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Metabolic Studies on Toxic Compounds

5. Identification of New Methylhippuric Acids

By

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With 4 Figures

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Introduction

The glycine conjugation of aromatic acids has been demonstrated as an important detoxication mechanism in various animal species, including man[4,5]. Past studies in this laboratory on comparative biotransformation of benzene homologues have shown that these compounds were oxidized to aromatic acids[1–3]. Because of the possible *in vivo* formation of glycine conjugates in various animal species fed with these chemicals, namely with 1,2,4- or 1,3,5-trimethylbenzenes, the synthesis of 3,5- and 3,4-dimethylhippuric acids was carried out and a method of identification was developed in this laboratory to detect these metabolites by thin-layer chromatography, ultraviolet and infrared spectroscopy.

Experimental

Chemicals. Starting materials were either C. P. chemicals or purified before use with ethanol and charcoal. Thionyl chloride and carbon tetrachloride were purified by fractional distillation. Benzene and toluene dried over sodium were used to avoid hydrolysis of the acid chlorides.

Melting Points. All melting points (uncorrected) were taken on a Reichert hot stage microscope.

Spectra. The ultraviolet spectra were recorded on a Cary recording spectrophotometer. The infrared spectra were obtained for all synthetic materials using Nujol mulls and a Perkin-Elmer Model 221 spectrophotometer.

Chromatography

Thin-layer chromatography was carried out on 4 × 9 cm and 20 × 20 cm glass plates coated with Camag Silica gel DS-5 (thickness: 250 microns).

Eastman Chromagram sheets coated with SiO_2 containing a fluorescent indicator were also used. Although ultraviolet light (2537 Å) was used to view all chromatograms previously dried in a fumehood free from acid fumes, the best results were obtained with the Chromagram sheets. The acids showed up well as compact violet spots on a red fluorescent background, and spraying with a dye such as bromocresol green did not increase the colors. However, it was necessary to spray the SiO_2 glass plates with bromocresol green (0.04% in ethanol) in order to obtain characteristic yellow spots on a blue background.

Table I. $R_f \cdot 100$ values for 3,4-dimethylbenzoic (3,4-DMBA), 3,4-dimethylhippuric (3,4-DMHA), 3,5-dimethylbenzoic (3,5-DMBA) and 3,5-dimethylhippuric acids (3,5-DMHA). The plates used above were coated with Silica gel DS-5 (CAMAG)

Solvent		3,4-DMBA		3,4-DMHA		3,5-DMBA		3,5-DMHA	
System	Composition V/V	4× 9 cm	20× 20 cm	4× 9 cm	20× 20 cm	4× 9 cm	20× 20 cm	4× 9 cm	20× 20 cm
1	Benzene 85 Pyridine 15	60	82.5	9.3	5.2	61	71	12	11
2	Hexane 95 Acetic acid 5	33	43.5	—	—	28	44.7	—	—
3	Toluene 80 Pyridine 20	69	78.5	9.3	6.5	68	77	14	19
4	Toluene 75 Pyridine 25	74	81	16	15	68	78	19	20
5	Toluene 70 Pyridine 30	74	84.5	28	16	80.5	75	29	27

Table II. $R_f \cdot 100$ values for 3,4-dimethylbenzoic (3,4-DMBA), 3,4-dimethylhippuric (3,4-DMHA), 3,5-dimethylbenzoic (3,5-DMBA) and 3,5-dimethylhippuric acids (3,5-DMHA). These compounds were spotted on Eastman Chromagram sheets, coated with SiO_2 containing a fluorescent indicator

Solvent		3,4-DMBA		3,4-DMHA		3,5-DMBA		3,5-DMHA	
System	Composition V/V	4× 9 cm	20× 20 cm	4× 9 cm	20× 20 cm	4× 9 cm	20× 20 cm	4× 9 cm	20× 20 cm
1	Benzene 85 Pyridine 15	59	79	9	9	67	74.5	5	12
2	Hexane 95 Acetic acid 5	44	80	—	—	52	85	—	—
3	Toluene 80 Pyridine 20	57	85	10	7	62	87	7	11
4	Toluene 75 Pyridine 25	76	86	12	8.5	72	84	23	8
5	Toluene 70 Pyridine 30	77	87	14	7	77	86	28	14

The $R_f \cdot 100$ values obtained are summarized in Tables I and II. These values are reported for a migration of 75 mm for the 4×9 cm plates or sheets and 100 mm for the larger ones. Quantities from 5 to 10 μg of each acid were used in spotting.

