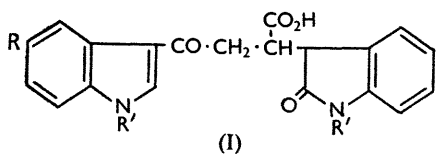
969. *The Chemistry of Bacteria. Part VI.* The Synthesis of a Trimethyl Derivative of the C_{20} Acid from Violacein.*

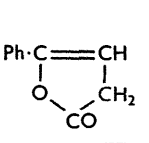
By C. B. Barrett, R. J. S. Beer, G. M. Dodd, and Alexander Robertson.

The synthesis of γ-(5-methoxy-1-methyl-3-indolyl)-α-(1-methyl-3-oxindolyl)-γ-oxobutyric acid (I; R = OMe, R′ = Me) has confirmed the structure (I; R = OH, R′ = H) assigned to the C_{20} acid [1] obtained by degradation of violacein.

The structure (I; R = OH; R′ = H) which was assigned to the C_{20} acid [1] obtained from violacein by treatment with alkali in the presence of zinc has now been confirmed by the synthesis of the trimethyl derivative (I; R = OMe, R′ = Me). In exploratory experiments it was found that the lactone (II), prepared by dehydration of β-benzoylpropionic acid with acetic anhydride, condensed readily with isatin, giving an intensely red product (III; R = H). This lactone was converted by mild alkaline hydrolysis into an unstable



(I)



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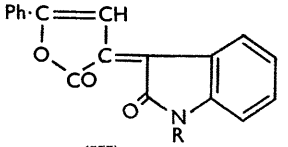

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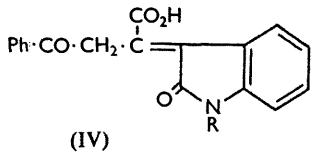

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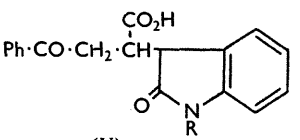

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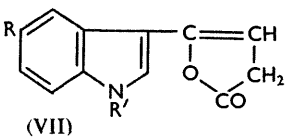

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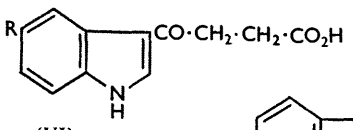

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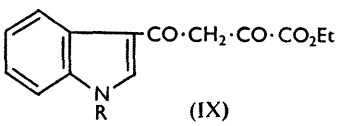

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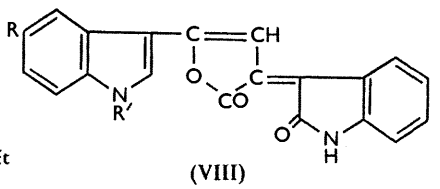

(VIII)

orange acid regarded as (IV; R = H) since in hot solvents it reverts to the red lactone (III; R = H). On reduction with zinc and alkali the acid (IV; R = H) gave β-benzoyl-α-3-oxindolylpropionic acid (V; R = H), a close analogue of the C_{20} acid. With hot acetic anhydride and sodium acetate, both compounds (IV; R = H) and (V; R = H) gave the red acetylated lactone (III; R = Ac) which was also obtained directly from (III; R = H) by acetylation. The conversion of the saturated acid (V; R = H) into compound (III; R = Ac) involves, not only acetylation and dehydration, but also oxidation and is exactly analogous to the behaviour of the C_{20} acid [1] under the same conditions.

An essentially similar series of experiments, with 1-methylisatin in place of isatin in the initial condensation, afforded the series (III), (IV), and (V) in which R = Me. When heated with acetic anhydride and sodium acetate, the colourless acid (V; R = Me) reverted to the red unsaturated lactone (III; R = Me).

* Part V, *J.*, 1957, 2227.

[1] Ballantine, Barrett, Beer, Boggiano, Clarke, Eardley, Jennings, and Robertson, *J.*, 1957, 2222.

