459. *The Chemistry of Bacteria. Part VIII.*[1] * *The Synthesis of Violacein and Related Compounds.*

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4-Hydroxy-2-(3-oxindolylidene)but-3-enoic lactones (III) are converted by the action of ammonia into the corresponding lactams. By this reaction and other related methods, violacein (I; R = R′ = R″ = H), the major pigment of *Chromobacterium violaceum*, and some simpler analogues have been synthesised. A second pigment, produced in small amounts by the bacterium, has been shown by synthesis to be deoxyviolacein (V; R = R′ = R″ = H).

Violacein, the major pigment of *Chromobacterium violaceum*, has been formulated[1] as 5-(5-hydroxy-3-indolyl)-3-(3-oxindolylidene)-2-oxopyrroline, the *trans*-form of which is represented by structure (I; R = R′ = R″ = H). In synthetical experiments we began with analogues in which the 5-hydroxyindole nucleus was replaced by phenyl.

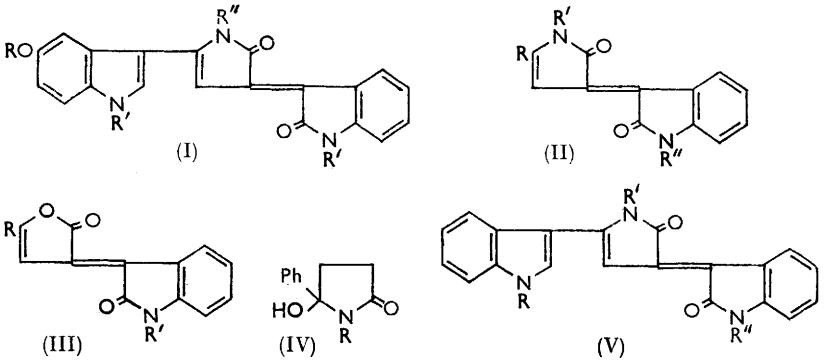
The simplest model, 3-(3-oxindolylidene)-2-oxo-5-phenylpyrroline (II; R = Ph, R′ = R″ = H), has been obtained by two related methods: first, from the readily available lactone[2] (III; R = Ph, R′ = H) by the action of ammonia in hot aqueous ethanol; and, secondly, by acid-catalysed condensation of isatin and β-benzoylpropionamide. This amide, prepared from 4-hydroxy-4-phenylbut-3-enoic lactone by treatment with ammonia, does not apparently exist in the cyclic form, 2-hydroxy-5-oxo-2-phenylpyrrolidine (IV; R = H), as suggested by Walton[3] since its ultraviolet absorption spectrum in alcohol is similar to that of acetophenone. The product derived from 4-hydroxy-4-phenylbut-3-enoic lactone and methylamine, on the other hand, resembles benzene in its absorption spectrum and must therefore be regarded as the hydroxypyrrolidine (IV; R = Me). The same conclusions have recently been reached by Cromwell and Cook[4] in an extensive study of the reactions of 4-hydroxy-4-phenylbut-3-enoic lactone with amines.

The hydroxypyrrolidine (IV; R = Me) condensed readily with isatin, yielding a mono-*N*-methyl derivative (II; R = Ph, R′ = Me, R″ = H) of the parent model compound, and the di-*N*-methyl derivative (II; R = Ph, R′ = R″ = Me) was similarly prepared from

* For a preliminary report see *Proc. Chem. Soc.*, 1958, 232.

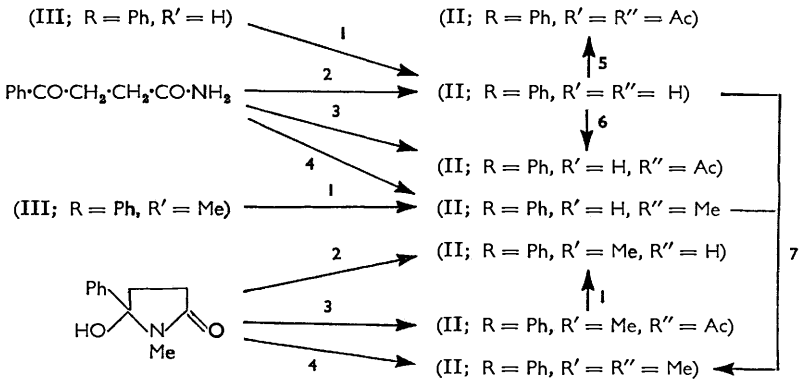
[1] Part VII, Ballantine, Barrett, Beer, Eardley, Robertson, Shaw, and Simpson, *J.*, 1958, 755.
[2] Barrett, Beer, Dodd, and Robertson, *J.*, 1957, 4810.
[3] Walton, *J.*, 1940, 438.
[4] Cromwell and Cook, *J. Amer. Chem. Soc.*, 1958, **80**, 4573.

1-methylisatin. Condensation of β-benzoylpropionamide and 1-methylisatin gave the second mono-*N*-methyl derivative (II; R = Ph, R′ = H, R″ = Me) which was also obtained from the lactone (III; R = Ph, R′ = Me) by the action of ammonia.



