Biopharmaceutical Studies on 2-(Aminoethanesulfonylamino)pyridine and Related Compounds I: Absorption, Excretion, Metabolism, and Preliminary Pharmacological Investigation

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Abstract □ 2-(Aminoethanesulfonylamino)pyridine (tauraminopyridine) and its nicotinoyl derivative showed analgesic and anti-inflammatory activities but not hypocholesteremic action, and the nicotinoyl derivative had more potent pharmacological actions with less toxicity than tauraminopyridine. It was also observed that a relatively large amount of nicotinoyltauraminopyridine was bound to serum proteins in rabbits and accumulated in rat organs.

Keyphrases □ 2-(Aminoethanesulfonylamino)pyridine and nicotinoyl derivative—biopharmaceutical studies, rabbits, rats □ Tauraminopyridine and nicotinoyl derivative—biopharmaceutical studies, rabbits, rats □ Nicotinoyltauraminopyridine—rabbit serum protein binding, presence in rat organs

Tauraminopyridine [2-(aminoethanesulfonylamino)-pyridine] was synthesized in a series of 2-aminoethanesulfonic acid derivatives. If taurine is liberated partially from tauraminopyridine and its derivatives *in vivo*, the surface activity of taurine must have some effect on the activity of the remaining components (2-aminopyridine and/or unchanged tauraminopyridine). It was established that the binding of the amino group with the 2-aminoethanesulfonyl group cannot be severed easily in rabbits, and liberation of taurine from the tauraminopyridine derivative will be examined later in man.

The present work describes some results of biopharmaceutical and pharmacological studies on tauraminopyridine and related compounds as a preinvestigation in order to begin clinical experiments as early as possible.

EXPERIMENTAL

Acute Toxicity—Each group of test animals consisted of five female and five male mice (dd strain, average weight 15 g.). Various doses of the chemical were dissolved in water, and the volume was limited to 0.1 ml./10 g. body weight for administration. The LD_{50} was calculated from the number of animals that died during 72 hr. after administration, according to the method of Litchfield and Wilcoxon (1). The experiment was carried out under the same conditions as described previously (2).

Tail Withdrawal Reflex in Mice—The same method of determining the analgesic potency as described in the paper of Ben-Bassat *et al.* (3) was used.

Effect of Tauraminopyridine Hydrochloride and Nicotinoyltauraminopyridine on Squirming and Capillary Permeability—Male dd strain mice, weighing about 20 g. each, were given the test compounds orally. The experimental condition was designed as described previously (2).

Effect on Hypocholesteremia Induced by Polyoxyethylene Ether[1]—Male rats of Wistar strain, weighing about 120 g. each, were used, and the experimental procedure was the same as described previously (4).

Anti-Inflammatory Activity—Inflammation was produced by injecting carrageenin (0.1 ml. of 2% solution) into the plantar surface of the rat hind paw. Test compounds were administered as aqueous solutions for tauraminopyridine hydrochloride and as water suspensions for nicotinoyltauraminopyridine, and their volume was kept constant at 0.1 ml./100 g. of body weight for intraperitoneal dosage. The experiment was carried out under the same conditions as reported previously (2).

Effect of Sodium Salicylate and Nicotinoyltauraminopyridine on Erythrocyte Lysis—The procedure used was the same as that described previously (5). Blood samples were obtained from male rats (Wistar strain, average weight 180 g.) by decapitation. The test compound was dissolved in phosphate buffer (pH 7.4).

Hemolytic Action *In Vitro*—Hemolytic action of nicotinoyltauraminopyridine on rat blood was determined by the method reported previously (5), using male rats (Wistar strain, average weight 300 g.).

Determination of Tauraminopyridine and Nicotinoyltauraminopyridine in Rabbit Blood—The test compounds were administered orally to male rabbits (average body weight about 2.5 kg.). Each group consisted of four rabbits. To 1 ml. of plasma, 4 ml. of 10% trichloroacetic acid was added. The supernate after centrifugation was filtered, and the absorbance of the filtrate was measured at 292 nm. for tauraminopyridine and at 294 nm. for nicotinoyltauraminopyridine. A mixture of 0.9 ml. of normal rabbit plasma and 0.1 ml. of the test compound solution was treated as just described to prepare a calibration curve.

