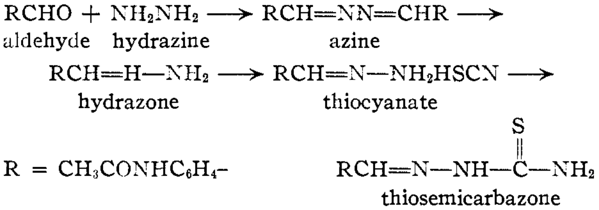
the present investigation was undertaken to see whether a thiosemicarbazone can be successfully developed from a hydrazone which can be prepared from an excess of hydrazine hydrate and an aldehyde. *p*-Acetaminobenzaldehyde thiosemicarbazone has thus been prepared by allowing *p*-acetaminobenzaldehyde hydrazone to react with ammonium thiocyanate.

Further attempts to prepare it *in situ* from a mixture of *p*-acetaminobenzaldehyde, excess hydrazine sulfate and ammonium thiocyanate in water also resulted in the formation of thiosemicarbazone in excellent yield. The reaction in this case proceeds through the formation of azine which is converted into its hydrazone in the presence of excess of hydrazine. This with thiocyanate gives the thiosemicarbazone after subsequent rearrangement of the thiocyanate derivative as



$$\underset{\text{aldehyde}}{\mathrm{RCHO}} + \underset{\text{hydrazine}}{\mathrm{NH_2NH_2}} \longrightarrow \underset{\text{azine}}{\mathrm{RCH{=}NN{=}CHR}} \longrightarrow$$

$$\underset{\text{hydrazone}}{\mathrm{RCH{=}H{-}NH_2}} \longrightarrow \underset{\text{thiocyanate}}{\mathrm{RCH{=}N{-}NH_2HSCN}} \longrightarrow$$

$$\mathrm{R = CH_3CONHC_6H_4{-}} \qquad \underset{\text{thiosemicarbazone}}{\mathrm{RCH{=}N{-}NH{-}\overset{\overset{S}{\|}}{C}{-}NH_2}}$$

Experimental

p-**Acetaminobenzaldehyde.**—The *p*-aminobenzaldehyde (Beard and Hodgson[7]) was acetylated in chloroform with acetic anhydride. It recrystallized from hot water in pale yellow needles, m.p. 158°.[8]

p-**Acetaminobenzaldehyde Hydrazone.**—*p*-Acetaminobenzaldehyde (7 g.) was refluxed with 75% hydrazine hydrate (20 cc.) in alcohol (40 cc.) for one hour. The clear solution was allowed to cool when colorless crystalline needles of hydrazone separated, m.p. 165–166°.

Anal. Calcd. for $C_9H_{11}N_3O$: N, 23.7. Found: N, 23.6.

It is of interest to note that when *p*-acetaminobenzalazine is heated with hydrazine hydrate in alcohol *p*-acetaminobenzaldehyde hydrazone is formed which when heated with water changes itself to *p*-acetaminobenzalazine, m.p. 315–316° as confirmed by a mixed m.p. with an authentic sample prepared by the method of Bernstein, *et al.*[9]

p-**Acetaminobenzaldehyde Thiosemicarbazone.**—(i) The hydrazone was suspended in water and acidified to congo red with concd. hydrochloric acid. Ammonium thiocyanate (15 g.) was then added and the mixture concentrated on a water-bath. Water was added and concentrated as before. This process was repeated until the residue was crystalline. It was then filtered and washed with hot water. Yellow crystals were obtained having m.p. 225–226° (dec.). There was no depression of m.p. when mixed with a sample prepared from the aldehyde and thiosemicarbazide; Bernstein, *et al.*,[9] reported a m.p. 223–224° (dec.).

Anal. Calcd. for $C_{10}H_{12}N_4OS$: N, 23.7. Found: N, 23.5.

This identification was confirmed by deacetylating when *p*-aminobenzaldehyde thiosemicarbazone was obtained m.p. 196° with no depression of m.p. with an authentic sample (Berstein, *et al.*).

(ii) To *p*-acetaminobenzaldehyde (8 g.) in alcohol (50 cc.) was added a 300-cc. solution of a mixture of hydrazine sulfate (20 g.) and sodium carbonate (8 g.) when an instantaneous precipitate[10] was formed. To this mixture was added ammonium thiocyanate (16 g.) in water (100 cc.) and the mixture heated under a reflux for 11 hours until the frothing completely subsided. The large flat crystals of *p*-acetaminobenzaldehyde thiosemicarbazone were collected; yield (8 g.) m.p. 226–227° (dec.) confirmed by a mixed m.p. with the above specimen.

