ALKALOIDS FROM *Papaver albiflorum* PACZ. subsp. *albiflorum* AND *P.* cf. *stevenianum* A. D. MIKHEEV*

Jiří SLAVÍK and Leonora SLAVÍKOVÁ

Department of Medical Chemistry and Biochemistry, J. E. Purkyně University, 662 43 Brno

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Papaver albiflorum PACZ. subsp. *albiflorum* (tetraploid, $2n = 28$; total alkaloid content 0·001%) afforded corytuberine, protopine and allocryptopine as dominant alkaloids; in addition, small amounts of mecambrine, thebaine, scoulerine, rhoeadine, papaverrubines D, C, A and E, berberine and coptisine were detected. *Papaver* cf. *stevenianum* A. D. MIKHEEV (hexaploid, $2n = 42$; total alkaloid content 0·066% and 0·015%, respectively) gave berberine as the principal alkaloid (0·065% and 0·013%, respectively) which was responsible for the yellow colour of latex. Corytuberine, allocryptopine, protopine and isocorydine were isolated as minor alkaloids and small amounts of corydine, mecambrine, thebaine, scoulerine, papaverrubines D, C, A and E, coptisine, *cis*-N-methylcanadinium hydroxide and N-methylthebainium hydroxide were detected. Alkaloid PCH 1, isolated recently as minor constituent from *Papaver rhoeas* var. *chelidonioides* O. KUNTZE, has been shown to be identical with isorhoeagenine.

The present work is a continuation of our previous communications on alkaloids in species of the genus *Papaver* growing on the Czechoslovak territory[1,2] and concerns the two further taxa, *Papaver albiflorum* subsp. *albiflorum* PACZ. and *P.* cf. *stevenianum* A. D. MIKHEEV. Both of them are annual herbs belonging to the section *Rhoeadium* SPACH (*Orthorhoeades* FEDDE).

P. albiflorum subsp. *albiflorum* is an East-European species which in Slovakia reaches the western border of the area. It is a tetraploid (chromosome number $2n = 28$)[3,4] with white petals and colourless or albescent latex. In a preliminary study of herbarium material from Central Slovakia (total alkaloid content 0·12%)[1] we isolated thebaine as the dominant alkaloid, together with traces of minor alkaloids of which we identified corytuberine and protopine. Considerably different results were obtained with greater amounts of plant material cultivated from seeds collected in Southwestern Slovakia. The total alkaloid content (0·001%) was two orders of magnitude lower and only traces of thebaine were present. Using the procedure commonly used in our investigations (see e.g. ref.[2]), we isolated from the strongly polar fraction corytuberine (0·0003%) as the main alkaloid of the plant. Column

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chromatography of nonquaternary weakly polar bases on alumina afforded protopine and allocryptopine; thin-layer chromatography detected negligible amounts of thebaine, papaverrubine D, C, A and E, rhoeadine and scoulerine. The quaternary protoberberine fraction contained traces of berberine and coptisine. The ripe capsules contained 0·008% of alkaloids, essentially the same as found in the whole plant. except that thebaine was the dominant alkaloid. In comparison with the plants from Central Slovakia[1] it seems probable that the studied population from Southwestern Slovakia either represents a different chemovariety or that the plant has lost almost completely its ability to accumulate thebaine when cultivated under artificial conditions. The composition, as well as quantitative content, of the other alkaloids is very similar in both populations.

P. stevenianum A. D. MIKHEEV (*P. dubium* auct. fl. cauc. et fl. ross. p.p., *P. nothum* auct. non STEV.) has been described as an independent species only in 1981 from the Cherson region of Ciscaucasia[5]. Until that time it was considered to be identical with the closely related *P. dubium* L. species. *P. stevenianum* is also related to the species *P. lecoquii* LAMOTTE[5]. The taxon studied in this paper was found by Kubát[6] in 1983 in Bratislava. Its botanical features[6] agree completely with those described for *P. stevenianum*[5]. It has yellow latex and red petals with a large dark violet spot on the basis. Like *P. stevenianum*[5], it is also a hexaploid[6] ($2n = 42$). Since the taxon studied in this communication has not been found so far on the Czechoslovak territory and no definitive decision on its very probable identity with *P. stevenianum* exists, we name it provisionally *P.* cf. *stevenianum* A. D. MIKHEEV. The fact that its distribution is limited to two geographically so distant localities is not well understandable. No data on alkaloids of this species are hitherto available.

