# ALKALOIDS FROM *Papaver setigerum* DC.*

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Received February 19, 1996
Accepted April 29, 1996

Two new benzylisoquinoline alkaloids of the papaverine type, setigerine (racemic α-methoxypapaverine, **1b**) and setigeridine (**1c**), were isolated from *Papaver setigerum* DC. as the minor constituents. Their structures were established by spectral data and X-ray crystallographic analysis. Papaverine (**1a**) was isolated as the major alkaloid of the plant (0.05 wt.%) besides of less amounts of morphine, codeine and rhoeadine. In a low yield, protopine, cryptopine, thebaine, rhoeagenine, and *N*-methylthebainium (as iodide) were obtained and small quantities of laudanosine, stylopine, isoboldine, scoulerine, papaverrubine A, B, C, D and E, coptisine, corytuberine, and magnoflorine were identified by thin layer chromatography.

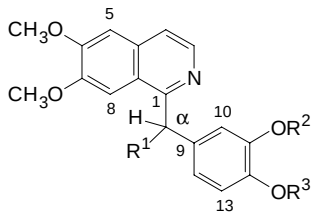
**Key words:** Benzylisoquinoline alkaloids; Setigerine; Setigeridine; *Papaveraceae*.

*Papaver setigerum* DC. (*Papaveraceae*) is an annual herb of the section *Meconium* SPACH (synonym: *Mecones* BERNH.) native to West and Central Mediterranean region and Canary Islands. It is closely related to *P. somniferum* L. of the same section[2], and sometimes confused with this owing to an erroneous description of *P. setigerum* in the classic Fedde's monography[2] (cf. ref.[3]). Significant differencies in the morphological features which can be used to distinguish both species were given by La Valva et al.[3]. Some discrepancies exist in the literature concerning the alkaloids contained in *P. setigerum*. According to the most authors[3,5,6], *P. setigerum* exhibits, when compared with *P. somniferum*, a relatively high content of papaverine and a very low content of morphine[4–6], codeine[5,6], and thebaine[5]. In the dry latex of the plants from Canary Islands, an appreciable amount of laudanosine was detected[6]. In contrary to this, La Valva et al.[3] found papaverine as the only one alkaloid in plants from South Italy and Mediterranean France. With respect to the botanical description, the taxon studied by Kleinschmidt[7] was probably a form of *P. somniferum* (cf. ref.[3]). It should also be pointed out that all investigations dealing with the alkaloids of *P. setigerum* have been carried out by means of analytical methods only, i.e., by paper[4,5,7], gas[3] and high-performance liquid chroma-

* Part XCV in the series Alkaloids of the *Papaveraceae*; Part XCIV: see ref.[1].

tography[6]. No reports on the isolation and characterization of the individual alkaloids were hitherto available.

As a part of an ongoing study of the *Papaveraceae* alkaloids, we investigated a population of *P. setigerum* which was grown from the seeds obtained from the Botanical Garden of Marseille. The morphological characters of the plants were in full accordance with those given by La Valva et al.[3]. Using the methanol extraction and isolation procedure universally adopted in our studies, we isolated the total alkaloids in a yield of 0.11 and 0.17% of dry whole plants and ripe capsules , respectively. Twelve alkaloids were separated in a crystalline form. The major alkaloid was papaverine (**1a**) (0.05 and 0.11%) in agreement with the literature data[3,5,6]. The content of morphine (0.027%, equally in both whole plants and capsules) was more than one order of magnitude lower than that in *P. somniferum*. It is accompanied with a lesser amount of codeine. Remarkable was the content of rhoeadine (0.006 and 0.010%) which has never been found either in *P. setigerum* or in *P. somniferum*. Its occurrence in *P. setigerum* indicates the close chemical affinity to *P. decaisnei* HOCHST. (ref.[8]).




**1**

| **1** | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| **a** | H | $CH_3$ | $CH_3$ |
| **b** | $OCH_3$ | $CH_3$ | $CH_3$ |
| **c** | $OCH_3$ | $CH_2$ | |

Successive crystallizations and column chromatography of the remaining bases afforded minor amounts of the known alkaloids protopine, cryptopine, thebaine and rhoeagenine, along with two new alkaloids and an alkaloid assigned PSG 1, m.p. 247 °C, incompletely characterized due to the scarcity of material.

