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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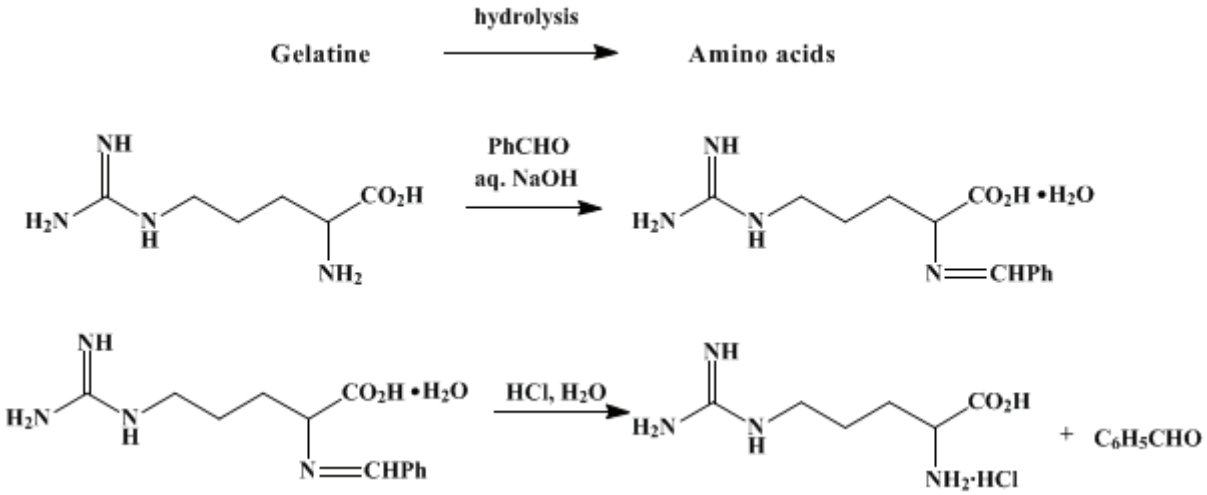
Organic Syntheses, Coll. Vol. 2, p.49 (1943); Vol. 12, p.4 (1932).

d-ARGININE HYDROCHLORIDE



Submitted by E. Brand and M. Sandberg.
Checked by H. T. Clarke and S. Graff.

1. Procedure

Benzylidenearginine, Method 1.—To 500 g. of gelatine (Note 1) is added 1.5 l. of concentrated hydrochloric acid (sp. gr. 1.19); the mixture is warmed on the steam bath for thirty minutes and boiled over a free flame for eight to ten hours (Note 2) under a reflux condenser provided with a trap for hydrogen chloride gas. The solution is concentrated to 400 cc. on the steam bath under reduced pressure, employing the apparatus shown in Org. Syn. Coll. Vol. I, **1941**, 427. The syrupy residue is then diluted with 500 cc. of distilled water and again concentrated to 400 cc. This process of dilution and evaporation is repeated twice more (Note 3). The final residue is dissolved in 500 cc. of hot distilled water and decolorized by adding 15 g. of decolorizing carbon and heating for ten minutes on the steam bath. The filtrate is chilled in an ice-salt bath and treated with 250–350 cc. of a 40 per cent solution of sodium hydroxide until slightly alkaline to litmus, keeping the temperature below 10°. An additional 70-cc. portion of 40 per cent sodium hydroxide solution is added, keeping the temperature below 5°; this is followed by the addition, in four portions, of 225 cc. of benzaldehyde, with vigorous shaking after each addition, the temperature being held below 5° throughout (Note 4). The addition of the benzaldehyde occupies about ten minutes.

The resulting emulsion is allowed to stand overnight in the refrigerator at 0–5°; the crystalline precipitate is filtered by suction and washed first with 80 cc. of ice-cold water in four portions, then with 50 cc. of a mixture of two volumes of ether and one volume of methyl alcohol, and, finally, with ether (Note 5) until the washings are colorless and free of benzaldehyde. After drying in a vacuum desiccator the product weighs 35–40 g.; it melts with decomposition at 206–207° (corr.).

