The Alkaloids of Perennial Rye-Grass (*Lolium perenne* L.). Part IV.[1] Isolation of a new Base, Perlolyrine; the Crystal Structure of its Hydrobromide Dihydrate, and the Synthesis of the Base

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A new alkaloid, perlolyrine, 1-(5-hydroxymethyl-2-furyl)-9*H*-pyrido[3,4-*b*]indole (6b), has been isolated from *Lolium perenne* L. (Graminae). The structure of the base was revealed by an *X*-ray analysis of its hydrobromide dihydrate $C_{16}H_{12}N_2O_2$,HBr,$2H_2O$ (space group $P\bar{1}$, a = 7·81, b = 10·55, c = 10·55 Å, α = 79·2 β = 72·9, γ = 78·8°), by use of the heavy-atom method, assisted by spectroscopic data. The final value of R over 2360 reflections is 0·129; an attempt to refine the structure in space group $P1$ failed. The alkaloid has been synthesised from tryptophan and 5-acetoxymethyl-2-formylfuran. The 3,4-dihydro- and 1,2,3,4-tetrahydro-derivatives have also been synthesised, starting from tryptamine. The ways in which perlolyrine and its reduced derivatives break up on electron impact are discussed. Perlolyrine is shown to be present in the dried grass, and is not an artefact of the working-up process.

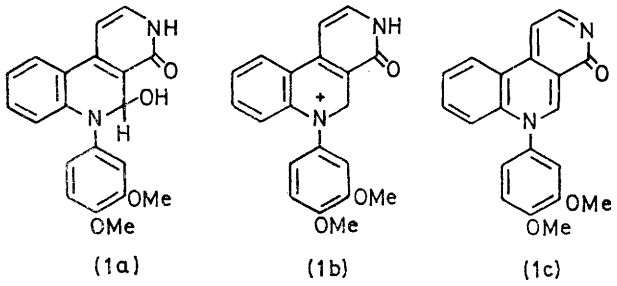
PAPER chromatography of the bases from perennial rye-grass showed that, in addition to the previously known perloline and perlolidine, several other fluorescent materials were present.[2] Their locations on the chromatogram were described by a series of 'Zone Numbers', and the material responsible for the fluorescence of Zone 4 is the subject of this paper; the name perlolyrine is suggested.

Several methods were tried for isolating the base on a preparative scale. Preparative paper chromatography proved impractical. Partition chromatography on a column, with an ethyl acetate–acetic acid–water mixture similar to that used for paper chromatography, followed by adsorption chromatography over alumina, gave enough of the base for its m.p. to be determined, and to allow recognition of the characteristic u.v. absorption spectrum of its solution in acid. However, as a general method for separating the bases present in the grass the process had two disadvantages. Though the dark-coloured bases were concentrated in the leading portions of the eluate, the portions of the eluate containing perlolidine and perlolyrine were coloured by them; and, although perlolidine (Zone 3), perlolyrine (Zone 4), and perloline (Zone 8) could be recognised on the column, it was difficult to relate regions on the column between the bands due to perlolyrine and perloline with zone numbers on a paper chromatogram. Extraction of the crude mixture of bases from solution in ether–methylene chloride using a series of buffers of progressively decreasing pH [nominal values 7, 6, . . ., −1 (*i.e.* concentrated hydrochloric acid)], followed by paper chromatography of each fraction, showed that each buffer removed a mixture of bases. After counter-current distribution (100 transfers) between the phases of an ethyl acetate–acetic acid–water mixture (5-1-5, v-v-v), every tube contained some of the dark tarry bases. However, a series of counter-current distributions between a series of aqueous buffers of progressively decreasing pH and a n-butanol–toluene mixture gave efficient separation. The process was monitored by u.v. spectroscopy, and in the first runs the spectra of the control fractions were examined over the range 200—450 nm. These gave some information about the behaviour and possible structures of other bases in the extracts of the grass. However, as the final isolation of perlolyrine was made from material that had been repeatedly used for trial separations, artefacts could have been present too; therefore, experimental details of the isolation are restricted to those that concern perlolyrine. During the trials at isolation it became clear that the base was unstable to concentrated acid, though only after much had been lost.

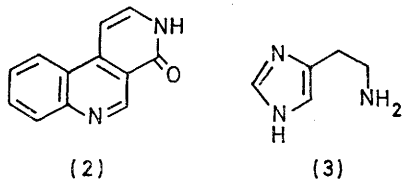
The small quantity of perlolyrine available meant that only physical methods could be used to investigate its structure. At the time, as now, the alkaloids of known structure occurring in the various *Lolium* species were perloline [base, (1a); cation, (1b), perlolidine (2), and histamine (3) in *L. perenne* L.,[1,3,4] annuloline (4) in *L. annuum* L. (Italian, or annual rye grass),[5] and loline (5a), lolinine (5b), and norloline (5c) in *L. cuneatum* L.[6]. The u.v. spectra of perlolyrine as the free base or the protonated cation were different from all the alkaloidal spectra listed by Sangster and Stewart,[7] including those of perloline, perlolidine, and annuloline. The distribution of the alkaloid between an organic phase and aqueous buffers of various pH values showed it to be a relatively weak base. The u.v. spectra of solutions of the base containing sodium hydroxide or triethylamine were identical, showing the base did not possess either a phenolic hydroxy-group, or the imino-group of a 2- or 4-pyridone. It was not possible to purify the free base adequately for mass spectrometry, so the fragmentation pattern was determined by addition of excess of triethylamine to a solution of the purified hydrobromide in methanol, and evaporation of the solution to dryness on

[1] Part III, M. A. Akhtar, W. G. Brouwer, J. A. D. Jeffreys, C. W. Gemenden, W. I. Taylor, R. N. Seelye, and D. W. Stanton, *J. Chem. Soc.* (*C*), 1967, 859.
[2] J. A. D. Jeffreys, *J. Chem. Soc.*, 1964, 4504.
[3] J. A. D. Jeffreys, G. A. Sim, R. H. Burnell, W. I. Taylor, R. E. Corbett, J. Murray, and B. J. Sweetman, *Proc. Chem. Soc.*, 1963, 171; G. Ferguson, J. A. D. Jeffreys, and G. A. Sim, *J. Chem. Soc.* (*B*), 1966, 454.
[4] H. D. Fowler, *Nature*, 1962, **193**, 582.
[5] R. S. Karimoto, B. Axelrod, J. Wolinsky, and E. D. Schall, *Phytochemistry*, 1964, **3**, 349.
[6] S. T. Akramov and S. Yu. Yunusov, *Chem. Natural Compounds*, 1965, **1**, 203, and references cited; *cf.* S. G. Yates and H. L. Tookey, *Austral. J. Chem.*, 1965, **18**, 53.
[7] A. W. Sangster and K. L. Stuart, *Chem. Rev.*, 1965, **65**, 69.

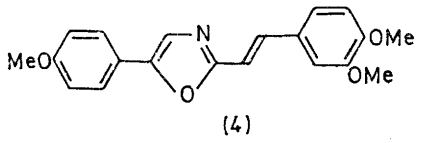
the probe of the mass spectrometer. An organic base was used to prevent permanent contamination of the probe, and a tertiary aliphatic amine was chosen as this was both a stronger base than perlolyrine and incapable of addition across a double bond. Perloline, and its



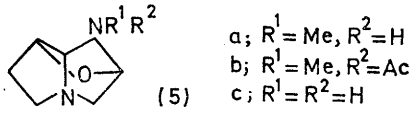
(1a) (1b) (1c)



(2) (3)



(4)

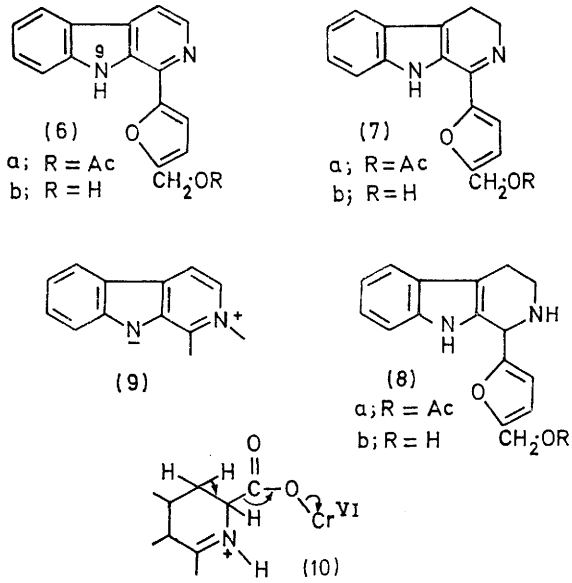


(5)

ethers, in solution are in equilibrium with the anhydronium base (1c) and water, or an alcohol.[2] Corresponding addition of an amine to an anhydronium base would give a species with an additional, non-skeletal nitrogen atom in the molecule; and such a species, if present, would be much more confusing than the corresponding one with the elements of an extra molecule of ethanol.

High-resolution mass-spectrometry revealed the molecular formula (M) of the base as $C_{16}H_{12}N_2O_2$; the fragmentation pattern showed ready loss of OH (peak for ion M — OH, and metastable peak), and ions corresponding to M — CHO, M — CH_3O, and M — C_2H_3O. Thus the molecule contained a hydroxy-group; and the peak for M — CHO suggested it contained a furanoid ring. The molecular formula revealed that the alkaloid was not produced by a variant of the biogenetic processes that give perloline or perlolidine; and, as it was an aromatic compound and thus likely to undergo extensive rearrangement before fragmentation, detailed study of the mass spectrum was judged unprofitable, and the structure was determined by *X*-ray crystallography (see later) which showed the base to be 1-(5-hydroxymethyl-2-furyl)-9*H*-pyrido[3,4-*b*]indole (6b).

This base, its 3,4-dihydro-derivative (7b), and its 1,2,3,4-tetrahydro-derivative (8b) have been synthesised from 5-acetoxymethyl-2-formylfuran (9a) and tryptophan (for perlolyrine) or tryptamine (for the reduced derivatives), by acid-catalysed ring-closure followed by oxidation where necessary and hydrolysis. The ring closure is formally analogous [8] to a Pictet–Spengler reaction.[9] [Such a reaction is reported [10] between tryptamine and 2-formylfuran to form a yellow product in low yield; as (8a) and (8b) are colourless, it is probably not that recorded in the literature, but the corresponding 1,2-dehydro-compound.]



(6) (7) (8) (9) (10)

Tryptophan and 5-acetoxymethyl-2-formylfuran reacted in acetic acid to give the ring-closed product [as (8a), but with a hydroxycarbonyl function at position 3] which was not isolated, but oxidised with acidified dichromate. This caused aromatisation of the newly formed ring and simultaneous loss of carbon dioxide; the conditions resemble those used in the conversion of tryptophan into harman.[11] The oxalic acid added during the working-up process forms a weak complex with the chromium(III) ions and, by holding the chromium temporarily in solution in the alkaline liquid, facilitated the isolation of the products. The process gave a mixture of the acetyl derivative (6a) of perlolyrine, and a smaller quantity of perlolyrine itself; their separation is described in the Experimental section. In addition, a variable but small quantity of a base was produced whose colours in solution and solubility relations [solubility in ether (red solution) < in water (yellow solution) < in chloroform (orange solution)] show it is an

[8] A. H. Jackson, B. Naidoo, and P. Smith, *Tetrahedron*, 1968, **24**, 6119.

