and incubated in a humidified oven at 75° for 15 min. An additional plate from each solvent system was sprayed with a silver nitrate–ammonium hydroxide mixture[4] and exposed to an intense source of ultraviolet light for 5 min. A third plate was sprayed with a 1% solution of diphenylamine in methanol and exposed to ultraviolet light (4).

To help identify the metabolites, 1 mmole of CPD and 1.25 mmoles of NaOH were placed in 100 ml. of water and shaken for 2 hr. at 37°. The aqueous solution was concentrated to 25 ml. and extracted three times with anhydrous ethyl ether. The ether extracts were combined and evaporated to dryness. The residue was dissolved in 5 ml. of ether and 10-μl. portions spotted on each plate.

RESULTS

Table I gives the R_f values of the urine samples and reference compounds in a benzene–ethyl acetate solvent system and a 2-propanol–ammonium hydroxide solvent system sprayed with various identifying reagents. Two spots appeared on each plate from each solvent system sprayed with the naphthoresorcinol reagent which visualizes glucuronides. Both spots appeared in the test urines not treated with β-glucuronidase. No spots appeared in either the control urines or the enzyme-treated test urines, suggesting that the metabolites of CPD are glucuronide conjugates. On plates sprayed with silver nitrate–ammonium hydroxide mixture which visualizes organic chlorides, spots appeared in both the untreated and treated test urines, indicating that organically bound chlorine is present.

However, the spots in the untreated urine did not correspond to those in the enzyme-treated urines. Finally, one plate from each solvent system was sprayed with diphenylamine which colors organic nitrate esters. Spots appeared in both the treated and untreated test urines but they did not correspond to each other.

The partial alkaline hydrolysis of CPD was used to produce a mixture of the two mono nitrate esters and the completely hydrolyzed 1-chloro-2,3-propanediol. The conclusive identification of which spot corresponds to which mono nitrate ester could not be made without very sophisticated instrumentation, and the introduction by synthesis of only one nitrate group into the molecule without two or a mixture of the mono nitrates is nearly impossible. The spots from the partially hydrolyzed CPD, which could only be a mixture of the mono nitrates, do indeed correspond to the spots from the urine hydrolyzed by β-glucuronidase.

[4] One part by volume of a 1% solution of silver nitrate in ammonium hydroxide is mixed with 1 part by volume of ethanol (4).

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Smooth Muscle and Cardiovascular Pharmacology of α-Elaterin-2-D-glucopyranoside Glycoside of *Citrullus colocynthis*

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The active principle present in the pulp of *Citrullus colocynthis* which exhibits cathartic abortifacient activity is not yet well established. The cardiovascular and smooth musculature activity of a recently identified glycoside, α-elaterin-2-D-glucopyranoside (coloside A) was therefore carried out to discover if it was the purgative or the ecbolic principle. Coloside A failed to stimulate the uterus, but elicited purgative properties. It demonstrated antihistaminic and antiacetylcholine-like activity on the intestinal musculature and exhibited negative chronotropic and negative inotropic activity in isolated mammalian and amphibian heart.

IN THE Ayurvedic system of medicine, the pulp of *Citrullus colocynthis* is recommended as a purgative (1) and is known to cause miscarriage when administered to pregnant women. The abortive activity of *C. colocynthis* is considered to be an indirect action, a manifestation of the cathartic activity of the drug produced by the congestion in the pelvic region (2). However, the possibility of some direct action on the uterus cannot be completely ruled out, since Stimpson (3) had shown that alcohol-free tincture of *C. colocynthis* produced increased tone and amplitude of contraction of isolated rabbit uterus. It is therefore likely that besides causing congestion in the pelvic region *C. colocynthis* possesses some principle which directly stimulates the uterus.

There is a controversy regarding the constituent responsible for its purgative action. From the water extract of the pulp, Walz (4) isolated a glycoside, colocynthin, which was believed to be the purgative principle. Subsequent workers, however, failed to isolate the glycoside from the water-soluble fraction. Power and Moore (5) after detailed chemical and pharmacological studies concluded that *C. colocynthis* does not contain any glycoside, and the purgative principle resided in the chloroform and ether-soluble resins and amorphous alkaloid (6–8). Lavie *et al.* (9) isolated a glycoside from the chloroform extract of the pulp of *C. colocynthis* which was identified by Khadem and Rahman (10, 11) to be α-elaterin-2-D-glucopyranoside. Since no systemic

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pharmacological studies have yet been made with this compound, the smooth musculature and cardiovascular pharmacology of α-elaterin-2-D-glucopyranoside was undertaken. The glycoside α-elaterin-2-D-glucopyranoside will be referred to as coloside A in the text.

