ON ALKALOIDS FROM *Papaver syriacum* BOISS. *et* BLANCHE*

J.SLAVÍK and L.SLAVÍKOVÁ

Institute of Medical Chemistry,
J. E. Purkyně University, 662 43 Brno

Received April 21st, 1975

From *Papaver syriacum* BOISS. et BLANCHE rhoeadine, isorhoeadine, rhoeagenine, thebaine, protopine, (±)-mecambrine, (−)-stylopine and (−)-β-stylopine methohydroxide were isolated and the presence of several papaverrubines and a small amount of coptisine, berberine and corysamine was also proved.

Alkaloids from *Papaver syriacum* BOISS. et BLANCHE have not yet been studied. *P. syriacum* BOISS. et BLANCHE is an annual from the section *Orthorhoeades* FEDDE, native in the Middle East. From plants cultivated on the territory of our country we isolated the sum of alkaloids in a 0·10% yield (per dry plant material) and separated from it rhoeadine as the main alkaloid. After fractionation of the remaining bases, using a procedure commonly used in our studies, we obtained thebaine, rhoeagenine and protopine, as well as a small amount of optically inactive alkaloid by direct crystallization. On the basis of its melting point, mass spectrum, UV spectrum and R_F values the last alkaloid was identified as (±)-mecambrine. This is the first case of the occurrence of a racemic form of this alkaloid in nature; the synthesis of (±)-mecambrine has been described[1,2]. From the non-crystallizing residue we further isolated by column chromatography on alumina isorhoeadine and (−)-stylopine, which were present in minute amounts only. In addition to this the presence of at least six papaverrubines was also demonstrated. Among them two are very probably identical with papaverrubines A (N-demethylisorhoeadine) and E (N-demethylrhoeadine). In the fraction of quaternary protoberberines coptisine was detected as the main component in addition to trace amounts of berberine and corysamine. From the fraction of quaternary alkaloids, which were isolated after their conversion to iodides by extraction with chloroform, (−)-β-stylopine methiodide (*cf.*[3]) was isolated and the presence of additional three alkaloids was demonstrated, which, however, could not be obtained crystalline.

The mentioned findings indicate a close biochemical relationship of the species *P. syriacum* with that group of species from the section *Orthorhoeades* which is characterized by the presence of rhoeadine as the main alkaloid in addition to a smal-

* Part LXI in the series Alkaloids of the *Papaveraceae*; Part LX: This Journal *41*, 285 (1976).

ler amount of protopine, rhoeagenine and other minor alkaloids. Representatives of this group are, for example, *P. rhoeas* L.[4,5] and *P. strigosum* (Bönningh.) Schur.[6,7].

EXPERIMENTAL

The melting points were determined on a Kofler block and they were not corrected. The mass spectra were recorded with an AEI-MS 902 spectrometer, the UV spectra on a Unicam SP 1800 instrument and the CD curves on a Roussel-Jouan Dichrograph CD 105. For thin layer chromatography both silica gel G (Merck) with gypsum (5 : 1) was used in combination with the systems cyclohexane–diethylamine 9 : 1 (S_1), cyclohexane–chloroform–diethylamine 7 : 2 : 1 (S_2), and ethanol–water–25% ammonia 15 : 9 : 1 (S_3), and Silufol UV 254 sheets (Kavalier, Votice) with the solvent system methanol–diethylamine 4 : 1 (S_4). Paper chromatography was carried out on paper Whatman No 1, descending manner, with 1-butanol–acetic acid–water 10 : 1 : 3 (S_5) and ethanol–water 2 : 1 (S_6). Papaverrubines were detected with concentrated hydrochloric acid fumes for 20 minutes (formation of purple spots), while the detection of quaternary protoberberines was carried out in UV light and the detection of other alkaloids with potassium iodoplatinate or with Dragendorff's reagent.

