UDC 615.778 : 615.784.6

93. Mitsuru Furukawa and Shigeshi Toyoshima : Researches on Chemotherapeutic Drugs Against Viruses. XXXV.*1 Syntheses and Antiviral Activity of Alkylated Ephedrine and its Related Compounds.

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As reported in the previous papers,[1] some compounds of alkylated ephedrine were found to possess inhibitory effects on Japanese B encephalitis virus. This finding suggested that the effect of those alkylated ephedrine derivatives might be due to alkyl group introduced onto the benzene ring of ephedrine, since ephedrine itself and its side-chain substitutes were found not to have any activity on the virus.

In addition of those studies, the authors[2] reported about the syntheses of 1-dialkylphenyl-2-aminopropanol derivatives, but have not published their antiviral activity, yet.

In succession of those findings, the authors synthesized 1-(methoxy-methylphenyl)-2-aminopropanol and N-substitutes of 1-dialkylphenyl-2-aminopropanol in these studies. This paper describes the syntheses of 1-(methoxy-methylphenyl)-2-aminopropanol and N-substitutes of 1-dialkylphenyl-2-aminopropanol, and the antiviral activity of these compounds.

Synthesis of 1-(2-Methoxy-5-methylphenyl)-2-aminopropanol Derivatives——*p*-Methylanisole, employed as the starting material, was condensed with propionic anhydride in the presence of aluminium chloride in carbon disulfide, and 2′-methoxy-5′-methylpropiophenone thus obtained was isonitrized with methyl nitrite. The resulting isonitroso derivative was reduced to the corresponding aminoketone by catalytic hydrogenation in the presence of palladium-carbon. The catalytic reduction of the aminoketone was, however, observed difficult to be carried out, though 2-aminopropiophenone is, in general, known to be readily converted into the corresponding aminoalcohol by catalytic hydrogenation. This fact was considered due to the steric hinderence with methoxyl group substituted at 2-position on benzene ring. Hereupon, this catalytic reduction of 2-amino-2′-methoxy-5′-methylpropiophenone was, with success, carried out under more drastic condition. The whole process of the synthesis is shown in Chatr 1.

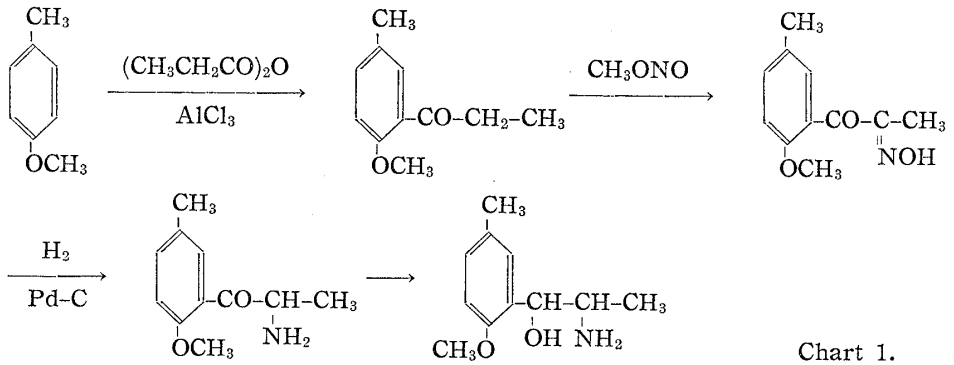

Chart 1.

*1 This paper constitutes a part of a series entitled "Researches on Chemotherapeutic Drugs against Viruses" by Takeo Ueda.

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1) T. Ueda, S. Toyoshima, K. Takahashi, M. Muraoka : Keio J. Med., 8, 199 (1959).
2) M. Furukawa, T. Ueda : This Bulletin, 8, 867 (1960).
3) W.H. Hartung, J.C. Munch : J. Am. Chem. Soc., **51**, 2262 (1929).

