the protected α,α-diamino compound; TLC R_f (E) 0.52; mp 145–147 °C; IR (KBr) 1735, 1683, 1647, 1519 cm^{-1}; NMR ($CDCl_3$) δ 7.75 (m, 1 H, NH), 7.31, 7.27, 7.25 (3 m, 15 H, arom), 6.26 (m, 1 H, NH), 5.48 (m, 1 H, NC*H*N), 5.16, 5.07 (2 s, 4 H, PhCH_2O), 3.57, 3.52 (2 s, 3 H, CH_3), 3.51 (m, 1 H, CO*CH*CO), 3.14 (m, 2 H, C_β H_2). Anal. ($C_{28}H_{28}N_2O_7$) C, H, N.

***N*-[(*RS*)-α-(Methoxycarbonyl)-β-phenylpropionyl]-α,α-diaminoacetic Acid (10).** Benzyl *N*-[(*RS*)-α-(methoxycarbonyl)-β-phenylpropionyl]-*N'*-(benzyloxycarbonyl)-α,α-diaminoacetate (27.55 mg, 0.55 mmol) was dissolved in MeOH. To this solution was added Et_3N (600 μL, 0.43 mmol), followed by 100 mg of 10% Pd/C. The hydrogenation was performed at atmospheric pressure with vigorous stirring. After 10 min, the mixture was filtered through a Celite pad and the solvent removed under reduced pressure to yield a glass. The residue was studied in NMR and IR and was tasted: IR (KBr) 1720, 1670, 1643, 1510 cm^{-1}; NMR ($CDCl_3$) δ 8.95 and 8.88 (2 d, 1 H, NH), 7.25 (m, 5 H, arom), 4.71 (2 overlapping d, 1 H, NC*H*N), 3.95 (m, 1 H, CO*CH*CO), 3.54 (s, 3 H, OCH_3), 3.03 (m, 2 H, C_β H_2).

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Peptide Sweeteners. 4. Hydroxy and Methoxy Substitution of the Aromatic Ring in L-Aspartyl-L-phenylalanine Methyl Ester. Structure–Taste Relationships

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A series of analogues of the dipeptide sweetener L-aspartyl-L-phenylalanine methyl ester having hydroxy and/or methoxy substitution on the aromatic ring was synthesized and tasted. The introduction of a methoxy group in the para position of the aromatic ring of the peptide sweetener is crucial to the reduction or destruction of the sweet taste. The effects of substituents in the ortho or meta position are not as pronounced. In the case of *o*-methoxy substitution, the resulting analogue is only slightly less sweet than the parent dipeptide sweetener.

Following the accidental discovery[4] that L-aspartyl-L-phenylalanine methyl ester (1) is intensely sweet, several groups have studied the structural requirements necessary for sweet taste. The zwitterionic N-terminal function must possess either a six- or five-membered zwitterionic ring as in L-aspartyl[4] or (*RS*)-α-aminomalonyl[5,6] residues, respectively. The C-terminal residue is more flexible in its structural requirements and can accommodate a large variety of amino acid esters[4–9] and amides.[10]

In order to maximize sweetness, it is necessary to have a certain dissimilarity in size of the hydrophobic groups located on the α carbon of the C-terminal residue. The orientation of these groups should correspond to the configurational arrangement found in L-phenylalanine methyl ester (1) such that the large group corresponds to the benzyl side chain and the small group corresponds to the methoxycarbonyl structure. Our previous studies[11,12] demonstrated the importance of the peptide bond which connects the N-terminal zwitterionic part and the C-terminal hydrophobic portion. Any modification of the peptide bond results in the complete loss of sweetness.

In this paper, we present a study of the effects of hydroxy and methoxy mono- and disubstitution at various positions on the aromatic ring in the dipeptide sweetener 1. Results from these compounds should refine our present understanding of the sweetener's hydrophobic portion.

