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

"BOUND" MORPHINE AND SEED DEVELOPMENT

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Abstract—Vigorous acid hydrolysis or pepsin digestion of ground poppy seeds led to the production of alkaloid-like substances including codeine. Fermentation of ground seeds or germination of whole seeds for 1 to 2 days also led to the production of similar compounds as well as papaverine. Radioactive morphine was fed to the latex of the capsule and the seeds collected later were germinated in similar conditions and the alkaloid-like substances shown to be radioactive. Furthermore the ovules were shown to contain radioactive alkaloid-like substances (without degradative treatment) as well as radioactive non-alkaloidal compounds similar to those already reported.[1] It is suggested that morphine is rapidly metabolised in the latex into a series of compounds some of which are alkaloid-like and others non-alkaloidal, ethanol insoluble, "bound" forms. The bound forms are stored in the seeds and broken down into smaller alkaloid-like substances during germination. An attempt was made to produce seeds deficient in these compounds by depriving the capsule of much of its latex at a critical stage in development. The seeds produced showed remarkable differences from controls; their germination rate and root hair and chlorophyll production were significantly reduced, thus indicating that the latex contents are essential to the production of viable seeds. The possible pharmacological effect of bound forms of alkaloids in poppy seeds is also discussed.

INTRODUCTION

IN A previous paper[1] we showed that radioactive morphine is rapidly metabolized in the latex into non-alkaloidal substances which are then translocated out of the latex. Some of these appear in the developing ovules and seeds and there is some evidence[1,2] that, as the seeds mature, the methanol soluble compounds decrease and more complex methanol insoluble substances accumulate. The two water soluble compounds of low R_f value reported[1] are probably of this latter type and for convenience we shall refer to them as "bound" forms of morphine. If such compounds are transferred from the latex to the seeds and stored there it is possible they will be used later during germination. The present paper is therefore concerned with establishing that such bound forms do occur in the seeds, investigating whether they are related to the morphine of the latex and if so, whether their presence is of significance to seed activity.

RESULTS

1. *Investigation of the "Bound" Alkaloids of the Seeds*

As already stated we assumed the bound forms would be complexes and therefore our first experiments were designed to break up such substances into smaller compounds soluble in non-polar solvents.

(*a*) ***Berlin seed.*** Samples of ripe seed from the 1966 Berlin poppy crop were subjected to

[1] J. W. FAIRBAIRN and S. EL-MASRY, *Phytochem.* **6**, 499–504 (1967).
[2] J. W. FAIRBAIRN and A. PATERSON, *Nature* **210**, 1163 (1966).

the following treatments: (*a*) vigorous acid hydrolysis, (*b*) acid-pepsin digestion at 37°, and (*c*) fermentation at 22°. Each sample was coarsely powdered and the first two exhausted with ethanol before treatment in order to remove possible traces of "free" alkaloids or alkaloid-like substances (see Discussion). After treatment the seeds were once more extracted with ethanol and the extract tested for the presence of alkaloid-like substances. In all samples one phenolic substance (M_1) and two non-phenolic substances (M_2 and M_3) were detected, and all gave positive colours with the well known alkaloid reagents, Dragendorff's (orange-red) and potassium iodoplatinate (blue-black). The substances were isolated in small quantity by band chromatography and their R_f values in four systems and their spectra in u.v. in acid and alkaline media were compared with those of morphine and codeine. The results given in Table 1 show that M_3 is codeine but that M_1, though very similar to morphine is not identical with it; M_2 is non-phenolic, like codeine, but differs from it in R_f value in systems I and II.

TABLE 1. PROPERTIES OF THREE ALKALOID-LIKE SUBSTANCES (M_1, M_2 AND M_3) OBTAINED FROM POPPY SEEDS BY DEGRADATIVE TREATMENT, COMPARED WITH THOSE OF MORPHINE AND CODEINE

Substance	R_f values in different systems*				λ max (nm)	
	I	II	III	IV	0·1 N HCl	NaOH
Morphine, (phenolic)	0·29	0·23	0·42	0·18	285	298
M_1 "phenolic"	0·29	0·23	0·42	0·04	266	266
M_2 "non-phenolic"	0·29		0·42		280	280
M_3 "non-phenolic"	0·46	0·37	0·60		285	285
Codeine, (non-phenolic)	0·46	0·37	0·60		285	285

* See "Experimental" for details.