The novel alkaloids, m.p. 148 °C and 209 °C, to which the trivial names setigerine and setigeridine were given, were separated by column chromatography on aluminium oxide. Both compounds were optically inactive and their UV spectra with absorption maxima at 206, 240, 281 and 328, and 208, 237, 282 and 328 nm, respectively, were similar to the spectrum of papaverine. The mass spectrum estimated the composition $C_{21}H_{23}NO_5$ and $C_{20}H_{19}NO_5$ for setigerine and setigeridine, respectively. The amounts of both alkaloids available were too small to perform the complete spectral analyses. For-

tunately, because of the excellent ability of setigerine to form well developed crystals, its structure could be established[9] by X-ray crystallographic analysis as α-methoxypapaverine (**1b**). The presence of two enantiomers was observed indicating that setigerine occurs in the plant in a racemic form.

While the mass spectrum of papaverine is relatively simple (cf. also ref.[10]), the spectra of setigerine and setigeridine are somewhat more complex. In the spectra of the new alkaloids, the molecular ion and the fragment ions M – $CH_3$, M – $OCH_3$ and the nitrogen-free ions of the benzyl moiety are the most abundant and common to both compounds. However, all these fragments in the spectrum of setigeridine were by 16 mass units lower than those in the spectrum of setigerine suggesting that two methoxy groups of setigerine are replaced by one methylenedioxy group in setigeridine. The fragment ions at *m/z* 324 and 310, occurring in the spectrum of setigeridine but absent in the spectra of both setigerine and papaverine, resulted probably from a methylenedioxy group by loss of a formyl group and carbon monoxide molecule from the $M^+$ and $M^+ - CH_3$ ions, respectively. The nitrogen-free fragment ion at *m/z* 165, of the composition $C_9H_9O_3$, corresponding to the fragment ion at *m/z* 181 in the spectrum of setigerine, indicated that the methylenedioxy unit is placed in the benzyl moiety of setigeridine. Hence, since the structure of setigerine has been established[9] as **1b**, the structure **1c** follows for setigeridine.

This is the first evidence of the occurrence of α-substituted papaverine-like alkaloids in nature.

From the strongly polar alkaloid fraction isolated after conversion into iodides, small amount of *N*-methylthebainium iodide was separated. In the mother liquors of the remaining fractions, thin layer chromatography revealed the presence of laudanosine along with minute quantities of stylopine, isoboldine, scoulerine, papaverrubines A (*N*-demethylisorhoeadine), B (*N*-demethylglaudine), E (*N*-demethylrhoeadine), D (porphyroxine) and C (epiporphyroxine) (cf. also ref.[11]), coptisine (cf. also ref.[12]), corytuberine and magnoflorine besides of several non-identified non-phenolic and phenolic alkaloids.

## EXPERIMENTAL

The melting points were determined on a Mettler F 51 apparatus and were uncorrected. Electron impact mass spectra (EI-MS) were performed on a Jeol MS D 100 spectrometer and Finnigan MAT TSQ 70 instrument. UV spectra were recorded in methanol on a Unicam SP 1800 apparatus and IR spectra in Nujol on a Specord 75 IR (Zeiss, Jena) spectrometer. Thin layer chromatography (TLC) was carried out using silica gel (Merck) and following solvent systems: cyclohexane–diethylamine 9 : 1 (S1), cyclohexane–chloroform–diethylamine 7 : 2 : 1 (S2) and 6 : 3 : 1 (S3), benzene–methanol 4 : 1 (S4), benzene–diethylamine 19 : 1 (S5), chloroform–ethanol–diethylamine 8 : 1 : 1 (S6), methanol–water–25% aqueous ammonia 15 : 3 : 1 (S7) and 1-propanol–water–85% formic acid 12 : 7 : 1 (S8). Silufol UV 254 plates (Kavalier, Czech Republic) and systems methanol–diethylamine 4 : 1 (S9) and 1 : 1 (S10) were used for quaternary protoberberines. The spots exhibiting fluorescence were detected in UV light (254 and 360 nm), the other spots with potassium hexaiodoplatinate(IV). The papaver-

rubines were made visible by 20 min exposure to vapours of concentrated hydrochloric acid (formation of purple spots).

Extraction and Isolation of the Alkaloids

The plants were cultivated at the Centre for Cultivation of Medicinal Plants, Medical Faculty, Masaryk University, Brno, from the seeds obtained from the Botanical Garden of Marseille. The culture was protected against contamination with any other species and its homogeneity carefully controlled. The plants were harvested at the stage of unripe capsules on July 18, 1988. Small amount of ripe capsules was collected separately. A herbarium specimen was deposited at our Institute. The plant material was dried at a room temperature.