[9] W. M. Whaley and T. R. Govindachari, 'The Pictet-Spengler Synthesis of Tetrahydroisoquinolines and Related Compounds,' in 'Organic Reactions,' John Wiley and Sons, Inc., New York, 1951, vol. VI.

[10] G. Hahn, L. Bärwald, O. Schales, and H. Werner, *Annalen*, 1935, **520**, 107.

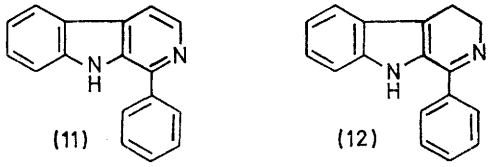
[11] W. O. Kermack, W. H. Perkin, and R. Robinson, *J. Chem. Soc.*, 1921, 1602.

anhydronium base of partial structure (9), but with at least one more aromatic ring fused on to it. It has not been further examined.

Hydrolysis of the ester (6a) with either acid or base was easy; and if the ester was not isolated, but the crude products from the cyclisation were warmed with dilute acid and the perlolyrine was precipitated as the hydrobromide, the alkaloid could be synthesised in 25—30% yield in 24 hr. Identity of the natural and synthetic bases was confirmed by identity of the three zero-layer *X*-ray diffraction photographs of their hydrobromides. Neither the natural nor the synthetic base melted sharply, apparently owing to the presence of two (or more) crystalline forms. Thus, when the synthetic base was heated rapidly it began to melt at 183° (Kofler); if the heating was interrupted before all the sample had melted and the stage allowed to cool, the molten material solidified. On reheating, at 183° the portion of the base previously unmelted fused first, at 183°, while crystals persisted to 187—190° in the part of the specimen that had melted and resolidified.

Condensation of tryptamine and 5-acetoxymethyl-2-formylfuran gave the acetyl ester of the 1,2,3,4-tetrahydro-derivative (8a) of perlolyrine, and this was hydrolysed to the corresponding alcohol (8b). Oxidation of the ester (8a) with acidified dichromate gave a base, a solution of which in acid had a u.v. spectrum different from that of perlolyrine; this base proved to be (7a). Hydrolysis gave the corresponding alcohol (7b). Oxidation of 1,2,3,4-tetrahydroisoquinolines often stops at the 3,4-dihydro-stage,[12] and generally the presence of a carboxy-group in the 3-position is necessary for the oxidation of derivatives of 1,2,3,4-tetrahydro-9*H*-pyrido-[3,4-*b*]indole to the fully aromatic material.[13,14] Aromatisation involving electron transfer from a carboxylate anion has been described;[15] under the conditions of this work (*ca.* M-sulphuric acid) the carboxy-group is not appreciably ionised, but electron transfer to the chromium *via* a complex with the carboxy-group [*e.g.* as (10)] seems as plausible as transfer *via* the nitrogen atom.[14] Solutions of the reduced compounds (8a) or (8b) turned red on exposure to light and air, and a solution in acid of the product, after being washed with ether, had an absorption maximum at 390 nm., indicating the presence of the dihydro-compound, (7a) or (7b) respectively. Analogous oxidations by air have been described.[16]

Spectral Data.—Ultraviolet spectra. Figure 1 shows the spectra of the alcohols (6b) and (7b) in dilute acid, and as the free bases; the spectrum of (7b) in water containing 5% by volume of methanol resembled that of the base in dilute acid. The spectra of (6b) and (7b) resemble those of their analogues with a benzenoid ring at the 1-position; $\lambda_{max.}$ (log ε): for (11) (free base in



ethanol; log ε not given) 238, 277, 294, 347, and 360;[17] for (12) (free base in ethanol), 231 (4·33), 247 (4·38), and 323 (4·12); and for (12) (perchlorate in ethanol), 253 (4·04), 276 (3·94), 287 (3·92), and 368 (4·34) nm.[18]

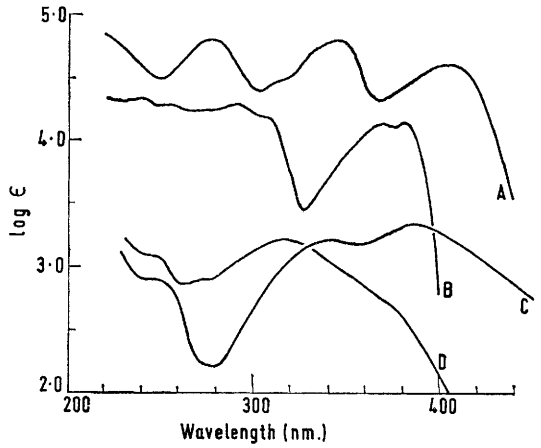


FIGURE 1 Absorption spectra of (6b) in M-sulphuric acid (A), and in methanol (B); and of (7b) in 2M-hydrochloric acid containing 5% of methanol (C), and in 0·1M-sodium hydroxide solution containing 5% of methanol (D). For curves (C) and (D), log ε (true) = log ε (graph) + 1·0. For curve (A), log ε (true) = log ε (graph) −0·5.

^1H *N.m.r.* The assignments of the signals to specific protons are made by comparison with data published by Varian[19] and with the results for some 1-aryl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indoles.[20] The shielding effect of N(2) on the proton attached to C(3′) seen for compounds (8a) and (8b) occurs with 2-aminomethylfuran. The presence of electron-withdrawing substituents at the 2′-position causes slight deshielding of the proton on C(4′), and a much larger downfield shift for the proton on C(3′); for compound (7b) the doublet expected for the latter proton cannot be identified among the signals from the aromatic protons. For compound (8a) and (8b) the signals from the protons on C(4) form a partly resolved multiplet centred near τ 6·85,

[12] H. T. Openshaw and H. C. S. Wood, *J. Chem. Soc.*, 1952, 391; N. J. Leonard and G. W. Leubner, *J. Amer. Chem. Soc.*, 1949, **71**, 3408.
[13] Th. Wieland and E. Neeb, *Annalen*, 1956, **600**, 161.
[14] A. P. Gray, E. E. Spinner, and C. J. Cavallito, *J. Amer. Chem. Soc.*, 1954, **76**, 2792.
[15] L. F. Fieser and M. J. Haddadin, *J. Amer. Chem. Soc.*, 1964, **86**, 2392.
[16] R. H. F. Manske, 'The Carboline Alkaloids' in 'The Alkaloids,' Academic Press, New York and London, 1965, vol. VIII, p. 47.
[17] B. T. Ho, W. M. McIsaac, W. Tansey, and K. E. Walker, *Canad. J. Chem.*, 1967, **45**, 2963; *cf.* C. K. Bradsher and E. F. Litzinger, jun., *J. Heterocyclic Chem.*, 1964, **1**, 168.
[18] A-M. M. E. Omar and S-i. Yamada, *Chem. and Pharm. Bull. Japan*, 1966, **14**, 856; almost identical figures are given by Y. Kanaoka, E. Sato, and Y. Ban, *ibid.*, 1967, **15**, 101.
[19] N. M. R. Spectra Catalog by N. S. Bhacca, L. F. Johnson, and J. N. Shoolery, Varian Associates, Palo Alto, California, 1962.
[20] W. A. Skinner and R. M. Parkhurst, *Canad. J. Chem.*, 1965, **43**, 2251.

while those on C(3) generate a broad singlet centred near τ 7·1; the whole region integrates for 4 protons for (8a), and for 5 protons for (8b). For the dihydro-compounds, (7a) and (7b), the signals due to the protons on C(3) and C(4) are similar, but the low solubility of (7b) caused it to give a poor record; those for (7a) appear symmetrical about τ 6·49, and resemble spectrum No. 5—20 for an A_2B_2 system in ref. 21. With the parameters H[C(3)] τ 5·99, H[C(4)] τ 6·99,[22] $J_{3,4}$ 42 Hz, $J_{AA'} = J_{AB} = 7$ Hz, $J_{BB'} = J_{AB'} = 14$ Hz, the high-field signals are observed (calculated) at: τ 6·86 (6·78, 6·81); 6·98 (7·01); 7·06 (7·08); 7·09 (7·11); 7·28 (7·26); and 7·34 (7·30).

Mass spectra. The patterns of break-up can be divided into two groups, those not involving loss of nitrogen, and those in which nitrogen is lost. The first group includes the degradations in which only the furanoid part of the molecule is affected. For the alcohols, the chief modes of fragmentation for which simple lines of fission can be drawn are given in Figure 2, and involve

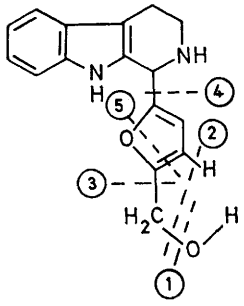


FIGURE 2 Possible modes of fragmentation of 1-(5-hydroxymethyl-2-furyl)-9*H*-pyrido[3,4-*b*]indole (6b)

loss of (1) HO; (2) H_2O; (3) CH_3O; (4) $C_5H_5O_2$; and (5) $C_2H_3O_2$. For their acetates, the fragment lost in the corresponding process with the same serial number contains the additional atoms C_2H_2O, *e.g.* process (1) involves loss of $C_2H_3O_2$. For these esters an ion (M — $C_2H_3O_2$) can be produced in two ways; either the loss of the fragment $C_2H_3O_2$, or (6), a process not listed above, loss of keten to give the alcohol which then loses HO. In the mass spectrum of 5-acetoxymethyl-2-formylfuran the strongest peak is due to the ion formed by loss of keten. The esters (6a), (7a), and (8a) each give a peak of the correct whole-number mass for the corresponding alcohol, but of low abundance; and the presence in the mass spectrum of (6a) of a metastable peak corresponding to the loss of an acetoxyl radical is evidence that for the esters process (1) is more important than process (6). Additionally, the esters can lose the acetyl cation C_2H_3O [process (7)]. The reduced compounds (7a), (7b), (8a), and (8b) can lose hydrogen, ultimately giving the fully aromatic system [(6a) or (6b) as appropriate], or one of its degradation products. Two other ions in the spectra of the alcohols are produced by rearrangement,[23] and involve loss of (8) CHO, and (9) CH_2O_2; metastable peaks in the spectrum of compound (6b) show that process (9) involves successive loss of H_2O and CO. Table 1 summarises the occurrences of these modes of fissure.

Most of the fragment ions shown by accurate mass-measurement to contain less than two nitrogen atoms have also lost oxygen, and the only simple and plausible process recognisable is loss of CH_3N, as $H_2C{=}NH$, from compounds (8a), and (8b). In their mass spectra are peaks for ions resulting both from simple loss of this fragment, and from loss of this species accompanied by one of the other processes numbered above. The results are summarised in Table 2.

