Metabolites from the Sponge *Pachymatisma johnstoni*; L-6-Bromohypaphorine, a New Amino-acid (and its Crystal Structure)

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6-Bromohypaphorine[1-trimethylammonio-3-(6-bromoindol-3-yl)propionate], tryptophan, uracil, thymine, cholest-4-en-3-one and 24-methylenecholest-4-en-3-one have been isolated from *P. johnstoni*. The structure and configuration of 6-bromohypaphorine were confirmed by *X*-ray analysis. 6-Bromohypaphorine and related compounds show anomalous mass spectra resulting from thermal reactions in the mass spectrometer.

Pyrolysis of hypaphorine gave a mixture of products including indole, 3-methylindole, and an indolyl-tetrahydrocarbazolecarboxylic acid. Pyrolysis of diethyl(indol-3-ylmethyl)ammonium chloride yielded indole, 3-methylindole, di-indol-3-ylmethane, and related cyclic and open-chain compounds containing three indole units.

Pachymatisma johnstoni is one of the larger British sponges, occurring in masses up to 50 cm across or more. Extraction of fresh material with acetone, removal of solvent, and further solvent extraction of the aqueous residue yielded, after extensive chromatography, a novel bromo-amino-acid together with a variety of other metabolites including glycolipids, steroids, and pyrimidines. The bromo-compound, isolated from the butanol fraction, was first detected on t.l.c. as a u.v.-absorbing spot which gave a violet colour with *p*-dimethylaminocinnamaldehyde. Other minor components of the same fraction were identified later as typtophan, uracil, and thymine. The pyrimidines have not been observed previously in sponges except in *Tedania ignis*.[1]

The combined evidence, particularly the rest of the n.m.r. spectrum (see Experimental section) is consistent with the indolyl amino-acid structure (1), but the ' osmometric molecular weight ' determined in water, was 882 ± 25 [*M* of (1) is 324], and there are triplet clusters in the mass spectrum *above m/e* 500 characteristic of ions containing two atoms of bromine (see Figure 1). This implied that the amino-acid was perhaps a trimer related to (1), and to solve this apparently more complex structure we turned to *X*-ray crystallographic analysis. However, it was immediately clear from the unit cell parameters that the molecule was monomeric, and further work established that the new amino-acid was 6-bromohypaphorine (2). (The ' osmometric molecular weight ' value can be explained by association, and the abnormal mass spectrum is discussed below.) L-Hypaphorine has been observed frequently in Leguminosae,[3] especially *Erythrina*,[4] but has not been reported in sponges, nor could we detect it in *P. johnstoni*. On the other hand the occurrence of 6-bromoindoles in marine organisms has been known since 1909 when 6,6'-dibromoindigotin was identified,[5] as an artefact, shown [6] later to be derived from the 6-bromoindoxyl sulphates (3). Recent examples are the metabolites (4; R = H or Me) from a sponge,[7a] and (5) from an acorn worm.[7b]

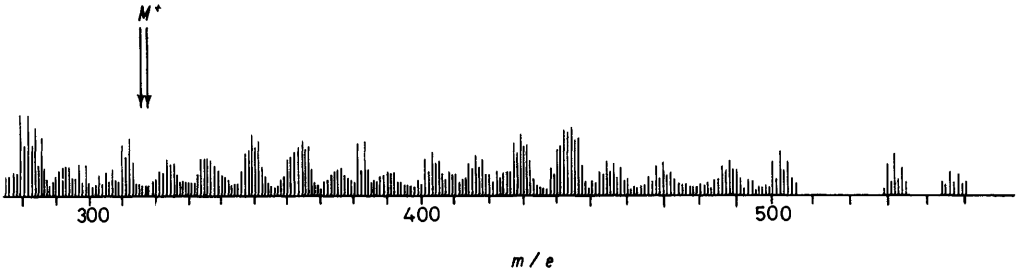
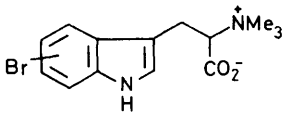


FIGURE 1 Mass spectrum of 6-bromohypaphorine above *m/e* 300

6-Bromohypaphorine.—The bromo-compound was sparingly soluble in water, and showed u.v. absorption (methanol) at 228, 285, and 294 nm very like that of tryptophan. A betaine structure was suggested by the formation of a hydrochloride with concomitant shift of a strong i.r. band from 1 626 (CO_2^-) to 1 740 cm^{-1} (CO_2H), an $\overset{+}{N}Me_3$ cation being indicated by a nine-proton singlet in the n.m.r. spectrum at δ 3.49 (in $CF_3{\cdot}CO_2H$), and three base peaks in the mass spectrum at *m/e* 116 ($Me_2\overset{+}{N}{=}CHCO_2Me$), 59 ($Me_3\overset{+}{N}$), and 58 ($Me_2\overset{+}{N}{=}CH$) (*cf.* ref. 2).

[1] V. Krishnamoorthy and R. H. Thomson, unpublished work.
[2] (*a*) N. Mandava, J. D. Anderson, and S. R. Dutky, *Phytochemistry*, 1974, **13**, 2853; (*b*) J. S. Fitzgerald, *Austral. J. Chem.*, 1963, **16**, 246.
[3] S. Ghosal, P. K. Banerjee, and S. K. Banerjee, *Phytochemistry*, 1970, **9**, 429.
[4] L. Marion in ' The Alkaloids,' eds. R. H. F. Manske and H. L. Holmes, vol. II, Academic Press, New York, 1952, p. 372.
[5] P. Friedländer, *Ber.*, 1909, **42**, 765.
[6] J. T. Baker and M. D. Sutherland, *Tetrahedron Letters*, 1968, 43; H. Fouquet and H.-J. Bielig, *Angew. Chem. Internat. Edn.*, 1971, **10**, 816.
[7] (*a*) G. E. Van Lear, G. O. Morton, and W. Fulmor, *Tetrahedron Letters*, 1973, 299; (*b*) T. Higa and P. J. Scheuer, *Naturwiss.*, 1975, **62**, 395.

