224. Synthesis of L-Propargylglycine and Derivatives[1])

by **Othmar Leukart, Mario Caviezel, Alex Eberle, Emanuel Escher, Aung Tun-Kyi** and **Robert Schwyzer**
Institut für Molekularbiologie und Biophysik
Eidgenössische Technische Hochschule, CH-8093 Zürich

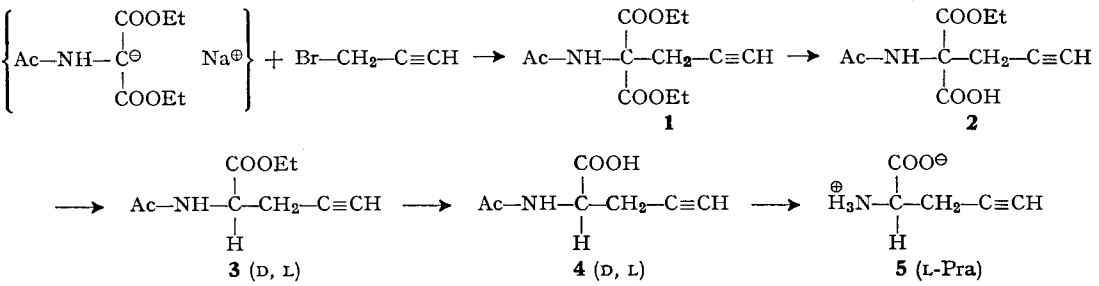
(15. VI. 76)

Summary. The chemical synthesis of L-propargylglycine (L-Pra) and of various derivatives is described. The new amino acid is a starting material for carboranylalanine (Car) and for tritiated norleucine.

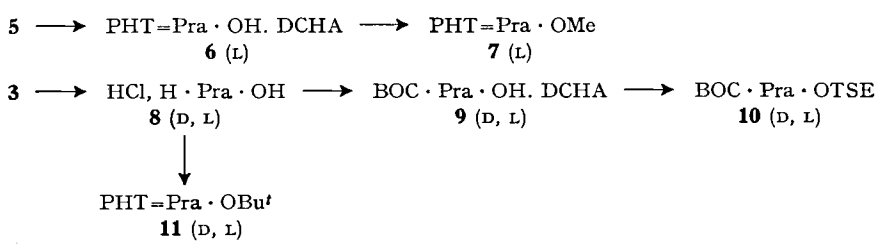
L-Propargylglycine (L-Pra) is a useful starting material for the preparation of L-carboranylalanine [2] and can, like L-allylglycine [3], serve for the preparation of tritium labelled norleucine and norleucine peptides.

The first step of the synthesis of L-Pra was the condensation of the sodium salt of diethyl α-acetamidomalonate and propargyl bromide (*Scheme 1*) to yield crystalline diethyl α-acetamido-α-propargylmalonate (**1**). This was converted by the usual procedures (*cf.* [1]) *via* monoethyl α-acetamido-α-propargylmalonate (**2**) and ethyl D,L-*N*-acetyl-propargylglycinate (**3**) to D,L-*N*-acetyl-propargylglycine (**4**). L-Propargylglycine (**5**) was obtained in excellent yield from **4** by stereospecific hydrolysis of the acetamide group with porcine kidney acetylase.

Scheme 1



Scheme 2



1) This work was supported by the *Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung* and is part of the doctoral thesis of *O. L.* Abbreviations according to [1]. For more convenience propargyl is used instead of 2-propynyl (IUPAC).

Some derivatives of L- and D,L-Pra were prepared according to *Scheme 2* by the usual procedures (*cf.* [1]); they all proved to be crystalline, well-defined products.

Experimental Part

M.p. were determined in open capillaries and are uncorrected. Microanalyses were carried out in the Organisch-chemisches Mikrolabor ETH (*W. Manser*). Optical rotations and IR. spectra (characteristic absorptions in cm^{-1}) were determined in our laboratories (Prof. *H. G. Weder*). The 'usual procedure' for extracting products from reaction mixtures consisted in repeated extraction of an aqueous phase with an organic solvent, washing the organic phase with an appropriate choice of basic, acidic, and neutral aqueous solutions, drying it with magnesium sulfate, and evaporating the solvent in a rotatory evaporator in a suitable vacuum.

Thin-layer chromatography was carried out with commercial silica gel plates. The solvent systems are (v:v): A = *n*-butanol/water/acetic acid 4:1:1, B = 2-propanol/pyridine/water 7:6:6, C = ethylacetate/methanol/acetic acid 7:2:1, D = chloroform, E = chloroform/methanol 9:1, F = chloroform/methanol/acetic acid 95:5:3.

Abbreviations are: DCCI = *N,N'*-dicyclohexylcarbodiimide, DCHA = dicyclohexylamine, DCU = *N,N'*-dicyclohexylurea, DMF = dimethylformamide.

