574. *Synthesis of* DL-*Canavanine.*[1]

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An advantageous synthesis of DL-canavanine from γ-butyrolactone is described.

CANAVANINE was isolated from jack bean (*Canavalia ensiformis*) by Kitagawa and his co-workers [2,3] who assigned formula (I) to it, and confirmed this by enzymic degradation to canaline. They established the structure of canaline as α-amino-γ-amino-oxybutyric acid (II) by catalytic hydrogenolysis to homoserine (α-amino-γ-hydroxybutyric acid) and

(I) $NH_2{\cdot}C(:NH){\cdot}NH{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}CH(NH_2){\cdot}CO_2H$ $NH_2{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}CH(NH_2){\cdot}CO_2H$ (II)

by reconversion of the latter into canaline and then into canavanine.[4] Direct degradation of canavanine to α-amino-γ-bromobutyric acid by means of hydrobromic acid,[5] and to homoserine and guanidine by bacterial cleavage [6] or hydrogenolysis [7] furnished additional proofs of its constitution.

Canavanine appears to be the only natural compound containing the guanidino-oxy-group. Structurally related to arginine, it appears to participate in enzymic reactions of arginine.[7-9] It was shown to inhibit the growth of some bacteria,[8] viruses,[10] and protozoa.[9]

[1] Presented, in part, before the XXXth Meeting of the Israel Chem. Soc., Jerusalem, April 1962; cf. *Bull. Res. Council Israel*, 1962, **11**, *A*, 36.
[2] Kitagawa and Tomiyama, *J. Biochem.* (*Japan*), 1929, **11**, 265.
[3] Kitagawa and Yamada, *J. Biochem.* (*Japan*), 1932, **16**, 339.
[4] Kitagawa, *J. Biochem.* (*Japan*), 1937, **25**, 23.
[5] Gulland and Morris, *J.*, 1935, 763.
[6] Kihara, Prescott, and Snell, *J. Biol. Chem.*, 1955, **217**, 497.
[7] Walker, *Arch. Biochem. Biophys.*, 1955, **59**, 233.
[8] Oginsky and Gehrig, *J. Biol. Chem.*, 1952, **198**, 799; Horowitz, *ibid.*, 1948, **174**, 371; Volcani and Snell, *ibid.*, p. 893; Kihara and Snell, *ibid.*, 1955, **212**, 83; Walker, *ibid.*, 1956, **218**, 549.
[9] Walker, *J. Biol. Chem.*, 1955, **212**, 207.
[10] Pilcher, Suike, and Smith, *Proc. Exp. Biol. Med.*, 1956, **88**, 79; Pearson, Lageborg, and Winzler, *ibid.*, 1952, **79**, 409.

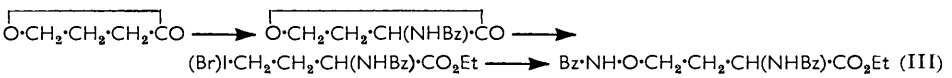
Although L-canavanine occurs in large amounts in jack bean and in *Colutea arborescens*,[11] its isolation is tedious and it is not readily accessible. α-*N*-Acetyl-L-canavanine has been reported to racemise and, after hydrolysis, DL-canavanine was then isolated as flavianate and as sulphate.[12] D-Canavanine was prepared from the DL-form by enzymic resolution with arginase.[12]

The total synthesis of canavanine is identical with that of canaline as regards building up the α-amino-γ-amino-oxybutyric acid skeleton. The amino- and carboxyl groups must then be protected before conversion of the 4-amino-oxy- into the 4-guanidino-oxy-group.

