310. *A Study of Melanin Formation by Use of* 2-(3 : 4-*Dihydroxy*-[3-^{14}C]*phenyl*)-, 2-(3 : 4-*Dihydroxy*[4-^{14}C]*phenyl*)-, *and* 2-(3 : 4-*Dihydroxy*[5-^{14}C]*phenyl*)-*ethylamine.*

By G. A. Swan and D. Wright.

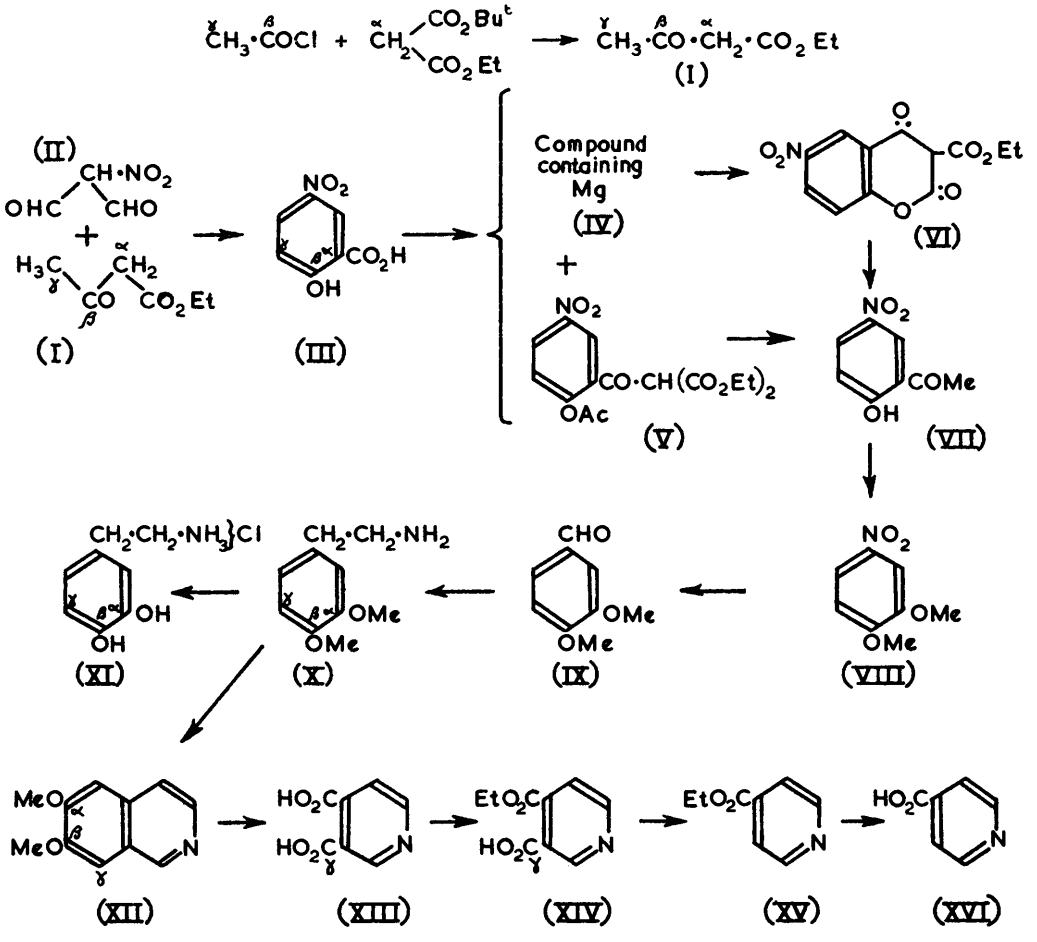
The compounds named in the title have been synthesised and used to study melanin formation. It was found that 29·8, 27·2, and 3·5% of the carbon dioxide evolved during the reaction arise from the 3-, 4-, and 5-positions, respectively of the benzene ring. A comparison of the specific activities of the melanins with those of their precursors suggests that approximately one unit in five in the polymer has undergone oxidative fission, with the loss of (probably) $C_{(4)}$, $C_{(5)}$, and $C_{(6)}$ of the indole nucleus. The results are in keeping with the structure of melanin suggested by Bu'Lock and Harley-Mason [1] (involving coupling of indole units between positions 3 and 7), with this oxidation occurring as a side-reaction.

