427. *The Chemistry of Bacteria. Part IV.* A C_{20}-Acid from Violacein.*

By J. A. Ballantine, C. B. Barrett, R. J. S. Beer, B. G. Boggiano, K. Clarke, Stephen Eardley, B. E. Jennings, and Alexander Robertson.

Reductive hydrolytic fission of violacein gives, in high yield, a colourless acid, $C_{20}H_{16}O_5N_2$, which on the basis of chemical and spectroscopic evidence is formulated as a γ-(5-hydroxy-3-indolyl)-α-oxindolyl-γ-oxobutyric acid (VII).

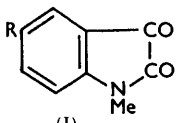
In earlier papers of this series it has been argued that violacein, the pigment of *Chromobacterium violaceum*, contains a 5-hydroxyindole nucleus, an oxindole nucleus, and probably a hydroxypyrrole residue. The present communication is concerned with the structure of a colourless acidic degradation product, $C_{20}H_{16}O_5N_2$, obtained from violacein by the action of alkali in the presence of zinc. If, as now seems likely, the molecular formula of violacein proves to be of the type C_{21}–N_3 (see Part III *), then only one carbon atom and one nitrogen atom are lost in the formation of this C_{20}-acid, a conclusion which is consistent with the high yield of the acid and with the simultaneous production of ammonia, identified as toluene-*p*-sulphonamide, during the degradation.

The behaviour of the C_{20}-acid with methylating agents showed that it is a phenolic acid containing two other weakly acidic replaceable hydrogen atoms. Thus with diazomethane the acid gave a phenolic methyl ester (a monomethyl derivative) which, rather surprisingly, was also formed by the action of methyl iodide and potassium carbonate in boiling acetone. With methyl sulphate and an excess of alkali the C_{20}-acid gave an acidic trimethyl derivative containing one methoxyl group, whilst the use of an excess of methyl sulphate, so that the reaction mixture finally became acidic, yielded a neutral tetramethyl derivative with two methoxyl groups. The latter compound is clearly the methyl ester of the trimethyl derivative, from which it was prepared by methyl iodide–potassium carbonate. The position of the methyl groups in the trimethyl derivative is established by the oxidation with potassium permanganate to 1-methylisatin (I; R = H) and 5-methoxy-1-methylisatin (I; R = OMe). The C_{20}-acid therefore contains the 5-hydroxyindole and oxindole residues believed to be present in violacein. Of the four carbon atoms unaccounted for, one is present as a carboxyl, and a second must occur as a carbonyl group since the tetramethyl derivative of the C_{20}-acid forms a 2 : 4-dinitrophenylhydrazone; the parent acid does not give satisfactory carbonyl derivatives. Further evidence that the C_{20}-acid contains a keto-acid system is provided by condensation of the trimethyl derivative and hydrazine with the loss of two molecules of water to give a non-acidic product which must be either a pyrazolone or a pyridazinone. The same compound is also obtained from the tetramethyl derivative with, in this case, the elimination of a molecule of water and of methanol. The properties of the C_{20}-acid are not those of a β-keto-acid. Thus, it is stable to hot alkali in the absence of oxygen and is not readily decarboxylated. Under vigorous conditions for decarboxylation, extensive decomposition occurs and the main product is 5-hydroxyindole. We consider that the C_{20}-acid is a γ-keto-acid and must contain system (II) with linked oxindole and 5-hydroxyindole nuclei, an arrangement which is in agreement with the absence of a *C*-methyl group (Kuhn–Roth determination).

The stability of the C_{20}-acid to alkaline hydrolysis indicates that the carbonyl group in expression (II) is not attached to the oxindole nucleus, since 3-acyloxindoles are alkali-sensitive. The carbonyl group must therefore be linked to the indole nucleus, in either the α- or the β-position. That it is attached to the β-position is indicated partly by the oxidation experiments described later and partly by spectroscopic studies. Thus, the 2 : 4-dinitrophenylhydrazone of the tetramethyl derivative is a deep red compound ($\lambda_{max.}$

* Part III, *J.*, 1954, 2679.

