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Studies on Hypolipidemic Agents. I. Synthesis and Pharmacological Properties of Nicotinic Acid–Ethanolamine Derivatives

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A series of nicotinic acid–ethanolamine derivatives was synthesized and evaluated pharmacologically and biochemically in mice and rats. Many compounds showed considerable hypolipidemic activity in two models: hypercholesterolemic mice and hypertriglyceridemic rats. The results clarified some structure–activity relationships; there was an increase in efficacy resulting from the introduction of alkyl or aryl groups on the nitrogen of the ethanolamine part. Furthermore, it was clearly shown that coupling of ethanolamines with nicotinic acid (NA) resulted in a marked decrease in toxicity. Among the compounds tested, 2-(*N*-isopropyl-*N*-nicotinoylamino)ethyl nicotinate (**20**) was found to possess more favorable pharmacological and toxicological profiles than NA. Studies on **20** indicated that its hypolipidemic effect might be attributable to NA released by hydrolysis *in vivo* of the ester linkage. After administration of **20**, the maximum serum level of free NA was approximately 4 times lower and the persistence of NA level in the serum was longer than that of the NA-treated group.

Keywords——nicotinic acid derivative; ethanolamine analogue; hypolipidemic activity; structure–activity relationship; acute toxicity; hypercholesterolemic mouse; hypertriglyceridemic rat; serum nicotinic acid level

It is now accepted that there is a causal relationship between the increase in plasma lipid levels and the development of atherosclerotic disease.[1] Hence, increased lipid level is said to be one of the most prominent risk factors for atherosclerotic vascular degeneration.[2] Hyperlipidemia in atherosclerotic patients, however, can't be adequately controlled by dietary regulation alone.[3] Thus, many well-tolerated hypolipidemic drugs are widely used for the improvement of hyperlipidemia associated atherosclerosis.[4]

Nicotinic acid (NA) is a drug well-known for its ability to lower the level of plasma lipids[5] and has been employed as one of four drugs in a long-term clinical trial (the Coronary Drug Project in the U.S.A.).[6] According to Carlson,[7] this drug is a powerful inhibitor of lipolysis and as a consequence of the inhibition of lipid mobilization from the adipose tissue it lowers the biosynthesis of triglyceride-rich lipoprotein in the liver.

Although the pharmacological actions of NA have been noted with great interest for a long time, its clinical usefulness is restricted by its side effects, such as flushing, pruritus, and gastro-intestinal complaints. An additional disadvantage of this drug is its low potency, 3—6 g per day being required for an effective therapy. For these reasons, many attempts have been made to find more effective and less toxic compounds than NA. For example, NA was esterified with various polyalcohols in the hope of prolonging and intensifying the activity (*e.g.*, pentaerythritol tetranicotinate).[8] Chemical modification of the structure of NA was also carried out (*e.g.*, pyridyl–carbinol and –acetic acid).[9,10]

On the other hand, Bondesson[11] has reported that analogues of 2-aminoethanols have

hypolipidemic activity in rats. However, the mechanism of the hypolipidemic action of the compounds remain unknown. It was anticipated that more useful hypolipidemic agents might be found among compounds with ester or amide–ester linkages between NA and ethanolamine analogues. This paper describes the synthesis, and pharmacological and biochemical investigations, of a series of nicotinic acid–ethanolamine derivatives.

Results and Discussion

Most of the new compounds listed in Table I were synthesized in a usual manner and subjected to hypolipidemic activity testing in mice and rats and to acute toxicity testing in mice.

Hypolipidemic Activity

As shown in Table II, many derivatives of this series showed hypolipidemic activity in both models. NA, used as a reference drug, lowered the serum cholesterol level by 16—25% at a dose of 0.5% in the diet in hypercholesterolemic mice. In this model, many test compounds showed considerable hypocholesterolemic activities; compounds **2**, **10**, **20** and **22** were more than twice as active as NA.

