[CONTRIBUTION FROM THE CHEMICAL LABORATORY, UNIVERSITY OF VERMONT]

Equilibrium in the System, Benzoic and Hippuric Acids, Glycine and Water

BY CHARLES L. INGERSOLL AND GEORGE H. BURROWS

Hippuric acid is readily hydrolyzed, yielding benzoic acid and glycine. Dessaignes[1] proved the formation of hippuric acid when benzoic acid and glycine are heated together in a closed tube.

It was shown by Wöhler in 1824 that benzoic acid introduced into the stomach gives rise to hippuric acid in the urine. Mutch,[2] whose work has been confirmed by Clementi,[3] studied the enzyme histozym, deemed responsible for this synthesis, and gave evidence that under its influence benzoic acid and glycine form hippuric acid and that in the opposite direction hippuric acid is largely but not completely hydrolyzed.

In the present work it is shown that a measureable equilibrium exists among these substances in aqueous solution, and this equilibrium has been determined at each of several temperatures and under somewhat varied conditions of concentration.

Materials and Experimental Details

Benzoic acid of reagent grade proved pure as nearly as could be judged through melting point and titration. Glycine of the Synthetical Laboratories likewise proved adequately pure. Hippuric acid of the highest grade obtainable was crystallized from water three times with centrifugal draining, after which the dried sample gave closely the theoretical titration value. The water used was of approximately 5×10^{-6} specific conductance at 25°.

Equilibrium was established through heating aqueous solutions of the substances in sealed Pyrex glass tubes. The heating of solutions of random concentrations for sufficient time to reach equilibrium led to slight decomposition. On this account, equilibrium was approached from each side by briefer heating of solutions that initially approximated closely the final concentrations. At suitable temperatures the equilibrium percentages of hippuric acid are quite small and this fact, on analytical grounds, determined the general range of concentrations selected.

The heating baths of boiling aniline, dimethylaniline, and xylene, gave temperatures of 184, 194 and 136°, respectively, constant within 0.5°.

Tubes containing solutions of initially precisely known composition were heated usually for approximately four hours, then quickly chilled, opened, and the contents analyzed, in order to ascertain the direction and extent of change toward equilibrium.

The method of analysis depended on the ready solubility of benzoic acid in benzene and the almost absolute insolubility of hippuric acid and of glycine in this solvent, and on the solubility of hippuric acid in acetone and the corresponding almost absolute insolubility of glycine in acetone. Hippuric acid and glycine thoroughly shaken with benzene, and also glycine shaken with acetone, in amounts comparable with those in our experiments, on filtering and evaporation of the solvents, did not leave weighable residues.

The solution to be analyzed, approximating 10 cc. and of known weight, was extracted five times with pure, neutral benzene, 45 cc. of benzene being used in each extraction, preliminary experiments having shown that this treatment gives practically complete removal of benzoic acid. The combined extracts were titrated with aqueous sodium hydroxide, using phenol red as indicator.

The aqueous solution deprived of benzoic acid was evaporated to dryness at 50° in a current of dry air. Evaporation at higher temperatures leads to slight formation of benzoic acid from hippuric acid and glycine. The dry residue was thoroughly extracted with five successive 10-cc. portions of pure acetone. After filtering, the hippuric acid in these combined extracts was titrated with standard base and phenol red. In both sets of extractions and titrations care was taken to prevent entrance of carbon dioxide from the air.

The final glycine contents of the solutions were calculated from the known initial compositions and the findings of benzoic and hippuric acids.

The following experiments, made to test the analytical method, give indication of its accuracy.

Benzoic acid taken, g.	Benzoic acid found, g. (extracted and titrated)	Difference, g.
0.1368	0.1365	0.0003
.1383	.1377	.0006

Hippuric acid and glycine were dissolved in water, the water was evaporated and the residue was extracted with acetone, as described above.

Hippuric acid taken, g.	Glycine taken, g.	Hippuric acid found, g.
0.0170	0.3633	0.01695

The results of the equilibrium measurements are given in Table I. The column headed "Loss" is in each instance the difference between the weight of benzoic acid taken and that found at equilibrium when the latter amount is increased or decreased by the decrease or increase, respectively, of hippuric acid, calculated as benzoic acid. This offers a check on the accuracy of the analytical work, and its low values give evidence that disturbing side reactions did not occur.

The equilibrium constant, $K = C_{\mathrm{BA}} \times C_{\mathrm{Gl}}/C_{\mathrm{HA}}$, is calculated on the basis of moles of each solute to

---

[1] Dessaignes, *Jahresber.*, **10**, 367 (1857).

[2] Mutch, *J. Physiol.*, **44**, 176 (1912).

