Biopharmaceutical Studies on Guaiacol Glycerol Ether and Related Compounds IV: Drug in Blood and Bile

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Abstract □ Blood levels of guaiacol glycerol ether mononicotinate in animals were determined, and it appears that the chemical is absorbed with pseudo-first-order reaction by passive transport. Guaiacol glycerol ether mononicotinate does not affect blood pressure and respiration. The compound has no effect on bile flow and the cholesterol value in bile, but it increases the amount of cholesterol excreted in the bile of hypercholesteremic rats receiving polyoxyethylene ethers intravenously. The effect of the metabolites of the compound on cholesterol content in bile was also investigated.

Keyphrases □ Guaiacol glycerol ether mononicotinate—biopharmaceutical studies □ Cholesterol excretion—guaiacol glycerol ether mononicotinate effect □ Colorimetric analysis—spectrophotometer

The hypocholesteremic effects of guaiacol glycerol ether (GGE) and guaiacol glycerol ether mononicotinate (GGE-MN) in animals have been reported (1). In a previous report (2), metabolites of GGE and GGE-MN in rabbit urine were discussed.

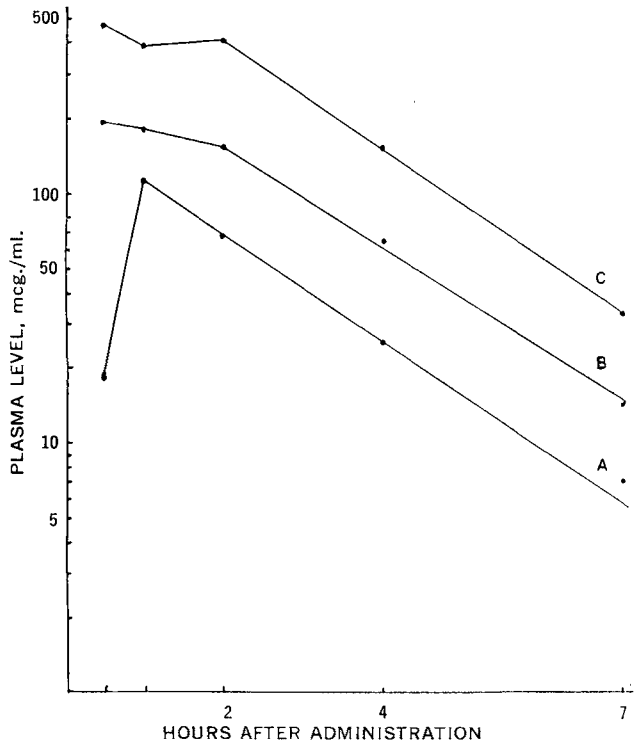

Figure 1—*Mean plasma level of GGE-MN after oral administration to mice at different doses. Key: A, 100 mg./kg.; B, 200 mg./kg.; and C, 300 mg./kg. GGE-MN was screened through 100 mesh and prepared in suspension form with water. Groups consisted of 10 male mice (dd strain); animals were killed according to the sampling schedule. At time of sacrifice, animals were bled completely, and the equivoluminal mixture of the blood from each group was reserved for analysis. All data concerned with mice show mean values of each group.*

The present study was designed to determine the blood levels of GGE-MN in animals and the effects of it and related compounds on bile in animals and the mechanism of hypocholesteremic action.

EXPERIMENTAL

Determination of GGE-MN in Blood (3)—To 1 ml. of plasma in an ice-cold centrifuge tube, 2 ml. of ethanol was added, and the mixture was stirred thoroughly. After the mixture stood for 15 min. in an ice-water bath, 0.5 ml. of the supernatant from centrifugation was mixed with 1 ml of ethanol, 2 ml. of ammonia buffer solution, and 3 ml. of 10% cyanogen bromide solution. The solution was held at room temperature for 15 min., and absorbance was determined at 400 mμ. A mixture of 0.9 ml. of normal animal plasma and 0.1 ml. of ethanolic GGE-MN solution of known concentration was treated as just described to prepare a calibration curve.

Ammonia buffer solution was prepared to make the equivoluminal mixture of 0.1 *M* NH_4OH, 0.5 *M* K_2HPO_4, and 2.0 *M* NH_4Cl.

Male mice (dd strain, mean body weight 20 g.), female rats (Wistar strain, mean body weight 200 g.), or male rabbits (mean body weight 2 kg.) were used in the study.

