2-(2-Aminophenyl)-4,5-methylenedioxybenzyl Alcohol (X).—A solution of 2-(2-nitrophenyl)-4,5-methylenedioxybenzyl alcohol (1.0 g.) in 100 ml. of absolute alcohol was hydrogenated in a Parr shaker for 20 min. at 32 p.s.i. over platinum dioxide. The solution was filtered and the colorless filtrate was concentrated at reduced pressure, leaving an oily residue of the amino alcohol. The picrate, crystallized from ethanol, crystallized with 1 mole of solvent and melted at 150–154°.

Anal. Calcd. for $C_{14}H_{13}NO_3 \cdot C_6H_3N_3O_7 \cdot C_2H_6O$: C, 50.97; H, 4.28; N, 10.81. Found: C, 51.28; H, 4.67; N, 11.12.

Ismine (I).—To a solution of the 2-(2-aminophenyl)-4,5-methylenedioxybenzyl alcohol prepared above, in 10 ml. of pyridine, was added 6 ml. of ethyl chlorocarbonate, and the solution was kept overnight at room temperature. The red solution was poured into 150 ml. of water and extracted with three 50-ml. portions of ether. The ether extracts were washed with 2% hydrochloric acid, dried, and concentrated. The residual oil, showing broad carbonyl absorption at 5.7–5.8 μ, was taken up in 100 ml. of dry tetrahydrofuran and reduced with a threefold excess of lithium aluminum hydride at reflux for 20 hr. The mixture was cooled, diluted with 100 ml. of tetrahydrofuran, and treated cautiously with saturated aqueous sodium sulfate to destroy the excess hydride. The inorganic salts were filtered and the filtrate was concentrated. The colorless oily residue crystallized on the addition of a seed crystal of ismine, yielding 0.36 g. (38%), m.p. 76–87°. Recrystallization from 30% ethanol gave material of m.p. 93.5–95°, not depressed by admixture with an authentic sample,[8] m.p. 95–97.5°. The infrared spectra of the synthetic and authentic samples were superimposable. The picrate of the synthetic material melted at 157–159°, not depressed by mixing with a sample of authentic ismine picrate, m.p. 162–164°.

6-Cyanopiperonal (XII).—A mixture of 6-bromopiperonal[13] (54.4 g.), ethylene glycol (15.5 g.), and *p*-toluenesulfonic acid (2.0 g.) in benzene (450 ml.) was refluxed with a Dean–Stark trap for 4 hr., during which time 4.3 ml. of water (calcd. 4.5 ml.) was collected. After standing overnight, the benzene solution was washed with 10% sodium bicarbonate, dried, diluted with 200 ml. of heptane, and concentrated. On cooling, 45.2 g. of colorless acetal, m.p. 58–64°, was collected. The infrared spectrum showed only a trace of carbonyl absorption, and the acetal was used without further purification.

A mixture of the acetal (24.15 g.), cuprous cyanide (10.0 g.), and pyridine (5.0 ml.) was heated at 185° for 16 hr. Benzene (100 ml.) and ammonium hydroxide (100 ml.) were added to the still warm solution, the lumps were broken up, and the salts were filtered. The filtrate was diluted with 400 ml. of ether, the layers were separated, and the organic layer was washed successively with dilute ammonium hydroxide (30 ml.), 3 *N* hydrochloric acid (two 30-ml. portions), 5% sodium bicarbonate (two 30-ml. portions), water (two 30-ml. portions), and saturated salt solution. Concentration of the dried solution gave 7.55 g. (39%) of 6-cyanopiperonal ethylene acetal, m.p. 85–92°.

The cyano acetal (3.19 g.) was stirred for 5 min. with 20 ml. of 5% hydrochloric acid at 40–50°. The solid was collected, washed with water, and dried, giving 2.55 g. (100%) of cyanoaldehyde. After recrystallization from benzene, the pale yellow crystals melted at 162–164°, and showed infrared bands at 4.47 and 5.92 μ.

