324. *The Chemistry of Bacteria. Part I. The Synthesis of Hydroxyindoles.*

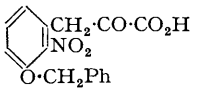
By R. J. S. Beer, Kenneth Clarke, H. G. Khorana, and Alexander Robertson.

The synthesis of 6- and 7-hydroxyindole by way of the benzyl ethers of the requisite *o*-nitrohydroxyphenylpyruvic acid is described. By application of Nenitzescu's reaction (*Ber.*, 1925, **58**, 1063) the synthesis of 4-hydroxyindole and of 5-hydroxy-2-methylindole together with a new synthesis of 5-hydroxyindole has been achieved from the corresponding 2 : β-dinitroacetoxystyrenes.

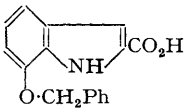
In the course of degradation experiments on the pigment, violacein, from *Chromobacterium violaceum* it became necessary to synthesise indoles hydroxylated in the benzene nucleus as reference compounds in attempts to identify two isomeric products, C_8H_7ON, obtained in small amounts, one of which gave a strong reaction with Ehrlich's reagent. Apart from studies on 5 : 6-dihydroxyindole and its derivatives in connection with melanin production and related topics (*e.g.*, Raper, *Biochem. J.*, 1927, **21**, 89; 1930, **24**, 239; Burton, *J.*, 1932, 546; Bergel and Morrison, *J.*, 1943, 48), comparatively little work has been recorded on the synthesis of the required type of hydroxyindole. Blaikie and Perkin (*J.*, 1924, **125**, 296) achieved the synthesis of 4-, 5-, and 7-methoxyindole by a general method, but were unable to demethylate their products, although Hoshino and Shimodaira (*Annalen*, 1935, **520**, 19) record the de-ethylation of bufotenine ethyl ether. Similarly, Burton and Stoves (*J.*, 1937, 1726) prepared 5- and 6-benzyloxyindole

by the Blaikie–Perkin route but did not succeed in removing the benzyl residues, whereas Bergel and Morrison (*J.*, 1943, 49) without reference to the original describe the preparation of Burton and Stoves's 5-benzyloxyindole-2-carboxylic acid which they succeeded in debenzylating by hydrogenolysis and in subsequently converting into 5-hydroxyindole. The use of the carboxyl groups in the α-position to inhibit the hydrogenation of the heterocyclic system finds a close parallel in the work of Foster and Robertson (*J.*, 1939, 921, 930) on the analogous coumarone-2-carboxylic acids.

In the present studies 6-*hydroxyindole* has been prepared by hydrogenolysis of 6-benzyloxyindole-2-carboxylic acid (Burton and Stoves, *loc. cit.*) followed by decarboxylation of the resulting 6-*hydroxyindole-2-carboxylic acid.*



(I.)



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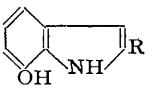
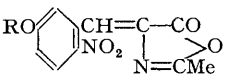


(III.)

For the synthesis of 7-*hydroxyindole* (III; R = H) by the same route, the condensation of 2-*nitro*-3-*benzyloxytoluene* with ethyl oxalate followed by hydrolysis of the product gave the pyruvic acid (I) which was characterised by the formation of the *oxime*, and was subsequently converted into (III; R = H) by way of the stages (II) and (III; R = CO_2H). Unlike the behaviour of 6-hydroxyindole-2-carboxylic acid which gave a 53% yield of the indole, the yield of (III; R = H) from (III; R = CO_2H) was extremely small. Attempts to synthesise 4-*hydroxyindole* from 2-*nitro*-6-*benzyloxytoluene* by this route were abandoned because when the latter reacted with ethyl oxalate in the presence of potassium ethoxide only small amounts of resinous material were obtained which had the properties of a pyruvic acid but which could not be purified economically. On being submitted to reduction and cyclisation the crude acid gave a product from which only a trace of a crystalline substance exhibiting an Ehrlich reaction was isolated.