Extraction and Isolation of Alkaloids

The plants were cultivated in the Experimental Botanical Garden of the Medical Faculty in Brno from seeds obtained from the botanical garden in Jeruzalem. They were collected during flowering and the unripe fruits stage on 19. 7. 1972 and then dried at room temperature. Dry, ground plants (5700 g) were extracted with five portions of cold methanol (totally 100 l). After evaporation of methanol by distillation the extract was triturated with 1% acetic acid and filtered. From the filtrate alkaloidal fractions *A*, *B*, *E* and *I* were obtained in the conventional manner[8,9]. In order to prevent decomposition of alkaloids sensitive to mineral acids acetic acid was used exclusively and all operations were carried out in the cold.

Fraction A (5·0 g; 0·088%) was dissolved in 500 ml of 1% acetic acid; the insoluble residue was filtered off under suction, washed with diluted ammonia solution and crystallized from chloroform–methanol. Rhoeadine (0·70 g) was obtained (total yield 1·31 g; 0·023%). The acid filtrate after rhoeadine was alkalized with ammonia and extracted with ether. After evaporation of the solvent 4·30 g of bases were obtained which were crystallized from chloroform–methanol to afford additional rhoeadine (0·60 g). The residue of bases was dissolved in 5% acetic acid, a saturated potassium chloride solution was added, and the mixture was extracted with chloroform. The mixture was thus separated to a fraction containing hydrochlorides (or acetates) soluble in chloroform (*AC*) and another insoluble in chloroform (*AD*). Both fractions were then separated[8] to non-phenolic bases (AC_1 and AD_1) and phenolic ones (AC_2 and AD_2). Crystallization of the bases AC_1 (1·14 g) from ether gave 0·27 g of thebaine (total yield 0·31 g; 0·005%), while 0·87 g of amorphous bases remained in the mother liquors which were then separated by column chromatography on alumina (in principle according to[5]). The column was prepared from 64 g of alumina for chromatography (Lachema), activated by heating (activity approximately II). The amorphous bases AC_1 (0·87 g) were dissolved in benzene, the same volume of light petroleum was added, and the mixture chromatographed. Fractions of 50 ml volume were collected. For elution benzene–light petroleum 1 : 1 was used first (150 ml, fractions 1—3), then benzene (200 ml, fractions 4—7), benzene–ether 2 : 1 (100 ml, fractions 8 and 9) and 1 : 1 (350 ml, fractions 10—16),

ether (100 ml, fractions 17, 18), ether–chloroform 9 : 1 (100 ml, fractions 19, 20), 2 : 1 (200 ml, fractions 21—24), and 1 : 1 (100 ml, fractions 25, 26), chloroform (100 ml, fractions 27, 28), chloroform–methanol 19 : 1 (100 ml, fractions 29, 30), 4 : 1 (200 ml, fractions 31—34), and 3 . 2 (50 ml, fraction 35). The composition of the fractions was controlled by thin-layer chromatography in S_1 and S_2 and the fractions of the same composition were combined. After evaporation fractions 1—7 did not leave a weighable residue. From fractions 8 and 9 (85 mg) isorhoeadine (15·0 mg; 0·0003%) and (—)-stylopine (3·9 mg; 0·00007%) were isolated by crystallization from methanol. Fraction 10 (35 mg) gave on crystallization from methanol 10·8 mg of rhoeadine, from fractions 11 and 12 (300 mg) thebaine (37·8 mg) and (±)-mecambrine (6·2 mg) were obtained by crystallization from ether. The amorphous residue contained in addition to both mentioned alkaloids an unidentified alkaloid as the main component, which had R_F 0·06 in S_1 and 0·45 in S_2. Fraction 13 (10 mg) was amorphous and in addition to alkaloids present in fractions 11 and 12 it also contained at least four additional alkaloids. On crystallization from ether fraction 14—18 (120 mg) gave 11·4 mg of protopine and 7·5 mg of a non-alkaloid substance of m.p. 143—145°C. The amorphous residue contained additional four unidentified alkaloids. Fraction 20—23 (195 mg) gave on crystallization from methanol 23·4 mg of rhoeagenine. Fraction 24—35 (78 mg) was amorphous and contained at least six unidentified alkaloids. The amorphous bases of all fractions turned red-brown to brown in air and became resinous. In all fractions the presence of papaverrubines was detected.

