SYNTHETIC UTILIZATION OF α-AMINO-γ-BROMOBUTYRIC ACID IN THE PREPARATION OF NON-CODED AMINO ACIDS; IR STUDY OF HOMOSERINE*

Zdenko PROCHÁZKA, Jorga SMOLÍKOVÁ, Petr MALOŇ and Karel JOŠT

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6

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α-Amino-γ-bromobutyric acid and its derivatives were utilized in the preparation of some non-coded (non-proteinogenic) amino acids and their derivatives. The bromine atom was replaced by hydrogen, hydroxyl, iodine and nitro, amino, and thiocyanato groups. Homolanthionine was prepared by two methods. Homoserine has been shown to exist in three crystalline modifications whose differences in the IR spectra indicate conformational changes.

Derivatives of α-amino-γ-bromobutyric acid** (ref.[2]) can be useful starting compounds for preparation of many non-proteinogenic amino acids and their derivatives. Methyl N^{α}-benzyloxycarbonylamino-γ-bromobutyrate[2] (*I*) was a key intermediate in the preparation of cystathionine derivatives[2–4], suitable for the synthesis of the so-called carba-analogues. The atom of bromine can be replaced by other functional groups in a nucleophilic substitution. Thus, action of potassium cyanide afforded a γ-cyanobutyric acid derivative[5] and reaction with benzyl selenol gave Se-benzylselenohomocysteine which was transformed into selenomethionine or selenoethionine[6]. Treatment with sodium salts of C-acids, sodium ethoxide, or Wurtz synthesis, lead to 1-amino-cyclopropanecarboxylic acid[7].

In the present communication we describe the substitution of bromine in α-amino-γ-bromobutyric acid derivatives by iodine, hydrogen, nitro group, hydroxyl and thiocyanato group. Finkelstein reaction (treatment with sodium iodide in acetone) with methyl N^{α}-benzyloxycarbonylamino-γ-bromobutyrate[2] (*I*) afforded the corresponding fully protected derivative of γ-iodobutyric acid *IV*. Complete substitution of bromine was achieved only after repeated conversion.

Reaction of sodium nitrite in dimethylformamide in the presence of urea (in order to increase the solubility of sodium nitrite) yielded methyl N^{α}-benzyloxycarbonyl-

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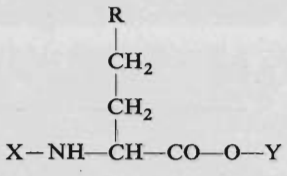
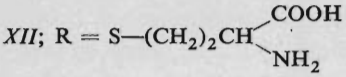
** The amino acids used in this paper are of the L-series. The nomenclature and symbols obey the published recommendations[1].

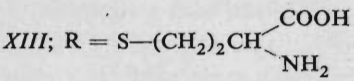
amino-γ-nitrobutyric acid (*V*). Substantially higher yields were achieved when a derivative of α-amino-γ-iodobutyric acid instead of α-amino-γ-bromobutyric acid was used as starting compound. The reaction mixture which was separated by chromatography on silica gel invariably contained the starting compound *I* and a side-product, N^{α}-benzyloxycarbonylaminobutyrolactone. (After the reaction, the starting compound *I* which was a mixture of derivatives of γ-bromobutyric and γ-chlorobutyric acids, was again enriched in the second component[7].) Butyrolactone was formed to a greater or lesser extent in all reactions of α-amino-γ-bromobutyric acid derivatives: derivatives containing free α-amino group afforded α-aminobutyrolactone. After saponification, the derivative *V* was converted into dicyclohexylammonium salt of N^{α}-benzyloxycarbonylamino-γ-nitrobutyric acid (*VI*) which was transformed into free α-amino-γ-nitrobutyric acid (*VII*) and also hydrogenolyzed over palladium to give α,γ-diaminobutyric acid (*VIII*).



I; R = Br, X = Z, Y = CH_3
II; R = Br, X = HCl.H, Y = CH_3
III; R = Br, X = HBr.H, Y = H
IV; R = I, X = Z, Y = CH_3
V; R = NO_2, X = Z, Y = CH_3
VI; R = NO_2, X = Z, Y = H.DCHA
VII; R = NO_2, X = H, Y = H
VIII; R = NH_2, X = H, Y = H
IX, R = H, X = H, Y = H
X; R = OH, X = H, Y = H
XI; R = SCN, X = Z, Y = CH_3



XII; X = Z, Y = CH_3



XIII; X = H, Y = H

Reductive removal of the bromine atom afforded α-aminobutyric acid (*IX*). The reduction of α-amino-γ-bromobutyric acid[2] (*III*) was accomplished by zinc in acetic acid or, better, by hydrogenolysis over palladium black, whereas the reduction with zinc in hydrochloric acid was not successful. Substantial amount of the arising α-aminobutyrolactone was separated on an ion exchange resin. On reaction with sodium hydroxide the derivative *III* was transformed into a mixture of α-amino-γ-hydroxy-butyric acid (homoserine, *X*) and the lactone.