Benzylidenearginine, Method 2.—The hydrolysis of 500 g. of gelatine and the removal of excess hydrochloric acid are conducted as described above. After treatment with 15 g. of decolorizing carbon the filtrate is diluted to 2.5 l., heated almost to boiling, and treated with 110 g. of 2,4-dinitro-1-naphthol-7-sulfonic acid ("flavianic acid") (Note 6) dissolved in 400 cc. of hot water. The mixture is boiled (Note 7) for about three minutes and diluted with boiling water to a total volume of 4 l. The mixture is cooled rapidly to 45° and then allowed to cool slowly to room temperature, with occasional stirring and vigorous scratching of the walls of the container. After standing for about two hours at room temperature (Note 8), practically all (Note 9) the arginine dinitronaphtholsulfonate should have separated in crystalline, readily filterable form. The product is filtered by suction and washed first with three 100-cc. portions of a 0.5 per cent solution of dinitronaphtholsulfonic acid and then with two 25-cc. portions of 95 per cent ethyl alcohol. After being dried in air, the product weighs 113–118 g. (Note 10);

it decomposes at 245–265° (Note 11).

One hundred grams (0.21 mole) of finely powdered arginine dinitronaphtholsulfonate is added, all at once, to 230 cc. of cold 2 *N* sodium hydroxide; the salt dissolves readily on agitation. There is then added *without delay* (Note 12) 35 g. (0.33 mole) of benzaldehyde, in four portions and with vigorous shaking, each portion being accompanied by 75 cc. of ice-cold water. During this process, benzylidenearginine separates as a crystalline cake. The mixture is allowed to stand at 15–20° for one to two hours, whereupon the product is filtered (Note 13) and washed successively with two to four 50-cc. portions of ice-cold water, three 20-cc. portions of a mixture of 20 cc. of methyl alcohol and 40 cc. of ether, and finally two 50-cc. portions of ether (Note 5). It is then dried in air. The yield is 39–43 g., corresponding to about 44–48 g. from 500 g. of gelatine (Note 10).

Arginine Hydrochloride.—A suspension of 50 g. (0.18 mole) of benzylidenearginine in 39 cc. of 5 *N* hydrochloric acid is heated in a boiling water or steam bath for forty-five minutes, with occasional shaking. The mixture is allowed to cool and is freed of benzaldehyde by shaking with three 100–150 cc. portions of ether. The aqueous solution is filtered if necessary, decolorized with 3 g. of decolorizing carbon, filtered, and concentrated on the water bath at 70° under reduced pressure until crystallization sets in. The residue is transferred from the flask with the aid of 25 cc. of hot 70 per cent ethyl alcohol; the arginine hydrochloride is precipitated by adding 300 cc. of absolute alcohol. After filtering the product, a further small quantity of crystalline hydrochloride is obtained by adding 300 cc. of ether to the mother liquor. The combined (Note 14) yield amounts to 33–34 g. (88–90 per cent of the theoretical amount). It melts at 220° (corr.) and exhibits a rotation of $[\alpha]_D^{25^\circ}$ = +12.2 to 12.3° (5 per cent in water).

2. Notes

1. The quality of gelatine is technically defined on the basis of its physical properties, and different samples vary widely in chemical composition. In checking, the highest yields (9.7–10.3 per cent of the weight of gelatine taken) of benzylidenearginine were secured from the "Bactogelatine" of the Digestive Ferments Company.
2. The biuret reaction is generally found to be negative after five hours.
3. The third distillate generally contains only 1–2 g. of hydrogen chloride. In checking this preparation on a larger scale, it has been found convenient to add the water continuously below the surface of the boiling syrup; this modification, which constitutes a steam distillation under reduced pressure, brings about a more rapid removal of the excess hydrochloric acid.
4. Unless the temperature is held below 5°, difficulty is experienced in emulsifying the benzaldehyde.
5. Benzylidenearginine is quite insoluble in ether but appreciably soluble in methyl alcohol and in water. Attempts to recrystallize it from the latter solvents lead to a product of inferior quality, owing to decomposition in solution. Impure or contaminated samples may be purified by hydrolysis with hot hydrochloric acid and reprecipitation with benzaldehyde after neutralization.
6. The free dinitronaphtholsulfonic acid can be prepared readily from commercial Naphthol Yellow S by treating a filtered saturated solution of the dye with three volumes of concentrated hydrochloric acid. The crystals which separate are washed with cold 20 per cent hydrochloric acid and dried, first in air and finally in a vacuum desiccator over solid sodium hydroxide.
7. The boiling prevents the precipitation of arginine diflavianate and minimizes the separation of the flavianates of other amino acids.
8. Crystallization is occasionally delayed, particularly in first runs when traces of arginine flavianate are not available in the atmosphere for spontaneous inoculation. In such cases it may be necessary to chill the solution in the refrigerator with occasional vigorous scratching.
9. The mother liquor, on long standing in the icebox, may deposit a second crop of crystals which appear to consist largely of sodium dinitronaphtholsulfonate and yield no arginine on further treatment. The filtrate thus obtained in Method 2 is suitable for the recovery of other amino acids, thereby differing from the corresponding mother liquor from Method 1.
10. This yield was obtained from a batch of gelatine from which 35–36 g. of benzylidenearginine was obtained by Method 1.
11. According to the literature,[1] pure arginine dinitronaphtholsulfonate melts at 258–260° with decomposition. The presence of moisture lowers the melting point considerably. If the mother liquor is allowed to stand for several days at 0–5°, some sodium dinitronaphtholsulfonate may crystallize.