Anal. Calcd. for $C_{10}H_{12}N_4OS$: N, 23.7. Found: N, 23.6.

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[7] Beard and Hodgson, *J. Chem. Soc.*, 4 (1944).

[8] Friedlander and Cohn, *Monatsh.*, **24**, 1, 87 (1903), reported a m.p. 161°.

[9] Bernstein, *et al.*, THIS JOURNAL, **73**, 906 (1951).

[10] Identified as the *p*-acetaminobenzalazine, m.p. 315–316° with no depression on mixed m.p. with an authentic sample. *Anal.* Calcd. for $C_{18}H_{18}N_4O_2$: N, 8.69. Found: N, 8.48.

Preparation and Properties of Pure Ammonium DL-Lactate[1]

BY E. J. COSTELLO AND E. M. FILACHIONE

RECEIVED OCTOBER 31, 1952

Ammonium lactate, which can readily be prepared by fermentation of carbohydrates in the presence of ammonia[2–4] is potentially an important primary fermentation product. Previous studies at this Laboratory have shown that ammonium lactate upon interaction with alcohols produces lactic ester and ammonia in high yields.[5–7] However, comparatively little information about the physical properties of ammonium lactate has been reported,[8–11] only the distillation of ammonium lactate in vacuum[10] and certain properties of aqueous ammonium lactate[11] having been recorded. Pure crystalline ammonium lactate, however, was not used in these investigations, and lactamide was most likely a contaminant.[12] This paper reports the preparation of pure crystalline ammonium lactate and the determination of various properties of this pure salt.

Experimental

Preparation of Ammonium Lactate.—The equivalence point of ammonium lactate was first determined as follows: An approximately 0.1 *N* lactic acid solution was prepared by diluting a high quality 20% lactic acid solution and refluxing for one day to completely remove any polylactic acid. A 25-cc. aliquot of this solution (0.098 *N*) was titrated potentiometrically with freshly standardized ammonium hydroxide (0.102 *N*). The change in *p*H in the vicinity of the equivalence point was pronounced, considerably more than in the titration of a weaker acid such as acetic acid. The equivalence point for ammonium lactate corresponded to a *p*H of 6.65.

The 80% edible grade DL-lactic acid of commerce (4 kg.) was diluted to approximately 20% concentration with water (12 kg.) and this diluted solution was heated under a reflux condenser for 24 hours at 85–90°. Thus the polylactic acid, present in concentrated lactic acid solutions, was almost completely hydrolyzed to lactic acid. The equilibrated 20% aqueous lactic acid solution was then neutralized with concentrated ammonium hydroxide to a *p*H of 7.0. The dilute ammonium lactate solution was concentrated

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[1] Article not copyrighted.

[2] H. C. Jansen, Dutch Patent 57,848 (July 15, 1946).

[3] L. H. C. Perquin, Dutch Patent 58,545 (Nov. 15, 1946).

[4] L. L. Kempe, H. O. Halvorson and E. L. Piret, *Ind. Eng. Chem.*, **42**, 1852 (1950).

[5] E. M. Filachione, E. J. Costello and C. H. Fisher, THIS JOURNAL, **73**, 5265 (1951).

[6] E. M. Filachione and E. J. Costello, *Ind. Eng. Chem.*, **44**, 2189 (1952).

[7] E. M. Filachione and C. H. Fisher, U. S. Patent 2,565,487 (Aug. 28, 1951).

[8] B. E. Brown and F. R. Reid, *Am. Fertilizer*, **95**, No. 12, 12 (1941).

[9] F. Groebe and O. Spengler, German Patent 680,660 (Aug. 17, 1939).

[10] R. Escales and H. Koepke, *J. prakt. Chem.*, **87**, 258 (1913).

[11] A. A. Dietz, E. F. Degering and H. H. Schopmeyer, *Ind. Eng. Chem.*, **33**, 1444 (1941).

[12] J. Wislicenus, *Ann.*, **133**, 257 (1865).