Reaction of potassium thiocyanate with the derivative *I* gave methyl N^{α}-benzyloxycarbonylamino-γ-thiocyanatobutyrate (*XI*) which was isolated from the reaction mixture by column chromatography on silica gel. Alkaline hydrolysis of the compound *XI* destroyed the thiocyanato group (as evidenced by disappearance of the SCN band at 2 165 cm^{-1} in the IR spectrum) and afforded, probably *via* the thiol, a derivative of homocystine; this was proved after acid hydrolysis of the intermediate by comparison with an authentic sample on an amino acid analyzer.

The alkylation of sodium sulfide with α-amino-γ-bromobutyric acid derivatives depended significantly on the nature of the derivative and on reaction conditions. With derivatives containing an unprotected amino group (*i.e.* α-amino-γ-bromobutyric acid hydrobromide (*III*) or methyl α-amino-γ-bromobutyrate hydrochloride (*II*, ref.[2])) the reaction course was far from unequivocal. When the derivative *I* and half equivalent of sodium sulfide were used, the fully protected homolanthionine was prepared which after acid hydrolysis and treatment with ion exchange resin afforded free homolanthionine (*XIII*); this, however, was invariably contaminated with homoserine and homocystine. Pure homolanthionine was obtained in higher yield by the following procedure. Homocystine was reduced with sodium in liquid ammonia and the product *in situ* alkylated at the sulfur atom with methyl N^{α}-benzyloxycarbonylamino-γ-bromobutyrate (*I*). The obtained partially protected derivative of homolanthionine *XII* afforded free homolanthionine on acid hydrolysis.

The CD spectra of α-amino-γ-nitrobutyric acid (*VII*) (Table I) exhibit a positive band in the region of the $n-\pi^*$ transition of carbonyl group (200 – 220 nm). On going from neutral to alkaline medium, this band displays a characteristic bathochromic shift (10 nm), accompanied by a significant intensity decrease[8]. Molar ellipticities of this maximum in acidic and alkaline media correspond approximately to the values published for norvaline[8]; we can thus assume that, at least in these media, the nitro chromophore contributes only negligibly to the dichroic absorption of the positive band. However, in a neutral buffer the intensity is about twice as large as that

TABLE I
CD Spectra of α-amino-γ-nitrobutyric acid (*VII*)

Solvent	$\lambda_{max}{}^{a}([\Theta]_{max}{}^{b})$	$\lambda_{max}([\Theta]_{max})$
0·01M-HCl	246 (— 100)	205 (+4 200)
Phosphate buffer pH 7·5	234 (—2 400)	205 (+6 300)
0·01M-NaOH	253 (— 480)	215 (+1 700)

[a] λ_{max} wavelength of the apparent maximum, nm; [b] $[\Theta]_{max}$ molar elipticity, deg cm^2 $dmol^{-1}$.

of norvaline. The positive sign of the band agrees with that of L-amino acid. The long-wavelength, relatively broad, negative band of lower intensity, whose dichroic

