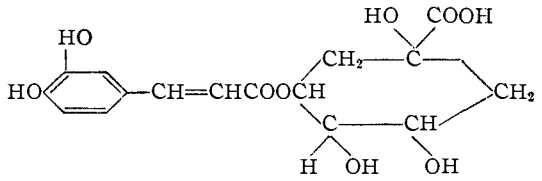
[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

Chlorogenic Acid and Respiration of Sweet Potatoes

BY GEORGE O. RUDKIN AND J. M. NELSON

Evidence has been presented by Boswell and Whiting,[1] Boswell[2] and Baker and Nelson[3] supporting the view that the oxidase tyrosinase[3a] serves as the terminal oxidase in a respiration chain operating in thin slices of potato tubers, *Solanum tuberosum*, when the latter is permitted to respire in water buffered with phosphate buffer at *p*H 5–6. Since tyrosinase is specific toward many *o*-dihydric phenols, it is reasonable to assume that the hydrogen carrier functioning next to the terminal oxidase is an *o*-dihydric phenol. Boswell and Whiting isolated a substance from potato tubers, which responded to the green ferric chloride test, characteristic of *o*-dihydric phenols and, when added to respiring potato slices, caused an increase in the rate of respiration. These investigators, however, failed to obtain their substance in sufficiently pure condition to further identify it. According to Robinson and Nelson,[4] *o*-dihydroxyphenylalanine or dopa may possibly play the role of a hydrogen carrier functioning next to the tyrosinase in the respiring slices of the common potato.

Since sweet potato roots, *Ipomoea batatas*, also contain a catecholase, closely related to tyrosinase,[4a] the present study was undertaken to isolate from the roots substances which when added to thin slices of the roots, respiring in water buffered with phosphate to *p*H 5–6, might increase both the rates of oxygen uptake and carbon dioxide given off. Two *o*-dihydric phenols have been isolated. One of these proved to be chlorogenic acid



and the other, component A, apparently a mixture of three compounds, one of which made up about three-fourths of the mixture and appeared to be closely related to chlorogenic acid.

When added to thin respiring sweet potato slices, 1 mg. of chlorogenic acid not only increased the rate of oxygen uptake but also increased the rate of carbon dioxide given off (Fig. 1). That the chlorogenic acid served as a hydrogen carrier in the respiration of the slices is supported by the following. In experiments using partly purified preparations of sweet potato oxidase, approximately two atoms of oxygen were consumed for each molecule of chlorogenic acid added (Fig. 2). This is in agreement with the theoretical volume of oxygen necessary to oxidize chlorogenic acid to the corresponding hydroxy quinone. The difference between the total volumes of oxygen consumed in the Warburg flask containing the respiring slices and 1 mg. of chlorogenic acid and the flask containing slices respiring alone (control), was 222 μl. during a period of three hours. The calculated volume of oxygen necessary for the oxidation of chlorogenic acid to the hydroxy-quinone stage is 62 μl. per mg. Thus, at least 160 μl. of oxygen must have been consumed in oxidizing something else. Since a corresponding increase in carbon dioxide evolution was noticed, it is reasonable to suppose that the oxygen reacted with hydrogen or its equivalent furnished by the preceding part of the respiratory chain. Furthermore, the specificity of the sweet potato oxidase for *o*-dihydroxyphenols, such as chlorogenic acid, is an indication that it functioned adjacent to the terminal oxidase.

As further evidence that the chlorogenic acid functioned in the respiratory chain, it may be pointed out that at the end of three and one-half hours the flask containing one mg. of chlorogenic acid with the slices showed practically no coloration. This lack of coloration was an indication that the chlorogenic acid was being alternately oxidized and reduced, since complete oxidation of the acid, as in the case of its enzymatic oxidation in which the quinone is not reduced, resulted in highly colored reaction mixtures. This coloration was probably due to the polymerization products of hydroxyquinone. The fact that the addition of chlorogenic acid to the respiring slices caused an increase in the rate of respiration suggests that the concentration of *o*-dihydric phenols was a rate-limiting factor in the respiration of the sweet potato slices, since it was possible, upon the addition of this particular hydrogen carrier, to observe an increase in the rate of respiration.

