The Chemistry of Bacteria. Part III. An Indolylpyrrylmethene from Violacein.*

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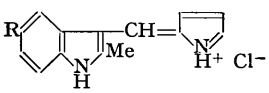
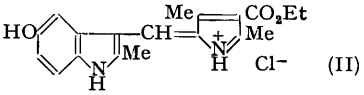
Degradation of acetylviolacein with hydriodic acid gives a yellow mono-acidic base $C_{13}H_{10}O_2N_2$, which forms deep red salts and is believed to be a 5-hydroxyindolyl-hydroxypyrrylmethene. From the properties of synthetic model compounds, it appears that the C_{13} base is most probably a β-hydroxy-pyrrylmethene and it is inferred that this type of system is also present in violacein.

By thermal decomposition of violacein and oxidation of its acetyl derivative, conveniently termed acetylviolacein, it was shown that the violacein molecule contained a 5-hydroxy-indole nucleus and an indole residue probably as oxindole (Part II *). From an examination of the product formed from acetylviolacein with hot hydriodic acid, it now appears that a hydroxypyrrole residue may also be present. This new degradation product, which was isolated as the deeply coloured, sparingly soluble hydriodide, is a yellow base, $C_{13}H_{10}O_2N_2$, best characterised as its red monohydrochloride and perchlorate. It formed a non-basic triacetyl derivative and, with methyl sulphate and alkali, yielded a monomethyl ether, $C_{13}H_9ON_2{\cdot}OMe$, which on acetylation furnished a diacetyl derivative and, like the parent base, gave rise to coloured salts. With aqueous sodium hydrogen carbonate, the base $C_{13}H_{10}O_2N_2$ formed an unstable sodio-derivative, readily decomposed with dilute acetic acid whilst an aqueous sodium hydroxide solution of the base soon became deep green, owing to aerial oxidation. With sodium dithionite (hydrosulphite) the green solution changed to red, reverting to green on aeration or treatment with hydrogen peroxide. In alkaline solution the methylated base was relatively stable to aerial oxidation.

From these results the product $C_{13}H_{10}O_2N_2$ appeared to be an indolylpyrrylmethene, a type of compound which apparently has not been described previously but which, by analogy with the well-known dipyrrylmethenes (pyrromethenes), would be expected to have properties similar to those exhibited by the base. The analytical data indicate that two hydroxyl groups are present and, from the results obtained in Part II (*loc. cit.*), it is reasonable to place one at the 5-position of the indole residue and the second in the pyrrole ring. To test this view an investigation on the preparation and properties of indolylpyrrylmethenes was initiated. Of the established methods for the synthesis of dipyrrylmethenes that which involved the condensation of a pyrrole-aldehyde with a second pyrrole residue appeared to be more generally applicable to the present case. Thus in some preliminary experiments the condensation of pyrrole-2-aldehyde with 2-methylindole by the action of alcoholic hydrogen chloride gave a deep red crystalline compound, presumably (I; R = H), which decomposed during the isolation process but which showed a similar ultra-violet absorption spectrum to the unstable product (I; R = OH) similarly obtained from 5-hydroxy-2-methylindole and the same aldehyde. In support of our hypothesis it was found

(I) 

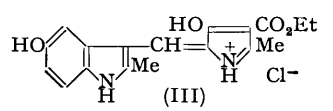
 (II)

that the absorption curves of the synthetic products showed a definite resemblance to that of the hydrochloride of the base $C_{13}H_{10}O_2N_2$. With pyrrole-2-aldehyde and 3-methylindole only a dark amorphous insoluble product was formed. The methene hydrochloride (II) was readily formed from 3-ethoxycarbonyl-2 : 4-dimethylpyrrole and 5-hydroxy-2-methylindole-3-aldehyde, which was conveniently prepared from 5-hydroxy-2-methylindole by the Gattermann reaction. The salt (II) was unstable in hot solvents and could not be recrystallised but with pure reactants it was isolated analytically pure from the reaction mixture.

* Part II, *J.*, 1949, 885.

