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(−)-HYDROXYCITRIC ACID—THE PRINCIPAL ACID IN THE FRUITS OF *GARCINIA CAMBOGIA* DESR.

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Abstract—The principal acid in the highly acidic fruits of *Garcinia cambogia* has been identified as (−)-hydroxycitric acid. This acid has not been encountered in nature before. Fruits of two other species of Garcinia were found to contain the same acid, and the leaves of *Hibiscus cannabinus*, (+)-allo-hydroxycitric acid, an isomer reported earlier in *Hibiscus sabdariffa*. Wide occurrence of hydroxycitric acid in nature is thus indicated.

INTRODUCTION

Very little information is available in literature about the hydroxycitric acids (four isomers are possible, since two asymmetric C atoms are present). Lippmann[1] reported the presence of an acid in beet juice which he suspected to be hydroxycitric acid; but the acid was optically inactive. Pawolleck[2] had synthesized a salt of the hydroxycitric acid earlier, and more recently Martius[3] succeeded in preparing all the four isomers, starting from *trans*-aconitic acid. Griebel[4,5] isolated a hydroxycitric acid from *Hibiscus sabdariffa* Linn. calyxes and identified it as (+)-allo-hydroxycitric acid lactone, described by Martius.[3]

The dried rind of the fruit of *Garcinia cambogia* Desr., popularly known as "Malabar Tamarind", is extensively used all over the west coast of South India for culinary purposes, and commercially for "Colombo curing" of fish. The fruit contains about 30 per cent acid calculated as citric acid on dry wt. basis. The organic acids present have been mistakenly identified in the past as tartaric and citric acids.[6,7] The evidence which has led to the identification of the acid as (−)-hydroxycitric acid is presented here. This acid has so far not been encountered in nature.

EXPERIMENTAL AND RESULTS

Both the dried rind of the fruit, as available commercially, and fresh fruits were used for the investigations. The fresh succulent fruit is about the size of an orange, with a thin skin and deep vertical furrows forming blunt lobes.

Paper Chromatography

Using extracts of the fruit, two predominant acid spots were found on chromatograms run in different solvent systems. The R_f values of unknowns were mostly so close to tartaric and citric acids (Table 1) that they were apt to be mistaken for them. A spray of 5% sodium

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[1] E. V. Lippmann, *Ber. Deut. chem. Ges.* **16**, 1078 (1883).

[2] A. Pawollek, *Liebigs Ann. Chem.* **178**, 155 (1875).

[3] C. Martius and R. Maue', *Z. physiol. Chem.* 33, 269 (1941).

[4] C. Griebel, *Z. Unters. Lebensm.* **77**, 561 (1939).

[5] C. Griebel, *Z. Unters. Lebensm.* **83**, 481 (1942).

[6] K. I. Kuriyan and K. C. Pandya, *J. Indian Chem. Soc.* **469**, 1931.

[7] A. Sreenivasan and R. Venkataraman, *Curr. Sci. (India)* **28**, 151 (1959).

metavanadate coloured the lower acid spot reddish orange; this reaction is supposed to be a characteristic of optically active tartic acid.[8] However, attempts at quantitative determination of tartaric and citric acids by A.O.A.C.[9] methods failed to give any potassium bitartrate or pentabromacetone precipitates, even when large aliquots were taken.

TABLE 1. R_fS OF ACID SPOTS

Spot	R_f in			
	BAW	BFW	EFW	PAW
No. 1 (free acid)	0·34	0·25	0·22	0·36
Tartaric	0·33	0·24	0·34	0·27
No. 2 (lactone)	0·46	0·42	0·49	0·26
Citric	0·45	0·40	0·48	0·18

BAW—*n*-Butanol:acetic:water (4:1:5), Whatman No. 1 paper.
BFW—*n*-Butanol:formic:water (4:1:5), Whatman No. 2 paper.
EFW—Ether:formic acid:water (10:1:5), Whatman 3MM paper.
PAW—Propanol:ammonia:water (6:3:1), Whatman No. 2 paper.

Column Chromatography

Gradient elution of acids in the fruit, juice, using a Dowex formate column[10] gave a pattern (Fig. 1) showing the largest peak in the position occupied by citric and isocitric acids (also malonic and pyruvic). However, the pooled fractions analysed for isocitric acid by the isocitric dehydrogenase method[11] showed complete absence of this acid. The other acids were ruled out by a consideration of the R_f values on chromatograms.

