906. *Compounds of Curcumin and Boric Acid. Part I. The Structure of Rosocyanin.*

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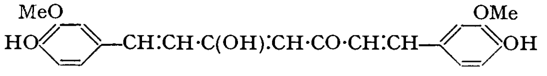
Rosocyanin, the compound formed when curcumin reacts with boric acid in the presence of a mineral acid, has been shown to contain two curcumin molecules co-ordinated around a boron atom, associated with one equivalent of an anion. The probable structural formula and electronic configuration of the molecule have been suggested on the basis of spectrophotometric, ion-exchange, and other studies.

THE red coloration when turmeric extract or its active principle curcumin reacts with boric acid is the basis of the most sensitive spot test for boron. This reaction is increasingly important in agricultural and metallurgical analysis, techniques based on that used by Naftel (*Ind. Eng. Chem., Anal.*, 1939, **11**, 407) being, in our opinion, the most satisfactory. Such methods are, however, still empirical and occasionally erratic. It is therefore desirable to know the structure of rosocyanin, the red compound formed.

Early work, *e.g.*, that by Schlumberger (*Bull. Soc. chim.*, 1866, **5**, 194) or Ivanow-

Gajowski (*Ber.*, 1872, **5**, 1103) with impure curcumin, or that by Clarke and Jackson (*Amer. Chem. J.*, 1908, **39**, 696) in which they assumed an incorrect formula for curcumin and believed rosocyanin to be an isomer of it, is of little value. We have been unable to repeat many of Clarke and Jackson's results and, in particular, find rosocyanin to contain boron. The present paper reports the application of, mainly, physicochemical techniques to supplement analytical findings.

The structure (I) put forward for curcumin by Heller (*Ber.*, 1914, **47**, 887, 2998; 1917, **50**, 1244) and Ghosh (*J.*, 1919, **115**, 292) has been confirmed by infra-red studies (see Part III, p. 4653). The presence of the enolic β-diketone group is significant as



$$\mathrm{HO{-}C_6H_3(MeO){-}CH{:}CH{\cdot}C(OH){:}CH{\cdot}CO{\cdot}CH{:}CH{-}C_6H_3(OMe){-}OH}$$

(I)

this group, or an amino-form (in which NH_2 replaces OH) occurs in all common absorptiometric reagents for boron, *e.g.*, quinalizarin derivatives, dianthrimide etc. This was recognised by Korenman (*Zh. An. Khimi*, 1947, **2**, Pt. 3, 153) who queried the current assumption that rosocyanin contained no boron and postulated that it consisted of a boron atom combined with one curcumin molecule.

As would be expected if the β-diketone group is the reactive unit, curcumin will not combine with boric acid if the carbonyl group is blocked by, for example, hydroxylamine. For less obvious reasons, there is no apparent reaction if the two phenolic hydroxyl groups are methylated, etc. It is hoped to study later the behaviour of substituted curcumin and allied compounds.

EXPERIMENTAL

Curcumin, prepared by British Drug Houses Ltd., had m. p. 181—182° and was probably >99·5% pure.

Rosocyanin.—Clarke and Jackson's method (*loc. cit.*) was modified as follows. To curcumin (4 g.) and boric acid (4 g.), dissolved in absolute alcohol (80 ml.), concentrated sulphuric (2 ml.) or hydrochloric acid (30 ml.) was added. After 3 hours' boiling under reflux the solution was poured into water (400 ml.), and the precipitated solid filtered off, dried at 100°, and extracted with ether until colourless. Pouring its solution in the minimum volume of cold pyridine into ten times the volume of benzene precipitated rosocyanin, which was filtered off, re-extracted with ether, and dried at 150°.

