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Papaver bracteatum Lindl.—a new plant source of opiates

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Certain strains of *P. bracteatum* Lindl. are reported to contain in the roots significant quantities of thebaine, which can be used for the production of codeine and other opiates. Results from plants raised from Iranian seeds and grown near London for 4 to 5 years are presented. The botanical and chemical characters correspond closely to those of the active strain Halle III described by Böhm (1970) and enable this plant to be clearly distinguished from the closely related *P. orientale* L. The thebaine content of the roots was 0·25% which is much lower than the value of 0·7 to 1·3% reported in Halle III roots. The fruiting tops, however, were also investigated and found to contain significant amounts of thebaine in field conditions. If tops were harvested in the summer and roots in the autumn, about 15 kg thebaine per hectare should be produced from our strain grown in the conditions described. This compares favourably with about 1 kg alkaloids per hectare from Turkish opium production or even the 3 kg per hectare from the high yielding Indian farms.

Although all known species of the Papaveraceae produce alkaloids (mostly isoquinoline type) very few produce morphine-like substances. Apart from small amounts in *Papaver setigerum* D.C. (Kleinschmidt, 1958), morphine itself only occurs in significant amounts in *P. somniferum* L. and this has long been the sole plant source for the production of morphine, codeine and other opiates. Because of the increase in illicit traffic in opiates steps have now been taken to discontinue opium production in Turkey: if a similar ban were applied elsewhere, there would be grave difficulties in meeting the demand for opiates for medicinal use. Reports therefore (see below) that a particular strain of *Papaver bracteatum* Lindl. contains large amounts of thebaine are of considerable interest, as thebaine can be fairly readily converted into codeine (Böhm, 1970). Furthermore, thebaine is used for the manufacture of the endoethenotetrahydrothebaines of outstanding analgesic potency (Bentley & Hardy, 1967; Haddlesey, Lewis & others, 1972) and of naloxone (British Patent, 1963) a narcotic antagonist and is therefore a starting material for some potentially useful drugs.

Papaver bracteatum Lindl. is closely related to *P. orientale* and is said to produce small amounts of isothebaine, thebaine, protopine, salutaridine and other minor alkaloids (Santavy, 1970). In 1963, Neubauer & Mothes reported on a chemical race, which they designated "Halle III", and which produced in the roots 0·7 to 1·3% alkaloids of which 98% was thebaine. Later work (Böhm, 1967) showed that other alkaloids occurred in the aerial parts of young plants but disappeared at maturity; however, two sub-races were detected, one containing thebaine only at maturity, the other "type E" containing thebaine plus alkaloid E. (later identified as alpinigenine, Guggisberg, Hesse & others, 1967). In 1967, Sharghi & Lalezari described a race growing wild in the mountains of Iran; in the latex they found 26% of thebaine (dry weight basis).

In this paper we report on the alkaloids of a variety of *P. bracteatum* from Iran which we have been growing in England for 4 to 5 years.

MATERIALS AND METHODS

Materials

A few seeds were obtained from Iran in 1967, sown in the greenhouse and the seedlings transplanted out of doors in the spring of 1968. Two of the plants flowered and examination of the capsule/stem latex showed that thebaine was the major alkaloid; 12·4% was present in the fresh latex, corresponding to about 25% of the dried latex, thus indicating that ours was an active strain. The number of plants was therefore increased by root division of one of the plants in succeeding years. Seeds were also collected from this original plant; sown in November 1970 and transplanted out of doors in April 1971: no flowers were obtained that summer, but large numbers in 1972, the second year of growth. Botanical and chemical characters of the original plants and those produced from them were examined in detail in order to characterize our strain satisfactorily and correlate the results with those of other workers.

Botanical characters

Our plants were distinguished from *P. orientale* plants by the following characters: (a) *Bud shape:* oval to oblong in contrast to the round shape in *P. orientale.* (b) *Bracts:* the flower buds are always closely surrounded by 5 to 8 imbricated bracts with coarsely toothed fringes: one at least of the bracts persists long after the sepals, petals and stamens have been shed. *P. orientale* buds, in contrast, are usually naked, protection being afforded by the hairy calyx. (c) *Calyx:* covered with coarse, closely appressed trichomes: in *P. orientale* the hairs are thinner, and more erect. (d) *Petals:* Lindley (1821) reported 5 free petals: we have found 6 normally present (occasionally 5); they are deep red with one or two prominent black blotches at the base. We confirmed that the petal pigments can be distinguished from those of *P. orientale* by chromatography and that they are surprisingly stable in contrast to *P. orientale* petals which quickly fade (Günther & Böhm, 1968). Our plants, though all ultimately derived from one seed, showed the two types of root-structure, one producing a few thick roots, the other numerous thinner interlocking ones, described by Böhm (1970).

We also examined the herbarium specimens at Kew and found that all 16 specimens labelled *P. bracteatum* had the distinguishing morphological characters already described for our plants. Furthermore, the petals of *P. orientale* plants had all faded, but there was an interesting difference among those of the *P. bracteatum* plants. Specimens from the Caucasus and Iran had retained a deep crimson purple colour, whereas those from Turkey had faded like the *P. orientale* petals.