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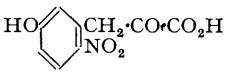

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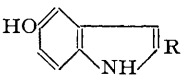

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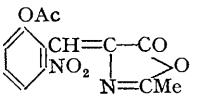

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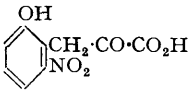
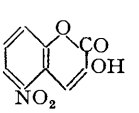


(VIII.)

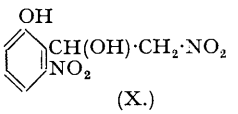


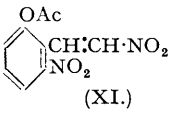
(IX.)

Where the requisite *o*-nitrobenzaldehyde is available, the azlactone method affords a convenient route to the required hydroxy-*o*-nitrophenylpyruvic acids for the synthesis of hydroxyindoles, and accordingly a new synthesis of 5-hydroxyindole-2-carboxylic acid (VI; R = CO_2H) was developed, the 5-keto-2-methyl-4 : 5-dihydro-oxazole from aceturic acid being used in place of the corresponding phenyl derivative because of the difficulties encountered in the hydrolysis of 5-keto-2-phenyl-4-(2′-nitrobenzylidene)-4 : 5-dihydro-oxazoles (cf. Burton, *J.*, 1935, 1265). Condensation of 2-nitro-5-hydroxybenzaldehyde with aceturic acid by means of sodium acetate and acetic anhydride gave the *azlactone* (IV; R = H) and its *acetate* (IV; R = Ac), either of which on hydrolysis with hot hydrochloric acid furnished the *pyruvic acid* (V) in good yield. Reduction of (V) and simultaneous cyclisation of the resulting amino-compound afforded 5-hydroxyindole-2-carboxylic acid, identical with a specimen obtained from its benzyl ether (*loc. cit.*). In a projected synthesis of 4-hydroxyindole from 2-nitro-6-hydroxybenzaldehyde by the same route, the *azlactone* (VII) was obtained in satisfactory yield, and, as would be expected, the pyruvic acid (VIII) formed by the acid hydrolysis of (VII) immediately cyclised to form 5-*nitro*-3-*hydroxycoumarin* (IX). We were unable, however, to discover the requisite conditions for the reduction of the pyruvic acid (VIII) regenerated from (IX) combined with the cyclisation of the resulting amino-acid to yield the 4-hydroxyindole-2-carboxylic acid.

In view of the failure to obtain 4-hydroxyindole by the foregoing methods, we investigated

the application of Nenitzescu's apparently little-known procedure for converting 2 : β-dinitrostyrene into indole (*Ber.*, 1925, **58**, 1063) to the synthesis of hydroxyindoles.


(X.)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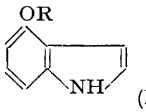
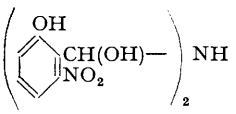
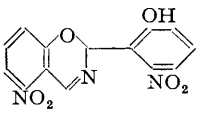

(XII.)

2-Nitro-6-hydroxybenzaldehyde was condensed with nitromethane by means of alcoholic potassium hydroxide, and the resulting crude nitro-alcohol (X) simultaneously dehydrated and acetylated with the formation of 2 : β-*dinitro*-6-*acetoxystyrene* (XI). Reduction of (XI) with iron filings and acetic acid gave rise to 4-*acetoxyindole* (XII; R = Ac) which on deacetylation by the methyl-alcoholic ammonia method furnished 4-*hydroxyindole* (XII; R = H). By an analogous series of reactions 5-hydroxyindole was prepared from 2-nitro-5-hydroxybenzaldehyde by way of 2 : β-*dinitro*-5-*acetoxystyrene* and 5-*acetoxyindole*, a procedure which avoids the wasteful decarboxylation process; the overall yield from the aldehyde was 35—40%. When nitromethane was replaced by nitroethane in the condensation with 2-nitro-5-hydroxybenzaldehyde, a somewhat lower yield of the homologous 2 : β-*dinitro*-5-*acetoxy*-β-*methylstyrene* was obtained which was successively converted into 5-*acetoxy*- and 5-*hydroxy*-2-*methylindole* by the standard method.