In the ester series (**1**—**16**), nicotinoyloxyethylamine hydrogen chloride (**1**), a simple representative compound in this series, possessed hypocholesterolemic activity almost equivalent to that of NA. Changing the amino function from primary to secondary or tertiary by the introduction of alkyl (**2**—**5**) or aryl (**13**) groups resulted in a slight increase in activity. Of the primary amine derivatives (**1**, **6**, **9**—**12**), a methyl substitution on the hydroxy carbon (**6**) completely abolished the activity, while introduction of alkyl groups on the amino carbon (**9**—**12**) retained or raised the activity of the parent compound (**1**), except in the case of **12**, which showed lower activity.

Among *N*-alkyl or aryl-substituted derivatives, an additional methyl substitution on the hydroxy carbon (**7**, **14**) or dimethyl substitution on the amino carbon (**15**) resulted in a decrease in activity as compared with the parent compounds (**2**, **13**).

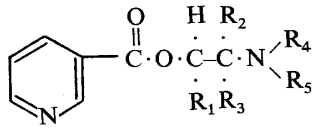
In the amide ester series (**18**—**34**), the activities of **20**, **22**, **29** and **31** were equal to or greater than that of NA. The compounds with a methyl on the hydroxy carbon (**24**, **32**) or dimethyl on the amino carbon (**33**) lost the activities of the parent compounds (**20**, **31**). However, the activities of **25** and **34**, in which a mono-methyl or -ethyl group was introduced on the amino carbon, were almost identical to that of NA.

The compounds were subjected to further testing in hypertriglyceridemic rats in order to examine the effect on triglyceride metabolism. When a fructose solution was provided as drinking water for 2 d, the triglyceride level in serum rose to about twice that in normal rats (see footnote to Table II). In this model, NA significantly lowered the serum triglyceride level by 37—47% at a dose of 300 mg/kg. The hypotriglyceridemic activities of **2**, **4**, **6**, **10**, **12**, **22**, **25** and **27** were much less than that of NA, while those of other compounds were equal to or greater than that of NA. However, no clear relationship could be found between the structures and hypotriglyceridemic activities of these compounds.

It is often the case that the degrees and spectra of hypolipidemic activities of different agents vary with the experimental animals and conditions used, because of the complexity of lipid metabolism. The sum of relative potencies (*RP*) in the present two models, therefore, is presented in Table II as total *RP* to indicate the general efficacy of each compound.

As judged from the total *RP*, the introduction of alkyl or aryl groups on the nitrogen resulted in a general increase in the activity (**1**→**2**, **3**, **13**; **6**→**7**, **8**, **14**; **22**→**24**; **27**→**34**), while lower alkyl groups introduced at the hydroxy carbon or amino carbon rather decreased the efficacy (**1**→**6**, **12**; **2**→**7**; **13**→**15**; **18**→**25**; **20**→**24**), although there were some exceptions.

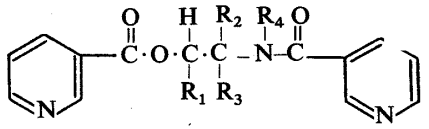
TABLE I. Physicochemical Properties of Nicotinic Acid–Ethanolamine Derivatives



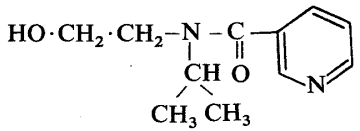
Compound No.	R_1	R_2	R_3	R_4	R_5	mp (°C)	Recrystn. solvent[a]	Yield (%)	Formula[b]	Analysis (%) Calcd (Found)		
										C	H	N
1	H	H	H	H	H	188—190	A	76	$C_8H_{12}Cl_2O_2N_2$	40.19 (40.19	5.09 5.21	11.72 11.50)
2	H	H	H	H	CH_3	164—167	A	76	$C_9H_{14}Cl_2O_2N_2$	42.71 (42.55	5.57 5.47	11.06 10.71)
3	H	H	H	CH_3	CH_3	187—190	A	61	$C_{10}H_{16}Cl_2O_2N_2$	44.96 (44.79	6.04 6.15	10.49 10.22)
4	H	H	H	C_2H_5	C_2H_5	132—134	A	61	$C_{12}H_{20}Cl_2O_2N_2$	44.73 (44.90	7.19 7.44	8.69 8.69)
5	H	H	H	H	n-C_4H_9	126—128	B	55	$C_{12}H_{20}Cl_2O_2N_2 \cdot 1H_2O$	45.96 (46.12	7.07 7.00	8.94 8.93)
6	CH_3	H	H	H	H	185—187	A	72	$C_9H_{14}Cl_2O_2N_2$	42.71 (42.50	5.57 5.62	11.06 10.95)
7	CH_3	H	H	H	CH_3	136—139	C	59	$C_{10}H_{16}Cl_2O_2N_2$	44.96 (45.02	6.04 6.20	10.48 10.54)

