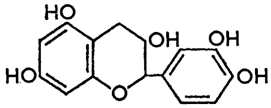
561. *Complex Formation between Copper and Some Organic Acids, Phenols, and Phenolic Acids occurring in Fruit.*

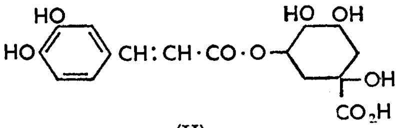
By C. F. Timberlake.

Cupric ions form complexes with (+)-catechin, chlorogenic acid, and caffeic acid.

Complex formation between cupric ions and catechol has been described.[1] Further potentiometric studies have shown that cupric ions can combine similarly with the *ortho*-diphenolic groups of (+)-catechin (I), chlorogenic acid (II),[2] and caffeic acid (III). In acid solutions (pH <4) cupric ions combine with the carboxyl group of chlorogenic acid; the stabilities of the copper complexes of chlorogenic and quinic acids (IV) are compared with that of the dihydroshikimic acid (V) [3] complex.



(I)



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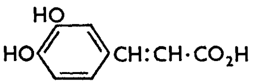
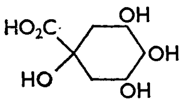
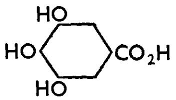


(III)

(IV)





(V)

Experimental.—Dilute solutions of the compounds (5 and 2·5 × 10^{-3}M) were titrated with alkali (5 × 10^{-2}N) at 25° with and without cupric chloride by the method previously described.[1] In the titrations without additions of potassium nitrate, pH was defined as equal to $-\log \{H^+\}$ where { } represents activity and ion-activity coefficients were evaluated from Davies's [4] equation. In the titrations with additions of potassium nitrate the volumes and concentrations of the solutions were such that ionic strengths were maintained relatively constant at 0·050—0·055; pH was then defined as equal to $-\log [H^+]$ where [ ] represents molar concentration.

(+)-*Catechin complexes.* As with catechol, graphs relating the pH of solution to the ratio of concentration of added base to concentration of copper (m_b/m_{Cu}) obtained on titration of (+)-catechin solutions containing cupric chloride with sodium hydroxide showed inflections at (m_b/m_{Cu}) = 2 and 4, corresponding to the neutralisation of 2 and 4 hydrogen ions per atom of copper present. The values of pK_1 and pK_2 calculated from points on the buffer

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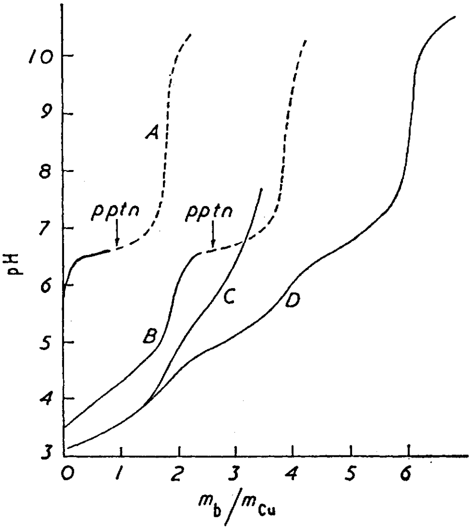
[1] Timberlake, *J.*, 1957, 4987.
[2] Moores, McDermott, and Wood, *Analyt. Chem.*, 1948, **20**, 620.
[3] Carr, Pollard, Whiting, and Williams, *Biochem. J.*, 1957, **66**, 283.
[4] Davies, *J.*, 1938, 2093.

ranges were constant where, (+)-catechin being regarded as a tetrabasic acid H_4A, $K_1 = [CuH_2A]\{H^+\}^2/f_2[Cu^{2+}][H_4A]$ and $K_2 = f_2[Cu(H_2A)_2^{2-}]\{H^+\}^2/[CuH_2A][H_4A]$. The mean values found were $pK_1 = 7{\cdot}80$ ($I \longrightarrow 0$), $pK_2 = 11{\cdot}39$ ($I \longrightarrow 0$).

Dihydroshikimic acid complex. Potentiometric titrations of dihydroshikimic acid solutions with alkali showed a small liberation of hydrogen ions in the presence of cupric chloride. When the carboxyl group had been neutralised, further additions of alkali precipitated copper hydroxide (curve *B*, Figure). The dissociation constant of the carboxyl group of dihydroshikimic acid (HA) was $4{\cdot}1 \times 10^{-5}$ ($p_cK = 4{\cdot}39$) at zero ionic strength and $5{\cdot}1 \times 10^{-5}$ ($p_cK = 4{\cdot}29$) at $I = 0{\cdot}05$. The mean values of the stability constant of complex CuA^+ calculated from several pH values during early parts of the titrations were $K_s = [CuA^+]/f_2[Cu^{2+}][A^-]$; $\log K_s = 2{\cdot}08$ ($I \longrightarrow 0$) and $K_s = [CuA^+]/[Cu^{2+}][A^-]$; $\log K_s = 1{\cdot}65$ ($I = 0{\cdot}05$).

