Phytochemistry, 1967, Vol. 6, pp. 499 to 504. Pergamon Press Ltd. Printed in England

THE ALKALOIDS OF *PAPAVER SOMNIFERUM* L.—V*.

FATE OF THE "END-PRODUCT" ALKALOID MORPHINE

J. W. FAIRBAIRN and S. EL-MASRY

School of Pharmacy, Brunswick Square, London, W.C.1

(*Received* 27 *September* 1966)

Abstract—Samples of morphine-U-^{14}C were fed to the phloem region of the pedicel just below developing capsules of *Papaver somniferum* L. Examination of the capsule latex at intervals after feeding showed that there had been a rapid absorption of the morphine into the latex which was in marked contrast to the rate for tyrosine. The morphine was rapidly metabolized in the latex to form two non-alkaloidal polar substances and the bulk of these were rapidly translocated out of the latex. They, and possibly other related derivatives, appeared in the pericarp and ovules and at later stages seemed to form methanol-insoluble substances or were metabolized as $^{14}CO_2$. Some of the fed morphine was localized at the site of injection for a time and was transformed into other Dragendorff positive substances, amino acids and sugars. These may be breakdown products produced by extra-laticiferous enzymes and therefore do not represent the normal metabolism of morphine.

INTRODUCTION

MOST workers investigating the biogenesis of alkaloids feel they have completed their task when the stages involved in the production of the major alkaloids in a plant are completely elucidated. The tacit assumption is made that these molecules represent the end-products of interesting metabolic processes and that they slowly accumulate during active growth and gradually disappear during senescence. However, recent work has shown that surprisingly rapid changes in the pattern of the major alkaloids of *Conium maculatum*[1] and *P. somniferum*[2] take place during active growth; furthermore the major alkaloids disappear in significant quantities at quite short time intervals, suggesting that they themselves are intermediaries in other, possibly important, metabolic processes. The rapid synthesis and disappearance of the alkaloids also explains the results reported by Stermitz and Rapoport[3] who found an otherwise inexplicable loss of morphine after feeding large quantities of thebaine to poppy plants. Work by Tso and Jeffrey[4,5] on *Nicotiana* plants also led them to suggest that tobacco alkaloids are in a dynamic state and do not represent mere "end-product" substances. They showed that when ^{14}C and ^{15}N labelled nicotine was fed to tobacco plants radioactivity was transferred to amino acids, sugars, pigments, organic acids and other plant metabolites. Transformations of one tobacco alkaloid to another also occurred. In order therefore to follow the "post-morphine" part of the story of poppy alkaloids we fed radioactive morphine to growing plants and attempted to study its subsequent fate. A preliminary report on work done in 1963–4 has already been published[6] and we now submit further experimental results based on later work.

* Part IV—Reference 6.

[1] J. W. FAIRBAIRN and P. N. SUWAL, *Phytochem.* **1**, 38 (1961).
[2] J. W. FAIRBAIRN, A. PATERSON and G. WASSEL, *Phytochem.* **3**, 577 (1964).
[3] F. R. STERMITZ and H. RAPOPORT, *J. Am. Chem. Soc.* **83**, 4045 (1961).
[4] T. C. TSO and R. N. JEFFREY, *Arch. Biochem. Biophys.* **80**, 46 (1959).
[5] T. C. TSO and R. N. JEFFREY, *Arch. Biochem. Biophys.* **92**, 253 (1961).
[6] J. W. FAIRBAIRN and A. PATERSON, *Nature* **210**, 1163 (1966).

RESULTS

1. *Entry of the Radioactive Morphine into the Capsule Latex*

Two batches of morphine-U-^{14}C were fed to two groups of poppy plants by the method already described.[2] This method enabled the alkaloidal solution to enter the phloem region of the pedicel just below the developing capsule. At stated intervals after feeding, the radioactivity of the latex from the capsule was determined and the results with one group of plants are compared in Fig. 1 with those obtained when radioactive tyrosine, of much higher specific activity, was fed to similar plants by the same method. Direct injection of tyrosine into the capsule was also tried but the proportion of radioactivity entering the latex was considerably less than when the first method was used. With the second group of plants no sample was drawn at 24 hr but one drawn at 26 hr showed that 15·6 per cent of the fed activity was present in the latex; all other samples exhibited low activity.

