recrystallized from EtOH to give pale yellow needles, mp 227—229°. *Anal.* Calcd. for $C_{21}H_{25}O_5N \cdot HClO_4$: C, 53.45; H, 5.55; N, 2.97. Found: C, 53.36; H, 5.50; N, 2.72. IR cm^{-1} (KBr): $\nu_{C=\overset{+}{N}H}$ 1655.

1,2,3,4-Tetrahydro-5,6,7-trimethoxy-1-(3,4-dimethoxybenzyl)isoquinoline (XXI)——To a suspension of 0.20 g of 3,4-dihydroisoquinoline (XX) perchlorate in 50 ml of MeOH was added portionwise 0.2 g of $NaBH_4$ with stirring at room temperature, and the stirring was continued for further 1 hr at room temperature. After refluxing for 1 hr, the reaction mixture was worked up as usual, and the benzene extract was dried over K_2CO_3 and the solvent was removed by distillation to leave 141 mg of 1,2,3,4-tetrahydroisoquinoline (XXI) as a pale yellow viscous syrup, whose oxalate was recrystallized from EtOH to give colorless needles, mp 205—207°. *Anal.* Calcd. for $C_{21}H_{27}O_5N \cdot C_2H_2O_4$: C, 59.60; H, 6.31; N, 3.02. Found: C, 59.21; H, 6.49; N, 2.87.

5,6,13,13a-Tetrahydro-2,3,4,10,11-pentamethoxy-8H-dibenzo[*a*,*g*]quinolizine (VIII)——1,2,3,4-Tetrahydroisoquinoline (XXI) hydrochloride (387 mg), prepared from the oxalate of XXI as usual, was mixed with 10 ml of 37% CH_2O and 10 ml of water, and the resultant mixture was heated on a water-bath for 2 hr. After cooling, the reaction mixture was basified with conc. NH_4OH aq. solution and extracted with benzene. The extract was washed with water and dried over K_2CO_3. Removal of the solvent afforded 238 mg of quinolizine derivative (VIII) as a pale yellowish-orange viscous syrup, which was recrystallized from EtOH to give colorless needles, mp 135—136°. *Anal.* Calcd. for $C_{22}H_{27}O_5N$: C, 68.55; H, 7.06; N, 3.63. Found: C, 68.86; H, 7.19; N, 3.44. IR cm^{-1} (KBr): ν_{max} 2720—2800 (Bohlmann bands). NMR (ppm) (CCl_4): 3.72 (9H, singlet, three OCH_3), 3.76 (3H, singlet, OCH_3), 3.78 (3H, singlet, OCH_3), 6.41 (2H, singlet, aromatic protons), and 6.49 (1H, singlet, aromatic proton).

Acknowledgement We are grateful to the Analytical Centers of Pharmaceutical Institute, Tohoku University and Tokyo College of Pharmacy for microanalyses and NMR determination. We also thank President Dr. M. Terasaka and Dr. S. Nagase of Tokyo College of Pharmacy for their grateful encouragement.

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UDC 615.31 : 547.466.2.04

Studies on Application of Amino Acid as Medicinal Agent. II.[1] Reaction of Amino Acid Ester with Difunctional Grignard Reagent

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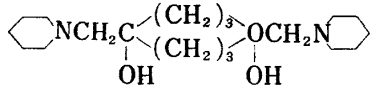
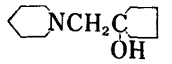
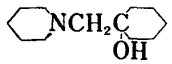
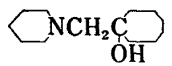
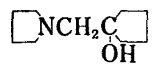
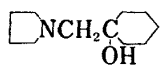
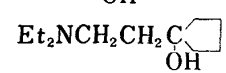
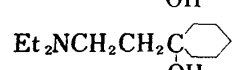
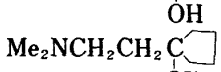
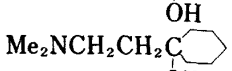
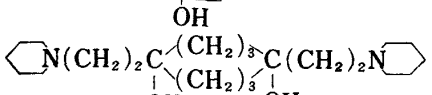
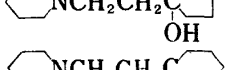
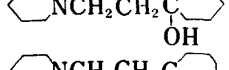
In the previous paper, in order to find non-narcotic analgesis, a number of substituted amino-*tert*-alcohol derivatives were synthesized by the reaction of various substituted amino acid esters with a variety of Grignard reagents. Later, we attempted to prepare cyclic amino-*tert*-alcohols. This paper deals with the reaction of N,N-disubstituted α- and β-amino acid ester with difunctional Grignard reagent containing polymethylene group.

