NaOH. Charcoal was then added and the solution was boiled for another five minutes, then filtered hot. The filtrate was acidified with 150 ml. of 2 *N* acetic acid and about 25 ml. of 2 *N* HCl. The yellow precipitate was allowed to settle out. After cooling it was filtered and washed with water, alcohol and ether. It was recrystallized three times from hot alkali and, after thorough washing with water, was dried at 150° *in vacuo*. The compound crystallized with one mole of water which was not lost by prolonged drying *in vacuo*. The light yellow crystals did not melt at 300°.

Anal. Calcd. for $C_7H_6O_3N_4 \cdot H_2O$ (212.0): C, 39.62; H, 3.77; N, 26.41. Found: C, 39.06; H, 3.76; N, 26.03. After 12 hr. of drying at 150°: C, 39.62; H, 3.61; N, 25.79.

4-Amino-6,7-dihydroxy-2-methylpyrimido-(4,5-b)-pyrazine (II).—2.37 g. (0.01 mole) of 2-methyl-4,5,6-triaminopyrimidine sulfate was mixed with a great excess, 9.0 g. (0.1 mole) of anhydrous oxalic acid and 2.68 g. (0.02 mole) of sodium oxalate. The mixture was then heated under vacuum (70 mm.) for three hours, gradually bringing the temperature up to 250°. The yellowish-brown residue was dissolved in 25 ml. of hot 2 *N* NaOH and diluted with distilled water to 100 ml. The solution was heated with charcoal while hot, then filtered into 50 ml. of 2 *N* HCl. After stirring and cooling, the precipitate was collected and this treatment was repeated twice more with just enough 2 *N* NaOH each time to bring about the solution of the pteridine. The final white precipitate was collected, washed with cold water until free of traces of hydrochloric acid and dried *in vacuo* at 100°. This pteridine has no water of crystallization. The resulting white powder did not melt at 300°.

Anal. Calcd. for $C_7H_7O_2N_5$ (193.7): C, 43.28; H, 3.60; N, 36.07. Found: C, 42.98; H, 3.50; N, 35.53.

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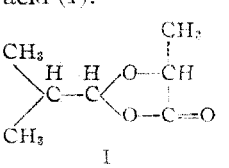
RECEIVED JUNE 2, 1950

Acid-catalyzed Esterification of Lactic Acid with β-Methallyl Alcohol[1]

BY NORMAN G. GAYLORD[2]

The attempted synthesis of β-methallyl lactate by refluxing excess β-methallyl alcohol with lactic acid in the presence of *p*-toluenesulfonic acid resulted in the isolation of a colorless liquid which was identified as lactic isobutylidene ether ester or isobutyrallactic acid (I).



I

I is insoluble in water and acids but dissolves in hot alkali. It is acidic to moistened litmus, decolorizes bromine water with liberation of hydrogen bromide, decolorizes permanganate but does not take up hydrogen over palladium-on-charcoal. Although it gives a questionable test with Brady reagent (2,4-dinitrophenylhydrazine–HCl) application of the standard procedure[3] for the preparation of the 2,4-dinitrophenylhydrazone gives a solid which was identified by analysis and mixed melting point as the derivative of isobutyraldehyde. Application of the procedure for the preparation of the *p*-phenylphenacyl ester of acids[4] gives a solid identified by analysis and mixed melting point as the derivative of lactic acid.

I could arise from an initial acid-catalyzed rearrangement of β-methallyl alcohol to isobutyraldehyde[5,6] followed by hemiacetal formation and dehydration to the lactone. I is analogous to the previously reported chloralides[7] and the formaldehyde, benzaldehyde[8] and acetone[9] derivatives of hydroxy acids.

The failure of the reaction of I with 2,4-dinitrophenylhydrazine to yield a derivative of lactic acid as well as the 2,4-dinitrophenylhydrazone of isobutyraldehyde, as has been reported[8] in the case of phenylhydrazine and the formaldehyde compounds of malic and tartaric acids, can be reconciled with the fact that, under the experimental conditions, no solid derivative was obtained from 2,4-dinitrophenylhydrazine and pure lactic acid.

The desired β-methallyl lactate can be prepared satisfactorily in the absence of a catalyst.[10]

Experimental

Isobutylidene Ether Ester of Lactic Acid.—Six hundred and thirty-six grams (6 moles) of 85% lactic acid, 1728 g. (24 moles) of β-methallyl alcohol, 8 g. of *p*-toluenesulfonic acid and 300 ml. of benzene were refluxed in a 3-l. round-bottomed flask fitted with a thermometer, mechanical stirrer and a vacuum-jacketed, silvered, fractionating column (45 cm. effective length, 12 mm. i.d.) packed with $^3/_{16}$" glass helices, topped by a water-cooled Dean–Stark tube (Barrett modification) and bulb reflux condenser. Refluxing was continued for 68 hours during which time the aqueous layer was withdrawn from the trap and the benzene continuously returned to the system. The acid catalyst was neutralized with 25 g. of anhydrous sodium acetate, and the Dean–Stark tube and reflux condenser were replaced by a total reflux, partial take-off distilling head. After separation of the forerun consisting of benzene, 380 g. of isobutyraldehyde and 860 g. of β-methallyl alcohol, the fraction boiling at 67–75° at 12 mm. was collected. This fraction was redistilled to yield 625 g. (72%) of product, b. p. 71.0–71.3° at 14 mm., $n^{24.8}D$ 1.4198, $d^{24.8}_4$ 1.0168.