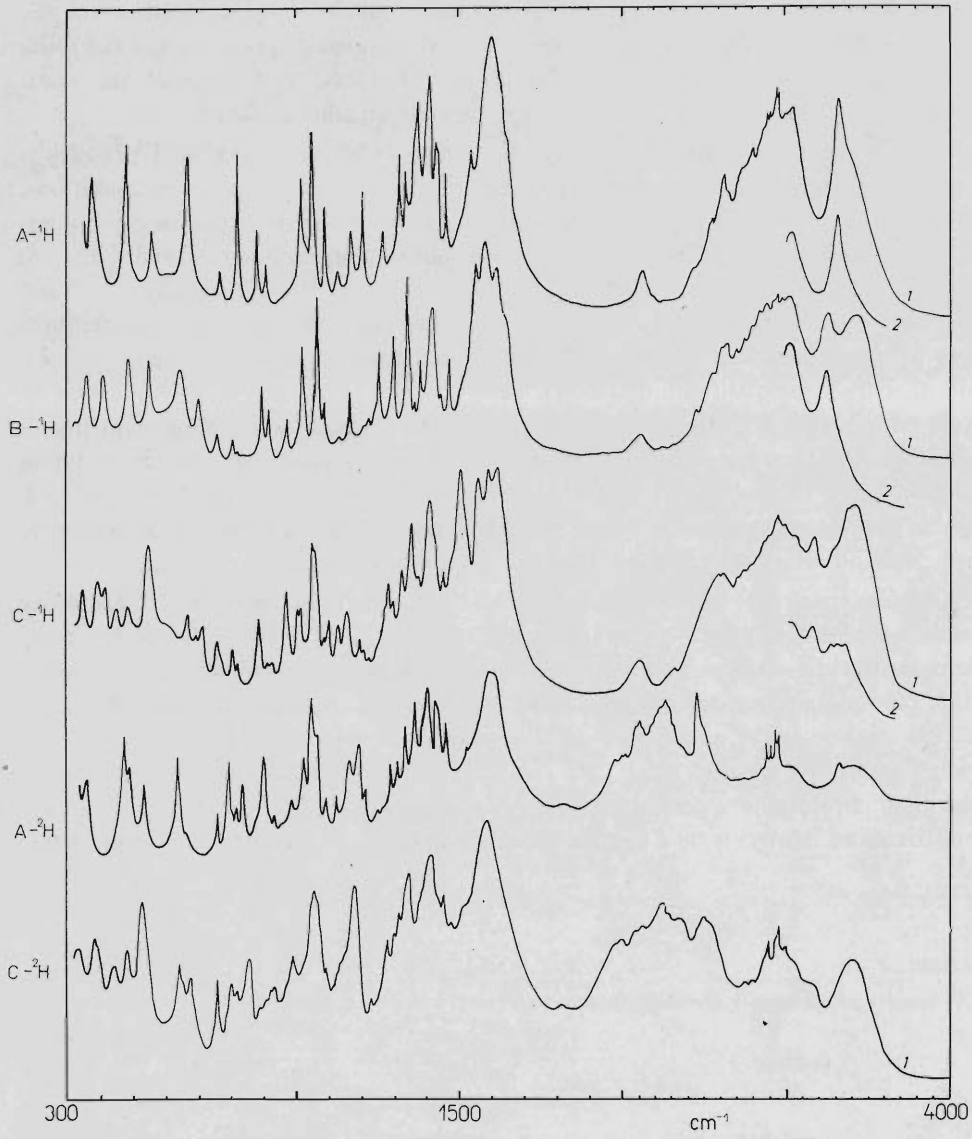


FIG. 1

IR spectra of the A-^{1}H, B-^{1}H and C-^{1}H forms of homoserine, $^{(+)}NH_3CH(CH_2CH_2OH)COO^-$, and the A-^{2}H and C-^{2}H forms of deuteriated homoserine, $^{(+)}(^2H_3)NCH(CH_2CH_2O^2H)COO^{(-)}$, *1* in KBr pellet, *2* in Nujol mull

absorption begins at 270–280 nm and whose apparent maximum ranges between 234 and 253 nm according to pH of the solution, could correspond to the nitro group $n-\pi^*$ transition. In such a case, however, its dichroic absorption maximum would be shifted hypsochromically in comparison with values published for other optically active compounds, containing nitro chromophore (280 nm in nitrosteroides; see ref.[9]). The band intensity is highest in neutral buffer. It is probable that the mentioned intensity increase is related with the intensity increase of the band due to the carboxyl $n-\pi^*$ transition as a result of mutual interaction.

During the study of IR spectra of homoserine (*X*) we have found that in some instances its spectrum agreed with the published one[10], whereas in other cases it was different, although the compound was beyond doubt identical, as evidenced by its rotation, and elemental as well as amino acid analysis. We observed thus three types of completely different spectra of crystalline homoserine (designated as A-^{1}H, B-^{1}H and C-^{1}H in Fig. 1) and further the spectrum of the amorphous state. The spectrum of B-^{1}H was identical with that already published.

All the crystalline modifications are stable if protected from moisture and do not change even after 6 months. When exposed to moisture, the types B-^{1}H and C-^{1}H are transformed into the type A-^{1}H, the type C-^{1}H much easier than B-^{1}H. During this transformation it is possible to observe the mixtures of both forms (B-^{1}H + + A-^{1}H or C-^{1}H + A-^{1}H). However, neither of these forms is a hydrate, as proved analytically as well as by the temperature dependence of the spectra, and water acts evidently only as a catalyst. On heating, the spectrum of the type A-^{1}H does not change below 145°C. Above this temperature, the spectrum begins to change into that of the C-^{1}H type, the transformation being complete at 157°C. During this transition the crystals undergo no visible change. The type C-^{1}H also can be prepared by crystallization of homoserine from water or by freeze-drying of its aqueous solution. The residue which exhibits a spectrum of the amorphous state is transformed during a short time interval into the type C-^{1}H, even directly in a KBr pellet. The same behaviour was observed with the B-^{1}H type which in the same temperature range was transformed into the type C-^{1}H.

All the spectra of homoserine reflect its zwitterionic structure. Largest differences were found in the region of OH stretching vibrations. The spectra of the three crystalline forms are so different that it is unlikely that the difference is due to three crystalline modifications, differing only in the arrangement in the unit cell[11,12]. The spectrum of the form C-^{1}H is far more complicated than spectra of the forms A-^{1}H and B-^{1}H.

In order to gain some information about the conformation of these crystalline types, we deuterated homoserine. We were able to obtain the relatively pure forms A-^{2}H and C-^{2}H (Fig. 1). All attempts to prepare the B-^{2}H form resulted in mixtures B-^{1}H + A-^{2}H, B-^{1}H + C-^{2}H or B-^{1}H with a small amount of B-^{2}H. The isotope exchange was accompanied by change of wavenumbers and intensities of most of the bands (Fig. 1). This change is more marked with the crystalline type A than

with the type C which is in accord with a different molecular geometry of these two types[13]. However, wavenumbers and intensities of some bands remained unchanged on deuteration or changed very little (shifts smaller than 10 cm^{-1}).

