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Biosynthesis of Sinigrin. IV. Syntheses of DL-Allylglycine (2-^{14}C,^{15}N), DL-Homomethionine(2-^{14}C,^{15}N), DL-Homomethionine(G-^{3}H),3-Methylthiopropionamide(1-^{14}C) and 4-Methylthiobutyramide(1-^{14}C)

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The syntheses of DL-allylglycine(2-^{14}C,^{15}N), DL-homomethionine(2-^{14}C,^{15}N), DL-homomethionine(G-^{3}H), 3-methylthiopropionamide(1-^{14}C) and 4-methylthiobutyramide(1-^{14}C) were described.

In a preliminary communication, we showed that homomethionine was a direct precursor of sinigrin, one of the mustard oil glucosides, in horseradish (*Armoracia lapathifolia* Gilib.).[2] The present paper describes the syntheses of labelled homomethionine and some labelled compounds used as the possible precursors of sinigrin.

DL-Allylglycine(2-^{14}C, ^{15}N) was obtained by mixing DL-allylglycine(2-^{14}C) (I) and DL-allylglycine(^{15}N) (II). The compound (I) was synthesized by the alkaline hydrolysis of methyl 2-acetamido-2-cyanopenten-(4)-ate(2-^{14}C) (III), which was obtained by the condensation of methyl acetamidocyanoacetate(2-^{14}C) (IV) and allyl bromide. Goering, *et al.* reported that allylglycine was formed by refluxing ethyl allylacetamidomalonate in concentrated hydrochloric acid, but the yield of allylglycine was low (34%), since 2-aminopentano-δ-lactone was produced by the lactonization of allylglycine.[3] However we observed that when III was refluxed in 10% sodium hydroxide for 5 hours, I was obtained quantitatively, and the prolongation of the reaction time of hydrolysis decreased the yield of I. DL-Allylglycine-(^{15}N) (II) was

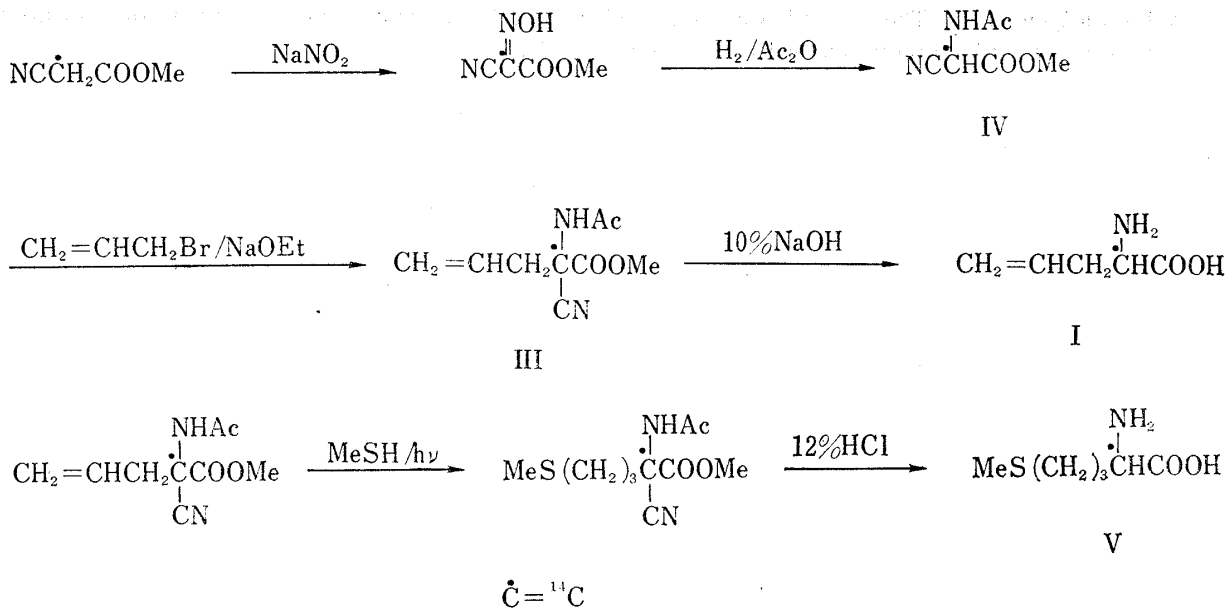

Chart 1. Synthesis of Allylglycine-2-^{14}C and Homomethionine-2-^{14}C

1) Location: *9-1, 4-Chome, Anagawa, Chiba-shi, Chiba.*
2) M. Matsuo and M. Yamazaki, *Biochem. Biophys. Res. Comm.*, **25**, 269 (1966).
3) H.L. Goering, S.J. Cristol, and K. Dittmer, *J. Am. Chem. Soc.*, **70**, 3310 (1948).

