ALKALOIDS FROM *Papaver rupifragum* BOISS. *et* REUT.*

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The following alkaloids were isolated from *Papaver rupifragum* BOISS. et REUT.: rhoeadine, protopine, rhoeagenine, magnoflorine and very small amounts of coptisine, cryptopine and (—)-stylopine. The presence of isorhoeadine, allocryptopine, corytuberine, papaverrubine A, B, C, D and E and corysamine was also detected.

Papaver rupifragum BOISS. et REUT. is a perennial plant from the section *Pilosa* PRANTL native to southern Spain, closely related to the species *P. atlanticum* BALL (*cf.*[1]). Little has been known of the alkaloids of this plant. Using thin-layer chromatography[1] up to eleven alkaloids were detected in it, of which rhoeadine and protopine have been identified. In addition to this the presence of papaverrubines A, B, E and D (ref.[1,2]) and of alkaloid R_1 (ref.[3]) undoubtedly identical with coptisine[4] has also been proved. From whole plants which were cultivated in the Experimental Garden in Brno we isolated a sum of bases in a 0·23% yield per dry weight and identified sixteen alkaloids. The dominant alkaloid was rhoeadine which represented almost the half of all bases present. In a smaller yield we also separated from the non-quaternary fraction protopine and rhoeagenine as further significant alkaloids. The amorphous residue of the bases from the mother liquors after the mentioned alkaloids was submitted to column chromatography on alumina. Cryptopine and (−)-stylopine were thus isolated in a very low yield. In addition to this, fractions were also obtained containing minimum amounts of isorhoeadine, allocryptopine and eleven papaverrubines of which papaverrubine A, B, C, D, and E were identified. The fraction of quaternary protoberberines was composed of coptisine with minute amounts of corysamine as an admixture. From the fraction of iodides of quaternary alkaloids we isolated magnoflorine iodide (0·005% per dry weight) and detected corytuberine[5] and a further unidentified alkaloid.

From the findings mentioned it is evident that *P. rupifragum* is not only botanically but also biochemically very closely related to the species *P. atlanticum* which displays practically the same spectrum of alkaloids[6–9]. Recently we also succeeded in isolat-

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ing[10] magnoflorine – the main component of the quaternary fraction from *P. rupifragum* – also from *P. atlanticum*. Papaverrubine B, present in *P. rupifragum* only is an exception, considered by Vent and coworkers[1] as the differentiating character between these two species.

EXPERIMENTAL

The melting points were determined on a Mettler FP 51 apparatus and they were not corrected. For thin-layer chromatography both silica gel G Merck and the solvent systems cyclohexane–diethylamine 9 : 1 (S_1), cyclohexane–chloroform–diethylamine 7 : 2 : 1 (S_2) and 4 : 5 : 1 (S_3), benzene–methanol 4 : 1 (S_4), benzene–acetone–methanol 7 : 2 : 1 (S_5), benzene–diethylamine 19 : 1 (S_6), chloroform–methanol–diethylamine 8 : 1 : 1 (S_7), ethanol–water–25% ammonia 15 : 9 : 1 (S_8), methanol–water–25% ammonia 15 : 3 : 1 (S_9) were used, as well as Silufol UV 254 (Kavalier) and the systems methanol–diethylamine 4 : 1 (S_{10}) and 1 : 1 (S_{11}). Paper chromatographies were carried out on Whatman paper No 1, in the descending manner, in the systems 1-butanol–acetic acid–water 10 : 1 : 3 (S_{12}) and ethanol–water 3 : 2 (S_{13}). The spots of fluorescing alkaloids were detected under UV light, the spots of papaverrubines in concentrated hydrochloric acid vapours after 20 minutes exposure (formation of purple spots), and the spots of other alkaloids with potassium iodoplatinate and Dragendorff's reagent. The isolated alkaloids were all identified by their melting point, mixture melting point or also IR and UV spectra and R_F values (by comparison with authentic samples).

Extraction and Isolation of Alkaloids

The plants were cultivated in the Experimental Garden of the Medical Faculty, Brno, from seeds obtained from various botanical gardens. Two-year old specimens were collected, including roots, in the stage of flowering and unripe fruits on June 16th, 1975. The material was dried at room temperature.

The dry ground material (5·36 kg) was extracted exhaustively with 15 l portions of methanol in the cold. Methanol was distilled off, the syrupy residue transferred into 5 l of 1% acetic acid and the insoluble matter was filtered off. From the filtrate the alkaloidal fractions *A*, *B*, *E* and *I* were obtained in the conventional manner[11,12].

