Stability, Absorption, Excretion, and Distribution of Nafiverine

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Abstract □ The stability of a new antispasmodic, nafiverine, in an aqueous solution and in rabbit plasma was investigated. Metabolites of nafiverine in blood, bile, and urine were determined quantitatively. The effectiveness of an intramuscular administration of nafiverine was comparable to that of an intravenous administration. One metabolite of nafiverine, *N*-(2-hydroxyethyl)-*N'*-[α-(naphthyl)propionyloxy-2-ethyl]piperazine, is a new compound and its synthesis is described.

Keyphrases □ Nafiverine—stability, absorption, excretion, and distribution, intramuscular, intravenous, and oral administrations, rats, rabbits, and mice □ Stability—nafiverine, aqueous solution and rabbit plasma □ Absorption—nafiverine, intramuscular, intravenous, and oral administrations, rats, rabbits, and mice □ Distribution—nafiverine, intramuscular, intravenous, and oral administrations, rats, rabbits, and mice □ Metabolism—nafiverine, rats, rabbits, and mice

Nafiverine[1], *N,N'*-di[α-(1-naphthyl)propionyloxy-2-ethyl]piperazine (I), is a new synthetic antispasmodic which shows low toxicity and a high myolytic activity on the smooth muscles of the ileum, uterus, and coronary vessels (1, 2). The present work investigated its stability in aqueous solution, absorption, excretion, and distribution.

EXPERIMENTAL[2]

α-(1-Naphthyl)propionic Acid (III)—A mixture of 80 g of sodium hydroxide and 800 ml of 50% ethanol was added to 61.2 g of dihydrochloride of I, and this mixture was stirred for 30 min at room temperature. After the addition of 250 g of ice to the reaction mixture, 190 ml of concentrated hydrochloric acid was added to make the mixture acidic. The mixture was diluted to 2 liters with water and was stored in a freezer. Crystals separated and were collected by filtration and recrystallized from ethanol as colorless scales (40 g), mp 147.5–148.5°. The melting point of III agreed with that reported by Blicke and Feldkamp (3).

Anal.—Calc. for $C_{11}H_{11}O_2$: C, 77.98; H, 6.04. Found: C, 77.98; H, 6.34.

***N,N'*-Di(2-hydroxyethyl)piperazine (IV)**—After filtration of III, the filtrate was made alkaline (pH about 10.0) by the addition of 5% sodium hydroxide solution and evaporated to dryness. The residue was extracted with 300 ml of chloroform, and the chloroform extract was evaporated to obtain about 9.3 g of crude IV. This residue was recrystallized from ethanol to colorless prisms, mp 134.0–135.5°; the melting point agreed with that reported by Pyman (4).

Anal.—Calc. for $C_8H_{18}N_2O_2$: C, 55.14; H, 10.41; N, 16.08. Found: C, 55.42; H, 10.61; N, 16.32.

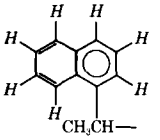
***N*-(2-Hydroxyethyl)-*N'*-[α-(1-naphthyl)propionyloxy-2-ethyl]piperazine (V)**—A solution of 17.6 g (0.08 mole) of α-(1-naphthyl)propionyl chloride (obtained by the treatment of III with thionyl chloride) in 100 ml of chloroform was gradually added dropwise during 2.5 hr to a refluxing mixture of 41.8 g (0.24 mole) of IV and 450 ml of chloroform. After evaporation of chloroform, the residue was mixed with dilute sodium bicarbonate solution to pH 9–10. This mixture was extracted with ether, and the ethereal solution was dried over anhydrous sodium sulfate after washing with a small amount of water. A yellowish oily substance (34.4 g) was obtained by evaporation of ether.

The oily residue was reacted with methanesulfonic acid in cold methanol, and the methanol was removed *in vacuo*. The residue thus obtained was mixed with 140 ml of water and stored in a refrigerator. A white precipitate (dimethanesulfonate of I) separated and was filtered. The filtrate was washed with ether and then extracted with ether after the medium was made alkaline with 8% sodium bicarbonate solution. From this ethereal solution, 12.4 g of crude V was obtained after evaporation of ether. The crude V was purified by TLC and identified as its methanesulfonate.

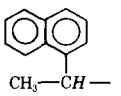
The TLC conditions were: adsorbent, Diatomite (Kieselgel G) in thickness of 1 mm; solvent, benzene–ethyl acetate–diethylamine (7:2:1); and R_f values (20°), 0 (III), 0.25–0.46 (V), and 0.53–0.57 (I).

Pure V (1.1 g) was obtained from 2.5 g of crude V by extraction of the corresponding spots with methanol.