(XIII.) 


(XIV.)

For condensing aromatic aldehydes with nitromethane, Rao and co-workers (*Helv. Chim. Acta*, 1929, **12**, 58) recommend the use of ammonium acetate in acetic acid, but when this reagent was applied to 2-nitro-6-hydroxybenzaldehyde the nitro-compound (X) was not formed. Instead, a good yield of a well-crystallised product, $C_{14}H_9O_6N_3$, was obtained which was phenolic and gave a *monoacetate*. Further, it was subsequently discovered that the nitromethane did not take part in the reaction, and therefore it seems reasonably certain that the substance is the *benz*-1 : 3-*oxazine* (XIV), probably formed by way of the intermediate benzaldehyde-ammonia compound (XIII).

Further experimental work on (XIV) and its homologues is in hand.

Experimental.

2-*Nitro*-5-*hydroxyphenylpyruvic Acid* (V).—An intimate mixture of 2-nitro-5-hydroxybenzaldehyde (5 g.), aceturic acid (6 g.), sodium acetate (6 g.), and acetic anhydride (15 ml.) was heated on the steam-bath for 1½ hours, kept for 10 days, and treated (agitate) with methanol (10 ml.) followed by water (5 ml.). 2 Hours later the solid (4·8—5·6 g.) was collected and dissolved in warm alcohol. On cooling, the solution deposited 5-*keto*-2-*methyl*-4-(2′-*nitro*-5′-*acetoxybenzylidene*)-4 : 5-*dihydro-oxazole* (IV; R = Ac) in yellow needles, m. p. 140°, after recrystallisation from the same solvent (Found : C, 53·9; H, 3·7; N, 9·8. $C_{13}H_{10}O_6N_2$ requires C, 53·8; H, 3·5; N, 9·7%). Concentration of the first alcoholic liquor and then dilution with water gave a pale yellow solid which on crystallisation from aqueous alcohol gave the *hydroxybenzylidene*-4 : 5-*dihydro-oxazole* (IV; R = H) in colourless needles, m. p. 173° (Found : C, 53·4; H, 3·4; N, 11·2. $C_{11}H_8O_5N_2$ requires C, 53·2; H, 3·2; N, 11·3%).

Concentrated sulphuric acid (20 ml.) was gradually added to water (25 ml.) containing a suspension of the foregoing mixed azlactones (4 g.), and the resulting solution boiled for 1 minute. On cooling, 2-*nitro*-5-*hydroxyphenylpyruvic acid* separated; it crystallised from concentrated hydrochloric acid–water (1 : 1) in colourless prisms (3·2 g.), m. p. 194° (decomp.), having a green ferric reaction in alcohol (Found : C, 48·1; H, 3·3; *M*, by titration, 223·6. $C_9H_7O_6N$ requires C, 48·0; H, 3·1%. *M*, 225). The same acid (1·35 g.), m. p. 194° (decomp.), was obtained when the azlactone mixture (2 g.) was boiled with 1·5N-hydrochloric acid (35 ml.) for 4—5 hours and the product isolated with ether. Oximation of the acid with hydroxylamine and aqueous sodium hydroxide for ¼ hour on the steam-bath gave the *oxime* which formed colourless needles, m. p. 181°, from aqueous alcohol (Found : C, 45·2; H, 3·5; N, 11·8. $C_9H_8O_6N_2$ requires C, 45·0; H, 3·3; N, 11·7%).

5-*Hydroxyindole* (VI; R = H).—Ferrous sulphate (44 g.) dissolved in water (48 ml.) was added to a solution of the foregoing pyruvic acid (5 g.) in aqueous ammonia (34 ml.; *d* 0·88) and water (14 ml.), and the mixture gently refluxed for 1½ hours. The mixture was filtered, the solid extracted several times with warm dilute aqueous ammonia (500 ml.), and the combined filtrates acidified and repeatedly extracted with ether. Evaporation of the dried ethereal extracts left 5-hydroxyindole-2-carboxylic acid (3 g.), m. p. 246° (decomp.), after purification from water, identical with a specimen prepared by route employed by Bergel and Morrison (*loc. cit.*). When a solution of the acid from either source (0·5 g.) in glycerol (5 ml.) was kept at 225—230°, carbon dioxide was evolved, and after 25 minutes the solution was cooled, diluted with water, and extracted several times with ether. The red residue left on the evaporation of the ether was extracted with light petroleum (b. p. 80—100°); on concentration the solution deposited

