Standards, Washington D.C., for his generous gift of α-allitol. Thanks are also due to Dr. Tetsuo Hiraoka, Sankyo Co., Ltd., for the measurement of NMR spectra.

Tokyo Biochemical Research Institute, Tahadaminami-cho, Toshima-ku, Tokyo, Japan

Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Tokyo, Japan

Morizo Ishidate (石倉善三)

Zenzo Tamura (田村善藏)

Toshio Kinoshita (木下俊夫)

Received September 27, 1964

UDC 547.595.2:582.972

Isolation of 4,5-Di-O-cafeylequinic Acid from Coffee Beans

In 1950, Barnes, et al.\textsuperscript{1} isolated "isochlorogenic acid" from coffee beans, but its 5-O-cafeylequinic acid structure\textsuperscript{1b} has remained in doubt.\textsuperscript{3} On the other hand, 4-O-cafeylequinic acid and 5-O-cafeylequinic acid structures, respectively, have since been assigned\textsuperscript{3} to neochlorogenic acid (III)\textsuperscript{b} and "band 510" (IV)\textsuperscript{9} isolated from artichoke leaves and coffee beans. It has further been shown that "isochlorogenic acid" is a mixture of three components.\textsuperscript{4,5}

\[
\begin{align*}
(\text{I}) & \quad \text{quinic acid; } R_3 = R_4 = R_5 = H \\
(\text{II}) & \quad \text{chlorogenic acid; } R_3 = \text{cafeyley; } R_4 = R_5 = H \\
(\text{III}) & \quad \text{neochlorogenic acid; } R_3 = \text{cafeyley; } R_4 = R_5 = H \\
(\text{IV}) & \quad \text{"band 510"; } R_3 = \text{cafeyley; } R_4 = R_5 = H \\
(\text{V}) & \quad 4,5\text{-di-O-cafeylequinic acid; } R_3 = R_4 = \text{cafeyley; } R_5 = H 
\end{align*}
\]

We have now isolated from unroasted Brazilian coffee beans a white powder to which a 4,5-di-O-cafeylequinic acid structure is assigned. The preliminary isolation and purification procedures were substantially the same as those described for "isochlorogenic acid."\textsuperscript{1b} The crude acid was further purified by three 50-plate counter-current-distributions between butyl acetate and phosphate buffer (one run at pH 5.7 followed by two runs at pH 4.8); this procedure removed a contaminant with λ\textsubscript{max}\textsuperscript{H₂O} 233 and 281 m\textsuperscript{λ} as well as some chlorogenic acid (crystalline). In this way an acid, referred to as Compound I in this paper, was obtained\textsuperscript{8} with m.p. 140° (decomp.), [\(\alpha\)]\textsubscript{D} \textsuperscript{172} (c=1.0, MeOH); C, 56.31; H, 5.26 (calcld. for C\textsubscript{34}H\textsubscript{22}O\textsubscript{12}.H\textsubscript{2}O: C, 56.17; H, 4.90); λ\textsubscript{max}\textsuperscript{H₂O} 330 m\textsuperscript{λ} (log ε

\textsuperscript{81} An outline of this work has been presented at the "Symposium on Recent Developments in Plant Polyphenols," Delhi, October 1964. Dr. J. Corse and co-workers at Western Research Laboratory, Albany, California, have recently identified "isochlorogenic acid c" (cf. ref. 6) as 4,5-di-O-cafeylequinic acid (private communication).

\textsuperscript{82} The powder was dried at 80° for 20 hours \textit{in vacuo}.

Although Compound I could not be crystallized, it gave only a single spot on thin-layer chromatography (t.l.c.) under various conditions (Table I). Alkaline-hydrolysis afforded caffeic acid and quinic acid.

