CCXVI. THE PREPARATION OF CANAVANINE FROM *CANAVALIA OBTUSIFOLIA*

By M. DAMODARAN and K. G. A. NARAYANAN

From the University Biochemical Laboratory, Chepauk, Madras

(*Received 1 September 1939*)

Our interest in canavanine arose under the same circumstances which in the first instance led to its discovery by Kitagawa & Tomita in jack bean [1929]. In studying some new sources of urease it was found that the seeds of *Canavalia obtusifolia* resembled jack bean not only in possessing high urease activity, but also in giving rise to very large amounts of "extra" urea on incubation with whole blood or liver tissue [Damodaran & Sivaramakrishnan, 1937]. The aqueous extract of the seed meal gave the ruby-red reaction with irradiated sodium nitroprusside characteristic of canavanine and after some preliminary trials the amino-acid was isolated in good yield from this new source.

Difficulty was experienced in obtaining homogeneous well crystallized preparations by adhering strictly to the procedure described by Kitagawa & Yamada [1932]. Their method consisted in extracting the fat-free jack bean meal with aqueous alcohol, concentrating the extract to a thick syrup and treating it with absolute alcohol. The crude canavanine thus precipitated was redissolved in water and converted into canavanine flavianate. From the twice-recrystallized flavianate the free base was liberated by barium hydroxide, taken up in aqueous alcohol and allowed to crystallize. It was found by these authors that the amino-acid thus obtained was contaminated with some persistent impurity. In a later publication Kitagawa [1937] therefore recommended the destruction of the impurity by digesting the first crude canavanine precipitate for several hours with 10% HCl. Gulland & Morris [1935], who also seem to have experienced the same difficulty, suggest the purification of the base liberated from the flavianic acid salt by conversion into the rufianate.

We have found that the simplest procedure for obtaining satisfactory yields of the pure product is to introduce a preliminary treatment of the crude canavanine solution with basic lead acetate.

Experimental

The seeds were freed from husk, ground finely and defatted with light petroleum. 1 kg. of the fat-free meal was extracted three times with 50% alcohol (1 l. alcohol per 250 g. meal with 2 hr. shaking each time). The extracts were combined, concentrated under reduced pressure to a syrup (400 ml.) and poured into about 5 l. of absolute alcohol with vigorous stirring. The crude canavanine was precipitated as a viscous mass. After standing in the refrigerator overnight the alcohol was decanted from the precipitate of crude canavanine, the sticky solid redissolved in 300 ml. of distilled water, and again treated with absolute alcohol as before. The reprecipitated material was dissolved in about $3\frac{1}{2}$ l. of water and treated with a saturated solution of basic lead acetate until

precipitation was complete. The precipitate was centrifuged off and washed with small quantities of warm water.

The combined filtrates were made acidic to Congo red by the addition of dilute H_2SO_4 ($N/5$) and after filtration from the lead sulphate, treated with excess of flavianic acid solution (200 g. in 500 ml. of water) with vigorous stirring. A heavy orange-yellow precipitate consisting of microscopic needles appeared in a few minutes. The mixture was left in the refrigerator overnight, filtered at the pump and the precipitate washed with ice-cold water. It was recrystallized twice from $1\frac{1}{2}$ l. of hot water. M.P. 212°, after previous browning at 190°.

The flavianate was dissolved in about 3 l. of hot water and a saturated solution of $Ba(OH)_2$ was slowly added with vigorous stirring until the mixture was strongly alkaline to litmus. The barium flavianate was filtered at the pump and washed three times with dilute $Ba(OH)_2$ solution, the precipitate being ground up each time with $Ba(OH)_2$ before being returned to the filter. Excess of Ba from the combined filtrates was removed by the addition of $N/10$ H_2SO_4 to *p*H 7. The Ba-free filtrate was golden yellow in colour and contained traces of flavianic acid. It was concentrated under reduced pressure to a volume of 600 ml., boiled with animal charcoal for a few minutes which removed the last traces of flavianic acid, the colourless solution freed quantitatively from H_2SO_4 using $N/10$ solutions of $Ba(OH)_2$ and H_2SO_4 and the filtrate concentrated under reduced pressure in an atmosphere free from carbon dioxide, to a volume of 300 ml. Concentration to a syrup is inadvisable as it renders crystallization difficult. To the solution obtained as above three volumes of 95 % alcohol were added and the mixture was left at 0° for a day. The canavanine which crystallized out in irregular prisms was filtered at the pump, washed with a small volume of ice-cold water and then with cold 75 % alcohol. A second lot of crystals was obtained by concentrating the mother liquor. The yield obtained was usually more than 20 g.

To follow the course of the fractionation quantitatively, in one experiment the total N and the urea produced by the action of liver extract was determined on the solution obtained at each stage. The canavanine present was calculated on the assumption that it was the only substance present responsible for urea formation. The results are given in Table I. The actual yield of crystalline canavanine was 23·5 g.

Table I

	Total-N (g.)	Urea-N (g.)	Canavanine present (g.)
Alcoholic extract from 1 kg. (dry wt.) of seed meal	21·56	7·42	46·3
Filtrate after treatment with basic lead acetate	15·38	6·44	40·1
Filtrate after decomposition of flavianate	10·36	5·13	32·0

For analysis the product was recrystallized three times from aqueous alcohol and dried at 100° *in vacuo* over P_2O_5. The M.P. of the recrystallized sample was 183°. (Found: C, 35·38; H, 6·82 (Weiler & Strauss); N, 30·61 % (micro-Kjeldahl). Calculated for $C_5H_{12}O_3N_4$: C, 34·1; H, 6·82; N, 31·8 %.) Gulland & Morris [1935] give C, 34·2; H, 6·8; N, 30·7 %. In the two papers of Kitagawa available here no elementary analysis is given. We find amino-N (micro-Van Slyke), 50·6 % of the total N in 5 min. at 30°.

SUMMARY

From the seeds of *Canavalia obtusifolia* the amino-acid canavanine can be readily prepared in pure condition in yields amounting to above 20 g. per kg. of the fat-free seed meal.

REFERENCES

Damodaran & Sivaramakrishnan (1937). *Biochem. J.* **31**, 1041.
Gulland & Morris (1935). *J. chem. Soc.* 763.
Kitagawa (1937). *J. Biochem., Tokyo,* **25**, 23.
—— & Tomita (1929). *Proc. imp. Acad. Japan,* **5**, 380.
—— & Yamada (1932). *J. Biochem., Tokyo,* **16**, 339.