186. *The Chemistry of Bacteria. Part II. Some Degradation Products of Violacein.*

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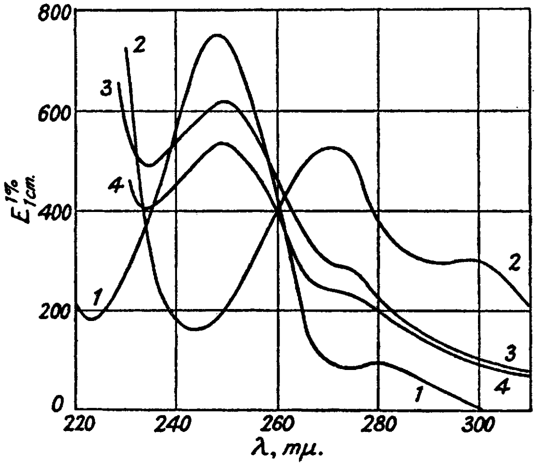
Thermal decomposition of violacein, from *Chromobacterium violaceum*, gives a mixture in small yield from which oxindole and 5-hydroxyindole, as its picrate, have been isolated. In agreement with this result it has been found that oxidation of the acetyl derivative of violacein gives a neutral fraction and a mixture of at least three acids from which *N*-acetylanthranilic acid and 2-acetamido-5-acetoxybenzoic acid have been isolated. From the neutral fraction of the oxidation product isatin has been separated. On the basis of these findings the violacein molecule is considered to contain an indole and a hydroxyindole residue.

THE intensely violet phenolic pigment violacein produced by *Chromobacterium violaceum* has been isolated in the amorphous form by a number of workers, *e.g.*, Hartley (*Sci. Proc. Roy. Dublin Soc.*, 1913, **14**, 63), Lasseur and Girardet (*Bull. Trimistriel. Soc. Sci., Nancy*, 1926, **3**, 1), Reilly and Pyne (*Biochem. J.*, 1927, **21**, 1059), and Tobie (*J. Bact.*, 1935, **29**, 223), who have studied the properties of the substance. As might be expected the results obtained with amorphous material have been inconclusive, and further, the colouring matters described by Sartory *et al.* (*Compt. rend.*, 1938, **206**, 950; *Bull. Sci. Pharm.*, 1938, **45**, 302) and by Friedheim (*Compt. rend. Soc. Biol.*, 1932, **110**, 352; *Arch. Sci. phys. nat.*, 1932, **14**, 125) appear to differ so considerably from violacein that it is doubtful if they are the same pigment. Crystalline violacein appears to have been first obtained by Kögl and Tönnis (unpublished work; see " Handbuch der Pflanzenanalyse ", 1932, Vol. III, Part 2, p. 1443) who like some of the earlier workers cultivated the organism in a liquid medium. Essentially the same procedure has been described by Strong (*Science*, 1944, **100**, 287), but the amount of the colouring matter obtained in this way is small, about 710 mg. from 65 l. of medium. In the course of an investigation of the chemistry and constitution of this pigment in these laboratories some improvement in the yield has been effected by the use of a solid medium, but the quantity available for investigation is limited and as it is hoped to improve still further the method of culture, full details will be published when the experiments directed to this end have been completed.

