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Papaver bracteatum Lindley: thebaine content in relation to plant development

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Four thebaine-rich varieties of *P. bracteatum* have been grown in the open over two seasons and the thebaine distribution in aerial parts examined to determine the most suitable source material for commercial production. The leaves contained only 0·1 to 0·15%; the capsules 0·5 to 3·0% and the bled latex 28 to 53%. The maximum for the latter occurred about 3–4 weeks after petal opening and during the day, at about 15.00 h. A product 'bractium' prepared exactly as opium from *P. somniferum* contained up to 55% thebaine and calculations from the 1974 results gave theoretical yields up to 58 kg of thebaine per hectare. However this is a very labour intensive method; furthermore bled latex only represents about 46% of the total thebaine of the capsule. In addition the pedicels contain significant amounts of thebaine, so that fruiting tops may be recommended as source material. In the capsule the thebaine content reaches a peak 3 to 4 weeks after petal opening and again two weeks later. At this fully ripe stage there is a theoretical yield of 50 kg per hectare. Two further advantages accrue from collection at this time: the ripe seeds can probably be used for similar purposes as poppy seed; and the pericarps at this stage contain no 'bound thebaine' (i.e., thebaine insoluble in MeOH : NH_4OH but soluble in acetic acid—in unripe capsules bound thebaine represents 18 to 36% of the total thebaine). There is some evidence that, as this perennial plant increases in age, the capacity for thebaine production seems to continue increasing. Storage of raw material, even in ideal conditions, led to a loss of thebaine of 12 to 20% in one year.

Although the first report on *P. bracteatum* as a commercial source of opiates recommended using the root as the starting material (Neubauer & Mothes 1963), Fairbairn & Hakim (1973) demonstrated the potential of the aerial parts, the use of which would have many agricultural advantages. The variation in thebaine content in relation to the development of the aerial parts therefore needed investigation. Böhm (1967) studied the changing pattern of alkaloids in the aerial parts during development by qualitative t.l.c. only. Later Aynehchi & Jaffarian (1973), using quantitative t.l.c., studied changes in the thebaine content of the root and aerial parts, including the capsule but not the latex, from plants growing wild in Iran during 1971. We have extended these studies with an examination of four thebaine-rich strains grown under controlled conditions in our experimental garden. Particular attention was paid to the latex as it seemed likely that, as with the closely related *P. somniferum* the alkaloids occur exclusively in this tissue; we also continued the investigation of the 'bound forms' of thebaine referred to by Fairbairn & Helliwell (1975).

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MATERIALS AND METHODS

Plants were cultivated in our experimental garden in North London from seeds of the following thebaine-rich strains: *MH-1*—from Iran (the one used in our previous work) (Fairbairn & Hakim, 1973). *MH-3*—seeds collected in the Alborz Mountains, Iran, at 2200 m and 36° 13′N; 51° 19′E in 1972. *UNB-2*—seeds supplied by the U.N. Division of Narcotics and originally from Iran. *ARYA-1*—from the Alborz Mountains (U.N. Report J/2, 1973).

The authenticity of the plants produced by each strain was checked by using the characters already summarized (Fairbairn & Hakim, 1973) and those given by Goldblatt (1974).

Thebaine content was determined by the g.l.c. method of Fairbairn & Helliwell (1975); in general, plant material was dried and powdered as described except when fresh weight determinations on latex were necessary. For this purpose fresh latex was transferred directly to 5% acetic acid containing 0·1% sodium metabisulphite, and subsequently extracted as for dried samples.

RESULTS

Leaves

Representative samples of leaves were collected and

their thebaine content measured at intervals from November 1973 to the end of July 1974. At this time of year the plants become dormant and the leaves, which then contain only traces of alkaloids, almost disappear until about September, when a rosette of new leaves is formed and remains over winter. The seasonal variations in thebaine content for three strains are shown in Fig. 1.

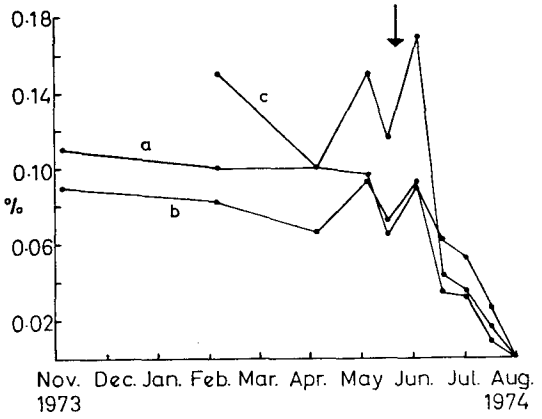

FIG. 1. Variation in leaf thebaine content (% dry weight) during one season's growth, based on samples from the three strains of plant, a—MH-3, b—UNB-2 and c—ARYA-I. Arrow indicates onset of flowering.

