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Phenylalanine Derivatives Enhancing Intestinal Absorption of Insulin in Mice

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The adjuvant effect of *N*-acyl-L- and D-phenylalanine derivatives on intestinal absorption of insulin was investigated in normal mice. The correlation between the chemical structural properties of the *N*-acyl moiety and the absorption-promoting activity was estimated from the glucose concentrations and the insulin levels in the blood of mice after oral combined administration of insulin and adjuvant. The chemical structural properties of *N*-acyl-phenylalanine derivatives necessary for adjuvant effect on intestinal absorption of insulin were as follows.

1. An aromatic ring is present, separated by two atoms from the acyl carbonyl group.
2. Either of X or Y is oxygen or X–Y is a double bond in Fig. 2.
3. The *N*-acyl moiety has small hydrophobic substituents, such as F, Cl, or Me at R_α, R_β, R_n and has an appropriate hydrophilic–hydrophobic balance of the overall molecule.

The use of these agents to enhance insulin absorption offers the possibility of a new approach to oral insulin therapy.

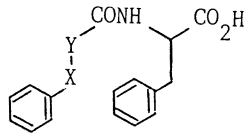
Keywords——insulin; intestinal absorption; adjuvant; *N*-acyl-phenylalanine

Introduction

Insulin is secreted from the pancreas and passes into the portal vein, through the liver, and reaches the peripheral veins. This physiological route of insulin may be reproduced by oral administration, if insulin can be absorbed from the duodenum or the small intestine into the portal vein and the liver can be exposed to high concentrations of insulin as in the normal physiological state. The difference of physiological effect between portal administration and peripheral injection of insulin remains unclear,[1] but it is important to develop an administration route similar to the physiological route of insulin.

The therapeutic administration of insulin is at present limited to the injection route because insulin is destroyed and poorly absorbed through the mucosal membrane in the gastrointestinal tract. Another route of administration of insulin, for instance oral administration, would offer advantages in terms of avoidance of patient's suffering from painful injections, as well as possibly fewer problems with antigenicity. Rectal administration[2] and nasal administration[3] of insulin has been considered as possible parenteral administration routes of insulin. Oral administration of insulin has also been attempted with ionic or nonionic surfactants,[4] protease inhibitors,[5] or nonsurfactant adjuvants.[6] Moreover, liposome-encapsulated insulin has been administered orally.[7] Recently, oral administration of azopolymer-coated insulin was reported.[8] However, these routes have not yet been proved to be practical for general use.

We have been interested in the development of methods for oral administration of



1: X=CH_2, Y=O
4: X=O, Y=CH_2
11: X-Y=-CH=CH-

Fig. 1. The Three Basic Structures of the *N*-Acyl Moiety of Phenylalanine Derivatives

insulin. We examined the adjuvant effect of about 300 *N*-acyl amino acids on intestinal absorption of insulin. We found that some *N*-acyl-phenylalanine derivatives, such as *N*-benzyloxycarbonyl-phenylalanine (**1**), *N*-phenoxyacetyl-phenylalanine (**4**) and *N*-cinnamoyl-phenylalanine (**11**), shown in Fig. 1, had an adjuvant effect.[9]

After that discovery, we examined many analogues, in order to elucidate the relationship between their structures and absorption-promoting effects.

We wish to report here the effects of substituents on the acyl moiety of *N*-acyl-L- and D-phenylalanine derivatives on the adjuvant effect in oral administration of insulin.

Chemistry

N-Acyl-phenylalanine derivatives were prepared from phenylalanine and appropriate carboxylic acid chlorides by means of the Schotten–Baumann reaction. They were also prepared from phenylalanine methylester hydrochloride and appropriate carboxylic acids by DCC–HOBt (*N*,*N*′–dicyclohexylcarbodiimide–1-hydroxybenzotriazole) coupling and subsequent hydrolysis of the methyl ester.

Physical data for the prepared compounds are summarized in Table IV.

Results and Discussion

Structure Activity Relationships

In the first screening of about 300 *N*-acyl amino acids, insulin was administered orally at a dose of 250 U/kg with 5000 mg/kg of *N*-acyl amino acid to normal ICR-CD1 mice. The extent of absorption was estimated from the reduction in blood glucose concentration. We found that *N*-benzyloxycarbonyl-phenylalanine (**1**) (referred to as type I), *N*-phenoxyacetyl-phenylalanine (**4**) (type II) and *N*-cinnammoyl-phenylalanine (**11**) (type III), show in Fig. 1, resulted in significant insulin absorption through the gastrointestinal tract. Interestingly, these three *N*-acyl moieties resemble each other in having an aromatic ring at two atoms distance from the carbonyl group.

A preliminary examination was made of the effects of substituents in the phenylalanine derivatives possessing the above three types of *N*-acyl groups, and the results are shown in Table I. In this examination, 250 U/kg of insulin and 200 to 600 mg/kg of *N*-acyl-phenylalanine derivatives were orally administered as a solution to normal ICR-CDl mice, and the decrease in serum glucose level was estimated.