Mass Spectrum of 6-Bromohypaphorine (2).—As already noted, the mass spectrum of 6-bromohypaphorine (2) includes clusters of weak peaks at high mass, *m/e* 548/546/544, 534/532/530, 504/502/500 (ratio *ca.* 1 : 2 : 1), *etc.* Attempted sublimation of (2) at 200 °C *in vacuo*



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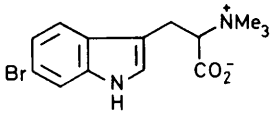

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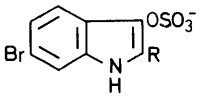

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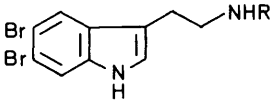

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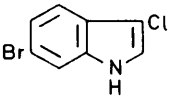

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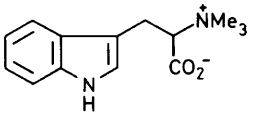

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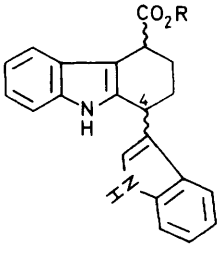
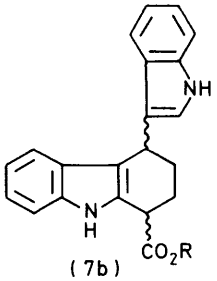
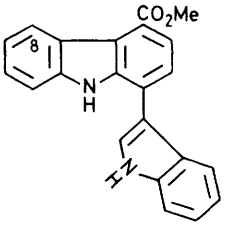


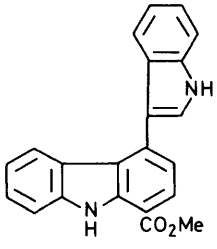
(7a)



(7b)



(8a)



(8b)

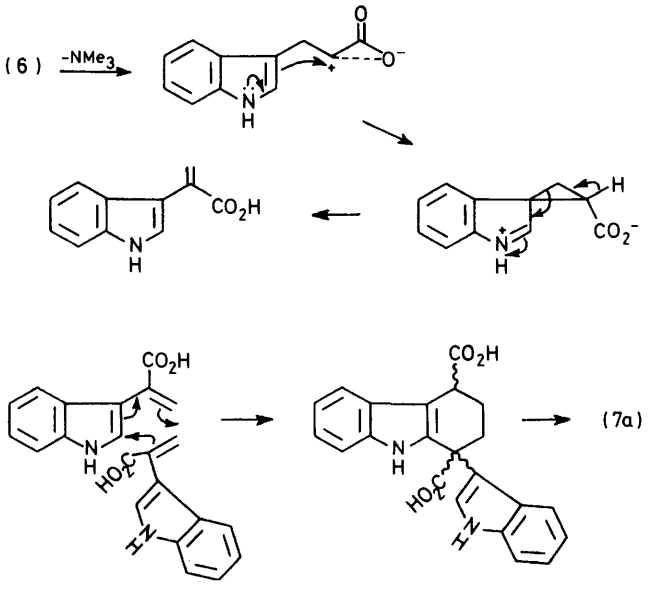
resulted in extensive decomposition, and gave low yields of a white solid mixture whose mass spectrum was qualitatively similar to that of (2) including ions at *m/e* 504/502/500. This indicates that the peaks at high mass in the spectrum of 6-bromohypaphorine result from thermal reactions on the probe of the mass spectrometer. (±)-5-Bromo- and -5,7-dibromohypaphorine, synthesised for comparison, behaved in the same way. The mass spectrum of 5-bromohypaphorine was virtually identical with that of (2) whereas the spectrum of the dibromo analogue showed peaks up to *m/e* 678/676/674/672/670. In the case of hypaphorine, with the temperature of the mass spectrometer source at 150 °C, the ion of highest mass, *m/e* 187, corresponds to $M^+ - NMe_3$, whereas ions at *m/e* 388, 370, 344, and 330 are observed if the source temperature is raised to 250 °C. These observations led us to examine the thermal decomposition of (the more readily available) hypaphorine in some detail.

Pyrolysis of (±)-*Hypaphorine.*—Heating hypaphorine (6) *in vacuo* at 270 °C for *ca.* 2 min give a complex mixture of products, in very small amounts. The principal components were indole and 3-methylindole, previously observed in the pyrolysis of tryptophan,[8] and a third indole derivative giving a violet colour with *p*-dimethylaminocinnamaldehyde. From its molecular formula, $C_{21}H_{18}N_2O_2$, and spectroscopic and other properties, this compound must be derived from two molecules of hypaphorine, contains nine aromatic and six aliphatic protons, a carboxy and two NH groups, and is pentacyclic. On methylation it gave two diastereoisomeric methyl esters. These compounds showed indolic u.v. absorption, and of the various possible structures (7a and b; R = Me) agree closely with the ^1H n.m.r. spectra. The chemical shifts of the NH and all the aromatic protons correspond to those of indole and 1,2,3,4-tetrahydrocarbazole, and the methylene protons (4 H) resonate as a single broad hump at δ 2.0—2.5. The splitting patterns of the methine proton signals which occur in the region δ 4 as a doublet of doublets (*J* 5 and 6 Hz) and a multiplet are particularly indicative of the arrangement shown in structures (7a and b; R = Me) but are not in good accord with the many alternative possibilities. Dehydrogenation of both methyl esters with dichlorodicyanobenzoquinone gave the same fully aromatic carbazole ester (8a or b). The ^1H n.m.r. spectrum of this compound is in general agreement with those of carbazole and indole. The low chemical shift of the proton doublet at δ 8.93 (*J* 7 Hz) can be rationalised by deshielding of H-8 by the carbonyl group at H-1 in structure (8a), implying that the precursor esters are diastereoisomers of structure (7a; R = Me). The formation of these products can be interpreted (*e.g.* see Scheme) more easily than the alternatives (7b), and accordingly we regard the pyrolysis product as (7a; R = H). The 'dimeric' peaks in the mass spectrum of hypaphorine can now be explained in terms of the Scheme where *m/e* 330 corresponds to structure (7a; R = H) and *m/e* 374 to the precursor dicarboxylic acid. Peaks at *m/e* 344 and 388 may arise from the methyl esters of (7a) and its precursor di-acid, formed by initial migration of one methyl group from nitrogen to oxygen in (6) and subsequent rearrangement and cycloaddition [supporting evidence for such a process may be found in the presence of a peak at *m/e* 116 ($Me_2\overset{+}{N}{=}CHCO_2Me$) in the same spectrum].

Pyrolysis of Diethyl(indol-3-ylmethyl)ammonium Chloride (11).—As gramine derivatives such as (9) and (10) also gave anomalous mass spectra, we then examined the pyrolysis of a salt of that type. Heating the tertiary

[8] G. P. Shulman and P. G. Simmonds, *Chem. Comm.*, 1968, 1040.

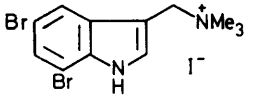
amine salt (11) at 250 °C gave mainly indole and 3-methylindole together with numerous minor compounds. In high-boiling solvents the proportion of indole and



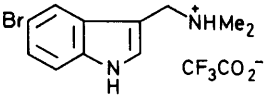
SCHEME

3-methylindole was markedly diminished, giving higher yields of other less volatile compounds, the highest (*ca.* 10%) being obtained in dimethyl sulphoxide at 180 °C. Of the many compounds formed we identified one dimeric and two trimeric indole derivatives; undoubtedly tetramers and higher oligomers were also present, the bulk of the product being polymer.

