Biochemical Pharmacology, Vol. 18, pp. 1553–1557. Pergamon Press. 1969. Printed in Great Britain

ALKALOIDS OF *MELODINUS SUAVEOLENS* AND THEIR EXCRETION AS A COMMON END-PRODUCT IN THE RAT

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(*Received* 28 *November* 1968; *accepted* 8 *January* 1969)

Abstract—Alkaloids extracted from *Melodinus suaveolens* in a preliminary, exploratory study appear to be α-methylene indolines. When the alkaloidal extract is injected into the rat, there is excreted in the urine a common end-product which appears to be an alkaloid different from any of the alkaloids of the injected extract.

IN HONG KONG extracts of local plants are employed extensively by the Chinese herbalist in the treatment of a variety of ailments. We have shown in an earlier paper that alkaloids from one of these plants influence certain metabolic activities of the mammalian cell,[1] and we have been engaging in experiments of an exploratory nature with another plant, *Melodinus suaveolens* Champ. (Apocynaceae) to enquire if there is a biochemical basis for the therapeutic action alleged by the herbalist.

Extracts of the fruit of *M. suaveolens* are prepared and prescribed by the Chinese herbalist in Hong Kong as a therapeutic agent when mild sedation is required. Although commonly called "Mountain Orange" because of the colour, shape and size of its fruit, the plant does not belong to the orange family. We have found that the seeds, leaves, bark and stems of this plant contain alkaloids and that the concentration in the seeds greatly exceeds that found in other plant parts.

Since the Chinese herbalist prepares an infusion of the fruit for his preparations, it was considered a reasonable conjecture that this infusion would contain alkaloids which might be responsible for the sedative action claimed.

As a preliminary step in our enquiry we have extracted the alkaloids from the seeds, separated the several alkaloids from one another by Thin Layer Chromatography (TLC), purified them and examined their Infrared (i.r.) and Ultraviolet (u.v.) spectra. From a consideration of the data we have deduced the alkaloids to be α-methylene indolines.

From a biological point of view, the first question requiring a satisfactory answer is "are these alkaloids metabolized by the animal", since any evidence for the biochemical activity of a substance is made more cogent if it is apparent that the substance in question has been engaged in chemical transformations within the animal. To attempt to answer this question we have examined by TLC the alkaloids excreted in the urine of the rat after an injection of the plant alkaloidal mixture, and compared the excretion pattern with the initial pattern of the plant mixture. From this comparison we have concluded that the alkaloids of *M. suaveolens* undergo transformation

in the rat, and are excreted as a common end-product. This paper reports the findings at this stage.

EXPERIMENTAL

Extraction

After air-drying, the seeds were minced by a meat grinder and stored in desiccators until required. The dried, minced seeds were extracted twice with light petroleum (b.p. 60°) and the combined light petroleum extract shaken with 250 ml of 2N HCl. An emulsion formed, which on standing overnight at room temperature, cracked so that the acidic layer could then be separated from the organic solvent. The latter was discarded. The seeds were then dried in warm air to remove any adhering organic solvent, soaked in 500 ml of 2N HCl for two days, filtered and tested for the presence of alkaloids by Mayer and Dragendorff reagents. This soaking and filtering procedure was repeated until the acid filtrate yielded a negative test with the alkaloidal reagents. The acid extracts were then combined, made alkaline with ammonia to pH 10 in an ice-bath, with constant shaking. A dark purple precipitate was formed. The alkaline solution was extracted with 250 ml chloroform three times and concentrated by evaporation under reduced pressure to a final volume of about 50 ml. This concentrated chloroform solution was shaken with 200 ml of 2N HCl, the acid layer removed and the extraction repeated with further portions of acid until all alkaloids had been removed from the organic layer. The combined acid extract was then adjusted to pH 10 with dilute ammonia solution, and the alkaloids were re-extracted with chloroform as before. This repeated extraction from the chloroform to the acid phase yielded a relatively pure alkaloidal mixture which could be used subsequently for injection in the experimental animal.

Separation of the alkaloids

TLC was used as a means of separating the alkaloids of the mixture. Silica gel G was employed as matrix for the glass plates, on which 50 μl of the chloroform mixture of alkaloids was placed as a spot. Of several solvent systems tested, the best separation of the alkaloids was obtained by the system cyclohexane:diethylamine 20:1. Nine spots were located by spraying with Iodoplatinate Reagent, but only eight were observed under u.v. light at 366 mμ.

