Absolute Configurations of Alkyleitric Acids

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The absolute configurations of five naturally occurring alkyleitric acids (1–5) have been determined. The relative configurations were elucidated by comparison with NMR spectra or GLC retention times of stereospecifically synthesised \( R^*,R^* \) esters and \( R^*,S^* \) esters (Fig. 1). The absolute configurations of 1–4 (2S,3S)\(^\dagger\) and 5 (2R,3S)\(^\dagger\) were determined by CD measurements on the natural acids and optically active synthetic acids (12–15) as their molybdate complexes. Previously reported molecular ellipticities for 2-alkylmalic acids must be corrected.

Five alkyleitric acids (1–5) have previously been isolated from or detected in natural sources and the determination of their absolute configurations is reported here. The methylcitic acids 1 and 5 are excreted in the urine of humans suffering from propionic acidemia, a rare disease leading to death in infancy.\(^1\) A methylcitic acid has also been found in the culture of *Candida lipolytica*\(^2\) and from the NMR and optical rotation data given\(^3\) we conclude it to be acid 1. (–)-Decyleitric acid (2) is a metabolite produced by a variant of *Penicillium simplicissimum*,\(^4\) norcaperatic acid (3) is probably responsible for the toxicity of *Cantharellus floccosus*,\(^5\) and the 1-monomethyl ester of the latter (caperatic acid*) is a common lichen acid. Agaric acid (4) is a hexadecyleitric acid occurring in *Polyporus officinalis*.\(^6\) Another representative of this structural class, butyleitric acid, has been assumed to be an intermediate in the biosynthesis of the fungal metabolite glauconic acid.\(^8\)

In order to determine the stereostructures of the natural alkyleitric acids we have synthesised such compounds stereospecifically from the epoxides 6 and 7 (Fig. 2) as well as from their racemic modifications. The absolute configurations of 6 and 7 are known\(^9\) since they have been correlated with the known\(^10\)–13 lactones of hydroxycitic acids. Evidence for a *trans* opening of the epoxide rings in 6 and 7 on reaction with lithium dialkylcuprate reagents comes from the fact that dimethyl epoxy-fumarate, on reaction with lithium dimethylcuprate, gives *erythro*-3-methylmalic acid. Consequently, 6 should yield (2S,3S)-alkyleitric acids and 7 should yield (2R,3S)-alkyleitric acids as shown in Fig. 2.

The relative configurations of 2–4 were determined by \(^1\)H NMR spectroscopy, using the synthetic \( R^*,R^* \) esters and \( R^*,S^* \) esters as reference materials; the spectral features used are depicted in Fig. 3. The trimethyl...


garcinia acid

\[
\begin{align*}
1. & \quad CH_3OH, CH_3COCl \\
2. & \quad CH_3SO_2Cl \\
3. & \quad CH_3COONa \\
\end{align*}
\]

hibiscus acid

\[
\begin{align*}
1. & \quad NaOH, H_2O \\
2. & \quad H^+ \\
\end{align*}
\]

\[
\begin{align*}
6 & \quad H_3COOC \\
7 & \quad H_3COOC \\
\end{align*}
\]

\[
\begin{align*}
LiR_2Cu & \quad LiR_2Cu \\
\end{align*}
\]

\[
\begin{align*}
COOH & \quad COOH \\
R & \quad R \\
3 & \quad 3 \\
H & \quad H \\
2 & \quad 2 \\
COOH & \quad COOH \\
\end{align*}
\]

\[
\begin{align*}
COOCH_3 & \quad COOCH_3 \\
R & \quad R \\
3 & \quad 3 \\
H & \quad H \\
2 & \quad 2 \\
\end{align*}
\]

\[
\begin{align*}
\text{12: } R &= CH_3 \\
\text{13: } R &= C_{10}H_{21} \\
\text{14: } R &= CH_3 \\
\text{15: } R &= C_{10}H_{21} \\
\text{8: } R &= CH_3 \\
\text{9: } R &= C_{10}H_{21} \\
\text{10: } R &= CH_3 \\
\end{align*}
\]

25,35

\[2R,35\]

Fig. 2. Synthetic scheme for the correlation of alkylcitric acids with lactones of hydroxcitic acids. The formulæ 8–15 are to be regarded as Fischer projections although the lowest numbered chain members are not placed at the top.