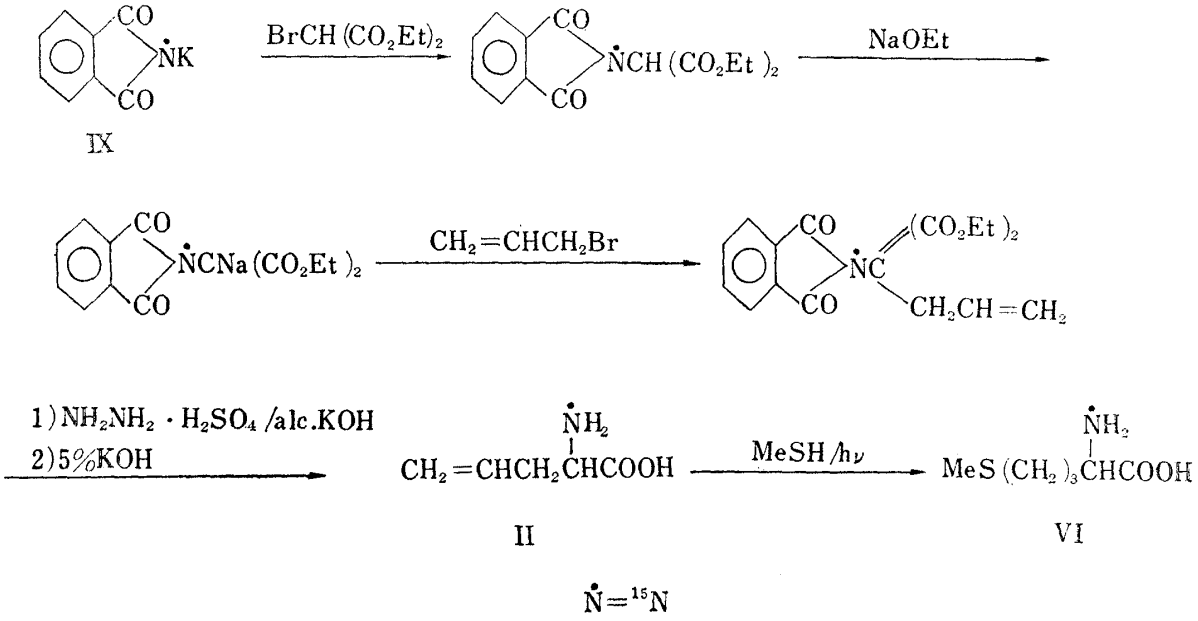

Chart 2. Synthesis of Allylglycine-^{15}N and Homomethionine-^{15}N

obtained by treatment of ethyl 2-carboethoxy-2-phthalimidopenten-(4)-ate(^{15}N), derived from diethyl sodium phthalimidomalonate (^{15}N) and allyl bromide, with hydrazine sulphate and 5% sodium hydroxide.

DL-Homomethionine(2-^{14}C, ^{15}N) was obtained by mixing DL-homomethionine(2-^{14}C) (V) and DL-homomethionine(^{15}N) (VI). (V and VI) were prepared by a slightly modified method of Kjaer and Wagner.[4] DL-Homomethionine(2-^{14}C) (V) was obtained by the hydrolysis of methyl 2-cyano-2-acetylamido-5-methylthiopentanoate(2-^{14}C), which was derived from III and methyl mercaptan. DL-Homomethionine(^{15}N) (VI) was synthesized by the addition of methyl mercaptan to II. The synthetic processes of I, II, V and VI were showed in Charts 1 and 2.

DL-Homomethionine(G-^{3}H) was synthesized by the Wilzbach method.

3-Methylthiopropionamide(1-^{14}C) (VII) and 4-methylthiobutyramide(1-^{14}C) (VIII) were synthesized by the similar method. The synthetic processes of VII and VIII were showed in Chart 3.