The intensely coloured 2-oxopyrroline (II; R = Ph, R′ = R″ = H) resembles violacein spectroscopically and in other respects, particularly in its sensitivity to alkalis and in its behaviour towards methylating and acetylating reagents. Both imino-groups in the analogue (II; R = Ph, R′ = R″ = H) are methylated by methyl sulphate–potassium carbonate, and violacein should therefore, under the same conditions, form a tetramethyl derivative in good agreement with the analytical data previously published.[1] Brief acetylation of the model compound gave a sparingly soluble mono-*N*-acetyl derivative which must have structure (II; R = Ph, R′ = H, R″ = Ac) since on methylation it afforded compound (II; R = Ph, R′ = Me, R″ = Ac) which was also obtained by condensation of 2-hydroxy-1-methyl-5-oxo-2-phenylpyrrolidine and isatin in boiling acetic anhydride. Prolonged acetylation of the analogue (II; R = Ph, R′ = R″ = H) furnished a benzene-soluble di-*N*-acetyl derivative. Violacein behaves similarly in that short-term acetylation yields mainly a triacetyl derivative, presumably (I; R = R′ = Ac, R″ = H), which on continued acetylation is converted into the more soluble tetra-acetylviolacein.

A further simple model of the violacein structure was provided by the phenol (II; R = *p*-hydroxyphenyl, R′ = R″ = H), prepared by the action of ammonia on 4-*p*-acetoxyphenyl-4-hydroxy-2-(3-oxindolylidene)but-3-enoic lactone (III; R = *p*-acetoxyphenyl, R′ = H). Like violacein, this compound undergoes a series of colour changes in alkaline solution and crystallises from acetone in a solvated form; its trimethyl derivative is unsolvated.



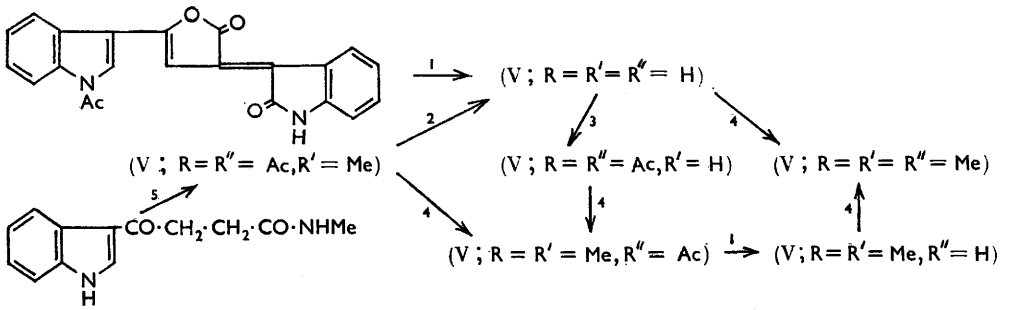
Reagents: 1, NH_3. 2, Isatin–MeOH–HCl. 3, Isatin–Ac_2O. 4, 1-Methylisatin–Ac_2O. 5, Ac_2O–NaOAc, 1 hr. 6, Ac_2O–NaOAc, 10 min. 7, Me_2SO_4–$COMe_2$–K_2CO_3.

Chart I.

The reactions involved in the preparation and inter-relation of the model oxindolylidene-2-oxopyrrolines are summarised in Chart 1. These experiments suggested that violacein was correctly formulated as (I; R = R′ = R″ = H) and served to indicate possible synthetical routes to the pigment and its close derivatives. The preparative method involving the replacement of the heterocyclic oxygen atom of lactones of type (III) by nitrogen was first applied in the indole series to the synthesis of 5-(3-indolyl)-3-(3-oxindolylidene)-2-oxopyrroline (V; R = R′ = R″ = H) which proved to be identical with a minor pigment of *Chromobacterium violaceum*. This product, termed deoxyviolacein, gave the expected trimethyl derivative on treatment with methyl sulphate–potassium carbonate, and acetylation yielded a di-*N*-acetyl derivative, regarded as (V; R = R″ = Ac, R′ = H). Unexpectedly, the latter derivative was converted by methyl sulphate–potassium carbonate into a mono-*N*-acetyl di-*N*-methyl derivative which must be formulated as (V; R = R′ = Me, R″ = Ac) since the infrared absorption spectrum of its hydrolysis product (V; R = R′ = Me, R″ = H) contains a peak at 3185 cm.$^{-1}$, characteristic of an oxindole rather than an indole NH group. Deacetylation under methylating conditions also occurred in the formation of a diacetyldimethylviolacein from triacetylviolacein. These deacetylations were apparently caused by the use of relatively large amounts of potassium carbonate; under normal conditions, triacetylviolacein gave the expected triacetylmethylviolacein (I; R = R′ = Ac, R″ = Me), and 5-(1-acetyl-3-indolyl)-3-(1-acetyl-3-oxindolylidene)-1-methyl-2-oxopyrroline (V; R = R″ = Ac, R′ = Me), obtained by condensation of isatin and γ-3-indolyl-*N*-methyl-γ-oxobutyramide in acetic anhydride, gave in the presence of an excess of potassium carbonate, the monoacetylidimethyl derivative (V; R = R′ = Me, R″ = Ac) mentioned above.

