Electrophilic Substitution in Pyrroles. Part 5.[1] Reaction of Dipyrrylmethanes with Arenediazonium Ions

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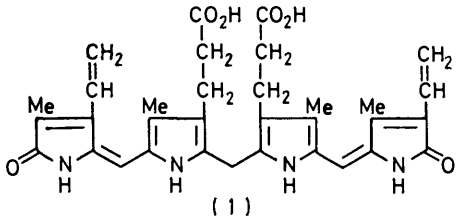
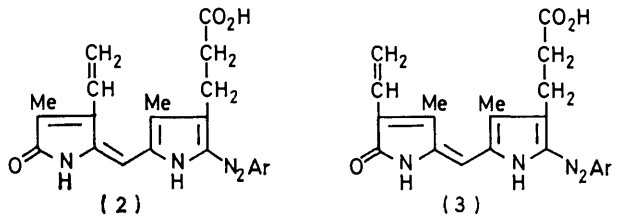
Dipyrrylmethanes react with arenediazonium ions in acid solution to form azopyrroles and formaldehyde. At low pH cleavage of the methylene bridge is brought about by attack of hydrogen ions, but at higher pH arenediazonium ions assume this role. In the reaction of benzylpyrrole, benzyl alcohol was detected as a product, suggesting formation of a benzyl carbonium ion. The relevance of these studies to the van den Bergh test for bilirubin is considered.

In the van den Bergh method [2,3] for the colorimetric determination of bilirubin in biological fluids bilirubin (1) reacts with arenediazonium ions (diazotised sulphanilic acid) in acid solution. The pyrrolic products of reactions have been identified as (2) and (3) [4] and cleavage of the central methylene bridge results in formation of formaldehyde.[5] There are many complications in the application of this apparently simple analytical procedure to biological fluids and, in an effort to understand and improve the test, we thought it would be of value to examine the mechanism of the reaction in some detail. The bilirubin molecule, with its many functional groups, is rather complex for study and so we approached the task by examining the reactions of relevant portions of the bilirubin molecule with arenediazonium ions. In previous papers [6,7] we described kinetic studies of reaction of simple pyrroles with several substituted benzenediazonium ions. We now turn to a series of compounds which are models for the central part of the bilirubin molecule, *viz.* 2,2′-dipyrrylmethanes (4). As far as we know, there have been no previous kinetic studies of the reaction between these compounds and arenediazonium ions although, in a qualitative study, Treibs and Kolm [8] report that azo-coupling occurs, with cleavage of the methylene bridge and formation of two molecules of pyrrole-2-azoarene. In this respect they resemble diphenylmethanes [9] and dinaphthylmethanes.[10]

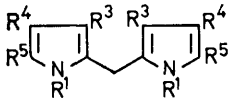


(1)



(2) (3)

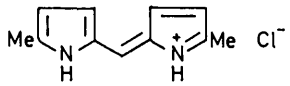
Ar = $C_6H_4SO_2OH$



(4) a; $R^1 = R^3 = R^5 = Me$, $R^4 = Et$
b; $R^3 = R^4 = R^5 = Me$, $R^1 = H$
c; $R^5 = Me$, $R^1 = R^2 = R^3 = R^4 = H$

RESULTS AND DISCUSSION

A number of routes are available for the synthesis of dipyrrylmethanes. In principle, all polysubstituted pyrroles, with free 2-positions, react with formaldehyde in acid solution to form 2,2′-dipyrrylmethanes, but this procedure is only successful when there are deactivating substituents (*e.g.* CO_2Et). When they are absent, polymerisation occurs. Thus, we were unable to repeat the reported [11] preparation of (4a) by this method. Two other methods,[12] both employing acidic conditions, were also found to be unsuccessful, except for pyrroles with deactivating substituents. However, we found two methods which were of general applicability. Reaction of 2-formylpyrrole with a 2-unsubstituted pyrrole in the presence of HCl gives a dipyrrylmethene salt *e.g.* (5),[13] which can be hydrogenated to the corresponding dipyrrylmethane.[14] The yield, however, is low. On the other hand, dipyrryl ketones (6) are relatively easy to synthesise [15] and can also be reduced to dipyrrylmethanes.[14]



