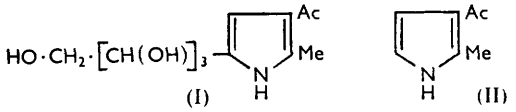
217. *Identification of Two Chromogens in the Elson–Morgan Determination of Hexosamines. A New Synthesis of 3-Methylpyrrole. Structure of the "Pyrrolene-phthalides."*

By J. W. Cornforth and (Mrs.) M. E. Firth.

The substance producing most of the colour with Ehrlich's reagent in the Elson–Morgan assay of hexosamines is shown to be 2-methylpyrrole; 3-acetyl-2-methylpyrrole is also formed. A synthesis of 3-methylpyrrole in four steps from 2-methylallyl chloride is described. Infrared spectra indicate that the condensation products of pyrroles with phthalic anhydride are benzo-[*f*]pyrrocoline-5 : 10-diones; several of these are described.

During the quarter-century which has elapsed since Elson and Morgan [1] published their method for the colorimetric determination of hexosamines, several modifications have increased its precision and specificity and have confirmed it as the method of choice for biochemical work; but in essence the procedure has not changed: the hexosamine is heated with acetylacetone in alkaline solution, and a purple-red colour is then generated by addition of Ehrlich's *p*-dimethylaminobenzaldehyde reagent.

Elson and Morgan assumed that glucosamine and acetylacetone condensed to yield 3-acetyl-2-methyl-5-(tetrahydroxybutyl)pyrrole (I) and that this substance reacts with *p*-dimethylaminobenzaldehyde, as pyrroles were known to do, to give the coloured product. Earlier, Pauly and Ludwig [2] condensed glucosamine with acetylacetone in concentrated alcoholic solution and obtained a crystalline product containing the pyrrole (I). Boyer and Furth [3] prepared the pure substance, showed that it gave a red colour with Ehrlich's reagent, and obtained evidence that the tetrahydroxybutyl group was absent from the coloured product.



The view that the trisubstituted pyrrole (I) is the chromogen was not challenged until 1951, when Schloss reported that a chromogen volatile with steam was formed in the initial reaction. On concentration of the alkaline liquid at low pressure and separate treatment of distillate and residual liquid with Ehrlich's reagent, the distillate gave a purplish-red solution absorbing maximally at 550 mμ and the residue an orange-red solution showing maximal absorption at 512 mμ. The volatile chromogen, Schloss reported, was unstable; he could not purify or identify it. From the non-volatile residue several fractions were prepared; of these, only one was both crystalline and chromogenic, and it was obtained in quantity too small for purification and analysis. Schloss concluded however that certainly two and probably three chromogens were present in the residue.

We succeeded in isolating the volatile chromogen from the distillate by way of an insoluble mercury complex and in purifying it by distillation at room temperature through magnesium perchlorate; later, a simpler isolation procedure was found. The general properties (including rapid oxidation in air) were suggestive of a pyrrole, and analysis indicated the formula C_5H_7N, which is that of a methylpyrrole. Condensation with phthalic anhydride gave a "phthalide" melting at 174°. Since the "phthalide" from 3-methylpyrrole was reported in Fischer and Orth's monograph [5] to melt at 157° whereas

[1] Elson and Morgan, *Biochem. J.*, 1933, **27**, 1824.
[2] Pauly and Ludwig, *Z. physiol. Chem.*, 1922, **121**, 176.
[3] Boyer and Furth, *Biochem. Z.*, 1935, **282**, 242.
[4] Schloss, *Anal. Chem.*, 1951, **23**, 1321.
[5] H. Fischer and Orth, "Die Chemie des Pyrrols," F. Enke, Stuttgart, 1933.

that from 2-methylpyrrole was given m. p. 215°, it was decided to prepare 3-methylpyrrole first for comparison; and the unsatisfactory nature of the known methods of synthesis prompted the development of a new process (see below). This pyrrole with phthalic anhydride gave two isomeric " phthalides," m. p. 223° and 169—170°. Mixture with the derivative from glucosamine did not depress the melting point of the more fusible compound; however, the infrared spectra of all three " phthalides " were different. A specimen of 2-methylpyrrole was then prepared by a ready Huang-Minlon reduction of the readily accessible [6] pyrrole-2-aldehyde. Its infrared spectrum was identical with that of the pyrrole from glucosamine and the melting points and infrared spectra of the derived " phthalides " were also identical. The melting points given in Fischer and Orth's monograph [5] should be transposed: the substances m. p. 215° and 157° were prepared by Dennstedt and Zimmermann [7] from 3-methylpyrrole and 2-methylpyrrole respectively.

