evolution), containing 1 mol chloroform (δ 7·25 p.p.m.) (Found: C, 32·0; H, 2·8; N, 6·15; O, 13·75, OMe, 6·3. $C_{24}H_{26}Br_4N_4O_6$,$CHCl_3$ requires C, 32·0; H, 2·9; N, 6·0; O, 13·65, OMe, 6·6%). The mother liquors were evaporated and the residue (2·1 g) was combined with the homoaerothionin (300 mg) from fractions 6—9 and converted into the diacetate. Fractions 15—21 afforded the amide (XVI) (3·5 g), m.p. 192—194° (lit.,[2] 193—195°) (from acetone) spectroscopically identical with literature data.[2]

Alkaline Treatment of Aerothionin and Homoaerothionin.—(*a*) Aerothionin (0·6 g) was boiled on a steam-bath with 3% methanolic potassium hydroxide (15 ml) and water (4·5 ml) for 2 h. After evaporation of the methanol, the aqueous solution was diluted to *ca.* 40 ml and acidified with acetic acid. The precipitated *oximinophenol* (VIII; R = H) (0·6 g) was chromatographically pure, and crystallised from ether–chloroform as prisms, m.p. 188·5—189·5° (Found: C, 34·9; H, 3·1; Br, 39·1; N, 7·1. $C_{24}H_{26}Br_4N_4O_8$ requires C, 35·2; H, 3·2; Br, 39·1; N, 6·9%). A sample crystallised from acetone–chloroform had m.p. 182—187° and contained 1 mol chloroform (Found: C, 31·5; H, 2·7;

[13] B. B. Stowe, *Prog. Chem. Org. Nat. Prods.*, 1959, **17**, 248; A. Ahmad and I. D. Spenser, *Canad. J. Chem.*, 1960, **38**, 1625; 1961, **39**, 1340.

[14] L. Bouveault and R. Locquin, *Bull. Soc. Chim. France*, 1904, [3], **31**, 1142; K. L. Waters, *Chem. Revs.*, 1947, **41**, 585.

[15] B. A. Tapper, E. E. Conn, and G. W. Butler, *Arch. Biochem. Biophys.*, 1967, **119**, 593.

[16] K. R. Hargreaves, A. McGookin, and A. Robertson, *J. Appl. Chem.*, 1958, **8**, 273.

N, 5·8; O, 13·4. $C_{24}H_{26}Br_4N_4O_8,CHCl_3$ requires C, 32·0; H, 2·9; N, 6·0; O, 13·65%), $\lambda_{max.}$ (EtOH) 292 nm (log ε 3·39), $\nu_{max.}$ (Nujol) 3340, 3200, 3070, 1647, 1616, and 1551 cm^{-1}; δ ($^2H_6$-acetone) 10·93 (2H, b, OH *), 7·99 (2H, m, $NHCH_2$), 7·59 (2H, s, ArH), 3·82 (10H, s, $ArCH_2$ and OCH_3), 3·40 (4H, m, $NHCH_2$), and 1·62 p.p.m. (4H, m, $NHCH_2CH_2$). The *tetra-acetate* crystallised from ether–benzene as prisms, m.p. 140—141° (Found: C, 39·5; H, 3·6; Br, 32·8; N, 5·8. $C_{32}H_{34}Br_4N_4O_{12}$ requires C, 39·0; H, 3·5; Br, 32·4; N, 5·7%), $\nu_{max.}$ (Nujol) 3365, 1797, 1760, 1675, 1623, and 1530 cm^{-1}; δ ($CDCl_3$) 7·48 (2H, s, ArH), 7·00 (2H, m, $NHCH_2$), 3·87 (4H, s, $ArCH_2$), 3·85 (6H, s, OCH_3), 3·37 (4H, m, $NHCH_2$), 2·33 and 2·18 (each 6H, s, CH_3CO), and 1·59 p.p.m. (4H, m, $NHCH_2CH_2$). The *tetramethyl derivative* (prepared with methyl iodide and silver oxide in boiling chloroform) crystallised from benzene–light petroleum (b.p. 60—80°) as needles, m.p. 128—129° [Found: C, 38·1; H, 4·1; Br, 36·7; N, 6·3%; *M*, 871·9107 (1 : 4 : 6 : 4 : 1 quintet). $C_{28}H_{34}{}^{79}Br_3{}^{81}BrN_4O_8$ requires C, 38·5; H, 3·9; Br, 36·6; N, 6·4%; *M*, 871·9092], $\nu_{max.}$ (Nujol) 3310, 1650, and 1536 cm^{-1}; δ ($CDCl_3$) 7·15 (2H, s, ArH), 6·75 (2H, b, $NHCH_2$), 3·88 (4H, s, $ArCH_2$), 3·94, 3·84, and 3·82 (each 6H, s, OCH_3), 3·35 (4H, m, $NHCH_2$), and 1·59 p.p.m. (4H, m, $NHCH_2CH_2$). The *tetrabenzyl derivative* [prepared by stirring the phenol (102 mg), benzyl chloride (0·5 g), sodium iodide (0·6 g), and anhydrous potassium carbonate (1 g) in dimethylformamide (2 ml) for 22 h] formed micro-crystals, m.p. 124·5—125·5° (from ethanol) (Found: C, 53·3; H, 4·5; Br, 27·2; N, 4·8. $C_{52}H_{50}Br_4N_4O_8$ requires C, 53·0; H, 4·3; Br, 27·1; N, 4·8%); $\nu_{max.}$ (Nujol) 3410, 1682, and 1500 cm^{-1}; δ ($CDCl_3$) 7·00—7·70 (22H, m, ArH), 6·70 (2H, b, $NHCH_2$), 5·13 (4H, s, $ArCH_2ON=$), 4·99 (4H, s, $PhCH_2OC\leqslant$), 3·94 (4H, s, $ArCH_2-C\leqslant$), 3·86 (6H, s, OCH_3), 3·20 (4H, m, $NHCH_2$), and 1·50 p.p.m. (4H, m, $NHCH_2CH_2$).