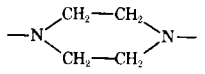
Dimethanesulfonate (VI) of V—Fifty milliliters of methanol containing 6.73 g of methanesulfonic acid (0.07 mole) was added gradually under ice cooling and stirring to a mixture of 12.4 g of V (0.035 mole) and 100 ml of methanol. Ethyl acetate was added to the reaction mixture until formation of turbidity and then methanol was added until the turbidity vanished. When the mixture was stored overnight in a refrigerator, colorless crystals of VI separated (14 g), mp 114–115°; NMR ($CDCl_3$)[3] of V: τ 1.78–2.63 (7H, m)



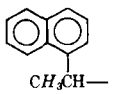
5.50 (1H, q, J = 7.0 Hz)



5.81 (2H, t, J = 5.5 Hz, —COOCH_2CH$_2$N<), 6.43 (2H, t, J = 5.3 Hz, >N—CH$_2$CH_2OH), 6.47 (1H, s, >N—CH$_2$CH$_2$OH), 7.54 (2H, t, J = 5.5 Hz, —COOCH$_2$CH_2N<), 7.57 (2H, t, J = 5.3 Hz, >NCH_2CH$_2$OH), 7.72 (8H, s)



and 8.33 (3H, d, J = 7.0 Hz)



IR (liquid film): ν_{COO} 1735 and ν_{OH} 3400 cm^{-1}.

Anal.—Calc. for $C_{23}H_{36}N_2O_9S_2$: C, 50.35; H, 6.61; N, 5.11. Found: C, 50.13; H, 6.63; N, 5.21.

Determination of I, II, V, and VI (Methyl Orange Method)—A mixture of 3 ml of water, 1.5 g of sodium chloride, 8 ml of chloroform, and 1 ml of plasma or aqueous solution containing I, II, V, or VI was shaken vigorously for 1 hr. Four milliliters of the chloroform layer after centrifugation was subjected to the methyl orange method (5). The chloroform layer was mixed with 3 ml of an equivolume mixture of 0.35% methyl orange solution and 1.75% boric acid solution, and the mixture was shaken for 10 min. Three milliliters of the chloroform layer after centrifugation was mixed with 0.5 ml of ethanol containing 2% sulfuric acid, and the absorbance of this mixture was determined at 520 nm. A mixture of 0.9 ml of plasma and 0.1 ml of a compound solution of known concentration was treated as described to prepare a calibration curve for plasma concentration.

Determination of IV (Folin Method)—The modified Folin method (6) was used to quantitate IV.

Determination of III (Formalin Method)—A mixture of 1 ml of plasma, 3 ml of 0.033 *N* HCl, 1.5 g of sodium chloride, and 8 ml

[1] Instituto De Angeli Co., Ltd., Italy.

[2] Nafiverine dimethanesulfonate (II) was obtained from the Instituto De Angeli Co., Ltd., Italy.

[3] The internal standard was tetramethylsilane.

Table I—Tabulation of Slope (k_1)[a], Heat of Activation, and Other Data Derived from Arrhenius Plots of Pseudo-First-Order Rates for Thermal Degradation of II and VI in Aqueous Solutions

	II	VI
Rate constant (k_1) at 40°	27.8×10^{-3}	17.1×10^{-3}
Rate constant (k_1) at 50°	99.4×10^{-3}	31.9×10^{-3}
Rate constant (k_1) at 60°	330.5×10^{-3}	57.6×10^{-3}
Heat of activation, ΔHa in kcal/mole	25.7	12.6
P[b]	16.4	7.1
Predicted rate constant k_1 at 25°	3.5×10^{-3}	6.2×10^{-3}

[a] The rate constant k_1 is in reciprocal days. [b] Log (frequency factor).

of ethyl acetate was shaken for 1 hr. After centrifugation, the evaporated residue from 4 ml of the ethyl acetate layer was reacted with 1 ml of 3% paraformaldehyde in concentrated sulfuric acid for 1 hr at room temperature. Absorbance of this mixture after the addition of 3 ml of concentrated sulfuric acid was measured at 640 nm. A mixture of 0.9 ml of plasma and 0.1 ml of III solution of known concentration was treated as described to prepare a calibration curve.

Stability Test of II and VI in Aqueous Solution—A 1% solution of II or a 0.3% solution of VI stored at 40, 50, or 60° was used for the stability tests.

To assay II or VI, one spot, having the same R_f value as the authentic sample of pure II or VI after development of 10 μl of the test solution by TLC, was shaken with a mixture of 3 ml of water, 1.5 g of sodium chloride, and 8 ml of chloroform for 1 hr. Four milliliters of the chloroform layer after centrifugation was treated by the methyl orange method to quantitate II or VI. The TLC solvent was ethyl acetate–benzene–ethylamine (7:5:1), the color developer was Folin reagent, and the adsorbent was Diatomite (Kieselgel G), 0.25 mm in thickness. The R_f values of I, III, IV, and V at 20° were 0.90, 0.03, 0.03, and 0.35, respectively.

Stability Test of II in Rabbit Plasma *In Vitro*—A mixture of 0.9 ml of rabbit plasma and 0.1 ml of 0.1% II solution was stored for 2 hr at 0 and 20°. One spot, having the same R_f value as the authentic sample of pure II after development of 0.1 ml of the mixture by TLC, was shaken with a mixture of 3 ml of water, 1.5 g of sodium chloride, and 8 ml of chloroform for 1 hr. After centrifugation, 4 ml of the chloroform layer was treated by the methyl orange method or Folin method to quantitate II, IV, and VI. The TLC solvent was ethyl acetate–benzene–diethylamine (2:7:1), the color developer was Folin reagent, and the adsorbent was Diatomite (Kieselgel G), 0.5 mm in thickness. The R_f values of the authentic samples at 20° were 0.01 (IV), 0.35 (VI), and 0.85 (II).