For isolation of 2-methylpyrrole from the glucosamine–acetylacetone reaction, the glucosamine concentration was several hundred-fold higher than is used analytically. To test the importance of 2-methylpyrrole as a chromogen at concentrations customary in analysis, a reaction mixture prepared according to a good analytical procedure [8] was concentrated at low pressure. Distillate and residual liquid were each diluted to the original volume of the reaction mixture, and the colours developed by Ehrlich's reagent were compared with the colours from samples of the original reaction mixture and of dilute aqueous solutions of 2-methylpyrrole. This experiment showed that when the colour in an Elson–Morgan analysis is read, as is customary, at 530 mμ, somewhat more than two-thirds of the light absorption is derived from a chromogen readily volatile with steam and giving with Ehrlich's reagent a colour indistinguishable from that given by 2-methylpyrrole. The results leave little doubt that this pyrrole is the principal chromogen in the Elson–Morgan analysis; the computed yield is around 10% of the theoretical. Incidentally, 2-methylpyrrole at a concentration of 0·1 p.p.m. in aqueous ethanol is easily detectable by Ehrlich's reagent.

Schloss [4] produced evidence for the presence of a second chromogen which reacted very slowly with Ehrlich's reagent to give a solution absorbing maximally at 512 mμ. Anastassiadis and Common [9] found that higher concentrations of acetylacetone suppressed formation of this chromogen; its nature remains unknown, but it can make little contribution to colour production during the customary short period of incubation with Ehrlich's reagent.

The presence of another chromogen in the reaction mixture was indicated by Dr. A. Gottschalk (Melbourne), who found in 1955 (personal communication) that condensation of alkaline acetylacetone and glucosamine followed by chromatography on paper with butanol–acetic acid gave a single purple spot of R_F 0·87 after spraying with Ehrlich's reagent and heating. The initial concentration of glucosamine was 10—20 times the usual analytical concentration. The material of R_F 0·87 was not detectable on paper by Schiff's reagent after treatment with periodate. When the reaction mixture was extracted by ether and the extract was concentrated, a little crystalline, Ehrlich-positive material was obtained which absorbed maximally at 245 mμ and on chromatography gave the purple spot of R_F 0·87. When the reaction mixture was concentrated at low pressure before chromatography, four weak spots of R_F 0·80, 0·67, 0·58, and 0·48 respectively appeared along with the spot of R_F 0·87 on spraying with Ehrlich's reagent.

Following this indication we extracted with ether the solution remaining after concentration of an Elson–Morgan reaction mixture. From the extract a crystalline substance was obtained which slowly gave a blue-purple colour ($\lambda_{max.}$ 570 mμ) with Ehrlich's reagent.

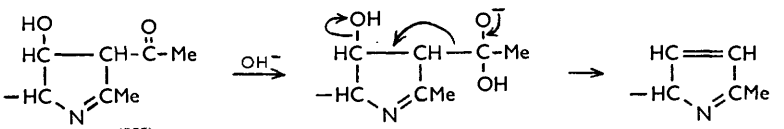
[6] Silverstein, Ryskiewicz, Willard, and Koehler, *J. Org. Chem.*, 1955, **20**, 668.
[7] Dennstedt and Zimmermann, *Ber.*, 1886, **19**, 2200.
[8] Nilsson, *Biochem. Z.*, 1936, **285**, 386.
[9] Anastassiadis and Common, *Canad. J. Chem.*, 1953, **31**, 1093.

The empirical formula, C_7H_9ON, indicated that the substance was 3-acetyl-2-methylpyrrole (II), a structure which Dr. Gottschalk had predicted, on the basis of his observations, for the substance of R_F 0·87, and which we confirmed by preparation of an identical substance by a Knorr synthesis from aminoacetaldehyde and acetylacetone. Dr. Gottschalk, to whom we are much indebted for communication and discussion of his results, found the material from both sources to have the expected R_F 0·87 in butanol–acetic acid.

3-Acetyl-2-methylpyrrole is presumably formed from glucosamine and acetylacetone even at analytical concentrations of glucosamine, and since it reacts with Ehrlich's reagent it can be regarded as a chromogen. However, quantitative tests showed that the colour produced under analytical conditions was several hundred times fainter than the colour from an equimolar amount of 2-methylpyrrole. Since the yield of 3-acetyl-2-methylpyrrole cannot be even ten times that (10%) of the 2-methylpyrrole formed in the glucosamine–acetylacetone reaction, it seems that no significant proportion of the colour in an Elson–Morgan analysis originates from 3-acetyl-2-methylpyrrole.

The results of paper chromatography, which point to 3-acetyl-2-methylpyrrole as the principal chromogen, are in apparent conflict with the evidence assigning that position to 2-methylpyrrole. However, 2-methylpyrrole would have been lost by evaporation from the paper before it was sprayed with Ehrlich's reagent. The remarkably intense spot given by 3-acetyl-2-methylpyrrole on paper, contrasting with its feeble chromogenic power in solution, is explicable if the strongly acidic environment built up on heating the sprayed paper caused some deacetylation [10] to the intensely chromogenic 2-methylpyrrole.