Latex

(a) *Variation with stage of development of capsule.* On a particular day, flowers, with petals just opened, from each strain were labelled Week 0 (W0), thus ensuring that all flowers used were of the same physiological age. Small samples of latex were drawn at stated intervals by pricking the ovary of developing capsules (about noon on each occasion) and removing the small amount of exuded latex from each capsule and pooling the drops for analysis. The results are shown in Fig. 2.

(b) *Variation during a 24 h period.* Small samples of latex were drawn, as described above at 2-hourly intervals during one complete day at Week 1 (W1) and at Week 3 (W3) in 1974, and at Week 3 (W3) only in 1975, using the UNB-2 strain. The results of analysis on a fresh weight basis and, in addition, on a dry weight basis in the 1975 samples, are given in Fig. 3.

(c) *Production of 'bractium'.* A dried product was produced from the latex in exactly the same way as opium is prepared from *Papaver somniferum.* In view of the results in Figs 2 and 3, the capsules were incised at the optimum time for high thebaine content, that is, two to four weeks after petal

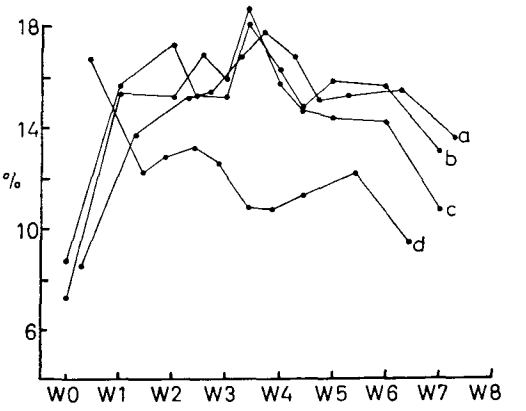

FIG. 2. Variation in thebaine content (% dry weight) of latex drawn from capsules during development from petal opening (W0) to maturity 7 weeks later (W7) using all 4 strains of plant. a—ARYA-I, b—UNB-2, c—MH-3, d—MH-1.

opening and between 14.00 and 15.30 h. A toothed tool, identical with that used in Turkey (Trease & Evans, 1972), was used for scarification and the exuded droplets were allowed to dry on the capsule overnight and scraped off next day to produce 'bractium'—the equivalent of opium. The samples were weighed and the moisture content determined

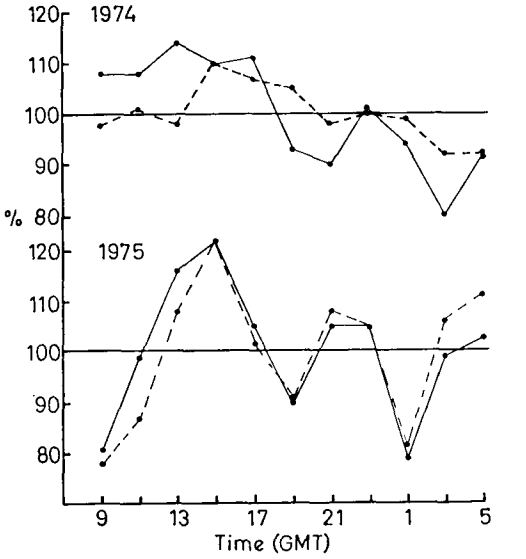

FIG. 3. Two-hourly variation in thebaine content of latex from capsules at W1 ●—● and W3 ●- -● in 1974. In 1975, samples were all drawn at W3 but thebaine contents on fresh ●—● and dry weight ●- -● bases were determined. UNB-2 strain used throughout. Daily averages for 1974: W1, 14·52%; W3, 15·06% thebaine. For 1975, W3 (fresh weight) 12·19%; W3 (dry weight) 35·23% thebaine. y axis—Percentage variation from daily average of thebaine content.

by drying at 105°. In some cases, a second scarification was made on the same capsules, a few hours after removing the bractium and the second crop collected next day. Batches were prepared from MH-1 and MH-3 in 1974 and from ARYA-I in 1975 and the results are given in Table 1. The scarified capsules of the 1975 batch were allowed to mature and were collected 7–8 weeks after petal fall and found to contain 0·57% thebaine (dry weight basis). The five samples collected in 1974 were stored in a desiccator in the dark. At the end of six months there were losses from 0 to 8% and at one year from 12 to 20% in thebaine content.