8	CH_3	H	H	H	Iso-C_3H_7	155—159	C	78	$C_{12}H_{20}Cl_2O_2N_2$	48.82 (48.99	6.83 7.09	9.49 9.23)
9	H	H	CH_3	H	H	124—127	C	64	$C_9H_{14}Cl_2O_2N_2$	42.71 (42.48	5.57 5.62	11.06 11.18)
10	H	CH_3	CH_3	H	H	180—183	C	45	$C_{10}H_{16}Cl_2O_2N_2$	44.96 (43.08	6.04 5.77	10.48 9.77)[c]
11	H	H	C_2H_5	H	H	172—173	C	51	$C_{10}H_{16}Cl_2O_2N_2$	44.96 (44.85	6.04 5.96	10.48 10.23)
12	H	H	*n*-C_3H_7	H	H	171—173	C	71	$C_{11}H_{18}Cl_2O_2N_2$	46.99 (46.75	6.45 6.51	9.96 9.69)
13	H	H	H	H	CH_2–C_6H_5	190—192	B	62	$C_{15}H_{18}Cl_2O_2N_2 \cdot 0.7H_2O$	52.70 (52.56	5.72 5.63	8.20 8.38)
14	CH_3	H	H	H	CH_2–C_6H_5	180—183	B	55	$C_{16}H_{20}Cl_2O_2N_2$	52.99 (55.73	5.87 5.95	8.16 7.96)
15	H	CH_3	CH_3	H	CH_2–C_6H_5	197—199	B	75	$C_{17}H_{22}Cl_2O_2N_2$	57.15 (57.50	6.21 6.34	7.84 7.52)
16	H	H	H	H	C_6H_{11}	184—187	B	54	$C_{14}H_{21}Cl_2O_2N_2$	59.05 (59.08	7.43 7.42	9.84 9.87)
17	H	H	H	H	Iso-C_3H_7	116—119	D	83	$C_{11}H_{18}Cl_2O_2N_2$	46.99 (47.56	6.45 6.31	9.96 9.27)[c]

(continued)



Compound No.	R_1	R_2	R_3	R_4	mp (°C)	Recrystn. solvent[a]	Yield (%)	Formula[b]	Analysis (%) Calcd (Found)		
									C	H	N
18	H	H	H	H	82—84	E	74	$C_{15}H_{15}O_3N_3$	63.15 (63.10	5.30 5.28	14.72 14.61)
19	H	H	H	C_2H_5	Viscous oil		70	$C_{16}H_{17}O_3N_3$	64.20 (64.30	5.73 5.85	14.03 14.20)
20 (Maleate)	H	H	H	Iso-C_3H_7	107—109	F	69	$C_{21}H_{23}O_7N_3$	58.74 (58.64	5.40 5.45	9.78 9.52)
21	H	H	H	*n*-C_4H_9	Viscous oil		24	$C_{18}H_{21}O_3N_3 \cdot 1/2H_2O$	64.27 (64.13	6.59 6.37	12.49 12.36)
22	CH_3	H	H	H	80—82	G	48	$C_{15}H_{15}O_3N_3$	63.15 (63.13	5.30 5.28	14.72 14.61)
23	CH_3	H	H	CH_3	Viscous oil		86	$C_{16}H_{17}O_3N_3$	64.20 (64.20	5.73 5.84	14.03 13.91)
24	CH_3	H	H	Iso-C_3H_7	63—66	H	76	$C_{18}H_{21}O_3N_3$	66.04 (65.77	6.47 6.51	12.83 12.76)
25	H	H	CH_3	H	139—142	C	61	$C_{15}H_{15}O_3N_3$	63.15 (63.28	5.30 5.48	14.72 14.39)
26	H	CH_3	CH_3	H	76—77	I	79	$C_{16}H_{17}O_3N_3$	64.20 (64.13	5.73 5.87	14.03 13.96)