By starting with indolyloxobutyric acids of type (VI) the synthetical method outlined above was obviously applicable to the preparation of compounds of type (I). In the first instance we elected, on grounds of availability, to employ the keto-acid (VI; R = H) derived from indole itself, and although this acid gave intractable products when heated with acetic anhydride, treatment with hot acetyl chloride afforded in good yield a crystalline product regarded as the lactone (VII; R = H, R′ = Ac). This lactone reacted smoothly with isatin in alcohol containing a trace of pyridine, giving the red condensation product (VIII; R = H, R′ = Ac) which, with zinc dust and alkali, was converted into γ-3-indolyl-α-3-oxindolyl-γ-oxobutyric acid (I; R = H, R′ = H).

With the 5-methoxyindolyloxobutyric acid [2] (VI; R = OMe) as starting material repetition of the synthetical procedure gave, by way of the intermediates (VII; R = OMe, R′ = Ac) and (VIII; R = OMe, R′ = Ac), the colourless acid (I; R = OMe, R′ = H) which on methylation yielded γ-(5-methoxy-1-methyl-3-indolyl)-α-(1-methyl-3-oxindolyl)-γ-oxobutyric acid (I; R = OMe, R′ = Me). This product was identical with the trimethyl derivative [1] of the C_{20} acid and was converted by the action of hot acetic anhydride containing sodium acetate into a red compound identical with the " magenta lactone ".[1] The structure (I; R = OH, R′ = H) for the C_{20} acid may therefore be regarded as firmly established.

An alternative route to compounds of type (I), which was explored without success, involved the condensation of an indolyl-αγ-dioxobutyric ester or acid, *e.g.*, (IX; R = H), with oxindole. A similar condensation, between pyruvic acid and oxindole, has been described by Julian *et al.*[3] Both 3-acetylindole and 3-acetyl-1-methylindole reacted readily with ethyl oxalate in the presence of sodium ethoxide, giving the expected products (IX; R = H) and (IX; R = Me); these were converted to the corresponding acids by alkaline hydrolysis, but neither the dioxobutyric esters nor the acids could be induced to condense in the desired manner with oxindole.

Experimental

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

4-Hydroxy-2-3′-oxindolylidene-4-phenylbut-3-enoic Lactone (III; R = H).—Isatin (6·0 g.) was added to a stirred solution obtained by heating β-benzoylpropionic acid [4] (7·0 g.) with acetic anhydride (8 ml.) on the steam-bath for 2 hr. and the mixture was then heated for a further 30 min. After being washed with a little hot alcohol, the crystalline *lactone* (4·5 g.) was sufficiently pure for use in the next stage. An analytical sample was crystallised from alcohol forming deep red needles, m. p. > 260° (Found: C, 74·7; H, 4·1; N, 4·6. $C_{18}H_{11}O_3N$ requires C, 74·7; H, 3·8; N, 4·8%). The same product was obtained by the interaction of the preformed lactone [5] of β-benzoylpropionic acid with isatin in hot ethanol containing a trace of piperidine.

β-Benzoyl-α-3-oxindolylpropionic Acid (V; R = H).—On being warmed 4-hydroxy-2-3′-oxindolylidene-4-phenylbut-3-enoic lactone (3 g.) slowly dissolved in a mixture of alcohol (20 ml.) and 2N-aqueous sodium hydroxide (12 ml.); the salt of the resulting acid was kept in solution by gradual addition of hot water (40 ml.). On acidification with acetic acid the cooled filtered solution deposited a sticky solid which became crystalline when rubbed. This acid readily reverted to the lactone in hot solvents; rapid recrystallisation from ethyl acetate–light petroleum furnished the acid in orange leaflets (2 g.), m. p. 133°, which gave unsatisfactory analytical data. A solution of the acid (2 g.) in 2N-aqueous sodium hydroxide (25 ml.) and water (20 ml.) was heated and stirred with zinc dust (1 g.) until the colour of the solution changed from orange to pale yellow. The filtered solution was cooled to 0° and carefully acidified, giving *β-benzoyl-α-3-oxindolylpropionic acid* which separated from ethyl acetate–light petroleum in colourless leaflets (1·3 g.), m. p. 185—186° (Found: C, 69·7; H, 5·0; N, 4·5. $C_{18}H_{15}O_4N$ requires C, 69·9; H, 4·9; N, 4·5%). The red solution obtained by heating β-benzoyl-α-3-oxindolylpropionic acid (0·5 g.) with acetic anhydride (4 ml.) and sodium acetate (0·5 g.) for 15 min. was cooled, and the following day the crystalline deposit was collected, washed