At next, attempts were made to obtain N-methyl derivative of 1-(2-methoxy-5-methylphenyl)-2-aminopropanol. The monomethylation of the compound was, however, observed difficult because of the ease of further methylation. Therefore, as the improved method, the reduction of 2-(methylbenzylamino)-2′-methoxy-5′-methylpropiophenone was conceived, which had been prepared by the condensation of 2-bromo-2′-methoxy-5′-methylpropiophenone with methylbenzylamine.[4] In practice, it was found that the objective compound, 1-(2-methoxy-5-methylphenyl)-2-methylaminopropanol was produced in only a low yield by the catalytic reduction and debenzylation of 2-(methylbenzylamino)-2′-methoxy-5′-methylpropiophenone. This finding suggested that the polymerization probably competed with the catalytic hydrogenation in this reaction. The whole process is illustrated in Chart 2.

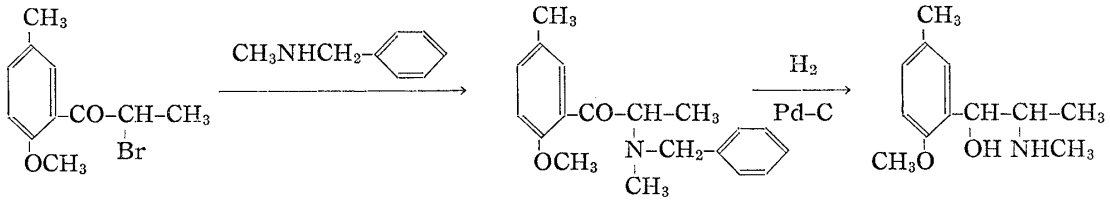


Chart 2.

Synthesis of 1-(3, 4-Dimethylpheny)-2-alkylaminopropanol——As described in the previous report,[2] there are the two geometrical isomers of 1-(3,4-dimethylphenyl)-2-aminopropanol, owing to the difference of the steric configuration. Therefore, there should exist also two geometrical isomers i.e. *erythro* and *threo* of 1-(3,4-dimethylphenyl)-2-monoalkylaminopropanol.

Higher homologs of 1-(3, 4-dimethylphenyl)-2-monoalkylaminopropanol than the butyl, were readily synthesized by the direct monoalkylation of two steric isomers of 1-(3,4-dimethylphenyl)-2-aminopropanol with alkyl halides.

However, this method of monoalkylation was observed unapplicable to the syntheses of the lower homologs, because of the ease of further alkylation. The treatment of 1-(3,4-dimethylphenyl)-2-aminopropanol with an slight excess of methyl iodide afforded [2-(3,4-dimethylphenyl)-2-hydroxyisopropyl]trimethylammonium iodide, while the alkylation with halides of ethyl and propyl gave tertiary amino derivatives. Hereupon, the

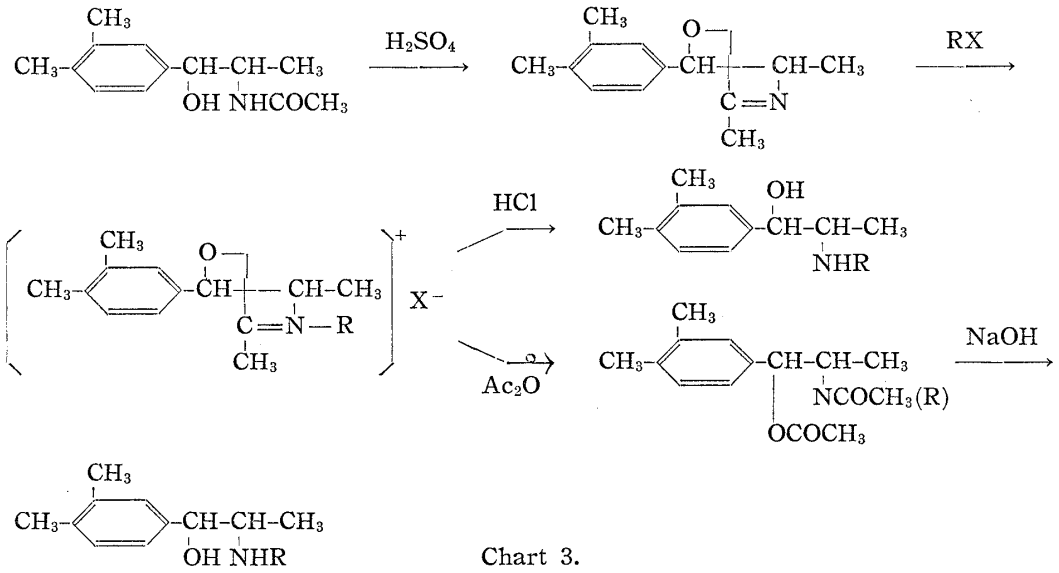
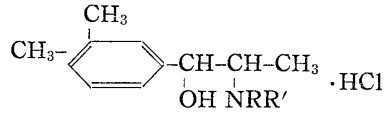