When 2-methylpyrrole is formed from glucosamine and acetylacetone, a tetrahydroxybutyl group is lost at some stage from the glucosamine moiety and an acetyl group from the acetylacetone moiety. The fission between $C_{(2)}$ and $C_{(3)}$ of the glucosamine chain, which also occurs during formation of 3-acetyl-2-methylpyrrole, will be discussed elsewhere in a more general context: it can be formulated in several ways, each essentially of the retroaldol type. Theoretically, loss of the acetyl group might have occurred from a pyrrole, from acetylacetone, or from some intermediate stage. The first two explanations are untenable; 3-acetyl-4-methylpyrrole,[11] for example, withstands 10% aqueous sodium hydroxide at 140°, and it was easily shown that 3-acetyl-2-methylpyrrole was not converted into the intensely chromogenic 2-methylpyrrole by heat at pH 9—10, as in an Elson–Morgan reaction mixture. Again, when acetone was substituted for acetylacetone in an Elson–Morgan analysis, the colour formed on addition of Ehrlich's reagent was very weak. The acetyl group must therefore be lost at an intermediate stage. Addition of hydroxyl ion to an intermediate such as (III) may initiate a concerted elimination as shown.



(III)

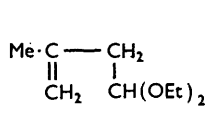
Synthesis of 3-Methylpyrrole.—4-Ethoxycarbonyl-3-methylpyrrole-5-carboxylic acid can be made in very poor yield [12] by a Knorr condensation of aminoacetone with ethyl oxaloacetate; the acid, when heated with concentrated aqueous potassium hydroxide, gives 3-methylpyrrole.[13] This appears to be the least inconvenient method hitherto described for preparing 3-methylpyrrole.

In the first step of the present synthesis, 2-methylallylmagnesium chloride reacts with

[10] H. Fischer and Bartholomäus, *Z. physiol. Chem.*, 1912, **80**, 6.
[11] H. Fischer, Sturm, and Friedrich, *Annalen*, 1928, **461**, 259.
[12] Piloty and Hirsch, *ibid.*, 1913, **395**, 70.
[13] H. Fischer and Rose, *ibid.*, 1935, **519**, 22.

ethyl orthoformate to form 3-methylbut-3-enal diethyl acetal (IV). This reaction gave Kritchevsky [14] a low yield of a product for which no analysis was given. From the data now available it can be estimated that this product contained about 25% of ethyl orthoformate. Our experience of Kritchevsky's procedure confirmed the low yield and the difficulty of purifying the product. Eventually the expedient of adding methylallyl chloride slowly to a stirred mixture of ethyl orthoformate and magnesium, in which reaction had been initiated by methyl iodide, gave a 54% yield of the acetal (IV). The purification was simplified by finding conditions, which did not affect the acetal, for hydrolysis of residual orthoformate. In a normal Tschitschibabin–Bodroux synthesis the Grignard reagent is prepared in ether, the orthoformate is added, and prolonged heating is required to complete the formation of acetal. In the present modification, ethyl orthoformate must participate as an ether in formation of the Grignard complex. The acetal may then be produced by an intramolecular reaction; if not, the high concentration of orthoformate seems at least to suppress the usually dominant side-reaction of the Grignard reagent with methylallyl chloride. In contrast, no improvement on Kritchevsky's yield was secured by adding methylallyl chloride to magnesium and ethyl orthoformate in dilute ethereal solution.

The unsaturated acetal reacted with perbenzoic or, better, with perphthalic acid to give the epoxide (V) in good yield. When this was treated with aqueous or methanolic ammonia the hydroxy-amino-acetal (VI) was formed; the postulated direction of addition



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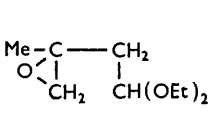

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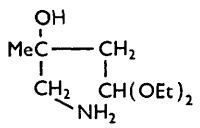

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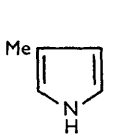

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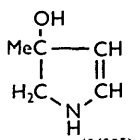


(VIII)

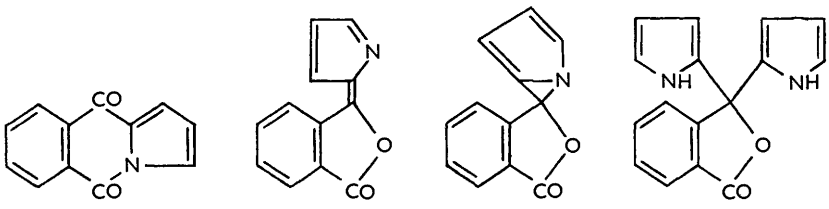
of ammonia is supported by well-known analogies and confirmed by what follows. When the acetal was dissolved in aqueous citric acid and distilled in steam, 3-methylpyrrole (VII) was isolated from the distillate in 39% yield. Probably the amino-aldehyde formed by acid hydrolysis of the acetal (VI) cyclises first to a hydroxypyrroline (VIII), which can be regarded as an intermediate in a hypothetical Knorr condensation of aminoacetone with acetaldehyde: it is presumed to give the pyrrole (VII) by spontaneous or acid-catalysed dehydration. The pyrrole is thus obtained in four stages from commercially available materials. Since large amounts of the final product were not required the last two stages were studied less extensively and conditions giving the best yields have probably not been found.