27	H	H	C_2H_5	H	80—82	E	92	$C_{16}H_{17}O_3N_3$	64.20 (64.39	5.73 5.81	14.03 13.84)
28	H	H	n-C_3H_7	H	97—99	E	96	$C_{17}H_{19}O_3N_3$	65.16 (64.88	6.11 6.02	13.40 13.17)
29	H	H	H	C_6H_5	102—104	E	30	$C_{20}H_{17}O_3N_3$	69.15 (69.31	4.93 5.00	12.10 11.85)
30	H	H	H	C_6H_{11}	Viscous oil		46	$C_{20}H_{23}O_3N_3$	66.28 (66.58	6.68 6.66	11.59 11.23)
31	H	H	H	CH_2–C_6H_5	Viscous oil		82	$C_{21}H_{19}O_3N_3$	69.79 (69.51	5.30 5.33	11.63 11.62)
32	CH_3	H	H	CH_2–C_6H_5	Viscous oil		28	$C_{22}H_{21}O_3N_3$	70.38 (70.07	5.64 5.91	11.19 10.94)
33	H	CH_3	CH_3	CH_2–C_6H_5	80—82	J	45	$C_{23}H_{23}O_3N_3$	70.93 (70.88	5.95 6.01	10.79 10.82)
34	H	H	C_2H_5	CH_2–C_6H_5	Viscous oil		40	$C_{23}H_{23}O_3N_3$	70.29 (70.03	6.00 5.92	10.69 10.41)
35					Viscous oil		60	$C_{11}H_{16}O_2N_2$	63.44 (63.70	7.74 7.90	13.45 13.70)

a) A=ethyl alcohol–water; B=dimethylformamide–tetrahydrofuran; C=ethyl alcohol; D=dimethylformamide; E=ethyl acetate; F=acetone–ethyl ether; G=ethyl acetate–hexane; H=ethyl acetate–isopropyl ether; I=acetone–isopropyl ether; J=ethyl ether–hexane.
b) The structure of each compound was identified by IR, nuclear magnetic resonance and MS as well as elemental analysis.
c) Although the analytical results were not as expected because of its high hygroscopicity, the chemical structure was supported by the spectra as mentioned in footnote *b*).

TABLE II. Pharmacological Properties of Nicotinic Acid–Ethanolamine Derivatives

Compounds No.	Hypocholesterolemic activity in mice[a]		Hypotriglyceridemic activity in rats[b]		Total *RP* (A+B)	LD_{50} (g/kg, *p.o.*) in mice[d]
	% reduction from control	*RP* (A)[c]	% reduction from control	*RP* (B)		
Nicotinic acid	15.6[e]—25.1[e]	1.0	37.2[e]—47.0[g]	1.0	2.0	4.5 (3.5—5.4)
1	17.6[e]	1.1	53.7[g]	1.1	2.2	>5.0
2	41.5[g]	2.7	23.4	0.5	3.2	>5.0
3	47.1[g]	1.9	39.5[f]	0.8	2.7	>5.0
4	30.5[g]	1.2	11.0	0.2	1.4	—
5	34.2[g]	1.4	43.0[g]	0.9	2.3	=5.0
6	0	0	24.0	0.5	0.5	—
7	18.0	0.8	40.7[g]	0.9	1.7	—
8	8.3	0.4	44.4[f]	0.9	1.3	—
9	22.3	1.0	36.0[f]	0.8	1.8	=5.0
10	30.6[g]	2.0	18.4	0.4	2.4	=5.0
11	30.5[f]	1.4	33.1[f]	0.7	2.1	>5.0
12	9.0	0.4	30.1	0.6	1.0	—
13	42.5[g]	1.7	43.1[f]	0.9	2.6	<5.0
14	38.6[g]	1.5	50.6[g]	1.1	2.6	=2.5
15	28.7[e]	1.1	39.3[g]	0.8	1.9	=2.5
16	16.1[e]	0.6	43.5[g]	0.9	1.5	—
18	16.9[e]	1.0	51.6[e]	1.4	2.4	<10.0
19	20.8[e]	1.2	51.5[e]	1.4	2.6	<10.0
20	37.4[g]	2.2	59.9[f]	1.6	3.8	≫10.0 (as free base)
21	17.0	0.7	54.9[e]	1.5	2.2	>5.0
22	37.6[g]	2.2	0	0	2.2	>5.0
23	4.4	0.2	64.3[f]	1.7	1.9	=5.0
24	28.7[f]	1.3	48.1[e]	1.3	2.6	=5.0
25	21.9[e]	1.0	6.9	0.2	1.2	—
26	7.5	0.5	52.1[e]	1.4	1.9	—
27	26.8[e]	1.6	0	0	1.6	—
28	11.2	0.5	60.8[f]	1.6	2.1	=5.0
29	22.6[e]	0.9	40.3	1.1	2.0	=5.0
30	16.6[e]	0.7	73.2[f]	2.0	2.7	>2.5
31	27.1[e]	1.1	52.9[e]	1.4	2.5	>2.5
32	0	0	39.0[f]	1.1	1.1	—
33	2.3	0.1	62.7[f]	0.7	1.8	<2.5
34	27.7[f]	1.1	62.4[f]	1.7	2.8	<2.5

