N-(Cyclohexylcarbonyl)-D-phenylalanines and Related Compounds. A New Class of Oral Hypoglycemic Agents. 2

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A series of analogues of N-(cyclohexylcarbonyl)-D-phenylalanine (**5**) have been synthesized and evaluated for their hypoglycemic activity. Relationships were studied between the activity and the three-dimensional structure of the acyl moiety, which was characterized by high-resolution ^1H NMR spectroscopy and MNDO calculations. The role of the carboxyl group of the phenylalanine moiety was also studied by comparing the activities of the enantiomers, the decarboxyl derivative, the esters, and the amides of the phenylalanine derivatives. Thus, the structural requirements for possessing hypoglycemic activity was elucidated and a highly active compound, N-[(*trans*-4-isopropylcyclohexyl)carbonyl]-D-phenylalanine (**13**) was obtained, which showed a 20% blood glucose decrease at an oral dose of 1.6 mg/kg in fasted normal mice.

N-Benzoylphenylalanines and their related compounds possessing hypoglycemic activity have been previously reported.[1] Of these compounds, N-(4-ethylbenzoyl)-, N-(4-isopropylbenzoyl)-, and N-(4-*tert*-butylbenzoyl)-D-phenylalanine showed more than a 20% blood glucose decrease at an oral dose of 10–25 mg/kg in normal fasted mice. The structural features for possessing activity were elucidated: the *R* configuration of the phenylalanine moiety was necessary, and alkyl groups, such as ethyl, isopropyl, and *tert*-butyl, substituted at the para position of the benzoyl moiety particularly enhanced the activity.

In further studies on the relationships between the structure of the acyl moiety and activity, N-(cyclohexylcarbonyl)-D-phenylalanine (**5**) was found to be more potent than N-benzoyl-D-phenylalanine. This result suggested that the planar structure (benzene ring) of the acyl moiety of N-benzoyl-D-phenylalanine derivatives was not always necessary for activity. Various analogues of **5** with modified cyclohexylcarbonyl groups were synthesized and evaluated for blood glucose lowering activity. A highly active compound, N-[(*trans*-4-isopropylcyclohexyl)carbonyl]-D-phenylalanine (**13**), which showed a 20% blood glucose decrease at an oral dose of 1.6 mg/kg in normal fasted mice was obtained. The three-dimensional structure of the acyl moiety was characterized by high-resolution ^1H NMR spectroscopy and semiempirical molecular orbital calculation (MNDO) in order to study the relationship between the three-dimensional structure of the acyl moiety and the activity. The role of the carboxyl group of the phenylalanine moiety was studied by comparing activities of the enantiomers, decarboxyl derivative, esters, and amides of **13**. The carboxyl group was found to be essential for hypoglycemic activity.

Chemistry

The alkylated cyclohexanecarboxylic acids were prepared by catalytic reduction of alkylated benzoic acids[2-4] (Scheme I). The reduction was conducted in acetic acid under 5 kg/cm^2 of hydrogen over PtO_2 at room temperature. The reduction of 4-alkylated benzoic acid gave a mixture of trans- and cis-4-alkylated cyclohexanecarboxylic acid in a ratio of 1:3. The mixture was esterified, and the major cis isomer of the ester was isolated by high-performance liquid chromatography (HPLC) with a YMC A-043 S-5 SIL (20 × 250 mm i.d.) column. The basic hydrolysis of the cis isomer of the ester provided the pure cis isomer of the 4-alkylated cyclohexanecarboxylic acid. The cis isomer of the ester was isomerized at 150 °C with 0.1 equiv of NaH. A mixture of trans- and cis-4-alkylated cyclohexanecarboxylic acid ester was given in a ratio of 6:1. The basic hydrolysis of the mixture, followed by crystallization, provided the pure trans isomer of 4-alkylated cyclohexanecarboxylic acid.

The N-substituted phenylalanine was synthesized by the active ester method[5,6] (Scheme II). The corresponding carboxylic acid was coupled with dicyclohexylcarbodiimide