It has been shown by Swan and Wright [2] that 96% of the carbon dioxide evolved during the formation of melanin from 2-(3 : 4-dihydroxyphenyl)ethylamine (cf. XI) originates in the breakdown of the benzene nucleus. No conclusion was, however, drawn regarding the relation of the specific activity of the melanin to that of the original amine, since the increases observed were only of the same order as the possible experimental error. If nearly all the evolved carbon dioxide arose from one particular carbon atom, and the experiment were made with 2-(3 : 4-dihydroxyphenyl)ethylamine labelled specifically with ^{14}C at that position, then the specific activity of the evolved carbon dioxide should be nearly eight times as great as that formed by combustion of the amine. Moreover, the activity of the resulting melanin should be considerably lower than that of the amine, provided that the molecules undergoing loss of carbon dioxide were in fact incorporated in or into the melanin. The use of specifically labelled compounds should therefore provide a test of the validity of the postulates put forward by Bruce [3] and Jolles,[4] in both of which the oxidative fission of the benzene nucleus is regarded as an essential step in

[1] Bu'Lock and Harley-Mason, *J.*, 1951, 703.
[2] Swan and Wright, *J.*, 1954, 381.
[3] Bruce, *Chem. and Ind.*, 1954, 310; *J. Appl. Chem.*, 1954, **4**, 469.
[4] Jolles, *Chem. and Ind.*, 1953, 845, 1367.

melanin formation. In our earlier work, we carried out our radioactivity measurements on " infinitely thick " layers of barium carbonate. In the experiments described in this paper, all measurements were made by the gas-counting method on carbon dioxide, which has led to some increase in accuracy and has enabled us to use compounds of much lower specific activity (approximately 0·2 μc/mmole).

In the course of experiments designed to introduce ^{14}C into a specific position of the benzene ring the condensation of acetone or ethyl acetoacetate with various malondialdehydes was investigated. In the case of ethoxycarbonylmalondialdehyde (cf. Prelog,



Würsch, and Königsbacher [5]) and cyanomalondialdehyde no condensation products could be isolated under a variety of conditions; and only a small yield of *p*-nitrophenol was obtained from acetone and nitromalondialdehyde (II) (cf. Hill and Torray [6]). Also benzoylacetone and acetylacetone failed to give the expected hydroxy-benzophenone or -acetophenone derivatives. However an almost quantitative yield of 5-nitrosalicylic acid (III) was obtained from the condensation of ethyl acetoacetate and nitromalondialdehyde and this was converted into 2-(3 : 4-dihydroxyphenyl)ethylamine in an eleven-stage synthesis. The acetylated acid chloride when condensed with ethoxymagnesium malonic ester gave two compounds (IV and V) both of which could be hydrolysed to 2-hydroxy-5-nitroacetophenone (VII) : one was presumably the benzoylmalonate derivative (V), while the other

[5] Prelog, Würsch, and Königsbacher, *Helv. Chim. Acta*, 1951, **34**, 258.
[6] Hill and Torray, *Ber.*, 1895, **28**, 2597.
[7] Anschütz, *Annalen*, 1909, **367**, 169.

appeared to be a magnesium derivative (IV) of ethyl 3 : 4-dihydro-6-nitro-4-oxocoumarin-3-carboxylate. (Anschütz [7] obtained ethyl 3 : 4-dihydro-4-oxocoumarin-3-carboxylate by reaction between 3 mols. of sodiomalonic ester and 1 mol. of acetylsalicyloyl chloride.) The above 2-hydroxy-5-nitroacetophenone could also be obtained from 5-nitrosalicylic acid by initially methylating the hydroxyl group, but the overall yield was lower. 4-Nitrocatechol was obtained in high yield from 2-hydroxy-5-nitroacetophenone by Dakin's method,[8] and methylation (to VIII) and reduction afforded 4-aminoveratrole. Several attempts to convert 4-aminoveratrole *via* 4-cyanoveratrole into veratraldehyde (IX) by application of Stephen's method [9] were unsuccessful. However by treatment of the diazonium compound with formaldoxime according to Beech's method [10] a 50% yield of veratraldehyde was obtained. An attempted preparation of 4-methoxybenzoylnitromethane by the application of Beech's method to methazonic acid gave only an azo-compound (cf. Kimich [11]). Veratraldehyde was then converted into 2-(3 : 4-dihydroxyphenyl)ethylamine (*via* X) as described by Swan and Wright.[2]

Thus by initially preparing ethyl acetoacetate (I) labelled in the α-, β-, and γ-position, specimens of 2-(3 : 4-dihydroxyphenyl)ethylamine hydrochloride (XI) were obtained labelled in the 3-, 4-, and 5-nuclear positions respectively. The radiochemical yields (from barium carbonate to dihydroxyphenylethylamine hydrochloride) achieved were 0·4, 1·3, and 1·1%, respectively. The results obtained when each of these compounds was converted into melanin are summarised in Table 1.

TABLE 1.

