Naturally Occurring Lactones and Lactames

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

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The absolute configuration of the hydroxycitric acid lactones, hibiscus acid and garcinia acid, is determined to be (2S,3R)- and (2S,3S)-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. The natural occurrence of hydroxycitric acid, apparently optically inactive, was reported in 1883 by Lippmann, and two diastereomeric γ-lactones of hydroxycitric acid, hibiscus acid and garcinia acid, have been isolated. Lewis and Neelakantan 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, Lewis 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 pK1 and pK2 values, the cis configuration being assigned to that isomer for which the pK2 value differs most from the pK1 value.
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-

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**Acta Chem. Scand. 23 (1969) No. 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 δ = 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,"\(^8\) 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 \(\gamma\)- and \(\delta\)-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.

<table>
<thead>
<tr>
<th>Compound</th>
<th>UV</th>
<th>CD</th>
<th>ORD</th>
<th>Absolute configuration at C-4 (^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\lambda_{\text{max}})</td>
<td>(\lambda_{\text{max}})</td>
<td>(\theta)</td>
<td>(\theta)</td>
</tr>
<tr>
<td>Hibiscus acid (2a)</td>
<td>216nm</td>
<td>215nm</td>
<td>+15099</td>
<td>232nm (^b)</td>
</tr>
<tr>
<td>Hibiscus acid dimethyl ester</td>
<td>216nm</td>
<td>214nm</td>
<td>+14525</td>
<td>230nm (^b)</td>
</tr>
<tr>
<td>Garcinia acid (2c)</td>
<td>216nm</td>
<td>210nm</td>
<td>+6614</td>
<td>233nm (^b)</td>
</tr>
<tr>
<td>Garcinia acid dimethyl ester</td>
<td>214nm</td>
<td>214nm</td>
<td>+8504</td>
<td>228nm (^b)</td>
</tr>
<tr>
<td>((-))-Isocitric lactone</td>
<td>214nm</td>
<td>213nm</td>
<td>-4197</td>
<td>232nm (^b)</td>
</tr>
<tr>
<td>((-))-Isocitric lactone</td>
<td>214nm</td>
<td>214nm</td>
<td>-4326</td>
<td>230nm (^b)</td>
</tr>
<tr>
<td>dimethyl ester</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

\(^b\) Also recorded by Lewis.\(^4\)

Table 2. Contribution of asymmetric \( \alpha \) - and \( \beta \)-carbon atoms to the molar rotation of the diastereomeric hydroxycitric acids.

<table>
<thead>
<tr>
<th>( \alpha )-Hydroxy acid</th>
<th>( [M]_D^{25} ) (degrees)</th>
<th>Molar rotation of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>in ( \text{H}_2\text{O} )</td>
<td>as Na-salt in ( \text{H}_2\text{O} )</td>
</tr>
<tr>
<td>( (2S,3R) )-2-Hydroxycitric acid (1a)</td>
<td>+91.5</td>
<td>+69.1</td>
</tr>
<tr>
<td>( (2S,3S) )-2-Hydroxycitric acid (1c)</td>
<td>−41.6</td>
<td>−56.2</td>
</tr>
</tbody>
</table>

* equal to \( [M]_D^{25} \)(\( \text{H}_2\text{O} \)) − \( [M]_D^{25} \)(salt)
negative Cotton effect of (−)-isocitric lactone ([2R,3S]-3-carboxy-2-hydroxy-
 pentandioic acid-2,5-lactone)\textsuperscript{12} (Table 1).

f. Inasmuch as all known \( \text{L}-\alpha \)-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 (\textit{cf.} Winitz, Birnbaum, and Greenstein\textsuperscript{13}).

One of the classical studies of Hudson\textsuperscript{14} 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 differ-
ence 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,\textsuperscript{14} amino acids containing
more than a single asymmetric centre,\textsuperscript{13} and the isocitric acids.\textsuperscript{13}

The rotation of the diastereomeric (2\( S \),3\( R \))-2-hydroxyxycitric acid (1a) and
(2\( S \),3\( S \))-2-hydroxyxycitic 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 \( \alpha \)-asymmetric centre to the molar rotations of both hydroxy-
cticric acids was calculated\textsuperscript{13} (\textit{cf.} Table 2) and the positive \( \alpha_{\text{H,O}} - \alpha_{\text{salt}} \) values
are indicative of the \( \text{L} \)-configuration for both acids.

