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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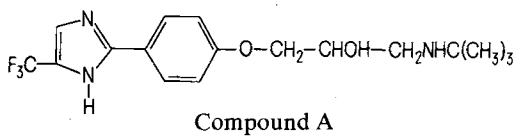
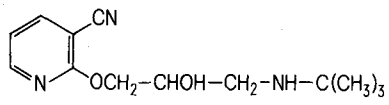
Summary. Two new β-adrenoceptor blocking drugs with acute antihypertensive and positive inotropic effects are described: Compound A (2-[4-(3-tert.butylamino-2-hydroxypropoxy)phenyl]-4-trifluoromethylimidazole) and MK-761 (2-(3-tert.butylamino-2-hydroxypropoxy)-3-cyanopyridine hydrochloride). In SH rats 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-

xy)-3-cyanopyridine hydrochloride (MK-761), in spontaneously hypertensive (SH) rats are described here.



Compound A



MK-761

Spontaneously hypertensive rats of the Wistar-Okamoto strain were purchased from Carworth Farms (Vincentown, NJ). Arterial pressure was recorded in conscious male rats of 290–350 g b.wt and 30–40 weeks of age by a direct technique involving cannulation of the caudal artery[4]. Mean arterial pressure and heart rate were printed at 0.5-h intervals through a data acquisition system (Data Graphics Corp., San Antonio, TX) by means of ASR-33 teletype units. Compound A was dissolved in diluted HCl; MK-761 and hydralazine were dissolved in distilled water. All drugs were administered in volumes of 2 ml/kg.

Both experimental compounds and hydralazine lowered arterial pressure in SH rats. The dose-response regression lines for compound A, MK-761 and hydralazine were calculated on the basis of experiments performed on different occasions but in the same laboratory and with the same technique; they are shown in figure 1. If the potency of hydralazine were equal to 1, the relative potency of compound A was 4.3 with 95% confidence limits of 2.1 and 13.1 and that of MK-761 3.4 with 95% confidence limits of 1.8 and 7.3. The potency of compound A did not differ significantly from that of MK-761; either of them was more potent than hydralazine. The onset and duration of action of the 3 compounds at 1 or 1.25 mg/kg p.o. are shown in figure 2. All 3 compounds had a rapid onset of action; the maximal effects of hydralazine and MK-761 were reached at 1 h after treatment while the maximal effect of compound A was obtained at 4 h after treatment. The antihypertensive effects of hydralazine and compound A were accompanied by cardiac acceleration while changes in heart rate seen with MK-761 did not differ significantly from those seen with 1% methylcellulose (figure 3).

At 8 mg/kg p.o. timolol, a β-adrenoceptor blocking drug[5], antagonized the acute antihypertensive effect of compound A, 0.312 m/kg p.o. but not of MK-761 at the same dose. At 2 mg/kg p.o. timolol did not significantly reduce the antihypertensive effect of compound A (table 1).

To study in vitro β-adrenoceptor stimulant and blocking effects of the 2 drugs, papillary muscles from the right veotricle of cat hearts were isolated in accordance with the technique of Cattell and Gold[6] and placed in a 100-ml bath containing solution C of Thorp and Cobbin[7]. The papillary muscles were stimulated electrically with square wave shocks of 4–10 msec duration, 6–10 V and 1-per-sec frequency from a Grass Model S-4A stimulator. Contractile force was recorded through a Statham UC-3 Universal transducing cell on a Honeywell Model 906 Visicorder. Positive inotropic effects on papillary muscles were used to estimate the intrinsic sympathomimetic activity of the 2 drugs. The effect was expressed as maximal increase in the contractile force within 30 min after addition of the test drug to the bath. To determine the involvement of the β-adrenoceptors in cardiac stimulant effect of the test compounds, other preparations were pretreated with timo-

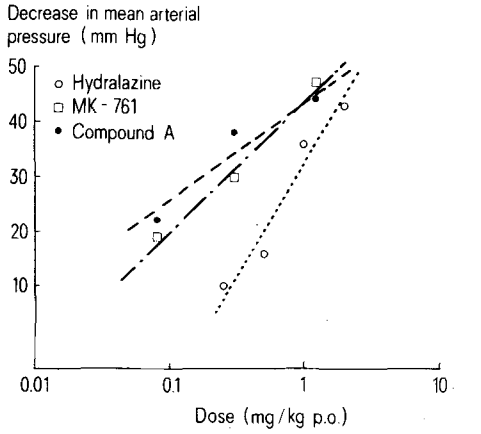


Fig. 1. Dose-response regression lines for the antihypertensive effects of compound A, MK-761 and hydralazine in SH rats. Maximal decreases in mean arterial pressure over a 24-h period after treatment are plotted against doses of the compounds. Average values for 5–13 observations at each dose level of each drug.