	Weight (mg.)		Radioactivity (as CO_2) (counts/min.)	
	(1)	(2)	(1)	(2)
2-(3 : 4-Dihydroxy[3-^{14}C]phenyl)ethylamine hydrochloride	72·2	76·4	1259	1259
$BaCO_3$ resulting from melanin formation	19·1	21·0	3010	2980
Melanin	23·8	22·5	1125	1132
Melanin (refluxed for 24 hr. with 2N-HCl)	—	—	1044	1070
2-(3 : 4-Dihydroxy[4-^{14}C]phenyl)ethylamine hydrochloride	71·9	71·9	1377	1377
$BaCO_3$ resulting from melanin formation	22·6	20·1	2830	3150
Melanin	29·0	25·06	1250	1282
Melanin (refluxed for 24 hr. with 2N-HCl)	—	—	1210	1160
2-(3 : 4-Dihydroxy[5-^{14}C]phenyl)ethylamine hydrochloride	76·0	72·9	498	498
$BaCO_3$ resulting from melanin formation	21·2	21·1	141	137
Melanin	27·8	26·0	510	505
Melanin (refluxed for 24 hr. with 2N-HCl)	—	—	518	510

That these compounds were truly specifically labelled was demonstrated by the conversion of some of the radioactive 2-(3 : 4-dimethoxyphenyl)ethylamine from each synthesis into 6 : 7-dimethoxy*iso*quinoline (XII), and the three specimens of the *iso*quinoline [12] were then oxidised to cinchomeronic acid (XIII). The cinchomeronic acid obtained from two of the samples of *iso*quinoline, *i.e.*, those synthesised from 2-(3 : 4-dimethoxy[3-^{14}C]phenyl)- and 2-(3 : 4-dimethoxy[4-^{14}C]phenyl)-ethylamine, were completely inactive whereas in the case of the cinchomeronic acid obtained from 2-(3 : 4-dimethoxy[5-^{14}C]phenyl)ethylamine all of the radioactivity was retained in the acid. As expected, this radioactivity was completely lost during the conversion [13] of the cinchomeronic acid into *iso*nicotinic acid (XVI) *via* the silver salt of 4-ethyl hydrogen cinchomeronate (XIV). These results (Table 2) show that no appreciable rearrangement had occurred at any stage of the syntheses, provided one makes the plausible assumption that interchange of $C_{(3)}$ and $C_{(4)}$ of the benzene nucleus does not occur.

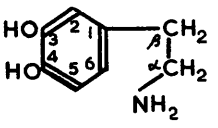
From the results given in Table 1 it can be calculated that 29·8, 27·2, and 3·5% of the evolved carbon dioxide originates in the 3-, 4-, and 5-position, respectively, of the 2-(3 : 4-dihydroxyphenyl)ethylamine. We have already shown [2] that 1·9 and 2·3% of the carbon dioxide originates in the α- and the β-position of the side chain, respectively. We therefore

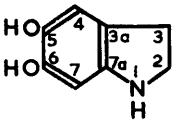
[8] Dakin, *Amer. Chem. J.*, 1909, **42**, 477.
[9] Stephen, *J.*, 1925, 1874.
[10] Beech, *J.*, 1954, 1297.
[11] Kimich, *Ber.*, 1877, **10**, 140.
[12] Späth and Polgar, *Sitzungsber. Akad. Wiss. Wien*, 1928, **137**, IIb, 1142.
[13] Strache, *ibid.*, 1890, **99**, IIb, 153.

TABLE 2.

	Radioactivity (as CO_2) (counts/min.)				
		Cinchomeronic acid		*iso*Nicotinic acid	
	6 : 7-Dimethoxy*iso*quinoline	Calc.	Found	Calc.	Found
[6-^{14}C]	763	0	2	—	—
[7-^{14}C]	1225	0	5	—	—
[8-^{14}C]	1136	1785	1770	0	2

infer that 35·3% arises from positions 1, 2, and 6 together. Assuming cyclisation to 5 : 6-dihydroxyindole to occur, it seems likely that only something of the order of 2% will arise from each of positions 1 and 6. (As two carbon atoms of the pyrrole ring remain intact, it appears probable that the entire pyrrole ring will remain unbroken; the small evolution of carbon dioxide from positions α, β, 1, 5, and 6 might be due to complete oxidation of a small amount of the amine.) This would imply that something of the order of 30% would originate from the 2-position. Unfortunately we have not yet proved this,





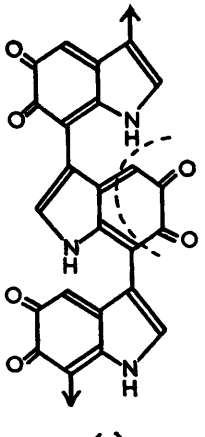
because the above synthesis is unsuited to the preparation of the required 2-(3 : 4-dihydroxy[2-^{14}C]phenyl)ethylamine—although it is hoped to do so later. However, if these figures are tentatively accepted, they would correspond in the case of 5 : 6-dihydroxyindole to the results in Table 3.

TABLE 3.

Position in 5 : 6-dihydroxyindole ...	2	3	3*a*	4	5	6	7	7*a*
% of total evolved CO_2	1·9	2·3	*ca.* 2 *	*ca.* 30 *	29·8	27·2	3·5	*ca.* 2 *

* Values marked thus have not been measured directly; they are merely approximate, hypothetical values.