From fraction AD_1 (0·55 g) 80 mg of rhoeagenine (total yield 105 mg; 0·002%) and 46 mg of protopine (total yield 57 mg; 0·001%) were obtained by crystallization from ether and methanol. From the remaining bases 7 mg of thebaine and 10·5 mg of (±)-mecambrine (total yield 16·7 mg; 0·0003%) were obtained after further purification by crystallization from ether. An amorphous residue (160 mg) was obtained which turned violet in air. In addition to mecambrine, thebaine and protopine the presence of two additional bases was detected in it (R_F values 0·04 and 0·10 in S_1, and 0·09 and 0·20 in S_2).

The phenolic fractions AC_2 (0·08 g) and AD_2 (0·16 g) were amorphous and darkened in air. According to thin-layer chromatography both fractions had practically identical composition and contained bases of R_F (in S_2) 0·08, 0·13, 0·18, 0·23, 0·32 and 0·41 in addition to two papaverrubines.

Fraction B contained 13·8 mg of yellow bases (0·00024%) which according to chromatography in S_4, S_5 and S_6 were composed mainly of coptisine (yellow spot in UV light, R_F 0·53, 0·45 and 0·12 resp.) in addition to a small amount of berberine (green-yellow in UV light, R_F 0·17, 0·59 and 0·16 resp.) and corysamine (green-yellow in UV light, R_F 0·09, 0·70 and 0·53 resp.). Fraction *E* (0·35 g) was amorphous. From fraction *I* (—)-β-stylopine methiodide (36·7 mg; 0·0006%) was obtained by crystallization from methanol. The residue (0·51 g) was amorphous and turned brown in air. In addition to traces of β-stylopine methiodide (R_F 0·44) it contained an alkaloid of R_F 0·27 in S_3 and a smaller amount of two additional alkaloids of R_F 0·35 and 0·40 in the same solvent.

Characterization of Alkaloids

Rhoeadine: from chloroform–methanol small needles, m.p. 252°C, undepressed on admixture with a preparation from *P. rhoeas*[4]. The reaction to papaverrubines was very weak. The R_F values (0·54 in S_1 and 0·86 in S_2) and the colour reaction with sulfuric acid (red-brown-olive green) were the same as with an authentic sample.

Isorhoeadine: long needles, m.p. 160—162°C (methanol or ether–hexane), undepressed with an authentic sample, readily soluble in ether, poorly soluble in methanol and hexane. UV spectrum:

λ_{max} (log ε) 242 nm (3·90), 293 nm (3·97), λ_{min} 230 nm (3·81) and 265 nm (3·13), identical with the literature data[10]. The R_F values (0·61 in S_1 and 0·89 in S_2) were identical with those of an authentic sample. The same is true of the reaction with conc. sulfuric acid (red-brown-olive green).

Rhoeagenine: minute prisms, m.p. 238—239°C (methanol), undepressed with a preparation prepared by hydrolysis of rhoeadine (see[4]). The R_F values (0·18 in S_1 and 0·55 in S_2) were identical with those of an authentic sample, as also was the colour reaction with conc. sulfuric acid (red-brown-olive green).

Thebaine: prisms of m.p. 190—191°C (ether), undepressed with an authentic sample. $[\alpha]_D^{22}$ $-214° \pm 2°$ (*c* 0·25, methanol). The mass and UV spectra were identical with the spectra from literature[11]. The R_F values (0·30 in S_1 and 0·64 in S_2) and the colour reactions (with conc. sulfuric acid, Erdmann's, Fröhde's and Marquis's reagents the colour was red, turning to orange-red, with conc. nitric acid the colour was yellow) were identical as with an authentic sample.

Protopine: m.p. 204—205°C (chloroform–methanol), undepressed with an authentic sample. R_F 0·37 (S_1) and 0·77 (S_2) and colour reactions identical as in the case of an authentic specimen.