From the literature it appeared that, of the simple hydroxypyrroles and their aldehydes required for the synthesis of methenes of type (I) with a hydroxyl group in the pyrrole residue, the few known compounds were not readily accessible and therefore the more readily prepared ethoxycarbonyl derivatives of α- and β-hydroxypyrroles were employed in exploratory work. Thus 5-hydroxy-2-methylindole-3-aldehyde reacted smoothly with 3-ethoxycarbonyl-4-hydroxy-2-methylpyrrole in cold alcoholic hydrogen chloride, giving the bright red 2′-(4′-ethoxycarbonyl-3′-hydroxy-5′-methylpyrryl)-3-(5-hydroxy-2-methylindolyl)-methene hydrochloride (III), the constitution of which was confirmed by its preparation from 5-hydroxy-2-methylindole and 4-ethoxycarbonyl-3-hydroxy-5-methylpyrrole-2-aldehyde. This compound resembled the salts of the C_{13} base from violacein in several respects, *e.g.*, it gave a sodio-derivative on treatment with aqueous sodium hydrogen carbonate, a monomethyl ether with methyl sulphate, and a yellow non-basic triacetyl derivative, solutions of which in pyridine, like those of the triacetyl derivative of the C_{13} base, showed a marked green fluorescence.

Attempts to condense 5-hydroxy-2-methylindole-3-aldehyde, with 2-hydroxy-, 4-ethoxycarbonyl-2-hydroxy-, or 3-ethoxycarbonyl-5-hydroxy-2-methyl-pyrrole by the same method were unsuccessful. No reaction occurred at room temperature and when the reaction mixtures were heated the product obtained in each case was 5 : 5′-dihydroxy-2 : 2′-dimethyldi-indolylmethene hydrochloride (IV) which was also formed from 5-hydroxy-2-methylindole and 5-hydroxy-2-methylindole-3-aldehyde in hot alcoholic hydrogen chloride or from 5-hydroxy-2-methylindole and formic acid.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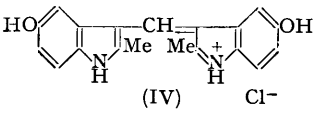
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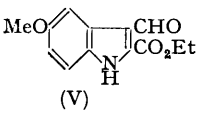
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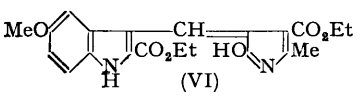
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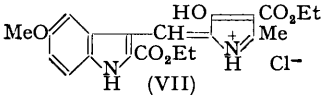
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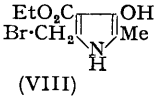
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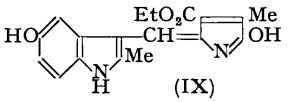
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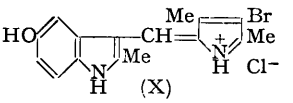
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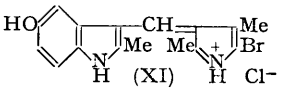
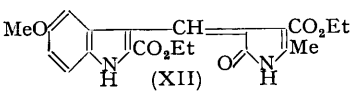

(XI)

The α-hydroxypyrroles are obviously less reactive than 3-ethoxycarbonyl-4-hydroxy-2-methylpyrrole and their failure to condense with 5-hydroxy-2-methylindole-3-aldehyde was attributed to the instability of the aldehyde in the hot reaction mixture. In attempting to overcome this difficulty 5-hydroxy-2-methylindole was heated with 4-ethoxycarbonyl-2-hydroxy-5-methylpyrrole-3-aldehyde but the di-indolylmethene (IV) was again formed, presumably by way of a di-indolylpyrrylmethene. That α-hydroxypyrroles are capable of reacting with indole-3-aldehydes in the desired manner was demonstrated by the successful preparation of 3′-(4′-ethoxycarbonyl-2′-hydroxy-5′-methylpyrryl)-3-(2-ethoxycarbonyl-5-methoxyindolyl)methene (VI) in warm alcoholic hydrogen chloride from 3-ethoxycarbonyl-5-hydroxy-2-methylpyrrole and 2-ethoxycarbonyl-5-methoxyindole-3-aldehyde (V), obtained from 2-ethoxycarbonyl-5-methoxyindole by Gattermann's method. As indicated by its separation from the hot acidic reaction mixture, the methene (VI) does not readily form salts and it does not react with aqueous sodium hydrogen carbonate. Prepared by the standard method from 3-ethoxycarbonyl-4-hydroxy-2-methylpyrrole and isolated as the hydrochloride, the related β-hydroxypyrrylmethene (VII) is definitely basic and also forms a sodio-derivative. A further example of a weakly basic indolyl-α-hydroxypyrrylmethene is afforded by 2′-(3′-ethoxycarbonyl-5′-hydroxy-4′-methylpyrryl)-3-(5-hydroxy-2-methylindolyl)methene (IX) which was prepared by heating 2-bromomethyl-3-ethoxycarbonyl-5-hydroxy-4-methylpyrrole (VIII) with an excess of 5-hydroxy-2-methylindole in methanol, according to another known dipyrrylmethene synthesis (see, *e.g.*, Fischer and Alder, *Z. physiol. Chem.*, 1931, **197**, 246).