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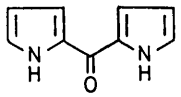
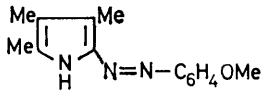


(6)

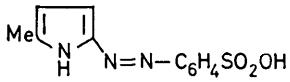
We confirmed that in acid solution, conditions similar to those used in the van den Bergh test, dipyrrylmethanes react with arenediazonium ions to form formaldehyde and a pyrrole-2-azoarene. The former was isolated as its dimedone and the latter was identified by its visible–u.v. spectra. The spectra of a number of azopyrroles had been recorded in a previous study.[6] The product of reaction between (4b) and 4-methoxybenzenediazonium ions was (7), and that from (4c) and diazotised sulphanilic acid was (8).

It is reasonable to assume that the above reactions are initiated by electrophilic attack on one of the pyrrole

rings. There are two electrophiles present in the reaction mixture, *viz.* arenediazonium ions and protons. Kinetic evidence allows us to determine which is responsible for cleavage of the methylene bridge. The rate of reaction was measured in a stopped-flow spectrophotometer by observation of the appearance of highly coloured azopyrroles. The reaction was found to be first order in appearance of product and there was no evidence for formation of azopyrrole in two steps, although two moles of azopyrrole are produced for every mole of dipyrrylmethane present. The variation of k_{obs} with diazonium ions concentration was found to be linear



(7)



(8)

with a positive intercept. The slope of this curve gives the second-order rate constant (k_1) for formation of the azopyrrole and the intercept is k_{-1}, the rate constant for the back reaction (see Scheme). The values of k_1 and k_{-1} are given in Table 1; the significant fact is that the values are the same as those for the monomeric pyrroles.[6] It is clear, then, that the species responsible for cleavage of the molecule is the hydrogen ion and the reaction observed in the spectrophotometer is the reaction of the pyrrole thus formed with arenediazonium ion. We propose the reaction mechanism shown in the Scheme. Although formation of pyrrole monomer occurs in two steps, its formation is so much faster than its reaction with arenediazonium ion that appearance of product is a single step process. We know from previous work [6] that reaction between pyrrole and an

TABLE 1

Data for the reaction of pyrroles and dipyrrylmethanes with (a) 4-methoxybenzediazionium ions and (b) diazotised sulphanilic acid in aqueous solution at 25°

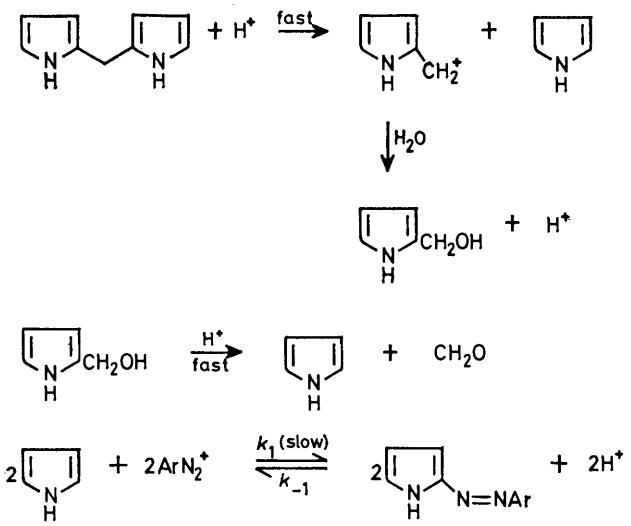
	Diazonium ion	k_1/l mol^{-1} s^{-1}	k_{-1}/l mol^{-1} s^{-1}
(4b)	(a)	4.7×10^4	6.8
3,4,5-Trimethylpyrrole	(a)	4.7×10^4	6.3
(4c)	(b)	5.7×10^2	2.9×10^2
2-Methylpyrrole	(b)	5.9×10^2	2.5×10^2

$[Dipyrrylmethane]_0 = 2.5 \times 10^{-5}$M; $[Pyrrole]_0 = 5 \times 10^{-5}$M; $[ArN_2^+] = 0.020$M; $[HCl] = 0.050$M.

arenediazonium ion is a reversible process. It was established by McDonagh and Assisi [16] that bilirubin (1) undergoes a reversible acid catalysed cleavage of the central methylene bridge, leading to formation of a mixture of bilirubin isomers. The first step of the proposed mechanism has, therefore, some precedent.