Scheme I

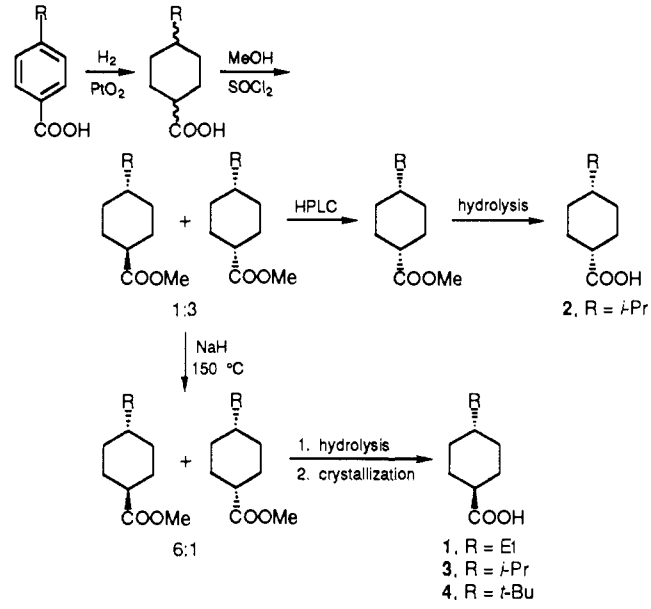

Table I. 4-Alkylcyclohexanecarboxylic Acids

compd	R	mp, °C	yield, %	formula[a]
1	CH_3CH_2	50–51	67	$C_9H_{16}O_2$
2[b]	$(CH_3)_2CH$		64	$C_{10}H_{18}O_2$
3	$(CH_3)_2CH$	96.5–97.5[c]	70	$C_{10}H_{18}O_2$
4	$(CH_3)_3C$	179–180	65	$C_{11}H_{20}O_2$

[a] Analyzed for C, H, and N; analytical results were within ±0.4% of the theoretical values. [b] Oil. [c] Literature[3] mp 94 °C.

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(1) Shinkai, H.; Toi, K.; Kumashiro, I.; Seto, Y.; Fukuma, M.; Dan, K.; Toyoshima, S. *J. Med. Chem.* **1988**, *31*, 2092.
(2) Cooke, R. G.; Macbeth, A. K. *J. Chem. Soc.* **1939**, 1245.
(3) Bose, A. K.; Manhas, M. S. *J. Org. Chem.* **1962**, *27*, 1244.
(4) Pylander, P. N.; Steele, D. R. *Engelhard Ind. Tech. Bull.* **1962**, *3*, 19.

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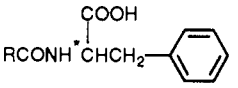
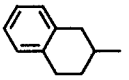
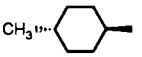
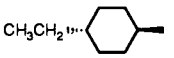
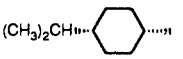
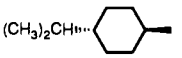
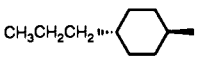
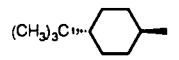
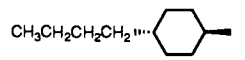
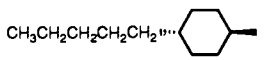
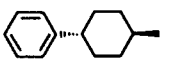
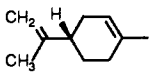
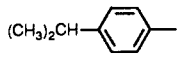
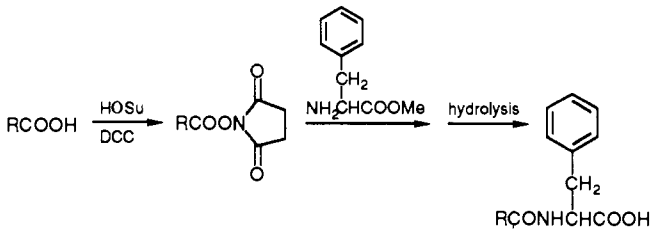
Table II. N-Substituted D-Phenylalanines



compd	R	confign at *C	mp, °C	$[\alpha]_D$, deg	yield, %	formula[a]	hypoglycemic activity[b]
5		D	170–171	−42.6[c]	65	$C_{16}H_{21}NO_3$	100.0
6		D	111–112.5	−35.2[d]	80	$C_{15}H_{19}NO_3$	50.0
7		D	109–110	−8.6[e]	73	$C_{17}H_{23}NO_3$	inactive[f]
8		D	179–181	+33.4[d]	50	$C_{17}H_{21}NO_3$	inactive[f]
9		D	88–89	−4.1[g]	82	$C_{20}H_{21}NO_3$	inactive[f]
10		D	124–125	−11.5[e]	43	$C_{17}H_{23}NO_3$	25.0
11		D	96–97	−11.1[e]	53	$C_{18}H_{25}NO_3$	25.0
12		D	111–112	−13.2[g]	66	$C_{19}H_{27}NO_3$	inactive[f]
13		D	127–128	−9.0[g]	65	$C_{19}H_{27}NO_3$	1.6
14		D	104–105	−8.8[g]	48	$C_{19}H_{27}NO_3$	25.0
15		D	158–159	−9.2[e]	49	$C_{20}H_{29}NO_3$	6.3
16		D	144–145	−7.5[g]	50	$C_{20}H_{29}NO_3$	25.0
17		D	130–130.5	−8.4[h]	79	$C_{21}H_{31}NO_3$	inactive[f]
18		D	142.5–143.5	+0.9[h]	69	$C_{22}H_{25}NO_3$	inactive[f]
19		D	109–110	−37.2[g]	44	$C_{19}H_{23}NO_3$	25.0
20		D	141–145	+25.5[g]	64	$C_{19}H_{21}N_1O_3 \cdot {}^1/_4H_2O$	25.0
tolbutamide							25.0

[a] Analyzed for C, H, and N; analytical results were within ±0.4% of the theoretical values. [b] Lowest dose in milligrams/kilogram causing more than a 20% blood glucose decrease (fasting mice, po). [c] In MeOH, c = 1.0%, at 25 °C. [d] In MeOH, c = 0.5%, at 22 °C. [e] In MeOH, c = 1.0%, at 23 °C. [f] Less than 20% blood glucose decrease at a dose of 100 mg/kg. [g] In MeOH, c = 1.0%, at 20 °C. [h] In MeOH, c = 1.0%, at 22 °C.