[2] Ballantine, Barrett, Beer, Boggiano, Eardley, Jennings, and Robertson, *J.*, 1957, 2227.
[3] Julian, Printy, Ketcham, and Doone, *J. Amer. Chem. Soc.*, 1953, **75**, 5305.
[4] Martin and Fieser, *Org. Synth.*, Coll. Vol. II, p. 81.

thoroughly with warm water, and then recrystallised from acetic acid, giving 2-(1-*acetyl*-3-*oxindolylidene*)-4-*hydroxy*-4-*phenylbut*-3-*enoic lactone* (III; R = Ac) in red needles (150 mg.), m. p. 220° (Found: C, 72·5; H, 4·1; N, 3·9. $C_{20}H_{13}O_4N$ requires C, 72·5; H, 3·9; N, 4·2%). The same product was obtained by the interaction of acetic anhydride and sodium acetate with β-benzoyl-α-3-oxindolylidenepropionic acid and with 4-hydroxy-2-3′-oxindolylidene-4-phenylbut-3-enoic lactone.

β-*Benzoyl*-α-(1-*methyl*-3-*oxindolyl*)*propionic Acid* (V; R = Me).—When isatin was replaced by 1-methylisatin in the condensation described above, the product (75% yield) was 4-*hydroxy*-2-(1-*methyl*-3-*oxindolylidene*)-4-*phenylbut*-3-*enoic lactone* (III; R = Me) which crystallised from alcohol in dark red needles, m. p. 182° (decomp.) (Found: C, 75·4, 75·0; H, 4·6, 4·6; N, 4·6. $C_{19}H_{13}O_3N$ requires C, 75·3; H, 4·3; N, 4·6%). On hydrolysis with alkali this lactone (5 g.) afforded β-*benzoyl*-α-(1-*methyl*-3-*oxindolylidene*)*propionic acid* (3·8 g.), forming yellow needles, m. p. 112°, from benzene–light petroleum (Found: C, 70·7; H, 4·8; N, 4·0. $C_{19}H_{15}O_4N$ requires C, 71·0; H, 4·7; N, 4·4%). In hot solvents this acid is rapidly converted into the original lactone; reduction of the compound with zinc dust in alkaline solution gave β-*benzoyl*-α-(1-*methyl*-3-*oxindolyl*)*propionic acid* which formed colourless needles, m. p. 151°, from ethyl acetate–light petroleum (yield 70%) (Found: C, 71·0; H, 5·2; N, 4·4. $C_{19}H_{17}O_4N$ requires C, 70·6; H, 5·3; N, 4·3%). With boiling acetic anhydride containing sodium acetate, the last acid (600 mg.) furnished 4-hydroxy-2-(1-methyl-3-oxindolylidene)-4-phenylbut-3-enoic lactone (160 mg.), m. p. 181° (decomp.), identical with the material already described.

4-(1-*Acetyl*-3-*indolyl*)-4-*hydroxy*-2-3′-*oxindolylidenebut*-3-*enoic Lactone* (VIII; R = H, R′ = Ac).—Hot freshly distilled acetyl chloride (15 ml.) was added to γ-3-indolyl-γ-oxobutyric acid [2] (0·5 g.) and the mixture then heated under reflux for 2 hr. The resulting yellow solution was evaporated to dryness in a vacuum and, on crystallisation from light petroleum (b. p. 100—120°), the residue afforded 4-(1-*acetyl*-3-*indolyl*)-4-*hydroxybut*-3-*enoic lactone* (VII; R = H, R′ = Ac) (0·42 g.) in pale yellow needles, m. p. 155° (Found: C, 69·5; H, 4·8; N, 5·6; Ac, 17·8. $C_{12}H_8O_2N{\cdot}CO{\cdot}CH_3$ requires C, 69·7; H, 4·6; N, 5·8; Ac, 17·8%). Heated for 30 min. with isatin (55 mg.) in alcohol (7 ml.) containing one drop of pyridine, this lactone (100 mg.) gave 4-(1-*acetyl*-3-*indolyl*)-4-*hydroxy*-2-3′-*oxindolylidenebut*-3-*enoic lactone* (120 mg.) which crystallised from acetone–light petroleum in deep red needles, m. p. 285° (Found: C, 71·0; H, 3·6; N, 7·2; Ac, 11·8. $C_{20}H_{11}O_3N_2{\cdot}CO{\cdot}CH_3$ requires C, 71·4; H, 3·8; N, 7·6; Ac, 11·6%).

