[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF ROCHESTER]

Degradation Studies on Apo- and Dehydro-apo-β-erythroidine[1,2]

BY M. F. GRUNDON AND V. BOEKELHEIDE

RECEIVED OCTOBER 19, 1951

The hydrogenation of apo-β-erythroidine in neutral solution has been shown to be a complex reaction involving disproportionation and yielding, among other products, a substance of empirical formula, $C_{15}H_{13}NO_2$. This product, named dehydro-apo-β-erythroidine, was found to have aromatic properties and to be formed readily by a simple dehydrogenation of apo-β-erythroidine. From the oxidation of dehydro-apo-β-erythroidine using alkaline permanganate, 7-carboxyisatin, 2-aminoisophthalic acid and 3,8-dicarboxy-4-hydroxyquinoline have been isolated and identified. A similar oxidation of apo-β-erythroidine yielded 7-carboxyisatin and 3,8-dicarboxy-4-hydroxyquinoline. The infrared spectra of apo-β-erythroidine and some related compounds are presented and it is pointed out that neither the infrared spectra of these compounds nor the results of the oxidation experiments are in accord with the structures advanced by Koniuszy and Folkers or by Lapiere and Robinson.

The preparation and properties of three derivatives of β-erythroidine, desmethoxy-β-erythroidine, apo-β-erythroidine and isoapo-β-erythroidine have been described in a previous communication.[3] In the previous work it was indicated that apo-β-erythroidine and isoapo-β-erythroidine both formed a common "tetrahydro" derivative. Further investigation has revealed the complexity of the hydrogenation reaction and has provided evidence that the previously designated tetrahydro derivative is actually a dihydro derivative. This change in designation is based on the results of further analyses and the fact that the compound absorbs three moles of hydrogen under acidic conditions to give an octahydro derivative. This same octahydro derivative also results when apo-β-erythroidine is subjected to prolonged hydrogenation in acid and it is presumably identical with that obtained by Koniuszy and Folkers.[4]

When an attempt was made to convert apo-β-erythroidine to its dihydro derivative by hydrogenation in neutral solution until a molar equivalent of hydrogen had been absorbed, the reaction mixture yielded three products: dihydro-apo-β-erythroidine, isoapo-β-erythroidine and a compound of empirical formula, $C_{15}H_{13}NO_2$. The last compound, which was a major product of the reaction, is obviously the result of dehydrogenation and has been named dehydro-apo-β-erythroidine. More conveniently, dehydro-apo-β-erythroidine was prepared by simply heating apo-β-erythroidine in ethanol in the presence of platinum oxide catalyst. Although dehydro-apo-β-erythroidine is not easily hydrogenated, it was reduced in acetic acid solution, whereby octahydro-apo-β-erythroidine was obtained. The fact that octahydro-apo-β-erythroidine is the ultimate hydrogenation product of each of these derivatives, as shown in Fig. 1, suggests that dehydro-apo-β-erythroidine, apo-β-erythroidine and isoapo-β-erythroidine all have the same basic carbon skeleton.

The lactone group, typical of β-erythroidine derivatives, is retained in dehydro-apo-β-erythroidine and the compound dissolves in warm aqueous alkali. When such an alkaline solution was oxi-

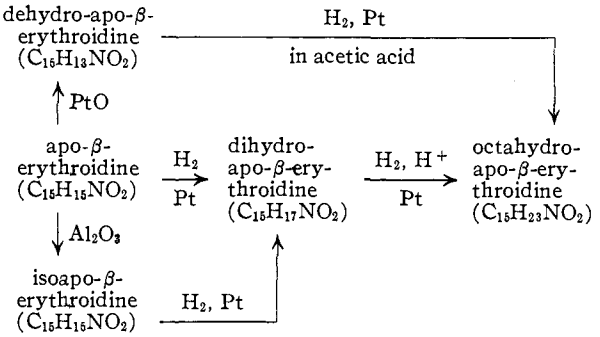
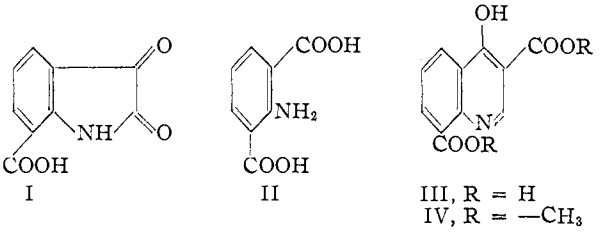


Fig. 1.

dized at room temperature with aqueous potassium permanganate three products were isolated, namely, 7-carboxyisatin (I), 2-aminoisophthalic acid (II) and 3,8-dicarboxy-4-hydroxyquinoline (III). The quinoline compound, together with 7-carboxyisatin,



was also obtained by a similar oxidation of apo-β-erythroidine.

To prove the identity of the 7-carboxyisatin, a sample of this material was prepared by the method of Waldmann.[5] Both the acid and its methyl ester were compared to the corresponding synthetic compounds and were shown to be identical by means of mixed melting point determinations and a comparison of infrared spectra.

Although 2-aminoisophthalic acid has been prepared previously by Noelting and Gachot,[6] its characterization was not adequate for our purposes. Also, their procedure, which started from 2-nitro-*m*-xylene, appeared rather involved and a new method was sought. Oxidation of a suitable quinoline derivative appeared to offer a plausible route, and because of the isolation of a quinoline derivative in the oxidation of apo-β-erythroidine, this approach had intrinsic interest. Ault, Hirst and Morton[7] have reported that treatment of isatin

(1) Aided by a Grant from the National Foundation for Infantile Paralysis, Inc.

(2) Paper IV in this series, for the preceding communication see V. Boekelheide and E. J. Agnello, THIS JOURNAL, **73**, 2386 (1951).

(3) G. L. Sauvage and V. Boekelheide, *ibid.*, **72**, 2062 (1950); *cf.* F. Koniuszy and K. Folkers, *ibid.*, **72**, 5579 (1950), also ref. 4.

(4) F. Koniuszy and K. Folkers, *ibid.*, **73**, 333 (1951).

(5) H. Waldmann, *J. prakt. Chem.*, **147**, 338 (1937).

(6) E. Noelting and C. Gachot, *Ber.*, **39**, 73 (1906).

(7) R. G. Ault, E. L. Hirst and R. A. Morton, *J. Chem. Soc.*, 1653 (1935).

with diazomethane gave 2,3-dihydroxyquinoline, and since 7-carbomethoxyisatin was at hand, this method was applied to obtain 2,3-dihydroxy-8-carbomethoxyquinoline. In contrast to isatin which gave only 2,3-dihydroxyquinoline and its 3-methyl ether, 7-carbomethoxyisatin gave three colorless products, namely, two isomeric compounds of empirical formula, $C_{10}H_7NO_4$ (A, m.p. 233–236°, and B, m.p. 178–179°) and a third product (C) of empirical formula $C_{11}H_9NO_4$. The relationship between A and C was readily shown by the fact that A was converted to C by further treatment with diazomethane. That A is 2,3-dihydroxy-8-carbomethoxyquinoline and C is its 3-methyl ether has been assumed on the basis of analogy and is supported by the fact that A showed approximately a molar-equivalent uptake of periodic acid, whereas B was unaffected. On treatment with ferric chloride solution, A gave a dark green color similar to that given by 2,3-dihydroxyquinoline, whereas B gave no color with ferric chloride. Also the infrared spectrum of A corresponds much more closely to that of 2,3-dihydroxyquinoline than does that of B (see Fig. 2). The structure of B was not established but it is probably 2,4-dihydroxy-8-carbomethoxyquinoline.

