859. *The Alkaloids of Perennial Rye-grass* (Lolium Perenne *L.*). *Part I. Perloline.*

By J. A. D. Jeffreys.

Perloline is shown to be a normal constituent of perennial rye-grass growing in Britain. Its isolation is described. Perloline is shown to exist in different solvents as an anhydronium base, a pseudo-base, or an ether thereof, as a cation, or as an anion.

In 1941 Melville and Grimmett reported the presence of a yellow, fluorescent alkaloid in perennial rye-grass (*Lolium perenne* L.) growing in New Zealand, and called this base Perloline.[1] The alkaloids of this grass have formed the subject of a number of Papers dealing with the extraction of perloline and other bases from the grass,[2] the estimation of alkaloids in the grass,[3] the characterisation and chemistry of perloline,[4] its toxicity and physiological effects,[5] its effect on plant growth,[6] and the effect of genetic differences on the perloline content of rye-grass herbage.[7] Besides perloline, a colourless fluorescent base has been characterised and called perlolidine;[2c,4e,8] a volatile base and a red, fluorescent basic fraction have been described,[2c,4b,9] the last two not being fully characterised. Surveys of other species of grass grown in New Zealand showed that perloline was also present in *Lolium temulentum* L., *Festuca arundinacea* Shreb., and *Setaria lutescens* (Weigel) F. T. Hubb; it has also been reported in *Lolium multiflorum* Lam., *Dactylis glomerata* L., and *Phleum pratense* L.[2c,4c] In *Lolium perenne* the alkaloid was present chiefly in the stems, and then only in the summer.

The writer has detected perloline spectroscopically in an extract from *L. perenne* growing wild in Leeds during May (but not during the previous December) and isolated it from this grass growing wild at Barnet, Hertfordshire during July, and on a much larger scale from hay from a pure strain of *Lolium perenne* grown at Auchincruive, Ayrshire, harvested in the early summer. Paper chromatography of the bases from the last two sources showed that there were more alkaloids in the grass than had been previously reported. The chromatogram could be divided into ten zones, one of which is perloline. Preparative chromatography on columns has shown that some of these zones contain more than one base. The overall yield of 490 mg. of perloline from 52 kg. of hay (10 p.p.m., corresponding to about 2 p.p.m. in the grass) is low compared with that reported from New Zealand. Some perloline may have been destroyed in the hay-making (another sample of this grass, dried by hot air, contained traces only of perloline); also, the hay contained basic material which, though not perloline, gave a yellow solution in chloroform, with a green fluorescence. If this material is present in the fresh grass, then the published colorimetric method for estimating perloline [3a] will give high results.

The workers in New Zealand published analyses for perloline and derivatives, and assigned to the base the formula $C_{40}H_{34}N_4O_7$ containing 2 basic nitrogen atoms, 4 methoxyl groups, and one acetylateable hydroxyl group. No degradation product of known

[1] Melville and Grimmett, *Nature*, 1941, **148**, 782.
[2] (*a*) Reifer and Bathurst, *New Zealand J. Sci. Technol.*, 1942, **24***B*, 17; (*b*) Grimmett and Melville, *ibid.*, p. 141; (*c*) Grimmett, *ibid.*, p. 151; (*d*) Mangan, *ibid.*, 1952, **33***B*, 245.
[3] (*a*) Bathurst and Raper, *New Zealand J. Sci. Technol.*, 1943, **24***B*, 161; (*b*) Clare and Morice, *ibid.*, 1945, **27***B*, 36.
[4] (*a*) Reifer and Bathurst, *New Zealand J. Sci. Technol.*, 1943, **24***B*, 155; (*b*) Shorland, *ibid.*, p. 159; (*c*) White and Reifer, *ibid.*, 1945, **27***B*, 38; (*d*) Metcalf, *ibid.*, 1947, **29***B*, 88; (*e*) Metcalf, *ibid.*, 1954, **35***B*, 473.
[5] (*a*) Cunningham and Clare, *New Zealand J. Sci. Technol.*, 1943, **24***B*, 167; (*b*) Raymond-Hamet, *Compt. rend.*, 1957, **245**, 1828.
[6] Fejer, *New Zealand J. Agric. Res.*, 1960, **3**, 734.
[7] Butler, *New Zealand J. Agric. Res.*, 1962, **5**, 158.
[8] (*a*) Reifer and White, *New Zealand J. Sci. Technol.*, 1945, **26***B*, 242; (*b*) Perrin, *ibid.*, 1957, **38***B*, 688.
[9] Shorland, White, and Grimmett, *New Zealand J. Sci. Technol.*, 1943, **24***B*, 179.

structure was identified; perloline on treatment with oxidising agents, or on storage of its hydrochloride, gave perlolidine. Later it was found that perloline had a single pK_a, and formed only a monohydrochloride, and the formula was revised to $C_{20}H_{18}N_2O_4$;[4d] the spectra of perloline and perlolidine have been discussed,[4e] and it was suggested that perlolidine is formed by removal of a dimethoxyphenyl group from perloline.