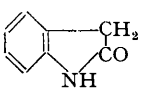
Comparatively little is known regarding the chemical nature of violacein, and the empirical formulæ proposed by Reilly and Pyne (*loc. cit.*) and by Tobie (*Proc. Soc. Exp. Biol. Med.*, 1936, **34**, 620), derived from analyses of the amorphous pigment, may be neglected. On the basis of their analytical results obtained with crystalline material, Kögl and Tönnis (*loc. cit.*) suggested the empirical formula $C_{35}H_{25}O_6N_5$ or $C_{42}H_{30}O_7N_6$ for violacein, whilst the analyses obtained by Wrede (*Z. physiol. Chem.*, 1934, **223**, 113; *Arch. exp. Path. Pharm.*, 1937, **186**, 532), who showed that the pigment formed comparatively stable crystalline addition compounds with a number of bases—aniline, chloroaniline, pyridine, and chloropyridine—point to the empirical formula $C_{42}H_{28}O_7N_6$ rather than $C_{35}H_{23}O_6N_5$. The analytical figures for specimens of violacein and its acetyl derivative prepared in this laboratory (unpublished work) are in agreement with those of Wrede (*loc. cit.*), but a decision regarding the empirical formula cannot be made profitably at this stage, more especially since the extremely low solubility of the compound in suitable solvents precludes accurate molecular-weight determinations. With violacein the results of nitrogen estimations are unexpectedly variable (cf. Wrede, *loc. cit.*). Whilst Kögl and Tönnis (*loc. cit.*) and Wrede (*loc. cit.*) have described the general properties of the crystalline compound and its acetate, no identifiable major degradation products have hitherto been reported other than the formation of traces of oils (pyrrolic ?) by distillation with zinc dust and other violent degradations reported by Wrede and by Tobie (*loc. cit.*), who also claimed to have obtained an indication of the presence of anthranilic acid by a colour reaction in a solution resulting from a hydrolytic process (*J. Bact.*, 1938, **35**, 11).

In 1938, Dr. T. S. Subramanian, working in this laboratory, observed that when violacein was heated with zinc dust in a vacuum a white sublimate was produced in small amount from which material, m. p. 112°, was separated. This product gave a strong Ehrlich reaction and had the composition C_8H_7ON. On reinvestigating this reaction we have found that when heated in a vacuum or in a slow stream of nitrogen, alone or mixed with zinc dust or copper powder, violacein decomposes vigorously, and a poor yield of a volatile crystalline product is obtained, the main component of which is oxindole (I). Oxindole was isolated by fractional crystallisation of the pyrolysis product, and in addition there was obtained a fraction, m. p. 102—103°, which had the composition and some of the properties of a hydroxyindole but did not appear to be identical with any of the isomeric synthetic hydroxyindoles (Part I, *J.*, 1948, 1605). Examination of the ultra-violet spectrum of this product indicated that, in all probability, it contained a considerable proportion of oxindole mixed with the compound responsible for the Ehrlich reaction, presumably a hydroxyindole. Though owing to lack of material it has not been possible to resolve this mixture, we have isolated from it a small amount of an orange-red picrate which corresponds in every respect with a sample of the picrate of authentic 5-hydroxyindole (II). Further, it has been observed that crystallisation of a mixture of 5-hydroxyindole (1 part) and oxindole (2 parts) gives rise to a product, m. p. 101—103°. This is not regarded as a genuine addition compound containing stoicheiometric amounts of the components because the melting point was slightly raised (to 103—104°) by recrystallisation. The absorption curve of the artificial mixture of oxindole and 5-hydroxyindole, m. p. 103—104°, cuts the curve for oxindole at the expected isobestic points. That of the pyrolysis product, m. p. 102—103°, is qualitatively very similar to the curve for the artificial mixture, but the absorption is slightly more intense, suggesting the presence of a third product with continuous end absorption rising gradually from 300 mμ to 230 mμ. When the pyrolysis product was treated directly with picric acid in benzene there was obtained a trace of a second deep red crystalline picrate, and this observation, in conjunction with the unpleasantly characteristic smell of the crude material (the hydroxyindoles are odourless), suggests that small amounts of at least one other indole are present (cf. Wrede, *loc. cit.*).

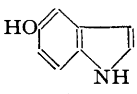


Absorption curves.

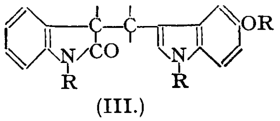
1. *Oxindole.*
2. 5-*Hydroxyindole.*
3. *Pyrolysis product, m. p.* 102—103°.
4. *Mixture of* 1 *and* 2, *m. p.* 103—104°.



(I.)



(II.)



(III.)

