

Naturally Occurring Lactones and Lactames

III. The Absolute Configuration of the Hydroxycitric Acid Lactones: Hibiscus Acid and Garcinia Acid

PER M. BOLL, ELSE SØRENSEN and ERIK BALIEU

*Chemical Laboratory II (General and Organic Chemistry), University of Copenhagen,
The H. C. Ørsted Institute, DK-2100 Copenhagen, Denmark*

The absolute configuration of the hydroxycitric acid lactones, hibiscus acid and garcinia acid, is determined to be (2*S*,3*R*)- and (2*S*,3*S*)-2-hydroxycitric acid-2,5-lactone, respectively. The relative configuration is determined from (a) titration and determination of the dissociation constants, (b) synthesis of the acids from *trans*- and *cis*-aconitic acid, (c) IR- and NMR-data. The absolute configuration is determined from (d) Hudson's lactone rule, (e) ORD- and CD-curves, and (f) calculation of partial molar rotations.

Hydroxycitric acid contains two centres of asymmetry and, correspondingly, there are four stereoisomeric forms, all prepared by Martius and Laue in 1941.¹ The natural occurrence of hydroxycitric acid, apparently optically inactive, was reported in 1883 by Lippmann,² and two diastereomeric γ -lactones of hydroxycitric acid, hibiscus acid^{3,4} and garcinia acid,⁵ have been isolated. Lewis and Neelakantan⁵ have tried to determine the absolute configuration, using Hudson's lactone rule and the exaltation of optical rotation by borax of free, non-lactonized garcinia acid. The latter experiment, considered by the authors as indicative of the relative configuration of the two hydroxyl groups, can hardly be considered informative, as rotation about the single bonds is free, and, furthermore, α -hydroxy acids form complexes with borax. In a recent personal communication, Lewis⁶ communicates that X-ray diffraction studies have provided the same relative configuration of the acids as reported in the present paper (Fig. 1).

Relative configuration. a. *Cis* and *trans* configurations of adjacent carboxyl groups on a ring system or about a double bond may be differentiated on the basis of relative difference in pK_1 and pK_2 values, the *cis* configuration being assigned to that isomer for which the pK_2 value differs most from the pK_1 value.^{7,8}

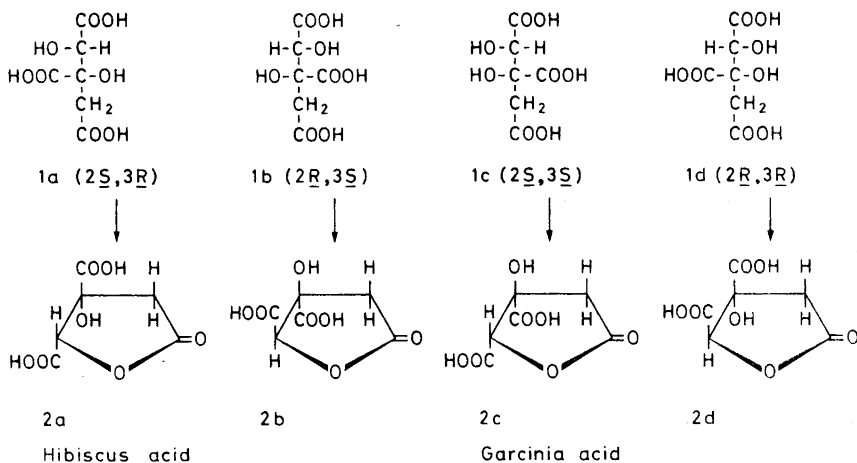


Fig. 1. Isomers of (a) 2-hydroxycitric acid and (b) the corresponding 2,5-lactones.