Collection of Rabbit and Rat Bile—Rabbits varying in weight from 3.0 to 3.5 kg. were anesthetized with urethan, 1 g./kg. s.c., following a fasting period of approximately 24 hr.

Blood pressure was recorded by a mercury manometer connected

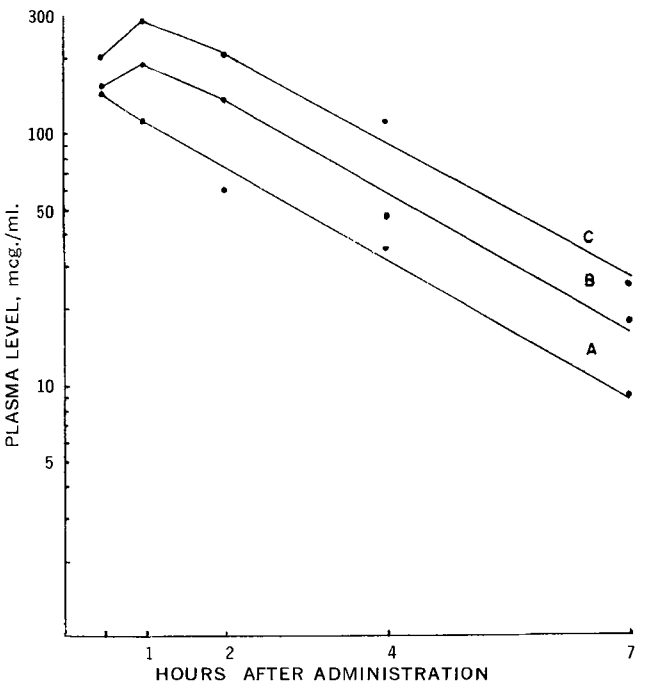

Figure 2—*Mean plasma level of GGE-MN after oral administration to rats at different doses. Key: A, 100 mg./kg.; B, 200 mg./kg.; and C, 300 mg./kg. GGE-MN was screened through 100 mesh and prepared in suspension form with water. Each female rat, five rats per group (Wistar), was killed according to the sampling schedule. At the time of sacrifice, each animal was bled completely and the blood was reserved for analysis.*

Table I—Pharmaceutical Formulas of GGE-MN Administered to Rabbits

Sample No.	Form	Composition
1	Capsule	GGE-MN,[a] 100 mg.; lactose, 125 mg.; and silicic acid anhydrous, 5 mg.
2	Capsule	GGE-MN, 100 mg.; microcrystalline cellulose,[b] 75 mg.; and silicic acid anhydrous, 5 mg.
3	Tablet	GGE-MN, 100 mg.; microcrystalline cellulose, 84 mg.; CMC-Na, 6 mg.; and talc, 10 mg.
4	Tablet	Film coated on the tablet of Sample 3 by cellulose acetate phthalate solution

[a] GGE-MN was screened through 100 mesh. [b] Avicel, Asahi Kasei Kogyo Co. Ltd., Japan.

to a cannula in a carotid artery, and respiration was recorded on a kymograph.

A polyethylene tube was inserted into a bile duct, and the bile was collected for 7.5 hr. following administration of the compound under continuous anesthesia using urethan. At the end of the 1st (control) hr., the compound was administered orally by a stomach tube.

Rats (Wistar strain), varying in body weight from 160 to 190 g., were treated by the same method as the rabbits.

Determination of GGE-MN in Rabbit and Rat Biles—One milliliter of bile instead of plasma was treated according to the method for determination of GGE-MN in blood after oral administration of GGE-MN by a stomach tube.

Collection of Rat Bile at Polyoxyethylene Ethers Hypercholesteremia—Male rats of Wistar strain, weighing from 160 to 190 g., were injected intravenously with 400 mg./kg. of tyloxapol[1] in distilled water, adjusting the volume to 0.2 ml./100 g. of body weight. The test sample was administered intraperitoneally immediately after injection of polyoxyethylene ethers. Collection