We were unable to isolate the alcohol (9) from the reaction mixture but Jackson *et al.*[17] were able to obtain an analogous indole compound (10) as a product in the reaction of 3,3'-di-indolylmethane with arenediazonium ions. Also, we were able to prepare a sample of the alcohol (11) by the borohydride reduction of the corresponding aldehyde. We found it to be an unstable compound which decomposed at room temperature on standing. It reacted with arenediazonium ions to form, as seen from the resulting spectra, pyrrole-2-azoarenes. As the 3-, 4-, and 5-positions are blocked, such a reaction must involve loss of the CH_2OH group. Kinetic studies were inconclusive and not reproducible.

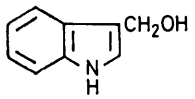
As cleavage is effected by hydrogen ions it should be



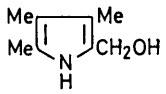
SCHEME

possible to observe this reaction in the absence of diazonium ions. We looked for the spectral changes in the u.v. region which might accompany conversion of a dipyrrylmethane to a pyrrole in acid solution in a stopped-flow spectrophotometer, but without success. Either the reaction is too fast for this technique or the spectral changes are too small for measurement by this method.

In the absence of hydrogen ions and the presence of diazonium ions reaction still occurs, but the details of the mechanism change. The dipyrrylmethane (4b) was mixed with 4-methoxybenzenediazonium tetrafluoroborate in a phosphate buffer of pH 7. Appearance of azopyrrole was followed by a stopped-flow method. The oscilloscope trace provided strong evidence that reaction occurs in two steps, which is reasonable if cleavage of the bridge occurs by attack of the diazonium ion on each



(10)

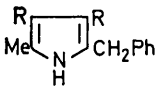


(11)

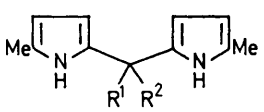
pyrrole ring. It was possible to obtain a second-order rate constant (2.5×10^3 l mol^{-1} s^{-1}) for the first reaction, but the data were not good enough to evaluate that of the second reaction. It was estimated to be about a quarter the rate of the first reaction. It should be noted that the rate constant is much smaller than that quoted for the same compound in Table 1, further proof that the rate-determining step had changed.

The effect of changing the acidity upon the rate of reaction of dipyrrylmethane and arenediazonium ions is not illuminating. For both (4b and c) the effect is the same as that upon reaction of 3,4,5-trimethyl- and 2-methyl-pyrrole. This provides rather ponderous confirmation of the proposed reaction scheme.

The most unexpected process in the Scheme is the expulsion in the first step of a primary carbonium ion which subsequently reacts with water to give an alcohol. The isolation of the analogous indolyl alcohol by Jackson *et al.*[17] provides strong support for the mechanism, but it would be of more direct relevance if a similar reaction could be found for a pyrrole compound. There is no reason why the carbonium ion should be part of a pyrrole compound and a 2-benzylpyrrole should react in the same way, with the advantage that the benzyl alcohol formed does not undergo further reaction and should be isolable. The benzylpyrrole (12a) was prepared and, on



(12) a; R = Me
b; R = H



(13) a; R^1 = H, R^2 = Me
b; R^1 = R^2 = Me

reaction with 4-methoxybenzenediazonium ions in acid solution, formed the azopyrrole (7), which was identified by its visible spectrum. Detection of benzyl alcohol, the other product of reaction, was more difficult as the solubility of (12a) is very low. We found it impossible to isolate and identify any benzyl alcohol in the reaction mixture by conventional means. However, by starting with ^{13}C labelled (12a), it was possible to prove formation of benzyl alcohol by an isotopic dilution technique. Unfortunately, the activity of benzyl alcohol isolated was only half what it should have been for complete conversion of (12a) to (7) and benzyl alcohol. It is difficult to see what, apart from benzyl alcohol, the benzyl cation could form in aqueous solution. We are unable to explain this inconsistency, but we can claim with confidence that the benzyl cation is a major product of reaction.

