[CONTRIBUTION FROM THE INSTITUTE OF PAPER CHEMISTRY]

Liriodenine, A Nitrogen-Containing Pigment of Yellow Poplar Heartwood (*Liriodendron tulipifera*, L.)

M. A. BUCHANAN AND E. E. DICKEY

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The trivial name "liriodenine" is proposed for a yellow pigment isolated from yellow poplar heartwood. Liriodenine has the composition $C_{17}H_9O_3N$, and a melting point of 282°. Oxidation with chromic acid yields an acid which is decarboxylated at the melting point to form benzo[g]quinoline-5,10-dione. A second pigment containing methoxyl as well as nitrogen was also isolated, but the quantity of this material was not sufficient for proper characterization.

Colorless alkaloids have been reported in the bark of the yellow poplar tree (family *Magnoliaceae*) but they were not well characterized.[1,2] More recently, a lignan diglucoside was isolated from the inner bark.[3] The extractives of the wood, on the other hand, have received but little attention. Yellow poplar heartwood is usually olive-yellow to olive-brown in color, but in trees of rapid growth, the color may be distinctly yellow. On exposure to air and light, the surface darkens. The color of the wood is of some importance in the lumber and is a disadvantage in groundwood pulp prepared from this species.[4,5]

The benzene-soluble extractives of the heartwood consist of a complex mixture of substances, some of which are highly colored. Paper chromatography results in a yellow spot with a bright yellow fluorescence. Attempts to isolate the substance responsible for the yellow spot led to a crystalline pigment, which, however, was not fluorescent. The trivial name "liriodenine" is proposed for this pigment, which melts at 282° and has the composition $C_{17}H_9O_3N$. Liriodenine is basic and is extracted from benzene solution with dilute hydrochloric acid along with a mixture of other colored materials. It is slightly soluble in benzene or chloroform, but is nearly insoluble in ethanol and ether. It is soluble in aqueous acids, and forms a stable rose-colored solution in concentrated sulfuric acid which changes to yellow on addition of potassium nitrate.

The low ratio of hydrogen to carbon in liriodenine suggested the presence of a condensed ring system, but distillation with zinc dust did not form any recognizable products. Tests for methoxyl and methylenedioxy groups were negative. An acetate was not formed under any of the usual acetylating conditions. The absence of hydroxyl groups was supported by the lack of any absorption band in the $3\ \mu$ region in hexachlorobutadiene mulls, and by the lack of shift in the ultraviolet spectrum in alkaline ethanolic solution. A monooxime, m.p. 271°, was formed on refluxing with hydroxylamine in pyridine solution, indicating that one of the oxygens was present as a carbonyl group.

Liriodenine was reduced by catalytic hydrogenation to a colorless material which readily reverted to the original material on exposure to air. When warmed with 1% sodium hydroxide containing a small amount of sodium hydrosulfite and some ethanol to promote solubilization, liriodenine formed a blood-red solution which returned to yellow on shaking in the air. It was slowly decolorized to a strongly fluorescent solution by boiling with a mixture of aqueous acetic acid, dilute hydrochloric acid, and tin. The behavior with reducing agents suggested a quinone grouping similar to that present in anthraquinone.

Liriodenine when treated with chromic acid in dilute sulfuric acid solution formed a red-colored insoluble material which was resistant to oxidation and which may have been a chrome lake. In more concentrated sulfuric acid at elevated temperatures, chromic acid oxidation resulted in the formation of water-soluble materials which were not isolated. By proper choice of conditions, it was possible to oxidize liriodenine to a water-insoluble monocarboxy acid $C_{14}H_7O_4N$ with a melting point of 335–336°. The acid decomposed at the melting point with the evolution of gas and the formation of a crystalline sublimate which was identified as benzo[g]quinoline-5,10-dione by comparison with a sample synthesized by the method of Clemo and Driver.[6] The infrared spectra of the two materials were identical, and there was no depression in the mixed melting point.

Identification of the sublimed needles permits a partial formulation of liriodenine as I and the acid obtained on oxidation as II. A melting point of 355–357° has been reported[7] for benzo[g]quinoline-4-carboxy-5,10-dione, and thus it seems likely that the carboxyl group is not in the 4- position.

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(1) J. U. Lloyd and C. G. Lloyd, *Pharm. Rundsch.*, **4**, No. 8, 169 (1886).

(2) P. Morel and P. Totain, *Assoc. franc. avance. sci. Congrés Nîmes*, 41 Session, 810 (1912).