3,5-Dimethylhippuric Acid

Thionyl chloride (5.5 ml, 0.073 *M*) in dry benzene (60 ml) is added slowly to a 50-ml pear-shape flask containing 5 g (0.033 *M*) of 3,5-dimethylbenzoic acid. The mixture is warmed at low heat over a period of 3 hours until the acid is completely dissolved. The thionyl chloride and benzene in excess are removed at room temperature by vacuum distillation. The acid chloride formed is added slowly to a 250-ml Erlenmeyer flask containing an aqueous solution of sodium glycinate (5 g, 0.066 *M* glycine in 50 ml of 10% NaOH). The mixture is shaken vigorously after each addition of acid chloride until the reaction is completed. Crushed ice is added to the solution which is acidified with concentrated HCl (congo red paper). The precipitate obtained is collected on a fritted filter, washed with distilled water, and dried overnight in a dessicator containing calcium sulphate. Crude yield: 90%. The material is boiled with carbon tetrachloride (30 ml) for 10 minutes and filtered after cooling. Recrystallization from water: ethanol (1 : 1 by vol.) gave a crystalline material melting at 174–175°. The ultraviolet and infrared spectra are shown in Figs. 1 and 2 with those of the corresponding benzoic acid.

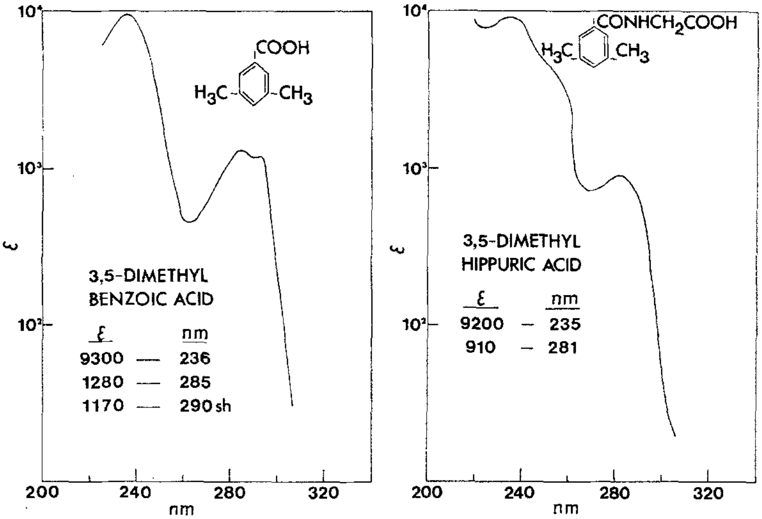

Fig. 1. Ultraviolet spectra of 3,5-dimethylbenzoic and 3,5-dimethylhippuric acids

Anal.*: Calcd. for $C_{11}H_{13}NO_3$: %C, 63.76; %H, 6.31; %N, 6.74;
Found: %C, 63.63; %H, 6.13; %N, 6.93.

3,4-Dimethylhippuric Acid

Thionyl chloride (7 ml, 0.097 *M*) in a mixture of dry solvents (30 ml benzene + 10 ml toluene) is added to 3,4-dimethylbenzoic acid (4 g, 0.027 *M*). The slurry obtained was refluxed with continuous stirring over a period of 7 hours. Solvents in excess was removed under vacuum at room temperature. The acid chloride formed was added slowly to an aqueous solution of sodium glycinate of similar composition to that described for the preparation of the 3,5-isomer. The crude compound was boiled with CCl_4 (20 ml) for 10 minutes and filtered after cooling. Crude yield: 42%. Recrystallization from water–ethanol (1 : 1 by vol.) white crystals melting at 139–141°. The ultraviolet and infrared spectra of this compound are compared respectively in Figs. 3 and 4 with those of 3,4-dimethylbenzoic acid.

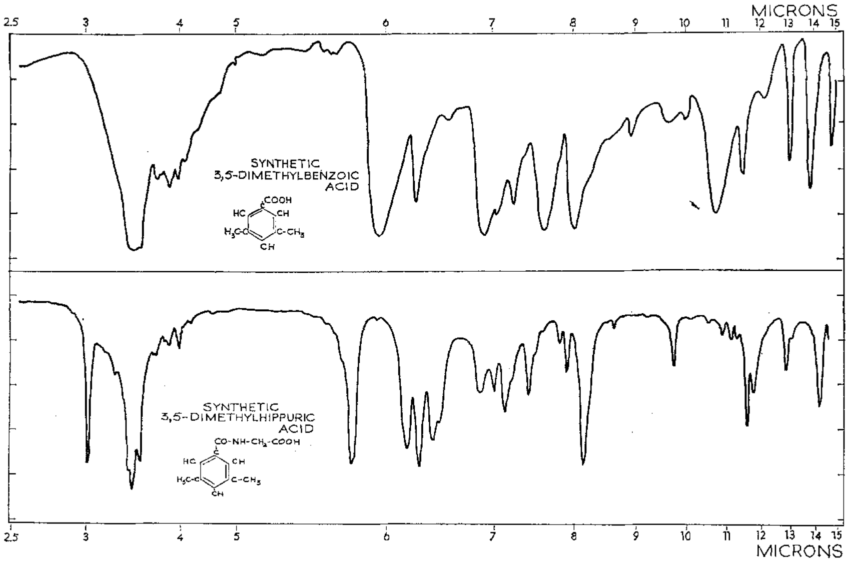

Fig. 2. Infrared spectra of 3,5-dimethylbenzoic and 3,5-dimethylhippuric acids

Anal.: Calcd. for $C_{11}H_{13}NO_3$: %C, 63.76; %H, 6.31; %N, 6.74;
Found: %C, 63.86; %H, 6.43; %N, 6.68.