The structural conversion at the C-terminal carboxylic group of the derivatives to amide (**2**, **6**) or ester (**5**), and the addition of phenylalanine to the C-terminal (**3**, **7**, **15**) showed no increase in the adjuvant activity. The results indicate the importance of the carboxylic group in these compounds for adjuvant activity. Phenylalanylphenylalanylphenylalanine (**16**), which was reported as an adjuvant for rectal absorption of Cefoxitin and Cefmetazol[10] and is structurally similar to our adjuvants, was ineffective for oral administration of insulin. On the other hand, introduction of substituents on the aromatic ring of the *N*-acyl moiety, except *m*-substituents (**12**), resulted in an increase in the adjuvant activity (**8**, **9**, **10**, **13**, **14**). This fact led us to make further studies of the structural transformation of the *N*-acyl moiety of the

TABLE I. Adjuvant Effect of *N*-Acyl-L-Phenylalanine Derivative after Oral Administration of Insulin (250 U/kg) in Mice ($n=5$)

Ar–X–Y–CO–NH–CH($CH_2C_6H_5$)–CO–Z

No.	Ar	–X–Y–	Z	Adjuvant dose (mg/kg)	Hypoglycemic effect[a] $-\Delta G$ (mg/dl)
1	C_6H_5	$-CH_2-O-$	OH	300	45.9[b]
2	C_6H_5	$-CH_2-O-$	NH_2	600	+
3	C_6H_5	$-CH_2-O-$	L-Phe	300	14.2
4	C_6H_5	$-O-CH_2-$	OH	200	22.9[c]
5	C_6H_5	$-O-CH_2-$	OCH_3	600	+
6	C_6H_5	$-O-CH_2-$	NH_2	600	12.9
7	C_6H_5	$-O-CH_2-$	L-Phe	300	4.2
8	p-ClC_6H_4	$-O-CH_2-$	OH	300	29.5[b]
9	1-$C_{10}H_7$	$-O-CH_2-$	OH	200	51.0[c]
10	2-$C_{10}H_7$	$-O-CH_2-$	OH	200	51.0[c]
11	C_6H_5	$-CH=CH-$[d]	OH	600	56.3[c]
12	m-$CH_3OC_6H_4$	$-CH=CH-$[d]	OH	300	+
13	p-$CH_3C_6H_4$	$-CH=CH-$[d]	OH	300	37.9[b]
14	p-ClC_6H_4	$-CH=CH-$[d]	OH	300	53.2[c]
15	p-ClC_6H_4	$-CH=CH-$[d]	L-Phe	300	17.9
16	L-Phe		L-Phe	600	+

a) Values are shown as decrease of blood glucose level compared with PBS-treated control at 30 min after administration. Significantly different from non-treated normal control group. *b*) $p<0.01$, *c*) $p<0.001$. *d*) Cinnamoyl moieties are *E* isomers. "+" means no hypoglycemic effect.

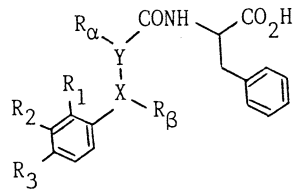


Fig. 2. *N*-Acyl Moiety of Phenylalanine Derivatives with Various Substituents

phenylalanine derivatives to improve the adjuvant activity.

Various substituents, namely R_α, R_β, R_n were introduced into the *N*-acyl moiety of phenylalanine derivatives as shown in Fig. 2. Table II shows the results obtained when 50 U/kg of insulin and 300 mg/kg of adjuvant were orally administered in solution to normal ICR-CDl mice.

Among the compounds with a type of *N*-acyl moiety similar to *N*-phenoxyacetyl (type II), *N*-(*p*-chlorophenoxacetyl)-phenylalanine (**8**) and *N*-naphthoxyacetyl-phenylalanines (**9**, **10**, **22**) have excellent adjuvant effects.

Among the *N*-cinnamoyl derivatives (type III), compounds with α-F (**39**), α-Me (**40**), α-Cl (**41**), β-Cl and *p*-Cl (**14**) were most effective and they produced over 40 mg/dl decrease in blood glucose concentration. However, the correlation between the position of the substituent and the effect was unclear. Introduction of a hydrophilic group, such as α-acetylamino (**42**), *m*-MeO (**12**) or 3,4-methylenedioxy (**35**), were ineffective. On the other hand, introduction of hydrophobic functional groups, such as *m*-Me, *m*-CF_3, *p*-Me and *p*-Et (**26**, **28**, **13**, **32**) was effective. However, introduction of more hydrophobic and bulkier groups, such as *p*-propyl, *p*-phenyl, *m*-phenoxy or *m*,*p*-dichloro (**33**, **34**, **29**, **36**) was ineffective.