(*b*) *Other seed varieties.* Samples of Halle, Tasmanian and a Commercial Blue seed were treated as before using the acid-pepsin digestion process mainly for convenience. All samples produced the three substances M_1, M_2 and M_3.

(*c*) *Germination methods.* Seeds of all varieties were placed on damp blotting paper for 24–36 hr during which the radicles burst through the testa and began elongating. At this stage no root hairs were formed nor had the cotyledons appeared so that *de novo* synthesis of alkaloids from exogenous substrates was unlikely to have taken place. The germinated seeds were then extracted with ethanol and the alkaloid-like substances separated. All samples produced M_1, M_2 and M_3. In addition, Tasmanian seeds produced two other Dragendorff positive substances, and comparatively large quantities of the other three substances. Seeds which were allowed to germinate for 48 hr also produced papaverine which was identified by R_f values and u.v. spectra in neutral, acid and alkaline media.

2. *Derivation of Bound Alkaloids from the Morphine of the Latex*

Although insufficient material was available for the elucidation of the structure of these alkaloid-like substances and their corresponding bound forms, their derivation from the morphine of the latex was indicated by the following experiments. Tritiated morphine ($3{\cdot}13 \times 10^5$ d.p.m./μM) was fed to living plants at the phloem region of the pedicel[3] about 1 week after petal fall. As already shown,[1] the morphine is translocated to the capsule latex

[3] J. W. FAIRBAIRN, A. PATERSON and G. WASSEL, *Phytochem.* **3**, 582 (1964).

in which it is rapidly metabolized and some of the newly formed compounds pass into the developing ovules. Samples of these ovules were examined at intervals and it was found that 11 days after feeding about 6 per cent of the fed radioactivity was present in an ethanolic extract of the ovules. Chromatographic examination showed the presence of a Dragendorff positive substance which was radioactive and had similar properties to, though not identical with, the compound M_1 (Table 1). Radioactivity was also present in other substances which gave no reaction with Dragendorff reagent and had zero R_f values. As the ovules and seeds matured the amount of ethanol soluble radioactive substances fell rapidly till, in the ripe seeds, they represented only 0·26 per cent of the fed activity. These results confirm that the smaller ethanol soluble derivatives of morphine newly arrived from the latex form larger molecules, which are no longer ethanol soluble, and which are stored in the seeds. These large molecules ("bound" forms), in turn, must be the source of the M series of compounds already obtained from the seeds. To confirm this, samples of the mature seeds were germinated for 24–36 hr and extracted with ethanol. Examination of the ethanol extract showed the presence of the morphine-like substance M_1 which was found to be radioactive; there was also some activity in the non-phenolic fraction.

Independent evidence for the close connection between morphine and these new substances has also been obtained by some work on a crude enzyme preparation from poppy which uses morphine as a substrate and produces *inter alia* these new compounds. We hope to publish details of this enzyme work later.

3. *Alkaloids and Seed Viability*

Although neither alkaloids as such, nor the latex in which they are produced, enter the developing ovules[4] it is evident from the work just described that bound forms of morphine (and possibly of other alkaloids) do pass from the latex into the ovule and are stored in the seed. An attempt was therefore made to test whether their presence was essential to seed viability by depriving the capsule of most of its latex during a critical stage in its development. Previous work[4] indicated that about two weeks after petal fall production of laticiferous vessels was at a maximum and quite marked changes in the alkaloidal pattern took place then.[5] Accordingly the capsules from a number of poppy plants were pricked with needles about 2 weeks after petal fall so as to bleed off as much latex as possible. The capsules were allowed to ripen and the seeds from them, and from a control group which had not been incised, were collected. Although both types of seed looked similar, tests on their viability showed quite astonishing differences. In the seeds from the bled capsules, the germination rate, root hair formation and cotyledon emergence were significantly less than that of the control seeds (Table 2).