431 mμ in $CHCl_3$) very similar in colour to the dinitrophenylhydrazone of 1 : 2 : 3 : 4-tetrahydro-9-methyl-4-oxocarbazole [1] ($\lambda_{max.}$ 436 mμ in $CHCl_3$). Moreover the ultraviolet absorption of the C_{20}-acid and its derivatives can be very satisfactorily interpreted as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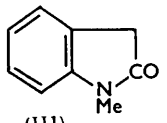
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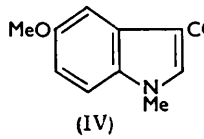
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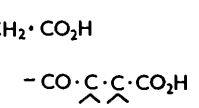

(II)

simple summations of absorptions due to oxindole and β-acylindole residues. As illustrated in the Table the absorption characteristics of an equimolar mixture of 1-methyloxindole (III) and γ-(5-methoxy-1-methyl-3-indolyl)-γ-oxobutyric acid [1] (IV) are almost identical with those of the tetramethyl derivative and, similarly, a mixture of 1-methyloxindole and the pyridazinone [1] derived from the acid (IV) shows virtually the same ultraviolet absorption as does the pyridazinone obtained from the trimethyl derivative of the C_{20}-acid. The differences between the ultraviolet absorption characteristics of α- and β-acylindoles are marked [1] and it is therefore possible to eliminate an α-acylindole structure for the C_{20}-acid.

	$\lambda_{max.}$ (mμ) (log ε)	$\lambda_{min.}$ (mμ) (log ε)
Equimolar mixture of (III) and (IV)	253 (4·36), 306 (3·95)	233 (3·93), 285 (3·85)
Tetramethyl deriv. of C_{20}-acid	255 (4·39), 308 (4·05)	287 (3·92)
Equimolar mixture of (III) and the pyridazinone from (IV)	265 (4·28), 278 (4·27), 327 (4·30)	244 (4·17), 272 (4·24), 290 (4·00)
Pyridazinone obtained from trimethyl-deriv. of C_{20}-acid	266 (4·28), 280 (4·26), 328 (4·30)	245 (4·18), 275 (4·23), 292 (4·01)

The infrared absorption spectra of the C_{20}-acid and its derivatives are consistent with formulation of the acid as a β-acylindole having attached carboxyl and oxindolyl groups. The tetramethyl derivative, for example, has four well-defined peaks in the carbonyl region at 1736, 1695, 1631, and 1610 cm.$^{-1}$, of which those at 1695 and 1610 cm.$^{-1}$ may be attributed to the oxindole system (oxindole, 1695, 1610 cm.$^{-1}$; 1-methyloxindole, 1695, 1605 cm.$^{-1}$); the peak at 1736 cm.$^{-1}$ is assigned to the carbonyl of the methoxycarbonyl group, and that at 1631 cm.$^{-1}$ to the carbonyl of a 1-methyl-3-acylindole (characteristic value,[1] 1620—1640 cm.$^{-1}$). The C_{20}-acid and its methyl ester have ill-defined peaks at *ca.* 3200 cm.$^{-1}$ but these cannot be assigned to NH groups in β-acylindole systems [1] since oxindole absorbs in the same region (3165 cm.$^{-1}$).

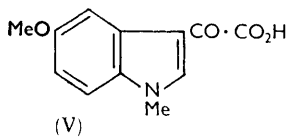
Early in these investigations it was observed that the C_{20}-acid was converted by hot acetic anhydride and sodium acetate into a non-acidic red product, the " red lactone," analyses of which indicated that three acetyl groups had been introduced and that a molecule of water and, probably, two atoms of hydrogen had been lost. Under similar conditions the trimethyl derivative of the C_{20}-acid also gave a red product (the " magenta lactone "), again with the loss of a molecule of water, the disappearance of acidic properties, and the probable loss of two hydrogen atoms. The tetramethyl derivative, with no free carboxyl group, was unattacked by the acetylating mixture, and the monomethyl ester gave a *colourless* product which could not be satisfactorily purified. Thus the formation of a neutral red product in these reactions requires the presence of a carboxyl group which is presumably involved in a cyclisation eliminating water.