Component A also appeared to take part in the respiration of the sweet potato slices. Since it also proved to be phenolic, it, too, when added to the respiring slices, probably functioned as a hydrogen carrier next to the terminal oxidase, in a manner similar to that of the chlorogenic acid. The fact that both the rates of oxygen uptake and carbon dioxide given off were increased over the rates of the control also suggested that it functioned in a manner similar to that of the chlorogenic acid.

The volume of oxygen necessary to carry out

(1) J. G. Boswell and G. C. Whiting, *Ann. Bot.* (NS), **2**, 847 (1938).
(2) J. G. Boswell, *ibid.*, **9**, 55 (1945).
(3) D. Baker and J. M. Nelson, *J. Gen. Physiol.*, **26**, 269 (1943).
(3a) Termed polyphenolase or catecholase by the first-mentioned investigators.
(4) E. S. Robinson and J. M. Nelson, *Arch. Biochem.*, **4**, 111 (1944).
(4a) It is hoped to discuss the nature of the oxidase in sweet potato roots more fully in a subsequent communication.

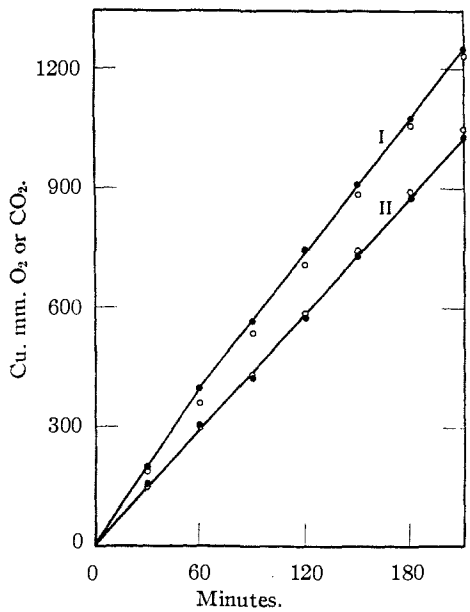


Fig. 1.—Curve I: showing the increase in rates of oxygen uptake (solid circles) and carbon dioxide given off (circles) when 1 mg. of chlorogenic acid was added to thirty-five sweet potato slices respiring in a phosphate buffer solution, *p*H 5.5. Curve II, control: rates of oxygen uptake (solid circles) and carbon dioxide given off by thirty-five sweet potato slices respiring in the absence of added chlorogenic acid.

the enzymatic oxidation of component A by means of sweet potato oxidase was found to be 56 μl. per mg. (Fig. 2). This oxidation was carried out under conditions like those for the enzymatic oxidation of chlorogenic acid. Although its complete identity is as yet unknown, this similarity, taken with the parallel behavior with chlorogenic acid on enzymatic oxidation, suggests that in taking

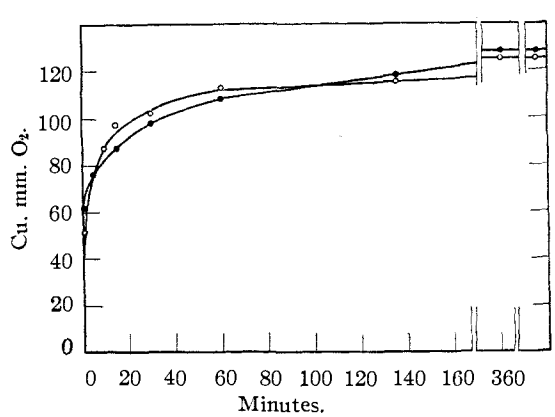


Fig. 2.—Curve (solid circles) shows the total oxygen uptake when 1.97 mg. of chlorogenic acid was oxidized enzymatically, by means of a crude preparation of sweet potato oxidase, in a phosphate buffer, *p*H 5.5; temperature 25°. Curve (circles) shows the total oxygen uptake when 2.12 mg. of component 2 of component A was enzymatically oxidized.

up 56 μl. of oxygen per mg. it too was oxidized to the hydroxyquinone stage, taking up two atoms of oxygen per mole.

When component A was oxidized by being added to respiring slices, as illustrated in Fig. 3, the flask containing five mg. of component A and respiring slices took up 843 μl. of oxygen above the control. Since the calculated volume of oxygen necessary to oxidize 5 mg. of component A to its final state of oxidation is about 280 μl., based on 56 μl. per mg. when oxidized enzymatically, it follows, as in the case of chlorogenic acid, that about 563 μl. of oxygen must have been consumed in oxidizing something else.