Distribution of II and Its Metabolites in Animal Tissues—

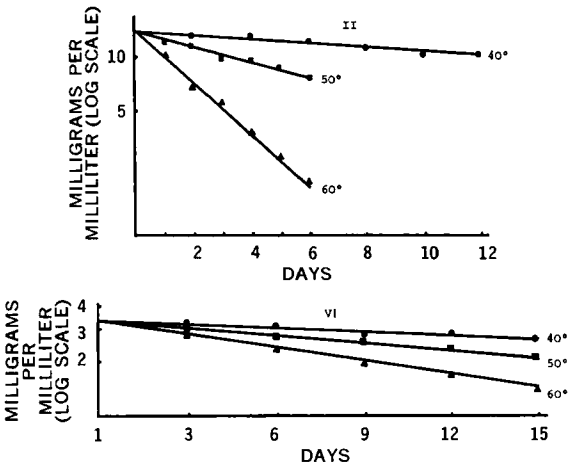


Figure 1—*Content of II or VI in aqueous solution. Initial concentration was: II, 14 mg/ml; and VI, 3.3 mg/ml. All data are the mean values of three experiments under the same conditions.*

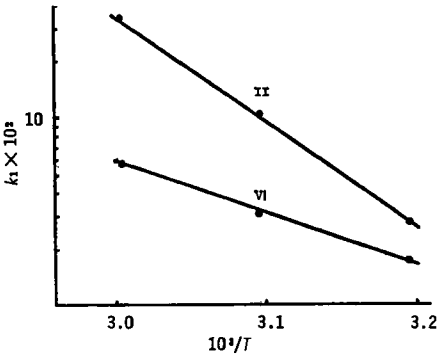


Figure 2—*Arrhenius plots of II and VI in aqueous solution. T = absolute temperature; and* k_1 *= decomposition rate constant in reciprocal days.*

Each group consisted of three male and three female rabbits (average weight 2.3 kg) or of five male and five female rats (Wistar strain, average weight 140 g). To rabbits and rats, 87 and 100 mg/kg of II were administered intramuscularly, respectively.

Eight organs (brain, lungs, heart, liver, kidneys, spleen, uterus, and testicle) were used for the determination of II–V. The sample sizes were: 4 g, brain and kidneys; 3 g, lungs and testicle; 2 g, heart;

Table II—Distribution of Metabolites I, III, IV, and V[a] of Nafiverine after Intramuscular Administration of Nafiverine Dimethanesulfonate to Rabbits

Organ	Hour	I		III		IV		V	
		mmoles/g	μg/g	mmoles/g	μg/g	mmoles/g	μg/g	mmoles/g	μg/g
Brain	2	0.12 ± 0.02	86 ± 14	0.19 ± 0.05	38 ± 9	1.14 ± 0.05	199 ± 9	0.05 ± 0.01	29 ± 8
	4	0.17 ± 0.01	120 ± 7	0.06 ± 0.01	11 ± 2	1.30 ± 0.16	226 ± 28	0.08 ± 0.01	46 ± 4
Lungs	2	0.10 ± 0.01	75 ± 6	0.54 ± 0.13	108 ± 26	1.41 ± 0.18	246 ± 31	0.06 ± 0.01	32 ± 7
	4	0.07 ± 0.02	51 ± 14	0.06 ± 0.01	11 ± 2	0.32 ± 0.20	56 ± 35	0.07 ± 0.00	39 ± 1
Heart	2	0.16 ± 0.00	117 ± 3	0.16 ± 0.01	31 ± 2	0.33 ± 0.11	57 ± 19	0.08 ± 0.01	42 ± 3
	4	0.17 ± 0.02	126 ± 19	0.05 ± 0.01	9 ± 2	0.22 ± 0.09	38 ± 16	0.08 ± 0.01	42 ± 6
Liver	2	—	—	0.10 ± 0.02	19 ± 4	0.58 ± 0.34	101 ± 58	—	—
	4	—	—	0.32 ± 0.26	62 ± 51	0.70 ± 0.14	121 ± 24	—	—
Spleen	2	0.23 ± 0.00	163 ± 2	0.65 ± 0.31	129 ± 62	1.29 ± 0.05	225 ± 9	0.10 ± 0.02	56 ± 12
	4	0.18 ± 0.02	128 ± 15	0.38 ± 0.10	76 ± 19	1.19 ± 0.22	206 ± 37	0.11 ± 0.05	61 ± 26
Kidneys	2	0.09 ± 0.02	65 ± 12	0.69 ± 0.08	137 ± 20	1.23 ± 0.25	214 ± 44	0.07 ± 0.01	36 ± 5
	4	0.10 ± 0.01	72 ± 9	0.71 ± 0.04	142 ± 9	0.93 ± 0.32	163 ± 56	0.06 ± 0.00	32 ± 3
Uterus	2	0.24 ± 0.05	174 ± 37	0.69 ± 0.14	138 ± 29	1.38 ± 0.14	240 ± 25	0.22 ± 0.06	118 ± 32
	4	0.14 ± 0.05	102 ± 41	0.43 ± 0.12	84 ± 23	1.12 ± 0.52	195 ± 91	0.08 ± 0.01	46 ± 6
Testicle	2	0.13 ± 0.04	94 ± 29	0.08 ± 0.06	15 ± 11	0.16 ± 0.05	28 ± 8	0.05 ± 0.01	28 ± 8
	4	—	—	0.01 ± 0.00	2 ± 0	0.04 ± 0.01	7 ± 2	—	—

[a] See text.