Chemical characters

Thebaine was found to be the major alkaloid at all stages of plant development and was identified by comparison with authentic material using co-chromatography in 12 t.l.c. systems, colour reactions and ultraviolet spectra. Alpinigenine was also found as a major alkaloid and was identified similarly by comparison with authentic material; in addition melting point, mass spectra and nmr spectra were compared. Since alpinigenine occurred in the aerial parts at all stages including the mature plant and

latex, our plants must have been the "type E" referred to by Böhm (1967). Minor alkaloids similar to those described by Böhm also occurred at the young stages of development. Quantitative analysis (see below) showed that in the capsule latex from our original plant 18·9% and in that from the second generation seedlings 18·2% of the total alkaloids was alpinigenine.

Thebaine content of capsule and stem latex and root at various stages of development

Sampling. Samples of *latex* were collected from a number of capsules of equivalent physiological age; the day on which petals were fully opened was taken as week 0. At suitable intervals a representative number of the capsules (from 16 to 35) were cut off and the latex flowing from them, and from the severed pedicels, collected separately into 0·1 M citric acid containing 0·01 M potassium metabisulphite. The exhausted capsules were also collected, the ovules removed and the pericarp blended with methanol. In another experiment 161 "*tops*" each consisting of a capsule plus 25–40 cm of attached pedicel were cut off from 8 four-year old plants.—Most of the capsules were at week 2 to 4 stage. The tops were allowed to lie in the open exposed to the sun for 3 h, after which they were cut up and dried in an oven at 50° for 2 days and then powdered. Finally, *roots* were collected in the autumn when the aerial parts had died: they were dried at 40–50° and powdered. All samples were stored at 4° till required for analysis.

Quantitative analysis. For *latex* samples the citric acid extracts were brought to pH 7·4 to 8·0 and extracted with successive quantities of $CHCl_3$ to exhaustion. The combined $CHCl_3$ extracts were washed with water, evaporated to dryness and the residue dissolved in $CHCl_3$ to a known volume. A suitable aliquot was chromatographed quantitatively, using silica gel GF_{254}: benzene–methanol (80:20). The solvent front was allowed to run to 16 to 17 cm: the plate was then dried and redeveloped in the same solvent for the same distance. The thebaine band was removed by micro-vacuum suction (Fairbairn & El-Masry, 1967) and the dried powder washed on to a sintered glass funnel with chloroform–methanol (80:20) and eluted to exhaustion with the same solvent. The combined eluates were evaporated to dryness, the residues dissolved in 0·1 N HCl to a suitable volume and the extinction at 285 nm (max) and 255–257 nm (min) determined. The percentage of thebaine was calculated using an E 1%, 1 cm value of 261: if the ratio of E values at 285 nm to that at 257 nm was less than 1·50 the result was rejected. The method was checked by using known amounts of thebaine solution and processing them in the same way. The recovery of thebaine was 98 to 102% with a ratio of E values of 1·75. Alpinigenine was determined in a similar manner.

The methanol extracts of the *pericarp* were filtered, the marc exhaustively extracted with methanol and the combined extracts reduced to small volume. The pH was brought to 2 with citric acid and colouring matter removed by shaking with ether: after raising the pH (8–9) the alkaloids were extracted into chloroform which was washed and processed as for the latex samples above. For *roots* and *tops* a known weight of the powder was exhausted with methanol–ammonia 0·88 (98:2) by shaking at room temperature for 1 h. This extract was treated as for the methanol extracts above, except that a preliminary removal of pigment from root samples was not necessary.

Table 1. *Thebaine content of latex and capsule during development from flower to fruit in plants in the 4th year.* Figures in brackets are percentage thebaine.

Time of collection	Yield of latex (*a*) Capsule	(*b*) Stem	Fresh wt of exhausted pericarp	Absolute amount of thebaine per "top"
W0†	35 mg (11·8%)	17 mg (13·4%)	2·3 g (0·074%)	8·11 mg
W1	58 mg (6·8%)	18 mg (8·2%)	n.d.	
W2	62 mg (4·9%)	7·5 mg (16·6%)	6·1 g (0·030%)	6·11 mg
W3	95 mg (6·9%)	38 mg (6·2%)	9·2 g (0·046%)	12·74 mg
W4	81 mg (6·9%)	24 mg (6·2%)	8·0 g (0·084%)	13·79 mg

†—Week 0 the stage at which the corolla is fully opened; W1, W2—weekly intervals afterwards.
n.d.—Not done.

RESULTS

Results for quantitative analysis of latex and capsules at various stages of development are given in Table 1 and for dried tops and for roots in Table 2.

Density of plants and capsules. In our first bed plants were 33 to 60 cm apart, each plant producing 22 capsules on average. In the second bed plants were 34 to 43 cm apart producing 17 capsules per plant.

Second generation seedlings. Plants grown from seeds of the original plant showed identical botanical and qualitative chemical characters to those of the parents: capsule latex collected at week 1 was found to contain 7·19% thebaine which is similar to the values for the parent plant given in Table 1.

