Acta Chemica Scandinavica B 28 (1974) 317—321

Synthesis and Resolution of Vinylglycine, a β,γ-Unsaturated α-Amino Acid

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DL-Vinylglycine (2-amino-3-butenoic acid) has been synthesized for the first time from ethyl 2-bromo-3-butenoate by amination and saponification, and by a Strecker synthesis from acrolein. D-Vinylglycine has been produced by treatment of the racemate with L-amino acid oxidase or baker's yeast. The spectroscopic properties and chemical stability of vinylglycine have been determined. Vinylglycine, the simplest β-γ-unsaturated α-amino acid, has previously been postulated as an intermediate in various enzymatic processes. The natural occurrence of other β-γ-unsaturated α-amino acids is briefly discussed.

A number of aliphatic α-amino acids possessing β,γ-unsaturation have been found in natural sources in recent years as indicated in Table 1. Several biological actions have been ascribed to this type of amino acid, and a potential role as amino acid antagonists can be envisioned. Thus, (*E*)-2(*S*)-amino-4-methoxy-3-butenoic acid (Ie, Table 1) is an antimetabolite, inhibiting growth of a *Bacillus* species [4,5] and of *Escherichia coli*.[5] Rhizobitoxine (If, Table 1) in low concentrations strongly inhibits ethylene production by *Sorghum* seedlings and induces chlorosis in new leaf tissues in many plants,[7] whereas the saturated analogue, dihydrorhizobitoxine,[8] does not seem to induce any chlorosis.[9]

Vinylglycine is the simplest β,γ-unsaturated α-amino acid. It has been postulated as an intermediate in the enzymatic conversion of homoserine to threonine [10] and α-ketobutyrate [11] but has not so far been encountered as a compound of natural derivation, and no report of its synthesis has appeared. It is the purpose of the present paper to describe a synthesis of DL- and D-vinylglycine and the chemical and spectroscopic properties of these compounds.

RESULTS

Syntheses. Synthesis of vinylglycine in reasonable yields was impeded by the instability of the compound upon treatment with heat, acid, or base (see below). Even if substantial amounts of vinylglycine could be produced, the isolation of pure material was always accompanied by great losses. The most satisfactory yields were obtained by stirring ethyl 2-bromo-3-butenoate with concentrated aqueous ammonia, followed by cold saponification and isolation of vinylglycine by use of a cation exchange resin. The synthesis of ethyl 2-bromo-3-butenoate has been reported previously.[12] Later an improved version of this synthesis has been described and it has been shown that on treatment of this substance with diethyl sodiomalonate it undergoes displacement of bromine without rearrangement.[13] Vinylglycine was also produced in a Strecker reaction performed essentially as described in an analogous synthesis of β-methylene-DL-norvaline (Ia, Table 1).[1] The dark brown reaction mixture contained six components reacting with ninhydrin besides vinylglycine. However, after a cumbersome isolation procedure involving ion exchange chromatography, pure vinylglycine was obtained in a yield of 1.1 %.

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Table 1. Naturally occurring β,γ-unsaturated α-amino acids with the general formula $R_3(R_2)C=C(R_1)CH(NH_3^+)COO^-$ (I).

	R_1	R_2	R_3	Configuration at C_2	Configuration at double bond	Occurrence	Ref.
Ia	CH_3CH_2	H	H	*S* (L)	–	*Lactarius helvus*	1
Ib	$CH_3CH_2CH_2$	H	H	*S* (L)	–	*Amanita vaginata* var. *fulva*	2
Ic	CH_2OH	CH_3	H	*S* (L)	*Z*	*Bankera fuligineoalba*	3
Id	CHO	CH_3	H	*S* (L)	?	*Bankera fuligineoalba*	3
Ie	H	CH_3O	H	*S* (L)	*E*	*Pseudomonas aeruginosa*	4,5
If	H	$CH_2(OH)CH(NH_2)CH_2O$	H	?	*E*	*Rhizobium japonicum*	6

Double bonds can be produced by elimination reactions from sulphonium compounds [14] and sulphoxides.[15] Therefore production of vinylglycine was attempted by thermal decomposition of *S*-methylmethionine, *S*-adenosylmethionine and methionine sulphoxide. However, homoserine, probably produced *via* the lactone, was the only reaction product which could be identified in a number of experiments under varying conditions of temperature and solvent. This negative result is in agreement with previous experiments on the degradation of these compounds. Thus *S*-methylmethionine on heating gives homoserine and dimethyl sulfide. [16,17] Vinylglycine has been postulated as an intermediate in the acid degradation of methionine sulphoxide but has not been identified in the reaction mixture.[18]

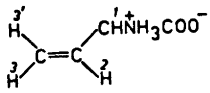
Resolution. Because of the instability of vinylglycine (see below) difficulties could be expected in attempts at chemical resolution of the racemate. Therefore the racemate was subjected to the action of L-amino acid oxidase,[19] which resulted in production of the levorotatory D-amino acid. However the optical purity obtained in this preparation was less than 25 % as indicated by the subsequent experiments.

