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Potential Antitumor *N*-Carbamoyl-*N'*-methyl-*N'*-nitroso Derivatives of Amino Acids

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Synopsis. *N*-Carbamoyl-*N'*-methyl-*N'*-nitroso derivatives of amino acids have been prepared by treating the amino acids with methyl isocyanate followed by nitrosation with sodium nitrite in dilute acetic acid solution.

An *N*-alkyl-*N*-nitrosoureido group seems to be an essential functional group for the appearance of biological activity in streptozotocin[1] and its analogues.[2-4] In all the compounds studied so far in our laboratory, the functional group was attached to a carbohydrate residue, which does not seem to have an inconvertible role in their biological activity. Therefore, it might be possible to replace the carbohydrate moiety by other groups without losing the antitumor activity.

In the present paper, we wish to report a preparation of *N*-carbamoyl-*N'*-methyl-*N'*-nitroso derivatives of amino acids.

Potassium cyanate[5] or aryl isocyanate[6,7] have been used to prepare carbamoyl derivatives of amino acids. Under similar reaction conditions, methyl isocyanate was used to prepare *N*-carbamoyl-*N'*-methyl derivatives of glycine, alanine, aspragine, phenylalanine and methionine in the present study. The *N*-carbamoyl-*N'*-methyl derivatives of phenylalanine and methionine were obtained only as the potassium salts. Acidification of the salts resulted in an intramolecular cyclization giving hydantoin derivatives, which had large negative optical rotations.[8] Formation of hydantoin from a carbamoyl derivative under more drastic conditions has been described in a literature.[5,9-11]

Nitrosation of the *N*-carbamoyl-*N'*-methyl derivatives was performed in aqueous acetic acid solution with sodium nitrite at 0 °C, and the structure of the product was determined by PMR. The spectrum of the product had a singlet at τ 6.8—6.9 corresponding to N–CH_3 protons, clearly indicating the absence of a proton on the nitrogen atom attached to the methyl group.

The antitumor activities will be reported in the near future in an appropriate journal.

Experimental

Glycine, L-alanine, L-asparagine, L-phenylalanine and L-methionine were purchased from Asahi Kasei. Melting points were determined in capillary tubes and are uncorrected. PMR spectra were measured at 60 and 100 MHz on a Varian A-60D and a JEOL JMN-MH 100 spectrometers with reference to tetramethylsilane as an internal standard and the peak positions are given in τ values.

General Procedure for N-*Carbamoyl*-N'-*methyl Amino Acids.* The amino acid was dissolved in an equivalent molar amount of 10% potassium hydroxide solution and methyl isocyanate was added to the solution under ice cooling. The mixture was agitated overnight at room temperature and the pH was brought to 2—3 with Amberlite IR-120 (H^+ type). The solution was evaporated under reduced pressure below 30 °C to give a crude product.

N-*Carbamoyl*-N'-*methyl-glycine* (**1**). The crude crystalline product was recrystallized from methanol to give **1**, mp 147—148 °C, in 81% yield.

Found: C, 36.44; H, 6.07; N, 21.53%. Calcd for $C_4H_8N_2O_3$: C, 36.36; H, 6.10; N, 21.20%.

N-*Carbamoyl*-N'-*methyl*-L-*alanine* (**3**). The crude product was crystallized from ethanol and recrystallized from the same solvent to give **3**, mp 137—138 °C, $[\alpha]_D^{16}$ +3.0° (*c* 1.0, water), in 56% yield.

Found: C, 40.79; H, 6.74; N, 19.07%. Calcd for $C_5H_{10}N_2O_3$: C, 41.09; H, 6.90; N, 19.17%.

N-*Carbamoyl*-N'-*methyl*-L-*asparagine* (**5**). The crude product was crystallized from ethanol to give **5**, mp 142—143 °C, $[\alpha]_D^{15}$ +7.5° (*c* 1.0, water), in 66% yield.

Found: C, 38.32; H, 5.95; N, 22.44%. Calcd for $C_6H_{11}N_3O_4$: C, 38.09; H, 5.86; N, 22.21%.

Potassium Salt of N-*Carbamoyl*-N'-*methyl*-L-*phenylalanine* (**7**). The reaction mixture was evaporated without the treatment with Amberlite IR-120. The residue was crystallized from ethanol and the crystalline product was recrystallized from the same solvent to give **7** as hygroscopic needles, mp 179 °C (dec.), $[\alpha]_D^{24}$ +34.2° (*c* 1.0, water), in 69% yield.

Found: C, 47.28; H, 5.14; N, 9.67%. Calcd for $C_{11}H_{13}N_2O_3K \cdot H_2O$: C, 47.47; H, 5.43; N, 10.06%.

Potassium Salt of N-*Carbamoyl*-N'-*methyl*-L-*methionine* (**9**). The mixture was evaporated without the treatment with Amberlite IR-120. The residue was crystallized from isopropyl alcohol and the product was recrystallized from the same solvent to give **9**, mp 162—164 °C, $[\alpha]_D^{22}$ +4.7° (*c* 1.0, water), in 91% yield.

Found: C, 34.19; H, 5.46; N, 11.46%. Calcd for $C_7H_{13}N_2O_3SK$: C, 34.41; H, 5.36; N, 11.46%.

5-Benzyl-3-methyl-hydantoin (L-*Phenylalanine hydantoin*) (**11**). A 2.3 g portion of **7** was dissolved in water (20 ml) and the solution was adjusted to pH 3 with Amberlite IR-120 (H^+ type) to give an oily precipitate. Ethanol was added to the mixture until the precipitate was dissolved. The ion exchange resin was removed by filtration and the filtrate was evaporated to give a crystalline residue. The residue was recrystallized from methanol to give 1.6 g (93%) of **11**, mp 164—166 °C, $[\alpha]_D^{21}$ −103° (*c* 1.0, ethanol). PMR ($CDCl_3$): τ 7.12 (s, 3H, NCH_3).