The dimer (5% yield) proved to be di-indol-3-ylmethane (12), identical with authentic material.[9] A trimer, $C_{27}H_{21}N_3$, was isolated (2.2%) as light-sensitive needles, decomp. >270°, which we regard as the tri-indole (13). It shows the u.v. absorption of an indole, and the mass spectrum includes significant peaks at *m/e*



(9)



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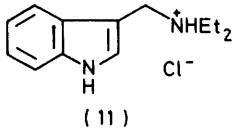

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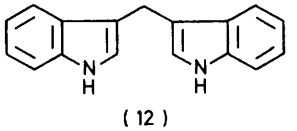


(12)

387(100%), 257(100%), and 130(15%) corresponding to M^+, $2/3M^+ - 1$, and $1/3M^+ + 1$, respectively, as found in the mass spectra of the tri-*N*-methyl derivative [10] and cycloveratrylene (15).[11] The ^{1}H n.m.r. spectrum shows signals for ArH, NH, and CH_2 only, in the ratio 4 : 1 : 2, and the ^{13}C n.m.r. spectrum confirms the presence of only one type of aliphatic carbon atom, eliminating the possibility of significant quantities of the isomer (14) being present. The trimer (13) can exist in either a crown (rigid) or saddle (flexible) conformation. The ^{1}H n.m.r. spectrum in [$^2H_6$]acetone at −5 °C (Figure 2) clearly

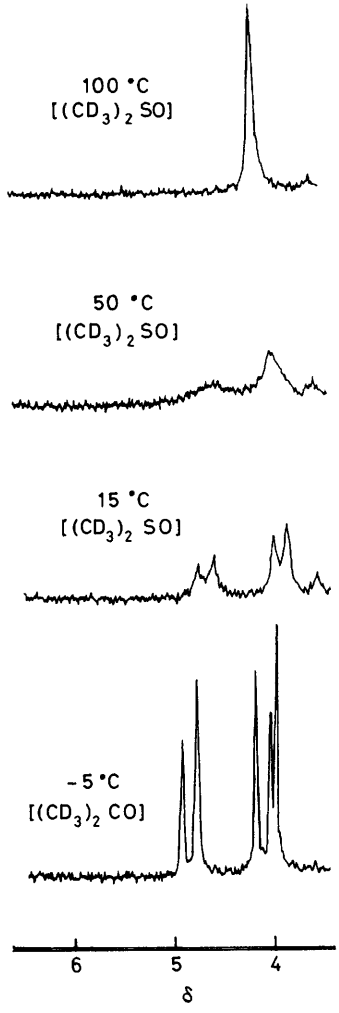


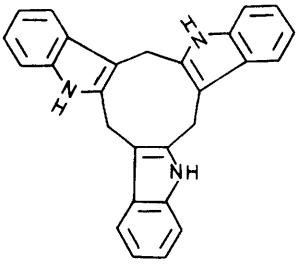
FIGURE 2 100 MHz ^{1}H N.m.r. spectrum of the trimer (13) below δ 6

shows the presence of the crown conformation, exhibiting two sharp symmetrical doublets (ratio 1 : 1) at δ 4.11 and 4.85 (*J* 14 Hz). A sharp singlet at δ 3.98 (*ca.* 1 H) indicates the presence of a small proportion (*ca.* 20%) of the saddle conformation. In [$^2H_6$]dimethyl sulphoxide at 15 °C the methylene protons resonate as two unequal doublets centred at δ 3.93 and 4.69 (*J* 14 Hz) which collapse to two unequal broad signals at 50 °C, and finally at 100 °C to a broad singlet at δ 4.12, indicating

[9] J. Thesing, *Chem. Ber.*, 1954, **87**, 692.
[10] J. Bergman, S. Högberg, and J.-O. Lindström, *Tetrahedron*, 1970, **26**, 3347.
[11] H. Erdtman, F. Haglid, and R. Ryhage, *Acta Chem. Scand.*, 1964, **18**, 1249.

that crown–saddle interconversion is rapid at this temperature.

The second trimer (2.9%) was obtained only as a gum, although homogeneous by t.l.c. It had the molecular formula $C_{26}H_{21}N_3$ and a much weaker molecular ion than (13) at *m/e* 375(13%), but it fragmented in similar fashion



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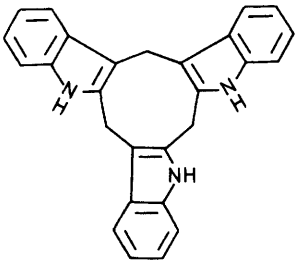

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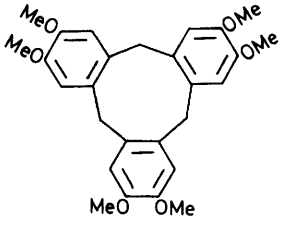

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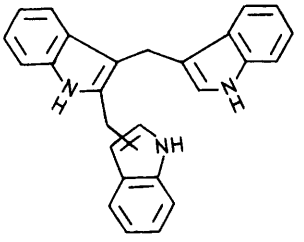

(16)

to give significant peaks at *m/e* 258(100%), 257(95%), and 130(10%). The n.m.r. spectrum again showed signals for ArH, NH, and CH_2 only, but in the ratio 14 : 3 : 4, and the methylene protons resonated as two singlets (each 2 H) at δ 4.25 and 4.32 [in $(CD_3)_2CO$; *cf.* δ 4.20 for di-indol-3-ylmethane in $(CD_3)_2CO$]. These data are consistent with partial structure (16), and although they do not distinguish decisively between the two possibilities the absence of any resonance around δ 6.5 (typical for H-3 in indoles) indicates that the undetermined methylene attachment is probably to C-3. In an attempt to relate compound (16) with (13) it was treated briefly with formaldehyde and sulphuric acid. T.l.c. of the resulting mixture showed that some cyclic trimer (13) had been formed. However, the result is ambiguous because di-indol-3-ylmethane (12) was also one of the products, and this may have been the precursor of (13) and not (16). It is known that di-indol-3-ylmethane (13) can undergo cleavage and rearrange to 2,3′-methylenedi-indole under acidic conditions (ref. 10 and references therein) but (16) did not react with formaldehyde in the absence of acid. All products identified in the pyrolysis of (11) can be accounted for in terms of mechanisms analogous to those proposed for the acid-catalysed conversion of 3-hydroxymethylindole into di-indol-3-ylmethane (12) [12] and the acid-catalysed cleavage of (12).[10] As all steps are probably reversible, numerous products could arise even at a low level of oligomer formation. Other mechanisms, including free-radical processes, can also be envisaged.