Isolation of Acid

Attempts to separate the two acids by fractional precipitation of the calcium or barium salts proved futile. Separation by quantitative paper chromatography by application of a concentrated band at the starting line gave two separate bands. But when these bands were eluted, concentrated and tested again, each gave the original two spots. Ether extraction of the mixture of acids showed a partial concentration of the upper spot, giving a clue that a lactone might be involved. On saponification of the mixture using excess alkali and passing through a cation-exchange resin (Zeocarb 215) the eluate showed only the lower spot. But on concentration over a water bath, the upper spot also appeared. On heating the concentrate over a water bath for several hours and keeping in a desiccator for some days, a crude crystalline material was obtained. This gave only one spot (upper) on chromatograms. On titration of this material with alkali, two different end-points were obtained, using phenolphthalein, in the cold and after boiling, characteristic of lactones. It was thus clear that the two spots on chromatograms are those of a γ-hydroxy acid and its lactone.

Isolation of the acid on a large scale was carried out by the following procedure. The dried rind was cooked with about three volumes of water in an autoclave ($10\ \mathrm{lb/in^2}$ for 15 min) and the extract filtered first through cloth and then paper. The filtrate was concentrated to

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[8] H. A. STAFFORD, *Amer. J. Bot.* **46**, 347 (1959).

[9] *Official and Tentative Methods of Analysis*, Assn. of Agr. Chem. Washington (1955).

[10] J. K. PALMER, *Bulletin* 589, Connecticut Agri. Exp. Station, New Haven, Conn (1955).

[11] J. R. STERN, S. P COLOWICK and N. O. KAPLAN, *Methods in Enzymol.* 3, 428 (1957).

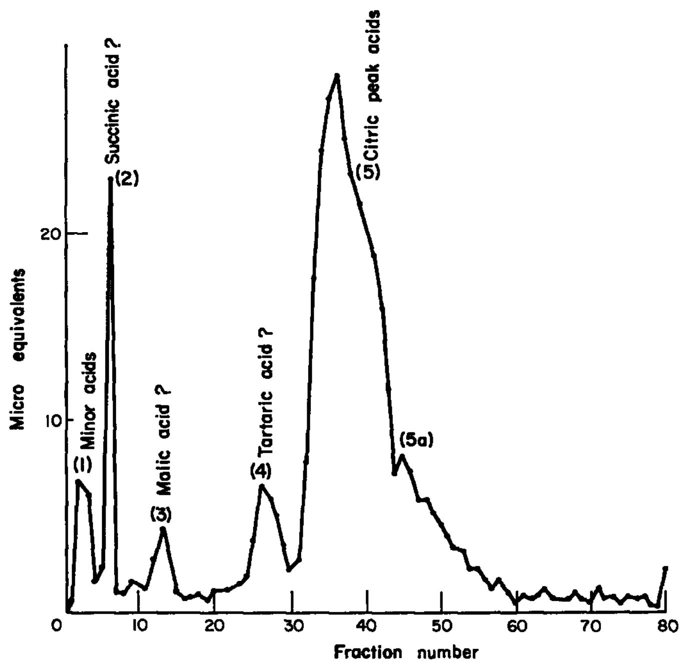


FIG. 1. DIAGRAM OF THE ION-EXCHANGE SEPARATION OF THE ORGANIC ACIDS OF *Garcinia cambogia* ON DOWEX 1 FORMATE COLUMN.

a small volume and treated with two volumes of alcohol to remove pectin. The clear filtrate was neutralized with alkali, when a viscous, dark, heavy liquid separated (it had been found by previous experience that this oily liquid is the highly hygroscopic alkali salt of the acid). The clear supernatant was decanted off and the oily liquid washed with 60 % alcohol several times. By repeated treatment with absolute alcohol, this could be dried to a pale yellow hygroscopic powder, which formed the pure alkali salt. Aqueous solutions of the alkali salt were passed through a cation-exchange resin (Zeocarb 215) for recovery of the acid. The acid itself could not be crystallized because evaporation resulted in formation of the lactone. The syrup obtained on evaporation of the eluate from the ion-exchange resin was dried on a water bath for several hours, and then in a vacuum oven at 100° for 8–10 hr. A crude crystalline mass was obtained after keeping in a desiccator for several days. Alternately, the material obtained by evaporation on a water bath could be seeded with a few crystals of the lactone. The crude crystalline material, on extraction and recrystallization from ether, gave clusters of small needle-shaped crystals of the lactone (Fig. 2).

Characteristics of the Acid

Some of the characteristics of the lactone and the acid are shown in Table 2. The rotation for acid was obtained by saponifying the lactone with excess alkali and adding an equivalent amount of HCl before taking the reading. The equivalent weight of pure lactone is 69, as determined by alkali titration or silver salt decomposition.