However, examination of the published analytical figures and spectra suggested that perloline salts could also be those of anhydronium base, of formula $C_{20}H_{16}N_2O_3$, or a multiple of this. The existence of this anhydronium base was confirmed by the change in the infrared spectrum (see Experimental section) when the ethanol solvate of perloline ethyl ether was heated *in vacuo*. The residual material had a spectrum more complex than that of the starting material; treatment of the anhydronium base with ethanol regenerated the original compound. The proton magnetic resonance spectrum of the anhydronium base in solution in deuterochloroform showed peaks at 6·12 and 6·02 τ

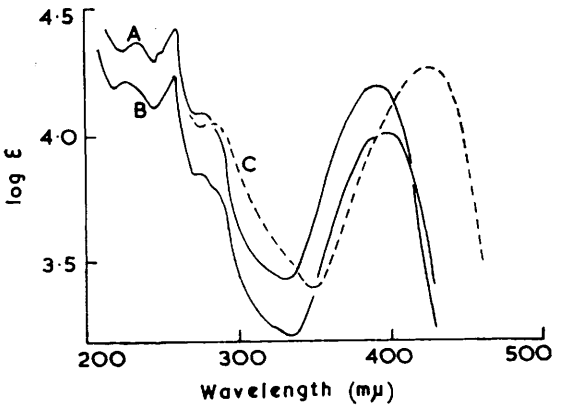


FIG. 1. Absorption spectra of (A) Perloline in 0·01N-hydrochloric acid; (B) *N*-Methylperloline in 0·01N-hydrochloric acid, log ε values displaced by −0·20; (C) *N*-Methylperloline bromide in chloroform, ε values calculated for a monohydrate.

corresponding to the two aromatic methyl ether groups; the other signals were at lower τ values, indicating the absence of any saturated aliphatic protons. Setting the combined areas of the *O*-methyl protons to six showed that this molecule contained 16 protons in all. Conclusive proof that the formula was based on C_{20} was not available till the *X*-ray analysis showed the space-group of the mercurichloride to be *Pnna*,[10] requiring eight equivalent molecules in a unit cell of weight 4150. Previously published molecular-weight measurements based on Raoult's Law were inconclusive, and an attempted determination of the molecular weight by mass spectrometry failed, due to the alkaloid's non-volatility.

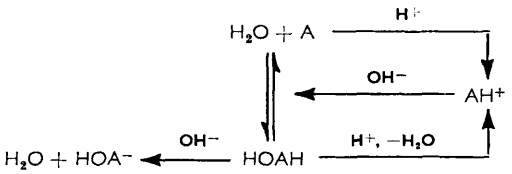
Perloline mercurichloride has been shown to be the salt of the anhydronium base (I);[10] other canonical forms can be drawn. Treatment of perloline or its ethers with excess of methyl iodide gives *N*-methylperloline iodide, which can be de-*O*-methylated while retaining the *N*-methyl group.[4c] Since the spectra of the cation from *N*-methylperloline and of the conjugate acid from perloline anhydronium base are nearly identical (Fig. 1), these ions are respectively (IV; R = Me) and (IV; R = H). This result agrees with the finding [11] that 1,4-dihydro-1-methyl-4-oxopyrimidine (II) is protonated on N(3), not on the carbonyl group (in this compound and in anhydroperloline the carbonyl group is similarly situated towards the nitrogen atoms). The work of Burnell and Taylor [10] shows that perloline is the pseudo-base (III; R = R′ = H), and hence that its ethers are (III; R = H, R′ = alkyl). It follows that *N*-methylperloline free base is (III; R = Me, R′ = H).

Perloline is difficult to recrystallise, and the base is more easily handled as its methyl ether which can be recrystallised from methanol; determinations of the equivalent weight,

[10] Jeffreys, Sim, Burnell, Taylor, Corbett, Murray, and Sweetman, *Proc. Chem. Soc.*, 1963, 171.
[11] Brown, Hoerger, and Mason, *J.*, 1955, 211.

and of the proton magnetic resonance spectrum, showed that this base crystallised unsolvated. By contrast, the erratic results of determinations of the equivalent weight, and of the loss in weight on heating of the ethyl ether, showed that this crystallised from ethanol with a molecule of ethanol of crystallisation, part of which was lost on exposure to the air. The spectrum of perloline ethyl ether in ethanol was calibrated by comparing the absorbance of a solution of the base in dilute acid with that of the methyl ether. The double melting points of perloline and its ethyl ether may be explained by assuming the higher one to be that of the anhydronium base.