(±)-*Mecambrine*: m.p. 191—192°C (ether); literature gives for a synthetic preparation m.p. 197—198°C (chloroform–ether)[1] or 200—201°C (ether)[2]. According to CD measurements the substance is optically inactive. The mass spectrum was identical with that of authentic (—)--mecambrine[12]. The UV spectrum was in agreement with the literature data[10,12]. The same is true of the R_F values (0·19 in S_1 and 0·56 in S_2) and characteristic colour reactions[12].

(—)-*Stylopine*: needles of m.p. 200—202°C (methanol), undepressed on admixture of an authentic sample (m.p. 201—202°C.) The R_F values (0·75 in S_1 and 0·96 in S_2) and the characteristic colour reaction with Erdmann's reagent (yellow-green, than blue-green and blue) were identical as in the case of a reference sample.

(—)-β-*Stylopine methiodide*: from methanol m.p. 297—300°C, undepressed with a sample prepared by methylation of (—)-stylopine[3]. The R_F values (0·44 in S_3 and 0·57 in S_5) were identical with those of a reference sample (α-form[3]: R_F 0·40 in S_3 and 0·75 in S_5). The same is true of the colour reaction with Erdmann's reagent, which was identical with the reaction of (—)-stylopine.

Papaverrubines. The presence of papaverrubines was demonstrated by heating with a 3·5% hydrochloric acid (purple coloration). A strong reaction of papaverrubines was obtained with the mother liquors after crystallization of rhoeadine, with the amorphous residue of the fraction AC_1 and the fraction AC_2, while a very weak reaction was obtained with fractions AD_1 and AD_2. In the non-phenolic fractions the presence of four papaverrubines was demonstrated by thin-layer chromatography, with the following R_F values: 0·03, 0·32, 0·37 and 0·49 in S_1 and 0·13, 0·69, 0·73 and 0·82 in S_2. In the phenolic fractions the presence of two papaverrubines was detected, with R_F 0·05 and 0·09 in S_1 and 0·32 and 0·46 in S_2. In both systems authentic samples of papaverrubine A and E had corresponding R_F values, 0·37 or 0·73, respectively.

We thank Dr V. Preininger, Department of Chemistry, Medical Faculty, Palacký University, Olomouc, for the samples of isorhoeadine and papaverrubine A and E, Dr L. Dolejš, Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague 6, for the mass spectra, Mrs H. Pilařová of the same Institute for the CD measurements and Mrs J. Bochořáková of our Institute for the measurement of the UV spectra.

REFERENCES

1. Kametani T., Sugi H., Shibuya S., Fukumoto K.: Tetrahedron *27*, 5375 (1971).
2. Horii Z., Uchida S., Nakashita Y., Tsuchida E., Iwata C.: Chem. Pharm. Bull. *22*, 583 (1974).
3. Slavík J., Slavíková L., Dolejš L.: This Journal *40*, 1095 (1975).
4. Slavík J.: This Journal *24*, 2506 (1959); Chem. Listy *52*, 1957 (1958).
5. Němečková A., Šantavý F.: This Journal *27*, 1210 (1962).
6. Šantavý F., Maturová M., Němečková A., Schröter H.-B., Potěšilová H., Preininger V.: Planta Med. *8*, 167 (1960).
7. Slavík J., Appelt J.: This Journal *30*, 3687 (1965).
8. Slavík J., Slavíková L.: This Journal *26*, 1836 (1961).
9. Slavíková L., Slavík J.: This Journal *31*, 3362 (1966).
10. Holubek J., Štrouf O.: *Spectral Data and Physical Constants of Alkaloids.* Vol. I, No 172 and No 268, and Vol. VI, No 746. Academia, Prague 1965 and 1970.
11. Wheeler D. M. S., Kinstle T. H., Rinehart K. L., jr: J. Amer. Chem. Soc. *89*, 4494 (1967).
12. Slavík J., Slavíková L.: This Journal *28*, 1720 (1963).

Translated by Ž. Procházka.