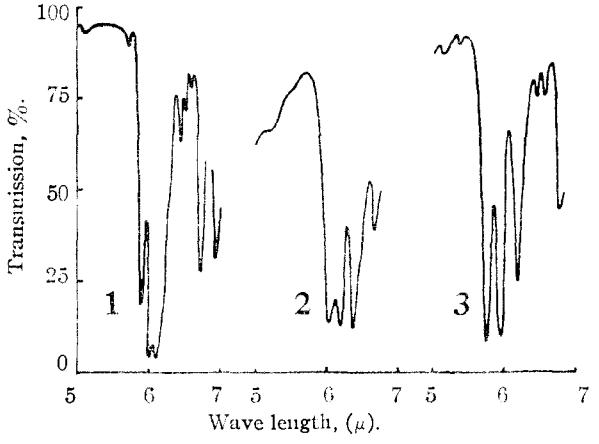
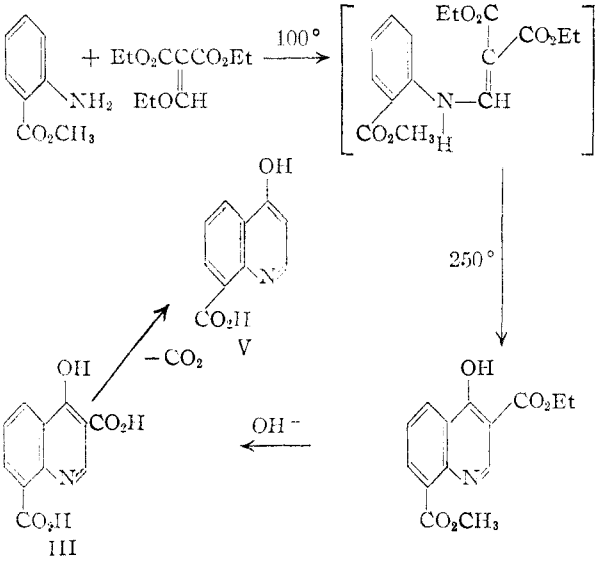


Fig. 2.—Infrared absorption spectra of 8-carbomethoxy-2,3-dihydroxyquinoline (1), 2,3-dihydroxyquinoline (2), and 8-carbomethoxy-2,4-dihydroxyquinoline (3). All in Nujol mull.

Compound A gave the corresponding acid on hydrolysis and this was oxidized by treating it in an alkaline solution with aqueous potassium permanganate. The major product of the reaction was 2-(N-oxalylamino)-isophthalic acid, although small quantities of 7-carboxyisatin and 2-aminoisophthalic acid were also obtained. The latter compound was obtained in quantity by the alkaline hydrolysis of 2-(N-oxalylamino)-isophthalic acid, and it was shown by a comparison of infrared spectra to be identical with the oxidation product (II) from dehydro-apo-β-erythroidine. The corresponding dimethyl esters were also prepared and shown to be identical by a mixed melting point determination.

The identity of the third oxidation product (III) was somewhat more difficult to deduce but was revealed by the following experiments. This oxidation product showed the properties of a dibasic acid and gave a dimethyl ester on reaction with diazomethane. Also, it underwent decarboxylation to give compound V ($C_{10}H_7NO_3$), when it was heated with quinoline and copper chromite. An examination of the infrared spectrum of compound V raised the possibility that it was either a 2- or 4-hydroxyquinoline derivative. In view of the probable relationship of compound V to the other oxidation products, a synthesis of 8-carboxy-4-hydroxyquinoline was undertaken using the Price–Roberts method,[8] as illustrated in the following scheme.



This method proved to be a fortunate choice, for not only was V found to be identical with 8-carboxy-4-hydroxyquinoline, but III was also identical with the intermediate dibasic acid, 3,8-dicarboxy-4-hydroxyquinoline. The identities of the natural and synthetic compounds were shown for both III and V by mixed melting point determinations on the corresponding esters and by comparison of the infrared spectra of both the acids and the esters.

In considering the significance of the oxidation products, I, II and III, in relation to the structures of apo-β-erythroidine and dehydro-apo-β-erythroidine it is important to decide whether they are formed by independent oxidation mechanisms or whether I and II result from subsequent oxidation of the quinoline derivative (III). Permanganate oxidation of III under the same conditions employed for apo-β-erythroidine proceeded extremely slowly. A small quantity of 2-(N-oxalylamino)-isophthalic acid was formed, but no 7-carboxyisatin was isolated. It seems likely, there-

TABLE I

	Wave length in μ						
Dehydro-apo-β-erythroidine	3.31	5.76	6.11		6.41	6.61	6.73
Apo-β-erythroidine		5.75	6.07	6.25	6.34		6.70
Isoapo-β-erythroidine	2.71	5.89	6.21	6.26	6.34		6.74
Dihydro-apo-β-erythroidine		5.79		6.25			6.74
Octahydro-apo-β-erythroidine		5.76					

(8) C. C. Price and R. M. Roberts, THIS JOURNAL, **68**, 1204 (1946); *cf.* B. Riegel, G. R. Lappin, B. H. Adelson, R. I. Jackson, C. J. Albisetti, Jr., R. M. Dodson and R. H. Baker, *ibid.*, **68**, 1264 (1946).

fore, that in the oxidation of the β-erythroidine derivatives the quinoline compound (III) and 7-carboxyisatin are formed independently.

A study of the infrared spectra of the β-erythroidine derivatives in the region 2.5–7.0 μ provides some information regarding the structure of these compounds. The positions of the significant absorption bands are specified in Table I and the spectra are given in Fig. 3.

Marion, Ramsey and Jones[9] in their investigation of infrared spectra of alkaloids, show that unassociated NH groups produce absorption bands in the region 2.86–3.08 μ, and, when associated, between 3.01 and 3.29 μ. None of the β-erythroidine derivatives under consideration absorb in this region, although the possibility remains that the band at 3.31 μ in dehydro-apo-β-erythroidine may be due to the presence of an NH grouping. However, Marion, Ramsey and Jones[9] found that in the eleven indole alkaloids that they studied, the N–H stretching bands occurred between 2.87 and 2.91 μ. The infrared data indicate that these β-erythroidine derivatives are all tertiary amines. In all these compounds, with the exception of isoapo-β-erythroidine the C=O stretching vibration of the lactone group appears in the region 5.75–5.79 μ. The position of this band at a higher wave length in isoapo-β-erythroidine may indicate that in this compound the C=O of the lactone group is in conjugation. The bands at 6.25–6.26 μ and at 6.70–6.74 μ in apo-β-erythroidine, isoapo-β-erythroidine and dihydro-apo-β-erythroidine seem to correspond to typical benzene ring absorption. The spectrum of dehydro-apo-β-erythroidine in this region appears somewhat anomalous. The 6.73 μ band is present but the bands at 6.11 and 6.61 μ are difficult to interpret, and, in fact, suggest the presence of a type of amide structure. The absorption bands at 6.34–6.41 μ, which occur in apo-, isoapo and dehydro-apo-β-erythroidine, correspond to similar bands appearing in the spectra of quinolines, indoles and related structures and may be attributed to the phenyl-N group.

(9) L. Marion, D. A. Ramsey and R. N. Jones, THIS JOURNAL, **73**, 305 (1951).

