

1017. *Sporidesmins. Part II.*¹ *The Structure of Degradation Products Related to 5-Chloro-6,7-dimethoxyisatin.*

By R. HODGES, J. W. RONALDSON, A. TAYLOR, and E. P. WHITE.

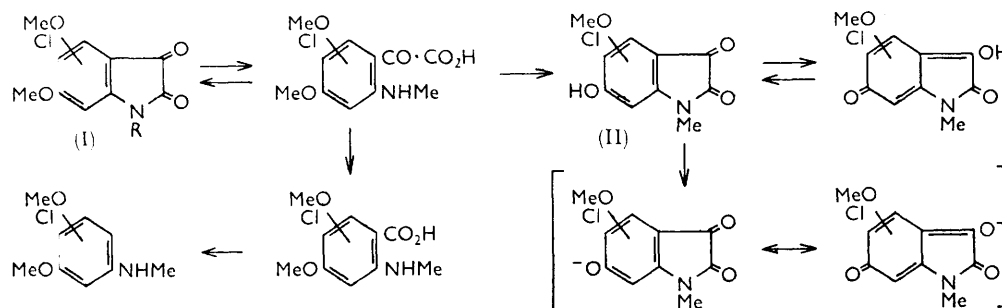
Solutions of sporidesmin in ethanol, when shaken with mercury, gave 5,5'-dichloro-6,6',7,7'-tetramethoxy-1,1'-dimethylindigo, which was converted in high yield into 5-chloro-6,7-dimethoxy-1-methylisatin on treatment with alkali. The isatin and 5-chloro-6,7-dimethoxyisatin were obtained after reaction of sporidesmin with manganese dioxide. Chlorination of 6,7-dimethoxyisatin gave the 5-chloro-derivative, identical with the degradation product.

WE reported ¹ the isolation in low yield of a red ketone $C_{11}H_{10}ClNO_4$ when sporidesmin and sporidesmin-B were subjected to alkaline degradation. The low yields were explained when it was shown that further degradation took place in hot alkaline solution. Two products were isolated, one a red phenolic ketone $C_{10}H_8ClNO_4$, and the other an acid, which was not obtained pure but provided a secondary aromatic base, $C_9H_{12}ClNO_2$, on pyrolysis. The proton magnetic resonance spectrum of the C_{11} -ketone confirmed the presence of two methoxyl groups and one *N*-methyl group, shown by analysis. Absorption due to an aromatic proton was also present. The C_{11} -ketone was readily soluble in cold aqueous alkali and was reprecipitated on acidification of such solutions. Back titration of alkaline solutions, potentiometrically, showed the presence of an acid, $pK_a \sim 3$, but solutions in aqueous ethanol were neutral. These results can best be explained by assuming the opening of a lactam in alkaline solution and its closure on acidification. This interpretation is confirmed by the spectroscopic properties of the C_{11} -ketone and its degradation products. The peaks at 221, 262, 319, and 435 $m\mu$ are typical of an isatin, as are the strong absorptions at 1736 and 1605 cm^{-1} .

Neutral solutions ($10^{-3}M$) of the C_{10} -phenolic ketone were bright-red. Solutions of pH 1 and 14 of the same concentration were colourless. The ultraviolet spectra of solutions of $pH < 2$ were similar to the spectra of isatins, whilst spectra of solutions of $pH > 13$ were similar to those of isatinic acids. There appeared in the spectra of solutions of $pH > 3$ a band at 405 $m\mu$ that became progressively more intense with increase of pH to a maximum

¹ Part I, Ronaldson, Taylor, White, and Abraham, *J.*, 1963, 3172; see also Hodges, Ronaldson, Taylor, and White, *Chem. and Ind.*, 1963, 42.

at approximately pH 6. Potentiometric titration showed the presence of an acidic group of pK_a about 4.5, thus the intense band ($\log \epsilon$ 4.14) in the visible spectrum was due to the anion of this acid. It followed that this abnormal absorption was due to a quinonoid form and therefore that the hydroxyl group occupied either the 4 or the 6 position. Such an orientation was supported by the stability of the isatinic acid in air in alkaline solution²



and also by the ease of hydrolysis of the methyl ether. The C=O stretching frequencies of (I; R = Me) and (II) in chloroform were 1736 and 1739 cm^{-1} , respectively, showing that strong intramolecular hydrogen bonds were absent and therefore that the hydroxyl group occupied the 6 position. The degradation reactions can be summarized as shown.

