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Essential Oil of *Anemopsis californica* Part II: Minor Constituents

DILIP R. SANVORDEKER and MADHUKAR G. CHAUBAL*

Abstract □ The minor constituents of the essential oil hydrodistilled from the roots and rhizomes of *Anemopsis californica* (Nutt.) Hook and Arn. (Fam. *Saururaceae*) have been isolated by gas chromatography on two stationary phases: polypropylene glycol adipate and polyethylene glycol. The isolated compounds were identified by the comparison of their IR spectra with those of pure authentic compounds. Esdragol, thymolmethylether, linalool, *p*-cymene, 1,8-cineol, *d*-limonene, camphene and α- and β-pinene have been shown to be present in the oil. In addition, the presence of two ketones, a *sec* alcohol, tentatively identified as 1-methyl-4-isopropyl-6-hydroxy-bicyclo (3:1:0) hexane, an ester, and six hydrocarbons is also indicated on the basis of the IR spectra of the corresponding fractions.

Keyphrases □ *Anemopsis californica* oil—minor constituents □ GLC—separation, identity □ IR spectrophotometry—identity □ NMR spectroscopy—identity

Methyleugenol is the major constituent of the essential oil hydrodistilled from the roots and rhizomes of *Anemopsis californica* (Nutt.) Hook and Arn. (Fam. *Saururaceae*). Thymol and piperitone have also been shown to be present in appreciable quantity (1). These three compounds together make up approximately 74% of the oil. The isolation and identification of nine minor constituents of the essential oil of *Anemopsis* is reported here.

EXPERIMENTAL

Extraction of Essential Oil—The essential oil used in the previous experiments (1) was used for the quantitative determination of the constituents. Additionally, 500 g. crude drug (1) + 3.5 l. water was also hydrodistilled in the apparatus described earlier (1). However the distillation was carried out in an interrupted manner. After the first short period of distillation, the flask was cooled and the collected oil removed and stored over anhydrous Na_2SO_4. Distillation was then resumed for another short period followed by cooling and removal of oil. A series of fractions were thus separately collected as shown in Table I.

Table I—Fractional Collection of Essential Oil of *A. californica*

Fraction No	Distillation Period, hr.	Volume Collected, ml.	Color of Fraction
1	2.5	2.5	Colorless
2	2.0	2.5	Pale yellow
3	3.75	4.0	Pale yellow
4	4.0	4.0	Light-green
5	3.75	4.0	Greenish-blue
6	7.50	8.0	Greenish-blue
7	6.0	6.0	Deep bright-blue
Total	29.25	31.0	

Gas Chromatography (GC)—The chromatograph, recorder, and column dimensions were described earlier (1). In addition, a recorder[1] with an integrator,[2] was used for quantitation of peaks. The chart-speed of this recorder was 2.54 cm. (1 in.)/3 min.

Packing—Two stationary phases polypropylene glycol adipate[3] and polyethylene glycol[4] were coated on acid-washed diatomite aggregate[5] 60–80 mesh, as previously described and packed in two separate columns. Each column was preconditioned overnight at 170° in a slow stream of helium. The operating conditions were as follows: carrier gas, helium, 75 ml./min., inlet pressure Column A, 20.5 p.s.i. and Column B, 28 p.s.i.; detector, 240°, 150 ma. filament current; injection port, 200°; outlet 180°; column temperature, Column A, 160°, isothermal and 110–170°, isothermal-linear, and Column B, 180°, isothermal and 110–190°, isothermal-linear. In the isothermal-linear mode the starting temperature of the column (110°) was maintained for 15 min. after injection of the sample and then raised to the final temperature for each column by manual programing.

