BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 46, 1847—1850 (1973)

Synthesis of Alanosine

Yoshikazu Isowa, Hideaki Kurita, Muneki Ohmori, Masanari Sato, and Kaoru Mori
Sagami Chemical Research Center, Nishiohnuma, Sagamihara, Kanagawa 229

(Received January 13, 1973)

DL-2-Amino-3-(*N*-tosyl-*N*-benzyloxyamino)propionic acid (DL-V) was synthesized starting from ethyl 2,3-dibromopropionate and *N*-tosyl-*O*-benzylhydroxylamine. L-2-Benzoylamino-3-(*N*-benzoyl-*N*-hydroxyamino)propionic acid anilide (L XIV) obtained *via* the enzymatic resolution of DL-2-benzoylamino-3-benzyloxyaminopropionic acid (DL-IX) was converted by acid hydrolysis to L-2-amino-3-hydroxyaminopropionic acid (L-II). The nitrosation product of the amino-hydroxyamino acid (L-II) was identical with alanosine (L-I).

Alanosine[1,2] (L-I) is an antimicrobial agent isolated from a Streptomyces. The structure[2] of L-I was determined to be a 3-*N*-nitroso-derivative of L-2-amino-3-hydroxyamino-propionic acid (L-II), one of the α-amino-ω-hydroxyamino acid.[3–5]

A route for the synthesis of L-II starting from methyl 2-acetamino-3-chloropropionate and hydroxylamine has been reported by Lancini *et al.*[6,7] The optically active amino-hydroxyamino acid (L-II) has been obtained by the acid hydrolysis of the cinchonine salt of L-2-benzoylamino-3-(*N*-benzoyl-*N*-hydroxyamino)propionic acid (L-XI). Nitrosation of L-II with sodium nitrite in the equimolar amount of cold M hydrochloric acid gave alanosine (L-I).

In our previous paper,[8] we described the syntheses of 5-(*N*-tosyl-*N*-benzyloxy)-DL-ornithine and 5-*N*-hydroxy-L-ornithine, a higher homolog of L-II. The present paper deals with the synthesis of L-II from DL-2-amino-3-(*N*-tosyl-*N*-benzyloxy-amino)propionic acid (DL-V), which was prepared from *N*-tosyl-*O*-benzylhydroxylamine (III) and ethyl 2,3-dibromopropionate. The reaction paths were outlined in Fig. 1.

Papain-catalyzed resolution[8–11] of DL-2-acetamino-3-(*N*-tosyl-*N*-benzyloxyamino)propionic acid (DL-VI) under the same conditions (38 °C, pH 6.1, or 7.3) as in the case of 2-*N*-acetyl-5-(*N*-tosyl-*N*-benzyloxy)-DL-ornithine in the presence of aniline was unsuccessful. But optically active 2-acetamino-3-(*N*-tosyl-*N*-benzyloxyamino)propionic acid anilide (VII), $[\alpha]_D^{23}$ −113.4° (*c* 1, chloroform) was obtained at 50 °C and pH 7.3 in spite of the bulkiness of the hydroxyamino-protecting tosyl and benzyl groups at the β-position of the molecule.

On the other hand, the detosylation product of the amino acid (DL-V), DL-2-amino-3-benzyloxyaminopropionic acid (DL-VIII), which was obtained from DL-V by the action of 36% hydrogen bromide–acetic

