Organic Syntheses

A Publication
of Reliable Methods
for the Preparation
of Organic Compounds

Working with Hazardous Chemicals

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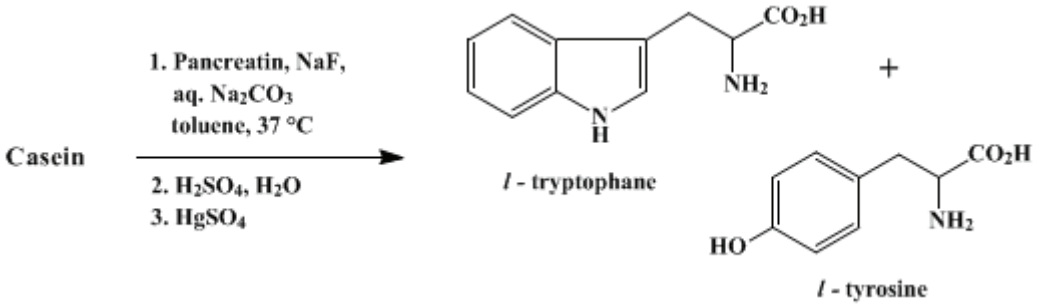
DOI:10.15227/orgsyn.010.0100

Organic Syntheses, Coll. Vol. 2, p.612 (1943); Vol. 10, p.100 (1930).

l-TRYPTOPHANE

[By-product, *l*-Tyrosine]



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1. Procedure

(*A*) *Tryptophane*.—In an 8-l. (2-gal.) bottle is placed 600 g. of commercial casein (coarse powder), which is then covered with about 3.2 l. of tap water at 37° (Note 1). The bottle is shaken until all the casein is moistened. A solution of 60 g. of anhydrous sodium carbonate (Note 2) and 6 g. of sodium fluoride (Note 3) in 1 l. of water at 37° is added. A thin paste of 20 g. of commercial pancreatin in 100 cc. of water (37°) is poured in. The mixture is covered with a layer of toluene (80 cc.), diluted to 6 l., stoppered, shaken thoroughly, and placed in a warm room or bath at 37°.

After four or five days, with daily shakings, most of the casein is in solution and chalky masses of tyrosine begin to separate. After five days, a second 20-g. portion of pancreatin in 100 cc. of water is added. After twelve days, the bottle is cooled in an icebox overnight and the undissolved material is filtered (Note 4) and reserved for the preparation of tyrosine.

The filtrate (6.9–7 l.) is measured into a 16-l. (4-gal.) stone jar, and for every liter there is added 163 cc. of dilute sulfuric acid (one volume of 95 per cent sulfuric acid and one volume of water, cooled to room temperature). The first part of the acid must be added cautiously on account of the liberation of carbon dioxide.

The tryptophane is precipitated by adding a solution of 200 g. of mercuric sulfate (Note 5) in a mixture of 1860 cc. of water and 140 cc. of 95 per cent sulfuric acid. After standing for twenty-four to forty-eight hours, the clear liquid is siphoned out and the yellow precipitate is filtered and washed (Note 6) with a solution of 100 cc. of concentrated sulfuric acid in 1.9 l. of distilled water containing 20 g. of mercuric sulfate, until the filtrate is colorless and Millon's test is atypical (Note 7); about 1.5 l. is necessary. The precipitate is washed with three successive 500-cc. portions of distilled water to remove most of the sulfuric acid.

The moist precipitate (120–130 g.) is suspended with mechanical stirring in 1.2–1.3 l. of distilled water, and a hot, 20 per cent aqueous solution of barium hydroxide is added until the mixture is permanently alkaline to phenolphthalein (about 120 cc. is required). A rapid stream of hydrogen sulfide is passed in with stirring until the mercury is completely precipitated (Note 8). The precipitate is filtered and washed with water until a sample of the washings gives a negative test for tryptophane with bromine water (Note 9). The barium is removed from the combined filtrate and washings by adding the exact amount of dilute sulfuric acid (Note 10) and filtering. The filtrate is concentrated under reduced pressure to about 80 cc.

The tryptophane is extracted from the aqueous solution by repeated shaking in a separatory funnel with 25-cc. quantities of *n*-butyl alcohol; water is added from time to time to keep the volume

approximately constant (Note 11). The butyl alcohol extract is distilled under reduced pressure. After the water present has distilled, the tryptophane precipitates in the distilling flask and may cause bumping. When all the water has been removed, as is indicated by non-formation of drops on the side of the condenser, the distillation is stopped and, after cooling, the tryptophane is filtered and washed with a little fresh butyl alcohol. Such extractions and distillations are continued until the quantities of tryptophane obtained are negligibly small (Note 11).