Binding of Nicotinoyltauraminopyridine with Rabbit Serum Proteins—Each group consisted of four male rabbits (average weight 2.3 kg.). Blood was taken at 1 and 2 hr. after 350 mg./kg. of nicotinoyltauraminopyridine was given orally. The plasma concentration of nicotinoyltauraminopyridine, including the free compound and the chemical bound to protein, was determined by the same method as for the determination of nicotinoyltauraminopyridine in rabbit blood.

To carry out the equilibrium dialysis of nicotinoyltauraminopyridine in blood, 5 ml. of water was placed outside of the Visking cellophane tubing in a standard tapered test tube; a mixture of 1 ml. of rabbit plasma and 1 ml. of water was placed inside the tubing and then dialyzed at 4° for 72 hr. After centrifuging the outside solution for 10 min., the supernate was filtered and the absorbance of the filtrate was measured at 294 nm. The values thus obtained show the concentration of free nicotinoyltauraminopyridine.

Determination of Nicotinoyltauraminopyridine in Rat and Mouse Blood—The test compound was given orally to male rats and mice. A mixture of 1 ml. of plasma and 1 ml. of 10% perchloric acid was stored at 37 ± 2° for 1 hr. and then mixed with 3 ml. of water. Absorbance of the supernate from centrifugation was measured at 262 nm.

A mixture of 0.9 ml. of normal animal plasma and 0.1 ml. of nicotinoyltauraminopyridine solution of known concentration was treated as just described to prepare a calibration curve.

Distribution of Nicotinoyltauraminopyridine in Rat Organs—To each male rat (Wistar strain), 400 mg./kg. of nicotinoyltauraminopyridine was administered orally; the animals were killed 0.5, 24, or 120 hr. after the administration. Each group consisted of three rats, and six organs (heart, lung, spleen, kidney, brain, and liver) were used for the determination of nicotinoyltauraminopyridine.

The sample of each organ was prepared from a pooled mixture of the same organs in one group. The total weight of the sample used was 2 g. of the heart, 3 g. of the lung, 1 g. of the spleen, 4 g. each of the kidney and brain, and 7.5 g. of the liver. These samples were

[1] USAN name is tyloxapol; Triton WR 1339.

Table I—Average Pain Reaction Time in Control Mice and Mice Given Intraperitoneal Injection of Tauraminopyridine Hydrochloride and Nicotinoyltauraminopyridine[a]

Compound	Dose, mg./kg.	Average Reaction as Recorded in Seconds after Injection: 15 min.	30 min.	60 min.	90 min.	120 min.	150 min.	PRT[b]
Control		1.5	1.8	2.0	1.9	1.9	1.8	1.8
Tauraminopyridine hydrochloride	200	1.7 (0/10)[c]	2.4 (5/10)	2.3 (6/10)	2.4 (6/10)	2.1 (3/10)	2.0 (2/10)	2.2
	250	2.7 (5/10)	2.5 (5/10)	2.6 (7/10)	2.5 (5/10)	2.4 (5/10)	2.4 (5/10)	2.5
	300	2.6 (5/10)	2.6 (8/10)	2.8 (9/10)	2.8 (6/10)	2.5 (5/10)	2.5 (4/10)	2.6
Nicotinoyltauraminopyridine	50	2.0 (3/10)	2.2 (4/10)	2.5 (4/10)	2.3 (6/10)	2.1 (5/10)	2.0 (4/10)	2.2
	75	2.6 (4/10)	2.8 (5/10)	2.7 (6/10)	2.6 (5/10)	2.0 (4/10)	2.2 (4/10)	2.5
	100	2.9 (6/10)	3.3 (7/10)	2.8 (5/10)	2.1 (4/10)	1.9 (3/10)	1.9 (3/10)	2.5

[a] Each group consisted of 10 female mice (dd strain, about 15 g. each in body weight). [b] PRT = average pain reaction time. [c] Numbers in parentheses are: number of effected mice/number of mice used.

homogenized with ethanol, using 6 ml. for the heart and lung, 5 ml. for the spleen, 7 ml. for the kidney and brain, and 9 ml. for the liver. A mixture of 1 ml. of the homogenate and 1 ml. of 10% perchloric acid was stored at 37 ± 2° for 1 hr. The supernate of the mixture, after centrifugation, was diluted with ethanol to make 1:30 for heart and lung, 1:15 for spleen, 1:50 for kidney and brain, and 1:100 for liver. The absorbance of the diluted homogenate solution thus obtained was measured at 262 nm.