γ-3-*Indolyl*-α-3-*oxindolyl*-γ-*oxobutyric Acid* (I; R = R′ = H).—On being agitated with zinc dust in nitrogen the orange solution of 4-(1-acetyl-3-indolyl)-4-hydroxy-2-3′-oxindolylidenebut-3-enoic lactone (100 mg.) in hot 1·5N-aqueous sodium hydroxide rapidly became pale yellow and 2 min. later the mixture was filtered, the residue was washed with water, and the combined filtrate and washings were cooled to −5°, made slightly acid (pH 6·5), and filtered. Acidification of the filtrate to pH 2 precipitated a white solid which on crystallisation from ethyl acetate–light petroleum and then from ethyl acetate gave γ-3-*indolyl*-α-3-*oxindolyl*-γ-*oxobutyric acid* in colourless plates (75 mg.), m. p. 235° (decomp.) (Found: C, 68·8; H, 4·8; N, 7·9. $C_{20}H_{16}O_4N_2$ requires C, 69·0; H, 4·6; N, 8·0%).

4-(1-*Acetyl*-5-*methoxy*-3-*indolyl*)-4-*hydroxy*-2-3′-*oxindolylidenebut*-3-*enoic Lactone* (VIII; R = OMe, R′ = Ac).—Treated with boiling acetyl chloride (10 ml.) for 2 hr., γ-(5-methoxy-3-indolyl)-γ-oxobutyric acid [2] (250 mg.) afforded 4-(1-*acetyl*-5-*methoxy*-3-*indolyl*)-4-*hydroxybut*-3-*enoic lactone*, which crystallised from light petroleum (b. p. 100—120°) in yellow needles (180 mg.), m. p. 159° (Found: C, 66·5; H, 4·8; N, 5·2. $C_{15}H_{13}O_4N$ requires C, 66·4; H, 4·8; N, 5·2%). This lactone (400 mg.) and isatin (220 mg.) in hot alcohol (15 ml.), containing one drop of pyridine, gave 4-(1-*acetyl*-5-*methoxy*-3-*indolyl*)-4-*hydroxy*-2-3′-*oxindolylidenebut*-3-*enoic lactone* (480 mg.), forming deep red needles from acetone, m. p. 310° (decomp.) (Found: C, 68·8; H, 4·3; N, 6·5. $C_{23}H_{16}O_5N_2$ requires C, 69·0; H, 4·0; N, 6·7%).

γ-(5-*Methoxy*-1-*methyl*-3-*indolyl*)-α-(1-*methyl*-3-*oxindolyl*)-γ-*oxobutyric Acid* (I; R = OMe, R′ = Me).—Reduction of 4-(1-acetyl-5-methoxy-3-indolyl)-4-hydroxy-2-3′-oxindolylidenebut-3-enoic lactone (150 mg.), dissolved in 1·5N-aqueous sodium hydroxide (15 ml.) at 100°, with zinc dust (300 mg.) in the absence of air gave γ-(5-methoxy-3-indolyl)-α-3′-oxindolyl-γ-oxobutyric acid (80 mg.) as an off-white amorphous powder which could not be satisfactorily crystallised. Addition of methyl sulphate (1·0 ml.), in portions (0·2 ml.), to a warm agitated solution of the oxobutyric acid (50 mg.) in 2N-aqueous sodium hydroxide (7 ml.) (in nitrogen) during 30 min. gave, after acidification of the cooled mixture, γ-(5-*methoxy*-1-*methyl*-3-*indolyl*)-α-(1-*methyl*-3-*oxindolyl*)-γ-*oxobutyric acid* which was washed with cold acetone and then crystallised from

