Organic Syntheses

A Publication
of Reliable Methods
for the Preparation
of Organic Compounds

Working with Hazardous Chemicals

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These paragraphs were added in September 2014. The statements above do not supersede any specific hazard caution notes and safety instructions included in the procedure.

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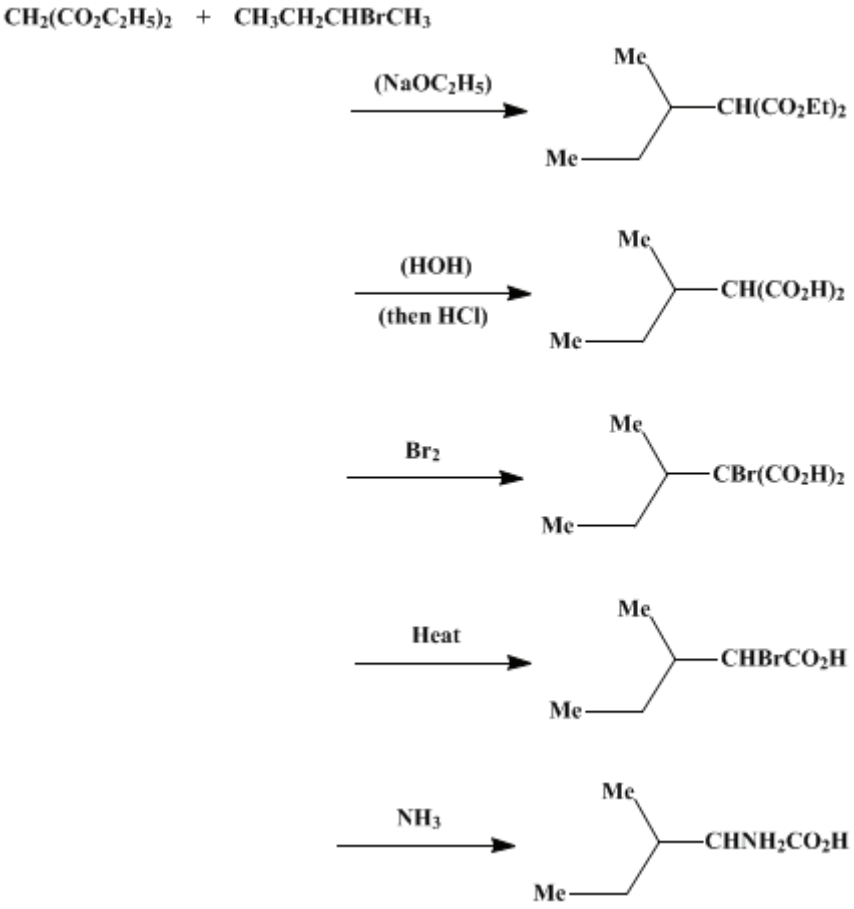
dl-ISOLEUCINE

[α-Amino-β-methylvaleric acid]



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1. Procedure

A. *Diethyl sec.-butylmalonate*. To 700 ml. of absolute ethanol in a 2-l. three-necked round-bottomed flask equipped with a long, wide-bore reflux condenser is added 35 g. (1.52 gram atoms) of sodium cut in pieces of suitable size. When all the sodium has reacted, the flask is placed on a steam cone and fitted with a mercury-sealed stirrer, a dropping funnel, and a reflux condenser bearing a calcium chloride tube (Note 1). The flask is heated, and 250 g. (1.56 moles) of diethyl malonate is added in a steady stream with stirring. After the ester addition, 210 g. (1.53 moles) of *sec*-butyl bromide is added at such a rate that the heat of reaction causes refluxing. The mixture is then stirred and refluxed for 48 hours. At the end of this time, the reflux condenser is exchanged for a downward condenser and the ethanol is removed by distillation (Note 2). The residue is treated with 200 ml. of water, shaken, and allowed to stand until the ester layer separates. The ester layer is separated from the aqueous layer and distilled from a 500-ml. two-necked flask fitted with a well-wrapped 18-in. Vigreux column. The fraction boiling at 110–120°/18–20 mm. is diethyl *sec*-butylmalonate, and it amounts to 274–278 g. (83–84%).

B. α-*Bromo*-β-*methylvaleric acid*. In a 2-l. three-necked round-bottomed flask equipped with a stirrer and dropping funnel and placed on a steam cone, 250 g. of technical potassium hydroxide is dissolved in 200 ml. of water. To the hot solution, 250 g. (1.16 moles) of diethyl *sec*-butylmalonate is added in a steady stream with vigorous stirring (Note 3). A tube connected to a vacuum line assists in

the removal of ethanol. The mixture is stirred and heated for 5 hours (Note 4), and then the contents of the flask are transferred to a beaker fitted with a stirrer and surrounded by an ice bath. The cooling is hastened by the addition of 50 g. of ice, and, when the temperature reaches 15°, technical hydrochloric acid is added at such a rate that the temperature does not rise above 20°. After the addition of about 250 ml. of acid, the monopotassium salt separates, necessitating stirring by hand until solution again occurs. When the solution is acid to Congo red (Note 5) it is transferred to a separatory funnel (Note 6).

The *sec*-butylmalonic acid is extracted with three 200-ml. portions of ether, and the combined extracts are dried over calcium chloride overnight. The ether solution is then decanted into a 2-l. three-necked flask fitted with a mercury-sealed stirrer, reflux condenser, and dropping funnel. Five milliliters of bromine is added at one time, and the solution is stirred until decolorized (Note 7). Then 50 ml. more bromine is added dropwise at such a rate that the ether refluxes gently. When all the bromine has been added, 200 ml. of water is added through the dropping funnel dropwise so as to produce no foaming or violent reaction.