The reaction was carried out using ethyl piperidino and pyrrolidinoacetate as α-amino acid ester, and ethyl N,N-dimethyl-β-amino, N,N-diethyl-β-amino, β-piperidino and β-pyrrolidinopropionate as β-amino acid ester. These amino acid esters were prepared by the reaction of ethyl chloroacetate and ethyl acrylate with the corresponding amines, respectively. While, difunctional Grignard reagent was prepared by treating polymethylenedihalide, containing more than two carbon atoms in polymethylene group, with magnesium in absolute ether

1) Part I: S. Hayashi, M. Furukawa, Y. Fujino, and T. Ohkawara, *Chem. Pharm. Bull.* (Tokyo), 17, 145 (1969).

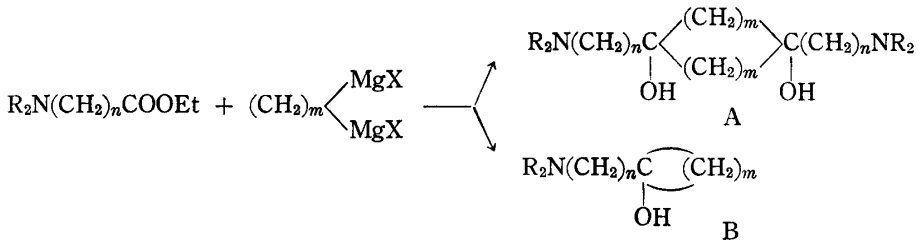
2) Location: *a*) *Oe-hon-machi, Kumamoto*; *b*) *Kashima-cho, Higashiyodogawa, Osaka.*

TABLE I

Structure	Formula	Mol. wt. calcd.	Rast	bp°C/mm	Yield %	Analysis % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
	$C_{20}H_{38}O_2N_2$	338.6	313.8	100/13	16	71.38	11.38	8.33	71.35	11.11	8.17
	$C_{11}H_{21}ON$	183.3	196.1	115/20	40	72.08	11.57	7.64	72.28	11.50	7.29
	$C_{12}H_{23}ON$	197.3	227.1	122/ 9	69	73.03	11.75	7.10	72.93	11.84	7.22
	$C_{13}H_{25}ON$	211.3	232.8	102/ 2.5	21	73.88	11.92	6.63	73.50	11.96	6.56
	$C_{10}H_{19}ON$	169.3	193.2	70/ 2.5	23	70.96	11.31	8.28	71.39	11.73	8.07
	$C_{11}H_{21}ON$	183.3	225.0	91/ 2.5	38	72.08	11.55	7.64	72.18	11.52	7.97
	$C_{11}H_{23}ON$	185.3	225.2	83/2	17	71.29	12.51	7.56	70.89	12.55	7.27
	$C_{12}H_{25}ON$	197.3	212.8	100/ 7.5	14	72.30	12.64	7.03	71.71	12.51	6.74
	$C_9H_{19}ON$	157.3	190.3	56/ 2	20	68.74	12.18	8.91	68.94	11.83	8.66
	$C_{10}H_{21}ON$	171.3	216.4	89/ 5.5	37	70.12	12.36	8.18	70.29	12.42	8.00
	$C_{22}H_{42}O_2N_2$	366.6	312.6	92/ 3.2	11	72.00	11.55	7.69	72.07	11.86	7.26
	$C_{12}H_{23}ON$	197.3	223.9	104/ 2.3	34	73.03	11.75	7.10	72.65	11.73	6.95
	$C_{13}H_{25}ON$	211.3	244.1	115/ 2.9	37	73.88	11.92	6.63	73.38	11.62	6.95
	$C_{14}H_{27}ON$	225.8	275.5	113/ 2.9	23	74.61	12.08	6.22	73.98	12.05	6.24