Better results were obtained by subjecting racemic vinylglycine to the asymmetric action of baker's yeast during fermentation of sucrose. In this way a sample of D-vinylglycine was obtained possessing an $[\alpha]_D$ of $-94°$ in water. This product was hydrogenated to give a sample of 2-aminobutyric acid, $[\alpha]_D - 16.6°$ in hydrochloric acid. If it is assumed that the reduction proceeded without racemization, the D-vinylglycine must have contained about 9 % of the L-isomer, since a rotation of $+20.1°$ in hydrochloric acid is reported for L-2-aminobutyric acid (*cf.* Ref.[20], p. 116).

The D-configuration of the levorotatory stereoisomer is established by its production in the action of L-amino acid oxidase on the racemate and its reduction to D-2-aminobutyric acid. Furthermore the amino acid obeys the Clough-Lutz-Jirgensons rule (*cf.* Ref.[20], p. 85), showing a negative shift in $[\alpha]_D$ on acidification. Circular dichroism measurements (see below) also agree with the assignment of configuration.

Properties of vinylglycine. The UV-spectrum of vinylglycine in water shows a shoulder at about 210 nm, which can be attributed to the carboxylate group, and increasing absorption towards 190 nm due to the C=C bond. In hydrochloric acid the position of the shoulder is shifted to about 220 nm, in agreement with the change of the carboxylate group into a free carboxyl group. Measurements of CD-curves for the D-isomer (with optical purity more than 90 %, see above) demonstrated a negative Cotton effect at 208 nm in water and

212 nm in hydrochloric acid. No indication of additional Cotton effects could be observed down to 192 nm in water and 197 nm in hydrochloric acid. The strong UV-absorption prevented measurements at lower wavelengths. The Cotton effects observed must be attributed to the carboxylate and carboxyl groups. The numerically rather high $\Delta\varepsilon$-values found (−6.7 in water, −4.9 in hydrochloric acid) indicate, however, an influence from the C=C bond (*cf.* Ref. 21).



The ^{1}H NMR-spectrum of vinylglycine shows as expected the α-proton as a doublet and the three vinylic protons as a complex pattern. The spectrum was simulated using the LAOCN3 program.[22] With the following values for chemical shifts and coupling constants, agreement was obtained with the measured spectrum both with regard to position and intensity of the peaks (for designation of atoms see formula): δ_1 4.29 ppm; δ_2 5.97; δ_3 5.48; $\delta_{3'}$ 5.48; $J_{1,2}$ 7.1 cps; $J_{2,3}$ 10.8 cps; $J_{2,3'}$ 16.7 cps; $J_{3,3'}$ −1.2 cps.

On chromatograms vinylglycine produces with ninhydrin a yellow colour which changes through a brownish shade into the normal purple. The colour change is that expected for an α-amino acid with an electrophilic substituent in the β-position.[23]

When heated in water vinylglycine is slowly decomposed with the concomitant production of 2-aminobutyric acid and ammonia. However traces of vinylglycine can still be observed after 168 h at 100° or 68 h at 120°. In 1 N hydrochloric acid vinylglycine is decomposed without the production of other ninhydrin-reactive compounds. After 50 h at 100° no vinylglycine remained. When heated in aqueous ammonia vinylglycine is decomposed with the production of unidentified ninhydrin-reactive compounds. After 22 h at 100° in 2 N ammonia all vinylglycine had disappeared, whereas no decomposition was observed during 168 h in 2 N ammonia at room temperature.

When vinylglycine was heated in aqueous solution with 2-keto-4-methylpentanoic acid, leucine could be identified in the reaction mixture by paper chromatography. This result indicates that the production of 2-aminobutyric acid from vinylglycine in water takes place by production of 2-ketobutyric acid followed by transamination.