The Equilibria between the Different Species in Solution.—(*a*) *General.* Here (A) refers to the anhydronium base (I); (AH^+) to its conjugate acid (IV; R = H); (HOAH) to perloline (III; R = R′ = H); (ROAH) to ethers (III; R = H, R′ = alkyl) of this pseudo-base; and (HOA^-) to the anion (V). For perloline, the relevant equilibria are (i) that between the pseudo- and anhydronium base and water; (ii) that between the anhydronium base and its conjugate acid; (iii) that between the pseudo-base and its anion. These are summarised below. The possible equilibrium (iv) between the carbinolamine and its aldehydic tautomer (VI) lies well on the side of the former, as perloline is inert towards reagents for a carbonyl group.[4c] If water is replaced by an alcohol, giving an ether of



perloline, equilibria of the types (i), (ii), and (iii) still apply; while for *N*-methylperloline possible equilibria are one of the type (iv), and that between (III; R = Me, R′ = H), water, a proton, and the ion (IV; R = Me). Equations can be written for similar equilibria in solutions of the anhydronium base in all protic solvents.

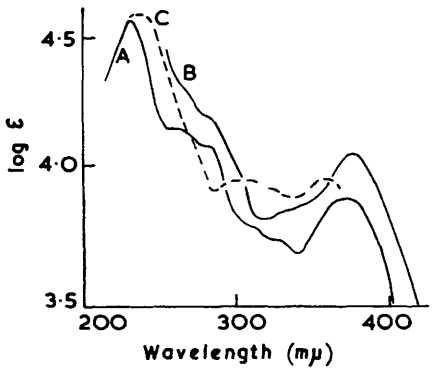


FIG. 2. Absorption spectra of (A) *N*-Methylperloline in 0·01N-sodium hydroxide solution; (B) *N*-Methylperloline in chloroform; log ε values displaced by +0·20; (C) Perloline in 5N-sodium hydroxide.

(*b*) *Between alcohols and the anhydronium base.* The ultraviolet absorption spectrum of *N*-methylperloline base is almost independent of the solvent (Fig. 2), showing that the same species is present in dilute alkali and in chloroform. By contrast, the spectra of perloline and its ethers are strongly solvent-dependent (Fig. 3). Perloline and its ethers, being of similar structure to *N*-methylperloline, would be expected to have similar ultraviolet spectra, with an absorption maximum near 380 mμ. This peak is seen in the spectrum of perloline methyl ether in methanol, and in that of perloline ethyl ether in ethanol, but not in that of perloline methyl ether in chloroform containing a small quantity of methanol. The latter spectrum is assigned to the anhydronium base (A) on the following evidence. The proton magnetic resonance spectrum of the anhydronium base in deuterochloroform showed a doublet at 6·02 and 6·12 τ due to the two aromatic methyl ether groups,

and a single signal at 2·98 τ assigned to the aromatic protons of the veratryl residue. Similarly, an aged solution of perloline methyl ether, present as the cation (AD^+), in deuterotrifluoroacetic acid (CF_3CO_2D) showed a doublet at 5·84 and 5·97 τ, a single signal at 2·62 τ, and an additional signal at 5·91 τ due to methyl trifluoroacetate (the freshly-prepared solution had an additional peak at 6·29 τ due to methanol; on standing this disappeared, while that at 5·91 τ grew more intense). However, a solution of perloline methyl ether (0·146M) in deuterochloroform gave a spectrum indicating that the ether had in part dissociated into methanol and the anhydronium base:

$$MeOAH \rightleftharpoons A + MeOH$$

This spectrum showed a doublet at 2·94 and 2·98 τ due to the aromatic protons of two veratryl groups in different environments; a quartet between 6·0 and 6·2 τ due to the protons of the four *O*-methyl groups of these veratryl residues; a signal at 6·54 τ due to methanol,[12] and one at 6·82 τ due to the protons of the aliphatic methyl ether group in the undissociated ether. The relative strengths of the signals at 6·54 and 6·82 τ were, respectively, 1·2 : 1·0, whence in deuterochloroform the instability constant $[A][MeOH]/[MeOAH] = 2·1 \times 10^{-1}$ If the instability constant is the same in chloroform as in deuterochloroform, then in