The purified fraction *A* (12·38 g) was predominantly crystalline. After repeated washing with methanol 10·96 g of a crystalline mixture of bases were obtained from which 5·84 g of rhoeadine (total yield 6·01 g; 0·11%), m.p. 253—254°C, 1·89 g of protopine (total yield 2·54 g; 0·047%), m.p. 208—209°C, and 0·55 g of rhoeagenine (total yield 1·44 g; 0·027%), m.p. 237—239°C, were obtained by systematic crystallization from chloroform–methanol. The residual amorphous bases (4·10 g) of fraction *A* were separated to fraction AC_1, AC_2, AD_1 and AD_2 using the procedure described in ref.[13]. On crystallization from chloroform–methanol and methanol alone further amounts of rhoeadine (0·17 g), rhoeagenine (0·34 g) and protopine (0·10 g) were obtained from fraction AC_1, and, in the same manner, rhoeagenine (0·55 g) and protopine (0·55 g) from fraction AD_1. In the mother liquors of fraction AC_1 11 papaverrubines could be detected by TLC in systems S_1, S_2, S_4, S_5 and S_6, of which papaverrubine A, B and E were identified in addition to a small amount of phenolic papaverrubines C and D which were found predominantly in fraction AC_2. The amorphous residues of bases of fractions AC_1 and AD_1 were combined (1·19 g) and separated on a column of 70 g of alumina (Reanal), activity about II. From the fraction eluted with benzene–ether 2 : 1 (—)-stylopine (1·6 mg), m.p. 198—200°C, was isolated by crystallization from methanol, and in the fraction eluted with benzene–ether 1 : 1 isorhoeadine was

identified by TLC in addition to the residues of rhoeadine. On elution with ether or ether–chloroform 4 : 1 the remaining part of protopine and rhoeagenine, respectively, were obtained. Elution with the mixtures ether–chloroform 4 : 1 and 1 : 1 gave cryptopine (yield after crystallization from methanol 2·4 mg), m.p. 217—219°C, and in the fraction eluted with ether–chloroform 1 : 1 allocryptopine was also detected by TLC. The phenolic fractions AC_2 (0·03 g) and AD_2 (0·08 g) remained amorphous. In AC_2 seven unidentified alkaloids (R_F in S_2 0·04, 0·11, 0·21, 0·31, 0·41, 0·44, 0·47) were detected in addition to the mentioned papaverrubines C and D, and in the fraction AD_2 six unidentified alkaloids (R_F in S_3 0·14, 0·22, 0·37, 0·50, 0·65 and 0·78).

From fraction *B*, converted to chlorides, 13·6 mg of colourless hydrochloride were obtained by crystallization from water (clusters), m.p. 245—246°C, R_F values 0·02 in S_1, 0·12 in S_2 and 0·38 in S_3, which was not identified. From the mother liquors 21·5 mg of the yellow base of coptisine with a negligible admixture of corysamine were obtained on alkalization with sodium hydroxide and extraction with ether.

Fraction *E* (0·89 g) contained predominantly non-alkaloidal substances and it remained amorphous. Small amounts of corytuberine could be detected in it by using TLC.

Fraction *I* afforded 0·28 g of magnoflorine iodide (0·0052%), m.p. 264—265°C, on crystallization from methanol. The amorphous residue (3·09 g) contained the rest of the magnoflorine and a small amount of corytuberine and other unidentified alkaloid in addition to non-alkaloidal substances.

R_F Values

In systems S_1 and S_2: allocryptopine 0·26, 0·68; cryptopine 0·27, 0·74; isorhoeadine 0·61, 0·89, papaverrubine A 0·34, 0·73; papaverrubine B 0·23, 0·67; papaverrubine C 0·13, 0·37; papaverrubine D 0·06, 0·29; papaverrubine E 0·34, 0·73; protopine 0·47, 0·82; rhoeadine 0·58, 0·88; rhoeagenine 0·19, 0·66; stylopine 0·64, 0·96. In systems S_4, S_5 and S_6: papaverrubine A 0·89, 0·85, 0·86; papaverrubine B 0·79, 0·79, 0·80; papaverrubine C 0·70, 0·76, 0·46; papaverrubine D 0·60, 0·67, 0·30; papaverrubine E 0·54, 0·58, 0·75. In systems S_7, S_8 and S_9: corytuberine 0·50, 0·86, 0·92; magnoflorine 0·00, 0·55, 0·42. In systems S_{10}, S_{11}, S_{12} and S_{13}: coptisine 0·49, 0·85, 0·54, 0·75; corysamine 0·14, 0·55, 0·19, 0·74.

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