Each organ of rats not receiving the chemical was treated as just described to prepare a control sample. The mixture of control homogenate and nicotinoyltauraminopyridine solution of known concentration was treated as described to prepare a calibration curve.

Collection of Rat Bile—Female rats (Wistar strain), varying in body weight from 200 to 245 g. each, were anesthetized with 1 g./kg. of urethan subcutaneously, following a fasting period of approximately 24 hr. A polyethylene tube was inserted into the bile duct, and the bile was collected for 7.5 hr. after administration of nicotinoyltauraminopyridine under continuous anesthesia with urethan. At the end of the 1st (control) hr., the compound was given orally.

Determination of Nicotinoyltauraminopyridine in Rat Bile—A mixture of 1 ml. of the rat bile and 1 ml. of 10% perchloric acid was stored at 37 ± 2° for 1 hr., and 0.5 ml. of this mixture was diluted with 9.5 ml. of water. The absorbance of this diluted solution was measured at 262 nm.

A mixture of 0.9 ml. of normal rat bile and 0.1 ml. of nicotinoyltauraminopyridine solution of known concentration was treated as described to prepare a calibration curve.

Urine Sample for Detection of Metabolites of 2-Aminopyridine, Tauraminopyridine, and Nicotinoyltauraminopyridine—*2-Aminopyridine*—Urine obtained from five female rabbits (average weight 2.2 kg.), each receiving 200 mg. of 2-aminopyridine orally every day for 5 days, was collected for 7 days. The total amount administered to five rabbits was 5 g., and 1270 ml. of urine was collected. After freeze evaporation of urine, 46 g. of a residue was obtained.

Table II—Effect of Test Compounds on Squirming and Capillary Permeability[a]

Compound	Oral Dose, mg./kg.	Reduction of Squirming, %	Inhibition of Squirming, ED_{50}, mg./kg.	Change in Permeability, %
Tauraminopyridine hydrochloride	100	28	194 (125–301)[b]	−2
	200	53		−31
	300	64		−27
Nicotinoyltauraminopyridine	100	19	184 (143–237)[b]	−30
	150	32		−27
	200	60		−19

[a] Percentages were calculated from the mean of groups consisting of 12 male mice to that of the controls (12 mice). [b] 95% confidence limits.

Tauraminopyridine—To four female rabbits weighing about 2.0 kg. each, 450 mg. of tauraminopyridine was given orally. Urine was collected for 2 days, and about 20 g. of the residue was obtained after freeze evaporation of 430 ml. of urine.

Nicotinoyltauraminopyridine—One gram of nicotinoyltauraminopyridine per rabbit was administered orally to female rabbits weighing about 2.3 kg. each. Urine was collected for 2 days, and about 230 ml. was excreted. About 14 g. of a residue was obtained after freeze evaporation.

Assay of Metabolites of Tauraminopyridine and Nicotinoyltauraminopyridine in Rabbit Urine—A mixture of 0.3 ml. of 5 *N* HCl and 200 mg. of the lyophilized residue of urine of rabbits receiving tauraminopyridine or nicotinoyltauraminopyridine was stored in an incubator at 37 ± 2° for 1 hr. After an addition of 0.3 ml. of 5 *N* sodium carbonate solution, 80 μl. of the centrifuged supernate was submitted to TLC and separated into four spots (20 μl. each). The four spots corresponding to tauraminopyridine and nicotinoyltauraminopyridine were each scraped off the TLC plate, using a mixed solvent of ethanol–28% ammonia–dioxane (2:1:4) for tauraminopyridine or methanol–28% ammonia (1:1) for nicotinoyltauraminopyridine, and extracted with 5 ml. of water on a boiling water bath for 3 min. The absorbance of the supernate after centrifugation was measured at 310 nm. for tauraminopyridine and at 308 nm. for nicotinoyltauraminopyridine.