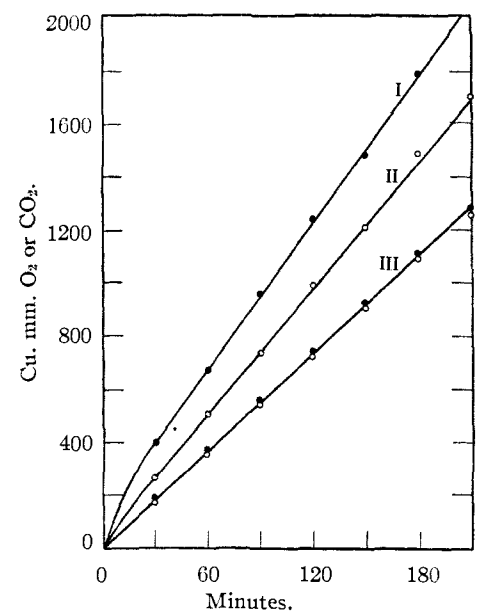


Fig. 3.—Curves I and II show the respective rates of oxygen uptake and carbon dioxide given off when 5 mg. of component 2 of component A was added to thirty-five respiring sweet potato slices. Curve III, control: rates of oxygen uptake (solid circles) and carbon dioxide given off (circles) by the respiring sweet potato slices without the addition of component 2 of component A.

Gorter[5] has claimed that chlorogenic acid occurs widely distributed in the plant kingdom, and Oparin[6] has suggested that it probably constitutes one of Palladin's respiratory chromogens. According to the latter, respiratory chromogens take part in the respiratory process by being oxidized by atmospheric oxygen to the respiratory pigment (quinone compounds). The latter then acts as a hydrogen acceptor and is reduced back to the chromogen by a hydrogen donator, an intermediate product of the respiratory process.[7]

If further work should confirm the view that both chlorogenic acid and component A can serve as hydrogen carriers, then it would follow that

(5) K. Gorter, *Ann.*, **358**, 327 (1908); **379**, 110 (1911).
(6) A. Oparin, *Biochem. Z.*, **124**, 90 (1921).
(7) W. Stiles and W. Leach, "Respiration in Plants," The Dial Press, New York, N. Y., 1932.

the flow of the protons or electrons from the metabolite to the terminal oxidase may pass through different hydrogen carriers operating next to the terminal oxidase.

Chlorogenic acid and component A showing a tendency to precipitate gelatin, yielding protocatechuic acid on alkaline hydrolysis, and component A giving a red phlobaphene product when boiling with mineral acids, all suggest a close relationship to the catechol tannins. Possibly the precursors of the tannins may turn out to be of significance in plant respiration.

Experimental

Fifty pounds of sweet potatoes were minced and extracted with 30 liters of acetone, filtered, and the acetone in the filtrate removed by distillation. The aqueous residue (3–4 liters) was filtered through infusorial earth to remove fats, carotenoids, etc. The clear aqueous filtrate was then treated with neutral lead acetate to precipitate the *o*-dihydric phenols. Due to other material which might also have been coprecipitated, the crude *o*-dihydric bodies were extracted from the lead precipitate by treating the latter with 5% aqueous acetic acid solution. After filtering the extract, the clear filtrate was treated with more lead acetate and made to *p*H 8.5 with ammonium hydroxide solution to reprecipitate the *o*-dihydric phenols. The yellow precipitate obtained was washed with water in the centrifuge and then suspended in water. To the suspension dilute sulfuric acid was added carefully until the yellow color had disappeared, indicating that the lead had been changed to lead sulfate. The latter was filtered off yielding a clear amber-colored filtrate. Since the compounds to be isolated appeared to be catechol derivatives and acidic, the latter filtrate was made 2 molar with potassium dihydrogen phosphate, the *p*H adjusted to 2 with sulfuric acid, and extracted with ethyl acetate. The ethyl acetate extract (2–3 liters) was next concentrated under reduced pressure to about 100 ml. On standing, inorganic salts tended to separate on the bottom of the flask. The supernatant liquid was decanted from the crystals into another flask, the phenolic compounds precipitated by the addition of petroleum ether and the precipitate formed separated by centrifugation. The supernatant liquid (850 ml.) was decanted and a small amount of benzene added to the residue. The organic solvents were then evaporated under reduced pressure and about 2 g. of a white, fluffy powder I, obtained.