As can be seen, the roots emerged very slowly and never reached more than half the number of those from the control seeds. Of those which emerged, root hair formation was also much slower than in the controls: some of those which did not produce root hairs gradually became brown at the tips and no further development occurred. Cotyledon emergence and development was also significantly reduced and it was noticed that, even in those from the bled seeds which did develop, the colour was reddish green at first in contrast with the good green colour of the control ones. Accordingly the chlorophyll content of the viable seedlings of each group was determined[6] and the results are given in Table 3.

[4] J. W. Fairbairn and L. D. Kapoor, *Planta Med.* **8**, 49 (1960).
[5] J. W. Fairbairn and G. Wassel, *Phytochem.* **3**, 253 (1964).
[6] D. I. Arnon, *Plant Physiol.* **24**, 1 (1949).

TABLE 2. COMPARISON OF VIABILITIES OF SEEDS FROM BLED CAPSULES AND FROM CONTROLS

Days after germination	Emergent roots (%)*		Roots possessing hairs (%)		Emergent cotyledons (%)		Fully opened cotyledons (%)	
	Control	Bled	Control	Bled	Control	Bled	Control	Bled
1 day	75	1	—	—	—	—	—	—
2 days	93	37	92	4	—	—	—	—
3 days	97	48	97	36	83	15	52	7
5 days	98	50	98	36	98	36	83	20
7 days	98	50	98	36	98	48	97	48

* Each figure represents the mean from 5 experiments each involving 50+50 seeds.

TABLE 3. CHLOROPHYLL CONTENT OF SEEDLINGS PRODUCED FROM NORMAL AND FROM BLED CAPSULES

Days after germination	μg/100 Seedlings			
	Chlorophyll *a*		Chlorophyll *b*	
	Normal	Bled	Normal	Bled
5 days	57·5	33·5	31·6	21·2
8 days	83·5	64·7	35·0	24·1
10 days	97·7	97·6	52·7	47·0

DISCUSSION

Bound Forms of Morphine in the Seeds

Our results show firstly that poppy seeds contain molecules (presumably larger than normal alkaloids) which on degradative treatment produce alkaloid-like substances, one of which is codeine. Although many workers report that the seeds contain no alkaloids, some occasionally report their presence.[7,8] However these reported alkaloids may originate from fragments of capsule wall, or even smears of latex, unavoidably admixed with the seeds during collection. To eliminate this source of error the samples of seed used in our work were picked over to remove capsule debris, then ground and extracted with ethanol to remove any remaining traces of "free" alkaloids. The new ethanol soluble substances reported are therefore all derived from bound forms stored in the seeds.

The relationship of some of these new compounds to morphine has been established by their close similarity in R_f values and u.v. spectra (Table 1). Even more convincing is the fact that, after feeding radioactive morphine to the latex, radioactive alkaloid-like substances and ethanol insoluble ("bound" forms) appeared in the ovules and seeds. Germination of the mature seeds led to the breakdown of the bound forms to produce radioactive alkaloid-like substances. It would seem therefore that morphine is rapidly metabolized in the latex[1] into a series of compounds, some of which are alkaloid-like and ethanol soluble and others non-alkaloidal, ethanol insoluble and probably fairly large molecules. Some of these are

[7] L. VAN ITALLIE, *Ann. Pharm. Franc.* **4**, 156 (1946).
[8] V. L. PREININGER, P. VRUBLOVSKY and V. L. STASTNY, *Pharmazie* **20**, 439 (1965).

transported to the developing seeds and stored in them mainly as the large molecules. During germination these compounds break down to form the smaller alkaloid-like substances. It is possible that some other alkaloids from the latex are also metabolized similarly since codeine and papaverine are detectable in germinated seeds before *de novo* synthesis is likely. These results therefore add considerably to the evidence we have previously produced that morphine is not an end-product substance but is metabolized into a series of new compounds.

