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25. Noboru Takahayashi, Yutaka Mio, Tatsushi Oka, Tsukasa Shima, Noboru Shimahara, and Miwako Iki: Synthesis of Actinomycin Analogs. I.*1

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It is well known that actinomycins are effective antibiotic but unexpectedly show strong toxicity. In 1956, the structure of actinomycin-C_3 was determined by Brockmann and his co-workers;[1] the chromophoric moiety bound with cyclic lactone of pentapeptide. On the other hand, Johnson, *et al.*[2] and Fujita[3] reported chemical structure of other actinomycins, and Hackmann, *et al.*[4] reported that actinomycins were hyperplasiastatic and had effect for Hodgikin's disease.[5]

A carcinogenetic activity of actinomycin-S[6] is interesting and it is probably due to its combination with deoxyribonucleic acid.[7] An analog of a chromophoric unit of actinomycins, hydroxyanthranilic acid, has carcinogenetic and carcinolytic activity, and antimycin, a structural analog of actinomycin chromophore, is a carcinostatic.[9] These facts suggested that cytostatic compounds could be expected from derivatives, which are analogous to the chromophoric moiety of actinomycins.

Actinomycin-C_3 was synthesized by Brockmann, *et al.*[10] and antimycic acid and its derivatives by Okumura, *et al.*,[11] while N-(3-hydroxyanthraniloyl)-glycine, a metabolite of *Bombyx mori*, was synthesized by Kikkawa, *et al.*[12] N-(1-Phenoxazinylcarbonyl)glycine ethyl ester was prepared by Predvoditeleva, *et al.*[13]

In the present investigation on the significance of the methyl groups of actinomycin chromophore, the syntheses of N-(2-nitro-3-methoxybenzoyl)-amino acids and their derivatives were carried out. 2-Nitro-3-methoxybenzoyl chloride was prepared by chlorination of carboxylic acid[14] compound with thionyl chloride. The amino acids or their methyl esters were treated with the above chloride in aqueous tetrahydrofuran at 0° to 5°. By these processes, glycine methyl ester, DL-alanine methyl ester, O-benzyl-L-serine methyl ester, L-threonine methyl ester, DL-threonine methyl ester, L-threonine, and O-benzyl-L-serine were derived to N-(2-nitro-3-methoxybenzoyl) compounds in 46~73% yield. The

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optically active compounds were not racemized in these reactions. Some of these compounds were hydrogenated in the presence of palladium-charcoal catalyst, producing 2-amino-3-methoxybenzoyl compounds.

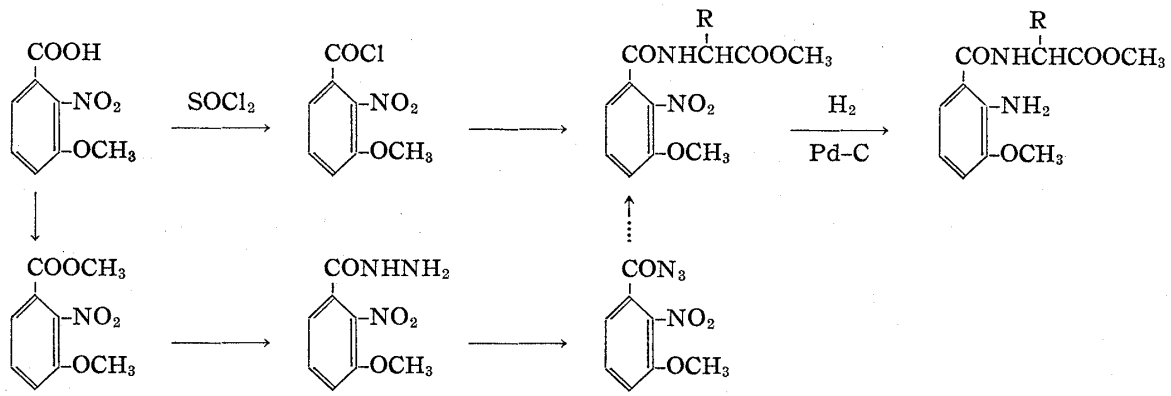


Chart 1.