The tryptophane so produced (7–8 g.) varies somewhat in quality in different runs. It is purified by recrystallization from 60 cc. of dilute alcohol (two volumes of 95 per cent alcohol to one volume of water), filtering from the hot solution an appreciable quantity of insoluble matter, and subjecting this to a second extraction with an additional 10 cc. of aqueous alcohol. The solution is decolorized by the addition of 1 g. of Norite and allowed to stand in the icebox; the silvery leaflets of tryptophane are filtered and washed successively with cold 70 per cent, 80 per cent, 95 per cent alcohol, and, finally, with a little ether. Less than half the tryptophane is obtained in each crystallization (Note 12). The yield of pure (Note 13) tryptophane is 4.0–4.1 g., together with under 0.1 g. of less pure product.

(*B*) *Tyrosine*.—The insoluble material (160–170 g.) obtained on filtering the digestion mixture (p. 612) is suspended in 320 cc. of water and 80 cc. of 36 per cent hydrochloric acid, and the mixture is boiled gently for thirty minutes (Note 14). After straining through cheesecloth, decolorizing with 6 g. of Norite (Note 15), and filtering hot (Note 16), the warm (60–70°) solution is shaken with three 20-cc. portions of benzene (Note 17) and heated to boiling (Note 18). A slight excess (120–150 cc.) of 28 per cent ammonia is cautiously added, and the mixture is allowed to stand overnight in the icebox. The crystalline product is then filtered and washed with three 40-cc. portions of ice-water. After drying, it weighs about 23 g. The mother liquor and washings are evaporated to about 200 cc., when a second crop is obtained, weighing slightly under 1 g.

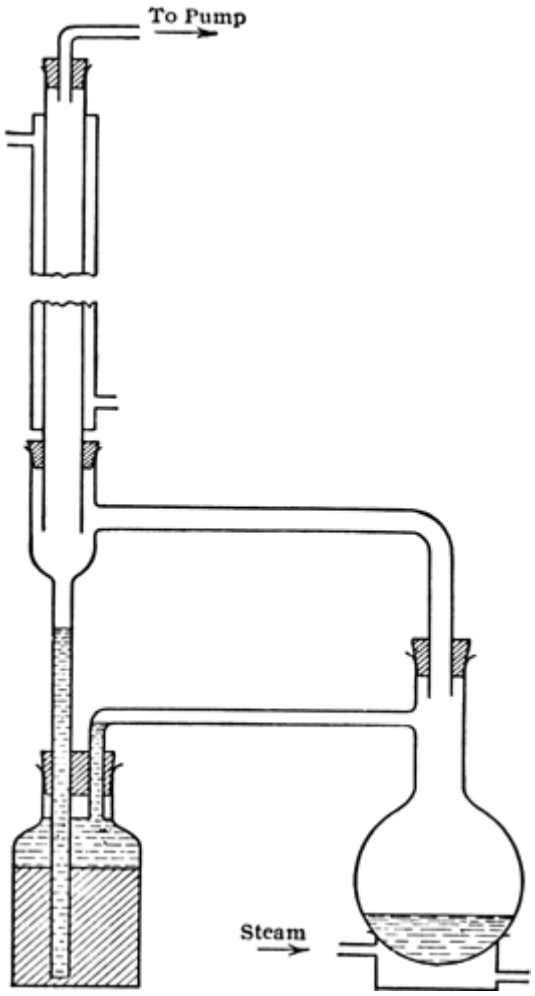
The combined product is suspended in 400 cc. of water and dissolved by adding 8 g. of sodium hydroxide in 20–30 cc. of water (Note 19); 2 g. of Norite is added, and the solution filtered. The residue is washed on the funnel with 20–30 cc. of hot distilled water. The filtrate is heated to boiling (Note 18) and treated with 13 cc. of hydrochloric acid (Note 20), when crystallization usually begins. The mixture is then acidified to litmus with acetic acid (Note 21) and allowed to stand overnight in the refrigerator. The resulting tyrosine is filtered and washed with ice-cold distilled water (130–150 cc. is necessary) until the washings are free of chloride. The product is dried in air or in a vacuum oven. The yield is 17.0–18.2 g. of pure white, silky needles of tyrosine. A second crop (about 0.5 g.) of a slightly less pure product may be obtained on concentrating the mother liquor to about 120 cc.