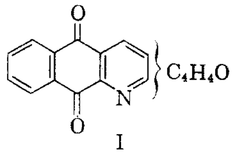
(3) E. E. Dickey, *J. Org. Chem.*, **23**, 179 (1958).

(4) S. D. Wells and J. D. Rue, U. S. Dept. Agr. Dept. Bull. No. 1485, 1927. 101 p.

(5) R. M. Kingsbury, F. A. Simmonds, and E. S. Lewis, *Tappi*, **32**, 273 (1949).

(6) G. R. Clemo and G. W. Driver, *J. Chem. Soc.*, 829 (1954).

(7) A. Etienne and A. Staehelin, *Bull. soc. chim. France*, 1954, 748; *Chem. Abstr.* **49**, 9620.



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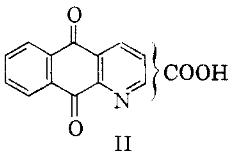
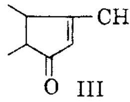


II

The benzo[g]quinoline-5,10-dione nucleus was previously demonstrated in the pigment phomazarine isolated form the fungus *Phoma terrestris* Hansen.[8,9] This pigment, like liriodenine, gave only a small amount of unidentified oil on zinc dust distillation and, on hydrogenation, gave a product readily oxidized to the original pigment.

The low ratio of hydrogen to carbon in liriodenine suggests that a fourth condensed ring is present, and the absorption band at 2952 cm.$^{-1}$ suggests the presence of a methyl group. The formation of a monooxime together with the lack of evidence for a hydroxyl group suggest that the third oxygen may be present as a carbonyl group. These conditions would be met by the presence of the ring structure III, but further evidence is needed to elucidate the nature of the fourth ring.



III

After working up the mother liquors to obtain the maximum yield of liriodenine, a chloroform solution was obtained which would not yield additional crystals but which still had a strong yellow coloration.

Evaporation of the solvent gave a dark-colored residue which on repeated crystallization from ethanol gave crystals of a second yellow to orange pigment. This second pigment forms the yellow spot with yellow fluoresence on paper chromatograms. Only small amounts of this pigment have been isolated, and the analytical data have been variable. Analysis for carbon and hydrogen, and estimation of the equivalent weight by titration suggest that the composition may be $C_{20}H_{17}O_5N$ with a molecular weight of 351. On the other hand, a single analysis for nitrogen and two of the methoxyl determinations suggest a molecular weight in the range of 320–330 with three methoxyl groups. At present, the composition of this pigment is still uncertain.

EXPERIMENTAL

Spectra. Infrared spectra (Fig. 1) were determined on potassium bromide wafers with the Perkin-Elmer Model 21 Spectrophotometer. Ultraviolet and visible spectra were determined with a Beckman Model DK-2 Spectrophotometer.

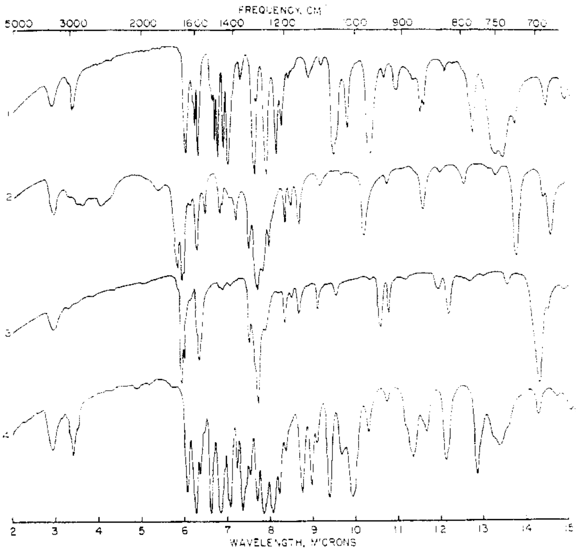


Fig. 1. Infrared absorption curves. I. Liriodenine. II. Acid obtained on oxidation. III. Sublimed needles (benzo[g]-quinoline-5,10-dione). IV. Second yellow pigment

Isolation. Sawdust prepared from yellow poplar heartwood was extracted with 3 l. benzene–95% ethanol 10:1 in a Soxhlet apparatus for 6 hr., and a second charge of sawdust was extracted with the same solvent. The extract from the two charges was concentrated under reduced pressure to about one-half volume. This removed the ethanol and caused the precipitation of some dark-colored material which was separated by filtration. The benzene solution was extracted with five 100-ml. portions of 1% hydrochloric acid. The combined acid solutions were neutralized by careful addition of solid sodium bicarbonate, and the liberated bases were extracted with chloroform. The residue obtained by evaporation of the chloroform was crystallized from chloroform using 4 ml. of solvent per g. of solid. The mother liquor solids were eluted from an alumina column with chloroform, and the eluent from the yellow band was collected. Crystallization of the solids from this fraction gave small amounts of additional yellow crystals. Average yields of the combined crystalline fractions were 0.08% (dry wood basis) from extraction of undried green sawdust, and 0.06% for seasoned lumber.