Diethyl α-acetamido-α-propargylmalonate (**1**). 16.4 g (0.71 g-atom) of sodium were dissolved in 400 ml of abs. ethanol under nitrogen. To this clear solution, 138.8 g (0.64 mol) of diethyl α-acetamidomalonate, dissolved in 800 ml of abs. ethanol, were added dropwise. The temp. was 60°. After 6 h the solvent was evaporated, and the oily residue treated with dry acetonitrile. The sodium salt of diethyl α-acetamidomalonate crystallized during this process; it was separated by filtration, washed with acetonitrile and ether, and dried: 153.4 g (100%), m.p. 145° (decomp.). This salt (0.64 mol) was dissolved in 600 ml of DMF and treated with 83.5 g (0.70 mol) of propargyl bromide (stirring at 80° under N_2). The temp. was raised to 120° and stirring continued for 3 h. After cooling the precipitated NaBr was removed by filtration, the solvent evaporated, and the residue dissolved in water and ether. The organic phase was washed thrice with water, dried, and evaporated. The residue crystallized from diisopropyl ether/petroleum ether: 125 g (77%) of **1**, m.p. 91–92°, Rf 0.68 (E), 0.85 (A).

$C_{12}H_{17}NO_5$ (255.27) Calc. C 56.46 H 6,71 H 5.49% Found C 56.36 H 6.79 N 5.58%

Monoethyl α-acetamido-α-propargylmalonate (**2**). A solution of 40.8 g (0.728 mol) of KOH in 100 ml of water and 1.52 l of ethanol was slowly added (15 min) to a solution of 124 g (0.485 mol) of **1** in 800 ml of ethanol. The resulting solution, 0.3 M with respect to KOH, was kept for 3 h at 20°. Excess alkali was neutralized with 121.5 ml (0.243 mol) of 2N HCl; after 15 h at 4° KCl was removed by filtration, and the filtrate evaporated. The residue was dissolved in 1.5 l of water at 0°, acidified to pH 2 with 2N HCl, and the precipitate gathered, washed, and dried: 96.6 g (88%) of **3**, m.p. 145° (decomp.), Rf 0.49 (A).

Ethyl D,L-N-*acetyl-propargylglycinate* (**3**). The solution of 96 g (0.423 mol) of **2** in 1 l of dioxane was boiled for 24 h (bath temp. 130°). The solvent was evaporated and the residue crystallized from a mixture of ethanol/diisopropylether/petroleum ether: 56.4 g (73%) of **3**, m.p. 73°, Rf 0.25 (D), 0.73 (E), 0.8 (A).

$C_9H_{13}NO_3$ (183.21) Calc. C 59.00 H 7.15 N 7.65% Found C 59.01 H 7.15 N 7.71%

D,L-N-*Acetyl-propargylglycine* (**4**). 3.12 g (17 mmol) of **3** were dissolved in 30 ml of ethanol and hydrolysed for 15 h at 20° with 1.12 g (20 mmol) of KOH in 2.5 ml of water and 25 ml of ethanol. The ethanol was then evaporated, and the residue acidified to pH 2 with 1N HCl. The product was extracted with ethyl acetate and crystallized from the same solvent: 2.6 g (99%), m.p. 137–139°, Rf 0.58 (A).

L-*Propargylglycine* (**5**). 20.9 g (135 mmol) of **4** were dissolved in 1.35 l of water. After adjustment of the pH to 7.5 with ammonia, 27 mg of porcine renal acylase I, and 101 mg of cobalt acetate were added. After 15 h at 37° more acylase (27 mg) was added, and the hydrolysis continued for 4 h. The reaction was stopped, and the acylase denatured by the addition of 10 ml of trifluoroacetic acid and heating at 50° for 10 min. The solution was cleared with charcoal, and it's pH adjusted to 7 with ammonia. Most of the solvent was evaporated, and the residue treated with much acetone. The first precipitate consisted of 6.085 g (80%) of **5**, m.p. 230° (decomp. beginning

at 210°), Rf 0.26 (A), $[\alpha]_D^{20} = -35.0°$ ($c = 1$, H_2O). The following fraction of 1.1 g, precipitating more slowly, was less pure.

L-N-*Phthalyl-propargylglycine dicyclohexylamine salt* (**6**). 1.15 g (10.2 mmol) of **5** were dissolved in a solution of 2.92 g (10.2 mmol) of $Na_2CO_3 \cdot 10H_2O$ in 10 ml of water. 2.23 g (10.2 mmol) of *N*-ethoxycarbonyl-phthalimide (*cf.* [1]) were added, and the mixture stirred for 15 h at 20°. The clear solution was partly evaporated, brought to pH 1–2, and extracted thrice with ethyl acetate. The organic phase was dried, partly evaporated, and treated with a solution of 1.85 g (10.2 mmol) of DCHA in diisopropyl ether. The crystalline precipitate was gathered, washed with ether, and dried: 4.02 g (93.5%) of **6**, m.p. 205°, Rf 0.33 (E), 0.66 (B), $[\alpha]_D^{20} = -29.4°$ ($c = 1$, EtOH).