TABLE I

PHYSICAL PROPERTIES OF AQUEOUS AMMONIUM LACTATE

Concn., % by wt.	n^{20}_D	n^{25}_D	n^{40}_D	d^{20}_4	d^{25}_4	d^{40}_4	Viscosity, cps. 20°	25°	40°
0.0	1.3330	1.3321			0.9971		1.01	0.8	
5.0	1.3407	1.3400	1.3383	1.0132	1.0155	1.0066	1.18	1.04	0.86
15.0	1.3576	1.3557	1.3540	1.0412	1.0385	1.0346	1.53	1.35	0.97
28.8	1.3786	1.3775	1.3756	1.0810	1.0788	1.0730	2.66	2.17	1.60
46.4	1.4064	1.4050	1.4037	1.1288	1.1249	1.1198	6.28	5.22	3.36
70.0	1.4416	1.4406	1.4379	1.1826	1.1808	1.1729	43.05	32.87	16.35
78.8	1.4543	1.4536	1.4503	1.2006	1.1984	1.1904	160.64	115.0	46.31

in a steam-heated laboratory-size glass circulating evaporator[13] operated at 30 mm. Because of the explosion hazard[14] resulting from mercury exposed to ammonia, the pressure was initially adjusted to 30 mm.; the manometer then was closed from the system and the pressure periodically checked by momentarily opening the mercury manometer to the system. The solution was evaporated until its temperature reached 70°. The concentrated ammonium lactate solution was then withdrawn from the evaporator, and the *p*H, which was approximately 6, was adjusted to 6.7 by addition of concentrated ammonium hydroxide. Analysis of the solution by the formol titration method[15] showed an ammonium lactate content of 84.5%. Analysis of the solution for total nitrogen[16] and for ammonia nitrogen by the magnesium oxide method[17] showed 11.1 and 11.0% nitrogen, respectively; thus the salt was essentially free of lactamide.

Crystalline Ammonium Lactate.—To 100 g. of 84.5% aqueous ammonium lactate was added 250 ml. of benzene and the stirred mixture was refluxed for 3.5 hours (pot temp., 79–85°) with continuous removal of water; a water separating trap was employed to separate water from its benzene azeotrope. Benzene was then evaporated in vacuum from the reaction mixture and the resulting sirupy liquid was stored in a refrigerator. Crystals slowly separated over a storage period of approximately one month and these were removed by filtration.

Once crystals were obtained it was more convenient to isolate the solid ammonium lactate as follows: 500 g. of 84.5% ammonium lactate solution was cooled in an ice-bath, after which seeds of crystalline ammonium lactate were added to the cooled solution. On filtration 268 g. (63% of the salt in solution) of crystalline ammonium lactate was collected.

At room temperature the crystalline salt was soluble in water, glycerol and 95% ethyl alcohol; slightly soluble in methanol; and insoluble in absolute ethyl, *n*-propyl, isopropyl and *n*-butyl alcohols, ether, acetone and ethyl acetate. With the exception of isopropyl alcohol, acetone and ether, the solid salt was soluble in these solvents at approximately their boiling temperatures. A saturated solution of ammonium lactate in water at 20°, analyzed by the formol titration method,[15] contained 224.7 g. of salt per 100 g. of water, corresponding to a 69.2% solution.

n-Propyl alcohol was found to be the best solvent for recrystallization of ammonium lactate. Two recrystallizations from *n*-propyl alcohol resulted in an anhydrous salt melting at 91–94°.

Anal. Calcd. for $C_3H_9O_3N$: NH_3, 15.90; lactic acid, 84.10. Found: NH_3, 15.78; lactic acid, 84.10.

Physical Properties of Aqueous Ammonium Lactate.—Solutions of various concentration were prepared by dissolving the pure crystalline salt in distilled water. The refractive index, density and viscosity were determined at 20, 25 and 40° by standard procedures. The results are shown in Table I.

Distillation of Ammonium Lactate.—Crystalline ammonium lactate, 51 g., was distilled in an alembic still[18] at 0.2 to 0.4 mm. and 38 g. of a colorless sirupy liquid was collected at 76.5 to 83°. (Care was taken as described above to prevent ammonia from contacting the mercury in the manometer.) In accordance with observations of previous investigators,[10] the distillate appeared to be a complex of equimolar amounts of ammonium lactate and lactic acid, $CH_3CHOHCO_2NH_4 \cdot CH_3CHOHCO_2H$. *Anal.* Calcd. for $C_6H_{15}O_6N$: N, 7.10; neut. equiv., 197.2. Found: N, 6.85; neut. equiv., 195.8. Formol titration showed an equivalent weight as an ammonium salt of 214 (theoretical value, 197.2).

The distillate was redistilled at various pressures in the range of 0.1 to 10 mm.; the following boiling points were observed: 74° at 0.14 mm., 89.5° at 0.50 mm., 99° at 0.75 mm., 102.5° at 1.00 mm., 130° at 4.20 mm., and 141° at 8.30 mm.