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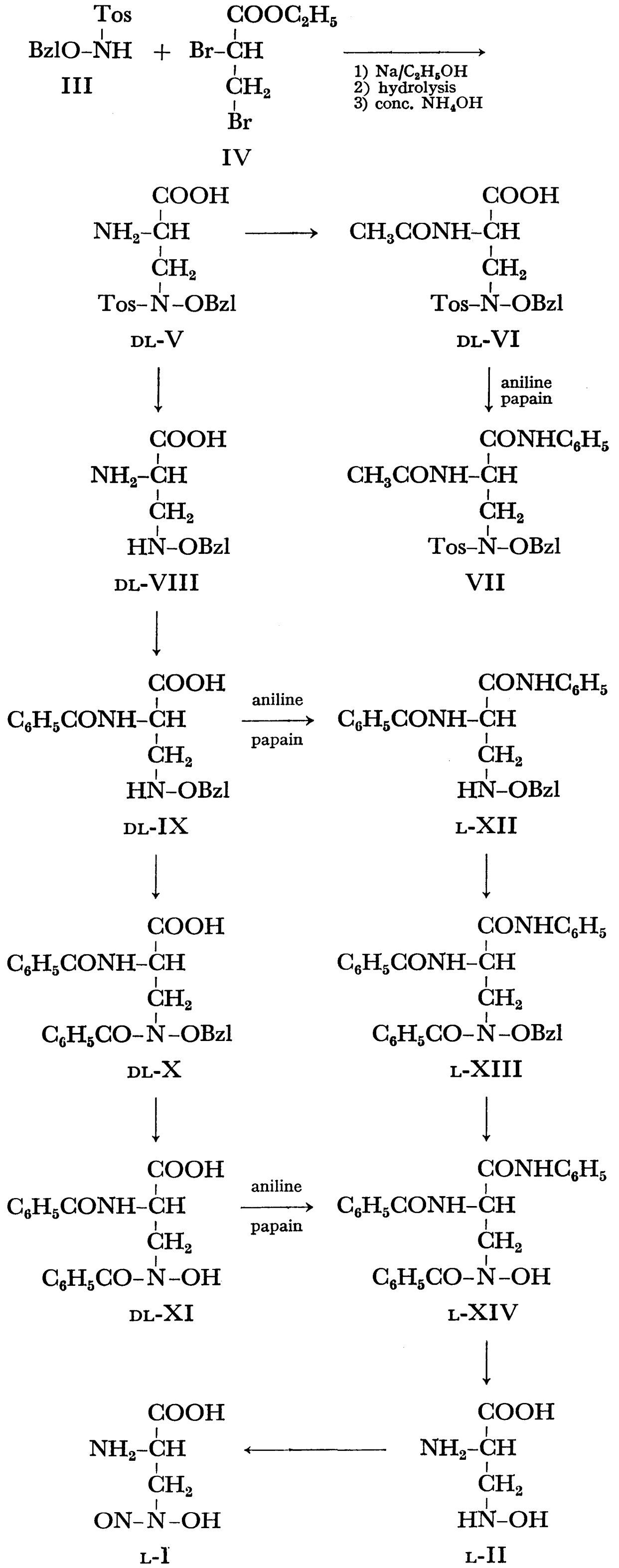

Fig. 1

acid in the presence of phenol, followed by the 2-*N*-benzoylation with *N*-benzoyloxysuccinimide, was resolved into derivatives of its optical isomers with the aid of cysteine-activated papain in the presence of aniline at 38 °C and pH 6.1; L-2-benzoylamino-3-benzyloxyaminopropionic acid anilide (L-XII), $[\alpha]_D^{26}$ −108.9° (*c* 1, chloroform) and D-2-benzoylamino-3-benzyloxyaminopropionic acid (D-IX), $[\alpha]_D^{26}$ −18.2° (*c* 1, acetic acid) were obtained.

3-*N*-Benzoylation of L-XII in pyridine with benzoyl chloride, followed by hydrogenation, yielded L-2-benzoylamino-3-(*N*-benzoyl-*N*-hydroxyamino)propionic acid anilide (L-XIV). The L-dibenzoyl derivative (L-XIV) was also obtained by resolution of DL-2-benzoylamino-3-(*N*-benzoyl-*N*-hydroxyamino)propionic acid (DL-XI), which was prepared by 3-*N*-benzoylation of monobenzoylamino acid (DL-IX) in ethyl acetate with benzoyl chloride in the presence of *N*-methylmorpholine and subsequent debenzylation.