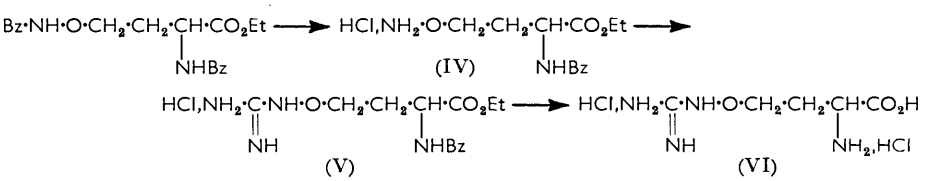
In a previous synthesis the copper complex of DL-canaline was treated with methylisourea, yielding 10—12% of DL-canavanine,[13] but no analytical data were given. Another approach [13] consisted in converting 5-(2-amino-oxyethyl)hydantoin, an intermediate in a DL-canaline synthesis, into 5-(2-guanidino-oxyethyl)hydantoin; it seems that the subsequent drastic alkaline hydrolysis required for the cleavage of the hydantoin accounts for the failure of this procedure; canavanine withstands alkaline hydrolysis for several hours [4] but is degraded to canaline by prolonged treatment with aqueous barium hydroxide.[14]

Regeneration of L-canavanine [4,15] from L-canaline was carried out on α-*N*-benzoylcanaline by means of methylisourea. The intermediate α-*N*-benzoylcanaline was prepared by dibenzoylation of canaline, conversion into the lactim (azlactone form), and very short hydrolysis with 10% sulphuric acid to remove only the ω-*N*-benzoyl group; liberation from the sulphate was carried out by barium hydroxide. Analytical data and yields were not given, and selectivity in the hydrolysis is not assured.

The synthesis described below makes use of an intermediate in canaline synthesis which is based on γ-butyrolactone. Re-examination in this laboratory of the preparation of α-benzamido-γ-butyrolactone, and its smooth conversion into ethyl α-benzamido-γ-bromo- or -γ-iodo-butyrate,[16] permitted use of these intermediates for the total syntheses of DL-canaline and DL-canavanine. Condensation of the halogeno-esters with sodium benzhydroxamate yielded ethyl α-benzamido-γ-benzamido-oxy-butyrate (III) in an overall yield of 28%,[17] which was increased to 39% in conditions which suppressed side reactions.



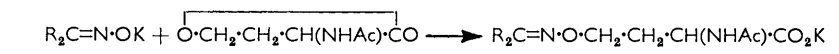
The ester (III) was selectively debenzoylated with ethanolic hydrogen chloride, to give ethyl α-benzamido-γ-amino-oxy-butyrate hydrochloride (IV) in 95% yield, and this was converted into α-*N*-benzoyl-DL-canavanine ethyl ester hydrochloride (V) by means of methylisothiourea sulphate or, better (70% yield), cyanamide. Hydrolysis with 18%



hydrochloric acid then yielded DL-canavanine dihydrochloride (VI) (80%), from which DL-canavanine (I) (87%) was liberated by use of triethylamine in ethanol. An overall yield of 18% of canavanine, based on γ-butyrolactone, was achieved.

[11] Pearson and Bell, *Biochem. J.*, 1955, **59**, 221.
[12] Nakatsu, *J. Biochem.* (*Japan*), 1959, **46**, 1343.
[13] Nyberg and Christensen, *J. Amer. Chem. Soc.*, 1957, **79**, 1222.
[14] Rinderknecht, *Nature*, 1960, **186**, 1047.
[15] Kitagawa and Takani, *J. Agric. Chem. Soc.* (*Japan*), 1935, **11**, 1077.
[16] Knobler and Frankel, *J.*, 1958, 1629.
[17] Knobler and Frankel, *J.*, 1958, 1632.

Preliminary results were recently obtained in this laboratory on alkylic opening of α-acylamino-γ-butyrolactone by means of oximes, leading to the appropriate canaline derivative:



This considerably improves the preparation of the intermediate (IV).

Experimental

Materials.—γ-Butyrolactone was obtained from Matheson Coleman and Bell, U.S.A. α-Bromo-γ-butyrolactone [18] (85—90%), α-benzamido-γ-butyrolactone [16] (60—65%), and ethyl α-benzamido-γ-bromo- and -γ-iodo-butyrate [16] (yields could be increased to 90%) were prepared as in the references cited.