(*b*) Crude homoaerothionin (180 mg) was treated as for aerothionin for 1 h, and acidified with 4M-hydrochloric acid. The precipitate (144 mg) was purified by p.l.c. on silica gel in ethyl acetate–benzene (1 : 1) to give the *phenol* (XV; R = H) as a gum which formed micro-crystals, m.p. 136·5—138° (from chloroform) (Found: C, 35·9; H, 3·6; N, 6·55. $C_{25}H_{28}Br_4N_4O_8$ requires C, 36·1; H, 3·5; N, 6·7%), $\lambda_{max.}$ (MeOH) 292 nm (log ε 3·79), $\nu_{max.}$ (Nujol) 3310, 1647, 1616, and 1551 cm^{-1}; δ ($^2H_6$-acetone) 11·00 (2H, b, OH), 7·90 (2H, b, $NHCH_2$), 7·59 (2H, s, ArH), 3·81 (10H, $ArCH_2$ and OCH_3), 3·38 (4H, m, $NHCH_2$), and 1·60 p.p.m. (6H, m, $CH_2CH_2CH_2CH_2CH_2$). The *tetramethyl derivative* was prepared from the crude phenol with methyl iodide and silver oxide in boiling chloroform, and purified by t.l.c. on silica gel in ethyl acetate–benzene (1 : 4). It separated from methanol as nodules, melting range 32—39° (Found: *M*, 883·9250. $C_{29}H_{36}H_4{}^{79}Br_4O_8$ requires *M*, 883·9268), $\nu_{max.}$ ($CHCl_3$) 3410, 1670, and 1523 cm^{-1}; δ ($CDCl_3$) 7·20 (2H, s, ArH), 6·77 (2H, bt, $NHCH_2$), 3·92 (4H, s, $ArCH_2$), 3·96, 3·87, and 3·85 (each 6H, s, OCH_3), 3·35 (4H, m, $NHCH_2$), and 1·63 p.p.m. (6H, m, $CH_2CH_2CH_2CH_2CH_2$).

Hydrolysis of the Phenols (VIII; R = H) *and* (XV; R = H).—(*a*) The phenol (VIII; R = H) (0·5 g) was suspended in 6M-hydrochloric acid (30 ml); the mixture was heated under reflux for 4 h, and then cooled, diluted with water (120 ml), and extracted with ether (3 × 150 ml). After removal of the solvent the residual oil (200 mg) was divided into two equal portions.

