

Purification and Characterization of an *S*-adenosyl-L-methionine:flavonoid 3'-*O*-methyltransferase from Leaves of *Trillium apetalon* Makino

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Z. Naturforsch. **54c**, 501–507 (1999); received February 1/April 22, 1999

Trillium apetalon, *S*-adenosyl-L-methionine:flavonoid 3'-*O*-methyltransferase, Quercetin, Isorhamnetin, Flavonoids

In the leaf extract of *Trillium apetalon* (Liliaceae) distributed in Japan, an enzyme was demonstrated which catalyzes a methyl group transfer from *S*-adenosyl-L-methionine (SAM) to the 3' position of quercetin and its glycosides. The enzyme (*Trillium* F3'OMT) was purified 433-fold with a yield of 0.2% by $(\text{NH}_4)_2\text{SO}_4$ precipitation and chromatographies of DEAE-cellulose, SAH-EAH-Sepharose 4B, Sephacryl S-200 and additional chromatofocusing. *Trillium* F3'OMT has a pH optimum of 7.0 and a pI of 5.3. The apparent molecular weight was estimated by Sephacryl S-200 to be about 78 kDa; SDS-PAGE profile showed that the enzyme was a dimer composed of MW 38 kDa 2 subunits. The enzyme activity was stimulated by EDTA and dithiothreitol (DTT), but strongly inhibited by *p*-chloromercuribenzoate (PCMB) and iodoacetate. The activity was moderately inhibited by Mg^{2+} and Zn^{2+} , and strongly inhibited by Co^{2+} , Mn^{2+} and Hg^{2+} . The apparent K_m values for quercetin and SAM were 10 μM and 3.6 μM , respectively. Lower substrate specificity of the glycosides compared with quercetin indicates that methylation precedes glycosylation in flavonoid biosynthesis of *T. apetalon*.