GC Fraction Collection and Identification of Constituents—The effluent peaks were collected in U-shaped traps fashioned from 22.86-cm. (9-in.) lengths of 0.31-cm. (0.125-in.) glass tubing. The straight, long arm of the trap was connected to the heated chromatograph

[1] Varian Aerograph model-20, Varian Aerograph Instruments Inc., Walnut Creek, Calif.
[2] Disc Chart, model-244, Disc Instruments Inc., Santa Ana, Calif.
[3] Reoplex-400, Union Carbide Corp., New York, N. Y.
[4] Carbowax-20M, Union Carbide Corp., New York, N. Y.
[5] Chromosorb-W, Johns-Manville Products Corp., New York, N. Y.

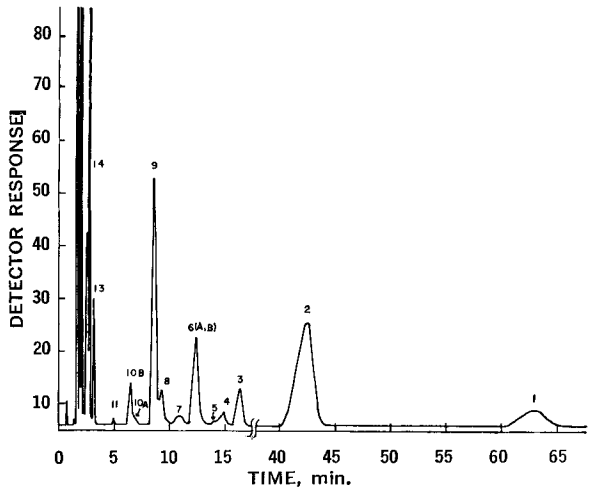

Figure 1—*Gas chromatogram of Fraction 1 collected during hydrodistillation of essential oil of* Anemopsis californica; *polypropylene glycol adipate, 160°, 4 μl., atten.: 2×.*

outlet with a short piece of tubing (Tygon). The outlet arm of the trap was bent and wrapped with a piece of film[6] which prevented excessive loss of fraction and also contamination by atmospheric moisture. The traps were chilled in a dry-ice bath. All the fractions were rechromatographed before spectral analysis. The following authentic samples were also purified by GC in the laboratory:

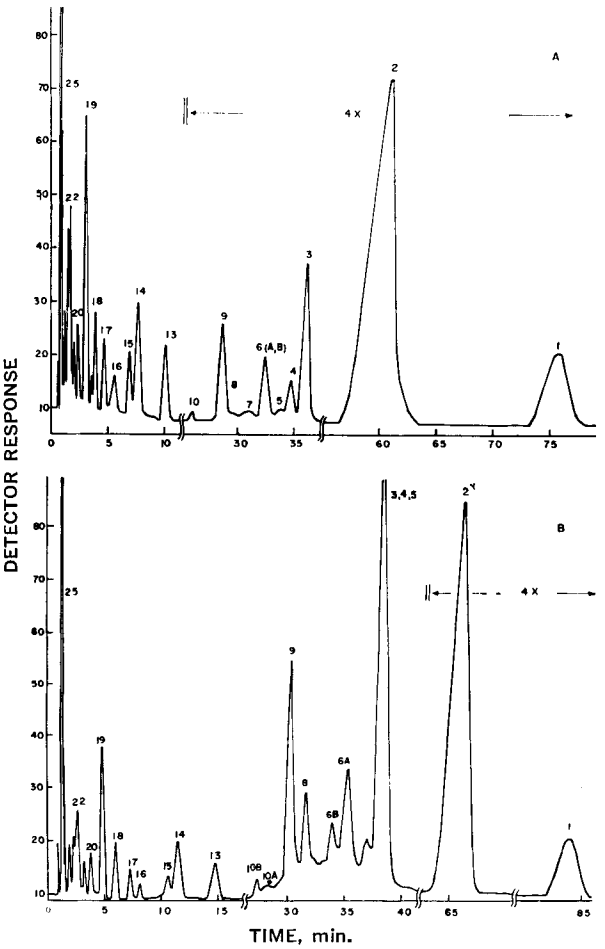

Figure 2—*Isothermal-linear gas chromatograms of hydrodistilled essential oil of* Anemopsis californica. *Key: A, polypropylene glycol adipate, 110–170°, 5 μl.; B, polyethylene glycol 110–190°, 5 μl.*

[6] Parafilm, Marathon Division of American Can Co., Neenah, Wisc.