The first portion was methylated with diazomethane in methanol in the usual way, followed by t.l.c. on silica gel plates in benzene. The product obtained from the band with R_F 0·2 was eluted with acetone and crystallised from carbon tetrachloride to give the *coumarin* (X; R = OMe), m.p. 210—211° (50 mg) (Found: C, 35·9; H, 2·05; O, 17·8. $C_{11}H_8Br_2O_4$ requires C, 36·25; H, 2·2; O, 17·6%), $\lambda_{max.}$ (MeOH) 286, 298, and 318 nm (log ε 4·07, 4·08, and 4·00); $\nu_{max.}$ (Nujol) 1740 and 1630 cm^{-1}; δ ($CDCl_3$) 7·56 (1H, s, ArH), 6·69 (1H, s, CH=C), and 3·93 p.p.m. (6H, ds, OCH_3, split into well separated singlets by addition of C_6D_6).

The second portion was kept in methanol–hydrogen chloride for 16 h at room temperature. After removal of solvent the residue was run on silica-gel plates in chloroform and the band with R_F 0·6 was eluted with acetone and crystallised from light petroleum (b.p. 40—70°) to give the methyl ester of (IX) (5 mg) identical with authentic material (see preceding paper).

Similar acid hydrolysis of the phenol (XV; R = H) (60 mg) gave (X; R = OMe) (identified by mixed m.p. and u.v. and i.r. spectroscopy) and the methyl ester of (IX) (identified by t.l.c. and g.l.c.).

Degradation of the Tetramethyl Derivative (VIII; R = Me). —(*a*) Compound (VIII; R = Me) (150 mg) was oxidised with potassium permanganate (4 g) in boiling 50% aqueous acetone (50 ml) for 3½ h. The manganese dioxide was filtered off, and washed with aqueous sodium hydrogen carbonate and acetone. After evaporation of the acetone, the combined filtrates were extracted with chloroform (recovered starting material, 67 mg), concentrated to *ca.* 10 ml and acidified with 4M-hydrochloric acid. The precipitate (37 mg) crystallised from chloroform to give 3,5-*dibromo*-2,4-*dimethoxybenzoic acid* as needles, m.p. 193° (Found: C, 31·9; H, 2·2; Br, 47·2; *M*, 337·8786. $C_9H_8{}^{79}Br_2O_4$ requires C, 31·8; H, 2·35; Br, 47·0%; *M*, 337·8791), $\nu_{max.}$ (Nujol) 3240—2060br, 1705, and 1675 cm^{-1}, δ [$CDCl_3$–($^2H_6$-acetone)] 8·15 (1H, s, ArH), 8·01 (1H, b, CO_2H), and 3·95 p.p.m. (6H, s, OCH_3).

(*b*) Compound (VIII; R = Me) (400 mg) was heated under reflux in methanol (7 ml) and water (1 ml) containing potassium hydroxide (2 g) for 21 h. After evaporation of the methanol, the solution was extracted with chloroform and acidified. The acidic product was taken into chloroform, evaporated, and esterified with excess of ethereal diazomethane. Preparative t.l.c. on silica gel in benzene then yielded methyl 3-(3,5-*dibromo*-2,4-*dimethoxyphenyl*)-2-*methoximinopropionate* (134 mg, 34%) which was distilled *in vacuo* and eventually crystallised, m.p. 64° (Found: C, 36·7; H, 3·5; Br, 37·9; N, 2·9%; *M*, 424·9301. $C_{13}H_{15}{}^{79}Br{}^{81}BrNO_5$ requires C, 36·7; H, 3·6; Br, 37·6; N, 3·3%; *M*, 424·9298), $\nu_{max.}$ (Nujol) 1724 cm^{-1}; δ ($CDCl_3$) 7·19 (1H, s, ArH), 3·92 (2H, s, $ArCH_2$), 4·07 (3H, s, OCH_3), and 3·85 (6H, s, OCH_3). Hydrolysis of the ester with M-sodium hydroxide in 50% aqueous methanol gave the *acid* (V; R = Me), as prisms, m.p. 104—104·5° (from benzene–light petroleum) (Found: C, 35·2; H, 3·2; Br, 38·8; N, 3·4%; *M*, 408·9159. $C_{12}H_{13}{}^{79}Br_2NO_5$ requires C, 35·1; H, 3·2; Br, 38·9; N, 3·4%; *M*, 408·9162), $\nu_{max.}$ (Nujol) 3340—2200br and 1700 cm^{-1}; δ ($CDCl_3$) 7·82 (1H, b, CO_2H), 7·22 (1H, s, ArH), 3·94 (2H, s, $ArCH_2$), 4·12, 3·91, and 3·90 p.p.m. (each 3H, s, OCH_3).

