bicarbonate solution and the alkaline layer acidified. The precipitated acid was purified by sublimation under low pressure. When the product (1.6 g.), m.p. 72–73°, was crystallized from water containing a few drops of acetic acid the melting point rose to 80°. Mixed m.p. with the acid previously described was undepressed.

δ-(o-tolyl)valeric acid (II). To a solution of sodium hydroxide (4 g.) in diethylene glycol (42 ml.) was added ethyl γ-2-methylbenzoyl butyrate (5.9 g.) followed by hydrazine hydrate (50%, 8.5 ml.). The mixture was refluxed on an oil bath kept at 140° for 1 hr. The system was then connected to a distilling arrangement and the temperature was raised to 200°. Brisk evolution of nitrogen set in, and a few milliliters of water distilled out. After 3 hr. the reaction mixture was cooled, diluted with water, and acidified with hydrochloric acid (1:1) in the cold. The precipitated white solid (3.9 g.) had m.p. 57–58° which rose to 58.5–59° on crystallization from petroleum ether (b.p. 40–60°).

Anal. Calcd. for $C_{12}H_{16}O_2$: C, 75.00; H, 8.33. Found: C, 75.10; H, 8.60.

1-Methylbenzosuber-5-one. (III). To a mixture of phosphorus pentoxide (153 g.) and phosphoric acid (89%, 97.5 ml.), maintained at 100°, δ-(*o*-tolyl)valeric acid (5 g.) was gradually added with stirring. In 7 min., the reaction mixture turned an amber color which gradually deepened. The temperature was maintained at 100° for 2 hr. Then the reaction mixture was decomposed with ice water, and allowed to stand for 15 min. The separated solid was filtered, and washed with dilute ammonia; yield, 4.9 g., m.p. 62–63°, which rose to 65° on sublimation in high vacuum and crystallization from methanol.

Anal. Calcd. for $C_{12}H_{14}O$: C, 82.76; H, 8.05. Found: C, 82.52; H, 8.11.

The *2,4-dinitrophenylhydrazone* crystallized from benzene–ethyl acetate mixture, m.p. 240°.

Anal. Calcd. for $C_{18}H_{18}O_4N_4$: C, 61.02; H, 5.08. Found: C, 61.12; H, 5.22.

6-Formyl-1-methylbenzosuber-5-one (IV). To an ice-cold suspension of sodium ethoxide from sodium (0.55 g.) and ethanol (1.4 ml.) in thiophene-free benzene (26 ml.) was added a mixture of compound III (2.1 g.) and ethyl formate (1.8 g.) in benzene (13 ml.) under nitrogen. After keeping overnight in a nitrogen atmosphere, the reaction mixture was decomposed with ice water. The benzene layer was separated and washed twice with 3% alkali, and mixed with the water layer. The combined aqueous solution was extracted once with ether, and then acidified with 80% acetic acid. The formyl derivative was extracted with ether and distilled to yield 1.8 g., b.p. 133°/0.4 mm. With ferric chloride it gave a reddish violet color turning greenish violet.

Anal. Calcd. for $C_{13}H_{14}O_2$: C, 77.23; H, 6.93. Found: C, 77.65; H, 7.12.

γ-(2-Carboxy-6-methylphenyl)butyric acid (V). (*a*) *By oxidation with permanganate:* To an ice-cold solution of the formyl derivative (IV, 1.65 g.) in 3% sodium hydroxide, powdered potassium permanganate (3.9 g.) was added slowly with stirring. After 2 hr., the solution was treated with sufficient hydrochloric acid (1:1) and saturated sodium bisulfite solution when a tarry mass separated. This was removed and washed several times by decantation with water and then dissolved in hot sodium bicarbonate solution. The alkaline solution was acidified and left in a refrigerator overnight. Black and white particles of solid separated, the latter melting at 129–130°. This was subjected to evaporative distillation and the distillate crystallized twice from benzene to yield 0.3 g., m.p. 136–136.5°.

(*b*) *By ozonolysis:* Sufficient ozonized oxygen was passed through a solution of the formyl derivative (IV, 0.2 g.) in a mixture of ethyl acetate (5 ml.) and glacial acetic acid (5 ml.) chilled in an ice-salt bath. Three such lots were combined and treated with water (4.5 ml.) and hydrogen peroxide (30%, 1.5 ml.). The mixture was then kept overnight. Ethyl acetate and acetic acid were removed under reduced pressure and the residue was treated with water and then taken up in ether. The ether solution was thoroughly extracted with saturated sodium bicarbonate solution. The combined bicarbonate solutions were acidified and extracted with ether. Removal of ether and trituration of the residue with petroleum ether (b.p. 40–60°) gave a solid having an indefinite melting point. Evaporative distillation followed by two crystallizations from benzene gave colorless crystals having m.p. 136–136.5° which was not depressed on admixture with the product prepared according to the method (a).

