Phytopharmacologic Studies of *Ipomoea muricata* Seeds

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A glycoside muricatin ($C_{28}H_{52}O_{11}$), isolated from the seeds of *I. muricata*, appears to consist of 4-*O*-L-rhamnopyranocyl-L-rhamnopyranose moiety attached glycosidically to (+)-11 hydroxyhexadecanoic acid. It is cardiac depressant, spasmolytic to the smooth musculature of gut, lowers the blood pressure of the anesthetized dog, and has no action on the respiration, uterus, skeletal muscle, and blood vessels of the frog.

UNDER the research program on systematic phytochemical and pharmacological investigations of the plants belonging to genus *Ipomoea* (5, 6, 9–11), studies on the seeds of *Ipomoea muricata* were undertaken in this department. *I. muricata* (family, Convolvulacae), commonly known as kaladana, is found in the Himalyan region from Kangra to Sikkim and on Dacan hills. Seeds of the plant are bitter, purgative, and used as a substitute for *I. hyderacea* and in powder form as febrifuge (2, 4). Leaves are used in apthous (8). In this communication, characterization and preliminary pharmacodynamic actions of the glycoside muricatin isolated from the seeds of *I. muricata* are reported.

MATERIALS AND METHODS

Chemical—The glycoside muricatin has been isolated from the petroleum ether extract of the authenticated seeds of *I. muricata* by the method of Mishra and Tewari (7).

A chemical examination of the seeds of this plant was also made by Mishra and Tewari (7). They reported an enzyme, a lipoid, an organic acid, a glucoside, $C_{30}H_{54}O_{13}.1^1/_2H_2O$, and a dark brown resinous mass. However, the glycoside muricatin (m.p. 108–109°, $[\alpha]_D^{25} - 44.5°$) isolated in the present study is a white crystalline solid. It was found to be soluble in water, methanol, ethanol, sparingly soluble in acetone, and insoluble in ether, benzene, and chloroform. The elemental analysis of muricatin indicated the empirical formula $C_{28}H_{52}O_{11}$. This water-soluble glycoside was a weak acid with neutralization equivalent 543. Acid hydrolysis yielded 2 moles of L-rhamnose and 1 mole of fatty acid. On the basis of physical and chemical studies, muricatin appears to consist of 4-*O*-L-rhamnopyranocyl-L-rhamnopyranose moiety attached glycosidically to (+)-11-hydroxyhexadecanoic acid.

Pharmacodynamic Studies of Muricatin—The pharmacodynamic actions of this water-soluble glycoside muricatin have been studied on various organ systems. In each case 10 experiments were performed to draw the inference.

To study the effect of muricatin on the frog's heart, perfusion of the frog heart *in situ* and isolated frog's heart was carried out according to the method of Bulbring, as quoted by Burn (1). The ventricular contractions were recorded on a slowly moving drum through a starling heart lever.

The effect of drug was studied on the isolated frog rectus abdominis muscle preparation (set up in a 5-ml. bath), according to the method described by Burn (1). Acetylcholine responses were obtained by offering it in a strength of 1:2,000,000 for 90 sec.

The effect of drug on carotid blood pressure, respiration, carotid occlusion response, and intestine *in situ* was studied on anesthetized mongrel dogs of either sex, weighing between 6 and 10 Kg. Sodium pentobarbital (35 mg./Kg. i.p.) was used as an anesthetic agent. Blood pressure was recorded by introducing an arterial cannula in the left carotid artery and connecting the cannula to a mercury manometer. Respiration was recorded by connecting the trachea with Mary's tambur. All the injections were made through the cannulated femoral vein. Auricular and ventricular contractions were registered in anesthetized dogs by the suspension method of Jackson (3). Intestinal movements in dogs were recorded through Jackson's enterograph.