Oxidative degradation of violacein has not so far yielded any identifiable product, probably because the phenolic system renders the molecule too sensitive, and on this account we have turned to a study of the degradation of acetylviolacein. From the mixture formed when the oxidation was effected with chromic oxide in hot acetic acid, a mixture of acidic substances was isolated from which *N*-acetylanthranilic acid was separated in comparatively large amount. On repeating the oxidation at room temperature a somewhat higher yield of a similar acidic mixture was obtained, of which the main component was again *N*-acetylanthranilic acid. A second acid was isolated but could not be obtained in an absolutely pure state. Esterification of this substance with diazomethane and subsequent distillation of the product in a high vacuum ultimately gave a pure specimen of the methyl ester, obtained in two crystalline forms, m. p. 103—104° and 106—107°, which from its analytical results appeared to be the diacetyl derivative of a methyl hydroxyanthranilate. From the colour reactions given by a solution of the deacetylated acid it appeared probable that the parent oxidation product was 2-*acetamido*-5-*acetoxybenzoic acid.* Accordingly *methyl* 2-*acetamido*-5-*acetoxybenzoate* was synthesised and found to be identical with the methyl ester of the degradation product. From the aqueous liquors left after the separation of the main acidic oxidation products there was isolated by continuous ethereal extraction a mixture which, in addition to a small quantity of mixed acids, contained isatin.

It seems probable, from the production of *N*-acetylanthranilic acid and isatin by oxidation of acetylviolacein and of oxindole by thermal decomposition of the parent pigment, that the violacein molecule contains an oxindole nucleus or one closely related to it, the nitrogen atom of which bears an acetyl group in acetylviolacein. There is, however, no direct evidence as yet that these degradation products have their origin in the same part of the molecule. Similarly, it is a reasonable hypothesis that the diacetyl derivative of 5-hydroxyanthranilic acid arises from a 5-acetoxy-1-acetylindole residue in acetylviolacein, and that it is this part of the molecule which affords 5-hydroxyindole in the pyrolysis of violacein itself, although it is, of course, conceivable that this product arises as the result of a rearrangement. The expression (III; R = H) represents one of the possible ways in which the two indole nuclei may be linked in the pigment. The acetyl derivative would then be (III; R = Ac).

In the course of earlier attempts to define the nature of the mixture, m. p. 112°, from the pyrolysis of violacein, this material was compared with the hydroxytolyl cyanides which have the same composition, although it seemed unlikely that the latter compounds would give a positive Ehrlich reaction. Of the ten possible members of this series five were known, and the preparation of the other five, *i.e.*, 3-*hydroxy*-, 4-*hydroxy*-, and 5-*hydroxy*-o-*tolyl cyanide*, 5-*hydroxy*-m-*tolyl cyanide*, and 3-*hydroxy*-p-*tolyl cyanide*, by standard procedures is described in the experimental section.

EXPERIMENTAL.

Pyrolysis of Violacein.—An intimate mixture of crystalline violacein (100 mg.) and zinc dust (1·0 g.) was heated in a small retort in a vacuum at 330°/0·1 mm. for 1½ hours, and a colourless product distilled on to the cooler part of the retort tube where the liquid solidified. The distillate (80—100 mg.) from 10 experiments was combined and fractionally crystallised from light petroleum (b. p. 60—80°), and the following two apparently homogeneous substances were isolated :

(*a*) A compound forming long needles (20 mg.), m. p. 126°, from light petroleum (b. p. 60—80°), undepressed on admixture with an authentic specimen of oxindole, m. p. 126—127°, with which its properties were identical [Found : C, 72·0; H, 5·8; N, 10·6; *M* (Rast), 139·1. Calc. for C_8H_7ON : C, 72·2; H, 5·3; N, 10·5%; *M*, 133]. The ultra-violet absorption spectrum of the specimen dissolved in alcohol shows $\lambda_{\max.}$ 248·5 mμ, $\varepsilon_{\max.}$ 9930, with a weak maximum at 280 mμ, ε 1260. This agrees well with the absorption curve recorded by Ramart-Lucas and Biquard (*Bull. Soc. chim.*, 1935, **2**, 1387).