Synthesis. The general synthetic approach used in this paper was to couple β-benzyl *N*-(benzyloxycarbonyl)-L-aspartate[13] to the appropriate aromatic-substituted phenylalanine methyl esters with either *N,N'*-dicyclohexylcarbodiimide (DCC)[14] or DCC and 1-hydroxybenzotriazole.[15]

The DL-*m*-hydroxyphenylalanine was resolved by fractional crystallization of the brucine salts following the procedure of Sealock.[16] The optical resolution of DL-*o*-hydroxyphenylalanine was performed on the corresponding

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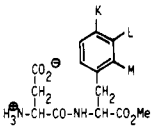
Table I. Qualitative Taste Evaluation of Aryl-Substituted Derivatives of L-Aspartyl-L-phenylalanine Methyl Ester



	Chirality of Residues		Substitution on the Phenyl Ring			Taste[a]
	N-terminal	C-terminal	K (para)	L (meta)	M (ortho)	
i) Phenylalanine analogs						
1	L	L	H	H	H	+++[b]
2	L	D	H	H	H	-[b]
3	L	DL	H	H	H	+++
ii) Tyrosine(p-Hydroxyphenylalanine) analogs						
4	L	L	OH	H	H	++[b]
5	L	D	OH	H	H	o
6	L	DL	OH	H	H	++
7	L	L	OMe	H	H	+[b]
iii) m-Hydroxyphenylalanine analogs						
8	L	L	H	OH	H	++
9	L	D	H	OH	H	o
10	L	DL	H	OH	H	++
11	L	L	H	OMe	H	++
12	L	DL	H	OMe	H	++
iv) o-Hydroxyphenylalanine analogs						
13	L	L	H	H	OH	++
14	L	D	H	H	OH	o
15	L	DL	H	H	OH	++
16	L	DL	H	H	OMe	+++
v) m,p-Dihydroxyphenylalanine analogs						
17	L	L	OH	OH	H	+
18	L	L	OMe	OH	H	o
19	L	L	OH	OMe	H	+
20	L	L	OMe	OMe	H	o

a) The compounds were tested by a volunteer taste panel from our laboratories. The panel was able to achieve reproducible taste intensities involving sucrose solutions and the parent dipeptide sweetener. All compounds were tasted well above the threshold. The taste intensities were related to an 8% sucrose solution. At least six double-blind tests were performed by the panel on each compound.

b) The taste intensities of these compounds are taken from Reference 4.

+ ~1 - 5 times as sweet as sucrose
++ ~10 - 40 times as sweet as sucrose
+++ ~80 - 200 times as sweet as sucrose
- bitter
o tasteless

ethyl ester which was subjected to enzymatic digestion by chymotrypsin.[17] The methyl esters were prepared from the corresponding amino acids either by Fischer esterification[18] or by using thionyl chloride.[19] The protecting groups were removed by catalytic hydrogenation in either methanol or acetic acid.[20]

Results and Discussion

The qualitative taste evaluations of the aryl-substituted dipeptide methyl esters (**1–20**) are summarized in Table I. In order for the dipeptide to be sweet, both chiral centers must have the L configuration. This requirement is observed for all the compounds listed in Table I. It is observed that if the L,L compound is sweet, the L,DL analogue is also sweet. The presence of the tasteless or slightly bitter isomer (L,D) in a diastereoisomeric mixture does not destroy the sweet taste of the other diastereomer (L,L) present in the mixture.

Monosubstitution of the phenyl residue either by a hydroxy or a methoxy group in any of the available positions, i.e., ortho, meta, and para, does not impair the perception of a sweet taste, though the taste intensity is less than the parent sweetener (**1**). Also among the monosubstituted compounds, a significant difference in their sweet intensity is observed, i.e., the *o*-methoxy derivative (**16**) is sweeter than the monosubstituted derivatives (**4**, **8**, **11**, and **13**), which in turn are sweeter than the *p*-methoxy compound (**17**).