by the usual manner. To the etheral solution of the Grignard reagent thus prepared was immediately added the amino acid ester, followed by mild reflux, at such a rate that ether boils gently, for half an hour. Then the reaction mixture was hydrolyzed with dilute hydrochloric acid, and the aqueous layer was made alkaline with ammonium hydroxide. The separated oily product was extracted with ether and purified by distillation.

In this reaction, it should be assumed to give rise to either type A or B of two possible cyclic compounds, as shown in the following chart.



Of polymethylenedimagnesium dihalide, ethylenedimagnesium dihalide (m=2) failed to react with amino acid esters. However, polymethylenedimagnesium dihalide containing three or more carbon atoms (m=3,4,5,6) were allowed to react with all of the amino acid esters employed to give the corresponding cyclic amino-*tert*-alcohols, type A or B. The elemental analysis can not determine whether the product should be A or B, because the elemental composition of A is the same as that of B. Also, it would be presumed to be difficult to distinguish them by their infrared (IR) and nuclear magnetic resonance (NMR) spectra. On the other hand, we could readily determine by measuring the molecular weight by Rast's method if the cyclic amino alcohol obtained was type A or B. The experimental and the calculated values of the molecular weight of these compounds obtained are shown in Table I.

As shown in the Table I, trimethylenedimagnesium dihalide (m=3) reacted with ethyl piperidinoacetate and ethyl piperidinopropionate to afford the type A of cyclic aminoalcohol, formed by condensation of each two molecules. On the other hand, polymethylenedimagnesium dihalides (m=4,5,6) containing more than three carbon atoms gave compounds of type B by the similar reaction with amino acid esters. Distillation of these compounds obtained was readily carried out under reduced pressure without dehydration. Additionally, only in the case of m=4, a little of higher boiling fraction was obtained in addition to the main product of type B. The structure of the product was not able to clarify, because of its extrem small quantity. However, from the IR spectrum, it was presumed to be type A compound. These experimental results suggested that the cyclic aminoalcohols smaller than five-membered ring were difficult to obtain in these reactions.

The pharmacological activities of these compounds obtained will be reported in the other paper.

Experimental

Ethyl Pyrrolidinoacetate——To 70 g (0.57 mole) of ethyl chloroacetate was added dropwise with cooling and stirring 80 g (1.14 mole) of pyrrolidine. The reaction mixture was allowed to stand overnight and then poured into ice water. The oily products separated were extracted with ether, washed with H_2O and dried over $CaCl_2$. After evaporation of ether, the residue was distilled under reduced pressure to give 45 g (51%) of colorless liquid boiling at 98—99°/23—24 mm. *Anal.* Calcd. for $C_8H_{15}O_2N$: C, 61.12; H, 9.62; N, 8.91. Found: C, 61.14; H, 9.56; N, 8.97.

Ethyl Piperidinoacetate——Prepared from 60.0 g (0.704 mole) of piperidine and 43 g (0.352 mole) of ethyl chloroacetate by the method of Bischoff,[3] bp 114°/28 mm. Yield was 47 g (78.3%).

Ethyl β-Dimethylaminopropionate——Prepared from 22.5 g (0.2 mole) of a 40% aqueous solution of dimethylamine and 10 g (0.1 mole) of ethyl acrylate by the method of Adamson,[4a] bp 65—66°/24 mm.