Table III—Distribution of Metabolites I, III, IV and V[a] of Nafiverine after Intramuscular Administration of Nafiverine Dimethanesulfonate to Rats

Organ	Hour	I		III		IV		V	
		mmoles/g	μg/g	mmoles/g	μg/g	mmoles/g	μg/g	mmoles/g	μg/g
Brain	1	—	...	0.12	23	0.03	5	—	...
	2	—	...	0.11	22	—	...	—	...
	6	—	...	0.03	6	—	...	—	...
Lungs	1	0.12	91	0.03	5	0.03	6	0.08	44
	2	—	...	0.13	25	0.06	11	—	...
	6	—	...	0.11	22	—	...	—	...
Heart	1	0.13	93	0.13	25	0.05	9	0.03	17
	2	0.09	65	1.32	263	0.21	37	0.03	16
	6	—	...	0.03	6	0.10	18	—	...
Liver	1	—	...	—	...	0.02	3	—	...
	2	—	...	—	...	0.08	14	—	...
	6	—	...	—	...	—	...	—	...
Spleen	1	0.08	58	0.06	11	0.04	7	0.05	25
	2	0.07	48	0.15	29	0.38	66	0.06	34
	6	—	...	—	...	—	...	—	...
Kidneys	1	0.11	79	0.11	22	0.25	44	0.08	42
	2	0.09	64	—	...	0.22	39	0.05	24
	6	—	...	—	...	0.10	17	—	...
Uterus	1	0.19	139	0.02	3	0.06	11	0.07	39
	2	—	...	—	...	0.32	55	—	...
	6	—	...	—	...	—	...	—	...
Testicle	1	—	...	0.06	11	0.02	3	—	...
	2	—	...	—	...	—	...	—	...
	6	—	...	—	...	—	...	—	...

[a] See text.

15 g, liver; and 1 g, spleen and uterus. These samples were homogenized with water, using 7 ml for brain, spleen, and kidney; 6 ml for lung, uterus, and testicle; and 8 ml for heart and liver.

The homogenate was centrifuged and 1 ml of the supernate was treated by the same method as used for the determination of III and IV.

For the determination of II and V, 4 ml of the supernate from the centrifuged homogenate was mixed with 1.5 g of sodium chloride and 8 ml of chloroform; this mixture was shaken vigorously for 1 hr. After centrifugation, 4 ml of the chloroform layer was evaporated, the residue was dissolved in 100 μl of ethanol, and 50 μl of the ethanol solution was submitted to TLC. The TLC and following procedures used were the same as described in *Stability Test of II in Rabbit Plasma* In Vitro. Organs of rabbits and rats not receiving any drug were treated in the same manner to serve as controls. Mixtures of control homogenate spiked with II, III, IV, or V solution of known concentration were treated as already described to prepare a calibration curve.

Collection of Rabbit Bile—Three female rabbits, 2.0–2.5 kg 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 II under continuous anesthesia with urethan. At the end of the 1st (control) hr, the compound was given intravenously, intramuscularly, or orally.

Determination of Metabolites of II in Rabbit Bile—To 1 ml of rabbit bile in an ice-cold centrifuge tube, 2 ml of ethanol was added and the mixture was stirred thoroughly. After standing for 30 min under cooling with ice water, 0.5 ml of the supernate from centrifugation was mixed with 0.5 ml of water. This mixture was used for the determination of III or IV by using the formalin method and Folin method.

Detection of Urinary Metabolites of II in Rabbits—Urine was collected for 24 hr from four female rabbits (average weight 3.2 kg) receiving 1000 mg of II orally. After freeze evaporation of urine, 500 mg of the residue was mixed with 2 ml of water, and 0.1 ml of the supernate after centrifugation of this mixture was submitted to TLC.

The TLC conditions were: adsorbent, Diatomite (Kieselgel G), 0.5 mm in thickness; distance developed, 15 cm; Solvent A, acetone–ethyl acetate–chloroform (3:2:1); Solvent B, benzene–ethyl acetate–diethylamine (7:2:1); Solvent C, chloroform–methanol–28% ammonia (10:4:1); Solvent D, benzene–acetone–methanol (7:2:1); and color developer, Folin reagent or saturated solution of potassium dichromate in concentrated sulfuric acid. The R_f values were: Solvent A, 0.93 (I), 0.71 (III), 0.01 (IV), and 0.07 (V); Solvent B, 0.85 (I), 0.01 (III), 0.07 (IV), and 0.46 (V); Solvent C, 0.98 (I),