Oxidation of the " red lactone " gave *N*-acetylanthranilic acid and isatin as the only identified products, but a similar oxidation of the " magenta lactone " afforded not only 1-methylisatin and 5-methoxy-1-methylisatin, but also 5-methoxy-1-methyl-3-indolylglyoxylic acid (V) which was isolated as its methyl ester. In view of this result it must be regarded as unlikely that in the C_{20}-acid the oxindole nucleus is directly attached to the α-position of the 5-hydroxyindole system as in (VI).

Of the two remaining possible structures (VII) and (VIII), to be considered for the

[1] Ballantine, Barrett, Beer, Boggiano, Eardley, Jennings, and Robertson, following paper.

C_{20}-acid, (VII) is preferable for two reasons. If violacein contains a hydroxyindolylpyrrylmethene structure,[2] the the γ-keto-acid system (II) is formed from the pyrrole ring and the methene carbon atom (with loss of one carbon atom and ammonia). The reconstruction of a normal pyrrylmethene from the keto-acid system of (VIII) is impossible; the carbon atom marked * would have to be quaternary in violacein and would effectively block the extensive conjugation necessary to produce the colour of the pigment. This objection does not apply to structure (VII). A second reason for rejecting structure (VIII) arises from a consideration of the nature of the red cyclisation products (lactones) obtained from the C_{20}-acid and its trimethyl derivative. On the basis of structure (VII) for the C_{20}-acid, these products can be satisfactorily formulated as (IX; R = Ac or Me), which



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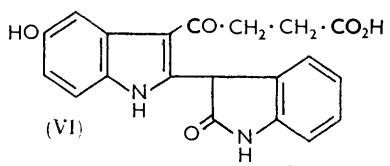

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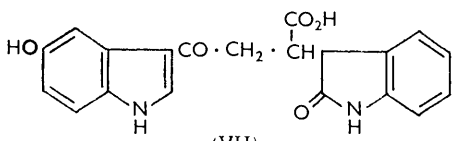

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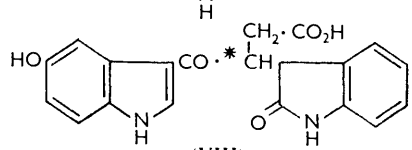

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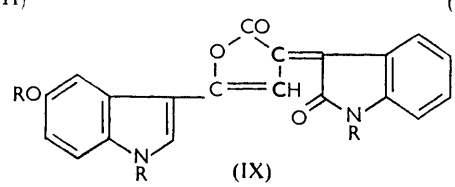

(IX)

accounts for the loss of acidic properties and for the observed dehydration; it is assumed that the oxidation permitted by the analyses brings about the conversion of an oxindolyl into an oxindolidene group, a change which should give rise to colour, especially in the environment represented by (IX). A study [3] of various model compounds, for example an analogue of (VII) in which the 5-hydroxyindole nucleus is replaced by a phenyl group, strongly supports these views.

We are unable to devise an equally satisfactory formulation for these products on the basis of structure (VIII).

Methyl 5-methoxy-1-methyl-3-indolylglyoxylate, required for comparison with the oxidation product described above, was synthesised by two independent methods. 5-Methoxyindole was readily converted into the glyoxylic acid by reaction with oxalyl chloride and hydrolysis of the intermediate acid chloride. With methyl sulphate and alkali the acid gave both 5-methoxy-1-methyl-3-indolylglyoxylic acid (V) and its methyl ester, which was also obtained from the glyoxylic acid by conventional esterification methods. The interaction of 5-methoxy-3-indolylglyoxylyl chloride and methanol furnished methyl 5-methoxy-3-indolylglyoxylate and with methyl sulphate this ester also yielded methyl 5-methoxy-1-methyl-3-indolylglyoxylate. The same product was prepared from 5-methoxy-1-methylindole by a Hoesch reaction with methyl cyanoformate.