These results could be readily explained on the basis of a polyindolequinone structure for melanin, involving linkage between the 3- and the 7-position: this was postulated by Bu'Lock and Harley-Mason [1] (cf. Horner and Spietschka [14]), and received support from the oxygen-uptake experiments and spectroscopic determinations on various alkylated dihydroxyindoles by Beer, Broadhurst, and Robertson,[15] although certain observations conflict with the similar experiments of Cromartie and Harley-Mason.[16] We have already put forward evidence that the evolution of carbon dioxide during the melanin formation is a side-reaction, due to the action of hydrogen peroxide which is formed during the primary reactions. It seems likely that the action of this hydrogen peroxide might be to split the linkage between positions 5 and 6 of the polyindolequinone, thus converting $C_{(5)}$ and $C_{(6)}$ into carboxyl groups. If these quinone residues are linked between positions 3 and 7, this oxidation might continue to position 4, and decarboxylation might result in the splitting off of $C_{(4)}$, $C_{(5)}$, and $C_{(6)}$ in some of the units (cf. *A*). However, as $C_{(7)}$ is involved in the polymeric linkage, it could not split off without the polymer's breaking down to smaller units.



(*A*)

The fact that the 2-(3 : 4-dihydroxyphenyl)ethylamine molecules which undergo loss of carbon dioxide are in fact incorporated in or into the melanin follows from a comparison of the specific activities of the carbon dioxide formed by combustion of the melanins and of the original amine hydrochlorides (Table 1). Also, the relative amounts of carbon dioxide arising from the different positions suggest that oxidation occurs mainly after, rather than before, cyclisation and polymerisation.

When the melanins were boiled for 16 hr. with dilute hydrochloric acid, they underwent some loss in weight (*ca.* 15%), a slight increase in carbon content and a slight change in

[14] Horner and Spietschka, *Annalen*, 1955, **591**, 1.
[15] Beer, Broadhurst, and Robertson, *J.*, 1954, 1947.
[16] Cromartie and Harley-Mason, *Chem. and Ind.*, 1953, 972.

specific activity. Longer boiling, however, appeared to bring about little further change (see Table 4). In the following considerations, the specific activities of the melanin samples which have been boiled for 24 hr. with acid are therefore used. Evaporation of the acid extract yielded a dark brown, gummy residue, which dissolved readily in water to give a solution which gave a dark precipitate when neutralised with ammonia.

TABLE 4

Compound	Radioactivity (counts/min.) of CO_2 produced on combustion
2-(3 : 4-Dihydroxy[4-^{14}C]phenyl)ethylamine hydrochloride	1320
Melanin (not refluxed)	1220
Melanin (refluxed for 3 hr. with 2N-HCl)	1167
,, (,, 24 hr. ,,)	1146
,, (,, 72 hr. ,,)	1140

The specific activities of the " purified " melanins can be explained if it is assumed that one indole unit in five in the polymer loses carbon from positions 4, 5, and 6. This would require the melanins obtained from 2-(3 : 4-dihydroxy[3-^{14}C]phenyl)- and 2-(3 : 4-dihydroxy[4-^{14}C]phenyl)-ethylamine to have 86·5% of the radioactivity of the corresponding amine hydrochlorides. The activities actually observed were 84 and 86·8%, respectively. In the case of 2-(3 : 4-dihydroxy[5-^{14}C]phenyl)ethylamine, the evolved carbon dioxide is of low specific activity, and the melanin formed, on combustion, yields carbon dioxide of slightly higher activity than that derived by burning the amine hydrochloride. The latter fact is in keeping with linkage at the 7-position of the indole nucleus.

It would be interesting to repeat the above experiments on the formation of melanin from the labelled precursors in the presence of catalase and of hydrogen peroxide. However, it is clear that the fission of the benzene ring, with evolution of carbon dioxide, should be regarded as a side-reaction, not an essential step in the primary formation of melanin, as suggested by Bruce [3] and by Jolles.[4]

EXPERIMENTAL

The radioactivity determinations were carried out as described by Swan,[17] and the oxidation of 2-(3 : 4-dihydroxyphenyl)ethylamine to melanin as described by Clemo, Duxbury, and Swan.[18]

Ethyl [α-^{14}C], [β-^{14}C], *and* [γ-^{14}C]*Acetoacetate* (I).—[*Me*-^{14}C]Acetic acid was prepared from [^{14}C]methyl iodide by the method of Cox, Turner, and Warne [19] and [*carboxy*-^{14}C]acetic acid by that of Lemmon.[20] Each was converted into [^{14}C]acetyl chloride as described by Cox and Turner.[21]