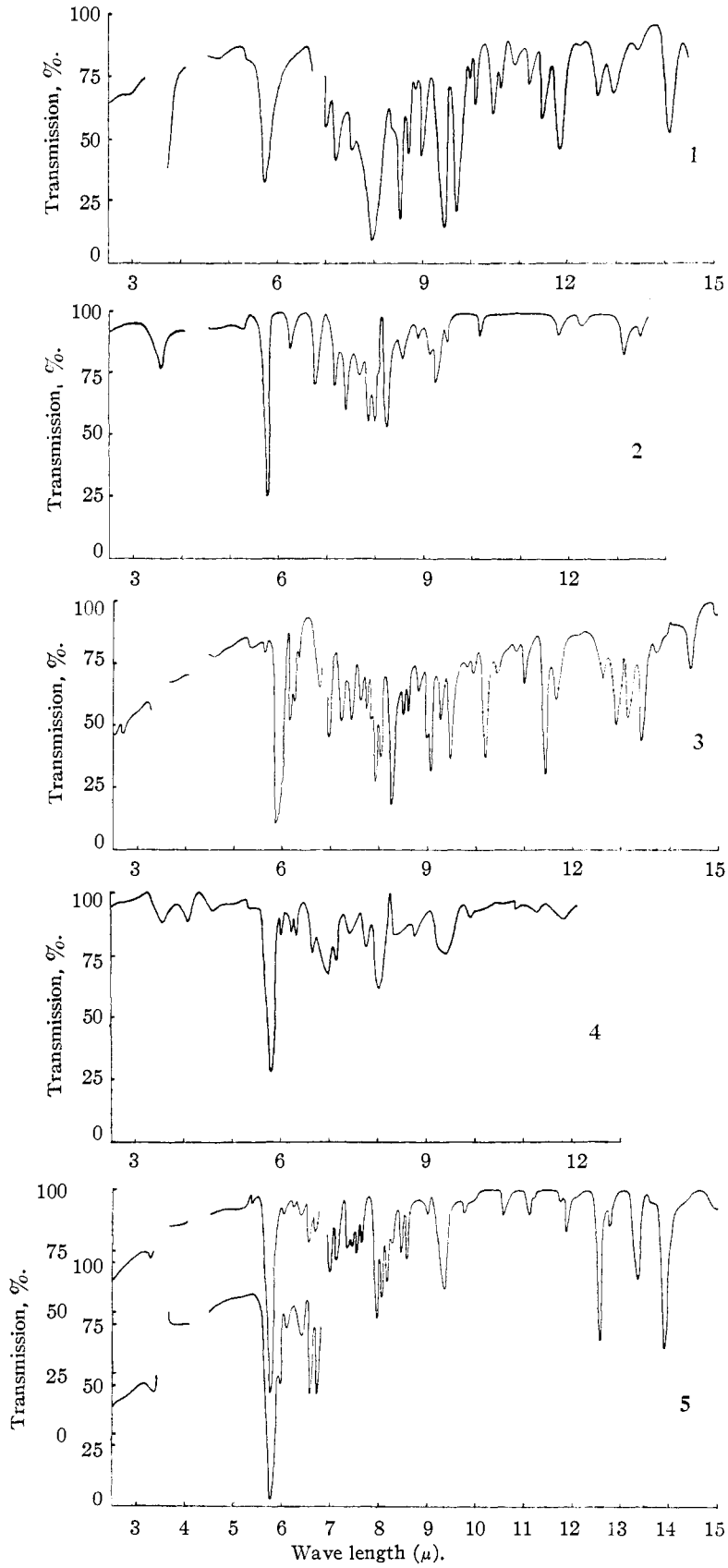
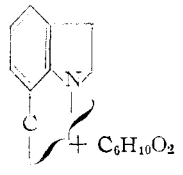
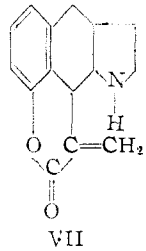


Fig. 3.—Infrared absorption spectra: 1, octahydro-apo-β-erythroidine (nujol mull); 2, dihydro-apo-β-erythroidine (chloroform); 3, isoapo-β-erythroidine (nujol mull); 4, apo-β-erythroidine (chloroform); 5, dehydro-apo-β-erythroidine (nujol mull, lower curve at twice the concentration).

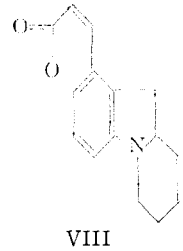
On the basis of our oxidation studies and the infrared spectra of these β-erythroidine derivatives, it would seem likely that apo-β-erythroidine should be represented by the partial structure (VI). The easy dehydrogenation of apo-β-erythroidine to its dehydro derivative would then represent the conversion of a dihydroindole to a true indole structure. Also, in support of this suggestion, dehydro-apo-β-erythroidine is much less basic than apo-β-erythroidine and does not form a hydrochloride or methiodide as does the apo derivative. In addition, dehydro-apo-β-erythroidine gives a positive Ehrlich test, which is not given by the apo derivative. The oxidation products are readily explainable on the basis of VI, including the quinoline derivative (III). This compound could result either from the presence of a reduced quinoline ring system in the apo-β-erythroidine molecule or by an indole–quinoline transformation of the type recently described by Witkop, Patrick and Rosenblum.[10] The former probability would appear to be more likely, since the positive Ehrlich test suggests that dehydro-apo-β-erythroidine has a free β-position.



VI



VII



VIII

Recently Koniuszy and Folkers[4] suggested structure VII as a possibility for apo-β-erythroidine and Lapiere and Robinson[11] have suggested structure VIII. It is apparent from our studies that neither of these suggestions is correct and that the true structure of the molecule must differ rather fundamentally. Although one can readily devise several possible structures for the complete apo-β-erythroidine molecule, based on VI as a partial structure, it would appear advisable to defer structural postulation until further experimental evidence is at hand. The position of the lactone group, which can no longer be considered to be linked to the aromatic ring, is most pertinent in this regard and obvious experiments designed to decide its location are underway.

In their publication Koniuszy and Folkers[4] concluded that apo-β-erythroidine contained a terminal methylene group since, on oxidation with acidic permanganate, it yielded formic acid. In view of the complete absence in the infrared spectrum of apo-β-erythroidine of the peaks ordinarily attributed to the terminal methylene group,[12] the formation of formic acid must be accounted for on some other basis. In support of this opinion subjection of apo-β-erythroidine to ozonolysis did not yield sufficient formaldehyde for detection as its dimedon derivative. As mentioned previously, neither apo-β-erythroidine nor its octahydro derivative show an absorption peak in the region typical of an N–H grouping. That both of these compounds are tertiary and not secondary amines was also borne out by their conversion to quaternary methiodide salts. Thus, the explanation that the conversion of desmethoxy-β-erythroidine to apo-β-erythroidine represents the cleavage of a β-amino lactone is not supported by our evidence.