DISCUSSION

Comparison with other strains

The botanical and qualitative chemical characters of our plants were very similar to those of the Halle III strain, but the thebaine content was significantly less. Neubauer & Mothes (1963) reported 0·7 to 1·3% in the dried roots: our plants contained only 0·25%. The dry weights of root per normal plant, however, were similar: about 52–57 g against their values of 40–45 g for roots collected in the autumn. They also report a thebaine content for the dry capsules of about 0·7% (Neubauer & Mothes, 1963, revised Table 4). From our Table 1 the value for Week 3 capsules is about 0·35% and for Week 4 capsules 0·5% (calculated on a dry weight basis), again giving significantly lower results than those of Halle III strain. These differences could be due to (*a*) different conditions of growth, (*b*) the fact that Halle III strain

Table 2. *Thebaine content of roots, and of tops (capsule with 25–40 cm of attached pedicel) collected in field conditions.*

Collection details	Dry weight (g)	Thebaine content (%)
Roots:		
1. Collected 25.6.71: 3 years old	52	0·25
2. Collected 6.8.71: 3 years old	57	0·24
Tops:		
3. Collected 29.6.72: 4 years old (161 tops)	561	0·15

appears to have come from Armenia or the Caucasus, whereas ours came from Iran, (*c*) different methods of assay; we found the method used by Böhm (Sakurai, 1960) gave variable results even with pure thebaine. However the most important factor may be the significant proportion of alpinigenine (about 20% of the total alkaloids of the latex) in our plants, which may be associated with decreased thebaine production.

Yields per hectare

Roots. Neubauer & Mothes (1963) give a figure of 34 kg thebaine per hectare. This assumes a density of 625 plants per 100 m² (40 cm apart), an average root dry weight of 70 g, with a mean thebaine content of about 0·8%. (The high weight of root per plant can only be obtained by using "disbudded" plants). Our variety of normal, not disbudded plants, gave per plant 55 g root containing 0·24% thebaine: at a density of 625 plants per 100 m² the yield per hectare would be 8·25 kg thebaine.

Importance of the aerial parts. Böhm (1970) rejects the use of the capsules partly because disbudding is necessary in order to increase root yields in the autumn. Disbudding, however, may be a labour-expensive operation and accordingly we have studied the thebaine content of the developing capsules and pedicels. From Table 1 it will be seen that the absolute amount of thebaine per top increases during the first three weeks of growth and then steadies off. If tops were collected at weeks 2 to 4, the average yield would be about 11 mg. At about 20 tops per plant (see our "Results") this would be 0·22 g per plant corresponding to 13·7 kg per hectare. Tops collected in more realistic conditions however contained only 0·15% thebaine (Table 2) corresponding to 5·2 mg per top; on this basis the yield would be 6·5 kg per hectare. This figure is about half of what would be expected from our results based on freshly collected latex, and may be partly a seasonal variation and partly due to decomposition of thebaine in the 161 tops during their deliberate exposure to the sun. We also found it extremely difficult, during the assay process, to free the extracts from interfering pigments and the extended procedure may have led to an artificially low result. The results, however, show that a significant yield of thebaine can be obtained from the fruiting tops; these could be harvested mechanically without excessive damage to the foliage leaves, so that root development would not be hindered. In fact the operation would be a form of disbudding and may well lead to increased root yield in the autumn.

Assuming the lower yield from the tops in field conditions, the combined results (root and tops) give a total of about 15 kg thebaine per hectare. Obviously, this would be much greater if a more active strain such as Halle III were used. But a yield of 15 kg per hectare compares favourably with the yields of alkaloid from opium production. In 1971 the average yield of opium in Turkey was 7 kg per hectare, which at 15% morphine-codeine content (dry weight basis) corresponds to about 1 kg alkaloids per hectare. The highest yields for opium in 1971 was from India and corresponds to about 3 kg alkaloids per hectare; the lowest from Bulgaria correspond to 0·1 kg per hectare, calculated on the same assumptions (United Nations 1971).

Taxonomic consideration

Although Lindley (1821) gave this plant specific rank, later botanists have questioned this and assume there is a continuous range of form between *P. orientale* and *P. bracteatum.* Thus Cullen (1965) states that both *P. bracteatum* and *P. orientale* "are widely cultivated as garden ornamentals under the name *P. orientale*, every transition

occurs between them". This opinion may be based on inability to recognise genuine *P. bracteatum*. The first sample we obtained from a botanical garden turned out to be *P. orientale*. Günther & Böhm (1968) state that they obtained many samples from botanical gardens but in nearly all cases the plants were *P. orientale*. In only five cases did they obtain genuine *P. bracteatum*, and these were all labelled "*P. orientale*"! These authors claim that genuine *P. bracteatum* has distinct characters which clearly distinguish it from *P. orientale*. Our own observations confirm most of their claims, especially the differences in calyx trichomes; the blood red colour of petals, whose stability and chromatographic behaviour are very distinct from those of *P. orientale*. We also consider the persistent bract a reasonably constant difference. These characters seem constant as they persist in the plants raised from seeds produced by our original plant.

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