2. *Examination of the Radioactive Latex of the Capsule*

(*a*) *At peak radioactivity* (24 hr after feeding: see Fig. 1). From the first batch of capsules about 14 mg of latex was collected when radioactivity was at its highest (24 hr). The latex was immediately transferred to aqueous acid; rendered alkaline and the alkaloids removed by extraction into ethyl acetate. Only 10 per cent of the radioactivity (equivalent to $0{\cdot}29 \times 10^4$ d.p.m./100 mg latex) was found in the ethyl acetate layer and of this the bulk was due to morphine. The aqueous layer contained the remainder of the activity (equivalent to $2{\cdot}59 \times 10^4$ d.p.m./100 mg latex), of this activity 90 per cent was located in two spots near the starting point of a two-dimensional paper chromatographic system (phenol/water (4:1) and *n*-butanol/acetic acid/water (5:1:2)). In the same system the normal amino acids of poppy latex[7] as well as the alkaloids were in positions well removed from the starting point.

(*b*) *At low radioactivity.* Three collections of latex from both batches were made; (*a*) 30 mg collected before the peak activity (activity $0{\cdot}21 \times 10^4$ d.p.m./100 mg latex), (*b*) 83 mg collected up to 3 hr after the peak (activity $0{\cdot}16 \times 10^4$ d.p.m./100 mg latex), and (*c*) 89 mg collected 1 week after the peak ($0{\cdot}028 \times 10^4$ d.p.m./100 latex). The three collections were bulked and treated as already described for the "peak" latex. Radioactivity equivalent to $0{\cdot}06 \times 10^4$ d.p.m/100 mg latex was found in the morphine and a similar amount in the two spots from the aqueous layer already referred to.

3. *Distribution of Radioactive Substances Throughout the Plant*

Since the newly-formed radioactive substances seemed to pass out rapidly from the latex of the capsule (Fig. 1) we decided to examine all organs of the plant, (*a*) immediately after the disappearance of most of the radioactivity from the capsule latex and (*b*) a few weeks later. Methanol extracts of the organs were prepared and examined; 36 per cent of the fed activity was found in the combined extracts from the plants collected earlier but only 11 per cent in those collected later. The distribution of this methanol-soluble radioactivity in the various organs is shown in Table 1. Paper chromatographic examination of the various extracts gave the following results.

In extracts from the *pericarp* at the earlier stage, and from which most of the latex had been removed, the bulk of the radioactivity was in the water-soluble substances of low R_f value already referred to. At the later stage, however, only about 40 per cent of the water-soluble radioactivity was located in these low R_f substances; the remainder was distributed among amino acids and related substances. In the immature *seeds*, or ovules, small quantities

[7] A. JABBAR and E. BROCKMANN-HANSSEN, *J. Pharm. Sci.* **50**, 406 (1961).

of methanol-soluble, radioactive substances were present but none were found in the mature seeds collected at the later stage. However after vigorous hydrolysis of these mature seeds significant amounts of water-soluble substances were found (Table 1). The *stems* contained the highests proportion of the fed radioactivity but this was all localized in the region of the

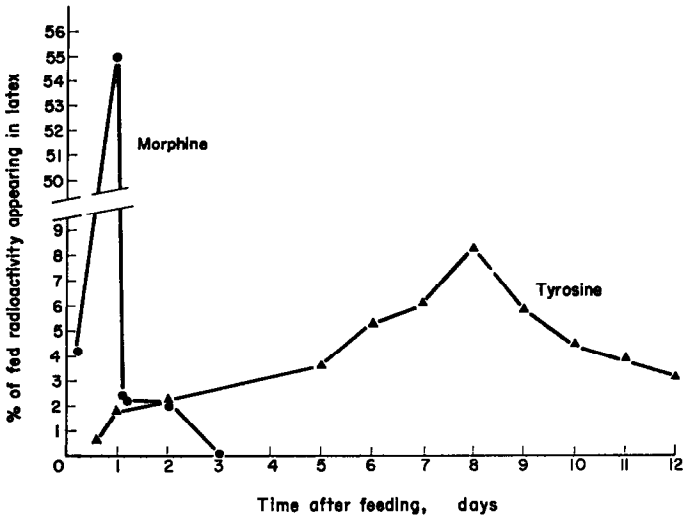

FIG. 1. RATES OF ABSORPTION OF MORPHINE AND TYROSINE FROM THE PHLOEM REGION OF THE PEDICEL INTO THE LATEX OF THE CAPSULE.