Bound Forms of the Alkaloids and Seed Viability

An important problem raised by this work is whether the new compounds have any metabolic significance. We have attempted to answer this, in the first place, by depriving fruits, during critical stages in their development, of the bulk of the latex in which the alkaloids and their derivatives are formed. In these circumstances the seeds produced have significantly reduced viability, especially in root hair and chlorophyll formation. Obviously factors other than the alkaloids or their derivatives may be involved but the results are sufficiently striking to warrant further investigation. Preliminary experiments indicate that the harmful effects produced by latex deprivation can be partly reduced by germinating the seeds in the presence of dilute alkaloidal solutions. We intend repeating these "replacement therapy" experiments, not only with alkaloids but with some of the newer bound forms when sufficient are available.

It should be noted that the bleeding experiments did not deprive the developing ovules entirely of latex as this was present in significant amounts during the first 14 days of ovule development before bleeding took place. This probably explains the fact that about one third of the seeds from bled capsules survived; however even the surviving seedlings showed diminished chlorophyll content. But as these seedlings develop they will produce alkaloids freely by *de novo* synthesis and this may explain why chlorophyll formation reaches normal after 10 days (Table 3). Some of these plants were grown on in the greenhouse, others were transferred out of doors in the early summer of 1967 and all have developed into normal healthy plants. It would seem therefore that the bound forms of alkaloids in the seed are of critical importance only in the early stages of development; later on the new plantlet synthesizes its own supplies independently. In any event it appears that the alkaloids and their derivatives are important to normal development in this plant.

Pharmacological Effect of the New Compounds

A further interesting point is raised by our results since poppy seed is a common article of diet in some parts of the world. Digestion in the stomach (acid-pepsin) may lead to the release of alkaloid-like substances which may exert pharmacological action. Recently Preininger, Vrublovsky and Stastny[8] reported on a patient who, after ingesting 3 to 4 tablespoonfuls of ground seed, found her normally unpleasant symptoms of irritability and general weakness disappeared and were followed by mild euphoria. An alkaline chloroform extract of poppy seeds yielded 0·005 per cent residue which appeared to contain (chromatography only) codeine, thebaine, and an unknown alkaloid-like substance, but no morphine. After incubation of the seeds at 37° for 48 and 72 hr, however, the alkaline chloroform extract yielded 0·37 and 0·27 per cent residue respectively; the above alkaloids and possibly morphine were detected in the residue. This fivefold increase after incubation is consistent with our conclusion that the seeds contain bound forms of the alkaloids or alkaloid-like substances. The authors state further that the amount of "alkaloid" detected could not account for the patient's experience and since we found that morphine-like substances, rather than morphine, are formed during digestion of the seeds, it may well be that the former are considerably more

active pharmacologically than morphine. Bentley and Hardy[9] have shown that modification of the otherwise inactive thebaine leads to compounds of fantastic analgesic potency; sometimes 7000 to 10,000 times as active as morphine. We hope to extract sufficient of our new compounds to test out the possibility that the morphine-like compounds have enhanced pharmacological activity.

EXPERIMENTAL

Poppy Seed Varieties Used

(*a*) Berlin, *Papaver somniferum* L. subsp. *eurasiaticum* var. *glaucum* Rothm.
Deep red coloured petals.

(*b*) Halle 4/2 A special strain produced at the Institut für Biochemie der Pflanzen, Halle, D.D.R.
White petals with a pink spot at the base.

(*c*) Tasmanian A special strain produced by Macfarlan Smith Ltd., Edinburgh, and grown in Tasmania.
White petals with a pink spot at the base.

(*d*) Blue seeds A commercial sample of seed.

T-Morphine

Highly active tritiated morphine was produced at the Radiochemical Centre, Amersham by a modification of the method of Kirby and Ogunkoya.[10] This was diluted with non-active morphine and recrystallized to produce morphine of suitable activity. One tritium remains at position 2 of the morphine molecule; in this position (ortho to the phenolic group) it is very stable. We checked this by passing known quantities of T-morphine through the various extraction, chromatographic and other processes used in the work. Significant losses only occurred after several hours exposure to strong acid or NaOH (0·1 N). We therefore took care never to leave extracts in these conditions for more than ½ to 1 hr.

Preliminary Treatment of the Seeds

About 20-g batches of seed from which capsule debris had been picked out, were crushed, extracted with 40, 30 and 30 ml portions of warm ethanol and dried. The dried marc was then treated by one of the following processes.