TABLE 1

Fragmentations, not involving loss of nitrogen, of compounds (6a), (6b), (7a), (7b), (8a), and (8b)

Process no.	Compound (6a)	(6b)	(7a)	(7b)	(8a)	(8b)	(10)
1	M^+, M^{++}	*, M^+	+	+	—	M^+	M^+
2	*, M^+, M^{++}	*, M^+	M^+	+	*, M^+	M^+	—
3	M^+	M^+	M^+	M^+	M^+	M^+	M^+
4	M^+	M^+	+	—	$M^{+\,a,b}$	$M^{+\,b}$	—
5	M^+	M^+	+	M^+	—	—	
6	—	n.a.	—	n.a.	—	n.a.	*, +
7	M^+	n.a.	M^+	n.a.	M^+	n.a.	
8	M^+	M^+	—	M^+	+	M^+	n.a.
9	M^+	M^+	—	M^+	—	—	n.a.

[a] Also for [(4) + loss of H_2]. [b] Also for [(4) + loss of $2H_2$].

M High-resolution mass-measurement on ion, the charge of which is indicated.

* Metastable peak observed for formation from parent molecule.

+ For ion of appropriate whole-number mass, ion current >10% of ion current for parent ion. — As above, but ion current <10% of that for parent ion. n.a. Not applicable.

TABLE 2

Loss of unit $H_2C{=}NH$ alone, or accompanied by other fragmentation processes *

Compound	Alone	Process (1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
(8a)	M^+	M^+	M^+	M^+		M^+	(1)	M^+		M^+
(8b)	$M^{+\,a}$	M^+		M^+		M^+	n.a.	n.a.		M^+

* Symbols as in Table 1.

[a] Also for [(4) and loss of H_2].

In addition, the spectrum of the alcohol (7b) shows ions formed by loss of CH_3N, and $CH_3N + C_2H_3O_2$, [*i.e.* accompanied by process (5)]; a low peak in the spectrum of (7a) occurs at the correct whole-number mass for loss of CH_3N. For the aromatic compounds the process corresponding to loss of CH_3N is loss of HCN; in the spectra of (6a) and (6b) there is a low peak due to an ion of the correct whole-number mass for loss of HCN + HO, and accurate mass-measurement discloses an ion from (6a) formed by loss of HCN + $C_2H_2O_2$ [process

[21] K. B. Wiberg and B. J. Nist, 'The Interpretation of N.M.R. Spectra,' Benjamin Inc., New York, 1962, p. 327.

[22] *Cf.* K. T. Potts, M. Kanaoka, T. H. Crawford, and J. W. Thomas, *J. Heterocyclic Chem.*, 1964, **1**, 297.

[23] *Cf.* G. Spiteller, *Adv. Heterocyclic Chem.*, 1966, **7**, 301.

(9)], and one from (6b) produced by loss of HCN + $C_5H_5O_2$ [process (4)].

Presence of Perlolyrine in the Grass.—The structure of perlolyrine revealed its genesis from tryptophan and 5-hydroxymethyl-2-formylfuran, but left uncertain whether the base was truly present in the dried grass, or produced through the action of acids and oxygen during the process of isolation and separation of the alkaloids. Using the synthetic base a reliable way was developed to isolate small quantities of the base, and to identify it by the *X*-ray powder diagram of its mercurichloride. A sample of the grass growing wild in Glasgow was collected in June 1968, dried in air at room temperature, milled, and extracted with benzene containing triethylamine; the subsequent treatment of the extract and isolation of the base are described in the Experimental section. It was considered that the process would give an extract free of proteins, amino-acids, and carbohydrate, and that the alkaline conditions used would inhibit both the coversion of any sugars into 5-hydroxymethyl-2-formylfuran, and the possible formation of perlolyrine or its precursors from any of this aldehyde already in the grass. Subsequent work has shown that the process does not remove the alkaloids efficiently from the grass. From the extract a small quantity of base was isolated which, after precipitation with mercury(II) chloride, gave an *X*-ray powder photograph matching that from synthetic perlolyrine so treated. Thus perlolyrine is not an artefact. It has also been identified among the alkaloids from two other samples of this grass, one of which contained only traces of perloline.

In 1960 a large-scale extraction of hay from this grass was made,[2] and the concentrated extract stored in sealed jars; the contents separated into a fluid aqueous layer and a gelatinous lipoid phase in roughly equal amounts. As far as possible, aliquot portions of these layers were taken from an untouched jar, and examined for perlolyrine, and an order-of-magnitude figure derived for the concentration of the base in this sample of hay. For the counter-current distribution of the bases (Run no. 37) the relevant figures are:

Tube no.	60	65	70
Extinction (1 cm.) at 407 nm.	0·27	0·33	0·19
Quantity (mg.) of perlolyrine in tube	0·136	0·162	0·096

To the degree of accuracy appropriate, the concentraton maximum can be taken at tube no. 65, which ideally would contain 8% of the base in the train of tubes.[24] As the quantity of extract used corresponded to *ca.* 2 kg. of hay, the figures give a concentration of the base of *ca.* 1 p.p.m. This figure is judged to be low, perhaps by a factor of 2, taking into account some sources of error, namely: overestimation if other bases are present which also absorb at 407 nm., underestimation due to loss (see Experimental), and the greater spread of the base through the train of tubes (observed with other materials in the machine used) than simple theory predicts.

The analytical behaviour of the dihydro- and tetrahydro-derivatives, (7b) and (8b), respectively, of perlolyrine has not been studied; and (8b), if present in the hay used for the large-scale extraction, would not have been recognised by the methods used. However, the characteristic absorption spectrum of the dihydro-base (7b), which is of similar intensity to that of perlolyrine, has not been seen among the spectra of the various fractions from the counter-current distributions; and it is unlikely that the concentration of this base in the hay used for the large-scale extraction exceeded 0·1 p.p.m.

EXPERIMENTAL

Spectral measurements: i.r., Perkin-Elmer 125, KCl discs; u.v., Perkin-Elmer 137; ^{1}H n.m.r., Perkin-Elmer R10, at 40 MHz, in deuteriochloroform unless otherwise stated; and mass, AEI MS9. Operations: m.p.s, Kofler block; chromatography, over alumina (Spence D, untreated; 40 ml. of the dry settled powder), with benzene to which 2% of volume of methanol had been added; counter-current distribution, between an aqueous buffer (pH specified; hydrochloric acid–citrate [25] if pH < 3, phosphate citrate [25] otherwise), and a mixture of n-butanol and toluene (1 : 2, by vol.). Unless otherwise specified, basification was done with sodium carbonate, and organic liquids were dried over sodium sulphate. The following operations occur frequently in the text, and when applied to a relatively pure substance, are given in abbreviated form. Extraction of a base from aqueous solution: the solution was, if necessary, rendered alkaline (alkali specified if not sodium carbonate), shaken with an organic solvent (nature, volume, no. of portions), which was washed (liquid, volumes, no. of portions), dried, filtered through sintered glass, and evaporated. Isolation of a base from aqueous solution: the solution was made alkaline, and was shaken with an organic solvent; this latter phase was shaken with aqueous acid, and the base was subsequently extracted, as described above.

Obtaining Perlolyrine from the Grass.—(a) *By partition chromatography.* A solution of the mixed alkaloids was run through a column as described in Part I of this series.[2] The fastest-moving materials were very dark, and were followed first by a band with a blue fluorescence under u.v. light due to perlolidine,[1] then by a band with a greenish-white fluorescence due to perlolyrine. The portions of the eluate containing the latter were shaken with dilute sulphuric acid, the aqueous layer was made alkaline, and the bases were passed successively into methylene chloride, sulphuric acid, and methylene chloride. The final solution was yellow, with a blue fluorescence. Evaporation of this solution gave a brown gum (0·0318 g. from *ca.* 44 kg. of hay, 0·720 mg./kg.) that partly crystallised on treatment with methanol. Paper chromatography (solvent system E) [26] revealed several other bases besides perlolyrine.

(b) *By counter-current distribution.* The bases from the grass, mainly residues from which perloline and perlolidine had been extracted, were placed in tube 0, and 101 transfers

[24] L. C. Craig and D. Craig in ' Technique of Organic Chemistry,' Interscience Publishers Ltd., London, 1955, vol. III, ch. II, pp. 242—332; J. A. D. Jeffreys, *Chem. and Ind.*, 1967, 1594.

[25] H. T. S. Britton, ' Hydrogen Ions,' Chapman and Hall Ltd., London, 4th edn., 1955, vol. I, pp. 353, 356.

[26] H. Schmidt, J. Kebrle, and P. Karrer, *Helv. Chim. Acta*, 1952, **35**, 1864.

(a mis-setting of the counter for 100) carried out. The contents of tubes no. 76 and upward were combined, the layers were separated, and the aqueous phase was basified and extracted with n-butanol (½ vol. of aqueous phase, 3×); this butanol was diluted with methylene chloride (3 vol.), the mixture was washed and added to the upper phase from the counter-current distribution. The combined solutions were evaporated to dryness and the residue was recycled with the next buffer. In successive runs the buffers had nominal pH-values of 6, 4, 3, and 2. For monitoring, after each run the contents of tubes no. $5n$ ($n = 0, 1, 2 \ldots, 15$) were evaporated under reduced pressure till all the organic phase was removed; the aqueous phase was made up to 13 ml. with distilled water, and the bases were isolated (stock, nearly saturated sodium carbonate, 10 ml.; CH_2Cl_2, 25 ml. × 3; H_2O, 13 ml.; stock 1M-sulphuric acid, 10 ml.; ether, 10 ml.). The volumes used are probably not critical, but strict volume control is necessary if the bases are to be located in the train of tubes. Initially, the u.v. spectrum of each sample was recorded over the range 220—390 nm., but for routine purposes measurement of the absorbance at 260 nm. proved adequate. The results of a typical run (No. 34) were:

Tube no.	10	15	20	25	30	35	40	45	50	55	60
100 × ($E_{1\ cm.}$ at 260 nm.)	8	9	21	35	27	25	37	28	17	9	9

The absorption spectrum of tubes 20, 25, and 30 was that of perlolidine; that of tubes 35, 40, 45, and 50, perlolyrine. The relevant runs can be summarised:

Run no.	23	33	37	34	35	28
pH of aqueous phase	3·10	3·13	2·61	1·99	1·94	1·86
100 × R_F of perlolidine	75	70	*	27	*	26
100 × R_F of perlolyrine	*	*	65	43	38	40

* Base not detected.

Perlolyrine in quantity can easily be located, for, under the conditions used, in u.v. light in the tubes containing the base the lower phase has a greenish fluorescence, the upper phase a purple one. No other base in the grass shows this pattern; the tubes containing perlolidine have a blue fluorescence in the lower layer and none in the upper layer.

The contents of the appropriate tubes were combined, concentrated under reduced pressure, and the base was isolated (ether) and chromatographed. Perlolyrine formed a colourless band with a violet fluorescence under u.v. light; the eluate had a blue fluorescence in daylight. Evaporation of this extract gave crystals, but small quantities of the base were better handled by evaporation of the eluate in the presence of a few ml. of 0·1M-hydrobromic acid, and filtration of the hot solution. On cooling, perlolyrine hydrobromide separated. The basic fraction from *ca.* 80 kg. of hay, after being stripped of perloline and perlolidine, then used in trials of methods for separating the other bases, yielded pure perlolyrine hydrobromide (7 mg.). It was known that much had been lost in the working-up process.