Table 1. *Production of 'bractium', an opium-like preparation from the unripe capsules.*

Strain	No. of caps scarified	Wt of bractium (g)	Moist. (%)	Thebaine content dry wt (%)
1974 MH-1	131; 1 scarif.	7·461	25·0	44·3
MH-1	74; 1st ,,	5·314	17·3	43·2
	2nd ,,	4·407	22·6	45·8
MH-3	31; 1st ,,	1·429	16·7	44·6
	2nd ,,	2·117	25·3	54·5
1975 ARYA-I	100; 1st ,,	2·398	9·0	32·7
	2nd ,,	1·289	14·3	37·6

Capsules

As the *expelled* latex may not represent the total latex content (and therefore the total thebaine) of the capsule, analyses of the thebaine content of expelled latex and that remaining in the bled capsule were carried out separately. At stated intervals after petal fall, several capsules of each variety were severed at the base and the expelled latex allowed to drain into tared collecting vessels. The expelled latex and bled capsules were weighed immediately then dried to constant weight at 105° and the thebaine content determined. The results for all varieties are given in Table 2.

Bound forms of thebaine were also determined in these samples by assaying for 'free' thebaine using MeOH/NH_4OH (98:2) as extracting solvent and then for 'free and bound' thebaine using the recommended solvent 5% acetic acid (Fairbairn & Helliwell, 1975) (Table 3).

Fruiting tops

Capsules with 15–20 cm of attached pedicel, from three strains, were collected at intervals, dried at 105° without removing the ovules or seeds and powdered. The results of analysis for % thebaine and absolute amounts are given in Table 4.

Table 2. *Thebaine content of (a) expelled latex and (b) the remaining capsule ('bled capsule') during maturation in 4 strains of plant in 1974.* Values are average mg/capsule. (W0 = time of petal opening).

Stage of maturity	(a) Expelled latex	(b) Bled caps.	Total theb.	Total dry wt (g)	Total theb. (%)
MH-1					
W0 + 2 days	4·3	5·6	9·9	0·587	1·70
+ 11 ,,	9·6	7·3	16·9	1·512	1·12
+ 16 ,,	8·6	6·5	15·1	2·189	0·69
+ 21 ,,	9·8	7·2	17·0	2·354	0·72
+ 30 ,,	10·6	9·9	20·5	2·854	0·72
UNB-2					
W0 + 6 days	9·9	12·7	22·6	1·296	1·74
+ 11 ,,	8·5	16·6	25·1	1·627	1·54
+ 16 ,,	9·9	13·5	23·4	2·310	1·01
+ 21 ,,	12·8	19·5	32·3	3·524	0·92
+ 28 ,,	10·8	18·7	29·5	4·092	0·72
MH-3					
W0 + 2 days	7·4	8·8	16·2	0·914	1·77
+ 8 ,,	11·0	12·8	23·8	1·810	1·32
+ 13 ,,	9·6	11·6	21·2	1·963	1·08
+ 21 ,,	9·1	14·9	24·0	2·740	0·88
+ 30 ,,	13·3	9·8	23·1	4·947	0·47
+ 42 ,,	9·6	8·2	17·8	2·855	0·62
+ 49 ,,	1·7	7·9	9·6	1·987	0·48
+ 56 ,,	No latex	32·7	32·7	6·050	0·54
ARYA-I					
W0 + 3 days	8·0	10·3	18·3	0·597	3·07
+ 6 ,,	6·4	6·0	12·4	0·470	2·64
+ 13 ,,	10·7	10·3	21·0	1·046	2·00
+ 21 ,,	15·3	11·2	26·5	2·634	1·00

Table 3. *Analyses of samples referred to in Table 2, for thebaine content (% dry weight) calculated as free* total† and bound‡ forms.*