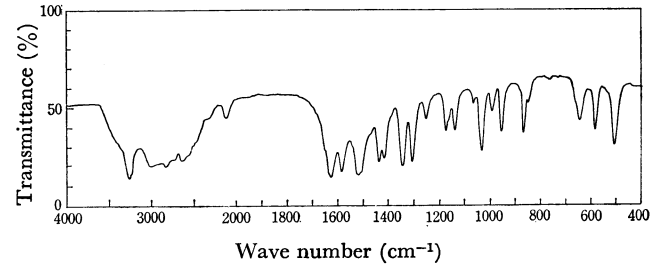

Fig. 2. L-2-Amino-3-hydroxyaminopropionic acid

Acid hydrolysis of the protected amino-hydroxyamino acid derivative (L-XIV) gave L-2-amino-3-hydroxyaminopropionic acid (L-II), which was identical, in respect of optical rotatory power, with an authentic sample obtained by another route.[6,7] The IR spectrum of L-II is shown in Fig. 2. Nitrosation of the amino-hydroxyamino acid (L-II) by the method reported by Lancini *et al.*,[7] gave L-2-amino-3-(*N*-nitroso-*N*-hydroxyamino)propionic acid, alanosine (L-I). The identity of the synthetic alanosine with natural one was confirmed by color reaction, optical rotation, and elemental analysis. The IR spectrum of L-I is shown in Fig. 3.

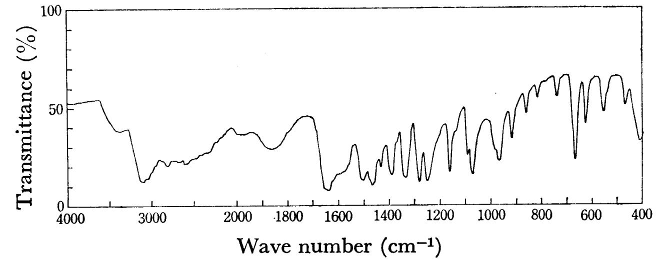

Fig. 3. L-2-Amino-3-(*N*-nitroso-*N*-hydroxyamino)propionic acid; alanosine

Experimental

Melting points were all determined with a Yanagimoto electric micromelting point apparatus, unless otherwise indicated, and are uncorrected. Optical rotations were measured with a Yanagimoto automatic polarimeter OR-50. Infrared spectra were recorded on a Hitachi EPI G-3 spectrophotometer as KBr disk. Nuclear magnetic resonance spectra were run on a Hitachi Perkin-Elmer R-20 High Resolution spectrometer, using tetramethylsilane as an internal standard. Concentration and evaporation of solutions were always carried out under reduced pressure.

DL-2-Amino-3-(N-tosyl-N-benzyloxyamino)propionic Acid (DL-V). To a solution of sodium (23 g, 1g-atom) in ethanol

(1 l) was added *N*-tosyl-*O*-benzylhydroxylamine (277 g, 1 mol). To the resultant mass, under ice-cooling and occasional shaking, ethyl 2,3-dibromopropionate (260 g, 1 mol) was added over a period of 2 hr. After stirring for 12 hr at room temperature, 5 M sodium hydroxide (210 ml) was added slowly at 0 °C and stirred for 12 hr at room temperature. Thereafter 2.5M sulfulic acid (*ca.* 210 ml) was added to the solution until pH 4 under ice-cooling and the solvent was evaporated. To the residue, ethyl acetate (1.5 l) and water (200 ml) were added, the organic layer was dried over sodium sulfate and the solvent was removed. To the viscous oily residue conc. aqueous ammonia (3 l) was added and the solution saturated with ammonia gas was stored at room temperature for 10 days. Thereafter the solution was concentrated. The crystalline deposit was collected and washed with water and methanol. Yield, 273 g (75%);mp 184—186 °C (decomp.). This crude product was used without recrystallization in the subsequent preparation. A part of the product was recrystallized from acetic acid–water to give an analytical sample, mp 187—188 °C (decomp.).