Experimental

Unless otherwise stated the light petroleum employed had b. p. 60—80°.

Ultraviolet absorption spectra refer to EtOH solutions (Unicam spectrophotometer), and infrared data were obtained with a Grubb-Parsons double-beam spectrometer and a paste of the compound in " Nujol."

[2] Beer, Jennings, and Robertson, *J.*, 1954, 2679.
[3] Unpublished results from this laboratory.

Degradation of Violacein with Zinc and Alkali.—A mixture of violacein (1·0 g.), zinc dust (2·0 g.), and 2N-aqueous sodium hydroxide (100 ml.) was heated under reflux in nitrogen for 45 min., then decanted from undissolved zinc, which was washed with a little water. The combined liquors were neutralised (pH 6·5—7) with concentrated hydrochloric acid and, after removal of the precipitated zinc salts, acidified, giving a pale yellow solid (*ca.* 700 mg.). Recrystallised from acetone–light petroleum (b. p. 40—60°), this gave the C_{20}-*acid* in small colourless needles (500—650 mg.), m. p. 252—254° (decomp.) (Found: C, 65·7; H, 4·7; N, 8·0%; equiv., 371, 368. $C_{20}H_{16}O_5N_2$ requires C, 65·9; H, 4·4; N, 7·7%; *M*, 364). In one experiment, the evolved basic gas was collected in 2N-hydrochloric acid and converted into its toluene-*p*-sulphonyl derivative which crystallised from ethyl acetate–light petroleum in colourless plates, m. p. and mixed m. p. with toluene-*p*-sulphonamide, 136°.

Alkyl Derivatives of the C_{20}-*Acid.*—A slight excess of ethereal diazomethane was added to a solution of the C_{20}-acid (200 mg.) in methanol (50 ml.), and 15 min. later the solvents were evaporated *in vacuo*. Trituration of the residue with aqueous sodium hydrogen carbonate left the *methyl ester* which on repeated recrystallisation from ethyl acetate–light petroleum formed colourless needles (170 mg.), m. p. 247—249° (Found: C, 66·1; H, 5·3; N, 7·5; OMe, 8·1, 8·4. $C_{19}H_{15}O_3N_2{\cdot}CO_2Me$ requires C, 66·7; H, 4·8; N, 7·4; OMe, 8·2%). The same ester (90 mg.) was obtained by heating the acid (200 mg.) with methyl iodide and potassium carbonate in boiling acetone.

Addition of methyl sulphate (2·5 ml.) portion-wise (0·5 ml.) to a warm agitated solution of the C_{20}-acid (250 mg.) in 2N-aqueous sodium hydroxide (17 ml.) (nitrogen atmosphere) during 45 min. gave, after acidification of the cooled mixture, the *trimethyl derivative* (250 mg.) which was washed with cold acetone and then crystallised from acetone, forming colourless plates (200 mg.), m. p. 266—267° (decomp.) [Found: C, 67·5, 67·7; H, 5·8, 5·5; N, 6·8; OMe, 7·6%; equiv., 404, 410. $C_{21}H_{18}O_2N_2(OMe){\cdot}CO_2H$ requires C, 68·0; H, 5·4; N, 6·9; OMe, 7·6%; *M*, 406].

Methylation of the C_{20}-acid (1·0 g.), in the presence of 2N-aqueous sodium hydroxide (30 ml.), with an excess of methyl sulphate (7·5 ml.) so that the mixture eventually became acidic, gave the neutral *tetramethyl derivative* (900 mg.) which separated from alcohol in colourless needles, m. p. 181° [Found: C, 68·5, 68·7; H, 6·0, 6·0; N, 6·45; OMe, 15·5. $C_{21}H_{18}O_2N_2(OMe){\cdot}CO_2Me$ requires C, 68·6; H, 5·7; N, 6·7; OMe, 14·8%]. The same product, m. p. and mixed m. p. 180—181°, was obtained by the action of methyl iodide and potassium carbonate on the trimethyl derivative in boiling acetone. The 2 : 4-*dinitrophenylhydrazone* crystallised from benzene–light petroleum in red prisms, m. p. 228—230° (decomp.) (Found: C, 59·6; H, 4·7; N, 13·7. $C_{30}H_{28}O_8N_6$ requires C, 60·0; H, 4·7; N, 14·0%).

