325. *Synthesis of* DL-*Canaline and Some of its Derivatives.*

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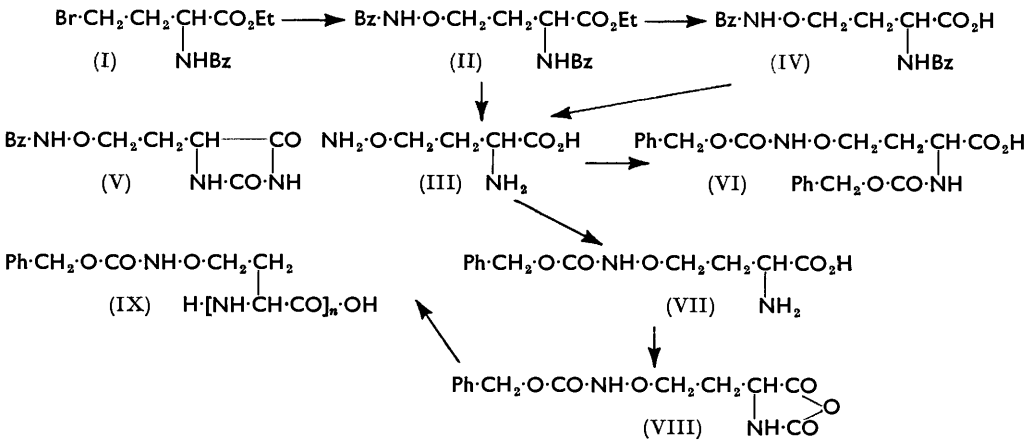
DL-Canaline was prepared in a 5-step synthesis from γ-butyrolactone in 21% overall yield. Conditions for introduction of the amino-oxy-group by means of benzhydroxamic acid and for the liberation of DL-canaline from its picrate and salts were worked out.

α-Benzyloxycarbonylamino-γ-benzyloxycarbonylamino-oxy- and γ-benzyloxycarbonylamino-oxy-derivatives of DL-canaline were prepared. The latter served for the preparation of its α-*N*-carboxy-anhydride which polymerised to a benzyloxylcarbonyl derivative of poly-DL-canaline.

DURING the last few years we have been working out convenient syntheses of canaline,[1] (α-amino-γ-amino-oxybutyric acid), canavanine (the γ-guanidinoxy-derivative), and related substances.

Canaline is produced by enzymic cleavage of canavanine which was detected in jack bean by Kitagawa and his co-workers who also succeeded in clarifying the structure of these two amino-acids.[2] By hydrogenation of the natural L-canaline, α-amino-γ-hydroxybutyric acid was obtained, from an appropriate derivative of which, on introduction of the amino-oxy-group, the L-canaline was finally regenerated. Although this procedure does not imply a practicable synthesis of canaline, the constitution was thus proved.



We report below a synthesis of DL-canaline based on ethyl DL-α-benzamido-γ-bromobutyrate [3] (I). This was condensed with benzhydroxamic acid in absolute ethanol in the presence of sodium ethoxide. The resulting ethyl DL-α-benzamido-γ-benzamido-oxybutyrate (II) (55%) was hydrolysed by dilute hydrochloric acid and DL-canaline (III) was obtained by treatment of the resulting DL-canaline dihydrochloride with triethylamine in aqueous ethanol in 85% yield (21% overall, based on γ-butyrolactone). The introduction of the amino-oxy-group by means of benzhydroxamic acid was also effected under milder conditions, in aqueous-alcoholic alkali or by ethanolic triethylamine.

A derivative with differently masked α-amino- and γ-amino-oxy-groups, the hydantoin (V), was obtained on condensation of benzhydroxamic acid with 5-2′-bromoethylhydantoin.[4]

[1] This work was completed before the publication of a synthesis in 7% overall yield of DL-canaline by Nyberg and Christensen (*J. Amer. Chem. Soc.*, 1957, **79**, 1222); cf. Y. Knobler, Ph.D. Thesis, Jerusalem, 1957; *Bull. Res. Council Israel*, 1957, **6**, 296.

[2] See Kitagawa, *J. Biochem.* (*Japan*), 1937, **25**, 23.