5-hydroxyindole, m. p. 107°, after sublimation and recrystallisation, identical with material prepared by the method of Bergel and Morrison (*loc. cit.*); yield, 0·25 g. of indole and 0·8 g. of unchanged acid from 2·5 g. of acid (Found: C, 72·3; H, 5·4. Calc. for C_8H_7ON: C, 72·2; H, 5·3%). Addition of a hot benzene solution of the hydroxyindole to picric acid in the same solvent gave a red precipitate of the *picrate* which separated from benzene–light petroleum (b. p. 60—80°) in elongated orange-red needles, m. p. 167° (Found: N, 15·3. $C_8H_7ON,C_6H_3O_7N_3$ requires N, 15·5%).

In view of the poor yields obtained in the decarboxylation process, a number of modifications of the method were investigated with unsuccessful results; *e.g.*, heating 5-hydroxyindole-2-carboxylic acid (*a*) in diphenyl ether at 210° for 10 minutes and then at 250° for 1 minute gave unchanged material, (*b*) with aniline at 184° gave a dark resin, (*c*) with boiling quinoline containing copper-bronze gave tar and a little unchanged acid, (*d*) at 250° in a vacuum gave a sublimate of acid.

6-*Hydroxyindole*.—2-Nitro-4-benzyloxyphenylpyruvic acid (18·4 g.) was prepared by condensation of 2-nitro-4-benzyloxytoluene (Burton and Stoves, *loc. cit.*) (24 g.) and ethyl oxalate (15 g.) in ether (350 ml.) with potassium ethoxide (from 4 g. of potassium) in the course of 18 hours, and hydrolysis of the resulting ester with warm 5% aqueous sodium hydroxide (250 ml.) during 1 hour. The aqueous layer was separated, washed twice with a little ether, and acidified with hydrochloric acid, yielding the pyruvic acid as an oil which solidified and then separated from dilute acetic acid in yellow plates (18·4 g.), m. p. 94° (Found: C, 60·9; H, 4·3; N, 4·3. Calc. for $C_{16}H_{13}O_6N$: C, 61·0; H, 4·1; N, 4·4%) (cf. Burton and Stoves, who give m. p. 89—90° for this acid and state that it forms a hydrate, for which they record analysis). The oxime separated from aqueous alcohol in colourless needles, m. p. 182° (decomp.) (Found: N, 8·4. $C_{16}H_{14}O_6N_2$ requires N, 8·5%). Simultaneous reduction and cyclisation of this acid gave 6-benzyloxyindole-2-carboxylic acid, m. p. 200° (decomp.) (Burton and Stoves give m. p. 185—186°) (Found: C, 71·8; H, 4·9; N, 5·2. Calc. for $C_{16}H_{13}O_3N$: C, 71·9; H, 4·9; N, 5·2%). Debenzylation of this benzyl ether (2 g.) with hydrogen and a 10% palladium–charcoal catalyst (2 g.) in acetic acid (100 ml.) was complete in 10 minutes, and ½ hour later the product was isolated by evaporation of the filtered solution in a vacuum and crystallised from water (charcoal), giving 6-*hydroxyindole-2-carboxylic acid* in glistening needles (0·9 g.), m. p. 236° (decomp.), which gave a red colouration with Ehrlich's reagent (Found: C, 61·1; H, 4·1; N, 8·0. $C_9H_7O_3N$ requires C, 61·0; H, 4·0; N, 7·9%). Methanol can be used as a solvent in place of acetic acid in the debenzylation process.