Furthermore, we examined the effect of structures similar to *N*-phenoxyacetyl (type II) and *N*-cinnamoyl (type III) structures. For example, the saturation of vinyl groups in

TABLE II. Adjuvant Effect of *N*-Acyl-L-phenylalanine Derivatives (300 mg/kg) after Oral Administration of Insulin (50 U/kg) in Mice ($n=5$)

Ar–X(R_β)–Y(R_α)–CO–NH–CH($CH_2C_6H_5$)–CO_2H

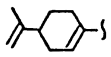
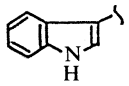
No.	Ar	–X(R_β)–Y(R_α)–	Hypoglycemic effect[a] $-\Delta G$ (mg/dl) 15 min	30 min
17	*p*-FC_6H_4	–CH_2–O–	70.2[d]	26.6[d]
18	*o*-ClC_6H_4	–O–CH_2–	21.9	+
19	*m*-ClC_6H_4	–O–CH_2–	29.9	+
20	*m*-$CF_3C_6H_4$	–O–CH_2–	15.7	14.8
8	*p*-ClC_6H_4	–O–CH_2–	42.2[e]	6.0
21	*o,m*-$(CH_3)_2C_6H_3$	–O–CH_2–	14.1	24.5[d]
9	1-$C_{10}H_7$	–O–CH_2–	38.2[e]	+
10	2-$C_{10}H_7$	–O–CH_2–	53.7[e]	21.2[e]
22	1-$C_{10}H_7$	–O–$CH(CH_3)$–[g]	15.7[d]	44.2[e]
23	1-$C_{10}H_7$	–O–$CH(CH_3)$–[g]	+	+
24	*o*-FC_6H_4	–CH=CH–[f]	0.7	+
25	*o*-ClC_6H_4	–CH=CH–[f]	12.2	+
12	*m*-$CH_3OC_6H_4$	–CH=CH–[f]	+	5.2
26	*m*-$CH_3C_6H_4$	–CH=CH–[f]	21.9[c]	0.6
27	*m*-ClC_6H_4	–CH=CH–[f]	13.3	30.7
28	*m*-$CF_3C_6H_4$	–CH=CH–[f]	29.4	22.4
29	*m*-$C_6H_5OC_6H_4$	–CH=CH–[f]	15.0	+
30	*p*-FC_6H_4	–CH=CH–[f]	+	+
13	*p*-$CH_3C_6H_4$	–CH=CH–[f]	37.5[c]	2.4
14	*p*-ClC_6H_4	–CH=CH–[f]	65.7[e]	40.3[e]
31	*p*-$CF_3C_6H_4$	–CH=CH–[f]	21.4	42.0[e]
32	*p*-$CH_3CH_2C_6H_4$	–CH=CH–[f]	30.1[d]	6.6
33	*p*-$CH_3CH_2CH_2C_6H_4$	–CH=CH–[f]	4.4	+
34	*p*-$C_6H_5C_6H_4$	–CH=CH–[f]	+	+
35	*m,p*-$CH_2O_2C_6H_3$	–CH=CH–[f]	16.4	14.5
36	*m,p*-$Cl_2C_6H_3$	–CH=CH–[f]	+	+
37	1-$C_{10}H_7$	–CH=CH–[f]	0.3	+
38	2-$C_{10}H_7$	–CH=CH–[f]	35.9[d]	0.9
39	C_6H_5	–CH=CF– (*Z*)	39.7[d]	26.8[b]
40	C_6H_5	–CH=C(CH_3)– (*E*)	42.3[e]	+
41	C_6H_5	–CH=CCl– (*Z*)	55.7[e]	38.4[e]
42	C_6H_5	–CH=C($NHCOCH_3$)– (*Z*)	+	+
43	*p*-$CH_3C_6H_4$	–CH=CF– (*Z*)	23.7	+
44	*p*-ClC_6H_4	–CH=CF– (*Z*)	25.3[d]	+
45	*p*-$CH_3C_6H_4$	–CH=CCl– (*Z*)	+	10.5
46	C_6H_5	–CCl=CH– (*Z*)	47.8[e]	34.3[d]
47	*p*-ClC_6H_4	–S–CH_2–	4.1	3.8
48		–O–CH_2–	29.2	20.5
49		–O–CH_2–	+	+
50	C_6H_5	–CH_2CH(Cl)– (*S*)	47.8[e]	6.5
51	C_6H_5	–CH_2–CH(Cl)– (*R*)	14.7	16.9
52		–CH=CH–	+	+
53		–CH=CH–	19.0	+
54		–CH=CH–	18.9	1.6
55		–CH=CH–	6.4	+

TABLE II. (continued)

No.	Ar	$-X(R_\beta)-Y(R_\alpha)-$	Hypoglycemic effect[a] $-\Delta G$ (mg/dl) 15 min	30 min
56		$-CH=CH-$	+	7.7

a) See Table II. Significantly different from non-treated normal control group. *b*) $p<0.05$, *c*) $p<0.02$, *d*) $p<0.01$, *e*) $p<0.001$. *f*) Cinnamoyl moieties are *E* isomers. "+" means no hypoglycemic effect. *g*) The *R* or *S* stereoisomer.