In the C-H stretching vibration region the spectral shape, typical for each of the crystalline forms, remained unchanged (A-^{1}H and A-^{2}H: 2 960 cm^{-1}, 2 945 cm^{-1}, 2 915 cm^{-1}, 2 890 cm^{-1}; for C-^{1}H and C-^{2}H: 2 950 cm^{-1}, 2 890 cm^{-1}); since deuteration improved the band resolution it was possible to observe some additional weak bands. The bands in the region 2 000–350 cm^{-1}, due to C-H deformation vibrations, skeletal and COO^- vibrations (ref.[13–19]), are listed in Table II. The first two vibration types can afford information about molecular conformation.

The large differences in wavenumbers and intensities of these deuteration-insensitive bands in the spectra of A and C are not likely to be due to a different strength of hydrogen bonds (in case of contribution of COO^- vibrations to some of the

TABLE II

Deuteration-insensitive bands in the region 2 000—350 cm^{-1} (in KBr pellets)

Type A			Type C		
wavenumber cm^{-1}	intensity[a]	$\Delta\nu^b$ cm^{-1}	wavenumber cm^{-1}	intensity[a]	$\Delta\nu^b$ cm^{-1}
1 465	m	0	1 478	sh	—3
1 439	m	0	1 450	w	0
1 410	s	—2	1 412	s	—1
1 374	m-s	—5	1 375	vw	+5
1 340	m	0	1 352	m-s	—7
1 172	w	—3	1 298	vw	0
1 130	w	—2	1 272	vw	+3
1 059	sh	+3	1 230	vw	0
1 050	s	+2	1 087	vw	+3
769	w	—8	1 059	s	0
481	m-s	+9	1 051	s	0
		—7	931	vw	0
			920	vw	0
			818	vw	+3
			809	w	—8
			760	w	—4
			446	w	—6
			349	w	—4

[a] Estimate for bands whose intensity did not change upon deuteration: s strong, m medium, w weak, vw very weak, sh shoulder; [b] shift of the given band on deuteration.

mentioned bands) or an analogous change in geometry, such as in case of the α-, β- and γ-forms of glycine[11,13]. Particularly the changes in the band shifts in the region 1 350–900 cm^{-1} indicate rather a conformational change on transition from one crystalline form into another one[16–19]. The complicated spectrum of the form C indicates that its unit cell contains obviously molecules of non-identical conformation[20,21].

Thus, it seems that in the case of homoserine we are dealing with a phenomenon, already described in the literature[12,18,22], *i.e.* a change of crystalline modification which is accompanied by a change of molecular conformation.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Analytical samples were dried for 24 h at room temperature and 150 Pa. Thin-layer chromatography was performed on silica gel-coated Silufol plates (Kavalier, Czechoslovakia) in the following systems: 2-butanol–98% formic acid–water (75 : 13·5 : 11·5) (S1), 2-butanol–ammonia–water (85 : 7·5 : 7·5) (S2), 1-butanol–acetic acid–water (4 : 1 : 1) (S3), 1-butanol–pyridine–acetic acid–water (15 : 10 : 3 : 6) (S4), n-heptane–tert-butyl alcohol–pyridine (5 : 1 : 1) (S5), 5% methanol in benzene (S8), 20% methanol in benzene (S9), 1-butanol–water–acetic acid (50 : 40 : 15) (S13), 2% methanol in benzene (S14), n-heptane–tert-butyl alcohol (3 : 1) (S16), 2-butanol–water (9 : 1) (S17). Paper electrophoresis was carried out in a moist chamber in 1M-acetic acid (pH 2·4) and in a pyridine–acetate buffer (pH 5·7) on Whatman 3MM paper; 20 V/cm, 60 min. Spots in the thin-layer and electrophoretical experiments were detected by ninhydrin reaction or chlorination method. The reaction mixtures were taken down on a rotatory evaporator at bath temperature 30—40°C under reduced pressure (water pump), dimethylformamide-containing mixtures at 150 Pa. Amino acid analyses were performed on an automatic two-column analyzer type 6020 (Development Workshops, Czechoslovak Academy of Sciences). Optical rotations were determined on a Perkin–Elmer instrument, type 141 MCA (Perkin–Elmer Corporation, Norwalk, USA). Column chromatography was performed on silica gel (particle size 30—60 μ). Anhydrous sodium sulfide was prepared by drying the crystalline nonahydrate over phosphorus pentoxide in a desiccator for at least 14 days and did not contain more than 0·5 equivalent of water.

