

## Isolation of *trans*-Aconitic Acid from *Asarum europaeum* L.

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From *Asarum europaeum* L. *trans*-aconitic acid could be isolated in such quantities (approximately 2.5 % of the dried crude drug), that the plant, according to Stout *et al.*<sup>1</sup> must be classified as an aconitate accumulator. According to the same authors most species of this category belong to the *Graminae* and only few to the *Dicotyledons*.

*trans*-Aconitic acid could be isolated as the dominant acid constituent from a cold prepared and acidified aqueous extract of fresh plant material. There is thus reason to assume that the *trans* form of the acid is a naturally occurring constituent and not an artefact.<sup>2</sup>

*Experimental.* 500 g dried and pulverized drug (consisting of leaves and rhizomes) was boiled for 4 h with 5 liters of distilled water, filtered, and the filtrate was concentrated *in vacuo* to 500 ml. The extract was acidified with 25 ml of concentrated hydrochloric acid and extracted several times with 500 ml diethyl ether in a separating funnel. The ether extracts were concentrated *in vacuo* and the crystalline residue (12.5 g) was recrystallized from acetone-benzene and ether. M.p. 195°C. (Found: C 41.2; H 3.40. Calc. for C<sub>8</sub>H<sub>6</sub>O<sub>7</sub>:

C 41.3; H 3.47). By titration with 0.01 N KOH the equivalent weight was determined to 58.0. Comparative examination of *trans*-aconitic acid and the isolated acid by means of TLC, UV absorption, IR and mass spectra showed that the acids were identical. Molecular weight determined by mass spectrometry was found to be 174.

*Chromatography.* Thin-layer chromatography separated well the *cis*- and *trans*-aconitic acids, whereas only one peak became visible on the gas chromatogram, probably due to the heating.<sup>3</sup> For the thin-layer chromatography was used cellulose Macherey Nagel MN 300, and as developing solvent 1-pentanol-formic acid (98 %)-water (50:50:2.5). For detection of the acid served *p*-dimethylaminobenzaldehyde, 4 % in acetic anhydride.<sup>4</sup> The reagent produces a red violet colour with *trans*-aconitic acid upon heating for 10 min at 110°C after spraying. The reaction is very sensitive: 0.1 µg gives a visible spot.

Under corresponding conditions *cis*-aconitic acid produces only light red colour. The reaction was negative with malic acid, citric acid, oxalic acid, succinic acid, tartaric acid, isocitric acid, malonic acid, quinic acid, chelidonic acid, chlorogenic acid, itaconic acid, and α-ketoglutaric acid.

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