Anal.[11] Calcd. for $C_7H_{12}O_3$: C, 58.31; H, 8.39; MR_D 35.87. Found: C, 58.14; H, 8.23; MR_D 35.77.

The 2,4-dinitrophenylhydrazone prepared in the usual way[3] was the derivative of isobutyraldehyde.

(1) Abstracted from a portion of the dissertation submitted in partial fulfillment of the requirements for the Ph.D. Degree, Polytechnic Institute of Brooklyn, June, 1950.

(2) E. I. du Pont de Nemours & Co., Yerkes Research Laboratory, Buffalo, N. Y.

(3) Shriner and Fuson, "The Systematic Identification of Organic Compounds," 3rd ed., John Wiley and Sons, Inc., New York, N. Y., 1948, p. 171.

(4) Ref. 3, p. 157.

(5) Sheshukov, *J. Russ. Phys.-Chem. Soc.*, **16**, 478 (1884).

(6) Tamele, Ott, Marple and Hearne, *Ind. Eng. Chem.*, **33**, 115 (1941); Hearne, Tamele and Converse, *ibid.*, **33**, 805 (1941).

(7) Städeler, *Ann.*, **61**, 101 (1847); **106**, 254 (1858); Wallach, *ibid.*, **193**, 1 (1878); Meldrum and Bhatt, *J. Univ. Bombay*, **3**, Pt. 2, 149 (1934); etc.

(8) van Ekenstein and de Bruyn, *Rec. trav. chim.*, **20**, 331 (1901); **21**, 310 (1902).

(9) Willstätter and Königsberger, *Ber.*, **56**, 2107 (1923); Oeda, *Bull. Chem. Soc., Japan*, **10**, 187 (1935).

(10) Fisher, Rehberg and Smith, THIS JOURNAL, **65**, 763 (1943).

(11) Microanalyses by Dr. Francine Schwarzkopf.

The *p*-phenylphenacyl ester prepared in the usual way[4] was the derivative of lactic acid.

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BROOKLYN 2, NEW YORK RECEIVED JULY 6, 1950

Several Derivatives of Acetyl-*dl*-phenylalanine[1]

BY VIVIAN GOLDENBERG, HARRY GOLDENBERG[2] AND A. D. MCLAREN

In a recent communication, MacAllister and Niemann[3] reported for the first time the hydrolysis of a hydrazide, *viz.*, nicotinyl-*l*-tyrosylhydrazide, by the proteolytic enzyme chymotrypsin. We are prompted to submit the preparation of another hydrazide, plus a thio ester, both of which are selectively attacked by beef chymotrypsin.

Experimental

Acetyl-*dl*-phenylalanine Hydrazide (APH).—APH was derived from acetyl-*dl*-phenylalanine ethyl ester (APEE),[4,5] prepared in turn by the condensation of acetic anhydride and *dl*-phenylalanine ethyl ester. APEE (1.5 g.) was dissolved in 5 ml. of absolute alcohol to which was added 0.62 ml. of 100% hydrazine hydrate. The solution was permitted to remain in a stoppered flask for 24 hours at room temperature, after which time 1.25 g. of the desired compound, m. p. 160.1–160.2° (uncor.), was precipitated out with a mixture of ether and petroleum ether. It was recrystallized from ethanol, from which it separated as needles.

Anal. Calcd. for $C_{11}H_{15}O_2N_3$: C, 59.70; H, 6.83; N, 18.99. Found: C, 59.64; H, 6.68; N, 18.79.

Unlike nicotinyl-*l*-tyrosylhydrazide, APH is hydrolyzed slowly by chymotrypsin under the conditions observed. After a 20-hr. incubation at 24.6° of an aqueous solution (*p*H 7.3) consisting of APH (0.1 *M*) and chymotrypsin (0.470 mg. nitrogen/ml.) in 0.05 *M* phosphate buffer, the extent of hydrolysis was found to be 24.2% by titration of the carboxyl groups liberated.[6] This value is based on an effective substrate concentration of 0.05 *M*, *i. e.*, it is presumed the *d*-form is not hydrolyzed. Blanks showed complete stability of APH, in the absence of enzyme, for a period of at least one day.