Certain hydroxypyrromethenes have been prepared from bromopyrromethenes by replacement of the nuclear bromine atoms with hydroxyl groups (cf. Lichenwald, *ibid.*, 1942, **273**, 118; Fischer and Reinecke, *ibid.*, 1939, **258**, 253). In an attempt to adapt this method, 2′-(4′-bromo-3′ : 5′-dimethylpyrryl)-3-(5-hydroxy-2-methylindolyl)- (X) and 3′-(5′-bromo-2′ : 4′-dimethylpyrryl)-3-(5-hydroxy-2-methylindolyl)-methene (XI) were synthesised by condensing 5-hydroxy-2-methylindole with 4-bromo-3 : 5-dimethylpyrrole-2- and 5-bromo-2 : 4-dimethylpyrrole-3-aldehyde respectively. Unfortunately the instability of these methenes in hot solvents precluded their use for the preparation of the corresponding hydroxy-compounds.

The remarkable difference in their degree of basicity serves to distinguish the two methenes (III) and VII) having a β-hydroxypyrrole residue from (VI) and (IX) with an α-hydroxypyrrole nucleus. Thus the methene hydrochlorides (III) and (VII) are relatively stable salts, whilst the methenes (VI) and (IX) separated as the free bases from acidic alcoholic solutions; this difference may be attributed to the existence of the α-hydroxypyrrole derivatives in the lactam form, *e.g.*, type (XII) (cf. Grob and Ankli, *Helv. Chim. Acta*, 1949, **32**, 2010; Davoll, *J.*, 1953, 3802). In this connexion the compounds (XIII) and (XV) formed by the condensation of *p*-dimethylaminobenzaldehyde with 3-ethoxycarbonyl-4-hydroxy-2-methyl- and 3-ethoxycarbonyl-5-hydroxy-2-methyl-pyrrole are of interest. Both products are yellow bases but, whereas (XIII) forms intensely violet salts, represented by the resonating ion (XIV), the α-hydroxypyrrole derivative (XV) gives only weakly coloured (orange) salts which are probably best represented by (XVI).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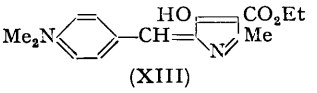
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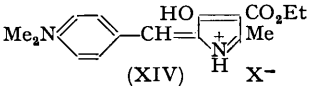
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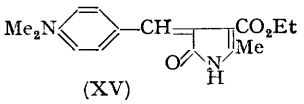
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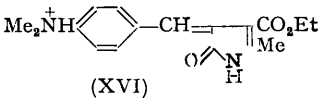
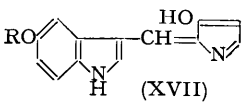

(XVI)

From the foregoing results it is clear that the properties of the C_{13} base from acetylviolacein are closely parallelled by those of the synthetic methene (III) and that the base does not contain an α-hydroxypyrrole residue. It therefore seems likely that the two probable structures for the C_{13} base are (XVII or XVIII; R = H) and hence the methyl ether of the base would be represented by (XVII or XVIII; R = Me) because on general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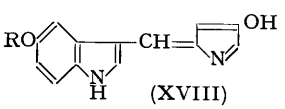
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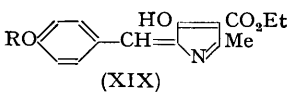
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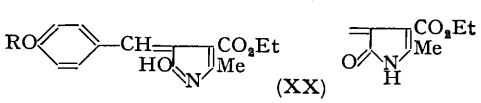
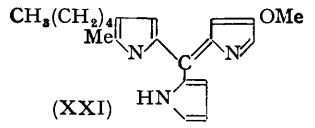

(XX)

grounds the hydroxyl group in the indole residue would be expected to be preferentially methylated. It was found that in model methylation experiments with 2′-(4′-ethoxycarbonyl-3′-hydroxy-5′-methylpyrryl)- (XIX; R = H) and 3′-(4′-ethoxycarbonyl-2′-hydroxy-5′-methylpyrryl)-1-*p*-hydroxyphenylmethene (XX; R = H), only the hydroxyl groups in phenyl residues were alkylated. The orientation of the methylation products (XIX and XX; R = Me) was confirmed by the synthesis of these methenes from anisaldehyde and the requisite pyrroles.