(*b*) A product obtained in very faintly yellow flakes (22 mg.), m. p. 102—103°, soluble in dilute aqueous sodium hydroxide and giving a greenish-yellow colouration, ultimately changing to brown, with aqueous ferric chloride (Found : C, 71·9, 71·9; H, 5·1, 5·7; N, 10·5. Calc. for C_8H_7ON : C, 72·2; H, 5·3; N, 10·5%). This material gave an intense red colouration, tinged with blue, with Ehrlich's reagent and a positive nitroprusside test for indoles. In spite of the close similarity to the hydroxyindoles (Part I, *loc. cit.*), a mixture of this material with either 4-hydroxy- (m. p. 98°), 5-hydroxy- (m. p. 107°), or 7-hydroxy-indole (m. p. 96°) showed a depression in m. p., and the ultra-violet absorption spectrum (see figure) was markedly different from those of the synthetic hydroxyindoles, and suggested that a high proportion of oxindole was present together with a much smaller amount of one or other of the isomeric hydroxyindoles.

When picric acid (27 mg.), dissolved in the minimum amount of warm benzene, was added to a solution of the material (17 mg.), m. p. 102—103°, in a small volume of the same solvent, a crystalline picrate began to separate almost immediately. On isolation, the compound (7 mg.) had m. p. 162° (decomp.) and was recrystallised with little loss from benzene mixed with a trace of light petroleum, forming clusters of elongated orange-red needles, m. p. 166—167° (decomp.) (bath preheated to 100°) after slight shrinking and darkening. This derivative corresponds in colour, crystal form, and habit with the authentic picrate of 5-hydroxyindole, m. p. 167° (decomp.), and a mixture of the two picrates had the same m. p. Both derivatives have a somewhat lighter colour than the picrates of the other hydroxyindoles which have been prepared (Part I, *loc. cit.*). The same mixture of products was obtained when zinc dust was replaced by copper powder and when the reaction was carried out in a slow stream of nitrogen at atmospheric pressure. Modification of the conditions in a variety of ways failed to increase the proportion of 5-hydroxyindole in the crude pyrolysis product.

Oxidation of Acetylviolacein with Chromium Trioxide.—(*A*) To boiling acetic acid (35 ml.) containing acetylviolacein (Wrede, *loc. cit.*) (200 mg.) in suspension a warm solution of chromic acid in acetic acid (approximately 3·5 ml. of a 10% solution) was gradually added until the original deep red colour changed to a clear green (5—10 minutes). After having been kept at room temperature for one hour the mixture was diluted with water (110 ml.) and extracted several times with ether. By this means a brown tar was isolated which was separated into benzene-soluble and benzene-insoluble fractions. The latter fraction was triturated with aqueous sodium hydrogen carbonate, the extract filtered to remove non-acidic resinous material, the filtrate acidified and exhaustively extracted with ether, and the combined dried extracts evaporated, leaving a small amount of an acidic product. Crystallised by slow evaporation of an ethyl acetate–light petroleum (b. p. 60—80°) solution, the material from five oxidation experiments gave an acid in short yellow needles, m. p. 214—218°, which was not further purified owing to lack of material but was converted into the methyl ester by means of diazomethane. This compound could be readily crystallised from benzene, forming colourless needles, m. p. 163° (Found : C, 55·9; H, 4·6; N, 6·5. Calc. for $C_{11}H_{11}O_5N$: C, 55·7; H, 4·7; N, 5·9%).

The benzene-soluble fraction from five experiments, dissolved in a little benzene, was treated with a little light petroleum (b. p. 60—80°), giving a pale yellow precipitate (*ca.* 70 mg.) from which two acids were separated by fractional crystallisation from light petroleum (b. p. 80—100°). After repeated purification from benzene the more soluble acid was obtained in colourless needles, m. p. 181°, and identified by comparison with an authentic specimen of *N*-acetylanthranilic acid, m. p. 185°; a mixture of the two specimens melted at 183—184° (Found: C, 60·6; H, 5·3; N, 7·5. Calc. for $C_9H_9O_3N$: C, 60·3; H, 5·0; N, 7·8%). Prepared by means of diazomethane, the methyl ester formed shining needles, m. p. 100°, undepressed on admixture with an authentic specimen, m. p. 100—101°.

The second acid isolated from the benzene soluble fraction was obtained in larger amount by method (*B*) and is described below.