Decarboxylation of the indolecarboxylic acid (0·5 g.) in glycerol at 220—230° during 20 minutes gave 6-*hydroxyindole* which was purified by crystallisation from light petroleum (b. p. 80—100°), followed by sublimation in a vacuum, and then recrystallisation, being ultimately obtained in colourless needles (0·2 g.), m. p. 125·5° (Found: C, 72·3; H, 5·4; N, 10·8. C_8H_7ON requires C, 72·2; H, 5·3; N, 10·5%). The *picrate* formed dark red needles, m. p. 154—156° (decomp.), from benzene (Found: N, 15·3%).

2-*Nitro*-3-*benzyloxyphenylpyruvic Acid* (I).—2-Nitro-3-hydroxytoluene (Gibson, *J.*, 1923, 1269) (5 g.) was benzylated with benzyl bromide (3·8 ml.) and excess of potassium carbonate in boiling acetone (100 ml.) during 2½ hours; on isolation the *ether* was distilled in a high vacuum and then crystallised from light petroleum (b. p. 40—60°), forming colourless needles (6·5 g.), m. p. 36° (Found: N, 5·6. $C_{14}H_{13}O_3N$ requires N, 5·8%). This compound (24 g.) was condensed with ethyl oxalate (15 g.) by means of potassium ethoxide (from 4 g. of potassium) by the method used for 2-nitro-4-benzyloxytoluene; the resulting pyruvic acid was obtained in yellow plates (15·5 g.), m. p. 125°, which gave an *oxime* forming colourless needles, m. p. 190—191°, from aqueous alcohol (Found: C, 58·3; H, 4·4; N, 8·6. $C_{16}H_{14}O_6N_2$ requires C, 58·2; H, 4·2; N, 8·5%).

7-*Hydroxyindole* (III; R = H).—Simultaneous reduction and cyclisation of the foregoing nitropyruvic acid (3·8 g.) gave 7-*benzyloxyindole-2-carboxylic acid* (II) which crystallised from dilute acetic acid in needles (2·2 g.), m. p. 164° (Found: C, 71·8; H, 5·0; N, 5·0. $C_{16}H_{13}O_3N$ requires C, 71·9; H, 4·9; N, 5·2%). Debenzylation of this ether (2 g.) by the hydrogenation method furnished 7-*hydroxyindole-2-carboxylic acid* (III; R = CO_2H) which formed needles (1 g.), m. p. 252°, from water, almost insoluble in light petroleum or benzene and readily soluble in alcohol or acetone (Found: C, 61·2; H, 4·1; N, 8·0%). Decarboxylation of this acid (0·5 g.) by the glycerol process gave 7-*hydroxyindole* which was purified by sublimation in a vacuum and recrystallisation from light petroleum (b. p. 40—60°), forming colourless prisms (20 mg.), m. p. 96°, moderately soluble in benzene or chloroform and readily soluble in alcohol or ethyl acetate (Found: C, 72·4; H, 5·5; N, 10·8%).

2 : β-*Dinitro*-6-*acetoxystyrene* (XI).—Potassium hydroxide (0·7 g.) dissolved in alcohol (10 ml.) cooled to − 10° was added in the course of 20 minutes to an agitated solution of 2-nitro-6-hydroxybenzaldehyde (1 g.) and nitromethane (0·4 g.) in alcohol kept at − 10°. The mixture was then carefully acidified with cooled hydrochloric acid, and after dilution with water the condensation product, which usually remained in solution, was isolated with ether, being obtained as a yellow oil which slowly solidified. When this material was gently warmed with sodium acetate (2 g.) and acetic anhydride (3 ml.) for 15 minutes, and water (20 ml.) subsequently added, 2 : β-*dinitro*-6-*acetoxystyrene* separated as a gum which on trituration with water formed a granular crystalline powder (1·35 g.), m. p. 128—130°. Crystallised from alcohol, the acetate formed light tan plates having the same m. p. (Found: C, 47·5; H, 3·0; N, 11·3. $C_{10}H_8O_6N_2$ requires C, 47·6; H, 3·2; N, 11·1%).

