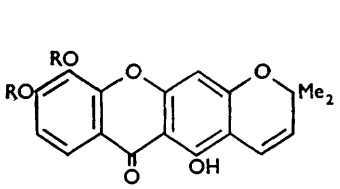
114. *An Investigation of the Gibbs Reaction and its Bearing on the Constitution of Jacareubin.*

By F. E. KING, T. J. KING, and L. C. MANNING.

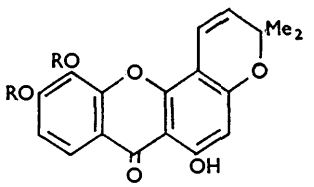
Experiments on the action of 2 : 6-dichlorobenzoquinone chloroimide on a variety of phenols have shown that the appearance of a colour (Gibbs reaction) does not invariably indicate the formation of an indophenol. The presence of this chromophore, which is diagnostic of unsubstituted CH *para* to a phenolic group, can be demonstrated by its characteristic absorption in the 500—700 mμ region. Since indophenol formation can thus be established in the case of 5 : 6-dimethyljacareubin, it is possible to decide between the alternatives (I; R = H) and (II; R = H) suggested for jacareubin in favour of the linear formulation (I; R = H).

THE formation of indophenols from 2 : 6-dichlorobenzoquinone chloroimide (Gibbs reagent) has been developed into a sensitive colour reaction which has been widely used for the detection of unsubstituted CH *para* to a phenolic group.[1,2,3] The test is best performed in a borate buffer of pH 9·24 and, if positive, results in a blue colour which Gibbs has shown may be quantitatively estimated by observing the light absorption at 610 mμ. Difficulties occur in applying the reaction to sparingly soluble and weakly acidic compounds, which do not dissolve in the borate buffer or in the alternative aqueous sodium hydrogen carbonate recently recommended by Schmid and Burger,[2] so ethanol has been added to the buffer to increase its solvent properties.[3] With very weak phenols, which only slowly combine with the reagent, direct visual interpretation of the result is confused by the gradual discoloration of the reagent at the required pH, and this complication is particularly marked when the condensation product imparts a less intense green-blue colour to the test solution.

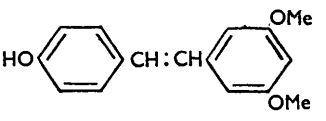
The ambiguities of the Gibbs reaction were apparent during its use in investigations of jacareubin, a deep yellow constituent of the heartwood of *Calophyllum brasiliense*.[4] It has been shown that the pigment is a trihydroxypyranoxanthone, but the evidence derived from degradations does not differentiate between the linear (I; R = H) and the angular structure (II; R = H). Since di-*O*-methyljacareubin has structure (I or II;



(I)



(II)



(III)

R = Me), it should be possible to settle the constitution by means of the Gibbs reaction. However, when carried out as hitherto described, the test proved indecisive owing to the

[1] Gibbs, *J. Biol. Chem.*, 1927, **72**, 649.
[2] Schmid and Burger, *Helv. Chim. Acta*, 1952, **35**, 932.
[3] Davidson, Keane, and Nolan, *Sci. Proc. Roy. Dublin Soc.*, 1943, **23**, 143.
[4] King, King, and Manning, *J.*, 1953, 3932.

sparing solubility of the ether. By experiments with a number of phenols it was shown that addition of the reagent in borate buffer to a dilute solution of a suitable phenol in *pyridine* gives a blue or blue-green colour within 10 seconds. With pyridine alone a muddy green solution was obtained in approximately 2 minutes. Di-*O*-methyljacareubin in pyridine rapidly gave a blue-green colour, thereby indicating the linear constitution (I) for jacareubin and its derivatives.

In order to establish the validity of this result 26 phenols of various types were submitted to the test under the new conditions; tri-*O*-methyljacareubin was included and gave the expected negative result. Colour reactions were obtained without exception from phenols unsubstituted in the *para*-position; in addition it has been shown that the reaction is not inhibited by *p*-halogen and *p*-carboxyl substituents, confirming observations by Davidson, Keane, and Nolan.[3] On the other hand, pterostilbene [5] (III) and 4 : 4′-dihydroxychalkone, though fully substituted in the *para*-position, also gave positive reactions. In view of this unexpected result, the colour reactions of all the compounds under investigation were submitted to examination in a spectrophotometer.