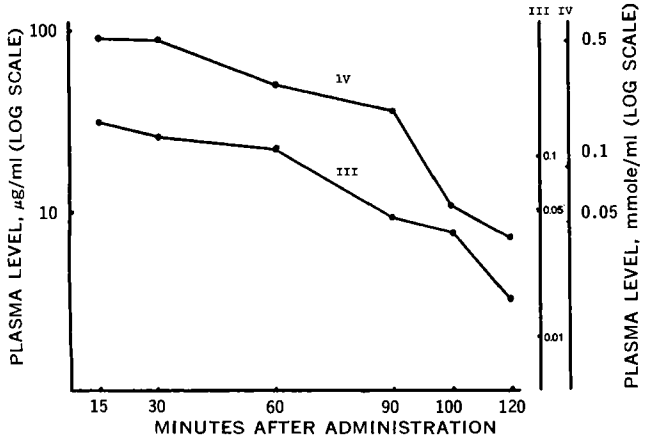


Figure 3—*Plasma level of metabolites of nafiverine dimethanesulfonate following intravenous administration of 50 mg to five female rabbits weighing about 2.4 kg each.*

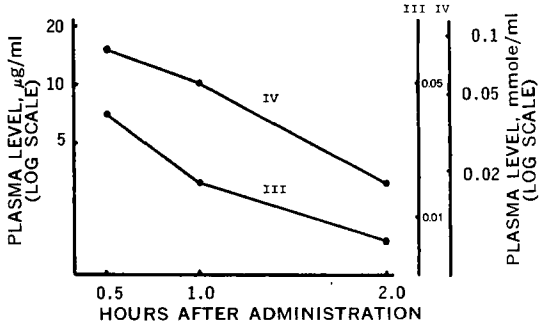


Figure 4—*Plasma level of metabolites of nafiverine dimethanesulfonate following intramuscular administration of 50 mg to five female rabbits weighing about 2.8 kg each.*

Table IV—Amount[a] of Metabolites III and IV[b] of Nafiverine Excreted in Female Rabbit Bile for 7.5 hr after Administration of Nafiverine Dimethanesulfonate

Route	Mean Body Weight, kg	Dose, mg	Metabolite	μg	% [c]	Bile Volume, ml/kg
Intravenous	2.3	50	III:	406 ± 131[d]	1.5 ± 0.5	16.1 ± 2.7[d]
			IV:	1790 ± 686	15.0 ± 5.8	
Intramuscular	2.3	50	III:	92 ± 50	0.3 ± 0.2	14.2 ± 6.5
			IV:	1211 ± 484	10.2 ± 4.1	
Oral	2.1	100	III:	—	—	14.9 ± 2.5
			IV:	—	—	
Control	2.3					19.8 ± 2.9

[a] Each group consisted of three rabbits. [b] See text. [c] Percent of III or IV was calculated from a ratio of 2 moles of excreted III or 1 mole of excreted IV to 1 mole of nafiverine dimethanesulfonate. [d] Mean value ± *SE*.

0.85 (III), 0.83 (IV), and 0.98 (V); and Solvent D, 0.96 (I), 0.52 (III), 0.02 (IV), and 0.39 (V).

Assay of Metabolites of II in Rabbit Urine—A mixture of 1 ml of water and 500 mg of the lyophilized residue of urine of rabbits receiving II in different doses and by different routes of administration was centrifuged, and 10 μl of the supernate was submitted to TLC. The two spots corresponding to III and IV were each scraped off from the TLC plate developed with a mixed solvent of chloroform–methanol–28% ammonia (10:4:1) and extracted with 2 ml of ethanol by standing for 30 min at room temperature.

After centrifugation, 1 ml of the supernate of the ethanolic extract was used for determination of III and IV. To assay III, the evaporated residue of the supernate was treated by the formalin method. In the determination of IV, the supernate was treated by the Folin method (6). The calibration curves of the two compounds were prepared separately by TLC as described by spotting the solution of the pure compound of a known concentration.

RESULTS AND DISCUSSION

The assay procedures used in the present work (*i.e.*, methyl orange method for I, II, V, and VI; Folin method for IV; and formalin method for III) are all very sensitive, and it was ascertained that 1 μg of any of the six compounds in the experimental conditions used is assayable by the respective assay method. Furthermore, III and IV do not show color reaction by the Folin method and the formalin method, respectively.

Since there are two ester bonds in the chemical structure of II, the stability of II in aqueous solution was examined to find the reason for its stability in animal blood. In the stability tests of II and VI in aqueous solutions, II and VI are not detected and assayed as themselves (dimethanesulfonate) but as I and V (free forms), respectively, by the TLC determination. From TLC of the test solutions with which the stability tests were performed, I, III, IV, and V were detected as the decomposition products of II; and III, IV, and V were detected as the decomposition products of VI.

From the assay results (Fig. 1) of unchanged II or VI, the kinetics of degradation of II and VI at 40, 50, and 60 ± 1° were interpreted on the basis of the pseudo-first-order reactions. Several constants (7) concerning the stabilities of II and VI (Table I) were derived from the results shown in Fig. 1; the Arrhenius plot is shown in Fig. 2.