Methyl N^{α}-Benzyloxycarbonylamino-γ-iodobutyrate (*IV*)

A solution of the compound *I* (ref.[2]; 2·0 g) and sodium iodide (1·2 g) in acetone (8 ml) was stirred at room temperature for 24 h. The separated sodium bromide was filtered, the filtrate taken down, and the residue partitioned between ether and water. The ethereal solution was washed with a 5% sodium thiosulfate solution and with water, dried over sodium sulfate and taken down. The residue was crystallized from benzene and light petroleum at 0°C, affording 1·9 g (84%) of the product; calculated: 33·64% I; found: 29·13% I. The product was dissolved in acetone (8 ml) and the whole procedure with sodium iodide was repeated, affording 1·55 g (68%) of the desired compound, m.p. 74—75°C; $[\alpha]_D$ —34·4° (*c* 0·5; dimethylformamide). R_F 0·43 (S5), 0·38 (S14), 0·65 (S8). For $C_{13}H_{16}INO_4$ (377·2) calculated: 41·40% C, 4·28% H, 3·71% N, 33·64% I; found: 41·75% C, 4·39% H, 3·90% N, 32·70% I. IR spectrum (KBr), cm^{-1}: 3 310 (vs), 1 693 (s), 1 545 (s), (O—CO—NH); 698 (m) ($C_6H_5CH_2$); 1 741 (s), 1 438 (m), 1 299 (s) ($COOCH_3$); in $CHCl_3$ 3 435 (m), 3 340 (w), 1 725 (vs), 1 712 (sh), 1 515 (s), 1 506 (sh), (O—CO—NH); 702 (m) ($C_6H_5CH_2$); 1 741 (sh), 1 440 (m), 1 238 (s), ($COOCH_3$). In further preparations

the product was not isolated and after filtration of the separated sodium bromide the second portion of sodium iodide was added.

Methyl N^{α}-Benzyloxycarbonylamino-γ-nitrobutyrate (*V*)

a) *From the derivative of γ-bromobutyric acid*: Sodium nitrite (3·4 g) and urea (1·9 g) were added to a solution of compound *I* (ref.[2]; 5·3 g) in dimethylformamide (30 ml) and the mixture was stirred for 24 h at room temperature. The solvent was removed *in vacuo* and the oily residue partitioned between ether and water. The product was taken up in ether and the ethereal extract washed with water, dried over sodium sulfate and taken to dryness. The residue was purified by chromatography on a column of silica gel (3 × 33 cm; eluant n-heptane–tert-butyl alcohol 3 : 1). The eluted material was detected by thin-layer chromatography (system S5). Fractions 180—300 ml contained the starting compound *I* and N^{α}-benzyloxycarbonylaminobutyrolactone (R_F 0·43 and 0·18, respectively, in S5), fractions 355—455 ml contained the desired product. In further fractions we found an unidentified product. Evaporation of the pertinent fractions gave 0·6 g (13%) of the chromatographically pure oily product; R_F 0·28 (S5), 0·35 (S16). The analytically pure product was obtained by crystallization from a mixture of tert-butyl alcohol–n-heptane–ether; m.p. 42—44°C; $[\alpha]_D$ —30·8° (*c* 0·2; methanol). For $C_{13}H_{16}N_2O_6$ (296·3) calculated: 52·70% C, 5·44% H, 9·45% N; found: 53·00% C, 5·58% H, 9·36% N. IR spectrum, cm^{-1}: 3 430 (m), 1 725 (vs), 1 713 (sh), 1 515 (s), 1 505 (sh) (O—CO—NH); 703 (w), (C_6H_5·.CH_2); 1 745 (sh), 1 440 (m), 1 240 (s) ($COOCH_3$); 1 565 (s), 1 556 (sh), 1 330 (m) (NO_2).

b) *From the derivative of γ-iodobutyric acid*: Sodium nitrite (0·56 g) and urea (0·48 g) were added to a solution of compound *IV* (1·52 g) in dimethylformamide (10 ml). The reaction mixture was worked up in an analogous manner as described for the procedure *a*); in addition, the ethereal extracts were washed also with a sodium thiosulfate solution. Yield 0·36 g (31%) of the oily product, chromatographically identical with the compound prepared under *a*).

Dicyclohexylammonium N^{α}-Benzyloxycarbonylamino-γ-nitrobutyrate (*VI*)

A 1M-NaOH solution (4 ml) was added to a solution of the compound *V* (0·6 g) in methanol (10 ml). After stirring for 1 h at room temperature, Dowex 50 W (30 ml; H^+-form) was added and the mixture was stirred for 30 min. The ion exchange resin was filtered off, washed with methanol and the filtrate was taken down and dried azeotropically (benzene). The residue was dissolved in methanol and dicyclohexylamine (0·4 ml) was added. The solvent was evaporated and the residue triturated with ether. The crystalline material was collected on filter, washed with ether and dried, yielding 0·31 g (30%) of the product, m.p. 148—152°C, which on crystallization from benzene and light petroleum melted at 150—153°C; yield 0·30 g (29%). $[\alpha]_D$ +5·8° (*c* 0·26; dimethylformamide). R_F 0.79 (S1), 0·30 (S2), 0·56 (S4), 0·03 (S5), 0·73 (methanol). For $C_{24}H_{37}N_3O_6$ (463·6) calculated: 62·18% C, 8·04% H, 9·06% N; found: 62·04% C, 8·03% H, 8·74% N.