Preparation of Dibutylammonium Lactate from Ammonium Lactate.—A mixture of 74% aqueous ammonium lactate (1 mole) and di-*n*-butylamine (1 mole) was refluxed for two hours to remove ammonia. On cooling in the refrigerator, a solid crystallized from the reaction mixture. The solid was separated and recrystallized from benzene to give 116 g. (53% yield) of dibutylammonium lactate, m.p. 76.5–78°. No attempt was made to recover material in the mother liquors. When the material was mixed with an authentic sample of dibutylammonium lactate[19] the melting point was not depressed.

Hygroscopic Properties of Ammonium Lactate.—The apparatus designed by Wink[20] was used in a constant temperature room at 25 ± 2° to determine the hygroscopicity of ammonium lactate. The procedure was substantially that described for determination of the hygroscopicity of lactamide derivatives.[21] A 2- to 4-g. sample of the salt, weighed to the nearest 0.2 mg., was distributed on glass wool mats in the dishes of the Wink apparatus. The samples were exposed to atmospheres of various relative humidity and weighed daily until the composition changed less than 0.1% in 24 hours. This composition, usually attained in one to two weeks, was taken as the equilibrium value. Saturated solutions of the following salts, relative humidity at 25° indicated in parentheses, were used to provide constant humidity[20–23]: potassium acetate (22%), magnesium chloride hexahydrate (33%), potassium nitrite (48%), magnesium nitrate hexahydrate (52%), sodium bromide (58%), sodium nitrite (64%) and sodium chloride (75%). Zero humidity was obtained with anhydrous calcium sulfate. The results are summarized in Table II and Fig. 1. For comparison, the curve for glycerol[24] is included in Fig. 1. The absorption curve for ammonium lactate (ABCD, Fig. 1) was obtained by exposing the crystalline ammonium lactate to the various relative humidities. The desorption curve (DCA, Fig. 1) was obtained by transferring equilibrium composition samples to vessels of lower relative humidity. All the equilibrium composition samples corresponding to the points on curve DCA of Fig. 1 were liquid. The liquid samples obtained at relative humidities

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[13] D. T. Mitchell, P. Shildneck and J. Dustin, *Ind. Eng. Chem., Anal Ed.*, **16**, 754 (1944).

[14] J. J. Sampey, *Chem. Eng. News*, **25**, 2138 (1947).

[15] A. Ronchese, *J. pharm. chim.*, **25**, 611 (1907); *Analyst*, **32**, 303 (1907).

[16] C. O. Willits, M. R. Coe and C. L. Ogg, *J. Assoc. Offic. Agr. Chemists*, **32**, 118 (1949).

[17] Association of Official Agricultural Chemists, "Official and Tentative Methods of Analysis," 6th ed., 2.29, p. 28, Washington, D. C., 1945.

[18] W. P. Ratchford and C. E. Rehberg, *Anal. Chem.*, **21**, 1417 (1949).

[19] W. P. Ratchford and C. H. Fisher, *J. Org. Chem.*, **15**, 317 (1950).

[20] W. A. Wink, *Ind. Eng. Chem., Anal. Ed.*, **18**, 251 (1946).

[21] W. P. Ratchford, *Ind. Eng. Chem.*, **42**, 1565 (1950).

[22] D. S. Carr and B. L. Harris, *ibid.*, **41**, 2014 (1949).

[23] R. H. Stokes and R. A. Robinson, *ibid.*, **41**, 2013 (1949).

[24] W. C. Griffin, *ibid.*, **37**, 1126 (1945).

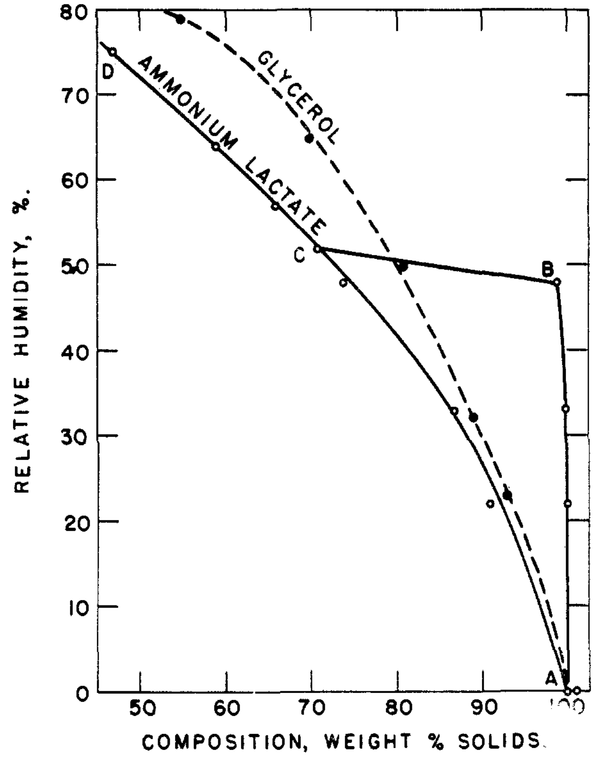

Fig. 1.—Hygroscopicity of ammonium lactate at 25°.

of zero and 33% crystallized when seeded with solid ammonium lactate. Although other equilibrium compositions were not tested in this manner, presumably all compositions between C and A of curve DCA (Fig. 1) would be supersaturated solutions of ammonium lactate.