Since the above powder appeared to be a mixture of closely related phenolic compounds, a small amount, 2.58 mg., was subjected to an 8-plate Craig counter distribution.[8] For the purpose 9 small separatory funnels, and 10-ml. portions of ethyl acetate and aqueous 2 *M* potassium dihydrogen phosphate solution, *p*H 4.7, as the two solvents, were used. Due to the components of the mixture all possessing a maximum absorption at about 3250 Å., it was possible to determine, by means of the Beckman spectrophotometer, the relative distribution of the components, as optical densities, in the respective funnels. The results are shown in Fig. 4.

In the light of the latter information, 2–3 g. of powder I was dissolved in aqueous 2 *M* potassium dihydrogen phosphate solution, *p*H 4.7, and crude component A extracted by means of ethyl acetate. The phosphate solution was then made *p*H 2 with dilute sulfuric acid and crude B extracted with ethyl acetate. The two ethyl acetate extracts were concentrated and crude components A and B obtained as white fluffy powders by means of the same procedure as already described.

Further Purification of Component B.—A small portion of the crude component B powder was again subjected to an 8 plate counter-current distribution, following the same procedure as used in obtaining the data shown in Fig. 4, except that in this case the phosphate solution was made *p*H 3.8 instead of 4.7. The results obtained are shown in Fig. 5.

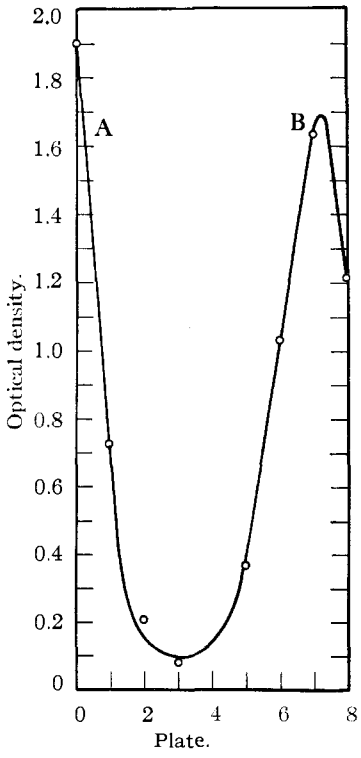


Fig. 4.

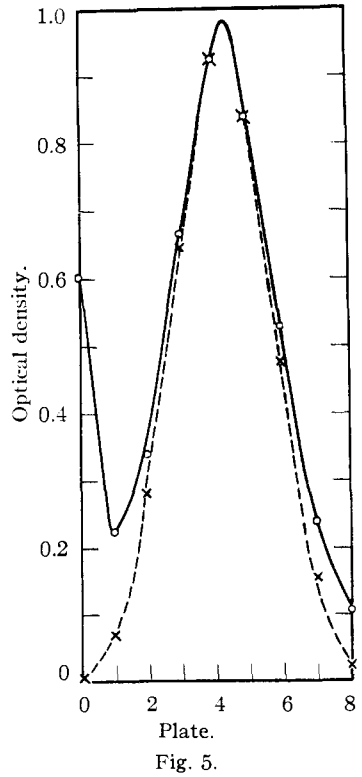


Fig. 5.

(8) L. C. Craig, *J. Biol. Chem.*, **155**, 519 (1944); **161**, 321 (1945).

Since the contents of funnels 3 to 8, Fig. 5, produced a nearly symmetrical curve it appeared that this part of the curve indicated that the solute contained in these five funnels might be practically a pure compound. The latter view is supported by the theoretical curve, broken line, Fig. 5, calculated by means of the equation of Craig and Williamson,[9] assuming that the peak of the experimental curve corresponds to a pure compound and selecting the two points on the latter curve corresponding to funnels 4 and 5 as basis for the calculation.

$$Tnr = \frac{nl}{rl\,(n-r)l}\left(\frac{1}{K+1}\right)^n K^r$$

Tr = fraction of the solute contained in the r^{th} plate (separatory funnel) of an n-plate system, and K = the distribution constant.[9a]

Isolation of Chlorogenic Acid.—The fact that component B had collected, as shown in Fig. 5, in practically pure condition in plates or funnels 3 to 8, while the impurities were retained in the first three plates, suggested the following procedure for isolating practically pure component B from the main portion of crude component B powder. For the purpose, three 1000-ml. separatory funnels, corresponding to plates 0, 1 and 2 in Fig. 5, were used. Each contained 500 ml. of ethyl acetate and 1 g. of the crude powder B was dissolved in the ethyl acetate contained in the first funnel. Three liters of aqueous 2 *M* potassium dihydrogen phosphate solution, *p*H 3.8, were passed, in 500-ml. portions, consecutively through the three funnels, after which the phosphate solution was acidified to *p*H 2 with dilute sulfuric acid and extracted with ethyl acetate. The ethyl acetate extract was then concentrated, following the same procedure as described earlier, and 0.6 g. of practically pure component B obtained as a white fluffy powder.