Purification of the alkaloids

In order to obtain greater quantities of the individual alkaloids, separation of the alkaloidal mixture was brought about by the use of Preparative Layer Chromatography (PLC). The bands which resulted were viewed under ultraviolet light, scraped off and eluted exhaustively with methanol. The methanolic solutions of each separate alkaloid were combined and evaporated under reduced pressure to about 10 ml. This process was repeated two or three times, or until a spot when tested in a thin layer plate yielded only one clearly defined spot on development—then it was considered pure. Although nine different alkaloids were separated, only six were obtained in a yield sufficient for our particular study. These were labeled A–F. The purified methanolic solution was evaporated to dryness by a current of warm air, and the residue dissolved in a small volume of warm ethyl acetate. On cooling pale yellow crystals were obtained which were quite deliquescent. The crystals were then kept either in a

desiccator over anhydrous calcium chloride, or dissolved in chloroform. These alkaloids were examined for their spectral characteristics.

Animal experiments

Male white rats weighing approximately 200 g were injected i.p., with 1 ml of a solution of the alkaloidal mixture dissolved in 1% HCl, and corresponding to 350 mg/kg body weight. A similar group of animals was used as a control. These were injected with 1 ml of 1% HCl. Urine was collected over a 24-hr period for 5 consecutive days following injection. The volume and pH of the urine was recorded, followed by acidification with $\frac{1}{10}$ volume of concentrated HCl to prevent bacterial contamination.

Extraction of alkaloids from urine

After centrifuging and filtering, the urine was autoclaved at 10–15 lb pressure for 1 hr, in order to hydrolyse any alkaloids which may have been excreted as glucuronides. The acid hydrolysate was filtered and made alkaline with ammonium hydroxide to pH 10 before extracting with chloroform. The extraction was repeated until all alkaloids were removed. The combined extracts were reduced to a small volume, dehydrated under pure anhydrous sodium sulphate, filtered and dried by evaporation in a vacuum desiccator over anhydrous calcium chloride. The dried product was dissolved in 0·1 ml chloroform of which 50 μl was applied as a spot to the chromatoplate. Urine from the animals injected with the 1% HCl only was given identical treatment.

RESULTS AND DISCUSSION

Six alkaloids of the nine demonstrated by TLC were isolated, purified and subsequently examined for their spectral characteristics by employing a Perkin–Elmer i.r. spectrophotometer and a Hilger–Watts spectrophotometer. Table 1 shows these values.

As far as we have been able to determine, no attempt has been made to isolate alkaloids from *M. suaveolens*, although considerable investigation has been carried

TABLE 1. PHYSICAL DATA OBTAINED ON ALKALOIDS OF *M. suaveolens*

Fraction	i.r. Absorption bands ($CHCl_3$) (cm^{-1})	u.v. $CHCl_3$ λmax. (mμ)	$R_f \times 100$
A	1750	293	78
	3700	329	
B	1720	292	67
	3600	327	
C	1750	280	50
	3600	328	
D	1750	286	38
	3600	329	
E	1720	299	25
	3600	324	
F	1750	290	15
	3600	327	
G	—	—	92
H	—	—	10
I	—	—	3

out on other species. In 1964 Raffauf[2] attempted to classify the Apocynaceae according to the chemical structure of their constituent alkaloids, and he reported that all the Melodinus alkaloids so far encountered were members of the indole class possessing an indoline chromophore, of which quebrachamine, is a suitable example. The following year Linde[3] published a report on the presence of fifteen indolic alkaloids which he had isolated from *M. australis*, and which proved to have chromophores of α-methylene indoline, dihydroindole (indoline), as well as indole. In the same year Bernauer, Englert and Vetler[4] reported the finding of an alkaloid obtained from *M. scandens* which was not an indole alkaloid, but which they assumed had been derived biosynthetically, by oxidation at C_2 and subsequent rearrangement, from a precursor which *was* an indole.

In our study, all the alkaloids isolated from *M. suaveolens* demonstrated bands in their i.r. spectra at about 1750 cm^{-1} and 3600 cm^{-1}, indicating the presence of carbonyl and hydroxy or amino groups, respectively.