Quinic acid complexes. Potentiometric titrations of quinic acid solutions with alkali showed a small liberation of hydrogen ions in the presence of cupric chloride. On addition of alkali beyond that necessary to neutralise the carboxyl group, considerable liberation of hydrogen ions occurred, indicating appreciable complex formation (curve *C*). There was no precipitate and the solution became green. At pH values above 8, readings were unsteady.



Potentiometric titration.

A. Titration of 40 ml. of solution containing $CuCl_2,2H_2O$ (0·00125M) and KNO_3 (0·05M) with 0·05N-NaOH.

B. Titration of 40 ml. of solution containing dihydroshikimic acid (0·0025M), $CuCl_2,2H_2O$ (0·00125M), and KNO_3 (0·05M) with 0·05N-NaOH.

C. Titration of 40 ml. of solution containing quinic acid (0·0025M), $CuCl_2,2H_2O$ (0·00125M), and KNO_3 (0·05M) with 0·05N-NaOH.

D. Titration of 40 ml. of solution containing chlorogenic acid (0·0025M), $CuCl_2,2H_2O$ (0·00125M) and KNO_3 (0·05M) with 0·05N-NaOH.

The dissociation constant of the carboxyl group of quinic acid (HA) was $2{\cdot}6 \times 10^{-4}$ ($p_cK = 3{\cdot}58$) at zero ionic strength and $3{\cdot}2 \times 10^{-4}$ ($p_cK = 3{\cdot}50$) at $I = 0{\cdot}05$. The mean values of the stability constant of complex CuA^+ calculated from several pH values during the early parts of the titrations were $\log K_s = 2{\cdot}66$ ($I \longrightarrow 0$) and $\log K_s = 2{\cdot}44$ ($I = 0{\cdot}05$). The ncreased stability of CuA^+ of quinic acid (α-OH) over that of dihydroshikimic acid (α-H) is in accord with the findings of Lloyd, Wycherley, and Monk[5] for other monocarboxylic acids. The dissociation constant of quinic acid has been reported as $2{\cdot}2 \times 10^{-4}$ at 9°.[6]

Chlorogenic acid complexes. During the early part of the titration of chlorogenic acid solution containing cupric chloride with alkali, liberation of hydrogen ions was similar to that occurring with quinic acid. However on addition of nearly one equivalent of alkali (with respect to chlorogenic acid) the pH depression was greater than that which occurred with quinic acid (curve *D*). Further addition of alkali gave two well-defined buffer regions separated by inflections at intervals of $(m_b/m_{Cu}) = 2$. These findings suggest the formation of five-membered ring complexes between cupric ions and the *ortho*-diphenolic groups of chlorogenic acid.

Chlorogenic acid being regarded as H_3A, practically all the copper must be present as CuA^- at the inflection where $(m_b/m_{Cu}) = 4$. On further additions of alkali, CuA^- is converted into CuA_2^{4-} since constant values of K_2 were obtained from points on the buffer range $m_b/m_{Cu} = 4$—6, indicating that complexes CuA^- and CuA_2^{4-} were the only ones present.

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[5] Lloyd, Wycherley, and Monk, *J.*, 1951, 1786.

[6] Heilbron, " Dictionary of Organic Compounds," Eyre and Spottiswoode, London, 1934.

The tabulated quantitative data were evaluated:

Dissociation constants of chlorogenic acid

	$I \longrightarrow 0$	$I = 0{\cdot}05$
Carboxyl group	${}_{c}K_1 = 2{\cdot}6 \times 10^{-4}$ ($p_cK_1 = 3{\cdot}59$)	${}_{c}K_1 = 3{\cdot}2 \times 10^{-4}$ ($p_cK_1 = 3{\cdot}50$)
First phenolic group	${}_{c}K_2 = 2{\cdot}6 \times 10^{-9}$ ($p_cK_2 = 8{\cdot}59$)	${}_{c}K_2 = 4{\cdot}5 \times 10^{-9}$ ($p_cK_2 = 8{\cdot}35$)

Gorter [7] has reported the dissociation constant (${}_{c}K_1$) of chlorogenic acid as $2{\cdot}2 \times 10^{-4}$ at 27°.