Rosocyanin is a dark green powder with a metallic reflex. It decomposes at *ca.* 250° without melting. It is insoluble in water or non-hydroxylic solvents, such as benzene, carbon tetrachloride, and ether, and gives in alcohols, deeply coloured solutions, although it is only about 0·01% soluble. The greatest solubility is in pyridine and molten phenol (0·5—1%) and in concentrated sulphuric or acetic acid, but the acid solutions are unstable. Rosocyanin solutions are slowly hydrolysed by acid or alkali if water is present. This is most pronounced with alkali, and we confirm Clarke and Jackson's findings that curcumin is the main product. In acid rosocyanin has a characteristic crimson-red colour which changes to blue-green on basification.

Analyses. Boron was determined on an aliquot of the solution obtained after wet oxidation of 0·1 g. of rosocyanin with hydrogen peroxide in 2·5N-sodium hydroxide, essentially by Naftel's method (*loc. cit.*); the remainder of the solution was used for the determination of chloride or sulphate. The sulphate was reduced by Luke's method (*Analyt. Chem.*, 1949, **21**, 1369) to hydrogen sulphide which was determined absorptiometrically as Lauth's violet (cf. Polson and Strickland, *Analyt. Chim. Acta*, 1952, **6**, 452). Chloride was titrated with mercuric nitrate with diphenylcarbazide as indicator. Control experiments were carried out on similar weights of pure curcumin.

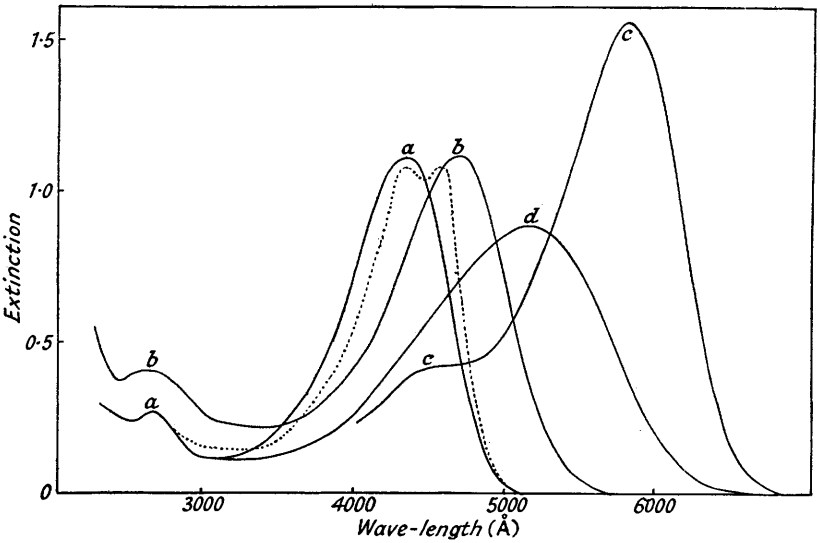
The curcumin content was determined after gentle hydrolysis. A dilute rosocyanin solution of known boron concentration was hydrolysed by warm 0·5N-alcoholic potassium hydroxide until the blue colour changed completely to yellow. The identity of the curcumin formed was confirmed, and its concentration estimated quantitatively, from the absorption spectrum of the resulting solution.

For direct cryoscopic determination of molecular weight phenol was the most suitable solvent, but even with it the extremely small depression (*ca.* 0·05°) necessitated the rigorous exclusion of moisture (by anhydrone and silica gel).

For *rosocyanin chloride* were found: C, 64·5; H, 5·25; B, 1·33; Cl, 4·3%; curcumin, mols. per atom of B, 2·1; *M*, 800 ± 150 ($C_{42}H_{38}O_{12}ClB$ requires C, 64·6; H, 4·9; B, 1·38; Cl, 4·5%; curcumin, mols. per atom of B, 2·0; *M*, 781).

For *rosocyanin sulphate hydrate* (stable at 150°) were found: C, 61·6; H, 5·45; SO_4'', 6·1%; curcumin, mols. per atom of B, 2·11; *M*, 590 ($C_{84}H_{74}O_{24}B_2,H_2SO_4,2H_2O$ requires C, 62·2; H, 4·95; SO_4'', 5·9%; curcumin, mols. per atom of B, 2; apparent *M*, 541). Molecular ratios are of greater significance (Found: B : curcumin : SO_4 = 2 : 4·22 : 1·02. Required: 2 : 4 : 1).