12. Delay in adding the benzaldehyde must be avoided or sodium dinitronaphtholsulfonate may crystallize; the water is added to prevent this. The presence of the excess of benzaldehyde also appears to help prevent this crystallization.
13. A sintered-glass suction filter is advantageous for collecting and washing the benzylidenearginine.
14. The over-all loss involved in the various steps may be estimated from the following experiment: 5.0 g. of arginine nitrate was converted through the dinitronaphtholsulfonate into benzylidenearginine and then back into arginine nitrate, when 4.2 g. was recovered.

3. Discussion

Arginine has been precipitated (*a*) in the form of its silver derivative at *p*H 10;[2] (*b*) as its dinitronaphtholsulfonate which is then decomposed by means of 33 per cent sulfuric acid,[1] by the combined action of hot dilute sulfuric acid and butyl alcohol,[3] by cold concentrated hydrochloric acid followed by aniline,[4] or by barium hydroxide.[5] Arginine has likewise been precipitated (*c*) in the form of its benzylidene derivative from solutions rendered alkaline with barium hydroxide or sodium hydroxide.[6] It has been separated as such (*d*) from protein hydrolysates by electrolysis under controlled *p*H.[7]

In the present directions, Method 1 is essentially that developed by Bergmann and Zervas;[6] Method 2 forms a combination of methods (*b* and *c*) and thus at once affords a product of high purity while avoiding the mechanical difficulties involved in the complete removal of the dinitronaphtholsulfonic acid from its arginine salt.

References and Notes

1. Kossel and Gross, Z. physiol. Chem. **135**, 167 (1924).
2. Kossel, ibid. **22**, 176 (1896–7); **25**, 165 (1898); Kossel and Kutscher, ibid. **31**, 165 (1900); Vickery and Leavenworth, J. Biol. Chem. **72**, 403 (1927); **75**, 115 (1927).
3. Pratt, ibid. **67**, 351 (1926).
4. Cox, ibid. **78**, 475 (1928).
5. Felix and Dirr, Z. physiol. Chem. **176**, 38 (1928).
6. Bergmann and Zervas, ibid. **152**, 282 (1926); **172**, 277 (1927).
7. Foster and Schmidt, J. Biol. Chem. **56**, 545 (1923); J. Am. Chem. Soc. **48**, 1709 (1926); Cox, King, and Berg, J. Biol. Chem. **81**, 755 (1929).

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

gelatine

arginine diflavianate

arginine flavianate

flavianic acid

ethyl alcohol,
alcohol (64-17-5)

sulfuric acid (7664-93-9)

hydrogen chloride,
hydrochloric acid (7647-01-0)

methyl alcohol (67-56-1)

ether (60-29-7)

aniline (62-53-3)

sodium hydroxide (1310-73-2)

benzaldehyde (100-52-7)

butyl alcohol (71-36-3)

decolorizing carbon (7782-42-5)

arginine (74-79-3)

barium hydroxide (17194-00-2)

Benzylidenearginine

arginine dinitronaphtholsulfonate

dinitronaphtholsulfonic acid

Arginine Hydrochloride (1119-34-2)

sodium dinitronaphtholsulfonate

arginine nitrate

D-Arginine hydrochloride (627-75-8)

2,4-dinitro-1-naphthol-7-sulfonic acid

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