THE ALKALOID COMPOSITIONS OF *Hypecoum erectum* AND *H. lactiflorum*

L. D. Yakhontova, O. N. Tolkachev,
M. N. Komarova, and A. I. Shreter

UDC 547.944/945

In recent years, plants of the genus *Hypecoum* have been a source of new alkaloids. M. Shamma and his colleagues have isolated from *Hypecoum parviflorum* Kar. et Kir (Pakistan) the previously undescribed bases peshawarine [1] and feruloyltyramine [2]. We have reported on the isolation from *H. erectum* L. of, together with protopine, the new alkaloids hypecorine, hypercorinine [3-6], and hyperectine [7-9] and the determination of their structures. Hyperectine is a spirobenzylisoquinoline alkaloid with a maleimide group, while hypecorine and hypecorinine belong to a new type of spiroaminoketal alkaloids.

Continuing our study of *H. erectum* growing in Transbaikalia* we have isolated from this plant the alkaloids coptysine and allocryptopine; in addition, we have isolated in the form of its hydrochloride corydamine, found previously in *Corydalis incisa* Pers. [10] and also 1-N-methylcanidine nitrate, mp 254-255°C (decomp.) [11].

It must be mentioned that protopine and 1-N-methylcanadine are present in the plant in the form of the nitrates, as was established by isolation of the nitrates of these alkaloids by extracting the plant raw material with methanol followed by their recrystallization from methanol. The protopine and 1-N-methylcanadine nitrates obtained in this way were identical with authentic samples prepared from the corresponding bases and nitric acid.

A comparative study of *H. erectum* growing in Transbaikalia and in the Mongolian Peoples' Republic showed that their alkaloid compositions were similar to one another. The main alkaloid, regardless of the growth region, was protopine, which amounted to 50% of the combined alkaloids. At the same time, the total amount of alkaloids in the *H. erectum* from Mongolia was 0.8%, and in that from Transbaikalia it was 3.5%.

The species *Hypecoum lactiflorum* Kar. et Kir. growing in the Western part of Mongolia, which is close to *H. erectum*, contained in its epigeal part 1.2% of combined alkaloids the qualitative composition of which was identical with that of the *H. erectum* alkaloids. However, in *H. lactiflorum* the main alkaloids were protopine, hypecorine, hypecorinine, and allocryptopine, present in approximately equal amounts and making up half of the total amount of alkaloids.

Thus, in the plants studied the total amounts of alkaloids and of their individual components depend on the growth site. The closeness of the chemical composition of *H. erectum* and *H. lactiflorum* indicates their genetic affinity, which is in harmony with the closeness of their botanical characteristics [12, 13].

LITERATURE CITED

1. M. Shamma, A. S. Rothenberg, G. S. Jayatilake, and S. F. Hussain, Heterocycles, 5, 41 (1976).
2. S. F. Hussain, B. Gozier, and M. Shamma, Phytochemistry, 21, No. 12, 2979 (1982).
3. L. D. Yakhontova, M. N. Komarova, M. E. Perel'son, K. F. Blinova, O. N. Tolkachev, Khim. Prir. Soedin., 624 (1972).
4. L. D. Yakhontova, M. N. Komarova, M. E. Perel'son, K. F. Blinova, and O. N. Tolkachev, Abstracts of Lectures at the 3rd Soviet—Indian Symposium on the Chemistry of Natural Compounds [in Russian], Tashkent (1973), p. 184.
5. L. D. Yakhontova, M. N. Komarova, O. N. Tolkachev, and M. E. Perel'son, Khim. Prir. Soedin., 491 (1976).

*The alkaloid composition of plants gathered in the flowering phase was studied.

All-Union Scientific-Research Institute of Medicinal Plants, Moscow. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 673-674, September-October, 1984. Original article submitted April 12, 1984.

