245. *Melanin and its Precursors. Part I. The Synthesis of 5 : 6 : 5′ : 6′-Tetrahydroxyindigo.*

By John Harley-Mason.

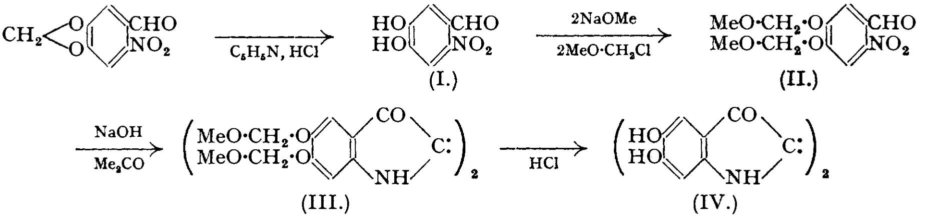
As previously reported briefly (*Nature*, 1947, **159**, 338) 5 : 6 : 5′ : 6′-*tetrahydroxyindigo* has been synthesised by the action of acetone and alkali on 2-*nitro*-4 : 5-*bis*(*methoxymethoxy*)-*benzaldehyde* and subsequent acid hydrolysis of the 5 : 6 : 5′ : 6′-*tetra*(*methoxymethoxy*)*indigo* thus obtained. Its properties are very unlike those of melanin, and it is further shown that melanin is unlikely to contain a quinone or semiquinone derived from it.

It has recently been suggested (Clemo and Weiss, *J.*, 1945, 702) that 5 : 6 : 5′ : 6′-tetrahydroxyindigo (IV) or a quinone or semiquinone derived from it by oxidation is an important constituent of melanin, the black pigment produced by the oxidation of tyrosine and 3 : 4-dihydroxyphenylalanine. It was of interest, therefore, to synthesise the tetrahydroxyindigo by an unambiguous route and to compare its properties with those of melanin.

Three earlier attempts to obtain this compound have been recorded. (1) Hayduck (*Ber.*, 1903, **36**, 2930) unsuccessfully attempted to demethylate a compound which he considered to be 5 : 6 : 5′ : 6′-tetramethoxyindigo; however, he showed later (*Ber.*, 1903, **36**, 3528) that his starting material was in fact the 6 : 7 : 6′ : 7′-isomer. (2) Clemo and Weiss (*loc. cit.*) attempted to demethylenate 5 : 6 : 5′ : 6′-bismethylenedioxyindigo (piperonal indigo) under various conditions, but obtained only alkali-insoluble products giving unsatisfactory analytical figures. (3) Parijs (*Rec. Trav. chim.*, 1930, **49**, 18) claimed that the compound can be obtained by the action of acetone and alkali on 6-nitroprotocatechuic aldehyde (I); he stated that " The addition of alkali to an acetone solution [of 6-nitroprotocatechuic aldehyde] afforded a solution of the corresponding indigo : subsequent addition of acid precipitated dark blue 5 : 6 : 5′ : 6′-tetrahydroxyindigo ". This claim could not be substantiated by the author, only the starting materials being recovered after the treatment described. Indeed, the failure is hardly surprising in view of the fact that Friedländer and Schenk (*Ber.*, 1914, **47**, 3040) found that 2-nitro-5-hydroxybenzaldehyde failed altogether to condense with acetone and alkali.