Acetylation of the C_{20}-*Acid.*—The acid (100 mg.), acetic anhydride (4 ml.), and sodium acetate (100 mg.) were heated under reflux for 15 min., and 12 hr. later the " *red lactone* " [4-(5-*acetoxy*-1-*acetyl*-3-*indolyl*)-2-(1-*acetyl*-3-*oxindolidene*)-4-*hydroxybut*-3-*enoic lactone*] was collected, washed with water, and crystallised from benzene, forming deep red needles (30 mg.), m. p. *ca.* 286° (decomp.) [Found: C, 66·7; H, 4·0; N, 5·8; Ac, 26·8. $C_{20}H_9O_4N_2(CO{\cdot}CH_3)_3$ requires C, 66·4; H, 3·8; N, 6·0; Ac, 27·4%]. Acetylation by the acetic anhydride–pyridine method (5 days at 0°) gave an inseparable mixture of red and colourless products.

The deep magenta solution obtained by heating a mixture of the trimethyl derivative (100 mg.) of the C_{20}-acid, anhydrous sodium acetate (100 mg.) and acetic anhydride (3 ml.) under reflux for 10 min. slowly deposited a neutral product (*ca.* 30 mg.), the " *magenta lactone* " [4-*hydroxy*-4-(5-*methoxy*-1-*methyl*-3-*indolyl*)-2-(1-*methyl*-3-*oxindolene*)*but*-3-*enoic lactone*], which crystallised from benzene in deep magenta plates with a green reflex, m. p. 268—270° (decomp.), and does not contain an acetyl residue (Found: C, 71·9; H, 7·2; N, 6·8. $C_{23}H_{18}O_4N_2$ requires C, 71·5; H, 4·7; N, 7·3%). Under the same conditions, the tetramethyl derivative of the C_{20}-acid was recovered unchanged.

Oxidation of the " Magenta Lactone " from the Trimethyl Derivative of the C_{20}-*Acid.*—A stirred solution of the lactone (880 mg.) in boiling acetone (1 l.) was cooled rapidly and, before crystallisation began, powdered potassium permanganate (1·3 g.) was added in portions. The precipitated manganese dioxide was removed and the dark red solid left after evaporation of the liquor was extracted with boiling water, giving a mixture of pale orange needles and clusters of red needles, which was separated in the first instance by chromatography from benzene on talc, but more successfully by careful fractional sublimation in a high vacuum. Thus were obtained (*a*) 1-methylisatin (bath-temp. 80—90°/0·005 mm.), m. p. 130°, which crystallised

from benzene–light petroleum in pale orange prisms, m. p. and mixed m. p. 134° (Found : C, 67·3; H, 4·6. Calc. for $C_9H_7O_2N$: C, 67·1; H, 4·4%) and (*b*) 5-methoxy-1-methylisatin (bath-temp. 100—110°/0·005 mm.), m. p. *ca.* 170°, which formed dark red needles, m. p. and mixed m. p. 175—176°, from benzene–light petroleum (Found : C, 62·7; H, 4·8. Calc. for $C_{10}H_9O_3N$: C, 62·9; H, 4·7%). The manganese dioxide precipitate was extracted with hot water (3 × 20 ml.) and, on acidification, the extracts afforded a pale brown acidic solid (100 mg.). More acidic material was obtained by continuous ether-extraction of the acidified aqueous extracts. The combined acidic fractions were esterified with ethereal diazomethane and on distillation the product gave two fractions : (*a*) a trace of colourless oil (bath-temp. 120—140°/0·01 mm.) and (*b*) a pale yellow oil (bath-temp. 140—180°/0·01 mm.) which became partly crystalline. Fraction (*b*) was redistilled (bath-temp. 130—140°/0·001 mm.) and, on crystallisation from acetone–light petroleum, then gave methyl 5-methoxy-1-methyl-3-indolylglyoxylate in pale yellow prisms, m. p. and mixed m. p. 133° (Found : C, 62·4; H, 5·3; N, 5·7; OMe, 25·7. Calc. for $C_{13}H_{13}O_4N$: C, 63·1; H, 5·3; N, 5·7; OMe, 25·1%).