Scheme II



(DCC) and *N*-hydroxysuccinimide (HOSu) to the phenylalanine ester. The synthesized ester was hydrolyzed by 2 N aqueous sodium hydroxide to the N-substituted phenylalanine.

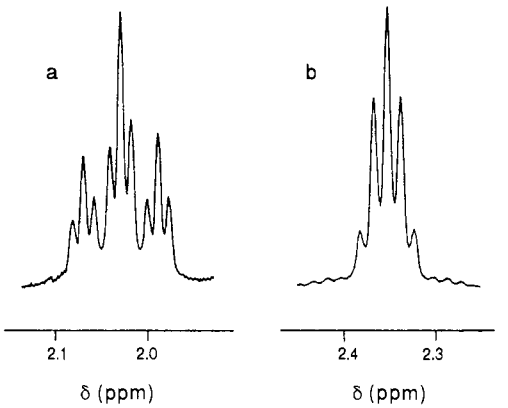


Figure 1. The ^1H NMR spectra of (a) the H1 of **13** (trans isomer) and (b) the H1 of **12** (cis isomer).

The amide compounds **25** and **26** were synthesized by coupling the succinimido ester of *N*-[(*trans*-4-isopropyl-

(5) Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M. *J. Am. Chem. Soc.* **1964**, *86*, 1839.
(6) Weygand, F.; Hoffmann, D.; Wüdnsch, E. *Z. Naturforsch. B* **1966**, *21*, 426.