EXPERIMENTAL

Coloside A (m.p. 156°) was isolated from the Indian variety of the pulp of *C. colocynthis* by the method described by Khadem and Rahman (11). The solution of the drug was made by dissolving a weighed quantity of the compound in a known minimal quantity of warm ethanol, and the volume was made up with warm water so that solution did not contain more than 10% of ethanol. Experiments were run simultaneously with the solution of coloside A and the solvent containing 10% ethanol. Since the control experiments with the solvents, *i.e.*, 10% ethanol, did not exhibit any pharmacological action, only the activity of coloside A is described here.

Smooth Muscle—The isolated uterus of rat, the intestine of rabbit, and the ileum of guinea pig were set up by the methods described by Smith (12) and Turner (13).

The interaction between coloside A and uterine stimulants, such as oxytocin 0.002 units/ml. and ergometrine 0.01 mg./ml., were studied on the isolated rat uterus suspended in oxygenated de Jalons solution as described by Burn (14).

Antiacetylcholine, antihistaminic, and antibarium chloride activity of the glycoside was determined both on rabbit intestine and guinea pig ileum. The doses employed and preparations used are shown in Table I.

TABLE I—DOSES AND PREPARATIONS

Drug	Rabbit Intestine, mcg./ml.	Guinea Pig Ileum, mcg./ml.
Acetylcholine	0.5	5
Histamine	5	0.1
Barium chloride	0.2	0.2

Cardiovascular System—The experiment of frog heart perfusion was carried out by Bulbring's method as described by Burn (15). For the Straub heart preparation the excised frog heart was prepared according to the Straub procedure as quoted by Gaddum (16). Perfusion of the isolated rabbit heart was performed by the modified method of Langendorff as described by Burn (17).

To study the effect of coloside A on the blood pressure and heart *in situ*, mongrel dogs of either sex, between 7–12 Kg. weight, were employed and anesthetized with morphine, 5 mg./Kg., followed 15 min. later by urethan, 1.4 Gm./Kg., both given intramuscularly. The left common carotid artery was cannulated and blood pressure was recorded on a slowly moving kymograph. The drug was administered through the cannulated right femoral vein. The chest was then opened under artificial respiration. A bent entomological pin passed near the tip of the ventricle and was attached to a Starling lever after passing through a pulley. Similar arrangements were made to record auricular beats.

RESULTS

Results described below are the average of three experiments.

Smooth Muscle—*Isolated Rat Uterus*—Coloside A did not produce any contractions of the rat uterus in concentration up to 0.1 mg./ml. On the other hand, it exhibited a uterine depressant property. Though the drug did not elicit any relaxation of the uterus in concentrations up to 0.1 mg./ml., it decreased the rate and amplitude of contraction. Coloside A also failed to alter the response produced by uterine stimulants such as oxytocin and ergometrine.

Isolated Rabbit Intestine and Guinea Pig Ileum—Coloside A did not produce any spasmogenic activity on the isolated intestine. Neither did it affect the rhythmic movements of rabbit intestine, nor did it produce any contraction of the guinea pig ileum suspended in Tyrode's solution up to 0.1 mg./ml. However, it exhibited antihistaminic and antiacetylcholine-like action which is described in Table II. Coloside A did not counteract the action of barium chloride on intestine.

TABLE II—ANTIACETYLCHOLINE AND ANTIHISTAMINIC ACTIVITY OF COLOSIDE A[a]

Dose, mg./ml.	Prepn.	Stimulant	Dose, mcg./ml.	% Inhibition
0.1	Rabbit intestine	Acetylcholine	0.5	43
0.1	Rabbit intestine	Histamine	5.0	92
0.05	Guinea pig ileum	Acetylcholine	5.0	27
0.1	Guinea pig ileum	Acetylcholine	5.0	49
0.05	Guinea pig ileum	Histamine	0.1	67
0.1	Guinea pig ileum	Histamine	0.1	90

[a] Three experiments were performed in each case.

