Biotransformation Studies on 1-Chloro-2,3-propanediol Dinitrate

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Metabolic studies on the coronary vasodilator, 1-chloro-2,3-propanediol dinitrate (CPD), show that two metabolites can be isolated from the urine of dogs receiving the compound orally. The data suggest that the biotransformation of CPD commences with the splitting of the nitrate ester forming a free hydroxyl group which is then conjugated with glucuronic acid. The probable metabolites are 1-chloropropyl-3-glucuronide-2-nitrate and 1-chloropropyl-2-glucuronide-3-nitrate. Upon treatment with β-glucuronidase, these metabolites correspond to the products from the partial hydrolysis of CPD, which could only be the mono nitrate esters.

IN THE TREATMENT of coronary insufficiency, glyceryl trinitrate is the standard coronary vasodilator with which others have been compared. Krantz *et al.* (1) have shown that the number of nitrate groups in the molecule is not the major factor in determining vasodilating potency. Activity was found to be due to the intact molecule and not to the hydrolysis products of the ester. Likewise, the oil/water coefficient was found to be a factor in determining potency.

In recent years the search has been directed to related compounds that show a greater specificity for the coronary arteries and provide a longer duration of action with less side effects. Toward this end, 1-chloro-2,3-propanediol dinitrate[1] (CPD) was investigated. Burgison *et al.* (2) have shown that CPD and glyceryl trinitrate are equipotent in increasing coronary flow in the Langendorff rabbit heart preparation, and the latter appears to evoke a lesser degree of depressor response in the dog. Further, they have shown that the chlorinated ester manifests more persistent activity, and clinical trials (3) have confirmed these findings.

Previous metabolic studies on coronary vasodilators in this laboratory have shown that no CPD could be found in the urine of dogs which had received the compound orally. The logical step in metabolism would be the hydrolysis of the nitrate ester to the free hydroxyl group and conjugation with glucuronic acid. It was also of interest to see if the chlorine atom was cleaved during biotransformation.

METHODS

Three female mongrel dogs were fasted for 24 hr., and the urine was then collected for an additional 24 hr. as a control. On the next day, the dogs were again fasted for 24 hr., then 6 mg. of CPD was administered orally, and the urine was collected for 24 hr. after administration of the compound. The test urines were evaporated to dryness at 40° *in vacuo*. The residues were dissolved in buffer (pH 4.5) consisting of 5.79 Gm. $CH_3COONa \cdot 3H_2O$ and 3.25 ml. of glacial acetic acid in 1 L. of distilled water. Each test urine was then divided into two parts. To one part β-D-glucuronide glucuronohydrolase (β-glucuronidase) from *Helix pomatia*[2] was added, after which each part was incubated at 37° for 2 hr. Each part was then evaporated to dryness at 40° *in vacuo* and the residues were extracted three times with 5-ml. portions of anhydrous ethyl ether and the ether extracts were combined. The volume was reduced to 5 ml. by spontaneous air evaporation. The control urines were treated similarly but without the enzyme β-glucuronidase. A 10-μl. portion of the control, of the enzyme-treated sample, and of the untreated urines of each dog were spotted on thin-layer chromatography plates coated with silica gel + fluorescein (0.25 mm.) which had been activated at 100° for 30 min. The plates were developed by ascending techniques with two solvent systems, one a 2-propanol–ammonium hydroxide (4:1) mixture, the second a benzene–ethyl acetate (4:1) mixture. One plate from each solvent system was sprayed with a 1,3-dihydroxynaphthalene (naphthoresorcinol)–trichloroacetic acid mixture[3]

TABLE I—COMPARISON OF URINARY METABOLITES OF CPD TO SELECTED REFERENCE COMPOUNDS

Material	Spray	Solvent System: Benzene–Ethyl Acetate (4:1)	Solvent System: 2-Propanol–Ammonium Hydroxide (4:1)
Control urine	[a]	No spots appeared	No spots appeared
Control urine	[b]	No spots appeared	No spots appeared
Control urine	[c]	No spots appeared	No spots appeared
Untreated urine	[a]	0.07, 0.10	0.30, 0.43
Untreated urine	[b]	0.07, 0.10	0.30, 0.43
Untreated urine	[c]	0.07, 0.10	0.30, 0.43
Treated urine[d]	[a]	No spots appeared	No spots appeared
Treated urine	[b]	0.17, 0.27	0.38, 0.53
Treated urine	[c]	0.17, 0.27	0.38, 0.53
CPD	[a]	No spots appeared	No spots appeared
CPD	[b]	0.67	0.87
CPD	[c]	0.67	0.87
CPD (partial alkaline hydrolysis)	[a]	No spots appeared	No spots appeared
	[b]	0.17, 0.27, 1.00	0.38, 0.53, 0.77
	[c]	0.17, 0.27	0.38, 0.53
Chloroacetic acid	[a]	No spots appeared	No spots appeared
Chloroacetic acid	[b]	0.23	0.47
Chloroacetic acid	[c]	No spots appeared	No spots appeared
3-Chloropropionic acid	[a]	No spots appeared	No spots appeared
	[b]	0.30	0.50
	[c]	No spots appeared	No spots appeared
3-Chloro-1,2-propanediol	[a]	No spots appeared	No spots appeared
	[b]	1.00	0.77
	[c]	No spots appeared	No spots appeared
3-Chloro-1-propanol	[a]	No spots appeared	No spots appeared
	[b]	1.00	0.40
	[c]	No spots appeared	No spots appeared

[a] Naphthoresorcinol–trichloroacetic acid. [b] Silver nitrate–ammonium hydroxide. [c] Diphenylamine–methanol. [d] Urine incubated with β-glucuronidase.

Received July 10, 1967, from Huntingdon Research Center, Towson, MD 21204

Accepted for publication August 21, 1967.

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[1] Supplied as Bangina by Organon, Inc.

[2] Boehringer Mannheim Corporation, GRD/ARS 15427.

[3] One part by volume of an alcoholic naphthoresorcinol solution (0.2 Gm. naphthoresorcinol dissolved in 100 ml. ethanol) is mixed with 1 part by volume of an aqueous 20% solution of trichloroacetic acid (4).

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

Received June 1, 1967, from the Department of Pharmacology, S.M.S. Medical College, Jaipur, India.
Accepted for publication August 14, 1967.