In the dog the common carotid arteries of both sides were exposed. Gentle pressure by putting bull dog clips on both the carotid arteries for 10 sec., at a point just below the bifurcation of carotid arteries into external and internal carotid arteries, brought about occlusion and rise in blood pressure. In the case of carotid occlusion studies in dogs, the blood pressure was recorded from the femoral artery.

The action of muricatin was also observed on electrically induced contractions (preganglionic stimulation) of the cat's nictitating membrane. Experiments were also performed to study the effect of the drug on blood pressure of the cat, following spinal transection at the C_2 level, using the method of Burn (1).

The effect of the drug was observed on isolated preparations of rabbit's and rat's ileum. The freshly removed segments were suspended in a 10-ml. isolated organ bath, containing Tyrode solution (NaCl, 8.0 Gm.; KCl, 0.2 Gm.; $MgCl_2$, 0.5 Gm.; $NaHCO_3$, 1.0 Gm.; Na_2HPO_4, 0.5 Gm.; distilled water, 1 L.) maintained at 37 ± 1° and freely oxygenated.

The action was also observed on isolated uterus of the gravid and nongravid albino rat, guinea pig, and rabbit. One of the uterine horns was suspended in a 10-ml. bath containing oxygenated modified Locke's solution (NaCl, 9.0 Gm.; KCl, 0.42 Gm.; $CaCl_2$, 0.06 Gm.; glucose, 0.5 Gm.; $NaHCO_3$, 0.5 Gm.; distilled water, 1 L.) at 32°. The effect of a single dose was recorded for 90 sec. and an interval of 5 min. was allowed between the successive doses.

Blood vessels of the frog were perfused with Ringer's solution (NaCl, 0.65%; KCl, 0.014%; $CaCl_2$, 0.012%; $NaHCO_3$, 0.02%; Na_2HPO_4, 0.001%; distilled water, 100 ml.) by introducing a cannula in the innominate artery and counting the drops of perfusate. Injections of the drug were made in the ventral lymph sac.

OBSERVATIONS

Muricatin in graded doses causes a decrease in rate, amplitude, and tone of the frog's heart *in situ* and isolated frog's heart. The repetition of higher doses produced tachyphylaxis. The cardiac depressant

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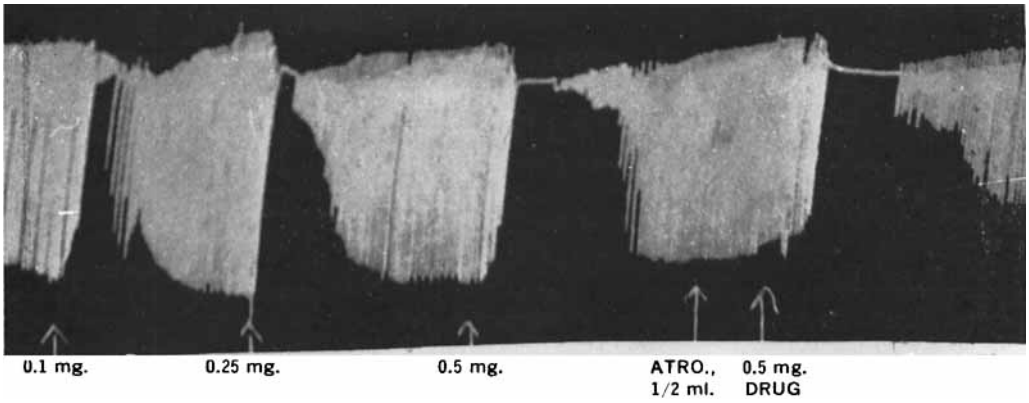

Fig. 1—Effect of muricatin on isolated frog's heart.

effect of the drug could not be blocked by pretreatment with atropine, which, however, blocked the activity of equidepressant doses of acetylcholine (Fig. 1).

