Journal of Natural Products
Vol. 53, No. 1, pp. 204-206, Jan-Feb 1990

DETECTION OF DITERPENOID ALKALOIDS IN APHIDS FEEDING ON *ACONITUM NAPELLUS* AND *ACONITUM PANICULATUM*[1]

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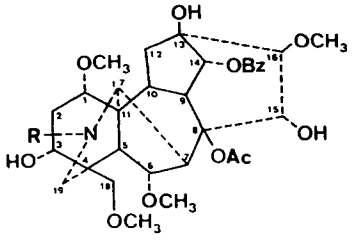
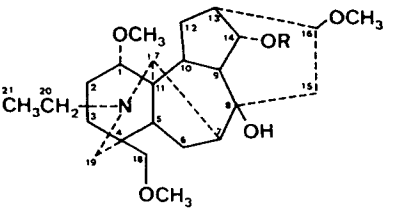
ABSTRACT.—Aconitine [**1**] was isolated from *Brachycaudus napelli* feeding on *Aconitum napellus*. The presence of talatisamine [**3**] and 14-*O*-acetyltalatisamine [**4**] was demonstrated in aphids of the same species feeding on *Aconitum paniculatum* ssp. *paniculatum*.

There have been reports of aphids which feed on alkaloidal plants and absorb quinolizidine alkaloids (2,3) and the indolizidine alkaloid swainsonine (4), as well as of aphids which are deterred by quinolizidine, indolizidine, and pyrrolizidine alkaloids (4).

The author observed that aphids which feed on *Aconitum napellus* L. (Ranunculaceae) cause, when accidentally in contact with his face, the same unpleasant feeling of numbness accompanied by itching and burning as does aconitine [**1**], a diterpenoid alkaloid. This observation was suggestive that the aphids were capable of accumulating significant amounts of aconitine in spite of its high toxicity. To verify this, aphids feeding on *A. napellus* purchased from a nursery were analyzed. The main alkaloids of this species reported in the literature include aconitine and/or mesaconitine [**2**] (5,6). Additionally, aphids feeding on *Aconitum paniculatum* Lam. ssp. *paniculatum* that had been transplanted from their natural habitat in the Engadin (Switzerland) to Basel were analyzed. Such plants had been shown previously to contain talatisamine [**3**] and 14-*O*-acetyltalatisamine [**4**] as the main alkaloids (7). Talatisamine is significantly less poisonous than aconitine (8,9).



1 R=Et
2 R=Me



3 R=H
4 R=Ac

The aphids formed colonies mainly on the leaf stalks of the upper part of the aconites. They were collected with the aid of a small, soft paintbrush, killed with $CHCl_3$ vapors in a test tube, and dried in a desiccator in vacuo. Prof. Dr. G. Lampel, Fribourg, was kind enough to identify the aphids as *Brachycaudus napelli* (Schrk. 1801) (Aphididae). [*B. napelli* is now recognized as a separate species from *Brachycaudus aconiti* (Mordw. 1928) (11,12).]

The aphids feeding on *A. napellus* were collected on July 27, 1986, dried in a desiccator, and extracted 28 months later. By this time the lipids covering their bodies had formed a white crystalline mass which dissolved readily in pentane. The defatted aphids were extracted with $CHCl_3$ at pH 9. The alkaloids of

[1]Communication No. 10 about *Aconitum*. For Communication No. 9, see A. Katz (1).

the crude $CHCl_3$ extract were separated by preparative chromatography on hptlc Si gel plates. From the 8 alkaloidal areas which resulted, the areas which corresponded to aconitine were scraped off, and the alkaloid was eluted with $CHCl_3$-Et_2NH (99:1) and further purified by crystallization. The crystals were shown to be aconitine by mp, R_f in three different solvent mixtures, and ms. Fabms showed the molecular ion, while eims displayed the features typical of aconitine [**1**] (10), i.e., no molecular ion, a base peak corresponding to $[M - HOAc - MeOH]^+$, and an $[M - MeOH]^+$ peak.

With reference to the dry wt of the aphids, the yield of pentane-soluble lipids amounted to 21.8%, of crude $CHCl_3$ extract to 4.6%, of crude aconitine to 1.1%, and of pure aconitine to 0.67%. In relation to the weight of the living aphids the yield was 5.7% of lipids, 1.2% of $CHCl_3$ extract, 0.28% of crude aconitine, and 0.18% of pure aconitine. These figures may vary with weather conditions before and during collection and are therefore less reliable than the figures relating to dry wt.