The spectrum of the simple dichloroindophenol has been determined by Gibbs,[1] and by Merrill, Spencer, and Getty,[6] who gave $\lambda_{max.}$ 605 mμ. The presence of substituents, particularly if unsaturated, has a marked effect on the absorption of the derived indophenol in the 600 mμ region, but for the monohydric phenols investigated by us the maxima lie between 590 mμ (*o*-cresol) and 665 mμ (tetra-*O*-methylquercetin). The products from two of the three dihydric phenols, however, have absorption maxima slightly outside this range. The nature of the phenol also noticeably influences the rate of formation and stability of the indophenol, as measured spectrophotometrically. Thus, with phenol, maximum absorption was observed in 2 hours, and decomposition was negligible during 24 hours, whereas with di-*O*-methyljacareubin maximum absorption was reached in 30 minutes, the intensity falling after a further 60 minutes to approximately 70% of the maximum value. In the case of dihydrodi-*O*-methyljacareubin the absorption band, which had attained a maximum value within 6 minutes, had disappeared after a further hour. Moreover, slight changes (up to 15 mμ) of $\lambda_{max.}$ with time were also observed, but in spite of these variations every compound unsubstituted in the position *para* to the phenolic hydroxyl group gave within 20 minutes a coloured product exhibiting (cf. Table 1) the typical indophenol absorption band. The coloured solutions obtained from pterostilbene and 4 : 4′-dihydroxychalkone, on the other hand, have no absorption peak in the 500—700 mμ region; they are therefore included in Table 2 with other phenols having no free activated *para*-position and not giving a colour reaction with the Gibbs reagent.

On the assumption of complete reaction at maximum absorption, extinction coefficients for some of the phenols are included in Table 1. It is to be expected that values will vary

TABLE 1. *Wavelengths* (mμ) *and intensities* (*in parentheses*).

Phenol, 605 (10,450)	*p*-Hydroxybenzoic acid, 610
1-Hydroxy-3 : 5 : 6-trimethoxyxanthone, 655 (10,000)	Methyl salicylate, 635
Di-*O*-methyljacareubin, 650 (11,000)	Methyl 5-bromosalicylate, 635
Dihydrodi-*O*-methyljacareubin, 650 (10,500)	*p*-Chlorophenol, 605
3 : 7 : 3′ : 4′-Tetra-*O*-methylquercetin, 665 (11,750)	*o*-Chlorophenol, 625
5-Hydroxy-3 : 7 : 3′ : 4′ : 5′-pentamethoxyflavone, 650	*o*-Cresol, 590
5-Hydroxy-7 : 4′-dimethoxy*iso*flavone, 630	Orcinol, 665
2-Hydroxy-ω : 4 : 6-trimethoxyacetophenone, 655	Resorcinol, 575
Vanillic acid, 610	Catechol, 675

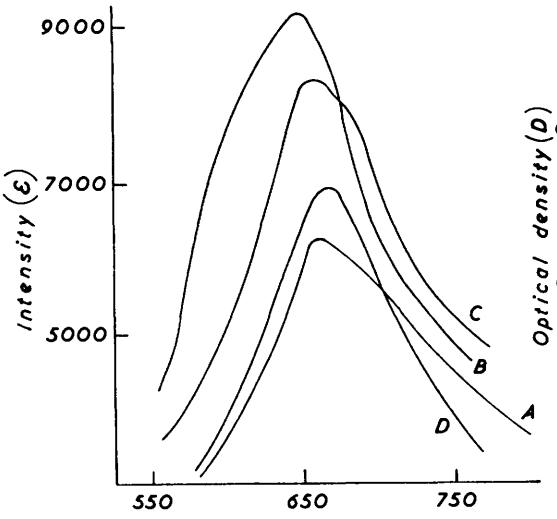
with pH, and this is demonstrated by the figure for indophenol (10,450) determined at pH 9·2 approx. (borate buffer) and for the pure sodium salt (18,000).[6] However, as they have been obtained by a uniform procedure at constant pH (9·2), the extinction coefficients given are believed to be strictly comparable and are in satisfactory agreement. For

[5] Spath and Schlager, *Ber.*, 1940, **73**, 1, 881; see also King, Cotterill, Godson, and King, *J.*, 1953, 3693.

