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ALKALOIDAL STORAGE, METABOLISM AND TRANSLOCATION IN THE VESICLES OF *PAPAVER SOMNIFERUM* LATEX

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Key Word Index—*Papaver somniferum*; Papareraceae; alkaloids; metabolism; morphine; radioactive precursors; translocation; vesicles.

Abstract—After centrifuging stem and capsule latex of *Papaver somniferum* at 1000 *g*, 95–99% of the alkaloids were found in the pellet, which consists mainly of "alkaloidal vesicles". The alkaloids appear to be stored in the vacuolar sap of the vesicles rather than membrane bound, and in this respect the vesicles behave as normal vacuoles. However evidence is produced to indicate that the stem latex and vesicles are translocated into the capsule during its rapid expansion after petal fall. During this time the morphine itself is being synthesized and metabolized in the vesicles (more rapidly in the stem vesicles than in those of the capsule) and the metabolites pass out of the latex into the pericarp with a significant amount appearing in the ovules. The vesicles are therefore not merely passive accumulators of alkaloids.

INTRODUCTION

PREVIOUS work has indicated that the alkaloids of *Papaver somniferum* are biosynthesized in the 1000 *g* fraction of the latex and almost certainly in the vacuolar particles which have been described by several workers.[1–5] In this present paper we investigate the suggestions made earlier[6] that the alkaloids occur exclusively in these organelles and that the stem latex is more active metabolically than the capsule latex. The problem of translocation of alkaloids into the developing capsule is also a subject of the present investigation. Because, as will be shown later, the alkaloids of the poppy plant seems to be exclusively associated with these organelles, we suggest the name "alkaloidal vesicles".

RESULTS

Alkaloids almost exclusively located in the vesicles

Separate samples of stem and capsule latex were diluted with mannitol buffer and centrifuged at 1000 *g* for 0·5–1 hr; the supernatant and pellet (re-suspended in fresh buffer) were re-spun. The alkaloidal spectrum of the combined pellets was investigated by TLC and the quantity of morphine present estimated. The final supernatant was

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filtered (to remove any remaining organelles) and the alkaloidal spectrum and morphine content determined. The pellets contained the normal poppy alkaloids and the supernatant contained morphine and codeine mainly, with traces of other alkaloids. The quantitative results given in Table 1 show that 95–99% of the morphine (and therefore of the other alkaloids) is contained in the pellet.

TABLE 1. DISTRIBUTION OF MORPHINE BETWEEN THE SUPERNATANT AND THE 1000 *g* PELLET OF EXPELLED STEM AND CAPSULE LATEX IN (a) NORMAL CONDITIONS (b) AFTER PLASMOLYSIS AND (c) AFTER ADDING EXOGENOUS NUCLEAR LABELLED ^{14}C MORPHINE (Wo = time of petal fall)

Sample		Time of collection	Morphine content		
			Supernatant	1000 *g* pellet	Proportion in pellet
(a) Normal					
1 Stem 1971	1·23 g	Wo + 2 days	2·0 mg	61·5 mg	96·6 %
2 Capsule 1971	1·16 g	Wo + 2 days	1·65 mg	36·0 mg	95·6 %
3 Capsule 1971	1·92 g	Wo + 6 days	2·48 mg	47·15 mg	95·0 %
4 Stem 1972	0·827 g	Wo + 15 days	2·15 mg	53·0 mg	96·1 %
5 Capsule 1972	1·346 g	Wo + 15 days	1·19 mg	127·5 mg	99·1 %
(b) Plasmolysed					
6 Stem 1972	1·123 g	Wo + 12 days	1st 75 mg	Traces	Traces only
(c) Exogenous ^{14}C morphine			2nd 8 mg		
7 Capsule 1972	1·58 g	Wo + 7 days	1230 cpm	25000 cpm	95·4 %
7·8 mg morphine added (30700 cpm)*					(of the radioactivity)
Incubated 2·5 hr					
8 Stem 1972	1·634 g	Wo + 2 days	9167 dpm	21000 dpm	70·0 %
7·1 mg added					(of the radioactivity)
(32500 dpm)			15·94 mg	71·87 mg	81·85 %
Incubated 4 hr			morphine	morphine	(of the morphine)

* cpm not corrected for quenching in this series.