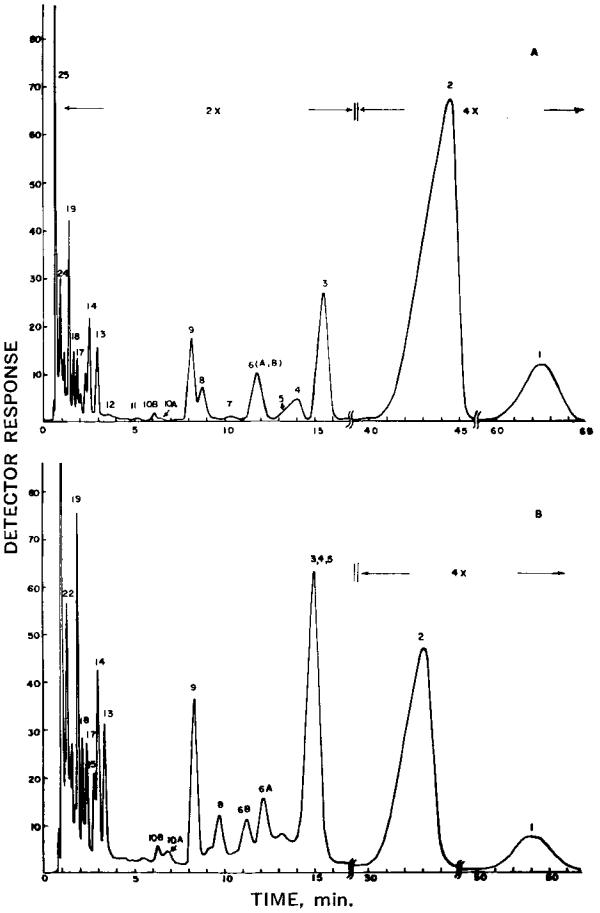

Figure 3—*Isothermal gas chromatograms of hydrodistilled essential oil of* Anemopsis californica. *Key: A, polypropylene glycol adipate, 160°, 5 μl.; B, polyethylene glycol, 180°, 4 μl.*

α-pinene and 1,8-cineol,[7] camphene and linalool,[8] *p*-cymene and δ-limonene.[9]

The identification of the constituents of the essential oil was based primarily on the comparison of their IR spectra with those of the GC-pure authentic samples and in a few cases comparison with published spectra. The IR spectra were obtained in simple KBr micro cells. Two thin, clear, 13-mm. pellets were pressed from approximately 150 mg. KBr each. One of the pellets was placed in the IR pellet holder[10] and 2–5 μl. of the sample was placed in its center. The second pellet was then placed on top and the holder cap carefully fastened. The NMR spectra were obtained in CCl_4 (Varian 60 MHz). TMS was the reference standard.

Retention Time (RT), Relative Retention Time (RRT) and Quantitation of Peaks—The RT values were measured from the time of injection = 0, to the center of each peak. The RRT values were based on that of δ-limonene = 1. The retention data were checked by the injection of the authentic samples. The peak quantitation was achieved by an integrator which was factory installed and calibrated.

RESULTS AND DISCUSSION

Fractionation of the essential oil prior to GC by the use of a high efficiency distillation column was not satisfactory. Further, the possibility of heat decomposition of the constituents and the production of artifacts had to be considered. The isolation of fractions during hydrodistillation of the oil of *A. californica* was most satisfactory. The oil was not exposed to further heat and GC of the

[7] Matheson Coleman & Bell Co., Los Angeles, Calif.
[8] Distillation Products Industries Inc., Rochester, N. Y.
[9] Fritzsche Brothers Inc., New York, N. Y.
[10] Perkin-Elmer Corp., Norwalk, Conn.