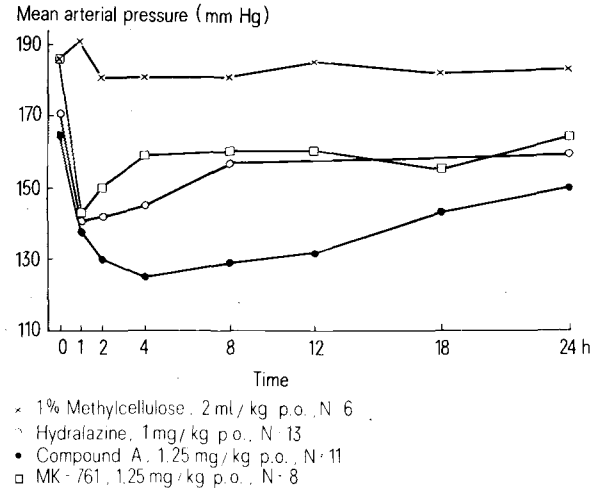


Fig. 2. Onset and duration of antihypertensive action of hydralazine, compound A and MK-761 in SH rats.

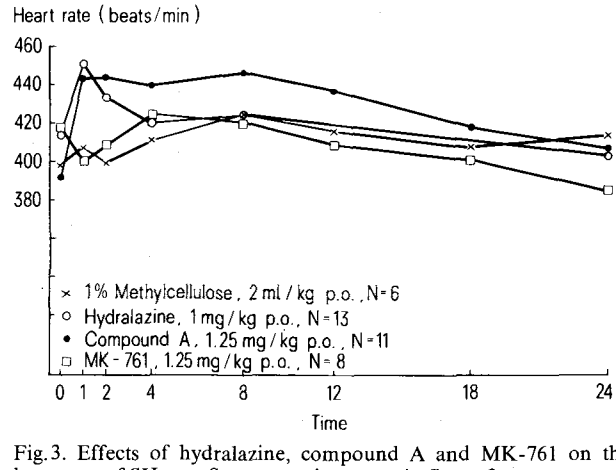


Fig. 3. Effects of hydralazine, compound A and MK-761 on the heart rate of SH rats. Same experiments as in figure 2.

Table 1. Antagonism of the antihypertensive action of compound A and and MK-761 by timolol in SH rats

Group No.	Compound	Dose mg/kg p.o.	No. of rats per group	Mean arterial pressure, mm Hg at hours after treatment 0	0.5	1	2	4	8	24
1	Saline	2 ml/kg	7	159	158	160	160	160	160	162
2	Compound A	0.312	5	165	153*	143*	144*	143*	140*	155
3	Timolol	2.0	4	163	165	162	163	165	167	167
4	Timolol	8.0	6	164	171	168	164	165	164	166
5	Timolol + compound A	2.0 0.312	4	171	159*	150*	151*	145*	155*	165*
6	Timolol + compound A	8.0 0.312	5	172	172	170	164	164	164	169
7	MK-761	0.312	8	180	160*	160*	172	177	175	162
8	Timolol + MK-761	8.0 0.312	4	174	169	159*	147*	148*	151*	140*

* Significantly different from control value at 0 time, $p < 0.05$.

Table 2. Effects on the contractile force of isolated cat heart papillary muscles – β-adrenoceptor stimulant activity

Treatment	Concentration (µg/ml)	No. of preparations	Contractile force Before treatment (mg ± SE)	Maximal increase during 30 min after treatment (mg ± SE)
Saline + saline	0.1 ml 0.1 ml	16	420 ± 29	0
Timolol + saline	0.2 0.1 ml	16	358 ± 29	0
Saline + compound A	0.1 ml 0.125	8	425 ± 46	+ 80 ± 15
Saline + compound A	0.1 ml 0.5	8	400 ± 29	+ 175 ± 15
Saline + compound A	0.1 ml 2.0	8	435 ± 43	+ 260 ± 39
Timolol + compound A	0.2 0.125	8	285 ± 42	0
Timolol + compound A	0.2 0.5	8	295 ± 30	+ 10 ± 7
Timolol + compound A	0.2 2.0	8	355 ± 46	+ 45 ± 19
Saline + MK-761	0.1 ml 0.125	8	415 ± 35	+ 45 ± 16
Saline + MK-761	0.1 ml 0.5	8	435 ± 44	+ 115 ± 16
Saline + MK-761	0.1 ml 2.0	8	425 ± 39	+ 205 ± 44
Timolol + MK-761	0.2 0.125	8	395 ± 31	+ 15 ± 7
Timolol + MK-761	0.2 0.5	8	350 ± 27	+ 95 ± 17
Timolol + MK-761	0.2 2.0	8	330 ± 30	+ 100 ± 20

Table 3. Effects on the contractile force of isolated heart papillary muscles from cats pretreated with reserpine. Average values for 8 preparations per treatment