The most promising approach to the tetrahydroxyindigo appeared to be the condensation of a derivative of 6-nitroprotocatechuic aldehyde, in which the hydroxyl groups were suitably protected, with acetone and alkali, and subsequent removal of the protecting groups. Since Friedländer and Schenk (*loc. cit.*) had observed that the tosyl derivative of 2-nitro-5-hydroxybenzaldehyde could be converted into the corresponding indigo with acetone and alkali, and that the tosyl groups could be removed subsequently, it seemed probable that a similar procedure would be applicable to 6-nitroprotocatechuic aldehyde. This compound was first prepared by Parijs (*loc. cit.*) by the action of acetyl nitrate on 6-nitropiperonal diacetate and subsequent hydrolysis. On repetition of this curious reaction, which appears to involve nitration of the methylene group, it was found that the yields obtained were very much lower than the 95% claimed by Parijs, and that even smaller yields were obtained in larger-scale runs. Accordingly, other methods of demethylenation of 6-nitropiperonal were examined. The compound proved remarkably resistant, and no satisfactory result could be achieved by heating with sulphuric acid of various concentrations, heating with hydrobromic acid in acetic acid to 150°, or by treatment with phosphorus pentachloride. Eventually it was found that the methylene group could be removed by heating with pyridine hydrochloride at 160—170°. This reaction is, of course, closely analogous to the demethylations using pyridine hydrochloride recorded by Prey (*Ber.*, 1942, **75**, 350) and appears to be the first case of the use of this reagent for demethylenation, as distinct from demethylation. It was found to be essential that the pyridine hydrochloride be as dry as possible or the yield was much reduced, presumably owing to the formation of formaldehyde which then condensed with the hydroxy-aldehyde. The reaction is also strongly exothermic and a considerable excess of pyridine hydrochloride must be used to prevent over-heating.

Treatment of 6-nitroprotocatechuic aldehyde with benzenesulphonyl chloride and alkali gave the *bisbenzenesulphonyl* derivative, which surprisingly, however, failed to react at all with acetone and alkali. An alternative protecting group was therefore sought. When the disodium salt of the dihydroxy-aldehyde was treated with two molecules of chloromethyl ether in benzene, 2-*nitro*-4 : 5-*bis(methoxymethoxy)benzaldehyde* (II) was obtained, and from it, 5 : 6 : 5′ : 6′-*tetra(methoxymethoxy)indigo* (III) in good yield by the action of acetone and alkali. The latter compound could be recrystallised unchanged from glacial acetic acid, but on addition of water and a trace of hydrochloric acid to the acetic acid solution, hydrolysis occurred, giving the required tetrahydroxyindigo. As it could not be recrystallised, the compound was purified by dissolving it in dilute aqueous alkali, filtering the intense permanganate-violet solution in the absence of air, and immediately precipitating the indigo by addition of acetic acid. Thus prepared, it formed a greenish-black powder which retained a molecule of water of crystallisation even after drying in a high vacuum. It was practically insoluble in the usual organic solvents, but was slightly soluble in pyridine, giving a blue solution. The identity of the product was confirmed (*a*) by acetylation with acetic anhydride in pyridine which gave a *tetra-acetyl* derivative which could be recrystallised from nitrobenzene, and (*b*) by methylation with methyl sulphate and alkali, giving 5 : 6 : 5′ : 6′-*tetramethoxyindigo*, identical with that obtained from the treatment of 6-nitroveratraldehyde with acetone and alkali.



On reduction with sodium hydrosulphite (dithionite) and alkali, the tetrahydroxyindigo gave a deep yellow vat, which reoxidised in air to give an intense violet solution, which however soon faded to brown with further absorption of oxygen.

The behaviour of melanin towards sodium hydrosulphite and alkali has been described by Figge (*Proc. Soc. Biol. Exp. Med.*, 1939, **41**, 127). Reduction was found to occur, giving a brownish leuco-compound which could be re-oxidised to melanin by potassium ferricyanide. This observation has been confirmed in the present work, and in addition it was found that the leuco-compound, like melanin itself, is insoluble in aqueous alkali. Tetrahydroxyindigo is thus quite different from melanin. Furthermore, a quinone or semiquinone derived by oxidation of the tetrahydroxy-compound would undoubtedly give on reduction with sodium hydrosulphite the same leuco-compound as that obtained from (IV) itself.

In the preparation of the aldehyde (II) a by-product was obtained which was soluble in alkali. Analysis indicated that this was a monomethoxymethyl derivative of 6-nitroprotocatechuic aldehyde. To determine the location of the methoxymethyl group, the compound was methylated with methyl sulphate and alkali, and then hydrolysed with acid. The product was 6-nitro*iso*vanillin (Pschorr and Stoehrer, *Ber.*, 1902, **35**, 4394), so the original compound was 2-*nitro*-4-*hydroxy*-5-*methoxymethoxybenzaldehyde*.

EXPERIMENTAL.