L-N-*Phthalyl-propargylglycine methyl ester* (**7**). 500 mg (1.18 mmol) of **6** were dissolved in 4 ml of methanol and treated with 295 mg (2.36 mmol) of dimethyl sulfate at room temp. according to *Keller* [4]. After 24 h the solution was evaporated and isolated as usual with ethyl acetate K_2SO_4/$KHSO_4$-solutions (5%, 2:1, *v/v*). The crude product was chromatographed on silica gel with chloroform. The main fraction was crystallized from petroleum ether: 215 mg (83%) of **7**, m.p. 112°, Rf 0.75 (E), $[\alpha]_D^{20} = -63.2°$ ($c = 1$, EtOH).

$C_{14}H_{11}NO_4$ (257.25) Calc. C 65.36 H 4.31 N 5.45% Found C 65.60 H 4.47 N 5.27%

D,L-*Propargylglycine hydrochloride* (**8**). 1.83 g (10 mmol) of **3** were boiled for 2 h in 30 ml of 2N HCl. After evaporation the residue was crystallized from 2-propanol/diisopropyl ether: 1.22 g (81.5%) of **8**, m.p. 195°, Rf 0.24 (A), 0.61 (B).

D,L-N-t-*Butoxycarbonyl-propargylglycine dicyclohexylamine salt* (**9**). 650 mg (5.75 mmol) of **8** were dissolved in 20 ml of water, and treated with a solution of 905 mg (6.33 mmol) of *t*-butoxycarbonylazide in 5 ml of dioxane according to *Schnabel* [5]. The product was isolated as usual with ethyl acetate: 1.23 g (100%) of D,L-*N*-*t*-butoxycarbonyl-propargylglycine, oil, Rf 0.83 (A). – IR.: 3420 (N—H), 3300 (C≡C—H), 1700 (C=O, amide).

300 mg (1.4 mmol) of this oil were dissolved in a small amount of diisopropyl ether, and treated with a solution of 255 mg (1.4 mmol) of DCHA in the same solvent: 500 mg of crystalline **9**, m.p. 147°.

$C_{22}H_{38}N_2O_4$ (393.55) Calc. C 66.97 H 9.71 N 7.10% Found C 67.05 H 9.73 N 6.99%

D,L-N-t-*Butoxycarbonyl-propargylglycine 2-(p-toluenesulfonyl)ethyl ester* (**10**). A solution of 325 mg (1.53 mmol) of **9** and 322 mg (1.61 mmol) of 2-(*p*-toluenesulfonyl)ethanol [6] in 5 ml of ethyl acetate was cooled to 0° and treated with 346 mg (1.68 mmol) of DCCI. After 30 min the mixture was kept at 20° for 15 h. DCU was removed by filtration and the product isolated from the filtrate by the usual extraction method with ethyl acetate, $NaHCO_3$ (5% solution), and K_2SO_4/$KHSO_4$-solutions (5%, 2:1, *v/v*). The oily product (640 mg) was chromatographed over silica gel with chloroform/methanol 9:1. The main fraction consisted of 450 mg (75%) of **10** that was slightly contaminated with DCU: oil, Rf 0.8 (E). – IR.: 3420 (N—H), 3300 (C≡C—H), 2100 (DCU), 1750 (C=O, ester), 1700 (C=O, amide).

D,L-N-*Phthalyl-propargylglycine t-butyl ester* (**11**). A solution of 350 mg (1.44 mmol) of D,L-*N*-phthalyl-propargylglycine and 3.6 ml of *t*-butyl alcohol in 5 ml of dry pyridine was cooled to –5° and treated dropwise with 0.16 ml (1.73 mmol) of $POCl_3$. After 15 min at –5° the mixture was kept for 1 h at 20°. The turbid, brownish mixture was extracted in the usual manner with ethyl acetate: 260 mg of crude product were obtained. This was chromatographed over 10 g of silica gel with chloroform. The residue of the main fraction was crystallized from petroleum ether: 120 mg of **11**, m.p. 97°, Rf 0.8 (E). – IR.: 3300 (C≡C—H), 1770, 1705 (C=O, phthalyl), 1730 (C=O, ester).

REFERENCES

[1] *E. Wünsch*, «Synthese von Peptiden», Bd. *15* von «*Houben-Weyl*, Methoden der organischen Chemie», E. Müller, Herausgeber, Georg Thieme Verlag, Stuttgart 1974.
[2] *O. Leukart, M. Caviezel, A. Eberle, E. Escher, A. Tun-Kyi & R. Schwyzer*, Helv. *59*, 2184 (1976).
[3] *R. Schwyzer & G. Karlaganis*, Liebigs Ann. Chem. *760*, 1298 (1973).
[4] *O. Keller*, Dissertation No. 5325, ETH Zürich (1974).
[5] *E. Schnabel*, Liebigs Ann. Chem. *707*, 188 (1967).
[6] *A. W. Miller & C. J. M. Stirling*, J. chem. Soc. [C] *1968*, 2612.