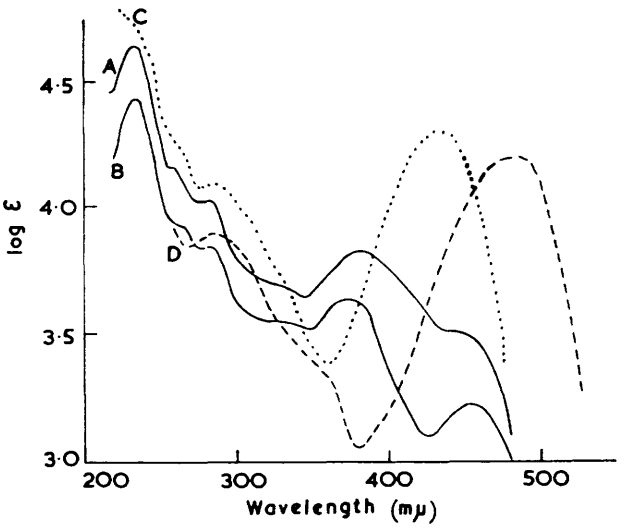


FIG. 3. Absorption spectra of (A) Perloline methyl ether in methanol; (B) Perloline ethyl ether in ethanol; log ε values displaced by −0·20; (C) Perloline in aqueous buffer, pH 11·21; (D) Perloline methyl ether ($1·043 \times 10^{-4}$M) in chloroform containing $9·93 \times 10^{-3}$ mole/l. methanol; log ε values displaced by +0·10.

chloroform $9·93 \times 10^{-3}$M in methanol and $1·043 \times 10^{-4}$M in perloline methyl ether the latter will be 95·4% dissociated, and spectrum D in Fig. 3 is thus essentially that of the anhydronium base. Spectrum A in Fig. 3 shows that perloline methyl ether is partly dissociated in methanol, though the equilibrium is on the side of the ether; the same is true of solutions in ethanol (Fig. 3) and isopropanol [4e] of the corresponding ethers. The ultraviolet absorption spectrum (not reproduced) of a solution of perloline ethyl ether in chloroform shows that this ether too is dissociated under these conditions. This is confirmed by the infrared absorption spectrum of such a solution, 34 peaks of which are listed in the Experimental section. Of these peaks, 6 are common to both solid perloline ethyl ether ethanolate and solid anhydroperloline; of the remainder, 14 peaks can be matched with those of anhydroperloline, 3 with those of perloline ethyl ether ethanolate, and 11 with neither compound.

(*c*) *Equilibria in aqueous solution.* When the possibility of ionisation, ignored in section

[12] Bhacca, Johnson, and Shoolery, "High Resolution N.M.R. Spectra Catalog," Varian Associates, Palo Alto, 1962, Spectrum No. 1.

(*b*), is taken into account, four species can be written. In aqueous solution these are the anhydronium base (A), the pseudo-base (HOAH), the cation (AH^+), and the anion (HOA^-). Their inter-relationships require three equilibria:

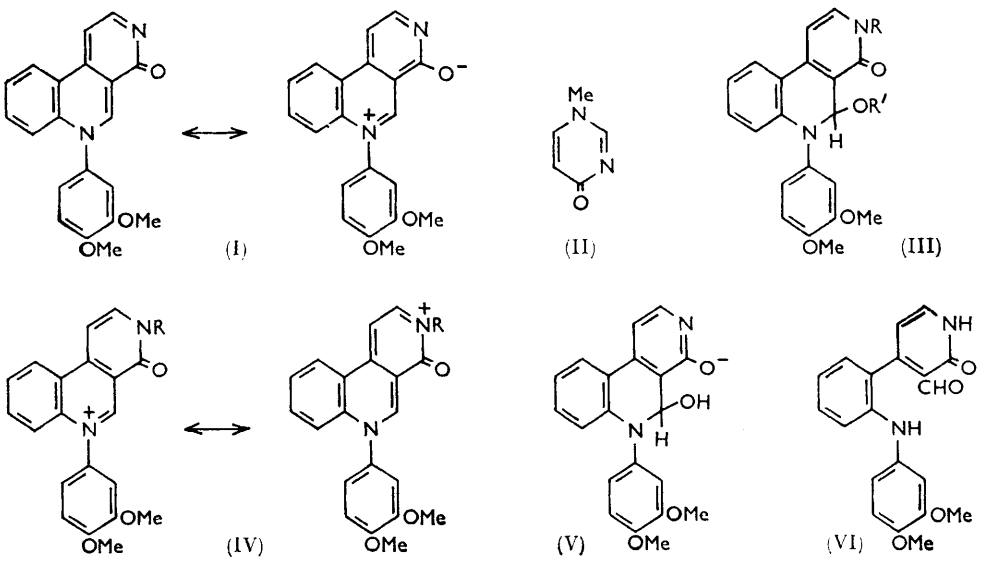
$$A + H_2O \rightleftharpoons HOAH \qquad K_h = [A]/[HOAH], \text{ as } [H_2O] \text{ is constant}$$

$$A + H^+ \rightleftharpoons AH^+ \qquad K_{a_1} = [H^+][A]/[AH^+]$$

$$H^+ + HOA^- \rightleftharpoons HOAH \qquad K_{a_2} = [H^+][HOA^-]/[HOAH] \text{ (ionisation of the pyridone function).}$$

As K_h is independent of the pH, the mixture of the two bases concerned is not distinguishable spectroscopically from a single base. Consequently, though values for K_{a_1} and K_{a_2} can be found spectrophotometrically, these are apparent (*e.g.*, $[H^+][A + HOAH]/[AH^+]$), and not true values. If K_h were known, the apparent pK_a values could be converted into true (concentration) ones by subtracting $\log(1 + 1/K_h)$ from pK_{a_1}, and $\log(1 + K_h)$ from pK_{a_2}.