Found: C, 64.58; H, 5.94; N, 13.79%. Calcd for $C_{11}H_{12}N_2O_2$: C, 64.69; H, 5.92; N, 13.71%.

3-Methyl-5-(methylmercapto)ethyl-hydantoin (L-*Methionine hydantoin*) (**12**). Compound **9** (6.0 g) was dissolved in water (50 ml) and the solution was adjusted to pH 3 with Amberlite IR-120 (H^+ type). The solution was evaporated under reduced pressure to give a syrup, which yielded crystals in 2-methoxyethanol at −20 °C. Recrystallization from the same solvent afforded 0.7 g (13%) of **12**, mp 88—89 °C, $[\alpha]_D^{17}$ −56.1° (*c* 1.0, ethanol). PMR ($CDCl_3$): τ 7.02 (s, 3H, NCH_3).

Found: C, 45.00; H, 6.36; N, 14.59; S, 17.33%. Calcd for $C_7H_{12}N_2O_2S$: C, 44.66; H, 6.43; N, 14.88; S, 17.03%.

General Procedure for Nitrosation. The *N*-carbamoyl-*N'*-methyl amino acid or its potassium salt was dissolved in aqueous acetic acid and sodium nitrite was added to the solution under ice cooling with agitation. The solution was settled overnight and afterwards treated with Amberlite IR-120 (H^+ type), the solution was lyophylized to give a crude product.

N-*Carbamoyl*-N'-*methyl*-N'-*nitroso-glycine* (**2**). The crude product was crystallized from chloroform to give **2**, mp 116—117 °C in 63% yield. PMR (C_5D_5N): τ 5.46 (d, 2H, CH_2), 6.86 (s, 3H, NCH_3); (C_5D_5N–D_2O): τ 5.32 (s, 2H, CH_2).

Found: C, 30.12; H, 4.43; N, 25.91%. Calcd for $C_4H_7N_3O_4$: C, 29.82; H, 4.38; N, 26.08%.

N-*Carbamoyl*-N'-*methyl*-N'-*nitroso*-L-*alanine* (**4**). The residue was crystallized from ether to give **4**, mp 85—87 °C, $[\alpha]_D^{15}$ +71° (*c* 1.0, chloroform) in 62% yield. PMR (C_5D_5N): τ 4.7—5.1 (m, 1H, CH), 6.84 (s, 3H, NCH_3); (C_5D_5N–D_2O): τ 5.08 (q, 1H, CH).

Found: C, 34.07; H, 5.16; N, 24.21%. Calcd for $C_5H_9N_3O_4$: C, 34.29; H, 5.18; N, 23.99%.

N-*Carbamoyl*-N'-*methyl*-N'-*nitroso*-L-*asparagine* (**6**). The crude product was crystallized from ethanol to give **6**, mp 139 °C, $[\alpha]_D^{26}$ +12° (*c* 0.5, water), in 61% yield. PMR (C_5D_5N): τ 4.2—4.5 (m, 1H, CH), 6.84 (s, 3H, NCH_3); (C_5D_5N–D_2O): τ 4.49 (t, 1H, CH).

Found: C, 33.25; H, 4.70; N, 25.52%. Calcd for $C_6H_{10}N_4O_5$: C, 33.03; H, 4.62; N, 25.68%.

N-*Carbamoyl*-N'-*methyl*-N'-*nitroso*-L-*phenlalanine* (**8**). A 0.5 g portion of **11** and sodium nitrite (0.21 g) were dissolved in cold water (10 ml) and a mixture of glacial acetic acid (0.2 ml) and ether (10 ml) was added to the solution cooled by ice with gentle agitation. After the mixture was stirred overnight at room temperature, the etheral layer was washed with water, dried over anhydrous sodium sulfate and evaporated. The residue was crystallized from ethyl acetate–*n*-pentane to give 0.23 g (51%) of **8**, mp 97—98 °C, $[\alpha]_D^{28}$ −18° (*c* 0.2, ethanol). PMR ($CDCl_3$): τ 4.7—5.1 (m, 1H, CH), 6.82 (s, 3H, NCH_3); ($CDCl_3$–D_2O): 4.92 (t, 1H, CH)

Found: C, 52.53; H, 5.21; N, 16.71%. Calcd for $C_{11}H_{13}N_3O_4$: C, 52.59; H, 5.21; N, 16.73%.

N-*Carbamoyl*-N'-*methyl*-N'-*nitroso*-L-*methionine* (**10**). **9** (1.0 g) was dissolved in water (6 ml), and the solution was added to a mixture of sodium nitrite (0.48 g), glacial acetic acid (0.4 ml) and water (3 ml) under ice cooling with agitation. After standing at room temperature overnight, the solution was treated with Amberlite IR-120 (H^+ type) and extracted with chloroform. The chloroform solution was dried over anhydrous sodium sulfate and evaporated. The residue was crystallized from acetone to give 780 mg (75%) of **10**, mp 103—104 °C, $[\alpha]_D^{22}$ +17° (*c* 1.0, chloroform). PMR ($CDCl_3$): τ 4.8—5.3 (m, 1H, CH), 6.81 (s, 3H, NCH_3); ($CDCl_3$–D_2O): τ 5.19 (t, 1H, CH).

Found: C, 35.97; H, 5.63; N, 18.12; S, 13.35%. Calcd for $C_7H_{13}N_3O_4S$: C, 35.74, H, 5.57; N, 17.86; S, 13.63%.

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