The partial-resolution method developed by Horeau\textsuperscript{15} for determining
the absolute configuration of optically active secondary alcohols employing
the reaction with racemic \( \alpha \)-phenylbutyric anhydride, has been discussed
with regard to its mechanism\textsuperscript{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 \( \alpha \)-phenylbutyric
anhydride, dextrorotatory \( \alpha \)-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\textsuperscript{15,18}:

\[
\begin{align*}
\text{S} & \rightarrow (+)-\alpha \text{-phenylbutyric acid (recovered)} \\
\text{H} & \text{O} \\
\text{L} & \\
\end{align*}
\]

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

\[
\text{COOH} \\
\text{S} \sim \text{CH}_2 \sim , \text{M} \sim \text{CH} \sim , \text{L} \sim \text{COOH}, \text{It can, of course, be discussed how reasonable this assignment of order of size is.}
\]

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

\textit{Acta Chem. Scand.} 23 (1969) No. 1
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 bromoresol 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 calyces (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°, [α]D$^25$ +112° (c=0.49, MeOH) (Found: C 43.96; H 4.51. Calc. for C$_{14}$H$_{16}$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): 1760 (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.), [α]$_D$$^25$ +110° (c=1.37, H$_2$O) (Lit.$^6$ m.p. 183°, [α]$_D$$^25$ +122°).

(28,3R)-2-Hydroxycitramide. Prepared by voluntarily evaporation of hibiscus acid dimethyl ester in liquid ammonia. Yield: 100%. M.p. 185–188°, [α]$_D$$^28$ +16.8° (c=0.25, H$_2$O) (Found: C 35.17; H 5.42; N 20.24. Calc. for C$_9$H$_{11}$N$_2$O$_6$: 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. [α]$_D$$^28$ +92.1° (c=0.30, MeOH). IR-spectrum (neat): 1755 (ester), 1790 (γ-lactone), ca. 3350 cm$^{-1}$ (OH, broad).

**Di-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 chloride in two portions, separated by an interval of 24 h. 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$_2$O$_5$, the NMR-spectrum of the residual oil indicated the presence of garcinia acid, non-lactonized hydroxyctic 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$_2$O$_5$ and...
treatment with ether and petrol ether at \(-80^\circ\) M.p. after recrystallization from ether 175\(^\circ\). 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.\(^{16}\) M.p. 128 - 130\(^\circ\), \([\alpha]_D^{25} = -24.8^\circ\) (c=0.23, \(\text{H}_2\text{O}\)) (Found: C 51.80; H 4.47; N 5.38. Calc. for \(\text{C}_{11}\text{H}_{14}\text{NO}_3\): C 52.17; H 4.38; N 5.59). \((-\)\)-alloisocitric lactone. Isolated from its pyridinium salt as an oil, \([\alpha]_D^{25} = -35.2^\circ\) (c=0.46, \(\text{H}_2\text{O}\)) (Lit.\(^{18}\) - 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-\(d_4\). All values are \(\delta\) [ppm] relative to TMS=0. Values in parentheses are the coupling constants in cps.

<table>
<thead>
<tr>
<th></th>
<th>(\alpha)</th>
<th>(\beta)</th>
<th>(\gamma)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\delta)</td>
<td>(\beta)</td>
<td>(\gamma)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>(\alpha)</th>
<th>(\beta)</th>
<th>(\gamma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hibiscus acid (2a)</td>
<td>d 3.10 (17.5)</td>
<td>d 3.56 (17.5)</td>
<td>s 5.10</td>
</tr>
<tr>
<td>Hibiscus acid</td>
<td>d 2.67 (17.5)</td>
<td>d 3.27 (17.5)</td>
<td>s 7.83 sharp s 3.82</td>
</tr>
<tr>
<td>Garlic acid (2c)</td>
<td>d 3.07 (17.5)</td>
<td>d 3.51 (17.5)</td>
<td>s 4.71</td>
</tr>
<tr>
<td>Garlic acid, dimethyl ester</td>
<td>d 2.67 (17.5)</td>
<td>d 3.16 (17.5)</td>
<td>s 7.0 broad s 3.76</td>
</tr>
</tbody>
</table>

Potentiometric titrations. About 50 mg of a particular lactone was dissolved in 10 ml of water and titrated at 25.5\(^\circ\) 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.\(^{19}\) At \(\bar{n}\) equal to 1.5, the pH is equal to \(pK_a\), and at \(\bar{n}\) equal to 0.5, pH is equal to \(pK_a^b\).

