Effect of Sumithion on carbohydrate levels and phosphorylase activity in *Sarotherodon mossambicus*

Glucose concentration (mg/100 ml of blood)				Glycogen content (mg of glucose/g wt of tissue)				Phosphorylase activity (μmoles of inorganic phosphate/mg protein/h)			
Tissue	Control	Experimental	Change (%)	Tissue	Control	Experimental	Change (%)	Tissue	Control	Experimental	Change (%)
Blood	71.83±2.45	91.50±2.88	+27.38	Muscle	1.12±0.285	0.95±0.167[b]	−15.17	Muscle phosphorylase 'a'	0.342±0.031	0.421±0.026	+23.09
								'ab'	0.849±0.045	0.910±0.066[b]	+7.18
				Liver	15.91±2.67	11.43±2.70[a]	−28.15	Liver phosphorylase 'a'	0.424±0.042	0.538±0.035	+27
								'ab'	0.912±0.056	0.972±0.048[b]	+ 6.57

Changes after pesticide treatment are statistically significant. $p<0.001$; [a] $p<0.01$; [b] Not significant. Values are expressed as mean±SD of 6 animals.

pounds[4]. The fall in muscle glycogen being insignificant, despite the fact that muscle phosphorylase is stimulated, suggests increased turnover of glycogen, and that the muscle glycogen does not contribute towards hyperglycemia in Sumithion-treated *S. mossambicus*. It is likely that the observed hyperglycemia is mediated by glucagon which acts mainly only on hepatic tissue. Increased production of glucagon and depressed insulin activity were reported earlier in chick embryos treated with malathion[10].

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Toxic substances produced by *Fusarium*. X. Concerning the malformation disease of mango

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Summary. Accumulation of mangiferin and degraded carotenoids, in response to *Fusarium moniliforme* infection, has been suggested to be responsible for the malformation disease of *Mangifera indica* L. (Anacardiaceae).

The malformation disease of mango (*Mangifera indica* L.), in India, has rendered many superior quality mango orchards, in the Northern States, completely abortive. The disease has also destroyed many nursery beds of grafted seedlings. *Fusarium moniliforme* was reported[2] as a causal organism for the malformation disease. The fungus was found to secrete a large number of mycotoxins[3] on the infected plant parts. Mango is not only a staple food for many people, but its various parts are also used for therapeutic purposes in popular medicine. The presence of mycotoxins in the various parts of the afflicted mango plant is thus a cause for alarm from a public health view-point.

The purpose of this investigation was to determine the mechanism of malformation with a view eventually to controlling the disease. We recently reported[3] that in healthy mango twigs, mangiferin (1,3,6,7-tetrahydroxyxanthone-C_2-β-D-glucoside) occurred only as a minor entity, while in malformed shoots and twigs, infected with *F. moniliforme*, its concentration was increased very significantly. This observation prompted us to examine the role, if any, of mangiferin in mango malformation.

Material and mtehods. The crown of 2 1-year-old mango plants (cv. *Banarasi Langra*) and, healthy inflorescence (on intact mature plants), at an early stage of differentiation, were intentionally infected with *F. moniliforme* var. *subglutinans* (CMI-IMI 225231). The estimation of mangiferin on the infected parts was accomplished by maceration of the plant tissues with ethyl alcohol, preparative layer chromatography of the alcoholic concentrates, and by UV spectrophotometry of the eluted zones corresponding to authentic mangiferin. The presence of fusaric acid, a normal toxic metabolite of fusaria, in the diseased shoots and inflorescence of mango was tested according to a published procedure[3]. In a separate experiment, the ability of this fungus to form fusaric acid, in in vitro cultures, was tested. Richard's medium was used and the inoculum was incubated at 21 °C for 21 days. The role of mangiferin in supressing the fusaric acid production by this fungus was examined. Mangiferin (1×10^{-5} M) was added to Richard's solution prior to the 4th subculture stage.

The effect of the application of a large concentration of mangiferin to *M. indica* was determined. Aqeuous suspension of mangiferin (1×10^{-4} M) was administered for 7 days to the apical buds of 2 1-year-old mango plants by a wick arrangement. Only distilled water was applied to the control plants. The plants were kept in a green house, under aseptic condition, for a period of about 3 months.

The effect of the culture filtrates of *F. moniliforme* on the disease development was investigated. The fungus was grown in Richard's medium at 21 °C for 21 days and the

liquid was collected by filtration under aseptic condition. 3 young mango shoots (each about 20 cm long) were cut and the cut ends were dipped in the culture filtrate (50 ml) in Erlenmeyer flasks. The set-up was incubated at 21 °C (humidity 90%). In the control, only distilled water was added. In another experiment, a tetracyclic triterpene, $C_{30}H_{50}O_3$ (1×10^{-4} M), of the elemolic acid type, obtained from a Basidiomycetes fungus, was added. The triterpene was previously found[4] to arrest the carotenoid production of a number of fusaria. Subsequently, the effect of this culture filtrate on the mango shoot and inflorescence was investigated.