Spectroscopically, three species can be distinguished: (1) over the pH range 0 — ~7; (2) in the pH range 11·1—11·4, where the optical densities at 433 and 359 mμ have a maximum and a minimum, respectively; (3) in concentrated (5N) sodium hydroxide solution. These three spectra were used as standards, and assigned, respectively, to the species (AH^+), (A + HOAH), and (HOA^-). Measurements of the spectra in the pH range 7·88—9·02 gave an apparent $pK_{a_1} = 8{\cdot}51 \pm 0{\cdot}02$ in agreement with the literature



value; measurements in 0·01—1·0N-sodium hydroxide solution gave an apparent $pK_{a_2} = 13{\cdot}43 \pm 0{\cdot}15$.

The ultraviolet absorption spectrum of perloline in water in the pH range 11·1—11·4 (Fig. 3) resembles that of the methyl ether in chloroform, though with $\lambda_{max.}$ at a shorter wavelength, and is not like that of the methyl ether in methanol. Perloline, therefore, dissociates more easily than its ethers, and in aqueous solution equilibrium lies on the side of the anhydronium base, rather than the pseudo-base. The value of $\lambda_{max.}$ for the long wave-length absorption band of the anhydronium base increases progressively with decrease in the dielectric constant of the solvent. A similar effect is seen in the case of the analogous ion

(IV; R = Me) (Fig. 1); and for these species the ratios ($\lambda_{max.}$ in chloroform)/($\lambda_{max.}$ in water) differ by less than 5%.

From data at present available K_h cannot be evaluated. An order-of-magnitude number can be obtained by assuming that the integrated intensity (= $\varepsilon_{max.} \Delta\nu$ where $\Delta\nu$ is the band-width in cm.$^{-1}$ at the point where $\varepsilon = \varepsilon_{max.}/2$) of the long wave-length absorption band of the anhydronium base is the same in water as in chloroform; the figure is 19. Using this value, the correction to pK_{a_1} is comparable with the experimental error, while pK_{a_2} becomes 12·11, which is of the same order of magnitude as that (11·62) of 2-pyridone.[13]

Experimental

The hay from *Lolium perenne* L., Strain S24 was obtained through the good offices of Dr. Manson of the Hannah Institute for Dairy Research, Auchincruive, Ayrshire, and the large-scale extraction was done by J. F. MacFarlane and Co. Ltd., Edinburgh. Unless otherwise stated, m. p.s were determined on a Kofler hot-stage apparatus; paper chromatography was by the ascending technique, with Karrer's solvent-system E;[14] infrared spectra were determined in KCl discs on a Grubb–Parsons spectrophotometer Type S4; ultraviolet and visible spectra on a Perkin-Elmer model 137 UV; equivalent weights were determined by potentiometric titration in ethanol–water (1 : 2 v/v) mixtures. The mass-spectrometric determination referred to above was carried out by Dr. Reed of the University of Glasgow, the proton magnetic resonance spectrum of anhydroperloline by Dr. Melera of Varian A.G., Zurich, on a Varian A-60, with tetramethylsilane as internal reference, and those of perloline methyl ether by Dr. Bladon of this department on a Perkin-Elmer Nuclear Magnetic Resonance Spectrometer at 40 megacycles, with tetramethylsilane as internal reference.

Isolation of Perloline.—The grass from Barnet. The grass was dried in air at room temperature, then a chloroform solution of the bases was prepared by a method similar to that described by Mangan,[2d] except that no centrifuge was used. This was extracted with 0·5N-sulphuric acid; the aqueous layer was basified with sodium carbonate and extracted with chloroform. The chloroform extract was then washed with water, evaporated to dryness, and the residue suspended in a phosphate buffer (pH 4) until required. The bases were separated by a method similar to (but less convenient than) that described below.

The hay from Auchincruive. Hay (about 100 kg., corresponding to about 500 kg. of fresh grass) from *Lolium perenne* L. strain S24 was dried at 60°, milled to pass a 2-mm. screen, and extracted with ethanol. The extract was concentrated to 25 l. and stored in jars holding *ca.* 1·8 l. On storage the extract separated into an aqueous and a lipoid phase in roughly equal amounts.

