

ADDITIONAL CONSTITUENTS OF *ORCHIS MILITARIS*

CORINNE APPOLONIA, ANDREW MARSTON, and KURT HOSTETTMANN\*

*Institut de Pharmacognosie et Phytochimie, Ecole de Pharmacie de l'Université,  
2 rue Vuillermet, CH-1005 Lausanne, Switzerland*

*Orchis militaris* L. (Orchidaceae) is found widely distributed on basic soils throughout central and northern Europe. The chemistry of this plant has not been extensively studied, apart from a report on the isolation of militarine and loroglossine (1). As we were able to obtain a certain quantity of this protected orchid from a site in the path of a road construction project, we undertook a more thorough investigation of its constituents using recently developed separation techniques.

## EXPERIMENTAL

PLANT MATERIAL.—*O. militaris* (whole plant) was collected near Sembrancher, Valais, Switzerland, during May 1982. A voucher specimen is deposited at the Institut de Pharmacognosie et Phytochimie, University of Lausanne.

EXTRACTION AND ISOLATION.—Fresh plant material was extracted with MeOH, and after evaporation of solvent, the extract was partitioned between CHCl<sub>3</sub> and H<sub>2</sub>O. The aqueous layer was then partitioned with *n*-BuOH. Preparative chromatography using an axially-compressed column (silica gel) of the CHCl<sub>3</sub> extract, followed by centrifugal tlc (2) led to the isolation of coumarin and 4-hydroxybenzyl methyl ether, both isolated for the first time from this plant. Coumarin was identified by comparing its spectral data with an authentic sample. The latter was identical in all respects (mp, ms, uv, <sup>1</sup>H nmr) with 4-hydroxybenzyl methyl ether isolated from the Asian orchid *Gastrodia elata* (3).

Droplet counter-current chromatography (dccc) (4) of the *n*-BuOH extract gave six fractions, and after various chromatographic manipulations of these fractions, the flavonol glycosides isoquercitrin and astragalín together with the glycosides melilotoside and militarine, were obtained. Melilotoside, first isolated from *Melilotus altissimus* (5), was characterized from <sup>1</sup>H nmr, <sup>13</sup>C nmr, and fabms.

Isoquercitrin, astragalín, and melilotoside were isolated for the first time from the plant. Militarine has previously been isolated from *O. militaris* (1) and, in view of the fact that 4-hydroxybenzyl methyl ether from *G. elata* is probably an artifact (3), it might be produced from militarine during the extraction procedure. Similarly, coumarin isolated from *O. militaris* may also represent an artifact of melilotoside.

Full details of the isolation and identification of the compounds are available on request to the senior author.

## ACKNOWLEDGMENTS

We wish to thank the Swiss National Science Foundation for financial support; Professor W.J. Richter, Ciba-Geigy A.G., Basel, for fabms measurements; and Mr. E. Anchisi, Alpine Botanical Garden, Champex, VS, Switzerland, for identification and collection of the plant material.

## LITERATURE CITED

1. A. Aasen, D. Behr, and K. Leander, *Acta Chem. Scand.*, **B 29**, 1002 (1975).
2. K. Hostettmann, M. Hostettmann-Kaldas, and O. Stichler, *J. Chromatogr.*, **202**, 154 (1980).
3. H. Taguchi, I. Yosioka, K. Yamasaki, and I.H. Kim, *Chem. Pharm. Bull.*, **29**, 55 (1981).
4. K. Hostettmann, M. Hostettmann, and A. Marston, *Nat. Prod. Reports*, **1**, 471 (1984).
5. J. Shinoda and M. Imaida, *J. Pharm. Soc. Jap.*, **54**, 617 (1934).

Received 17 December 1985