The dried, whole plants (4.08 kg) were exhaustively extracted in the cold with methanol (total 160 l). After removal of the solvent, the dark green sirupy residue was extracted with 1% acetic acid (total 1.1 l) until a negative Mayer test was achieved and filtered. The combined acid filtrates were made alkaline with the aqueous sodium carbonate solution and extracted several times with ether (fraction A) and then with chloroform containing 20 vol.% of ethanol (fraction E). The remaining alkaline aqueous layer was then adjusted to pH value about 6, saturated aqueous potassium iodide solution was added and the mixture was extracted several times with chloroform containing 20 vol.% of ethanol (fraction I).

Crude fraction A was purified by the usual acido-basic procedure and the purified bases were crystallized from methanol. Rhoeadine (0.21 g) crystallized first followed by a crop of papaverine (1.58 g). The remaining bases were dissolved in 1% acetic acid, saturated aqueous potassium chloride solution was added and the mixture extracted with chloroform, thus separated into the chloroform-extractable (AC) and non-extractable (AD) hydrochloride fraction. These were divided into a non-phenolic ($AC_1$ and $AD_1$) and phenolic ($AC_2$ and $AD_2$) fraction by partitioning between ether and 5% aqueous sodium hydroxide solution. Crystallizations of the fraction $AC_1$ gave a further portion of papaverine (0.38 g), and rhoeadine (16.6 mg) along with crude setigeridine contaminated with some protopine and rhoeadine (9.5 mg). Residual bases (0.74 g) were amorphous. TLC in S1–S5 proved small amounts of papaverrubine A, B, and E besides of other alkaloids. Fraction $AD_1$ when crystallized from methanol yielded protopine (168.2 mg), rhoeagenine (55.0 mg), cryptopine (10.3 mg), codeine (isolated as sparingly soluble sulfate; base 0.27 g), alkaloid PSG 1 (2.3 mg; prisms, m.p. 246–247 °C, $R_F$ value 0.55 in S1), and 55.7 mg of amorphous bases.

The remaining amorphous bases of the fraction $AC_1$ and $AD_1$ were pooled and subjected to column chromatography on aluminium oxide (Reanal, according to Brockmann, activity about II) with benzene. The fractions eluted with benzene–ether (1 : 3) contained small amounts of stylopine (TLC in S1–S5). Elution with ether yielded crude setigerine (11.6 mg). Fractions eluted with ether–chloroform (3 : 1) gave rhoeadine (4.4 mg), papaverine (38.8 mg), and setigeridine (1.3 mg). Rhoeagenine (13.1 mg), thebaine (16.1 mg) and protopine (9.2 mg) were obtained from the fractions eluted with ether–chloroform (1 : 1). In the mother liquor, laudanosine was identified as the main component (TLC in S1–S5). The elution with chloroform gave rhoeagenine (3.6 mg) and cryptopine (1.0 mg). The fractions eluted with chloroform–methanol (9 : 1 to 1 : 1) did not crystallize and contained several non-identified alkaloids (TLC S1–S5).

The phenolic fraction $AD_2$ (0.25 g) afforded morphine (21.2 mg) by crystallization from methanol, fraction $AC_2$ (0.14 g) was amorphous. TLC in S1–S5 revealed the presence of small amounts of isoboldine, scoulerine, papaverrubines D and C and several non-identified alkaloids.

Fraction E was crystallized from methanol to give morphine (1.07 g). The remaining bases were converted into sulfates and crystallized from methanol yielding codeine sulfate (base, 0.20 g). In the mother liquor, trace amount of coptisine was detected (TLC in S9 and S10). The fraction I purified

in the usual manner and crystallized from methanol gave *N*-methylthebainium iodide (3.6 mg). Small amounts of corytuberine, magnoflorine and coptisine were identified in the mother liquors (TLC in S6–S10).

The dry ripe capsules (40 g) were processed in the same manner as the whole plants. Crystallization of the fraction A from methanol afforded rhoeadine (6.6 mg), papaverine (41.7 mg) and protopine (5.3 mg). In the amorphous residue (5.4 mg), the presence of codeine, isoboldine and scoulerine was proved (TLC in S1–S5). From the fraction E, morphine (10.9 mg) was obtained by crystallization from methanol and the presence of coptisine was detected (TLC in S9 and S10). In the fraction I, *N*-methylthebainium salt, corytuberine, magnoflorine and coptisine were identified (TLC in S6–S10).

Characterization of the Alkaloids Isolated

The individual alkaloids were identified by their melting points, mixed melting points, UV and IR spectra and EI-MS, respectively, and TLC behaviour in comparison with the reference samples. The yields of isolated alkaloids in wt.% of the dry whole plants and dry ripe capsules are given in parentheses. The presence of alkaloid detected only by TLC is denoted +, its absence –.