FIG. 1. *Absorption spectra of curcumin and its derivatives in* 2·0 × 10^{-5}M-*solution* (1-*cm. cell*).
a, Curcumin. b, Curcumin in excess of alkali. c, Phenolic solution containing acid. d, Curcumin in dilute alkali. ----, *Beryllium–curcumin complex.*



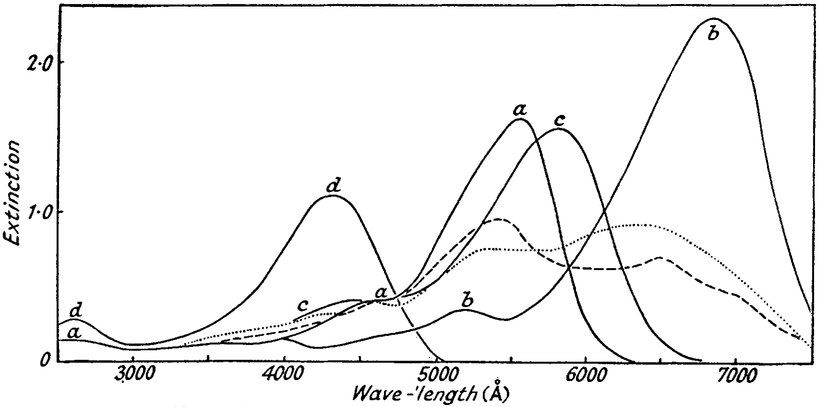


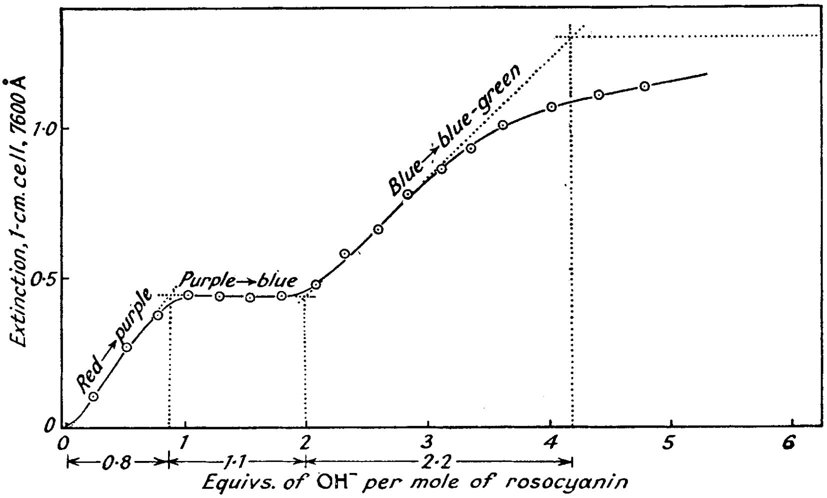
FIG. 2. *Absorption spectra of* 1·0 × 10^{-5}M-*rosocyanin* (1-*cm. cell*).
a, Acid rosocyanin; ----, *first neutralisation stage;* . . . ., *second neutralisation stage. b, Fully alkaline rosocyanin. c, Curcumin in acidic phenol* (2·0 × 10^{-5}M). *d, Curcumin in neutral alcohol* (2 × 10^{-5}M).

Absorption Spectra.—These were measured with a Uvispek spectrophotometer, at the concentrations shown, with 1-cm. cells and absolute ethyl alcohol as solvent. Spectra for 2 × 10^{-5}M-curcumin solutions are shown in Fig. 1. The two spectra of the alkaline solutions correspond respectively to the blood-red solution obtained when only a trace of alkali is present and to the more orange solution found with excess of alkali. Curcumin also becomes red in acidic alcohol, especially under nearly anhydrous conditions. In dry molten phenol containing a trace of a strong acid (*e.g.*, perchloric) this colour change is most marked, the solution being reddish-purple. The purple solutions decompose fairly rapidly, and the spectrum shown by curve *c* is only approximate.

