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(54) Title: PREPARATION OF 4-AMINO-2,4-DIOXOBUTANOIC ACID

(57) Abstract: A process for synthesizing 4-amino-2,4-dioxobutanoic acid involves reacting diethyl oxalate with sodium ethoxide in ethanol to form a reaction mixture, and afterward adding 2-cyano-3-hydroxy-butenedioate to the reaction mixture and allowing a re-
action to proceed under conditions suitable to form a first reaction product of the formula diethyl-2-cyano-3-hydroxy-butenedioate,
and then isolating the cyano-3-hydroxy-butenedioate, and afterward reacting the diethyl-2-cyano-3- hydroxy-butenedioate with
aqueous sodium hydroxide under conditions suitable to form 4-amino-2,4-dioxobutanoic acid.



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PREPARATION OF 4-AMINO-2,4-DIOXOBUTANOIC ACID

STATEMENT REGARDING FEDERAL RIGHTS

This invention was made with government support under Contract No. DE-AC52-06NA25396 awarded by the U.S. Department of Energy. The government has
10 certain rights in the invention.

FIELD OF THE INVENTION

The present invention relates to the preparation of 4-amino-2,4-dioxobutanoic acid.

BACKGROUND OF THE INVENTION

15 The compound 4-amino-2,4-dioxobutanoic acid is a metabolite found in plants (see, for example: Ta et al., "Utilization of the Amide Groups of Asparagine and 2-Hydroxysuccinamic Acid by Young Pea Leaves," Plant Physiology, July 1984, vol. 75, pp. 527-530, incorporated by reference). Meister reported in "Preparation and Enzymatic Reactions of the Keto Analogues of Asparagine and Glutamine," J. Biol.
20 Chem., vol. 200, (1953), pp. 571-589, which is incorporated by references, a process for synthesizing 4-amino-2,4-dioxobutanoic acid. This preparation was on a small scale and was expensive because it required crude snake venom.

SUMMARY OF THE INVENTION

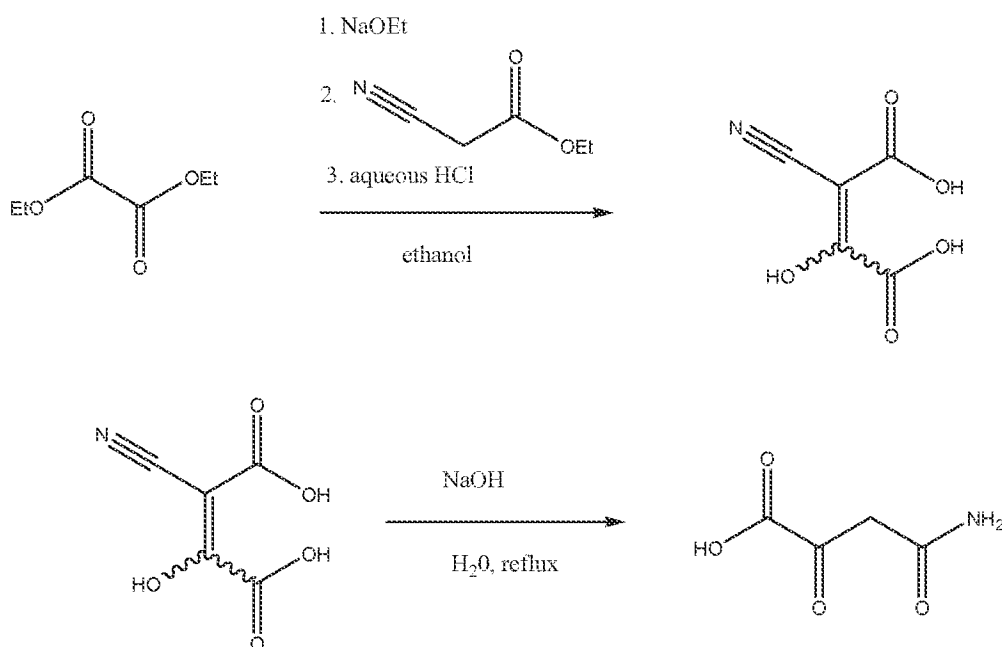
The present invention provides a process for synthesizing 4-amino-2,4-
25 dioxobutanoic acid involves reacting diethyl oxalate with sodium ethoxide in a suitable solvent to form a reaction mixture, and afterward adding 2-cyano-3-hydroxy-butenedioate to the reaction mixture and allowing a reaction to proceed under conditions suitable to form a first reaction product of the formula diethyl-2-cyano-3-hydroxy-butenedioate, and isolating said first reaction product of cyano-3-hydroxy-
30 butenedioate, and then reacting said first reaction product of diethyl-2-cyano-3-hydroxy-butenedioate with aqueous sodium hydroxide under conditions suitable to form 4-amino-2,4-dioxobutanoic acid.