Stage of maturity	Expelled latex Free	Expelled latex Total	Bled capsule Free	Bled capsule Total	Bound: total (%) Bled cap.	Bound: total (%) Whole cap.
MH-1						
W0 + 2 days	35·9	35·6	0·45	0·97	54	30
+ 11 ,,	34·0	34·1	0·14	0·49	71	31
+ 16 ,,	31·2	31·5	0·15	0·30	51	22
+ 21 ,,	32·6	32·7	0·11	0·31	65	27
+ 30 ,,	35·1	34·5	0·19	0·35	46	22
UNB-2						
W0 + 6 days	33·9	34·2	0·67	1·08	33	19
+ 11 ,,	36·3	36·3	0·70	1·04	33	22
+ 16 ,,	32·6	32·4	0·34	0·59	42	24
+ 21 ,,	35·8	36·1	0·23	0·56	59	36
+ 28 ,,	30·4	31·0	0·24	0·46	48	30
MH-3						
W0 + 2 days	42·6	42·8	0·66	0·98	33	18
+ 8 ,,	38·8	38·2	0·27	0·72	63	34
+ 13 ,,	35·6	35·7	0·30	0·60	50	27
+ 21 ,,	30·7	30·4	0·31	0·55	44	27
+ 30 ,,	34·3	34·0	0·10	0·20	50	21
+ 42 ,,	27·9	28·0	0·14	0·29	52	24
+ 49 ,,	n.d.	35·6	0·38	0·40	5	4
+ 56 ,,	no latex	—	0·53	0·54	2	2
ARYA-I						
W0 + 3 days	46·8	47·1	0·83	1·78	54	30
+ 6 ,,	46·5	46·9	0·68	1·31	48	23
+ 13 ,,	52·6	52·2	0·40	1·00	60	30
+ 21 ,,	44·0	44·2	0·26	0·43	40	17

* Free—thebaine extracted by MeOH/NH_4OH (98:2).
† Total—thebaine extracted by acetic acid 5%.
‡ Bound—total—free.

Table 4. *Thebaine content of* P. bracteatum *'fruiting tops' collected during the period of capsule maturation.*

Sample		Average dry wt of one 'aerial top' (g)	Thebaine in sample %	Thebaine per 'aerial top' (mg)
UNB-2				
W0	+ 14 days	4·08	0·92	37·6
	+ 20 ,,	5·63	1·00	56·3
	+ 28 ,,	7·67	0·35	26·8
	+ 36 ,,	9·02	0·41	37·0
MH-3				
W0	+ 10 days	3·10	1·16	36·0
	+ 16 ,,	6·67	0·49	32·7
	+ 21 ,,	6·73	0·39	26·3
	+ 32 ,,	5·83	0·32	18·7
ARYA-I				
W0	+ 10 days	2·68	1·35	36·2
	+ 21 ,,	4·12	0·64	26·3
	+ 26 ,,	4·83	0·57	27·6
	+ 41 ,,	7·50	0·56	42·0

DISCUSSION

Cultivation possibilities

This examination of the thebaine content of the aerial parts as possible economic sources of the drug suggests the following conclusions:

Leaf. This does not seem to be a promising source material as the thebaine content is only about 0·1 to 0·15%; furthermore, most leaves grow close to the ground and are not easily harvested. It is interesting that the percentages of thebaine we found are higher than those reported by Aynehchi & Jaffarian (1973) for leaves of plants growing in their natural habitat, in which only traces of thebaine were found except in one collection which contained 0·09%.

Latex. Thebaine contents of 28 to 53% were found in latex (Table 3) thus confirming values reported earlier (Fairbairn & Helliwell, 1975, 34–36%; Cheng, 1972, 45%; Sharghi & Lalezari, 1967, 26%). For three strains we found the maximum percentage to occur 3–4 weeks after petal opening (Fig. 2, Table 3). For strain MH-1 however, while the maximum absolute amount occurs at a similar time, the maximum percentage occurs much earlier; this confirms results previously reported for this strain (Fairbairn & Hakim, 1973). We also found variation within one day with the maximum percentage occurring about 15.00 h (UNB-2 strain Fig. 3) and this may be related to the ambient temperature which is usually highest then. These 2-hourly variations in thebaine content are ±20 % of the daily mean value and are significantly less than those for morphine, codeine and thebaine in *Papaver somniferum* (approximately ±90%, Fairbairn & Wassel, 1964). But the low morning values followed by a peak at about 15.00 h are similar to thebaine and codeine values found with *P. somniferum* and is in marked contrast to the morphine content which normally has a distinct peak before noon (Fairbairn & Wassel, 1964). Vágujfalvi (1973) also studied these fluctuations in alkaloid content in *P. somniferum* and *P. orientale* and found wide variation in the amounts present according to the individual plant, but usually there were distinct changes around midday and also after midnight.

Bractium. With a knowledge of peak yield times we prepared samples of dried latex ('bractium') at the optimum time for thebaine content and obtained samples containing 43 to 55% thebaine in 1974. In 1975, the values were 33 to 38% and the yield per plant was also significantly less (Table 1). This was almost certainly due to the dry and hot summer when the flow of latex is much less than from plants growing in well watered soil. Another factor may have been that these plants were only in their first full year of flowering.