Synthetic Compounds

3,5-*Dibromo*-2,4-*dimethoxybenzoic Acid* (IV).—Bromine (3·5 g, 0·022 mol) in acetic acid (10 ml) was added to ethyl 2,4-dihydroxyphenylglyoxylate [16] (2·1 g, 0·01 mol) in the

* One OH signal was not observed.

same solvent (10 ml) and stirred at room temperature for 30 min. Dilution with water (200 ml) precipitated the 3,5-*dibromo-derivative* (2·83 g, 77%) which crystallised from aqueous ethanol as yellow needles, m.p. 117·5—118·5° (Found: C, 32·8; H, 2·4; Br, 43·6. $C_{10}H_8Br_2O_5$ requires C, 32·6; H, 2·2; Br, 43·4%), $\nu_{max.}$ (Nujol) 3490, 1715, and 1640 cm^{-1}. Treatment with methyl iodide–silver oxide–chloroform gave the dimethyl ether (ν_{CO} 1740 and 1680 cm^{-1}) as an oil which was hydrolysed in boiling 5% potassium hydroxide in 50% aqueous ethanol during 15 min to give 3,5-*dibromo*-2,4-*dimethoxyglyoxylic acid* (III), m.p. 143—144° (from benzene) (Found: C, 33·0; H, 2·4; Br, 43·4%; *M*, 365·8746. $C_{10}H_8{}^{79}Br_2O_5$ requires C, 32·6; H, 2·2; Br, 43·4%; *M*, 365·8740), $\nu_{max.}$ (Nujol) 3240—2060, 1731, 1720sh, and 1680 cm^{-1}. The acid (150 mg) in 10% aqueous sodium hydroxide (9 ml) was treated with hydrogen peroxide (100 vol.; 1·5 ml) for 1 h. Acidification with 4M-hydrochloric acid gave 3,5-*dibromo*-2,4-*dimethoxybenzoic acid* (138 mg) which formed needles, m.p. 188—189° (from benzene) undepressed on admixture with that described above; both samples had identical i.r. spectra.

When ethyl 2,4-dimethoxyphenylglyoxylate [17] (2·4 g, 0·01 mol) in carbon tetrachloride (10 ml) was treated with bromine (3·8 g, 0·024 mol) only monobromination occurred even after the mixture had been refluxed 1 h. On cooling *ethyl* 5-*bromo*-2,4-*dimethoxyphenylglyoxylate* separated, and was recrystallised from ethanol as needles, m.p. 116—117° (2·14 g, 68%) (Found: C, 45·4; H, 4·3; Br, 24·9. $C_{12}H_{13}BrO_5$ requires C, 45·5; H, 4·1; Br, 25·2%), $\nu_{max.}$ (Nujol) 1730 and 1645 cm^{-1}. A similar reaction in refluxing pyridine also gave the monobromo-compound (76%). Alkaline hydrolysis gave the *acid*, m.p. 182·5—183·5° (from benzene) (Found: C, 41·7; H, 2·9; Br, 27·4. $C_{10}H_9BrO_5$ requires C, 41·6; H, 3·1; Br, 27·6%), $\nu_{max.}$ (Nujol) 3220—2140, 1715, and 1650 cm^{-1}; δ [$CDCl_3$–($^2H_6$-acetone)] 7·97 (1H, s, 6-H), 7·70 (1H, bs, OH), 6·69 (1H, s, 3-H), 4·01 and 3·93 p.p.m. (each 3H, s, OCH_3). Oxidative decarboxylation with alkaline hydrogen peroxide (as above) afforded 5-bromo-2,4-dimethoxybenzoic acid as needles, m.p. 201—202° (lit.,[18] m.p. 195—196°).