The once crystallized product usually melted in the range of 275–280°. It was purified to a melting point of 282° by further recrystallizations from chloroform, and by chromatography on an alumina column using chloroform as the eluting solvent.

Anal.[10] Calcd. for $C_{17}H_9NO_3$: C, 74.18; H, 3.30; N, 5.09; O, 17.44; molecular weight 275.2. Found: C, 74.17, 73.94; H, 3.28, 3.36; N, 5.07, 5.08; O, 17.52; molecular weight (by an ebullioscopic method using chloroform as solvent) 270.

$\lambda_{\max}^{C_2H_5OH}$ 247.4 mμ (log ϵ 4.22), 268.2 mμ (log ϵ 4.13), 309.2 mμ (log ϵ 3.62), 413 mμ (log ϵ 3.82). $\lambda_{\min}^{C_2H_5OH}$ 257.9 mμ (log ϵ 4.08), 291.9 mμ (log ϵ 3.51), 340 mμ (log ϵ 3.16), 455–700 mμ (log ϵ 0.0). $\lambda_{\max}^{0.1N\ HCl\ in\ C_2H_5OH}$ 256.7 mμ (log ϵ 4.33), 277.3 mμ (log ϵ 4.26), 329 mμ (log ϵ 3.67), 392 mμ (log ϵ 3.69), 455 mμ (log ϵ 3.58). $\lambda_{\min}^{0.1N\ HCl\ in\ C_2H_5OH}$ 268.7 mμ (log ϵ 4.20), 307 mμ (log ϵ 3.53), 362 mμ (log ϵ 3.55), 426 mμ (log ϵ 3.52), 545–700 mμ (log ϵ 0.00).

Oxime. A 0.257-g. portion of liriodenine purified to a melting point of 280–281° was refluxed for 0.5 hr. with 10 ml. of pyridine and 0.267 g. of hydroxylamine hydrochloride. The crystals dissolved after heating for 10 min. The cooled reaction mixture was stirred into 100 ml. of 2*N* acetic acid. The yellow precipitate was filtered, washed with water, and

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(8) F. Kögl and J. Sparenburg, *Rec. trav. chim.*, **59**, 1180 (1940).

(9) F. Kögl and F. S. Quackenbush, *Rec. trav. chim.*, **63**, 251 (1944).

(10) Except for the first sample of the second pigment, all analyses were by Huffman Microanalytical Laboratories, Wheatridge, Colo.

dried. The yield was 0.267 g. After crystallizing two times from *n*-butanol, the melting point was 271°.

Anal. Calcd. for $C_{17}H_{10}N_2O_3$: C, 70.34; H, 3.47; N, 9.65. Found: C, 69.68; H, 3.34; N, 9.45; residue from combustion 0.5%.

Oxidation with chromic acid. Liriodenine (0.1 g.) was dissolved in 8 ml. of 1:1 (v./v.) concd. sulfuric acid–water by warming, and the solution was diluted with 3 ml. of water. After cooling to room temperature, 0.2 g. of chromic oxide dissolved in 1 ml. of water and 4 ml. of 1:1 sulfuric acid–water was added gradually over a 1-hr. period. After standing at room temperature for 15–16 hr., the solution was diluted with 40 ml. of water and was heated in a steam bath for 1 hr. The final reaction mixture was cooled to room temperature, and the resulting light-colored product filtered and washed with water. The oxidation product was dissolved in dilute ammonium hydroxide and was reprecipitated by acidification with hydrochloric acid. The yield was 0.08 g. The crude product had a melting point of about 330°, and on melting formed a sublimate of yellow crystals. The material was soluble in hot formic, acetic, and nitric acids, but satisfactory conditions for recrystallization were not found. It was purified by dissolving in 2% ammonium hydroxide, heating the solution, and neutralizing with hydrochloric acid. After several such treatments, the melting point was 335–336° on rapid heating.