Ethyl DL-α-*Benzamido-γ-benzamido-oxy-butyrate.*—Benzhydroxamic acid (10·86 g.) in absolute ethanol (70 ml.) was added to a solution of sodium ethoxide in absolute ethanol (from 1·65 g. of sodium in 70 ml.). To the mixture containing some precipitated sodium benzhydroxamate, dry ethyl α-benzamido-γ-iodo- (14·44 g.) or -γ-bromo-butyrate [16] (12·56 g.) (recrystallised from ethanol–water or benzene–light petroleum) in absolute ethanol (70 ml.) was added. Stirring and heating to the b. p. caused dissolution. The solution was stirred at 55—65° for 24 hr., filtered, divided into several portions, and cooled to −5°. Water was added to cloudiness; then an additional 3—4 volumes were added portionwise, with cooling and scratching. After storage overnight at 0°, filtration and washing with cold water gave ethyl α-benzamido-γ-benzamido-oxybutyrate [17] (11·7 g., 80%), m. p. 122—124°.

Variations of this procedure gave less satisfactory yields.

α-N-*Benzoyl*-DL-*canaline Ethyl Ester Hydrochloride* (IV).—Ethyl α-benzamido-γ-benzamido-oxybutyrate (10·8 g.) was refluxed with 15% ethanolic hydrogen chloride (100 ml.) during 4 hr. The solution was cooled and the product was precipitated carefully by addition of dry ether to turbidity and keeping the mixture in the cold overnight. When collected and recrystallised from a small amount of ethanol or ethanol–ether, the *hydrochloride* (IV) (8·7 g., 95%) had m. p. 162—163°, gave a positive Jaffé's test and a negative ninhydrin test, and had $\nu_{max.}$ (in Nujol) 3335s, 2705s, 2040w, 1725vs, 1640vs, 1540s, 1405m, 1337s, 1310m, 1227s, 1190m, 1105m, 1065w, 1040m, 1030m, 917m, 697s, cm.$^{-1}$ (Found: C, 51·5; H, 6·2; N, 9·3; Cl, 11·9; OEt, 14·9.. $C_{13}H_{19}ClN_2O_4$ requires C, 51·6; H, 6·3; N, 9·25; Cl, 11·7; OEt, 14·9%).

α-N-*Benzoyl*-DL-*canavanine Ethyl Ester Hydrochloride* (V).—To a solution of the α-*N*-benzoylcanaline ethyl ester hydrochloride (IV) (6 g.) in absolute ethanol (90 ml.), cyanamide (1·68 g.) was added. The solution was refluxed for 5 hr. The solvent was evaporated to dryness *in vacuo*, then kept in the vacuum at 80—85° for 30 min., and the residue was dissolved in absolute ethanol (20 ml.), cooled, treated with dry ether to turbidity, and kept for 2—3 days at 0°. An oily hydrochloride separated; it gave with sodium nitroprusside, activated by sunlight, a positive test for the guanidino-oxy-group. A second crop (crystalline) was obtained by addition of ether to the filtrate and storage in the cold for 1—2 weeks. Recrystallised from ethanol–ether, the *hydrochloride* (V) (4·8 g., 70%) had m. p. 154—156°, $\nu_{max.}$ (in Nujol) 3225s, 1725m, 1665m, 1640s, 1565s, 1515s, 1300m, 1265w, 1220s, 1190m, 1150m, 1100w, 1020m, 725s, 695s, cm.$^{-1}$ (Found: C, 48·15; H, 6·1; N, 16·3; Cl, 9·7; OEt, 13·05. $C_{14}H_{21}ClN_4O_4$ requires C, 48·8; H, 6·1; N, 16·25; Cl, 10·3; OEt, 13·1%). A *monopicrate*, m. p. 154—156°, was obtained (Found: N, 18·3; OEt, 8·6. $C_{20}H_{28}N_7O_{11}$ requires N, 18·3; OEt, 8·5%).

An attempt, to bring cyanamide into reaction with the free α-*N*-benzoylcanaline ester obtained by use of silver carbonate and hydrogen sulphide led to a less pure product and to lower yield (45%).