3-(3,5-*Dibromo*-2,4-*dimethoxyphenyl*)-2-*methoximino-propionic Acid* (V; R = Me).—3,5-Dibromo-2,4-dimethoxybenzaldehyde (6·48 g), acetylglycine (2·34 g), fused sodium acetate (1·2 g), and acetic anhydride (5·1 g) were heated under reflux at 200° for 2 h; the cooled, solidified melt was digested with water and filtered. The resultant azlactone was hydrolysed by refluxing it with 75% aqueous acetone for 17 h. The acetone was removed and the oily suspension was extracted with sodium hydrogen carbonate to give the *acetamidocinnamic acid* (VII; R = Me) (3·6 g, 43%) as needles, m.p. 220—221° (from chloroform) (Found: C, 36·6; H, 3·3; Br, 37·8; N, 3·4. $C_{13}H_{13}Br_2NO_5$ requires C, 36·9; H, 3·1; Br, 37·8; N, 3·3%), $\nu_{max.}$ (Nujol) 3340—2100, 3210, 1690, and 1645 cm^{-1}, δ [$CDCl_3$–($^2H_6$-Me_2SO)] 9·40 [1H, b, OH or NH (one not observed)], 7·78 (1H, s, 6′-H), 7·30 (1H, s, 3-H), 3·87 and 3·82 (each 3H, s, OCH_3), and 2·00 p.p.m. (3H, s, C–CH_3). The acetamido-acid (1·5 g) was boiled with M-hydrochloric acid in 75% aqueous tetrahydrofuran (THF) for 17 h. After removal of the THF the mixture was basified with sodium hydrogen carbonate and extracted with ether. Acidification of the aqueous layer gave a precipitate which crystallised deom chloroform to give 3,5-*dibromo*-2,4-*dimethoxyphenylpyruvic acid* (VI; R = Me), m.p. 164—167° (0·76 g, 56%) (Found: C, 34·9; H, 2·5; Br, 41·8. $C_{11}H_{10}Br_2O_5$ requires C, 34·6; H, 2·6; Br, 41·6%), $\nu_{max.}$ 3300—2000, 1690, 1650sh, and 1630sh cm^{-1}. In chloroform solution the pyruvic acid exists in keto and enol forms, the latter predominating in the ratio *ca.* 9 : 2; δ [$CDCl_3$ + 2 drops ($^2H_6$-Me_2SO)], enol form 8·44 (s, 6-H), 8·15 (b, OH), 6·75 (s, C*H*=C), 3·89 and 3·83 (s, OCH_3); keto form, 6-H not observed, 8·15 (b, OH), 4·14 (s, $ArCH_2$), 3·80 p.p.m. (s, OCH_3), the second OCH_3 signal underlies a methoxy-peak of the enol form. (Phenylpyruvic acid itself is also a keto-enol mixture in chloroform solution.) The pyruvic acid (573 mg) and *O*-methylhydroxylamine hydrochloride (750 mg) were kept in water (10 ml) containing sodium hydrogen carbonate (882 mg) for 16 h at room temperature. Acidification followed by chloroform extraction gave an oil which was treated with ethereal diazomethane. Distillation *in vacuo* gave the methyl ester (290 mg) identical (i.r., n.m.r., and t.l.c.) with that described above. Hydrolysis, as before, gave the propionic acid (V; R = Me), m.p. 104—105°, identical (mixed m.p. and i.r.) with that derived from (VIII; R = Me).

2-*Benzyloximino*-3-(2-*benzyloxy*-3,5-*dibromo*-4-*methoxy-phenyl*)*propionic Acid* (V; R = $PhCH_2$).—2-Benzyloxy-3,5-dibromo-4-methoxybenzaldehyde was condensed with acetylglycine, as above, and the resulting azlactone was hydrolysed in hot aqueous acetone to give the *acetamido-cinnamic acid* (VII; R = $PhCH_2$) which separated from chloroform as prisms, m.p. 214—215° (Found: C, 45·5; H, 3·4; Br, 32·0; N, 3·1. $C_{19}H_{17}Br_2NO_5$ requires C, 45·7; H, 3·4; Br, 32·0; N, 2·8%), $\nu_{max.}$ (Nujol) 3230, 3300—2100, 1692, 1655, and 1510 cm^{-1}; δ [$CDCl_3$ + ($^2H_6$-Me_2SO)] 8·75 1H, s, OH or NH), 7·75 (1H, s, 6′-H), 7·48 (6H, m, $C_6H_5CH_2$ and CH=C), 4·97 (2H, s, $C_6H_5CH_2$), 3·90 (3H, s, OCH_3), and 2·04 p.p.m. (3H, s, CH_3CO). Hydrolysis, as above, gave the *pyruvic acid* (VI; R = CH_2Ph) which crystallised from benzene as prisms, m.p. 150—152° (Found: C, 44·3; H, 3·1; Br, 34·6. $C_{17}H_{14}Br_2O_5$ requires C, 44·6; H, 3·1; Br, 34·9%), $\nu_{max.}$ (Nujol) 3300—2100, 1690, and 1655 cm^{-1}; δ [$CDCl_3$ + 3 drops ($^2H_6$-Me_2SO), in this solvent the compound was almost entirely in the enol form] 10·72 (2H, b, enol and acid HO), 8·50 (s, enol 6-H), 7·55 (5H, m, $C_6H_5CH_2$), 6·83 (s, enol CH=C), 4·92 (2H, s, $C_6H_5CH_2O$), 4·10 (s, enol $ArCH_2CO$), and 3·92 p.p.m. (3H, s, OCH_3). Treatment of the pyruvic acid with *O*-benzylhydroxylamine hydrochloride in the usual way gave the *benzyloximino-derivative* (V; R = $PhCH_2$) as needles, m.p. 161·5—162·5° (from ethanol) (Found: C, 50·9; H, 3·7; Br, 28·3; N, 2·5. $C_{24}H_{21}Br_2NO_5$ requires C, 51·2; H, 3·8; Br, 28·4; N, 2·5%), $\nu_{max.}$ (Nujol) 3300—2300 and 1700 cm^{-1}; δ ($CDCl_3$) 7·40 [12H, m, ArH + (?)HO], 5·25 (2H, s, $PhCH_2ON$), 5·02 (2H, s, $PhCH_2OAr$), and 3·90 p.p.m. (5H, s, OCH_3 and $ArCH_2$).