There is some evidence that as the plants grow older their capacity for thebaine production increases. Thus the MH-1 variety in its fourth year of flowering (1972) produced approximately 8% thebaine in the fresh latex (Fairbairn & Hakim, 1973). Whilst this same variety, in its sixth year, produced approximately 12% thebaine (Fig. 2). This corresponded to about 36% thebaine on a dry weight basis, and in the optimum conditions used for producing bractium, about 45% thebaine (dry weight basis) was obtained from this variety (Table 1). Similarly, roots from 1-year old plants growing in Iran contained only 0·7% thebaine whereas 'old wild roots' contained 1·7 to 2·1% (U.N. Report 1973 J/1). Bractium production is obviously labour-intensive but may be very suitable in regions of cheap peasant labour and poor transport facilities. Calculations based on results in Table 1 show that yields can be surprisingly high. Thus in 1974 the 105 plants (MH-1 and MH-3) which were twice scarified yielded bractium corresponding to 4·854 g thebaine. The theoretical yield based on 625 plants per 100 m² and 20 capsules per plant (Fairbairn & Hakim, 1973) would be 58 kg per hectare. For the 131 once scarified plants however, the yield would be only 24 kg per hectare. Even after removing bractium the capsules continue to grow and when ripe in one experiment they contained as much as 0·57% thebaine. Significant losses of thebaine in bractium occur after 1 year's storage.

Whole capsules. The harvesting of whole capsules is preferable, especially for mechanised cultivation. Furthermore, over the whole period of capsule maturation the expelled latex only accounts for 46% of the total thebaine in the capsule (average of 21 collections, Table 2). Taking the additional thebaine into account the maximum percentage in the capsule occurs shortly after petal opening in all 4 strains, but the maximum absolute amount per capsule occurs 3 to 4 weeks after petal opening (Table 2). However the work with strain MH-3 indicates a second maximum at the very ripe stage when the latex has dried up. Although the percentage thebaine is lower than at earlier stages, this is largely due to the increase in seed weight; if seeds were removed the percentage thebaine would be 2 or 3 times higher.

Fruiting tops. The stems have been shown to contain significant amounts of thebaine (Aynehchi & Jaffarian, 1973; Cheng, 1972) so we repeated our earlier work on the fruiting tops (capsule plus 20–30 cm of pedicel; Fairbairn & Hakim, 1973) and confirmed that this product would be a suitable source of thebaine, as it can be conveniently harvested. Results (Table 4) show that a peak of thebaine content occurs during the first 3 weeks, but in samples UNB-2 and ARYA-I there is again evidence of a second peak 5 to 6 weeks after petal opening. As would be expected, the values are on the whole higher than those for capsules alone (Table 2) but the two sets of results are not comparable because they were based on different plants. The amounts in the fruiting tops are significantly higher than those reported earlier (Fairbairn & Hakim, 1973) and can be accounted for mainly by the use of better strains. Even higher yields may be obtained as the plants grow older.

For *commercial production* our results indicate that the fruiting tops collected about 5 to 7 weeks after petal opening may be the best material for harvesting especially if mechanization is used. Taking the average yield per top for UNB-2 at week 5 (37 mg) and ARYA-I at week 6 (42 mg) the theoretical yield, calculated on the basis previously used (Fairbairn & Hakim, 1973), would be about 50 kg thebaine per hectare, although obviously in field conditions it may be less. A further advantage of harvesting at this stage would be the production of seed which seems to have very similar composition to ordinary poppy seed (U.N. Report 1974 J/15) and could be used for the same purposes. At the end of several years the large amount of root formed could clearly be used as an additional source of thebaine.

'Bound' form of thebaine. The results in Table 3 confirm those of Fairbairn & Helliwell (1975) in that all the samples of bled latex yielded all its thebaine to MeOH/NH_4OH. In contrast, all samples of capsule, from which the latex had been bled, yielded substantially more thebaine to 5% acetic acid than to MeOH–NH_4OH. This additional 'bound' thebaine represented 30 to 70% of the thebaine in the bled capsule and 18 to 36% of that in the whole capsule. Obviously if capsule material is used as a source of thebaine this additional yield when an acid extracting solvent is used will be significant. However, if fully ripe capsules are used (e.g. MH-3 at 7 or 8 weeks after petal fall; Table 3), practically no bound forms are present, this might be a further advantage of harvesting at this stage.

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