Stability constant of complex CuH_2A^+, *calculated from several* pH *readings during the early parts of the titrations.*

$$K_s = [CuH_2A^+]/f_2[Cu^{2+}][H_2A^-];\ \log K_s = 2{\cdot}72\ (I \longrightarrow 0)$$
$$K_s = [CuH_2A^+]/[Cu^{2+}][H_2A^-];\ \log K_s = 2{\cdot}42\ (I = 0{\cdot}05)$$

Equilibrium constant of the reaction $CuA^- + H_2A^- \rightleftharpoons CuA_2^{4-} + 2H^+$

$$K_2 = f_4[CuA_2^{4-}]\{H^+\}^2/f_1^2[CuA^-][H_2A^-];\ pK_2 = 11{\cdot}4\ (I \longrightarrow 0)$$
$$K_2 = [CuA_2^{4-}][H^+]^2/[CuA^-][H_2A^-];\ pK_2 = 10{\cdot}3\ (I = 0{\cdot}05)$$

The activity equilibrium constant K_2 is probably subject to some error since it involves the activity coefficient of the quadrivalent ion CuA_2^{4-}.

The similarity of the stability constants of the copper complexes of quinic and chlorogenic acids indicates that at low pH values (<4) complex formation between cupric ions and the *ortho*-diphenolic groups of chlorogenic acid is very small.

The complexes existing with CuA^- in the buffer region at $(m_b/m_{Cu}) = 2$—4 have not yet been defined. In 1 : 1 mixtures of chlorogenic acid and cupric chloride ($2{\cdot}5 \times 10^{-3}$M), precipitation occurred midway along the buffer region in the vicinity of pH 5. The precipitated material contained 15·5% of copper. The calculated copper content of CuA^- or HACu, in which the carboxyl group is un-ionised, is 15·0%. The precipitate therefore may have been neutral HACu. If this were so, HACu should ionise according to equation $HACu \rightleftharpoons H^+ + CuA^-$ so that additions of base in this region neutralise the hydrogen ions liberated with formation of CuA^-. However, attempts to evaluate the experimental data by use of equations based on the presence of Cu^{2+}, CuH_2A^+, HACu, and CuA^- in various combinations did not give very consistent figures for the constants concerned, possibly owing to neglect of any complexes formed with the quinic acid part of the chlorogenic acid molecule in this pH region.

Support for the formation of HACu is obtained by consideration of Schwarzenbach's [8] work, who found that both copper and iron formed complexes containing an un-ionised sulphonic acid group with Tiron (catechol-3 : 5-disulphonic acid). The ionisation of the ferric complex $Fe[C_6H_2O_2SO_3(SO_3H)] \rightleftharpoons H^+ + Fe[C_6H_2O_2(SO_3)_2]^-$ occurs in acid solution.[9]

Caffeic acid complexes. Potentiometric titrations of solutions of caffeic acid (H_3A) and cupric chloride also indicated the formation of complexes CuA^- and CuA_2^{4-}. Consistent values of the stability constant of complex CuH_2A^+ were not obtained, probably owing to combination of cupric ions with the *ortho*-diphenolic groups of caffeic acid during the early stages of the titration, when the pH values were considerably higher than those found with chlorogenic acid. The tabulated quantitative data were evaluated.

Dissociation constants of caffeic acid

	$I \longrightarrow 0$	$I = 0{\cdot}05$
Carboxyl group	${}_{c}K_1 = 2{\cdot}4 \times 10^{-5}$ ($p_cK_1 = 4{\cdot}62$)	${}_{c}K_1 = 3{\cdot}2 \times 10^{-5}$ ($p_cK_1 = 4{\cdot}49$)
First phenolic group	${}_{c}K_2 = 8{\cdot}5 \times 10^{-10}$ ($p_cK_2 = 9{\cdot}07$)	${}_{c}K_2 = 1{\cdot}7 \times 10^{-9}$ ($p_cK_2 = 8{\cdot}76$)

The equilibrium constant of reaction $CuA^- + H_2A^- \longrightarrow CuA_2^{4-} + 2H^+$ is $pK_2 = 12{\cdot}1$ ($I \longrightarrow 0$); $pK_2 = 11{\cdot}0$ ($I = 0{\cdot}05$). The activity equilibrium constant is probably subject to some error since it involves the activity coefficient of the quadrivalent ion CuA_2^{4-}.

The natures of the ionic species in equilibrium with CuA^- during titration to the first

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[7] Gorter, *Annalen*, 1911, **379**, 110.

[8] See Bjerrum, Schwarzenbach, and Sillén, "Stability Constants of Metal-Ion Complexes, with Solubility Products of Inorganic Substances. Part 1. Organic Ligands." Special Publication No. 6, The Chemical Society, London, 1957, Table 200.

[9] Willi and Schwarzenbach, *Helv. Chim. Acta*, 1951, **34**, 528.

inflection are still uncertain. Precipitation occurred at certain concentrations ($2{\cdot}5 \times 10^{-3}$M-caffeic acid and $1{\cdot}25 \times 10^{-3}$M-cupric chloride) suggesting that neutral CuHA containing a non-ionised carboxyl group may be formed.

It is considered that the solutions of chlorogenic acid and caffeic acid contained mainly the *trans*-isomers of these compounds.[10]

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[10] Williams, *Chem. and Ind.*, 1955, 120.