An attempted condensation of isatin and γ-3-indolyl-γ-oxobutyramide in acetic anhydride failed because under these conditions the amide was converted into γ-(1-acetyl-3-indolyl)-γ-oxobutyronitrile (ν_{CN}, 2262 cm.$^{-1}$), a complication which was not encountered in the analogous condensations with β-benzoylpropionamide.

Chart 2 summarises the reactions involved in the preparation of deoxyviolacein and its derivatives.



Reagents: 1, NH_3. 2, KOH, then NH_4Cl–NH_3. 3, Ac_2O–NaOAc. 4, Me_2SO_4–K_2CO_3–$COMe_2$. 5, Isatin–Ac_2O.

Chart 2.

Synthesis of violacein methyl ether (I; R = Me, R′ = R″ = H) was achieved easily, but in rather low yield, by the action of ammonia on 4-(1-acetyl-5-methoxy-3-indolyl)-4-hydroxy-2-(3-oxindolylidene)but-3-enoic lactone [2] (III; R = 1-acetyl-5-methoxy-3-indolyl, R′ = H). Fission of the ether, with hydrogen bromide in acetic acid, gave a phenol identical with natural violacein, and further methylation furnished tetramethylviolacein. A less direct comparison of synthetic and natural materials was afforded by degradation of violacein methyl ether with hydriodic acid, which yielded a crystalline hydriodide identical with that previously obtained from acetylated violacein.[5] The nature

[5] Beer, Jennings, and Robertson, *J.*, 1954, 2679.

of this degradation product, formerly thought to be an indolylpyrrylmethene, will be discussed elsewhere.

Treatment of violacein methyl ether with methyl sulphate–potassium carbonate gave only a low yield of tetramethylviolacein; the major product was an insoluble salt-like material identical in properties with a compound occasionally obtained by methylation of violacein. Further experiments showed that, with a large excess of potassium carbonate, violacein gives mainly the salt-like product which separates from the reaction mixture and is therefore not converted into the tetramethyl derivative. This by-product could not be recrystallised but on acetylation afforded diacetylmethylviolacein (I; R = Me, R′ = Ac, R″ = H), also obtained by acetylation of synthetic monomethylviolacein. It seems probable that, in the presence of relatively large amounts of potassium carbonate, the alkali-sensitive oxindole ring of violacein (or of its methyl ether) is opened and that the acid thus generated then forms a potassium salt.

TABLE 1. *Ultraviolet and visible absorption spectra (in ethanol).*

Compound	$\lambda_{max.}$ (mμ) (log ε)
β-Benzoylpropionamide	240(4·01), 278(2·97)
(IV; R = Me)	250(2·23), 256(2·31)
(II; R = Ph, R′ = R″ = H)	270(4·42), 330(3·79), 490(4·20)
(II; R = Ph, R′ = R″ = Me)	272(4·38), 340(3·88), 502(4·09)
(II; R = *p*-HO·C_6H_4, R′ = R″ = H)	270, 352, 539 *
(II; R = *p*-MeO·C_6H_4, R′ = R″ = Me)	269(4·39), 349(4·07), 513(4·19)
(V; R = R′ = R″ = H)	260(sh), 366, 562 *
(V; R = R′ = R″ = Me)	272(4·29), 377(3·92), 558(4·23) †
(I; R = Me, R′ = R″ = H)	262, 374, 582 *
Violacein (natural)	260, 376, 585 *
,, (synthetic)	261, 378, 585 *
Tetramethylviolacein	270(4·31), 382(3·88), *ca.* 590(4·29)
,, ,, (synthetic)	271(4·33), 383(3·89), *ca.* 590(4·32)

* Low solubility precludes accurate measurement of intensity.
† Measured in ethyl acetate solution.

TABLE 2. *Infrared absorption spectra (Nujol mulls).*

	Compound	NH region (cm.$^{-1}$)	C=O region (cm.$^{-1}$)
Phenyl-oxopyrrolines	(II; R = Ph, R′ = R″ = H)	3195	1681s, 1653m
	(II; R = Ph, R′ = H, R″ = Me)	3205	1678s
	(II; R = Ph, R′ = Me, R″ = H)	3205	1712w, 1684s, 1647m
	(II; R = Ph, R′ = R″ = Me)	—	1684s
	(II; R = Ph, R′ = H, R″ = Ac)	3205	1740m, 1715s, 1690s
	(II; R = Ph, R′ = Me, R″ = Ac)	—	1733s, 1715s 1698s
	(II; R = Ph, R′ = R″ = Ac)	—	1718sh, 1706s, 1689sh
	(II; R = *p*-MeO·C_6H_4, R′ = R″ = Me)	—	1692w, 1667s
Indolyl-oxopyrrolines	(V; R = R′ = R″ = H)	3448, 3195—3125	1692s, 1669s
	(V; R = R′ = R″ = Me)	—	1701w, 1672s
	(I; R = R′ = R″ = H)	3100—3330	1690s, 1665s
	(I; R = R′ = R″ = Me)	—	1695w, 1681s

s = strong, m = medium, w = weak, sh = shoulder.