Acid Hydrolysis

Heated in 50 ml 2 N HCl in a boiling water bath for 2 hr. About 100 ml ethanol was then added, the mixture filtered and the residue washed with 2 × 50 ml ethanol. The combined filtrates were evaporated to small bulk under vacuum and the non-phenolic and phenolic alkaloids separated by the method described below.

Acid Pepsin Digestion

Incubated at 37° for 5 hr with 50 ml of Acid Pepsin Solution (British Pharmacopoeia 1963). About 100 ml ethanol was added and the alkaloids separated as described below.

Fermentation

Crushed seeds, which had not previously been treated with ethanol, were moistened with water and incubated at 22° for 3 days. They were then extracted with ethanol and the alkaloids separated as described below.

Extraction Methods

Troublesome emulsions, especially when extracting seeds, occurred unless the following procedure was used. Seedlings and ovules were blended with warm ethanol several times, filtered and the combined filtrates evaporated almost to dryness; the ethanol extracts from treated seeds, already referred to, were also evaporated to small volume. These residues were extracted with N HCl, filtered and the acid filtrate solution shaken with ethyl acetate to remove pigments, etc. The filtrate was made alkaline with ammonia and the alkaloids extracted into chloroform/isopropanol (3:1) and the combined organic solvent layer washed with water and evaporated to dryness. The residue was dissolved in N HCl, filtered, brought to pH 12 with NaOH and the non-phenolic

[9] K. W. BENTLEY and D. G. HARDY, *Proc. Chem. Soc.* 220 (1963).
[10] G. W. KIRBY and L. OGUNKOYA, *J. Chem. Soc.* 6914 (1965).

alkaloids extracted with chloroform. The aqueous layer was then brought to pH 8 with phosphoric acid and the phenolic alkaloids extracted with chloroform/isopropanol (3:1). Both organic solvent layers were washed with water, evaporated to dryness, and each residue dissolved in methanol for chromatographic examination of the non-phenolic and phenolic alkaloids. For preparative work System II (below) was used and the compounds eluted from the separated bands with chloroform/ethanol (9:1).

Chromatographic Systems

Plates, 250 μ thick; Systems I, II and III slurried with ethanol and dried at room temperature, System IV slurried with water and activated at 105°. Tanks were allowed to equilibrate with the solvent for half an hour before use. Room temperature (19–22°) was used throughout; running time about 45 min so that solvent front travelled 15 to 17 cm.

System I

Adsorbent: Kieselguhr G (Merck)/Silica gel H (Merck) (1:1).

Solvent: acetone/toluene/ethanol/ammonia (0·880) (20:20:3:1), freshly prepared.

System II

Adsorbent: In this system the lower part of the plate was spread with Kieselguhr G and the remainder with a mixture of Kieselguhr G and Silica gel H. This enabled the morphine, which normally has very low R_f values, to rise well above the starting line on which impurities were retained. Once the Kieselguhr/Silica gel mixture was reached the morphine did not move much further but the remaining alkaloids continued to rise and were well separated from each other. The dual plates can be conveniently prepared by using a "gradient-mixture spreader" (Desaga),[11] or a similar device. A slurry of Kieselguhr G (5 g with 10 ml ethanol) was poured into one end of the spreader for about a quarter of its length; the remainder of the spreader was filled with a slurry of Silica gel H and Kieselguhr G (7·5 g each in 35 ml ethanol). Five plates were then prepared in the normal way.

Solvent: ethyl acetate/benzene/ammonia (0·880) (80:10:2·5), freshly prepared.

System III

Adsorbent: Plates prepared as for System II.

Solvent: as in System I.

System IV

Adsorbent: Silica gel G (Merck).

Solvent: methanol.

Chlorophyll Determination

The chlorophyll was extracted from the seedlings by maceration with 80 per cent acetone for 2 hr in the dark. The extinctions of the extract at 663 nm and 645 nm were determined and the chlorophyll content calculated according to the method by Arnon.[6]

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[11] E. STAHL, *Angew. Chem. Intern.* 3, 784 (1964).