When sporidesmin in ethanol solution was shaken with mercury a blue degradation product, $\text{C}_{22}\text{H}_{20}\text{Cl}_2\text{N}_2\text{O}_6$, that provided the C_{11} -isatin on alkaline hydrolysis, was obtained. The spectroscopic and analytical data for the blue product suggested that it was the indigo corresponding to the C_{11} -isatin. Indigos are unstable under strongly alkaline conditions³ and 1,1'-dimethylindigo is known to be degraded in mild alkaline media.⁴ We have been unable to find, in the literature, a report of the formation of an isatin from this reaction; generally, anilines and anthranilic acids⁵ have been the products isolated. We therefore prepared 1,1'-dimethylindigo⁶ and showed that it was converted into 1-methylisatin after treatment with alkali. The reason for the ease of hydrolysis of 1,1'-dimethylindigos is not clear though presumably the ability of unmethylated indigos to form anions contributes to their stability.

The C_{11} -isatin was also obtained in variable yield from sporidesmin by reaction of benzene solutions with mercury or manganese dioxide. From the latter reaction a by-product, $\text{C}_{10}\text{H}_8\text{ClNO}_4$, was isolated that had an N-H stretching mode in the infrared and gave a transient violet colour⁷ when its solution was made alkaline. The oxidation of the methyl in *N*-methyl groups by manganese dioxide is known,⁸ and the isatin was therefore assigned the formula (I; R = H). This product was not obtained when (I; R = Me) was treated with manganese dioxide, hence the isatin (I; R = H) represents an alternative mode of degradation of sporidesmin.

While the orientation of the remaining aromatic substituents was being determined by synthetic studies we were informed of X-ray crystallographic results⁹ which showed that sporidesmin was a 5-chloro-6,7-dimethoxyindoline. Hence the isatin (I; R = H) having

² Hahn and Tulus, *Ber.*, 1941, **74**, 505.

³ Fischer and Hahn, *Z. physiol. Chem.*, 1913, **84**, 260; Beilstein, 1st suppl., XXIV, p. 372.

⁴ Weinstein and Wyman, *J. Amer. Chem. Soc.*, 1956, **78**, 4007.

⁵ Friedlaender and Schrenk, *Ber.*, 1910, **43**, 1972; Fritzsche, *J. prakt. Chem.*, 1840, **20**, 453; 1841, **23**, 67.

⁶ Ettinger and Friedlaender, *Ber.*, 1912, **45**, 2075.

⁷ Kohn and Ostersetzer, *Monatsh.*, 1916, **37**, 25.

⁸ Henbest and Thomas, *Chem. and Ind.*, 1956, 1097; Hight and Wildman, *J. Amer. Chem. Soc.*, 1955, **77**, 4399.

⁹ Fridrichsons and Mathieson, *Tetrahedron Letters*, 1962, 1229.

this substitution pattern was synthesized by chlorination¹⁰ of 6,7-dimethoxyisatin.^{11,12} The intermediate was prepared¹³ from 2,3-dimethoxyaniline¹⁴ itself obtained by a Schmidt reaction on 2,3-dimethoxybenzoic acid, an intermediate more readily available than that used by Robinson.¹¹ The synthetic isatin (I; R = H) was identical with that obtained from sporidesmin. After treatment of its potassium salt with methyl iodide it provided an *N*-methyl derivative that was identical with the degradation product (I; R = Me) of sporidesmin.

EXPERIMENTAL

Proton magnetic resonance spectra were determined for deuteriochloroform solutions on a Varian A-60 instrument; we are indebted to Dr. R. J. Abraham for these measurements and to Dr. J. S. Shannon for mass spectra. Alumina was of activity IV.

5,5'-Dichloro-6,6',7,7'-tetramethoxy-1,1'-dimethylindigo.—(a) Sporidesmin benzene solvate (1.6 g.), ethanol (40 ml.), water (80 ml.), and mercury (5 ml.) were shaken together for 10 days at room temperature. The insoluble part was collected, washed with 30% aqueous ethanol, dried, and washed with hot benzene until no further blue solution passed through the filter. Evaporation of the benzene filtrate gave blue rods (0.57 g.), m. p. 190–195°. Further indigo could be obtained by extraction of the aqueous ethanol washings with chloroform. Chromatography on alumina gave the indigo, eluted with benzene as a leading blue zone. *5,5'-Dichloro-6,6',7,7'-tetramethoxy-1,1'-dimethylindigo* separated as blue rods, m. p. 206°, on slow concentration, using a hot-air blower, of benzene solutions that had been diluted with 2 volumes of ethanol (Found: C, 53.9, 54.9; H, 4.4, 4.6; Cl, 15.1; N, 5.6, 5.45; O, 21.2; OMe, 24.7; *N*-Me, 7.1%; *M* (mass spectroscopy), 478, 480. $C_{22}H_{20}Cl_2N_2O_6$ requires C, 55.1; H, 4.2; Cl, 14.8; N, 5.85; O, 20.5; OMe, 25.9; *N*-Me, 6.3%; *M*, 479, λ_{max} (ether) 218, 259, 304, 360, 647 $m\mu$ ^{4,6} (log ϵ 4.56, 4.37, 4.49, 4.14, 4.42), ν_{max} (Nujol, KBr) 1644 (C=O), 1598 cm^{-1} , τ 2.51 (intensity 2, aromatic H), 6.03 (intensity 12), 6.30 (intensity 6).

