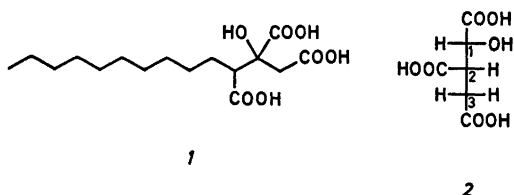


Short Communications

(–)-Decylcitric Acid and (+)-Isocitric Acid as Metabolites from *Penicillium spiculisporum* — a CorrectionSVANTE BRANDÄNGE,^a STAFFAN JOSEPHSON,^a ANDERS MÄHLÉN,^b LARS MÖRCH^a and STAFFAN VALLÉN^a^a Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, S-104 05 Stockholm, Sweden and ^b Department of Chemistry, National Bacteriological Laboratory, S-105 21 Stockholm, Sweden

The isolation of two diastereomeric metabolites, (+)-decylcitric acid and (–)-decylcitric acid, from a strain of *Penicillium spiculisporum* (ATCC 24792) was reported in 1968.¹ We here report that of these only (–)-decylcitric acid is a metabolite from the fungus and that the substance considered to be (+)-decylcitric acid is a mixture of (–)-decylcitric acid (1) and (+)-isocitric acid (2).



In the previous work¹ two spots were obtained on TLC which were ascribed to (+)-decylcitric acid ($R_F=0.25$) and (–)-decylcitric acid ($R_F=0.44$). Working on the absolute configurations of alkylcitric acids we investigated a product available in a small amount from the previous work. This product showed $[\alpha]_{578}^{22} + 1.9^\circ$ (c 1.8, acetone), compared with $[\alpha]_{578}^{20} + 2.4^\circ$ reported for the assumed (+)-decylcitric acid. Esterification with methanol and conc. sulfuric acid gave a product which contained (NMR, GLC-MS) trimethyl isocitrate and trimethyl decylcitrate as major components in approximately equimolar amounts and dimethyl isocitrate lactone in a few mol per cent.

The separation of decylcitric and isocitric acids is difficult to achieve by fractional crystal-

lisation but is easily performed by a partition of a mixture of the methyl esters between water and light petroleum. Using this technique it was found that the crude, acidic fermentation product contained the two acids in approximately equimolar amounts. Hydrolysis of the trimethyl decylcitrate yielded only (–)-decylcitric acid. The spot with the R_F -value 0.25 previously ascribed to (+)-decylcitric acid should thus be ascribed to isocitric acid. Authentic (\pm)-isocitric acid shows this mobility. The absolute configuration of the trimethyl isocitrate was determined by its conversion to (–)-dimethyl isocitrate lactone,^{2,3} a crystalline compound derived from (+)-isocitric acid (2) of known absolute configuration (1*R*, 2*S*).⁴

The (1*S*, 2*S*) diastereomer of 2, but not 2 itself, has previously been found as a metabolite from *Penicillium* species.⁵ The (1*R*, 2*S*) isomer 2 which has been isolated from many plants is the one formed in the citric acid cycle.⁶

Experimental. Melting points are corrected. Optical rotations were measured on a Perkin-Elmer 141 polarimeter and NMR spectra on a Varian XL-100 instrument. GLC operations were carried out using a Perkin-Elmer 900 chromatograph equipped with a JXR column (3 % on Gas-Chrom Q, 100–120 mesh, 0.2 × 180 cm). For the simultaneous analysis of trimethyl decylcitrate and esters of isocitric acid and its lactone a temperature programme was used: initial temp. 100 °C, heating 10 °C/min to 210 °C.

Separation of the fermentation products. The crude fermentation product¹ (5.0 g) was treated (reflux, 4 days) with methanol (700 ml) and conc. sulfuric acid (10 ml). Sodium hydrogen carbonate solution was added under ice-cooling until pH 5–6 was reached. The methanol was evaporated and the resulting two-layered system (100 ml) was extracted five times with light petroleum and then ten times with chloroform-ethanol (3:2). The combined petroleum layers were washed once with water and then dried (Na_2SO_4). Evaporation of the solvent followed by drying in a vacuum desiccator left trimethyl decylcitrate (3.6 g), at least 95 % pure (NMR, GLC). ¹H NMR, $\delta(\text{CDCl}_3)$: 3.87 (s, 1 H), 3.79 (s, 3 H), 3.69 (s, 3 H), 3.65 (s, 3 H), 3.17, 3.01, 2.76 and 2.60 (AB-spectrum, 2 H), 2.80–2.56 (1 H, probably four signals), 2.00–0.70 (21 H). The combined chloroform-ethanol layers were dried (Na_2SO_4) and then concentrated. Removal of

inorganic salts by extraction of the residue twice with chloroform followed by evaporation of the solvent left an oil (1.7 g), which to over 95 % extent consisted of a mixture of trimethyl isocitrate and dimethyl isocitrate lactone in the approximate ratio 10:1 (NMR).