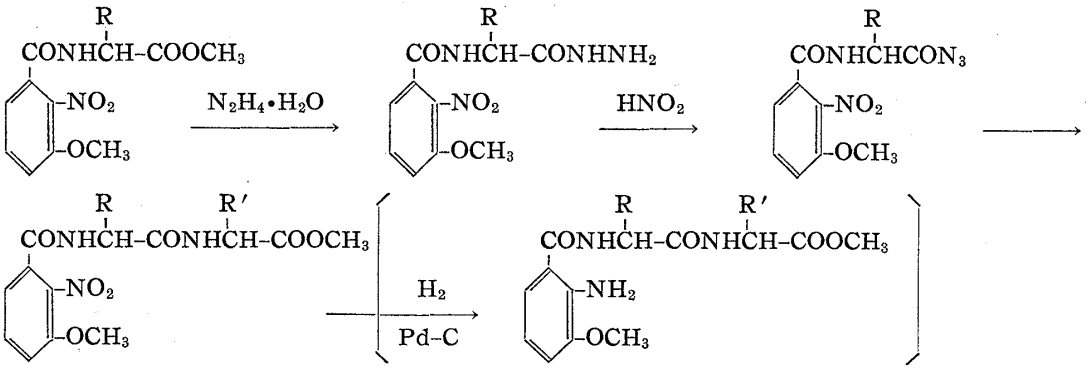


Chart 2.

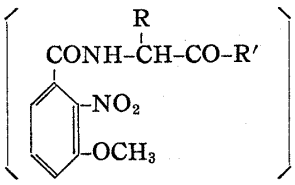
2-Nitro-3-methoxybenzoic acid was also esterified with methanol saturated with hydrogen chloride. This methyl ester and N-(2-nitro-3-methoxybenzoyl)-glycine methyl ester were derived to hydrazides, which were treated with nitrous acid at a low temperature (−5° to 0°). Thus 2-nitro-3-methoxybenzoyl azide as crystals and N-(2-nitro-3-methoxybenzoyl)-glycyl azide as amorphous were respectively obtained. The former azide was treated with L-threonine methyl ester, but only slightly viscous syrup was obtained. N-(2-Nitro-3-methoxybenzoyl)-glycyl-DL-valine methyl ester was prepared from the latter azide with DL-valine methyl ester at room temperature.

The ultraviolet absorption spectra of these synthesized products were taken in 95% ethanol. The N-(2-nitro-3-methoxybenzoyl) compounds have absorption maxima at 220 and 292~294 mμ, and the 2-amino compounds at 217, 331, and 340 mμ. The solution of the amino compounds showed fluorescence.

Experimental

General Method for N-(2-Nitro-3-methoxybenzoyl)-amino Acid Methyl Esters from 2-Nitro-3-methoxybenzoyl Chloride——A mixture of 0.01 mole of 2-nitro-3-methoxybenzoic acid and 12 g. of $SOCl_2$ was refluxed gently for 1 hr. and the excess of $SOCl_2$ was removed in a reduced pressure. The residue was dissolved in absolute tetrahydrofuran and this solution was added in small portions to an amino acid methyl ester solution (prepared from 0.01 mole of hydrochloride with 10 ml. of 1*N* NaOH solution) with stirring and ice cooling. During the reaction, the solution was kept alkaline to Thymol Blue with 2*N* NaOH solution. The stirring was continued for 1 hr. after the addition of acid chloride. The reaction mixture was extracted with Et_2O, the Et_2O layer was dried over Na_2SO_4,