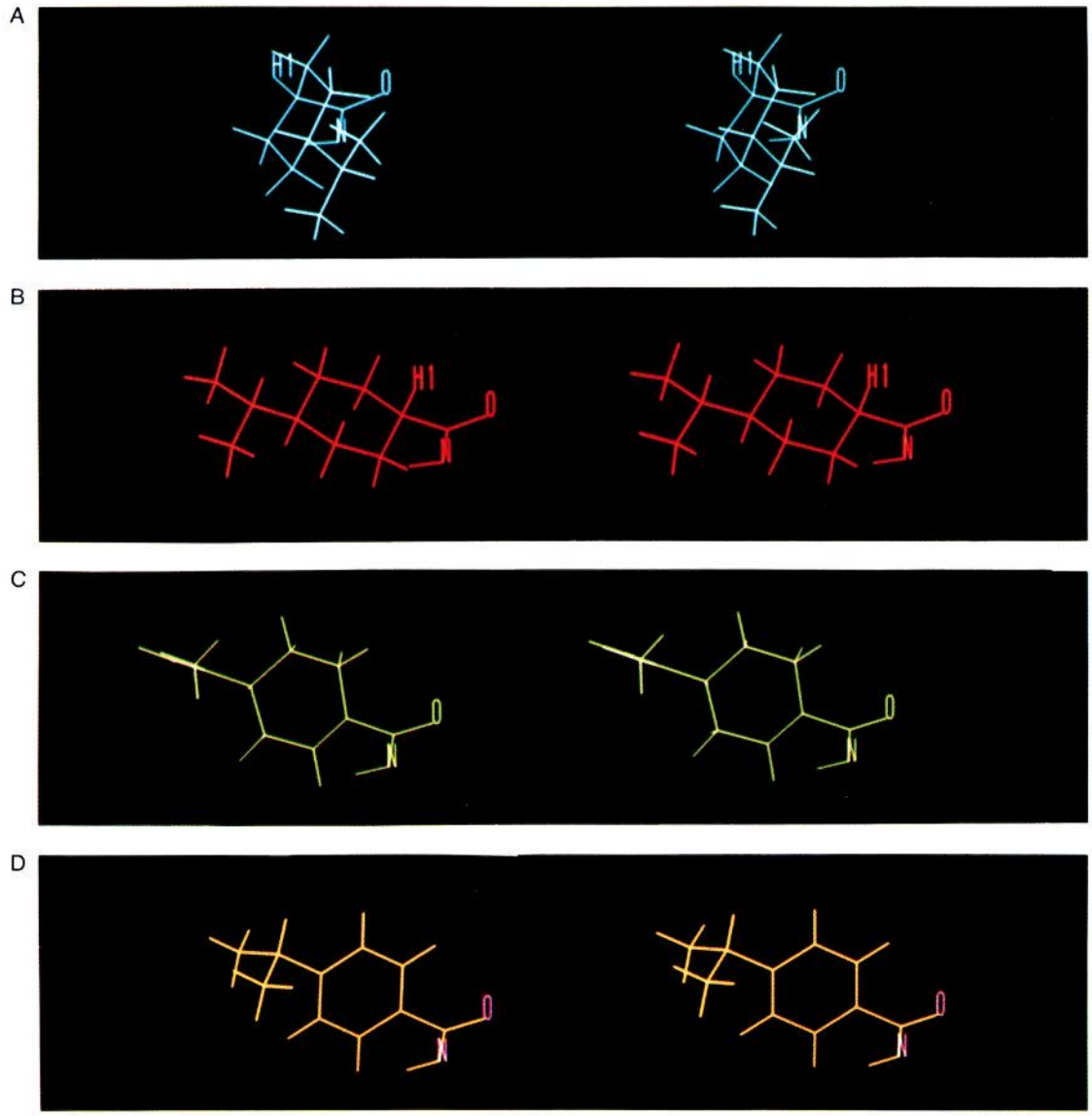


Figure 2. Stereoview of the minimum-energy structures: (A) **12**, (B) **13**, (C) **19**, and (D) **20**. Only acyl moieties are shown for clarity.

cyclohexyl)carbonyl]-D-phenylalanine to ammonia and diisopropylamine, respectively.

Computational Method

Compounds **12** and **13** contain an amide structure. Compounds **19** and **20** contain an amide and an unsaturated structure. High-quality force field parameters for the amide and the unsaturated structure of these compounds were not available to us. Therefore, we have not performed molecular orbital calculations but MNDO calculations on these compounds in order to obtain the minimum-energy structures. The calculations were performed on a VAX 11/750 computer using the AMPAC semiempirical molecular orbital package (QCPE program 506, MNDO method).[7] The SYBYL molecular modeling system was used for molecular building, fitting, and volume manipulation.

(7) Dewar, M. J. S.; Thiel, W. J. *J. Am. Chem. Soc.* **1977**, *99*, 4899, 4907.

Results and Discussion

Table II summarizes the results of the synthetic study of N-substituted D-phenylalanines and their hypoglycemic activities. Cyclohexyl compound **5** and cyclopentyl compound **6** were active, but cycloheptyl compound **7** was inactive. 2,3-Disubstituted cyclohexyl compounds (norbornane compound **8** and tetrahydronaphthalene compound **9**) were inactive. The effect of the acyl moiety depended on their bulkiness: the size of six- and less than six-membered ring which had no substituents was suitable for possessing activity, although the introduction of C1–C4 alkyl groups (methyl, **10**; ethyl, **11**; isopropyl, **13**; *n*-propyl, **14**; *tert*-butyl, **15**; and *n*-butyl, **16**) to the trans 4-position of the cyclohexane ring enhanced the activity. The introduction of C5-C6 alkyl groups (*n*-pentyl, 17; phenyl, 18) decreased the activity. Among the C1–C4 alkyl groups, C3 (isopropyl) and C4 (*tert*-butyl) sizes particularly enhanced the activity, but *n*-propyl (C3 size) and *n*-butyl (C4 size) groups were less effective than isopropyl and *tert*-butyl groups. The difference in these activities was considered to be attributed to the difference in both the volume and length of the alkyl substituent. The most