[3] Knobler and Frankel, preceding paper.

[4] Livak, Britton, VanderWeele, and Murray, *J. Amer. Chem. Soc.*, 1945, **67**, 2218.

Alternatively the ester (II) was hydrolysed in aqueous potassium hydroxide to the acid (IV) which was debenzoylated by aqueous sulphuric acid, the acid being then removed as barium salt. However, the DL-canaline thus obtained contained impurities from which it could not be freed conveniently.

We desired to polymerise DL-canaline by the Leuchs anhydride method; first we tried unsuccessfully to obtain the *N*-carboxy-anhydride (VIII) by the action of thionyl chloride on the di(benzyloxycarbonyl) derivative (VI) of DL-canaline. The *N*-carboxy-anhydride (VIII) was however obtained on treatment, with carbonyl chloride, of the derivative (VII) which resulted preferentially from the condensation of DL-canaline with 1 mol. of benzyl chloroformate. Heating the anhydride *in vacuo* yielded the benzyloxycarbonyl derivative (IX) of poly-DL-canaline.

Experimental

M. p.s were determined in a Fisher–Johns apparatus. The ascending method of paper-chromatography was used (80% phenol).

Ethyl DL-α-*Benzamido-γ-benzamido-oxybutyrate* (II).—(a) *By condensation of benzhydroxamic acid with ethyl* DL-α-*benzamido-γ-bromobutyrate* (I). To a solution of sodium ethoxide made from sodium (1·65 g.) and absolute ethanol [dried over $Mg(OEt)_2$] (70 ml.) was added a solution of benzhydroxamic acid (9·9 g.) in absolute ethanol (70 ml.). To the well-stirred suspension of the benzhydroxamic salt thus obtained was added a solution of ethyl α-benzamido-γ-bromobutyrate (I) (12·56 g.) in absolute ethanol (70 ml.). The stirred mixture was kept at 55—65° for 10—12 hr. The solution was cooled, filtered, and cooled at 0°. Water was added gradually to incipient cloudiness, and the mixture kept at 0° for crystallisation, and filtered; and addition of water, etc., was repeated. The crystals were dried (P_2O_5), washed with dry ether, and again dried (P_2O_5 *in vacuo*). The pure *ester* (8·15—8·9 g., 55—60%) had m. p. 122—124° (if ethyl α-benzamido-γ-iodobutyrate [3] was used the yield was only 40—45%) (Found: C, 64·7; H, 6·0; N, 7·6; OEt, 12·2. $C_{20}H_{22}O_5N_2$ requires C, 64·9; H, 6·0; N, 7·6; OEt, 12·2%).

(b) *By condensation of benzhydroxamic acid with ethyl α-benzamido-γ-iodobutyrate.* (i) Ethyl DL-α-benzamido-γ-iodobutyrate [3] (36·1 g.) in ethanol (200 ml.) was added to a mixture of benzhydroxamic acid (20·56 g.) in ethanol (400 ml.) with potassium hydroxide (8·4 g.) in water (50 ml.). Water (350 ml.) was added, most of the precipitated potassium benzhydroxamate dissolving. The mixture was heated with stirring at 40—45° for 18—20 hr., then cooled, and filtered, and the bulk of the ethanol was removed under reduced pressure. An oil separated which partly crystallised. The mixture was cooled to complete precipitation. Water was removed by decantation and the crude product washed with water, warmed at 40—50°, then cooled at 0°. Generally a crude white solid was obtained which, after drying (P_2O_5) and washing with dry ether, was pure enough for further reaction. If a crude soft product was obtained, purification could not be effected solely by washing with dry ether: reprecipitation from aqueous ethanol solution was necessary. Soft material obtained was dissolved in ethanol and precipitated as described under (a). The pure substance (15 g., 40%), had m. p. 122—124° (if ethyl DL-α-benzamido-γ-bromobutyrate was used, the yield was much poorer) (Found: C, 64·6; H, 6·1; N, 7·5; OEt, 12·2%).