The powder was crystallized from water, by seeding, and proved to be chlorogenic acid having a m. p. 208–209° cor. The crystals were quite soluble in ethyl alcohol, moderately in water, but practically insoluble in ether. An ammoniacal solution of the acid turned green on autoxidation, while an aqueous solution of the acid, made alkaline with sodium hydroxide, turned reddish brown on autoxidation. The latter behavior also takes place when the acid undergoes aerobic oxidation enzymatically. The green color appears to be due to some reaction involving ammonia.

Analysis of the Crystalline Chlorogenic Acid.—Dried *in vacuo* for five hours over phosphorus pentoxide at 110°. Calcd. for $C_{16}H_{18}O_9$: C, 54.24; H, 5.12. Found: C, 54.52; H, 5.23. Analysis of the pentacetyl derivative, m. p. 178.5–179.5° cor. Calcd. for $C_{26}H_{28}O_{14}$: C, 55.31; H, 5.02. Found: C, 55.34; H, 5.24.

Further Purification of Crude Component A.—Since crude component A was more complex than crude B, 1.43 mg. was subjected to a counter current distribution, employing a special apparatus,[10] consisting of 24 plates. Ethyl acetate and aqueous 2 *M* potassium dihydrogen-sodium mono-hydrogen phosphate solution, *p*H 6.3, were used as the solvents. The results obtained are shown in Fig. 6. The two peaks suggest the presence of two components, 1 and 2, and the shoulder of the curve, between plates 6 and 12 indicates the presence of a third component, 3.

To obtain information concerning the relative magnitude of component 3, two theoretical curves were calculated for components 1 and 2, by means of the Craig and Williamson equation. For component 1, the values for T_1 and T_2 were the optical densities of plates 1 and 2, and for component 2, the optical densities of plates 15 and 16. These calculated curves are represented by the broken curves in Fig. 6. By plotting the differences between the experimental curve and the two calculated curves, the middle curve (— — —) in the graph, corresponding to plates 3 to 16 and having a peak at plate 9, was obtained. The latter curve indicates that component 3 was present in its maximum concentration in plate 9.

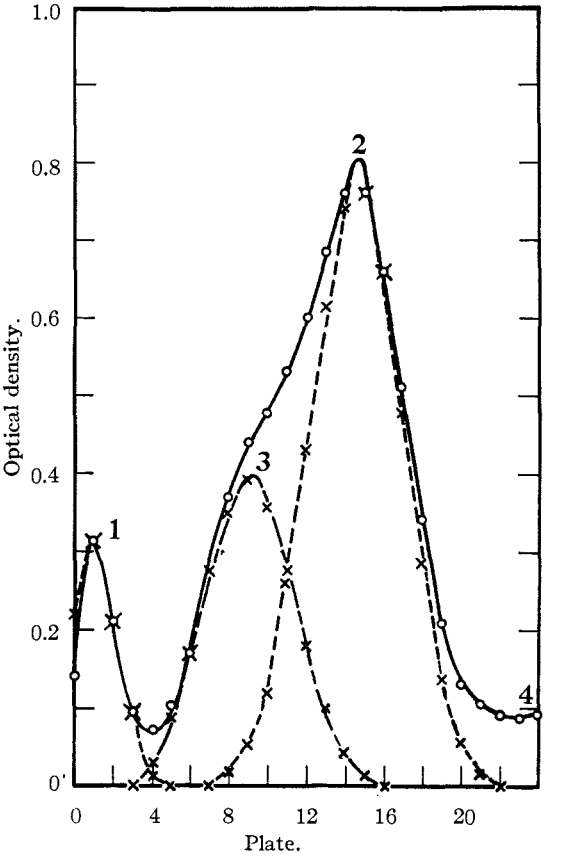


Fig. 6.