One sample of latex was plasmolysed by dilution with water; this simple operation resulted in the whole of the morphine (and other alkaloids) passing into the supernatant (Table 1). In a further experiment radioactive morphine was added to freshly collected capsule latex which was then diluted with 4 vol. of mannitol buffer and kept in ice for 1·5 hr. After a further 1 hr at room temp. it was centrifuged at 1000 *g* for 1 hr in order to deposit all the pellet, and the results in Table 1 show that 95% of the radioactivity was now in the combined pellets. Similar work was done on a further sample of stem latex, but only 70% of the radioactivity was found in the pellet. The morphine content of the pellet and supernatant was therefore determined and 81·85% of the morphine was found in the pellet (Table 1). These results indicate that the stem and capsule latex may differ in metabolic activity as has already been suggested:[6] this possibility was therefore next investigated.

Relative activity of stem and capsule latex

At various times samples of stem and capsule latex were collected simultaneously from the same plants and transferred to separate tubes containing radioactive DOPA (DL-3-(3,4-dihydroxyphenyl) alanine-2-^{14}C. After a suitable interval an extract was made and fractionated between water and organic solvent; the proportion of radioactivity in the organic solvent was taken as a measure of the metabolic change of the DOPA. Suitable controls were also carried out. In two further experiments radioactive morphine

was fed in similar circumstances; metabolic activity was measured by the proportion of radioactive non-morphine produced. The two controls used gave a value of 1·7% conversion against an average of 13·3% conversion in the DOPA experiments. The *relative* activities of the stem latex, taking the capsule latex as 1·0, were 2·1, 1·0, 1·0, 1·3, 3·5 and 0·2 in the DOPA experiments and 1·5 and 8·3 in the morphine experiments.

Translocation of morphine from pedicel to developing capsule

During the last 7 yr we have frequently fed radioactive morphine to the pedicel (stem) and found radioactive morphine and its metabolites in the capsule latex shortly afterwards. The highest amounts were 55 and 15·6% of the fed morphine[7] after 1 day. Later experiments (unpublished) gave figures varying from 8 to 15% of the fed morphine in the first 3 days. In a further experiment T-morphine was fed to the pedicel of some plants about 3 cm below the capsule base. After 1 hr detectable quantities of radioactivity (0·6% of fed radioactivity) were found in the capsule latex drawn from about 2 cm above the capsule base; after 3 and 5 hr 1·68 and 6·18% of the fed radioactivity was present. In another experiment T-morphine was fed to the stem about 29 cm below the capsule and significant quantities (1·86% of fed radioactivity) were present in the capsule latex after 4 hr.

These figures relate to the radioactivity in the expelled capsule latex only; our earlier work indicated that the metabolites were translocated from the latex to other parts of the capsule. To confirm this, radioactive morphine (^{14}C nuclear labelled) was fed to the pedicels below developing capsules and the radioactivity present in various parts determined a few days later. Three separate batches of plants were used during 1969 and 1970; the recovery of radioactivity in the various extracts was 33, 33 and 37% respectively. About 60, 33 and 36% of the recovered radioactivity was found in the capsule and the distribution of this radioactivity in the capsule tissues is shown in Table 2. In the 1969 experiments (No. 1) the total radioactivity in all the extracts (stem and capsule) was $72{\cdot}83 \times 10^3$ dpm with only $14{\cdot}06 \times 10^3$ dpm in the morphine, thus indicating that about 80% of the morphine had been metabolized.

TABLE 2. DISTRIBUTION OF RADIOACTIVITY IN THE CAPSULE TISSUES AFTER FEEDING ^{14}C-NUCLEAR LABELLED MORPHINE TO THE STEM *ca* 5 cm BELOW THE CAPSULE

Sample	Radioactivity in the capsule			
	Absolute (dpm $\times 10^{-3}$)	Latex (%)	Bled pericarp (%)	Ovules (%)
(1) 35 plants (1969)*	42·07	17	63	20
(2) 16 plants (1970)	10·24	15	54	31
(3) 18 plants (1970)	11·44	23	56	21

* In this experiment about 80% of the recovered activity was non-morphine substances.