The lipoid phase from two jars was removed in boiling carbon tetrachloride (one portion of 4 l., then 5 of 2 l.), and the non-aqueous phase washed with 0·5N-hydrochloric acid (0·5 l., then 1 l.), the aqueous layers combined, and washed with carbon tetrachloride (2 l.). The combined aqueous phases were half-saturated with sodium chloride, basified with sodium carbonate, and extracted with chloroform (4 l.); the chloroform layer was washed three times with 2N-HCl (0·5 l.), and re-used. After 12 cycles only traces of basic material were being removed. The acidic extracts were combined, washed with chloroform, saturated with sodium chloride, basified with sodium carbonate solution, and re-extracted with chloroform. This was washed with distilled water, which removed some yellow material, the chloroform dried (sodium sulphate), filtered through sintered glass (not filter paper: see below), and evaporated to dryness on a water-bath under reduced pressure, leaving the non-volatile bases (3·46 g.). Volatile bases which passed over with the last portion of the chloroform were extracted therefrom with dilute hydrochloric acid, and the extract stored.

Separation of the bases. (*a*) *By paper chromatography.* After a number of trials, Karrer's solvent system E[14] was found suitable. The R_F values of the bases are not fully reproducible, as they depend on the composition of the mixture of bases, and the loading of the paper; nevertheless, 10 zones could be distinguished on most of the chromatograms, with the approximate R_F values given in the Table.

[13] Albert and Hampton, *J.*, 1954, 505.
[14] Schmid, Kebrle, and Karrer, *Helv. Chim. Acta*, 1952, **35**, 1864.

Zone No.	100 × R_F Paper No.: 1	3MM	17	Fluorescence under u.v. light
1	100—85	100—80	100—95	Yellow-white
2	85—75	80—70	95—90	Dull
3	75—60	70—60	—	Blue
4	60—50	60—45	—	Greenish-white
5	50—40	45—30	—	Dull
6	40—25	30—20	—	Blue
7	20—15	20—15	—	Blue
8	15—08	15—10	20—13	Dull; yellow in daylight
9	06—04	08—07	13—10	Blue
10	04—00	07—00	10—00	White

Whatman No. 17 paper had a low resolving power; on it zones 3 and 4, and 5—7 appeared homogeneous. Small quantities of perloline and other bases were isolated by chromatography on either No. 17 paper or No. 3MM. The mixed bases were streaked along the paper at a loading of 0·10—0·15 mg. cm.$^{-1}$ for No. 3MM and 0·3 mg. cm.$^{-1}$ for No. 17; at the end of these runs the mixture was distributed over the whole paper. When the starting material was a fairly pure compound the loading was 0·1–0·2 times the values given above.

(*b*) *On a column.* The bases above (3·46 g.) were dissolved in a mixture of the upper phase (20 ml.) and the lower phase (10 ml.) of an ethyl acetate–water–acetic acid mixture (1·0 : 1·12 : 0·18 v/v), and the solution made into a paste with Hyflo Super-cel (15 g.). This was applied to the top of a column of Hyflo Super-cel (360 g.) carrying 240 ml. of the lower phase of the above mixture, and which was capped with a protecting layer of fine sand. Development with the upper phase of this mixture was continued until zones 8 and 9 were separate; the column was then drained, extruded, and zone 8 dissected out, and the base removed by stirring the column section with a mixture of chloroform and methanol (3 : 1 v/v) containing excess of ammonia. The liquid was filtered through sintered glass, well washed with distilled water, dried (sodium sulphate), filtered through sintered glass, and evaporated to dryness on a water-bath under reduced pressure. The red-brown gum that remained was taken up in ethanol (10 ml.); on standing, perloline ethyl ether ethanolate (274 mg.) separated, m. p. 257—258° (decomp.), (243° capillary).

To date, extracts representing 52 kg. of this hay have been worked up in portions, yielding 11·77 g. of non-volatile bases, and 490 mg. of perloline (weights of samples weighed as perloline ethyl ether have been recalculated to allow for the difference in composition). In general, the yield of total bases has been close to 230 mg./kg. hay; that of perloline has varied between 4 and 17 mg./kg. The corresponding figures for the grass collected at Barnet are: total bases, about 195 mg./kg. dry grass; perloline, 23 mg./kg.

Perloline.—This was obtained either by precipitating an aqueous solution of one of its salts with sodium hydrogen carbonate, or on addition of ethyl acetate to a solution in aqueous pyridine of perloline or the anhydronium base. In each case the base formed small needles, m. p. in the range 154—173°; it may decompose at this temperature, or it may solidify (this is usual) and remelt in the range 248—258° (decomp.) (lit.,[4c] m. p. 181°). (Found: equiv. wt., 345, 353. Calc. for $C_{20}H_{18}N_2O_4$, equiv. wt., 350) $\nu_{max.}$ 3378sh, 3268s, 3096m, 2967w, 2907s, 2825m, 1637s, 1563m, 1524w, 1504s, 1471m, 1439m, 1412w, 1351m, 1328w, 1304s, 1253s, 1224s, 1202s, 1170m, 1124m, 1111sh, 1081w, 1036sh, 1024s, 968s, 929m, 878m, 801m, 775w, 764m, 751s, 731m, 710w, cm.$^{-1}$; pK_a (determined spectrophotometrically in a series of borate buffers) 8·52 ± 0·02: lit.,[4d] 8·54 ± 0·04. The solubility of this base in different solvents, and the fluorescence fitted the descriptions by previous workers.[2b,4a] Passage of a solution in chloroform of the base, or one of its ethers through a filter paper results in appreciable loss, as the base is strongly adsorbed on cellulose; it can be removed therefrom with dilute acid.