Chart 3.

4) H. Takamatsu, Y. Minaki : Yakugaku Zasshi, **76**, 1234 (1956).

following special method was found available for the syntheses of the lower homologs. The lower homologs were, with success, synthesized by the ring cleavage after the alkylation of *threo*-2,4-dimethyl-5-(3,4-dimethylphenyl)oxazoline, which had been prepared through the ring closure of 1-(3,4-dimethylphenyl)-2-acetamidopropanol with concentrated sulfuric acid,[5] with alkyl halide. This special synthetic process is shown in Chart 3.

The compound synthesized by the two methods are listed in Table I.

TABLE I. 

R	R′	From	m.p. (°C)	Mol. formula	N% Calcd.	N% Found
C_2H_5	H	*erythro*	235	$C_{13}H_{22}ONCl$	5.74	5.86
〃	〃	*threo*	218	〃	5.74	5.82
〃	C_2H_5	*erythro*	203	$C_{15}H_{26}ONCl$	5.15	5.37
〃	〃	*threo*	202～203	〃	5.15	5.28
C_3H_7	H	*erythro*	232	$C_{14}H_{24}ONCl$	5.43	5.26
〃	〃	*threo*	231	〃	5.43	5.23
〃	C_3H_7	*erythro*	143～145 (free)	$C_{17}H_{29}ON$	5.31	5.18
C_4H_9	H	*erythro*	222～223	$C_{15}H_{26}ONCl$	5.15	5.08
〃	〃	*threo*	203～204	〃	5.15	5.22
C_5H_{11}	〃	*erythro*	222～223	$C_{16}H_{28}ONCl$	4.89	4.83
〃	〃	*threo*	211～212	〃	4.89	4.85
C_6H_{13}	〃	*erythro*	218～219	$C_{17}H_{30}ONCl$	4.67	4.71
〃	〃	*threo*	209～210	〃	4.67	4.66
C_8H_{17}	〃	*erythro*	232～233	$C_{19}H_{34}ONCl$	4.27	4.33
〃	〃	*threo*	218～219	〃	4.27	4.33
$C_{10}H_{21}$	〃	*erythro*	211～212	$C_{21}H_{38}ONCl$	3.93	4.11
〃	〃	*threo*	202～203	〃	3.33	4.14