Table II—Composition of the Hydrodistilled Oil of *Anemopsis californica*

Peak No.	Compd.	% v/v	Retention Data: Polyester Vinyl Plasticizer 110° RT[a]	RRT[b]	160° RT	RRT	Polyethylene Glycol 110° RT	RRT	180° RT	RRT
1	Thymol	13.8	—	—	62.3	26.3	—	—	54.0	18.9
2	Methyleugenol	57.0	—	—	43.5	26.3	—	—	34.0	11.9
3	Piperitone	8.0	—	—	16.4	6.8	—	—		
4	Ketone?	1.80	—	—	14.7	6.1	—	—	15.5	5.3
5	Unknown	0.70	—	—	13.6	5.7	—	—		
6A	*sec*-Alcohol[c]?	1.8	—	—	12.2	5.2	—	—	12.1	4.2
6B	Esdragol[c]	1.32	—	—			—	—	11.2	3.9
7	Ketone (cyclic?)	0.34	—	—	10.8	4.6	—	—	—	—
8	Ester (cyclic, formate?)	1.50	—	—	9.0	3.8	—	—	9.8	3.4
9	Thymolmethylether[c]	2.96	—	—	8.5	3.6	—	—	8.3	2.85
10A	Unknown	0.20	—	—	6.3	2.6	—	—	6.9	2.4
10B	Linalool	0.32	—	—	6.1	2.58	—	—	6.3	2.2
11	Unknown	Trace	—	—	5.1	2.16	—	—	5.6	1.95
12	Unknown	Trace	—	—	3.6	1.5	—	—	4.5	1.6
13	*p*-Cymene	1.1	9.9	1.48	3.2	1.24	14.7	1.4	3.35	1.12
14	1,8-Cineol	1.35	7.55	1.09	2.5	1.26	11.5	1.1	3.0	1.05
15	*d*-Limonene	0.93	6.9	1.0	2.36	1.0	10.6	1.0	2.8	1.0
16	Unknown	0.1	5.6	0.81	1.9	0.81	8.3	0.78	2.3	0.81
17	β-Pinene	0.4	4.7	0.68			7.3	0.69		
18	Camphene	0.44	3.95	0.57	1.6	0.67	6.08	0.57	2.1	0.74
19	α-Pinene	1.4	3.08	0.45	1.5	0.62	4.9	0.46	1.95	0.68
20			2.4	0.35	1.25	0.53	3.85	0.36	1.65	0.58
21			2.05	0.30	1.1	0.45	3.20	0.30	1.50	0.53
22			1.8	0.26	1 0	0.44	2.65	0.25	1.3	0.46
	Hydrocarbons	4.5								
23			1.6	0.20	0.98	0.43	2.21	0.21	1.5	0.4
24			1.2	0.18	0.80	0.36	1.9	0.18		
25			0.98	0.14	0.70	0.30	1.25	0.11	1.0	0.32

[a] RT = Retention Time, min. [b] RRT = Relative Retention Time (*d*-limonene reference). All compounds identified by IR spectra. [c] NMR in addition to IR identification.

first two fractions showed satisfactory enrichment of the compounds of interest. Fraction 1 (Table I) was especially rich in the compounds eluted in the first 15 min. of injections (Fig. 1). This fraction was therefore used for the collection of the peaks for identification. Polypropylene glycol adipate (2, 3) and polyethylene glycol (4, 5) have been effectively used to elucidate the composition of essential oils. These papers also give the retention data for some of the constituents isolated from the oil of *A. californica*. These two stationary phases were therefore used in this work. The isothermal-linear programmed temperature technique gave good resolution of the peaks of interest and allowed convenient condensation of the fractions under each peak. Figure 2 shows these chromatograms for both the stationary phases. The retention data were obtained by operation in the isothermal mode. These chromatograms are shown in Fig. 3. The composition of the oil, determined to date, is shown in Table II, which also gives the retention data.