acetone, forming colourless plates (45 mg.), m. p. 266° (decomp.) (Found: C, 68·0; H, 5·4; N, 6·8. $C_{23}H_{22}O_5N_2$ requires C, 68·0; H, 5·4; N, 6·9%). This product was shown to be identical with the trimethyl derivative [1] of the C_{20} acid from violacein by mixed m. p. determination, by comparison of ultraviolet and infrared absorption spectra, and by its conversion by hot acetic anhydride and sodium acetate into 4-hydroxy-4-(5-methoxy-1-methyl-3-indolyl)-2-(1-methyl-3-oxindolylidene)-but-3-enoic lactone, identical in infrared absorption and other respects with the " magenta lactone ".[1]

Ethyl γ-3-Indolyl-αγ-dioxobutyrate (IX; R = H).—A mixture of ethyl oxalate (3·2 g.) and 3-acetylindole [6] (3·2 g.), alcohol (40 ml.), and sodium ethoxide (from 0·5 of sodium) was heated under reflux for 10 hr., concentrated in a vacuum, and acidified with 2N-sulphuric acid at 0°. The addition of ice-cold water completed the precipitation of *ethyl γ-3-indolyl-αγ-dioxobutyrate* which separated from aqueous alcohol in yellow leaflets (2·9 g.), m. p. 192° (Found: C, 64·8; H, 5·1; N, 5·5. $C_{14}H_{13}O_4N$ requires C, 64·9; H, 5·0; N, 5·4%).

Ethyl γ-(1-Methyl-3-indolyl)-αγ-dioxobutyrate (IX; R = Me).—3-Acetylindole (5 g.), in acetone (50 ml.), was mixed with potassium hydroxide (30 g.) in water (18 ml.), then methyl sulphate (25 ml.) was gradually introduced to the agitated mixture during 30 min. 30 Min. later the mixture was poured into water, and the product, *3-acetyl-1-methylindole*, recrystallised from light petroleum forming colourless feathery needles (4·8 g.), m. p. 109—110° (Found: C, 76·3; H, 6·2; N, 8·1. $C_{11}H_{11}ON$ requires C, 76·3; H, 6·4; N, 8·1%). A solution of 3-acetyl-1-methylindole (3·5 g.) and ethyl oxalate (3·2 g.) in benzene (25 ml.) was heated with sodium ethoxide (from 0·3 g. of sodium) for 7 hr. The yellow salt was collected, washed with benzene and ether, and decomposed with hot aqueous acetic acid. On cooling, the solution deposited *ethyl γ-(1-methyl-3-indolyl)-αγ-dioxobutyrate* which crystallised from acetic acid in yellow needles (3·6 g.), m. p. 122° (Found: C, 66·3; H, 5·5; N, 5·1. $C_{15}H_{15}O_4N$ requires C, 65·9; H, 5·5; N, 5·1%).

γ-3-Indolyl-αγ-dioxobutyric Acid.—Ethyl γ-3-indolyl-αγ-dioxobutyrate (200 mg.), alcohol (5 ml.), and 2N-aqueous sodium hydroxide (0·5 ml.) were heated on the steam-bath for 10 min. and poured into water (30 ml.). Acidification then gave the *acid* which separated from acetic acid in yellow leaflets (150 mg.), m. p. 220 (Found: C, 62·1; H, 3·7; N, 6·4. $C_{12}H_9O_4N$ requires C, 62·3; H, 3·9; N, 6·1%). Similarly prepared, *γ-(1-methyl-3-indolyl)-αγ-dioxobutyric acid* crystallised from acetic acid in yellow needles, m. p. 120° (Found: C, 64·0; H, 4·8; N, 5·3. $C_{13}H_{11}O_4N$ requires C, 63·7; H, 4·5; N, 5·7%).

The analyses were performed by Mr. A. S. Inglis, M.Sc., and his associates in this Department.

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[5] Kugel, *Annalen*, 1898, **299**, 54.
[6] Saxton, *J.*, 1952, 3592.