Regarding the two peaks of the experimental curve (Fig. 6) as representing components 1 and 2 in their purest condition and the peak of the middle curve as component 3 in its purest condition, it became possible, by virtue of the values of their respective distribution constants, K[10a] given in the table below, to calculate the relative amounts (per cent.) of each of the three components present in crude A. By adding the Tr values, corresponding to the three calculated curves in Fig. 6, it was found that the amounts of components 1, 2 and 3 were, respectively, 10.1, 56.3 and 27.4%.

With the above information in hand, the following procedure was adopted for the further purification of component A. The latter was considered as distributed in an eight-plate system. The same solvents and *p*H were used as for the data shown in Fig. 6, and the phosphate solution was taken as the migrating layers. By treating each of the three components, 1, 2 and 3 as separate solutes, and substituting their respective distribution constants, K, mentioned above, in the Craig and Williamson equation, the resulting values for Tr, multiplied by the per cent. of each component in crude A, would give the amounts of each component in the different plates. As can be seen in the table, components 2 and 3 occurred mostly in plates 2 to 5, practically all of component 1, having migrated to plates 6, 7 and 8.

(9) L. C. Craig and B. Williamson, to be published.

(9a) The above equation only applies to distributions in which the upper phase migrates. When the lower phase migrates then $1/K$ should be substituted for K.

(10) L. C. Craig, *J. Biol. Chem.*, **155**, 519 (1944).

(10a) The values of K for components 1 and 2 were those used in calculating the two theoretical curves in Fig. 6. K for component 3 was calculated by using the optical densities of plates 8 and 10, located on the curve for the third component.

TABLE I

CALCULATED DISTRIBUTION OF COMPONENTS 1, 2 AND 3, CONTAINED IN CRUDE COMPONENT A, WHEN SUBJECTED TO AN EIGHT-PLATE COUNTER-CURRENT DISTRIBUTION, USING ETHYL ACETATE AND AN AQUEOUS 2 M KH_2PO_4–Na_2HPO_4 SOLUTION, pH 6.3

Plate no.	0	1	2	3	4	5	6	7	8
Tr[a]	$\frac{K^8}{(K+1)^8}$	$\frac{8K^7}{(K+1)^8}$	$\frac{28K^6}{(K+1)^8}$	$\frac{56K^5}{(K+1)^8}$	$\frac{70K^4}{(K+1)^8}$	$\frac{56K^3}{(K+1)^8}$	$\frac{28K^2}{(K+1)^8}$	$\frac{8K}{(K+1)^8}$	$\frac{1}{(K+1)^8}$
					Amounts of Component 1 K = 0.058				
$Tr \times 0.101$	0.0000	0.0000	0.0000	0.0000	0.0000	0.00069	0.00602	0.02967	0.06394
					Amounts of Component 2 K = 1.54				
$Tr \times 0.563$	.01028	.05340	.12138	.15764	.12791	.06645	.02158	.0040	.00032
					Amounts of Component 3 K = 0.611				
$Tr \times 0.272$	.0000	.00015	.00873	.02686	.058469	.07657	.06266	.02929	.00599

[a] From Craig and Williamson equation.

According to the above, it became evident that it was possible to purify crude component A to the extent of removing most of component 1. Accordingly crude A was subjected to the distribution treatment, using 250 mg. of the latter at a time, 50-ml. portions of ethyl acetate and 2 M potassium dihydrogen–sodium monohydrogen phosphate solution, pH 6.3, and nine 200-ml, separatory funnels. The lower phosphate layers were removed from funnels 2, 3, 4 and 5, acidified to pH 2 with sulfuric acid and extracted with ethyl acetate. This ethyl acetate extract was then combined with the top ethyl acetate layers from the same funnels, the combined ethyl acetate extract concentrated and 750 mg. of a white fluffy powder obtained in the usual manner. Judging from the values given in the table, the powder consisted approximately of 0.10, 73 and 26% of component 1, 2 and 3, respectively. No further attempt in purification of crude A was undertaken.

The above powder, 750 mg., consisting mainly of component 2, was hydrolyzed by refluxing with 12% aqueous sodium hydroxide solution. Upon acidification, a precipitate settled out in crystalline form. This, when recrystallized from water, gave 310 mg. of caffeic acid, m. p. 195–196° cor.

Analysis of Caffeic Acid.—Calcd. for $C_9H_8O_4$: C, 59.95; H, 4.48. Found: C, 60.02; H, 4.67.