EXPERIMENTAL

^{1}H NMR spectra were measured on a JEOL-C-60 HL instrument. Chemical shifts are given in ppm downfield from sodium 2,2,3,3-tetradeuterio-3-(trimethylsilyl)-propionate. Infra-red spectra (in KBr) were recorded on a Perkin-Elmer Model 337 instrument. UV-spectra were measured in 1 mm cells on a Perkin-Elmer Model 402 instrument. Optical rotations were determined on a Perkin-Elmer Model 141 polarimeter in 1 dm tubes. CD-curves were recorded on a Roussel-Jouan Dichrograph CD 185 using 2 mm cells and concentrations of approximately 0.3 mg/ml. Microanalyses were performed by Mr. G. Cornali and his staff.

Paper chromatography was performed by the descending technique on Whatman No. 1 paper in A, BuOH−HOAc−H_2O [12 : 3 : 5 (v/v/v)], and B, PhOH−H_2O−conc. NH_3 [120 : 30 : 1 (w/v/v)]. The following R_F-values were found: Vinylglycine: A, 0.35; B, 0.66. 2-Aminobutyric acid: A, 0.47; B, 0.71.

DL-*Vinylglycine by Strecker synthesis.* The synthesis was performed as a modification of a standard procedure.[1,24] A solution of acrolein (28 g), NH_4Cl (64 g), and KCN (25 g) in water (270 ml) was kept at 5° for 30 min and at room temperature for 2 1/2 h. Conc. HCl (200 ml) was added and the solution concentrated by heating under a stream of nitrogen. After cooling, filtration, further concentration to 150 ml and filtration (to remove inorganic salts) the solution was applied to a strongly acidic cation exchange resin (Amberlite IR 120, 1 × 37 cm, H^+-form). After washing with water (300 ml) the column was eluted with ammonia (250 ml, 2 N). The eluate was concentrated to dryness (2.0 g), dissolved in water and passed through a column of deactivated carbon (1 × 5 cm). The effluent from the carbon was evaporated to dryness (1.75 g), the residue dissolved in water and the solution applied to a strongly acidic cation exchange resin (Dowex 50W × 8, 1.2 × 75 cm, particle size 45−70 μ, 3-chloropyridinium form). The column was washed with water (150 ml) and eluted with aqueous 3-chloropyridine (0.25 M). Fractions of 12 ml each were collected. Fractions 91−109 were concentrated to dryness to give chromatographically pure vinylglycine (480 mg, 1.1 %). Final purification was accomplished by passage of an aqueous solution through a small carbon column, concentration to dryness, redissolution in water (0.5 ml) and precipitation with ethanol (2 ml) to give colourless DL-vinylglycine (58 mg). (Found: C 47.51; H 6.99; N 13.70. Calc. for $C_4H_7NO_2$: C 47.52; H 6.98; N 13.85). IR:

ν_{max}(KBr) 2080 cm^{-1} (medium), 1660 (very strong), 1585 (vs), 1520 (vs), 1420 (vs), 1395 (vs), 1340 (vs), 1280 (strong), 1160 (m), 1120 (s), 1080 (m), 1040 (m), 990 (s), 935 (vs), 915 (vs), 880 (m), 790 (vs), 660 (s), 610 (m), 515 (m), 470 (s).

DL-*Vinylglycine from ethyl 2-bromo-3-butenoate.* Ethyl 2-bromo-3-butenoate [12,13] (74 g) was stirred with concentrated aqueous ammonia (450 ml) for 20 h. After addition of NaOH (30 %, 45 ml) the solution was kept at 5° for 14 days and at −15° for another 14 days. The solution was then applied to a cation exchange resin (Amberlite IR 120, 5 × 40 cm, H^+-form). The resin was washed with water (15 l). and eluted with aqueous pyridine (1 M, 2 l) All fractions showing a positive reaction with ninhydrin were pooled and evaporated to dryness, leaving a brown residue (13.6 g) which was dissolved in water (100 ml) and passed through a column of Sephadex G-10 (2.5 × 90 cm). The column was eluted with water, 6 ml fractions being collected. Fractions 25 – 51 contained vinylglycine as the major component. The residue (8.5 g) from fractions 36 – 51 was dissolved in a small amount of water and absolute ethanol was added. On storage in the icebox overnight a slightly yellow product precipitated (1.3 g). A second crop (1.2 g, total yield 6.6 %) was obtained on concentration of the mother liquor to near dryness followed by addition of absolute ethanol. The product was identical with the product from the Strecker synthesis as judged by paper chromatography and IR- and ^1H NMR-spectroscopy.