In the beginning, we investigated orientationally the voucher specimen of the plants found by Kubát at the natural locality. From the quaternary nonphenolic protoberberine fraction we isolated berberine in the form of its crystalline chloride as the dominant alkaloid in a relatively high yield (0·065%); undoubtedly, its presence was responsible for the yellow colour of latex. In the fraction of weakly polar bases (content below 0·001%) we detected by thin-layer chromatography corydine, protopine and allocryptopine as the main alkaloids and in the strongly polar fraction we found corytuberine, *cis*-N-methylcanadinium hydroxide and N-methylthebainium hydroxide. In the second phase of our study, we investigated greater amount of material from plants that had been cultivated from the seeds gathered at the natural locality. Also in this case we found berberine as the dominant alkaloid (0·013%), the second dominant alkaloid being corytuberine (0·001%) which also is commonly present in the related taxa of the *P. dubium* agg. group[1,2]. Of the nonquaternary bases (less than 0·001%) we isolated allocryptopine and small amounts of protopine and isocorydine. Thin-layer chromatography detected mecambrine, thebaine, scoulerine, rhoeadine and papaverrubines D and C, together with traces of papaverrubines A

and E. In the mother liquors after crystallization of berberine chloride we detected small amount of coptisine.

The quantitative as well as some qualitative differences in the alkaloid composition between the wild and monoculture-cultivated plants are not surprising: we have already observed similar differences with several species. From the biochemical standpoint, the hexaploid *P.* cf. *stevenianum* is most close to the tetraploid species *P. albiflorum* subsp. *austromoravicum* KUBÁT and *P. lecoquii* LAMOTTE, whereas it has practically no common biochemical features with the hexaploid *P. dubium* L.

The alkaloid PCH 1, isolated recently[2] as minor constituent from *Papaver rhoeas* var. *chelidonioides* O. KUNTZE, m.p. 205°C, has been additionally shown by mass and UV spectrum, mixture melting point and chromatography, to be identical with isorhoeagenine that we had previously isolated[7] from *P. rhoeas* L.

EXPERIMENTAL

Melting points were determined on a Mettler FP 51 instrument and are uncorrected. Mass spectrum was measured on a Jeol MSD 100 spectrometer, IR spectra in Nujol on a Specord 75 IR (Zeiss, Jena) spectrometer and UV spectra in methanol on a Unicam SP 1800 instrument. Thin-layer chromatography (TLC) was performed on silica gel G (Merck): weakly polar bases in the systems cyclohexane–diethylamine (9 : 1), cyclohexane–chloroform–diethylamine (7 : 2 : 1 and 6 : 3 : 1), benzene–methanol (4 : 1) and benzene–acetone–methanol (7 : 2 : 1); quaternary alkaloids and corytuberine in methanol–water–25% ammonia (15 : 3 : 1), ethanol–water–25% ammonia (15 : 9 : 1) and 1-propanol–water–85% formic acid (12 : 7 : 1). Quaternary protoberberines were also chromatographed on Silufol UV 254 (Kavalier) in methanol–diethylamine (4 : 1 and 1 : 1). Fluorescing alkaloids were detected by UV light, other alkaloids with potassium iodoplatinate. Papaverrubines were detected by 20 minutes' exposure to concentrated hydrochloric acid vapours (purple spots). The isolated alkaloids were identified by their melting points, mixture melting points, IR, UV and (in some cases) mass spectra, and thin-layer chromatographic comparison with reference samples.

Extraction and Isolation of Alkaloids

The plants were cultivated at the Center for Cultivation of Medicinal Plants of the Medical Faculty, J. E. Purkyně University, Brno, from the seeds collected at the following natural localities: *P. albiflorum* subsp. *albiflorum* at Moravský Ján and *P.* cf. *stevenianum* in Bratislava (collected by Dr K. Kubát). The plants were harvested in 1984 and 1985 at the stage of flowering and unripe fruits and were dried at room temperature. The dried ground plant material was exhaustively extracted with cold methanol in a percolator. After evaporation of the solvent, the extract was worked up as usual (see e.g. ref.[2]) to give alkaloid fractions A (weakly polar nonquaternary bases), B (quaternary nonphenolic protoberberines), and I (strongly polar alkaloids as iodides).