Spectroscopic data for some of the compounds described in this paper are collected in Tables 1 and 2.

EXPERIMENTAL

Claims for identity of specimens prepared by different routes are based on infrared absorption spectra.

2-Hydroxy-1-methyl-5-oxo-2-phenylpyrrolidine (IV; R = Me).—As described by Walton,[3] 4-hydroxy-4-phenylbut-3-enoic lactone was converted by methylamine into the pyrrolidine, which formed colourless needles, m. p. 132—135° (Found: C, 69·2; H, 6·7; N, 7·3. Calc. for

$C_{11}H_{13}O_2N$: C, 69·1; H, 6·8; N, 7·3%), but with ammonia the product was β-benzoylpropionamide, prisms (from water), m. p. 123—125° (Found: C, 67·7; H, 5·9; N, 7·8. Calc. for $C_{10}H_{11}O_2N$: C, 67·8; H, 6·2; N, 7·9%).

3-(3-*Oxindolylidene*)-2-*oxo*-5-*phenylpyrroline* (II; R = Ph, R′ = R″ = H).—A solution of isatin (0·8 g.) and β-benzoylpropionamide (1·0 g.) in methanol (10 ml.) containing concentrated hydrochloric acid (2 ml.) was heated under reflux for 30 min. The sparingly soluble 3-(3-*oxindolylidene*)-2-*oxo*-5-*phenylpyrroline* separated from acetone in small dark crystals with a green sheen (1·3 g.), m. p. >300° (Found: C, 75·0; H, 4·1; N, 9·9. $C_{18}H_{12}O_2N_2$ requires C, 75·0; H, 4·2; N, 9·7%). The same oxopyrroline (0·5 g.) was prepared by passing ammonia into a boiling suspension of 4-hydroxy-2-(3-oxindolylidene)-4-phenylbut-3-enoic lactone [2] (1·0 g.) in 90% ethanol (750 ml.) for 3 hr. (Found: C, 74·9; H, 4·5; N, 9·7%).

3-(1-*Acetyl*-3-*oxindolylidene*)-2-*oxo*-5-*phenylpyrroline* (II; R = Ph, R′ = H, R″ = Ac).—The foregoing oxopyrroline (1·0 g.), heated with acetic anhydride (25 ml.) and sodium acetate (1·0 g.) for 10 min., gave 3-(1-*acetyl*-3-*oxindolylidene*)-2-*oxo*-5-*phenylpyrroline* (0·6 g.), forming dark red-brown needles, m. p. >300° from acetone (Found: C, 72·6; H, 4·3; N, 8·9. $C_{20}H_{14}O_3N_2$ requires C, 72·7; H, 4·3; N, 8·5%). This product (0·9 g.) was also obtained by heating isatin (0·8 g.) with β-benzoylpropionamide (1·0 g.) in acetic anhydride (4 ml.) for 30 min. (Found: C, 72·7; H, 4·3%).

3-(1-*Methyl*-3-*oxindolylidene*)-2-*oxo*-5-*phenylpyrroline* (II; R = Ph, R′ = H, R″ = Me).—Prepared by heating 1-methylisatin (0·8 g.) and β-benzoylpropionamide (1·0 g.) with acetic anhydride (4 ml.) for 30 min., 3-(1-*methyl*-3-*oxindolylidene*)-2-*oxo*-5-*phenylpyrroline* formed dark brown needles (1·0 g.), m. p. >300°, from acetone (Found: C, 75·4; H, 4·5; N, 8·9. $C_{19}H_{14}O_2N_2$ requires C, 75·5; H, 4·7; N, 9·3%). The same product (0·7 g.) separated when ammonia was passed into a boiling suspension of 4-hydroxy-2-(1-methyl-3-oxindolylidene)-4-phenylbut-3-enoic lactone [2] (1·0 g.) in 90% ethanol (Found: C, 75·3; H, 4·9; N, 9·3%).

1-*Methyl*-3-(3-*oxindolylidene*)-2-*oxo*-5-*phenylpyrroline* (II; R = Ph, R′ = Me, R″ = H).—Isatin (0·8 g.) and 2-hydroxy-1-methyl-5-oxo-2-phenylpyrrolidine (1·0 g.), heated in methanol (10 ml.) containing concentrated hydrochloric acid (2 ml.) for 30 min., gave 1-*methyl*-3-(3-*oxindolylidene*)-2-*oxo*-5-*phenylpyrroline* which crystallised from ethyl acetate in dark brown needles (0·9 g.), m. p. 254° (Found: C, 75·7; H, 4·6; N, 9·1. $C_{19}H_{14}O_2N_2$ requires C, 75·5; H, 4·6; N, 9·3%).