Koniuszy and Folkers report in their publication that apo-β-erythroidine melts at 144° and is optically active ($[\alpha]^{25}D$ +26.6°). Since these properties are not in agreement with those which we previously found, we have re-examined this compound. In our hands the preparation of apo-β-erythroidine, whether accomplished by their procedure or ours, gives, as previously reported,[3] a compound melting at 132–132.5° and having no appreciable optical activity. After repeated crystallization from a methanol–ethyl ether solution or when purified on a Florisil column, apo-β-erythroidine is obtained as white crystals, whereas isoapo-β-erythroidine is a bright yellow. The difference in color of apo- and isoapo-β-erythroidine, as well as other evidence including the infrared spectra, makes it seem quite unlikely that the lower melting point of our preparation is due to partial isomerization to the isoapo derivative.[13]

Experimental[14]

Octahydro-apo-β-erythroidine.—A solution of apo-β-erythroidine (960 mg.) in warm ethanol (100 cc.) was treated with hydrochloric acid (1 cc.). The cold solution was added to platinum oxide (400 mg.), prereduced in ethanol (50 cc.). Hydrogenation proceeded at room temperature and atmospheric pressure, and, after 3 hours, 180 cc. of hydrogen was absorbed. Fresh catalyst (100 mg.) was added, and after a further 8 hours, hydrogenation was complete, when a total of 370 cc. of hydrogen had been taken up. The solvent was removed from the filtered solution, water (50 cc.) was added, and the solution was made alkaline with sodium bicarbonate. By extraction with chloroform (6 portions of 20 cc. each), a slightly brown gum was obtained which was refluxed with hexane (50 cc.). A gummy residue (A) remained. The hexane solution, on cooling, deposited colorless plates of octahydro-apo-β-erythroidine (378 mg.), m.p. 128–131°, raised by recrystallization from hexane to 135–135.5°. Concentration of the hexane filtrate gave 17 mg. more of this material, m.p. 128–131°. The total yield of octahydro-apo-β-erythroidine was 395 mg. (40%).

Anal. Calcd. for $C_{15}H_{23}NO_2$: C, 72.30; H, 9.30; N, 5.63. Found: C, 72.15; H, 9.52; N, 5.93.

Complete evaporation of the hexane solution gave a yellow oil which was distilled at 1 mm., and a colorless oil (247 mg.) collected between 135 and 150° (bath temperature). Investigation of this product is proceeding.

The hexane-insoluble material (A) was heated with benzene (20 cc.), and the benzene-insoluble residue crystallized from ethanol–ethyl ether in colorless plates (24 mg.), m.p. 213–217°, raised by recrystallization from the same solvent to 224–226°. This compound is being investigated further.

The methiodide of octahydro-apo-β-erythroidine was prepared by refluxing an ethanol solution of octahydro-apo-β-erythroidine with methyl iodide for 12 hours. It was obtained in 52% yield as white plates, m.p. 264–265°,

(10) B. Witkop, J. B. Patrick and M. Rosenblum, THIS JOURNAL, **73**, 2641 (1951).

(11) C. Lapiere and R. Robinson, *Chem. and Ind.*, **30**, 650 (1951).

(12) D. Barnard, L. Bateman, A. J. Harding, H. P. Koch, N. Sheppard and G. B. Sutherland, *J. Chem. Soc.*, 915 (1950).

(13) The difference in properties of our preparation of apo-β-erythroidine and that of Koniuszy and Folkers (ref. 4) raises the possibility that the two preparations represent different compounds.

(14) Analyses by Miss Claire King and the Micro-Tech Laboratories. All melting points are corrected. The infrared spectra were recorded by Mr. Carl Whiteman, using a Perkin-Elmer instrument, Model 12 B.

raised by recrystallization from ethanol to 265–266°. The compound slowly discolored in air.

Anal. Calcd. for $C_{16}H_{26}NO_2I$: C, 48.98; H, 6.73. Found: C, 49.11; H, 6.70.

Partial Hydrogenation of Apo-β-erythroidine.—Apo-β-erythroidine (1.05 g.), dissolved in ethanol (200 cc.), was added to prereduced platinum oxide (0.4 g.) suspended in ethanol (50 cc.). Hydrogenation was carried out at room temperature and atmospheric pressure until 140 cc. of hydrogen had been absorbed (100 cc. of hydrogen was required for the saturation of one double bond). The solvent was removed at room temperature from the filtered solution, and the resulting yellow gum dissolved in boiling ethanol. The solution, on cooling, deposited pale yellow crystals (520 mg.), m.p. 125–129° (A). The filtrate (B) was kept for further investigation.

The solid (A) crystallized from ethanol in colorless needles (170 mg.), m.p. 161–164°. The mother liquors (C) from this crystallization were retained. By further recrystallization from ethanol the compound (A) was obtained in long colorless rectangular plates, m.p. 173–173.5°, which proved to be dehydro-apo-β-erythroidine.

Anal. Calcd. for $C_{15}H_{13}NO_2$: C, 75.27; H, 5.47; N, 5.85. Found: C, 75.56, 75.39; H, 5.66, 5.72; N, 6.12.

Concentration of the ethanol solution (C) gave pale yellow prisms (210 mg.), m.p. 140–165°. This material, disssolved in benzene, was chromatographed on Florisil. The first pale yellow band was eluted with benzene. Evaporation of this benzene solution and crystallization of the residue from ethanol gave colorless needles (32 mg.), m.p. 155–160°, alone or mixed with dehydro-apo-β-erythroidine. Elution with ether and evaporation of the solvent yielded a residue, which crystallized from ethanol in pale yellow prisms (103 mg.), m.p. 153–154°. Colorless rectangular prisms of the same melting point were obtained by further recrystallization from ethanol.

Anal. Calcd. for $C_{15}H_{17}NO_2$: C, 74.05; H, 7.05; N, 5.76. Found: C, 73.98, 74.03; H, 7.21, 7.37; N, 5.65.

This compound, now regarded as dihydro-apo-β-erythroidine, was shown by a mixed melting point determination to be identical with the derivative described by Sauvage and Boekelheide as tetrahydro-apo-β-erythroidine.[3]

The solution (B) was concentrated to 5 cc. and kept at 0° for 48 hours. Yellow prisms separated (180 mg.), m.p. 110–125° (D). Concentration of the filtrate to 2 cc. and refrigeration gave yellow crystals (8 mg.), m.p. 142–144°, not depressed by mixing with isoapo-β-erythroidine. By evaporation of the filtrate and submitting a benzene solution of the residue to chromatographic separation on Florisil, a yellow band was formed and eluted with benzene. Removal of the solvent gave a yellow gum, crystallizing from ethanol in yellow crystals (70 mg.), m.p. 101–119°. This solid, combined with fraction (D), crystallized from ethanol in pale yellow prisms (130 mg.), m.p. 129–132°, with softening at 113°, undepressed by mixing with apo-β-erythroidine.

Dehydro-apo-β-erythroidine.—A solution of apo-β-erythroidine (3.2 g.) in ethanol (100 cc.) was heated under reflux in the presence of platinum oxide (100 mg.) for 3 hours. The hot solution was filtered and, on cooling, deposited almost colorless rectangular plates (1.6 g., 51% yield), m.p. 160–162°. Recrystallization from ethanol raised the melting point to 171.5–172.5°, not depressed by mixing with dehydro-apo-β-erythroidine. Dehydro-apo-β-erythroidine dissolves in warm aqueous alkali, and by acidification is recovered quantitatively. It gives a deep purple color in the Ehrlich test for indoles. Attempts to prepare a hydrochloride or methiodide resulted in complete recovery of dehydro-apo-β-erythroidine.

The solution obtained by removal of the crude product was evaporated and the residue, dissolved in benzene (15 cc.), chromatographed on a Florisil column. The first deep yellow band was eluted with benzene and the solution yielded a yellow gum, which separated from ethanol in yellow crystals (0.42 g.), m.p. 118–122° (A). Concentration of the filtrate gave pale yellow crystals (0.06 g.), m.p. 134–136° (B). Complete evaporation of the filtrate and recrystallization of the residue from ether gave large yellow prisms (0.08 g.), m.p. 136–142° alone or mixed with isoapo-β-erythroidine.