It must be emphasized that our yield of crystalline alkaloids was small and that as a consequence examination of their infrared spectra was not as revealing in terms of structure as we had hoped. However, the u.v. analysis, requiring much smaller amounts of material, yielded absorption data which, from an examination of "Interpretation of the Ultraviolet Spectra of Natural Products" by Scott[5] led us to conclude that the alkaloids possessed the α-methylene indoline chromophore, with its two bands, at λ max 293 and 320 mμ.

When engaged in experiments with animals we were careful to ensure that our method of extraction of alkaloids from urine did not interfere with or destroy the plant alkaloids themselves. Thus we added an aliquot of the plant alkaloid mixture to voided urine and carried out the same extraction procedure before applying the final organic extract as a spot to the Silica gel chromatoplate. When stained with Iodoplatinate Reagent the nine spots appeared in positions identical with those alkaloids of the plant mixture. Similar extractions of urine collected following injection of the dilute hydrochloric acid (used as a solvent for the alkaloids) to the control group of animals revealed no alkaloidal spots on a chromatogram. We have in this way established that neither the method of extraction nor the injection of the acid solvent affected the excretion of the end-product of the plant alkaloid mixture.

We were interested in the observation that the alkaloids were well tolerated by the animal, and appeared to be non-toxic, since this seemed to bear out the claim made by the Chinese herbalist on the absence of side-effects of his plant preparation when ingested by his patients.

When one examines Fig. 1, which is a typical drawing of our chromatograms, it is evident that none of the alkaloids present in the original injected mixture are excreted by the rat. One spot only appears on all our plates, and this spot has an R_f value different from any value corresponding to the original plant alkaloids.

We have considered the possibility that metabolic transformations of the alkaloids could yield products which are non-alkaloidal in character. However, an examination of all our chromatograms under u.v. light has led us to the conclusion that the plant alkaloids are transformed *in vivo* to another alkaloid and excreted as such.

At this stage in our investigations we have no knowledge of the nature of the common end-product excreted, except that it appears to be another alkaloid. It is well known that most drugs are transformed in the body to yield pharmacologically

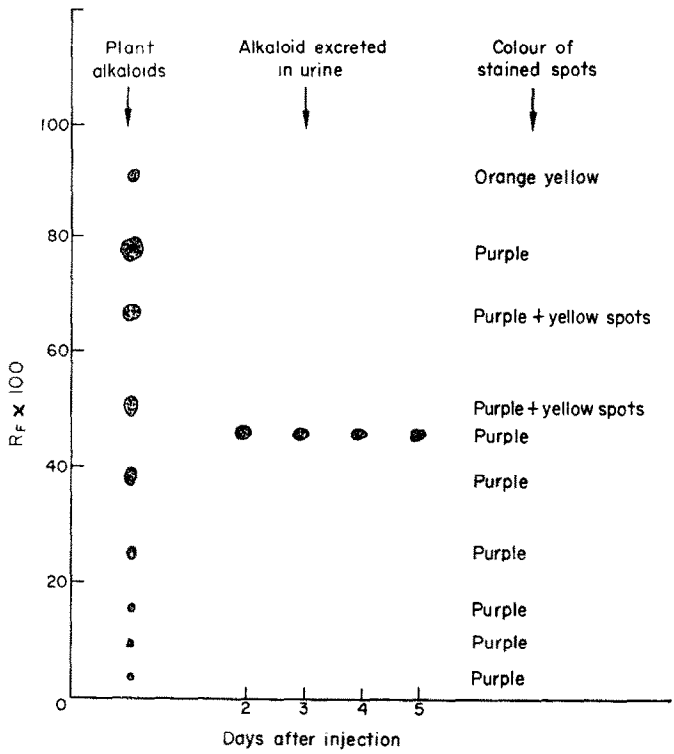

FIG. 1. Drawing of a typical Silica gel G thin layer chromatogram, developed in cyclohexane:diethylamine 20:1 in which is demonstrated the relative positions of the alkaloids extracted from *M. suaveolens* and the common end-product, a different alkaloid, which is excreted in urine following injection of the whole plant alkaloidal extract.

inactive metabolites or active products, a variety of biochemical changes being involved. We are currently engaged in experiments to enquire into the nature of the biochemical changes which are involved in the transformation of these alkaloids.

Acknowledgements—The authors are indebted to the Eli Lilly Company for a grant of money which helped to support this investigation.

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