Anal. Calcd. for $C_{12}H_{14}O_4$: C, 64.86; H, 6.31. Found: C, 64.80; H, 6.16.

CALCUTTA, INDIA

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, FRESNO STATE COLLEGE]

A Comparison of Rates of Precipitation of Substituted Hippuric Anilides Formed by Papain-Catalyzed Reactions between Hippuric Acid and Substituted Anilines at Approximately *p*H 4.6

JOHN LEO ABERNETHY, JOHN NAKAMURA, AND BRO. MYRON COLLINS

Received September 18, 1957

The rates of precipitation of twelve substituted hippuric anilides have been studied, in papain-catalyzed reactions between hippuric acid and substituted anilines at pH ≅ 4.6. Six of these are new compounds. A comparison of these rates permits a reasonable interpretation of results in terms of steric hindrance, electrostatic effects and resonance. In the absence of steric effects, the reaction of substituted anilines appears to increase with increasing basicity.

Preliminary to a series of enzymatic resolutions being instigated in this laboratory, it was important to study the relative rates of precipitation of a few well-chosen substituted hippuric anilides, formed by papain-catalyzed reactions between hippuric acid and appropriately substituted anilines. The general procedure given by Bergmann and Fraenkel-Conrat,[1] as subsequently adapted by

(1) M. Bergmann and H. Fraenkel-Conrat, *J. Biol. Chem.*, 119, 707 (1937).

Bennett and Niemann[2] and others, was followed. L-Cysteine was used as a promotor.

$$C_6H_5CONHCH_2COOH + NH_2C_6H_4X \xrightarrow[pH \cong 4.6;\ 40°]{\text{papain, L-cysteine}} C_6H_5CONHCH_2CONHC_6H_4X + H_2O$$

Reactions between acylated amino acids and aniline,[1] or phenylhydrazine[1] or even substituted anilines[3] have been reported without regard to effects of the position of substituents on the benzene nucleus of the base reactant. A few studies have been made with respect to dependence of yield[1,4] on *p*H, which have revealed that amide formation usually proceeds fastest in the *p*H range of about 4.5 to 5.0. All of the reactions in the present investigation were carried out at *p*H $\cong$ 4.6, with convenient usage of an acetic acid–sodium acetate buffer.

Since the substituted anilines were not all of equal molar solubility, five were selected for graphic comparison from the entire group studied. All five had an initial concentration of 0.05 mole in 250 ml. of buffered solution. These were the *o*-, *m*- and *p*-aminophenols (Fig. 1) and the *m*- and *p*-amino-

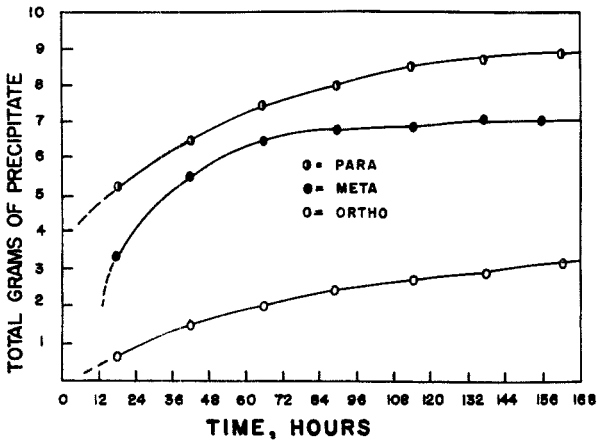


Fig. 1. Comparative rates of precipitation of hippuric *o*-, *m*- and *p*-hydroxyanilides formed from hippuric acid and aminophenols. 0.050 mole hippuric acid; 0.050 mole aminophenol; 1.00 g. L-cysteine hydrochloride monohydrate; 0.50 g. Schwarz Papain; buffered at *p*H $\cong$ 4.6, HOAc–NaOAc; total volume of solution, 250 ml.

acetophenones (Fig. 2). Although the rates are qualitative, there is sufficient consistency to draw certain conclusions. Hydroxyl ortho to the amino group provides steric hindrance and decreases the rate of reaction, compared with the same substit-