The β-hydroxypyrrylmethene system in formulæ (XVII) and (XVIII) recalls the tripyrrylmethene structure (XXI) assigned to the bacterial pigment prodigiosin (Wrede and Rothhaas, *Z. physiol. Chem.*, 1934, **226**, 95) and the possibility that violacein may be a 5-hydroxyindolyl-hydroxypyrryl-oxindolylmethene [*e.g.*, type (XXII), $C_{21}H_{15}O_3N_3$ or an oxidised form of this, *e.g.*, (XXIII), $C_{21}H_{13}O_3N_3$] has not been overlooked. Structures of

this type, which are consistent with the results of oxidative degradations (unpublished work and Part II, *loc. cit.*), would account for the formation of 5-hydroxyindole and oxindole on pyrolysis and of an indolylpyrrylmethene, of type (XVII) or (XVIII), with hydriodic acid. On this basis the empirical formula $C_{42}H_{30}O_7N_6$ suggested by Wrede (see Part II, *loc. cit.*) for violacein could perhaps be accommodated by supposing the crystalline pigment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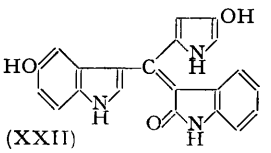
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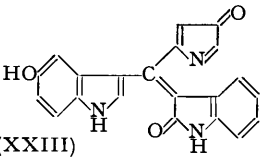
(XXIII)

to be a molecular complex of two C_{21} molecules, *e.g.*, $C_{21}H_{15}O_3N_3,C_{21}H_{13}O_3N_3,H_2O$, of the quinhydrone type but we prefer to reserve further discussion of possible structures until we have been able to interpret satisfactorily the results of other degradative work to be described in a later communication.

Experimental

Degradation of Acetylviolacein with Hydriodic Acid.—A mixture of acetylviolacein (Part II, *loc. cit.*) (0·2 g.), hydriodic acid (*d* 1·7; 10 ml.), and acetic acid (10 ml.) was heated under reflux (oil-bath at 130°) for 1½ hr. The resulting sparingly soluble, deep bronze, crystalline methene hydriodide (0·125 g.), m. p. >320°, which could not be satisfactorily recrystallised, was dissolved in warm 2N-sodium hydrogen carbonate (24 ml.), and the warm solution then treated with hot dilute acetic acid, giving the methene base as an insoluble brownish-yellow powder. This product (0·1 g.) slowly dissolved in boiling alcohol (30 ml.) containing concentrated hydrochloric acid (6 drops), giving a deep red solution which later deposited the *hydrochloride* in very deep red needles (a second crop was obtained by concentrating the solution ; total yield 0·1 g.), m. p. >320° (Found : C, 59·5; H, 4·1; N, 10·7. $C_{13}H_{10}O_2N_2$,HCl requires C, 59·4; H, 4·2; N, 10·7%). Light absorption in alcohol : $\lambda_{max.}$ 280, 308 mμ (log ε 4·16, 4·01) ; $\lambda_{min.}$ 255, 299, 340 mμ (log ε 3·89, 3·96, 3·46). On being kept, an alcoholic solution of the hydrochloride (0·1 g.) which had been diluted with distilled water (35 ml.) deposited the base as a *hydrate* in pale yellow needles, m. p. >320° (Found, in specimen dried in a vacuum at 80° for 1 hr. : C, 64·0; H, 4·8; N, 11·2. $C_{13}H_{10}O_2N_2,H_2O$ requires C, 63·9; H, 4·9; N, 11·5%). The *perchlorate* crystallised from alcohol in dark red needles, m. p. >320° (Found : C, 47·5; H, 3·4; N, 8·9. $C_{13}H_{10}O_2N_2,HClO_4$ requires C, 47·8; H, 3·4; N, 8·6%).

A mixture of the methene hydriodide (0·1 g.), acetic anhydride (5 ml.), and fused sodium acetate (0·2 g.) was heated under reflux for 15 min. and diluted with water (25 ml.). The yellow amorphous *acetyl* derivative, which eventually separated, was insoluble in ordinary solvents. Recrystallised from aqueous pyridine, this formed yellow needles containing solvent of crystallisation, m. p. *ca* 223—226° after sintering (Found, in a specimen dried in a vacuum at 70° for ½ hr. : C, 66·9; H, 4·8; N, 9·6. $C_{19}H_{16}O_5N_2,C_5H_5N$ requires C, 66·9; H, 4·9; N, 9·7%). Addition of water to a solution of the compound in acetic anhydride gave a solvent-free specimen which on repetition of the process had m. p. >320° [Found : C, 64·8; H, 4·6; N, 7·6; Ac, 35·6. $C_{13}H_7O_2N_2(CO{\cdot}CH_3)_3$ requires C, 64·8; H, 4·55; N, 8·0; Ac, 36·6%]. Light absorption in alcohol : $\lambda_{max.}$ 265 mμ (log ε 4·42) ; $\lambda_{min.}$ 250, 330 (log ε 4·33, 3·73) ; infl. at 289, 309 mμ (log ε 4·06, 4·01).