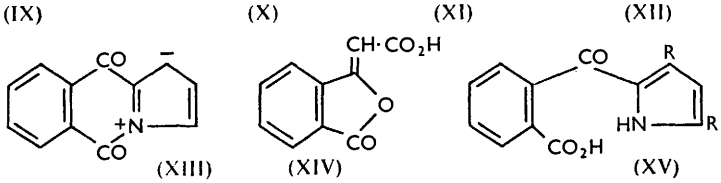
Structure of the "Pyrrolene-phthalides."—These compounds are usually made by heating pyrroles with phthalic anhydride in acetic acid at 180—190°. After their discovery [15] in 1884 their structure was discussed at intervals for half a century and eventually was settled to the somewhat premature satisfaction of Oddo,[16] H. Fischer,[17] and their schools. Three formulæ (IX, X, XI) have chiefly been considered.

Ciamician and Dennstedt [15] preferred (XI) to (IX), principally because they failed to induce reaction with hydroxylamine and V. Meyer [18] had had similar failures with known lactones. Ciamician [19] later preferred structure (X). Oddo [15] favoured (X) because he could obtain "pyrrolene-phthalide" by boiling pyrrophthalein (presumably XII) with hydrochloric acid, and he regarded this as evidence that both compounds have a lactone ring, though he recognised that this ring was probably broken at an early stage of the

[14] Kritchevsky, *J. Amer. Chem. Soc.*, 1943, **65**, 487.
[15] Ciamician and Dennstedt, *Ber.*, 1884, **17**, 2957.
[16] Oddo, *Gazzetta*, 1925, **55**, 242.
[17] H. Fischer and Orth, *Annalen*, 1933, **502**, 238.
[18] V. Meyer, *Ber.*, 1883, **16**, 1781; 1884, **17**, 817.
[19] Ciamician, *Ber.*, 1904, **37**, 4239.

reaction. Fischer and Orth [17] preferred (X) because " pyrrolene-phthalides " are yellow. None of these arguments appears impressive by modern standards: in particular a yellow colour, though decisive against (XI), is to be expected in a compound of classical structure (IX) in which quinonoid states (*e.g.*, XIII) must participate. Infrared spectrometry provides a crucial test. Grove and Willis [20] found the C=O stretching frequency of 3-methylenephthalide at 1780 cm.$^{-1}$; in phthalylideneacetic acid (XIV),





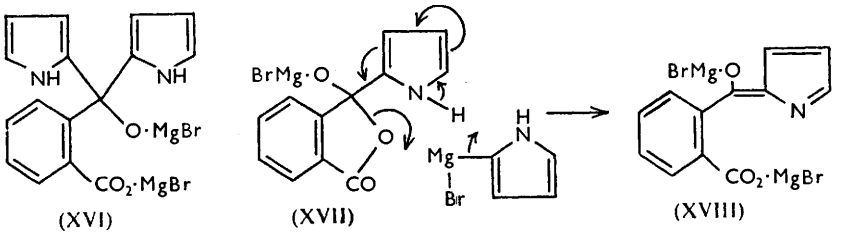
probably a closer model for (X), the stretching frequency of the lactone C=O was at 1800 cm.$^{-1}$. Thus if structure (X) is correct, " pyrrolene-phthalides " should absorb strongly near 1800 cm.$^{-1}$; if structure (IX) is correct the spectra should show the amide I band near 1700 cm.$^{-1}$ (cf. the Raman spectrum of 1-acetylpyrrole,[21] where the C=O stretching frequency is at 1716 cm.$^{-1}$), and a band near 1650 cm.$^{-1}$ due to the other carbonyl group (the C=O absorption of pyrrole-2-aldehyde, measured in dilute solution to reduce intermolecular hydrogen-bonding, was found at 1660 cm.$^{-1}$; cf. also the Raman spectrum of 2-acetylpyrrole [21]). In fact, no absorption near 1800 cm.$^{-1}$ was observed in our " pyrrolene-phthalides," which all showed a strong band at 1705—1708 cm.$^{-1}$ and another at 1650—1655 cm.$^{-1}$. This finding is consistent only with structure (IX); the " pyrrolene-phthalides " are therefore benzo[*f*]pyrrocoline-5 : 10-diones. The ultraviolet absorption of 1 : 3-dimethylbenzo[*f*]pyrrocoline-5 : 10-dione, incidentally, resembles that of many anthraquinones.

The stability and crystallising power of benzopyrrocolinediones makes them useful, as Fischer and Orth [5] have remarked, for characterising the simpler pyrroles. The poor yields of the reaction with phthalic anhydride are offset by a simple and effective technique of isolation described in the Experimental section. As indicated earlier, mixed melting points are not always reliable means of identification, but the infrared spectra are characteristic and rich in detail. The two isomerides from 3-methylpyrrole were easily separated by crystallisation.