Perlolyrine.—Pale yellow crystals were prepared by evaporation of the eluate from a chromatographic column; after washing with ether, these had m.p. 183° (not sharp), subliming from 150°. Exposure of the eluate from the column to strong sunlight caused it to turn yellow, and show a red fluorescence. Salts: the hydrochloride, hydrobromide, and hydriodide can all be recrystallised from dilute (0·1—1M) solutions of the appropriate acid. *Hydrochloride*, yellow needles, m.p. 204—233° (decomp.); *hydrobromide* (a) from dilute hydrobromic acid, yellow to brown blades, stable in air but breaking up on drying at *ca.* 15 mm. over silica gel; on heating, the crystals break up at 70—80°, turn red-brown at 230—240°, lose their birefringence at 255°, and do not melt below 280°; (b) from methanol–ether, brown needles, phase change 150—160°, breaking up with partial melting, 180°, birefringence lost, 240—245°; *hydriodide*, brown prisms breaking up on drying *in vacuo* over silica, losing birefringence at 228°, but not melting below 275°: *mercurichloride* [from mercury(II) chloride solution and a solution of the base in dilute hydrochloric acid], clusters of minute yellow prisms, very sparingly soluble in 2M-hydrochloric acid, losing birefringence at 208°, black but unmelted at 268°.

Fragmentation pattern. Excess of triethylamine was added to a solution of the hydrobromide in methanol, and the mixture was evaporated in air on the probe of the mass spectrometer. The parent ion had *m/e* 264·0903, corresponding to $C_{16}H_{12}N_2O_2$ (calculated 264·0899) and prominent fragmentation peaks at *m/e* 247·0872 ($C_{16}H_{11}N_2O$, 247·0871), 235·0854 ($C_{15}H_{11}N_2O$, 235·0771), 233·0708 ($C_{15}H_9N_3O$, 233·0715), 218, 205·0768 ($C_{14}H_9N_2$, 205·0766), 167, and 147·0493 ($C_{10}H_6N$, 140·0500). A metastable ion *m*/e* 231·09 corresponded to the change 264 ⟶ 247 (calc. *m*/e*, 231·18).

5-Acetoxymethyl-2-formylfuran (cf. *ref.* 27).—Phosphoryl chloride (28 ml., 0·31 mole) was cooled (acetone–carbon dioxide) and when most had solidified, dimethylformamide (2 portions of 5 ml. each) was added with stirring. After an initial rise to 20° the temperature was lowered to −5° and more dimethylformamide (54 ml.) was added. The temperature was allowed to rise during 2 hr. to 5°, then the cooling bath was removed and stirring was continued for 4 hr.; the mixture was cooled, and 2-acetoxymethylfuran [28] (46·6 g., 0·333 mole) was added between −10 and −20°. The mixture was kept below −5° for 1 hr., then allowed to warm spontaneously; 18 hr. later the mixture was poured on to ice (500 g.) and ether (175 ml.), and sodium carbonate (anhydrous 120 g.) in the minimum quantity of warm water was added. After equilibration and separation, the aqueous phase was extracted with ether (100 ml. × 6); the combined ether layers were washed (100 ml. × 3), dried, and evaporated to a black oil (56·3 g.) which later solidified. Extraction with light petroleum (b.p. 40—60°) gave a mixture of crystals and oil; the latter was removed by rapid washing with ether. Recrystallisation from light petroleum (b.p. 40—60°) gave the aldehyde (29·8 g., 60%) as colourless or pale yellow crystals, m.p. 56° (lit., m.p. 55—56°); τ 0·3 (CHO), 2·75 (d, *J* 4 Hz, 3-H), 3·39 (d, *J* 4 Hz, 4-H), 4·85 (H_2C), and 7·90 (Ac).

1-(5-Acetoxymethyl-2-furyl)-9H-pyrido[3,4-b]*indole* (6a).—Stock solutions were made of (i) potassium dichromate (50 g.), concentrated sulphuric acid (40 ml.), water (to 500 ml.), (ii) oxalic acid dihydrate (50 g.) in water (to 250 ml.), (this solution has to be used warm as it is super-saturated at room temperature); and (iii) sodium disulphite (50 g.) in water (to 300 ml.). Tryptophan (1·02 g.), 5-acetoxymethyl-2-formylfuran (0·84 g.), and glacial acetic acid (25 ml.) were heated on a steam bath for 20 min.; solution was complete in a few minutes and after 20 min. the liquid was usually dark brown. It was then poured into a boiling mixture of water (500 ml.) and the dichromate solution (40 ml.). Boiling was continued for 1 min., then the disulphite solu-

[27] Okuzumi, *Nippon Kageku Zasshi*, 1958, **79**, 1371.
[28] *Org. Synth.*, Coll. Vol. I, p. 285.

tion (25 ml.) was added, followed by oxalic acid solution (50 ml.). This was cooled and anhydrous sodium carbonate (50 g.) in water (200 ml.) was added, and the liquid was shaken with ether (100 ml. × 6). The ether solution, usually red or purple with a blue fluorescence in daylight, was washed (water) until the washings, at first yellow with a greenish-blue fluorescence, were colourless. The washed ether extract afforded a variable quantity (0·3—0·4 g.) of basic material consisting of a mixture of the ester (6a) with a smaller quantity of the corresponding alcohol (6b). Prolonging the time of oxidation to 5 min. or the addition of 0·5M-sulphuric acid (5 ml.) to the acetic acid reduced the yield of basic material. Treatment of the crude basic product with ethyl acetate caused the ester to separate first as thin brown tablets, followed some hours later by the alcohol (6b) as radiating masses of needles, yellow if pure but usually dark. Recrystallisation of the tablets from ethyl acetate gave 1-(5-*acetoxymethyl*-2-*furyl*)-9H-*pyrido*-[3,4-b]*indole* as yellow-brown tablets, m.p. 161°, resolidifying on cooling (Found: *m/e*, 306·1002; C, 70·8; H, 4·7; N, 9·2. $C_{18}H_{16}N_2O_3$ requires *M*, 306·1004; C, 70·6; H, 4·6; N, 9·2%); τ 0·1 (HN) 1·57 (d, *J* 4·8 Hz, 3-H), 1·80—3·00, (Ar); 2·82 (d, *J* 4·0, 3′-H); 3·43 (d, *J* 4·0 Hz, 4′-H); 4·78 (6′-H_2), and 7·90 (Ac); ν_{HN}, 330·0vs; ν_{CO}, 173–4vs; $\nu_{C=C}$, ν_{CN} in range 170·0—150·0, 162·4m, 161·5vw, 160·7m, 156·5m, 155·5m, 153·3w, 151·7vw, 150·4vw., mm^{-1}.

1-(5-*Hydroxymethyl*-2-*furyl*)-9H-*pyrido*[3,4-b]*indole* (6b).—This compound accompanies its acetyl ester in the preparation described above, and the compounds can be separated by hand-picking; it can be obtained if a solution of the ester in methanol, mixed with an equal volume of concentrated ammonia, stands in the dark for a few days; the base is then isolated more quickly as follows. The preparation of the ester (6a) was followed, as far as the removal of the red base from the ether solution. The ether was then extracted with 0·6M-dilute hydrobromic acid (40 ml. × 5); the aqueous layer, which contained a brown gum, and sodium bromide (40 g.), was warmed till a solution was obtained, then left at room temperature overnight. The precipitate was washed with 0·6M-hydrobromic acid, and dried over silica at atmospheric pressure. The crystals broke up and continuously lost weight; after 5 days they weighed 0·44 g. (*ca.* 25%). A portion was recrystallised from 0·05M-hydrobromic acid, and a single crystal was selected. The (0*kl*) Weissenberg photograph, and the (*hk*0) and (*h*0*l*) precession photographs were identical with those of the hydrobromide of perlolyrine. The free base, 1-(5-*hydroxymethyl*-2-*furyl*)-9H-*pyrido*[3,4-b]*indole* crystallised from benzene or ethyl acetate as yellow needles. When heated, these crystals sublimed at 150° and melted at 183—190°; from methanol, the base formed brown needles, breaking up between 75 and 90°, losing birefringence at 110°, subliming from 155°, melting at 183—190°; pK_b spectrophotometrically in a series of phosphate–citrate buffers), 5·70 ± 0·10 [Found: *m/e* 264·0901; (from benzene) C, 72·7; H, 4·6. $C_{16}H_{12}N_2O_2$ requires *M*, 264·0899; C, 72·3; H, 4·6%]; τ (C_5D_5N, range −1 to 10) 1·30 (d, *J* 5·6 Hz, 3-H), 1·97 (d, *J* 4·8 Hz, 4-H), 1·7—2·6, (Ar), 2·75 (d, *J* 4·0 Hz, 3-H), 3·30 (d, *J* 4·0 Hz, 4′-H) and 5·24 (6′-H_2) neither the HN, nor the HO signals could be identified; ν_{HN} 346·5s; $\nu_{C=C}$, $\nu_{C=N}$ in range 170·0—150·0; 162·5s, 160·5m, 156·4s, 155·5m, 153·0w, and 151·7vw $mm.^{-1}$; $\lambda_{max.}$ (log ε), in M-sulphuric acid 221 (4·34), 275 (4·32), 342 (4·31), and 408 (4·17); in methanol, 238 (4·36), 253 (4·28), 274 (4·26), 292 (4·30), 300infl (4·23), 367 (4·14), and 381 (4·17) nm.

Tryptamine.—This was prepared from tryptophan, by decarboxylation in refluxing diphenyl ether,[29] except that for each 1 g. of tryptophan 10 g. of diphenyl ether were used; no nitrogen was used; the diphenyl ether solution was poured, while almost boiling into 0·4M-hydrochloric acid (2 vol.); the process gave two liquid layers. The overall yield of basic material was 60% ± 5. When poured on to ice, the diphenyl ether layer solidified; allowing the diphenyl ether solution to cool before acid treatment resulted in oxidation of the amine.

1-(5-*Acetoxymethyl*-2-*furyl*)-1,2,3,4-*tetrahydro*-9H-*pyrido*-[3,4-b]*indole* (8a).—(a) Tryptamine (0·961 g.) and 5-acetoxymethyl-2-formylfuran (0·967 g.) in benzene (150 ml.) were refluxed on a steam-bath for 2 hr., then 10 ml. of benzene were distilled off, and the solution was cooled and stirred for 30 min. with M-hydrochloric acid (100 ml.). The aqueous phase was briefly yellow; then a brown resin separated. The benzene layer was washed (water, 50 ml., 25 ml.) and rejected; the washings were added to the resin, which dissolved to give a cloudy solution. This was shaken with ether (100 ml.) and the ether was rejected. The base was extracted (ether, 150 ml., 50 ml. × 2; water, 10 ml. × 3). Concentration to 24 ml. gave the crude amine (0·447 g. 25%); the mother liquor contained some tryptamine.