TABLE II

HYGROSCOPICITY OF AMMONIUM LACTATE AT 25°

Relative humidity, %	Equilibrium compn., % NH_4 lactate Absorp.[a]	Desorp.[b]	Relative humidity, %	Equilibrium compn., % NH_4 lactate Absorp.[a]	Desorp.[b]
0	100	101	52	71	..
22	100	91	57	66	..
33	100	87	64	59	59
48	99	74	75	47	..

[a] Crystalline salt exposed to the various relative humidities. [b] Equilibrium compositions, obtained by absorption, transferred to atmospheres of lower relative humidity.

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An Expression for Gradient Elution

BY ARTHUR CHERKIN, FRANK E. MARTINEZ AND MAX S. DUNN

RECEIVED SEPTEMBER 29, 1952

In discussing column chromatography Tiselius[1] has said that "one of the most important practical problems of chromatography is to eliminate tailing as far as possible." One solution to the problem is gradient elution, which has been found to reduce tailing and to improve fractionation of compounds adsorbed on columns.[1-4] In such elution the concentration of elutant is increased smoothly as elution proceeds, with the effect of accelerating the tail by a higher concentration of elutant than is present at the front.

In the usual apparatus, a relatively concentrated stock solution of elutant is added dropwise to a mixing reservoir fitted with a magnetic stirrer and partly filled with a dilute solution of elutant. A side outlet near the bottom of the reservoir is joined to the top of the chromatographic column. Since the only openings in the reservoir are the inlet and outlet, inflow and outflow rates are equal.

In using gradient elution, it is helpful to be able to pre-determine the initial and final concentrations of elutant entering the column and the total volume of eluate. To this end, the relationships of the variables involved were expressed as a differential equation which upon solution gave the general expression

$$C/C_0 = (e^K - 1)/e^K$$

where C = concentration of elutant in solution leaving reservoir; C_0 = concentration of elutant in stock solution entering reservoir; K = ratio of the volume of eluate collected to the volume of diluent in the mixing reservoir.

The values of C/C_0 obtained for different values of K are plotted in Fig. 1, which emphasizes the fact that for a linear change in elutant concentration the value of K should not exceed unity. That is to

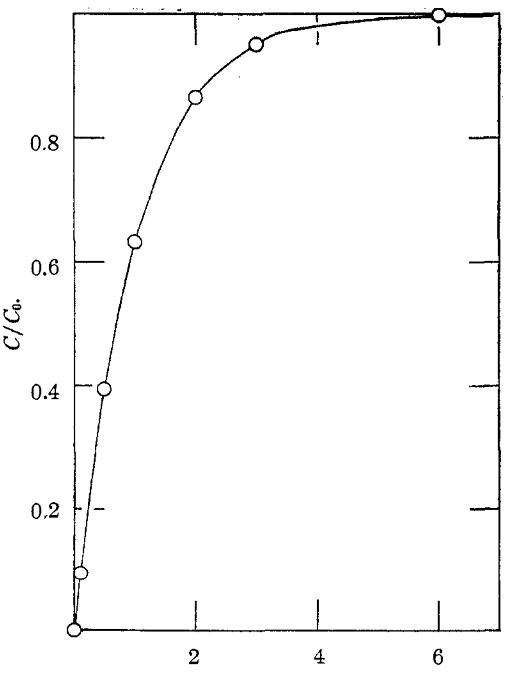

Fig. 1.—Relationship of $C/k \cdot C_0$ (ratio of concentration of elutant in solution leaving mixing reservoir to its concentration in solution entering reservoir) to K (ratio of volume of solution which has entered reservoir to volume of diluent originally placed there).

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(1) A. Tiselius, *Endeavour*, **11**, 5 (1952).

(2) H. Busch, R. B. Hurlbert and V. R. Fields, *J. Biol. Chem.*, **196**, 717 (1952).

(3) K. O. Donaldson, V. J. Tulane and L. M. Marshall, *Anal. Chem.*, **24**, 185 (1952).

(4) L. Hagdahl, R. J. P. Williams and A. Tiselius, *Arkiv. Kemi*, **4**, 193 (1952).