As for the meta,para disubstituted phenylalanine analogues, sweet taste is observed in the compounds which possess a hydroxyl group at the para position (*m*,*p*-dihydroxy and *m*-methoxy-*p*-hydroxy analogues, compounds **17** and **19**, respectively), while those having a *p*-methoxy substituent (*m*-hydroxy-*p*-methoxy and *m*,*p*-dimethoxy analogues, **18** and **20**, respectively) are tasteless.

Since the substituents we chose are small and removed from the two chiral centers, we will assume that we have not changed the overall backbone conformations of the dipeptides. Thus, we feel that these analogues selectively probe the steric and electronic environment close to the aromatic ring.

As to the nature of the interaction of any sweetener molecules with receptor sites, various concepts have been proposed. Hydrogen-bond formation was introduced by Shallenberger and Acree,[21] hydrophobic interactions were mentioned by Ariyoshi,[22] and dispersion forces (Van der Waals forces) were discussed by Kier.[23]

We introduced substituents in the hydrophobic segment of the sweetener such as the hydroxy and/or methoxy groups. These substitutions may substantially affect the complementarity required for an effective tastant–receptor interaction. Our qualitative taste evaluation showed that the introduction of hydroxy and/or methoxy groups reduces the intensity of taste but maintains the character of the sweet taste.

We attribute the decreased sweetness by monohydroxy or monomethoxy substitution (in **4**, **7**, **8**, **11**, **13**, and **16**) compared to the parent compound **1** to the introduction of steric effects, which reduce the effective interaction of the aromatic moieties with the hydrophobic site of the receptor. This effect is more pronounced in the para position, where the hydroxy analogue (**4**) is sweeter than the methoxy analogue (**7**). The opposite tendency is observed in the case of ortho substitution, where the *o*-methoxy analogue (**16**) is much sweeter than the *o*-hydroxy analogue (**13**). This indicates that the *o*-methoxy group can interact with the taste receptor site quite efficiently without any additional significant steric hindrance. The reduced sweetness of the *o*-hydroxyphenylalanine (**13**) compared to the *o*-methoxy analogue (**16**) may be attributed to unfavorable interactions arising from the phenolic hydroxyl located adjacent to the peptide bond.

It is interesting that a similar tendency in order of sweetness as found in the methoxy-substituted analogues (**16** > **11** > **7**) was observed in the series of L-aspartyl-DL-α-aminomalonic acid methylcyclohexyl methyl diesters[6,7] (**21–23**); i.e., the 2-methylcyclohexyl ester (**21**) is sweeter than the 3-methylcyclohexyl derivative (**22**), which is sweeter than the 4-methylcyclohexyl derivative (**23**).

In the meta,para disubstituted phenylalanine analogues (**17–20**), which have no ortho substituent, the reduction of sweetness caused by the increase in the size of the aromatic moiety is observed more clearly. Addition of the *p*-hydroxy group to the sweet meta monosubstituted

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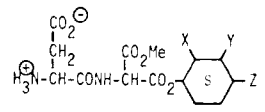

21 X=Me Y=Z=H

22 Y=Me X=Z=H

23 Z=Me X=Y=H

analogues (8 and 11) results in the less sweet disubstituted derivatives, i.e., *m,p*-dihydroxy and *m*-methoxy-*p*-hydroxy analogues (**17** and **19**), respectively. Replacement of the *p*-hydroxy group in compounds **17** and **19** by *p*-methoxy gives *m*-hydroxy-*p*-methoxy and *m,p*-dimethoxy analogues (**18** and **20**), which results in complete loss of sweetness. The combination of the larger methoxy group in the para position with any substitution, hydroxy or methoxy, in the meta position creates a structure which is too bulky to interact effectively at the receptor site. Thus, the sweet taste is lost.

In summary, to determine if our observations are general, we are preparing numerous other substituents at different positions about the aromatic ring, such as *o,o'*-, *o,m*-, and *o,m'*-disubstituted analogues. We expect at least the ortho,meta analogue to be quite sweet if our size generalizations are valid. We are also synthesizing other derivatives which have alkyl substituents (methyl, ethyl, etc.) on the aromatic ring.

