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ALKALOID BIOSYNTHESIS AND METABOLISM IN AN ORGANELLE FRACTION IN *PAPAVER SOMNIFERUM**

J. W. FAIRBAIRN and M. DJOTÉ

The School of Pharmacy, University of London, 29/39 Brunswick Square, London, W.C.1

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Abstract—The pellet produced by centrifuging suitably diluted latex at 1000–3000 *g* has been shown capable of synthesizing morphine from dihydroxyphenylalanine *in vitro* and preliminary studies indicate that a lutoid-like organelle is involved in this synthesis. Evidence is also produced to show that the further metabolism of morphine (described earlier[3]) takes place in the stem latex and its 3000 *g* fraction rather than in the capsule latex.

INTRODUCTION

OUR EARLIER work has shown that the isolated latex from unripe capsules of *Papaver somniferum* L. can synthesize alkaloids *in vitro*[1] and indicated that this activity may be associated with a heavy fraction obtained by centrifuging coagulated latex.[2] Improved techniques have now enabled us to separate a heavy fraction from non-coagulated latex and this has been used to reinvestigate the possibility that a special organelle is associated with the biosynthesis of alkaloids in this plant. Previous work has also shown that morphine is rapidly metabolized in the living plant into a series of new compounds[3] and a further object of this present work was to study whether this is also effected in the same organelle fraction.

RESULTS

Latex Fractions Fed with Radioactive DOPA

In the first two experiments 2–3 g of latex was mixed with mannitol buffer to about 6 ml and pellets were collected after spinning for 10 min each at 1000 *g*, 3000 *g* and 11,000 *g*. The three pellets and the final supernatant were incubated for 6–7 hr with a radioactive mix containing 2-^{14}C-DOPA (DL-3-(3,4-dihydroxyphenyl)alanine-2-^{14}C) and co-factors, then acidified and brought to the boil to stop further enzyme action. After filtration and alkalinization each fraction was extracted with organic solvent, and examined. Those from the 1000 *g* and 3000 *g* pellets were significantly radioactive and contained abundant alkaloid; that from the 11,000 *g* pellet contained no alkaloids nor radioactivity. The organic solvent extract of the supernatant contained no alkaloids but had a small amount of radioactivity. Controls, in which the latex was killed by boiling in acid before adding the radioactive precursor, had no radioactivity in the organic solvent extract of any of the three pellets. As there seemed to be no difference between the 1000 *g* and 3000 *g* pellet a third experiment was performed in which a 3000 *g* pellet alone was prepared and the crude alkaloids extracted as previously. The crude

* Part IX in the series "The Alkaloids of *Papaver somniferum* L."; for Part VIII, see Ref. 2.

[1] J. W. FAIRBAIRN, M. DJOTÉ and A. PATERSON, *Phytochem.* 7, 2111 (1968).
[2] J. W. FAIRBAIRN, J. M. PALMER and A. PATERSON, *Phytochem.* 7, 2117 (1968).
[3] J. W. FAIRBAIRN and S. EL MASRY, *Phytochem.* 6, 499 (1967).

alkaloidal fraction from the three experiments were combined and the morphine separated, recrystallized and its specific activity, as well as that of the picrate and diacetylmorphine formed from it, were determined. The latter was diluted with an equal weight of non-radioactive diacetylmorphine and the mixture recrystallized from ethanol and the specific activity of the diluted diacetylmorphine determined. The results are given in Table 1.

TABLE 1. RADIOACTIVITIES OF MORPHINE AND ITS DERIVATIVES FORMED BY INCUBATING DL-β-(3,4-DIHYDROXYPHENYL)ALANINE-α-^{14}C (DOPA) WITH A 3000 *g* PELLET OF POPPY LATEX

	M.p.	λ_{max} and λ_{min} (in MeOH) (nm)	Radioactivity (dpm/mM × 10^{-2})
Morphine	247–249° (decomp.)	max. 287 min. 264	193
Morphine, picrate	161–162°	max. 213, 292 (minor)* min. 278, 300	195
Diacetylmorphine (two batches)	170°	max. 214, 283 min. 253	(a) 153 (b) 162
Diluted diacetylmorphine (dilution × ½)		max. 214, 283 min. 253	82·4

* Wrong figures given in previous paper 1.

Several further batches of latex were diluted with buffer and centrifuged and the supernatant examined. In no instance were alkaloids detected except when the organelles were deliberately plasmolysed by dilution with water or by autolysis after standing at room temperature.

Morphine Metabolism in Stem and Capsule Latex

In most of our previous experiments the capsules had been cut off at their base and the latex exuding from the base of the capsule and from the top of the severed stem (pedicle) had been used. However, some preliminary experiments showed that the proportion of morphine to other alkaloids was significantly less in the stem latex than in the capsule latex. Accordingly samples of stem and capsule latex were collected separately from the same plants and incubated with T-morphine for 24 hr. The water-soluble radioactive products were then alkalinized and fractionated between organic solvent (alkaloids) and aqueous layers (non-alkaloids). Suitable controls using latex which had been killed with NHCl were also prepared. The radioactivities of the various fractions are shown in Table 2. Autoradiographs from each aqueous fraction were prepared and only in that from the stem latex was there distinct evidence of a discrete radioactive spot, and this had a low R_f. In the capsule and control samples no discrete spots were visible.

In a further experiment, 3000 *g* pellets were prepared separately from stem and capsule latex and incubated with T-morphine. Once more a significantly higher amount of radioactivity occurred in the aqueous fraction from the stem sample (7·8 per cent) than from the capsule latex (4·1 per cent). Autoradiography showed that a discrete spot (of low R_f) only occurred in the stem latex sample.