Peaks 1 and 2 were thymol and methyleugenol, respectively. Polypropylene gylcol adipate showed a resolution of Peaks, 3, 4, and 5 (Fig. 1, 2a, 3a). Polyethylene glycol showed only one peak in this region (Fig. 2b, 3b). Fraction under Peak 3 from the polyethylene glycol column was therefore condensed and rechromatographed on the adipate column resulting in the resolution of Peaks 3, 4, and 5 (Fig. 4). Peak 3 was piperitone. The IR of Peak 4 was indicative of an α-β unsaturated six-membered ketone which could be cyclic or acyclic (strong absorption: 1665 cm.$^{-1}$) with an isopropenyl substituent (medium absorption: 1420 cm.$^{-1}$ and strong absorption: 885 cm.$^{-1}$). Peak 5 could not be characterized for lack of sufficient sample.

Peak 6 initially collected from polypropylene glycol adipate gave an IR spectrum indicative of an aromatic alcohol. However, the NMR spectrum showed that the fraction was a mixture of at least two compounds. Rechromatography on the adipate did not

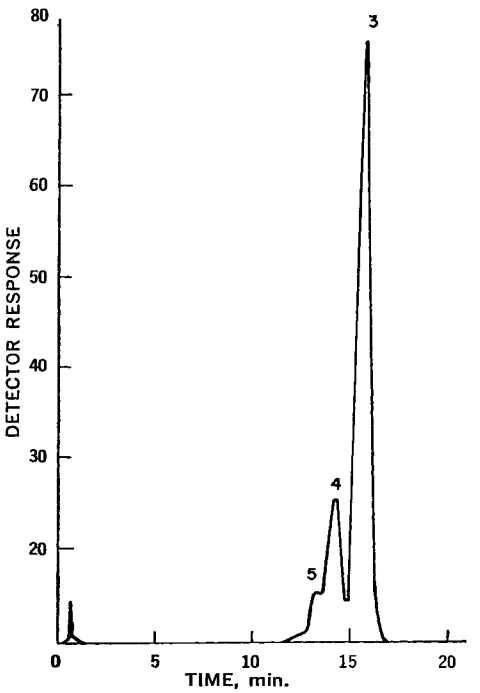

Figure 4—*Resolution of Peak 3 from polyethylene glycol after rechromatography on polypropylene glycol adipate, 160°, 2 μl., atten.: 2×.*

Table III—NMR Data for Peak 6A

TMS p.p.m.	Splitting	Coupling Constant J	Number of Protons	Assignment[a]
0.85	Doublet	3	6	1°-alkyl (—CH(CH$_3$)$_2$)
1.05	Singlet	—	3	1°-alkyl (—CH$_3$)
1.2	Multiplet	—	2	2°-OH (—CH$_2$—)
1.45	Singlet	—	1	2°-OH (C—OH)
1.55	Multiplet	—	1+1	3°-alkyl (—CH—)
1.75	Triplet	—	2	2°-alkyl (—CH$_2$—)
2.0	Multiplet	—	1	3°-alkyl (—CH)
3.65	Doublet	4	1	CH—OH
			Total protons 18	

[a] Possible structure:  1-methyl-4-isoproyl-6-hydroxy-bicyclo (3:1:0) hexane.

lead to resolution of the mixture. When the fraction collected from the adipate was rechromatographed on polyethylene glycol resolution into two peaks, 6A and 6B, was observed (Fig. 5). Peak 6A condensed as a white crystalline solid, m.p. 76–77°, uncorrected (Koffler Block). The IR spectrum of this compound (Fig. 6) indicated it to be a secondary dimeric alcohol (medium absorption: 3615, 3450, and 1052 cm.$^{-1}$) with an isopropyl substitution (medium absorption, doublet: 1385 and 1360 cm.$^{-1}$). The NMR spectrum (Fig. 7) shows features given in Table III, which also shows the possible structure for the compound, arrived at from the IR and NMR data. Further work on confirmation of the structure is in progress. Peak 6B gave an IR spectrum identical with that published for esdragol (estragol, methylchavicol, isoeugenol) (5, 6). The NMR spectrum also gave values as published for esdragol (7).