α-Amino-γ-nitrobutyric Acid (*VII*)

Dowex 50W (6 ml, H^+-form) and water (6 ml) were added to a solution of the derivative *VI* (0·3 g) in ethanol (20 ml). After stirring for 30 min, the ion exchange resin was filtered off, washed with ethanol and the filtrates were taken down and azeotropically dried (benzene). A 35% solution of HBr in acetic acid (0·4 ml) was added to the residue and after 10 min the reaction mixture was diluted with ether. After standing in a refrigerator, the precipitate was separated by centri-

fugation, stirred several times with ether with subsequent decantation of the supernatant. The material was crystallized from methanol–ether, affording 100 mg (67%) of the hydrobromide, m.p. 131—133°C. For $C_4H_8BrN_2O_4$ (229·0) calculated: 20·98% C, 3·96% H, 12·23% N, 34·89% Br; found: 21·01% C, 3·87% H, 12·14% N, 34·16% Br. The free base was liberated using Dowex 50W (5 ml column, H^+-form), eluted with 10% aqueous pyridine and after evaporation of the solvent crystallized from water and ethanol. The obtained product decomposed above 200°C, $[\alpha]_D$ +35·8° (*c* 0·17; 1M-HCl). $E_{2.4}^{Gly}$ 0·59, $E_{5.7}^{His}$ 0·00; R_F 0·35 (S13). On an amino acid analyzer, its elution time was the same as that of Ser (buffer pH 3·3, for conditions see ref.[23]), the colour yield of the ninhydrin reaction, related to Leu, was 0·158. For $C_4H_8N_2O_4$ (148·1) calculated: 32·44% C, 5·44% H, 18·91% N; found 33·07% C, 5·47% H, 18·73% N. IR spectrum in KBr (cm^{-1}): 3 055 (sh), 3 020 (s), 2 960 (s), 2 925 (s), 2 855 (sh), 2 755 (m), 2 620 (m), 2 425 (w), 2 105 (w), 1 615 (s), 1 588 (s), 1 564 (vs), 1 509 (m), 1 440 (w), 1 420 (m), 1 381 (w), 1 359 (m), 1 320 (w), 1 310 (w), 1 280 (w), 1 223 (sh), 1 214 (w), 1 174 (sh), 1 165 (w), 1 139 (ww), 1 099 (w), 1 047 (sh), 1 040 (w), 1 015 (w), 968 (sh), 960 (w), 913 (w), 880 (w), 825 (m), 772 (w), 627 (sh), 610 (w), 509 (m), 495 (sh), 388 (w), 378 (sh).

α,γ-Diaminobutyric Acid (*VIII*)

The derivative *VI* (0·14 g) in ethyl acetate was mixed with 0·5M-H_2SO_4, the organic layer containing the liberated acid was taken down and the residue was dissolved in methanol containing few drops of water and acetic acid. The solution was hydrogenated over Pd black (from 1 g of $PdCl_2$) for 3 h. The catalyst was filtered off and the filtrate taken to dryness; $E_{2.4}^{Gly}$ 1·62, $E_{5.7}^{His}$ 1·32, R_F 0·15 (S13). The product was pure according to the amino acid analysis and its chromatographic behaviour was identical with that of an authentic sample. 3-Naphthalene sulfonate, m.p. 214—216°C (after two crystallizations from water); reported[24] m.p. 215°C.

α-Aminobutyric Acid (*IX*)

a) *By hydrogenolysis.* The compound *III* (ref.[2]; 1·3 g) was hydrogenated in methanol (40 ml) over Pd-black (from 1 g of $PdCl_2$); the pH of the solution was maintained at about 8·0 by addition of 1M-NaOH (total amount about 10 ml). After 4 h (the hydrogen consumption had already ceased) the catalyst was filtered off and the filtrate was taken down at room temperature. The reaction mixture contained two products, $E_{2.4}^{Gly}$ 2·17 and 0·83, $E_{5.7}^{His}$ 1·28 and 0·00, which are probably α-aminobutyrolactone and α-aminobutyric acid. The residue, dissolved in several ml of water, was applied onto a column of Dowex 50W (H^+-form, 60 ml). After washing with water, the product was eluted with 10% aqueous pyridine. From the beginning of appearance of the product in the eluate (schlieren), 100 ml of the eluate were collected. After evaporation of the solvent, the residue was crystallized from water and ethanol, affording 0·24 g (48%) of the product, m.p. 236—238°C; $[\alpha]_D$ +41·3° (*c* 0·49, acetic acid). Reported[10] $[\alpha]_D$ +43·3° (*c* 1·0, acetic acid). $E_{2.4}^{Gly}$ 0·83, $E_{5.7}^{His}$ 0·00. For $C_4H_9NO_2$ (103·1) calculated: 46·60% C, 8·80% H, 13·59% N; found: 46·37% C, 8·76% H, 13·36% N. The identity of the product with α-aminobutyric acid was also proved by comparison with the published IR spectrum[10]. When the experiment was performed in anhydrous methanol in the absence of base, the hydrogenation was very sluggish (72 h); although the product was obtained in a higher yield, its rotation was lower.