Steroids.—The ethereal extract from *P. johnstoni* contained a mixture of steroids which were mainly cholest-4-en-3-ones. Two of the principal components were identified as cholest-4-en-3-one and its 24-methylene derivative. Cholest-4-en-3-ones have been isolated recently from the sponge *Stellata clarella*.[13]

EXPERIMENTAL

Extraction of Pachymatisma johnstoni.—Fresh sponge, collected off the Isle of Man, was squeezed to remove the bulk of the water, and left in acetone for several days. The solvent was then removed *in vacuo* and the residual aqueous suspension was extracted successively with ether, ethyl acetate, and butan-1-ol. The ethereal extract gave a brown oil (27.9 g) (from 4.5 kg of damp sponge), part of which was separated by p.l.c. on silica gel in chloroform–light petroleum (b.p. 60—80 °C) (2 : 1). The main u.v.-absorbing band (R_F 0.6) was recovered, and the material was passed down a column of Sephadex LH-20 in chloroform–methanol (2 : 1). The main band showed $\lambda_{max.}$ (C_6H_{12}) 232 nm, $\nu_{max.}$ (KBr) 2 940, 1 680, 1 650, and 1 620 cm^{-1}, δ ($CDCl_3$) 0.72, 0.97, 1.08, aud 1.19 (all s), 0.8—2.40 (m), 4.70 (d), and 5.75 (s); g.l.c. (OV-17; 270 °C) revealed four major components. Component 1 was identical [mass spectrum and g.l.c. (t_R 17.5 min)] with cholest-4-en-3-one; component 2 was identical [mass spectrum and g.l.c. (t_R 23.2 min)] with 24-methylene-cholest-4-en-3-one; components 3 (M^+ 412; t_R 31.3 min) and 4 (M^+ 412; t_R 37.6 min) were related cholest-4-en-3-ones but were not identified.

The butanolic extract yielded a pale brown resin (32 g) which was dissolved in methanol (60 ml); the solution was centrifuged and passed down a column (60 × 5 cm) of Sephadex LH-20 in methanol, in three separate portions, and 10 ml fractions were collected. Fractions 1—7 gave a mixture of glycolipids (24.7 g) which afforded on acidic hydrolysis a mixture of fatty acids, glucose, and galactose, but were not examined further. Fractions 8—10 were concentrated and rechromatographed on Sephadex LH-20. After partial removal of solvent, crystals (A) (153 mg) separated and a pale brown gum (B) (291 mg) was recovered from the mother liquor. The gum (B) was subjected to t.l.c. on cellulose plates in ethyl acetate–methanol–water (100 : 17 : 13); two bands separated which gave a blue-violet colour with *p*-dimethylaminocinnamaldehyde. The less mobile band, after gel filtration, afforded a pale yellow gum (1 mg), M^+ 204, $\lambda_{max.}$ (MeOH) 225, 283, and 290 nm, R_F value identical with that of tryptophan. The second band, after t.l.c. on silica gel in the same solvent system, and further gel filtration in methanol, yielded 6-bromohypaphorine (14 mg). A larger quantity (47 mg) was obtained from the crystals (A) *via* the nitrate as described for 5-bromohypaphorine. An attempt to purify (A) by t.l.c. on silica gel in ethyl acetate–methanol–water, as above, was not successful but small amounts of uracil and thymine were separated, and were identified (u.v. and mass spectra, and t.l.c.) by comparison with authentic samples.

L-6-*Bromohypaphorine* [1-*trimethylammonio*-3-(6-*bromoindol*-3-*yl*)*propionate*] (2) formed rods, decomp. 275—280° (Found: C, 51.9; H, 5.5; Br, 22.9; N, 8.1. $C_{14}H_{17}BrN_2O_2$ requires C, 51.7; H, 5.3; Br, 24.6; N, 8.6%), $[\alpha]_D^{15}$ +58° [MeOH–CF_3CO_2H (8 : 1)], $\lambda_{max.}$ (MeOH) 228, 285, and 294

[12] W. A. Remers in ' Indoles,' Part I, ed. W. J. Houlihan, Wiley–Interscience, New York, 1972, pp. 203—204.
[13] Y. M. Sheikh and C. Djerassi, *Tetrahedron*, 1974, **30**, 4095.

nm (log ε 4.51, 3.77, and 3.72), $\nu_{max.}$ (KBr) 3 050br, 1 626, 1 570w, 1 538w, 1 485, 1 455, 1 445, 1 427, 1 417w, and 1 410 cm^{-1}, δ ($CF_3 \cdot CO_2H$) 3.49 (9 H, s, $\overset{+}{N}Me_3$), 3.64 (2 H, d, *J* 8 Hz, $CH_2 \cdot CH$), 4.50 (1 H, t, *J* 8 Hz, $CH_2 \cdot CH$), 7.20 (1 H, s, indole H-2), 7.33 (2 H, ABq, *J* 8 Hz, indole H-4 and -5), and 7.57br (1 H, s, $W_{\frac{1}{2}}$ 2.5 Hz, indole H-7), *m/e* (70 eV; 190 °C) 550(0.01%), 548(0.02), 546(0.02), 544(0.01), 534(0.1), 532(0.2), 530(0.1), 504(0.15), 502(0.3), 500(0.15), 490(0.1), 488(0.15), 486(0.1), 472(0.1), 470(0.15), 468(0.15), 458(0.1), 456(0.15), 454(0.15), 452(0.1), 450(0.4), 445(0.5), 444(1.3), 443(1.0), 442(1.0), 441(0.5), 440(0.4), 431(0.4), 429(1.0), 427(0.5), 418(0.2), 416(0.4), 414(0.3), 405(0.2), 403(0.4), 401(0.2), 383(0.3), 381(0.3), 364(0.2), 363(0.3), 362(0.4), 361(0.3), 360(0.3), 349(0.6), 347(0.5), 336(0.3), 334(0.4), 326(0.07, M^+), 325(0.1), 324(0.1, M^+), 323(0.3) (below *m/e* 320 only peaks of ⩾5% intensity are listed) 267(20), 265(20), 224(8), 223(45), 222(35), 221(45), 220(20), 210(45), 208(45), 197(5), 195(7), 143(20), 142(26), 141(75), 140(18), 130(9), 129(22), 128(8), 117(5), 116.0709(100) ($C_5H_{10}NO_2$ requires 116.0711), 115(85) 114(37), 113(20), 103(5), 102(100), 89(10), 88(9), 87(6), 75(6), 70.5(15), 63(17), 62(6), 60(6), 59(100), 58(100), 57(20), and 56(11). The hydrochloride was obtained by dissolution in concentrated hydrochloric acid followed by rapid removal of solvent *in vacuo*; $\nu_{max.}$ (KBr) 3 320br, 3 160br, 1 740, 1 620w, and 1 595 cm^{-1}.