4-*Hydroxyindole* (XII; R = H).—The reaction initiated by gently warming a mixture of 2-β-dinitro-6-acetoxystyrene (0·5 g.), iron filings (2 g.), alcohol (4 ml.), and acetic acid (4 ml.) until there was a vigorous evolution of hydrogen was allowed to continue for 10—12 minutes (a longer reaction period decreased the yield) with occasional heating. After filtration to remove insoluble material (wash several times with a little warm alcohol) the filtrate was diluted with water, basified with sodium hydrogen carbonate, and repeatedly extracted with ether. Evaporation of the combined, dried extracts left a pale greenish gum from which 4-*acetoxyindole* (0·1—0·12 g.) was isolated by extraction with excess of warm light petroleum (b. p. 60—80°), and crystallised from the same solvent, forming glistening needles, m. p. 100° (Found: C, 69·1; H, 5·4; N, 8·2. $C_{10}H_9O_2N$ requires C, 68·6; H, 5·1; N, 8·0%).

A solution of this acetate (0·1 g.) in methanol (3 ml.) was saturated at 0° with ammonia and then kept at below 5° for 16 hours. After the removal of the ammonia and the solvent in a vacuum followed by the addition of water (50 ml.), the product was isolated with ether. Extraction of this material with several

portions of boiling light petroleum (b. p. 80—100°) and concentration of the combined extracts gave 4-*hydroxyindole* in elongated needles (yield, 70—80%), m. p .98°, unchanged on recrystallisation (Found : C, 72·0; H, 5·1; N, 10·3%). A mixture of this substance and its acetate had m. p. 75°. The *picrate* separated from benzene in red needles, which on being heated became partly yellow at 150° and then charred at 180° (Found : N, 15·2%). This indole, which is readily soluble in the usual organic solvents except light petroleum, gives a dark blue colour with alcoholic ferric chloride, and red solution exhibiting a strong green fluorescence with Ehrlich's reagent in the cold. On being warmed, the latter solution acquires a bluish tint, becomes turbid, and then deposits a green precipitate. On being kept a solution of the substance in tap water becomes blue.

2 : *β-Dinitro-5-acetoxystyrene* was prepared in almost theoretical yield from 2-nitro-5-hydroxybenzaldehyde by the method employed for the isomeric 6-acetoxy-derivative. Crystallised from alcohol, the substance formed straw-coloured needles, m. p. 118—119° (Found : C, 47·9; H, 3·2; N, 10·9%). Reduction of this nitrostyrene furnished 5-*acetoxyindole* which separated from light petroleum (b. p. 60—80°) in a mass of lustrous micaceous plates, m. p. 113—114°; yield, 50—55% (Found : C, 68·8; H, 5·0; N, 7·7%). Deacetylation of this derivative by the alcoholic-ammonia process gave 5-hydroxyindole, identical with an authentic sample.

2 : *β-Dinitro-5-acetoxy-β-methylstyrene.*—2-Nitro-5-hydroxybenzaldehyde (1 g.) was condensed with nitroethane (0·5 g.) by the procedure employed for nitromethane, but the product from the acetylation process was less pure and in some experiments did not solidify completely. The crude *acetoxystyrene* (0·8—0·9 g.) was triturated with a little alcohol, and the resulting granular product (0·7 g.) was crystallised from the same solvent, forming pale brownish hexagonal plates, m. p. 88—89° (Found : C, 49·4; H, 4·0; N, 10·2. $C_{11}H_{10}O_6N_2$ requires C, 49·6; H, 3·8; N, 10·5%).

5-*Hydroxy-2-methylindole* —Reduction of the foregoing styrene (0·5 g.) with iron filings and acetic acid furnished 5-*acetoxy-2-methylindole* which separated from light petroleum (b. p. 80—100°) in colourless needles (0·09—0·1 g.), m. p. 128—130° (Found : C, 69·6; H, 5·9; N, 7·8. $C_{11}H_{11}O_2N$ requires C, 69·8; H, 5·8; N, 7·4%). Deacetylation of this acetate gave 5-*hydroxy-2-methylindole* (yield, 80%) which formed colourless, elongated, slender needles, m. p. 133—134°, giving a brownish-purple colouration with alcoholic ferric chloride and a very intense red colouration with Ehrlich's reagent in the cold (Found : C, 73·6; H, 6·0; N, 9·5. C_9H_9ON requires C, 73·5; H, 6·1; N, 9·5%). The *picrate* crystallised from benzene in slender red needles, m. p. 157—158° (decomp.) (Found : N, 14·4. $C_9H_9ON,C_6H_3O_7N_3$ requires N, 14·9%).