3-(1-*Acetyl*-3-*oxindolylidene*)-1-*methyl*-2-*oxo*-5-*phenylpyrroline* (II; R = Ph, R′ = Me, R″ = Ac).—3-(1-Acetyl-3-oxindolylidene)-2-oxo-5-phenylpyrroline (500 mg.) was heated under reflux for 24 hr. with acetone (150 ml.), potassium carbonate (500 mg.), and methyl sulphate (3 ml.). The residue obtained by concentration of the filtered solution was triturated with water. After purification by chromatography on silica in benzene, 3-(1-*acetyl*-3-*oxindolylidene*)-1-*methyl*-2-*oxo*-5-*phenylpyrroline* formed almost black needles (300 mg.), m. p. 248° (Found: C, 73·0; H, 4·6; N, 8·3. $C_{21}H_{16}O_3N_2$ requires C, 73·3; H, 4·7; N, 8·1%), identical with a sample (Found: C, 73·2; H, 4·6; N, 7·9%) obtained by condensation of isatin and 2-hydroxy-1-methyl-5-oxo-2-phenylpyrrolidine in hot acetic anhydride. Deacetylation, with ammonia in boiling ethanol, gave 1-methyl-3-(3-oxindolylidene)-2-oxo-5-phenylpyrroline (Found: C, 75·3; H, 4·8; N, 9·4%).

1-*Methyl*-3-(1-*methyl*-3-*oxindolylidene*)-2-*oxo*-5-*phenylpyrroline* (II; R = Ph, R′ = R″ = Me).—Prepared by reaction of 1-methylisatin (0·8 g.) and 2-hydroxy-1-methyl-5-oxo-2-phenylpyrrolidine (1·0 g.) in boiling acetic anhydride (4·0 ml.) for 30 min., 1-*methyl*-3-(1-*methyl*-3-*oxindolylidene*)-2-*oxo*-5-*phenylpyrroline* formed dark red needles (0·9 g.), m. p. 203°, from ethyl acetate (Found: C, 75·8; H, 5·1; N, 9·1. $C_{20}H_{16}O_2N_2$ requires C, 75·9; H, 5·1; N, 8·9%). The same product (1·0 g.) was obtained when 3-(3-oxindolylidene)-2-oxo-5-phenylpyrroline (1·0 g.) was heated under reflux for 24 hr. with acetone (400 ml.), methyl sulphate (4 ml.), and potassium carbonate (1·0 g.). After purification by chromatography on neutral alumina, the methylated compound formed red-brown needles (0·9 g.), m. p. and mixed m. p. 203° (Found: C, 75·9; H, 5·0; N, 9·0; O, 9·8. Calc. for $C_{20}H_{16}O_2N_2$: O, 10·1%). Methylation of 3-(1-methyl-3-oxindolylidene)-2-oxo-5-phenylpyrroline similarly gave the dimethyl compound, m. p. and mixed m. p. 203°.

1-*Acetyl*-3-(1-*acetyl*-3-*oxindolylidene*)-2-*oxo*-5-*phenylpyrroline* (II; R = Ph, R′ = R″ = Ac).—3-(3-Oxindolylidene)-2-oxo-5-phenylpyrroline (1·0 g.) was heated under reflux with acetic anhydride (30 ml.) and sodium acetate (1·0 g.) for 1 hr., and the crude product obtained after treatment of the reaction mixture with water was extracted with benzene (Soxhlet). The

benzene-insoluble fraction (500 mg.) was 3-(1-acetyl-3-oxindolylidene)-2-oxo-5-phenylpyrroline. The benzene solution was passed through a silica gel column and concentrated; 1-*acetyl*-3-(1-*acetyl*-3-*oxindolylidene*)-2-*oxo*-5-*phenylpyrroline* separated in dark red needles (200 mg.), m. p. 260° (Found: C, 71·3; H, 4·2; N, 7·8. $C_{22}H_{16}O_4N_2$ requires C, 71·0; H, 4·3; N, 7·5%).

4-p-*Acetoxyphenyl*-4-*hydroxy*-2-(3-*oxindolylidene*)*but*-3-*enoic Lactone*.—Prepared by heating isatin (7·0 g.) and 4-*p*-acetoxyphenyl-4-hydroxybut-3-enoic lactone [6] (10 g.) for 30 min. in ethanol containing pyridine (1 ml.), this *compound* crystallised from acetone in deep red needles (13 g.), m. p. >300° (Found: C, 69·1; H, 3·7; N, 4·2. $C_{20}H_{13}O_5N$ requires C, 69·2; H, 3·8; N, 4·0%).

5-p-*Hydroxyphenyl*-3-(3-*oxindolylidene*)-2-*oxopyrroline* (II; R = *p*-hydroxyphenyl, R′ = R″ = H).—When ammonia was passed for 5 hr. into a boiling solution of the foregoing lactone (10 g.) in 95% ethanol (1500 ml.), the violet microcrystalline *oxopyrroline* (7·0 g.) was precipitated. Purification by Soxhlet extraction with acetone gave a solvated sample (cf. violacein), m. p. >300° (Found: C, 70·5; H, 4·3; N, 8·4. $C_{18}H_{12}O_3N_2,\frac{1}{2}C_3H_6O$ requires C, 70·3; H, 4·5; N, 8·4%). The oxopyrroline was readily soluble in dilute aqueous sodium hydroxide, giving a violet solution which rapidly became dark green; heating changed the colour of the solution to deep red, and acidification then precipitated an unstable yellow solid.