Anal. Calcd. for $C_9H_5NO_3$: C, 61.72; H, 2.88; N, 8.00. Found: C, 61.89; H, 2.98; N, 8.07.

(13) A. Oelker, *Ber.*, **24**, 2592 (1891).

Nuclear Magnetic Resonance Spectra and Stereochemistry of the Antibacterial Principle from *Haematoxylon braziletto*[1]

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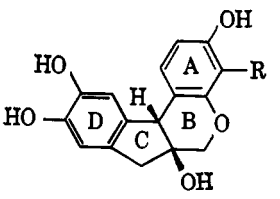
Aqueous extracts of the red heartwood of *Haematoxylon braziletto* gave brazilin (Ia), identified as its tetraacetate. N.m.r. spectroscopy confirmed its structure and that of the related haematoxylin (Ib) as containing an additional hydroxy group at C-4. The reactions of Ia indicate rings B and C to be *cis* fused, and optical rotatory dispersion shows the stereochemistry of Ia and Ib to be identical.

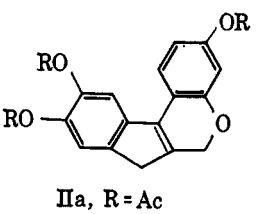
In Mexico and Lower California, sticks of the red heartwood of *Haematoxylon braziletto* have been employed for centuries for addition to drinking water for man and animals. The wood derives its name from the word *braza*, meaning fiery red, and has no geographic connection with Brazil or with the common brazilwood of the literature, *Caesalpinia echinata* or *Caesalpinia brasilensis*.

The antibacterial activity of the heartwood extracts has been previously reported.[2] The aqueous extracts, on isolation with ether, afforded a phenolic material which was acetylated at room temperature and gave an acetyl derivative shown by thin layer chromatography to consist of more than 60% of one compound, m.p. 149–151°. It was found to be identical by mixture melting point, infrared spectrum, and thin layer chromatography with a specimen of brazilin tetraacetate, m.p. 149–151°, prepared from brazilin (Ia) isolated from the South American tree *Caesalpinia echinata*.

It was of interest to examine the stereochemistry of brazilin and of the closely related haematoxylin (Ib), isolated from *Haematoxylon campechianum*. The



Ia, R=H
b, R=OH



IIa, R=Ac
b, R=Me

structures of both brazilin and haematoxylin as polyhydroxybenzindenopyrans are known from the work of Perkin and Robinson,[3] but the stereochemical cor-

(1) Supported in part by a grant (HE-5881) from the National Institutes of Health, U. S. Public Health Service.

(2) R. Pratt and Y. Yuzuriha, *J. Am. Pharm. Assoc., Sci. Ed.*, **48**, 69 (1959).

(3) (a) W. H. Perkin and R. Robinson, *J. Chem. Soc.*, **91**, 1073 (1907); (b) R. Robinson in "Chemistry of Carbon Compounds," Vol. IV, part B, E. H. Rodd, Ed., Elsevier Publishing Co., Inc., New York, N. Y., 1959, p. 1005; (c) R. Robinson, *Bull. soc. chim. France*, 125 (1958).