Specimens of ethyl acetoacetate labelled in the α-, β-, and γ-position respectively were all prepared by condensation of acetyl chloride with *tert.*-butyl ethyl malonate (one or other being appropriately labelled) as described for ethyl [β-^{14}C]acetoacetate by Dauben and Bradlow.[22] Small amounts of ethyl acetoacetate were present in the benzene distillate; these were recovered by washing it with sodium hydroxide solution, the extract being used in a subsequent condensation with nitromalondialdehyde. Considerable amounts (*ca.* 20%) of acetylmalonic ester (b. p. 110—115°/14 mm.) remained after the distillation of the ethyl acetoacetate. The 2 : 4-dinitrophenylhydrazone of the former was obtained from ethanol as yellow needles, m. p. and mixed m. p. 144—145° (Found: C, 47·4; H, 4·95; N, 14·55. Calc. for $C_{15}H_{18}O_8N_4$: C, 47·1; H, 4·7; N, 14·65%).

Attempts to convert acetylmalonic ester into ethyl acetoacetate following the general methods of Riegel and Lilienfeld,[23] Eisner, Elvidge, and Linstead,[24] and Brändström [25] were unsuccessful.

Diethyl [α-^{14}C]*Malonate.*—This was required for the preparation of ethyl [α-^{14}C]acetoacetate.

[17] Swan, *J.*, 1955, 1039.
[18] Clemo, Duxbury, and Swan, *J.*, 1952, 3464.
[19] Cox, Turner, and Warne, *J.*, 1950, 3167.
[20] Lemmon, in " Isotopic-Carbon " by Calvin, Heidelberger, Reid, Tolbert, and Yankwich, Chapman and Hall, London, 1949, p. 178.
[21] Cox and Turner, *J.*, 1950, 3176.
[22] Dauben and Bradlow, *J. Amer. Chem. Soc.*, 1952, **74**, 5204.
[23] Riegel and Lilienfeld, *ibid.*, 1945, **67**, 1273.
[24] Eisner, Elvidge, and Linstead, *J.*, 1950, 2223.
[25] Brändström, *Acta Chem. Scand.*, 1951, **5**, 820, 1413.

A solution of potassium cyanoacetate, prepared from [*Me*-^{14}C]acetic acid (4 g.) by Ropp's procedure,[26] was evaporated to dryness and was refluxed for 1 hr. with ethanol (14 ml.) and sulphuric acid (10 ml.; *d* 1·84). Water was then added, and the ester was extracted with ether and distilled (4·75 g., 45%).

This ester was converted into *tert.*-butyl ethyl [α-^{14}C]malonate by the method of Breslow, Baumgarten, and Hauser.[27] The conversion of diethyl [α-^{14}C]- into ethyl hydrogen [α-^{14}C]-malonate was carried out in two runs since the average chemical yield over the two stages was only *ca.* 60%. The above diethyl [α-^{14}C]malonate (4·75 g.) was diluted with inactive material (5·25 g.) and converted into ethyl hydrogen [α-^{14}C]malonate as described by Breslow *et al.*[27] From this first run, the mother-liquors from which the monopotassium salt had crystallised and also those from the extraction of the half ester were taken to dryness. The residue was then refluxed for 2 hr. with sulphuric acid (5 ml.; *d* 1·84), ethanol (10 ml.), and diethyl malonate (6 g.). Water was added and the ester was extracted, distilled (10·1 g.), and again converted into ethyl hydrogen [α-^{14}C]malonate. By this process a greater radiochemical yield was obtained.

5-Nitrosalicyclic Acid (III).—This was prepared by Hill's method.[28] Ethyl acetoacetate (5·65 g.) and sodium nitromalondialdehyde monohydrate (8·55 g., 1·3 mols.) were added to a mixture of 10% sodium hydroxide solution (21·5 ml.) and water (68 ml.). The resulting solution was kept for 4 days at room temperature and then acidified, giving the acid (7·55 g., 95%; m. p. 223—224°). When only 1 mol. of nitromalondialdehyde was used the yield was 65%. An equally good yield of 5-nitrosalicyclic acid was obtained by first allowing the ethyl aceto-acetate to stand with sodium hydroxide solution (1 mol.) for 24 hr. before the addition of the nitromalondialdehyde. This suggests that hydrolysis occurs before condensation.

Methyl 2-Methoxy-5-nitrobenzoate.—5-Nitrosalicyclic acid is difficult to methylate (Simonsen and Rau [29]). Thus, refluxing 5-nitrosalicyclic acid with 6 mols. of dimethyl sulphate in dry toluene for 10 hr. in the presence of excess of sodium hydrogen carbonate (cf. Clemo and Swan [30]) gave mainly methyl 5-nitrosalicylate and 12% of dimethylated material.

Complete methylation was effected by using diazomethane (0·625 g., 2·5 mols.) in ether (50 ml.) with 5-nitrosalicyclic acid (1 g.) in ether at 0° for 2 days. The crystals (0·5 g.; m. p. 100°) were then collected and the filtrate on evaporation gave a further 0·6 g., m. p. 98°.