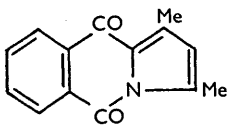
Ingraffia [22] reported the formation in small quantity of a *colourless* substance, which he formulated as the dimethyl analogue of (X), from the reaction of 2 : 4-dimethylpyrrylmagnesium bromide with phthalic anhydride. By a similar process with pyrrylmagnesium bromide, Oddo and Mingioia [23] had obtained traces of the yellow " pyrrolene-phthalide." The main product of each reaction was an oxo-acid (XV; R = H or Me). On repeating Ingraffia's experiment we obtained a colourless product which proved to be a magnesium salt of the acid (XV; R = Me); the earlier observation was presumably in error. The stoicheiometry of the Grignard reaction is of some interest, for Oddo and Mingioia reported

[20] Grove and Willis, *J.*, 1951, 877.
[21] Bonino and Chiorboli, *Atti Accad. naz. Lincei, Rend. Classe sci. fis., mat. nat.*, 1951, **10**, 104.
[22] Ingraffia, *Gazzetta*, 1934, **64**, 714.
[23] Oddo and Mingioia, *ibid.*, 1925, **55**, 235.

that about half the phthalic anhydride was recovered when equimolar amounts of the reactants were taken; yet when two equivalents of pyrrole to one of phthalic anhydride were used, about half the pyrrole was recovered. Oddo suggested that the first product of the reaction is (XVI) and that this loses pyrrole at an unspecified stage, but a more likely explanation is that the initial adduct (XVII) donates a proton to a second equivalent of pyrrylmagnesium bromide (the process is not necessarily cyclic as depicted here) to give the enolate (XVIII) which would react no further.



(XVI) (XVII) (XVIII)



(XIX)

The oxo-acid (XV; R = Me) was converted into the benzopyrrocolinedione (XIX) by the known technique[15] of heating it with slightly ammoniacal water. This benzopyrrocolinedione was identical with a specimen prepared directly from 2 : 4-dimethylpyrrole and phthalic anhydride. On mild alkaline hydrolysis the oxo-acid (XV; R = CH_3) was regenerated (cf. ref. 15).

Experimental

M. p.s marked (K) were taken on a Kofler block and are corrected.

2-Methylpyrrole and 3-Acetyl-2-methylpyrrole from Glucosamine.—An aqueous solution (800 ml.) of D-glucosamine hydrochloride (21·6 g.) was added to an aqueous solution (2200 ml.) containing sodium carbonate (106 g.), acetylacetone (19·6 ml.; redistilled, b. p. 66—67°/80 mm.) and N-hydrochloric acid (200 ml.). The mixture (pH 9·75) in two portions was heated on steam-baths under reflux condensers. Heating was continued for 20 min. after the temperature became steady. The deep orange solutions were cooled below 30°, combined, and distilled at 20 mm. (bath 50°; receiver cooled in ice-salt), until the distillate gave no significant colour with Ehrlich's reagent (0·5 g. of *p*-dimethylaminobenzaldehyde in 20 ml. of ethanol and 5 ml. of concentrated hydrochloric acid).

The distillate (450 ml.) was saturated with salt and extracted with ether (6 × 40 ml.), which was then shaken once with 2N-sodium hydroxide and once with water. The combined aqueous and alkaline extracts were re-extracted with ether (2 × 10 ml.). The combined ethereal solutions were dried ($CaCl_2$) at 0° under nitrogen and evaporated at −10°/30—40 mm. The residue was distilled twice at room temperature and 1 mm. pressure, the vapour passing over magnesium perchlorate and condensing in a bulb at −70°. The product (650 mg., 8%) was a clear colourless mobile liquid (Found: C, 73·9; H, 8·7; N, 17·4. Calc. for C_5H_7N: C, 74·1; H, 8·6; N, 17·3%), b. p. 138—146°/766 mm. when distilled in a nitrogen atmosphere from a small flask. The product remained colourless in nitrogen at −5° but darkened rapidly in air. The infrared spectrum was identical with that of authentic 2-methylpyrrole (see below).

The residual liquids after collection of aqueous distillate from two runs were combined and heated on a steam-bath for 45 min., then distilled as before. The pyrrole was precipitated as a mercury complex by adding hot saturated aqueous mercuric chloride to the distillate. The solid was suspended in N-sodium hydrogen carbonate and decomposed by hydrogen sulphide. The product was extracted with ether and purified as above, to give 2-methylpyrrole (40 mg.) (Found: N, 17·2%). The infrared spectrum was identical with that of the main product.

The aqueous reaction mixture from which 2-methylpyrrole had been distilled was extracted continuously with ether for 24 hr. The product (*ca.* 1 g.) remaining after evaporation of ether under nitrogen was distilled at 0·6—0·7 mm. The partly solid distillate (b. p. ~110—115°) was sublimed at <100°/0·5 mm. The somewhat oily sublimate was suspended in 1 : 1 ether–light petroleum (b. p. 40—60°) and dry ether was added until the undissolved material was wholly crystalline. The collected solid (130 mg.) had m. p. 93—94° (K). Recrystallisation from light petroleum (b. p. 60—80°) gave white needles, m. p. 94—95° (K), of 3-*acetyl*-2-*methylpyrrole* (II) (Found: C, 68·4; H, 7·2; N, 11·5. C_7H_9ON requires C, 68·3; H, 7·3; N, 11·4%), ν (in KCl) 1620 cm.$^{-1}$, ν (in CCl_4) 1660 cm.$^{-1}$ (C=O).