Cardiovascular System—*Frog Heart Perfusion*—Coloside A diminished the force of contraction of the ventricle and reduced the heart rate. A dose of 1 mg. decreased the amplitude of contraction and heart rate by 35 and 15%, respectively. Results are shown in Table III.

TABLE III—EFFECT OF COLOSIDE A ON THE FROG HEART PERFUSION[a]

Dose, mg.	% Decrease in Amplitude	% Decrease in Heart Rate	Recovery, Time, min.
0.25	0	0	0
0.50	12	7	12
1.00	36	16	22

[a] Three experiments were performed in each case.

Straub Heart Preparation—When a dose of 1:10,000 of the drug was administered to a Straub heart preparation, the amplitude of contraction was decreased by 70%, and the heart rate was reduced by 25%. At 1:10,000 concentration, it produced a mid-diastolic arrest (Table IV).

TABLE IV—EFFECT OF COLOSIDE A ON THE STRAUB HEART PREPARATION[a]

Dose	% Decrease in Amplitude	% Decrease in Heart Rate
1:100,000	11	0
1:10,000	70	23
1:1000	Mid-diastolic arrest	Mid-diastolic arrest

[a] Three experiments were performed in each case.

Isolated Rabbit Heart—The glycoside exhibited a negative chronotropic and a negative inotropic effect and reduced the coronary flow. At a 3-mg. dose it produced a mid-diastolic arrest (Table V).

TABLE V—EFFECTS OF COLOSIDE A ON THE ISOLATED RABBIT HEART[a]

Dose, mg.	% Decrease in Amplitude	% Decrease in Coronary Flow	% Decrease in Heart Rate
0.05	37	14	23
0.3	Mid-diastolic arrest	Mid-diastolic arrest	Mid-diastolic arrest

[a] Three experiments were performed in each case.

Dog Heart In Situ—Coloside A had no effect on this preparation up to 5 mg./Kg. dose level.

Dog Blood Pressure—A dose of 5 mg./Kg. of the drug failed to produce any significant hypotensive action.

DISCUSSION

The pulp of *C. colocynthis* is well-known for its cathartic and abortifacient activity. But the chemical constituents responsible for the purgative and ecbolic activity have not yet been definitely identified. A controversy exists regarding the nature of the cathartic principle. No compound has so far been isolated which exhibits uterine stimulant properties. An attempt was therefore made to distinguish and to isolate in pure form the cathartic and oxytocic principles of the drug.

Coloside A has been found to possess purgative properties.[1] However, it did not produce any contraction of the rat isolated uterus. Thus, coloside A is the cathartic principle without any oxytocic activity. It exhibited a uterine depressant property and decreased the rate and amplitude of contraction, and demonstrated antihistaminic and antiacetylcholine activity to confirm the finding of Hollander (18), who reported that a solution of colocynth blocked the parasympathetic nerve endings. It does not possess a direct depressant activity on the intestine, as it failed to counteract the response of barium chloride.

The drug produced a negative chronotropic and a negative inotropic effect on isolated mammalian and amphibian hearts. Similar activity was reported on the rabbit heart by Stimpson (3) in the alcoholic extract of the pulp of *C. colocynthis*. Since coloside A is a steroidal glycoside and shows cardiac depressant properties, this drug may be a cardiotonic. Further work on this aspect would be fruitful.

Coloside A did not have cathartic activity when administered intraperitoneally, although orally it produced drastic purgation. Since the drug is believed to bring about purgation by irritation of the intestine, one possibility is that when it is given by a route other than oral, the concentration of the glycoside in the lumen of the intestine is not sufficiently high to exhibit any activity. However, Gaddum (19) has suggested that bile is necessary for the activity of the pulp of *C. colocynthis*. It is plausible to assume that the active principle of *C. colocynthis* forms some complex with the bile to manifest its activity. Erspamer and Paolini (20) have shown that antihistaminics slow down the purgative response of *C. colocynthis*. Surprisingly enough coloside A has been found to be an antihistaminic. It is probable that coloside A is an antagonist at the histaminic receptors with affinity and no intrinsic activity, while the complex is an agonist and possesses both affinity as well as intrinsic activity on the same receptors.

[1] Unpublished observations.

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