By titration and measurement of conductivity the acid proved to be a γ-hydroxy tribasic acid.

FIG. 2. CRYSTALS OF HYDROXYCITRIC ACID LACTONE.

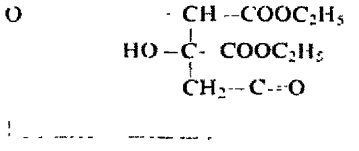
TABLE 2. COMPARISON OF "GARCINIA" ACID WITH "HIBISCUS" ACID

Property	Hibiscus acid (lactone)	Garcinia acid (lactone)
m.p.	183	178
$(\alpha)_D^{20}$	+122	+100
For free acid	+ 31	− 20
After saturation with Borax	+ 31	− 92
Sublimation	+	+
Crystal shape	Needles	Needles
Hygroscopicity	High	Slight
Solubility	High in water and alcohol; slight in ether	High in alcohol and water, fair in ether
Action of dehydrogenase (cucumber seed or liver homogenate) on free acid	Negative	Negative
Paper chromatography R_fs in:		
BFW* –Acid	0·15	0·24
Lactone	0·39	0·42
PAW—Acid	0·35	0·36
Lactone	0·26	0·26
Metavandate spray		
Acid spot	Yellow	Reddish orange
Lactone spot	Yellow	Yellow
Quinine salt, m.p.	227 decomp.	215° decomp.

* See Table 1.

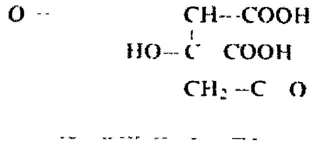
The molecular weight of lactone from alkali titres is 190; of the acid, 208.

The i.r. spectra of the ethyl ester (b.p. = 178° at 2 mm), showed ester groups (5·41–5·76 μ), OH groups (2·74–2·79 μ) and a γ-lactone (5·62–5·68 μ). The ester analysed for $C_{10}H_{14}O_7$ (Found: C, 49·2; H, 6·0. $C_{10}H_{14}O_7$ required: C, 48·78; H, 5·73%.) and had a saponification value of 686. The lactone gave analysis (Found: C, 38·2; H, 3·4. $C_6H_6O_7$ required: C, 37·9; H, 3·15%) for $C_6H_6O_7$. Based on this, the ester was given formula (I) and the lactone formula (II).



$$\begin{array}{l} O-CH-COOC_2H_5 \\ \quad\;\; HO-C-COOC_2H_5 \\ \quad\;\; CH_2-C=O \end{array}$$

(I)



$$\begin{array}{l} O-CH-COOH \\ \quad\;\; HO-C-COOH \\ \quad\;\; CH_2-C=O \end{array}$$

(II)

Since more than one (OH) group is present in the free acid molecule, a test for viscinal (OH) groups was made by measuring the exaltation of rotation produced by borax. $(\alpha)_D^{20}$ for free acid = −20°; after saturation with borax = −92°, which is clearly indicative of "the situation of the two hydroxyl groups in the same plane and on the same side of the carbon atoms to which they are bound".[12]

Since it was now evident that the acid from Garcinia is one of the hydroxycitric acids, (+)-allo-hydroxycitric lactone was prepared from the calyxes of *Hibiscus sabdariffa* fruits (according to the procedure recommended by Griebel)[4] for purposes of comparison. That the two acids are different is clear from the properties listed in Table 2. The properties of "Garcinia" acid closely resembled those of (−)-hydroxycitric acid described by Martius.[3]

A sample of our lactone was examined by Dr. C. Martius. It was found that the lactone from *Garcinia cambogia*, the lactone of synthetic (−)-hydroxycitric acid and the mixture of both had the same melting point. Paper chromatography also showed the identity of "Garcinia" acid and synthetic (−)-hydroxycitric acid.

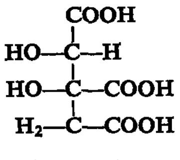
We have also examined the acid composition of a few related plants. The fruits of *Garcinia atroviridis* Griff and *Garcinia indica* Choisy, which are widely used for culinary purposes in India and Malaya, were found to contain (−)-hydroxycitric acid as the principal acid. *Hibiscus cannabinus* Linn. and *Hibiscus furcatus* Roxb. leaves were found to contain (+)-allo-hydroxycitric acid as the principal acid. The mildly acidic leaves of these two plants are also used for culinary purposes in India. The fruit calyxes in these were, however, poor in acid content.