On methylation by acetone–potassium carbonate–methyl sulphate this oxopyrroline (1·0 g.) gave 5-p-*methoxyphenyl*-1-*methyl*-3-(1-*methyl*-3-*oxindolylidene*)-2-*oxopyrroline* (0·65 g.) which formed violet needles, m. p. 188°, from acetone (Found: C, 73·0; H, 5·2; N, 8·3. $C_{21}H_{18}O_3N_2$ requires C, 72·8; H, 5·2; N, 8·1%).

5-(3-*Indolyl*)-3-(3-*oxindolylidene*)-2-*oxopyrroline* (V; R = R′ = R″ = H).—When ammonia was passed into a suspension of 4-(1-acetyl-3-indolyl)-4-hydroxy-2-(3-oxindolylidene)but-3-enoic lactone [2] (4·0 g.) in 90% ethanol (300 ml.) for 4 hr., 5-(3-*indolyl*)-3-(3-*oxindolylidene*)-2-*oxopyrroline* separated as a black amorphous powder (2·7 g.). After purification by chromatography on alumina in acetone, the oxopyrroline was obtained as small dark crystals with a green reflex, m. p. >300° (Found: C, 73·5; H, 4·1; N, 12·6. $C_{20}H_{13}O_2N_3$ requires C, 73·4; H, 4·0; N, 12·8%). This product (0·5 g.) was converted by methyl sulphate (1 ml.) and potassium carbonate (1·5 g.) in boiling acetone (100 ml.) in 8 hr. into an amorphous blue solid which was purified by chromatography on alumina in ethyl acetate. This *trimethyl derivative* separated from ethyl acetate as a royal-blue microcrystalline powder (0·31 g.) m. p. >300° (Found: C, 74·6; H, 5·1; N, 11·2. $C_{23}H_{19}O_2N_3$ requires C, 74·8; H, 5·2; N, 11·4%).

Prepared by heating the parent oxopyrroline (0·72 g.) for 10 min. with acetic anhydride (15 ml.) and sodium acetate (1·0 g.) the *diacetyl derivative* crystallised from acetone (Soxhlet) in minute dark red needles (0·80 g.), m. p. >300° (Found: C, 70·2; H, 4·2; N, 10·3. $C_{24}H_{17}O_4N_3$ requires C, 70·1; H, 4·2; N, 10·2%). Methylation using an excess of potassium carbonate converted this diacetyl derivative (0·35 g.) into 3-(1-*acetyl*-3-*oxindolylidene*)-1-*methyl*-5-(1-*methyl*-3-*indolyl*)-2-*oxopyrroline* which formed purple-red needles (0·13 g.), m. p. 268—271°, from benzene after purification by chromatography on silica (Found: C, 72·2; H, 5·0; N, 10·8. $C_{24}H_{19}O_3N_3$ requires C, 72·5; H, 4·8; N, 10·6%).

1-*Methyl*-5-(1-*methyl*-3-*indolyl*)-3-(3-*oxindolylidene*)-2-*oxopyrroline* (V; R = R′ = Me, R″ = H).—Ammonia was passed into a suspension of 3-(1-acetyl-3-oxindolylidene)-1-methyl-5-(1-methyl-3-indolyl)-2-oxopyrroline (100 mg.) in boiling ethanol for 2 hr. The crude *dimethyl derivative* was purified by chromatography in benzene solution on alumina and then crystallised from benzene in purple needles (20 mg.), m. p. 285—290° (Found: C, 74·1; H, 4·7; N, 12·0. $C_{22}H_{17}O_2N_3$ requires C, 74·4; H, 4·8; N, 11·8%). Further methylation of this product by the usual method gave the trimethyl derivative described above.

Derivatives of γ-(3-*Indolyl*)-γ-*oxobutyric Acid*.—4-(1-Acetyl-3-indolyl)-4-hydroxybut-3-enoic lactone [2] (0·50 g.), heated with aqueous ammonia (*d* 0·88; 30 ml.) for 1 hr., gave γ-(3-*indolyl*)-γ-*oxobutyramide* (0·385 g.) which formed colourless prisms, m. p. 214°, from alcohol (Found: C, 66·7; H, 5·4. $C_{12}H_{12}O_2N_2$ requires C, 66·7; H, 5·6%), $\lambda_{max.}$ (in EtOH) 242, 298 mμ (log ε 4·09 3·86), $\lambda_{min.}$ 228, 272 mμ (log ε 3·73, 3·77). The corresponding *methylamide* crystallised from acetone in colourless prisms, m. p. 207° (Found: C, 68·2; H, 6·2; N, 11·8. $C_{13}H_{14}O_2N_2$ requires C, 67·8; H, 6·1; N, 12·2%), $\lambda_{max.}$ 241, 298 mμ (log ε 4·16, 4·15), $\lambda_{min.}$ 229, 272 mμ (log ε 3·84, 3·86).

γ-3-Indolyl-γ-oxobutyramide (250 mg.), heated with acetic anhydride (10 ml.) for 1 hr., gave γ-(1-*acetyl*-3-*indolyl*)-γ-*oxobutyronitrile* (200 mg.) which formed colourless needles, m. p.