0009-3130/84/2005- 0645$08.50

6. O. N. Tolkachev, L. D. Yakhontova, N. N. Margvelashvili, M. E. Perelson, Tenth International Congress of Biochemistry, Hamburg (1976), p. 620.
7. M. E. Perel'son, D. A. Fesenko, L. D. Yakhontova, O. N. Tolkachev, Abstracts of Lectures at the 3rd Moscow Conference on Organic Chemistry and Technology [in Russian], All-Union Mendeleev Chemical Association, Organic Chemistry Section (1982), p. 110.
8. M. E. Perel'son, G. G. Aleksandrov, L. D. Yakhontova, D. A. Fesenko, O. N. Tolkachev, and M. N. Komarova, 7th Soviet-Indian Symposium on the Chemistry of Natural Compounds. Abstracts of Lectures [in Russian], Tbilisi (1983), p. 67.
9. G. G. Alexandrov [Aleksandrov], O. N. Tolkachev, M. E. Perel'son, D. A. Fesenko, and L. D. Yakhontova, Second International Conference on Chemistry and Biotechnology of Biologically Active Natural Products, Budapest (1983), p. 243.
10. G. Nonaka and I. Nishioka, Chem. Pharm. Bull., 21, No. 7, 1410 (1973).
11. K. I. Kuchkova, I. V. Terent'eva, and G. V. Lazur'evskii, Khim. Prir. Soedin., 1410 (1967).
12. P. M. Krylov, The Flora of Western Siberia [in Russian], Tomsk, No. 6 (1931), p. 1229.
13. M. G. Popov, The Flora of the USSR [in Russian], Moscow (1937), p. 573.

ISOLATION OF PYROPHOSPHATASE FROM COTTON-PLANT SHOOTS

B. O. Beknazarov and M. N. Valikhanov

UDC 547.992

Inorganic pyrophosphatase (EC 3.6.1.1) catalyzes the reversible hydrolysis-synthesis reaction of PP_i. This enzyme is assigned a fundamental role in the phosphorus metabolism of plants but the methods for its isolation and purification, particularly from the tissues of higher plants, are imperfect [1-3]. The aim of the present work was to isolate pyrophosphatase from the cotton plant and to investigate some of its properties.

The pyrophosphatase was isolated from three-day shoots of a cotton plant of variety 108-F. The protein extract was obtained by homogenizing the cotyledons of the shoots in a cooled mortar with a glass pestle in a small volume of Tris-HCl buffer, pH 7.2. Then the homogenate was centrifuged at 10,000g for 30 min in the cold (2-4°C). The proteins were precipitated with the aid of cooled acetone. They were collected by centrifugation and the precipitate was resuspended in the initial buffer and was recentrifuged under the conditions described above. The supernatant obtained was dialyzed against distilled water for 16-18 h.

The protein extract was concentrated by ultrafiltration through Amicon membranes, which pass proteins with a molecular weight of up to 1 kD under a pressure of gaseous nitrogen (3-4 atm). The protein extract was subjected to gel filtration on a 1.9 × 70 cm column filled with Sephadex C-100 (Sweden), and equilibrated with the initial buffer, rate of elution was 6.6 ml/h. The proteins were chromatographed on a 1.3 × 30 cm column filled with DEAE-cellulose (Serva) which had been suspended in and equilibrated with the initial buffer in an NaCl gradient at a rate of elution of 6 ml/h. Rechromatography of the enzyme extract was carried out on a column of DEAE-cellulose DE-52 (Whatman, United Kingdom) under the same conditions. The yield of protein was recorded with the aid of a Uvicord-III instrument (LKB) at 280 nm.

In parallel, the protein in the samples was determined by the Lowry method [4]. The substrate used was $Na_4P_2O_7 \cdot 10H_2O$ (Sigma). The pyrophosphatase activity was measured from the formation of organic phosphate, the amount of which was determined by the method of Weil-Malherbe and Gree [5] with the aid of Spectromom-195 and 402 instrument (Hungary). The stages of the purification of the enzyme are shown below:

V. I. Lenin Tashkent State University. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 674-675, September-October, 1984. Original article submitted March 26, 1984.

0009-3130/84/2005-0646$08.50