Found: C, 55.96; H, 5.23; N, 7.56%. Calcd for $C_{17}H_{20}N_2O_5S$: C, 56.03; H, 5.53; N, 7.69%.

DL-2-Acetamino-3-(N-tosyl-N-benzyloxyamino)propionic Acid (DL-VI). Crude amino acid (DL-V) (164.1 g, 0.456 mol) was mixed with glacial acetic acid (1.5 l) and acetic anhydride (60 ml) was added dropwise under stirring. After the clear solution was resulted, it was stirred for 6 hr at room temperature and then evaporated. The syrupy residue was taken up in a minimum quantity of ethyl acetate and the solution was chilled for 12 hr in a refrigerator. The resulting solid was collected by filtration. Yield, 161.0 g; mp 152—153 °C. Recrystallization from ethyl acetate gave an analytical sample, mp 156—157 °C.

Found: C, 56.11; H, 5.36; N, 6.88%. Calcd for $C_{19}H_{22}N_2O_6S$: C, 56.14; H, 5.46; N, 6.89%.

Resolution of DL-2-Acetamino-3-(N-tosyl-N-benzyloxyamino)propionic Acid (DL-VI); 2-N-Acetamino-3-(N-tosyl-N-benzyloxyamino)propionic Acid Anilide (VII). To the solution of the acetylamino acid (DL-VI) (20.3 g, 50 mmol) in 0.5M sodium hydroxide (300 ml) was added 0.5 M citric acid and the solution was adjusted to pH 7.3—7.35. The resultant clear solution was diluted to 500 ml and aniline (10 ml) was added together with papain (5 g) and L-cysteine hydrochloride monohydrate (5 g). The mixture was incubated at 50 °C for 48 hr. The precipitated crystals were collected, washed with 7% aqueous ammonia and water and dried. Recrystallization from *n*-propyl alcohol gave colorless crystals. Yield, 5.8 g (48.5%); mp 209—210 °C; $[\alpha]^{20}_{D}$ −110.2° (*c* 1, chloroform). Another recrystallization from the same solvent afforded an analytical sample, mp 211—212 °C and $[\alpha]^{23}_{D}$ −113.4° (*c* 1, chloroform).

Found: C, 62.05; H, 5.42; N, 8.79%. Calcd for $C_{25}H_{27}N_3O_5S$: C, 62.35; H, 5.65; N, 8.73%.

The above filtrate was adjusted to pH 1 with conc. hydrochloric acid and the deposited crystals were collected, washed with water and dried. Recrystallization from ethyl acetate gave colorless crystals, 7.45 g, mp 150—151 °C, which was identified DL-acetylamino acid (DL-VI) from the fact that this compound and the hydrolysis product, 2-amino-3-(*N*-tosyl-*N*-benzyloxyamino)propionic acid, were optically inactive and this compound gave the anilide (VII) under the above-described condition.

DL-2-Amino-3-benzyloxyaminopropionic Acid (DL-VIII). To a solution of phenol (54 g) and 36% hydrogen bromide-acetic acid (300 ml) in a glass-stoppered bottle, was added DL-V (64.5 g, 0.18 mol). After stirring for 84 hr at room temperature, the solution was evaporated to dryness at 45 °C. The residual crystals were triturated with ether and then dissolved in water (300 ml). The solution was adjusted to pH 7 with 7% aqueous ammonia under ice-cooling. After the removal of the precipitated crystals, the filtrate was concentrated to a small bulk and deposited crystals were collected. Yield, 20.55 g (54%); mp 206—210 °C (decomp.). An analytical sample was obtained by recrystallization from water, mp 210—211 °C (decomp.). IR; 3240 cm^{-1}. NMR (CF_3COOH): δ 4.46 (d, 2H), 5.12 (t, 1H), 5.53 (s, 2H), and 7.50 (s, 5H). Ninhydrin reaction was positive and triphenyltetrazolium negative.

