placed in the Naucleeae are good sources of oxindole and heteroyohimbine alkaloids and thus it seemed possible that a knowledge of the alkaloids in other members of the Naucleeae might assist in unravelling generic relationships. To this end, over 100 Naucleeae leaf samples from herbarium material were screened for alkaloids and species from the following genera were examined:

Adina, *Anthocephalus*, *Breonia*, *Cephalanthus*, *Metadina*, *Myrmeconauclea*, *Nauclea* (*Sarcocephalus*) and *Neonauclea*.

The results indicate that oxindoles or heteroyohimbines are not widespread in the rest of the tribe, only being found in several species of *Cephalanthus* and one of *Neonauclea*. The majority of the samples of *Neonauclea*, together with those of *Nauclea*, *Metadina* and *Myrmeconauclea*, contained pyridino-indolo-quinolizidinone alkaloids, also reported from species of *Mitragyna* and *Uncaria* (Phillipson, Hemingway & others, 1974). However, the latter two genera have morphological and anatomical affinities with the Cinchoneae which also includes genera producing heteroyohimbines and oxindoles. Inclusion of *Cephalanthus* in the Naucleeae has been questioned (Bremekamp, 1966) and considering its isolated position perhaps it would be better placed in a separate tribe. The presence and nature of the alkaloids of *Mitragyna* and *Uncaria* tends to support the taxonomic idea that these genera together with *Cephalanthus*, stand apart from the rest of the Naucleeae and that their exclusion would result in a taxonomically homogeneous tribe. Hence differences in alkaloid content proved useful in assessing the taxonomic relationships, although further information is required on the distribution of indole and quinoline alkaloids, especially in members of the Cinchoneae as well as of the Naucleeae.

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REFERENCES

BAKHUIZEN, VAN DEN BRINK, Jr., R. C. (1970). *Taxon*, **19**, 468–480.
BREMEKAMP, C. E. B. (1966). *Acta Bot. Neerl.*, **15**, 1–33.
HEGNAUER, R. (1973). "*Chemotaxonomie der Pflanzen*", Vol. 6, p. 138, Birkhäuser Verlag, Basel und Stuttgart, and references therein.
PHILLIPSON, J. D., HEMINGWAY, S. R., BISSET, N. G., HOUGHTON, P. J. & SHELLARD, E. J. (1974). *Phytochemistry*, **13**, 973–978.

Methylating and demethylating enzymes in *Papaver somniferum* latex

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Tracer work has established that the formation of the hydrophenanthrene phthalideisoquinoline and benzylisoquinoline groups of alkaloids involves a number of methylation and demethylation steps. Spenser (1966) reported that methionine was the most efficient donor for both *O*- and *N*-methyl groups of the alkaloids.

The presence of methylating enzymes was investigated in whole latex as well as latex which was fractionated to 1000 $g \times 30$ min and supernatant fractions. In the preliminary experiments the latex was incubated with methionine (^{14}C-methyl) in presence and in absence of co-factors intended to shift the equilibrium to the formation of *S*-adenosyl-L-methionine together with norlaudanosoline. The activity extracted using chloroform was taken as a measure of the total organic soluble methylation products (including alkaloids), formed by the latex at the end of the incubation period. The extraction was done after preliminary precipitation of the latex protein by acid followed by readjustment of the pH to 8·0–8·5. The radioactivity in the organic layer was found to be dependent on the amount of latex used and was considerably increased by inclusion of the co-factors in the incubation mixture implying preliminary formation of *S*-adenosyl-L-methionine which was more efficient in this respect as methyl donor.

On repeating the experiments with *S*-adenosyl-L-methionine (methyl ^{14}C) it was found that the activity in the organic layer was considerably increased by inclusion of norlaudanosoline

and it was mainly in the supernatant fraction. This was confirmed by a final experiment in which the 1000 *g* and supernatant fractions were incubated together with *S*-adenosyl-L-methionine (methyl ^{14}C) and norlaudanosoline followed by extraction of the total alkaloids from both fractions and subsequent autoradiography after development by t.l.c. using acetone–toluene–ethanol–ammonia (conc.) (10:10:3:½) (Antoun, 1974). Radioactive reticuline, codeine, thebaine and papaverine were found. Narcotine was not present indicating either that the enzymes involved in its formation were not active at the time of collection of latex or that its biosynthesis occurs outside the laticiferous vessels.

This preliminary work has shown that poppy latex was capable not only of carrying out the necessary methylation reactions of the alkaloids, but also capable of demethylating thebaine to codeine and possibly morphine.

REFERENCES

ANTOUN, M. D. (1974). Ph.D. Thesis (University of London), pp. 137–142; 149–151.
SPENSER, I. D. (1966). *Lloydia*, **29**, 71.

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Preliminary characterization of the histamine releasing activity of cotton dust

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Aqueous extracts of cotton dust possess histamine releasing activity (HRA) which is believed to play an important role in the aetiology of the acute symptoms of the occupational disease, byssinosis (Nicholls, Nicholls & Bouhuys, 1967). In this preliminary study an attempt has been made to characterize the chemical nature of this pharmacological activity.

HRA was determined by an *in vitro* technique using pig lung (Nicholls, Evans & others, 1973). The extractability of HRA from cotton dust by various solvents decreased in the order; distilled water > aqueous ethanol (50%, v/v) > aqueous ethanol (75%, v/v) > methanol > acetone > di-ethyl ether. The ether extract possessed about one-fifth of the HRA of the water extract. About one-third of the HRA of aqueous extracts of the dust was dialysable through a cellulose (Visking) membrane. The HRA of aqueous extracts of dust was extensively absorbed onto a weak cationic exchange resin. In aqueous extracts of cotton dust the activity was stable to boiling at neutral and alkaline but not acidic pH values.

Thin layer chromatography on cellulose plates in butan-1-ol, acetic acid, water (4,1,5, v/v) separated the HRA into three areas of R_F 0·73(A), 0·54(B) and 0·17(C) respectively. Spot A was lime-green in colour and gave a positive reaction for phenols and tannins (ferric chloride-ferricyanide reagent; Smith, 1960). The absorption spectrum and chromatographic mobility of this spot were identical to those of the flavonoid, rutin which has previously been found in the cotton plant (Greensmith, 1969). Rutin released histamine from pig lung *in vitro*.

Spot B possessed a chromatographic mobility and staining reaction to ninhydrin (crimson) identical to that of trimethylamine which has been also found in the cotton plant (Greensmith, 1969). This amine was found to be a histamine releasing agent with lung tissue.

Spot C was a yellow-brown colour which gave a whitish fluorescence under light at 350 nm and stained with ninhydrin. The material isolated from this area of the t.l.c. plate was insoluble in ether but readily soluble in water. It was precipitated from aqueous solution by addition of acetone to 70% (v/v). Hydrolysis of this fraction with acid followed by paper and thin layer chromatography demonstrated the presence of several amino acids and sugars. Hydrolysis also destroyed the HRA of this material. It is suggested that Spot C, which is the main histamine releasing component of cotton dust, is a polysaccharide-protein complex. Full characterization of this material is in progress.

Thus it is evident that cotton dust contains several agents of diverse chemical composition which are capable of releasing histamine.