TABLE 1. DISTRIBUTION OF METHANOL-SOLUBLE, RADIOACTIVE SUBSTANCES IN THE PLANT AFTER FEEDING C-14 MORPHINE; EXPRESSED AS % OF TOTAL RADIOACTIVITY RECOVERED

Interval after feeding	Total radioactivity in the acid methanol extract (d.p.m.)	Capsule		Stem	Leaf	Root
27 hr	$1{\cdot}873 \times 10^{4}$*	(i) Exuded latex 12·4% (ii) Pericarp + residual latex 21·1% (iii) Ovules 2·8%	36·3%	54·4%	8·5%	0·8%
24 days	$1{\cdot}128 \times 10^{4}$†	(i) Pericarp + latex (none exuded) 58·5% (ii) Seeds 14·2%‡	72·7%	17·9%	2·7%	6·6%

* Represents 36% of the fed radioactivity.
† Represents 11% of the fed radioactivity.
‡ After hydrolysis of the extract in 2 N HCl at 98° for 1 hr.

injection site: about a quarter of this localized activity was due to unchanged morphine and other Dragendorff positive compounds soluble in ethyl acetate; the remainder was distributed among amino acids, sugars and related compounds with only a small amount in the low R_f substances already referred to. Extracts from the *leaf* and *root* were not examined as they were of very low activity.

DISCUSSION

The history of the radioactive morphine as it passes from the point of entry in the pedicel to the seeds shows some remarkable features.

The *absorption* into the phloem region of the pedicel and transfer to the laticifers of the capsule is much more rapid than for tyrosine (Fig. 1), even though the latter is a smaller molecule and, because of the higher radioactivity of the material we had, the weight of tyrosine fed was only about $\frac{1}{80}$ of that of the morphine. The most obvious explanation is that competition for the metabolically important tyrosine from other tissues of the plant allows only a small proportion to enter the laticifers. In contrast, morphine has little metabolic "interest" outside the latex and so the bulk passes rapidly into the laticifers. In the second series the proportion of *fed* activity entering the capsule latex was considerably less than in the first series shown in Fig. 1. This may be partly due to the fact that we had missed the "peak" because samples were drawn at 26 hr and not 24 hr after feeding, but variation in absorption of the solution from the feeding cups due to variations in sunlight, temperature, wind etc. is also an important factor. In the first series, at time 24 hr, 93 per cent of the morphine had disappeared from the feeding cups whereas in the second series, at time 26 hr, only 53 per cent had disappeared. The proportion of radioactivity in the capsule latex, based on the amount actually *entering* the plant, for the first series at time 24 hr was therefore 59 per cent and for the second series at time 26 hr 30 per cent.

An even more striking fact is the rapid *transformation* of the morphine into non-alkaloidal substances once it enters the capsule latex. This would account for the rapid "disappearance" of morphine already reported in our earlier work[2] and clearly indicates that morphine is not an end-product. The newly formed compounds are neither amino acids nor alkaloids but are probably fairly polar substances judging from their position on the chromatograms. That the transformation takes place within the latex itself is clearly indicated by the fact that at peak activity 90 per cent of the radioactivity of the latex is in the form of non-morphine substances.

A third interesting feature is the rapid *translocation* of the newly formed substances out of the latex of the capsule (Fig. 1). That this is not due to translocation of latex *in toto* from the capsule to other parts of the plant is indicated by the results given in Table 1. In the first series the radioactivity retained in the pericarp and ovules is considerably more than that found in the exuded latex obtained by exhaustive tapping of the capsule wall. (Estimation of the alkaloids left in the exhausted capsule showed that the residual latex was much less than the exuded.) In the second series no latex could be obtained by incision as the capsules were brown and dry, yet a high proportion of the recovered activity was present in this almost latex free pericarp. The fact that very little of the activity appears in the leaves or roots (Table 1) also confirms that there is practically no downward passage of latex.

