Isolation of trans-Aconitic Acid from the Moss Mnium affine

Abstract. The water-soluble substance occurring in high concentration (up to 6 percent of the dry weight) in *Mnium affine* Bland. and forming the main fraction of the plant acids there, has been identified as *trans*-aconitic acid by nine different criteria. Evidence is presented in favor of the concept that the *trans* form in this case is the natural isomer and not an artifact derived from *cis*-aconitic acid. Information is given on the distribution of aconitic acid in other mosses and in liverworts.

Investigation of moss extracts undertaken to find the "spectrum" of normally occurring acids, which in turn might provide clues to the origin of oxalic acid and the role of the oxalic acid oxidase characteristic of all Bryales (*1*), revealed the presence in *Mnium affine* Bland. of an acid different from tartaric acid, glycolic acid, and any of the Krebs-cycle acids. The R_F of the unknown compound in butanol, 80 percent formic acid, and water (4:1:1) was found to be 0.77, which is higher than that of succinic acid and is surpassed only by that of fumaric acid. It is a well-known fact that the *trans* isomers of unsaturated acids exhibit larger R_F values than their *cis* counterparts, and since the unsaturated nature of the unknown compound could easily be demonstrated, the assumption that it was the *trans* form of some plant acid lay close at hand.

The organic acid fraction of *M. affine* was thereupon isolated by subjecting acidified aqueous extracts of the fresh moss to ether-extraction in a Kutscher-Steudel apparatus for 46 hours; from 1 kg (wet weight) of material approximately 4 g of solids were obtained after evaporation of the ether. After being dissolved in water, the residue was subjected to filter-paper chromatography on very thick sheets with butanol, 80 percent formic acid, and water (4:1:1) as the running-fluid; the fastest-moving fraction was collected, and it yielded 3.6 g of the unknown acid in the form of a yellowish-brown solid.

Titration-curves of material thus isolated revealed a buffering capacity over such a wide range that there was no escape from the conclusion that it was a tribasic acid. The identity with titration curves of *trans*-aconitic acid was almost complete (Fig. 1). The substance was obtained in very pure form by a ten-times repeated process of dissolving it in water and extracting it with ether. The yield, after the final product was dried over phosphorus pentoxide, was 3.0 g; R_F values were identical with those of *trans*-aconitic acid—namely, 0.77 with butanol, 80 percent formic acid, and water (4:1:1 by volume), 0.70 with ether, acetic acid and water (15:3:1 by volume), and 0.63 with *n*-amyl alcohol and 5*N* formic acid (1:1 by volume). Elemental analysis showed 41.66 percent C and 3.72 percent H, versus 41.21 and 3.63 percent, respectively, for authentic *trans*-aconitic acid. The melting point was 195°C versus 196° for *trans*-aconitic acid. The two compounds also proved to be identical in ultraviolet and infrared absorption (*2*), vulnerability to potassium permanganate oxidation, x-ray diffraction, and behavior on columns of Dowex 1 × 10 ion-exchange resin.

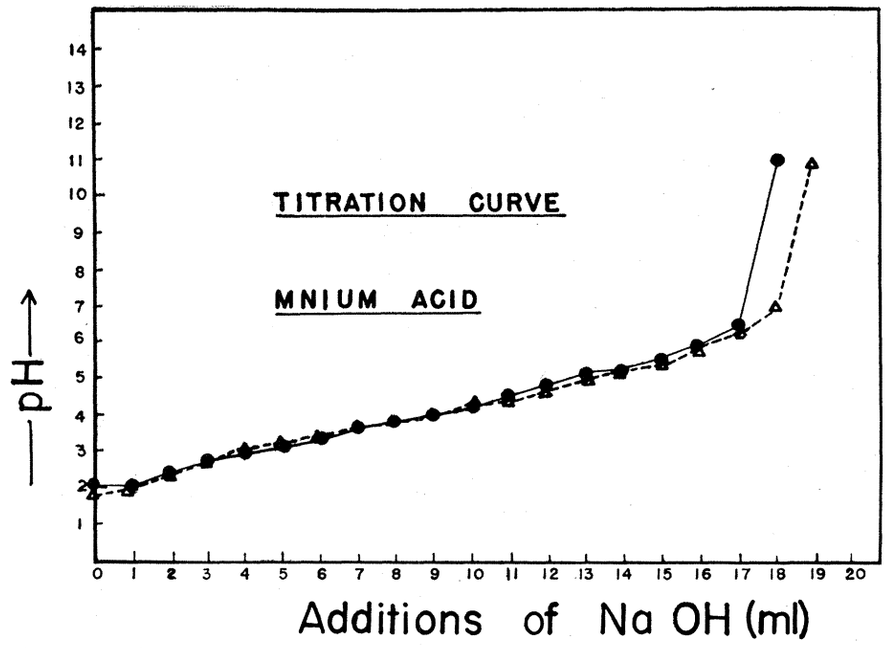

Fig. 1. Titration curves for the acid isolated from *Mnium affine* (broken line) and for authentic *trans*-aconitic acid (solid line).

The main point to be considered is whether the *trans*-aconitic acid in *Mnium affine* is an artifact produced from *cis*-aconitic acid initially present. In the horsetail *Equisetum telmateja*, where the bulk of the acid is *cis*-aconitic acid, the conversion to the *trans* form could indeed be achieved smoothly and almost quantitatively by the simple expedient of drying the plant material overnight in a forced-draft oven at 60° to 70°C. In spite of this, we must conclude tentatively and reluctantly, on the basis of the following considerations, that the natural form in *Mnium affine* is the *trans*-isomer. The addition of *cis*-aconitic acid to *Mnium affine* which is in the process of being ground in water results in the production of considerable quantities of citric acid and some isocitric acid as a result of the action of the very powerful moss aconitase; yet, high levels of citric plus isocitric acid were never observed in extracts of *Mnium affine* not thus treated. Furthermore, in contrast to what is seen in horsetails, extracts of fresh *Mnium affine* prepared in the cold and at low *p*H (obtained by adding trichloroacetic acid or sulfuric acid) failed to reveal *cis*-aconitic acid; yet, the latter compound can be recovered at least in part when added under these circumstances.

A possible taxonomic significance of aconitic acid in plants seems to be excluded by the observation that its occurrence is very erratic; thus, although present in some other moss genera, it is absent from the two *Mnium*-species *M. glabrescens* and *M. menziesii*. In the liverworts, aconitic acid forms a very important component of the acid fraction in *Marchantia polymorpha*, but it is lacking in *Porella navicularis* and *Scapania irrigua* (*3*).

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References and Notes

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