Biosynthetic and metabolic activity of the latex and vesicles

To confirm that the above changes take place in the latex and vesicles *in vitro* studies were next carried out. Biosynthetic activity was tested for by feeding ^{14}C-DOPA to the isolated latex and the 1000 *g* pellet (which on one occasion was washed three times

[7] FAIRBAIRN, J. W. and EL-MASRY, S. (1967) *Phytochemistry* **6**, 499.

with buffer to remove traces of supernatant). The pellet was usually incubated with additional co-factors but this was not done with the whole latex. Metabolic activity was tested for by incubating with ^{14}C-nuclear labelled morphine and determining the amount of conversion to radioactive non-morphine substances. The results are shown in Table 3.

TABLE 3. METABOLIC ACTIVITY *in vitro* OF LATEX AND VESICLES (PELLET) AS MEASURED BY CONVERSION OF (a) ^{14}C-DOPA TO RADIOACTIVE MORPHINE AND (b) ^{14}C-MORPHINE TO NON-MORPHINE SUBSTANCES. INCUBATION TIME ABOUT 18 hr IN ALL SAMPLES EXCEPT No. 11 WHICH WAS 4 hr

Sample	Stage when collected Wo = petal fall	Metabolic activity		
(a) *^{14}C-DOPA fed*		Sp. act. of the morphine (dpm/mM × 10^{-2})		
1 Pellet from capsule latex (washed three times)	Wo + 10 days	323		
2 Pellet from stem/capsule	Wo + 10 days	117		
3 Pellet from stem/capsule	Wo + 10 days	23		
4 Pellet from stem/capsule (no co-factors)	Wo + 10 days	34		
5 Control (killed pellet)	Wo + 10 days	34		
6 Whole latex, stem	Wo + 3 days	678		
7 Whole latex, stem	Wo + 8 days	886		
(b) *^{14}C-Morphine fed*		Radioactive non-morphine substances (% of fed radioactivity)		
		(i) $CHCl_3$/IPA soluble	(ii) Water soluble	(iii) Total
8 Stem pellet	Wo + 18 days	22·5	16·7	39·2
9 Stem pellet	Wo + 18 days	26·2	7·9	34·1
10 Stem latex	Wo + 18 days	15·3	12·0	27·3
11 Stem latex	Wo + 2 days	5·4	11·5	16·9

Increase in volume of pericarp during development

The capsules increase rapidly in size after petal fall (Wo) reaching a maximum at Wo + 2 weeks;[8] an attempt was made to quantify these observations by measuring the diameter of the capsule and the thickness of the pericarp (capsule wall) at various stages of development. The average of several determinations showed that at Wo the rad. was 6·75 mm and the pericarp thickness 0·75 mm. Eight days later the values were 16·0 and 1·23 mm respectively, and at Wo + 14 days the figures were 16·6 mm and 1·30 mm respectively. Assuming the capsules are spheres these figures correspond to an increase in vol. of the pericarp (which contains the bulk of the laticifers)[8] from 366 to 3424 mm^3 in 8 days and to 3968 mm^3 after 14 days.

Morphine content of successive drops of latex exuded from the severed stem

Severed stems continue to exude latex for some time after cutting. To check whether this was due to upward flow of "whole" latex or merely a flow of latex significantly

[8] FAIRBAIRN, J. W. and KAPOOR, L. D. (1960) *Planta Med.* **8**, 49.

diluted with cell sap from surrounding tissues, successive exudations were analysed for morphine content and the results are shown in Table 4.

TABLE 4. ANALYSES OF SUCCESSIVE COLLECTIONS OF LATEX OOZING FROM THE CUT END OF THE PEDICEL

Time Collection	Wt of latex (mg)	Alkaloidal content (%)		
		Morphine	Codeine	Thebaine
1971				
Wo + 6 days				
t_0	600	6·5	2·3	1·9
t_0 + 20 min	478	7·3	2·4	1·4
1972				
Wo + 10 days				
t_0	124	4·54	n.d.	n.d.
t_0 + 5 min	198	4·10	n.d.	n.d.
t_0 + 15 min	178	4·00	n.d.	n.d.
t_0 + 35 min	258	4·46	n.d.	n.d.