NN′-*Bis*-(2-*methoxyimino*-3-*phenylpropionyl*)-1,4-*diamino-butane* (II; R = Me).—Phenylpyruvic acid (1·25 g) was kept with *O*-methylhydroxylamine hydrochloride (1·25 g) in aqueous sodium hydrogen carbonate for 16 h and then acidified to give 2-*methoxyimino*-3-*phenylpropionic acid* which formed needles, m.p. 91—92° (from benzene–light petroleum) (Found: C, 62·4; H, 5·6; N, 7·5. $C_{10}H_{11}NO_3$ requires C, 62·2; H, 5·7; N, 7·3%), $\nu_{max.}$ (Nujol) 3360—2100 and 1708 cm^{-1}; δ ($CDCl_3$) 10·63 (1H, s, OH), 7·22 (5H, s, ArH), 4·07 (3H, s, OCH_3), and 3·88 p.p.m. (2H, s, $ArCH_2$). Boiling the acid (0·58 g) with an excess of thionyl chloride

[17] J. W. Clark-Lewis, J. A. Edgar, and K. Moody, *J. Chem. Soc.*, 1966, 1221.

[18] M. G. S. Rao, C. Srikantia, and M. S. Iyengar, *J. Chem. Soc.*, 1929, 1578.

gave the acid chloride [$\nu_{max.}$ (film) 1750 cm^{-1}] which was stirred with 1,4-diaminobutane (0·13 g) in pyridine (5 ml) for 15 h. Acidification with 4M-hydrochloric acid gave the *diamide*, m.p. 132—133° (from benzene–light petroleum) (0·59 g, 88%) (Found: C, 65·9; H, 7·1; N, 12·8. $C_{24}H_{30}N_4O_4$ requires C, 66·1; H, 6·6; N, 12·5%); $\nu_{max.}$ (Nujol) 3295, 1673, 1657, and 1530 cm^{-1}; δ ($CDCl_3$) 7·4—7·0 (10H, m, ArH), 6·73 (2H, b, $NHCH_2$), 3·95 (6H, s, OCH_3), 3·90 (4H, s, $ArCH_2$), 3·25 (4H, m, $NHCH_2$), and 1·50 p.p.m. (4H, m, $NHCH_2CH_2$).