The calibration curves of the two compounds were prepared separately by TLC as already described by spotting the solution of the pure corresponding compound of a known concentration. The data obtained from this procedure show the amount of total compound, including free and conjugated forms, in urine.

To assay the free form of tauraminopyridine or nicotinoyltauraminopyridine in urinary metabolites, 200 mg. of the lyophilized residue of urine of rabbits receiving the compound was diluted with 0.6 ml. of water instead of 0.3 ml. of 5 *N* HCl and 0.3 ml. of 5 *N* sodium carbonate solution. Eighty microliters of the centrifuged supernate of the diluted solution was treated by the same procedure as the total compound, including free and conjugated forms of the compound in urine.

Table III—Effect on the Edema Induced by Subplantar Injection of Carrageenin[a]

Compound	Dose, mg./kg.	Mean Increase in Paw Volume by Edema, Mean Value ± *SE*	Inhibition, %
Control		0.695 ± 0.063	—
Tauraminopyridine hydrochloride	200	0.583 ± 0.282	16
	300	0.338 ± 0.251	51
Nicotinoyltauraminopyridine	100	0.584 ± 0.237	16
	200	0.572 ± 0.262	18
	300	0.388 ± 0.283	44

[a] Each group consisted of 10 male rats.

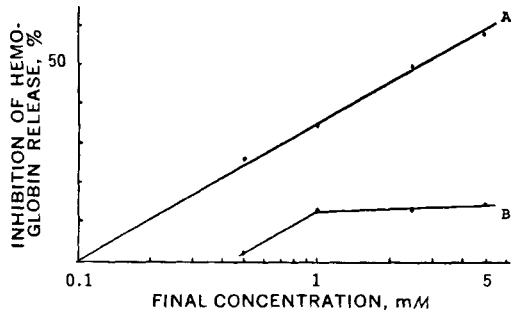


Figure 1—*Inhibition of heat-induced hemolysis. Key: A, sodium salicylate; and B, nicotinoyltauraminopyridine.*

2-Acetamidopyridine—The compound was synthesized according to the method reported by Camps (6). A mixture of 7 g. of 2-aminopyridine and 50 ml. of acetic anhydride was heated on a boiling water bath for 2 hr. About 6 g. of sodium bicarbonate was added to this mixture after an addition of 80 ml. of water. The residue obtained from evaporation of the reaction mixture was recrystallized from a mixed solvent of benzene and ligroine. About 2.6 g. of 2-acetamidopyridine, m.p. 69–71°, was obtained.

RESULTS

The LD_{50}'s (95% confidence limits) of 2-aminopyridine, tauraminopyridine hydrochloride, and nicotinoyltauraminopyridine after intraperitoneal administration to mice are 43 (37–48), 490 (439–547), and 3800 mg./kg. (3378–4275 mg./kg.), respectively. No significant difference in LD_{50} was observed between male and female mice.

The ED_{50} of tauraminopyridine hydrochloride 60 min. after intraperitoneal administration and that of nicotinoyltauraminopyridine 30 min. after intraperitoneal administration, as determined by the tail withdrawal reflex in mice, were 193 (158–235) and 69 mg./kg. (48–99 mg./kg.), respectively, with 95% confidence limits (Table I). The analgesic activity of the nicotinoyl derivative was about three times greater than that of tauraminopyridine in the tail withdrawal reflex, but little difference was observed between the two compounds in the ED_{50} of the squirming tests (Table II). The reduction of the permeability response did not approach 50% over the range of doses that inhibited squirming and, therefore, there is no corresponding ED_{50} for the permeability effect of both compounds.

Anti-inflammatory activities of tauraminopyridine and its nicotinate are shown in Table III. Both compounds showed some anti-inflammatory effect on edema induced by carrageenin, but the relatively large standard error indicates some inaccuracy in the test procedure.