[3] Clementi, *C. A.*, **18**, 1133 (1924).

1000 g. of water. To economize space, initial amounts of substances are not quoted. Inspection of them would show that at each temperature the equilibrium was approached from both sides.

In the experiments at 136°, benzoic acid alone was directly determined in the equilibrium solutions, the hippuric acid as well as the glycine finally present being calculated from the amounts of benzoic acid found and the known initial compositions of the solutions. On this account, less reliance is placed on the 136° values.

TABLE I

Temp., °C.	Equilibrium amounts in grams H_2O	B. A.	H. A.	Gl.	Loss	K
184	12.124	0.1172	0.0125	0.3116	0.0001	4.71
184	10.554	.1164	.0158	.3482	.0005	4.75
184	11.185	.1197	.0112	.2522	.0000	4.71
194	11.804	.1208	.0153	.3009	.0005	3.94
194	10.054	.1226	.0176	.3003	.0003	3.98
194	9.929	.1230	.0191	.2997	.0003	3.80
194	9.800	.1243	.0190	.3013	.0000	3.94
136	11.241	.1316	.0053	.3030		13.09
136	11.042	.1319	.0053	.3031		13.36

The average values of the constants at 184 and 194°, namely, 4.72 and 3.92, give the following value for the heat of the reaction in aqueous solution

$$C_6H_5CONHCH_2COOH + H_2O = C_6H_5COOH + CH_2NH_2COOH; \quad \Delta H = -7850 \text{ cal.}$$

The constant at 136° calculated from the values at 184 and 194° is 13.06 as against the directly determined average value of 13.23.

The free energies of benzoic acid and of glycine being known, as well as their solubilities, and that of hippuric acid, in water at 25°, it is possible to calculate the approximate value of the free energy of hippuric acid. This is not done here, in absence of heat capacities and activities needed for calculation of an accurate value.

Summary

The equilibrium in aqueous solution between benzoic acid, hippuric acid, glycine and water has been measured at 136, 184 and 194°.

BURLINGTON, VERMONT RECEIVED NOVEMBER 30, 1937

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF DUKE UNIVERSITY]

Equilibria in Cadmium Iodide Solutions[1]

BY ROGER G. BATES AND WARREN C. VOSBURGH

The equilibria in solutions of cadmium iodide have been investigated by McBain, Van Rysselberghe and Squance[2] and by Riley and Gallafent[3] with differing results. This investigation was undertaken to check the previous results by an electromotive force method that does not have the objection of liquid junction potentials. The electromotive force of the cell

$$Cd(Hg)/CdI_2(m)/CdI_2(m),\ Hg_2I_2/Hg \qquad (I)$$

was measured with varying amounts of either cadmium sulfate or potassium iodide added to the electrolyte, and with a cadmium iodide molality of 0.01 or 0.02. In cells in which the potassium iodide concentration was large, a cuprous iodide electrode was substituted for the mercurous iodide electrode. From the results, together with the stoichiometrical activity coefficients for cadmium iodide,[4] equilibrium constants were calculated for the following reactions

$$CdI_2 \rightleftarrows Cd^{++} + 2I^- \qquad (1)$$

$$CdI_2 \rightleftarrows CdI^+ + I^- \qquad (2)$$

$$CdI^+ \rightleftarrows Cd^{++} + I^- \qquad (3)$$

$$CdI_3^- \rightleftarrows Cd^{++} + 3I^- \qquad (4)$$

$$CdI_4^{=} \rightleftarrows Cd^{++} + 4I^- \qquad (5)$$

Experimental Methods

The apparatus, experimental methods and the preparation of most of the materials have been described in previous papers.[4,5] A cadmium sulfate solution about 0.8 m was prepared from thrice recrystallized cadmium sulfate and standardized by the evaporation to dryness of weighed samples and heating the residue at 500°. For the preparation of the cell electrolytes that contained cadmium sulfate, the calculated quantities of a carefully standardized cadmium iodide solution and the above cadmium sulfate solution were weighed and diluted by weight to the desired molality. The electrolytes that contained potassium iodide were made by dissolving weighed portions of recrystallized and carefully dried potassium iodide[5] in 0.01 or 0.02 m cadmium iodide solutions. A 1% copper amal-

---

[1] Part of a thesis submitted by Roger G. Bates in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Graduate School of Arts and Sciences of Duke University, June, 1937.

[2] McBain, Van Rysselberghe and Squance, *J. Phys. Chem.*, **35**, 1006 (1931).

[3] Riley and Gallafent, *J. Chem. Soc.*, 522 (1932).

[4] Bates and Vosburgh, THIS JOURNAL, **59**, 1585 (1937).

[5] Bates and Vosburgh, *ibid.*, **59**, 1188 (1937).