(b) Tryptamine (0·800 g.) and 5-acetoxymethyl-2-formylfuran (0·840 g.) in acetic acid (25 ml.) were heated for 30 min. on a steam-bath; the solution became black. It was poured into water (500 ml.) and the basic material was isolated (CH_2Cl_2, 100 ml. × 3; 0·1M-sulphuric acid, 300 ml.; ether, 50 ml. × 2). The crude product (0·688 g.) after two recrystallisations from ethyl acetate gave the pure base (0·376 g., 25%). From ethyl acetate, 1-(5-*acetoxymethyl*-2-*furyl*)-1,2,3,4-*tetrahydro*-9H-*pyrido*[3,4-b]*indole* formed colourless plates, m.p. 148° (Found: *m/e*, 310·1318; C, 69·4; H, 6·0; N, 8·8. $C_{18}H_{18}N_2O_3$ requires *M*, 310·1317; C, 69·7; H, 5·9; N, 9·0%); τ 1·8br [HN(indole)], 2·2—3·0, (Ar), 3·64 (d, *J* 3·2 Hz, 4′-H), 3·86 (d, *J* 3·2 Hz, 3′-H), 4·74 (6′-H_2), 6·8 (m, 4-H_2), 7·1 (m, 3-H_2), and 6·4—7·6 [HN(2)]; ν_{HN} 330·0s; $\nu_{C=O}$ 173·4vs; $\nu_{C=C}$ in range 170·0—150·0 $mm.^{-1}$ none.

1-(5-*Hydroxymethyl*-2-*furyl*)-1,2,3,4-*tetrahydro*-9H-*pyrido*-[3,4-b]*indole* (8b).—The ester (8a) (0·137 g.) was dissolved in a warm mixture of methanol (5 ml.) and concentrated ammonia (1 ml.), and the solution was stored in the dark for a fortnight, then poured into water (100 ml.), and the base was extracted (ether, 75 ml. × 3; water, 5 ml. × 2; $MgSO_4$); evaporation left an oil that crystallised from benzene, the crude base (0·100 g., 85%) having m.p. 155—159°. Recrystallisation from benzene gave 1-(5-*hydroxymethyl*-2-*furyl*]-1,2,3,4-*tetrahydro*-9H-*pyrido*[3,4-b]*indole* as white prisms, m.p. 161° (Found: *m/e*, 268·1205; C, 72·1; H, 6·0. $C_{16}H_{16}N_2O_2$ requires *M*, 268·1212; C, 71·6; H, 6·0%); τ (C_5D_5N) −1·7br [HN(indole)], 2·0—3·0 (Ar), 3·75 (q, probably two doublets, at 3·7, *J* 3 Hz, 3′-H), 3·8 (*J* 3 Hz, 4′-H), 4·52br (1-H), 5·26 (6′-H_2), 6·85 (m, 4-H_2), 7·10br (3-H_2), and 8·70br [HN(2)]; ν_{HN}, 332·0m; ν_{HN}, ν_{HO} 317·0s, b and 310·5m $mm.^{-1}$; $\lambda_{max.}$ (log ε) in MeOH, 227(4·37), 279(3·70), and 291(3·61) nm. Solutions of the base in organic solvents turn red on exposure to light and air.

1-(5-*Acetoxymethyl*-2-*furyl*)-3,4-*dihydro*-9H-*pyrido*[3,4-b]-*indole* (7a).—The ester (8a) (0·308 g.) in acetic acid (20 ml.)

[29] D. H. R. Barton, G. W. Kirby, R. H. Prager, and E. M. Wilson, *J. Chem. Soc.*, 1965, 3990.

was oxidised with the stock dichromate solution (20 ml.) by the method described earlier. Neither the ethereal solution of the crude base nor its solution in acid was noticeably fluorescent, and none of the base giving a red solution in ether was formed. The crude basic product (0·207 g.) was a yellow gum; the part of this soluble in hot petroleum (b.p. 60—80°) was chromatographed in ether, and appeared as a single yellow band on the column. The eluate was recrystallised by addition of petroleum (3 vol.) (b.p. 40—60°) to its solution in ether, concentration to incipient crystallisation, and rapid filtration through a No. 4 sinter. At 5°, 1-(5-*acetoxymethyl*-2-*furyl*)-3,4-*dihydro*-9H-*pyrido*[3,4-b]*indole* separated as pale yellow prisms, m.p. 132°. The base, dry or in contact with a solvent, is stable in the dark in a closed vessel, but darkens on exposure to light, and if moist becomes gummy. It is very soluble in the common solvents, except petroleum (Found: *m/e*, 308·1161; C, 70·1; H, 5·3%); τ 0·35 (HN), 2·1—3·0, (Ar), 2·90 (d, *J* 3·5 Hz, 3′-H), 3·44 (d, *J* 3·5 Hz, 4′-H), 4·78 (6′-H_2), 6·0 (m, 3-H_2), 7·0 (m, 4-H_2), and 7·87 (Ac); ν_{HN} 348·0w; $\nu_{C=O}$ 174·5vs; $\nu_{C=O}$, $\nu_{C=N}$ in range 170·0—150·0, 160·5m, 161·5w, 158·0s, 152·7s, and 155·3s mm.$^{-1}$.

1-(5-*Hydroxymethyl*-2-*furyl*)-3,4-*dihydro*-9H-*pyrido*-[3,4-b]*indole* (8b).—The ester (7a) (0·071 g.) was dissolved in M-hydrochloric acid (5 ml.) and the solution kept for 90 min. on a steam-bath; the base was extracted (ether, 50 ml. × 3; water, 10 ml. × 3) to give the crude product (0·052 g., 82%). Recrystallisation from benzene gave 1-(5-*hydroxymethyl*-2-*furyl*)-3,4-*dihydro*-9H-*pyrido*[3,4-b]*indole* as pale yellow prisms (0·040 g.), m.p. 180—190° (decomp., efferv.). (Found: *m/e*, 266·1075; C, 71·7; H, 5·3. $C_{16}H_{14}N_2O_2$ requires *M*, 266·1055; C, 72·2; H, 5·3%); τ(C_5D_5N; poor quality, consequent on the compounds low solubility) −1·9br (HN); 2·0—3·0 (Ar + 3′-H); 3·45 (d, *J* 4 Hz, 4′-H); 5·26, (6′-H_2); 6·0 (m, 3′-H_2); and 7·1 (m, 4-H_2); ν_{HN} 354·4m, sharp; ν_{HO} ca. 317·0s,br; $\nu_{C=C}$, $\nu_{C=N}$ 159·8m, 157·5m, and 154·0s,br, mn.$^{-1}$: $\lambda_{max.}$ (log ε) in 2N-hydrochloric acid containing 5% by volume of methanol, 245infl. (3·89), 342(4·20), and 390(4·30); in 0·1N-NaOH containing 5% by volume of methanol, 245infl. (4·11), 275 infl. (3·90), and 317(4·21) nm. The base is very sparingly soluble in the common organic solvents. On a paper chromatogram in Karrer's solvent system E[26] the base shows a yellow spot, R_F 0·20, with a yellow fluorescence under u.v. light; the colour and fluorescence disappear on exposure to ammonia vapour; pK_a (from the pH of a solution of the base in water containing 5% by volume of methanol), *ca.* 8·1. This base is also one of the products of the oxidation of the ester (8a).

Analytical Behaviour of Synthetic Perlolyrine.—Partition coefficients. Solutions of the base in aqueous methanol were equilibrated with benzene, carbon tetrachloride, and methylene chloride, and the concentrations of the base in the two phases determined spectrophotometrically. The values of *P* (= concentration in non-aqueous phase/concentration in aqueous phase) are approximately:

% MeOH by volume at start	*P* (benzene)	*P*(CCl_4)	*P*(CH_2Cl_2)
75	Not determined	0·3	Not determined
50	2	0·8	10
25	8	6	25
10	13	10	30

Chromatography. The base (1·09 mg.) in benzene containing 2% by volume of methanol (5 ml.) was chromatographed and the eluate was collected in 20 ml. portions. A small quantity of a yellow, non-fluorescent substance appeared in fraction 4 (this material usually accompanies perlolyrine); the progress of the base was followed by measurement of the absorbance at 260 nm.

Fraction no.	11	12	13	14	15	16	17	18	19	20	21	22
Fluorescence *	—	vw	w	st	st	st	w	w	vw	—	—	—
Quantity in fraction †	—	3	15	26	24	17	9	6	4	2	1	—
Total amount eluted †	—	3	18	44	68	85	94	100	104	106	107	107

* In daylight. † Units of 10 μg.

The Search for Perlolyrine.—(a) *In the product from the large-scale extraction.* *Ca.* ¼ l. of the aqueous phase (pH, 4·31), and a similar volume of the lipoid phase were taken; the quantities correspond to *ca.* 2 kg. of hay, 8 kg. of fresh grass. To the aqueous phase disodium hydrogen phosphate (55 g.) was added; the pH rose to 6·0, and the liquid was shaken out with benzene (250 ml. × 5). The lipoid phase was treated with methanol (300 ml.), carbon tetrachloride (600 ml.), and water (100 ml.). The mixture separated into two phases and a sludge between them. The sludge was treated with methanol (250 ml.), water (50 ml.), and carbon tetrachloride (500 ml.); again, a sludge separated at the interface, and was treated with the same quantities of solvents. The carbon tetrachloride layers were combined, washed with methanol–water (5–1, v–v; 200 ml., 300 ml., 300 ml.), and these washings shaken with carbon tetrachloride (100 ml. × 3). The sludge and the carbon tetrachloride layers were rejected: the aqueous–methanolic phase occupied *ca.* 1·65 l. To this (550 ml.), water (1·5 l.) containing sodium phosphate (Na_2HPO_4,$12H_2O$; 17 g.) was added; shaking the mixture with benzene gave a stable emulsion. This broke when, after adding the rest of the aqueous phase, anhydrous sodium carbonate (285 g.) in water (4 l.) was added, and the solution was extracted, in portions, twice with about one third of its volume of benzene.

The benzene extracts from the two parts (aqueous and lipoid layers) of the original extract were combined (*ca.* 6 l.), washed (M-sodium carbonate, 500 ml.), dried, concentrated under reduced pressure to 600 ml., and shaken with 2M-hydrochloric acid (50 ml. × 4); the acid layer was filtered, and the benzene layer was rejected. The bases were extracted (ether, 150 ml. × 3; water, 10 ml. × 5), to give a pale brown gum (0·103 g.). During the operations of this paragraph *ca.* one quarter of the bases was lost.

Counter-current distribution of the bases (Run no. 37) was done at pH 2·61; under u.v. light, tubes 56—70 showed the fluorescence colours characteristic of perlolyrine. The contents of tubes nos. 5*n* (*n* = 0, 1, 2, . . ., 16) were evaporated to remove the upper phase; 2M-hydrochloric acid (10 ml.) was added to each; the solutions were made up to 20 ml. and filtered, and the absorption spectra were measured. Perlolyrine was detected in tubes 60, 65, and 70; the results are given above.

(b) *In the grass collected in June* 1968. *L. perenne* at an early flowering stage was collected locally, dried several days in air at room temperature and then with hot air (temp. 60°), and was milled to give a powder (483 g.). This was extracted with boiling benzene containing triethylamine [3 l. + 65 ml.; (2·5 l. + 10 ml.) × 4]; the extracts were filtered and evaporated under reduced pressure to a total volume of 750 ml. Attempted washing with water (100 ml.) produced a stable emulsion so sufficient anhydrous

sodium sulphate was added to absorb all the water; the liquid was filtered, the filter cake was washed with benzene, the benzene portions were combined and dried by azeotropic distillation to a final volume of 1 l. This solution was stirred 90 min. with alumina (Spence D, untreated; 100 g.), then filtered.