The aphids from *A. paniculatum* ssp. *paniculatum* were collected on June 13 and July 24, 1986. They were killed and dried as the above-mentioned aphids. Extraction was undertaken 1 year later. In contrast to the extraction procedure described for the aphids from *A. napellus*, these aphids were not defatted prior to extraction. The $CHCl_3$ extract was applied to a Si gel hptlc plate, and the lipids were separated from the alkaloidal area with cyclohexane/$CHCl_3$. The alkaloids which migrated only slightly were scraped off and separated by hptlc. Six alkaloidal areas could be differentiated in the chromatogram. Two of them were isolated. One, with R_f 0.21, corresponded to talatisamine [**3**], the other, with R_f 0.31, to 14-*O*-acetyltalatisamine [**4**]. After acetylation the alkaloid with R_f 0.21 also displayed the R_f value of 14-*O*-acetyltalatisamine. Attempts to crystallize the isolated alkaloids were not successful, a difficulty encountered earlier when isolating small amounts of talatisamine or its acetate.

Considering the food plant of these aphids and the unambiguous result with aphids from *A. napellus*, the R_f values, obtained with four different solvent mixtures, can be regarded as acceptable evidence of the identity of the isolated alkaloid with 14-*O*-acetyltalatisamine [**4**]. Consequently this also shows the presence of talatisamine [**3**] in the aphids. The total yield of 14-*O*-acetyltalatisamine, including the acetylated talatisamine, was 0.34% of the dried aphids and 0.08% of live aphids.

It is noteworthy that *A. napellus*, which may be protected by poisonous secondary metabolites against predators (grazing cattle avoid *A. napellus*[2]) serve as a feeding ground for specialized predators which have developed resistance to the plant poison.

According to Wink *et al.* (2), the accumulation of quinolizidine alkaloids in aphids suggests that these alkaloids are transported in the phloem, and Dreyer *et al.* (4) conclude that, because the pea aphid is a phloem feeder, swainsonine must be transported in the phloem. From the fact that *Brachycaudus cardui* (L.) is known to be a phloem feeder (13) we may infer that *B. napelli* also is a phloem feeder, and hence that the alkaloids **1**, **3**, and **4** are transported in the phloem. However, one has to take into consideration that it seems to be controversial whether phloem-feeding aphids exploit only phloem cells or also parenchyma cells (13). Further work is planned to investigate whether the aphids metabolize aconite alkaloids or whether they only store and possibly excrete them.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—

[2]Personal communication by Prof. Dr. med. vet. Marcel Wanner, Veterinary Hospital, Zürich, Switzerland.

Mp's were determined on a Kofler hot-stage and are corrected. Mass spectra were obtained on a VG 70-SE mass spectrometer at 70 eV by eims and fabms. Matrix for fabms was thioglycerin.

Aphids from A. napellus.—One batch had a live weight of 965 mg and a dry wt of 252 mg; the second batch had a dry wt of 378 mg.

Aphids from A. paniculatum *ssp.* paniculatum.—One batch had a live weight of 737 mg and a dry wt of 156 mg, the second batch 1090 mg and 282 mg, respectively.

Voucher specimens of the aphids from both aconite species are deposited in the collection of Prof. G. Lampel, Institut de Zoologie, Université de Fribourg, CH-1700 Fribourg/Pérolles, Switzerland.

PHYSICAL DATA OF ISOLATED ACONITINE [**1**].—The yield was 1.7 mg. Both the isolated and the authentic aconitine [**1**] prepared in this laboratory showed the mp 180–191°. They also showed identical fabms $[M]^+$ 646 (100%), 630, 614, 600, 586, 572, 556, 540; and eims $[M-MeOH]^+$ 614, 585, 570, 554 (100%), 536, 400, 266, 202, 178, 161, 149, 129, 105.

Full details of isolation and identification are available from the author.

ACKNOWLEDGMENTS

The author is grateful to Prof. Dr. G. Lampel, Institut de Zoologie, Département d'Entomologie, Université de Fribourg, CH-1700 Fribourg/Pérolles, Switzerland, for identifying the aphids and for discussing questions referring to taxonomy and feeding behavior of aphids; to Mr. Ch. Quiquerez, ms Laboratory, Sandoz A.G., Basel, Switzerland, for the measurement of mass spectra, and to Prof. Dr. Marcel Wanner, Veterinary Hospital, Zürich, Switzerland, for his information concerning cattle and *A. napellus*.

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Received 6 July 1989