Fraction A separated from ethanol in slightly yellow prisms, m.p. 125–127°, not depressed by mixing with apo-β-erythroidine.

Fraction B, when recrystallized from methanol–ethyl ether, was obtained in colorless cubes, m.p. 148–150°, alone or mixed with dihydro-apo-β-erythroidine.

Hydrogenation of Dehydro-apo-β-erythroidine.—Dehydro-apo-β-erythroidine (220 mg.), dissolved in glacial acetic acid (20 cc.), was added to platinum oxide (200 mg.), prereduced in acetic acid (10 cc.). Hydrogenation proceeded at atmospheric pressure and room temperature until 86 cc. of hydrogen had been absorbed in 23 hours (103 cc. of hydrogen were required for the absorption of 4 moles of hydrogen). The filtered solution was concentrated to small volume at 40–50° and the residue treated with water (50 cc.). Any remaining acetic acid was neutralized with sodium bicarbonate, and by extracting the solution with ether (5 portions of 20 cc. each), a brown gum was obtained. The gum was extracted with portions of boiling hexane (3 portions of 30 cc. each), and the combined hexane solution concentrated to 15 cc. Colorless needles separated (48 mg.), m.p. 113–120°, and were obtained from ethanol in colorless plates (15 mg.), m.p. 132–134°, undepressed by mixing with octahydro-apo-β-erythroidine.

Hydrogenation of Dihydro-apo-β-erythroidine.—A solution of dihydro-apo-β-erythroidine (40 mg.) in water (10 cc.) containing concd. hydrochloric acid (0.2 cc.) was added to prereduced platinum oxide (100 mg.) suspended in water (20 cc.). Hydrogenation was carried out at room temperature and atmospheric pressure until no more hydrogen was absorbed (13 cc. in 4.5 hours, 12 cc. was required for the saturation of three double bonds). The mixture was filtered and the catalyst washed with water. The filtrate, made alkaline with sodium bicarbonate, was extracted with chloroform (8 portions of 30 cc. each). Evaporation of the chloroform gave a brown gum, which was extracted with portions of boiling hexane (3 portions of 10 cc. each). The hexane solution, when concentrated, deposited colorless crystals (15 mg.), m.p. 132–134°. By recrystallization from hexane the compound was obtained in the form of colorless rectangular plates, m.p. 135° alone, or mixed with octahydro-apo-β-erythroidine.

Potassium Permanganate Oxidation of Dehydro-apo-β-erythroidine. (A) Isolation of 3,8-Dicarboxy-4-hydroxyquinoline.—Dehydro-apo-β-erythroidine (1.00 g.) was heated on a steam-bath with 15% aqueous potassium hydroxide (15 cc.) until a clear solution resulted. After dilution with water (40 cc.), the solution was treated at room temperature with small portions of 3% aqueous potassium permanganate until a slight excess was present (275 cc. in 10 hours). The mixture was saturated with sulfur dioxide and, after the addition of hydrochloric acid (10 cc.), extracted repeatedly with ether (10 portions of 70 cc. each). The ether solution was washed with water (4 portions of 20 cc. each), concentrated to 50 cc. and refrigerated. A colorless solid precipitated, and crystallized from ethanol in long colorless needles (32 mg.), m.p. 360°, with some decomposition beginning *ca.* 310°.

By comparison of the infrared spectra the compound was shown to be 3,8-dicarboxy-4-hydroxyquinoline (III) (see below).

Anal. Calcd. for $C_{11}H_7NO_5$: C, 56.65; H, 3.03. Found: C, 56.69, 56.73; H, 3.01, 3.03.

The dimethyl ester of the above acid was prepared as follows. The dicarboxylic acid (35 mg.) was suspended in ether (5 cc.) and allowed to react with excess ethereal diazomethane for 10 minutes. The ether solution was concentrated to 3 cc., and the solid material (28 mg.), m.p. 242–245°, crystallized from aqueous methanol in colorless needles (26 mg.), m.p. 219–221°. Recrystallization raised the melting point to 222–224°.

A mixed melting point determination indicated its identity with 3,8-dicarbomethoxy-4-hydroxyquinoline (IV).

Anal. Calcd. for $C_{13}H_{11}NO_5$: C, 59.76; H, 4.24; N, 5.36; -OMe (2), 23.76. Found: C, 59.65, 59.53; H, 4.12, 4.22; N, 5.35; -OMe, 21.83.

(B) **Isolation of 7-Carboxyisatin.**—After removal of the 3,8-dicarboxy-4-hydroxyquinoline as indicated in part (A), the ether solution from which it separated was concentrated to 5 cc. and again refrigerated. This gave orange crystals (19 mg.), m.p. 255–258°, with decomposition, crystallizing from aqueous ethanol in tight clusters of orange needles (13

mg.), m.p. 274–276° dec., not depressed by admixture with 7-carboxyisatin (m.p. 277–279°).[6]

The infrared spectrum of this compound was identical with that of 7-carboxyisatin.

Anal. Calcd. for $C_9H_5NO_4$: C, 56.56; H, 2.64; N, 7.33. Found: C, 56.21; H, 2.71; N, 7.37.

The methyl ester of this oxidation product was prepared by heating 13 mg. of the acid on a steam-bath with a solution of anhydrous hydrogen chloride in methanol. After removal of the solvent, the residual methyl ester crystallized from methanol in large orange-red prismatic needles, m.p. 192.5–195°, not depressed by mixing with an authentic sample of 7-carbomethoxyisatin (m.p. 194–195°).[6] The identity of the two samples was further confirmed by a comparison of their infrared spectra.

(C) Isolation of 2-Aminoisophthalic Acid.—After removal of the 7-carboxyisatin, the ether filtrate was evaporated, and trituration of the residue with ethyl acetate gave an almost colorless solid, crystallizing from aqueous ethanol in slightly yellow blades (15 mg.), subliming away at 267–269°. The infrared spectrum was identical with that of an authentic sample of 2-aminoisophthalic acid (*vide infra*).

Anal. Calcd. for $C_8H_7NO_4$: C, 53.01; H, 3.89. Found: C, 53.23; H, 4.25.

The dimethyl ester of this oxidation product was prepared by treating a suspension of 10 mg. of the acid in ether with excess ethereal diazomethane. After 15 minutes the solvent was removed and the residue crystallized from aqueous methanol in colorless needles (9 mg.), m.p. 100–101.5°, with softening at 95°. The analytical sample melted at 101–101.5°, alone or mixed with dimethyl 2-aminoisophthalate.

Anal. Calcd. for $C_{10}H_{11}NO_4$: C, 57.42; H, 5.30; -OMe (2), 29.67. Found: C, 57.22; H, 5.31; -OMe, 29.30.

(D) Decarboxylation of the Oxidation Product from (A).—A mixture of the dicarboxylic acid (120 mg.), quinoline (1 cc.) and copper chromite (5 mg.) was heated at 210–220° for 45 minutes. During the course of the reaction the acid dissolved, effervescence occurred and, finally, solid material separated from the hot quinoline solution. The cooled mixture was treated with 10% hydrochloric acid (5 cc.), and the solid, obtained by filtration, was refluxed with ethanol (50 cc.). The insoluble material was suspended in water, sodium bicarbonate was added, and the solid (50 mg.) was recovered from the filtered alkaline solution by acidification. The product crystallized from water (300 cc.) in clusters of pale yellow flat prisms (40 mg.), decomposing at 349–352°, with sublimation beginning at *ca.* 285°.