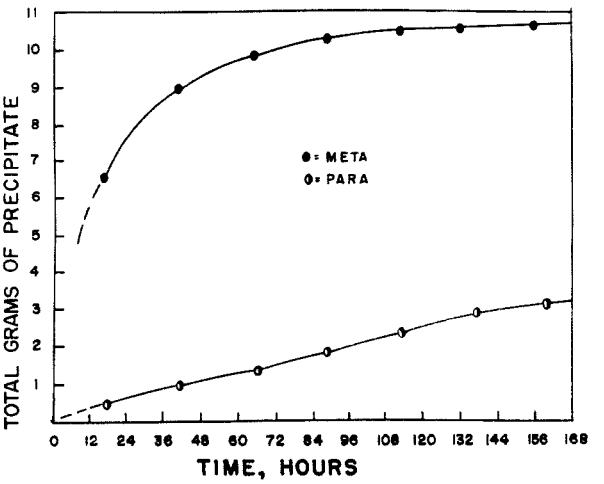
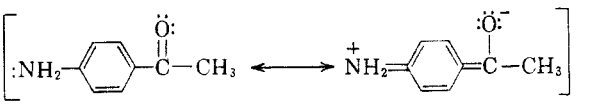


Fig. 2. Comparative rates of precipitation of hippuric *m*- and *p*-acetylanilides formed from hippuric acid and aminoacetophenones. 0.050 mole hippuric acid; 0.050 mole aminoacetophenone; 1.00 g. L-cysteine hydrochloride monohydrate; 0.50 g. Schwarz papain; buffered at *p*H $\cong$ 4.6, HOAc–NaOAc; total volume of solution, 250 ml.

uent in a meta or para position (Fig. 1). If the hydroxyl is para the reaction is faster than if it is meta, since with the hydroxyl in the para position resonance tends to keep the electron pair available on nitrogen for amide formation. *m*-Aminoacetophenone reacts faster than *p*-aminoacetophenone because the acetyl group is an electron-attracting group and electrons are withdrawn from the amino group by a resonance effect when the acetyl is para, but not when it is meta.



A comparison between *p*-aminophenol and *m*-aminoacetophenone shows that the latter reacts at a somewhat faster rate in these experiments. The implication made by these qualitative rate comparisons is that the reactivity of the substituted anilines is proportional to their basicities in the absence of steric effects in similarly substituted compounds.

Although methyl *p*-aminobenzoate was reported elsewhere[3] not to undergo a reaction with hippuric acid, it did under the conditions of our experiment. The low yield can be attributed to the low solubility of methyl *p*-aminobenzoate. Methyl anthranilate and *p*-nitroaniline were very low in solubility, anthranilic acid was of moderate solubility and both metanilic acid and sulfanilic acid were of substantial solubility. None reacted with hippuric acid. Six new substituted hippuric anilides are reported here. Two substituted anilines not previously investigated gave no product under conditions of this study.

(2) (a) E. L. Bennett and C. Niemann, *J. Am. Chem. Soc.*, **70**, 2610 (1948); (b) *J. Am. Chem. Soc.*, **72**, 1798 (1950); (c) *J. Am. Chem. Soc.*, **72**, 1800 (1950).

(3) E. Waldschmidt-Leitz and K. Kuhn, *Z. physiol. Chem.*, **285**, 23 (1950).

(4) Unpublished results from this laboratory.