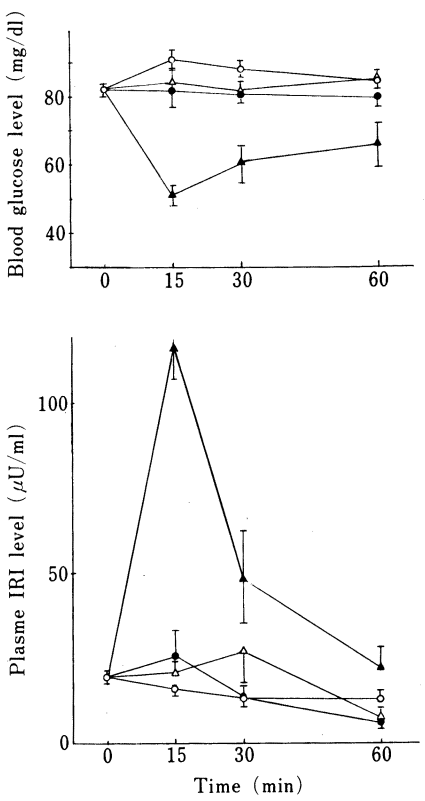


Fig. 3. Hypoglycemic Effect with Compound **39** in Mice ($n=5$)

○, PBS, control group 0.2 ml/10 g; ●, insulin alone 50 U/kg; ▲, **39** 300 mg/kg + insulin 50 U/kg; △, **39** 300 mg/kg.

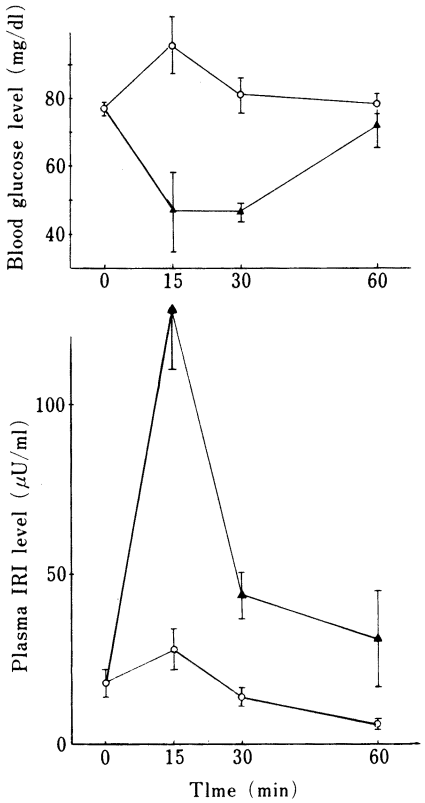


Fig. 4. Hypoglycemic Effect with Compound **46** in Mice ($n=5$)

○, PBS, control group; ▲, **46** 300 mg/kg insulin 50 U/kg.

cinnamoyl derivatives did not increase the adjuvant effect, namely *N*-(3-phenyl-1-chloropropionyl)-phenylalanines (**50**, **51**) were less effective than *N*-(α-chlorocinnamoyl)-phenylalanine (**41**). The saturation of the aromatic ring of the naphthoxyacetyl or cinnamoyl moiety (**48**, **49**, **52**, **53**) resulted in less effect than that of the original structure. 3-Heterocyclic-vinyloxy compounds (**55**, **56**) were not effective.

To confirm that the absorption of insulin was well reflected by the hypoglycemic effect, the increase in serum immunoreactive insulin level was estimated. Two typical results are

TABLE III. Adjuvant Effect of *N*-Acyl-D-phenylalanines Derivatives (300 mg/kg) after Oral Administration of Insulin (50 U/kg) in Mice ($n=5$)

Ar–X(R_β)–Y(R_α)–CO–NH–CH($CH_2C_6H_5$)–CO_2H

No.	Ar	–X(R_β)–Y(R_α)–	Hypoglycemic effect $-\Delta G$ (mg/dl) 15 min	30 min
57	*p*-ClC_6H_4	–CH=CH–	61.5[b)]	26.8[b)]
58	C_6H_5	–CH=CF– (*Z*)	30.3[b)]	23.3[a)]

Significantly different from non-treated normal control group. *a*) $p<0.02$, *b*) $p<0.001$.

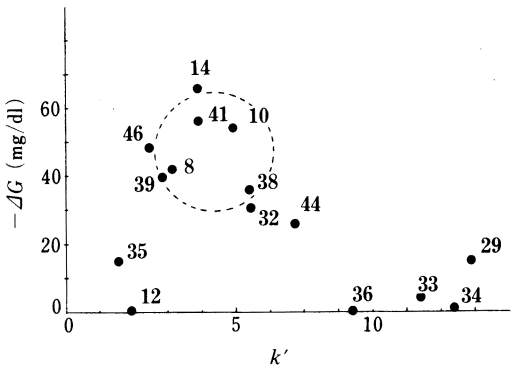


Fig. 5. The Relationship of the Hypoglycemic Effect ($-\Delta G$) and the Retention Factor k'

shown in Fig. 3 and Fig. 4. In both cases, the serum immunoreactive insulin level was remarkably increased at 15 min after the administration. The decrease of serum glucose level can be attributed to the increase in serum insulin level. No hypoglycemic effect was observed on control administration of insulin alone or adjuvant alone, as shown in Fig. 3.