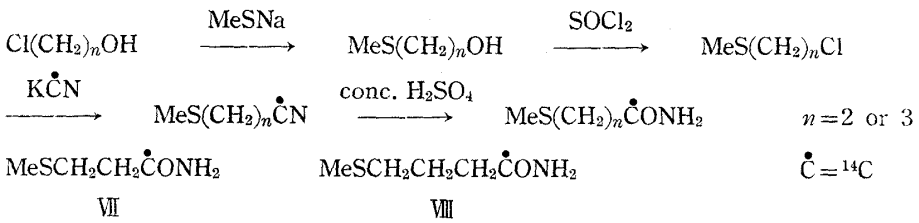

Chart 3. Synthesis of 3-Methylthiopropionamide-1-^{14}C and 4-Methylthiobutyramide-1-^{14}C

Experimental

DL-Allylglycine(2-^{14}C)——Methyl acetamidocyanoacetate (2-^{14}C) (540 mg) and allyl bromide (0.4 ml) were dissolved in EtOH (15 ml) containing Na (80 mg). The mixture was refluxed for 2 hr in dried atmosphere. After the solvent was removed *in vacuo*, the residue was extracted with anhyd. ether. Ether was evaporated *in vacuo*. Recrystallization of the product from 80% EtOH gave methyl 2-acetamido-2-cyanopenten-

4) A. Kjaer and S. Wagner, *Acta Chem. Scand.*, **9**, 721 (1955).

(4)-ate(2-^{14}C) (III). It was refluxed in 10% NaOH (15 ml) for 5 hr. The reaction mixture was acidified with conc. HCl (4.5 ml) and evaporated to dryness *in vacuo*. The residue was extracted by hot abs. EtOH and concentrated *in vacuo*. When the residue dissolved in the solution of 80% EtOH (30 ml) and pyridine (1 ml), was added to ether (150 ml), the crude I (394 mg) was separated. DL-Allylglycine(2-^{14}C) (I) was passed through a Dowex 50 (H^+) column and eluted with 2.5% NH_4OH. The effluent (50 ml) was collected and evaporated to dryness *in vacuo*. The addition of EtOH to the residue dissolved in small quantity of water yielded pure I (175 mg). mp 241—244° (decomp.). *Anal.* Calcd. for $C_5H_9O_2N$: C, 52.00; H, 7.87; N, 12.22. Found: C, 52.16; H, 7.87; N, 12.16. The yield from IV was 56%.

DL-Allylglycine(^{15}N)——Diethyl sodium phthalimidomalonate(^{15}N) (1.3 g) prepared by the reaction of potassium phthalimide(^{15}N) (IX) and diethyl bromomalonate, was dissolved in abs. EtOH (5 ml) and added to allyl bromide (0.38 ml). The mixture was refluxed for 2 hr, filtered and concentrated *in vacuo*. The residue was added to a mixture of hydrazine sulphate (1.3 g) and 1 N alc. KOH (22 ml), and refluxed for 2 hr. After the most of EtOH was removed under reduced pressure, 5% KOH (10 ml) was added to the mixture and refluxed for 1 hr. After cooling the solution was brought to pH 4 with AcOH, heated on the water bath for 15 min, filtered and concentrated *in vacuo*. The residue was dissolved in water (20 ml) and passed through a Dowex 50 (H^+) column. The effluent eluted with 5% NH_4OH, was collected and evaporated *in vacuo*. DL-Allylglycine(^{15}N) (II) (158 mg) was obtained by dissolving the residue in a small amount of water and addition of EtOH. The yield from IX was 22%.

DL-Homomethionine(2-^{14}C)——Unpurified methyl 2-acetamido-2-cyanopenten-(4)-ate(2-^{14}C), derived from IV (159 mg, 43 μCi/mM) by a method described above, was dissolved in EtOH (1 ml) and added to methyl mercaptan in a quarz tube. Moreover benzoyl peroxide (1 mg) and mercuric acetate (4 mg) were added to the mixture. The reaction vessel was saturated with oxygen, stopped tightly by a rubber stopper and exposed to ultraviolet light (a high presssure mercurry lamp, 200 W; Taika-rika Co., Ltd., Tokyo) at 0° for 7 hr. The solution was filtered and the filtrate was concentrated *in vacuo* below 50°. The residue was dissolved in 12% HCl (5 ml) and refluxed for 2 hr. The mixture was evaporated to dryness *in vacuo* and the residue was passed through a Dowex 50 (H^+) column. The effluent (50 ml) eluted with 2.5% NH_4OH was collected and concentrated *in vacuo*. DL-Homomethionine(2-^{14}C) (V) (39 mg) was obtained by dissolving the residue in a small amount of water and addition of EtOH. mp 250—252° (decomp.). *Anal.* Calcd. for $C_6H_{13}O_2NS$: C, 44.10; H, 7.90; N, 8.59. Found: C, 43.90; H, 7.90; N, 8.96. The yield from IV was 24%.