a) The plasma cholesterol values of control and normal groups in four experiments with a total of 32 mice averaged 246 ± 9 and 138 ± 6 mg/dl, respectively.
b) The serum triglyceride values of control ($n=24$) and normal ($n=10$) groups in two experiments averaged 182 ± 12 and 78 ± 2 mg/dl, respectively.
c) The relative potency (*RP*) is defined as the ratio of activity of test compounds and nicotinic acid under the same conditions in each experimental model.
d) The compounds were administered orally at doses of 2.5, 5.0 and 10.0 g/kg.
Significantly different from the control by Student's *t*-test (*e*) $p<0.05$, *f*) $p<0.01$, *g*) $p<0.001$).

Acute Toxicity

Along with the improvement of hypolipidemic activities, there was a considerable decrease in toxicity in the case of most compounds, as compared with NA (Table II). For example, the LD_{50} value in *per os* administration of **20** was much higher than 10 g/kg (as free base), which is very different from that of a mixture of NA and *N*-isopropylethanolamine

TABLE III. LD_{50} Values in Mice of Compound **20** and Related Compounds

Treatment	LD_{50} (g/kg, *p.o.*)
Compound **20**	$\geqslant 10.0$ (as free base)
Mixture[a]	$=2.5$
N-Isopropylethanolamine	<1.25
Nicotinic acid	4.5 (3.5—5.4)

a) The mixture is composed of 2 eq of nicotinic acid and 1eq of *N*-isopropylethanolamine.

TABLE IV. Hypotriglyceridemic Activity of Compound **20** and Chemically and Biologically Related Compounds in Hypertriglyceridemic Rats

Treatment	Structure[a]	Dose[b] (mg/kg)	Serum triglyceride (mg/dl)	
Normal[c]			132±18	
Control			205±23	
Compd. **20**	$R_1OCH_2CH_2N \cdot R_1$ R_2	304 (as free base)	82±8	(60%)[d]
Compd. **35**	$HOCH_2CH_2N \cdot R_1$ R_2	202	196±16	(4%)[g]
Compd. **17**	$R_1OCH_2CH_2NH \cdot R_2$	202 (as free base)	54±8	(73%)[i]
Mixture A[e]	$2R_1OH +$ $HOCH_2CH_2NHR_2$	239 100	61±4	(70%)
Mixture B[e]	$R_1OH +$ $HOCH_2CH_2NHR_2$	119 100	74±2	(64%)[i]
N-Isopropyl-ethanolamine	$HOCH_2CH_2NHR_2$	100	89±9	(56%)[i]
Nicotinic acid	R_1OH	239 100	112±16 144±13	(46%) (30%)[f,h]