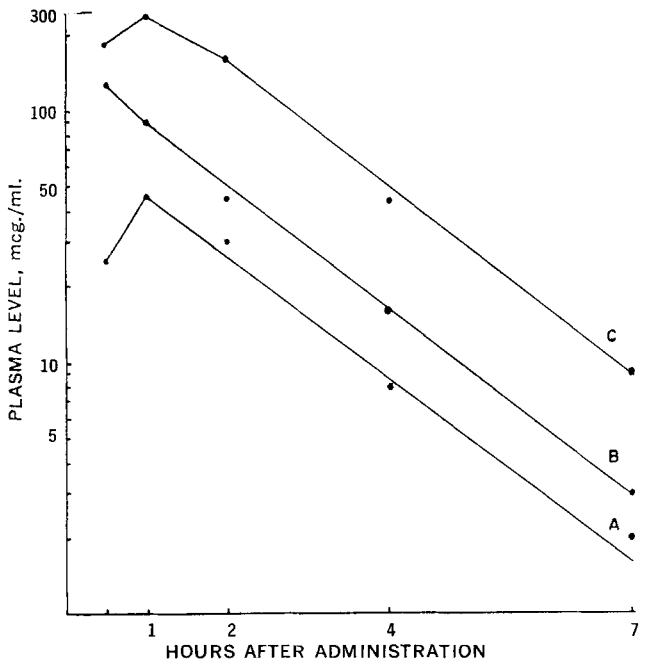

Figure 3—*Mean plasma level of GGE-MN after oral administration of water suspension to rabbits at different doses. Key: A, 50 mg./kg.; B, 150 mg./kg.; and C, 250 mg./kg. GGE-MN was screened through 100 mesh and prepared in suspension form with water. Four male rabbits weighing about 2 kg. each were used.*

[1] Triton WR-1339, Rohm & Haas Co., Philadelphia, Pa., supplied by Winthrop Laboratories, New York, N. Y.

Table II—Effect of GGE-MN and Its Related Compounds or Mixtures on Cholesterol Content in Bile of Rats[a] Receiving Polyoxyethylene Ethers (400 mg./kg.) Intravenously

Sample	Dose	Total Bile Volume for 7.5 hr. (ml./kg.), Mean Value ±SE[b]	Total Cholesterol in Bile (mcg./kg.), Mean Value ±SE[b]
No Polyoxyethylene Ethers Given			
No drug		21.3 ± 3.1	1730 ± 445
GGE-MN	300 mg./kg. (i.p.)	28.8 ± 6.4	2020 ± 421
GGE-MN	300 mg./kg. (orally)	19.4 ± 4.3	1578 ± 312
Polyoxyethylene Ethers, Given i.p.			
Control (no drug)		29.8 ± 4.8	5398 ± 536
GGE-MN	300 mg./kg.	26.7 ± 3.9	6083 ± 191
GGE-MN	200 mg./kg.	28.5 ± 8.4	5939 ± 1513
GGE	300 mg./kg.	27.2 ± 2.9	7479 ± 2780
GGE	1 mmole/kg.	25.8 ± 4.0	5043 ± 231
GGE	0.7 mmole/kg.	27.9 ± 6.5	5097 ± 770
Nicotinic acid	1 mmole/kg.	24.6 ± 6.8	6366 ± 964
Nicotinic acid	0.7 mmole/kg.	26.7 ± 8.1	6642 ± 1034
Equimolar mixture of GGE and nicotinic acid	1 mmole/kg.	23.0 ± 3.4	5385 ± 647
Equimolar mixture of GGE and nicotinic acid	0.7 mmole/kg.	22.6 ± 9.1	5658 ± 1130
β-(4-Hydroxy-2-methoxyphenoxy) lactic acid	1 mmole/kg.	26.3 ± 4.2	6998 ± 2889
(4-Hydroxy-2-methoxyphenoxy) acetic acid	1 mmole/kg.	27.2 ± 3.0	7186 ± 1666
Mesoinositol hexanicotinate	0.3 mmole/kg.	21.6 ± 6.6	5717 ± 804
2,6-Pyridine dimethanol bis-(*N*-methylcarbamate)	0.8 mmole/kg.	21.8 ± 6.6	7605 ± 1560

[a] Male rats (Wistar strain), weighing 160–200 g., were used. Each group consisted of five rats. [b] *SE* = standard error.

of bile in each group (five rats per group) was started 18 hr. after injection of the polyoxyethylene ethers (4, 5).

Assay of Cholesterol—Cholesterol in bile was determined quantitatively by the method of Zak (6).

Bilirubin in Rabbit Bile—Bilirubin concentration in bile was determined by the method of Jendrassik and Cleghorn (7).

Nicotinic Acid, Mesoinositol Hexanicotinate, and 2,6-Pyridine Dimethanol Bis(*N*-methylcarbamate)—These compounds were commercially available.