Treatment of the hydrochloride (IV) with *S*-methylisothiouronium sulphate [19] resulted in a 10% yield of the hygroscopic *sulphate* of α-*N*-benzoyl-DL-canavanine ethyl ester (Found: C, 47·5; H, 6·1; N, 14·8; S, 4·7. $C_{28}H_{42}N_8O_{12}S$ requires C, 47·05; H, 5·9; N, 15·7; S, 4·5%).

DL-*Canavanine Dihydrochloride* (VI).—The oily ester hydrochloride (V) (3·5 g.) was refluxed

[18] Livak, Britton, VanderWeele, and Murray, *J. Amer. Chem. Soc.*, 1945, **67**, 2218.
[19] Fuller and King, *J.*, 1947, 963.

in 18% hydrochloric acid (60 ml.) for 4 hr. The solution was cooled, and benzoic acid removed by filtration and by three extractions with ether; the mother-liquor was evaporated to dryness *in vacuo* on a water-bath, and the residue was kept in a vacuum at 90° for 30 min. After dissolution in absolute ethanol (20 ml.) and filtration, the product was precipitated with dry ether. The very hygroscopic *dihydrochloride* (2·1 g., 80%) gave a positive ninhydrin test and a positive colour reaction for the guanidino-oxy-group with sodium nitroprusside in sunlight (Found: C, 23·9; H, 6·0; N, 23·0; Cl, 27·0. $C_5H_{14}Cl_2N_4O_3$ requires C, 24·1; H, 5·6; N, 22·5; Cl, 28·5%).

Treatment of the dihydrochloride (VI) (0·25 g.) with triethylamine (0·25 ml.) in ethanol gave *canavanine monohydrochloride*, m. p. 190°, $\nu_{max.}$ (in Nujol) 3450m, 3125s, 2080w, 1690s, 1600s, 1335s, 1300m, 1270m, 1150m, 1110m, 1030s, 980m, 960m, 925m, 855w, 833w, 780m, 725m, cm.$^{-1}$ (Found: C, 28·2; H, 6·4; N, 26·05; Cl, 16·5. $C_5H_{13}ClN_4O_3$ requires C, 28·2; H, 6·1; N, 26·35; Cl, 16·7%).

To a solution of the dihydrochloride (0·5 g.) in water (5 ml.), a solution of flavianic acid (2·5 g.) in water (10 ml.) was added; the yellow precipitate was dissolved by heating, then the hot solution was filtered and cooled. The precipitated yellow *diflavianate* (1·75 g., 100%) melted at 210—220° (Found: C, 36·9; H, 3·2; N, 14·1; S, 8·0. $C_{25}H_{24}N_8O_{19}S_2$ requires C, 37·3; H, 3·0; N, 13·9; S, 8·0%).

On addition of a saturated solution of flavianic acid to a solution of free canavanine a diflavianate was gradually precipitated which had a somewhat higher m. p. (228—230°) (Found: C, 36·8; H, 3·2; N, 14·1%).

DL-*Canavanine* (I).—The dihydrochloride (1·25 g.) was dissolved in absolute ethanol (20 ml.), and triethylamine (2·5 ml.) was added. The white precipitate was dissolved by heating (sometimes with addition of a few drops of water); the solution was kept at room temperature for a few days while crystals of pure canavanine separated. When washed with absolute ethanol and dried at room temperature, the *base* (0·76 g., 78%) melted at 180—182°. A ninhydrin test and a test for the guanidino-oxy-group were positive. The product had R_F (in 80% phenol; ninhydrin) 0·50—0·52 and $\nu_{max.}$ 3510w, 3335s, 3050s, 2130m, 1640—1515vs, 1390s, 1770s, 1312s, 1165m, 1065s, 1015s, 995m, 943m, 926w, 877w, 833m, 775—770s cm.$^{-1}$ (Found: C, 34·5; H, 6·6; N, 31·85. $C_5H_{12}N_4O_3$ requires C, 34·1; H, 6·8; N, 31·8%).

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