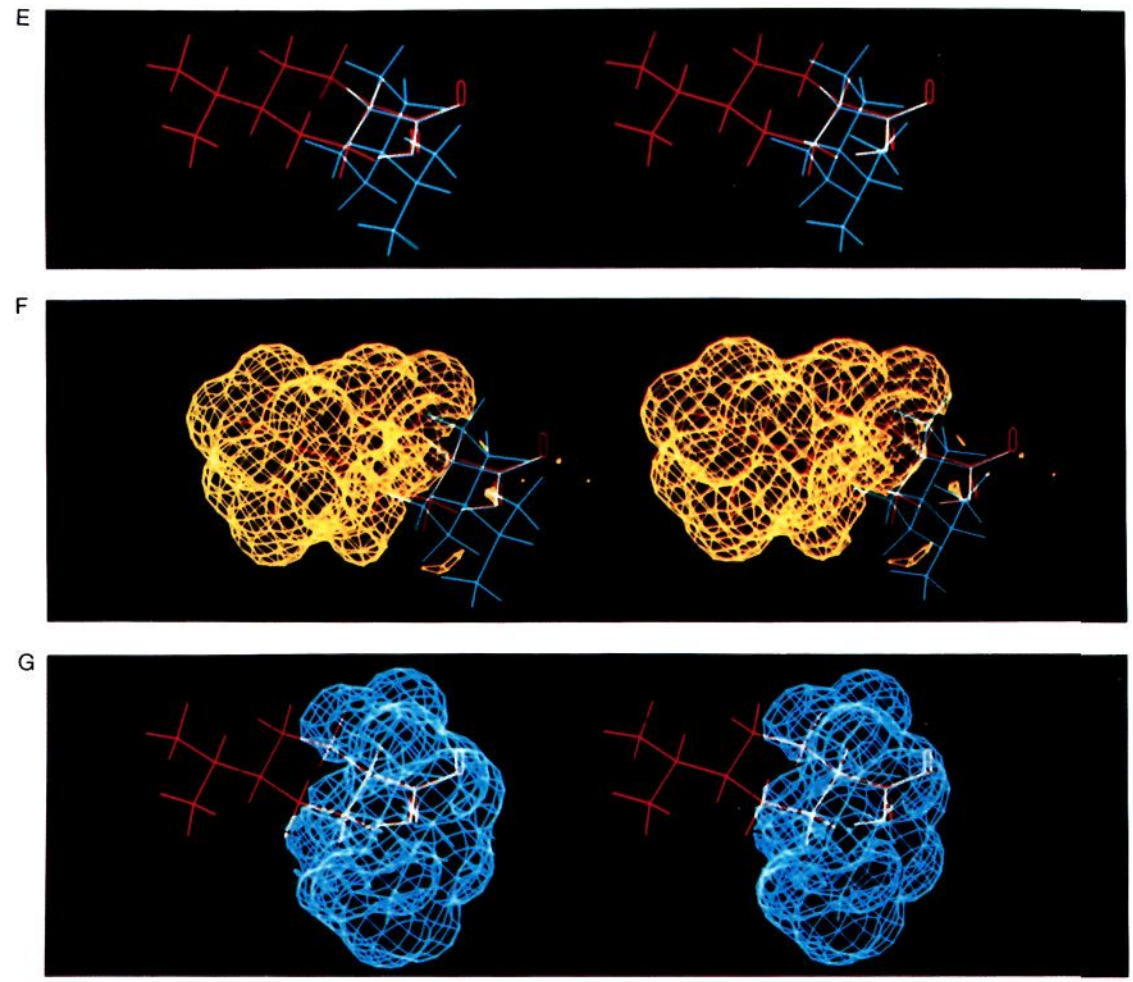


Figure 3. Stereoview of the overlap of (E) **12** and **13**. The van der Waals volume of the low active analogue (**12**) was subtracted from the volume of the high active compound (**13**) (F, yellow mesh region), and the volume of **13** was substracted from the volume of **12** (G, blue mesh region). Only acyl moieties are shown for clarity.

effective substituent, the isopropyl group, is considered to have the appropriate volume (C3 size) and length (about 4.5 Å). As seen in Table II, (*trans*-4-isopropylcyclohexyl)carbonyl compound **13** showed the highest potency among these analogues. Although (*cis*-4-isopropylcyclohexyl)carbonyl compound **12**, L-4-isopropenyl-1-cyclohexenecarbonyl compound **19**,[8] and 4-isopropylbenzoyl compound **20** were similar to **13** in molecular size and two-dimensional structure, their activities were unexpectedly different from that of **13**. **12** was inactive, while **19** and **20** were $^1/_{16}$ as potent as **13**.

The relative stereochemistry for **12** and **13** was studied by high-resolution ^{1}H NMR spectroscopy and semiempirical molecular orbital calculations (MNDO). In saturated H–C–C–H′ fragments the value of the coupling constant ($J_{HH'}$) depends critically on the H–C–C–H′ dihedral angle (Karplus rule).[9] Therefore, the proton signals at the 1-position on the cyclohexane ring of the *cis*-4-isopropyl compound **12** were compared with that of the *trans*-4-isopropyl compound **13** (Figure 1). The values of the coupling constant of *trans*-4-isopropyl compound **13** were 12 and 3.6 Hz and that of the cis-4-isopropyl compound **12** was 4.2 Hz. These values showed that the H1 of the *trans*-4-isopropyl compound **13** was axial and that of the *cis*-4-isopropyl compound **12** was equatorial. The semiempirical molecular orbital calculations (MNDO) and the computer graphic representations of **12** and **13** were carried out with the SYBYL molecular modeling system. The minimum-energy structures of **12** and **13** are shown in Figure 2. For comparison, the superimposing of **12** and **13** and the difference of the van der Waals volume between the low-activity analogue **12** and the highly active compound **13** are presented in Figure 3. The van der Waals volume of **12** was subtracted from the volume of **13** (yellow mesh region), and the volume of **13** was subtracted from the volume of **12** (blue mesh region). The result shows a great steric difference. The semiempirical molecular orbital calculations with the MNDO program on **19** and **20** were also carried out, and the three-dimensional structures of the acyl moiety of **19** and **20** were compared with that of **13**. The minimum-energy structures of **19** and **20** are shown in Figure 2. In the case of **13**, the cyclohexyl ring and the amide plane cross at a right angle. On the other hand, the torsional angle in compound **19** is 54° between the cyclohexene ring and amide plane, and the torsional angle in compound **20** is 30° between the benzene ring and amide plane. We suggest that the difference in the torsional angle between the ring structure and amide plane causes the difference in

(8) L-4-Isopropenyl-1-cyclohexenecarboxylic acid (L-perillic acid), which was a starting material of **19**, was available from Janssen Chimica Co.