Potentiometric titration of hibiscus acid (2a) yielded a pK_1' of 2.40 and a pK_2' of 3.82. For garcinia acid (2c), the corresponding values were 1.80 and 3.75 (Fig. 2). The *cis* isomer is the more strongly acidic as judged by comparison of the constants for the first dissociation. The greater spread between the first and second dissociation constants also assigns the *cis* configuration to garcinia acid.

b. This stereochemical conclusion was further supported by synthesizing hydroxycitric acid from both *trans*- and *cis*-aconitic acid by oxidation with silver chlorate and osmium tetroxide. *trans*-Aconitic acid yielded, by *cis*-hydroxylation, exclusively DL-garcinia acid, whereas somewhat impure *cis*-

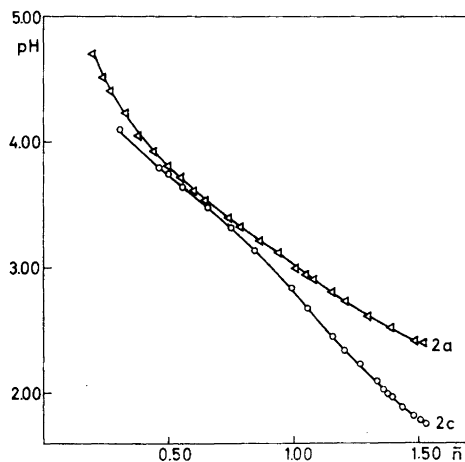


Fig. 2. Potentiometric titration of 47.6 mg of hibiscus acid (2a) (neutr. equiv. 94.8, theory 95.1) and of 70.5 mg of garcinia acid (2c) (neutr. equiv. 95.9, theory 95.1).

aconitic anhydride gave a mixture of DL-hibiscus acid and DL-garcinia acid, both syntheses in accordance with the above assignment.

c. Further support of the relative configuration is to be found in the NMR- and IR-spectra of the dimethyl ester of hibiscus acid (2a). A very sharp signal for the tertiary hydroxyl is observed in the NMR-spectrum at $\delta=6.84$ ppm and in the IR-spectrum at 3400 cm^{-1} , indicative of hydrogen bonding between the hydroxyl group and the adjacent *cis*-positioned carboxyl group. The corresponding signals for the dimethyl ester of garcinia acid (2c) are broad and their position less well defined.

Absolute configuration. d. The configuration of the hydroxyl groups constituting the alcohol part of a lactone has a major influence on the rotation of the latter compound. The "Hudson lactone rule",⁹ in its qualitative form stipulates that a lactone is more dextrorotatory than the free acid if the hydroxyl group involved in lactone formation is positioned on the right side in the Fischer projectional formula. Conversely, the lactone will be more levorotatory than the acid if the hydroxyl group is on the left side. This rule, shown applicable to the isocitric acids,¹⁰ indicates that both diastereomeric hydroxycitric acids possess the 2*S*-configuration (*cf.* Fig. 1 and Experimental) as already concluded by Lewis and Neelakantan.⁵ Hence, the absolute configurations of hibiscus acid and garcinia acid are as depicted in 2a and 2c, respectively.

e. It has recently been shown that the optical rotatory dispersion curves of (*S*)-4-carboxy-4-butanolide, (*S*)-4-carboxy-4-pentanolide, and several structurally similar necic acid γ - and δ -lactones, exhibit Cotton effects, the sign of which defines the configuration at the carbon atom bound to the heterocyclic oxygen atom. Thus, a positive Cotton effect signifies the *S*-configuration at this carbon atom and *vice versa*.¹¹

The CD as well as the ORD curves of hibiscus acid (2a) and garcinia acid (2c) exhibit positive Cotton effects indicating a 4*S*-configuration (2*S* of the free, non-lactonized acids). In agreement with this finding is the observed

Table 1. Spectroscopic data on different citric acid derivatives.