No significant difference between L- and D-derivatives of phenylalanine was observed in the oral insulin absorption enhancing effect. Two typical results for D-phenylalanine derivatives are shown in Table III. These results suggest that the physico-chemical properties of *N*-acyl moiety such as hydrophobicity rather than the stereochemistry of the phenylalanine moiety are important for the adjuvant effect.

Relationship of the Hypoglycemic Effect and the Hydrophobicity

The lipid–water partition character of adjuvants might be important, because adjuvants should interact with the membrane at the intestinal tract. Kakeya *et al.*[11] reported an examination of the rectal absorption-promoting activity of *N*-straight chain fatty acid substituted phenylalanine derivatives and other carboxylic acids on sodium ampicillin in rats. They found that the optimal log *P* value of carboxylic acid exerting the maximum rectal absorption-promoting effect was in the range of 4.2 to 4.8. We chose their three compounds, *N*-hexanoyl-($\log P_{oct}=2.11$, k' in our experiment was 1.89), *N*-octanoyl-($\log P_{oct}=3.11$, $k'=6.66$) and *N*-decanoyl-L-phenylalanine ($\log P_{oct}=4.11$, $k'=23.88$), which resemble our compounds, as internal standards for the high performance liquid chromatography (HPLC) study. The hydrophilic–hydrophobic balance of compounds can be predicted by HPLC study.[3c] We studied the hydrophobicity of adjuvants in terms of the reverse-phase HPLC retention factor k'.

The relationship of the hypoglycemic effect ($-\Delta G$) and the retention factor k' of some compounds is shown in Fig. 5. It became clear that the compounds with remarkable absorption-promoting activity had a retention factor k' value between 1.89 to 6.66, or were

eluted between *N*-hexanoyl-L-phenylalanine and *N*-octanoyl-L-phenylalanine in HPLC. More hydrophilic or hydrophobic compounds were less effective. These results suggest the importance of appropriate hydrophilic–hydrophobic balance of the overall molecule.

Protease-Inhibiting Activity

In intestinal absorption of insulin, it is necessary to avoid degradation of insulin by intestinal proteases. From this point of view, chymotrypsin inhibitors such as FK-488[5] have been used as adjuvants for intestinal absorption of insulin. We also examined the protecting effect of some compounds prepared here against chymotrypsin digestion. The IC_{50} values of phenylalanine derivatives against chymotrypsin were of the order of 10^{-3} M, which is lower by four orders than the value with FK-488 (7×10^{-7} M). No clear relationship was found between the chymotrypsin-inhibitory and absorption-promoting activities. However, at very high concentration, the adjuvants inhibit chymotrypsin activity completely.

Conclusion

The mechanisms by which our compounds promote insulin absorption in the intestine remain unclear, but some chemical structural properties of *N*-acyl-phenylalanine derivatives necessary for adjuvant effect on the intestinal absorption of insulin were revealed.

The properties are as follows.