Each value represents the mean ±S.E. of 6 animals.
a) R_1 and R_2 indicate nicotinoyl and isopropyl functions, respectively.
b) The doses of all groups other than nicotinic acid were calculated as equimolar in *N*-isopropylethanolamine contained.
c) Normal drinking water was given to the rats of this group.
d) Values in parenthesis represent the reduction from control group.
e) The molar ratios of nicotinic acid and *N*-isopropylethanolamine in mixtures A and B were 2:1 and 1:1, respectively.
The statistical significance of the data was determined by the combination of one-way layout and two-tailed test, and is designated by the *p* values (*f*) $p<0.05$, *g*) $p<0.01$ *vs.* comp. **20**, *h*) $p<0.05$, *i*) $p<0.01$ *vs.* comp. **35**).

equimolar with **20** or of the latter alone (Table III). Thus, acute toxicological studies showed that the safety range of **20** is much wider than that of NA. It is noteworthy that the coupling of ethanolamines and NA resulted in a marked decrease in toxicity. This is consistent with the finding reported by Sirtori,[12] that is, BR-931, an ethanolamine derivative of Wy-14643 [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthioacetic acid],[13] retained most of the activity but little of the toxicity of the parent compound. These observations led us to focus our interest on 2-(*N*-isopropyl-*N*-nicotinoylamino)ethyl nicotinate (**20**), as a substance with the most favorable pharmacological and toxicological profiles among the present compounds.

Metabolite in Serum after Administration of 20

Compound **20** is an amide–ester, which is expected to be highly susceptible to hydrolysis by esterase in the intestinal lumen or blood. Thus, we examined the biotransformation of **20** in

order to elucidate the active principle of hypolipidemic action and to identify the reason for the decrease in toxicity. The main metabolite was detected in the serum during 1/4—6 h after treatment with **20**, but the unchanged compound **20** was not found. This metabolite, isolated by the procedure described in the experimental section, showed complete identity in thin layer chromatographic behavior and infrared (IR) and mass spectra (MS) with the synthetic authentic compound, *N*-isopropyl-2-hydroxylethyl nicotinamide (**35**): IR ν_{max}^{film} cm^{-1}: 3400 (OH), 1630 (CON). MS *m/e*: 208 (M^+), 165 ($M^+ - C_3H_7$). *Rf*: 0.72 (the solvent system is described in the experimental section). From a preliminary experiment *in vitro*, it was found that the ester linkage of **20** was not hydrolyzed in either artificial gastric juice (pH 1.2) or intestinal fluid (pH 7.5) in the absence of a digestive enzyme such as pepsin or pancreatin (data not shown). From these experiments, it became clear that **20** was rapidly hydrolyzed into NA and the counterpart **35** by enzymatic action *in vivo*.

Hypolipidemic Activity of the Metabolite

The amide **35** as well as compounds chemically and biologically related to **20** were examined for activity in the hypertriglyceridemic model. The results are shown in Table IV. Although *N*-isopropylethanolamine, NA and their mixture showed a remarkable hypotriglyceridemic activity, **35**, a main metabolite of **20**, did not show such activity. These results indicated that the activity of *N*-isopropylethanolamine was greatly diminished by the formation of an amide with NA. On the bases of the biotransformation of **20** and a comparison of the hypotriglyceridemic activities of derivatives of **20**, the activity of **20** may be attributable to NA released by hydrolysis of the ester linkage *in vivo*.

Serum NA Level after Administration of 20

We next surveyed the serum nicotinic acid level after oral administration of the compound. The time–course of nicotinic acid level in serum after oral administration of **20** as well as that after administration of nicotinic acid is shown in Fig. 1. After administration of NA, the serum NA level increased rapidly, reached the maximum level (C_{max}) after 30 min and then declined promptly to reach a very low level after 5 h. On the other hand, the C_{max} of free NA in the case of **20** was approximately 4 times lower, but T_{max} and the persistence of NA level were longer as compared with the NA group.