Wo = petal fall, n.d. = not done.

DISCUSSION

Storage of the alkaloids

The results in Table 1 show that 95–99% of the alkaloids were found in the vesicle fraction after centrifugation. Although the vesicles are fairly robust, as even after three washings most of them seem to be intact,[5] obviously some must have been damaged during manipulation and these would release their alkaloids. This may well account for the 1–5% of alkaloids found in the supernatant, so that in the intact latex all the alkaloids must be stored in the vesicles. The plasmolysis experiment further shows that they are stored in the vacuolar sap rather than being membrane bound. The alkaloids in *Chelidonium majus* latex seem also to be stored in the vacuolar sap of the vesicles, though only 70% were found in the vesicle fraction after centrifugation, possibly due to the greater fragility of the latter.[9]

Addition of about 10% exogenous morphine (radioactive) to capsule latex led to rapid absorption into the vesicles (Table 1). However with the stem latex about 30% of the total radioactivity (9167 dpm) still remained in the supernatant, which also contained a significant amount of morphine (15·94 mg). The specific activity of the added morphine was 4580 dpm/mg so that the supernatant must have contained 2 mg of hot morphine and 13·94 mg of cold morphine. Since normally not more than 5% of the pellet morphine (in this instance 71·87 mg) passes into the supernatant during manipulation, the maximum cold morphine that should have been present is 3·59 mg. The difference (10·35 mg) therefore represents an unusual leakage of morphine from the vesicle and this may be due to "a possible interference between the vacuolar membrane and free (exogenous) alkaloids" as is thought to take place in *Chelidonium* latex.[9] However this leakage did not take place with the capsule latex and may therefore represent another difference between stem and capsule vesicles (see later). Perhaps the latter contains maturer vesicles with more robust walls.

[9] MATILE, PH., JANS, B. and RICKENBACHER, R. (1970) *Biochem. Physiol. Pflanzen.* **161**, 447.

At least one function of the vesicles therefore seems to be absorption and storage of alkaloids, and in this sense they behave as normal vacuoles.

Translocation of the alkaloids

The ^{14}C-morphine experiments confirm our long experience that morphine is translocated from the stem to the developing capsule. In the T-morphine experiment described detectable quantities had travelled 5 cm in 1 hr and significant quantities had travelled through about 30 cm of stem in 4 hr. Since the alkaloids are almost exclusively contained in the vesicles it is possible that the latter themselves move up into the capsule carrying the alkaloids with them. This could be partly effected by an *in toto* movement of latex from stem to capsule and some of our observations seem to confirm that this is possible.* During the first 8 days of capsule development the volume of the pericarp increased about tenfold, at the time when the density of the laticifers in the pericarp is also increasing.[8] This rapid increase in capacity for latex may therefore be satisfied mainly by upward flow from the stem. In good growing conditions this upward flow can be seen when the stem is severed; even after removal of the first and successive accumulating drops on top of the cut stem, others form rapidly, sometimes for a period of 1 hr or more. That this continuing flow is not due to transfer of water from the surrounding phloem into the severed laticifers is indicated by the results shown in Table 3. During the 35 min after cutting off the top of the stem, latex continued to flow regularly without any dilution of the morphine content. Thus the vesicles may function not only for absorption and storage of alkaloids, but also partly for translocation; however our *in vitro* studies indicate that, in addition, they may play a more active role.

Biosynthesis and metabolism of the morphine

The results in Table 3 confirm our previous ones[6] that the pellet can biosynthesize DOPA to morphine *in vitro* even, as in sample 1, after washing three times to remove traces of supernatant. However this biosynthetic activity varies considerably and in samples 3 and 4 is absent whether co-factors are present or not. Böhm *et al.*[4] record similar fluctuations in the ability of different samples of latex to convert tyrosine to morphine.