(*B*) Acetylviolacein (1 g.) suspended in acetic acid (250 ml.) was treated with a 5% solution of chromium trioxide in acetic acid (27 ml.), and the mixture kept for 20—24 hours with occasional shaking and filtered to remove a little unchanged pigment. The green concentrate (*ca.* 25 ml.) left on evaporation of the filtrate under reduced pressure was treated with water (250 ml.) and repeatedly extracted with ether, giving extract (*X*). The residual aqueous solution was then further exhausted with ether in a continuous extraction apparatus, giving extract (*Y*).

Evaporation of the extract (*X*) left a reddish-brown gum which was thoroughly triturated with 2N-sodium hydrogen carbonate (30 ml.), leaving a dark viscous undissolved residue (30—35 mg.). The filtered hydrogen carbonate solution was acidified and repeatedly extracted with ether (the residual aqueous liquor was finally combined with the liquor in the continuous extractor). Evaporation of the dried extracts left a gum which was digested with successive small portions of boiling light petroleum (b. p. 80—100°), the later portions of solvent containing an increasing proportion of benzene. In this manner four main fractions were obtained from the gum: (*a*) extracted with light petroleum, (*b*) with light petroleum containing 25% benzene, (*c*) with equal parts of light petroleum and benzene, and (*d*) a benzene-insoluble residue. On cooling, each extract deposited colourless crystalline material, and as the crops from (*a*) and (*b*) were found to be similar they were combined and designated substance (M_1) (130 mg. and 15 mg. obtained by concentration of the mother liquor); (*c*) gave (M_2) and (*d*) the residue (M_3).

The material M_1, m. p. *ca.* 135—140°, was found to contain *N*-acetylanthranilic acid and a smaller amount of a second acid. As the separation of the components by crystallisation proved difficult, the mixture was esterified with diazomethane and the mixed methyl esters (200 mg.) distilled in a high vacuum at 0·02 mm., giving 3 fractions. The first fraction, obtained with the bath at 110—120°, consisted of colourless crystals (110 mg.), m. p. 94—96°, which on recrystallisation from light petroleum (b. p. 60—80°) had m. p. 98—100°, undepressed on admixture with authentic methyl *N*-acetylanthranilate. The second fraction, which distilled with bath temperature about 140°, formed colourless crystals (47 mg.), m. p. *ca.* 89°, with previous sintering, and then m. p. 100—101° after recrystallisation from light petroleum (yield, 30 mg.). The third fraction, bath temperature 140—160°, was obtained in colourless crystals (23 mg.), m. p. 98—100°, and on recrystallisation from light petroleum gave a substance, m. p. 104—106°. The recrystallised products from the second and third fractions consisted mainly of the methyl ester of M_2 with which they were ultimately identified.

The product M_2 had m. p. about 176° after sintering at about 165°, and on recrystallisation formed an apparently homogeneous mass of colourless flakes which on being heated in a capillary tube shrank slightly at 150°, softened at 165°, and melted at 175—182°. This behaviour was not materially affected by numerous crystallisations from benzene or toluene, and, presumably owing to loss of solvent of crystallisation, the originally clear crystals became opaque on being kept at room temperature. When a little of the parent acid was deacetylated by being heated with a little N-hydrochloric acid, the solution gave a positive test for a primary amine (diazotisation), and the basified solution had a strong blue fluorescence. Prepared by means of ethereal diazomethane, the *methyl* ester formed clusters of colourless needles, m. p. *ca.* 100°, from light petroleum (b. p. 60—80°), and was then purified by distillation in a vacuum, bath temperature 150—160°/0·05 mm. On recrystallisation from light petroleum (b. p. 60—80°) the distillate gave methyl 2-acetamido-5-acetoxybenzoate in flat needles or plates, m. p. 106—107°, undepressed on admixture with a synthetic specimen [Found in specimen dried in a high vacuum at 40°: C, 57·3; H, 5·1; N, 5·6; OMe, 11·3; Ac, 42·0. $C_7H_4O_2N(OMe)Ac_2$, requires C, 57·4; H, 5·2; N, 5·6; OMe, 12·4; Ac, 34·2%].