Production of partially racemic D-*vinylglycine by the action of* L-*amino acid oxidase on the racemate.* DL-Vinylglycine (200 mg) was dissolved in 500 ml of 0.2 M Tris (pH 7.5), and L-amino acid oxidase [50 mg, from *Crotalus adamanteus* venom (Sigma No. A-9378)] was added. The flask was stoppered and placed in a water bath at 37°. After shaking for 17 h, the stopper was removed and the shaking continued for 3 h. The solution was concentrated and applied to a cation exchange resin (Amberlite IR 120, 2.5 × 30 cm, H^+-form). The resin was washed with water (1100 ml) and eluted with aqueous pyridine (0.5 M). The fractions containing the amino acid were pooled and concentrated to a brown, sticky residue. Further purification was accomplished by ion exchange on a small column of Dowex 50W × 8 resin, preparative paper chromatography (solvent A) and passage through a small carbon column to give a colourless sample (136 mg), $[\alpha]_D^{23} - 23°$ (*c* 0.93, H_2O).

Production of D-*vinylglycine by the action of baker's yeast on the racemate.* DL-Vinylglycine (200 mg) and sucrose (7 g) were dissolved in water (50 ml), and baker's yeast (5 g) was suspended in the mixture by stirring. The suspension was set aside at room temperature for two days, and the liquid was decanted through a 1 cm layer of Filtercel. The filtrate was concentrated and applied to a cation exchange resin (Amberlite IR 120, 1.3 × 17 cm, H^+-form). The column was rinsed with water, and vinylglycine was eluted with aqueous pyridine (0.5 M), 5 ml fractions being collected. Fractions 8 – 12, which contained vinylglycine, were combined and evaporated to dryness. The residue (104 mg) was dissolved in a few ml of water and passed through a filter having a small layer of carbon. The filtrate was evaporated to dryness, leaving a colourless sample of D-vinylglycine (78 mg), m.p. 216 – 218° (decomp.), $[\alpha]_D^{22} - 93.8°$ (*c* 1.5, H_2O). The paper-chromatographic behaviour and IR- and ^1H NMR-spectra were identical with those observed for the racemate. A sample of D-vinylglycine with $[\alpha]_D^{22} - 83.1°$, produced in a preliminary experiment, revealed, dissolved in hydrochloric acid, an $[\alpha]_D^{22} - 95°$ (*c* 0.3, 2 M HCl).

D-*2-Aminobutyric acid by reduction of* D-*vinylglycine.* An aqueous solution of D-vinylglycine (29 mg) $[\alpha]_D - 93.8°$ was hydrogenated with platinum on carbon (30 mg, 10 % Pt) as a catalyst. Hydrogen uptake was completed in less than one hour, as demonstrated by paper chromatography. The catalyst was removed by filtration, and the filtrate was evaporated to dryness to give colourless and chromatographically pure D-2-aminobutyric acid (33 mg), $[\alpha]_D^{21} - 16.6°$ (*c* 0.9, 2 N HCl).

DL-*2-Aminobutyric acid and ammonia by heating an aqueous solution of* DL-*vinylglycine.* DL-Vinylglycine (100 mg) in water (10 ml) was heated in a sealed tube for 4 days at 120°. Ammonia could be identified in the reaction mixture by the use of Nessler's reagent. The solution was taken to dryness, dissolved in water and applied to a small column of cation exchange resin (Dowex 50W × 8, H^+-form). After flushing with water, neutral amino acids were eluted with aqueous pyridine (1 M). 2-Aminobutyric acid, which was the main component, was purified by passage through a small carbon column, preparative paper chromatography and a second carbon treatment. A colourless, crystalline sample (18 mg) was obtained. The paper-chromatographic behaviour and ^1H NMR-spectrum were identical with those observed for an authentic sample of L-2-aminobutyric acid. Small deviations in the IR-spectra must have been due to the difference between the racemate and the enantiomer.

Acknowledgements. The authors are indebted to Dr. P. Laur, Danish Technical University, for the recording of the CD-curves. We are further indebted to Professor M. G. Ettlinger, University of Copenhagen, for valuable discussions and suggestions, for calling our attention to Ref. 13, and for providing us with a copy. The simulation of the ^1H NMR-spectrum of vinylglycine was kindly performed by Dr. C. E. Olsen of this department.

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Received November 14, 1973.