Results and discussion. In the fungal infected portion of the apical buds, the concentration of mangiferin was considerably increased (about 3–5-fold over the control). The concentration of mangiferin was maximum in the cortical cells surrounding the fungus-infested ones. Its concentration gradually declined in areas away from the fungal infected zones. In the infected inflorescence also, the concentration of mangiferin was dramatically increased (by about 10-fold over the control) within a period of about 4 weeks. The fungal infection and the concomitant increase in the amount of mangiferin are, therefore, biochemically related.

Mangiferin was earlier shown[5–7] to produce significant anti-*Fusarium* actions. In the present study, another noteworthy observation was that fusaric acid, a normal metabolite of fusaria, was absent in the infected mango shoots and inflorescence, while other fusarial metabolites e.g. 12,13-epoxy-trichothecenes, produced by the fungus in vitro, were present. The fungus, however, regained its ability to produce fusaric acid (8.5 mg/l) in vitro at the 4th successive stage of subculture. Addition of mangiferin (1×10^{-5} M), just prior to the 4th subculture stage, again arrested the formation of fusaric acid by this strain (CMI-IMI 225231) of the fungus. These observations are consistent with the reported[8] localized nature of the *F. moniliforme* infection of mango; the ingress of the fungal hyphae, presumably, being obstructed by the presence of abundant quantity of mangiferin. It further tends to suggest that the fungus proliferates through route(s) other than the xylem vessel.

Although increased product of mangiferin by the host impedes the normal growth and metabolism of *F. moniliforme*, it was not entirely without adverse side effects on the plant elaborating it. Thus the typical malformation syndrome, appearance of a large number of rudimentary leaves mingled with sterile flowers, seemed to be due to high concentration of mangiferin. This contention was supported from the following facts. In the 1-year-old mango plants into which aqueous solution of mangiferin was administered, a large number of branchlets with small leaves were emerged from the mangiferin-treated zone. The symptom was strikingly similar to the bushy growth of shoots observed in the malformation disease. The control plants did not produce such a symptom.

The culture fluid of the fungus caused complete abscission of the tender mango leaves when the shoots assumed the shape of a 'witche's broom'. This again is a common symptom of the malformation disease. The ability to cause abscission was not observed in the culture fluid treated with the tetracyclic triterpene. Xanthophylls, e.g. zeaxanthin and violaxanthin, which are liberally produced[3] by this strain of the fungus, were practically absent in the triterpene-treated culture fluid. In view of the fact that abscisic acid is derived, in vivo, from carotenoids[9,10] e.g. zeaxanthin and violaxanthin, this observation would seem to indicate the role, at least in part, of abscisic acid in the malformation of mango. The metabolic excursions reported above suggest that accumulation of mangiferin, in response to *F. moniliforme* infection, and secretion of carotenoid entities or moieties by the fungus are responsible for the malformation disease of mango.

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Antihypertensive and cardiac effects of two novel β-adrenoceptor blocking drugs

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Summary. Two new β-adrenoceptor blocking drugs with acute antihypertensive and positive inotropic effects are described: Compound A (2-[4-(3-tert.butylamino-2-hydroxypropoxy)phenyl]-4-trifluoromethylimidazole) and MK-761 (2-(3-tert.butylamino-2-hydroxypropoxy)-3-cyanopyridine hydrochloride). In SH rats both compounds, given orally, lowered arterial pressure and were more potent than hydralazine. The antihypertensive effect of compound A but not of MK-761 was antagonized by timolol. Both compounds had positive inotropic activity on cat heart papillary muscles; these effects were antagonized by timolol. The pretreatment of animals with reserpine greatly reduced the positive inotropic effect of MK-761 but not of compound A. The acute antihypertensive and positive inotropic effects of compound A are likely to be at least partially due to stimulation of β-adrenoceptors, e.g. intrinsic sympathomimetic activity. The effects of MK-761 on the same parameters appear to be mediated by different mechanisms.

In the search for β-adrenoceptor blocking drugs with acute antihypertensive activity, we studied a series of substituted trifluoromethylimidazoles and related compounds. Structure activity studies with a large series of related compounds are being reported elsewhere[2,3]. The antihypertensive effects of 2 selected compounds, 2-[4-(3-tert.butylamino-2-hydroxypropoxy)-phenyl]-4-trifluoromethylimidazole (compound A) and 2-(3-tert.butylamino-2-hydroxypropo-