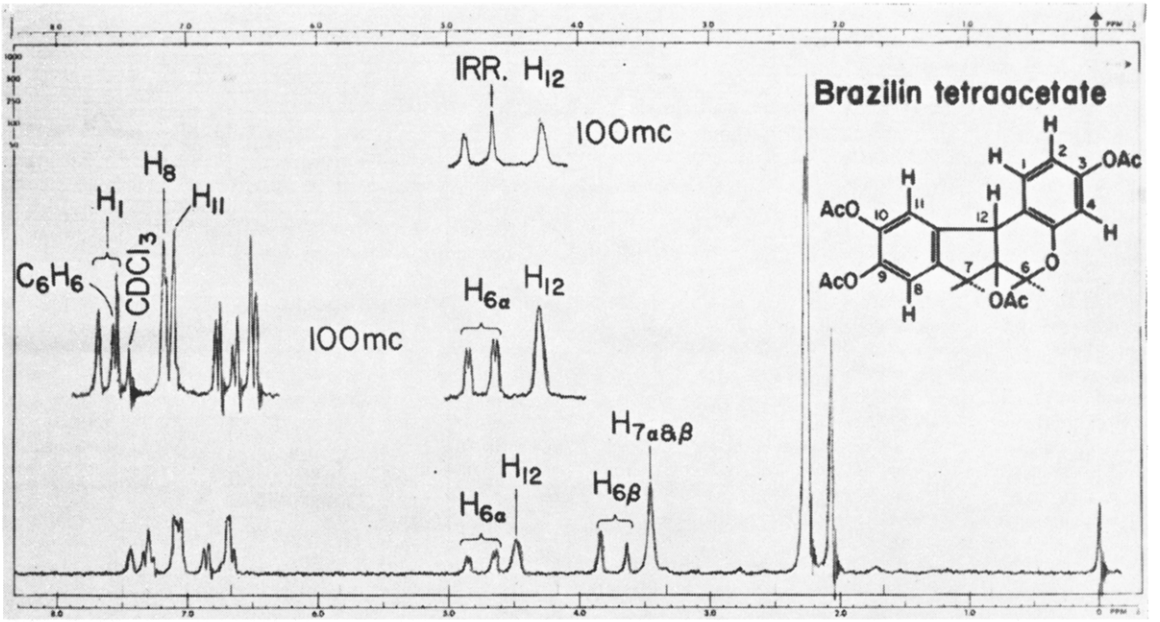


Figure 1.—N.m.r. spectrum of brazilin tetraacetate in deuteriochloroform solution.

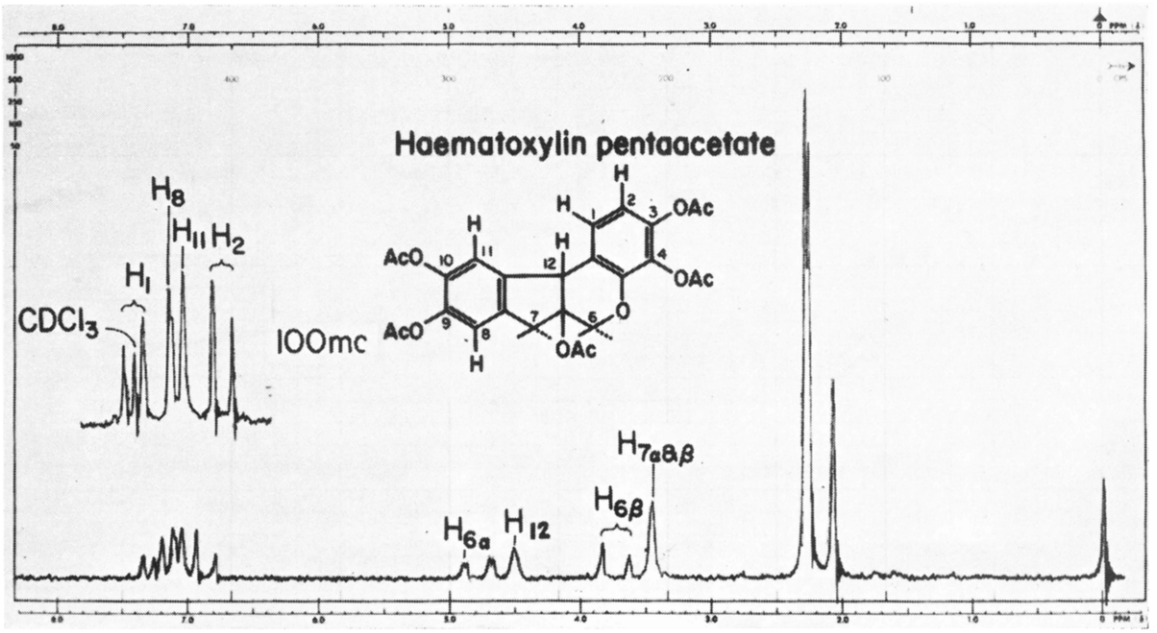


Figure 2.—N.m.r. spectrum of haematoxylin pentaacetate in deuteriochloroform solution.