The ether layer containing the bromomalonic acid is separated from the aqueous layer, and the ether is removed by distillation from a steam cone. The residual liquid is decarboxylated by refluxing for 5 hours in a 500-ml. round-bottomed flask on an oil bath heated to 130°. The bromo acid is then separated from the small amount of water and distilled. The material distilling at 125–140°/18–20 mm. is α-bromo-β-methylvaleric acid (Note 8). The yield is 150.5 g. (66.7%).

C. *dl-Isoleucine*. One hundred and fifty grams (0.77 mole) of α-bromo-β-methylvaleric acid is added to 645 ml. of technical ammonium hydroxide (sp. gr. 0.90) in a 1.5-l. round-bottomed flask. A stopper is wired in, and the flask is allowed to stand at room temperature for a week (Note 9). The stopper is removed and the mixture heated on a steam cone overnight to remove ammonia. The aqueous solution is concentrated under reduced pressure until bumping becomes violent (about 300 ml.). The mixture is then cooled to 15° and the crystals are collected on a filter. The crystals are washed with 40 ml. of ethanol and dried. The filtrate is again concentrated to about 150 ml., and a second crop of crystals is obtained. This second crop is washed with 25 ml. of water and then with 25 ml. of 95% ethanol. The yield of crude product is 65 g.

The isoleucine is recrystallized by dissolving it in 850 ml. of water heated to 95° on a steam cone. The solution is decolorized by treatment with a gram of Norit for 30 minutes and is then filtered hot. To the hot solution is added 425 ml. of 95% ethanol. and the flask is placed in an ice chest overnight. The yield of pure product is 38 g. An additional crop of 12 g. may be obtained by concentrating the mother liquors from the recrystallization to about 100 ml. and adding an equal volume of ethanol. This second crop is washed with 10 ml. of cold water and 10 ml. of cold ethanol. The total yield is 50 g. (49%). The product decomposes at 278–280° in a sealed evacuated capillary (Note 10).

2. Notes

1. The submitters carried out the preparation on a run ten times this size, using a 12-l. round-bottomed flask. After the sodium had reacted, the flask was fitted with a stopper containing the stirrer and two angle tubes connected respectively to a reflux condenser and a dropping funnel. The time allowed for the various reactions to take place was the same as for the smaller run. The percentage yields of the various products were practically identical.
2. On a run ten times this size, the submitters distilled the ethanol into another 12-l. flask connected by means of an adapter and fitted with the wide-bore reflux condenser originally used. The sodium necessary for a second run was added to the second flask as the ethanol distilled into it; on the large run this took 4–6 hours.
3. The ester is added quite slowly at first, until the reaction gets under way, and then more rapidly.
4. Water is added if necessary to keep the mass from solidifying.
5. About 400 ml. of acid is required.
6. For a run ten times this size, the solution is transferred to a 12-l. round-bottomed flask fitted with a stopper containing a large stop-cock which barely pierces the stopper and a glass tube which reaches to the bottom of the flask. A flask so fitted can be used as a large separatory funnel. The stopper is wired when the flask is upturned.
7. It is important that the first 5-ml. portion react completely; otherwise the bromination will not go

smoothly and the solution may foam out of the condenser.
8. .If the decarboxylation was not complete it will be finished here. Occasionally it is some time before a good water pump will maintain constant pressure.
9. On a larger run, the submitters allowed 350 g. of the bromoacid and 1.5 l. of ammonium hydroxide in a 3-l. flask to stand for a week. The contents of four such flasks, including any solid material, were combined in a 12-l. round-bottomed flask and heated on a steam cone overnight.
10. The product obtained in this manner has the calculated amount of amino nitrogen.

3. Discussion

dl-Isoleucine has been prepared by the reduction and subsequent hydrolysis of ethyl α-oximino-β-methyl-*n*-valerate;[2] by the action of aqueous ammonia on α-bromo-β-methyl-*n*-valeric acid;[3] and from ethyl *sec*-butylbromomalonate by saponification, decarboxylation, and amination.[4] The procedure described above is a combination of these last two methods.

References and Notes

1. These directions are the result of the efforts of many men who have worked on the preparation of isoleucine at the University of Illinois.
2. Bouveault and Locquin, *Compt. rend.*, **141**, 115 (1905); *Bull. soc. chim. France*, (3), **35**, 965 (1906).
3. Ehrlich, *Ber.*, **41**, 1453 (1908); Brasch and Friedmann, *Beitr. Chem. Physiol. Path.*, (2) 376 (1908).
4. Romburgh, *Rec. trav. chim.*, **6**, 150 (1887).

Appendix
Chemical Abstracts Nomenclature (Collective Index Number); (Registry Number)

ethanol (64-17-5)

calcium chloride (10043-52-4)

hydrochloric acid (7647-01-0)

ammonia (7664-41-7)

ether (60-29-7)

bromine (7726-95-6)

sec-Butyl bromide (78-76-2)

Norit (7782-42-5)

potassium hydroxide (1310-58-3)

sodium (13966-32-0)

ammonium hydroxide (1336-21-6)

diethyl malonate (105-53-3)

α-Bromo-β-methylvaleric acid,
α-bromo-β-methyl-n-valeric acid (42880-22-8)

α-Amino-β-methylvaleric acid

isoleucine,
DL-Isoleucine (73-32-5)

bromomalonic acid

sec-butylmalonic acid

Diethyl sec-butylmalonate,
Diethyl sec.-butylmalonate (83-27-2)

ethyl α-oximino-β-methyl-n-valerate

ethyl sec-butylbromomalonate

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