6-*Nitroprotocatechuic Aldehyde.*—6-Nitropiperonal (25 g.) and anhydrous pyridine hydrochloride (50 g.) were mixed and heated to 150° in an oil-bath. Commencement of the exothermic reaction was indicated by the appearance of bubbles in the liquid, and the temperature was kept below 170° by removing the flask from the oil-bath when necessary. When the reaction had subsided, the mixture was heated at 170° for 0·5 hour. After cooling the mass was boiled with water (300 c.c.) with the addition of charcoal, cooled, and filtered through charcoal to remove tar. Concentrated hydrochloric acid (10 c.c.) was added to the filtrate which was then extracted with ether in a continuous extractor. Evaporation of the ether yielded crude 6-nitroprotocatechuic aldehyde which was purified by recrystallisation from water; 9—12 g., m. p. 203°.

Bisbenzenesulphonyl derivative. 6-Nitroprotocatechuic aldehyde (2 g.) was dissolved in 10% aqueous sodium hydroxide (30 c.c.) and shaken with benzenesulphonyl chloride (4 c.c.). When the reaction was complete, the solid *product* was collected and recrystallised from benzene–light petroleum; m. p. 159° (Found: C, 49·5; H, 3·0. $C_{19}H_{13}O_9NS$ requires C, 49·3; H, 2·8%).

2-*Nitro*-4 : 5-*bis*(*methoxymethoxy*)*benzaldehyde* (II).—6-Nitroprotocatechuic aldehyde (5·5 g.) was dissolved in methanol (30 c.c.) and a solution of sodium methoxide prepared by dissolving sodium (1·4 g.) in methanol (30 c.c.) was added. The solvent was distilled off in a vacuum under nitrogen, leaving the disodium salt as a deep violet cake. Dry benzene (60 c.c.) was added, and the cake was broken up in order to obtain a fine suspension of the salt. A solution of pure redistilled chlorodimethyl ether (4·9 g.) in dry benzene (15 c.c.) was next added. Reaction proceeded, and the mixture became warm. After an hour's heating under reflux on the water-bath, the mixture was cooled and filtered from the separated sodium chloride. The filtrate was extracted with 2% sodium hydroxide solution until the aqueous layer was only faintly yellow, the benzene solution separated, dried (Na_2SO_4), and distilled down to 10 c.c. Light petroleum (b. p. 60—80°) (20 c.c.) was added to the hot solution. After standing overnight in the ice-chest, the 2-*nitro*-4 : 5-*bis*(*methoxymethoxy*)*benzaldehyde* which had separated was collected and recrystallised from benzene–petrol. It formed pale yellow prisms, m. p. 100—101° (Found: C, 48·5; H, 4·7. $C_{11}H_{13}O_7N$ requires C, 48·8; H, 4·8%).

The alkaline extract was acidified with acetic acid, and after standing overnight at 0°, the precipitate was collected and recrystallised from dilute methanol; pale yellow needles, m. p. 110°, were obtained (Found: C, 47·2; H, 4·3. $C_9H_9O_6N$ requires C, 47·7; H, 4·0%). That this compound was 2-*nitro*-4-*hydroxy*-5-*methoxymethoxybenzaldehyde* was proved as follows. The compound (1 g.) was dissolved in 5% sodium hydroxide solution (10 c.c.), and methyl sulphate (1 c.c.) added. After being heated at 80° with occasional shaking for 0·5 hour, the mixture was cooled and the solid product filtered off. It was hydrolysed by boiling for 1 hour with dilute hydrochloric acid, and the 6-nitro*iso*vanillin thus obtained was collected and recrystallised from dilute alcohol; m. p. and mixed m. p. 187° (Pschorr and Stoehrer, *loc. cit.*, give m. p. 189°).

5 : 6 : 5′ : 6′-*Tetra*(*methoxymethoxy*)*indigo* (III).—2-Nitro-4 : 5-bis(methoxymethoxy)benzaldehyde (2 g.) was dissolved in acetone (8 c.c.), and 10% sodium hydroxide solution (1 c.c.) added. After an hour, water (30 c.c.) and 10% sodium hydroxide solution (5 c.c.) were added with stirring. Next day the precipitated *indigo* was collected and washed with water and alcohol. On recrystallisation from glacial acetic acid it formed deep green needles, containing a molecule of acetic acid, which was lost on drying at 110° in a vacuum (Found: C, 55·7; H, 4·8. $C_{24}H_{26}O_{10}N_2,CH_3{\cdot}CO_2H$ requires C, 55·6; H, 5·3%. Found, in material dried at 110°: C, 56·7; H, 5·4. $C_{24}H_{26}O_{10}N_2$ requires C, 57·2; H, 5·2%).