respondence of the two compounds has not been established. In addition, the use of n.m.r. spectroscopy offered a means of confirming the location of the hydroxyl groups in the structure of Perkin and Robinson.

The n.m.r.[4] spectra in deuteriochloroform solution of brazilin tetraacetate and haematoxylin pentaacetate are shown in Figures 1 and 2. Both spectra were identical in the region of the aliphatic hydrogens (3.0 to 5.0 p.p.m.) where five hydrogens were located. A signal at δ 3.45 arises from the two hydrogens at C-7, where the deshielding effect on the 7β-H (*cis* to acetoxy) of the carbonyl group of the adjacent acetate is felt much more than in the case of the 7α-H, and has brought the 7β-H further downfield than it has the 7α-H, so that the chemical shifts are now very close.

(4) N.m.r. spectra were determined using a Varian A-60 spectrometer. Chemical shifts (δ) are given in p.p.m. downfield from an internal tetramethylsilane standard.

The hydrogens at C-6 are not equivalent, indicating that the pyran ring (ring B) exists in either a half-chair or a half-boat conformation (inversion of this ring results in a change from axial to equatorial for these hydrogens). They exhibit geminal coupling with $J_{6\alpha,6\beta}$ = 12.5 c.p.s. The 6β-H (equatorial) shows a doublet at 3.73 p.p.m. ($J_{6\alpha,6\beta}$ = 12.5 c.p.s.) while the 6α-H (axial) has slight coupling through 4σ-bonds to the hydrogen on C-12 with a dihedral angle close to 180 or 0° (Courtauld models) between the atoms, causing its doublet at δ = 4.75 p.p.m. to show splitting with $J_{6\alpha,12}$ = 2 c.p.s. The resonance at 4.50 p.p.m. is due to the hydrogen at C-12, and the existence of coupling between this and the 6α-H was verified by double irradiation at the frequency of the former, which caused the doublet (J = 2 c.p.s.) at 4.65 p.p.m. to degenerate into a singlet. (Since the chemical

TABLE I

CHEMICAL SHIFTS AND COUPLING CONSTANTS[a]

Compd.	Me of aromatic acetate	Me of aliphatic acetate	H at C-1	H at C-2	H at C-4	H at C-8	H at C-11
Brazilin tetraacetate	2.26 (9)	2.06 (3)	7.35 (1) $J_{1,2} = 8$	6.85 (1) $J_{1,2} = 8$ $J_{2,4} = 2$	6.69 (1) $J_{2,4} = 2$	7.10 (1)	7.04 (1)
Haematoxylin pentaacetate	2.26 (12)	2.06 (3)	7.35 (1) $J_{1,2} = 8$	6.85 (1) $J_{1,2} = 8$	...	7.10 (1)	7.04 (1)

[a] δ in p.p.m., J in c.p.s.; number of protons in parentheses.

shifts of the 6α-hydrogen signals are close, only that part of the signal at 4.65 p.p.m. was observed.)