The powder gave the green ferric chloride test, characteristic of o-dihydroxyphenols, and had the same shaped absorption curve that is characteristic of simple phenolic compounds, a maximum absorption (in water) occurring at 3250 Å. The fact that on alkaline hydrolysis it gave a large yield of caffeic acid strongly suggests that component 2 of the mixture resembled closely chlorogenic acid in chemical constitution. As further evidence of its similarity to chlorogenic acid, it was found to give the same greenish colored solution on auto-oxidation of its ammoniacal solution, as shown by chlorogenic acid. Like the latter, it showed the characteristic tannin property of precipitating gelatin. It responded to the phlobaphene test by yielding a red product when heated with mineral acid, and also gave a faint Wilson boric acid test for flavones.[11]

Preparation of Sweet Potato Slices.—Sweet potatoes in good, firm condition with no signs of decay were used. The slices were prepared by first cutting off the two ends of the sweet potato, then passing a sharp cork borer longitudinally through the root. This last operation was repeated as many times as the area of the surface permitted. The root was then cut horizontally by means of a microtome. In this way, several hundred round disks or slices, 1.2 cm. in diameter and 400 millimicrons thick were obtained from the same root. The slices were placed immediately in running, cold tap water and washed for twelve to one hundred hours. During the washing, the slices were continuously in motion and thereby thoroughly mixed. In this way, it was found possible to obtain lots, consisting of 30–35 slices, which were shown to be very uniform or equivalent with respect to respiration measurements.

A Warburg respirometer was used to follow the rate and amount of oxygen uptake and carbon dioxide evolution. The carbon dioxide liberated was determined according to the direct method of Dixon.[12] The temperature of the manometer bath was 25°. The capacity of the Warburg reaction flasks was about 50 cc. Thirty-five slices were used in the flask with 2 ml. of $M/5$ phosphate buffer, pH 5.2. Filter paper moistened with 0.2 ml. of a 20% potassium hydroxide solution, for absorbing the carbon dioxide as it was formed, was placed in the center wells. The total reaction volume of the flasks was made up to 11 cc. with water. One ml. of a solution of chlorogenic acid or the "A" fraction was added at zero time to the slices from a side arm after a fifteen-minute temperature equilibrium period.

Preparation of Sweet Potato Tyrosinase.—About 1450 g. of sweet potatoes (yellow variety) was homogenized in a Waring blendor six times with acetone. The powder was dried in air and suspended in 3500 ml. of $M/5$ secondary phosphate. The suspension was stirred for several hours and then filtered. The pH of the filtrate was 7.3. This had an activity of 16 Miller–Dawson catecholase units per ml.[13] or a total of 56,000 M. D. units. The aqueous phosphate solution was treated with 2 volumes of cold acetone. The resultant precipitate was filtered and suspended in water. This was stirred for one hour and then filtered. The filtrate contained a total of 58,000 M. D. catecholase units. The latter filtrate was made 0.6 saturated with ammonium sulfate. The precipitate resulting from the precipitation with ammonium sulfate was filtered and dissolved in 20 ml. of water. This was filtered. This same filtrate was found to contain 750 M. D. catecholase units per ml., and was used in diluted form.

Acknowledgments.—The authors wish to thank the United States Sugar Corporation, Clewiston, Florida, for furnishing some of the sweet potatoes used in the present study.

Summary

1. Two substances have been isolated from sweet potatoes. One of these has been shown to be chlorogenic acid, and the other to be closely related to chlorogenic acid.

2. It has been shown that chlorogenic acid and the other phenolic compounds, designated as

(11) C. W. Wilson, THIS JOURNAL, **61**, 2303 (1939).

(12) M. Dixon, "Manometric Methods," Cambridge University Press, 2nd ed., 1943.

(13) W. H. Miller, M. F. Mallette, L. J. Roth and C. R. Dawson, THIS JOURNAL, **66**, 514 (1944).

component A, can play the role of a hydrogen carrier next to the terminal oxidase in a respiratory chain of the sweet potato.