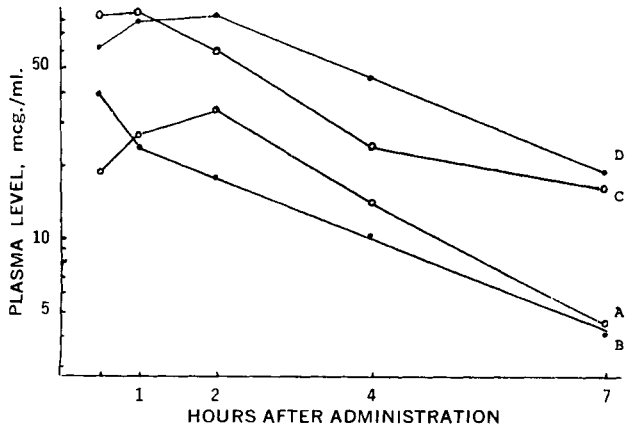


Figure 2—*Mean plasma level of tauraminopyridine or nicotinoyltauraminopyridine after oral administration to rabbits. Key: A, 175 mg./kg. of tauraminopyridine hydrochloride; B, 250 mg./kg. of nicotinoyltauraminopyridine; C, 350 mg./kg. of nicotinoyltauraminopyridine; and D, 450 mg./kg. of nicotinoyltauraminopyridine.*

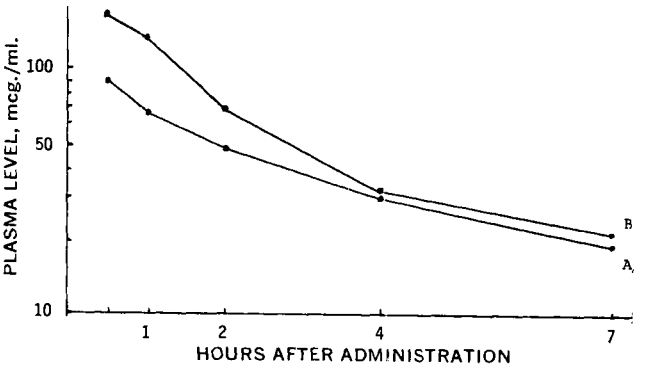


Figure 3—*Mean plasma concentration of nicotinoyltauraminopyridine after oral administration of 400 mg./kg. Each group consisted of five male rats (Wistar strain, about 170 g. each in body weight) or 10 male mice (dd strain, about 20 g. each in body weight). Animals were decapitated at the sampling time, and each animal was bled completely. An equivolume mixture of blood from each animal was used for analysis. Key: A, rats; and B, mice.*

No effect of nicotinoyltauraminopyridine on hypercholesteremia induced by polyoxyethylene ethers was observed after its intraperitoneal administration as 100-, 200-, and 300-mg./kg. doses.

Nicotinoyltauraminopyridine inhibited the erythrocyte lysis slightly in the heat-induced erythrocyte lysis test (5) *in vitro* (Fig. 1). Therefore, it appears unsuitable to consider a relationship between the protective effect of nicotinoyltauraminopyridine on heat-induced erythrocyte lysis and anti-inflammatory action of the chemical on rat edema induced by carrageenin. Sodium salicylate was used for comparison.

Nicotinoyltauraminopyridine showed no hemolytic action at 50, 100, and 200 mcg./ml. in final concentrations. Hemolytic action of saponin[2] was determined for comparison; the values were 8.0, 39.6, 78.5, and 99.6% at 40, 80, 120, and 160 mcg./ml. in final concentrations, respectively.

Colorimetric determination of tauraminopyridine and its nicotinoyl derivative was attempted by the modified Folin method (7), the potassium ferricyanide and ferric nitrate method (7), and other methods that are being used widely in determination of pyridoxine derivatives, but none was applicable for these two compounds. Therefore, these chemicals were assayed by UV analysis.

Blood levels of tauraminopyridine and its nicotinoyl derivative at different doses in rabbits were determined, and the results are shown in Fig. 2. Blood levels probably indicate the total amount, including the compound bound to serum proteins, since the experiments were made with the consideration that the binding of the chemical with serum proteins can possibly be severed by the action of trichloroacetic acid. Free nicotinoyltauraminopyridine in rabbit blood was determined by equilibrium dialysis. It was found that a relatively large amount of the compound (58.5 and 74.1% in the mean values at 1 and 2 hr. after the administration, respectively) is in a bound form. Whether the compound bound to serum proteins is useful or not to animals should be investigated extensively.