2. Notes

1. Tryptic action is more rapid if all water used is at 37°. Distilled water is not necessary at this stage.
2. This is a considerable excess of sodium carbonate. Smaller quantities might be satisfactory.
3. The sodium fluoride probably inhibits the action of the oxidases.
4. This filtration may be slow. Büchner funnels of 20-cm. diameter are best used; the material from a single filling is allowed to suck dry and the filter paper then changed.
5. Approximately this quantity of mercuric sulfate is necessary to precipitate the tryptophane completely, as judged by the Hopkins-Cole glyoxylic acid test.
6. This washing is to remove tyrosine, which is precipitated as a mercury compound somewhat more soluble than the tryptophane precipitate. The mercuric sulfate addition tends to reduce the tryptophane solubility.
7. A persistent red color is always obtained in the filtrates, but the final color is distinctly different from that due to tyrosine.
8. Excess hydrogen sulfide must remain in the solution after standing. A sample of the filtrate, after acidifying with acetic acid, should give a copious black preciptate with lead acetate.
9. The bromine water test is somewhat more satisfactory for pure tryptophane than the glyoxylic acid test. Hydrogen sulfide may interfere (owing to sulfur formation) and must be boiled out first. The solution to be tested must be acid with acetic acid.
10. This amount is best determined in a 20-cc. aliquot sample, employing 2 per cent sulfuric acid in a buret.

11. In checking, it was found satisfactory to extract in a continuous apparatus (Fig. 21). Extraction is continued until the liquid in the flask begins to bump on account of the separation of solid; a new charge of butyl alcohol is then employed, about five charges being necessary. This process is repeated until the residue after a three-hour period of extraction fails to give the red color, characteristic of tryptophane, with bromine water. The time necessary, of course, depends upon the rate of boiling; in checking, it was found to be twenty-eight to thirty hours.

Fig. 21



12. The recrystallization of the crude tryptophane is an extremely troublesome process. Not only must a less soluble by-product be removed, but the mother liquors contain a more soluble, gummy impurity in considerable proportion. After collecting each crop, the mother liquor must be evaporated to a small volume on the steam bath and treated with a double volume of alcohol. This process is repeated until no further crystals are obtained, but only a gum.
13. The purity of the tryptophane has been checked by the optical rotation ($[\alpha]_D = -28$ to $-33°$) and by analysis for amino nitrogen (6.8–6.9 per cent) by Van Slyke's method.
14. The boiling acid solution hydrolyzes protein material that otherwise greatly retards filtration.
15. The Norite used in this preparation is insufficient to decolorize the solutions completely but gives a white final product.
16. All the filtrations in the purification of tyrosine, except possibly the last, are best done on a 20-cm. Büchner funnel. Whenever charcoal is used, kieselguhr may be employed to obtain a clear filtrate.
17. Benzene extracts traces of substances, probably fatty acid, that retard filtration and greatly alter the quality of the final product.
18. The tyrosine crystallizes in long, silky needles, easy to filter, if the solution is neutralized at the boiling point.
19. A small amount of flocculent impurity remains undissolved.

20. The hydrochloric acid is added to provide chloride ion as an index of complete washing.
21. Tyrosine is very slightly soluble in all concentrations of acetic acid. Therefore any excess of acetic acid does not redissolve the tyrosine.

3. Discussion

The above procedure for preparing tryptophane is an adaptation from the methods of Hopkins and Cole,[1] Dakin,[2] and Onslow.[3]

Tyrosine, as a primary product, may be readily prepared by hydrolyzing silk with hydrochloric acid, neutralizing the acid with sodium hydroxide, and finally acidifying with acetic acid.

This preparation is referenced from:

- Org. Syn. Coll. Vol. 2, 325

References and Notes

1. Hopkins and Cole, J. Physiol. **27**, 418 (1902).
2. Dakin, Biochem. J. **12**, 302 (1918).
3. Onslow, ibid. **15**, 392 (1921).

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

bromine water

dilute alcohol

aqueous alcohol

alcohol (64-17-5)

sulfuric acid (7664-93-9)

hydrochloric acid (7647-01-0)

acetic acid (64-19-7)

ammonia (7664-41-7)

Benzene (71-43-2)

ether (60-29-7)

sodium hydroxide (1310-73-2)

hydrogen sulfide (7783-06-4)

sodium carbonate (497-19-8)

sulfur (7704-34-9)

mercury (7439-97-6)

carbon dioxide (124-38-9)

butyl alcohol,
n-butyl alcohol (71-36-3)

Norite (7782-42-5)

toluene (108-88-3)

lead acetate

barium hydroxide (17194-00-2)

tyrosine,
L-Tyrosine (60-18-4)

mercuric sulfate (7783-35-9)

barium (7440-39-3)

phenolphthalein (77-09-8)

glyoxylic acid (298-12-4)

Tryptophane,
l-TRYPTOPHANE (73-22-3)

sodium fluoride (7681-49-4)