Muricatin (1 mg./Kg.) caused a sharp fall in blood pressure (Fig. 2) without having any significant action on respiration of anesthetized dogs. This effect was also noticed in cats. Atropine sulfate (2 mg./Kg.) partially blocked this hypotensive effect of muricatin. But unlike acetylcholine, the drug did not give any pressure response when given after administration of atropine and eserine sulfate (1 mg./Kg.). This hypotensive effect in cats persists to the same degree even after spinal transection at the C_2 level. The hypotensive effect was more marked in experimentally induced hypertension in dogs with norepinephrine infused at the rate of 20 drops/min. at a concentration of 1:1000. Muricatin inhibited both auricles and ventricles *in situ* in dogs. The drug had no significant effect on the dog's carotid occlusion reflex and ileum *in situ* nor on electrically induced contractions of the cat's nictitating membrane.

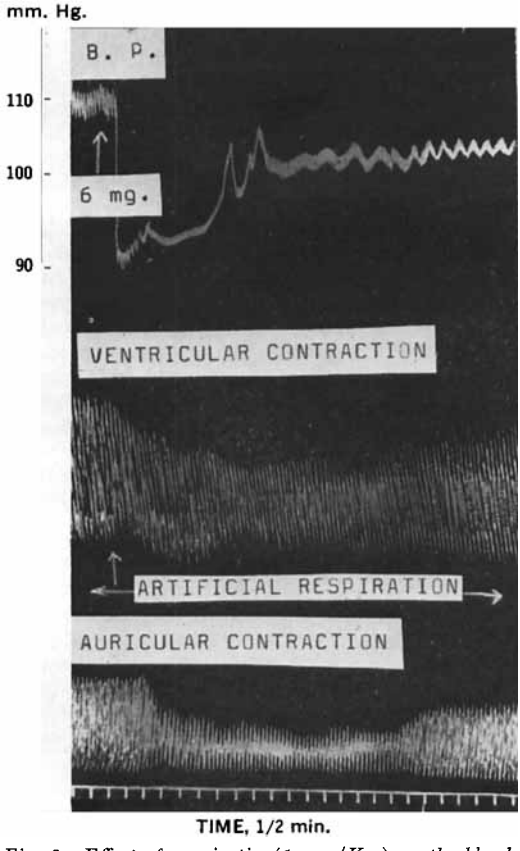

Fig. 2—Effect of muricatin (1 mg./Kg.) on the blood pressure and auriculo ventricular contractions of a female dog (6 Kg.) anesthetized with pentobarbital.

On isolated rabbit and rat ileum, muricatin in all the doses tried exhibited a spasmolytic activity. In these doses this was quite marked in magnitude. The antispasmodic activity of muricatin against spasms induced by posterior pituitary, acetylcholine chloride (0.2 mcg./ml.), histamine diphosphate (0.2 mcg./ml.), barium chloride (0.1 mg./ml.), and 5 HT (0.4 mcg./ml.) in isolated ileum of rabbit and rat has been studied. A 0.5-mg. quantity (in a bath of 10 ml.) of the drug antagonized these spasms (Fig. 3). The spasmolytic activity of papaverine was also of similar pattern, but it was about 4 to 5 times stronger in activity than muricatin in this respect.

The drug failed to have any effect on uterine strips of the various species tried and did not antagonize the spasm of the uterus induced by posterior pituitary. On the frog rectus muscle the drug had no action at any concentration. It did not affect the acetylcholine induced contractions. Muricatin had no effect on the perfused blood vessels of the frog at any concentration.

CONCLUSIONS

Ipomoea provides many plants which are employed in the Ayurvedic system of medicine. A glycoside has been isolated from *I. carnea* (12), but its pharmacological properties have not been reported.

The glycoside muricatin isolated from *I. muricata* in the present communication is water-soluble and thermostable. It shows a characteristic depressant action on the frog's heart. In older work on glycosides this was the principal effect on which attention was focused. In the initial stages fuller contractions in systole occur; various arrhythmias develop later (13).