Compound	UV		CD		ORD		Absolute configuration at C-4 ^a
	$\lambda_{\text{max}}^{\text{MeOH}}$	$\log \epsilon$	$\lambda_{\text{max}}^{\text{MeOH}}$	$[\theta]$	$\lambda_{\text{max}}^{\text{MeOH}}$	$[\Phi]$	
Hibiscus acid (2a)	216nm	2.20	215nm	+15099	232nm ^b	+10400	<i>S</i>
Hibiscus acid dimethyl ester			214nm	+14525	230nm	+10887	
Garcinia acid (2c)	216nm	2.22	210nm	+6614	233nm ^b	+4095	<i>S</i>
Garcinia acid dimethyl ester			214nm	+9504	228nm	+4885	
(-)-Isocitric lactone	214nm	2.15	213nm	-4197	232nm	-3058	<i>R</i>
(-)-Isocitric lactone dimethyl ester			214nm	-4326	230nm	-3578	

^a Equivalent to C-2 in the free, non-lactonized acids.

^b Also recorded by Lewis.⁶

Table 2. Contribution of asymmetric α - and β -carbon atoms to the molar rotation of the diastereomeric hydroxyctic acids.

	[M] _D ²⁵ (degrees)		Molar rotation of:						
	in H ₂ O	as Na-salt in H ₂ O	α in H ₂ O	α_{salt} in H ₂ O	$\alpha_{\text{H}_2\text{O}} - \alpha_{\text{salt}}$ in H ₂ O	β in H ₂ O	β_{salt} in H ₂ O	$\beta_{\text{H}_2\text{O}} - \beta_{\text{salt}}$	$\alpha_{\text{H}_2\text{O}} - \alpha_{\text{salt}}$ + $\beta_{\text{H}_2\text{O}} - \beta_{\text{salt}}$ *
L-Hydroxy acid									
(2 <i>S</i> ,3 <i>R</i>)-2-Hydroxyctic acid (1a)	+91.5	+69.1	+25.0	+6.5	+18.5	+66.5	+62.7	+3.9	+22.4
(2 <i>S</i> ,3 <i>D</i>)-2-Hydroxyctic acid (1c)	-41.6	-56.2	+25.0	+6.5	+18.5	-66.6	-62.7	-3.9	+14.6

* equal to [M]_D²⁵(H₂O) - [M]_D²⁵(salt)

negative Cotton effect of (–)-isocitric lactone ((2*R*,3*S*)-3-carboxy-2-hydroxy-pentandioic acid-2,5-lactone)¹² (Table 1).

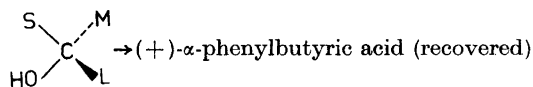
f. Inasmuch as all known L- α -hydroxy acids become more dextrorotatory on addition of acid to their sodium salts, the determination of the rotation of a hydroxy acids as salt and as free acid affords a convenient physical method for determining its configuration (*cf.* Winitz, Birnbaum, and Greenstein¹³).

One of the classical studies of Hudson¹⁴ was concerned with the testing of the validity of the van't Hoff theory of optical superposition in the sugar group. Hudson formulated rules (rules of isorotation) to show that the difference in the rotational values between isomeric sugars, which differ only in their configuration about a given asymmetric centre, is a constant. In its full generalization as applied to all substances, the hypothesis is definitely unsound; nevertheless, it has been shown that the hypothesis holds in first approximation for a large number of carbohydrates,¹⁴ amino acids containing more than a single asymmetric centre,¹³ and the isocitric acids.¹³

The rotation of the diastereomeric (2*S*,3*R*)-2-hydroxycitric acid (1a) and (2*S*,3*S*)-2-hydroxycitric acid (1c) may be considered to be a function of the sum of the partial rotations of each centre. The contribution (partial molar rotation) of the α -asymmetric centre to the molar rotations of both hydroxycitric acids was calculated¹³ (*cf.* Table 2) and the positive $\alpha_{\text{H}_2\text{O}} - \alpha_{\text{salt}}$ values are indicative of the L-configuration for both acids.