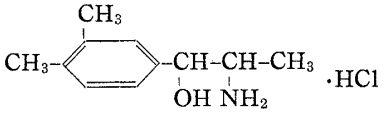
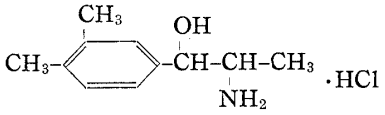
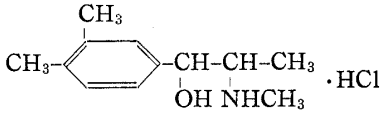
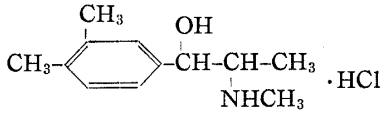
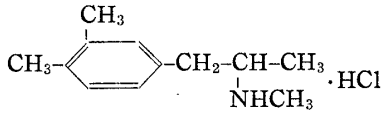
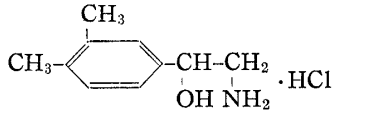
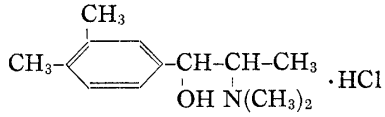
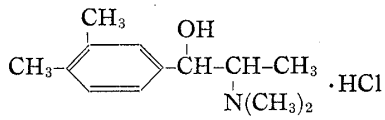
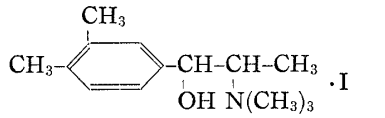
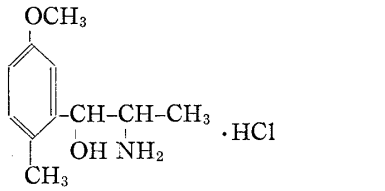
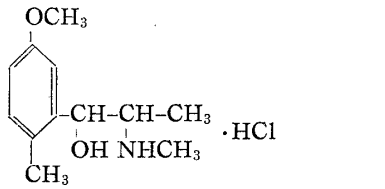
Screening Tests with Compounds Related to 1-Alkylphenyl-2-aminopropanol. Primary Screening Tests in Jap-B—HeLa Cells System——The Nakayama strain of Japanese B encephalitis virus was used for the test. Virus dilution of the Nakayama strain in concentration of 10^{-2}～10^{-3} was added into assay tubes containing HeLa Cells added with the maximal non-toxic dose of the test compound, and the concentration of the compound in the mixture was adjusted at 10^{-3}～10^{-4} mole. After the content of the tube was incubated at 37°, for 3 days, 0.03 cc. of the content was inoculated intracerebrally into groups of mice of 8～10 g. in body weight. After daily observation of these mice for 2 weeks, the mortality was calculated for the treated and untreated groups. The experimental results were shown in Table II.

As can be seen from Table II, [2-(3,4-dimethylphenyl)-2-hydroxyisopropyl]trimethylammonium iodide and 1-(2-methoxy-5-methylphenyl)-2-aminopropanol hydrochloride were considered as the significant.

Secondary Screening Tests *in vivo*——The two significant compounds were rescreened as to their activity *in vivo* on the Nakayama strain. $10^{-2.2}$ virus dilution (corresponding to $1\times LD_{50}$) was inoculated into groups of mice of 8～10 g. in body weight, and 72 hours later, 1/2～1/3 dose of LD_{50} of each test compound was injected intraperitoneally into groups of mice with a single dose. After daily observation of mice for two weeks, the mortality was calculated for the treated and untreated groups. The experimental results are shown in Table III.

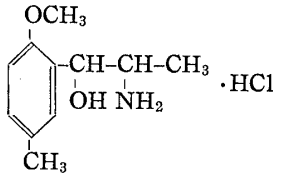
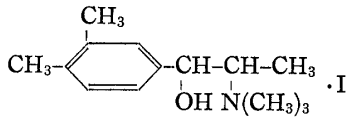
5) T. Taguchi, M. Kojima : Yakugaku Zasshi, **74**, 1293 (1954).

Table II.