(±)-5-*Bromohypaphorine*.—5-Bromogramine was prepared from 5-bromoindole, dimethylamine, and formaldehyde (*cf.* ref. 14); m.p. 159—161° (lit.,[15b] 162°), and converted into 5-bromotryptophan by published methods.[15] It sintered at 258°; m.p. 273—276° (decomp.) (lit.,[15b] 251°) (Found: M^+, 282.0003. $C_{11}H_{11}{}^{79}BrN_2O_2$ requires 282.0003), *m/e* 284(1%), 282(1), 210(100), 208(100), and 129(30). Treatment of the amino-acid with methyl iodide and sodium hydroxide gave the crude methyl ester methiodide, which was hydrolysed with aqueous 1% sodium hydroxide.[2b,16] The solution was cooled in ice, and acidified (pH 2) by careful addition of 8M-nitric acid. The nitrate was collected, washed with cold, dilute nitric acid, and redissolved in aqueous 5% sodium hydroxide (12 ml); the solution was adjusted to pH 4 with saturated potassium dihydrogen phosphate solution, and taken to dryness *in vacuo*. The residue, after fractional crystallisation from methanol, was passed through Sephadex LH-20, and again crystallised from methanol to give *5-bromohypaphorine* as rods, m.p. 225—227° (decomp.) (Found: C, 51.5; H, 5.3; Br, 24.6; N, 8.4. $C_{14}H_{17}BrN_2O_2$ requires C, 51.7; H, 5.3; Br, 24.6; N, 8.6%), $\nu_{max.}$ (KBr) 3 420br, 3 230br, 1 630, 1 490w, 1 465, and 1 455 cm^{-1}, δ ($CF_3 \cdot CO_2H$) 3.46 (9 H, s, $\overset{+}{N}Me_3$), 3.54 (2 H, d, *J* 8 Hz, $CH_2 \cdot CH$), 4.47 (1 H, t, *J* 8 Hz, $CH_2 \cdot CH$), 7.20 (1 H, s, indole H-2), 7.31 (2 H, s, indole H-6 and -7), and 7.46br (1 H, s, $W_{\frac{1}{2}}$ 2.5 Hz, indole H-4); mass spectrum indistinguishable from that of 6-bromohypaphorine (above).

(±)-5,7-*Dibromohypaphorine*.—To 5,7-dibromoisatin (61 g) and sodium borohydride (21 g) in dry tetrahydrofuran (500 ml) cooled to −78 °C under nitrogen, boron trifluoride–ether (135 ml) in the same solvent (150 ml) was added, dropwise, with stirring over 30 min. After stirring overnight, the solution was allowed to warm to 0 °C, and stirring was continued for a further 24 h. Ice was added until evolution of hydrogen had ceased; the suspension was then poured into water (1 l) and extracted with ether (700 ml) and then chloroform (2 × 200 ml). The residue from the combined extracts was dissolved in chloroform and chromatographed on a column of silica gel (5 × 30 cm) in light petroleum–chloroform (1 : 3). Elution of a fast-running band afforded a pale yellow oil which solidified, and was sublimed *in vacuo* to give *5,7-dibromoindole*, m.p. 69—70° (no m.p. in ref. 17) (50%), δ ($CDCl_3$) 6.49 (1 H, dd, *J* 2.5 and 3 Hz, simplified to d, *J* 3 Hz, on exchange with $CF_3 \cdot CO_2$-D, H-3), 7.13 (1 H, t, *J* 3 Hz, simplified to d, *J* 3 Hz, with $CF_3 \cdot CO_2D$, H-2), 7.42 (1 H, d, *J* 1.5 Hz, H-6), 7.66 (1 H, dd, *J* 1.5 and 0.8 Hz, simplified to d, *J* 1.5 Hz with $CF_3 \cdot CO_2D$, H-4), and 8.20br (1 H, exchanged with $CF_3 \cdot CO_2D$, NH), *m/e* 277(40%, M^+), 275(100, M^+), 273(40, M^+), 196(16), 194(16), 138(6), 137(13), 136(6), 115(50), and 114(5). Mannich reaction of 5,7-dibromoindole with dimethylamine and formaldehyde, as above, gave *5,7-dibromogramine* (*5,7-dibromo-3-dimethylaminomethylindole*) as rods, m.p. 128—131° (from acetone) (60%) (Found: M^+, 331.9351. $C_{11}H_{12}{}^{79}Br^{81}BrN$ requires 331.9347); δ ($CDCl_3$) 2.27 (6 H, s, NMe_2), 3.56 (2 H, s, CH_2), 7.09 (1 H, d, *J* 2 Hz, H-2), 7.43 (1 H, d, *J* 2.5 Hz, H-6), 7.78 (1 H, d, *J* 2.5 Hz, H-4), and 8.80br (1 H, NH); δ ($CF_3 \cdot CO_2D$) 3.08 (6 H, s, NMe_2), 4.59 (2 H, s, CH_2), 7.64 (1 H, s, H-2), 7.54 (1 H, d, *J* 2 Hz, H-6), and 7.72 (1 H, d, *J* 2 Hz, H-4); *m/e* 334(10%), 332(30), 330(10), 291-(7), 290(40), 289(15), 288(100), 287(10), 268(40), 210(5), 209(8), 208(8), 207(8), 118(10), and 117(10).

Alternatively, treatment of 5,7-dibromoindole under Mannich conditions with diethylamine gave N-(*5,7-dibromoindol-3-ylmethyl*)*diethylamine* as rods, m.p. 141—143° (from acetone) (62%) (Found: M^+, 357.9678. $C_{13}H_{16}{}^{79}Br_2N_2$ requires 357.9679), δ (CCl_4) 1.05 (6 H, t, *J* 7 Hz, $CH_2 \cdot CH_3$), 2.51 (4 H, q, *J* 7 Hz, $CH_2 \cdot CH_3$), 3.63 (2 H, s, $CH_2 \cdot N$), 7.07br (1 H, d, *J* 2 Hz, indole H-2), 7.42 (1 H, d, *J* 2 Hz, indole H-6), 7.79 (1 H, d, *J* 2 Hz, indole H-4), and 8.10br (1 H, NH); *m/e* 362(4%), 360(8), 358(4), 347(1), 345(2), 343(1), 290(35), 288(70), 286(35), 209(6), 208(4), 207(6), 128(13), 127(9), 72(50), and 58(100). This amine was converted, as before, into *5,7-dibromotryptophan*, decomp. 259° (from aq. HOAc) (23%) (Found: C, 36.5; H, 2.8; Br, 43.9; N, 7.5%; M^+, 359.9106. $C_{11}H_{10}{}^{79}Br_2N_2O_2$ requires C, 36.5; H, 2.8; Br, 44.2; N, 7.75%; *M*, 359.9108), δ ($CF_3 \cdot CO_2D$) 3.60—3.72 (2 H, m, $CH_2 \cdot CH$), 4.71 (1 H, t, *J* 6 Hz, $CH_2 \cdot CH$), 7.38 (1 H, s, 90% exchanged after 14 days, H-2), 7.52 (1 H, d, *J* 1.5 Hz, H-6), and 7.66 (1 H, d, *J* 1.5 Hz, H-4).