2-Methoxy-5-nitrobenzoic Acid.—The methyl ester (0·5 g.) was boiled for 5—10 min. with sodium hydroxide solution (10%; 5 ml.), and the resulting solution was cooled and acidified, giving the acid (0·45 g.; m. p. 148°).

2-Acetoxy-5-nitrobenzoic Acid.—This was prepared by the method of Grimme and Schmitz [31] in 96% yield.

2-Acetoxy-5-nitrobenzoyl Chloride.—The acid (7·65 g.) was vigorously stirred at 80° with thionyl chloride (13·5 ml.) in benzene (27 ml.). After 1—2 hr. dissolution was complete; the thionyl chloride was removed and the residue distilled in a short-path still [90%; b. p. 150° (bath)/0·01 mm.]. In the isotopic synthesis inactive " chaser " (0·35 g.) was used; the yield of acid chloride was then 7·6 g. (cf. Grimme and Schmitz [31]).

Ethyl 2-Acetoxy-5-nitrobenzoylmalonate (V).—Magnesium (0·93 g.) was treated with dry ethanol (5·3 ml.), diethyl malonate (6·9 g.), and carbon tetrachloride (0·15 ml.). When reaction slackened, ether (27 ml.) was added and the whole was refluxed for 1 hr., all the magnesium dissolving. The above acid chloride (7·6 g.) in benzene (28 ml.) was added dropwise with stirring during 5 min. and, after being warmed for 2 hr. and then cooled, the solution was treated with water (30 ml.). The mixture was stirred for 5 min. and then shaken with chloroform (60 ml.). After removal of some insoluble by-product, the chloroform was separated and the aqueous portion extracted with more chloroform (50 ml.). The combined washings were dried (Na_2SO_4), and the solvent was evaporated, leaving a yellow glass (V) (9·7 g.).

The material (IV) (3·5 g.) which was insoluble in water and chloroform contained magnesium. It was boiled for 2 hr. with hydrochloric acid (2N; 50 ml.), and the magnesium-free compound (2·75 g.; m. p. 204°), *ethyl 3 : 4-dihydro-6-nitro-4-oxocoumarin-3-carboxylate* (VI) was filtered off; it crystallised from chloroform as white needles, m. p. 205—206° (Found: C, 51·35; H, 3·35. $C_{12}H_9O_7N$ requires C, 51·6; H, 3·25%).

[26] Ropp, *J. Amer. Chem. Soc.*, 1950, **72**, 4459.
[27] Breslow, Baumgarten, and Hauser, *ibid.*, 1944, **66**, 1286.
[28] Hill, *Amer. Chem. J.*, 1900, **24**, 1.
[29] Simonsen and Rau, *J.*, 1917, **111**, 220.
[30] Clemo and Swan, *J.*, 1944, 274.
[31] Grimme and Schmitz, *Ber.*, 1951, **84**, 734.

2-Hydroxy-5-nitroacetophenone (VII).—(*a*) Ethyl 2-acetoxy-5-nitrobenzoylmalonate (V) (9·7 g.) was treated with water (19·5 ml.), sulphuric acid (9·7 ml.; *d* 1·84), and acetic acid (68 ml.), and the whole refluxed for 3—4 hr. White crystals separated soon after the addition of the acid but after 3 hr. little solid was present. Water (90 ml.) was then added and the mixture boiled for a further 2 hr. On cooling, the solution deposited the ketone (2·9 g.) as needles, m. p. 99—100°. To the filtrate was added water (50 ml.), and a further 0·2 g. of product gradually separated. Small amounts of material could be recovered by chloroform extraction of the mother-liquors.

The chroman (VI) (2·75 g.) was refluxed with sodium hydroxide solution (10%; 27 ml.) for 80 min., all dissolving. Acidification of the cooled solution caused the evolution of carbon dioxide and precipitation of the ketone (1·75 g.; m. p. 97—99°). Crystallisation from ethanol (20 ml.) gave pure material (1 g.; m. p. 99—100°), and concentration of the mother-liquors gave a further 0·3 g. of m. p. 99°. The combined yield of ketone (4·4 g.) represents 78% yield from the acid chloride.

When hydrolysis of ethyl 2-acetoxy-5-nitrobenzoylmalonate with more dilute acetic acid was attempted, a mixture of the chroman and ketone resulted.

(*b*) 2-Methoxy-5-nitroacetophenone (Mathieson and Newbery [32]) (0·2 g.) was dissolved in benzene (8 ml.), and aluminium chloride (0·2 g.) added (cf. Dilthey and Schumacher [33]). The mixture was refluxed for 8 hr., and then treated with water and a few drops of hydrochloric acid. The benzene layer was separated and the aqueous layer extracted with benzene. The benzene extracts were washed with dilute sodium hydroxide solution, which was then acidified and re-extracted with benzene. Evaporation of the dried (Na_2SO_4) solution yielded crystals (0·13 g.), m. p. 88—90°. Recrystallisation from aqueous ethanol gave material m. p. 95—98° (undepressed by the addition of material obtained by Allan and Loudon's method [34]).