The filtrate was shaken with 2N-hydrochloric acid (50 ml. × 5), the aqueous phase was washed with ether (50 ml. × 4), and the base was isolated (NH_3; ether, 50 ml. × 3, then CH_2Cl_2, 50 ml. × 2; 2N-ammonia, 10 ml.); the ether extract yielded the crude base (2·6 mg.). The alumina was suspended in benzene (100 ml.), made into a column, and washed with benzene containing 2% by volume of methanol (1 l., the quantity theoretically needed to elute all the perlolyrine). The eluate was shaken with 2N-hydrochloric acid (50 ml. × 6), and the bases were isolated (NH_3; ether, 50 ml. × 4, then CH_2Cl_2, 50 ml. × 4; water, 25 ml. × 2). Evaporation of this ether extract, and the two portions of methylene chloride gave further crude base (11·2 mg.). The combined bases (13·4 mg.) were submitted to countercurrent distribution at pH 2·57. The contents of tubes nos. 57—78 were combined, concentrated to 200 ml., and the bases were extracted (CH_2Cl_2, 50 ml. × 4; H_2O, 50 ml.) and chromatographed. A band of violet fluorescence moved from the column, and fractions 12 and 13 had a weak violet fluorescence. Fractions 11—16 were combined, concentrated to 1 ml., and the bases were separated from some yellow, non-basic material by its being shaken with 2N-sulphuric acid (0·5 ml. × 2), then extracted (saturated sodium carbonate, 0·5 ml., ether, 0·5 ml. × 4), the ether was evaporated, and the residue was taken up in 2N-hydrochloric acid (1 drop). Addition of mercury(II) chloride solution (1 drop) was followed by the slow separation of a yellow precipitate, which was collected by centrifugation down a capillary tube (i.d., 0·3 mm.) of Lindemann glass which was then sealed. An *X*-ray photograph of this material showed no powder lines, but after the specimen had been recrystallised by immersion in boiling water, and cooling during several hours, it displayed the same powder lines as an authentic specimen of perlolyrine mercurichloride.

Crystal Data.—Perlolyrine hydrobromide dihydrate, $C_{16}H_{12}N_2O_2,HBr,2H_2O$; $M = 381·22$. Triclinic, $a = 7·81$, $b = 10·55$, $c = 10·55$ Å, $\alpha = 79·2$, $\beta = 72·9$, $\gamma = 78·8°$, $U = 807$ Å^3, D_m (flotation in acetone–iodobenzene) = $1·558 \pm 0·005$, $Z = 2$, $D_c = 1·569$, $F(000) = 388$. Space group, $P\bar{1}$. Absorption coefficient for *X*-rays ($\lambda = 1·5418$ Å), $\mu = 38·7$ cm.$^{-1}$. Prisms elongated along *a*, with {100} and {010} prominent were obtained by crystallisation from dilute (*ca.* 0·1M) hydrobromic acid. Crystal dimensions: 0·8 × 0·07 × 0·03 mm.^{3}.

Experimental Measurements.—The unit-cell parameters were obtained from oscillation, rotation, and Weissenberg photographs of a crystal rotated about *a*, taken with Cu-K_α radiation ($\lambda = 1·5418$ Å); and from precession photographs of a second crystal mounted with a^* parallel to the dial axis using Mo-K_α radiation. Parameters calculated from the two types of photographs differed by less than 0·2%. The intensity data (layers 0—7*kl*) were collected by means of equi-inclination multiple-film Weissenberg exposures and estimated visually. In all, 2360 reflections were used, of which 550 were unobserved.

Determination of the Structure.—The space group was thought at first to be $P1$. Tests with a micropolarimeter suggested that a solution of the salt in methanol had a small, but non-zero dextrorotation. Also the reflections of the zero layer were divided into three ranges of sin θ/λ (as for the theoretical moments test; see below), and the $N(z)$ values obtained for each range. The weighted averages for the three ranges gave values appropriate to $P1$. Therefore, I_0 for unobserved reflections was assessed at half the locally observable minimum,[30] and the data, after correction for time-of-exposure, and polarisation and rotation factors, were used for a three-dimensional Patterson synthesis. This gave approximate co-ordinates for the end of the bromide–bromide vector; and, whilst the space group was assumed to be $P1$, one bromide ion was held at the origin. A Sim-weighted [31] Fourier map was computed, and atoms of each molecule in the unit cell were identified at all 13 sites of the pyridoindole system, and at sites (1′) and (2′). Most of the alkaloids in grasses derive from tryptophan; hence, the atom sites so far found, together with the known fluorescence of 9*H*-pyrido[3,4-*b*]indoles and the u.v. spectra of these compounds and their 3,4-dihydro-derivatives enabled the nitrogen atoms to be located and the degree of hydrogenation of the ring-system to be recognised. An isolated peak between N(2) and a bromide ion was recognised as the oxygen of one of the four water molecules. The atoms at sites (1′) weighted as oxygen, the others appropriately, were introduced into a structure-factor calculation with 230 planes; atoms weighted as carbon, at the sites of the remaining peaks in the Fourier map, were entered successively, and those that caused R to fall by at least 0·002 were also accepted for a second round of structure-factor and Fourier synthesis calculations with all the data. The Fourier map revealed the positions of every non-hydrogen atom, and the fragmentation pattern enabled the $-CH_2OH$ group to be identified. However, examination of the map suggested that the oxygen in the furan ring might have been wrongly placed. Structure factors were computed with all the five atoms weighted as carbon, and F_o and F_c maps were derived. Comparison showed the original choice of the oxygen atoms had been correct. Termination-of-series corrections were applied, individual temperature parameters assigned (previously, U for the bromide ions had been set at 0·045, and for all other atoms at 0·05), and another cycle of structure-factor calculations were carried out, followed by adjustment of parameters and then by two rounds of block-diagonal least-squares refinement of positional and isotropic temperature factors with individual layer scaling factors, and all reflections equally weighted. The parameters from the second round were used for a structure-factor calculation ($R = 0·133$) and a difference-Fourier synthesis. The last F_o map had given peak heights: at Br^-, 62; at O, N, C, 10·8—6·8; at the highest spurious peak, 3·6 eÅ^{-3}. The difference map had its highest peaks at bromide ion sites (6·2), and next highest (1·4 eÅ^{-3}) at light atom sites. The values of ($|F_o| - |F_c|$) were scrutinised, and 75 reflections with large Δ-values were examined. Of these, 19 had been wrongly indexed and 55 had been affected by a common error in scaling; no reason was found to alter the remaining value. Correction of these errors, followed by another least-squares cycle, gave $R = 0·132$. Hydrogen atoms were introduced into the calculation and, in the absence of a programme to do this, calculations were done by hand. These revealed that the bond lengths C(5′)–C(6′) in the two molecules were grossly different, as were the bond lengths C(6′)–O(7′); other bond

[30] W. C. Hamilton, *Acta Cryst.*, 1955, **8**, 185.
[31] G. A. Sim, *Acta Cryst.*, 1959, **12**, 813; *ibid.*, 1960, **13**, 511.

lengths were not examined. Audit of the unit-cell parameters revealed no error; theoretical moments of intensity[32] were evaluated for the zero- and first-layer reflections, revealing the space group as $P\bar{1}$ (Table 3). The values of the

TABLE 3

Layer	Range of sin θ/λ	No. of data	⟨z²⟩ obs.	Calc. (P1)	Calc. (P$\bar{1}$)
0 k l	0·13—0·30	47	2·94	1·75	2·27
	0·30—0·41	50	1·84	1·69	2·07
	0·41—0·50	52	1·80	1·67	2·01
	Weighted means		2·18	1·70	2·11
1 k l	0·13—0·24	52	2·10	1·78	2·32
	0·24—0·30	42	2·92	1·73	2·19
	0·30—0·37	60	2·22	1·70	2·10
	0·37—0·42	53	2·01	1·68	2·04
	0·42—0·46	46	2·71	1·67	2·01
	Weighted means		2·36	1·71	2·13

structure factors for the unobserved reflections were multiplied by 2/3;[30] atom co-ordinates were referred to a new origin at an inversion centre, and the atoms of the asymmetric unit entered with [(new i^{th} parameter) = 0·5 × sum (|i^{th} parameter|for both molecules)]. R rose to 0·157. Two further rounds of least-squares refinement, as above, brought R to 0·1452; introduction of hydrogen atoms [except those on O(7′) and O(W2), whose positions were indeterminate] at their calculated positions, with temperature factors = (temperature factor of heavier atom) × 1·25 gave R = 0·1437; this was considered a significant fall.[33] Two further least-squares cycles, as above, followed by five cycles of block-diagonal least-squares refinement of positional and temperature factors of all atoms including hydrogen, with anisotropic temperature factors for the bromide ion, and of a single overall scaling factor, with individually weighted reflections and a partial shift factor of 0·8 gave R = 0·129.

The quantity minimised during this refinement was $\Sigma w\Delta^2$, where $\Delta = \left||F_o| - G|F_c|\right|$, and the scaling factor a, for $F_o = 1/G$. The weighting scheme used in the last five rounds was of the form $\sqrt{w} = (p_1 + |F_o| + p_2|F_o^2| + p_3|F_o|^3)^{-\frac{1}{2}}$; values of the parameters were adjusted between rounds at first, but during rounds four and five they had values p_1 = 40·0, p_2 = −0·04, p_3 = 0·00168. Scattering factors were taken from the following sources: Br^-;[34a] O, N, C (valence);[35a] H.[34b] The co-ordinates and temperature factors from the last least-squares round were used for a final structure-factor calculation; observed and calculated structure factors are listed in Supplementary Publication No. SUP 20008 (15 pp., 1 microfiche).* Table 4 summarises the convergence of the refinement and the final data anslysis.

TABLE 4

Convergence of the refinement

Least-squares round no.	R overall	$R' = \Sigma w\Delta^2/\Sigma w(aF_o^2)$
4	0·1298	0·0255
5	0·1294	0·0254

Average values of $w\Delta^2$ in round 5

Range of $\|F_o\|$	0—6	6—12	12—24	24—48	48—96	>96
⟨wΔ²⟩	0·112	0·099	0·090	0·109	0·101	0·066
No. of values	615	672	691	322	58	2
Range of sin θ/λ	0·0—0·2	0·2—0·4	0·4—0·6	0·6—0·8		
⟨wΔ²⟩	0·155	0·096	0·100	0·092		
No. of values	101	754	1460	45		

Final structure-factor calculation with output from round 5

Layer h	0	1	2	3	4	5	6	7	Overall
R	0·125	0·120	0·125	0·109	0·128	0·140	0·168	0·180	0·1290
No. of data	221	397	351	328	315	310	232	206	2360

DISCUSSION

The unit cell chosen is the morphological one, and is also the Dirichelet-reduced cell.[36] The Delaunay-reduced cell[35b] has parameters: a_R = 10·55, b_R = 13·42, c_R = 10·99 Å α_R = 123·00, β_R = 92·58, γ_R = 129·63°, the matrix for the transformation being $(0\ 1\ 0/0\ 1\ \bar{1}/1\ 0\ \bar{1})(a/b/c/) = (a_R/b_R/c_R)$.