TABLE I

SUBSTITUTED HIPPURIC ANILIDES FROM HIPPURIC ACID AND SUBSTITUTED ANILINES

Substituted Aniline Reactant	Reaction Product	Melting Point of Product, °C.	Yield of Product in Grams for Consecutive Periods of Incubation					
			0–18 hr.	18–42 hr.	42–66 hr.	66–90 hr.	90–114 hr.	114–138 hr.
m-Phenylenediamine	Hippuric *o*-Aminoanilide	260–262°[4]	1.4904	1.8545	0.5185	0.2259	0.2238	0.1350
p-Phenylenediamine	Hippuric *p*-Aminoanilide	326°[4]	2.305	2.103	0.667	0.372	0.205	0.140
o-Aminophenol	Hippuric *o*-hydroxyanilide	202–203°	0.462	1.105	0.563	0.327	0.238	0.209
m-Aminophenol	Hippuric *m*-hydroxyanilide[a]	223–225°	3.2715	2.3599	0.8683	0.3086	0.1006	0.0729
p-Aminophenol	Hippuric *p*-hydroxyanilide	243–244°	5.264	1.320	0.820	0.605	0.379	0.251
Anthranilic acid	No Precipitate		Similarly methyl anthranilate, metanilic acid,[a] sulfanilic					
m-Aminobenzoic acid	*m*-Hippuramidobenzoic acid[a]	283–284°	3.2731	0.8647	0.4106	0.2412	0.1384	0.1004
p-Aminobenzoic acid	*p*-Hippuramidobenzoic acid	278–279°	0.704	0.925	0.085	0.027	0.0140	0.0130
m-Aminoacetophenone	Hippuric *m*-acetylanilide[a]	229–230°	6.558	2.460	0.777	0.395	0.145	0.128
p-Aminoacetophenone	Hippuric *p*-acetylanilide[a]	198–199°	0.5552	0.5140	0.4174	0.3648	0.3458	0.2558
o-Anisidine	Hippuric *o*-methoxyanilide	120–121°	0.0000	1.6551	0.8012	0.6235	0.4330	0.3342
p-Anisidine	Hippuric *p*-methoxyanilide[a]	217–218°	4.8200	2.3905	1.2587	0.6905	0.4684	0.3196
Methyl *p*-aminobenzoate	Methyl *p*-hippuramidobenzoate[a]	194–195°	0.2959	0.2856	0.2286	0.2115	0.1403	0.1201

[a] Prepared or studied for the first time.

EXPERIMENTAL

Activation of papain. The papain used in this investigation was supplied by the Schwarz Laboratories, Mount Vernon, N. Y. A modification of the method of Grassmann[5] and Bennett and Niemann[2b] for activation of the enzyme was employed. Fifty grams of papain was ground rapidly to a paste in a mortar with a few milliliters of cold water and was then stirred mechanically with 200 ml. of water in an ice bath for 4 hr. The solution was removed by suction filtration. Hydrogen sulfide was passed into the filtrate, surrounded by an ice bath, for 18 hr. Suspended matter was removed by centrifugation at 2000 r.p.m. for 20 min. The papain was precipitated once by addition of enough methanol to give a 70 volume % of solution, followed by centrifuging for 20 min. at 2000 r.p.m. The precipitate was dried over phosphorus pentoxide in a vacuum desiccator and then crushed lightly to a powder. The coarse powder was stored in stoppered vials, kept in a large, air-tight, brown bottle, fitted with a screw cap, and refrigerated at about 5°.

Procedure for rate studies. A sodium acetate (0.25*M*)–acetic acid (0.25*M*) buffer was used. For each reaction, 0.050 mole of hippuric acid, 0.050 mole of the substituted aniline, 1.00 g. of L-cysteine hydrochloride monohydrate, and 0.50 g. of papain were employed, with enough buffer to make 250 ml. of solution. The *p*H was adjusted to and maintained at 4.6. Usually the hippuric acid and substituted aniline were dissolved in hot buffer solution, which was cooled to 40°. Then L-cysteine and papain were added, after first being dissolved in about 5 ml. of buffer, with further addition of enough buffer to give a total volume of 250 ml. The solution was filtered, followed by incubation at 40°. Filtration was then carried out at the end of 18 hr., subsequently at the end of each 24 hr. for 6 days, and finally 7 days later. The precipitate for each period was dried and weighed, and then the total amount of precipitate was collected and recrystallized from a suitable solvent. In general, two or more recrystallizations, with the use of decolorizing carbon, were necessary to bring the sample to sufficient purity for nitrogen analysis. Melting points were corrected in the usual way for those determined in a potassium sulfate–sulfuric acid bath. Other melting points were determined by means of a Fisher-Johns melting point apparatus. Nitrogen analyses were determined at the Oakwold Laboratories, Alexandria, Va., and The Microchemical Specialties Company, Berkeley, Calif. Results are summarized in Table I.

Acknowledgments. This work was supported by funds from the Research Corporation and a grant-in-aid from the Society of Sigma Xi and RSEA. Mr. David R. Schwarz of the Schwarz Laboratories, Mount Vernon, N. Y., generously donated the papain used in these experiments. Professor Charles D. Hurd of Northwestern University offered helpful suggestions with regard to possible systems of correct nomenclature of the hippuric anilides. Indebtedness is expressed to Professor Carl Niemann of the California Institute of Technology for help, through private communications, with respect to appropriate treatment of the papain. Mr. Jimmie Jimkawa drew the graphs and other assistance was given by Mr. Jerome Blank and Mr. Herbert Chelner.

FRESNO, CALIF.