Attempts to convert ethyl 2-methoxy-5-nitrobenzoylmalonate (Mathieson and Newbery [32]) directly into 2-hydroxy-5-nitroacetophenone by acid hydrolysis were unsuccessful.

The m. p. of 2-hydroxy-5-nitroacetophenone has been given as 98—99° (Allan and Loudon [34]), 99·5° (Joshi and Singh [35]), and 111—112° (Lindemann and Romanoff [36]). We found that material prepared by Allan and Loudon's method required to be crystallised several times before a melting point of 98—99° was attained.

4-Nitrocatechol.—2-Hydroxy-5-nitroacetophenone (4·4 g.) was dissolved in N-sodium hydroxide (24·5 ml.), and hydrogen peroxide (1·07 g., 1·3 mols.) in water (25 ml.) was added, in an atmosphere of nitrogen. The mixture was initially warmed to 40° and then N-sodium hydroxide (16·5 ml.) was added during 100 min. Soon after the beginning of the reaction starting material began to be precipitated, but the addition of extra alkali produced a very deeply coloured solution. The reaction mixture was kept overnight and, after the addition of more alkali (7 ml.) during 30 min., hydrochloric acid was added. Unchanged ketone (0·97 g.) was collected and washed with water and the combined filtrates were extracted with ether (70 ml.). The dried (Na_2SO_4) solution was evaporated, leaving 4-nitrocatechol, m. p. 168°. Recrystallisation from benzene–methanol gave yellow needles, m. p. 171—172°. By treating the recovered ketone with hydrogen peroxide a total yield of 3·5 g. of 4-nitrocatechol (93%) was obtained.

4-Nitroveratrole (VIII).—Crude 4-nitrocatechol (3·5 g.) in ether (40 ml.) was treated with a solution of diazomethane (3·15 g.) in ether (210 ml.), then kept at 0° for 2 days, and the solution was filtered and evaporated. The residue was taken up in benzene (60 ml.) and washed with dilute sodium hydroxide solution. The residue from the dried (Na_2SO_4) benzene was recrystallised from ethanol, giving 4-nitroveratrole (2·7 g.; m. p. 96—97°) and a further 0·3 g. (m. p. 94°) were obtained from the mother-liquors (73%).

4-Aminoveratrole.—4-Nitroveratrole (3 g.) in methanol (60 ml.) was hydrogenated at 50°/55 atm. in the presence of Raney nickel for 2 hr. The catalyst was filtered off and the methanol removed in a stream of nitrogen. The residue was distilled (b. p. 159°/14 mm.), giving the amine as a white solid, m. p. 88° (2·15 g., 86%).

3 : 4-*Dimethoxybenzaldehyde* (IX).—4-Aminoveratrole (2·2 g.), dissolved in a mixture of hydrochloric acid (3·3 ml.; *d* 1·18) and water (9 ml.), was diazotised by treatment with sodium nitrite (1·05 g.) in water (2 ml.). The solution was then made neutral to Congo-red by addition

[32] Mathieson and Newbery, *J.*, 1949, 1133.
[33] Dilthey and Schumacher, *Annalen*, 1906, **344**, 300.
[34] Allan and Loudon, *J.*, 1949, 821.
[35] Joshi and Singh, *J. Amer. Chem. Soc.*, 1954, **76**, 4993.
[36] Lindemann and Romanoff, *J. prakt. Chem.*, 1929, **122**, 214.

of crystalline sodium acetate (1·3 g.). A 10% solution of formaldoxime was prepared according to Beech's directions[10] and to this was added a solution of copper sulphate (0·4 g.), sodium sulphite (0·06 g.; anhyd.), and crystalline sodium acetate (10 g.) in water (12 ml.). The diazonium solution was introduced below the surface of the stirred formaldoxime solution at 10° during 20 min. The mixture was stirred at 10° for 1 hr. after which hydrochloric acid was added until the mixture was acid to Congo-red. Ferric alum (17·5 g.) was added and the whole refluxed for 1 hr. After cooling, the mixture was extracted with ether, the extract was washed with saturated sodium hydrogen carbonate solution, dried (Na_2SO_4), and evaporated. The residue was distilled, giving the aldehyde (1·2 g., 50%), b. p. 140—150° (bath)/2 mm.