As shown by TLC, I and V did not appear in animal blood following administration of II. Plasma levels of Metabolites III and IV after administration of II are shown in Figs. 3–7. In Fig. 3, blood levels of the metabolites were determined until 2 hr after administration of II. Figure 4 shows that both III and IV disappeared 3 hr after their administration; in Fig. 5, IV vanished 7 hr after administration, although zero values could not be plotted on logarithmic paper. In rats (Fig. 6), both III and IV disappeared 4 hr after administration. In mice (Fig. 7), III and IV vanished 8 and 4 hr, respectively, after administration.

To see some of the reasons for the absence of I and V in animal blood, the stability of II in rabbit plasma was investigated *in vitro*. The methyl orange method can be used to quantitate both I and V; therefore, the remaining percent of I is the amount of a mixture of I and V in plasma. The remaining percentages of I at 0.5, 1, and 2 hr after storage of a mixture of rabbit plasma and II at 20 ± 1° were 45.2, 12.4, and 1.1%, respectively. On the other hand, the remaining percentage of I at 2 hr after storage of the same mixture at 0° was 97.7%. In addition, V was detected by TLC in a mixture of plasma and II stored at 20° for 0.5 and 1 hr, but V could not be

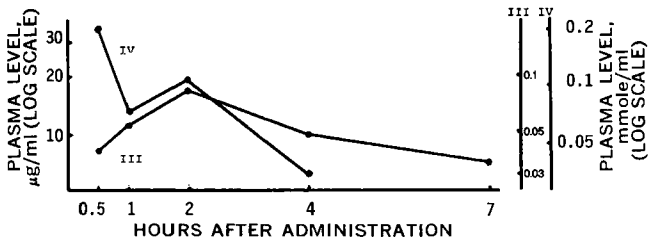


Figure 5—*Plasma level of metabolites of nafiverine dimethanesulfonate following oral administration of 100 mg to three female rabbits weighing about 2.3 kg each.*

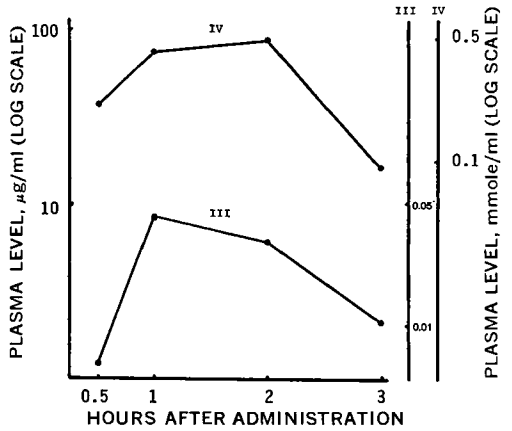


Figure 6—*Plasma level of metabolites of nafiverine dimethanesulfonate following intramuscular administration of 100 mg to five female rats (Wistar strain, average weight 135 g).*

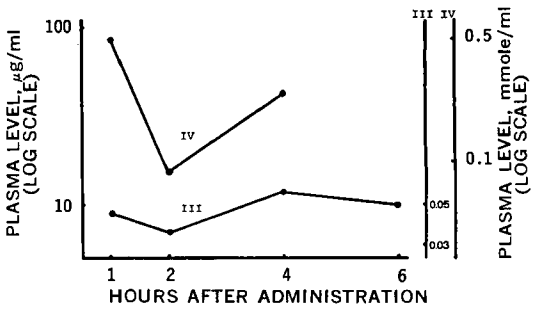


Figure 7—*Plasma level of metabolites of nafiverine dimethanesulfonate following intramuscular administration of 100 mg to 10 male mice (dd strain, average weight 20 g).*

Table V—Excretion of Metabolites III and IV[a] of Nafiverine after Administration of Nafiverine Dimethanesulfonate to Rabbits

Rabbits Used	Mean Body Weight, kg	Sex	Route	Dose, mg	Metabolite	Excreted Amount, mg 0–24 hr	24–48 hr	48–72 hr	72–96 hr	Total	Excretion[b], %
4	3.2	Male	Oral	1000	III	75.2	18.9	1.8	—	95.9	17.6
					IV	16.5	7.2	3.2	2.7	29.6	12.4
3	2.2	Female	Oral	100	III	5.8	7.7	0.4	0.2	14.1	25.8
					IV	2.3	2.7	0.4	—	5.4	22.7
4	3.0	Male	Intramuscular	900[c]	III	14.0	58.6	34.5	3.9	111.0	22.6
					IV	10.1	19.7	13.1	5.1	48.0	22.4
3	3.3	Female	Intramuscular	50	III	5.1	1.1	0.3	—	6.5	23.8
					IV	4.1	1.5	1.1	1.3	8.0	67.2
3	2.8	Female	Intravenous	50	III	5.6	1.2	0.5	—	7.3	26.7
					IV	5.2	1.6	1.3	—	8.1	68.0