(5) W. Grassmann, *Biochem. Z.*, **279**, 131 (1935).

TABLE I (*Cont'd*)

SUBSTITUTED HIPPURIC ANILIDES FROM HIPPURIC ACID AND SUBSTITUTED ANILINES

Reaction in Hours		Solvent for Recrystallization	Nitrogen Analyses		Total Yield, Grams	Percentage Yield	Color of Product
138–162 hr.	162–380 hr.		% Calcd.	% Found			
0.0982	0.4076	Ethanol	15.51	See Ref. 4	4.9539	36.75	White
0.0850	0.5430	Ethanol	15.51	15.56	6.1500	45.62	White
0.161	0.725	Ethanol	10.37	10.45	3.7900	28.00	White
0.0026	0.0000	Ethanol or Methanol	10.37	10.26	6.9844	51.69	Cream
0.177	0.653	Ethanol	10.37	10.38	9.4690	70.09	White
acid, and *p*-nitroaniline[a] gave no precipitate							
0.0791	0.3305	Glacial HOAc; then addition of water	9.39	9.29	5.4380	36.47	Cream
0.0120	0.153	Glacial HOAC; then addition of water	9.39	9.32	1.9330	14.34	White
0.091	0.3162	Ethanol or acetophenone	9.46	9.40	10.8702	73.35	Cream
0.2111	1.1169	Ethanol	9.46	9.34	3.7810	25.51	White
0.2266	1.1611	Ethanol	9.86	9.87	5.2347	36.81	Cream
0.2437	1.0495	Ethanol	9.86	9.99	11.2409	79.05	White
0.0941	0.3942	Ethanol	8.97	8.96	1.7703	11.71	White

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, OREGON STATE COLLEGE]

Psoralene I: Certain Reactions of Xanthotoxin*

MERVIN E. BROKKE AND BERT E. CHRISTENSEN

Received May 6, 1957

A number of the chemical reactions of 9-methoxypsoralene and its derivatives are described. These include nitration, halogenation, reduction, thionation, demethylation, ozonation, and other degradation procedures. Degradation studies and unequivocal synthesis show that bromination occurs at the four position. The structures of the 2,3-dihydropsoralene derivatives were established by a comparison of the ultraviolet absorption spectra of psoralene and coumarin derivatives. Thionation of the 9-methoxypsoralenes proceeds in a manner analogous to that reported for coumarins.

In 1911, Priess[1] discovered a new piscicide in an alcoholic extract of *fagara zanthoxyloides Lam.* to which he gave the name zanthotoxin. Later Thoms[2] after determining the structure of this compound renamed it xanthotoxin and in 1936 Spath reproduced it synthetically.[3]

In addition to its toxic action on fish[4] xanthotoxin (9-methoxypsoralene or 9-methoxyfuro[3,2-*g*]coumarin I) has since been shown to possess a molluscicidal activity.[5] When administered in large doses to mammals it was found to produce fatty degeneration of the liver and adrenal hemorrhage,[6] while in humans the compound has found medical acceptance for the treatment of leukodermia.[7] The most recent applications have made use of the fact that I alters the erythermal response to ultraviolet light,[8a,b,c] a property which has been used clinically to prevent sunburn.[8a] There is some evidence that

* This work was supported in part by grants from the Division of Research Grants and Fellowships, National Institutes of Health, Public Health Service. Published with the approval of the Monographs Publications Committee, Oregon State College as Research Paper No. 327, School of Science, Department of Chemistry.

(1) H. Priess, *Ber. Pharm. Ges.*, **21**, 227 (1911).
(2) H. Thoms, *Ber.*, **44**, 3325 (1911).
(3) E. Spath and M. Pailer, *Ber.*, **69**, 767 (1936).
(4) E. Spath and F. Kuffner, *Monatsh.*, **69**, 75 (1936).
(5) A. Schönberg and N. Latif, *J. Am. Chem. Soc.*, **76**, 6208 (1954).
(6) A. Elwi, *J. Roy. Egypt. Med. Assoc.*, **33**, 773 (1950).
(7) I. Fahmy and H. Abu-Shady, *Quart. J. Pharm. and Pharmacol.*, **21**, 499 (1948).
(8) (a) A. Lerner, *J. Invest. Dermatol.*, **20**, 299 (1953). (b) A. Griffin, M. O'Neal, and T. Fitzpatrick, *Congress of intern. biochem.*, Brussels 1955, 121. (c) L. Musajo, G. Rotighiero and G. Caporale, *Chemica e industria*, **35**, 13 (1953).