b) *With zinc in an acidic medium.* Zinc powder (0·80 g) was added in portions to a hot solution of compound III (ref.[2]; 0·79 g) in 6M-HCl (6 ml). The reaction mixture was boiled for 5 min, filtered and the filtrate applied on a column of Dowex 50W (H^+-form, 25 ml). After washing with water the product was eluted with 10% aqueous pyridine (40 ml of the eluate were taken after appearance of the product). The eluate was taken down and the residue crystallized from

aqueous ethanol, affording 40 mg (8%) of a product which had the same electrophoretic mobility as the compound obtained under *a*) but exhibited a lower rotation $[\alpha]_D$ +27·0° (*c* 0·50, acetic acid). When 99% acetic acid (10 ml) was used instead of 6M-HCl, the same procedure afforded 61 mg (12%) of product of the same electrophoretical mobility; $[\alpha]_D^{25}$ +39·3° (*c* 0·51; acetic acid). The reduction with zinc powder was in both cases accompanied with a large amount of a basic product, probably α-aminobutyrolactone which was eluted from the ion exchange resin more slowly than the product and was thus separated.

α-Amino-γ-hydroxybutyric Acid (Homoserine) (*X*)

A solution of compound *III* (ref.[2]; 1·3 g) in methanol (10 ml) and 1M-NaOH (12 ml) was stirred for 3 h at room temperature and then applied on a column of Dowex 50W (H^+-form, 90 ml). After washing with water, the product was eluted with 10% aqueous pyridine (200 ml after appearance of the product in the eluate), the eluate was taken down and the residue crystallized from water and ethanol, affording 0·3 g (26%) of the product; $E_{2.4}^{Gly}$ 0·77, $E_{5.7}^{His}$ 0·00, $[\alpha]_D$ +20·4° (*c* 0·5, 1M-HCl), $[\alpha]_D$ −8·4° (*c* 0·5, water). Reference[10] reports $[\alpha]_D$ +18·3° (2M-HCl) and −8·8° (water). For $C_4H_9NO_3$ (119·1) calculated: 40·33% C, 7·62% H, 11·76% N; found: 40·24% C, 7·45% H; 11·96% N. The identity with homoserine was also proved by analysis of a mixture with an authentic material in the amino acid analyzer.

Methyl N^{α}-Benzyloxycarbonylamino-γ-thiocyanatobutyrate (*XI*)

Potassium thiocyanate (0·6 g) was added to a solution of compound *I* (ref.[2]) (1·0 g) in dimethylformamide (4 ml). After stirring for 24 h at room temperature, the mixture was taken down, the residue partitioned between ether and water, and the product was taken up in ether. The ethereal extract was washed with water, dried over sodium sulfate and taken down. The residue was purified by chromatography on a silica gel column (2 × 16 cm, eluant 2% methanol in benzene). The eluted material was detected by thin-layer chromatography (S14). The desired product was present in fractions 120—170 ml. Evaporation afforded 0·20 g (22%) of an oily residue, $[\alpha]_D$ −36·7° (*c* 0·3, methanol); R_F 0·32 (S5), 0·49 (S8), 0·24 (S14); for $C_{14}H_{16}N_2O_4S$ (308·4) calculated: 54·53% C, 5·23% H, 9·08% N; found 54·81% C, 5·19% H, 8·85% N. IR spectrum, cm^{-1}: 3 435 (m), 3 340 (w), 1 725 (vs), 1 712 (sh), 1 515 (s), 1 505 (sh) (O—CO—NH); 705 (w), $C_6H_5CH_2$); 1 743 (sh), 1 440 (m), 1 240 (s), ($COOCH_3$); 2 165 (m) (SCN).

α-Methyl N^{α}-Benzyloxycarbonylhomolanthionate (*XII*)

Homocystine[25] (0·48 g) was reduced with sodium in liquid ammonia (100 ml). The derivative *I* (ref.[2]; 2·0 g) was added, the solution was stirred for 5 min and freeze-dried in vacuum of a water pump. The residue was dissolved in 0·1M-HCl, the solution washed with ether, evacuated and filtered through a column of Dowex 50W (H^+-form; 30 ml). The product was eluted with 10% aqueous pyridine, the eluate acidified to pH 4—5 with acetic acid and freeze-dried. The residue was dissolved in 20 ml of the lower phase of the system butanol–benzene–pyridine–0·1% acetic acid (6 : 2 : 1 : 9) and applied to the first two tubes of the countercurrent distribution machine (an all glass apparatus made in the glass-blowing workshop of this Institute; 20 tubes for 10 ml of the upper and 10 ml of the lower phase). After 18 transfers of the upper phase, the content of the tubes was acidified with acetic acid (1 ml) and detected by paper electrophoresis. The content of the tubes 12—20 was combined, concentrated and freeze-dried. The residue was crystallized from a mixture of water, methanol and ether, affording 0·7 g (46%) of the product, m.p. 182·5—183°C. $[\alpha]_D$ −7·2° (*c* 0·2; water); $E_{2.4}^{Gly}$ 0·43; $E_{5.7}^{His}$ 0·00; R_F 0·35 (S1), 0·07 (S2), 0·27

(S3), 0·54 (S4), 0·07 (S9), 0·24 (S17), 0·52 (methanol). For $C_{17}H_{24}N_2O_6S$ (384·4) calculated: 53·11% C, 6·29% H, 7·29% N, 8·34% S; found: 53·07% C, 6·25% H, 7·18% N, 7·91% S.