Perloline Methyl Ether.—Pale yellow needles, m. p. 248—252° (decomp.) from a solution of the anhydronium base in a small quantity of methanol; solubility in methanol at room temp., *ca.* 1·5 mg./ml. (Found: equiv. wt., 369, 371. $C_{21}H_{20}N_2O_4$ requires equiv. wt., 364) $\nu_{max.}$, 3367w, 3257w, 3115m, 3040sh, 2915s, 2825s, 1631vs, 1597s, 1565s, 1529m, 1502vs, 1479w, 1441m, 1414w, 1351m, 1326w, 1299s, 1271sh, 1252s, 1229s, 1200m, 1176w, 1163m, 1155w, 1133w, 1105m, 1081sh, 1075m, 1062m, 1043s, 1020m, 1010w, 977m, 957w, 934s, 917s, 903s, 879m, 855w, 828w, 806s, 799w, 782m, 777m, 763sh, 753vs, 734w, 725w, 709m. cm.$^{-1}$

Perloline Ethyl Ether Ethanolate.—Prepared as the methyl ether, but with ethanol in place of methanol. Brown tablets, solubility in ethanol at room temp., *ca.* 2 mg./ml.; m. p. in the range 243—263° (decomp.), mostly 243—245°; one sample in a capillary tube in a metal block pre-heated to 237° melted at once, effervesced, solidified, and re-melted at 248—250° (decomp.) lit.,[4c] m. p. 252°. (Found: equiv. wt., 395—422; loss in weight on heating at 140°/3 mm., 15·1—19·0%; $C_{22}H_{22}N_2O_4$ requires equiv. wt., 378, and loss of one mol. ethanol, 12·2%; $C_{22}H_{22}N_2O_4 \cdot C_2H_6O$ requires equiv. wt., 424, loss in weight for two molecules of ethanol, 21·8%) $\nu_{max.}$ 3344 broad s, 3279sh, 2959w, 2907s, 2817w, 1637s, 1618w, 1600sh, 1560s, 1529m, 1504s, 1481m, 1456w, 1439w, 1412sh, 1395sh, 1339m, 1302s, 1274sh, 1259sh, 1250s, 1227s, 1215sh, 1205w, 1179w, 1166m, 1159sh, 1126m, 1081m, 1049sh, 1033sh, 1020s, 977m, 963m, 929m, 885w, 852 broad w, 830w, 811 broad s, 785w, 761 broad vs, 749s, 707m; in chloroform, $\nu_{max.}$ 3448w, 3096w, 2976s, 2857sh, 1645vs, 1637vs, 1605m, 1575s, 1541s, 1513m, 1488m, 1471sh, 1344s, 1312vs, 1282sh, 1267m, 1250m, 1170w, 1155m, 1136m, 1125m, 1106w, 1092sh, 1087m, 1059m, 1038sh, 1022s, 982w, 936w, 886w, 881w, 849m, 832w, 815w cm.$^{-1}$.

Perloline Anhydronium Base.—Perloline ethyl ether ethanolate was heated at 190°/15 mm. for 90 sec. The anhydronium base was left as a brown powder, m. p. 242—244° (decomp.). (Found: C, 71·6, 71·5; H, 4·9, 4·8. $C_{20}H_{16}N_2O_3$ requires C, 72·3, H, 4·85%) $\nu_{max.}$ (Nujol mull) 3067sh, 1631s, 1600m, 1570s, 1543s, 1513s, 1427m, 1359m, 1344s, 1333w, 1314vs, 1282w, 1266m, 1252w, 1233m, 1218m, 1199w, 1183w, 1171w, 1152sh, 1142s. 1126s, 1079m, 1058s, 1038m, 1025s, 982m, 973w, 946w, 933m, 894m, 888m, 874w, 864w, 833s, 822s, 810s, 777s, 767vs, 745m, 736m, 727sh, 706m cm.$^{-1}$: τ 6·12, 6·02 (2 × OCH_3); 3·12, 3·05 (1H); 2·98 (3H); 2·58, 2·53 (1H); 2·37, 2·28, 2·20, (2H); 1·63, 1·57, 1·47, 1·37 (2H); 0·83 (1H). A solution of this material in ethanol was darker than that of the starting material, indicating slight decomposition: pumping off the solvent left crystals whose infrared spectrum was the same as that of perloline ethyl ether ethanolate.