Table I. N-(2-Nitro-3-methoxybenzoyl)-amino Acids and their Methyl Esters



No.	Amino acid residue (R)	Sufix (R′)	Formula	Crystal form[a]	Yield (%)	m.p. (°C)	Analysis (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
1[b]	H (Gly)	OCH_3	$C_{11}H_{12}O_6N_2$	Colorless prisms	46	116	49.26	4.51	10.44	49.21	4.46	10.18
2[c]	CH_3 (DL-Ala)	OCH_3	$C_{12}H_{14}O_6N_2$	Colorless prisms	60	104	51.06	5.00	9.93	50.96	5.10	9.84
3	CH_3-CH(OH) (DL-Thr)	OCH_3	$C_{13}H_{16}O_7N_2$	Colorless prisms	73	144	50.00	5.17	8.97	50.14	5.29	8.28
4[d]	CH_3-CH(OH) (L-Thr)	OCH_3	$C_{13}H_{16}O_7N_2$	Colorless needles	51	158.5～160	50.00	5.17	8.97	49.80	5.08	8.75
5	$C_6H_5CH_2$-O-CH_2- (L-Ser)	OCH_3	$C_{19}H_{20}O_7N_2$	Slightly yellow prisms	60	84.5～85.5	56.18	5.19	7.21	56.73	5.02	7.11
6[e]	$C_6H_5CH_2$-O-CH_2- (L-Ser)	OH	$C_{18}H_{18}O_7N_2$	Colorless small prisms	51	90	57.75	4.85	7.48	57.41	5.02	7.29
7[f]	CH_3-CH(OH) (L-Thr)	OH	$C_{12}H_{14}O_7N_2$	Colorless prisms	50	154	48.33	4.73	9.39	48.31	5.07	9.06

a) Recrystallized from methanol or ethanol.
b) UV λ_{max}^{EtOH} mμ (log ε): 220 (4.06), 292 (3.38).
c) UV λ_{max}^{EtOH} mμ (log ε): 220 (4.03), 294 (3.36).
d) $[\alpha]_D^{23}$ −96° (c=1, MeOH)
e) $[\alpha]_D^{20.5}$ −36° (c=0.25, MeOH)
f) $[\alpha]_D^{23}$ −73° (c=1, MeOH)

and the solvent mixture of Et_2O and tetrahydrofuran was evaporated in a reduced pressure. The residue was purified by recrystallization from MeOH or EtOH (cf. Table I).

General Method for N-(2-Nitro-3-methoxybenzoyl)-amino Acids from 2-Nitro-3-methoxybenzoyl Chloride——A solution of 2-nitro-3-methoxybenzoyl chloride (prepared from 0.01 mole of 2-nitro-3-methoxybenzoic acid with 12 g. of $SOCl_2$) in tetrahydrofuran and a solution of 0.01 mole of amino acid in 10 ml. of 1*N* NaOH solution were treated as above. The reaction mixture was extracted with Et_2O, the aqueous layer was acidified with 10% HCl, and extracted with Et_2O. The solvent was removed and the residue was washed with water. The product was recrystallized from benzene, MeOH, or EtOH (cf. Table I).

Methyl 2-Nitro-3-methoxybenzoate——A solution of 2 g. of 2-nitro-3-methoxybenzoic acid dissolved in 20 ml. of MeOH saturated with dry HCl was refluxed for 1 hr., and the solvent and HCl were evaporated to dryness in a reduced pressure. The residue was recrystallized from MeOH to colorless plates, m.p. 136～137°. Yield, 1.9 g. (89%). *Anal.* Calcd. for $C_9H_9O_5N$: C, 51.19; H, 4.30; N, 6.59. Found: C, 51.38; H, 4.37; N, 6.59.