β-(4-Hydroxy-2-methoxyphenoxy)lactic Acid and (4-Hydroxy-2-methoxyphenoxy)acetic Acid—The compounds were described in the previous paper (2).

RESULTS AND DISCUSSION

It was described previously (3) that unchanged GGE-MN in blood can be determined when barbituric acid buffer solution instead of ammonia buffer solution is used (8, 9).

When GGE-MN is ingested orally, unchanged GGE-MN and free nicotinic acid as the hydrolysis product of GGE-MN were detected in rabbit plasma (3). In the present work, the blood level of GGE-MN was determined as total drug including unchanged GGE-MN and nicotinic acid liberated from GGE-MN by using an ammonia buffer for the determination of GGE-MN.

GGE-MN seems to be absorbed with pseudo-first-order reaction by passive transport in mice and rats, since the blood level peak is almost proportional to the dose when the logarithm of the blood level is plotted against time from plasma data of GGE-MN (Figs. 1 and 2).

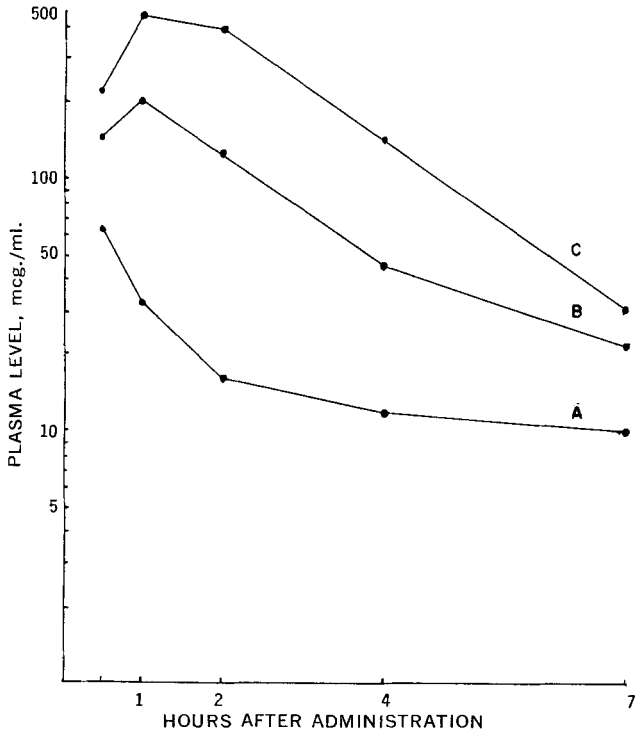

Figure 4—*Mean plasma level of GGE-MN after oral administration of Sample 1 to rabbits at different doses. Key: A, 50 mg./kg.; B, 150 mg./kg.; and C, 250 mg./kg. GGE-MN was screened through 100 mesh and prepared in suspension form with water. Four male rabbits weighing about 2 kg. each were used. Capsule contents were removed and used in water suspension form for ingestion.*

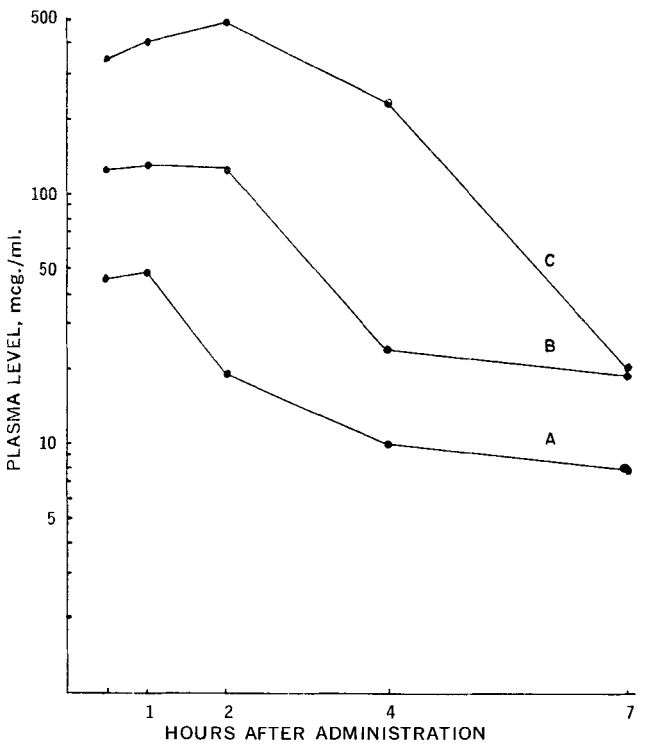

Figure 5—*Mean plasma level of GGE-MN after oral administration of Sample 2 to rabbits at different doses. Key: A, 50 mg./kg.; B, 150 mg./kg.; and C, 250 mg./kg. GGE-MN was screened through 100 mesh and prepared in suspension form with water. Four male rabbits weighing about 2 kg. each were used. Capsule contents were removed and used in water suspension form for ingestion.*