2-p-*Methoxyphenylazo-2-nitroethanaldoxime*.—A neutral solution of *p*-methoxybenzenediazonium chloride (from *p*-anisidine, 3·1 g., 1 mol.) was run with stirring during 15 min. into a solution of methazonic acid (3·8 g., 1·5 mols.), copper sulphate (1·25 g.), sodium sulphite (0·1 g.; anhyd.), and hydrated sodium acetate (20 g.) in water (30 ml.) kept at 15°. A brown precipitate was produced, and after being kept overnight the mixture was acidified and filtered. The dry material (7·6 g.) was only partially soluble in ether; the ether-insoluble material contained copper but this was decomposed by shaking it with ether and dilute hydrochloric acid, giving material identical with that obtained from the initial extract. This *azo-compound* (5 g.) crystallised from ethanol as dark red prisms, m. p. 163° (Found : C, 45·5; H, 4·3; N, 23·4. $C_9H_{10}O_4N_4$ requires C, 45·4; H, 4·2; N, 23·5%).

Cyanomalondialdehyde.—A solution of sodium cyanomalondialdehyde was prepared by Uhle and Jacobs's method.[37] A mixture of cyanoacetaldehyde diethyl acetal (0·6 g.), ethyl formate (0·42 g.), and ether (2·5 ml.) was added to powdered sodium (0·1 g.). After 12 hr. at room temperature the sodium salt was extracted with water (1 ml.), and the solution was made just acid with dilute nitric acid. A concentrated aqueous solution of silver nitrate (0·75 g.) was added and the silver salt was quickly filtered and washed with a little water. The dry salt (0·5 g.) was suspended in ether, and dry hydrogen chloride was bubbled through it for 6 hr. The silver chloride was removed and the ether distilled, leaving the aldehyde (0·2 g.). This material gave, with aniline, an immediate precipitate, which when crystallised from ethanol had m. p. 129° (Uhle and Jacobs[37] give 132°).

o-*Carboxy*-N-3′-*cyanopropylbenzamide*.—When γ-phthalimidobutyronitrile was set aside in ethanolic sodium hydroxide it was converted into the *amide*, which separated from water as colourless crystals, m. p. 114° (Found : C, 62·0; H, 5·5; N, 11·95. $C_{12}H_{12}O_3N_2$ requires C, 62·05; H, 5·2; N, 12·05%). This compound was the major product obtained on the attempted formylation of γ-phthalimidobutyronitrile, in the presence of sodium ethoxide in ethanol. Attempts to bring about a condensation with ethyl formate under alcohol-free conditions were also futile.

6 : 7-*Dimethoxy*isoquinoline (XII).—This was prepared from 2-(3 : 4-dimethoxyphenyl)-ethylamine essentially as described by Späth and Polgar[12] except that the dehydrogenation was carried out as follows : 3 : 4-Dihydro-6 : 7-dimethoxy*iso*quinoline (0·36 g.) and palladium black (0·12 g.) were heated together in a bulb distillation apparatus at 180°/11 mm. After 30 min. more palladium (0·06 g.) was added and heating was continued for a further 30 min. The *iso*quinoline was then distilled from the palladium as an almost colourless liquid [0·33 g., b. p. 140—150° (bath)/0·1 mm.]. The product, in benzene, was boiled with charcoal and the residue from the filtered and evaporated solution was converted into the hydrochloride by the addition of concentrated hydrochloric acid. The crude, dry product was dissolved in a little ethanol; the hydrochloride (0·3 g.; m. p. 220°) separated on the addition of warm acetone. The regenerated base separated from benzene–light petroleum (b. p. 60—80°) as needles (0·2 g., 55%), m. p. 93°.

Cinchomeronic Acid (XIII).—6 : 7-Dimethoxy*iso*quinoline (172 mg.) was heated for 3 hr. with hydrochloric acid (2 ml.; *d* 1·18) at 160°. The whole was taken to dryness, nitric acid (2 ml.; *d* 1·42) added, and the mixture was then heated on the steam-bath for 12 hr. The nitric acid was evaporated and the crude cinchomeronic acid was crystallised from 0·5N-nitric acid (yield : 134 mg., 88%; m. p. 267°).

4-*Ethyl Hydrogen Cinchomeronate* (XIV).—This was prepared from the acid in 75% yield by Strache's method.[13] A neutral solution of the ammonium salt was prepared by treatment of the half ester (0·1 g.) with ammonia (1 mol.) in water (2 ml.); and the silver salt (0·14 g.) separated on the addition of a concentrated solution of silver nitrate (0·1 g.).

iso*Nicotinic Acid* (XVI).—The above silver salt was heated in a bulb distillation apparatus

[37] Uhle and Jacobs, *J. Org. Chem.*, 1945, **10**, 76.

at atmospheric pressure. Decomposition occurred at about 180° and then the bath temperature was gradually raised to 300°. The distillate (XV) was refluxed with N-sodium hydroxide (1 ml.) for 30 min. and the solution was exactly neutralised with acetic acid. A concentrated solution of copper acetate (50 mg.) was added and after a few minutes the copper salt was filtered off, washed with water, and decomposed with hydrogen sulphide, the copper sulphide removed, and the filtrate evaporated, giving the acid (14 mg.), m. p. 310°.

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