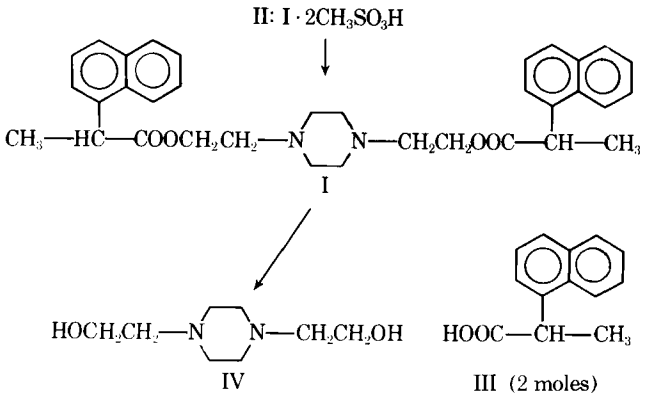
[a] See text. [b] Percent of III or IV was calculated from a ratio of 2 moles of excreted III or 1 mole of excreted IV to 1 mole of nafiverine dimethanesulfonate. [c] Nafiverine dimethanesulfonate (300 mg) was given three times every 2 hr to a rabbit.

found in the mixture stored at 0 or 20° for 2 hr. These facts revealed that I is unstable and rapidly hydrolyzed in rabbit plasma at 20°. As shown in Tables II and III, Metabolites I, III, IV, and V are distributed in animal organs but none of these metabolites is found 24 hr after intramuscular administration of II to rabbits and rats. It is interesting that a relatively large amount of metabolites of II was distributed in the uterus (rabbits and rats) but not in testicles.

The amount of metabolites of I excreted in rabbit bile during 7.5 hr after administration by different routes is shown in Table IV. No metabolites of I were found using the methyl orange method, and this fact supports the absence of I and V. Metabolites III and IV were found as the metabolites of I in rabbit bile. About 17% of the administered II was excreted in rabbit bile after its intravenous administration, while about 11% of the administered II was excreted in bile after its intramuscular administration. On the other hand, no metabolites were found after oral administration. It is desirable to examine the reasons for the considerable differences in the amount of metabolites excreted by different routes of administration. Compound II showed no effect on the volume of rabbit bile excreted during 7.5 hr.

Urine collected from rabbits receiving II was lyophilized and the residue was examined by TLC for the presence of metabolites. Urine was collected for 4 days after administration of II, since urine collected from 96 to 120 hr after the administration contained no chemical or its metabolites, as evidenced by TLC. Two metabolites, III and IV, were detected in rabbit urine by TLC. Two spots representing III and IV were separated and identified by using Solvent C for TLC. A micromelting-point determination of the mixture of evaporated residue of ethanolic extract (obtained from 5–10 spots having the same R_f value) and an authentic sample was used for identification.

To detect some metabolites present in conjugated form in rabbit urine, the lyophilized residue of urine was hydrolyzed with 5 *N* hydrochloric acid under the same conditions as reported for aminoethanesulfonylpiperidine (8). After neutralization with 5 *N* sodium carbonate solution, the mixture was submitted to TLC, using Solvents A, B, C, and D. No metabolites except III and IV were found, and no increase was found in the amounts of III and IV excreted. Therefore, III and IV in rabbit urine were directly determined by using the formalin method and the Folin method, respectively, as described under *Experimental*.



Scheme I

As shown in Table V, the fact that about 68% of IV was excreted after intravenous administration compared to about 67% after intramuscular administration shows that the availability of II after intramuscular administration is not different from that after intravenous administration. The ratio of the amount of IV to that of III in rabbit urine was near 1 after oral administration of II and intramuscular administration of 900 mg of II, while the ratio was above 2.5 after intramuscular administration of 50 mg of II and intravenous administration of II. The reason for these changes in the ratio according to the dose or the route of administration should be investigated.

In the present work, the main fate of II in animals should be as shown in Scheme I.

SUMMARY

1. Stabilities of nafiverine dimethanesulfonate and *N*-(2-hydroxyethyl) - *N'*-[α-(1-naphthyl)propionyloxy-2-ethyl]piperazine dimethanesulfonate were measured. The latter is a new compound and its synthesis was described.

2. Nafiverine could not be detected in animal blood but was present in animal tissues after the administration of its dimethanesulfonate.

3. In animal blood, bile, and urine, two metabolites, α-(1-naphthyl)propionic acid and *N,N'*-di(2-hydroxyethyl)piperazine, were detected and assayed.

4. Absorption of nafiverine dimethanesulfonate was almost the same by intramuscular administration as by intravenous administration on the basis of its excretion in rabbit urine.

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Spasmolytic Constituents of *Cedrus deodara* (Roxb.) Loud: Pharmacological Evaluation of Himachalol

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Abstract □ Himachalol has been identified as the major antispasmodic constituent in the wood of *Cedrus deodara*. The pharmacological studies of himachalol on various isolated smooth muscles (guinea pig ileum, rabbit jejunum, rat uterus, and guinea pig seminal vesicle) and against different agonists (acetylcholine, histamine, serotonin, nicotine, and barium chloride) indicated spasmolytic activity similar to that of papaverine. It was a more potent antagonist of barium chloride-induced spasm of guinea pig ileum than papaverine but less effective in reverting a similar spasm of rabbit jejunum and had no relaxing effect alone. In the conscious immobilized cat, intragastric administration of himachalol or papaverine (100 mg/kg) produced equal inhibition of carbachol-induced spasm of the intestine, lasting about 2 hr, but himachalol had a faster onset of action. Himachalol was devoid of spasmolytic effect on the bronchial musculature of guinea pig but was 3.3 times more potent than papaverine in antagonizing epinephrine-induced contraction of the guinea pig seminal vesicle. Intravenous injection of himachalol (3–10 mg/kg) in the cat produced a dose-dependent fall in blood pressure and an increased femoral blood flow.