TABLE 2. DISTRIBUTION OF RADIOACTIVITY (cpm) BETWEEN ORGANIC SOLVENT SOLUBLE AND WATER-SOLUBLE COMPOUNDS AFTER FEEDING T-MORPHINE TO STEM AND CAPSULE LATEX *in vitro*

	Stem	Capsule	Control
Organic solvent fraction	$7{\cdot}1 \times 10^4$ (63%)	$7{\cdot}2 \times 10^4$ (84%)	$9{\cdot}4 \times 10^4$ (85%)
Aqueous fraction	$4{\cdot}1 \times 10^4$ (37%)	$1{\cdot}4 \times 10^4$ (16%)	$1{\cdot}6 \times 10^4$ (15%)

Approximately $11{\cdot}7 \times 10^4$ cpm fed to each sample. Percentages (in brackets) represent proportion of total activity of the acid extract after incubation.

DISCUSSION

The most important result of this work is the demonstration that a heavy fraction of the latex is capable of synthesizing morphine from DOPA *in vitro*. The variety of poppy used and the experimental conditions were approximately similar to those used in our work on whole latex[1] and it is interesting to note that the specific activity of the morphine is similar in both sets of experiments. (Table 2 of Ref. 1 and Table 1 of this paper.) This strongly suggests that all the enzymes necessary are present in the heavy fraction, and that no significant factors are contained in the supernatant. In other words, the biosynthesis may be carried out entirely in one or more organelles. This is partly confirmed by a preliminary electron microscopical study of the heavy fraction by Dr. P. B. Dickenson (Natural Rubber Producers Research Association) which shows that the fraction consists mainly of thin-walled organelles resembling the lutoids of *Hevea brasiliensis* latex. If further work confirms that these organelles are the site of synthesis, this will be the first demonstration that the complicated synthesis of such an alkaloid as morphine takes place in a specialized organelle. Furthermore, since alkaloids were never found in the supernatant it can be assumed that all the opium alkaloids are produced and retained in the organelles of the heavy fraction. After deliberate plasmolysis, however, the alkaloids are readily released into the supernatant, suggesting they are not firmly attached to membranes but stored within the lutoid-like organelles. It is known that the lutoids of *Hevea*, being single membrane organelles, are very sensitive to osmotic changes.

One of our major interests is the subsequent metabolism of morphine after its formation and our work with T-morphine indicates that this also takes place in the heavy fraction, but mainly in that of the "stem" (which would include latex moving up from the leaves) rather than that of the capsule. In all our experiments with T-morphine it appears that some of the tritium is transferred to water or water-soluble compounds. However, comparison with suitable controls (e.g. Table 2) shows that a much higher proportion of tritium occurs in the water-soluble fractions after incubation with stem latex, or its heavy fractions, than with those of the capsule. That this is due to increased productions of water-soluble morphine metabolites is confirmed by autoradiography. Further confirmation comes from some preliminary enzyme studies. A crude enzyme preparation which used morphine as a substrate has been isolated from stem latex and seedlings but not from the capsule latex.

EXPERIMENTAL

Centrifugation and Incubation

Latex was drawn from spring-sown plants (Halle variety[4]) 1–2 weeks after petal fall by decapitating the capsules and pipetting the expelled latex from the cut ends of the capsule or stem directly into centrifuge tubes (nylon, 8 ml capacity) kept in ice. About 1 hr later the latex in each tube was carefully diluted with about three times its volume of cold mannitol buffer (mannitol, 0·4 M; phosphate buffer pH 7·0; total molarity about 0·5 M). The diluted latex was spun at 1000 *g* for 10 min in a refrigerated centrifuge (0–4°), the supernatant removed and respun at 3000 *g* for 15 min. The supernatant was again removed and spun at 11,000 *g* for 15 min. Each of the three pellets and the final supernatant were separately incubated at room temperature for 6–7 hr with 1 ml of the following radioactive "mix" (sucrose 0·25 M, $MgCl_2$ 0·5 mM, ATP 5 mM, co-enzyme A 0·5 mM, NAD 0·5 mM, glutathione 0·5 mM, mannitol 0·12 M, DL-β-(3,4-dihydroxyphenylalanine)α^{14}C (DOPA), all in phosphate buffer at pH 7·0. Molarity 0·5 M approximately. Radioactivity $4{\cdot}27 \times 10^6$ dpm/ml.)

Extraction and Radioactivity of the Morphine

After incubation, 2 ml N HCl was added to each tube and the contents heated to boiling point to coagulate the latex, and then cooled and filtered. The filtrate was washed with water, the combined filtrates made alkaline with excess N NaOH and the non-phenolic alkaloids removed by shaking with $CHCl_3$. The aqueous layer was brought to pH 8–8·5 and the phenolic alkaloids extracted with several portions of $CHCl_3$/isopropanol (3:1). The combined $CHCl_3$/isopropanol layers were dried over Na_2SO_4 and evaporated to dryness *in vacuo*. The residue was dissolved in 0·1 N HCl and dilute ammonia solution cautiously added until turbidity. After standing in the refrigerator overnight the precipitated alkaloids were filtered and the residue washed with ether to remove phenolic alkaloids other than morphine. The morphine was recrystallized, its picrate and its diacetyl derivative prepared and constants determined as previously described.[1]

Radioactivities were determined in a scintillation counter (Packard Tricarb Model 3003) or, for the picrate, in the Nuclear Chicago Gas Flow Counter, Model 1105. Autoradiographs were prepared by exposing thin-layer chromatograms for 10–15 min in a Spark Chamber (Pullan[5]) fitted with a polaroid camera.

[4] J. W. FAIRBAIRN and S. EL MASRY, *Phytochem.* 7, 181 (1968).

[5] B. R. PULLAN, in *Quantitative Paper and Third-Layer Chromatography* (edited by E. J. SHELLARD), Academic Press, New York (1968).