Treatment	Concentration (µg/ml)	Contractile force Before treatment (mg ± SE)	Maximal increase during 30 min after treatment (mg ± SE)
Saline	0.1 ml	420 ± 35	0
Compound A	0.125	420 ± 43	+ 90 ± 31
Compound A	0.5	430 ± 32	+ 180 ± 39
Compound A	2.0	405 ± 37	+ 190 ± 40
Saline	0.1 ml	420 ± 35	0
MK-761	0.125	400 ± 37	0
MK-761	0.5	425 ± 42	+ 25 ± 20
MK-761	2.0	400 ± 13	+ 35 ± 19

lol, 0.2 µg/ml, 30 min prior to the test compound. The concentration of timolol was selected on the basis of previous experiments to produce nearly maximal β-blockade. The reduction of the positive inotropic activity of the test drugs by timolol was considered an evidence for β-adrenoceptor stimulant activity of the test drugs. The results are summarized in table 2. Compound A and MK-761 increased contractile force at 0.125, 0.5 and 2.0 µg/ml; the increase produced by compound A was more pronounced than that with MK-761 at the same concentrations. The calculation of relative potency for cardiac stimulant activity of the 2 drugs revealed that MK-761 was 0.4 times as potent as compound A. The 95% confidence limits of the relative potency were 0.2 and 0.9. Timolol almost completely abolished the positive inotropic effect of compound A and significantly reduced that of MK-761. In preparations from cats pretreated with reser-

Table 4. Antagonism of isoproterenol (1 μg/ml)-induced increase in the contractile force of isolated cat heart papillary muscles by compound A or MK-761 (β-adrenoceptor blocking activity)

Treatment	Concentration (μg/ml)	No. of preparations	Contractile force Before treatment (mg ± SE)	Increase after isoproterenol (mg ± SE)
Saline	0.1 ml	14	538 ± 76	+ 994 ± 113
Compound A	0.03	6	427 ± 72	+ 1040 ± 169
Compound A	0.125	6	487 ± 77	+ 647 ± 168
Compound A	0.5	6	473 ± 88	+ 213 ± 63
MK-761	0.03	8	1125 ± 257	+ 790 ± 132
MK-761	0.1	12	747 ± 135	+ 667 ± 93
MK-761	0.3	8	818 ± 130	+ 245 ± 32
MK-761	0.7	8	728 ± 128	+ 140 ± 19
MK-761	1.0	8	600 ± 92	+ 75 ± 19

pine, 0.5 mg/kg i.p., 16–20 h prior to experiments, compound A had positive inotropic activity similar to that observed in normal cats (tables 2 and 3) whereas the positive inotropic effect of MK-761 was nearly abolished by pretreatment with reserpine. This suggests that the positive inotropic effects of MK-761 on papillary muscles from normal cats do not involve direct stimulation of β-adrenoceptors by MK-761 but are mediated by release of catecholamines from the heart muscle.

The β-adrenoceptor blocking activity of the test compounds was estimated by their ability to antagonize the positive inotropic effect of isoproterenol, 1 μg/ml, also on papillary muscles. Test compounds were added at various concentrations to the bath 30 min prior to isoproterenol. Compound A as well as MK-761 reduced the positive inotropic effect of isoproterenol in dose-dependent manner (table 4). There was no significant difference in the potency of the 2 drugs as β-adrenoceptor blocking agents.

The above-described results suggest that both test compounds are effective β-adrenoceptor blocking drugs with acute antihypertensive and cardiac stimulant effects. The cardiac stimulant and acute antihypertensive effects of compound A are likely to be due, at least in part, to direct β-adrenoceptor stimulant activity (ISA) whereas the cardiac stimulant and the acute antihypertensive effects of MK-761 are more likely to be due to a mechanism or mechanisms other than direct stimulation of β-adrenoceptors. This is suggested by the inability of timolol to reduce the antihypertensive effects of MK-761 in SH rats and by reduction of the cardiac stimulant activity of MK-761 by reserpine.

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Failure to identify 'thrombocytolysin' (a spasmogenic factor released from platelets by immunoreaction) with anaphylatoxin

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Summary. Horse platelets release an unidentified smooth muscle contracting substance after lysis by antiserum and complement. Since the active factor (thrombocytolysin) does not produce tachyphylactic response of the guinea-pig ileum it seems that it is not related to anaphylatoxins.

Anti-platelet serum (APS), in the presence of fresh guinea-pig or rabbit serum as a source of complement, releases from washed horse platelets a factor that contracts guinea-pig ileum segments in vitro and causes hypotension in atropinized rabbits[1,2]. A few min after mixing the complete system at 38 °C, there is agglutination of the platelets which settle at the bottom of the tube. As lysis of the platelets progresses a clear supernatant is formed above a transparent viscous mass. Time-course experiments showed that a smooth muscle activity can be detected in aliquots taken from the supernatant. The activity increases, reaches a plateau and eventually drops after 2 h incubation. After contrifugation, the clear, supernatant can be boiled a few min with 2 volumes of ethanol without affecting the activity which is still present in the aqueous phase after removal of the coagulated proteins. An active crude powder was prepared by evaporation of the protein-free filtrate in vacuo at 50 °C (or freeze-drying) which remains active at −20 °C for at least 3 years.

The active principle(s) is water soluble and heat stable in neutral or acidic solutions but is rapidly destroyed by boiling at pH 9 or higher. It is insoluble in organic solvents.

The nature of this smooth muscle active factor has not yet been determined. Pharmacological studies showed that 'thrombocytolysin' (T) is different from histamine, acetylcholine, adenylic acid, ADP, tyramine, bradykinin, 5-HT