Acetyl-*dl*-phenylalanine Thio Ethyl Ester (APTEE).—Acetyl-*dl*-phenylalanine (5 g., 0.024 mole), thoroughly dried in a vacuum desiccator for a week over phosphorus pentoxide, was suspended in about 30 ml. of acetyl chloride. Phosphorus pentachloride (5.1 g., 0.024 mole) was added. The flask was immediately stoppered tightly and placed in an ice-bath, whereupon the acetyl-*dl*-phenylalanine rapidly dissolved. After the solution had remained at room temperature for three hours, it was vacuum distilled to dryness with total exclusion of moisture. Anhydrous ether was quickly poured on the residue, and the solution was again vacuum distilled to remove acetyl chloride and phosphorus oxychloride. The crude acyl halide was further evacuated by pump for half an hour. Then about 40 ml. of ethyl mercaptan was added rapidly. Immediately a vigorous ebullition developed, and the residue went into solution. By the next day, some white material had precipitated out. The entire mixture was taken to dryness. The residue was slightly yellow and very hygroscopic. Water was added, the resulting suspension was chilled, made alkaline with bicarbonate, and stirred for five minutes with ether. The mixture separated into two layers; the ether layer was removed and dried over magnesium sulfate. The ether was removed by vacuum distillation to give a residue which still appeared somewhat yellow and hygroscopic. It was dried in a vacuum desiccator for several days. The residue was then washed with petroleum ether several times to remove the colored hygroscopic impurity. The crude thio ester (yield 3.2 g. or 53%) was dissolved in boiling ethanol, the solution cooled, and diluted with water. On standing in the ice-box, white needles crystallized out, m. p. 92–93° (uncor.).

Anal.[7] Calcd. for $C_{13}H_{17}O_2NS$: C, 62.13; H, 6.82; S, 12.76. Found: C, 62.05; H, 6.78; S, 12.89.

APTEE (0.03 *M*) and chymotrypsin[8] (0.048 mg. nitrogen/ml.) were incubated at 25° and an initial *p*H of 7.6 in a 50% alcoholic solution containing 0.006 *M* phosphate buffer. Within one minute an intense odor of ethyl mercaptan developed, accompanied by a drop in *p*H due to liberated carboxyl groups. The presence of mercaptan was confirmed by a positive nitroprusside test, which was carried out in weakly basic solution to avoid hydrolysis of APTEE. No spontaneous hydrolysis of the substrate could be detected in the absence of enzyme.

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BROOKLYN, NEW YORK RECEIVED MAY 9, 1950

The Hydration of 2-Heptyne[1]

BY G. F. HENNION AND C. J. PILLAR[2]

The hydration of an unsymmetrically situated non-terminal carbon–carbon triple bond has been studied previously in only three cases. Stearolic acid[3] was reported to form 9-keto- and 10-ketostearic acids, by treatment with sulfuric acid, in a ratio of 42.4:57.6. In the same way, 9-undecynoic acid yielded 59% 9-keto and 41% 10-keto product; hydration with acetic acid and mercuric acetate altered the ratio considerably, giving the 9-keto and 10-keto isomers in a ratio of 46:54.[4] 2-Pentyne (the only hydrocarbon reported) yielded 2- and 3-pentanones in nearly equal amounts by the sulfuric acid method.[5]

Since there had been previously described[6] a simple catalytic method for triple bond hydrations in high yield, it was decided to apply this procedure to 2-heptyne. A mixture of the two ketones, 2-heptanone and 3-heptanone, was easily

(1) From the M.Sc. (June, 1949) and Ph.D. (November, 1949) Theses of Vivian Goldenberg and Harry Goldenberg, respectively, of the Polytechnic Institute of Brooklyn, New York.

(2) National Institutes of Health Predoctoral Fellow, 1947–1949.

(3) R. V. MacAllister and C. Niemann, THIS JOURNAL, **71**, 3854 (1949).

(4) APEE, m. p. 68.0–68.6°, was synthesized in excellent yield and high purity according to the directions of E. Fischer (*Ber.*, **37**, 2495 (1904)) for the preparation of the related compound, chloroacetyl-*l*-tyrosine ethyl ester.

(5) E. Cherbuliez and P. Plattner (*Helv. Chim. Acta*, **12**, 324 (1929)) reported a m. p. of 68°.

(6) W. Grassmann and W. Heyde, *Z. physiol. Chem.*, **188**, 32, (1929).

(7) Microanalyses of APH and APTEE by F. Schwarzkopf, Elmhurst, L. I.

(8) Once crystallized chymotrypsin was used to study the catalyzed hydrolysis of both APH and APTEE.

(1) Paper LV on substituted acetylenes; previous paper, THIS JOURNAL, **72**, 3542 (1950).

(2) Rev. Conrad J. Pillar, O.S.B., St. Benedict's College, Atchison, Kansas.

(3) Robinson and Robinson, *J. Chem. Soc.*, 2204 (1926).

(4) Sherrill and Smith, *ibid.*, 1501 (1937).

(5) Mowat and Smith, *ibid.*, 19 (1938).

(6) Thomas, Campbell and Hennion, THIS JOURNAL, **60**, 718 (1938).