Rosocyanin absorption spectra (Fig. 2) were the same for chloride and sulphate. All solutions were 1 × 10^{-5}M with respect to combined boron and hence comparable with those used for Fig. 1, in that they were 2 × 10^{-5}M with respect to combined curcumin. When red acidic solutions of rosocyanin are made alkaline they first became purple and then an ink-blue before finally assuming the blue-green colour of the fully alkaline form. As it is almost impossible to obtain very dilute solutions of these two intermediate forms absolutely free from one another, the broken lines in Fig. 2 are only approximate.

*Titrations with Alkali.**—Fig. 3 shows the curve obtained by titrating 500 ml. of a 1·5 × 10^{-4}M-solution (nearly saturated) of rosocyanin chloride in absolute methyl alcohol with alcoholic potassium hydroxide in a rapid stream of carbon dioxide-free nitrogen. Changes taking place during the titration were followed absorptiometrically, small samples being taken by using the nitrogen to blow liquid from the flask through a fine-bore tube into an absorption cell which was immediately stoppered. The best results were obtained when dry alcohol was used but the shape of the curve varied with the wave-length at which the absorption was measured. Fig. 3 refers to a wave-length of 7600 Å, and the attendant colour changes are noted there. With titrations observed at 7400 Å (the shortest practicable wave-length) the final rise in extinction showed a slight inflection, indicating that two slightly different ionisations were taking place. The reaction was very sluggish towards the end of the titration, and maximum extinction was obtained on addition of a few ml. of N-alkali.

FIG. 3. *Titration of rosocyanin* (1·5 × 10^{-4}M) *with alkali in methyl alcohol.*



The ionisations taking place during this titration could be characterised, with respect to their relative strengths, by noting the pH of an aqueous solution containing a few drops of alcoholic rosocyanin as an acid-base " indicator." By taking the pH of half-colour changes to correspond to the pK values of the respective ionisations, the following values for ionisation constants were found: crimson-red ⟶ purple, *ca.* 3 × 10^{-7} (one H^+ ion); purple ⟶ blue, *ca.* 2 × 10^{-8} (one H^+ ion); blue ⟶ blue green, *ca.* 5 × 10^{-10} (two H^+ ions).

Behaviour of Rosocyanin on Ion-exchange Resins.—In alcoholic solutions containing only a trace of acid the crimson-red form of rosocyanin rapidly entered a cation-exchange resin, changing the latter to red, but could be removed by increasing the acidity of the alcoholic phase with perchloric acid. Thus the compound showed the properties of a cation, as would be expected from the fact that an equivalent of anion is always found associated with it in the solid state. The charge on the cation was obtained graphically (Strickland, *Nature*, 1950, **169**, 620, 738), by using the equation, $\log [\varepsilon/(\varepsilon_0 - \varepsilon)] = x \log C_A + K$, where C_A is the concentration of perchloric acid in the alcoholic phase, ε_0 the extinction of a solution before, and ε its extinction after, equilibration of a constant volume (55 ml.) with a constant weight (2·0 g.) of sulphonated

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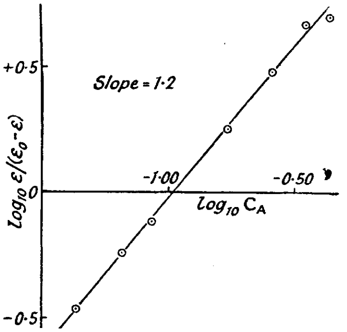
* Absorptiometric titrations of curcumin with alkali, by a similar technique, showed that the blood-red alkaline form resulted from simultaneous ionisation of both phenolic groups (K = *ca.* 3 × 10^{-9}) followed by the less ready ionisation of the enolic hydrogen atom (K = *ca.* 1 × 10^{-11}) to give the orange-red solution.

polystyrene resin in the H^+ form. The slope x of the line, which should equal the charge on the rosocyanin cation, is seen from Fig. 4 to approximate to unity.