Keyphrases □ *Cedrus deodara*—spasmolytic constituents, pharmacological evaluation of himachalol □ Himachalol—spasmolytic constituent of *C. deodara*, pharmacological evaluation □ Spasmogens—pharmacological evaluation of himachalol □ Medicinal plants—pharmacological evaluation of himachalol from *C. deodara*

During the biological screening of Indian plants for the presence of active substances, it was observed that a 50% ethanol extract from the wood of *Cedrus deodara* (Roxb.) Loud (N.O. Pinaceae)[1] possessed significant antispasmodic activity (1). Detailed studies were undertaken, and the present article describes the identification of the major spasmolytic constituent as the known sesquiterpene himachalol (I) (2, 3) and its pharmacological evaluation.

EXPERIMENTAL

Extraction and Identification of Himachalol—The alcoholic extract of the plant wood (10 kg) was separated into petroleum ether-soluble, chloroform-soluble, water-soluble, and water-insoluble fractions. The petroleum ether-soluble fraction (510 g) showed an enhancement of antispasmodic activity, and it was subjected to chromatography over alumina (10 kg) in hexane solution.

The progressive elution of the column by solvents with increasing polarity and the biological evaluation of the resultant eluates led to the isolation of a fraction (65.3 g), which was eluted with hexane–benzene (1:1). This fraction was dissolved in acetonitrile (80 ml) and allowed to stand in a deep freeze when a crystalline deposit was obtained. It was filtered and recrystallized as colorless rhombuses (23 g), mp 67°. It was found to be homogeneous by TLC and GLC and exhibited antispasmodic activity.

This crystalline substance, $C_{15}H_{26}O$ (M^+ 222), was an unsaturated sesquiterpene alcohol; IR (KBr): 3320, 1130, 1025, 1650, and 862 cm^{-1}; NMR ($CDCl_3$): 0.86, 1.0 (3H, each s, two quaternary CH_3), 1.23 (3H, s, one CH_3 attached to a carbon linked to an oxygen), 1.65 (3H, one vinylic CH_3), and 5.55 (1H, d, J = 5 Hz, one olefinic proton) ppm. On the basis of physical and spectral data, it was identified as himachalol (2, 3).

Acute Toxicity—Mice of either sex, 15–25 g, were divided into groups of 10 each. They were deprived of food for 16 hr and administered graded doses of himachalol and papaverine orally or intraperitoneally. Himachalol was used as a suspension in gum acacia, and papaverine hydrochloride was used as an aqueous solution.

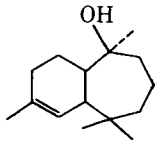
The volume of the oral and intraperitoneal injections never exceeded 0.2 and 0.1 ml/10 g of body weight, respectively. The mortality over the next 72 hr was recorded, and the LD_{50} value was calculated by the probit analysis method (4).

***In Vitro* Spasmolytic Activity**—*Guinea Pig Ileum*—Sections of ileum (4–5 cm long) were suspended in an organ bath of 16-ml capacity, containing aerated Tyrode solution at 35–36°. Acetylcholine chloride (1×10^{-8} g/ml), histamine acid phosphate (2.5×10^{-8} g/ml), serotonin (5×10^{-7} g/ml), nicotine sulfate (5×10^{-7} g/ml), and barium chloride (2×10^{-5} g/ml) were used as spasmogens and left in contact with the tissue for 15–20 sec. Contractions (1:6 magnification; 1 g tension) in response to spasmogen alone and in the presence of various concentrations of himachalol or papaverine were recorded using a frontal writing lever on a smoked drum.

An alcoholic solution of himachalol was added to the bath 1 min before the addition of the spasmogen and was washed out 15–20 sec later with two changes of bath solution at a 1-min interval. The concentration causing 50% reduction of contraction was calculated by plotting the log molar concentration and percent inhibition curve. Papaverine in an aqueous solution was used as the reference standard. The effect of different concentrations of himachalol and papaverine on the cumulative dose–response curve of the ileum to histamine was also studied using the Van Rossum and Van Den Brink (5) technique.

Rabbit Jejunum—Jejunum pieces (5–6 cm long) were set up as described for the guinea pig ileum. The effect of himachalol and papaverine was studied on the tone, motility, and barium chloride (2×10^{-5} g/ml)-induced spasm of the intestine.

Rat Uterus—Virgin rats, 100–230 g, were given stilbestrol (1



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[1] The plant sample was identified by Dr. B. Gupta and Mr. B. N. Mehrotra of the Botany Unit, Central Drug Research Institute, Lucknow, India. A voucher specimen (No. 27) has been preserved in the institute herbarium.