(9) Karplus, M. *J. Chem. Phys.* **1959**, *30*, 11.

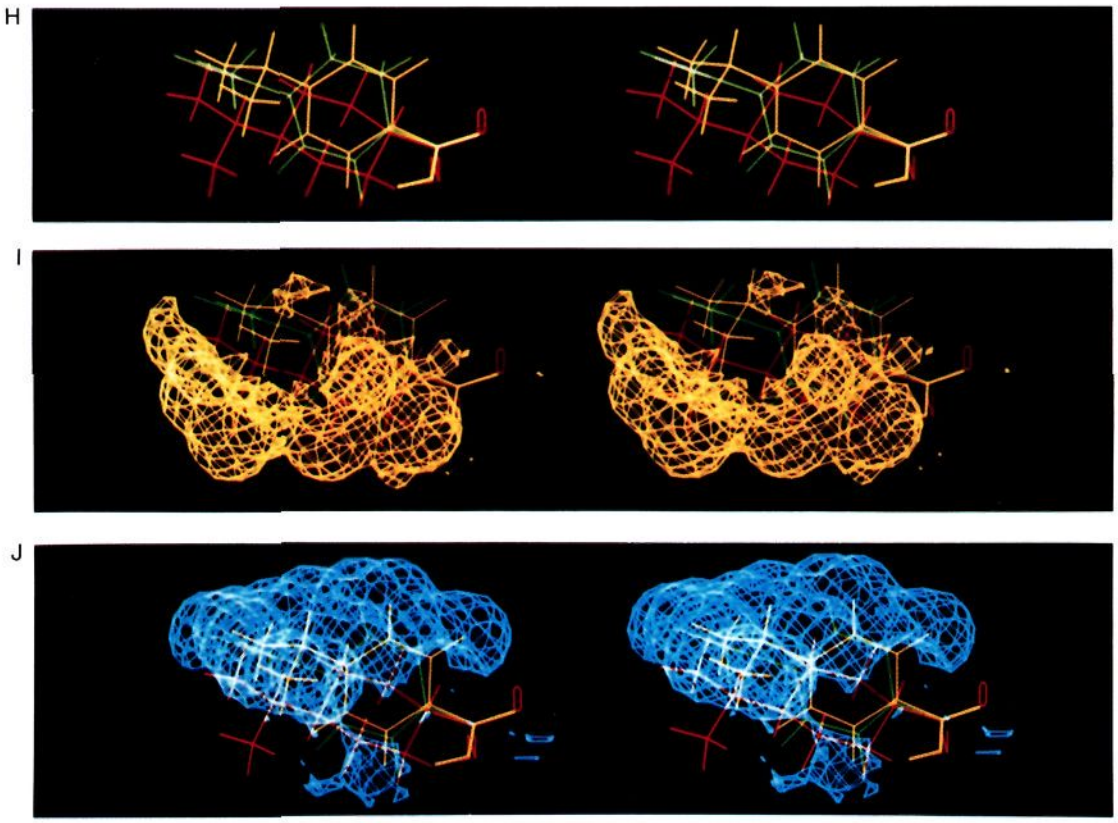
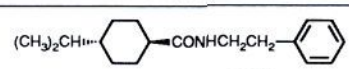
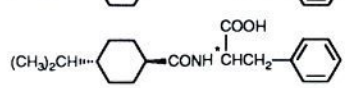
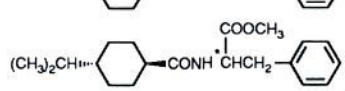
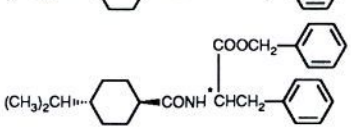
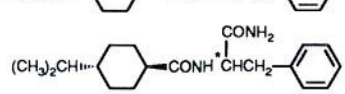
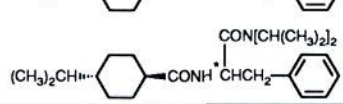


Figure 4. Stereoview of the overlap of (H) **13**, **19**, and **20**. The combined van der Waals volume of the low active analogues (**19** and **20**) was substracted from the volume of the high active compound (**13**) (I, yellow mesh region), and the volume of **13** was substracted from the combined volume of **19** and **20** (J, blue mesh region). Only acyl moieties are shown for clarity.