The results for the *stem* are interesting as more than half of the recovered activity in the first series (Table 1) was present in this region. The ethyl acetate soluble fraction contained radioactivity not only in the morphine but in one or two other Dragendorff positive spots which may represent breakdown products of morphine. The water-soluble fraction contained radioactivity in substances quite different from those in the capsule latex, such as amino acids and sugars. They may represent breakdown products of morphine produced by extra-laticiferous enzymes and therefore do not represent the normal metabolism of morphine. The results for the second series indicate that these abnormal substances are translocated and may explain the appearance of radioactive amino acids etc. in the pericarp at the later stage of development.

As the plants developed, the amount of radioactive substance soluble in methanol fell rapidly (Table 1, footnote). This may be due to ultimate breakdown to CO_2 or to the formation of methanol-insoluble substances. Previous work[6] indicated that the latter may take place in the seeds and we found that the ripe seeds (second series, Table 1) contained no methanol-soluble radioactive substance but a significant proportion after acid hydrolysis.

A tentative picture of the situation therefore is that morphine soon after its formation in the latex, is converted into more polar substances. These are rapidly translocated from the laticifers to other tissues of the pericarp and some reach the developing ovules (incidentally no laticifers pass into the ovules[8]). Later, these newly formed substances are converted into less soluble substances which may play an important part in the further development of the plant.

MATERIALS AND METHODS

Papaver somniferum L. sub. sp. *eurasiaticum* var. *glaucum* Rothm. plants were used throughout.

Radioactive morphine. Capsules from autumn-sown plants were fed with generally labelled L-tyrosine ^{14}C, by the method already described.[2] 1 week after petal fall (week 1). Batches of latex were drawn from the capsules 6, 7, and 8 days later, immediately transferred to 0·1 N HCl and filtered. The filtrate was made alkaline (pH 7–8), extracted with ethyl acetate and the latter evaporated to dryness. The morphine was separated from the residue as calcium morphinate and purified.[9] It was re-crystallized as the free base several times and its purity established by m. pt., R_f values in several chromatographic systems and by radioisotope dilution. The specific activity was $4{\cdot}98 \times 10^6$ d.p.m./mM.

Examination of the latex. The alkaloidal solution (in water, pH 5–6) was fed to the scraped pedicel by the method already described.[2] Samples of latex were drawn from the capsule, at stated intervals, by pricking and transferring the exuded drops to small tared stoppered flasks containing acid methanol (methanol 70, 0·3 N HCl 30). After filtering, the filtrate was diluted with water, made alkaline (pH 7–8) and extracted with ethyl acetate.

The *ethyl acetate layer* (containing the alkaloids) was evaporated to dryness, the residue dissolved in methanol and chromatographed on paper using (*a*) butanol/acetic acid/water (5:1:2) where the R_f of morphine is about 0·45 and (*b*) phenol/water (4:1) where the R_f of morphine is 0·9. After location the morphine spots were cut out and their radioactivity compared with that of the original ethyl acetate layer, using an end-window counter (Nuclear Chicago Gas Flow Counter). In both systems 95 per cent of the radioactivity was present in the morphine spots.

The *aqueous layer* was concentrated to small volume and suitable aliquots were subjected to two-dimensional chromatography using phenol/water (4:1) followed by butanol/acetic acid/water (5:1:2). Small areas were cut from the paper and examined for radioactivity by the method already referred to. One area (3 cm diameter) at the starting point contained 65 per cent and another slightly above contained a further 25 per cent of the activity in the original aqueous layer. Control chromatograms of the alkaloids and commonly occurring amino acids of the poppy latex showed that none of these were in the position of the above two spots.

[8] J. W. FAIRBAIRN and L. D. KAPOOR, *Planta Med.* **8**, 49 (1960).
[9] *International Pharmacopoeia*, Vol. 1, 1st ed., p. 162 (1951).

Whole Plant

At suitable intervals after feeding a whole plant was dug up and divided into seeds, pericarp, stems, leaves and roots, and each part macerated separately with methanol for several days. The filtrates were concentrated, fractionated, chromatographed and the radioactivity of the alkaloids, amino acids and sugars determined as already described for the latex. The extracts of the seeds collected 2½ weeks after feeding contained no radioactivity; consequently a fresh batch was heated in 2 N HCl in a boiling water bath for 1 hr, and filtered. The filtrate was neutralized and its radioactivity determined.

Acknowledgements—We would like to thank Mr. L. Guglielmi for valuable assistance in the cultivation of the poppy plants. This work forms part of a thesis to be presented by one of us (S. El-M.) for the award of a Ph.D. degree of the University of London.