Esters of 2–4 gave \(^1\)H NMR spectra containing the wide-spread AB spectrum in Fig. 3a, demonstrating these acids to be either \(R,R\) or \(S,S\). The NMR differences shown in Fig. 3 have been shown to be retained to a large extent in the spectra of the corresponding unesterified acids. The NMR results obtained here allow conclusions to be drawn about the configurations of alkylcitric acids described in the literature. Comparisons of the \(^1\)H NMR data from our methylcitric acids with those given \(^1\) for the methylecitric acid from \textit{Candida lipolytica} indicate that the latter should belong to the \(R^*,R^*\) pair, although differences in chemical shifts were apparent (see below). This conclusion is supported by comparison of the magnitude of the optical rotations of the trimethyl ester \(^5\) of the \textit{Candida lipolytica} acid with the rotations of 8 and 10 (see below). The NMR chemical shifts published for a crystalline methylecitric acid,\(^1\) synthesised by a Reformat-ský reaction,\(^1\) clearly show that this acid is the \(R^*,S^*\) isomer. The ethylcitric acid which is commercially available should on the same grounds be \(R^*,S^*\).\(^1\) It is also possible to distinguish between diastereomeric methylecitric acids by GLC of their trimethyl esters. Using this technique the methylecitric acids obtained from the urine of a patient with propionic acidemia\(^1\) were shown to be present as diastereomers in the approximate ratio 2:1. The retention times of the major and minor peaks were the same as those of the synthetic \(R^*,R^*\) and \(R^*,S^*\) isomers respectively.

The absolute configurations of 1–5 were studied by CD measurements on the natural and the synthetic acids 12–15 as molybdate (VI) complexes.\(^1\) The acids 1–4 showed CD

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The co-occurrence of 1 and 5, with 5 having the 2R configuration, is remarkable since the biosynthesis of the latter requires a re attack on oxaloacetate. Addition of acetyl-CoA at the re face has, however, been found to occur in a few anaerobic bacteria.17

Alkylcitric acids have previously been synthesised from diethyl alkylxaloacetates and Reformatsky reagents in yields ranging from 3 to 15%,14-16 By using instead the lithium ester enolate of (−)-menthyl acetate we obtained a 45% yield of the mixed ester of ethylcitric acid, containing R*,S* and R*,R* isomers in the approximate ratio 10:1. In an analogous synthesis, methylcitric acid was obtained in a 30% yield and only esters belonging to the R*,S* pair could be detected (GLC, NMR). According to a CD investigation of the ethylcitric acids, the 2S,3R isomer was formed in approximately 5% enantiomeric excess.

Correction. The molecular ellipticities of 2-alkylmalic acids reported in two previous papers17,18 are ten times too large.

EXPERIMENTAL

Epoxide ring opening reactions and isolations of the alkylcitric acids were performed as described previously.14 The acidic hydrolysis procedure12 gave a (2S,3S)-methylcitric acid which was partly (10%) isomerized and so an alkaline method was used in this case for preparation of the free acid. The ester was treated with 0.5 M sodium hydroxide solution (25 °C, 4 days) and after washing with ether, the solution was passed through a Dowex 50W-X8 (H+)-column and evaporated to dryness. The crude acid obtained was investigated directly by CD and, after methylation with diazomethane, by GLC. All CD data given below refer to maxima or minima obtained for the alkylcitric acids in aqueous solutions (unless otherwise stated) (pH 3.4 – 3.7) containing molybdate(VI). Further CD conditions have been described previously.12 Analytical GLC was performed on a Perkin-Elmer 900 instrument equipped usually with a JXR column (3% on Gas-Chrom Q, 100 – 120 mesh, 0.2 x 150 cm); for the trimethyl methylcitrate an ECNSS-M column (3% on Chromosorb W, 100 – 120 mesh) was used. Preparative GLC was performed on an Aerograph 1400 instrument using an ECNSS-M column (3% on Chromosorb W, 100 – 120 mesh, 0.3 x 360 cm) for trimethyl methylcitrates and an SE-30 column (2% on Chromosorb W, 60 – 80 mesh, 0.3 x 150 cm) for trimethyl decylicrates. Optical rotations were measured on a Perkin-Elmer 141
polarimeter, NMR spectra were recorded on a Varian XL-100 instrument and mass spectra (GLC-MS) on a Varian MAT 311 spectrometer.