Anal. Calcd. for $C_{10}H_7NO_3$: C, 63.49; H, 3.74; neut. equiv., 189. Found: C, 63.90; H, 4.02; neut. equiv., 189.

The methyl ester of the decarboxylation product was prepared by suspending 14 mg. of the acid in ether (5 cc.) and treating this with excess ethereal diazomethane. After 20 minutes, the solvent was removed and the residual gum extracted with several portions of boiling hexane. The methyl ester (4 mg.) crystallized from the combined hexane extracts and, after recrystallization from hexane, was obtained as colorless rectangular prisms, m.p. 137.5–138°, not depressed by admixture with 8-carbomethoxy-4-hydroxyquinoline.

The ester in aqueous ethanol solution gave an orange color with ferric chloride.

Anal. Calcd. for $C_{11}H_9NO_3$: C, 65.02; H, 4.47. Found: C, 64.83; H, 4.24.

Potassium Permanganate Oxidation of Apo-β-erythroidine.—Apo-β-erythroidine (2 g.) was dissolved in warm 15% aqueous potassium hydroxide (30 cc.), the solution diluted with water (100 cc.), and 3% aqueous potassium permanganate added in small portions until the pink color persisted for 3–4 hours (total, 600 cc. in 24 hours). The solution was saturated with sulfur dioxide, and, after the addition of hydrochloric acid (10 cc.), extracted with ether (10 portions of 100 cc. each). When the ether solution was concentrated to 50 cc., a colorless solid separated and was obtained as colorless needles (50 mg.), m.p. > 360°, from aqueous ethanol. Comparison of the infrared spectra showed that this compound was identical with 3,8-dicarboxy-4-hydroxyquinoline.

The dimethyl ester, prepared with diazomethane, crystallized from aqueous methanol in long needles, m.p. 218–221° alone, or mixed with the dimethyl ester of 3,8-dicarboxy-4-hydroxyquinoline.

After removal of the dicarboxylic acid, concentration of the ether filtrate to 10 cc. gave orange crystals (65 mg.), m.p. 226–239° with decomposition, which were extracted with boiling chloroform (100 cc.). The chloroform soluble portion crystallized from aqueous methanol in tight cluster, of orange needles (33 mg.), m.p. 271° with decompositions and with darkening beginning at *ca.* 240°. This was shown by a mixed melting point determination, and by a comparison of the infrared spectra to be identical with 7-carboxyisatin.

3-Carbethoxy-8-carbomethoxy-4-hydroxyquinoline.—A mixture of methyl anthranilate (5 g.) and diethyl ethoxymethylenemalonate (7.2 g.) was heated on a steam-bath for 14 hours. The viscous red oil did not crystallize, and without purification, diphenyl ether (50 cc.) was added and the solution heated at 250° for 30 minutes, and finally refluxed vigorously for 10 minutes. The cooled solution was diluted with hexane (150 cc.). The sticky solid, which precipitated, crystallized from ethyl acetate in colorless plates (3.30 g., 35% yield), m.p. 158–161°. The analytical sample was obtained from ethyl acetate as colorless blades, m.p. 161.5–162°.

Anal. Calcd. for $C_{14}H_{13}NO_5$: C, 61.09; H, 4.76. Found: C, 61.31; H, 4.82.

3,8-Dicarboxy-4-hydroxyquinoline.—The diester (231 mg.) was heated on a steam-bath for 2 hours with 1 *N* sodium hydroxide solution (10 cc.). The cold solution was acidified, and the precipitated solid crystallized from aqueous ethanol in long colorless needles (184 mg., 94% yield), m.p. >360°, but with some darkening *ca.* 300° and considerable decomposition *ca.* 340°.

Anal. Calcd. for $C_{11}H_7NO_5$: C, 56.65; H, 3.03; N, 6.01. Found: C, 56.62; H, 3.48; N, 6.14.

3,8-Dicarbomethoxy-4-hydroxyquinoline.—To a suspension of the dicarboxylic acid (235 mg.) in ether (30 cc.) was added an ether solution of diazomethane (prepared from 1 g. of nitrosomethylurea). After 12 hours the mixture was concentrated to 5 cc. and the solid removed and extracted with aqueous sodium bicarbonate. The material which remained insoluble (53 mg.), crystallized from aqueous methanol in colorless needles, m.p. 223–224°.

Anal. Calcd. for $C_{13}H_{11}NO_5$: C, 59.78; H, 4.25; -OMe (2), 23.76. Found: C, 59.81; H, 4.53; -OMe, 23.23.

8-Carboxy-4-hydroxyquinoline.—3,8-Dicarboxy-4-hydroxyquinoline (800 mg.) was heated under reflux with quinoline (5 cc.) in the presence of copper chromite (15 mg.) for 30 minutes. The crude 8-carboxy-4-hydroxyquinoline (185 mg.) was obtained from the reaction mixture as described in the decarboxylation of the natural material. It was heated with water (200 cc.), and the solid collected. The aqueous solution, on cooling, deposited colorless plates (25 mg.). By recrystallization from water the analytical sample was obtained, m.p. >360°, but with decomposition beginning at 358°.

The solid, which did not dissolve in water, was refluxed with water (200 cc.), sufficient sodium bicarbonate was added to produce a clear solution, and the solution was acidified at the boiling point. A further quantity of 8-carboxy-4-hydroxyquinoline (110 mg.) crystallized when the solution cooled. The total yield was 135 mg. (21%).

Anal. Calcd. for $C_{10}H_7NO_3$: C, 63.49; H, 3.73. Found: C, 63.36; H, 3.95.

8-Carbomethoxy-4-hydroxyquinoline.—The above acid (62 mg.), suspended in ether (10 cc.) was treated with an ethereal solution of diazomethane. After 12 hours unchanged acid (21 mg.) was removed by filtration, and the ether filtrate evaporated. The residual yellow gum was refluxed with several portions of hexane. The combined hexane solution, on cooling, deposited colorless needles (22 mg.), m.p. 127–135°, raised by recrystallization from hexane to 139.5–140.5°.

Anal. Calcd. for $C_{11}H_9NO_3$: C, 65.02; H, 4.46. Found: C, 65.29; H, 4.49.

The Reaction of Diazomethane with 7-Carboxyisatin (A) Isolation of 8-Carbomethoxy-2,3-dihydroxyquinoline.—A solution of 7-carboxyisatin[6] (2.0 g.) in 50 cc. of acetone was treated with an ethereal solution of diazomethane (prepared from 5.0 g. of nitrosomethylurea). After one hour the solv-

ent was removed, and the residue, on crystallization from methanol, gave 300 mg. of colorless plates, m.p. 224–226°. The filtrate was reserved and treated as indicated in (B). Further crystallization of the solid from methanol gave white plates, m.p. 233–236°.

These crystals gave a dark green color with ferric chloride in ethanol. Also, they were converted by further treatment with diazomethane to white needles, m.p. 163–163.5°, identical with the compound isolated in part (C). When compound A was treated with periodic acid, 0.83 molar equivalent of the reagent was consumed in five hours.

Anal. Calcd. for $C_{11}H_9NO_4$: C, 60.28; H, 4.14; CH_3O–, 14.16. Found: C, 60.37; H, 4.22; CH_3O–, 14.00.