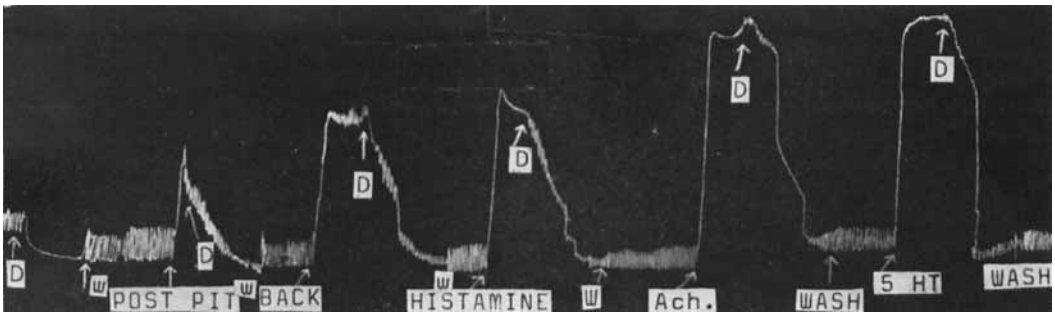

Fig. 3—Effect of muricatin on isolated rabbit ileum. Key: D, *drug (0.5 mg.).*

The blood pressure lowering property of muricatin is a characteristic response, which is partially blocked by atropinization. But this lowering of blood pressure persists even after spinal transection at the C_2 level. Therefore, it can be inferred that the lowering of blood pressure may be due to a myocardial depressant property of muricatin as evidenced by the observations on auricular and ventricular contractions.

The drug shows its benignity toward other organ systems of the body except a direct potent spasmolytic effect on gastrointestinal smooth musculature. In general, the pattern of relaxant and spasmolytic activity of muricatin was similar to that of papaverine. The difference was that the former was a weaker relaxant and spasmolytic compared to the latter. It therefore appears that the mechanism of action of muricatin on ileum is a papaverine-like, direct, nonspecific musculotropic action.

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Testing of Tablets with Prolonged Action. Enzyme Activity During the Modified Half-Change Method

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The enzyme activities of pepsin, pancreatic lipase, trypsin, and amylase were studied. It was found that the activity of the enzymes in freshly prepared artificial gastric fluid and in artificial intestinal fluid decreases during heating from room temperature to 37° and by keeping at 37°. To maintain proper enzyme levels during an 8-hr. interval when testing tablets with prolonged action, a modified half-change method is suggested. A survey of enzyme levels when using this modified method is given.

MANY SUBSTANCES used in tableting as excipients and fillers, such as zein, starch, stearates, and others, are digestible by gastrointestinal enzymes. Therefore, the possible effect of enzymes on disintegration and/or drug dissolution of oral tablets in *in vitro* tests must be taken into consideration, particularly in the case of procedures of several hours' duration applicable to sustained-release medication. In prolonged-release medication forms the drug is often embedded in synthetic fats such as glyceryl monostearate (1), glyceryl myristate (1), glyceryl palmitostearate (2), *etc.* Such glyceryl fatty acid esters are hydrolyzed by pancreatic lipase (3). The enzymatic hydrolysis depends on the length of the carbon chain of the fatty acids (4). The liberation of the active principle embedded in the tablet follows a characteristic inverted curve, because beside diffusion of the drug, digestion of the tablet base material takes place after 3 hr. when optimal pH conditions are reached for the enzyme activity (5). Gelatin, used as a binder in wet granulation as well as in direct compression, is liquified by trypsin (6), while casein (7) and peptides used for coatings (8) are digested. Amylose, suggested as a dry binder for direct compression (9), is digested by amylase. Gelatin, casein, and other peptides are also digested by pepsin (10).

Studying the stability of gastrointestinal enzymes in artificial gastric and intestinal fluid, it was found that the activity of the enzymes was partly destroyed during the time needed to heat the test solutions from room temperature to 37°, and while

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