In an embodiment, a process for synthesizing 4-amino-2,4-dioxobutanoic acid
35 includes the steps of reacting diethyl oxalate with sodium ethoxide in ethanol solvent to form a reaction mixture, then adding 2-cyano-3-hydroxy-butenedioate to the reaction mixture. After allowing the ingredients to react, the reaction mixture was extracted using dichloromethane and water. The aqueous layer was separated from the

dichloromethane layer, acidified, and extracted with additional dichloromethane. The dichloromethane layers were combined. Removal of the dichloromethane left diethyl-2-cyano-3-hydroxy-butenedioate. The diethyl-2-cyano-3-hydroxy-butenedioate was combined with aqueous sodium hydroxide to form a reaction mixture that was subjected to conditions suitable for forming 4-amino-2,4-dioxobutanoic acid.

DETAILED DESCRIPTION

This invention is concerned with the synthesis of 4-amino-2,4-dioxobutanoic acid and derivatives thereof. The reaction sequence begins by reacting sodium metal with an alcohol to form a sodium alkoxide. The synthesis was demonstrated using ethanol as the alcohol, which reacted with sodium to form sodium ethoxide. However, it should be understood that other suitable alcohols may also be used (methanol, ethanol, and the like). After forming the sodium ethoxide in ethanol solution, diethyl oxalate was added, and afterward, ethyl cyanoacetate was added. An acidic workup resulted in 2-cyano-3-hydroxy-butenedioate which was isolated as a pale yellowish solid. Without further purification, the 2-cyano-3-hydroxybutenedioate was heated in the presence of aqueous sodium hydroxide. The chemical reactions summarized below.



The details of the synthesis now follow. The synthesis of diethyl 2-cyano-3-hydroxy-butenedioate began by fitting a dry 5-liter Morton flask with a reflux

5 condenser. Absolute ethanol (1040 milliliters) was added to the flask under nitrogen, and sodium metal (35.2 g, 1.53 moles, 1.0 equivalent) was placed into the absolute ethanol also under nitrogen while an ice-water bath was used to cool the flask. After the mixture was stirred for about 6 hours, the ice water bath was removed and the reaction was brought to room temperature. The temperature rose briefly to about
10 30°C. After about 24 hours, the sodium metal had completely dissolved. Diethyl oxalate (219.2 grams, 1.5 moles, 1.0 equivalent) was added neat in a single portion to the flask. While the reaction mixture was stirring, a solution of ethyl cyanoacetate (169.7 grams, 1.5 moles, 1.0 equivalent) in absolute ethanol (1000 milliliters) was added dropwise at room temperature to the reaction mixture. The addition, which was
15 made at a rate of 1 drop every 2 to 3 seconds, took about 2 ½ days to complete. Afterward, the reaction mixture was extracted with dichloromethane (1000 milliliters) and deionized water (1000 milliliters). The aqueous layer was extracted with an additional 100 milliliters dichloromethane. The resulting aqueous layer had a pH of about 8-9. The layers were separated. The aqueous layer was acidified to a pH of
20 about 1 with 6 M HCl. The acidic aqueous layer was then extracted with dichloromethane (1000 milliliters) and the layers were separated. The organic layer was evaporated by rotary evaporator to yield diethyl 2-cyano-3-hydroxy-butenedioate as a pale yellowish solid (304 grams, 95%) that was used without any further purification.