3) C.A. Bishoff, *Ber.*, **31**, 2840 (1898).
4) *a*) D.W. Adamson, *J. Chem. Soc.*, **1949**, 144. *b*) B. Flürscheim, *J. Prakt. Chem.*, **68**, 350 (1903).

Yield was 11 g (75.9%). *Anal.* Calcd. for $C_7H_{15}O_2N$: C, 57.90; H, 10.42; N, 9.65. Found: C, 57.65; H, 10.45; N, 9.52.

Ethyl β-Piperidinopropionate——Prepared from 64 g (0.8 mole) of piperidine and 40 g (0.4 mole) of ethyl acrylate by the method of Adamson,[4a] bp 127—128°/32 mm. Yield was 63.5 g (88.2 %). *Anal.* Calcd. for $C_{10}H_{19}O_2N$: C, 64.83; H, 10.34; N, 7.56. Found: C, 64.96; H, 10.42; N, 7.69.

Polymethylenedimagnesium Dihalide——Magnesium turnings (0.2 atom) were covered with 20 ml of ether and a small amount of polymethylene dihalide was added and warmed to iniciate the reaction. If the reaction was not iniciated, a catalytic amount of iodine or ethyl bromide was added to the reaction mixture. As soon as the reaction was iniciated, the remainder of the halide (total 0.1 mole) was gradually added at such a rate that the mixture boils gently. Then the mixture was gently warmed until magnesium turnings were completely dissolved. The solution of polymethylenedimagnesium dihalide thus obtained was immediately used to the next reaction.

General Procedure for Synthesis of Cyclic Amino-*tert*-alcohol——To a ethereal solution of 0.1 mole of polymethylenedimagnesium dihalide prepared by the procedure described above, a solution of 0.025 mole of disubstituted amino acid ester was added at such a rate that the reaction mixture boils continuously. After addition, the mixture was gently refluxed for 30 min to complete the reaction, and then treated with 15% aqueous solution of HCl containing ice pieces. The aqueous layer was separated and made alkaline with aqueous NH_4OH solution. The separated oily product was extracted with ether, washed with H_2O and dried over Na_2SO_4. After removal of ether, the residue was purified by distilation under reduced pressure. Oxalic acid salt of piperidinomethylcyclopentan-1-ol was prepared by treating piperidinomethylcyclopentan-1-ol with the equimolar amount of oxalic acid in water. Prisms, mp 123—124°.

Acknowledgement We are grateful to Miss. H. Sato for infrared analyses and Miss K. Ogata for elemental analyses.

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UDC 612.398.145.014.46

Inhibition and Stimulation of the Biosynthesis of Protein and Nucleic Acid. IV.[1] Effect of 2-Amino-1,4-naphthoquinone Imine on the Biosynthesis of Nucleic Acid and Protein in Ehrlich Mouse Ascites Tumor Cells *in Vitro*

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(Received October 5, 1968)

In the previous papers dealing with the biological action of aminoquinone derivatives, it was shown that, among some derivatives, 2-amino-1,4-naphthoquinone imine-HCl (ANQI) had an intensive action to inhibit the synthesis of nucleic acid and protein, especially of DNA, in Ehrlich mouse ascites tumor cells *in vitro*,[1] and that this action was assumed to be attributable to its interacting activity with the moieties of purine bases in nucleic acid, particularly in native DNA.[3]

The present investigation was undertaken to clarify the details in the mode of inhibition of *in vitro* DNA, RNA, and protein synthesis in the tumor cells by ANQI. From the data described below, the following assumption may be possible; the inhibition of RNA and protein synthesis is not a primary action of ANQI, but a phenomenon being led secondarily by the inhibition of DNA synthesis.

1) Part III: S. Okada, *Chem. Pharm. Bull.* (Tokyo), **17**, 105 (1969).
2) Location; *Oshika, Shizuoka.*
3) S. Okada, *Chem. Pharm. Bull.* (Tokyo), **17**, 113 (1969).