**Table I. Silica Gel Thin-layer Chromatography**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Rf-Value</th>
<th>Chlorogenic acid</th>
<th>Compound I</th>
</tr>
</thead>
<tbody>
<tr>
<td>AcOH</td>
<td>0.54</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td>AcOEt-AcOH-H₂O (9:2:2)</td>
<td>0.77</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>BuOH-EtOH-H₂O (4:1:5)</td>
<td>0.67</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>CHCl₃-H₂O-AcOH (2:1:1)</td>
<td>0.9</td>
<td>0.84</td>
<td></td>
</tr>
</tbody>
</table>

*The upper phase was used.*

Potentiometric titrations carried out on 33% ethanol solutions of quinic acid (I), chlorogenic acid (II) and Compound I (V) clearly showed that the neutralization equivalent of Compound I is larger than that of chlorogenic acid (found 363, calc. 354) and is 536 (calcd. for monohydrate, 534).

The titrations also showed that whereas chlorogenic acid consumed only one mole of alkali in the pH range of 8 to 11 (where only one of the hydroxyl groups of the caffeic acid moiety is ionized), Compound I consumed two equivalents in the same pH range. This evidence suggests that Compound I is a dicaffeate. Furthermore, the pKₐ'

![Fig. 1: Nuclear Magnetic Resonance spectra of (a) Quinic Acid, (b) Chlorogenic Acid and (c) Compound I in DMSO-d₆ and DMSO-d₆/D₂O](image-url)

Only part of the spectra is shown for the latter solvent system. The peaks at ca. 2.5 p.p.m. are due to the solvent. Measured at 60 Mc. values in p.p.m. relative to TMS.
values of quinic acid, chlorogenic acid and Compound I are, respectively, 4.15, 4.25 and 4.25 (at 20°); the fact that the pKα' of Compound I is identical with that of chlorogenic acid clearly shows that neither of the caffeoyl groups is attached to the C₃-OH and also suggests that the conformation of the quinic acid moiety is the same in the two compounds, i.e., C₁-COOH is equatorial.⁷

The hydroxyl group at C₃ also is not involved in the ester linkage since Compound I when dissolved in acetone containing 1% dry hydrogen chloride and left at room temperature for several days was partly converted into a lactone; the product showed a distinct infrared band at 1787 cm⁻¹ (γ-lactone, KBr), and on silica gel t.l.c. gave two spots, Rf 0.19 (starting material) and 0.53 in a solvent system of chloroform:ethanol (10:1). Lactonization is known to occur between the C₅-OH and C₁-COOH in quinic acid⁹ and 5-O-cafeoylquinic acid⁹ and is accompanied by ring inversion.

It can therefore be concluded that Compound I is 4,5-di-O-cafeoylquinic acid, and this is fully supported by the nuclear magnetic resonance data (Fig. 1 and Table II).

**Table II. Chemical Shifts and Coupling Constants of Quinic Acid (I), Chlorogenic Acid (II) and Compound I (V) (in DMSO-d₆)**

<table>
<thead>
<tr>
<th></th>
<th>H₃</th>
<th>H₄</th>
<th>H₅</th>
<th>H₆₃, H₆₄</th>
<th>H₆α)</th>
<th>H₆β)</th>
<th>H₆γ</th>
<th>H₆δ</th>
<th>H₆ε</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I)</td>
<td>3.7~4.0</td>
<td>3.26(q)</td>
<td>3.7~4.0</td>
<td>1.8~1.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>J₆₃ 8.3</td>
<td>J₆₄ 3.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(II)</td>
<td>5.11</td>
<td>3.59(q)</td>
<td>3.95</td>
<td>1.9</td>
<td>6.18</td>
<td>7.47</td>
<td>7.10</td>
<td>7.04</td>
<td>6.83</td>
</tr>
<tr>
<td></td>
<td>J₆₃ 7.0</td>
<td>J₆₄ 2.5</td>
<td></td>
<td></td>
<td>J₆β 16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(V)</td>
<td>3.3</td>
<td>4.0(q)</td>
<td>5.2</td>
<td>2.1</td>
<td>6.32</td>
<td>7.54</td>
<td>7.06</td>
<td>7.04</td>
<td>6.79</td>
</tr>
<tr>
<td></td>
<td>J₆₃ 11.5</td>
<td>J₆₄ small</td>
<td></td>
<td></td>
<td>J₆β 16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The quartet at 3.26 p.p.m. in Fig. 1a with a large (8.3 c.p.s., ax-ax) and a small splitting (3 c.p.s., ax-eq) is assignable to the C₃-H; the appearance of the C₃-H signal as a quartet in DMSO shows that the coupling between the C₃-H and the C₆-OH proton is unusually small (it as generally 4~5 c.p.s.¹⁰). In chlorogenic acid (Fig. 1b) the signal of the C₃-H is shifted downfield by ca. 1.2 p.p.m. because of the ester linkage. The olefinic protons of the caffeoyl moiety in chlorogenic acid appear as a clear AB quartet (16 c.p.s., Jₛᵣₑ₉) which straddles the aromatic proton pattern, and convincing evidence for the di-O-cafeoyl structure in Compound I (Fig. 1c) is provided by the two sets of AB quartets both of which straddle the aromatic proton pattern.