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The 4-amino-2,4-dioxobutanoic acid was synthesized using the 2-cyano-3-hydroxy-butenedioate prepared as described above. A 5-liter Morton flask was equipped with an air condenser. Diethyl 2-cyano-3-hydroxy-butenedioate (214.2 grams, 1.00 mole, 1.0 equivalent) was dissolved in aqueous sodium hydroxide
30 (1.5 M, 2000 milliliters, 3.0 equivalents) at room temperature in the flask while stirring the contents of the flask. After about one minute, a heating mantle was placed underneath the flask. Using the heating mantle, the flask was heated sufficiently for reflux while the reaction mixture was stirred. After about 4 ½ hours at reflux, the heat was removed and the reaction mixture was allowed to cool to room temperature and
35 was stirred overnight at room temperature. The reaction solution was placed into an ice-water bath and acidified using 6M HCl until the pH of was equal to about 1. Solids formed after about 5 minutes. The solids were filtered. The colorless solids (27 grams) were analyzed by NMR and were found to include carbonate (¹³C-NMR

5 $\delta=162$ ppm). The bulk of the water was evaporated using a rotary evaporator at about 40°C. The solids were stirred in acetone (1000 milliliters) and then filtered. The acetone was then removed under a vacuum using a rotary evaporator followed by a vacuum pump to yield the 4-amino-2,4-dioxobutanoic acid in good yield (how much?) as a viscous yellow oil.

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Although the present invention has been described with reference to specific details, it is not intended that such details should be regarded as limitations upon the scope of the invention, except as and to the extent that they are included in the accompanying claims.

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WHAT IS CLAIMED IS:

1. A process for synthesizing 4-amino-2,4-dioxobutanoic acid, comprising:
reacting diethyl oxalate with sodium alkoxide in a suitable alcohol solvent form a reaction mixture, and afterward
adding 2-cyano-3-hydroxy-butenedioate to the reaction mixture and allowing a reaction to proceed under conditions suitable to form a first reaction product of the formula diethyl-2-cyano-3-hydroxy-butenedioate,
isolating said first reaction product of cyano-3-hydroxy-butenedioate,
reacting said first reaction product of diethyl-2-cyano-3-hydroxy-butenedioate with aqueous sodium hydroxide under conditions suitable to form 4-amino-2,4-dioxobutanoic acid.
2. A process for synthesizing 4-amino-2,4-dioxobutanoic acid, comprising:
reacting diethyl oxalate with sodium ethoxide in a suitable solvent to form a reaction mixture, and afterward
adding 2-cyano-3-hydroxy-butenedioate to the reaction mixture, and afterward
extracting the reaction mixture with a suitable organic solvent and water to form an organic layer and an aqueous layer,
then separating the aqueous layer from the organic layer,
then acidifying the aqueous layer and extracting the acidified aqueous layer with additional suitable organic solvent to form a second aqueous layer and a second organic layer,
then combining the combining the first and second organic layers, and
isolating diethyl-2-cyano-3-hydroxy-butenedioate from the organic layers,
then reacting the diethyl-2-cyano-3-hydroxy-butenedioate with aqueous sodium hydroxide under conditions suitable to form 4-amino-2,4-dioxobutanoic acid.
3. The process of claim 3, wherein the suitable organic solvent for extracting the reaction mixture is dichloromethane.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2014/021620

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07C 209/00 (2014.01)

USPC - 564/124

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) - C07C 69/00, 209/00, 209/04, 209/44, 209/54 (2014.01)

USPC - 560/301, 564/123-126, 129

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

CPC - C07C 209/00, 209/04, 209/44, 209/54 (2014.02)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Orbit.com, Google Patents, Public AppFT and PatFT, Google Scholar

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4,336,397 A (CRAGOE JR et al) 22 June 1982 (22.06.1982) entire document	1-3
A	VERBIC et al. An LFER study of the protolytic equilibria of 4-aryl-2,4-dioxobutanoic acids in aqueous solutions. J. Serb. Chem. Soc. 72(12):1201-1216, 2007. [retrieved on 22 May 2014]. Retrieved from the Internet. <URL: http://www.doiserbia.nb.rs/img/doi/0352-5139/2007/0352-51390712201V.pdf >. entire document	1-3
A	TOMASSINI et al. Inhibition of cap (m7GpppXm)-dependent endonuclease of influenza virus by 4-substituted 2, 4-dioxobutanoic acid compounds. Antimicrob. Agents Chemother. 38 (12):2827-2837, 1994. [retrieved on 22 May 2014]. Retrieved from the Internet. <URL: http://aac.asm.org/content/38/12/2827.full.pdf >. entire document	1-3

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Further documents are listed in the continuation of Box C.

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Date of the actual completion of the international search

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Date of mailing of the international search report

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