The kinetics of reaction of (12a) with 4-methoxybenzenediazonium ions and of (12b) with diazotised sulphanilic acid were then determined. The second order rate constants, k_1 and k_{-1}, for reaction in 0.050M-HCl were 4.59×10^4 and 6.3 l mol^{-1} s^{-1} for the former and 5.55×10^2 and 2.3×10^2 for the latter. These are the same as for the reactions of 3,4,5-trimethyl-pyrrole and 2-methyl-pyrrole (see Table 1) and show that, for benzylpyrroles as well, fission of the molecule, by attack of a hydrogen ion, precedes reaction with the diazonium ion. Although the benzyl cation is not a favourable leaving group, its rapid and irreversible reaction with water to give benzyl alcohol promotes reaction.

In view of the ready reaction of arenediazonium ions at the 3(4)-positions of pyrrole,[6] it is possible that with 3,4-unsubstituted dipyrrylmethanes there could be reaction without cleavage. However, the only product obtained from the reaction of (4c) with diazotised sulphanilic acid was (8). Introduction of methyl groups on the methylene bridge, to give (13a and b), changed neither the pyrrolic products of reaction nor the mechanism. This is shown by the data in Table 2. The other

TABLE 2

Data for the reaction of dipyrrylmethanes with diazotised sulphanilic acid in acid solution at 25 °C

	10^2k_1/l mol^{-1} s^{-1}	10^2k_{-1}/l mol^{-1} s^{-1}
(4c)	5.65	2.9
(13a)	5.70	2.1
(13b)	5.58	2.1
2-Methylpyrrole	5.87	2.5

[ArN_2^+] = 0.020M; [HCl] = 0.050M.

product of reaction is, of course, acetaldehyde or acetone, which were isolated as the dimedone derivatives.

In the next paper in this series the relevance of these studies to the reactions of bilirubin will be discussed.

EXPERIMENTAL

Material.—Amines were recrystallised or distilled before use and converted into arenediazonium salts by reaction with $NaNO_2$ and HCl.

The Grignard reagent of 2,3,4-trimethylpyrrole was prepared by the method of McCay and Schmidt.[18] To this was added phosgene in toluene and, after stirring, the mixture was poured onto ice. After filtration the crystals of *3,3′,4,4′,5,5′-hexamethyl-2,2′-dipyrryl ketone* were washed with ethanol and ether, and recrystallised from ethanol (yield 40%), m.p. 250—252° (lit.,[19] 253—255°). A solution of this (7 g) in boiling ethanol (100 ml) and morpholine (4 ml) was treated with sodium borohydride (6 × 2 g), added in portions over 3 h. Water (6 ml) was added 15 min after addition of each portion of borohydride. The mixture was refluxed for a further 2 h, cooled, and poured into water (200 ml). After extraction with ether (4 × 30 ml) the extracts were washed with water, dried ($MgSO_4$), and the solvent removed. The residue was extracted with petrol and the solution concentrated to give crystals of *3,3′,4,4′,5,5′-hexamethyl-2,2′-dipyrrylmethane* (4b) (1.5 g, 23%), m.p. 85—87°, M^+ 230, δ($CDCl_3$) 1.98 (6 H, s), 2.02 (6 H, s), 2.17 (6 H, s), and 3.93 (2 H, s) (Found: C, 78.2; H, 10.0; N, 12.25. $C_{15}H_{22}N_2$ requires C, 78.2; H, 9.65; N, 12.15%).

2-Methylpyrrole (3 g) was formylated at the 5-position by the method of Silverstein [20] (yield 1.58 g, 46%), m.p. 67° (lit.,[21] 68°). To a portion (4 g) in dry ether (400 ml) was added 2-methylpyrrole (3 g). The solution was stirred and HCl bubbled through for 5 min to give red crystals of dipyrrylmethene hydrochloride, which were filtered off and washed with ether (5.7 g, 62%), m.p. 130° (decomp.). The hydrochloride (3 g) was dissolved in absolute methanol (100 ml) and, after addition of Pd–charcoal, the mixture was hydrogenated at room temperature and atmospheric pressure for 48 h. Most of the methanol was removed and the residual solution added to water (200 ml). After ether extraction, the extracts were dried ($MgSO_4$) and the solvent removed. The residue was put on an alumina column and eluted with 50 : 50 v/v ether–methanol. Removal of the solvent gave crystals of *5,5′-dimethyl-2,2′-dipyrrylmethane*

(4c) which were recrystallised from petroleum (0.4 g, 15%) m.p. 84—86°, *m/e* 174 (M^+), δ($CDCl_3$) 2.15 (6 H, s), 3.88 (2 H, s), 5.87 (2 H, s), and 6.19 (2 H, s) (Found: C, 75.45; H, 8.4; N, 15.65. $C_{11}H_{14}N_2$ requires C, 75.8; H, 8.1; N, 16.1%).