DISCUSSION

Martius[3] emphasized the importance of hydroxycitric acid in plant and animal metabolism at a time when it was still not known to occur in nature. A survey of organic acids in a large number of fruits belonging to the Garcinia genus would doubtless reveal the presence of hydroxycitric acid in many of them besides those reported here. It is also quite likely that the other two isomers (+)-hydroxycitric acid and (−)-allo-hydroxycitric acid would be found in this and other genera. The occurrence of isomers of hydroxycitric acid in nature may thus be quite wide.

Configuration

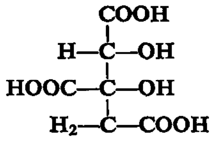
The exaltation of optical rotation by borate in "Garcinia" acid is clearly indicative of "vicinal" (OH) groups. The allo-hyroxycitric acid from *H. sabdariffa* does not show any such increase of rotation. If Hudson's lactone rule is applicable to the hydroxycitric acids (it is shown to be applicable to the isocitric acids[13]), then the absolute configuration of "Garcinia" acid should be (III). Correspondingly, those of the other isomers should be (IV), (V)



$$\begin{array}{r@{}c@{}l} & COOH & \\ HO- & C & -H \\ HO- & C & -COOH \\ H_2- & C & -COOH \end{array}$$

(—)-Hydroxycitric acid
Lactone dextro
"Garcinia acid"

(III)



$$\begin{array}{r@{}c@{}l} & COOH & \\ H- & C & -OH \\ HOOC- & C & -OH \\ H_2- & C & -COOH \end{array}$$

(+)— Hydroxycitric acid
Lactone levo

(IV)

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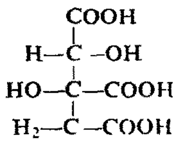
[12] J. Boeseken, *Rec. Trav. chim.* 34, 96 (1915).

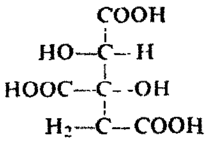
[13] H. Katsura, *Chem. & Ind. (London)* 1188 (1960).

and (VI). Confirmation of this by optical rotatory dispersion and X-ray diffraction studies would, of course, be necessary.



(—)-allo-Hydroxycitric acid
Lactone levo

(V)



(+)—allo-Hydroxycitric acid
Lactone dextro.
"*Hibiscus acid*"

(VI)

R_M Values

Bate-Smith and Westall[14] have shown that in several series of polyhydroxy compounds, the addition of each (OH) group decreases the R_M value $[(R_M = \log(1/R_f - 1)]$ of the compounds by an approximately constant amount. Hulme[15] found this to be true in a related series of acids like succinic, malic and tartaric acids. It is interesting to note that this is not obeyed in the series tricarballylic, citric and hydroxycitric acids (Table 3).

TABLE 3. R_M VALUES OF TRICARBALLYLIC ACID SERIES

Acid	BFW		PAW	
	R_M	ΔR_M	R_M	ΔR_M
Tricarballylic	−0·31		0·50	
		0·49		0·13
Citric	0·18		0·63	
		0·25		0·38
Hydroxycitric	0·43		0·25	
		0·57*		0·36*
Allo-hydroxycitric	0·75		0·27	

* Between allo-hydroxycitric and citric acid.

Colour Reaction with Metavandate

Since the R_f of hydroxycitric acid is very close to tartaric acid in the common solvent systems and it also gives a reddish orange colour with metavandate, it would not be sufficient to depend on these two data for identification of optically active tartaric acid in plants, as has been done previously.[6]

Biochemistry

The mode of formation of the acid in the plant cell would be a very interesting study. Martius[3] found that only one of the four isomers (+-hydroxycitric acid) could be readily dehydrogenated using cucumber seed or liver homogenate as source of the enzyme. The presence of large quantities of the inactive isomers in plants may then be due to the absence of

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[14] E. C. BATE-SMITH and R. G. WESTALL, *Biochim Biophys. Acta* **4**, 427 (1950).

[15] A. C. HULME, *J. Exp. Botany* **2**, 298 (1951).

enzymes which could oxidize them further, as in the case of (+)-tartaric acid.[16] This has to be verified by experimentation. Martius[3] makes the assumption that in the formation of hydroxy glutamic acid, (+)-hydroxycitric acid has an importance at least corresponding to that of citric or isocitric acids, for the endogenous formation of glutamic acid. It may be possible to prove the biological importance of this substrate, at least in plants.

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[16] H. B. Vickery and J. K. Palmer, *J. Biol. Chem.* **207**, 275 (1954).