To carry out animal experiments, it would be helpful to know the blood level of a compound. Therefore, blood concentrations of nicotinoyltauraminopyridine in rats and mice (Fig. 3) were determined without any special objective.

The distribution of nicotinoyltauraminopyridine in rat organs 0.5 hr. after its oral administration to three groups of rats was 210 ± 21 mcg./g. in the heart, 625 ± 83 mcg./g. in the lung, 522 ± 75 mcg./g. in the spleen, 584 ± 91 mcg./g. in the kidneys, 233 ± 38 mcg./g. in the brain, and 415 ± 73 mcg./g. in the liver (mean ± standard error). An attempt was made to find a method for quantitative analysis of nicotinoyltauraminopyridine in the homogenates of rat organs, but no better procedure than spectrophotometry in the UV region was found. Spectrophotometry in the UV region is not a good procedure for determining the distribution of a compound in animal organs, because the experimental values of control organs of animals not receiving the chemical varied in some cases. It was unexpectedly found that the amount of the compound in

[2] Merck.

Table IV—TLC[a] of Metabolites of 2-Aminopyridine, Tauraminopyridine, and Nicotinoyltauraminopyridine in Rabbit Urine

Material	Solvent	Color Developer	R_f Assumed Substance	Color of Spot
2-Aminopyridine–urine[b]	A	S	0.46 2-aminopyridine	Reddish brown
			0.24	Gray
			0.06	Black
2-Aminopyridine	A	S	0.46	Reddish brown
2-Acetamidopyridine	A	S	0.68	Brown
2-Hydroxypyridine	A	S	0.04	Brown
2-Aminopyridine–urine[b]	B	ID	0.80 2-aminopyridine	Yellowish brown
2-Aminopyridine	B	ID	0.80	Yellowish brown
Tauraminopyridine–urine[b]	C	S	0.07 tauraminopyridine	Reddish brown
Tauraminopyridine	C	S	0.07	Reddish brown
Nicotinoyltauraminopyridine–urine[b]	A	ID	0.04 nicotinoyltauraminopyridine	Yellowish brown
Nicotinoyltauraminopyridine	A	ID	0.04	Yellowish brown
Tauraminopyridine–urine	D	S	0.27 tauraminopyridine	Reddish brown
Tauraminopyridine	D	S	0.27	Reddish brown
Nicotinoyltauraminopyridine–urine	E	ID	0.82 nicotinoyltauraminopyridine	Yellowish brown
Nicotinoyltauraminopyridine	E	ID	0.82	Yellowish brown

[a] Adsorbent: Diatomite (Kieselgel G), 0.25 mm. in thickness. Solvent A: ethylacetate–ligroine–methanol (10:10:1). Solvent B: methanol–28% ammonia–chloroform (4:1:1). Solvent C: *N,N'*-dimethylformamide–isopropyl alcohol (1:2). Solvent D: ethanol–dioxan–28% ammonia (2:4:1). Solvent E: methanol–28% ammonia (1:1). Color developer S: 7% silver nitrate solution. Color developer ID: diluted iodine tincture (JP VII). [b] These urine samples were prepared by the methods described in the text.

tissues of rat organs is higher 24 hr. after administration than that 0.5 hr. after administration. Nicotinoyltauraminopyridine in rat tissues 24 hr. after its oral administration to three groups was 450 ± 52 mcg./g. in the heart, 1123 ± 75 mcg./g. in the lung, 1248 ± 160 mcg./g. in the spleen, 912 ± 83 mcg./g. in the kidneys, 404 ± 62 mcg./g. in the brain, and 438 ± 65 mcg./g. in the liver (mean ± standard error). Although a more precise method for the determination of the compound in animal organs is required, it is noteworthy that nicotinoyltauraminopyridine has a tendency to accumulate in animal organs. On the other hand, the compound was not detected in any of the rat organs 120 hr. after its administration.

Nicotinoyltauraminopyridine excreted in rat bile during 7.5 hr. after oral administration in a 400-mg./kg. dose to each of five rats was 5780 ± 792 mcg./kg. (mean value ± standard error), and the volume of rat bile excreted was not affected by the administration of the compound.

