The parameters of the thermal vibrations indicate a normal rigid molecule. The radial distribution curve does not seem to contain contributions from more than one conformation.

Further refinements of the structure are in progress.

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The Content of *trans*-Aconitic Acid in *Asarum europaeum* L. Determined by Means of a Chromatogram Spectrophotometer

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In a previous investigation on the non-volatile acids of *Asarum europaeum* L. large amounts of *trans*-aconitic acid were detected.[1]

It has for long been known that the metabolic active form of aconitic acid is the *cis*-isomer, and this might account for the fact that this compound rarely cumulates in the tissue. In certain monocotyledons, like grasses and the sugar cane, *trans*-aconitic acid has been found in considerable amounts, for which reason Stout *et al.*[2] have coined the term "aconitate accumulators" for plants that contain more than 1 % of *trans*-aconitate on a dry weight basis.

The enzyme aconitase reacts specifically with the *cis*-isomer. This might result in the cumulation of the *trans*-isomer. In some plants, however, it has been observed that the *trans*-isomer is converted into the *cis*-isomer and thus brought into the TCA-cycle.[3]

trans-Aconitic acid has been reported to possess anti cancer effect *in vitro*,[4] and to inhibit respiration in the rat kidney cortex and in liver slices, which suggests the *trans*-isomer to be an aconitase competitor in the TCA-cycle.[5]

Based on the main constituents of the essential oil, Stahl and Jork [6,7] divided the species *Asarum europaeum* L. into four chemical races. It was found of interest to investigate if there existed corresponding differences in the *trans*-aconitate content of the four races, and to which extent the content of *trans*-aconitic acid in the different parts of the plant is influenced by the age, and by the state of development of the leaves. The investigation revealed that there are no remarkable differences in the *trans*-aconitic acid content in the four different races of the *Asarum europaeum* L., but the content of the acid is so high (up to 11 % dry weight) that the plant, according to Stout *et al.*[8] should be classified as an aconitate cumulator.

Experimental. Leaves, stems, and rhizomes of the plant were dried for 2 h at 70°C and pulverized. Dried and thoroughly mixed powder of the different samples, corresponding to 1 g of the fresh plant, was mixed with 1 ml of 25 % hydrochloric acid and 4 g of Silica gel (0.2 – 0.5 mm). The mixture was transferred to a glass column and extracted with 250 ml of acetone, whereupon the extract was evaporated to dryness *in vacuo*, and the residue dissolved in 25.00 ml of acetone. (1 ml of the solution corresponds to 40 mg of fresh plant.) 2 – 10 µl of the solution were applied on the thin-layer plates and chromatographed with 2 – 5 µg of pure *trans*-aconitic acid dissolved in acetone as a reference sample. The plates were coated with layers of cellulose Macherey Nagel MN 300. The thickness was increased to 500 µ, which caused a better reflection of the background. Pentanol-formic acid (98 %)-water (50:50:2.5) served as developing solvent. The plates were dried after the development for 1/2 h at 110°C. The acid appeared as a dark spot

Table 1. The content of *trans*-aconitic acid in per cent of fresh plant in the four chemical races of *Asarum europaeum* L.

Part of plant	*trans*-Isoeugenol-methyl ether race	Sesquiterpene alcohol race	*trans*-Isoelemicin race	Asaron (*trans*-iso-asaron race)
Leaves	1.6	1.7	1.7	1.7
Stems	1.0	1.0	1.0	0.9
Rhizomes	0.6	0.4	0.7	0.6

Table 2. The content of *trans*-aconitic acid in leaves of different weights (corresponding to different stages of development).

Number of samples	I	II	III	IV	V	VI
Weight of the leaves, g	0.098	0.135	0.208	0.468	0.772	1.589
trans-Aconitic acid found, mg	0.6	1.0	2.0	7.0	17.5	12.5
Per cent in fresh plant	0.6	0.7	1.0	1.5	2.3	0.8
Per cent calculated on dry weight basis	3.2	4.2	4.5	5.9	11.0	3.2

in short wave UV light. The intensity of the spots was measured by means of a Chromatogram Spectrophotometer Zeiss PMQ II [9] at 230 nm. The content of *trans*-aconitic acid in the spots was calculated on the basis of the Kubelka Munks function [10] and a calibration curve plotted with pure *trans*-aconitic acid as a standard. The results of the investigation are shown in Tables 1 and 2.

As will appear from the tables no significant differences in the content of *trans*-aconitic acid in the four chemical races could be observed. For this part of the investigation plants of nearly the same size were used, and the dried powder of the different samples (leaves, stems, and rhizomes) was thoroughly mixed before the investigation. A considerable variation was found in the content of *trans*-aconitic acid in relation to weight and size of the plants. The content of the acid in the leaves increases till the leaves have reached a certain degree of development, and then it drops gradually.

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