Trimethyl isocitrate: ^1H NMR, $\delta(\text{CDCl}_3)$: 4.39 (d, 1 H, $J=3$ Hz), 3.9–3.3 (11 H), 3.05–2.52 (2 H, AB part of ABX spectrum, $J_{AB}=17$ Hz, $J_{AX}=7$ Hz, $J_{BX}=8$ Hz).

(-)-Decylcitric acid. The ester obtained in the extraction with light petroleum was hydrolysed with sodium hydroxide solution (2 M, reflux overnight). After washing with ether and acidification, the decylcitric acid was extracted with ether. The ether solution was dried (Na_2SO_4) and concentrated, and the residue was recrystallised first from water and then from acetone-light petroleum affording (-)-decylcitric acid, m.p. 135–139 °C, $[\alpha]_{D}^{25} -21^\circ$ (c 1.7, acetone). Lit.¹ value: $[\alpha]_{D}^{20} -10.9^\circ$ (c 1.8, acetone).

Characterisation of (+)-isocitric acid. The mixture of trimethyl isocitrate and dimethyl isocitrate lactone obtained in the chloroform-ethanol extraction was distilled at 150 °C (0.3–0.4 kPa) giving dimethyl isocitrate lactone, free from trimethyl isocitrate (GLC). Recrystallisation from methanol gave a sample with m.p. 106–107 °C and $[\alpha]_{D}^{25} -65^\circ$ (c 1.8, methanol). Lit.² m.p. 108.5–109 °C and lit.³ $[\alpha]_{D} -65^\circ$ (methanol). ^1H NMR, $\delta(\text{CDCl}_3)$: 5.08 (d, 1 H, $J=8$ Hz), 3.9–3.5 (7 H, including two 3 H singlets at 3.78 and 3.74), 3.19–2.56 (AB part of an ABX spectrum, 2 H, $J_{AB}=17$ Hz, $J_{AX}=9$ Hz, $J_{BX}=9$ Hz).

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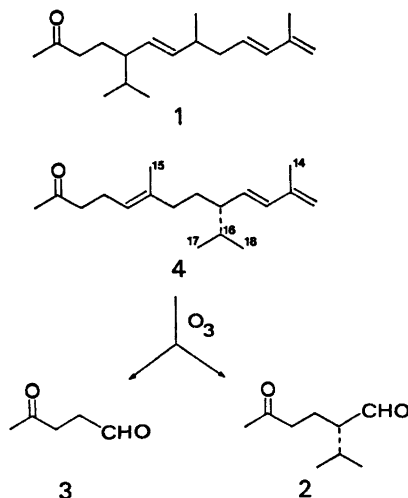
Tobacco Chemistry 37. The Absolute Configuration of Prenylsolanone, (9S)-6,12-Dimethyl-9-isopropyltrideca-5E,10E,12-trien-2-one, a Nor-thunberganoid of *Nicotiana tabacum* L.

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Recent studies have revealed that both the tobacco diterpenoids of the thunbergane type and several of the tobacco volatiles which are structurally reminiscent of the thunberganoids, possess the same absolute configuration, *S*, at the carbon atom carrying the isopropyl group thus strengthening the hypothesis that such diterpenoids act as precursors of the smaller molecules.^{1–4} In the present communication we wish to report the chirality of another tobacco nor-thunberganoid, prenylsolanone.



In a preliminary report 8,12-dimethyl-5-isopropyltrideca-6,10,12-trien-2-one (*1*) was proposed⁵ as the structure of a new tobacco constituent isolated from the carbonyl fraction of the essential oil of *Nicotiana tabacum* L. The carbon skeleton of this ketone (*1*) indicated that it was a nor-thunberganoid and a determination of the chirality of the isopropyl-bearing carbon atom would throw further light on this question. Ozonolysis of the ketone furnished the expected ketoaldehyde, (2*S*)-5-oxo-2-isopropylhexanal (*2*), but the second product, 4-oxopentanal (*3*) was inconsistent