P. albiflorum PACZ. subsp. *albiflorum*

The whole dried plant (3·41 kg) was extracted. The amorphous fraction A (175 mg), containing considerable amount of nonalkaloid compounds, was chromatographed on a column of neutral

alumina according to Brockmann (Reanal, 54 g, activity approximately II). Elution was carried out with the following solvents or their mixtures in the order: benzene–ether–chloroform–methanol; 25 ml fractions were collected. Chloroform fractions 43—47 on crystallization from methanol afforded protopine (4·0 mg; 0·00012%), prisms, m.p. 207—208°C, and allocryptopine (2·1 mg; 0·00006%), prismatic needles, m.p. 160—161°C. The other fractions contained predominantly nonalkaloidal, in part crystalline compounds, along with negligible amounts of the following alkaloids, identified by TLC: thebaine, mecambrine, scoulerine, rhoeadine and papaverrubines D, C, A and E. In fraction B (less than 1 mg) we identified berberine and traces of coptisine. Fraction I on crystallization from methanol afforded corytuberine hydriodide (11·6 mg; 0·00025% as base), lustrous leaflets, m.p. 207—209°C.

P. cf. *stevenianum* A. D. MIKHEEV

The whole dried plant (7·1 kg) was extracted. Fraction A was separated in the usual manner (see e.g. ref.[2]) into chloroform-extractable (AC) and chloroform-nonextractable (AD) hydrochlorides which were further separated into nonphenolic (AD_1) and phenolic (AD_2) fractions. The fraction AC (196 mg) contained predominantly nonalkaloidal compounds. Column chromatography on alumina (21 g) under conditions described above (ether–chloroform 4 : 1 to 1 : 4, fractions 36—57) gave, after crystallization from ether, isocorydine (1·1 mg; 0·00002%), m.p. 183—184°C. The other, mainly nonalkaloidal, fractions have been shown (TLC) to contain traces of rhoeadine, papaverrubine A and E, and another unidentified alkaloid. Fraction AD_1 on crystallization from methanol afforded allocryptopine (13·1 mg; 0·0002%), prismatic needles, m.p. 160—161°C, and protopine (5·0 mg; 0·00007%), prisms, m.p. 207—208°C. In addition to the above-mentioned alkaloids the amorphous residue (12·5 mg) contained (TLC) small amounts of mecambrine, thebaine and two unidentified alkaloids. In fraction AD_2 (17·5 mg) scoulerine and papaverrubines D and C were detected by TLC. Crystallization of fraction B from dilute hydrochloric acid furnished 0·99 g of berberine chloride (0·013% as base) as fine yellow needles, m.p. 206—207°C. Bases, recovered from the mother liquor (0·04 g) contained, besides berberine, small amount of coptisine (TLC). Crystallization of fraction I from methanol gave 96·2 mg of corytuberine hydriodide (0·001% as base), lustrous leaflets, m.p. 208—210°C (methanol–ether); IR spectrum: ν(OH) 3 490 cm^{-1}. In the mother liquor small amount of an unidentified alkaloid was detected.

A voucher specimen (19 g) of the plant collected at the natural locality was treated in the same manner as described. Fraction A (yield less than 0·001%) contained corydine, allocryptopine and protopine. Crystallization of fraction B from dilute hydrochloric acid gave berberine chloride (12·4 mg; 0·065%); some coptisine was also detected. Corytuberine, *cis*-canadine methohydroxide and thebaine methohydroxide were identified in fraction I.

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Note added in proof: Recently, in collaboration with Professor V. A. Chelombit'ko and Dr. A. D. Mikheev, we studied alkaloids of species *Papaver stevenianum* A. D. MIKHEEV from a natural locality near Pyatigorsk, Ciscaucasia, U.S.S.R. The material contained berberine as the principal alkaloid; of the minor alkaloids we identified corytuberine, allocryptopine and protopine in amounts proportional to those found in *P.* cf. *stevenianum* of the Czechoslovak origin. This means that both the studied taxa have identical alkaloid profile (Chelombit'ko V. A., Slavík J., Slavíková L., Mikheev A. D., unpublished results).

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