The difference between stem and capsule latex also shows inconsistencies. The results for the relative activity of the stem latex (capsule latex = 1·0) show that in either experiments the stem latex was significantly more active than the capsule (1·3–8·3 times) on five occasions, thus confirming an earlier observation[6] (stem 2·3). However on two occasions there was no difference in activity and in the remaining experiment the stem latex was significantly less active (0·2). These fluctuations may be due to unusual upward surges of active stem latex into the capsule possibly occasioned by changes in climatic conditions. Such changes would also explain the variable *in vitro* biosynthetic activity of different samples of expelled latex. Electron microscopical studies of the pellet and young laticifers[5] indicate that very young vesicles have smooth walls but later develop two to three electron-dense caps, some of which show distinct zonation. It may be that only in the latter does biosynthesis and metabolism of the alkaloids take place and that the varying activity of latex samples depends on the proportion of these zonated vesicles present.

* D. VAGUJFALVI (*Bot. Közlem* 1970, **57**, 113) came to a similar conclusion based on results of analyses of latex at different levels of the stem.

Significant metabolism of morphine takes place even *in vitro* (17–40% conversion, Table 3) and more so *in vivo*. Results from Experiment 1 (Table 2) showed that 80% of the recovered radioactivity were non-morphine substances. This confirms our previous work[7] and the more recent work of Miller *et al.*[10] who found about 50% non-morphine radioactivity after feeding radioactive morphine to the plant. Our previous work also indicated that most of the metabolites were translocated out of the latex into the pericarp and ovules. This is confirmed by the results of the three experiments in Table 2. Of the total radioactivity of $63{\cdot}75 \times 10^3$ dpm found in the capsules only 18% occurred in the latex; 60% occurred in the bled pericarp and 22% in the ovules (in which no alkaloids were detectable). A further function of the vesicles seems therefore to be the biosynthesis and metabolism of the alkaloids.

To summarize, the alkaloids are synthesized and stored in the alkaloidal vesicles and translocated to the rapidly developing capsule partly by an *in toto* upward movement of the latex. During this time biosynthesis may continue and further metabolism of the morphine occurs. The morphine metabolites, unlike the alkaloids, are translocated out of the capsule latex with quite a significant proportion appearing in the ovules.

EXPERIMENTAL

In vitro work. Halle or Hungarian varieties of poppy[11] were used and the latex drawn and centrifuged as already described;[6] experience showed that spinning at 1000 *g* × 1 hr was sufficient to deposit all the vesicles. Radioactive DOPA (DL-3(3,4-dihydroxyphenyl)alanine-2-^{14}C) was incorporated as previously described; nuclear radioactive morphine was prepared from plants[12] previously fed with generally labelled L-tyrosine ^{14}C and purified to constant radioactivity. It was dissolved in EtOH and suitable aliquots added to glass tubes and the solvent evaporated under N_2. Whole latex or 0·5 M mannitol suspensions (pH 7) of the pellet were added directly to the dry morphine in the tubes and allowed to incubate for several hr, sometimes with suitable co-factors.[6]

In vivo work. Radioactive morphine (^{14}C already described or 2-^{3}H-morphine) was fed by the cup method[12] to the pedicels of plants *ca* 1–2 weeks after petal fall and after a suitable interval the capsules were cut off and the latex exuding from the severed capsules and stems collected separately. Where necessary the bled stems were collected from the severed end to a point just below the feeding cup. The bled capsules were also collected and the ovules separated. All materials were extracted with methanol and the radioactivity of the extracts determined using a scintillation counter. In experiment No. 1 (Table 2) each extract, in addition, was fractionated to separate the H_2O soluble (nonalkaloids) substances from the $CHCl_3$–isopropanol soluble substances (alkaloids). The latter were further fractionated to separate phenolic alkaloids (unchanged morphine) from the non-phenolic alkaloids and the radioactivity of each fraction determined; by this means the total non-morphine substances were determined (see Table 3). For the successive collections (Table 4) 10 capsules were severed simultaneously and the latex flowing from the top of the cut pedicel collected immediately. Further latex continued to ooze out and samples were collected at the intervals stated.

Analytical methods. The morphine and other alkaloids were determined, when necessary, by a suitable adaptation of the quantitative TLC method.[13] For specific activity determinations morphine was crystallized to constant activity, occasionally after dilution with cold morphine.

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