2-Nitro-3-methoxybenzoic Acid Hydrazide——In a three-necked flask equipped with a mechanical stirrer and reflux condenser, 1.7 g. of methyl 2-nitro-3-methoxybenzoate was dissolved in 20 ml. of MeOH and the solution was boiled to reflux. Under stirring, a solution of 4.6 ml. of 85% N_2H_4-H_2O in 20 ml. of MeOH was poured into the ester solution. The stirring and refluxing was continued for 2 hr., and the reaction mixture was cooled in an ice box for 24 hr. The product was collected by filtration. The mother liquor was concentrated to half the original volume in a reduced pressure with cooling, the second crop of crystals thus obtained was combined with the first crop, washed with Et_2O and H_2O, and dried over P_2O_5. 1.6 g. of white crystals, m.p. 174～174.5°, were obtained from MeOH. Yield, 94%. *Anal.* Calcd. for $C_8H_9O_4N_3$: C, 45.50; H, 4.27; N, 19.90. Found: C, 45.99; H, 4.46; N, 19.81.

2-Nitro-3-methoxybenzoyl Azide——In a 200 ml. three-necked flask equipped with a mechanical stirrer was placed a solution of 0.5 g. of 2-nitro-3-methoxybenzoic acid hydrazide dissolved in a mixture of 20 ml. of dioxane and 5 ml. of dimethylformamide. The solution was cooled at −5°

to 0° with continued stirring, 1.2 ml. of 6*N* HCl and 0.51 ml. of cold 5*N* $NaNO_2$ were added in small portions. After 0.5 hr., 16.3 ml. of 1*N* $NaHCO_3$ was added carefully into the reaction mixture and after additional 15 min., 60 ml. of water at −3° to 0°. Slightly grey crystals that precipitated out were collected in an ice cold sintered glass filter, washed with cold water, and dried in vacuum at 0° for about 48 hr. Yield, 0.47 g. (89%), m.p. 115~116° (decomp.). *Anal.* Calcd. for $C_8H_6O_4N_4$: C, 43.25; H, 2.72; N, 25.22. Found: C, 42.66; H, 2.78; N, 24.80.

N-(2-Nitro-3-methoxybenzoyl)-glycine Hydrazide——2 g. of N-(2-nitro-3-methoxybenzoyl)glycine methyl ester and 50 ml. of 10% $N_2H_4 \cdot H_2O$ were treated as for 2-nitro-3-methoxybenzoic acid hydrazide. Colorless needles, m.p. 161°, were obtained by recrystallization from MeOH. Yield, 1.6 g. (80%). *Anal.* Calcd. for $C_{10}H_{12}O_5N_4$: C, 44.78; H, 4.51; N, 20.88. Found: C, 44.47; H, 4.50; N, 20.71.

N-(2-Nitro-3-methoxybenzoyl)-glycyl Azide——A solution of 1.6 g. of N-(2-nitro-3-methoxybenzoyl)-glycine hydrazide dissolved in a mixture of 52 ml. of dioxane and 13 ml. of dimethylformamide was cooled to −5° to 0° with continued stirring, and were added in small portions 1.2 ml. of 6*N* HCl and 1.33 ml. of cold 5*N* $NaNO_2$. After 1 hr., 42.4 ml. of 1*N* $NaHCO_3$ was added carefully into the reaction mixture and after additional 15 min., 50 ml. of H_2O at −3° to 0°. The AcOEt solution was kept at 5° for 15 hr., washed with ice-cold water, 2*N* HCl, $NaHCO_3$, and water, and dried over Na_2SO_4 in ice box. The solvent was removed in vacuum with cooling. A crude product, m.p. 68°, was obtained. Yield, 0.9 g. (58%).