The anhydronium base can be obtained as a red gum by adding a slight excess of alkali to an aqueous solution of a perloline salt, extracting the base with chloroform, drying the extract, and evaporating it to dryness under reduced pressure. The residue can be converted into perloline or one of its ethers by treatment with the appropriate solvent.

Perloline Perchlorate.—Minute yellow crystals, almost insoluble in cold water, m. p. 270° (decomp.) on slow heating, 280—290° (decomp.) on rapid heating; lit.,[4c] 280° (decomp.).

Perloline Mercurichloride.—(*a*) Red tablets separating when solutions of mercuric chloride and of perloline hydrochloride in dilute hydrochloric acid were mixed; m. p. in the range 198—222° (efferv., decomp.); lit.,[4c] red crystals, m. p. 199—201°; $\nu_{max.}$ 3571sh, 3472s, 3425sh, 3205m, 3058m, 2933w, 2841w, 1672s, 1616s, 1563sh, 1541w, 1531w, 1506s, 1453sh, 1441m, 1416sh, 1401sh, 1361m, 1337m, 1261s, 1238s, 1217s, 1202m, 1174w, 1136m, 1124m, 1095w, 1071w, 1022s, 976w, 889 broad m, 861w, 821sh, 813s, 797sh, 779w, 763s, 749m, 704m cm.$^{-1}$.

(*b*) Recrystallisation of the red form from distilled water gave yellow crystals, m. p. 238° (decomp., sintering from 218°), sometimes mixed with red tablets; recrystallisation of the red form from methanol gave yellow crystals, m. p. 258° (decomp., sintering from 230°; lit.,[4c] recrystallisation of the red form from water gave yellow crystals, m. p. 265°).

Methylperloline.—Perloline or one of its ethers was dissolved in chloroform, excess of methyl iodide added, and the solution stored in darkness until the liquid, which turned red, no longer had a visible fluorescence. Paper chromatography showed that some unchanged perloline was still present. The solution was evaporated to dryness, the residue taken up in aqueous ethanol, the solution acidified with dilute sulphuric acid, washed with methylene chloride, and the non-aqueous phase discarded. The aqueous layer was basified with sodium carbonate solution, and extracted with methylene chloride; this extract was washed with water and evaporated. The residue was taken up in commercial chloroform and the solution run through a column of untreated Spence alumina. Perloline remained at the top of the column, while methyl perloline moved down the column as a pale yellow band. Recrystallisation of this gave (i) from methanol (ethanol), almost colourless rhombs, m. p. 150° (143—149°), darkening from 190°, solidifying, and remelting at 226—232° (226—228°); (ii) from ethyl acetate, needles, m.p. 180—235°; $\nu_{max.}$ 3390sh, 3289s, 3967w, 2907s, 2825m, 1730w, 1642vs, 1590sh, 1570vs, 1538sh, 1502s, 1468sh, 1449s, 1410m, 1362m, 1325m, 1302m, 1267sh, 1256m, 1239 broad s, 1208m, 1190sh, 1171m, 1149w, 1125m, 1103m, 1088m, 1024 broad s, 980s, 945s, 920m, 864m, 851w, 845w, 812m, 795w, 782w, 763m, 747 broad s, 707w cm.$^{-1}$; (iii) on precipitation from dilute acid with ammonia, a microcrystalline solid, m. p. 185—238° (lit.,[4c] 199—201°). Paper chromatography showed a

single spot with R_F value = (R_F value of perloline) × 1·4 ± 0·1; solutions of this base in organic solvents, unlike those of perloline, are not noticeably fluorescent.

Methylperloline Bromide.—A solution of methylperloline in excess of 2N-hydrobromic acid was concentrated by standing it *in vacuo* over silica gel till crystals separated. On adding ethyl acetate to this suspension the solid dissolved, and bright yellow crystals separated, m. p. 108—140°, solidifying at *ca.* 170° and remelting at 275—285° (decomp.); when methanol was added to a suspension of the crystals, m. p. 108—140°, in ethyl acetate they dissolved, and pale yellow plates separated, which lost their birefringence at 272—310°, depending on the rate of heating; crystallised from water, the salt formed yellow flat needles which underwent a phase change at 51—53°, turned black and lost their birefringence at 285—290°, but which did not melt below 325°. The infrared spectra of both types of crystal showed a strong peak in the hydroxyl region, and the ε-values in Fig. 1 are calculated on the assumption that this salt is a monohydrate, as is the corresponding iodide.[4c]

Methylperloline Iodide.—Prepared as described in the literature, and recrystallised from water, this formed needles, m. p. 190—200°, solidifying, and remelting at 257° (decomp., efferv.); (lit.,[4c] crystallised from acetone, m. p. 259, 261°).

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