Treatment of 5,7-dibromotryptophan with methyl iodide and base, as above, gave 5,7-dibromohypaphorine as a white solid, $\nu_{max.}$ (KBr) 3 400br, 1 640, 1 590sh, 1 480, and 1 410 cm^{-1} [$\nu_{max.}$ (KBr) (after brief treatment with concentrated hydrochloric acid) 3 400br, 2 950br, 1 740, 1 625, 1 565, 1 475, and 1 415 cm^{-1}], δ ($CF_3 \cdot CO_2D$) 3.55 (9 H, s, $^+NMe_3$), 3.65 (2 H, d, *J* 6 Hz, $CH_2 \cdot CH$), 4.50 (1 H, t, *J* 6 Hz, $CH_2 \cdot CH$), 7.32 (1 H, s, H-2), 7.52 (1 H, d, *J* 1.5 Hz, H-6), and 7.63 (1 H, d, *J* 1.5 Hz, H-4), *m/e* 678(0.12%), 676(0.16), 674(0.17), 672(0.15), 670(0.12), 664(0.15), 662(0.25), 660-(0.40), 658(0.30), 656(0.15), 634(0.16), 632(0.27), 630(0.33), 628(0.30), 626(0.19), 620(0.19), 618(0.42), 616(0.70), 614-(0.50), 612(0.25), 605(0.50), 603(1.5), 601(2.0), 599(1.2), 597(0.40), 591(0.40), 589(1.0), 587(2.0), 585(1.5), 583(0.30) (below *m/e* 580 only peaks with ⩾5% intensity are listed), 317(5), 315(42), 313(5), 303(5), 301(8), 299(10), 141(7), 140(5), 102(7), 59(95), and 58(100).

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[15] D. G. Harvey, *J. Chem. Soc.* (*a*) 1958, 3760; (*b*) 1959, 473.
[16] P. van Romburgh and G. Barger, *J. Chem. Soc.*, 1911, **99**, 2068.
[17] H. Sirowej, S. A. Khan, and H. Plieninger, *Synthesis*, 1972, 84.

Pyrolysis of Hypaphorine (6).—Hypaphorine was prepared according to refs. 2*b* and 16 and purified *via* the nitrate; decomp. 253—255° (lit.,[2b] 246°; lit.,[3] 260°; lit.,[4] 255°), *m/e* 388(0.15%), 374(0.2), 344(0.2), 330(0.3), 303(1.5), 286(0.5), 285(0.7), 271(0.4), 269(0.3), 257(0.3), and 246(0.13, M^+) (below *m/e* 240 only peaks of intensity >5% are listed), 201(6), 187(40), 144(6), 143(95), 142(60), 131(5), 130(100), 116.0709 ($C_5H_{10}NO_2$ requires 116.0711) (17), 115(55), 102(30), 59(67), 58(100), and 57(5). Hypaphorine (3 × 5 g) was heated at 270 °C *in vacuo* until fusion was complete (*ca.* 2 min). The resulting dark brown glass was dissolved in acetone–methanol (1 : 1) and chromatographed on a column (6 × 60 cm) of silica gel in chloroform. Early fractions contained substantial amounts of indole and 3-methylindole (t.l.c. identification only). Chloroform–methanol (4 : 1) eluted a dark brown band containing a major component positive to *p*-dimethylaminocinnamaldehyde, which was purified by passage through a Sephadex LH-20 column in chloroform–methanol (1 : 1) and t.l.c. on silica gel plates in chloroform–acetone (4 : 1), followed by extraction from ether with sodium hydrogen carbonate solution. Acidification gave a white solid (33 mg) (7a; R = H) which was methylated (Me_2SO_4–K_2CO_3–Me_2CO) under nitrogen, in the dark. The resulting oily mixture of methyl esters was separated by t.l.c. on silica gel in chloroform, in subdued light, and the esters were then passed, separately through a column of Sephadex LH-20 in methanol. The more mobile epimer of *methyl 1,2,3,4-tetrahydro-4-indol-3-ylcarbazole-1-carboxylate* (7; R = Me) was a buff solid, m.p. 183—187° (13 mg) (Found: M^+, 344.1521. $C_{22}H_{20}N_2O_2$ requires *M*, 344.1524), $\lambda_{max.}$ (MeOH) 224, 282, and 290 nm (log ε 4.88, 4.30, and 4.24), δ ($CDCl_3$) 2.00—2.56 (4 H, m, CH_2·CH_2), 3.73 (3 H, s, OMe), 4.02 (1 H, m, Ar_2CH·CH_2), 4.38 (1 H, dd, *J* 5 and 6 Hz, CH_2·CH·CO_2Me), 6.78 (1 H, d, *J* 2 Hz, indole H-2), 6.90—7.65 (8 H, m, ArH), and 7.70br and 7.98br (each 1 H, NH), *m/e* 345(5%), 344(30), 286(7), 285(50), 283(7), 169(7), 168(100), 167(10), and 132(5). The less mobile *epimer* (12 mg) (Found: M^+, 344.1521) [still containing 5% of the other epimer (n.m.r.)] showed the same $\lambda_{max.}$ and mass spectrum as the other epimer, and δ ($CDCl_3$) 1.98—2.54 (4 H, m, CH_2·CH_2), 3.76 (3 H, s, OMe), 4.06 (1 H, t, *J* 6 Hz, Ar_2CH·CH_2), 4.48 (1 H, m, CH_2·CH·CO_2Me), 6.71 (1 H, d, *J* 2 Hz, indole H-2), 6.96—7.60 (8 H, m, ArH), and 7.65br and 8.04br (each 1 H, exchanged with CD_3OD, NH).

The less mobile ester (12 mg) in dioxan (4 ml) was boiled under reflux with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (50 mg) for 15 min in the dark; the product was poured into ethyl acetate (20 ml), and the intensely blue fluorescent solution was extracted twice with an aqueous solution (20 ml) containing sodium sulphite (0.5 g) and sodium hydroxide (50 mg). After washing, removal of solvent, and gel filtration (Sephadex LH-20 in methanol), *methyl 4-(indol-3-yl)carbazole-1-carboxylate* (8a) crystallised from chloroform–methanol in pale yellow needles (8 mg), m.p. 222—223° (Found: M^+, 340.1209. $C_{22}H_{16}N_2O_2$ requires *M*, 340.1211), $\lambda_{max.}$ (MeOH) 224, 256, 273, 313, and 368 nm (log ε 4.93, 4.47, 4.56, 4.36, and 4.24), $\nu_{max.}$ (KBr) 3 436, 3 318, 1 700, and 1 602 cm^{-1}, δ ($CDCl_3$) 4.10 (3 H, s, OMe), 7.08—7.73 (9 H, m, ArH), 7.99 (1 H, d, *J* 7 Hz, ArH), 8.93 (1 H, d, *J* 7 Hz, ArH) (but *not* coupled to signal at 7.99), and 8.73br and 9.35br (each 1 H, exchanged with CD_3OD, NH), *m/e* 341(20%), 340(100), 339(5), 309(10), 281(8), 280(10), 279(6), 170(5), and 140(11). Similar treatment of the more mobile ester (1 mg) gave the same product (t.l.c.).