(b) Sporidesmin benzene solvate (0.117 g.) was dissolved in methanol (2 ml.), and hydrochloric acid (20% aqueous, 2.5 ml.) was added. The solution, when heated under reflux, became orange, red, and finally (5 hr.) blue. The solution was evaporated and the leading blue zone, eluted from alumina with benzene, recrystallized as blue rods, m. p. 191°, having an infrared spectrum identical with that of the above indigo.

(c) Sporidesmin benzene solvate (0.22 g.) was intimately mixed with sodium dithionite (3 g.), water (10 ml.) was added, and the stirred mixture boiled for 10 min. On cooling, a crystalline solid (0.11 g.), m. p. 184°, $[\alpha]_D^{20}$ -26.4° , whose infrared spectrum was identical with that of sporidesmin, separated. The filtrate was made just alkaline with sodium hydroxide solution and heated under reflux for 1.5 hr. The residue after evaporation was triturated with ethyl acetate and the blue-green solution evaporated. After chromatography on alumina, blue rods (15 mg.), m. p. 192–193°, undepressed on admixture with the above indigo, were obtained. The product was difficult to separate from small quantities of a yellow gum.

5-Chloro-6,7-dimethoxy-1-methylisatin.—(a) The above indigo (0.57 g.), methanol (45 ml.), water (4 ml.), and sodium hydroxide solution (30%, 4 ml.) were shaken together at room temperature for 24 hr. The remaining indigo was dissolved by adding more methanol and sodium hydroxide solution and heated until the colour of the indigo disappeared. Extraction with chloroform gave the indigo (50 mg.). The raffinate was acidified and extracted with chloroform, giving (I; R = Me) (0.425 g.). Recrystallization gave red rods, m. p. 144° undepressed on admixture with the C_{11} -ketone¹ (Found: OMe, 24.0; *N*-Me, 5.1%; *M* (Rast), 220 (mass spectrometry), 254, 256. Calc. for $C_{11}H_{10}ClNO_4$: 2OMe, 23.0; *N*-Me, 5.1%; *M*, 255.5), λ_{max} (ethanol or 0.1*N*-hydrochloric acid) 221, 262, 319, 435 $m\mu$ (log ϵ 4.23, 4.10, 3.58 2.81), λ_{max} (0.1*N*-sodium hydroxide) 246, 277, 377 $m\mu$ (log ϵ 4.31, 3.90, 3.58), ν_{max} (KBr, Nujol, chloroform) 1736, 1605 cm^{-1} , no absorption in the O–H or N–H stretching region, τ 2.69 (intensity 1), 6.03, 6.14, 6.58 (all intensity 3). A solution of the ketone in a known excess of standard alkali

¹⁰ Ginsberg, *J. Amer. Chem. Soc.*, 1951, **73**, 702.

¹¹ Gulland, Robinson, Scott, and Thornley, *J.*, 1929, 2933.

¹² Kohn and Ostersetzer, *Monatsh.*, 1913, **34**, 791; Borsche and Jacobs, *Ber.*, 1914, **47**, 362.

¹³ Sandmeyer, *Helv. Chim. Acta*, 1919, **2**, 234; Marvel and Hiery, *Org. Synth.*, Coll. Vol. I, 1944, p. 327.

¹⁴ Mason, *J. Amer. Chem. Soc.*, 1947, **69**, 2241.

was titrated with acid potentiometrically. The curve obtained showed the presence of an acid (pK_a ca. 3.5) of equivalent weight ~ 265 .