On being powdered in warm 2N-sodium hydrogen carbonate (30 ml.) the hydroiodide (0·18 g.) of the methene formed a clear yellow solution which on cooling deposited the yellow microcrystalline sodio-derivative (0·15 g.), soluble in hot aqueous sodium hydrogen carbonate and insoluble in ether. With warm water or acetic acid it gave the parent base.

Methylation of the C_{13} *Base.*—Methyl sulphate (0·52 ml.) was added gradually to a vigorously agitated solution of the aforementioned hydriodide (0·3 g.) in 2N-sodium hydroxide (30 ml.) containing sodium dithionite (0·3 g.), and the mixture subsequently acidified with acetic acid, giving the methylated methene, the *hydrochloride* of which separated from alcoholic hydrochloric acid in deep red needles (0·18 g.), m. p. >320° [Found : C, 60·6; H, 4·2; N, 10·2; OMe, 11·9. $C_{13}H_9ON_2(OMe)$,HCl requires C, 60·75; H, 4·7; N, 10·1; OMe, 11·2%]. The *perchlorate* formed deep red needles, m. p. >320°, from alcohol [Found : N, 8·5. $C_{13}H_9ON_2(OMe),HClO_4$ requires N, 8·2%]. Acetylation of the methylated methene gave the *diacetyl* derivative which

separated from alcohol in yellow needles, m. p. *ca* 300° (decomp.) (Found: OMe, 10·0. $C_{17}H_{13}O_3N_2$·OMe requires OMe, 9·6%).

5-Hydroxy-2-methylindole-3-aldehyde.—A solution of 5-hydroxy-2-methylindole (Beer, Clarke, Davenport, and Robertson, *J.*, 1951, 2029) (2 g.) in ether (30 ml.), containing hydrogen cyanide (10 ml.), at −5° was saturated with hydrogen chloride and kept at 0° overnight. A solution of the resulting colourless aldimine hydrochloride in water (40 ml.) was carefully neutralised with dilute aqueous ammonia, boiled for 5 min., and then allowed to cool, giving *5-hydroxy-2-methylindole-3-aldehyde* which separated from methanol in colourless needles (1·0 g.), m. p. 275° (decomp.) (Found: C, 68·5; H, 5·1. $C_{10}H_9O_2N$ requires C, 68·6; H, 5·1%). The 2 : 4-*dinitrophenylhydrazone* crystallised from acetic acid in deep red needles, m. p. 291° (decomp.) (Found: C, 54·0; H, 3·8. $C_{16}H_{13}O_5N_5$ requires C, 54·1; H, 3·7%).

2-Ethoxycarbonyl-5-methoxyindole-3-aldehyde.—The Gattermann reaction with 2-ethoxycarbonyl-5-methoxyindole (3 g.) (Hughes and Lions, *J. Proc. Roy. Soc. N.S.W.*, 1937, **71**, 475) gave an aldimine hydrochloride which decomposed in hot water to *2-ethoxycarbonyl-5-methoxyindole-3-aldehyde*, forming pale yellow needles (1·7 g.), m. p. 240°, from alcohol (Found: C, 62·9; H, 5·2; N, 5·6. $C_{13}H_{13}O_4N$ requires C, 63·1; H, 5·3; N, 5·6%).

Indolylpyrrylmethenes from Pyrrole-2-aldehyde.—(*a*) When two drops of a saturated alcoholic solution of hydrogen chloride were added to 2-methylindole (0·11 g.) and pyrrole-2-aldehyde (0·1 g.) in the same solvent (3 ml.), a deep red colour developed rapidly and, upon addition of ether, red needles of the unstable methene hydrochloride separated. The absorption spectrum of the methene salt was measured as quickly as possible on the freshly prepared alcoholic solution: $\lambda_{max.}$ 272, 317 mμ; $\lambda_{min.}$ 240, 298 mμ.

(*b*) The condensation product of pyrrole-2-aldehyde and 5-hydroxy-2-methylindole in alcohol was also unstable. Light absorption (of the reaction mixture): $\lambda_{max.}$ 278, 318 mμ; $\lambda_{min.}$ 236, 297 mμ.