The benzene-insoluble residue M_3, collected from a number of oxidations, was extracted with aqueous sodium hydrogen carbonate, giving on acidification of the extract a small amount of an acid which separated from methanol–benzene in faintly coloured rectangular prisms, m. p. 230—231° (decomp.) after sintering about 227°. After having been sublimed at 160°/0·02 mm. this acid had m. p. 235—236° (decomp.). With ethereal diazomethane the partially purified acid gave a methyl ester which formed almost colourless needles, m. p. 150—165°, and was then separated from traces of a more volatile ester by sublimation at 140—145°/0·02 mm., being obtained as a colourless crystalline compound, m. p. 165—166° after sintering at 158°.

The extract (*Y*) (from 3 g. of acetylviolacein) was evaporated under reduced pressure, and a solution of the residue in a little methanol was treated with 2N-sodium hydrogen carbonate (50 ml.). The mixture was repeatedly extracted with ether, giving a non-acidic fraction, and from the aqueous hydrogen carbonate an acidic fraction was isolated after acidification with hydrochloric acid. On being heated in a small sublimation retort at 160—180°/0·001 mm. the non-acidic gum gave an orange-red crystalline sublimate (45 mg.), m. p. 196° after sintering, which on recrystallisation from benzene with little loss of material gave isatin in orange-red prisms, m. p. 199—200°, unchanged on further crystallisation or admixture with an authentic specimen, m. p. 201—202° (Found: C, 65·4; H, 3·4. Calc. for $C_8H_5O_2N$: C, 65·3; H, 3·4%). The acidic fraction was esterified with diazomethane and the ester distilled in a vacuum, giving several different esters in amounts too small to permit further investigation at present.

2-*Acetamido*-5-*acetoxybenzoic Acid*.—Acetylation of 5-hydroxyanthranilic acid (Limpricht, *Annalen*, 1892, **263**, 224) (0·2 g.), dissolved in 2% aqueous sodium hydroxide (10 ml.), with acetic anhydride (0·35 g.) at 0° in the course of a few minutes and subsequent addition of a slight excess of hydrochloric acid gave the *diacetyl* derivative (0·3 g.), m. p. 188—189° (Found in material dried in a high vacuum at 80°: C, 56·1; H, 4·6. $C_{11}H_{11}O_5N$ requires C, 55·7; H, 4·6%). Like those of the acid from acetylviolacein, the crystals of the synthetic diacetate became opaque on being kept. Esterification of this acid (0·12 g.) with a slight excess of ethereal diazomethane gave the *methyl* ester, forming from light petroleum (b. p. 60—80°), clusters of colourless needles, m. p. 103—104°, which on sublimation at 150—160°/0·02 mm. and subsequent recrystallisation had the same m. p. 103—104° (Found in material dried in a high vacuum at 40°: C, 57·5; H, 5·2. $C_{12}H_{13}O_5N$ requires C, 57·4; H, 5·2%). This ester was observed to exist in two crystalline forms; *viz.*, the form in needles, m. p. 103—104°, which was usually obtained, and the form in plates or flattened needles, m. p. 106—107°, originally obtained by inoculating a warm solution of the lower-melting form with a natural specimen obtained by the oxidation of violacein. When a mixture of the two forms, which begins to melt at 103°, was kept at this temperature for a few minutes, cooled, and then heated, it melted at 107°, but when a mixture of the two forms was melted completely and allowed to solidify it then had m. p. 102—103°.

A specimen of the ester obtained from violacein behaved in exactly the same manner.

(With D. J. McKeand.)

3-*Hydroxy*-o-*tolyl Cyanide*.—A solution of 3-amino-*o*-tolyl cyanide (Gabriel and Tieme, *Ber.*, 1919, **52**, 1079) (2 g.) in dilute sulphuric acid (20 ml.), kept at 0°, was treated with slightly more than one molecular proportion of aqueous sodium nitrite, the excess of nitrite destroyed with a little urea, and the diazonium sulphate solution added dropwise to boiling sulphuric acid (35 ml. of concentrated acid and 70 ml. of water). After isolation the product was crystallised from benzene–light petroleum (b. p. 60—80°), and the *cyanide* obtained in colourless prisms; m. p. 110—111° (Found: N, 10·7. C_8H_7ON requires N, 10·5%).