The metabolic pathway of 2-aminopyridine, tauraminopyridine, and nicotinoyltauraminopyridine was examined by using TLC (Table IV). The fate of the major portion of administered pyridine in the body is assumed to be as shown in Scheme I (8).

After administration of 2-aminopyridine, which has the fundamental nucleus of tauraminopyridine, to rabbits, unchanged compound and two unknown compounds were detected as the urinary metabolites. The chemical structure of these unknown compounds could not be established. In the case of the administration of tauraminopyridine or its nicotinoyl derivative, only the unchanged chemical was found; nevertheless, efforts were made to select suitable mixed solvents for TLC. It is interesting that the binding of the amino group with the 2-aminoethanesulfonyl group cannot be severed easily in rabbits after administration of tauraminopyridine or its nicotinoyl derivative. Additionally, it was found that tauraminopyridine or nicotinoyltauraminopyridine exists more in the unknown conjugated form than in the free form. The ratio of conjugated form to the total tauraminopyridine or the nicotinoyl derivative was about 55 or 89%, respectively. Whether the conjugated form is the ethereal sulfate or glucuronide or others was not examined, and the total amounts of these chemicals excreted could not be calculated because of incomplete collection of urine.

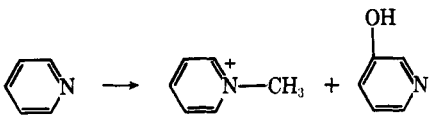
DISCUSSION

Phenyramidol[2-(β-hydroxyphenethylamino)pyridine] has a chemical structure similar to tauraminopyridine, having a pyridylamino nucleus. Phenyramidol is an analgesic which is claimed also to provide muscle relaxation by an interneuronal blockade action; it is recommended for the treatment of pain associated with musculoskeletal disorders and dysmenorrhea (9).

Some pharmacological activities of phenyramidol, reported by O'Dell *et al.* (10), are as follows: $LD_{\varepsilon 0}$ in mice, 450 mg./kg. i.p.; ED_{50} on writhing produced by phenyl-*p*-quinone, 65 mg./kg. orally; and ED_{50} on paralysis in 50% of mice, 185 mg./kg. i.p. These values for phenyramidol cannot be compared with activities of nicotinoyltauraminopyridine in the present work because of different experimental conditions, but they may be helpful when pharmacological actions of the nicotinoyl derivative are investigated extensively.

Nausea, epigastric distress, drowsiness, pruritus, and skin rash have been reported (9) as untoward effects of phenyramidol. The toxicity (LD_{50}) of nicotinoyltauraminopyridine is much less than that of phenyramidol, and the analgesic effect of the nicotinoyl derivative seems to be less than that of phenyramidol.

Although a comparison of the action of tauraminopyridine derivatives with that of phenyramidol was not the direct purpose of the present work, pharmacological activities of nicotinoyltauraminopyridine with lower toxicity are of interest for clinical trials. However, it should be borne in mind that this compound accumulates in rat organs, and more extensive work on this point is desired.



Scheme I

SUMMARY

1. Although 2-aminopyridine and tauraminopyridine are fairly toxic in mice, nicotinoyltauraminopyridine shows very low toxicity (LD_{50}).

2. The analgesic activity of nicotinoyltauraminopyridine is stronger than that of tauraminopyridine in the tail withdrawal reflex in mice, but little difference was observed between the two compounds in the $ED_{\varepsilon 0}$ of the squirming tests in mice.

3. Tauraminopyridine and its nicotinoyl derivative reveal anti-inflammatory effects to rats on edema induced by carrageenin, with relatively large standard errors.

4. Nicotinoyltauraminopyridine shows no hemolytic action on rat blood.

5. Nicotinoyltauraminopyridine is absorbable from the animal intestine, and a relatively large amount of the compound in blood exists in a bound form with serum protein.

6. After the administration of tauraminopyridine or its nicotinoyl derivative to rabbits, no metabolites except the unchanged compound were found qualitatively in urine by using TLC.