This may be due either to slow release of NA by hydrolysis of the ester in the intestinal

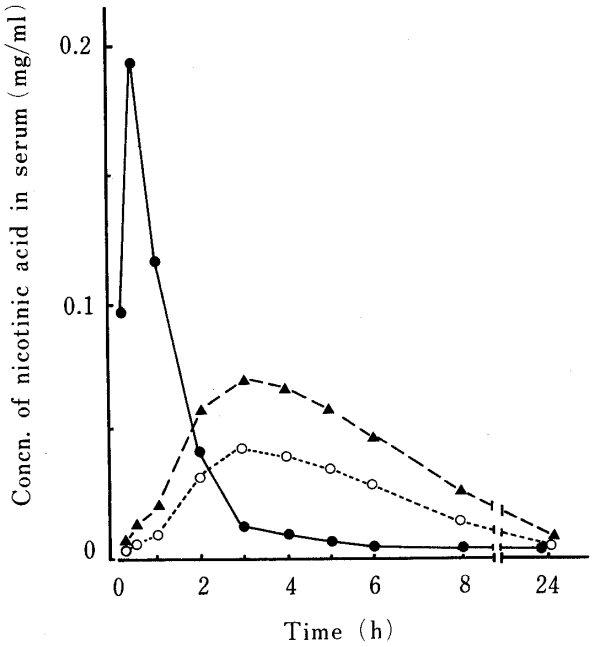


Fig. 1. Serum Levels of Nicotinic Acid in Rats Following Oral Administration of Nicotinic Acid (●—●) or Compound **20** (▲---▲, Total; ○---○, Free) at a Dose of 100 mg/kg as Nicotinic Acid

Each point represents the mean for five rats. The coefficient of variation of each point is less than 10%.

lumen or to slow absorption of compound **20** from the gut followed by immediate enzymatic hydrolysis of the ester linkage in the blood or liver. Such mechanisms would account for the lower toxicity and sustained effective serum level of free NA.

Conclusion

The present studies revealed that **20** had the most favorable pharmacological and toxicological profiles among the compounds tested, and that amide and ester formation between NA and *N*-isopropylethanolamine improved the pharmacological and toxicological properties of NA itself by changing the bioavailability of NA. Namely, the initial rapid rise of serum NA level, which is unavoidable with the use of free NA, is reduced when compound **20** is used. This may be directly related to the decrease in toxicity and sustained hypolipidemic action observed with **20** as compared with NA.

Experimental

Chemistry——Melting points were determined with a Yanagimoto micro-melting point apparatus and are uncorrected. IR spectra were determined with a Hitachi model 215 spectrometer. MS were measured with a JEOL 01SG mass spectrometer.

General Procedure for the Preparation of Ester Type Compounds (1—17)——An ethanolamine analogue (0.03—0.06 mol) was dissolved in 1,2-dichloroethane (30—60 ml) and then dry HCl gas was passed through the solution so as to convert the amine into its hydrochloride salt. After the addition of nicotinoylchloride–hydrogen chloride (1 eq), the mixture was stirred and heated (50—80 °C) for 4—8 h. Cooling gave a solid, which was separated and recrystallized from an appropriate solvent. Compounds obtained by this method are summarized in Table I together with their physicochemical data.

General Procedure for the Preparation of Amide–Ester Type Compounds (18—34)——An ethanolamine analogue (0.03—0.06 mol) and triethylamine (20—30 ml) were dissolved in chloroform or 1,2-dichloroethane (20—30 ml). To this mixture, nicotinoylchloride–hydrogen chloride (2 eq) was added under ice cooling during 10—15 min, and then the whole was refluxed for 4 h. The precipitated triethylamine HCl was filtered off. The filtrate was washed with saturated sodium chloride solution, treated with a small amount of activated carbon, and concentrated to dryness to give a brown viscous oil, which was triturated under cooling in an appropriate solvent. The crude solid was recrystallized. Those products, which could not be crystallized by the usual method, were purified by silica gel chromatography. Only compound **20** was obtained as the maleate (salt). Compounds obtained by this method are summarized in Table I together with their physicochemical data.