The assignments of the protons at C-6 and C-7 are based on the fact that protons on a carbon atom attached to oxygen are more deshielded than benzylic protons. For example, the benzylic protons in 2-phenethyl acetate and β-phenylpropionaldehyde resonate at 2.93 and 2.97 p.p.m., respectively,[5] while the methylene protons adjacent to the phenolic oxygen in *p*-bromophenetole and *p*-phenetidine appear at 3.93 p.p.m. in both cases, and that of 2-phenoxyethanol at 3.97 $\pm$ 0.1 p.p.m.[5]

The chemical shift data for the methyl protons and for the aromatic protons are summarized in Table I, which also shows the coupling constants wherever applicable and the number of hydrogens in each peak in parentheses. The chemical shifts of the C-2 and C-4 hydrogens are very close, both being adjacent to the oxygen function at C-3, with that at C-4 at slightly higher field due to additional *ortho* shielding by the oxygen of the pyran ring. The hydrogen at C-1, which is in the *meta* position to the oxygen at C-3, is not shifted with respect to the hydrogens of benzene at δ 7.32. In the case of the C-8 hydrogen, some long-range coupling of less than 1 c.p.s. is shown, which disappeared on double irradiation at the frequency of the C-7 hydrogen, showing its origin to be splitting by the latter. The absence of *ortho* or *meta* coupling by either the C-8 or the C-11 hydrogen confirms their *para* orientation in ring D in both spectra, while the absence of the signal for the C-4 hydrogen in the spectrum of haematoxylin pentaacetate and the existence of *ortho* coupling, but no *meta* coupling, for the C-2 hydrogen resonance supports the location of the additional oxygen function in haematoxylin as C-4, and also the structure of brazilin as 7,12-dihydrobenz[*b*]indeno[1,2-*d*]-pyran-3,6a,9,10(6H)-tetrol (Ia).

The spectra of brazilin and haematoxylin, run in trifluoroacetic acid–deuteriochloroform, were identical except for the number of hydrogens in the aromatic multiplet (six and five, respectively), and only that of haematoxylin is shown (Figure 3). The two signals for the C-7 hydrogens are now nonequivalent and well separated because of the smaller deshielding effect of the adjacent hydroxyl group compared with that of the acetoxy group and appear at 2.85 and 3.25 p.p.m. as a broad quartet ($J_{7\alpha,7\beta} = 17$ c.p.s.). The C-6 hydrogen resonances are also nonequivalent and form an AB system at 3.80 and 4.17 p.p.m. ($J_{6\alpha,6\beta} = 12$ c.p.s.). Here the chemical shifts of the two hydrogens at C-6 are closer than in the acetoxy compound, again because the deshielding effect of the adjacent hydroxyl group is less than that of the acetate group in the previous spectra, where the axial hydrogen at C-6 (6α) had moved further downfield on acetylation of the adjacent tertiary hydroxyl than had the equatorial hydrogen (6β).

Of the two possible conformations for ring B, we believe the half-chair form to be the more likely (requiring the 6α-H to be axial upwards) since it relieves steric repulsion between the hydrogens at C-1 and C-11 which is evident in the half-boat conformation (Courtauld models) of ring B.

The resonance for the hydrogen at C-12 is at 4.17 p.p.m., and overlaps one of the peaks of the quartet due to the C-6 hydrogens. In the aromatic region the hydrogens at C-1 and C-2 form an AB system at 6.62 and 6.82 p.p.m. with $J_{1,2} = 7$ c.p.s., while the C-8 and C-11 hydrogen signals coincide in a singlet at 6.73 p.p.m.

Since this work was completed, a synthesis of racemic brazilin has been reported[6] which further confirms structure Ia. The nature of the ring fusion of rings B and C seems established as *cis*[7] for the following reasons: (1) the aliphatic tertiary acetate is resistant to boiling acetic anhydride; (2) the dehydrated substance II has been synthesized, both in the form of its triacetate[8] (IIa) and of its trimethyl ether[9] (IIb), and found to be stable; and (3) brazilin tetraacetate may be induced to lose acetic acid in a catalyzed pyrolysis[10] to give the same olefinic triacetate. It remained to establish the stereochemical correspondence of brazilin and haematoxylin. These show $[\alpha]_D$ +121° and +99°, respectively. The lack of dehydration of haematoxylin pentaacetate with boiling acetic anhydride, and the virtual identity of the optical rotatory dispersion curves of the two acetates (Figure 4)[11] confirm their stereochemical correspondence in having the *cis* B/C ring junction. The O.R.D. curves are in excellent agreement with the ultraviolet absorption (λ_{max} 274 and 275 mμ, respectively, with a shoulder at 283 mμ in both cases).