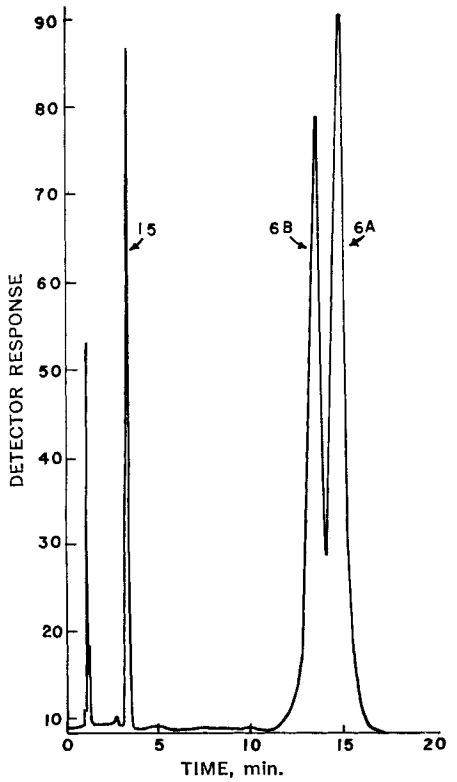

Figure 5—*Resolution of Peak 6 from polyester vinyl plasticizer after rechromatography on polyethylene glycol. δ-Limonene (15) reference. Hexane as solvent. Polyethylene glycol. 160°, 1 μl., atten.: 2×.*

Peak 7 has been tentatively identified as a cyclic ketone (strong absorption: 1735 and 1235 cm.$^{-1}$) with an isopropyl substituent. Peak 8 was indicated to be a cyclic ester, most probably a formate (strong absorption: 1727 and 1173 cm.$^{-1}$) with an isopropyl group.

GC on sucrose[11] had previously yielded an aromatic ether designated as Peak 12 (1). This ether is Peak 9 in the chromatograms shown here. The IR spectrum of this compound in KBr showed that the previously observed absorption band for an isolated unsaturation (1620 cm.$^{-1}$) was spurious. The NMR spectrum of this compound showed the following: aromatic quartet (δ = 6.8); aromatic —OCH$_3$ (δ = 3.8); one tertiary alkyl proton septet (δ = 3.3); one benzylic singlet (δ = 2.3); and a primary alkyl doublet (δ = 1.2).

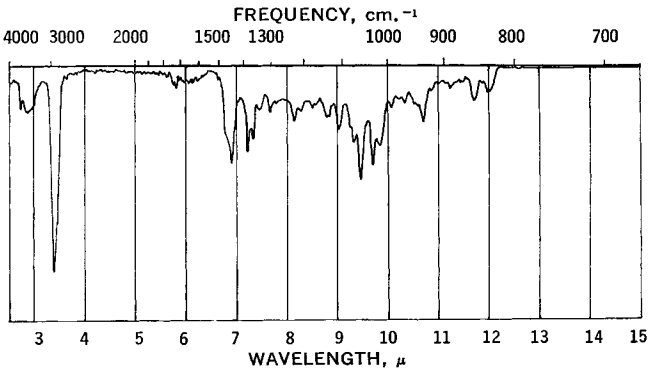

Figure 6—*IR spectrum of Peak 6A of essential oil of* A. *californica, from polyethylene glycol.*

[11] Hyprose-SP80, The Dow Chemical Co., Midland, Mich.