8-Carboxy-2,3-dihydroxyquinoline was prepared by heating 21 mg. of compound A with 1 cc. of a 15% aqueous potassium hydroxide solution for 3 hours on the steam-bath. On acidification of the reaction mixture a solid separated. This, after three crystallizations from ethanol, gave 8 mg. of white needles, subliming away at 315–320°.

Anal. Calcd. for $C_{10}H_7NO_4$: C, 58.54; H, 3.44. Found: C, 58.60; H, 3.88.

(B) **Isolation of 8-Carbomethoxy-2,4-dihydroxyquinoline (?).**—When the filtrate from part (A) was concentrated, a solid separated and was collected on the filter. The filtrate again was saved and reserved for part (C). Crystallization of this solid from methanol gave 220 mg. of white needles, m.p. 178–179°.

This product did not give a color with ferric chloride, did not react with 2,4-dinitrophenylhydrazine, and after five hours consumed only 0.06 molar equivalent of periodic acid.

Anal. Calcd. for $C_{11}H_9O_4$: C, 60.28; H, 4.14; CH_3O–, 14.16. Found: C, 60.36; H, 4.44; CH_3O–, 14.48.

(C) **Isolation of 8-Carbomethoxy-2-hydroxy-3-methoxyquinoline.**—Complete evaporation of the filtrate from part (B) gave a brown gum which dissolved in boiling ethyl acetate (10 cc.). This solution, when kept at 0°, deposited 240 mg. of colorless crystals, m.p. 159–160°. By recrystallization from ethyl acetate and then from hexane, the compound was obtained as fine white needles, m.p. 163–164°.

This product did not give a color with ferric chloride solution, did not react with 2,4-dinitrophenylhydrazine reagent, and only consumed 0.17 molar equivalent of periodic acid in 5 hours.

Anal. Calcd. for $C_{12}H_{11}NO_4$: C, 61.80; H, 4.76; CH_3O–, 26.61. Found: C, 61.85; H, 4.84; CH_3O–, 23.82.

8-Carboxy-2-hydroxy-3-methoxyquinoline was prepared from compound C. Although C was not soluble in sodium carbonate, it was soluble in sodium hydroxide and was hydrolyzed by heating 50 mg. of the material for two hours with 3 cc. of 1 *N* sodium hydroxide solution. Acidification of the reaction mixture gave a solid acid which, on crystallization from ethanol, yielded 32 mg. of white crystals, m.p. 274–276°. Further recrystallization of the material from ethanol gave a sample melting at 282–284°.

Anal. Calcd. for $C_{11}H_9NO_4$: C, 60.28; H. 4.14. Found: C, 60.17; H, 4.20.

The reaction of diazomethane with 7-carboxyisatin did not always give the same yield of products, and in cases where some of the 8-carbomethoxy-2,3-dihydroxyquinoline was converted to the 3-methyl ether, the following separation procedure was preferable.

7-Carboxyisatin (490 mg.) was treated with diazomethane as described above. The residue obtained by evaporation of the solvent crystallized from methanol, and the solid so obtained crystallized from benzene, whereby 8-carbomethoxy-2,3-dihydroxyquinoline was obtained as colorless plates (33 mg.), m.p. 234–236°. Evaporation of the benzene filtrate and crystallization from methanol gave 8-carbomethoxy-2,4-dihydroxyquinoline (?) in colorless needles (220 mg.) m.p. 166–169°, raised by recrystallization from the same solvent to 174–175°.

2-(N-Oxalylamino)-isophthalic Acid.—A solution of 300 mg. of 8-carbomethoxy-2,3-dihydroxyquinoline in 5 cc. of a 5% aqueous potassium hydroxide solution was heated on the steam-bath for 2 hours. The solution was then diluted with 20 cc. of water and treated with portions of 3% aqueous potassium permanganate until an excess was present (18 cc. of permanganate solution was required). The mixture was clarified with sulfur dioxide, 3 cc. of concd. hydrochloric acid was added, and the resulting solution was extracted 10 times with 10-cc. portions of ether. The yellow gum, obtained by evaporation of the ether, dissolved in 10 cc. of warm water, and when this solution was cooled, 15 mg. of orange needles, m.p. 245–265°, separated. The identity of these crystals was established by the fact that, on recrystallization from water, they melted at 274–277° dec., not depressed by admixture with an authentic sample of 7-carboxyisatin. When the filtrate from the initial separation of the orange crystals was concentrated to 3 cc., there was deposited 6 mg. of white plates, subliming away at 250°. Esterification of this material with diazomethane gave dimethyl 2-aminoisophthalate (*vide infra*). The filtrate from the separation of the 2-aminoisophthalic acid was concentrated to 2 cc. and allowed to stand at 0°. There separated 114 mg. of slightly yellow prisms, m.p. *ca.* 300°. A further crystallization from water gave a sample melting at 308–309° dec.

Anal. Calcd. for $C_{10}H_7NO_7 \cdot H_2O$: C, 44.29; H, 3.35. Found: C, 44.77; H, 3.25.

The trimethyl ester of 2-(N-oxalylamino)-isophthalic acid was obtained by means of diazomethane. On recrystallization from hexane it gave colorless needles, m.p. 137–138°.

Anal. Calcd. for $C_{13}H_{13}NO_7$: C, 52.88; H, 4.44; CH_3O– (3), 31.53. Found: C, 53.08; H, 4.58; CH_3O–, 32.28.

2-Aminoisophthalic Acid.—A solution of 87 mg. of 2-(N-oxalylamino)-isophthalic acid in 3 cc. of a 1 *N* aqueous sodium hydroxide solution was heated on a steam-bath for 4 hours. The cold solution was acidified with acetic acid, and the yellow precipitate (35 mg.) was crystallized from methanol to give 27 mg. of pale yellow plates, subliming above 260° and finally disappearing at 274°.

Anal. Calcd. for $C_8H_7NO_4$: C, 53.04; H, 3.90. Found: C, 53.29; H, 4.15.

The dimethyl ester was prepared using diazomethane and was obtained, after crystallization from methanol, as colorless needles, m.p. 100–101°.

Anal. Calcd. for $C_{10}H_{11}NO_4$: C, 57.41; H, 5.30; CH_3O– (2), 29.67. Found: C, 57.22; H, 5.31; CH_3O–, 29.30.

Dimethyl 2-(N-Acetylamino)-isophthalate.—A solution of 20 mg. of dimethyl 2-aminoisophthalate in 1 cc. of acetic anhydride was boiled under reflux for four hours. The major portion of the acetic anhydride was removed and 1 cc. of water was added. The precipitated solid dissolved in warm methanol and, on cooling, the solution deposited 3 mg. of starting material, m.p. 99–100°. Concentration and refrigeration of the filtrate gave 9 mg. of colorless needles, m.p. 85–86°. A sample, recrystallized from water, melted at 86.5–87°.

Anal. Calcd. for $C_{12}H_{13}NO_5$: C, 57.37; H, 5.21. Found: C, 57.55; H, 5.25.

Potassium Permanganate Oxidation of 3,8-Dicarboxy-4-hydroxyquinoline.—A solution of 500 mg. of 3,8-dicarboxy-4-hydroxyquinoline in 30 cc. of a 3% aqueous potassium hydroxide solution was treated with 1-cc. portions of a 3% aqueous potassium permanganate solution until the mixture remained pink for 4 hours (35 cc. was added over a 26-hr. period). Saturation of the solution with sulfur dioxide caused the precipitation of 190 mg. of a colorless solid. This was shown to be starting material by its melting point (above 360°) and by its conversion to the corresponding dimethyl ester, m.p. 222–224°, by means of diazomethane.