4-*Hydroxy*-o-*tolyl Cyanide*.—4-Amino-*o*-tolyl cyanide (Borsche and Wagner-Roemmich, *Annalen*, 1941, **546**, 277) (5 g.) was diazotised in sulphuric acid, and the resulting sulphate converted into 4-*hydroxy*-o-*tolyl cyanide* by the procedure employed for the 3-hydroxy-compound. The product formed colourless needles (1 g.), m. p. 100—101°, from light petroleum (b. p. 60—80°) (Found: C, 72·4; H, 5·4; N, 10·2. C_8H_7ON requires C, 72·2; H, 5·3; N, 10·5%). Acetylation by the pyridine–acetic anhydride method gave the *acetyl* derivative, which crystallised from light petroleum (b. p. 60—80°) in needles, m. p. 63·5—64° (Found: C, 68·6; H, 5·2; N, 7·9. $C_{10}H_9O_2N$ requires C, 68·6; H, 5·1; N, 8·0%).

5-*Hydroxy*-o-*tolyl Cyanide*.—The *oxime* of 5-hydroxy-*o*-tolualdehyde (Gattermann, *Annalen*, 1907, **357**, 322) formed needles, m. p. 151—152°, from water (Found: C, 63·9; H, 5·9; N, 9·3. $C_8H_9O_2N$ requires C, 63·6; H, 6·0; N, 9·3%). Dehydration of this compound (1 g.) with acetic anhydride gave 5-*acetoxy*-o-*tolyl cyanide* which crystallised from light petroleum (b. p. 60—80°) in prisms (0·7 g.), m. p. 45·5—46·5° (Found: C, 68·6; H, 5·1; N, 7·7%). On deacetylation, this acetate (0·7 g.) furnished the *cyanide* (0·5 g.), which formed prisms, m. p. 135—136°, from benzene–light petroleum (b. p. 60—80°) (Found: C, 72·2; H, 5·5; N, 10·7%).

5-*Hydroxy*-m-*tolyl Cyanide*.—The *acetyl* derivative of 5-hydroxy-*m*-toluic acid (Meldrum and Perkin, *J.*, 1909, **95**, 1896) formed colourless needles, m. p. 107·5—109·5° (Found: C, 61·5; H, 5·2. $C_{10}H_{10}O_4$ requires C, 61·9; H, 5·2%). The acid chloride was prepared from the compound (2 g.) with phosphorus pentachloride (2·1 g.) in chloroform (30 ml.) and converted into the *amide*, which separated from water in needles (1·5 g.), m. p. 139—140° (Found: C, 61·9; H, 5·5; N, 6·8. $C_{10}H_{11}O_3N$ requires C, 62·2; H, 5·7; N, 7·3%). Dehydration of the amide (1 g.) with boiling phosphorus oxychloride (0·5 g.) containing sodium chloride (0·5 g.) for 30 minutes gave the acetoxy-cyanide which on deacetylation furnished 5-*hydroxy*-m-*tolyl cyanide* (0·3 g.), which formed needles, m. p. 103·5—104·5°, from benzene–light petroleum (Found: C, 71·9; H, 5·3; N, 10·2%).

3-*Hydroxy*-p-*tolyl Cyanide*.—Dehydration of the oxime (Chuit and Bolsing, *Bull. Soc. chim.*, 1906, **35**, 134) (2 g.) of 3-hydroxy-*p*-tolualdehyde with boiling acetic anhydride (15 ml.) during 4½ hours gave 3-*acetoxy*-p-*tolyl cyanide* (1·1 g.) which formed needles, m. p. 58·5—59·5°, from light petroleum (b. p. 60—80°) (Found: C, 68·7; H, 5·1; N, 8·1%). On deacetylation with dilute sodium hydroxide this acetate gave the *hydroxy-cyanide* which separated from benzene–light petroleum (b. p. 60—80°) in needles, m. p. 125° (Found: N, 10·4%).

The ultra-violet absorption spectra of the compounds recorded have been determined by Professor R. A. Morton of this University, to whom we express our thanks.

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