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

\([\alpha]_D^{25}\)

\([\alpha]_D^{25}\) c

<table>
<thead>
<tr>
<th></th>
<th>([\alpha]_D^{25})</th>
<th>([\lambda]_D^{25})</th>
<th>c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hibiscus acid (2a)</td>
<td>+110</td>
<td>+209</td>
<td>1.37</td>
</tr>
<tr>
<td>(2(S),3(R))-2-Hydroxyxcitric acid (1a) (^a)</td>
<td>+44.0</td>
<td>+81.5</td>
<td>0.16</td>
</tr>
<tr>
<td>Trisodium (2(S),3(R))-2-hydroxyxycitrate</td>
<td>+33.2</td>
<td>+69.1</td>
<td>0.63</td>
</tr>
<tr>
<td>Garcinia acid (2c)</td>
<td>+100 (^b)</td>
<td>+190</td>
<td>0.63</td>
</tr>
<tr>
<td>(2(S),3(S))-2-Hydroxyxcitric acid (1c) (^a)</td>
<td>-20.0 (^b)</td>
<td>-41.6</td>
<td>0.63 (^b)</td>
</tr>
<tr>
<td>Trisodium (2(S),3(S))-2-hydroxyxycitrate</td>
<td>-27.0</td>
<td>-56.2</td>
<td>0.63 (^b)</td>
</tr>
</tbody>
</table>

\(^a\) Trisodium salt neutralized with 3 equivalents of HCl.

\(^b\) Calculated on the basis of free acid.

_Acta Chem. Scand._ 23 (1969) No. 1
LACTONES AND LACTAMES III

Partial resolution method. Hibiscus acid dimethyl ester: The reaction was carried out according to Mabry et al.11 with 107 mg (0.5 mmol) of the alcohol and 464 mg (1.5 mmol) of the racemic \( \alpha \)-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) 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 \( \alpha \)-phenylbutyric acid was checked using NMR and IR.

Yield of \( \alpha \)-phenylbutyric acid:

<table>
<thead>
<tr>
<th></th>
<th>mg</th>
<th>mmol</th>
<th>%</th>
<th>([\alpha]_{D}^{25})</th>
<th>optical resolution in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>263.4</td>
<td>1.60</td>
<td>64</td>
<td>+0.29</td>
<td>1.5 ((c=7.68), benzene)</td>
</tr>
<tr>
<td>2</td>
<td>267.7</td>
<td>1.63</td>
<td>65</td>
<td>+0.51</td>
<td>2.6 ((c=3.35), benzene)</td>
</tr>
<tr>
<td>3</td>
<td>258.1</td>
<td>1.57</td>
<td>63</td>
<td>+2.03</td>
<td>10.5 ((c=2.06), benzene)</td>
</tr>
<tr>
<td>4</td>
<td>242.1</td>
<td>1.48</td>
<td>59</td>
<td>+1.36</td>
<td>7.1 ((c=2.10), benzene)</td>
</tr>
</tbody>
</table>

\((2S,3R)-2\)-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 \( \alpha \)-phenylbutyric anhydride in 10 ml of pyridine. The heterogenic mixture was stirred at room temperature for 24 h. The rotations measured of the recovered \( \alpha \)-phenylbutyric acid in benzene were as follows: \([\alpha]_{D}^{0} +0.003 \ (c=0.538)\), \(-0.003 \ (c=1.00)\), \(+0.001 \ (c=0.538)\).

Acknowledgements. The authors are indebted to Dr. Eugenia Davis for measuring the CD and ORD curves and to Drs. Y. S. Lewis and H. B. Vickery for generous samples of garcinia acid and of (\(-\))-isocteric lactone and its dimethyl ester, respectively.

LITERATURE

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6. Lewis, Y. S. Personal communication.

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