Synthesis of 3-*Acetyl*-2-*methylpyrrole.*—Aminoacetal (3 g.) with water (1 ml.) was cooled in ice–salt and added dropwise with shaking to hydrochloric acid (18 g.; *d* 1·18). After 5 hr. at room temperature the solution was neutralised to methyl-orange by addition of sodium hydrogen carbonate. Acetylacetone (1·39 g.) was added at once and the pH was brought near to 10 by sodium hydroxide. After 24 hr. at 5° the mixture was saturated with salt and extracted with ether (6 × 15 ml.), which was washed once with saturated salt solution, dried ($MgSO_4$), and evaporated. Sublimation below 100°/0·5 mm. and two crystallisations from light petroleum (b. p. 60—80°) gave white needles, m. p. 94—95° alone or mixed with the product from glucosamine (Found: C, 68·3; H, 7·3; N, 11·7%). The infrared spectra of the two products, in KCl and in CCl_4, were identical. No semicarbazone or 2 : 4-dinitrophenylhydrazone could be prepared from 3-acetyl-2-methylpyrrole.

Preparation of 2-*Methylpyrrole.*—Pyrrole-2-aldehyde (5 g.) was added at room temperature to a mixture of potassium hydroxide (10 g.; pellets), 90% hydrazine hydrate (7·5 ml.), and diethylene glycol (100 ml.), from which 2—3 ml. of water had been distilled. After being refluxed for 15 min. the mixture was heated under a take-off condenser so that 2-methylpyrrole (with some water, hydrazine, and glycol) slowly distilled. After 4—5 hr. the condensate was only weakly Ehrlich-positive. To the total distillate (*ca.* 25 ml.) a little water was added; the lower layer was saturated with salt and extracted with ether (4 × 7·5 ml.). The combined upper layers were dried ($MgSO_4$), the ether was removed at −10°/30—40 mm., and the residue was distilled in nitrogen to give 2-methylpyrrole (3·65 g., 86%), b. p. 148°/755 mm. A redistilled sample was analysed (Found: C, 73·9; H, 8·9; N, 17·5%).

From 2-acetylpyrrole (1·5 g.), 2-ethylpyrrole (0·81 g.; b. p. 65°/20 mm.) was similarly obtained.

Formation of 2-*Methylpyrrole at Analytical Concentrations.*—Glucosamine hydrochloride (250 μg.) in water (5 ml.) was mixed with 5 ml. of a solution of acetylacetone (1 ml.) in aqueous 0·5N-sodium carbonate (50 ml.). This mixture was heated for 25 min. in a stoppered flask immersed in a bath at 95—100°. After being cooled and shaken the flask was opened and two 1 ml. samples (O_1, O_2) were withdrawn. The remainder was concentrated at 20 mm. (receiver at −15°). The thawed distillate (slightly under 4 ml.; a pilot experiment had established that this volume contained all the volatile chromogen) was diluted to 8 ml. with water and a 1 ml. portion (D) was taken. The residue (slightly >4 ml.) was also diluted to 8 ml. with water, and two 1 ml. samples (R_1, R_2) were taken. A solution (P) of 2-methylpyrrole (2·45 μg.) in water (1 ml.) was prepared by suitable dilutions.

Samples O_1, D, R_1, and P were treated with ethanol (5 ml.) followed, after mixing, by Ehrlich's reagent (0·5 ml. of a solution containing 0·5 g. of *p*-dimethylaminobenzaldehyde in 6 ml. of ethanol and 6 ml. of concentrated hydrochloric acid). Samples O_2 and R_2 received 0·5 ml. of 1 : 1 ethanol–acid instead of Ehrlich's reagent. A control (B) was prepared from water (1 ml.), ethanol (5 ml.), and Ehrlich's reagent (0·5 ml.). The solutions were left for 1—2 hr. at room temperature for development of colour. Solutions O_2 and R_2 showed no significant difference from B when examined at 530 or 540 mμ. Solutions O_1, D, R_1, and P were then measured, with O_2 as control. Results are shown in the Table.

λ	Optical densities				λ	Optical densities			
(mμ)	O_1	D	R_1	P	(mμ)	O_1	D	R_1	P
500	0·089	0·051	0·038	0·144	540	0·186	0·139	0·040	0·375
510	0·120	0·070	0·050	0·203	544	—	0·141	—	0·386
520	0·154	0·098	0·060	0·269	545	0·185	0·141	0·030	0·386
530	0·179	0·121	0·056	0·323	550	0·176	0·138	0·025	0·377
535	0·183	0·132	0·050	—	560	0·125	0·100	0·020	0·287

Two solutions (1 ml.) of 3-acetyl-2-methylpyrrole (200 μg. and 10 μg.) in water were treated with ethanol and Ehrlich's reagent as above. After 1 hr. the stronger solution was very pale pink (O.D. 0·05 at 540—560 mμ). After 1 week the stronger solution had become deep purple ($\lambda_{max.}$ 570 mμ); the weaker solution was very pale pink.