5 : 6 : 5′ : 6′-*Tetrahydroxyindigo* (IV).—The indigo (III) (1·5 g.) was dissolved in boiling glacial acetic acid (40 c.c.). Water (50 c.c.) was added to the boiling solution with vigorous stirring, giving a greenish suspension. Concentrated hydrochloric acid (1 c.c.) was next added, and the mixture heated on a boiling water-bath for 1 hour with stirring, the greenish tetramethoxymethoxy-compound being converted into the black tetrahydroxyindigo. After cooling, this was collected and dissolved in cold 1% sodium hydroxide solution under nitrogen, rapidly filtered from impurities, and the intense violet filtrate at once acidified with dilute acetic acid. The black precipitate was collected and thoroughly washed with water, alcohol, and ether (Found: C, 55·9; H, 3·6; N, 8·1. $C_{16}H_{10}O_6N_2,H_2O$ requires C, 56·0; H, 3·5; N, 8·15%).

5 : 6 : 5′ : 6′-*Tetra-acetoxyindigo.*—The *tetrahydroxyindigo* (0·2 g.) was suspended in pyridine (5 c.c.), and acetic anhydride (5 c.c.) added. The mixture was heated for 0·5 hour at 100° with stirring. After cooling, the *product* which had separated was filtered off and recrystallised from nitrobenzene in which it was sparingly soluble. It formed small dark blue prisms with an intense coppery lustre and decomposed without melting (Found: C, 58·0; H, 3·8; N, 5·5. $C_{24}H_{18}O_{10}N_2$ requires C, 58·3; H, 3·65; N, 5·7%).

5 : 6 : 5′ : 6′-*Tetramethoxyindigo.*—(a) *From* 6-*nitroveratraldehyde.* 6-Nitroveratraldehyde (2 g.) was dissolved in acetone (5 c.c.) and 5 drops of 10% sodium hydroxide solution added. After an hour, water (30 c.c.) and more 10% alkali (5 c.c.) were added, and after standing overnight the dark greenish precipitate of the *indigo* was collected and extracted with boiling alcohol. It was then recrystallised from nitrobenzene; yield 0·4 g. (The yield was not improved by heating the reaction mixture.) (Found: C, 62·5; H, 4·8. $C_{20}H_{18}O_6N_2$ requires C, 63·0; H, 4·7%). On cooling, the alcoholic extract deposited yellow plates. These were collected and recrystallised from alcohol (charcoal); m. p. 172°. Analysis indicated that this compound was 2-*nitro*-4 : 5-*dimethoxybenzylideneacetone* (Found: C, 56·7; H, 5·2. $C_{12}H_{13}O_5N$ requires C, 57·1; H, 5·2%).

(b) *From* (IV). The tetrahydroxyindigo (0·4 g.) was dissolved in 5% sodium hydroxide solution (5 c.c.) under nitrogen, and methyl sulphate (0·8 c.c.) added. The mixture was heated at 80° for 1 hour with frequent shaking, air being excluded. After cooling, the tetramethoxyindigo was collected, washed with alcohol, and recrystallised from nitrobenzene.

That the products from (a) and (b) were identical was shown by the fact that both could be oxidised to the same product, namely, 5 : 6-dimethoxyisatin.

The indigo (0·2 g.) was stirred with nitric acid (*d* 1·3) (1·5 c.c.) and warmed on the water-bath for a few minutes until reaction was complete. After cooling, the product was filtered off, extracted with dilute sodium hydroxide, filtered from impurities, and the isatin precipitated from the filtrate by the addition of hydrochloric acid; yellow-orange prisms from ethyl acetate, m. p. 250—253° with preliminary darkening (Found: C, 56·3; H, 4·6. Calc. for $C_{10}H_9O_4N$: C, 56·6; H, 4·35%). Hahn and Tulus (*Ber.*, 1941, **74**, 518) give m. p. 250—252°.

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