(b) Sporidesmin benzene solvate (50 mg.) and manganese dioxide¹⁵ (0.5 g.) were heated under reflux in benzene (5 ml.) for 80 min. The manganese dioxide was extracted with chloroform and all the benzene-soluble products adsorbed on silica gel¹ (10 g.). Elution with benzene-ether (19:1) gave the ketone (I; R = Me), m. p. 143° (9.1 mg.), whilst further elution with benzene-ether (4:1) yielded 5-chloro-6,7-dimethoxyisatin, red needles, m. p. 217—219° (sublimation) (Found: C, 49.95; H, 3.25. $C_{10}H_8ClNO_4$ requires C, 49.7; H, 3.35%), λ_{max} (methanol) 218, 257, 318 m μ (log ϵ 4.31, 4.19, 3.83), ν_{max} (Nujol, KBr) 3200, 1740, 1610 cm^{-1} . The benzene-insoluble products from the above reaction were sublimed, giving crystals of methyloxamide (0.8 mg.), m. p. 232—234° (sealed tube), having the same infrared spectrum as an authentic specimen.

(c) Sporidesmin benzene solvate (0.1 g.), benzene (10 ml.), and mercury (8 g.) were shaken together for 4 days at room temperature. The product (47 mg.) was chromatographed on alumina (10 \times 0.5 cm.) in benzene and the orange zones collected as described previously,¹ giving a total of recrystallized isatin (I; R = Me) of 13 mg., m. p. 143°.

(d) 5-Chloro-6,7-dimethoxyisatin (50 mg.), dry tetrahydrofuran (10 ml.), and potassium *t*-butoxide (28 mg.) were set aside at room temperature until the isatin had been converted into its violet potassium salt (20 min.), and methyl iodide (50 μ l.) was added. The mixture was evaporated after standing for 40 hr. at room temperature, and treated with dilute hydrochloric acid (5 ml.) and benzene (10 ml.). The acid layer was washed with benzene and the combined benzene extracts evaporated. The residue in benzene was chromatographed on silica gel as described above. The 1-methylisatin (35 mg., m. p. 140—142°, mixed m. p. 143°) was eluted with ether-benzene (1:19) immediately after a very small yellow band.

2,3-Dimethoxyaniline.—2,3-Dimethoxybenzoic acid¹⁴ (8.1 g.) in chloroform (60 ml.) was treated with a solution (0.8N, 60 ml.) of hydrazoic acid in chloroform.¹⁶ To the vigorously stirred solution sulphuric acid (d 1.84, 7 g.) was added and the mixture heated under reflux until no more nitrogen was given off. Ice (50 g.) was added, the aqueous phase separated, and the chloroform phase washed with 2N-hydrochloric acid (50 ml.). The basified, combined aqueous phases were extracted with ether and the dry (Na_2SO_4) extract saturated with hydrogen chloride. The precipitated hydrochloride (2.1 g.), m. p. 189°, which gave a positive diazo-reaction, was collected.

6,7-Dimethoxyisatin.—2,3-Dimethoxyaniline hydrochloride (1.1 g.) in water (1 ml.) was treated with chloral hydrate (1 g.) and the resulting solution saturated with sodium sulphate decahydrate. The mixture was added to hydroxylamine hydrochloride (1.36 g.) in water (1 ml.) and the resulting solution heated to boiling. After 2 min. an oil separated that crystallised on cooling. The crystals (0.35 g., m. p. 145°) were dried (0.05 mm. over H_2SO_4), added to concentrated sulphuric acid (6 ml.) at 40°, the mixture heated for 10 min. at 80°, poured on to ice, and the orange solution extracted 6 times with ethyl acetate. The dry combined extract (0.133 g.) was heated at 120°/0.05 mm., and a brown oil condensed on the walls of the sublimation tube. At 160°/0.05 mm. the isatin (60 mg., m. p. 197—198°) sublimed. It separated from ethanol-water (19:1) as orange needles, m. p. 204° (lit.¹⁰ 207°) (Found: C, 58.4; H, 4.75. Calc. for $C_{10}H_8NO_4$ C, 58.0; H, 4.35%), λ_{max} (EtOH) 206, 219, 256, 347 m μ (log ϵ 4.41, 4.32, 4.10, 3.97), ν_{max} (KBr) 3200, 1745, 1620 cm^{-1} .