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* The microanalyses reported in this article were carried out by Galbraith Laboratories, Knoxville, Tennessee, U. S. A.

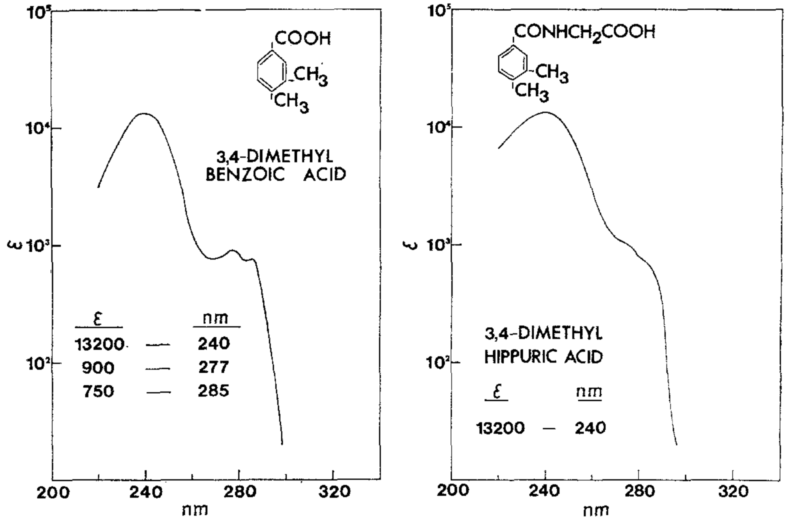

Fig. 3. Ultraviolet spectra of 3,4-dimethylbenzoic and 3,4-dimethylhippuric acids

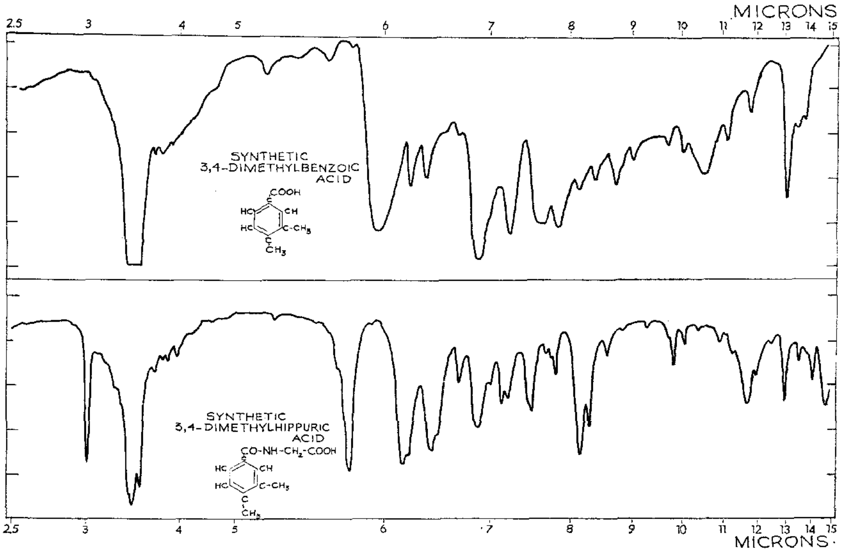

Fig. 4. Infrared spectra of 3,4-dimethylbenzoic and 3,4-dimethylhippuric acids

Summary

Metabolic Studies on Toxic Compounds

Two new aromatic acids, 3,4- and 3,5-dimethylhippuric acids, have been prepared by reacting acid chlorides of corresponding benzoic acids with sodium glycinate. Their physico-chemical properties and spectro-

scopic patterns (ultraviolet and infrared) have been described in this report as well as a method of identification by thin-layer chromatography.

Zusammenfassung

3,4- und 3,5-Dimethylhippursäure, zwei neue aromatische Säuren, wurden durch Einwirkung der entsprechenden Benzoesäurechloride auf Natriumglycinat hergestellt. Ihre physikalisch-chemischen Eigenschaften, ihre UV- und IR-Spektren angegeben und ihr Nachweis durch Dünnschichtchromatographie beschrieben.

Résumé

Deux nouveaux acides aromatiques, les acides diméthyl-3, 4- et diméthyl-3, 5-hippuriques ont été synthétisés en faisant réagir les chlorides des acides benzoïques correspondants sur le glycinate de sodium. Leurs propriétés physico-chimiques et leurs caractéristiques spectroscopiques (dans l'ultraviolet et l'infrarouge) ont été décrites dans ce rapport ainsi qu'une méthode d'identification par chromatographie sur couche mince.

References

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