Oxidation of the "Red Lactone" from the C_{20}*-Acid.*—Slow addition of potassium permanganate (235 mg.) in acetone (30 ml.) to a stirred cold solution of the lactone (210 mg.) in the same solvent (200 ml.) gave isatin, m. p. and mixed m. p. 198—200°, isolated from the acetone solution, and *N*-acetylanthranilic acid, isolated by water-extraction of the manganese dioxide formed in the oxidation and identified by conversion into the methyl ester, m. p. and mixed m. p. 97—98°.

Oxidation of the Trimethyl Derivative of the C_{20}*-Acid.*—Potassium permanganate (1·0 g.), in acetone (200 ml.), was added slowly to a stirred solution of the trimethyl derivative of the C_{20}-acid (1·0 g.) in the same solvent (1200 ml.) at 40°. The precipitated manganese dioxide was collected and washed with acetone, and the filtrate and washings were combined, decolorised with sulphur dioxide, and evaporated. A solution of the residue in benzene was washed with aqueous sodium hydrogen carbonate, dried, and evaporated, leaving bright crystals which by careful distillation in a pistol were resolved into 1-methylisatin (82 mg.) and 5-methoxy-1-methylisatin (71 mg.).

Attempted Decarboxylation of the C_{20}*-Acid.*—The mixture obtained by heating the C_{20}-acid (500 mg.) and copper bronze in glycerol (10 ml.) to 260—270° for 10 min. was diluted with water (40 ml.), filtered, and extracted with ether. The brown gum left on evaporation of the ether was boiled with hot light petroleum and, on cooling, the extract deposited 5-hydroxyindole (60 mg.) in colourless needles, m. p. and mixed m. p. 106—107°. On sublimation (bath-temp. 180°/0·001 mm.) the residue, insoluble in light petroleum, afforded a crystalline product, which separated from acetone–light petroleum in colourless cubes (15 mg.), m. p. 265° (Found : C, 69·2; H, 5·5; N, 7·9%). The latter product, which formed a deep red 2 : 4-dinitrophenylhydrazone and a crystalline acetyl derivative, m. p. *ca.* 190°, was also obtained in small yield in other attempted decarboxylations.

Interaction of the Trimethyl Derivative of the C_{20}*-Acid and Hydrazine.*—The colourless *pyridazinone* (200 mg.), obtained by heating a solution of the trimethyl derivative (200 mg.) in alcohol (15 ml.) with 90% hydrazine hydrate (1 ml.) for 8 hr., separated from 95% alcohol in long colourless needles, m. p. 242° (Found : C, 68·5; H, 5·6; N, 13·8. $C_{23}H_{22}O_3N_4$ requires C, 68·6; H, 5·5; N, 13·9%). The same product was formed in a similar reaction with the tetramethyl derivative of the C_{20}-acid.

5-Methoxy-3-indolylglyoxylic Acid.—Prepared from ethyl 5-methoxyindole-2-carboxylate,[4] 5-methoxyindole-2-carboxylic acid [5] was decarboxylated to 5-methoxyindole. Addition of oxalyl chloride (0·75 ml.) to a solution of 5-methoxyindole (0·70 g.) in ether (15 ml.) gave 5-methoxy-3-indolylglyoxylyl chloride [6] (1·0 g.) as an orange-red solid, m. p. 130°. A solution of this compound (0·50 g.) in warm 2N-aqueous potassium hydroxide (100 ml.) was cooled and acidified with dilute hydrochloric acid; crystallised from ethyl acetate, the resulting *5-methoxy-3-indolylglyoxylic acid* formed yellow prisms (300 mg.), m. p. 248° (Found : C, 60·2; H, 4·3; N, 6·1. $C_{11}H_9O_4N$ requires C, 60·3; H, 4·1; N, 6·4%).