However, if the rosocyanin solution was equilibrated with the alkali-metal form of a cation exchanger it no longer behaved as if it were a cation and no detectable amount entered the resin phase. Only on addition of excess of acid to the alcohol, with a corresponding partial conversion of the resin into the H^+ form, did exchange with the rosocyanin ion take place. If a resin containing rosocyanin was treated with alcoholic sodium perchlorate, the hydrogen ions on the resin were largely replaced by sodium ions, but the red colour remained and very concentrated solutions of the sodium salt were required to remove even traces of rosocyanin back into the alcoholic phase. If the resin containing rosocyanin was treated with alcoholic potassium hydroxide, the hydrogen ions were again replaced and the rosocyanin on the resin became ink-blue, but was still very stubbornly retained.

As was to be expected, the crimson-red form of rosocyanin would not enter an anion-exchanger, but the blue alkaline form entered very slightly into the resin phase, indicating that it was anionic. Quantitative work was impossible owing to the comparatively rapid hydrolysis of rosocyanin in the alkaline solutions employed.

FIG. 4. *Charge on rosocyanin cation.*

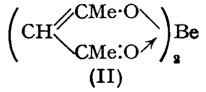


Comparison of Boron and Beryllium Complexes.—Both the acetylacetone and curcumin complexes of beryllium are readily prepared by treating freshly precipitated beryllium hydroxide with neutral alcoholic solutions of the diketones. These reactions proceed at a reasonable rate even in the presence of considerable amounts of water. It is known that the beryllium complex of acetylacetone has a similar spectrum to the free ketone (Adam, Booth, and Strickland, *Analyt. Chim. Acta,* 1950, 462) and this is so for the beryllium curcumin complex (cf. Fig. 1), which in the solid state is almost indistinguishable from curcumin, although it has a much higher m. p. (260°).

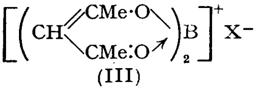
Combination of boric acid with acetylacetone or curcumin requires the more vigorous conditions brought about by adding the boron as trichloride. Reaction between boric acid and curcumin in neutral alcohol is negligibly slow, although it becomes appreciable if the solutions are concentrated and strongly acidic. It occurs more rapidly if the curcumin solution is first reddened by addition of acid. In acidified molten phenol, where, as mentioned above, the curcumin solution is purple red and has an absorption spectrum similar to that of rosocyanin (Fig. 2), the reaction with even μg.-quantities of boron is complete within a few seconds. On dilution of such solutions with alcohol the purple-red colour reverts to yellow in the absence of boron but remains red if boric acid is present for a few seconds before dilution.

DISCUSSION

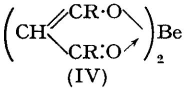
The presence of the enolic β-diketone group in curcumin suggests that its complexes with beryllium and boron would be analogous in composition to the corresponding acetylacetone derivatives, $Be(Aca)_2$ or $B(Aca)_2X$, where X is a monobasic anion (Dilthey,



(II)



(III)



(IV)

[**R** = **4** : **3** : 1-HO·C_6H_3(OMe)·CH:CH·]

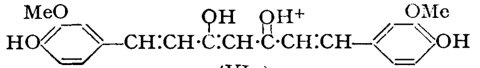
Annalen, 1906, **344**, 300). By analogy with the beryllium compound (II), Dilthey's boron complex would be expected to be (III). Such a structure (IV) almost certainly exists for the beryllium–curcumin derivative. As expected, its absorption spectrum in the visible region resembles that of curcumin, in acid or alkaline solution. The latter solutions are too unstable for quantitative work but the spectrum found is very similar to that of the first alkaline form of curcumin.

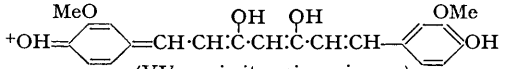
Clearly a configuration analogous to (IV) cannot apply to rosocyanin, although analytical data are in satisfactory agreement with its being the curcumin equivalent of (III), and molecular weight determinations show that no polymerisation takes place. The boron–curcumin derivative also behaves as a univalent cation in alcoholic solution.