(*c*) On the addition of alcoholic hydrogen chloride to a solution of pyrrole-2-aldehyde and 3-methylindole, a red colour was produced but almost immediately was obscured by the separation of an amorphous dark brown solid.

2′-(4′-Ethoxycarbonyl-3′-hydroxy-5′-methylpyrryl)-3-(5-hydroxy-2-methylindolyl)methene (III).—(*a*) A solution of 5-hydroxy-2-methylindole-3-aldehyde (0·2 g.) and 3-ethoxycarbonyl-4-hydroxy-2-methylpyrrole (Fischer and Loy, *Z. physiol. Chem.*, 1923, **128**, 75) (0·2 g.) in alcohol (50 ml.) containing saturated alcoholic hydrogen chloride (0·2 ml.) was concentrated until crystals appeared. The resulting *methene hydrochloride* crystallised from alcohol containing a trace of hydrogen chloride in bright red needles (0·28 g.), m. p. 232—234° (decomp.) (Found: C, 59·4; H, 5·35; N, 7·5. $C_{18}H_{18}O_4N_2$,HCl requires C, 59·5; H, 5·25; N, 7·7%). Light absorption in ethanol: $\lambda_{max.}$ 267, 301 mμ (log ε 4·10, 3·99); $\lambda_{min.}$ 245, 296 mμ (log ε 3·74, 3·89).

(*b*) The same compound, with identical properties and absorption spectrum, was obtained in rather low yield by the condensation of 5-hydroxy-2-methylindole and 4-ethoxycarbonyl-3-hydroxy-5-methylpyrrole-2-aldehyde (Fischer and Loy, *loc. cit.*) in a similar manner.

Prepared by the action of methyl sulphate (0·4 ml.) on a solution of the foregoing methene hydrochloride (0·3 g.) in 2N-sodium hydroxide (10 ml.) containing sodium dithionite (0·2 g.), the methyl ether of this methene was purified as its *perchlorate*, which formed red needles (0·21 g.), m. p. 228° [Found: C, 51·9; H, 4·7. $C_{18}H_{17}O_3N_2(OMe),HClO_4$ requires C, 51·75; H, 4·8%].

With hot acetic anhydride and sodium acetate the methene hydrochloride (0·2 g.) was converted into the *triacetyl* derivative, which separated from benzene in yellow rectangular prisms (0·15 g.), m. p. 210—212° [Found: C, 63·95; H, 5·3. $C_{18}H_{15}O_4N_2(CO{\cdot}CH_3)_3$ requires C, 63·7; H, 5·3%].

The following indolylpyrrylmethenes were prepared by the same general method.

2′-(4′-Ethoxycarbonyl-3′ : 5′-dimethyl-pyrryl)-3-(5-hydroxy-2-methylindolyl)methene (II), from 5-hydroxy-2-methylindole-3-aldehyde (0·2 g.) and 3-ethoxycarbonyl-2 : 4-dimethylpyrrole (0·2 g.), was obtained as the *hydrochloride* in bright red needles which could not be recrystallised; $\lambda_{max.}$ 246 mμ (log ε 3·94), $\lambda_{min.}$ 234, 300 mμ (log ε 3·87, 2·99) (Found: C, 63·0; H, 5·6. $C_{19}H_{20}O_3N_2$,HCl requires C, 63·2; H, 5·8%).

3′-(4′-Ethoxycarbonyl-2′-hydroxy-5′-methylpyrryl)-3-(2-ethoxycarbonyl-5-methoxyindolyl)methene (VI), from 2-ethoxycarbonyl-5-methoxyindole-3-aldehyde (0·2 g.) and 3-ethoxycarbonyl-5-hydroxy-2-methylpyrrole (0·17 g.), formed pale orange prisms (0·35 g.), m. p. 272—274°, from alcohol, $\lambda_{max.}$ 277, 302 mμ (log ε 4·06, 4·15), $\lambda_{min.}$ 260, 284, 340 mμ (log ε 3·98, 4·02, 3·84) (Found: C, 63·5; H, 5·4. $C_{21}H_{22}O_6N_2$ requires C, 63·3; H, 5·5%). This was also prepared with piperidine as the condensing agent.