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Species Differences in the Biotransformation of a New Antiarrhythmic Agent: Disopyramide Phosphate

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Abstract □ The biotransformation of disopyramide phosphate [4-diisopropylamino-2-phenyl-2-(2-pyridyl)butyramide phosphate] was studied in rats and dogs using the ^{14}C-labeled compound and in man using the unlabeled drug. Within 72 hr., 78.7 ± 1.4% of the administered radioactivity was recovered in the urine of dogs after oral administration and 44.1 ± 3.4% was recovered in the urine of rats after intraperitoneal administration. In dogs, 17.3 ± 3.4% of the urinary radioactivity was associated with the unchanged compound, 12.4 ± 1.6% with 4-isopropylamino-2-phenyl-2-(2-pyridyl)-butyramide, and 29.2 ± 2.6% with 3-phenyl-3-(2-pyridyl)-2-pyrrolidone; 18.0 ± 3.3% was present as a water-soluble conjugate which, on acid hydrolysis, gave the pyrrolidone as the major aglycone. In rats, the urinary radioactivity was predominantly (80.9 ± 2.3%) associated with the unchanged disopyramide. In this species the major metabolic pathway was aryl hydroxylation, giving two phenolic compounds, one of which was identified as 4-diisopropylamino-2-(*p*-hydroxyphenyl)-2-(2-pyridyl)butyramide. These phenolic metabolites were predominantly excreted in the bile as conjugates. In man, 56% of the administered drug was excreted unchanged in the urine while 4% was present as the secondary amine. The structural assignments of the metabolites were based on their detailed spectroscopic analysis and by comparison of their chromatographic properties with authentic samples.

Keyphrases □ Disopyramide phosphate biotransformation—species differences, rat, dog, man □ Biotransformation, disopyramide phosphate—species differences, rat, dog, man □ Urinary excretion—disopyramide phosphate biotransformation, rat, dog, man □ Pharmacokinetics, species differences—disopyramide phosphate biotransformation, rat, dog, man

Disopyramide phosphate[1] [4-diisopropylamino-2-phenyl-2-(2-pyridyl)butyramide phosphate, I, Scheme I] is an antiarrhythmic agent (1, 2) which has been shown to be useful in the clinic (3). Recent studies from these laboratories (4) showed marked species differences in the pharmacokinetic profiles of this drug in the rat, dog, and man. The purpose of the present investigation was to determine if the species differences also existed in its biotransformation. The presence of an *N,N*-diisopropyl group in disopyramide makes this compound of particular interest for metabolic studies. The isopropyl group can be expected to undergo two separate metabolic pathways: aliphatic hydroxylation to give a primary or tertiary alcohol, or *N*-dealkylation to give a primary or secondary amine (5). In this article, studies on the major route of elimination and the biotransformation of ^{14}C-disopyramide phosphate in rat and dog are presented. The urinary excretion of the unchanged compound and a metabolite from man given the unlabeled compound is also described.

MATERIALS AND METHODS

^{14}C-Labeled Disopyramide Phosphate[2]—The ^{14}C-labeled drug was prepared from phenyl(2-pyridyl)acetonitrile-1-^{14}C (50.26 μc./mg.)[3] by the method of Cusic and Sause (6). ^{14}C-Disopyramide phosphate had a specific activity of 19.5 μc./mg., and its radiochemical purity (determined by TLC) was 99.2%.

Measurement of Radioactivity—All samples were counted in a liquid scintillation spectrometer[4]. For counting aqueous solutions (urine and bile) and extracts containing polar compounds, Bray's scintillation solution (7) was used; for counting samples scraped from thin-layer chromatograms, the scintillation solution of Snyder and Smith (8) was used. Chemical quenching was corrected by the

[1] Disopyramide phosphate is also known as Norpace, SC-7031 phosphate, and SC-13957. USAN chemical name is α-[2-(diisopropylamino)-ethyl]-α-phenyl-2-pyridineacetamide.

[2] ^{14}C-Disopyramide phosphate was prepared by Mr. D. J. Zitzewitz.

[3] Obtained from Amersham/Searle Corp., Arlington Heights, IL 60005

[4] Mark I, Nuclear-Chicago Corp., Des Plaines, IL 60018