[6] Swain, Todd, and Waring, *J.*, 1944, 548.

199°, from methanol (Found: C, 69·6; H, 5·0. $C_{14}H_{12}O_2N_2$ requires C, 70·0; H, 5·0%), ν_{CN} 2262 cm.$^{-1}$. Hydrolysis of the nitrile with hot aqueous 2N-sodium hydroxide afforded γ-(3-indolyl)-γ-oxobutyric acid, m. p. and mixed m. p. 236°.

5-(1-*Acetyl*-3-*indolyl*)-3-(1-*acetyl*-3-*oxindolylidene*)-1-*methyl*-2-*oxopyrroline* (V; R = R″ = Ac, R′ = Me).—γ-(3-Indolyl)-*N*-methyl-γ-oxobutyramide (250 mg.) condensed with isatin (160 mg.) in boiling acetic anhydride, giving 5-(1-*acetyl*-3-*indolyl*)-3-(1-*acetyl*-3-*oxindolylidene*)-1-*methyl*-2-*oxopyrroline* (380 mg.) as black prisms, m. p. 275—280°, from benzene (Found: C, 70·9; H, 4·3; N, 9·6. $C_{25}H_{19}O_4N_3$ requires C, 70·6; H, 4·5; N, 9·9%). A suspension of this product (100 mg.) in ethanol (50 ml.) containing potassium hydroxide (1 ml. of 50% aqueous solution) was heated under reflux (nitrogen atomosphere) for 4 hr. After addition of ammonium chloride, ammonia was passed into the hot solution for 3 hr., and the solution was then concentrated to *ca.* 10 ml. Addition of water precipitated a black amorphous solid, which was purified by chromatography on alumina in acetone, giving 5-(3-indolyl)-3-(3-oxindolylidene)-2-oxopyrroline (12 mg.), identical with the sample previously described.

5-(1-Acetyl-3-indolyl)-3-(1-acetyl-3-oxindolylidene)-1-methyl-2-oxopyrroline (100 mg.) was converted in 4 hr. by methylation (excess of potassium carbonate) into 3-(1-acetyl-3-oxindolylidene)-1-methyl-5-(1-methyl-3-indolyl)-2-oxopyrroline (40 mg.), m. p. 265—270° (Found: C, 72·4; H, 4·8%), identical with the sample obtained by methylation of the diacetyl derivative of 5-(3-indolyl)-3-(3-oxindolylidene)-2-oxopyrroline.

Isolation of Deoxyviolacein.—The acetone mother-liquor obtained in the previously described [1] isolation of violacein gave, on concentration, a black viscous oil which was extracted repeatedly with benzene and then with ethyl acetate. Chromatography on alumina with, in turn, ethyl acetate, chloroform, and acetone as developing solvents separated deoxyviolacein as a mobile bright blue band from the strongly absorbed violacein. Evaporation of the acetone solution gave a black residue which separated from ethyl acetate as an amorphous blue-black solid (31 mg. from 80 trays), m. p. >300°, sparingly soluble in ethyl acetate, in acetone, and in ethanol giving bluish-purple solutions. Deoxyviolacein was insoluble in cold aqueous sodium hydroxide, but dissolved in alcoholic sodium hydroxide forming an emerald-green solution which rapidly became dark red.

By using the method employed for the methylation of violacein,[1] deoxyviolacein (50 mg.) was converted into the *trimethyl derivative*, which separated from ethyl acetate as a small dark blue crystals (35 mg.), m. p. >300° (Found: C, 74·7; H, 5·3; N, 11·3%), identical with the synthetic trimethyl derivative of 5-(3-indolyl)-3-(3-oxindolylidene)-2-oxopyrroline described above.

5-(5-*Methoxy*-3-*indolyl*)-3-(3-*oxindolylidene*)-2-*oxopyrroline* (I; R = Me, R′ = R″ = H).—Passage of ammonia for 4 hr. through a boiling suspension of 4-(1-acetyl-5-methoxy-3-indolyl)-4-hydroxy-2-(3-oxindolylidene)but-3-enoic lactone [2] (0·50 g.) in 90% ethanol gave the *oxopyrroline* as black crystals (75 mg.), m. p. >310°, which were purified by extraction (Soxhlet) with dry acetone (Found: C, 70·5; H, 4·2; N, 11·6. $C_{21}H_{15}O_3N_3$ requires C, 70·6; H, 4·2; N, 11·8%). The same product was obtained in *ca.* 4% yield by treatment of an ice-cold saturated solution of violacein in methanol with excess of diazomethane for 4 days.