(ii) To benzhydroxamic acid (8·9 g.) in absolute ethanol (150 ml.) was added triethylamine (6·5 g.) followed by ethyl DL-α-benzamido-γ-iodobutyrate (18·5 g.) in ethanol (75 ml.). The mixture was warmed at 50—55° and stirred for 15—16 hr., then cooled and filtered, and the filtrate was worked up as described under (a), but the time required for crystallisation was much longer. The ester (II) (8·3 g., 45%) melted at 123—124° (Found: C, 64·7; H, 6·0; N, 7·6; OEt, 12·2%).

DL-α-*Benzamido-γ-benzamido-oxybutyric Acid* (IV).—Ethyl DL-α-benzamido-γ-benzamido-oxybutyrate (II) (11·1 g.) was refluxed for 2 hr. with 0·5% aqueous sodium hydroxide (250 ml.). The solution was cooled, filtered, and acidified with 10% hydrochloric acid to Congo-red. The mixture, from which an oil separated, was warmed, shaken, allowed to cool, and chilled at 0° until the oil became semisolid. The supernatant layer was removed by decantation and the precipitate washed with water. The mother-liquor and washings were concentrated *in vacuo* and a further crop of substance was recovered. The combined crude products were dissolved in alcohol, and a little ether was added, followed by an excess of light petroleum. On cooling, the *acid* (IV) separated, having m. p. 158—160° (9·2—9·8 g., 90—95%) (Found: C, 63·1; H,

5·3; N, 8·1. $C_{18}H_{18}O_5N_2$ requires C, 63·1; H, 5·3; N, 8·2%); it gave negative ninhydrin and Jaffe's tests and hydrolysis with 10% hydrochloric acid liberated DL-canaline and two equivalents of benzoic acid.

DL-*Canaline* (DL-α-*Amino-γ-amino-oxybutyric Acid*) (III).—(a) The γ-benzamido-oxy-ester (II) (11·1 g.) was refluxed with 12% hydrochloric acid (400 ml.) during 4 hr., with frequent shaking. The solution was cooled to 0°, and the precipitated benzoic acid filtered off. The filtrate was extracted with ether, and the aqueous solution concentrated *in vacuo*. The residue was dissolved in water (20 ml.), and again evaporated *in vacuo* to a syrup and kept in a vacuum at 35—40° for a further hour. This procedure was repeated in order completely to remove traces of hydrochloric acid. It was then dissolved with careful warming in 85% ethanol (200 ml.), decolorised with Norite, and filtered. The clear filtrate was warmed in a water-bath to incipient boiling and basified (litmus) with triethylamine. The solution, which on occasions was faintly cloudy, was allowed to cool to room temperature and an excess of absolute ethanol added to incipient precipitation. The mixture was kept at 0° for 2 days during which a white granular precipitate settled. The supernatant liquid was removed by decantation and the precipitate dried (P_2O_5). The crystals were rinsed with absolute ethanol, collected, and kept in a well-stoppered flask. They melted at 175—180° with decomposition. The crude DL-canaline dissolved in ethanol at 70—80° on addition of water; the solution was then filtered through a preheated funnel, absolute ethanol added to cloudiness, and the whole was kept at 0°. The crystals were filtered off and dried (P_2O_5; m. p. 190—195°). The mother-liquor was concentrated to half its volume and to the hot solution were added small amounts of triethylamine and absolute ethanol to cloudiness. Storage at 0° gave a second crop of crystals (total yield, 3·4 g., 85%). The compound gave positive ninhydrin and Jaffe's tests and a red colour with ferric chloride. Paper chromatography gave R_F 0·77 (Found: C, 35·8; H, 7·6; N, 21·0. Calc. for $C_4H_{10}O_3N_2$: C, 35·8; H, 7·5; N, 20·9%).

(b) The acid (IV) (2·5 g.) was refluxed with 10% sulphuric acid (100 ml.) with frequent shaking. The solution was chilled at 0°, freed from benzoic acid by filtration, and extracted with ether. The aqueous solution was diluted, made slightly alkaline with barium hydroxide, filtered, and neutralised with very dilute sulphuric acid. The filtered solution was concentrated *in vacuo*, and the resulting semisolid material dissolved in ethanol and precipitated with light petroleum. The compound (0·9 g., 92%), m. p. 185—200°, gave positive ninhydrin and Jaffe's tests and had R_F 0·77 (Found: C, 35·0; H, 8·0; N, 20·3%).