Pyrolysis of Diethyl(indol-3-ylmethyl)ammonium Chloride (11).—The tertiary amine was prepared from indole, formaldehyde, and diethylamine under Mannich conditions,[14] as rods, m.p. 105—107° (from acetone) (lit.,[18] 105°). The *hydrochloride* (11) crystallised from methanol–ether as leaflets, decomp. 165—168° (Found: C, 65.3; H, 8.3; Cl, 14.7; N, 11.7. $C_{13}H_{19}ClN_2$ requires C, 65.4; H, 8.0; Cl, 14.85; N, 11.7%), *m/e* 260(0.1%), 259(0.1), 258(0.1), 257(0.1), 256(0.1), 245(0.2), 219(0.5), 203(0.5), 202(5), 201(0.7), 187(1.5), 131(10), 130(100), 129(3), 117(0.6), 113(2), and 112(0.5). The hydrochloride (21 g) in dimethyl sulphoxide (200 ml) was heated in an oil-bath at 180 °C, with rapid stirring, until a sample gave a thick, curdy precipitate with water (*ca.* 7 min). The solution was poured into water (800 ml) and the resulting suspension was filtered. The solid was dissolved in acetone (100 ml) to which ether (600 ml) was added, precipitating a polymeric brown gum. The solvents were removed, and the residue was chromatographed in subdued light on a column of silica gel in chloroform–light petroleum (b.p. 60—80 °C) (3 : 1); 30 ml fractions were collected.

The mother liquor from fraction 8 (below) was combined with fractions 6 and 7 and concentrated to give a pink solid which crystallised from methanol to give di-indol-3-ylmethane (12) (550 mg, 5.0%), m.p. 164—166°, identical (t.l.c., n.m.r., and mixed m.p.) with authentic material; δ [$(CD_3)_2CO$] 4.20 (2 H, t, *J* 0.9 Hz, CH_2), 6.88—7.68 (10 H, m, ArH), and 9.8br (2 H, exchanged with CD_3OD, NH); *m/e* 247(10%), 246(100, M^+), 245(95), 243(5), 217(5), and 130(8).

Fractions 8—12 deposited *5,6,11,12,17,18-hexahydrocyclonona*[1,2-b:4,5-b′:7,8-b″]*tri-indole* (13) as a crystalline solid (256 mg, 2.2%), which was purified further by fractional precipitation from acetone with chloroform, and crystallised from pyridine–methanol as needles, decomp. >270° (Found: C, 83.8; H, 5.5; N, 10.6%; M^+, 387.1738. $C_{27}H_{21}N_3$ requires C, 83.7; H, 5.5; N, 10.8%; *M*, 387.1735), $\lambda_{max.}$ (MeOH) 230, 284, and 292 nm (log ε 5.01, 4.50, and 4.45), $\nu_{max.}$ (KBr) 3 400, 1 620w, 1 463, and 1 435 cm^{-1}, δ_H [$(CD_3)_2SO$] (*a*) at 100 °C, 4.12br (6 H, s, $W_{\frac{1}{2}}$ 5 Hz, CH_2), 6.80—7.00 (6 H, m, ArH), 7.10—7.26 and 7.65—7.80 (each 3 H, m, ArH), and 10.43br (3 H, exchanged with CF_3·CO_2D, NH), (*b*) at 15 °C, 3.93 (2.5 H, d, *J* 14 Hz, CH_2), 4.69 (3.5 H, d, *J* 14 Hz, CH_2), 6.80—7.00 (6 H, m, ArH), 7.10—7.26 and 7.83—8.06 (each 3 H, m, ArH), and 10.70br (3 H, NH), (*c*) in $(CD_3)_2CO$ at −5 °C, 3.98 (1.0 H, s, CH_2), 4.11 (2.5 H, d, *J* 14 Hz, CH_2), 4.85 (2.5 H, d, *J* 14 Hz, CH_2), 6.90—7.60 (9 H, m, ArH), 7.83—7.98 (3 H, m, ArH), and 10.20br (3 H, NH), δ_C [$(CD_3)_2CO$; 40 °C] (proton-decoupled) 22.03, 108.73, 111.20, 118.22, 119.32, 121.14, 129.46, 135.46, and 135.96; *m/e* 388(15%), 387(100, M^+), 386(12), 385(5), 373(5), 372(30), 270(13), 269(20), 258(14), 257(100), 256(100), 255(20), 245(5), 243(25), and 130(15).

Fractions 16—19 gave a gum which was purified by repeated gel filtration in methanol in subdued light. The resulting gum (16) (320 mg, 2.9%) was homogeneous (t.l.c.) but would not crystallise; δ [$(CD_3)_2CO$] 4.25 and 4.32 (each 2 H, s, CH_2), 6.80—7.75 (14 H, m, ArH), and 9.35br, 9.47br, and 9.60br (each 1 H, NH); *m/e* 375(13%, M^+), 259(12), 258(100), 257(95), 256(20), 245(7), and 130(10). The trimer (16) (76 mg) in methanol (2 ml) containing formaldehyde (40% aq.; 2 drops) and concentrated sulphuric acid (1 drop) was boiled under reflux for 2 min. T.l.c. showed the presence

[18] H. Hellman, *Z. physiol. Chem.*, 1949, **284**, 163.

of the trimer (13), di-indol-3-ylmethane (12), and many other compounds.

Crystal Structure of 6-Bromohypaphorine.—The cell dimensions and symmetry were determined by oscillation and Weissenberg photographs (Cu-K_α radiation) and refined and confirmed on a Hilger–Watt four-circle diffractometer.

Crystal data. $C_{14}H_{17}BrN_2O_2$, $M = 325$. Orthorhombic, $a = 12.452(2)$, $b = 10.625(1)$, $c = 10.602(1)$ Å, $U = 1\,403$ Å^3, $D_m = 1.54$, $D_c = 1.539$ g cm^{-3}, $Z = 4$, $F(000) = 664$. Space group $P2_12_12_1$ (from systematic absences); μ(Mo-K_α) $= 31.3$ cm^{-1}.