cis-Epoxide 6. (±)-threeo-Trimethyl hydroxycitrate was prepared by osmium tetroxide hydroxylation of trans-trimethyl acenitrate.4 Resolution gave the dimethyl ester of (±)-garcinonic acid which was then hydrolysed according to Martius et al.4 Resolution of the racemic acid with chinchonine4 gave, after one recrystallisation, a salt from which garcinonic acid could be obtained (85% optically pure). The cis-epoxide 6 was prepared from garcinonic acid according to Guthrie et al.4 and it gave 

\( [\alpha]_D^{25} = -27.9^\circ \) (c 1.0, methanol) showing an 81% optical purity; lit.4 value: 

\( [\alpha]_D^{25} = -34.6^\circ \) (c 1.07, methanol). H NMR (CDCl₃): δ 3.78 (s, 6 H), 3.72 (s, 3 H), 3.67 (s, 1 H), 3.25, 3.08, 2.83, and 2.66 (AB spectrum, 2 H). MS (m/e, relative intensity): M⁺ = 31 = 201(8), 174(33), 173(46), 145(39), 141(56), 114(45), 113(100), 75(28), 59(87).

trans-Epoxide 7. The trans-epoxy acid was prepared by epoxidation of trans-acetoin acid. Resolution with chinchonidine4 gave, after one recrystallisation, a salt from which the (+)-epoxy acid could be obtained in 81% optical purity. It showed 

\( [\alpha]_D^{25} = +51^\circ \) (c 1.1, water) compared to the lit.3 value: 

\( [\alpha]_D^{25} = +63.1^\circ \) (c 1.0, water). This acid was esterified with diazomethane to give 7, 

\( [\alpha]_D^{25} = +133^\circ \) (c 1.8, chloroform). H NMR (CDCl₃): δ 3.90 (s, 1 H), 3.81 (s, 3 H), 3.78 (s, 3 H), 3.70 (s, 3 H), 3.11 (s, 2 H). MS (m/e, relative intensity): M⁺ = 31 = 201 (11), 174 (9), 173 (92), 145 (36), 141 (100), 114 (9), 113 (97), 75 (47), 59 (98).

(28,38)-Trimethyl methylcitrate (8) was obtained in 60% yield after preparative GLC. 

\( [\alpha]_D^{25} = +9.7^\circ \) (c 0.3, methanol) for the sample being 81% optically pure. Reported for the trimethyl ester of the methylcitric acid from *Candida lipolytica*: 

\( [\alpha]_D^{25} = +12^\circ \) (c 1.5, methanol). MS (m/e, relative intensity): M⁺ = 248 (not observed), 189 (12), 157 (100), 115 (61), 101 (27), 89 (28), 59 (55). H NMR (CDCl₃): δ 3.98 (s, 1 H), 3.82 (s, 3 H), 3.70 (s, 3 H), 3.67 (s, 3 H), 3.14, 2.08, 2.88 and 2.72 (AB spectrum, 2 H), 2.85 (q, 1 H, J = 7 Hz), 1.20 (d, 3 H, J = 7 Hz). The corresponding acid 12 showed (nm, \([\alpha]_D\times 10^3\) : 275, −0.90; 249, +1.5; 233, −1.1. 

(28R,38S)-Trimethyl methylcitrate (10) was also obtained in 60% yield after preparative GLC. 