Methyl 5-Methoxy-1-methyl-3-indolylglyoxylate.—(*A*) A solution of potassium hydroxide (1·5 g.) in water (4·5 ml.) was added to 5-methoxy-3-indolylglyoxylic acid (0·30 g.) in acetone (30 ml.), followed by methyl sulphate (2·5 ml.) added in 20 min. with constant shaking.

[4] Hughes and Lions, *J. Proc. Roy. Soc., N.S.W.*, 1937, **71**, 475.
[5] Blaikie and Perkin, *J.*, 1924, **125**, 296.
[6] Speeter and Antony, *J. Amer. Chem. Soc.*, 1954, **76**, 6209.

20 Min. later the colourless solid which had separated at the interface of the two layers was collected and dissolved in water. On acidification the solution gave *5-methoxy-1-methyl-3-indolylglyoxylic acid* which crystallised from ethyl acetate in bright yellow needles (0·20 g.), m. p. 194° (Found : C, 61·5; H, 4·8; N, 6·1. $C_{12}H_{11}O_4N$ requires C, 61·8; H, 4·7; N, 6·0%). On evaporation the acetone-rich layer from the methylation mixture left an oil which slowly solidified in contact with cold water. Recrystallised from ethyl acetate–light petroleum this afforded *methyl 5-methoxy-1-methyl-3-indolylglyoxylate* in yellow prisms (0·075 g.), m. p. 133—134° (Found : C, 63·1; H, 5·3; N, 5·9; OMe, 24·7. $C_{13}H_{13}O_4N$ requires C, 63·2; H, 5·3; N, 5·7; OMe, 25·1%). The same ester was obtained by the action of diazomethane on the glyoxylic acid or by evaporation of a solution of the acid in methanol containing a trace of concentrated hydrochloric acid.

(*B*) By the action of methanol (4 ml.) in ether (35 ml.) 5-methoxy-3-indolylglyoxylyl chloride (0·50 g.) was converted into *methyl 5-methoxy-3-indolylglyoxylate* which formed yellow plates (0·45 g.), m. p. 255°, from acetone (Found : C, 61·7; H, 4·9; N, 6·0. $C_{12}H_{11}O_4N$ requires C, 61·8; N, 4·7; N, 6·0%). With methyl sulphate in an aqueous acetone solution of potassium hydroxide this ester (0·3 g.) gave methyl 5-methoxy-1-methyl-3-indolylglyoxylate (0·23 g.), m. p. and mixed m. p. 134°.

(*C*) A solution of 5-methoxy-1-methylindole [7] (1·0 g.) and methyl cyanoformate (1·5 ml.) in ether (10 ml.) at 0° was saturated with hydrogen chloride and kept for 12 hr. at 0—5°. The crystalline ketimine hydrochloride was collected, and decomposed with warm very dilute aqueous sodium carbonate; the resulting methyl 5-methoxy-1-methyl-3-indolylglyoxylate, crystallised from acetone–light petroleum, had m. p. and mixed m. p. 134°.

5-Methoxy-1-methylisatin.—*5-Methoxy-1-methylisatin* was prepared by methylation of 5-methoxyisatin,[8] in 50% aqueous methanol (40 ml.), with methyl sulphate (2·5 ml.) and sufficient 2N-aqueous sodium hydroxide to maintain neutrality, and was isolated with benzene. It separated from water and then from benzene–light petroleum in dark red needles (0·07 g.), m. p. 175—176° (Found : C, 62·5; H, 4·8. $C_{10}H_9O_3N$ requires C, 62·8; H, 4·7%).

UNIVERSITY OF LIVERPOOL. [*Received, November 26th*, 1956.]

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