Ethylmagnesium bromide, prepared from bromoethane (10.8 g), was added dropwise to a solution of 2-methylpyrrole (5.8 g) in ether (10 ml) at 0°. After addition of benzyl chloride (12.7 g) stirring was continued for 5 h, when NH_4Cl solution (0.1M, 25 ml) was added over 1 h. The mixture was separated, the aqueous layer extracted with ether, and the combined ether solutions dried ($MgSO_4$). The solvent was removed and the residue distilled to give *2-methyl-5-benzylpyrrole* (12b) (3.5 g, 29%), b.p. 90—95° at 0.1 mmHg, *m/e* 171 (M^+), δ($CDCl_3$) 2.10 (3 H, s), 3.07 (2 H, s), 5.85 (1 H, s), 6.10 (1 H, s), and 7.00 (5 H, s) (Found: C, 83.45; H, 6.85; N, 7.2. $C_{12}H_{13}N$ requires C, 84.15; H, 7.65; N, 8.2%).

2,3,4-*Trimethyl-5-benzylpyrrole* (12a) (4.1 g, 35%) was prepared in a similar manner using 2,3,4-trimethylpyrrole (8 g), b.p. 101—105° at 0.1 mmHg, *m/e* 199 (M^+), δ($CDCl_3$) 1.98 (3 H, s), 2.04 (3 H, s), 2.19 (3 H, s), 3.00 (2 H, s), and 7.00 (5 H, m) (Found: C, 83.4; H, 8.25; N, 6.8. $C_{14}H_{17}N$ requires C, 84.3; N, 8.6; N, 7.05%). The radioactively labelled material was prepared from ^{14}C-labelled benzyl chloride.

5,5′-Dimethyl-2,2′-dipyrrylethane (13a) and 5,5′-dimethyl-2,2′-dipyrrylpropane (13b) were prepared by the method of Treibs *et al.*[22]

2-Hydroxymethyl-5-methylpyrrole was prepared from 2-formyl-5-methylpyrrole by the method of Silverstein *et al.*[20] It was too unstable to characterise satisfactorily.

Isotropic Dilution Experiment.—An aqueous solution of diazotised sulphanilic acid (12 g; an excess) was added to a solution of [^{14}C]-2,3,4-trimethyl-5-benzylpyrrole (6 g; 1.77×10^8 c.p.m. mol^{-1}) in ethanol (100 ml) with stirring. After 3 h stirring unlabelled benzyl alcohol (11.6 g) was added to the mixture. After addition of water (100 ml) and extraction with ether the organic layer, containing most of the benzyl alcohol, was removed and dried ($MgSO_4$). The solvent was removed and the residue purified on an alumina column. Elution with chloroform gave benzyl alcohol, which was purified by distillation. The activity of the material was 1.28×10^7 c.p.m. mol^{-1}. After allowing for the dilution this is 52% of the anticipated activity. A Beckman LS 100 liquid scintillation counter was used.

Isolation of the Dimedone Derivative of Formaldehyde.—Compound (4b) (0.2 g) was dissolved in 50 : 50 v/v dichloroethane–methanol (100 ml) and added to benzenediazonium chloride (0.5 g) in water (20 ml). The mixture was stirred for 2 h and then evaporated to dryness and the solvents distilled into a solution of dimedone (0.2 g) in methanol (50 ml). The dimedone solution was refluxed for 30 min and evaporated to dryness. The residue was purified by column chromatography to yield the dimedone derivative of formaldehyde (0.16 g, 32%), m.p. 182—184° (lit.,[23] 183°).

Isolation of the Dimedone Derivative of Acetaldehyde.—The above procedure was repeated with (14a) to give the dimedone derivative of acetaldehyde (0.1 g, 28%), m.p. 138—140° (lit.,[23] 141°).

Kinetics.—A 'Canterbury' stopped-flow spectrophotometer and an SP 500 Unicam spectrophotometer were used for the kinetic studies. Rate constants were calculated by the method of Swinbourne.[24]

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