Pharmacological Evaluation——Male Sprague–Dawley rats and ICR male mice were purchased from Charles River, Japan, Inc. They were fed with an ordinary chow diet (Nippon Clea, CE-II) and kept in air-conditioned rooms (25±1 °C, 50—60% relative humidity) with alternating 12 h periods of light and dark. Unless otherwise stated, the test compounds were suspended or dissolved in 1% carboxymethylcellulose solution at a concentration such that the administration volume was 0.5 ml per 100 g of body weight per day.

The hypolipidemic effect of test compounds was calculated according to the following formulae.

$$\text{reduction percent } (T_t) = C - T/C \times 100\ (\%)$$

$$\text{relative potency } (RP) = T_t/T_n$$

where

C = serum lipid level of the untreated hyperlipidemic group
T = serum lipid level of groups treated with test compounds
T_t = reduction percent of test compound groups
T_n = reduction percent of nicotinic acid group

Values for serum lipids level in treated animals were compared with those obtained in untreated animals run simultaneously.

Hypocholesterolemic Effect in Mice——Male mice (9—11 g) were allocated to experimental groups of 8 animals. The mice were fed with a high cholesterol diet consisting of 1% cholesterol, 0.1% cholic acid, 0.5% test compound and 98.4% commercial diet (Nippon Clea, powdered). A control group received a high cholesterol diet without the addition of any test compound. The experimental diets were given for 7 d *ad libitum*. The blood was then collected from the tail vein in heparinized microcapillary tubes and centrifuged for 3 min in a microcapillary centrifuge to obtain the

plasma. The total cholesterol level was determined by the *o*-phthalaldehyde method (cholesterol assay kit from Wako Pure Chemicals).

Hypotriglyceridemic Effect in Hypertriglyceridemic Rats——Hypotriglyceridemic activity was studied by a slight modification of the method of Dalton.[14] Male rats (210—220 g) were allocated to experimental groups of 6 animals. The rats had free access to standard pellets and 10% fructose solution as drinking water for 2 d. The test compounds were administered immediately after the provision of fructose solution, and the second and third doses were administered 24 and 42 h after the first dose, respectively. Six hours after the last dose, a blood sample for serum triglyceride determination was obtained by decapitation of the animals. Triglyceride levels were determined accordingly to the method of Van Handels and Zilversmit.[15]

Acute Toxicity in Mice——Male mice (26—29 g) were fasted for 18 h prior to the experiment and divided into experimental groups of 8 animals. The compounds suspended or dissolved in 5% gum acacia were given orally to mice in a volume of 2 ml per 100 g of body weight. The compounds were administered at doses of 2.5, 5.0 and 10.0 g/kg. The animals were observed for 3 d and the mortality was recorded. LD_{50} values were calculated according to the method of Litchfield and Wilcoxon.[16]

Identification of a Main Metabolite in Serum——Male rats (300—330 g) were allocated to experimental groups of 3 animals. Blood samples were obtained by decapitation at 1/4, 1/2, 1, 2, 4 and 6 h after a single oral administration at 500 mg/kg to rats fasted for 18 h prior to the experiment. The serum samples (10 ml) were extracted three times with 30 ml of chloroform. The combined extracts were dried over anhydrous sodium sulfate and evaporated to dryness *in vacuo*. The residue was dissolved in a small amount of chloroform, applied to silica gel plates (Kieselgel GF_{254}, Merck) and developed with chloroform–ethanol–pet. ether (5 : 4 : 1, v/v). The resulting chromatogram was visualized under ultraviolet light (2536 Å). The main band was scraped off and extracted with ethanol, then the solvent was evaporated off *in vacuo* to give a viscous oil, the structure of which was identified by comparison of the physicochemical data (*Rf* value, IR and MS spectra) with those of a synthesized, authentic sample.

Determination of Nicotinic Acid in Serum——Male rats (230—250 g) were fasted for 18 h prior to the experiment and divided into experimental groups of 5 animals. The rats were given nicotinic acid or compound **20** in an oral dose of 100 mg/kg as nicotinic acid. Blood samples were obtained by decapitation at 1/4, 1/2, 1, 2, 3, 4, 5, 6, 8 and 24 h after administration. The concentrations of free and total (free + amide) nicotinic acid in serum derived from the drugs were determined by a slight modification of the method of Swaminathan.[17]

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