The partial-resolution method developed by Horeau¹⁵ for determining the absolute configuration of optically active secondary alcohols employing the reaction with racemic α -phenylbutyric anhydride, has been discussed with regard to its mechanism^{16,17} and should be applicable also for determination of the configuration of tertiary alcohols.

When the dimethyl ester of hibiscus acid was reacted with α -phenylbutyric anhydride, dextrorotatory α -phenylbutyric acid could be extracted from the reaction solutions with a sodium bicarbonate solution. Therefore the absolute configuration is represented by the following formula, where L, M, and S indicate the relative sizes of the substituent group, namely large, medium and small^{15,18}:



The problem remaining is the assignment of relative size to the substituent groups. By accepting the *R*-configuration for the β -carbon of hibiscus acid, the substituent groups have to be arranged in the following order of size:

COOH
|

S~CH₂~, M~CH~, L~COOH. It can, of course, be discussed how reasonable this assignment of order of size is.

If the triamide of (2*S*,3*R*)-2-hydroxycitric acid prepared from hibiscus acid was reacted with α -phenylbutyric anhydride apparently no resolution occurred.

The hibiscus acid used in this investigation was isolated from *Hibiscus sabdariffa* L. as its dimethyl ester, but this is probably to consider as an artifact, since methanolic hydrogen chloride was used in extracting the plant material. The ester was identified from elemental analysis, IR- and NMR-spectroscopy, and saponification equivalents.

EXPERIMENTAL

Microanalyses were carried out in the microanalysis department of this laboratory by Mr. Preben Hansen and his staff.

Infrared spectra were recorded on a Perkin-Elmer Infracord model 137. Optical rotatory dispersion curves and circular dichroism curves were measured with a Cary 60 spectropolarimeter.

Thin-layer chromatography (TLC). The chromatograms were run on 8 × 10 cm plates with a 0.25 mm layer of silica gel (Kieselgel H Merck) in butanol-formic acid-water (6:1:3). The compounds were detected with bromocresol green indicator.

Preparative layer chromatography (PLC). The plates were 20 × 100 cm with a thickness of 2.5 mm of Kieselgel H Merck. Solvent system and detection reagent as above. The fractions were extracted in a Soxhlet apparatus with ether for about 10 h.

Hibiscus acid dimethyl ester. With the purpose of isolating anthocyanins as well as hibiscus acid, the dried, ground calyxes (1200 g) of *Hibiscus sabdariffa* L. fruits were extracted at room temperature for 68 h with 3.6 l of methanol containing 1.5 % of hydrogen chloride. The extraction was repeated for 22 h, this time with 2 l of the hydrogen chloride containing methanol. To the pooled methanol extracts 13 l of ether were added and the colouring matter was deposited as a dark red syrupy mass. It was dissolved in 800 ml of methanolic hydrogen chloride (1 %) and again precipitated by the addition of 2.5 l of ether. The pooled ether extracts were evaporated and dissolved in 1 l of methanol. Upon cooling 42.0 g of colourless crystals were collected. Recrystallized from propanol the m.p. was 128–129°, $[\alpha]_D^{25} +112^\circ$ ($c=0.49$, MeOH) (Found: C 43.96; H 4.51. Calc. for $C_6H_{10}O_7$: C 44.04; H 4.62).

When dissolved in excess of standard N NaOH and immediately titrated with standard N HCl an equivalent weight of 108 was obtained. When dissolved in excess of standard base, refluxed for 3 h and then titrated with standard N HCl an equivalent weight of 74 was obtained. Apparently the molecular weight is in the range of 216 (2×108) to 222 (3×74).

IR-spectrum (KBr): 1750 (ester), 1800 (γ -lactone), 3400 cm^{-1} (OH, sharp).