Anal. Calcd. for $C_{14}H_7O_4N$: C, 66.41; H, 2.79; N, 5.53. Found: C, 66.33; H, 2.83; N, 5.60.

$\lambda_{max}^{C_2H_5OH}$ 254 mμ (log ε 4.49), 325 mμ (log ε 3.50). $\lambda_{min}^{C_2H_5OH}$ 305 mμ (log ε 3.41).

Small amounts of the sublimed needles were obtained by mixing 10 mg. of the oxidation product with 20 mg. of precipitated calcium carbonate and heating in a 3-inch test tube to 335°. After resubliming, the needles melted at 275–277°. Benzo[g]quinoline-5,10-dione synthesized by the method of Clemo and Driver[6] melted at the same temperature, and the mixed melting point was not depressed.

$\lambda_{max}^{C_2H_5OH}$ 250 mμ (log ε 4.49), 326 mμ (log ε 3.46). $\lambda_{min}^{C_2H_5OH}$ 304 mμ (log ε 3.55).

Isolation of the second yellow pigment. In the isolation of liriodenine, the mother liquors were rechromatographed on alumina columns by eluting with chloroform as long as additional liriodenine could be crystallized from the material eluted as a yellow band. The mother liquors from these operations still had a very strong yellow color. They were combined, evaporated to dryness, and the solids crystallized from 95% ethanol to give a small amount of orange needles (0.01% dry wood basis). Further crystallizations from ethanol and from benzene gave a constant melting point of 235–236°.

Anal. Found: C, 68.54, 68.01; H, 4.90, 4.96; methoxyl, 33.5, 33.5; equivalent weight by titration, 359, 360.

$\lambda_{max}^{C_2H_5OH}$ 211 mμ (a 91.4), 245 mμ (a 86.5), 273 mμ (a 91.2), 355 mμ (a 30.3).

$\lambda_{max}^{C_2H_5OH}$ 228 mμ (a 62.5), 257 mμ (a 61.2), 318 mμ (a 18.2), λ 500–700 mμ (a 0.0).

The remaining crystals were recrystallized by dissolving in a small volume of chloroform and adding two to three volumes of low boiling petroleum ether. The resulting crystals melted at 235–236°.

Anal. Found: C, 68.60, 68.56; H, 4.77, 4.91; methoxyl, 28.8. Additional crystals melting at 235–236° were obtained from the mother liquors from isolation of the first product by crystallizing the mother liquor solids once from ethanol and once from benzene.

Anal. Found: C, 68.99; H, 4.93; N, 4.30; methoxyl, 28.3.

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APPLETON, WIS.

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

Some New Reactions and Reaction Products of Apogossypol and Desapogossypol[1]

DAVID A. SHIRLEY AND PEGGY W. ALLEY[2]

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The previously unreported desapogossypol was prepared by demethylation of the hexamethyl ether. Desapogossypol was converted to the hexaacetate, to desapogossypolone tetraacetate and to hydrodesapogossypolone octaacetate. The hexalllyl ether of apogossypol has been prepared and carried through a Claisen rearrangement which involved two *ortho*- and two *para*-rearrangements in each molecule. Epoxidation of apogossypolone tetramethyl ether and desapogossypolone tetramethyl ether gave in both cases the corresponding 2,3,2′,3′-diepoxy derivatives.

Gossypol (I), the principal pigment of cottonseed, must be chemically altered or removed during the processing of cottonseed for most uses. There is potentially available about 30,000 tons of gossypol per year from cotton produced in the United States. The large amount of gossypol available, the reactive nature of the molecule, and its deleterious effects in cottonseed processing and utilization are the reasons for a continuing program in these laboratories on the chemistry of gossypol and closely related derivatives. Our earlier work has involved a study of the reduction of gossypol with lithium aluminum hydride[3] and the formation of gossypol anils with a wide variety of primary amines.[4]

Apogossypol (II), apogossypol hexamethyl ether

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(1) A report of work conducted under contract with the U. S. Department of Agriculture and authorized by the Research and Marketing Act. The contract is being supervised by the Southern Utilization Research and Development Division of the Agricultural Research Service.

(2) Post-doctoral Research Fellow, 1958–59.

(3) D. A. Shirley and W. C. Sheehan, *J. Am. Chem. Soc.*, **77**, 4606 (1955).

(4) (a) D. A. Shirley and W. C. Sheehan, *J. Org. Chem.*, **21**, 251 (1956); (b) P. W. Alley and D. A. Shirley, *J. Org. Chem.*, **24**, 1534 (1959).