Found: C, 57.16; H, 6.68; N, 13.54%. Calcd for $C_{10}H_{14}N_2O_3$: C, 57.13; H, 6.71; N, 13.33%.

DL-2-Benzoylamino-3-benzyloxyaminopropionic Acid (DL-IX). a) To the suspension of DL-VIII (2.1 g, 10 mmol) in methanol (20 ml) were added 40% Triton B (4.7 ml) and *N*-benzoyloxy succinimide[12] (2.2 g, 10 mmol). After stirring for 12 hr at 50 °C, the solvent was removed and the residual oil was dissolved in water (30 ml). The water layer, after extracted with ethyl acetate, was acidified with conc. hydrochloric acid under cooling to pH 3.5. The resulting oil was scratched after standing in an ice box for 5 hr and then crystallized. Yield, 0.7 g (22.3%). Recrystallization from much water gave an analytical sample, mp 124—126 °C. NMR (CF_3COOH): 4.28 (2H), 5.32 (3H, benzylmethylene and α-methine protons), 7.49 and 7.68 (13H), and 8.16 (d, 1H, amide proton). Ninhydrin and triphenyltetrazolium test were negative.

Found: C, 65.02; H, 5.79; N, 8.93%. Calcd for $C_{17}H_{18}N_2O_4$: C, 64.95; H, 5.77; N, 8.91%.

b) The resulting oil was crystallized by adding a part of the product obtained as a) under stirring at room temperature. Yield, 64%; mp 143—147 °C. A part of the product was recrystallized from water to give an analytical sample, mp 147—149 °C. IR: 3370, 3250 cm^{-1}. NMR spectrum in trifluoroacetic acid was identical with that of the low-melting benzoylamino acid obtained as a). Ninhydrin test was negative. For the subsequent reaction this high-melting benzoylamino acid was used.

DL-2-Benzoylamino-3-(N-benzoyl-N-benzyloxyamino)propionic Acid (DL-X). To the stirred solution of DL-IX (6.28 g, 20 mmol) and *N*-methylmorpholine (2.22 g, 22 mmol) in ethyl acetate (100 ml) was added benzoyl chloride (3.08 g, 22 mmol) in ethyl acetate (20 ml) over 15 min at room temperature. The deposited crystals were collected after 12 hr and washed with ether and water. Yield, 6.84 g (81.5%); mp 175—177 °C. An analytical sample was obtained by recrystallization from ethyl acetate–ether, mp 182—183 °C.

Found: C, 68.53; H, 5.33; N, 6.79%. Calcd for $C_{24}H_{22}N_2O_5$: C, 68.89; H, 5.30; N, 6.70%.

DL-2-Benzoylamino-3-(N-succinyl-N-benzyloxyamino)-propionic Acid. To the stirred solution of the benzyloxyamino acid (DL-IX) (0.94 g, 3 mmol) and *N*-methylmorpholine (0.31 g, 3 mmol) in ethyl acetate (20 ml) was added succinic anhydride (0.3 g, 3 mmol). After 3 hr, water (20 ml) was added to the clear solution and the water layer was adjusted to pH 3 with 1 M hydrochloric acid. The precipitated oil was stored in an ice box for 12 hr and the resulting crystals were collected. Yield, 0.7 g (56.5%); mp 154—155 °C. Recrystallization from ethyl acetate–*n*-hexane gave an analytical sample, mp 153—154 °C. IR: 3300, 1710, 1650, 1525 cm^{-1}.

Found: C, 60.60; H, 5.24; N, 6.73%. Calcd for $C_{21}H_{22}N_2O_7$: C, 60.86; H, 5.35; N, 6.76%.

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DL-*2-Benzoylamino-3-(*N*-benzoyl-*N*-hydroxyamino)propionic Acid (*DL-*XI).* DL-X (4.18 g, 10 mmol) in methanol (130 ml) was hydrogenated in the presence of 5% palladium-charcoal (0.5 g). After 36 hr, the catalyst was removed and the filtrate was evaporated. The residual oil was dissolved in hot water and the crystalline debenzylation product was collected. Yield, 2.7 g (82.5%); mp 173—175 °C (lit,[7] mp 170 °C). Ferric chloride test was positive.