(5) (a) N. S. Bhacca, L. F. Johnson, and J. N. Shoolery, "N.M.R. Spectra Catalog," Vol. 1, Varian Associates, Palo Alto, Calif., 1962, No. 198, 208, 261; (b) N. S. Bhacca, D. P. Hollis, L. F. Johnson, and E. Pier, "N.M.R. Spectra Catalog," Vol. 2, Varian Associates, Palo Alto, Calif., 1963, No. 506, 529.

(6) O. Dann and H. Hofmann, *Ann. Chem.*, **667**, 116 (1963).

(7) W. B. Whalley, Symposium on Vegetable Tannins, Cambridge, England, April 1956, p. 151; Society of Leather Trades Chemists, Croydon, 1956.

(8) J. Herzig and J. Pollak, *Chem. Ber.*, **38**, 2166 (1905); **39**, 265 (1906).

(9) (a) W. H. Perkin, J. N. Ray, and R. Robinson, *J. Chem. Soc.*, 2094 (1927); 1504 (1928); (b) A. W. Gilbody and W. H. Perkin, *ibid.*, **81**, 1040 (1902).

(10) W. D. Ollis, private communication in ref. 3b, p. 1021.

(11) Optical rotatory dispersion curves were determined in 95% ethanol at 25° using a Bendix Model 460-C spectropolarimeter.

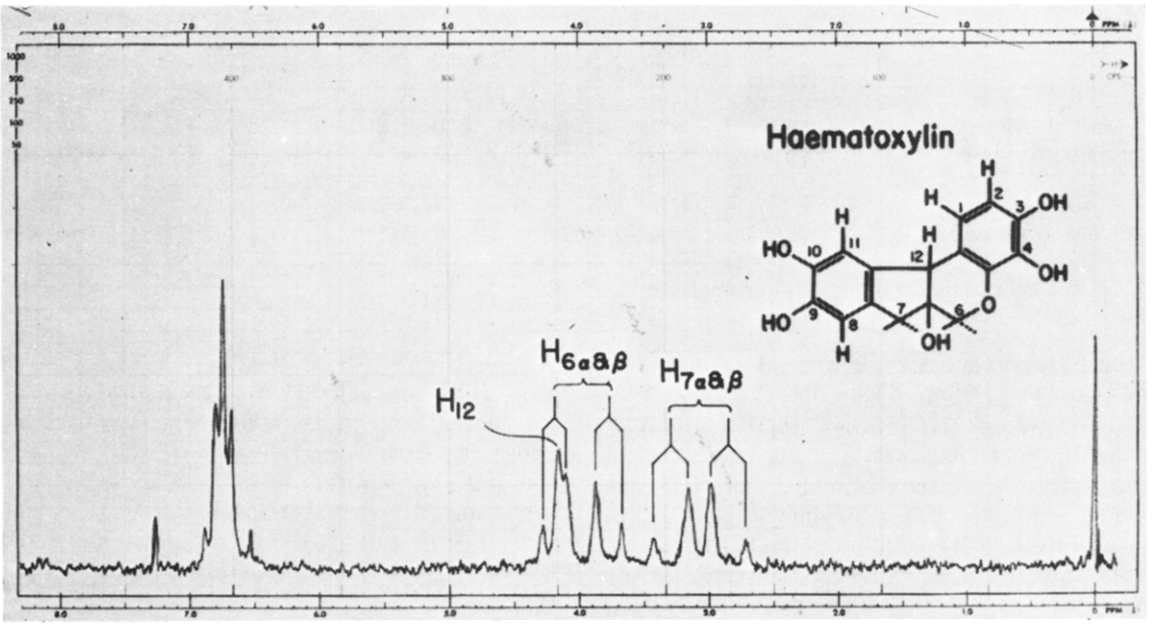


Figure 3.—N.m.r. spectrum of haematoxylin in trifluoroacetic acid–deuteriochloroform mixture.