Table III. *N*-[(*trans*-4-Isopropylcyclohexyl)carbonyl]phenylalanine Derivatives

compd	structure	confign at *C	mp, °C	$[\alpha]_D$, deg	yield, %	formula[a]	hypoglycemic activity[b]
21			134–135		66	$C_{18}H_{27}N_1O_1$	inactive[c]
22		L	130–131	+9.4[d]	56	$C_{19}H_{27}N_1O_3$	100.0
23		D	137–138	+8.8[d]	52	$C_{20}H_{29}N_1O_3$	3.1
24		D	129–130.5	+8.4[e]	58	$C_{26}H_{33}N_1O_3$	25.0
25		D	219–220	+1.6[f]	52	$C_{19}H_{28}N_2O_2$	inactive[c]
26		D	116.5–117	+2.8[e]	66	$C_{25}H_{40}N_2O_2$	100.0

[a] Analyzed for C, H, and N; analytical results were within ±0.4% of the theortical values. [b] Lowest dose in milligram/kilogram causing more than a 20% blood glucose decrease (fasting mice, po). For potency of reference compound (tolbutamide), refer to Table II. [c] Less than 20% blood glucose decrease at a dose of 100 mg/kg. [d] In MeOH, c = 1.0%, at 20 °C. [e] In MeOH, c = 1.0%, at 23 °C. [f] In DMF, c = 1.0%, at 23 °C.

activity. For comparison, the superimposing of **13**, **19**, and **20** and the difference of the van der Waals volume between **13**, **19**, and **20** are shown in Figure 4. The combined van der Waals volume of the low-activity analogues **19** and **20** was subtracted from the volume of highly active compound **13** (yellow mesh region), and the volume of **13** was subtracted from the combined volumes of **19** and **20** (blue mesh region). For possession of good activity, the existence of the yellow mesh region and/or the absence of the blue mesh region may be necessary.

Table III shows the effect of the changes in the carboxyl group on hypoglycemic activity. Decarboxyl compound **21** was inactive. In spite of having the carboxyl group, the L-phenylalanine derivative **22** was $^1/_{70}$ as potent as the

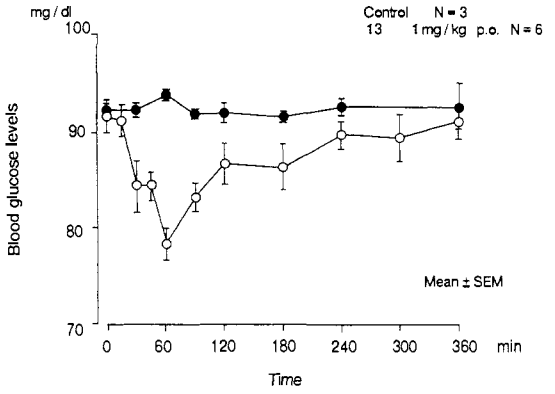


Figure 5. Blood glucose level vs time after oral administration of **13** was evaluated in a group of six male beagle dogs. Compound **13** was given orally in a gelatin capsule. A control group was treated in parallel with only a gelatin capsule. Blood samples were collected from the cephalic vein over a 0–6-h period. The blood glucose content was determined enzymatically by the glucose-oxidase-peroxidase method.

D-phenylalanine derivative **13**. Not only the carboxyl group but also the *R* configuration was necessary for possessing activity. Methyl ester **23** exhibited good efficacy. Benzyl ester **24** also maintained activity but was less potent than **23**. Amides **25** and **26** lost or extremely decreased the activity.

We elucidated the structural requirements for the hypoglycemic activity of the N-substituted D-phenylalanines and selected compound **13** as the best, which showed more than a 20% blood glucose decrease at an oral dose of 1.6 mg/kg in normal fasted mice. Blood glucose level vs time after oral administration of **13** in normal fasted dogs is shown in Figure 5. Further reports are in preparation on the mechanism of action of these compounds.

Experimental Section

All melting points were taken on a Yanagimoto micro melting point apparatus and are uncorrected. Analytical data of the compounds listed in the tables obtained for specified elements are within ±0.4% of the theoretical values unless otherwise noted. Optical rotations were measured on a JASCO DIP-140 digital polarimeter with a 10-cm cell. IR spectra were measured as KBr tablets with a JASCO IR-810 infrared spectrophotometer. ^1H NMR spectra of $CDCl_3$, CD_3OD, $(CD_3)_2SO$, or $(CD_3)_2CO$ solutions [internal $(CH_3)_4Si$, δ 0] were recorded on a Varian EM-390 spectrometer or a Varian XL-300 spectrometer.