[6] Merrill, Spencer, and Getty, *J. Amer. Chem. Soc.*, 1948, **70**, 2460.

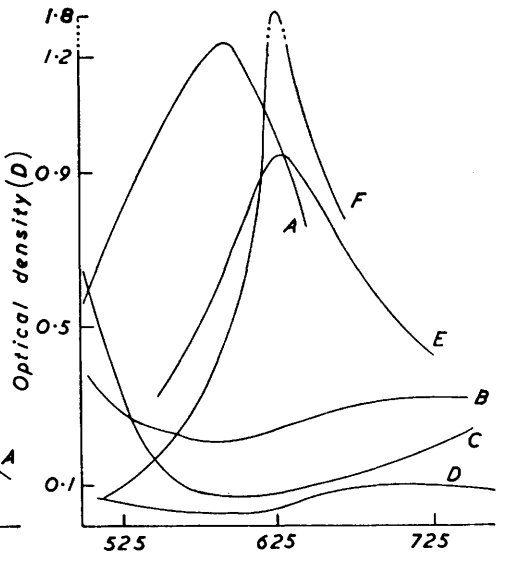
unstable indophenols it was found that, after reaction to a maximum value, the observed optical density (*D*) decreased linearly with time, and the values quoted in Table 1 were obtained by extrapolation of the descending linear part of the *D*–time curve to zero, the *D* value so obtained being used in the calculation of ε.

FIG. 1.



A, *Di*-O-*methyljacareubin*.
B, *Dihydrodi*-O-*methyljacareubin*.
C, 1-*Hydroxy*-3 : 5 : 6-*trimethoxyxanthone*.
D, 3 : 7 : 3′ : 4′-*Tetramethylquercetin*.

FIG. 2.



A, o-*Cresol*.
B, p-*Cresol*.
C, *Pterostilbene*.
D, 7-*Hydroxy*-5 : 4′-*dimethoxy*isoflavone.
E, 5-*Hydroxy*-7 : 4′-*dimethoxy*isoflavone.
F, *Methyl salicylate*.

Fig. 1 reproduces the light-absorption curves for those compounds (except phenol) examined quantitatively (constructed from data recorded at the moment of maximum intensity). Absorption curves typical of those obtained by the qualitative procedure are shown in Fig. 2, including those relating to the anomalous phenol pterostilbene and two others from Table 2.

TABLE 2. *Compounds giving negative tests.*

7-Hydroxy-5 : 4′-dimethoxy*iso*flavone	Acetovanillone
4 : 4′-Dihydroxy-3 : 3′-dimethoxystilbene	Aesculetin
Pterostilbene	Demethylsuberosin [7]
4 : 4′-Dihydroxychalkone	*p*-Cresol

EXPERIMENTAL

Determinations were carried out with a Unicam model S.P. 500 spectrophotometer. The pyridine used was of "AnalaR" quality and the dichlorobenzoquinone chloroimide was crystallised to constant m. p.

Qualitative Procedure.—A small quantity (1—3 mg.) of the liquid or powdered substance to be tested was placed in a measuring cylinder (25 ml.) and dissolved in pyridine (1 ml. pipette). To the solution was added a freshly prepared solution of Gibbs reagent (5 ml.) in pyridine (20—30 mg./15 ml.) from a burette and the mixture was diluted to 20 ml. with sodium borate buffer (pH 9·2) and well mixed. The absorption spectrum was determined between 10 and 20 min. after mixing, a precisely similar solution of the chloroimide in pyridine–borate being used in the solvent cell of the spectrophotometer. Any necessary dilution was carried out by using the aqueous buffer solution.

[7] King, Housley, and King, *J.*, 1954, 1392.

Quantitative Procedure.—This was carried out essentially as above but the test substance (approx. 1 mg.) was accurately weighed and the solutions were made in calibrated flasks. The time of mixing was noted, and determinations of the position and intensity of the absorption maximum were repeated until a steady value was attained or the rate of decrease of intensity was approximately constant.

THE UNIVERSITY, NOTTINGHAM. [*Received, September 6th,* 1956.]