2′-(4′-Ethoxycarbonyl-3′-hydroxy-5′-methylpyrryl)-3-(2-ethoxycarbonyl-5-methoxyindolyl)-methene (VII), from 2-ethoxycarbonyl-5-methoxyindole-3-aldehyde (0·2 g.) and 3-ethoxycarbonyl-4-hydroxy-2-methylpyrrole (0·17 g.), separated from alcohol containing a little hydrochloric acid as the *hydrochloride* in orange needles (0·3 g.), m. p. 198—200° (decomp.), $\lambda_{max.}$ 299 mμ (log ε 4·27); $\lambda_{min.}$ 274 mμ (log ε 4·06) (Found: C, 57·8; H, 5·4. $C_{21}H_{22}O_6N_2$,HCl requires C, 58·0; H, 5·3%).

2′-(4′-Bromo-3′: 5′-dimethylpyrryl)-3-(5-hydroxy-2-methylindolyl)methene (X), from 4-bromo-3: 5-dimethylpyrrole-2-aldehyde (Fischer and Ernst, *Annalen*, 1928, **447**, 148) (0·2 g.) and 5-hydroxy-2-methylindole (0·15 g.), was obtained as the *hydrochloride* in orange needles (0·3 g.), m. p. 282° with darkening at 220°, which could not be recrystallised, $\lambda_{max.}$ 258, 289, 326 mμ (log ε 3·79, 3·97, 3·82), $\lambda_{min.}$ 244, 267, 302 (log ε 3·59, 3·63, 3·65) (Found: C, 52·2; H, 4·3. $C_{16}H_{15}ON_2Br$,HCl requires C, 52·2; H, 4·4%).

3′-(5′-Bromo-2′: 4′-dimethylpyrryl)-3-(5-hydroxy-2-methylindolyl)methene (XI), from 5-bromo-2: 4-dimethylpyrrole-3-aldehyde (Fischer and Zeile, *Annalen*, 1928, **462**, 221) (0·4 g.) and 5-hydroxy-2-methylindole (0·3 g.), was obtained as the *hydrochloride*, in deep red prisms (0·15 g.), m. p. 171°, $\lambda_{max.}$ 275, 313 mμ (log ε 4·14, 3·86), $\lambda_{min.}$ 252, 300, 340 (log ε 3·91, 3·79, 3·49) (Found: C, 52·1; H, 4·2; N, 7·4. $C_{16}H_{15}ON_2Br$,HCl requires C, 52·2; H, 4·4; N, 7·6%).

Di-3-(5-*hydroxy*-2-*methylindolyl*)*methene* (IV).—(*a*) In alcoholic hydrogen chloride at room temperature a mixture of 5-hydroxy-2-methylindole (0·2 g.) and 5-hydroxy-2-methylindole-3-aldehyde (0·2 g.) gave *di*-3-(5-*hydroxy*-2-*methylindolyl*)*methene hydrochloride* which formed red needles (0·38 g.) (from alcohol), m. p. 320° (Found: C, 66·3; H, 5·1; N, 8·3. $C_{19}H_{16}O_2N_2$,HCl requires C, 66·5; H, 5·0; N, 8·2%). Light absorption in alcohol: $\lambda_{max.}$ 283 mμ (log ε 4·36); $\lambda_{min.}$ 244 mμ (log ε 3·61).

(*b*) The same product was obtained by heating a solution of the indole-aldehyde in 5% alcoholic hydrogen chloride for 30 min., and by the action of hydrogen chloride on a solution of 5-hydroxy-2-methylindole and formic acid in ether.

(*c*) The di-indolylmethene was the only product isolated from condensations carried out under the standard conditions (*i.e.*, with alcoholic hydrogen chloride) between 5-hydroxy-2-methylindole-3-aldehyde and 3-ethoxycarbonyl-5-hydroxy-2-methylpyrrole (Fischer and Müller, *Z. physiol. Chem.*, 1923, **132**, 75), 5-acetoxy-3-ethoxycarbonyl-2-methylpyrrole, 4-ethoxycarbonyl-2-hydroxypyrrole (Grob and Ankli, *Helv. Chim. Acta*, 1949, **32**, 2021), 2-acetoxy-3-acetyl-4-ethoxycarbonylpyrrole, 3-acetoxy-4-ethoxycarbonyl-2-hydroxypyrrole (Grob and Ankli, *loc. cit.*), and 2-hydroxypyrrole (Langenbeck and Boser, *Ber.*, 1951, **84**, 526). The condensation of 5-hydroxy-2-methylindole with 4-ethoxycarbonyl-2-hydroxy-5-methylpyrrole-3-aldehyde (Fischer and Müller, *loc. cit.*) also led to the di-indolylmethene.