***cis*-4-Isopropylcyclohexanecarboxylic Acid (2).** Cuminic acid (10 g, 61 mmol) was hydrogenated in acetic acid (50 mL) in the presence of platinum oxide (0.5 g) under 5 kg/cm^2 of hydrogen at room temperature. The reaction mixture was continuously shaken for 2 h. The acetic acid from the reaction mixture was distilled off under reduced pressure, and 9.5 g of the mixture of *cis*- and *trans*-4-isopropylcyclohexanecarboxylic acid in a ratio of 3:1 was obtained by distillation (113–116 °C, 1 mmHg). This mixture was esterified, and the methyl *cis*-4-isopropylcyclohexanecarboxylate (7.0 g, 39 mmol) was purified by HPLC with YMC A-043 S-5 SIL (20 × 250 mm) which was developed by a mixture of *n*-hexane and 1,2-dichloroethane in a ratio of 75:25 at a flow rate of 9.9 mL/min. This methyl ester was dissolved in 45 mL of methanol and hydrolyzed by 45 mL of 2 N aqueous sodium hydroxide at room temperature for 10 min. After the methanol was evaporated, the aqueous residue was acidified and then extracted with chloroform. The organic layer was dried with anhydrous magnesium sulfate and filtered and the filtrate was concentrated to give **2**.

***trans*-4-Substituted-cyclohexanecarboxylic Acid (1, 3, and 4).** A typical run (**3** in Table I) was as follows. The mixture of methyl *cis*- and *trans*-4-isopropylcyclohexanecarboxylate in a ratio of 3:1 (10 g, 56 mmol) was isomerized in the presence of 60% sodium hydride (0.22 g, 5.6 mmol) at 150 °C without solvent for 2 h. The mixture (9.4 g) of methyl *trans*- and *cis*-4-isopropylcyclohexanecarboxylate in a ratio of 6:1 was obtained by distillation (64 °C, 0.7 mmHg). This methyl ester was dissolved in 42 mL of methanol and hydrolyzed by 42 mL of 2 N aqueous sodium hydroxide at 20 °C for 10 min. The solution was acidified and the powdery precipitate was filtered. The product was recrystallized from aqueous methanol. The data are given in Table I.

N-Acylated Phenylalanines and Related Compounds (5–24). A typical run (**10** in Table I) was as follows. *trans*-4-Methylcyclohexanecarboxylic acid (10.0 g, 70 mmol) and HOSu (8.9 g, 77 mmol) were mixed in 200 mL of chloroform, followed by the addition of DCC (15.9 g, 77 mmol). This suspension was stirred for 3 h. The urea was filtered off, acetic acid (5 mL) was added, and the solution was stirred for 1 h. This solution was washed with saturated aqueous sodium bicarbonate and water, dried with anhydrous magnesium sulfate, and filtered. This filtrate was added to the solution of D-phenylalanine methyl ester (12.5 g, 70 mmol) in 100 mL of chloroform and stirred at 25 °C for 1 day. This mixture was washed with 1 N hydrochloric acid and water, dried with anhydrous magnesium sulfate, and filtered. The filtrate was concentrated to an oil. This oil was hydrolyzed in 50 mL of methanol and 20 mL of 2 N sodium hydroxide solution. After methanol was evaporated, the aqueous residue was adjusted to pH 2 with 1 N hydrochloric acid. The powdery precipitate was filtered and washed with water. The product was recrystallized from aqueous methanol. The data are given in Table II.

Amides 25 and 26. A typical run (**25** in Table II) was as follows. *N*-[(*trans*-4-Isopropylcyclohexyl)carbonyl]-D-phenylalanine (6.2 g, 20 mmol) and HOSu (2.5 g, 20 mmol) were mixed in 200 mL of chloroform, and DCC (4.4 g, 22 mmol) was added. This suspension was stirred for 1 day. The reaction mixture was worked up as described for the previous procedure. Ammonia water (29%, 2 mL) was added to the solution of the succinimido ester of **13** in 200 mL of chloroform and stirred at 25 °C for 1 day. This mixture was washed with 1 N hydrochloric acid and water, dried with anhydrous magnesium sulfate, and filtered. This filtrate was concentrated to a powder. The powder was recrystallized from ethyl acetate. The data are given in Table II.

Pharmacological Methods. The biological tests were carried out on groups of five ICR-CD1 mice (male, 5 weeks old, 18 h fasted). The test compounds were given orally as suspension in 0.5% (carboxymethyl)cellulose–0.14 M sodium chloride solution. The total volume was 1 mL/kg. A control group was treated in parallel with an equal volume of 0.5% (carboxymethyl)cellulose–0.14 M sodium chloride solution. To determine the lowest dose causing more than a 20% blood glucose decrease, the test compounds were applied in doses of 100.0, 50.0, 25.0, 6.3, 3.1, and 1.6 mg/kg. After 1 h blood samples were taken from the carotid. The blood glucose content was determined enzymatically by the glucose-oxidase-peroxidase method.[10] The statistical evaluation of the results utilized the Student's *t* test ($p < 0.05$).

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(10) Huggett, A.; Nixon, D. A. *Lancet* **1957**, 368.