Table 5 shows the values of the C(5′)–C(6′) and C(6′)–O(7′) bond lengths in the two molecules during the determination of the structure and its refinement in space group $P1$. The two sets of values, initially similar and reasonable, diverged during the Fourier refinement, and least-squares treatment aggravated the divergence. Refinement to a false minimum has been observed in other cases.[37] Nonetheless, it is disturbing since, though

TABLE 5

Bond lengths in the system C(5′)–C(6′)–O(7′) during the analysis in space group $P1$

	Molecule 1		Molecule 2	
	C(5′)–C(6′)	C(6′)–O(7′)	C(5′)–C(6′)	C(6′)–O(7′)
2nd Fourier	1·46	1·38	1·49	1·33
3rd Fourier	1·45	1·36	1·51	1·26
4th Fourier	1·44	1·40	1·51	1·24
Least-squares cycle 1	1·42	1·44	1·52	1·22
Least-squares cycle 2	1·40	1·49	1·57	1·20
Least-squares cycle 3	1·40	1·50	1·62	1·19

* See note about Supplementary Publications in Notices to Authors, No. 7 *J. Chem. Soc.*, (*C*), 1970, issue no. 9.

[32] F. Foster and A. Hargreaves, *Acta Cryst.*, 1963, **16**, 1124, 1133.

[33] W. C. Hamilton, *Acta Cryst.*, 1965, **18**, 502.

[34] (*a*) D. T. Cromer and J. T. Waber, *Acta Cryst.*, 1965, **18**, 104; (*b*) R. F. Stewart, E. R. Davidson, and W. T. Simpson, *J. Chem. Phys.*, 1965, **42**, 3175.

[35] (*a*) 'International Tables for X-Ray Crystallography,' Kynoch Press, Brimingham, 1962, vol. III, p. 202; (*b*) *ibid.*, 1952, vol. I, p. 530.

[36] V. Balashov and H. D. Ursell, *Acta Cryst.*, 1957, **10**, 582.

[37] F. Leung and S. C. Nyburg, *Chem. Comm.*, 1969, 137; G. Ferguson, J. G. Sime, J. C. Speakman, and R. Young, *Chem. Comm.*, 1968, 162; R. Parasarathy, *Science*, 1968, **161**, 179.

an unsuitable origin was chosen, phase symmetry about the true origin could be expected, on theoretical grounds,

TABLE 6

Final positional and temperature parameters and, in parentheses, their standard deviations, for the non-hydrogen atoms

Atom	x/a	y/b	z/c	U_{iso} (Å^2)
Br$^-$	0·24118(18)	0·17122(12)	0·11691(13)	*
C(1)	0·30662(132)	0·61388(92)	0·47661(96)	0·0321(20)
N(2)	0·33406(112)	0·64680(78)	0·34360(83)	0·0342(18)
C(3)	0·31066(150)	0·57084(104)	0·26179(110)	0·0410(24)
C(4)	0·24652(154)	0·45341(107)	0·31605(113)	0·0433(25)
C(4a)	0·21408(136)	0·41424(94)	0·44973(99)	0·0345(21)
C(4b)	0·14472(139)	0·29969(97)	0·54012(101)	0·0353(22)
C(5)	0·09091(166)	0·19106(116)	0·51983(122)	0·0490(27)
C(6)	0·03337(171)	0·09813(120)	0·63184(126)	0·0506(28)
C(7)	0·03493(170)	0·11813(118)	0·75825(124)	0·0511(28)
C(8)	0·08712(161)	0·22574(112)	0·78186(117)	0·0464(26)
C(8a)	0·14573(150)	0·31668(104)	0·66970(110)	0·0412(24)
N(9)	0·20452(111)	0·43530(78)	0·66395(82)	0·0334(18)
C(9a)	0·24325(129)	0·49592(91)	0·53424(97)	0·0313(20)
O(1′)	0·29123(100)	0·67372(70)	0·68770(74)	0·0412(17)
C(2′)	0·33630(133)	0·70339(93)	0·55135(98)	0·0333(21)
C(3′)	0·41114(158)	0·81470(111)	0·51118(116)	0·0447(26)
C(4′)	0·41193(177)	0·85719(123)	0·63079(129)	0·0549(30)
C(5′)	0·33366(153)	0·77390(107)	0·73510(112)	0·0427(25)
C(6′)	0·29295(207)	0·76388(147)	0·88381(150)	0·0663(36)
O(7′)	0·38776(172)	0·65654(119)	0·94045(125)	0·0910(34)
O(W1)	0·45536(132)	0·86950(93)	0·18357(98)	0·0667(24)
O(W2)	0·19252(227)	0·45622(156)	−0·06105(162)	0·1231(48)
H(2)	0·35546	0·75959	0·31270	−0·0145
H(3)	0·31582	0·62351	0·15934	0·1065
H(4)	0·21779	0·40522	0·27718	0·0561
H(5)	0·13158	0·16761	0·43415	0·0362
H(6)	0·00701	0·01683	0·60143	0·0583
H(7)	−0·01358	0·04721	0·86396	0·0274
H(8)	0·09119	0·23427	0·90015	0·0214
H(9)	0·20789	0·47321	0·72788	0·0717
H(3′)	0·46438	0·84383	0·41585	0·0294
H(4′)	0·43380	0·94712	0·62730	0·0071
H(6′)	0·18609	0·74284	0·89756	0·1994
	0·45107	0·80906	0·93129	0·0563
H(W1)	0·33514	0·96025	0·18504	−0·0014
	0·43355	0·84683	0·12371	0·0399

* For the bromide ion an anisotropic temperature factor was used, of the form

$$\exp[-2\pi^2(U_{11}h^2a^{*2} + U_{22}k^2b^{*2} + U_{33}l^2c^{*2} + 2U_{12}hka^*b^* + 2U_{13}hla^*c^* + 2U_{23}klb^*c^*)];$$

the final values were: $U_{11} = 0{\cdot}0625$; $U_{22} = 0{\cdot}0578$; $U_{33} = 0{\cdot}0552$; $2U_{12} = -0{\cdot}0288$; $2U_{13} = -0{\cdot}0387$; $2U_{23} = 0{\cdot}0025$.

to be preserved. In practice, refinement of the structure of potassium dichromate in both space-groups $P1$ and $P\bar{1}$ has given the same atomic parameters.[38] Evidently, such agreement is not automatic.

Table 6 shows the final positional and thermal parameters, and, except for hydrogen, their standard deviations. Values for the hydrogen atoms are given, as these were included in the refinement; they are not considered physically meaningful. Table 7 gives interatomic distances and valency angles, and Table 8 some out-of-plane distances.

Figure 3 shows one molecule and its environment, viewed down the a^* axis. This is the first derivative of 9*H*-pyrido[3,4-*b*]indole that has been the subject of a three-dimensional *X*-ray analysis; the parent compound (norharman) has been studied only in projection.[39] In the benzene ring the average C–C bond length, 1·39 Å, agrees with the usual value. The valency angles at C(5) and C(8) are somewhat less than 120° (average, 117°); the other four angles are greater than this. Analogous distortion, present in a benzene ring fused to a five-membered ring containing a sulphur atom,[40] also

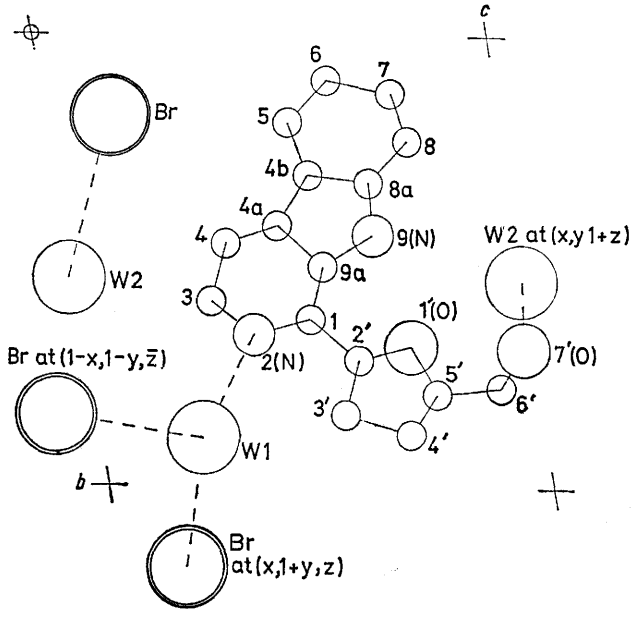


FIGURE 3 Perlolyrine hydrobromide. The environs of one molecule, viewed down the a^*-axis

occurs generally among indoles, the means of the angles at the corresponding positions being (to the nearest 1°) 117° in 3-indolylacetic acid;[41a] 116° in *N*(b)-glycyltryptophan;[41b] 116° in tryptophan hydrochloride;[41c] 114° in hunterburnine;[41d] and 113° in cleavamine.[41e] The pyrrole ring shows the expected symmetry; the bond C(4a)–C(4b), 1·48 Å, is of similar length to that (1·486 Å) in fluorene.[42] In the pyridine ring the valency angle at N(2), 124·3°, is almost identical with that (124·5°) in pyridoxine hydrochloride, and shows the usual increase in valency angle that occurs when an (sp^2)-hybridised nitrogen atom is protonated.[43] The other bond lengths and valency angles agree with previously measured analogues.[44] Within experimental error both the pyridoindole and furan ring systems are planar, and in the crystal the two systems are nearly coplanar.

Within the crystal the molecules are set in parallel

[38] J. K. Brandon and I. D. Brown, *Canad. J. Chem.*, 1968, **46**, 933.

[39] L. Bhattacherjee, *Proc. Nat. Inst. Sci. India*, 1963, **29***A*, 460.

[40] J. C. J. Bart, *J. Chem. Soc.* (*B*), 1968, 376; and references cited.

[41] (*a*) I. L. Karle, K. Britts, and P. Gum, *Acta Cryst.*, 1964, **17**, 496; (*b*) R. A. Pasternak, *ibid.*, 1956, **9**, 341; (*c*) T. Takigawa, T. Ashida, Y. Sasada, and M. Kakudo, *Bull. Chem. Soc. Japan*, 1966, **39**, 2369; (*d*) J. D. M. Asher, J. M. Robertson, and G. A. Sim, *J. Chem. Soc.*, 1965, 6355; (*e*) N. Camerman and J. Trotter, *Acta Cryst.*, 1964, **17**, 384.

[42] D. M. Burns and J. Iball, *Proc. Roy. Soc.*, 1955, *A*, **227**, 200.

[43] F. Hanic, *Acta Cryst.*, 1966, **21**, 332.

[44] 'Tables of Interatomic Distances and Configurations in Molecules and Ions,' Chemical Society Special Publication No. 11, 1958, Supplement, *ibid.*, No. 18, 1965.