5-*Nitro*-3-*hydroxycoumarin* (IX).—Interaction of 6-nitrosalicylaldehyde (Ashley, Perkin, and Robinson, *J.*, 1930, 395) (1 g.), aceturic acid (0·8 g.), sodium acetate (0·8 g.), and acetic anhydride (3 ml.) on the steam-bath during 1 hour, with subsequent addition of water, gave a flocculent precipitate of 5-*keto*-4-(2′-*nitro*-6′-*acetoxybenzylidene*)-2-*methyl*-4 : 5-*dihydro-oxazole*, forming yellow needles (1·3 g.), m. p. 180°, from alcohol (Found : C, 54·0; H, 3·5; N, 9·6. $C_{13}H_{10}O_6N_2$ requires C, 53·8; H, 3·5; N, 9·7%).

Hydrolysis of the azlactone (1 g.) with 1·5N-hydrochloric acid (35 ml.) on the steam-bath for 6 hours furnished 5-*nitro*-3-*hydroxycoumarin* which separated from the cooled mixture as a yellow flocculent precipitate and then crystallised from benzene–light petroleum (b. p. 60—80°) in pale yellow needles (0·49 g.), m. p. 206—207° (Found : C, 52·4; H, 2·2; N, 6·4. $C_9H_5O_5N$ requires C, 52·2; H, 2·4; N, 6·8%). This substance gave a 2 : 4-dinitrophenylhydrazone which separated from acetic acid in slender orange needles which melted above 250°.

6-*Nitro*-2-*benzyloxytoluene*, prepared by the potassium carbonate method, forming needles, m. p. 62°, from aqueous alcohol (Found : N, 5·7%).

Stability of Hydroxyindoles.—Absolutely pure specimens of the monohydroxyindoles are relatively stable in air, and, with the exception of 4-hydroxyindole, in aqueous solution. A dilute aqueous solution of the latter indole deposits a bluish precipitate in the course of 12 hours. In 1—2% aqueous sodium hydroxide 4- and 5-hydroxyindole give greenish-blue and pale reddish colourations which change respectively to deep blue-green and deep red-purple in the course of 20 minutes. The colourless dilute alkaline solutions of 5-hydroxy-2-methyl- and 6-hydroxy-indole assume faint green and pale bluish tints respectively in the course of 20 minutes. On being kept for longer periods all the alkaline solutions become darker and then finally change to dark brown.

1′ : 2″-*Dinitro*-6″-*hydroxy*-2-*phenyl*-5 : 6-*benz*-1 : 3-*oxazine* (XIV).—On being kept, a mixture of 2-nitro-6-hydroxybenzaldehyde (1 g.), acetic acid (0·5 ml.), ammonium acetate (0·5 g.), and alcohol (5 ml.) gradually deposited the *oxazine* in long orange needles (0·85 g.) which on recrystallisation from alcohol formed orange yellow cubes, m. p. 204—205°, after sintering at 200° (Found : C, 53·3; H, 2·9; N, 13·1. $C_{14}H_9O_6N_3$ requires C, 53·3; H, 2·9; N, 13·3%). This compound is slightly soluble in cold and readily soluble in warm aqueous sodium hydroxide forming a yellow solution, and with alcoholic ferric chloride gives an orange colouration slowly changing to red. On being refluxed with sodium acetate (2 g.) and acetic anhydride (5 ml.) for 1 hour the metoxazine (1 g.) gave the *acetate* which formed glistening needles (1·1 g.), m. p. 164°, from alcohol (Found : C, 54·0; H, 3·3; N, 11·5. $C_{16}H_{11}O_7N_3$ requires C, 53·8; H, 3·1; N, 11·8%).

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