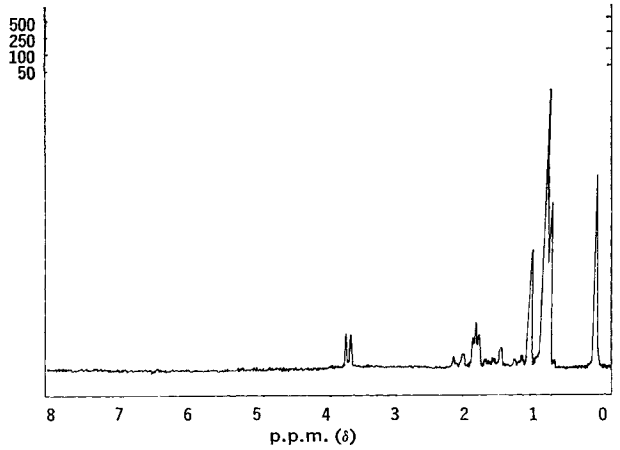

Figure 7—*NMR spectrum of Peak 6A of essential oil of* Anemopsis californica *from polyethylene glycol.*

The coupling constant (J = 9.1) also suggested an isolated aromatic proton. Thus the substitution pattern was seen to be 1:2:4. Among the possible structures, thymolmethylether was the immediate choice on biogenetic basis; thymol had been shown to be present in the oil in appreciable quantities. The IR and NMR spectra of synthetic thymolmethylether were identical with those of Peak 9.

Other compounds identified from the essential oil are given in Table II. Among these, β-pinene was identified by the comparison of peak IR with a published spectrum (8). The total fraction under Peaks 20 to 25 was collected in CCl_4. The IR spectrum of this sample showed that these six compounds are hydrocarbons.

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Biopharmaceutical Studies of Aminoethanesulfonylphenetidine I

SHUN-ICHI NAITO and KAZUO FUKUI

Abstract □ Blood levels of aminoethanesulfonylphenetidine (taurinophenetidine) in rabbits were determined and binding of the drug with rabbit serum protein *in vivo* and *in vitro* was examined. Metabolites of taurinophenetidine in rabbit urine were separated into four compounds, *p*-aminophenol, *p*-acetamidophenol, *p*-phenetidine, and unchanged taurinophenetidine. Metabolites of acetophenetidine were also investigated. Glucuronide in urine of rabbit ingesting taurinophenetidine is the conjugated form of *p*-aminophenol.

Keyphrases □ Aminoethanesulfonylphenetidine—biochemistry □ Metabolites, aminoethanesulfonylphenetidine—rabbit plasma, urine □ TLC—separation, identification □ Colorimetric analysis—spectrophotometer

Aminoethanesulfonic acid which is expected to separate from aminoethanesulfonylphenetidine when ingested, is one of the essential amino acids, in human subjects and has also surface-active property. It is widely known that *p*-phenetidine has antipyretic and analgesic actions, nevertheless, its toxicity must be severe. Aminoethanesulfonylphenetidine, the condensation product of aminoethanesulfonic acid and *p*-phenetidine, was prepared in order to obtain a more potent chemical than acetophenetidine with less side effects. Detailed actions of this new chemical cannot be predicted from the chemical structure without extensive research, since it is often observed that small changes in a chemical structure change pharmacological and biochemical behaviors of the chemical essentially, additionally, and extensively.

In the previous paper (1), stabilities of aminoethanesulfonylphenetidine (hereafter abbreviated as taurinophenetidine) stored at different temperature and pH were reported. It might be said that taurinophenetidine is stable in either solution or powder form to temperature, pH or moisture.

The purpose of this paper is to discuss some biopharmaceutical aspects of taurinophenetidine.

EXPERIMENTAL

Metabolites of Taurinophenetidine in Blood—A mixture of 0.5 ml. of rabbit plasma, taken 0.5 hr. (peak time of blood level) after