(c) The ester (II) (11·1 g.) was hydrolysed as described under (a). The syrupy hydrolysate was dissolved in little water and an excess of 5% ethanolic picric acid was added. The solution was heated for 20 min. at near-boiling temperature, filtered through a hot funnel, and kept for 2 days at 0°. The dipicrate (11·0 g., 62%), recrystallised from water, had m. p. 190° (Found: C, 32·3; H, 2·8; N, 19·0. Calc. for $C_{16}H_{16}O_{17}N_8$: C, 32·4; H, 2·7; N, 18·9%).

This dipicrate (5·9 g.) was shaken with 10% sulphuric acid (60 ml.) in a warm-water bath until the liberated canaline dissolved as the hydrogen sulphate, leaving an insoluble precipitate (picric acid). The mixture was cooled and filtered and the dissolved picric acid extracted with ether. DL-Canaline, liberated from the sulphate as described under (b) and purified as described under (a), had m. p. and R_F as above (0·53 g., 40%) (Found: C, 36·0; H, 7·6; N, 21·0%).

5-2′-*Benzamido-oxyethylhydantoin* (V).—To a solution of sodium ethoxide, from sodium (1·5 g.) in absolute ethanol (50 ml.), benzhydroxamic acid (8·9 g.) in absolute ethanol (75 ml.) was added with stirring. A solution of 5-2′-bromoethylhydantoin [4] (10·3 g.) in absolute ethanol (75 ml.) was added and the mixture stirred at 55—60° for 10—12 hr. The solution was then concentrated *in vacuo*, water was added, and the whole extracted continuously with ethyl acetate. The ethyl acetate was removed *in vacuo*, the residue dried (P_2O_5), and the hydantoin (V) (4 g., 30%) was obtained as hygroscopic crystals. The aqueous mother-liquor was evaporated *in vacuo* and the residue extracted with portions of cold absolute ethanol. After evaporation *in vacuo*, repeated treatment with absolute ethanol, and removal of the ethanol a semicrystalline mass was left, which when dried (P_2O_5) yielded a further amount of the *compound* (2·6 g., 20%) (Found: C, 54·0; H, 5·2; N, 16·0. $C_{12}H_{13}O_4N_3$ requires C, 54·7; H, 4·9; N, 15·9%).

DL-α-*Benzyloxycarbonylamino-γ-benzyloxycarbonylamino-oxybutyric Acid* (VI).—To DL-canaline (III) (4 g.) in water (200 ml.) pyridine (40 ml.) was added. To the stirred solution (0°) benzyl chloroformate (12 g.) was added in small portions. Stirring was continued for an

additional hour at 0°, then for another hour at room temperature. The mixture was kept in cold water for several hours until the oil in the emulsion coagulated. The mixture was extracted 7—8 times with ether, and the combined ethereal extracts were dried ($MgSO_4$) and distilled *in vacuo*, to leave a viscous residue of crude di(benzyloxycarbonyl)-DL-canaline. This was dissolved in a large quantity of ether and precipitated with light petroleum as an oil. This was separated and dissolved in ethanol, and the mixture filtered, concentrated *in vacuo*, and cooled. Light petroleum was added, the product separating as an oil. Crystallisation could only be induced by cooling in acetone–carbon dioxide. The *product* was first dried on a porous plate and then in a vacuum over phosphoric oxide, to a glass (8 g., 66%), which gave negative ninhydrin and Jaffe's tests (Found: C, 59·5; H, 5·3; N, 6·9. $C_{20}H_{22}O_7N_2$ requires C, 60·0; H, 5·5; N, 7·0%).