Hibiscus acid. Hibiscus acid dimethyl ester (4.50 g) was refluxed in 50 ml of 3 N hydrochloric acid for 6 h. The solution was concentrated *in vacuo* to a syrup, which for crystallization was stored over Drierite for 2 months. Recrystallized from ethanol-toluene, the m.p. was 182–183° (decomp.), $[\alpha]_D^{25} +110^\circ$ ($c=1.37$, H_2O) (Lit.⁵ m.p. 183°, $[\alpha]_D^{20} +122^\circ$).

(2S,3R)-2-Hydroxycitramide. Prepared by voluntarily evaporation of hibiscus acid dimethyl ester in liquid ammonia. Yield: 100 %. M.p. 185–188°, $[\alpha]_D^{25} +16.8^\circ$ ($c=0.25$, H_2O) (Found: C 35.17; H 5.42; N 20.24. Calc. for $C_6H_{11}N_3O_5$: C 35.12; H 5.40; N 20.48).

Garcinia acid dimethyl ester. Garcinia acid (60 mg) was esterified with methanolic hydrogen chloride. Yield: 68 mg of ester, which could not be induced to crystallize. $[\alpha]_D^{25} +92.1^\circ$ ($c=0.30$, MeOH). IR-spectrum (neat): 1735 (ester), 1790 (γ -lactone), ca. 3350 cm^{-1} (OH, broad).

DL-Garcinia acid. To a mixture of *trans*-aconitic acid (0.05 mol) in 250 ml of water and 2.5 ml of a 1 % aqueous solution of osmium tetroxide was added 3.2 g (0.0165 mol) of silver chlorate in two portions, separated by an interval of 24 h.¹⁹ After 48 h, the silver chloride was removed by filtration (1.657 g, 70 %) and the filtrate was evaporated *in vacuo*. After drying over P_2O_5 , the NMR-spectrum of the residual oil indicated the presence of garcinia acid, non-lactonized hydroxycitric acid, and aconitic acid. TLC: R_F 0.33 and 0.87 (garcinia acid: R_F 0.35; aconitic acid; R_F 0.89) PLC of 1.5 g of the synthetic mixture yielded 217 mg of an oil, which became crystalline on drying over P_2O_5 and

treatment with ether and petrol ether at -80° . M.p. after recrystallization from ether 175° . The NMR-spectrum established the identity of the compound as pure DL-garcinia acid.

DL-Hibiscus acid. *cis*-Aconitic anhydride (7.8 g, 0.05 mol) admixed *trans*-aconitic anhydride was reacted as under DL-garcinia acid to give, after PLC, a mixture of DL-hibiscus acid and DL-garcinia acid as judged from the NMR-spectrum.

Pyridinium salt of (–)-alloisocitric lactone. Prepared by isomerization of (–)-isocitric lactone in pyridine.²² M.p. $128-130^{\circ}$, $[\alpha]_D^{25} -24.8^{\circ}$ ($c=0.23$, H_2O) (Found: C 51.90; H 4.47; N 5.38. Calc. for $C_{11}H_{11}NO_6$: C 52.17; H 4.38; N 5.53).

(–)-alloisocitric lactone. Isolated from its pyridinium salt as an oil, $[\alpha]_{D_{25}} -35.2^{\circ}$ ($c=0.46$, H_2O) (Lit.¹³ -40.5). From its NMR-spectrum some diethyl ether seemed to be present in the compound.

Nuclear magnetic resonance spectra were recorded on a Varian A 60A instrument in dimethyl sulfoxide- d_6 . All values are δ [ppm] relative to TMS=0. Values in parentheses are the coupling constants in cps.

