Antifungal Activity of some Constituents of *Murraya koenigii* Spreng

In the course of our investigations on the antimicrobial properties of some plant isolates, we reported the antifungal and antibacterial properties of some natural coumarins[1–3]. These coumarins are new additions to the already known antibiotic substances or preparations from plant sources[4,5]. Recently we have isolated and characterized several crystalline constituents of the stem-bark of *Murraya koenigii* Spreng. (Fam. Rutaceae) an Indian medicinal plant of repute[6,7]. Of these murrayanine $C_{14}H_{11}O_2N$, m.p. 168°, has been tentatively formulated as 3-formyl-1-methoxy carbazole[8], while girinimbine[9] $C_{18}H_{17}ON$, m.p. 176°, and mahanimbine[10] $C_{23}H_{25}ON$, m.p. 94–95°, optical rotation D 26.5 = +45.4 (chloroform), have been found to be pyrano-carbazole derivatives.The stem-bark of the plant is used in the indigenous system of medicine against eruptions and bites of poisonous animals. It was therefore of interest to examine the antimicrobial properties of the compound isolated from the stem-bark of *M. koenigii*. In the present communication the antifungal action of murrayanine, girinimbine and mahanimbine on some human pathogenic fungii is reported. The compounds were tested against the fungii *Microsporum gypseum, Microsporum audouini, Trychophyton rubrum, Nocardia asteroids, Epidermophyton floccosum* and *Candida albicans*. The results on the antibiotic action of the compounds against some test bacteria and plant pathogenic fungii were not promising and therefore the data are not reported.

The experiments were carried out by the usual agar-cup assay method using Sabouraud's agar medium. After incubation at 37°C for 120-150 h, the inhibition zones produced by the compounds were measured. As the test materials were not soluble in water, 30 mg of each were dissolved in 10 ml of a mixture of ethanol and ethylene glycol (1:4). The results of assay are presented in Table I. The minimum inhibitory concentration (MIC) was determined, using dilute solution of the materials in the assay procedure. This is shown in Table II. It will be evident from the Tables that the activity of the compounds is species selective. The highest activity has been shown by girinimbine against *N. asteroids*.

The constituents of *M. koenigii*, therefore, may be regarded as new additions to the list of already known antibiotics derived from higher plants, although the inhibitory power of these compounds is limited[5]. The compounds belong to a hitherto unknown group of plant products built up on the carbazole skeleton. Some synthetic carbazole derivatives have also been found to be quite active against the above fungii. Carbazole and N-methyl carbazole have, however, no inhibitory action against these fungii. More results will be reported shortly.

Table I. Results of assay

Test organisms	Zone of inhibition in mm		
	Girinimbine	Murrayanine	Mahanimbine
Microsporum gypseum	20	14	12
Microsporum audouini	18	–	–
Trichophyton rubrum	16	10	12
Nocardia asteroids	24	–	20
Epidermophyton floccosum	–	–	–
Candida albicans	9	9	9

Table II. Minimum inhibitory concentration (MIC) of the substances in μg/ml

Test organisms	Girinimbine	Murrayanine	Mahanimbine
Microsporum gypseum	300	3000	3000
Microsporum audouini	300	–	–
Trichophyton rubrum	300	3000	3000
Nocardia asteroids	30	3000	300

Zusammenfassung. Es wird über die antifungale Wirksamkeit der Murrayanine, Girinimbine und Mahanimbine, drei aus *Murraya koenigii* Spreng. isolierte Carbazolderivate, berichtet.

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The Effect of Diet on the Hemolymph Amino Acid Constituents of the Larvae and Pupae of *Sarcophaga ruficornis* (Fabricius): (Diptera)

The hemolymph of a number of dipterous insects, for example *Musca* (PRATT[1], PRICE[2]), *Gastrophilus* (LEVENBOOK[3]) and *Calliphora* (FINLAYSON and HAMMER[4], HACKMAN[5]), have been analysed by paper partition chromatography and a wide variation has been found in their amino acid constituents. These variations have been

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related to taxonomic differences in various members of *Culex pipens* complex (MICKS[6]); fresh tissues of several species of fishes (BUZZATI et al.[7]) and *Aelosoma hemprichi* and *A. variegatum* (AUCLAIR et al.[8]). BENASSI et al.[9] have further suggested that these variations may be due to the differences in physiological and ecological conditions of the insects. However, no attempt has been made, as yet, to study the effect of the varying physiological conditions on the amino acid constituents of the hemolymph of the same species. In this paper, the amino acid constituents of the hemolymph of larvae and pupae of *Sarcophaga ruficornis*, reared on two widely different diets, viz. meat and dog biscuits, have been reported.