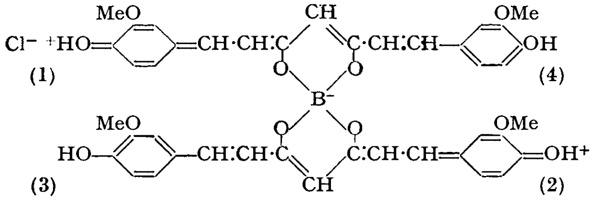
Nevertheless, rosocyanin cannot be prepared in the same way as the beryllium complex and its solutions have a totally different absorption spectrum in acid and alkali. The blood-red solution of curcumin in alkali must arise from a hybrid of structures such as (V*a*—*b*), and the resonance of normal and quinonoid forms is the only reasonable explanation for the profound colour change in acid solutions, shown in its most marked form in acidified molten phenol. In the latter case the phenol probably assists the change in a manner not yet understood, as a permanent effect is soon observed, solutions fading and losing the properties of curcumin when diluted with alcohol; for a short time, however, the effect of acid is reversible and must consist of the addition of a proton to the carbonyl group of the curcumin molecule to give a resonant hybrid of such structures as (VI*a*—*c*). As this red form of curcumin reacts so readily with boric acid it is reasonable to suppose that esterification of three central hydroxyl groups and migration of the hydrogen atom from the fourth lead sto (VII). Methylation, etc., of the phenolic groups in curcumin would inhibit the formation of conjugated structures (V), so these curcumin derivatives do not become red in acid or react with boron.


(V*a*)


(V*b*)

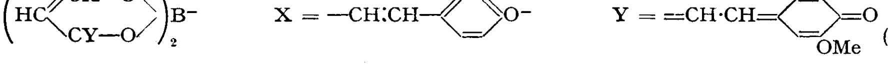

(VI*a*)


(VI*b*; *c* is its mirror image)


(VII)

The formulation of the main "stationary state" structure of rosocyanin as (VII) explains adequately most of the properties of this compound, especially the absorption spectra of solutions of various acidities. We may assume, for the sake of description, that a hydrogen atom of $^{+}$HO: group (1) is the first to ionise, taking with it the positive charge associated with the whole molecule. This occurs quite readily ($K = ca.\ 3 \times 10^{-7}$) and explains why, although rosocyanin behaves as if it were a univalent cation when equilibrated with a resin in the H^{+} form, it shows negligible exchange with a Na^{+} form. Inside the matrix of the latter the concentration of hydrogen ions is very small, and the rosocyanin molecule on entering the resin phase would dissociate to give a neutral molecule and no exchange could take place. However, once associated with a $SO_2{\cdot}O^-$ group on the resin, the rosocyanin is held comparatively strongly by some form of hydrogen bonding. The second hydrogen atom to ionise under the influence of alkali, also fairly readily, would be (2) ($K = ca.\ 2 \times 10^{-8}$). Finally the two remaining phenolic groups ionise almost simultaneously, these groups being rather more weakly acidic than when in the original curcumin.

The oscillator strength, *f*, of the main absorption band of rosocyanin at 5550 Å has a value almost exactly double that of curcumin at 4275 Å (1·6 : 0·8), indicating virtual degeneracy in the absorption of the two curcumin chains combined in this complex (VII).


(VIII)

Similarly, only one main band of long wave-length appears when all the hydrogen atoms have been ionised (VIII), each half of the molecule again having similar transition energies, with an *f* value of 1·8 for the whole unit. When, however, hydrogen ion (1) is removed from (VII), each half of the molecule is markedly different in structure and two bands appear which may be *x* and *y* bands of a two-dimensional oscillator. The rosocyanin molecule, with its two linear oscillators of variable structure held at approximately right angles in space, may prove a useful compound with which to study the effect of constitution on light absorption, especially when considered from the viewpoint of the Lewis–Calvin treatment (*Chem. Reviews*, 1939, **25**, 273).

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