NN′-*Bis*-(2-*benzyloximino*-3-*phenylpropionyl*)-1,4-*diaminobutane* (II; R = CH_2Ph).—Treatment of phenylpyruvic acid with *O*-benzylhydroxylamine hydrochloride gave the *benzyloximino-derivative* as needles, m.p. 83—84° (from benzene–light petroleum) (Found: C, 71·6; H, 5·5; N, 4·9. $C_{16}H_{15}NO_3$ requires C, 71·4; H, 5·6; N, 5·2%). Conversion into the acid chloride and reaction of this with 1,4-diaminobutane in pyridine gave the *diamide* as needles, m.p. 121—122° (from benzene–light petroleum) (Found: C, 73·1; H, 6·3; N, 9·4. $C_{36}H_{38}N_4O_4$ requires C, 73·2; H, 6·5; N, 9·5%), $\nu_{max.}$ (Nujol) 3300, 1650, and 1525 cm^{-1}; δ ($CDCl_3$) 7·25 (10H, m, ArH), 6·73 (2H, bt, $NHCH_2$), 5·22 (4H, s, $PhCH_2O$), 3·97 (4H, s, $PhCH_2C$), 3·25 (4H, m, $NHCH_2$), and 1·50 p.p.m. (4H, m, $NHCH_2CH_2$).

NN′-*Bis*-[3-(3,5-*dibromo*-2,4-*dimethoxyphenyl*)-2-*methoxyiminopropionyl*]-1,4-*diaminobutane* (VIII; R = Me).—The acid (V; R = Me) (154 mg) was converted into the acid chloride (ν_{CO} 1750 cm^{-1}) in the usual way and stirred with 1,4-diaminobutane (16 mg) in pyridine (2 ml) for 15 h. Acidification with 4M-hydrochloric acid and chloroform extraction afforded the *diamide* (83 mg, 51%) which was purified on silica-gel plates in ethyl acetate–benzene (3 : 7); it crystallised from benzene–light petroleum as needles, m.p. 130—131° [Found: *M*, 871·9081 (1 : 4 : 6 : 4 : 1 quintet). $C_{28}H_{34}{}^{79}Br_3{}^{81}BrN_4O_8$ requires 871·9092], identical (mixed m.p., t.l.c., i.r., and n.m.r.) with that derived from aerothionin.

NN′-*Bis*-[3-(3,5-*dibromo*-2,4-*dimethoxyphenyl*)-2-*methoxyiminopropionyl*]-1,5-*diaminopentane* (XV; R = Me).—This compound was prepared in the usual way using the chloride of the acid (V; R = Me) and 1,5-diaminopentane (24 mg) in pyridine (4 ml). Preparative t.l.c. afforded the *diamide* (68 mg, 33%) as an oil which separated from methanol in nodules, m.p. 35—39° (Found: *M*, 883·9293. $C_{29}H_{36}{}^{79}Br_4N_4O_8$ requires 883·9268), identical (mixed m.p., t.l.c., i.r., and n.m.r.) with that derived from homoaerothionin.

NN′-*Di*-(*acetamidoacetyl*)-1,4-*diaminobutane* (XI).—Ethyl acetamidoacetate (14·5 g, b.p. 158°/17 mm) and 1,4-diaminobutane (4·4 g) were heated at 145° for 1 h. Ethanol distilled off and the *tetra-amide* precipitated, m.p. 247—248° (from water) (Found: C, 50·0; H, 7·5; N, 19·5. $C_{12}H_{22}N_4O_4$ requires C, 50·3; H, 7·7; N, 19·6%), $\nu_{max.}$ (Nujol) 3300sh, 3285, and 1640 cm^{-1}; δ (D_2O, Bu^tOH as internal reference) 2·63 (4H, s, $NHCH_2CO$), 1·96 (4H, m, $NHCH_2$), 0·82 (6H, s, CH_3CONH), and 0·28 p.p.m. (4H, m, $NHCH_2CH_2$).

3-*Acetamido*-6,8-*dibromo*-7-*methoxycoumarin* (X; R = NHAc).— 3,5-Dibromo-2-hydroxy-4-methoxybenzaldehyde (3·0 g) was converted into the acetate with cold acetic anhydride/pyridine (17 h), and this oil (ν_{CO} 1775 and 1695 cm^{-1}) was heated at 200° with acetylglycine (1·2 g) and sodium acetate (0·8 g) in acetic anhydride (2·6 g) for 1 h. Solid began to deposit in a few min. After evaporation of the acetic anhydride the residue was boiled with 75% aqueous acetone for 11 h; the acetone was then removed and the aqueous suspension was digested with sodium hydrogen carbonate and chloroform. The insoluble *coumarin* (1·15 g, 30%) was collected and crystallised from Me_2SO–water to give needles, m.p. 292—294° (Found: C, 37·0; H, 2·3; Br, 41·0; N, 3·3. $C_{12}H_9Br_2NO_4$ requires C, 36·9; H, 2·3; Br, 40·9; N, 3·6%), $\nu_{max.}$ (Nujol) 3340, 1720, and 1690 cm^{-1}, δ ($^2H_6$-Me_2SO) 8·39 (1H, s, 5-H), 7·90 (1H, s, 4-H), 3·88 (3H, s, OCH_3), and 2·17 p.p.m. (3H, s, CH_3CON).