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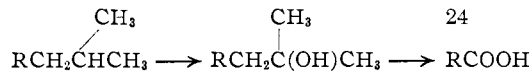
[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, JEFFERSON MEDICAL COLLEGE]

The Preparation of C-27 Steroids from Bile Acids. I. Coprostanetetrol-3(α),7(α),12(α),25

BY W. H. PEARLMAN[1]

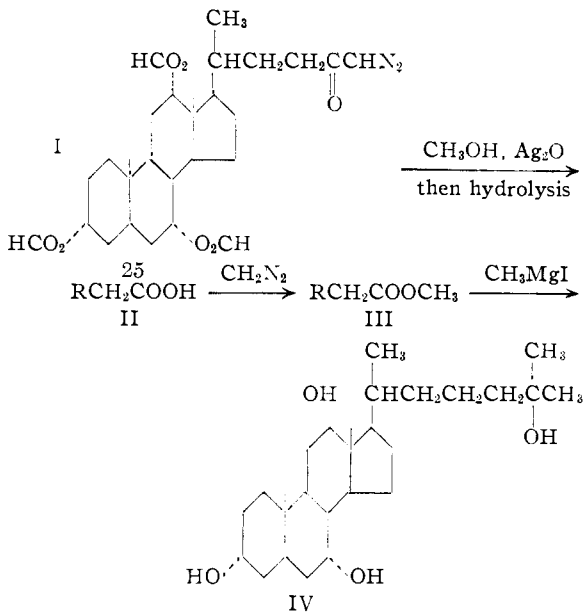
The preparation of coprostanetetrol-3(α),7(α),12(α),25 was considered desirable for the study of the intermediary metabolism involved in the biological conversion of cholesterol to cholic acid.[2] One of the metabolic routes obviously requires the removal of the terminal isopropyl group in the side-chain of the cholesterol skeleton. On the basis of chemical analogy, the oxidative attack *in vivo* may very well begin with the introduction of oxygen on carbon atom 25 and proceed as follows



R represents the rest of the steroid molecule as *e. g.*, in cholic acid.

According to this hypothesis, an immediate metabolic precursor of cholic acid might conceivably be coprostanetetrol-3(α),7(α),12(α),25. The preparation of the latter substance from cholic acid is described in this paper. It is noteworthy that the C-27 steroid, scymnol, which like coprostanetetrol-3(α),7(α),12(α),25 possesses a nucleus identical[3] with that of cholic acid, has been isolated from shark bile.[4] Similar polyhydric alcohols of the cholestane and ergostane series have been found to occur in toad bile.[5] A search for scymnol and related products in mammalian bile has thus far been unsuccessful, however.[6]

25-Homocholic acid (II), m. p. 219.5–220°, was prepared from cholic acid by the Arndt–Eistert method; the intermediary triformyl diazoketone (I), m. p. 134.5–135°, was first described by Ruzicka, *et al.*,[7] who had prepared it from the triformyl cholyl chloride of Cortese and Baumann.[8,9] The methyl ester of 25-homocholic acid (III), m. p. 166–167° (another form melts at 150–151°) on treatment with methylmagnesium iodide yielded coprostanetetrol-3(α),7(α),12(α),25 (IV),[10] m. p. 188–189°; the tetracetyl derivative melted at 142.5°.[11] Other methods for the extension of the side-chain in bile acids have been previously reported[11,12] but these do not provide a means for the introduction of oxygen on carbon 25 in the terminal isopropyl group.



R represents the rest of the molecule as in cholic acid.

Experimental[13]

25-Homocholic Acid (II).—The triformyl derivative of 25-diazo-25-homocholanetriol-3(α),7(α),12(α)-one-24 was prepared from triformyl cholyl chloride in a manner essentially that described by Ruzicka, *et al.*[7] The product thus obtained was purified by chromatography and crystallized from ethyl acetate–petroleum ether as clusters of plates, melting at 134.5–135° with decomposition (*cf.* m. p. 128–129°).[7]

(1) Aided by a grant from the National Cancer Institute.

(2) K. Bloch, B. N. Berg and D. Rittenberg, *J. Biol. Chem.*, **149**, 511 (1943).

(3) H. Ashikari, *J. Biochem. (Japan)*, **29**, 319 (1939); W. Bergmann and Wm. T. Pace, THIS JOURNAL, **65**, 477 (1943).

(4) Cited by L. F. Fieser, "The Chemistry of Natural Compounds Related to Phenanthrene," Reinhold Publishing Corp., New York, N. Y., 1937.

(5) Cited by H. Sobotka and E. Bloch, *Ann. Rev. Biochem.*, **2**, 45 (1943).

(6) W. H. Pearlman, THIS JOURNAL, **66**, 806 (1944).

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(13) All melting points are corrected.