\( [\alpha]_D^{25} = -17.7^\circ \) (c 0.75, methanol) for the sample being 81% optically pure. MS: very similar to that of 8. H NMR (CDCl₃): δ 3.98 (s, 1 H), 3.80 (s, 3 H), 3.68 (s, 6 H), 2.95 (q, 1 H, J = 7 Hz), 2.90 (s, 2 H), 1.26 (d, 3 H, J = 7 Hz). The corresponding acid 14 showed (nm, \([\alpha]_D\times 10^3\) : 280, −0.8; 249, +1.5; 233, −0.7; 218, +0.4; 205, −1.1. 

The methylcitric acid from *Candida lipolytica* showed 1 in D₂O δ 1.17 (3 H, d), 2.61 (1 H, q), 2.10 (3 H, s), 1.05 (3 H, d).
2.73 (2 H, q, J = 15.6 Hz). Our acid ID showed (in D₂O with sodium 3-(trimethylsilyl)propanesulfonate as internal reference): δ 1.20 (3 H, d), 2.90 (1 H, q), 2.91 and 3.17 (AB spectrum, 2 H, J = 16 Hz), whereas acid 14 showed δ 1.25 (3 H, d), =2.98 (1 H, obscured by the AB spectrum), 2.95 and 3.01 (AB spectrum, 2 H, J = 16 Hz). The spectra of these acids in alkaline D₂O solution did not show greater resemblance to the spectra of the acids. In our opinion, however, only the AB spectrum from 12 but not that of 14 resembles a 1:3:3:1 quartet. The specific rotation of the trimethyl ester of the natural acid is [α]D²⁰ +12° (c 1.5, methanol) and this value is in good agreement with that obtained for δ (being 81% optically pure): +9.7°. It differs clearly from that obtained for 10 (also 81% optically pure): −17.7°.

(−)-Decylcitrin acid, showed (nm, [θ] x 10⁴): 273, −2.4; 250, +2.3; 234, −3.2; 217, +3.0. With H₂O·THF (1:1) as solvent values of 285, −0.2; 249, +1.1; 225, −1.3; 210, +0.7 were obtained.

Norasperic acid (3), obtained by hydrolysis of cerasipic acid, showed in H₂O·THF (1:1) (nm, [θ] x 10⁴): 282, −0.4; 247, +0.6; 225, −0.7.

Agaricic acid (4) was purchased from E. Merck and was recrystallised from ethanol. It showed in H₂O·THF (1:1) (nm, [θ] x 10⁴): 284, −0.3; 249, +1.2; 227, −1.5; 210, +0.8.

Trimethyl ethylcitrate was synthesised from diethyl ethylxilaoacetate and the lithium ester enolate of (−)-menthyl acetate in analogy with previously performed condensations with (−)-menthyl acetate. Diethyl (−)-menthyl citrate was obtained in 45% yield (NMR, piperonal as internal standard). Hydrolysis and subsequent methylation with diazomethane gave trimethyl ethylcitrate containing (RS,SR) and (RR,SS) isomers in the approximate ratio 10:1 (GLC, NMR). An analytical sample of the same composition was obtained by preparative GLC and showed [α]D²⁰ +2.1° (c 2.7, chloroform).

M+ = 262 (not observed), 203 (11), 171 (100), 129 (75), 112 (30), 111 (33), 87 (28), 59 (36). CD values obtained (nm, [θ] x 10⁴): 267, +0.2; 249, −0.4; 232, +0.2. These values show that the 2S,3R isomer predominates over its enantiomer.

Dimethyl erythro-3-methylmalate. Dimethyl trans-2,3-epoxybutanedioate was prepared from the corresponding diacid and diazomethane. It was then treated with lithium dimethylcuprate (as described for 6 and 7), giving a product indistinguishable (GLC-MS) from an authentic sample of dimethyl erythro-3-methylmalate. The latter was obtained from the bis(p-bromophenacyl) ester of alkaline hydrolysis, acidification and reaction with diazomethane. The erythro and threo isomers were well separated on the ECNSS-M GLC column.

REFERENCES


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