After removal of the dicarboxylic acid, extraction of the aqueous filtrate with ether gave a yellow gum, yielding pale orange crystals (4 mg.) by trituration with ether. The ether filtrate (A) was kept for further investigation. The solid crystallized from ethanol in colorless needles, m.p. >360°. The ethanol filtrate gave a trace of a red 2,4-dinitrophenylhydrazone.

The ether solution (A) was evaporated, the resultant gum treated with diazomethane in ether solution, and the solvent removed. Boiling hexane extracted colorless needles (3 mg.), m.p. 134–136°, not depressed by mixing with the trimethyl ester of 2-(N-oxalylamino)-isophthalic acid.

Ozonolysis of Apo-β-erythroidine.—A solution of 150 mg. of apo-β-erythroidine in 10 cc. of chloroform was subjected for two hours to the action of a stream of oxygen containing 4 millimoles of ozone (by previous standardization). The exit gases were passed through two traps each containing 15 cc. of water. When the water from the traps was added to a hot solution of 250 mg. of dimethyldihydroresorcinol in 50 cc. of water, no precipitate was formed, thus indicating the absence of formaldehyde.

The non-volatile portion of the reaction mixture, which did not contain apo-β-erythroidine, is still under investigation.

Apo-β-erythroidine.—Apo-β-erythroidine was prepared by the method of Sauvage and Boekelheide[3] as pale yellow crystals, m.p. 129.5–130.5°, and obtained by recrystallization from ethanol as large colorless prisms, m.p. 132–132.5°; $[\alpha]^{26}D$ +1.0 (*c* 1.5, benzene). After four crystallizations from methanol–ethyl ether the melting point was unchanged. The observed rotation is within the experimental error of the determination and does not establish optical activity.

Apo-β-erythroidine (1.8 g., pale yellow, m.p. 129.5–130.5°) was also purified by dissolving it in benzene (50 cc.) and passing the resultant solution over a Florisil column (80 × 30 mm.). A broad pale yellow band appeared and was eluted with benzene (600 cc.). Evaporation of the solvent and crystallization of the residue from ethanol gave colorless prisms (1.04 g.), m.p. 132–132.5°.[15]

(15) A Florisil column was first used by Dr. G. L. Sauvage for the purification of apo-β-erythroidine.

ROCHESTER, N. Y.

[CONTRIBUTION FROM THE COATES CHEMICAL LABORATORY OF LOUISIANA STATE UNIVERSITY]

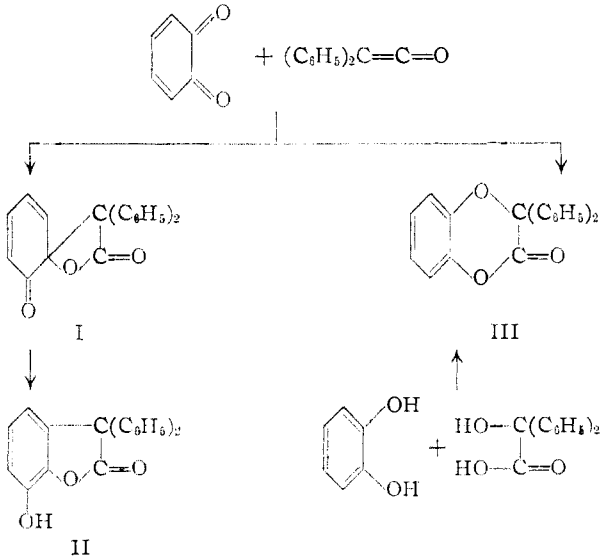
The Addition of Diphenylketene to *o*-Benzoquinone

BY J. L. E. ERICKSON AND J. M. DECHARY

The addition of diphenylketene to *o*-benzoquinone yields diphenyl-*o*-hydroxyphenoxyacetic acid lactone which is identical with the substance obtained by fusing catechol with benzilic acid, but which is described erroneously in the literature as 2,3-dihydroxytriphenylacetic acid lactone. The latter substance may be obtained, however, by the demethylation of 2,3-dimethoxytriphenylacetic acid which can be synthesized by conventional methods. An independent synthesis of diphenyl-*o*-hydroxyphenoxyacetic acid lactone is described whereby hydrogen chloride is eliminated from catechol and diphenylchloroacetyl chloride.

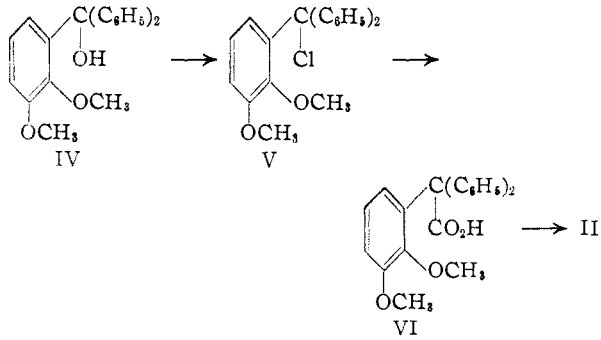
It has been observed that diphenylketene adds to *p*-benzoquinone to yield a colorless monolactone[1] which can be isolated, although upon heating, it decomposes into diphenyl-*p*-quinomethane and carbon dioxide. This monolactone undergoes rearrangement when illuminated in the solid state, or in boiling benzene, and is transformed into the isomeric benzene derivative, 2,5-dihydroxytriphenylacetic acid lactone.[2]

If the behavior of *p*-benzoquinone is typical, one might expect the reaction between diphenylketene and *o*-benzoquinone to furnish the analogous monolactone (I) which should either decompose into diphenyl-*o*-quinomethane, or rearrange into 2,3-dihydroxytriphenylacetic acid lactone (II).



It was found, however, that when *o*-benzoquinone in benzene suspension was treated with an excess of diphenylketene, the primary addition product (I) was not obtained. Instead, there was isolated a colorless substance which was identical with the product obtained from catechol and benzilic acid, and which is described in the literature[3] as 2,3-dihydroxytriphenylacetic acid lactone (II). The substance gave no color with ferric chloride and was insoluble in dilute alkaline solution, behavior which seemed inconsistent with structure (II) as well as with the possible isomeric 3,4-dihydroxytriphenylacetic acid lactone.

In consequence of these observations and for comparison with the product obtained from diphenylketene and *o*-benzoquinone, II was synthesized by the following sequence of reactions: 2,3-Dimethoxytriphenylcarbinol (IV), prepared from methyl 2,3-dimethoxybenzoate and phenyllithium, was converted by means of acetyl chloride into the corresponding chloromethane (V). When V was treated in ether–benzene solution with 1% sodium amalgam, followed by carbonation and hydrolysis of the sodium salt, 2,3-dimethoxytriphenylacetic acid (VI) was obtained. Demethylation of VI by hydriodic acid gave the lactone (II). Compounds II, V, and VI have not been previously reported.



As distinguished from the substance (m.p. 136–137.5°) obtained by either the reaction of diphenylketene with *o*-benzoquinone, or from catechol and benzilic acid, II is quite soluble in dilute alkali,

(1) H. Staudinger, *Ber.*, **41**, 1355 (1908).
(2) H. Staudinger, *Ann.*, **380**, 248 (1911).
(3) H. von Liebig, *Ber.*, **41**, 1648 (1908).