To select a proper pharmaceutical formulation of GGE-MN for clinical trials, several formulations were prepared as shown in Table I. Blood levels in rabbits when these pharmaceuticals were administered orally were compared with the blood level when a water suspension of GGE-MN was given, and the results are shown in Figs. 3–6. Formulations of Samples 3 and 4 seemed to be unsuitable, as compared to the water suspension or capsule form. Sample 1 appeared better than Sample 2, because there was less fluctuation in blood level of GGE-MN.

The bile from rabbits was collected under continuous urethan anesthesia, and arterial blood pressure and respiration were recorded on a kymograph. It was ascertained that GGE-MN and GGE have no effect upon arterial blood pressure, respiration, bile flow, and total bile volume excreted for 7.5 hr., as compared to untreated control rabbits.

When 300 mg./kg. of GGE-MN was administered orally to rabbits and rats under continuous anesthesia for 7.5 hr., no GGE-MN was observed in the bile within experimental error, and no variation of bilirubin concentration was recognized compared to control animals.

The effect of GGE-MN, GGE, or the equimolar mixture of GGE and nicotinic acid on cholesterol content in rabbit bile was determined, but it was observed that these three samples had no effect on the cholesterol content in bile.

In previous reports (1, 2), GGE-MN and the metabolites, namely, β-(4-hydroxy-2-methoxyphenoxy)lactic acid and (4-hydroxy-2-methoxyphenoxy)acetic acid, showed hypocholesteremic effect on hypercholesteremia caused by intravenous injection of polyoxyethylene ethers to rats.

In the present work, the effect of GGE-MN and its related compounds or the mixtures on cholesterol content in the bile of rats receiving polyoxyethylene ethers was investigated; the results are shown in Table II. No effect on the cholesterol value in bile was observed when GGE-MN was given orally or intraperitoneally to normal rats.

Compounds that clearly increased the amount of cholesterol excreted in the bile of hypercholesteremic rats receiving polyoxyethylene ethers intravenously were 300 mg. (1 mmole)/kg. of GGE-MN, 0.7 mmole/kg. of nicotinic acid, 0.8 mmole/kg. of 2,6-pyridine dimethanol bis(*N*-methylcarbamate),[2] and 1 mmole/kg. of (4-hydroxy-2-methoxyphenoxy)acetic acid, at 95% confidence limit.

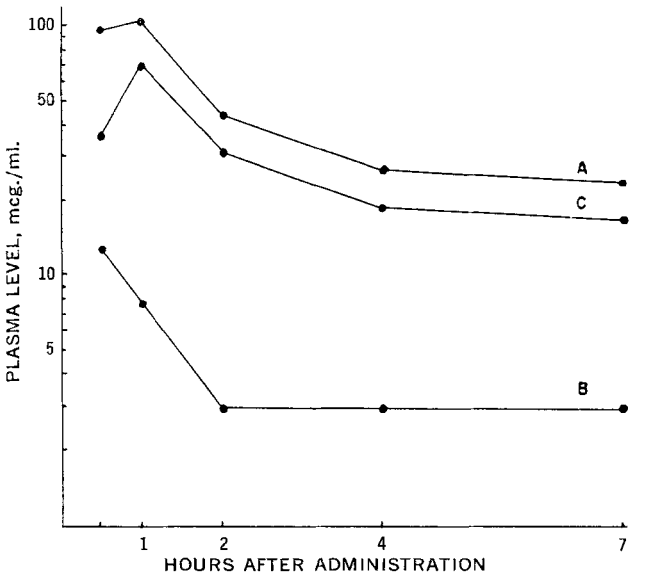

Figure 6—*Mean plasma level of GGE-MN after oral administration of Samples 3 and 4 to rabbits. Key: A, Sample 3, corresponded to 150 mg./kg. of GGE-MN; B, Sample 4, corresponded to 50 mg./kg. of GGE-MN; and C, Sample 4, corresponded to 150 mg./kg. of GGE-MN. GGE-MN was screened through 100 mesh and prepared in suspension form with water. Four male rabbits weighing about 2 kg. each were used. A mixture of 20 ml. of water and tablets after disintegration was used for ingestion.*

[2] Anginin, Banyu Pharmaceutical Co. Ltd., Japan.