Homolanthionine (*XIII*)

a) The compound *XII* (300 mg) was refluxed with azeotropic hydrochloric acid (15 ml) for 1 h and the solution was shaken with ether and taken down. The excess of the hydrochloric acid was removed by a repeated evaporation with water. The residue was dissolved in a small amount of water and applied on a column of Dowex 50W (H^+-form; 10 ml). After washing with water, the product was eluted with 10% aqueous pyridine. Evaporation of solvent and crystallization from water and ethanol afforded 120 mg (65%) of the product. $E_{2.4}^{Gly}$ 0·75, $E_{5.7}^{His}$ 0·00; R_F 0·22 (S13). On an amino acid analyzer the elution time was the same as that of Ile (buffer pH 4·3, for conditions see ref.[23]). $[\alpha]_D$ +37·5° (*c* 0·2; 1M-HCl); ref.[12] reports +37·3° (1N-HCl). For $C_8H_{16}N_2O_4S$ (236·3) calculated: 40·67% C, 6·83% H, 11·86% N; found: 40·55% C, 6·94% H, 11·52% N. IR spectrum (KBr), cm^{-1}: 3 040 (sh), 2 990 (s), 2 945 (s), 2 925 (s), 2 860 (m), 2 740 (m), 2 620 (m), 2 490 (sh), 2 305 (vw), 2 125 (w), 1 607 (sh), 1 586 (vs), 1 515 (s), 1 449 (sh), 1 441 (w), 1 404 (s), 1 349 (m), 1 339 (m), 1 325 (m), 1 272 (w), 1 241 (w), 1 202 (vw), 1 191 (w), 1 151 (w), 1 065 (vw), 1 031 (vw), 1 018 (vw), 980 (w), 958 (vw), 925 (vw), 895 (vw), 872 (w), 843 (vw), 835 (sh), 768 (w), 746 (sh), 697 (vw), 658 (w), 544 (m), 455 (w), 430 (sh), 413 (w), 360 (w).

b) Anhydrous sodium sulfide (0·27 g) was added to a solution of the compound *I* (ref.[2], 2·0 g) in dimethylformamide (15 ml) under nitrogen. After stirring for 24 h at room temperature, the mixture was taken down, the residue partitioned between water and ether, and the product was taken up in ether. The ethereal solution was washed with water, dried over sodium sulfate and taken down. The residue appeared to be a mixture of two compounds: the starting compound *I* (R_F 0·43 (S5), 0·65 (S8)) and a compound (R_F 0·25 (S5), 0·34 (S8)) which corresponded to the fully protected homolanthionine derivative. In addition, the residue contained minor amounts of compounds of R_F 0·20, 0·16 and 0·13 (S8). The residue was purified by chromatography on a column of silica gel (1·6 × 15 cm, eluant 5% methanol in benzene). The compounds were detected by thin-layer chromatography on silica gel (S8). Fractions 10—30 ml contained a mixture of both compounds, fractions 30—47 ml contained the desired product. IR spectrum, cm^{-1}: O—CO—NH: 3 435, 1 723, 1 512; $C_6H_5CH_2$: 701; $COOCH_3$: 1 740, 1 440, 1 239. Fractions 10—47 ml were combined, taken down and hydrolyzed by reflux with azeotropic hydrochloric acid (10 ml) for 1 h. The reaction mixture was washed with ether and taken to dryness, the residue was dissolved in a small amount of water and applied on a Dowex 50W column (H^+-form, 20 ml). After washing the column with water, the product was eluted with 10% aqueous pyridine (50 ml of the eluate were collected after appearance of the product in the eluate), the eluate was taken down and the residue crystallized from water and ethanol, affording 90 mg (13%) of the product, $[\alpha]_D$ +37·5° (*c* 0·5, 1M-HCl). According to the amino acid analysis, the product consisted of homolanthionine (78%), homoserine (15%) and homocystine (7%). In other experiments, the content of homolanthionine ranged between 73% and 90%.

Spectral Measurements

The CD spectra of the acid *VII* were taken on a Roussel Jouan CD 185/II instrument. The measurements were made with solutions of concentration about $1·5.10^{-3}$ mol l^{-1} at 25°C in 0·02 to 1 cm quartz cells. IR spectra were measured on a UR 20 (Carl Zeiss, Jena) spectrophotometer, unless stated otherwise. The spectra of α-amino-γ-nitrobutyric acid, homolanthionine and homoserine were taken on a Perkin–Elmer spectrometer, model 580; accuracy ±1·5 cm^{-1}. Each of the crystal modifications of homoserine was measured, in addition to KBr, also in a Nujol and

florube mull, in order to eliminate the effect of pressure and temperature in the preparation of the KBr pellet. The dependence of the crystal modifications on temperature was studied on a Kofler block and simultaneously the behaviour of the crystals was observed. After achieving the desired temperature, the sample was rapidly cooled and then its spectrum was taken in the normal manner. The deuteration was carried out by freeze-drying with D_2O or by crystallization from a mixture of D_2O and C_2H_5OD.

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