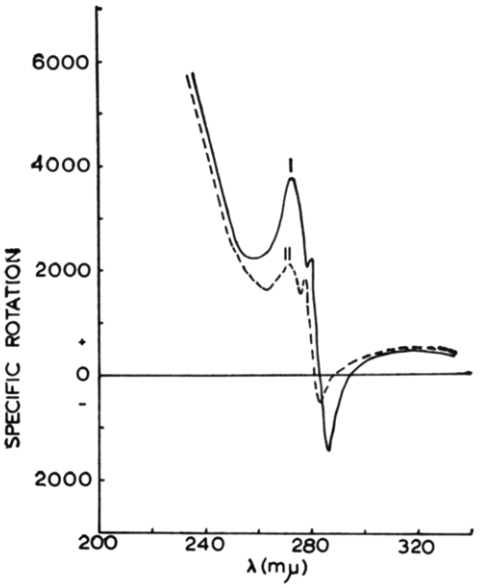


Figure 4.—Optical rotatory dispersion of brazilin tetraacetate (curve I, ———) and haematoxylin pentaacetate (curve II, – – – –).

Experimental

Extraction and Isolation.—The red heartwood of *Haematoxylon braziletto* was powdered with a rasp, and 10 g. of this powder was shaken with 50 ml. of distilled water for 12 hr. The extract was filtered, and the filtrate was acidified with 1 *N* hydrochloric acid. The combined filtrates from seven such extractions were thoroughly extracted with ether, and the ether solution was dried over anhydrous calcium chloride. Removal of solvent at room temperature left 1.1 g. (1.6%) of reddish amorphous residue.

Acetylation of the Crude Extract.—A solution of 0.55 g. of the crude extract in 20 ml. of acetic anhydride was treated with 5 mg. of *p*-toluenesulfonic acid and kept for 36 hr. at room temperature. The solution was then treated with ice–water, neutralized with sodium bicarbonate, and extracted with ether. The dried (sodium sulfate) ethereal extracts on evaporation left 0.6 g. of white solid which was chromatographed on a silica gel column. Elution with 9:1 benzene–chloroform gave 400 mg. of white crystals: m.p. 149–151°; ν_{max} 1767 (C=O of phenol acetate), 1739 (C=O of aliphatic acetate), and 1212 cm.$^{-1}$ (C–O of phenol acetate). Thin layer chromatography on silica using chloroform as solvent gave a single spot, R_f 0.4. The material was identified as brazilin tetraacetate (authentic sample,[12] m.p. 149–151°) by mixture melting point, identity of infrared spectra, and the thin layer chromatogram. The rotatory dispersion (*c* 0.083, 95% ethanol) was $[\alpha]_{333}$ +390°, $[\alpha]_{286}$ −1418° (trough), $[\alpha]_{281}$ +2208° (peak), $[\alpha]_{279}$ +2055° (trough), $[\alpha]_{278}$ +3759° (peak), $[\alpha]_{260}$ +2249° (trough), and $[\alpha]_{238}$ +5490°.

Haematoxylin pentaacetate showed the following rotatory dispersion (*c* 0.092, 95% alcohol): $[\alpha]_{333}$ +398°, $[\alpha]_{283}$ −459° (trough), $[\alpha]_{278}$ +1795° (peak), $[\alpha]_{276}$ +1552° (trough), $[\alpha]_{278}$ +2090° (peak), $[\alpha]_{260}$ +1701° (trough), and $[\alpha]_{235}$ +5689°.

(12) C. Liebermann and O. Burg, *Chem. Ber.*, **9**, 1883 (1876).