TABLE IV. Physical Properties

No.	mp (°C) (Crystn. solvent)[a]	$[\alpha]_D$ (*c*, solvent,[b] °C)	No.	mp (°C) (Crystn. solvent)[a]	$[\alpha]_D$ (*c*, solvent,[b] °C)
3	163—164, (A)	−1.40 (1.0, A, 17)	**31**[c]	222—224, (A)	−18.60 (1.6, A, 20)
4	137—138, (A)	+14.01 (0.5, B, 24)	**32**	136—137, (A)	−34.50 (1.5, A, 26)
5	60—63, (B)	−9.40 (0.5, A, 20)	**33**	121—122, (B)	−36.20 (1.8, A, 15)
6	188—191, (A)	+4.35 (1.0, A, 20)	**34**	232—233, (E)	−50.60 (1.7, A, 26)
7	178.5—179.5, (A)	−6.20 (0.5, A, 24)	**35**	178—180, (A)	−53.00 (0.8, A, 23)
8	144—145, (B)	+10.74 (0.5, A, 25)	**36**	147—148, (F)	−33.39 (0.5, A, 24)
9	182—185, (C)	+10.74 (1.2, A, 20)	**37**	220—221, (A)	+70.60 (1.1, C, 20)
10	177—178, (C)	+28.0 (1.1, A, 30)	**38**	218—219, (A)	+34.80 (1.0, C, 20)
12	119—121, (B)	−25.50 (0.8, A, 21)	**39**	145—148, (A)	−45.40 (1.2, A, 20)
13	135—137, (A)	−35.89 (1.0, A, 25)	**40**	103—107, (F)	−61.50 (0.86, A, 20)
14	221—224, (A)	−31.02 (1.0, A, 30)	**41**[d]	84—87, (F)	−4.10 (2.8, A, 20)
15	234 (dec.), (A)	−22.54 (0.05, A, 24)	**42**	205—207, (A)	−2.85 (1.0, A, 24)
17	92—93, (A)	−4.80 (0.5, A, 24)	**43**	163—163.5, (A)	−51.20 (0.5, A, 25)
18	165—166, (B)	+11.01 (1.0, A, 24)	**44**	155—160, (B)	−53.17 (1.0, A, 24)
19	143—144, (B)	+9.79 (1.0, A, 24)	**45**	114—115, (G)	−0.65 (1.0, A, 24)
20	158—159, (B)	+7.35 (1.0, A, 24)	**46**	122.5—123.5, (G)	−7.11 (1.0, A, 24)
21	175.5—176.5, (B)	+22.45 (1.0, A, 24)	**47**	177—178, (B)	+34.13 (1.0, A, 24)
22	144—146, (D)	+41.20 (1.0, A, 21)	**48**	123—124, (B)	+19.35 (1.0, A, 24)
23	115—116, (D)	−40.10 (1.1, A, 21)	**49**	105—106, (B)	+18.64 (1.0, A, 24)
24	132—133, (B)	−17.90 (0.88, A, 24)	**50**	114—115, (G)	+24.58 (1.0, A, 24)
25	126—127, (B)	+1.66 (1.0, A, 24)	**51**	116.5—117.5, (G)	+17.57 (1.0, A, 24)
26	141—142, (B)	−28.60 (1.7, A, 15)	**52**	136—137, (B)	+15.27 (1.0, A, 24)
27	146—147, (B)	−27.20 (1.7, A, 15)	**53**[e]	155—160	−135.24 (0.5, A, 24)
28	158—160, (C)	−19.10 (1.0, A, 20)	**54**	74—77, (B)	+6.94 (0.5, A, 24)
29	121—122, (B)	−28.70 (1.7, A, 30)	**55**	172—173, (B)	−58.24 (0.5, A, 24)
30	144—146, (B)	−21.06 (0.5, A, 24)	**56**[f]	197—205	+35.80 (0.5, A, 24)

a) A, MeOH–H_2O; B, AcOEt–*n*-hexane; C, EtOH–H_2O; D, $CHCl_3$–petroleum ether; E, acetone–H_2O; F, EtOH–AcOEt–*n*-hexane; G, AcOEt–petroleum ether. *b*) A, EtOH; B, MeOH; C, 0.1% NaOH : MeOH = 50 : 50. *c*) Formula $C_{19}H_{16}F_3N_1O_3 \cdot$ MeOH. *d*) Formula $C_{18}H_{16}Cl_1N_1O_3 \cdot 1/2$ EtOH. *e*) Cyclohexylamine salt. *f*) Dicyclohexylamine salt.

1. *N*-Acyl-phenylalanine derivatives which show an adjuvant effect have an aromatic ring at two atoms distance from the acyl carbonyl group.

2. Either of X or Y is oxygen or X–Y is a double bond in Fig. 2.

3. The *N*-acyl moiety has small hydrophobic substituents, such as F, Cl or Me at R_α, R_β, R_n, and an appropriate hydrophilic–hydrophobic balance of the overall molecule is necessary for the absorption-promoting effect.

Experimental

Melting points were determined with a Yanaco micro melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were determined on a Varian EM-390 90 MHz spectrometer in $CDCl_3$ or Me_2SO-d_6 solution for all compounds and were consistent with the assined structure. Infrared (IR) spectra were taken on the JASCO IR-810 spectrophotometer. Optical rotations were measured on a JASCO DPI-140 polarimeter. Elemental analyses were carried out with a Yanaco MT-3. The analitical results obtained for C, H and N were within $\pm 0.3\%$ of the theoretical values.

HPLC was performed on a Hitachi 635 with a YMC-A311 (ODS 6 × 100 mm) column with a mobile phase composed of 60% MeOH/40% 0.02 M phosphate buffer, pH 7.0, at 25 °C. The elution rate was 1 ml/min (100 kg/cm^3) and detection was carried out at ultraviolet (UV) 220 or 260 nm. We calculated the retention factor k' for each *N*-acyl phenylalanine derivative according to the formula

$$k' = (t_R - t_0)/t_0$$

in which t_0 = retention time of the solvent front and t_R = retention time of the *N*-acyl-phenylalanine derivatives.

Chemicals——L-Phenylalanine and D-phenylalanine were products of Ajinomoto Co. Commercially unavailable carboxylic acids were prepared in the laboratory. Aromatic substituted cinnamic acids were prepared following Koo *et al.*[12] Phenoxyacetic acids were prepared following Palmer and Kester.[13] α-Fluorocinnamic acids, α-chlorocinnamic acids and β-chlorocinnamic acids were prepared following Flkik,[14] Field and Carile[15] and Youssef and Abdel-Maksoud,[16] respectively.