5-Chloro-6,7-dimethoxyisatin.—6,7-Dimethoxyisatin (0.1 g.) was dissolved in *t*-butyl alcohol (6 ml.) at 25—30°, and *t*-butyl hypochlorite¹⁷ (0.5 ml.) was added. After $\frac{1}{2}$ hr. at 40° the mixture was evaporated and the residue (90 mg.), in benzene, run on to a silica gel (50 g.) column (13 \times 1 cm.). A dark red zone was eluted with ether-benzene (1:4), followed by the main yellow band. The latter (40 mg., m. p. 206—207°), recrystallized from ethanol, had m. p. 212°, and was identical with the product from manganese dioxide oxidation of sporidesmin.

5-Chloro-6-hydroxy-7-methoxy-1-methylisatin.—5-Chloro-6,7-dimethoxy-1-methylisatin (0.31 g.) in 5N-sodium hydroxide (5 ml.) was heated at 90° for 3 hr. The cooled solution was extracted with chloroform and the raffinate just acidified with dilute sulphuric acid, a red precipitate being formed. This was extracted into chloroform, adsorbed on silica gel (10 \times 1.5 cm.), and a diffuse red zone eluted with chloroform. 5-Chloro-6-hydroxy-7-methoxy-1-methylisatin separated

¹⁵ Mancera, Rosenkranz, and Sondheimer, *J.*, 1953, 2189.

¹⁶ Wolff, *Org. Reactions*, 1946, 3, 307.

¹⁷ Chattaway and Backeberg, *J.*, 1923, 123, 2999.

from methanol as red needles, m. p. 221° (Found: C, 49.3; H, 3.6; Cl, 14.6; N, 5.7; O, 26.6; OMe, 11.3; *N*-Me, 6.4. $C_{10}H_8ClNO_4$ requires C, 49.8; H, 3.3; Cl, 14.7; N, 5.8; O, 26.6; OMe, 12.8; *N*-Me, 6.4%); solutions in ethanol-water (1:1) had pH 3.8, and a value 240 was obtained as the equivalent weight when such a solution was titrated with standard alkali, and a value 4.5 for the pK_a of the acid. Ultraviolet spectra were determined in water-ethanol (1:1) at the pH values listed in the Table, and also at pH 6; the latter spectrum was identical with that obtained at pH 11. In the Table, bands in parentheses are inflections. [ν_{\max} . (Nujol) 1724, 1610, (chloroform) 1739, 1608 cm^{-1}]. Further elution of the silica-gel column with methanol

pH	1.0	1.0	1.0	3.75	3.75	3.75	3.75	3.75	11.0	11.0	11.0	11.0	14.0	14.0
λ_{\max} . (m μ)	208	265	346	228	277	(284)	404	(465)	228	284	405	(465)	(250)	339
log ϵ	4.43	4.28	3.84	4.45	3.95		3.92		4.59	4.00	4.14		3.8	3.70

removed a red zone and the eluate had a strong blue fluorescence. Evaporation gave a gum (0.17 g.) which was distilled at 170–200° (bath)/30 mm. for 3 hr. The colourless oily distillate (24 mg.), λ_{\max} . (methanol, and methanol + NaOH) 215, 253, 303 m μ (log ϵ 4.41, 4.10, 3.37), λ_{\max} . (methanol + HCl) 255 (infl.), 270 m μ (infl.) (log ϵ ca. 3.2, 3.2), ν_{\max} . (film) 3550, 1600, 1500, 1470 cm^{-1} , provided a picrate, m. p. 131° (from water). The base reacted with nitrous acid but gave no dyestuff when the reaction mixture was treated with alkaline R-salt. On warming it with acetic anhydride, 4-chloro-2,3-dimethoxy-*N*-methylacetanilide was obtained, colourless rods (from water), m. p. 75° (Found: C, 53.6; H, 6.0; N, 5.95%; *M* (mass spectrometry) 243, 245. $C_{11}H_{14}ClNO_3$ requires C, 54.2; H, 5.2; N, 5.75%; *M*, 243.5), ν_{\max} . (Nujol) 1650 cm^{-1} (*N*-acetyl C=O).

1-Methylisatin.—1,1'-Dimethylindigo ^{4,6} (31 mg.) in methanol (5 ml.) was shaken with 15% sodium hydroxide (0.9 ml.) for 2 hr. at room temperature. The product (27 mg.), extracted from acid solution by chloroform, was adsorbed on silica gel in benzene and the main yellow zone eluted with benzene-ether (9:1). 1-Methylisatin formed yellow needles (12 mg.) (from aqueous ethanol), m. p. 130°, identical (m. p., mixed m. p., i.r. spectrum) with an authentic specimen.⁷

RUAKURA ANIMAL RESEARCH STATION,
HAMILTON, NEW ZEALAND.

[Received, January 21st, 1963.]