Intensity measurements were made on a crystal of approximate dimensions $0.2 \times 0.25 \times 0.2$ mm, rotating about the c axis, by 2θ–ω scans out to $\theta = 25°$ (Mo-K_α radiation, graphite monochromator). Each measurement of a reflection was followed immediately by the measurement of the Friedel pair of that reflection; 1 086 reflections with net count $>3\sigma$ were deemed observed.

The structure was solved by the heavy atom method. Intensities were corrected for Lorentz and polarisation effects but not for absorption. The structure was refined by the block-diagonal least-squares method with anisotropic temperature factors to an R value of 6.6% with no correction for anomalous dispersion. At this stage anomalous dispersion corrections were introduced and calculation for the two enantiomers gave values for R of 6.0 and 7.7%. The configurational assignment based on these results was confirmed by comparison of the relative intensities of 45 appropriate reflections and their Friedel pairs, all of which differed in the correct sense. The configuration of 6-bromohypaphorine was thus established as being that of the usual naturally occurring amino-acids.

Refinement was continued with anomalous dispersion correction and with all the hydrogen atoms (located from a difference map) included. Final refinement was performed by full-matrix least-squares with hydrogen refined isotropically and the heavier atoms anisotropically. A weighting analysis showed that unit weights were appropriate. At convergence R was 0.04.

Table 1 shows the final atom co-ordinates and Table 2 gives the bond angles not involving hydrogen. Standard deviations of dimensions not involving hydrogen lay in the ranges 0.006—0.009 Å and 0.38—0.58°. Dimensions involving hydrogen were all within 3σ of expected values with the exception of H(C5) which was shorter than expected, presumably owing to errors caused by the proximity of the bromine atom. Figure 3 shows the crystallographic numbering and the bond-lengths not involving hydrogen. Observed and calculated structure factors and thermal parameters are listed in Supplementary Publication No. SUP 22032 (9 pp.).†

Computations were carried out by using the X-Ray '70 programs adapted for the ICL 1906A computer.

† For details of Supplementary Publications see Notice to Authors No. 7, *J.C.S. Perkin I*, 1976, Index issue.

TABLE 1

Fractional co-ordinates of atoms (H $\times 10^3$, others $\times 10^4$) with standard deviations in parentheses; hydrogen atoms are numbered with reference to the atom to which they are attached

Atom	x/a	y/b	z/c
Br(1)	4 001(1)	8 660(1)	5 847(1)
N(1)	5 848(4)	5 423(4)	2 724(4)
C2	6 503(4)	4 607(5)	2 913(6)
C3	6 748(4)	4 270(4)	4 138(6)
C4a	6 196(4)	5 267(5)	4 808(5)
C4	6 074(5)	5 603(3)	6 081(5)
C5	5 455(5)	6 631(6)	6 357(6)
C6	4 923(4)	7 291(6)	5 410(6)
C7	5 022(4)	6 994(5)	4 177(6)
C1a	5 645(4)	5 953(5)	3 860(6)
H(C2)	683(5)	380(6)	238(5)
H(C4)	646(5)	517(5)	671(6)
H(C5)	537(5)	698(7)	699(7)
H(C7)	464(4)	750(5)	355(5)
H(C8)	785(4)	293(4)	414(5)
H(C8)′	780(5)	361(7)	534(6)
H(C9)	633(4)	251(4)	599(4)
H(C11)	747(7)	191(8)	780(8)
H(C11)′	846(6)	236(7)	699(7)
H(C11)″	853(5)	105(6)	757(5)
C8	7 449(4)	3 268(5)	4 694(6)
C9	6 798(4)	2 175(5)	5 225(5)
C10	6 084(4)	1 592(4)	4 175(5)
N2	7 505(3)	1 155(4)	5 831(5)
C11	7 995(6)	1 623(6)	7 026(6)
C12	8 421(5)	757(6)	4 985(8)
C13	6 827(5)	19(6)	6 123(7)
O1	5 106(3)	1 638(4)	4 381(4)
O2	6 543(3)	1 184(5)	3 267(6)
H(N1)	553(4)	568(5)	211(5)
H(C12)	806(5)	44(6)	408(7)
H(C12)′	894(5)	142(6)	474(5)
H(C12)″	872(4)	13(5)	538(5)
H(C13)	672(7)	−22(8)	522(8)
H(C13)′	627(5)	34(5)	668(6)
H(C13)″	730(6)	−57(6)	663(6)

TABLE 2

Bond angles not involving hydrogen, in degrees, with standard deviations in parentheses

Atoms	Angle	Atoms	Angle
C1a–N1–C2	108.2(5)	C7–C1a–C4a	120.5(5)
N1–C2–C3	111.1(5)	C4a–C1a–N1	109.0(4)
C2–C3–C4a	107.7(5)	C3–C8–C9	112.1(4)
C2–C3–C8	126.2(5)	C8–C9–C10	110.2(4)
C8–C3–C4a	127.1(5)	C8–C9–N2	112.7(4)
C3–C4a–C1a	104.9(5)	N2–C9–C(10)	110.1(4)
C3–C4a–C4	135.2(5)	C9–C(10)–O1	114.9(5)
C1a–C4a–C4	119.9(5)	C9–C10–O2	116.9(4)
C4a–C4–C5	118.0(5)	O1–C10–O2	128.2(5)
C4–C5–C6	121.1(6)	C9–N2–C11	110.7(4)
C5–C6–C7	122.4(6)	C9–N2–C12	112.5(5)
C5–C6–Br1	119.7(5)	C9–N2–C13	109.3(4)
Br1–C6–C7	117.9(5)	C11–N2–C12	106.8(5)
C6–C7–C1a	118.0(6)	C11–N2–C13	108.8(5)
C7–C1a–N1	130.5(5)	C12–N2–C13	108.7(4)

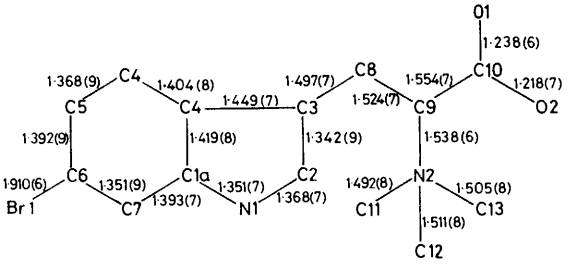

FIGURE 3 Crystallographic numbering and bond lengths for 6-bromohypaphorine

We thank Dr. J. H. Gough for preliminary work on the sponge extracts, and Professor C. Djerassi for a sample of 24-methylenecholest-4-en-3-one; we are particularly grateful to Dr. N. S. Jones, Marine Biological Station, Port Erin, for collecting *Pachymatisma johnstoni*. One of us (W. D. R.) thanks the Commissioners for the Exhibition of 1851 for a Scholarship.

[6/2080 *Received*, 11*th November*, 1976]