General Procedure for the Preparation of *N*-Acyl-phenylalanines——Procedure A (Schotten–Baumann Reaction): A carboxylic acid (69 mmol) was added to 100 ml of $SOCl_2$, and the reaction solution was heated at 90 °C for 3 h. The reaction solution was cooled, and the solvent was removed *in vacuo*. Then the residue was dissolved in 80 ml of acetone.

Phenylalanine (80 mmol) was dissolved in 43 ml of 2 N NaOH solution (86 mmol), then 77 ml of water and 240 ml of acetone were added. The reaction solution was cooled to 0 °C, then an acetone solution of carboxylic acid chloride and 38 ml of 2 N NaOH solution (76 mmol) were alternately added with stirring for 20 min, maintaining the solution at pH 9 to 11. The reaction solution was stirred for 30 min at room temperature, then the reaction solution was acidified with 2 N HCl solution and water was added. The precipitate was collected by filtration and then washed with water. *N*-Acyl-phenylalanine was recrystallized from an appropriate solvent.

Procedure B (DCC–HOBt Method): A carboxylic acid (30 mmol) and phenylalanine methylester hydrochloride (33 mmol) were dissolved in 250 ml of dioxane or CH_2Cl_2 and the reaction solution was maintained at 0—5 °C. Et_3N (33 mmol), HOBt (36 mmol) and DCC (36 mmol) were added, and the reaction solution was stirred at room temperature for 14 h. AcOH (4 ml) was added, and the reaction solution was stirred for 2 h at room temperature. Then the precipitate was filtered off. The solvent was removed *in vacuo* and the residue was dissolved in 250 ml of AcOEt. The organic layer was washed with 5% $NaHCO_3$ solution (150 ml × 3), brine (100 ml), 0.5 N HCl solution (150 ml × 3) and brine (100 ml). The organic layer was dried over magnesium sulfate and the solvent was removed *in vacuo*. The residue was filtered through silica gel (250 ml) with AcOEt–*n*-hexane, and the solvent was removed *in vacuo*. The residue was dissolved in 100 ml of MeOH and 44 ml of 1 N NaOH solution was added. The reaction solution was stirred for 30 min at room temperature, then the reaction solution was acidified with concentrated HCl and diluted with water. The precipitate was collected and *N*-acyl-phenylalanine was recrystallized from an appropriate solvent.

Insulin Absorption-Promoting Activity of *N*-Acyl-phenylalanine Derivative Preparations in Fasted ICR-CDl Mice——Preparation: Insulin (Connaught Co., Ltd., U.S.A. Zn-insulin, specific activity 23.3 U/mg) 300 Unit was dissolved in 3 ml of 0.05 N HCl solution and 27 ml of water was added (final concentration was 10 U/ml, pH 3). An *N*-acyl-phenylalanine derivative (600 mg) was dissolved in a small amount of 0.1 N NaOH solution and the solution was neutralized with 0.1 N HCl solution. The solution was diluted with phosphate buffer (0.05 M, pH 7.5) to a volume of 20 ml (final concentration was 30 mg/ml, pH 8.0). Insulin solution, *N*-acyl-phenylalanine derivative solution and 0.05 M phosphate buffer were mixed to a total volume of 0.2 ml at a specified mixing ratio.

Animal Experiment: Female ICR-CDl mice (6—7 weeks old, Charles River, Japan) were fasted for 16—18 h and 0.2 ml of preparation was orally administered. Blood samples were obtained from the infraorbital vein 15, 30 and

60 min after administration of preparations. Serum was separated by centrifugation at 3000 rev/min and serum glucose was determined by the glucose oxidase/peroxidase method. Serum IRI level was determined by double antibody radioimmunoassay using the Insulin RIA kit (Eiken, Japan). Each level was compared with that in a phosphate buffer control group.