*Papaverine* (**1a**, 0.049; 0.107): prismatic needles, m.p. 147–148 °C (methanol). EI-MS, *m/z* (%): 339 (72), 338 (100), 324 (77), 308 (18), 279 (8), 167 (9), 149 (15), consistent with the literature data[10]. UV spectrum: $\lambda_{max}$, nm (log ε): 207 (4.44), 238 (4.90), 280 (3.94), 315 (3.66), 327 (3.74); $\lambda_{min}$, nm (log ε): 213 (4.34), 264 (3.86), 305 (3.49), 319 (3.63), IR spectrum and TLC values in S1–S5 are in accordance with those of an authentic sample.

*Morphine* (0.027; 0.027): prisms, m.p. 253–254 °C (methanol), $[\alpha]_D^{24}$ –131° ± 3° (*c* 0.13, methanol). UV and IR spectra as well as the $R_F$ values in S3 and S6 agreed with those of a reference sample.

*Codeine* (0.012; +): prisms, m.p. 156–157 °C (ether), $[\alpha]_D^{24}$ –138°± 3° (*c* 0.15, methanol). UV and IR spectra and the $R_F$ values in S1–S5 were identical to those of an authentic sample.

*Rhoeadine* (0.0065; 0.017): thin needles, m.p. 255–256 °C (chloroform–methanol). UV and IR spectra as well as the $R_F$ values were identical to those of a reference sample from *P. rhoeas* L.

*Protopine* (0.0044; 0.013): prisms, m.p. 209–210 °C (chloroform–methanol). Identity was confirmed by UV, IR and the $R_F$ values in S1–S5.

*Rhoeagenine* (0.0018; –): small prisms, m.p. 241–242 °C (methanol). UV and IR spectra and the $R_F$ values in S1–S5 were identical to those of an authentic sample.

*Thebaine* (0.0004; –): prisms, m.p. 195–196 °C (ether), the identity being confirmed by UV, IR and the $R_F$ values in S1–S5.

*Cryptopine* (0.00028; –): prisms, m.p. 221–222 °C (chloroform–methanol). Identification by UV and IR spectra and the $R_F$ values in S1–S5.

*Setigerine* (**1b**, 0.00028; –): the crude product recrystallized from methanol gave pure compound (4.4 mg), well developed flat prisms, m.p. 151 °C sharply, $[\alpha]_D^{20}$ 0° ± 3° (*c* 0.06, chloroform). EI-MS, *m/z* (%): 369 (85), 354 (100), 338 (70), 324 (37), 280 (6), 181 (49). UV spectrum: $\lambda_{max}$, nm (log ε): 206 (4.39), 240 (4.76), 281 (3.83), 315 sh (3.68), 328 (3.78); $\lambda_{min}$, nm (log ε): 214 (4.25), 266 (3.78), 306 (3.58). The $R_F$ values 0.29 in S1 and 0.69 in S2.

*Setigeridine* (**1c**, 0.00015; –): small prisms, m.p. 208–209 °C (methanol), $[\alpha]_D^{20}$ 0° ± 3° (*c* 0.04, chloroform). HR EI-MS, *m/z* (composition, %): 353 ($C_{20}H_{19}NO_5$, 75), 338 ($C_{19}H_{16}NO_5$, 77), 324 ($C_{19}H_{18}NO_4$, 74), 322 ($C_{19}H_{16}NO_4$, 83), 310 ($C_{18}H_{16}NO_4$, 56), 294 ($C_{18}H_{16}NO_3$, 44), 279 ($C_{18}H_{17}NO_2$, 43), 165 ($C_9H_9O_3$, 100). UV spectrum: $\lambda_{max}$, nm (log ε): 208 (4.35), 237 (4.64), 282 (4.03), 328 (4.03); $\lambda_{min}$, nm (log ε): 215 (4.29), 267 (4.00), 303 (3.99); no change in 0.02 M methanolic solution of sodium hydroxide. The $R_F$ values 0.16 in S1 and 0.57 in S2.

*N-Methylthebainium iodide* (0.00006; +): prisms, m.p. 219–220 °C (methanol–ether), either alone or in admixture with an authentic sample[13]. The $R_F$ values in S6–S8 are identical to those of the co-chromatographed reference specimen.

*We are grateful to Dr V. Hanus, J. Heyrovsky Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, Prague, for the recording of the mass spectra, and to Mrs J. Bochorakova of our Institute for the measurement of UV and IR spectra and for the technical assistance.*

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