Found: C, 62.18; H, 4.90; N, 8.38%. Calcd for $C_{17}H_{16}N_2O_5$: C, 62.19; H, 4.91; N, 8.53%.

Resolution of DL-*2-Benzoylamino-3-benzyloxyaminopropionic Acid (*DL-*IX);* L-*2-Benzoylamino-3-benzyloxyaminopropionic Acid Anilide (*L-*XII) and* D-*2-Benzoylamino-3-benzyloxyaminopropionic Acid(*D-*IX).* To the suspension of DL-IX (9.42 g, 30 mmol) in 0.5 M sodium hydroxide (160 ml) was added 0.5 M citric acid and the solution was adjusted to pH 6.15. The resulting clear solution was diluted with water to 500 ml. Aniline (6 ml) was added to the solution together with papain (6 g) and L-cysteine hydrochloride monohydrate (6 g). The mixture was incubated at 38 °C. After 30 hr, the deposited L-anilide (L-XII) was collected, washed with water and recrystallized from *n*-propyl alcohol. Yield, 4.87 g (83.5%); mp 129—131 °C; $[\alpha]^{26}_{D}$ −108.9° (*c* 1, chloroform). IR: 3280, 1670 1630 cm^{-1}.

Found: C, 71.15; H, 6.03; N, 10.85%. Calcd for $C_{23}H_{23}N_3O_3$: C, 70.93; H, 5.95; N, 10.79%.

The above filtrate, combined with the washings was adjusted to pH 3.5 with conc. hydrochloric acid. The precipitated D-amino acid (D-IX) was collected, washed with water and recrystallized from ethyl acetate. Yield, 2.45 g (52%); mp 147—149 °C; $[\alpha]^{26}_{D}$ −18.2° (*c* 1, acetic acid).

Found: C, 64.89; H, 5.77; N, 8.93%. Calcd for $C_{17}H_{18}N_2O_4$: C, 64.95; H, 5.77; N, 8.91%.

L-*2-Benzoylamino-3-(*N*-benzoyl-*N*-benzyloxyamino)propionic Acid Anilide (*L-*XIII).* To the solution of the benzyloxyamino compound (L-XII) (3.89 g, 10 mmol) in pyridine (30 ml) was added benzoyl chloride (1.82 g, 13 mmol) under ice-cooling. After stirring for 12 hr at room temperature, the solvent was evaporated. The residual oil was taken up in ethyl acetate (100 ml) and washed with 1 M hydrochloric acid and water. The organic layer was dried over sodium sulfate and then evaporated. The residual crystals were recrystallized from *n*-propyl alcohol. Yield, 3.80 g (77%); mp 173—174 °C; $[\alpha]^{30}_{D}$ −82.6° (*c* 0.5, chloroform).

Found: C, 72.94; H, 5.45; N, 8.66%. Calcd for $C_{30}H_{27}N_3O_4$: C, 73.00; H, 5.51; N, 8.51%.

L-*2-Benzoylamino-3-(*N*-benzoyl-*N*-hydroxyamino) propionic Acid Anilide (*L-*XIV).* a) L-XIII (4.9 g, 5 mmol) in methanol (500 ml) was hydrogenated in the presence of 5% palladium-charcoal (0.5 g) at room temperature. After 72 hr, the catalyst was removed and the filtrate was evaporated. The residual crystals were recrystallized from *n*-propyl alcohol. Yield, 2.88 g (71.5%); mp 192—193 °C; $[\alpha]^{31}_{D}$ +55.2° (*c* 0.5, chloroform). IR: 3300, 3110, 1670, 1635 cm^{-1}. Ferric chloride test was positive.

Found: C, 68.19; H, 5.35; N, 10.72%. Calcd for $C_{23}H_{21}N_3O_4$: C, 68.47; H, 5.25; N, 10.42%.