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References

1) *a*) S. Baruh, *Am. J. Med. Sci.*, **269**, 25 (1975); *b*) R. W. Stevenson, J. A. Parsons and K. G. M. M. Alberti, *Clinical Endocrinology*, **8**, 335 (1978); *c*) R. W. Stevenson, J. A. Parsons and K. G. M. M. Alberti, *Metabolism*, **30**, 745 (1981); *d*) R. W. Stevenson, J. A. Parsons and K. G. M. M. Alberti, *Am. J. Physiol.*, **244**, E190 (1983); *e*) U. Fisher, R. A. Rizza, L. D. Hall, R. E. Westland, M. W. Haymond, A. H. Clemens, J. E. Gerich and F. J. Service, *Diabetes*, **31**, 579 (1982).
2) *a*) T. Nishihata, J. H. Rytting, T. Higuchi and L. Caldwell, *J. Pharm. Pharmacol.*, **33**, 334 (1981); *b*) A. Kamada, T. Nishihata, S. Kim, M. Yamamoto and N. Yata, *Chem. Pharm. Bull.*, **29**, 2012 (1981); *c*) S. Kim, A. Kamada, T. Higuchi and T. Nishihata, *J. Pharm. Pharmacol.*, **35**, 100 (1983); *d*) T. Nishihata, J. H. Rytting, K. Kamada, T. Higuchi, M. Routh and L. Caldwell, *ibid.*, **35**, 148 (1983); *e*) T. Nishihata, G. Liversidge and T. Higuchi, *ibid.*, **35**, 616 (1983); *f*) T. Nishihata, S. Kim, S. Morishita, A. Kamada, N. Yata and T. Higuchi, *J. Pharm. Sci.*, **72**, 280 (1983).
3) *a*) S. Hirai, T. Ikenaga and T. Matsuzawa, *Diabetes*, **27**, 296 (1978); *b*) K. Morimoto, K. Morisaka and K. Kamada, *J. Pharm. Pharmacol.* **37**, 134 (1985); *c*) G. S. Gordon, A. C. Moses, R. D. Silver, J. S. Flier and M. C. Carey, *Proc. Natl. Acad. Sci. U.S.A.*, **82**, 7419 (1985).
4) E. Touitou, M. Donbrow and A. Rubinstein, *J. Pharm. Pharmacol.*, **32**, 108 (1980).
5) *a*) S. Fujii, T. Yokoyama, K. Ikegaya and N. Yokoo, *Rinsho Kagaku*, **13**, 7 (1984); *b*) N. Yokoo, F. Sato, J. Kirihara, T. Yokoyama, K. Ikegaya, M. Nagakura and S. Fujii, *ibid.*, **14**, 8 (1985); *c*) S. Fujii, T. Yokoyama, K. Ikegaya, F. Sato and N. Yokoo, *J. Pharm. Pharmacol.*, **37**, 545 (1985); *d*) S. Fujii, E. Hattori, M. Hirata, K. Watanabe and H. Ishihama, Japan. Kokai Tokkyo Koho, 595115 (1984) [*Chem. Abstr.*, **98**, 221810*q* (1983)].
6) T. Nishihata, J. H. Rytting, A. Kamada and T. Higuchi, *Diabetes*, **30**, 1065 (1981).
7) *a*) H. M. Patel and B. E. Ryman, *Biochem. Soc. Trans.*, **5**, 1739 (1977); *b*) H. M. Patel, R. W. Stevenson, J. A. Parsons and B. E. Ryman, *Biochem. Biophys. Acta*, **716**, 188 (1982).
8) M. Saffran, S. Kumar, C. Savariar, J. C. Burnham, F. Williams and D. C. Neckers, *Science*, **233**, 1081 (1986).
9) *a*) S. Toyoshima, T. Seto, K. Fukushima and I. Kumashiro, Japan. Kokai Tokkyo Koho, 58189121 (1983) [*Chem. Abstr.*, **100**, 56844*d* (1984)]; *b*) K. Fukushima, *Diabetes* (Japan), **25**, 1276 (1982); *c*) K. Fukushima and S. Toyoshima, "In Current and Future Therapies with Insulin," N. Sakamoto and K. G. M. M. Alberiti, eds., Excerpta Medica, Amsterdam-Oxford, 1983, p. 257; *d*) K. Fukushima, Y. Ohbayashi and S. Toyoshima, "In Current topics in Clinical and Experimental Aspects of Diabetes Mellitus," N. Sakamoto and S. Baba, eds., Excerpta Medica, Amsterdam-Oxford, 1985, p. 489; *e*) K. Fukushima and S. Toyoshima, *Igakuno Ayumi* (Japan), **138**, 59 (1986); *f*) K. Fukushima, *Diabete J.*, **14**, 43 (1986); *g*) K. Fukushima, K. Toi, I. Kumashiro and S. Toyoshima, *J. Japan Diabetes Society*, **28**, No. 3, F107, 416 (1985); *h*) K. Fukushima, Y. Yamaki, K. Toi, I. Kumashiro and S. Toyoshima, *J. Japan Diabetes Society*, **29**, Supplement 2, C103, 204 (1986); *i*) K. Fukushima, Y. Yamaki, Y. Amino and K. Toi, *J. Japan Diabetes Society*, **30**, Supplement 1, P1-303, 442 (1987).
10) T. Nishihata, C.-S. Lee, M. Yamamoto, J. H. Rytting and T. Higuchi, *J. Pharm. Sci.*, **73**, 1326 (1984).
11) K. Nishimura, Y. Nozaki, A. Yoshimi, S. Nakamura, M. Kitagawa, N. Kakeya and K. Kitao, *Chem. Pharm. Bull.*, **33**, 282 (1985).
12) J. Koo, M. S. Fish, G. N. Walker and J. Blake, "Organic Synthesis," Coll. Vol. IV, John Wiley and Sons, Inc., New York, 1963, p. 327.
13) C. S. Palmer and E. B. Kester, "Organic Synthesis," Coll. Vol. I, John Wiley and Sons, Inc., New York, 1932, p. 75.
14) E. Flkik, *Bull. Soc. Chim. Fr.*, 2258 (1964).
15) L. Field and C. G. Carile, *J. Org. Chem.*, **26**, 3170 (1961).
16) A.-H. H. Youssef and H. M. Abdel-Maksoud, *J. Org. Chem.*, **40**, 3227 (1975).