2′-(3′-*Ethoxycarbonyl*-5′-*hydroxy*-4′-*methylpyrryl*)-3-(5-*hydroxy*-2-*methylindolyl*)*methene* (IX).—On being heated under reflux for 4 hr. a solution of 5-hydroxy-2-methylindole (0·3 g.) and 2-bromomethyl-3-ethoxycarbonyl-5-hydroxy-4-methylpyrrole (Fischer and Adler, *Z. physiol. Chem.*, 1931, **197**, 269) (0·3 g.) in methanol (4 ml.) became bright red and on cooling slowly deposited the *methene*. Recrystallised from methanol, this product formed red plates (0·15 g.), m. p. 189° with darkening from 120°, $\lambda_{max.}$ 286 mμ (log ε 4·14), $\lambda_{min.}$ 258 mμ (log ε 4·06) (Found: C, 66·0; H, 5·7; N, 8·5. $C_{18}H_{18}O_4N_2$ requires C, 66·3; H, 5·5; N, 8·6%).

p-*Dimethylaminophenyl*-2′-(4′-*ethoxycarbonyl*-3′-*hydroxy*-5′-*methylpyrryl*)*methene hydrochloride* (XIV), from *p*-dimethylaminobenzaldehyde and 3-ethoxycarbonyl-4-hydroxy-2-methylpyrrole, separated from alcoholic hydrogen chloride in violet needles, m. p. 162—164° (Found: N, 8·0. $C_{17}H_{20}O_3N_2$,HCl requires N, 8·3%).

With 3-ethoxycarbonyl-5-hydroxy-2-methylpyrrole (0·5 g.) *p*-dimethylaminobenzaldehyde (0·44 g.) gave the *hydrochloride* of *p*-dimethylaminophenyl-3′-(4′-ethoxycarbonyl-2′-hydroxy-5′-methylpyrryl)methene (XVI), forming orange prisms (0·8 g.), m. p. 207—209°, from alcohol containing a little hydrochloric acid (Found: N, 8·0; Cl, 11·2. $C_{17}H_{20}O_3N_2$,HCl requires N, 8·3; Cl, 10·9%).

Similarly *p*-hydroxybenzaldehyde (0·7 g.) and 3-ethoxycarbonyl-4-hydroxy-2-methylpyrrole (0·9 g.) gave 2′-(4′-*ethoxycarbonyl*-3′-*hydroxy*-5′-*methylpyrryl*)-p-*hydroxyphenylmethene* (XIX; R = H) in clusters of bright yellow needles (1·3 g.), m. p. 278° (decomp.) (Found: C, 66·2; H, 5·7; N, 5·4. $C_{15}H_{15}O_4N$ requires C, 66·0; H, 5·5; N, 5·1%). Prepared with boiling acetic anhydride and sodium acetate, the *diacetyl* derivative separated from 95% alcohol in yellow rectangular plates, m. p. 212—214° (Found: N, 3·8. $C_{19}H_{19}O_6N$ requires N, 3·9%). With methyl sulphate and aqueous sodium hydroxide the methene (XIX) gave 2′-(4′-*ethoxycarbonyl*-3′-*hydroxy*-5′-*methylpyrryl*)-p-*methoxyphenylmethene* (XIX; R = Me), forming yellow plates, m. p. 220°, from alcohol identical with a specimen (0·58 g.) prepared by the condensation of

3-ethoxycarbonyl-4-hydroxy-2-methylpyrrole (0·4 g.) and *p*-anisaldehyde (0·4 g.) in alcohol (24 ml.) and concentrated hydrochloric acid (2 drops) (Found: C, 66·3; H, 5·7; N, 4·8. $C_{16}H_{17}O_4N$ requires C, 66·9; H, 5·9; N, 4·9%).

3′-(4′-*Ethoxycarbonyl*-2′-*hydroxy*-5′-*methylpyrryl*)-p-*hydroxyphenylmethene* (XX; R = H), from *p*-hydroxybenzaldehyde (0·7 g.) and 3-ethoxycarbonyl-5-hydroxy-2-methylpyrrole (0·9 g.), separated from alcohol in yellow needles (1·4 g.), m. p. 224—226° (Found: N, 5·1. $C_{15}H_{15}O_4N$ requires N, 5·1%). The *methyl ether* (XX; R = Me) formed bright yellow cubes, m. p. 194°, from alcohol (Found: C, 66·7; H, 5·9; N, 4·8. $C_{16}H_{17}O_4N$ requires C, 66·9; H, 5·9; N, 4·9%). This was identical with the methene obtained when *p*-hydroxybenzaldehyde was replaced by anisaldehyde in the foregoing condensation.

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