3-Methylbut-3-enal Diethyl Acetal.—Ethyl orthoformate (90 ml.) and magnesium (35 g.) were stirred and heated at 60°. 2-Methylallyl chloride (*ca.* 2 ml.; freshly distilled) was added, followed by a little methyl iodide. A yellow colour soon appeared; as it faded, an exothermic reaction set in, and cooling was required to keep the temperature below 70°. 2-Methylallyl chloride (49·5 ml.) was then added at a rate which maintained a temperature of 60° (5—6 drops per min.; 4½ hr.) without artificial heating or cooling. Next day the flask was cooled in ice, and saturated aqueous ammonium chloride (*ca.* 40 ml.) was added dropwise until the mixture set solid; the cake was collected on a filter and washed well with ether. The filtrate was evaporated at low pressure and the residue was stirred with water (90 ml.) for 9 hr. The lower layer was saturated with salt and extracted twice with ether. Distillation of the united upper layers gave the *acetal* (IV) (45 g.), b. p. 58—60°/18—19 mm. A redistilled sample, b. p. 56°/17 mm., was analysed (Found: C, 68·6; H, 11·8. $C_9H_{18}O_2$ requires C, 68·3; H, 11·5%); it had b. p. 162°/745 mm., n_D^{21} 1·4155.

3 : 4-Epoxy-3-methylbutanal Diethyl Acetal.—The above product (13·2 g.) in ether (20 ml.) was cooled in ice and treated gradually with ethereal M-perphthalic acid (85 ml.), then allowed to warm and kept below 30° by occasional cooling until reaction subsided. Next day phthalic acid was removed by filtration and extraction of the filtrate with aqueous sodium hydrogen carbonate. The dried ($MgSO_4$) solution was distilled to give the *epoxy-acetal* (V) (10·9 g.), b. p. 82—86°/18 mm. A redistilled sample, b. p. 83—84°/17 mm., was analysed (Found: C, 61·8; H, 10·5. $C_9H_{18}O_3$ requires C, 62·0; H, 10·4%).

4-Amino-3-hydroxy-3-methylbutanal Diethyl Acetal.—The epoxy-acetal (3 g.) and methanolic ammonia (20 ml.; saturated at 0°) were kept at 37° for 24 hr. Distillation gave the *amino-acetal* (VI) (1·95 g.), b. p. 130°/17 mm. (Found: C, 56·5; H, 10·9; N, 7·2. $C_9H_{21}O_3N$ requires C, 56·6; H, 11·0; N, 7·3%), giving slowly a purple colour with Ehrlich's reagent. Aqueous ammonia, either at 100° for 3½ hr. or at room temperature for 48 hr., also opened the epoxide ring; the best yield of amino-acetal was 65%.

3-Methylpyrrole.—The amino-acetal (1·5 g.) was added to a solution of citric acid (4·5 g.) in water (400 ml.), and the solution was distilled, with one interruption to add more water, until the Ehrlich reaction of the distillate became weak. Isolation of 3-methylpyrrole from the distillate (400 ml.) followed the procedure (above) by which 2-methylpyrrole was obtained from glucosamine. The pyrrole (200 mg., 29%) was a clear colourless liquid, b. p. 142—143°/760 mm., which darkened rapidly in air (Found: C, 74·1; H, 8·7; N, 16·5. Calc. for C_5H_7N: C, 74·1; H, 8·6; N, 17·3%). A somewhat better yield (38%) was obtained by dissolving the amino-acetal (1 g.) in water (10 ml.) and citric acid (3 g.) and distilling the whole in steam until 400 ml. of distillate had been collected. A mercury complex of the pyrrole was formed when the amino-acetal (250 mg.) was kept with ammonium acetate (450 mg.), 0·5N-acetic acid (2·5 ml.), and mercuric chloride (900 mg.) at 40° for 2 days with occasional shaking.

General Procedure for Preparing Benzopyrrocolinediones ("*Pyrrolene-phthalides*").—The pyrrole (*x* g.) and phthalic anhydride (10*x* g.) were mixed with acetic acid (15*x* ml.) in a tube which was then cooled, constricted, evacuated, sealed, and heated for 2 hr. at 180—190°. The dark brown product was boiled with water, and the black residue was extracted with hot ethanol. The ethanolic filtrate was taken to dryness; the residue was treated with thiophen-free benzene and filtered. The filtrate after concentration was put on a column of alumina (35*x* g.; pretreated with methyl formate and prepared in benzene). On development with benzene a yellow band descended and was collected separately on elution. Evaporation gave the crystalline benzopyrrocolinedione.