Attempted Preparation of an α-N-*Carboxy-anhydride.*—Di(benzyloxycarbonyl)-DL-canaline (VI) (4 g.) was shaken with freshly distilled thionyl chloride (15 g.) and warmed to 40°. After 0·5 hr., dry light petroleum was added and the mixture warmed to 40—50°, till the layer beneath the petroleum became homogeneous. The petroleum was removed by decantation and replaced with a fresh portion of the same solvent. The mixture was refluxed at 50—55° for 0·5 hr. The bulk of the petroleum was decanted and the remainder removed in a vacuum. The viscous residue was dissolved in dry ethyl acetate and precipitated with dry light petroleum, then heated at 60—70° *in vacuo* during 2 hr. The product gave negative ninhydrin and Jaffe's tests and contained chlorine. No free α-amino-nitrogen was detected by the Van Slyke method. The crude product was heated *in vacuo* for 3 hr. at 80—90°. The residue solidified in the cold. The compound is insoluble in water, ether, and light petroleum; it dissolves easily in acetic acid, less so in ethanol. It was extracted with several portions of ethanol, and the filtrate decolorised with Norite. The filtered solution was concentrated *in vacuo* and the residue dried (P_2O_5). A semisolid product (1·3 g., 34%) remained. According to its analysis it seemed to be the lactam form of the di(benzyloxycarbonyl)-DL-canaline (2-benzyloxycarbonyl-4-benzyloxycarbonylamino-3-oxo*iso*oxazine) (Found: C, 62·4; H, 5·2; N, 7·3. $C_{20}H_{20}O_6N_2$ requires C, 62·5; H, 5·2; N, 7·3%). This cyclisation is analogous to that of αγ-di(benzyloxycarbonylamino)butyric acid.[5]

DL-α-*Amino-γ-benzyloxycarbonylamino-oxybutyric Acid* (VII).—To DL-canaline (III) (5 g.) in water (150 ml.) pyridine (30 ml.) was added. The mixture was stirred at 0° and benzyl chloroformate (6·4 g.) was added during 0·5 hr. The mixture was stirred with cooling for a further hour, then for 1 hr. at room temperature, and kept at 0° for several hours. It was twice extracted with ethyl acetate and the cooled aqueous layer was shaken with little ethanol and much ether. The ethereal layer was kept at 0°; a small quantity of white crystals was obtained. The aqueous layer was kept at 0° for several days, until crystals separated. The *benzyloxycarbonyl derivative* (6·5 g., 65%) of DL-canaline had m. p. 208—210°: it is fairly soluble in water but insoluble in ethanol and ether (Found: C, 53·7; H, 5·7; N, 10·4. $C_{12}H_{16}O_5N_2$ requires C, 53·7; H, 6·0; N, 10·4%).

DL-γ-*Benzyloxycarbonylamino-oxy-*α-*carboxyaminobutyric Anhydride* (VIII).—The acid (VII) (5·4 g.) was dispersed in dry dioxan (75 ml.) at 40—45° and stirred vigorously whilst carbonyl chloride, dried over sulphuric acid, was passed through it for 20—25 min. after complete dissolution. The solvent was removed *in vacuo* at 50—55°, and the residue dissolved in ethyl acetate and precipitated with light petroleum. The supernatant layer was removed by decantation and the process repeated as above. The residual *anhydride* dried (P_2O_5) to a viscous oil (4 g., 68%) [Found: C, 52·2; H, 4·7; N (Van Slyke), 4·8, (Kjeldahl) 9·5. $C_{13}H_{14}O_6N_2$ requires C, 53·0; H, 4·8; N, 4·8, 9·6%].

Poly-(DL-α-*amino-γ-benzyloxycarbonylamino-oxy-butyric Acid*) (IX).—The anhydride (VIII) (2·7 g.) was heated for 3 hr. in a vacuum at 60—90°. Carbon dioxide was evolved, and the material became viscous, finally spongy. The product was yellow-brown, translucent, and insoluble in water and other usual solvents, but dissolved in a great excess of dimethylformamide. Because of its extreme insolubility its chain length could not be determined. A biuret colour was not easily distinguishable owing to the colour of the polymer itself. The *polymer* (1·8 g., 72%) softened above 200° and darkened with decomposition above 250° [Found: C, 57·2; H, 5·8; N, 10·8. $(C_{12}H_{14}O_4N_2)_n$ requires C, 57·6; H, 5·6; N, 11·2%].

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[5] Wilkinson, *J.*, 1951, 104.