Since the infrared spectrum of Compound I is very similar to that shown in the paper by Barnes, et al.,¹¹ it is conceivable that the major constituent of "isochlorogenic acid" is 4,5-di-O-cafeoylquinic acid (Compound I).

---


The authors are greatly indebted to Professor I. Uritani, Department of Agriculture, Nagoya University, for originally suggesting this work and his continued interest, and to Dr. J. Corse, Western Research Laboratory, Albany, California, for sending us copies of their two manuscripts pertaining to "isochlorogenic acid c" prior to publication. This work has been supported by a Plant Phenolics Grant-in-Aid from the Ministry of Education.

Department of Chemistry, Tohoku University, Sendai, Japan

Yasuo Inoue (井上康男) Shohei Aoyagi (青柳象平) Koji Nakanishi (中西哲爾)

Received November 2, 1964

UDC 547.91.05: 582.38

21-Episerratenediol, Isolation and its Structure

This communication is concerned with the constitution and stereochemistry of a triterpene-diol, 21-episerratenediol, which we have isolated from Lycopodium serratum Thunb. var. Thunbergii Makino (Lycopodiaceae) along with serratenediol, serratenediol-3-acetate and two new triterpenoids.

The structure of serratenediol and its 3-acetate, two major triterpenoid constituents, were already elucidated as formulated by I and II, respectively.1,2

\[
\begin{align*}
&\text{I : } R=H \\
&\text{II : } R=\text{COCH}_3 \\
&\text{III }
\end{align*}
\]

Separation of minor triterpenoids was conveniently effected by repeated chromatography of derived acetates over silica gel and alumina. The physical constants of the three new triterpenoids and their acetates are listed in the Table. The nuclear magnetic resonance spectrum\(^*1\) of A-diacetate indicated the presence of a trisubstituted

<table>
<thead>
<tr>
<th>Alcohol</th>
<th>Acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) 21-Episerratenediol C_{20}H_{25}O_3, m.p. 289°~290°</td>
<td>C_{20}H_{25}O_3, m.p. 225°~229° [\alpha]_D^{29} = -29° (c = 2.06, CHCl_3)</td>
</tr>
<tr>
<td>(B) Serratriol C_{20}H_{25}O_3, m.p. 335°~336°</td>
<td>C_{20}H_{25}O_3, m.p. 245°~247° [\alpha]_D^{29} = +21° (c = 1.9, CHCl_3)</td>
</tr>
<tr>
<td>(C) Tohogenol C_{20}H_{25}O_3·\frac{1}{2}H_2O, m.p. 242°~244°</td>
<td>C_{20}H_{25}O_3, m.p. 305°~306° [\alpha]_D^{29} = +28° (c = 1.6, CHCl_3)</td>
</tr>
</tbody>
</table>

\(^*1\) NMR spectra were taken on Varian A-60 machine in CDCl_3 with Me_4Si as an internal standard by Mr. T. Shingu, Kyoto University, to whom we are indebted.