DL-Homomethionine(^{15}N)——The solution of DL-allylglycine(^{15}N) in water (3 ml) was added to methyl mercaptan (100 mg) in a quarz tube. Benzoyl peroxide (1 mg) and mercuric acetate (4 mg) were added to the mixture. The reaction vessel was saturated with oxygen and irradiated by an ultraviolet lamp at 0° for 5 hr. The reaction mixture was filtered and concentrated *in vacuo*. DL-Homomethionine(^{15}N) (VI) (55 mg) was obtained by the recrystallization from diluted EtOH. The yield from II was 76%.

DL-Homomethionine(G-^{3}H)——DL-Homomethionine (100 mg) was sealed with tritium gas (1 Ci) for 24 days in an ampule. The labelled material was dissolved in water (10 ml) and water was removed *in vacuo*. The treatment was repeated 5 times. The residue was purified by passing through a Dowex 50 (H^+) column and eluting with 2.5% NH_4OH. Recrystallization from diluted EtOH gave pure DL-homomethionine (G-^{3}H) (34.9 mg). The specific activity of it was 2.56 mCi/mM.

3-Methylthiopropionamide(1-^{14}C)——Ethylene chlorohydrin (40 g) was added to 20% CH_3SNa (200 g), stirred at room temperature for 1.5 hr and then refluxed gently for 30 min. After cooling, the mixture was extracted with ether. The ether solution was dried over anhyd. Na_2SO_4 and distilled under reduced pressure. β-Methylthioethanol (23 g) was obtained (bp 95—98° (60 mmHg)). $SOCl_2$ (12 g) in $CHCl_3$ (10 ml) was added dropwise to β-methylthioethanol (9.2 g) in $CHCl_3$ (10 ml) with stirring. After all the $SOCl_2$ solution had been added, stirring was continued for 45 min. The mixture was concentrated *in vacuo* and distillated under reduced pressure. β-Methylthioethyl chloride (5.7 g) was obtained (bp 77° (97 mmHg)).[5] β-Methylthioethyl chloride (133 mg)in 80% EtOH (1.5 ml) was added to K^{14}CN (65 mg, 250 μCi/mM) in a sealed tube. This was heated on the boilling water bath for 10 hr and evaporated to dryness *in vacuo*. The residue was extracted with ether (40 ml). The extract was dried over anhyd. Na_2SO_4 and concentrated *in vacuo*. Conc. H_2SO_4 (0.5 ml) was added to the residue at 0°. The mixture was allowed to stand at room temperature for 2 hr, neutralized with 28% NH_4OH and extracted with AcOEt (20 ml). The extract was dried over anhyd. Na_2SO_4 and evaporated *in vacuo*. Recrystallization from a mixture of MeOH and petr. ether gave VII (32.7 mg). mp 59°. *Anal.* Calcd. for C_4H_9ONS: C, 40.33; H, 7.62; N, 11.75. Found: C, 40.42; H, 7.66; N, 11.91. The yield from K^{14}CN was 27%.

4-Methylthiobutyramide(1-^{14}C)——γ-Methylthiopropyl chloride, derived from propylene chlorohydrin by such a similar method as β-methylthioethyl chloride was prepared, was added to K^{14}CN (65 mg, 250 μCi/mM) in 80% EtOH (1.5 ml). The mixture was heated in a sealed tube on a boilling water bath for 10 hr. The product was hydrolyzed with conc. H_2SO_4 (0.5 ml). 4-Methylthiobutyramide(1-^{14}C) (57 mg) was obtained. mp 79—80°. *Anal.* Calcd. for $C_5H_{11}ONS$: C, 45.08; H, 8.32; N, 10.52. Found: C, 44.97; H, 8.16; N, 10.42. The yield from K^{14}CN was 43%.

5) W.R. Kirner, *J. Am. Chem. Soc.*, **50**, 2451 (1928).

It was examined by dilution analysis and thin-layer chromatoscanner that all the compounds described above were radiochemically pure. The radioactivities were measured by using Tri-Carb liquid scintillation spectrometer, series 314 EX (Packard Instrument Company, Inc.).

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