Benzylation of 3,5-*Dibromo*-2-*hydroxy*-4-*methoxybenzaldehyde*.—(*a*) The aldehyde (4·65 g), benzyl chloride (1·9 g), sodium iodide (2·3 g), and anhydrous potassium carbonate (16 g) were stirred for 20 h in dimethylformamide (20 ml). The precipitate obtained on dilution with water was chromatographed on a column of silica gel in benzene to give 2-*benzyloxy*-3,5-*dibromo*-4-*methoxybenzaldehyde* (5·1 g, 85%) which crystallised from benzene–light petroleum as needles, m.p. 92—92·5° (Found: C, 44·8; H, 2·8; Br, 40·2. $C_{15}H_{12}Br_2O_3$ requires C, 45·0; H, 3·0; Br, 40·0%), $\nu_{max.}$ (Nujol) 1687 cm^{-1}; δ ($CDCl_3$) 9·94 (1H, s, CHO), 7·99 (1H, s, 6-H), 7·40 (5H, s, $C_6H_5CH_2$), 5·13 (2H, s, $PhCH_2O$), and 3·97 p.p.m. (3H, s, CH_3).

(*b*) The aldehyde (2·33 g), benzyl chloride (1·9 g), sodium iodide (1·14 g), and anhydrous potassium carbonate (8 g) were heated under reflux in acetone (50 ml) for 9 h. Filtration and evaporation left a residue which was chromatographed on a column of silica gel in benzene to give 4-(2-*benzyloxy*-3,5-*dibromo*-4-*methoxyphenyl*)-4-*hydroxybutan*-2-*one* as prisms, m.p. 98—99° (from benzene–light petroleum) (1·61 g, 47%) (Found: C, 47·5; H, 4·3; Br, 35·0. $C_{18}H_{18}Br_2O_4$ requires C, 47·2; H, 4·0; Br, 34·9%), $\nu_{max.}$ (Nujol) 3420 and 1694 cm^{-1}; δ ($CDCl_3$) 7·70 (1H, s, 6′-H), 7·43 (5H, s, $C_6H_5CH_2$), 5·32 (1H, m, 4-H), 5·15 and 4·91 (2H, q, *J* 10·5 Hz, $C_6H_5CH_2$), 3·91 (3H, s, OCH_3), 3·45 (1H, d, *J* 3·5 Hz, OH), 2·78 (1H, s) and 2·68 (1H, d, *J* 2·5 Hz, CH_2CO), and 2·09 p.p.m. (3H, s, CH_3CO).

(*c*) The aldehyde (1·17 g), benzyl chloride (0·95 g), sodium iodide (0·57 g), and anhydrous potassium carbonate (4 g) were stirred for 2 h at 100° in dimethylformamide (10 ml). Dilution with water and chloroform extraction gave an oil which was chromatographed on silica gel in benzene–light petroleum (1 : 1). The first fraction (0·1 g) was a dibenzyloxyaldehyde and later fractions (0·8 g) were mixtures of this with the desired monobenzyl derivative (t.l.c.). Crystallisation of the mixture from benzene–light petroleum afforded 2,4-*dibenzyloxy*-3,5-*dibromobenzaldehyde*, m.p. 121—124° (0·16 g; overall 14%) (Found: C, 52·7; H, 3·2; Br, 33·5. $C_{21}H_{16}Br_2O_3$ requires C, 52·9; H, 3·4; Br, 33·5%), $\nu_{max.}$ (Nujol) 1686 cm^{-1}; δ ($CDCl_3$) 9·96 (1H, s, CHO), 8·03 (1H, s, 6-H), 7·40 (10H, m, ArH), and 5·14 p.p.m. (4H, s, $ArCH_2$).

Two of us (K. M. and R. H. T.) are greatly indebted to Messrs. Elliott Smith and J. W. McBeth for collecting *V. thiona* and to the Nuffield Foundation for financial support. We also thank the S.R.C. Physicochemical Measurements Unit, Aldermaston, for mass spectra.

[1/1005 *Received, June 18th,* 1971]