Structure formula	Max. non-toxic conc. (M)	Dead mice/total used mice			
		10^{-3}		10^{-4}	
		Treated	Untreated	Treated	Untreated
	10^{-4}	5/5	5/5		
	10^{-4}	5/5	5/5		
	10^{-4}	5/5	5/5		
	10^{-4}	5/5	5/5		
	10^{-4}			4/5	5/5
	10^{-4}			4/5	5/5
	10^{-4}			5/5	5/5
	10^{-4}			4/5	5/5
	10^{-4}	5/5	5/5	1/5	5/5
	10^{-4}	5/5	5/5	2/5	5/5
	10^{-4}			4/5	5/5

As can be seen from Table III, it may be said that the intraperitoneal administration of the dose corresponding 1/3 LD_{50} of [2-(3,4-dimethylphenyl)-2-hydroxyisopropyl]-trimethylammonium iodide was significantly effective on the Nakayama strain, while 1-(2-methoxy-5-methylphenyl)-2-aminopropanol hydrochloride, uneffective.

Table III.

Compound	LD_{50} in mice (mg./kg.)	Singtle dose administered (mg./kg.)	Results: Treated	Results: Untreated
	42 (ip)	21 (ip)	7/14	6/20
		14 (ip)	9/15	
		7 (ip)	4/14	
	81 (ip)	40 (ip)	6/25	7/28
		26 (ip)	2/27	

Direct Virus-inactivating Action——The direct virus-inactivating action of [2-(3,4-dimethylphenyl)-2-hydroxyisopropyl]trimethylammonium iodide was examined as follows: 10^{-2} virus dilution of the Nakayama strain and 1 mg./cc. of the compound were mixed in a test tube. After the incubation at 22° for 24 hours, 0.03 cc. of the mixture was inoculated intracerebrally into mice. After the daily observation of mice for 14 days, the mortality of the treated group was compared with that of the control groups. The experimental results are shown in Table IV. From Table IV, it may be said that the compound did not show any direct virus-inactivating activity or has extremely weak effectiveness.

Table IV. Virus Inactivating Action of [2-(3,4-Dimethylphenyl)-2-hydroxyisopropyl]trimethylammonium Iodide on Jap-B.

Virus dilution	Virus control	Treated group	Virus dilution	Virus control	Treated group
10^{-5}	4/4	5/5	10^{-7}	0/5	1/5
10^{-6}	4/4	4/5	10^{-8}	0/5	0/5

Moreover, all of the above compounds were examined as to their activity on Type-1 Mahoney strain of polio virus and Type-1 strain of adeno virus, by tissue culture method. However, all of the compounds were found inactive against the both viruses.

As described above, the authors examined the activity of the compounds related to 1-alkylphenyl-2-aminopropanol on the Nakayama strain of Japanese B encephalitis virus, Type-1 Mahoney strain of polio virus and Type-1 strain of adeno virus, and found that [2-(3,4-dimethylphenyl)-2-hydroxyisopropyl]trimethylammonium iodide was effective on only the Nakayama strain, and this effect was not due to its virus-inactivating activity. However, the pharmacological properties as to their sympathomimetic effect remain as the problem to study. The work on these problems will be reported in another paper.

Experimental

General Procedure for Preparation of *dl-erythro-* or *threo*-1-(3,4-Dimethylphenyl)-2-alkylaminopropanol Hydrochloride Lower Homologs——A mixture of 0.01 mole of *threo*-2,4-dimethyl-5-(3,4-dimethylphenyl)oxazoline and 0.01 mole of alkyl halide in 10 cc. of anhyd. benzene was refluxed on a steam bath for 1 hr. After removal of the benzene, the residue was respectively converted to the two geometrical isomers regarding to the method shown by Taguchi and Kojima.

Higher Homologs——0.01 mole of *dl-erythro-* and *threo*-1-(3,4-dimethylphenyl)-2-aminopropanol in anhyd. EtOH added excess K_2CO_3 were respectively treated with alkyl bromide for 2 hr. under refluxing. Dry HCl was introduced into the filtered solution and the product that appeared on cooling or concentration was collected by filtration and recrystallized from EtOH. Analytical data of the compounds obtained thus are summarized in Table I.