N-(2-Nitro-3-methoxybenzoyl)-glycyl-DL-valine Methyl Ester——To 0.4 g. of valine methyl ester hydrochloride dissolved in 15 ml. of tetrahydrofuran, 0.33 ml. of triethylamine was added. The white crystals of triethylamine hydrochloride were filtered off and the filtrate was cooled with a freezing agent. To a solution of 0.5 g. of azide dissolved in cold tetrahydrofuran the former solution was added and the mixture was kept at 0° for 1 hr. and then at room temperature overnight. The solvent was evaporated and dried up in vacuum below 30°. The residue was recrystallized from MeOH to colorless prisms, m.p. 172.5~173.5°. Yield, 0.38 g. (56%). UV λ_{max}^{EtOH} mμ (log ε): 220 (4.07), 292 (3.36). *Anal.*Calcd. for $C_{16}H_{21}O_7N_3$: C, 52.31; H, 5.76; N, 11.44. Found: C, 52.22; H 5.80; N, 11.61.

Reaction of 2-Nitro-3-methoxybenzoyl Azide with L-Threonine Methyl Ester——To a solution of 4.5 g. of L-threonine methyl ester hydrochloride dissolved in 5 ml. of dried dioxane, 0.33 ml. of triethylamine was added cautiously with stirring. The colorless precipitate of triethylamine hydrochloride was filtered off, the dioxane solution of L-threonine methyl ester was cooled to 0°, 0.47 g. of 2-nitro-3-methoxybenzoyl azide was added in small portions with stirring, and the mixture was stood overnight at 0°. The solvent was evaporated to dryness in a reduced pressure below 30°. A slightly green viscous syrup was obtained and it was treated with MeOH and EtOH, but did not solidify.

N-(2-Amino-3-methoxybenzoyl)-glycine Methyl Ester——To a solution of 1.0 g. of N-(2-nitro-3-methoxybenzoyl)-glycine methyl ester in 50 ml. of MeOH, 0.5 g. of Pd-C (5%) was added and the mixture was shaken in H_2 atmosphere for 15 min. After the calculated amount of H_2 had been absorbed, the catalyst was filtered off and the filtrate was concentrated to dryness in a reduced pressure. The residue was purified by recrystallization from MeOH to slightly pink leaflets, m.p. 81~83°. Yield, 0.85 g. (96%). UV λ_{max}^{EtOH} mμ (log ε): 217 (4.16), 331 (3.65), 340 (3.63). *Anal.* Calcd. for $C_{11}H_{14}O_2N_2$: C, 55.45; H, 5.92; N, 11.76. Found: C, 55.45; H, 6.01; N, 12.28.

N-(2-Amino-3-methoxybenzoyl)-DL-alanine Methyl Ester——A solution of 1 g. of N-(2-nitro-3-methoxybenzoyl)-DL-alanine methyl ester and 0.5 g. of Pd-C in 100 ml. of MeOH was shaken in H_2 atmosphere. After absorption of 235 ml. of H_2, the reduction was completed. The fluorescent solution was filtered and the solvent was evaporated in a reduced pressure. Recrystallization of the reddish oily residue from Et_2O gave colorless needles, m.p. 56°. The yield was about theoretical. UV λ_{max}^{EtOH} mμ (log ε): 217 (4.14), 331 (3.59), 340 (3.52). *Anal.* Calcd. for $C_{12}H_{16}O_2N_4$: C, 57.13; H, 6.39; N, 11.07. Found: C, 57.01; H, 6.43; N, 11.03.

The other nitro compounds shown in Table I were also hydrogenated as above and oily or viscous syrupy products were obtained. Succeeding work on the reduction products and syntheses of N-(2-nitro-3-benzyloxybenzoyl)-peptide and their hydrogenated compounds will be described in a later paper.

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Summary

In the course of investigation on the synthesis of analog of actinomycin, L-threonine, O-benzyl-L-serine, and methyl esters of glycine, DL-alanine, L-threonine, DL-threonine, and O-benzyl-L-serine were acylated with 2-nitro-3-methoxybenzoyl chloride or azide. Some of these products were catalytically hydrogenated to 2-amino-3-methoxybenzoyl compounds. N-(2-Nitro-3-methoxybenzoyl)-glycyl-DL-valine methyl ester was synthesized from N-(2-nitro-3-methoxybenzoyl)-glycyl azide.

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