On the contrary, 0.7 mmole/kg. and 1 mmole/kg. of GGE did not increase the amount of cholesterol excreted in bile.

Other compounds such as 1 mmole/kg. of β-(4-hydroxy-2-methoxyphenoxy)lactic acid, 200 mg./kg. of GGE-MN, 300 mg./kg. of GGE, 1 mmole/kg. of nicotinic acid, 0.3 mmole/kg. of meso-inositol hexanicotinate,[3] and the equimolar mixture of GGE and nicotinic acid at 0.7- and 1-mmole/kg. doses increased the amount of cholesterol excreted in bile for some rats but did not alter that for other rats in the same group, compared with that of the control group.

On the other hand, all compounds used showed no effect on the total volume of bile excreted, compared with the control group, at 95% confidence limit.

It is interesting that (4-hydroxy-2-methoxyphenoxy)acetic acid, one of the metabolites of GGE and GGE-MN, has the effect of increasing cholesterol in bile in hypercholesteremia caused by intravenous injection of polyoxyethylene ethers. GGE-MN increased the excretion of cholesterol in bile at 1 mmole/kg., but nicotinic acid, one of the metabolites of GGE-MN, also has similar action at the same dose. Therefore, some question still remains whether the increasing effect of GGE-MN on excretion of cholesterol in bile is due to that of GGE-MN itself and/or to the metabolites of GGE-MN *in vivo*. The data concerned with mesoinositol hexanicotinate and 2,6-pyridine dimethanol bis(*N*-methylcarbamate) were determined for comparison purposes, since these compounds are now widely being used as hypocholesteremic agents.

[3] Hexanicite, Yoshitomi Pharmaceutical Co. Ltd., Japan.

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Chlorpromazine Metabolism I: Quantitative Fluorometric Method for 11 Chlorpromazine Metabolites

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Abstract □ Eleven chlorpromazine metabolites have been reacted with dimethylaminonaphthyl sulfonyl chloride to obtain fluorescent products. The reaction has been standardized and adapted to quantitative determination of nanogram amounts of the metabolites. A procedure for application to biological fluids has been developed. Addition and recovery experiments on urine and plasma indicate that the method is applicable to the study of the pharmacokinetic aspects of chlorpromazine metabolites in human subjects.

Keyphrases □ Chlorpromazine—metabolism □ Metabolites, chlorpromazine—quantitative determination □ 5-Dimethylaminonaphthalene-1-sulfonyl Cl–chlorpromazine metabolites reaction—fluorescence □ TLC—separation, identification □ Fluorometry—analysis

Despite a great deal of work done with chlorpromazine (CPZ), the significance of its biotransformation relative to its therapeutic response is not clear. The pharmacokinetics of CPZ and its metabolites in humans remain largely unexplored, possibly because of the limitations in sensitivity and precision of the analytical methods applied to the study of CPZ metabolism. Only recently (1–5) have the analytical methods been developed that offer desirable sensitivity for quantitating CPZ and its metabolites. However, their adaptation to routine determinations in clinical research is limited because these methods can quantitate only a few of the known metabolites (6).

The metabolites listed in Table I possess primary and secondary amino groups and/or hydroxy groups, all of which are capable of reacting with 5-dimethylaminonaphthalene-1-sulfonyl chloride (DNS, dansyl) to yield fluorescent products. Dansyl has been routinely used as an end-group detector in the study of protein structure; its application for quantitation has, to date, remained limited only to amino acids (7). It has also been reported useful as a reagent for detection of some alkaloids (8).

A preliminary account of the dansylation reaction and separation of the dansylated metabolites of CPZ has already been reported (9). This paper deals with the development and standardization of the reaction between 11 CPZ metabolites and DNS. The method has been adapted to quantitative determinations of nanogram quantities of the metabolites. Addition and recovery experiments on aqueous solutions, urine, and plasma are also included.

EXPERIMENTAL

Materials and Equipment—The following were used: 5-dimethylaminonaphthalene-1-sulfonyl chloride (dansyl, DNS), purity grade[1]; acetone, benzene, dichloromethane, dimethyl sulfoxide, di-

[1] Fluke-Buchs, Switzerland.