5-(5-*Hydroxy*-3-*indolyl*)-3-(3-*oxindolylidene*)-2-*oxopyrroline*; *Violacein* (I; R = R′ = R″ = H).—The solution obtained by heating 5-(5-methoxy-3-indolyl)-3-(3-oxindolylidene)-2-oxopyrroline (215 mg.) with hydrogen bromide dissolved in acetic acid (200 ml. of 5% solution) for 3 hr. was concentrated to small bulk and neutralised by addition of saturated aqueous sodium hydrogen carbonate. The precipitated black product (130 mg.) had the characteristic properties of violacein; in contrast to the starting material, it dissolved in aqueous sodium hydroxide, giving a green solution which rapidly became brownish-red on being heated. After purification by extraction (Soxhlet) with acetone, the product was obtained as black crystals with the requisite visible, ultraviolet, and infrared absorption spectra (Found: C, 69·55; H, 4·0; N, 11·3. Calc. for $C_{20}H_{13}O_3N_3,\frac{1}{2}C_3H_6O$: [1] C, 69·3; H, 4·3; N, 11·3%).

Heated with methyl sulphate (1·2 ml.), potassium carbonate (1·5 g.), and acetone (250 ml.) for 24 hr., the synthetic violacein methyl ether (105 mg.) gave mainly the insoluble partially methylated product (70 mg.) described below together with a small amount of tetramethylviolacein; [1] the latter was obtained in improved yield by methylation of the oxopyrroline with methyl sulphate in alkaline aqueous acetone, and crystallisation from ethyl acetate gave bronze needles, m. p. and mixed m. p. 220° (Found: C, 72·0; H, 5·6; N, 10·2. Calc. for $C_{24}H_{21}O_3N_3$: C, 72·2; H, 5·3; N, 10·5%).

Acetylation of Violacein.—The solution obtained by heating violacein (1·0 g.), sodium acetate (1·0 g.), and acetic anhydride (25 ml.) under reflux for 30 min. was poured into water, and the resulting solid extracted with benzene (Soxhlet). After purification by chromatography on silica, the benzene extract yielded *tetra-acetylviolacein* as shining brownish-red needles (250 mg.), m. p. >300° [Found: C, 65·5; H, 4·1; N, 8·4. $C_{20}H_9O_3N_3(CO\cdot CH_3)_4$ requires C, 65·8; H, 4·1; N, 8·2%]. The benzene-insoluble material was purified by repeated extraction (Soxhlet) with acetone, yielding *triacetylviolacein* as dark brown needles with a green reflex (500 mg.), m. p. >300° [Found: C, 66·1; H, 4·2; N, 8·8; O, 20·6. $C_{20}H_{10}O_3N_3(CO\cdot CH_3)_3$ requires C, 66·5; H, 4·1; N, 9·0; O, 20·4%].

Methylation of Violacein.—Violacein (500 mg.), heated for 18 hr. with methyl sulphate (2 ml.), potassium carbonate (1·0 g.), and acetone (300 ml.), gave the previously described [1] tetramethyl derivative (330 mg.), which crystallised from benzene in bronze needles, m. p. 128° (remelting at 220°). When a large excess (10 g.) of potassium carbonate was used, the main product was a dark acetone-insoluble compound which could not be satisfactorily purified but on treatment with acetic anhydride and sodium acetate afforded a crystalline *acetyl derivative*, dark needles with a green reflex, m. p. 315° (from acetic anhydride) [Found: C, 67·4, 67·7; H, 4·5, 4·1; N, 9·5. $C_{21}H_{13}O_3N_3(CO\cdot CH_3)_2$ requires C, 68·0; H, 4·3; N, 9·5%]. The same diacetylmethylviolacein was obtained by acetylation of 5-(5-methoxy-3-indolyl)-3-(3-oxindolylidene)-2-oxopyrroline obtained synthetically.

Methylation of Triacetylviolacein.—Triacetylviolacein (250 mg.), potassium carbonate (650 mg.), and methyl sulphate (1 ml.), heated under reflux in acetone (250 ml.) for 24 hr., gave an amorphous product which was purified by chromatography on silica with benzene and then benzene–chloroform (2 : 1) as eluting solvents. The resulting *triacetylmethylviolacein* formed dark needles (210 mg.), m. p. 257—258° [Found: C, 66·7; H, 4·5; N, 8·9; Ac, 27·1. $C_{21}H_{12}O_3N_3(CO\cdot CH_3)_3$ requires C, 67·1; H, 4·4; N, 8·7; Ac, 26·9%]. Repetition of the experiment but with excess of potassium carbonate (4·0 g.) afforded a *diacetyldimethylviolacein* (200 mg.) as minute prisms, m. p. 275° [Found: C, 68·8; H, 4·8; N, 9·5. $C_{22}H_{15}O_3N_3(CO\cdot CH_3)_2$ requires C, 68·6; H, 4·7; N, 9·2%].

The foregoing triacetylmethylviolacein (200 mg.), dissolved in boiling ethanol, was treated with ammonia for 2 hr. The dark residue obtained by evaporation of the ethanol was purified by chromatography on neutral alumina in ethyl acetate and thus afforded a monomethyl derivative of violacein, 5-(5-*hydroxy*-3-*indolyl*)-1-*methyl*-3-(3-*oxindolylidene*)-2-*oxopyrroline*, which crystallised from acetone in dark needles with a red reflex (100 mg.), m. p. >300° (Found: C, 70·2; H, 4·8; N, 11·0. $C_{21}H_{15}O_3N_3,\frac{1}{2}C_3H_6O$ requires C, 70·0; H, 4·6; N, 10·9%).

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