2′-Methoxy-5′-methylpropiophenone——65 g. of propionic anhydride (0.5 mole) was added slowly with stirring to the mixture of 54 g. of *p*-methylanisole (0.5 mole) and 143 g. of powdered anhyd. $AlCl_3$ (1.1

mole) in 200 cc. of CS_2. Stirring was continued for further 3 hr. after the completion of addition. The reaction mixture was poured into crushed ice and seperated oil was extracted with Et_2O. The Et_2O layer was washed with dil. NaOH solution, next with H_2O and dried over $CaCl_2$. After the solvent was distilled off, the residue was distilled *in vacuo*. Yield. 57 g. (65 %). $b.p_{15}$ 142°. Colorless liquid.

2,4-Dinitrophenylhydrazone——Yellow plates from EtOH. m.p. 110°. *Anal.* Calcd. for $C_{17}H_{18}O_5N_4$; N, 15.64. Found. N, 15.35.

2-Isonitroso-2′-methoxy-5′-methylpropiophenone——Dry HCl was passed through the solution of 35.6 g. of 2′-methoxy-5′-methylpropiophenone in 140 cc. of 17.1 g. dry Et_2O, while methyl nitrite, evolved by adding dropwise dil. H_2SO_4 solution into the mixture of 16 g. of sodium nitrite, 10.6 cc. of MeOH and 10 cc. of H_2O, was introduced into the stirred solution. During the introduction mixture was kept to reflux gently. After allowing to stand overnight, the mixture was repeatedly extracted with cold dil. NaOH solution. The extract was poured slowly, with stirring, into conc. HCl solution containing pieces of ice. The precipitate was collected by filtration, washed with H_2O and recrystallized from benzene. Yield. 32.5 g. (85.7 %). Colorless prisms, m.p. 100°. *Anal.* Calcd. for $C_{11}H_{13}O_3N$; N, 6.76. Found. N, 6.50.

2-Amino-2′-methoxy-5′-methylpropiophenone Hydrochloride——A solution of 12 g. (0.06 mole) of 2-isonitroso-2′-methoxy-5′-methylpropiophenone in 150 cc. of anhyd. EtOH containing excess of dried HCl was reduced in the presence of Pd-C catalyst, until calculated amount of H_2 was absorbed. The catalyst was filtered off and the product separated on concentration and cooling was collected by filtration and recrystallized from EtOH. Colorless needless.

1-(2-Methoxy-5-methylphenyl)-2-methylaminopropanol Hydrochloride——To a solution of 18 g. of 2′-methoxy-5′-methylpropiophenone in 100 cc. of benzene, 16 g. of Br_2 was added dropwise with stirring. After stirring for 1.5 hr. on a steam bath, the reaction mixture was washed with dil. K_2CO_3 solution, and dried over Na_2SO_4. To a mixture of the filtered solution and 14 g. of K_2CO_3 in 10 cc. of H_2O, 12 g. of N-methylbenzylamine was added with stirring. The mixture was warmed with stirring on a steam bath for 10 hr., the benzene layer was separated and washed with H_2O. After evaporation of benzene, the residue was dissolved in 50 cc. of MeOH containing excess of dry HCl. The solution was shaken in H_2 atmosphere in the presence of Pd-C catalyst at 70° under pressure. After removal of the catalyst by filtration, the filtrate was concentrated and the product was recrystallized from EtOH. Colorless needless. m.p. 182°. *Anal.* Calcd. for $C_{12}H_{20}O_2NCl$; N, 5.70. Found. N, 5.63.

The authors express their deepest thanks to United States Army, Research and Development Group (Far East) for the kind support to these studies.

Summary

In order to find antiviral compound, alkyl group was introduced into the neurotropic structure of ephedrine and activity of the resulting alkyl derivatives were examined against the Nakayama strain of Japanese B encephalitis virus, Type-1 Mahoney strain of polio virus and Type-1 strain of adeno virus. Among these derivatives, [2-(3,4-dimethylphenyl)-2-hydroxyisopropyl]trimethylammonium iodide and 1-(2-methoxy-5-methylphenyl)-2-aminopropanol showed *in vitro* effect, and only the former exerted *in vivo* activity on the Nakayama strain.

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