3-Methylbenzo[*f*]pyrrocoline-5 : 10-dione [98 mg.; m. p. 171—174° (K)], from 2-methylpyrrole (600 mg.), after two recrystallisations from light petroleum (b. p. 40—60°), formed yellow needles, m. p. 173—174° (K) (Found: C, 73·6; H, 4·3; N, 6·7. Calc. for $C_{13}H_9O_2N$: C, 73·9; H, 4·3; N, 6·6%). Dennstedt and Zimmermann [7] gave m. p. 157° with previous softening. The infrared spectrum (in KCl) was featureless above 1750 $cm.^{-1}$; this was true also of the other benzopyrrocolinediones examined. Bands were observed at 1708 and 1655 $cm.^{-1}$.

A mixture of 1- and 2-methylbenzo[*f*]pyrrocoline-5 : 10-diones (57 mg.) was obtained from 3-methylpyrrole (200 mg.). It was recrystallised from enough ethanol to prevent separation of

the more soluble isomeride; the yellow crystals were washed with light petroleum (b. p. 60—80°) and recrystallised from a little ethanol. 1(or 2)-Methylbenzo[*f*]pyrrocoline-5 : 10-dione formed yellow needles (18 mg.), m. p. 223° (K) (Found: C, 73·9; H, 4·5; N, 6·6. Calc. for $C_{13}H_9O_2N$: C, 73·9; H, 4·3; N, 6·6%). Dennstedt and Zimmermann [7] gave m. p. 215° with previous softening for what is presumably the same product. The mother-liquor and washings from the first recrystallisation were evaporated and the residue was recrystallised from light petroleum (b. p. 40—60°), to give 2(*or* 1)-*methylbenzo*[f]*pyrrocoline*-5 : 10-*dione* (13 mg.) as yellow needles, m. p. 169—170° (K) (Found: C, 73·8; H, 4·5; N, 6·7%). Both isomerides showed ν (in KCl) 1708 and 1655 cm.$^{-1}$.

1-*Ethylbenzo*[f]*pyrrocoline*-5 : 10-*dione* (43 mg.) from 2-ethylpyrrole (364 mg.) formed yellow needles, m. p. 114°, after sublimation *in vacuo* and crystallisation from methanol (Found: C, 74·6; H, 5·0; N, 5·9. $C_{14}H_{11}O_2N$ requires C, 74·7; H, 4·9; N, 6·2%).

1 : 3-*Dimethylbenzo*[f]*pyrrocoline*-5 : 10-*dione*.—(i) The directions of Ingraffia [21] for condensation of 2 : 4-dimethylpyrrylmagnesium bromide (from 6·4 g. of 2 : 4-dimethylpyrrole) and phthalic anhydride (5 g.) in ether were followed. The solid obtained by filtration after decomposition of the mixture with ice and carbon dioxide proved to be soluble in water with a negligible residue and was a magnesium salt of the acid obtained as below. The aqueous filtrate was extracted thrice with ether and acidified. The magnesium salt was dissolved in aqueous ammonium chloride and also acidified. The precipitates were combined (7·2 g.); crystallisation of a portion from methanol (charcoal)–water under nitrogen gave very pale pink crystals, m. p. 195—196·5° (decomp.), of 2-*o*-carboxybenzoyl-3 : 5-dimethylpyrrole (XV; R = Me) (Ingraffia [22] gave m. p. 188°) (Found: C, 68·9; H, 5·2; N, 5·9. Calc. for $C_{14}H_{13}O_3N$: C, 69·1; H, 5·35; N, 5·8%). This acid on being warmed with Ehrlich's reagent developed a cherry-red colour. The acid (100 mg.) was boiled under reflux with water (2 ml.) and ammonia (5 drops; *d* 0·88). After 1·5 hr. the product was collected and recrystallised from a little ethanol, to give 1 : 3-*dimethylbenzo*[f]*pyrrocoline*-5 : 10-*dione* (27·5 mg.), m. p. 181—183° (Found: C, 74·3; H, 4·8; N, 6·4. $C_{14}H_{11}O_2N$ requires C, 74·7; H, 4·9; N, 6·2%), ν (in KCl) 1705, 1650 cm.$^{-1}$; $\lambda_{max.}$ (in EtOH) 378, 318, 267, 237 mμ (log ε 3·67, 3·71, 4·28, 4·42 respectively).

(ii) This dimethylpyrrocolinedione was also obtained on heating 2 : 4-dimethylpyrrole with phthalic anhydride by the standard procedure and was identified by m. p. (181—182·5°), mixed m. p., and infrared spectrum with the sample obtained as above. A specimen (52·5 mg.) was heated with 2N-sodium hydroxide (2 ml.) until dissolution was complete (1 hr.) and the cooled solution was then acidified. The product, crystallised from methanol–water, had m. p. 195—196° (decomp.) alone or mixed with the oxo-acid (XV; R = Me) obtained as above (Found: C, 69·0; H, 5·4; N, 5·9%).

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