	αC		βC		γC	
	H _A	H _B	OH	COOCH ₃	H	COOCH ₃
Hibiscus acid (2a)	d 3.10 (17.5)	d 3.56 (17.5)	—	—	s 5.10	—
Hibiscus acid dimethyl ester	d 2.67 (17.5)	d 3.27 (17.5)	s 7.83 sharp	s 3.82	s 5.40	s 3.72
Garcinia acid (2c)	d 3.07 (17.5)	d 3.51 (17.5)	—	—	s 4.71	—
Garcinia acid, dimethyl ester	d 2.67 (17.5)	d 3.16 (17.5)	s 7.0 broad	s 3.76	s 5.03	s 3.70

	H _A	H _B	H	COOCH ₃	H	COOCH ₃
	(–)-Isocitric lactone	q 2.64 (17)	q 2.77 (17)	q 3.68 (8)	—	d 5.00 (8)
(–)-Isocitric lactone, dimethyl ester	q 2.75 (17.5)	q 2.90 (17.5)	q 3.92 (8)	s 3.70	d 5.33 (8)	s 3.65
(–)-alloisocitric lactone	q 2.64 (17)	q 2.80 (17)	m 3.4	—	d 4.95 (4)	—

Potentiometric titrations. About 50 mg of a particular lactone was dissolved in 10 ml of water and titrated at 23.5° under nitrogen with standard 0.1 N sodium hydroxide, using a model pH meter 22 (Radiometer).

pK_a values were obtained from a plot (Fig. 2) of pH vs. \bar{n} using the Bjerrum treatment and considering H^+ as the ligand.²⁰ At \bar{n} equal to 1.5, the pH is equal to pK_1 and at \bar{n} equal to 0.5, pH is equal to pK_2 .⁹

Polarimetric measurements. The specific rotations were measured in water on a Perkin-Elmer polarimeter model 141.

	$[\alpha]_D^{25}$	$[M]_D^{25}$	c
Hibiscus acid (2a)	+110	+209	1.37
(2 <i>S</i> ,3 <i>R</i>)-2-Hydroxycitric acid (1a) ^a	+44.0	+91.5	0.16
Trisodium (2 <i>S</i> ,3 <i>R</i>)-2-hydroxycitrate	+33.2	+69.1	0.63
Garcinia acid (2c)	+100 ⁵	+190	
(2 <i>S</i> ,3 <i>S</i>)-2-Hydroxycitric acid (1c) ^a	–20.0 ⁵	–41.6	
Trisodium (2 <i>S</i> ,3 <i>S</i>)-2-hydroxycitrate	–27.0	–56.2	0.63 ^b

^a Trisodium salt neutralized with 3 equivalents of HCl.

^b Calculated on the basis of free acid.

Partial resolution method. Hibiscus acid dimethyl ester: The reaction was carried out according to Mabry *et al.*²¹ with 107 mg (0.5 mmol) of the alcohol and 464 mg (1.5 mmol) of the racemic α -phenylbutyric anhydride in 5 ml of pyridine. Four experiments were performed, in which the reaction mixture was (1) set aside at room temperature for 24 h, (2) was refluxed for 2 h and then set aside at room temperature for 24 h, (3) refluxed for 12 h and then set aside at room temperature for 4 days, (4) set aside at room temperature for 20 days.

The purity of α -phenylbutyric acid was checked using NMR and IR.

Yield of α -phenylbutyric acid:

	mg	mmol	%	$[\alpha]_D^{25}$	optical resolution in %
1.	263.4	1.60	64	+0.29	1.5 ($c=7.68$, benzene)
2.	267.7	1.63	65	+0.51	2.6 ($c=3.35$, benzene)
3.	258.1	1.57	63	+2.03	10.5 ($c=2.06$, benzene)
4.	242.1	1.48	59	+1.36	7.1 ($c=2.10$, benzene)

(2*S*,3*R*)-2-Hydroxycitramide: Three experiments carried out as above with 102.5 mg (0.5 mmol) of the triamide and 310 mg (1.0 mmol) of racemic α -phenylbutyric anhydride in 10 ml of pyridine. The heterogenic mixture was stirred at room temperature for 24 h. The rotations measured of the recovered α -phenylbutyric acid in benzene were as follows: $\alpha_D +0.003$ ($c=0.538$), -0.003 ($c=1.00$), $+0.001$ ($c=0.538$).

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