Materials and methods. The adult flies of *S. ruficornis* were maintained at room temperature in a wire gauge cage and were fed on banana and sugar. A piece of meat in a small petri dish was provided for larviposition and was taken out of the cage after 1 h. Half of the larvae so obtained were transferred to a jar containing moistened dog biscuits suitable for rearing. The dog biscuits were prepared for rearing as described by FRINGES[10], with some modifications. Two or three dog biscuits were placed in a jar and covered with water and allowed to stand for about 3 min. The water was then thrown out and the biscuits were squeezed lightly with the palms to press out the required amount of water. The other half of the larvae was transferred to a jar containing pieces of buffalo meat for feeding. As the maggots of the same batch were transferred to both meat and dog biscuits, they were approximately of the same age. When the maggots were ready for pupation, they were removed to jars containing moist sawdust in which they finally pupated.

For the preparation of chromatograms of the hemolymph, the larvae were taken out of their respective media, washed separately with distilled water and dried on blotting paper. The larvae were then punctured with a needle and bled onto a small strip of chromatographic paper (Whatman No. 1). Four maggots fed on the same diet were used for preparing one chromatogram.

The pupae from dog biscuits and meat were taken out on the third day of their pupal period; thus they were approximately of the same age. Hemolymph of twelve pupae was sufficient for one chromatogram.

Two-dimensional ascending chromatograms were prepared in *n*-butanol:acetic acid:water (120:30:60), and phenol:water (4:1), as described earlier (SINGH[11]). The amino acids were located by 0.2% ninhydrin in water-saturated *n*-butanol and identified by comparing them with chromatograms of known amino acids.

Results and discussion. The results obtained are presented in the Table. A wide difference was observed in the amino acid constituents of the blood of the larvae reared on dog biscuit and meat. In the hemolymph of larvae from dog biscuits, sixteen amino acids were present; proline, alanine, and glutamine were in high concentrations; besides which, an unidentified spot was found in between leucines and valine. In the hemolymph of the larvae fed on meat, this spot and lysine, glutamine, and taurine were absent while threonine was in high concentration. The hemolymph so analysed was from larvae of the same age and, therefore, it may be concluded that the differences in the amino acid constituents were due to differences in the diet of the two groups of the larvae.

So far the hemolymph constituents of three meat-feeding dipterous species, viz. *Calliphora augar* (HACKMAN[5]), *C. erythocephala* (FINLAYSON and HAMER[4]), and *Lucilia cuprina* (author's unpublished work), have been studied, but it is difficult to establish any correlation between them on the basis of their diet.

The blood of *S. ruficornis* pupae analysed from the two different diets shows no difference in the amino acid constituents. This lack of effect of diet on the amino acids of the pupal blood is firstly because they belong to a non-feeding stage, and secondly because, probably during the period of histolysis and histogenesis, the amino acids assume a more specific and definitive character[12].

Free amino acids in the hemolymph of larvae and pupae of *S. ruficornis*

Amino acids	*S. ruficornis* larvae		*S. ruficornis* pupae	
	Dog biscuit	Meat	Dog biscuit	Meat
Leucines	+	+	+	+
Valine	+	+	+	+
Tryptophan	+	++	–	–
Proline	++	++	++	++
Tyrosine	+	+	++	++
Alanine	++	+	+	+
Arginine	+	+	+	+
Lysine	+	–	+	+
Histidine	+	+	+	+
Glutamine	++	–	+	+
Glycine	+	+	+	+
Serine	+	+	+	+
Taurine	+	–	+	+
Glutamic acid	+	+	+	+
Aspartic acid	+	+	–	–
Threonine	–	++	–	–
Unknown	+	–	–	–

++ high contentration; + medium concentration; – absent.

Zusammenfassung. Es werden zwei Zuchtgruppen von *Sarcophaga ruficornis*-Larven hinsichtlich ihres Aminosäuregehaltes des Blutes untersucht. Den auf Fleisch gezüchteten Tieren fehlt Lysin, Glutamin und Taurin, während diese Aminosäuren bei den auf Hundebisquit aufgezogen Larven vorhanden sind, ihnen dagegen Threonin fehlt. Der Aminosäuregehalt im Blut der Puppen beider Zuchtgruppen ist gleich.

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