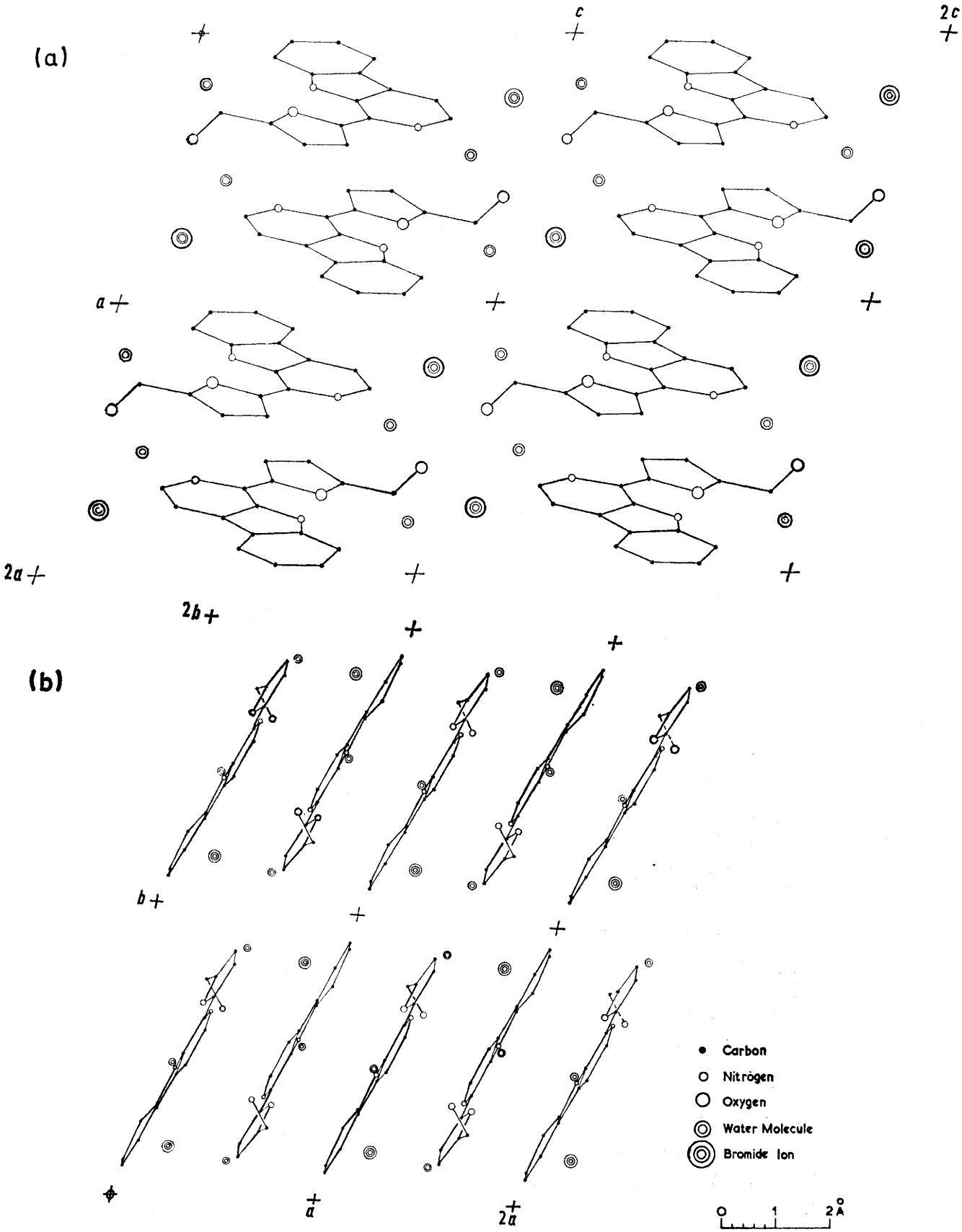


FIGURE 4 Perlolyrine hydrobromide. The crystal structure viewed (a) down the *b*-axis and (b) down the *c*-axis

sheets, with adjacent pairs related by an inversion centre, at the normal van der Waals separation for aromatic systems of *ca.* 3·4 Å. Figures 4(a) and (b) show the molecular packing viewed down the *b* and *c* axes respectively. For a dihydrate, the crystals are both unusually insoluble in water, and stable to air. The water molecules are involved in two systems of hydrogen bonds. One of these involves N(2), O(W1), and two bromide ions; the O · · · Br distances are normal.[45] The other system involves a bromide ion and four hydroxy-groups; the clustering is markedly asymmetric, as all the Br · · · O vectors lie within one hemisphere. The bond Br · · · H–O(7′) is rather long. In addition, near approaches O(W2) · · · O(W2) and O(W2) · · · O(7′) suggest alternative hydrogen bonds. The contacts listed involving O(W2) imply that this water molecule acts as the hydrogen source of three hydrogen bonds, and the relatively high temperature factor for this atom may be due to its being distributed between two alternative sites, one each for the bonding Br · · · H–O(W2)–H · · · O(W2), and Br · · · H–O(W2)–H · · · O(7′).

TABLE 7

Selected valency parameters

Bond lengths	(Å)	Bond lengths	(Å)
C(1)–N(2)	1·34	C(8)–C(8a)	1·40
C(1)–C(9a)	1·39	C(8a)–N(9)	1·40
N(2)–C(3)	1·35	N(9)–C(9a)	1·37
C(3)–C(4)	1·39	C(1)–C(2′)	1·43
C(4)–C(4a)	1·36	O(1′)–C(2′)	1·37
C(4a)–C(9a)	1·44	O(1′)–C(5′)	1·38
C(4a)–C(4b)	1·48	C(2′)–C(3′)	1·35
C(4b)–C(5)	1·37	C(3′)–C(4′)	1·37
C(4b)–C(8a)	1·41	C(4′)–C(5′)	1·34
C(5)–C(6)	1·41	C(5′)–C(6′)	1·49
C(6)–C(7)	1·39	C(6′)–O(7′)	1·37
C(7)–C(8)	1·36		

Largest standard deviation, 0·020 for C(6′)–O(7′).
Smallest standard deviation, 0·012 for O(1′)–C(2′).
Mean standard deviation, 0·015.

Valency angles (deg.)

(*a*) Internal angles in the various rings

Pyridine ring		Benzene ring	
C(1)	119·2	C(4b)	120·7
N(2)	124·3	C(5)	118·1
C(3)	119·5	C(6)	119·7
C(4)	119·5	C(7)	123·6
C(4a)	119·8	C(8)	116·2
C(9a)	119·2	C(8a)	121·6
Pyrrole ring		**Furan ring**	
N(9)	109·3	O(1′)	106·1
C(9a)	109·2	C(2′)	111·1
C(4a)	106·0	C(3′)	105·3
C(4b)	105·8	C(4′)	108·2
C(8a)	109·7	C(5′)	115·9

(*b*) Other valency angles

C(9a)–C(1)–C(2′)	123·8	O(1′)–C(5′)–C(6′)	115·9
N(2)–C(1)–C(2′)	118·6	C(4′)–C(5′)–C(6′)	134·8
C(1)–C(2′)–C(3′)	131·3	C(5′)–C(6′)–O(7′)	113·6
C(1)–C(2′)–O(1′)	117·6		

Largest standard deviation, 1·22 for C(5′)–C(6′)–O(7′).
Smallest standard deviation, 0·78 for C(2′)–O(1′)–C(5′).
Mean 0·95.

(*c*) Intramolecular interactions

N(2) · · · C(3′)	2·97	O(1′) · · · O(7′)	2·94
C(9a) · · · O(1′)	2·84	C(4′) · · · O(7′)	3·52
N(9) · · · O(1′)	2·80		

(*d*) Hydrogen bonds

N(2) · · · O(W1)	2·78	Br · · O(W2)	3·25
O(W1) · · · Br^{X}	3·35	Br · · · O(W1^{III})	3·35
O(W1) · · · Br^{IV}	3·40	Br · · · O(W1^{IV})	3·40
O(W2) · · · O(7′^{I})	2·84	Br · · · O(7′^{V})	3·56
O(W2) · · · O(W2^{II})	2·94		

(*e*) Interaction angles at the bromide ion

O(W2) · · · Br · · · O(W1^{III})	154°	O(W1^{III}) · · · Br · · · O(W1^{IV})	79°
O(W2) · · · Br · · · O(W1^{IV})	76	O(W1^{III}) · · · Br · · · O(7′^{V})	98
O(W2) · · · Br · · · O(7′^{V})	67	O(W1^{IV}) · · · Br · · · O(7′^{V})	68

TABLE 7 (*Continued*)

(*f*) Intermolecular contacts <3·6 Å

O(7′) · · · C(10^{IV})	3·25	C(6) · · · C(4′^{III})	3·50
C(4b) · · · C(3′^{V})	3·36	C(9a) · · · C(2′^{VII})	3·51
C(1) · · · C(5^{VII})	3·37	C(1) · · · C(4b^{VII})	3·51
C(3) · · · C(8a^{VII})	3·43	C(3) · · · C(8^{VII})	3·51
C(4a) · · · C(4a^{VII})	3·44	N(9) · · · N(2^{V})	3·52
N(9) · · · C(4^{VII})	3·45	N(2) · · · C(6^{VII})	3·52
N(2) · · · C(5^{VII})	3·45	N(2) · · · C(4b^{VII})	3·54
C(9a) · · · C(1^{V})	3·46	O(7′) · · · C(3^{VIII})	3·55
C(9a) · · · C(4a^{VII})	3·47	C(5) · · · C(2′^{VII})	3·56
C(4a) · · · C(2′^{V})	3·49	C(9a) · · · C(4b^{VII})	3·56
C(4a) · · · C(3′^{V})	3·49	C(1) · · · C(1^{IV})	3·57
O(7′) · · · C(4^{VIII})	3·49	O(1′) · · · C(4^{IX})	3·59
C(4) · · · C(9a^{VII})	3·50		

Superscripts refer to the following equivalent positions relative to the reference molecule at x, y, z:

I	x,	y,	$-1+z$	VI	x, y, $1+z$
II	$\bar{x}$,	$1-y$,	$\bar{z}$	VII	$\bar{x}$, $1-y$, $1-z$
III	x,	$-1+y$,	z	VIII	$1+x$, $1+y$, $1+z$
IV	$1-x$,	$1-y$,	$\bar{z}$	IX	$\bar{x}$, $\bar{y}$, $\bar{z}$
V	$1-x$,	$1-y$,	$1-z$	X	x, $1+y$, z

TABLE 8

Out-of-plane distances (Å)

Plane (1)		Plane (2)	
C(1)	±0·00	O(1′)	−0·01
N(2)	+0·02	C(2′)	0·02
C(3)	−0·02	C(3′)	0·01
C(4)	−0·01	C(4′)	−0·02
C(4a)	−0·02	C(5′)	0·01
C(4b)	+0·01	C(1)	−0·01
C(5)	+0·03	C(6′)	0·01
C(6)	+0·03		
C(7)	±0·00		
C(8)	−0·01		
C(8a)	−0·03		
N(9)	−0·03		
C(9a)	±0·00		
C(2′)	+0·05		

Dihedral angle between planes, 6·5°.

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