When this reaction was performed in the presence of 10% palladium-charcoal at 40 °C, the deoxygenated product of L-XIV, L-2,3-dibenzoylaminopropionic acid anilide was obtained. Mp 257—258 °C (methanol). This compound gave a negative ferric chloride test and was sparingly soluble in chloroform, ethyl acetate, *n*-propyl alcohol and methanol.

Found: C, 70.78; H, 5.42; N, 10.60%. Calcd for $C_{23}H_{21}N_3O_3$: C, 71.30; H, 5.46; N, 10.85%.

b) To the solution of the benzoylamino acid (DL-XI) (1.64 g, 5 mmol) in 0.5 M sodium hydroxide (30 ml) was added 0.5 M citric acid and the solution was adjusted to pH 6.15. The resultant clear solution was diluted to *ca.* 60 ml and aniline (1 ml) was added together with papain (0.5 g) and L-cysteine hydrochloride monohydrate (0.5 g). The mixture was incubated at 38 °C for 30 hr. The deposited crystals were collected and washed with water and ether. Recrystallization from *n*-propyl alcohol with the aid of charcoal gave an analytical sample. Yield, 0.45 g (44.5%); mp 193—194 °C; $[\alpha]^{29}_{D}$ +57.6° (*c* 0.5, chloroform).

Found: C, 68.40; H, 5.30; N, 10.21%.

L-*2-Amino-3-hydroxyaminopropionic Acid (*L-*II).* The protected amino-hydroxyamino acid (L-XIV) (1.7 g, 5 mmol) was stirred with 6M hydrochloric acid (100 ml) at 90—95 °C for 8 hr. After standing at room temperature for 3 hr, the deposited crystals were removed and the filtrate, after extracted with ether, was evaporated. Water (20 ml) was added and evaporated. The residual oil was dissolved in water (5 ml) and neutralized with 7% aqueous ammonia under cooling. Ethanol was added until the solution was cloudy. The precipitated crystals were collected, after the solution was stored in an ice box for 5 hr. Yield, 260 mg (43.5%). An analytical sample was prepared by recrystallization from water–ethanol. Mp 160—161 °C (decomp.) in a sealed capillary in a liquid bath (lit,[7] mp 161—163 °C (decomp.)); $[\alpha]^{27}_{D}$ +12.6° (*c* 0.5, M hydrochloric acid) (lit,[7] $[\alpha]_{D}$ +15.7°) IR: Fig. 2 in the text. Triphenyltetrazolium and ninhydrin test were positive.

Found: C, 30.26; H, 6.63; N, 22.97%. Calcd for $C_3H_8N_2O_3$: C, 30.00; H, 6.71; N, 23.33%.

L-*2-Amino-3-(*N*-nitroso-*N*-hydroxyamino)propionic Acid; Alanosine (*L-*I).* To a stirred clear solution of the amino-hydroxyamino acid (L-II) (240 mg, 2 mmol) in M hydrochloric acid (2 ml), sodium nitrite (138 mg, 2 mmol) was slowly added at 0 °C. After 30 min, the precipitated crystals were collected and washed with ethanol and ether. Yield, 190 mg (63.7%); mp 196—198 °C (decomp.). An analytical sample was obtained by recrystallization from 1 M sodium hydroxide–acetic acid. Mp 196—197 °C (decomp.) (lit,[7] mp 190 °C (decomp.); $[\alpha]^{27}_{D}$ −43.2° (*c* 0.5, 0.1 M sodium hydroxide) (lit,[7] $[\alpha]_{D}$ −46.0°). IR: Fig. 3 in the text. Ninhydrin and ferric chloride test were positive and the characteristic reaction of the *N*-nitroso group[13] with sulphanilic acid and α-naphtylamine was also positive. Triphenyltetrazolium test was negative.

Found: C, 24.00; H, 4.95; N, 28.19%. Calcd for $C_3H_7N_3O_4$: C, 24.16; H, 4.73; N, 28.18%.

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