Experimental Section

Melting points were taken on a Thomas-Hoover apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 141 with a 10-cm water-jacketed cell. L-*m*-Hydroxy-*p*-methoxyphenylalanine was donated graciously by Dr. J. Meienhofer from Hoffmann-LaRoche, Inc., and L-*m*-methoxy-*p*-hydroxyphenylalanine was obtained from Calbiochem. DL-*o*-Hydroxy- and DL-*m*-hydroxyphenylalanines were obtained from Sigma. All other amino acid derivatives were obtained from Bachem. Where analyses are indicated only by symbols of the elements, the analytical values were within ±0.4% of the theoretical values.

Amino Acid Methyl Esters. D-Tyrosine Methyl Ester Hydrochloride. This compound was prepared according to Schröder.[24]

***N*-Formyl-L-*m*-hydroxyphenylalanine.** This compound was prepared following the procedure of Sealock et al.:[16] mp 147–149 °C, lit.[16] mp 148–149 °C; $[\alpha]^{25}_D$ +43.8° (*c* 1, H_2O), lit.[16] $[\alpha]^{27}_D$ +45.7° (*c* 1, H_2O).

***N*-Formyl-D-*m*-hydroxyphenylalanine.** This compound was prepared following the procedure of Sealock et al.:[16] mp 148–150 °C, lit.[16] mp 146–148 °C; $[\alpha]^{25}_D$ −43.5° (*c* 1, H_2O), lit.[16] $[\alpha]^{27}_D$ −44.7° (*c* 1, H_2O).

L-*m*-Hydroxyphenylalanine Methyl Ester Hydrochloride. The amino acid derivative *N*-formyl-L-*m*-hydroxyphenylalanine (178 mg, 0.85 mmol) was dissolved in 12% hydrochloric acid and heated at 100 °C for 4 h. Removal of the solvent under reduced pressure gave a residue which was taken up in methanol and subsequently treated with dry HCl. Removal of the solvent under reduced pressure gave a residue which was crystallized from methanol–ether to yield the corresponding methyl ester hydrochloride as colorless needles: yield 193 mg (98%); mp 193–194 °C.

D-*m*-Hydroxyphenylalanine Methyl Ester Hydrochloride. Following the preceding procedure for the L isomer, the corresponding ester of the D isomer was obtained in a quantitative yield, mp 194–195 °C.

L-*o*-Hydroxyphenylalanine. This compound was prepared following the procedure of Tong et al.:[17] mp 232–235 °C dec; $[\alpha]^{25}_D$ −25.4° (*c* 1.6, 1 N HCl), lit.[17] $[\alpha]^{25}_D$ −26.8° (*c* 2, 1 N HCl).

D-*o*-Hydroxyphenylalanine. This compound was prepared following the procedure of Tong et al.:[17] mp 234–236 °C dec; $[\alpha]^{25}_D$ +26.7° (*c* 2, 1 N HCl), lit.[17] $[\alpha]^{25}_D$ +25.4° (*c* 2, 1 N HCl).

L-*o*-Hydroxyphenylalanine Methyl Ester. This compound was prepared as described above for L-*m*-hydroxyphenylalanine methyl ester hydrochloride. The crude ester hydrochloride was taken up in water, neutralized with $NaHCO_3$, and extracted into chloroform. The organic layer was dried over $MgSO_4$, and the residue obtained after removal of the solvent under reduced pressure was used without further purification.

D-*o*-Hydroxyphenylalanine Methyl Ester Hydrochloride. This compound was prepared as described above for L-*m*-hydroxyphenylalanine methyl ester hydrochloride. The crystalline ester hydrochloride was obtained in a quantitative yield, mp 169–172 °C.

L-*m*-Methoxyphenylalanine Methyl Ester Hydrochloride. To a solution of *N*-formyl-L-*m*-hydroxyphenylalanine (260 mg, 1.24 mmol) in methylene chloride/methanol (40 mL, 1:1) was added a large excess of a freshly prepared ethereal solution of diazomethane. After the solution was left standing for 3 days, the insoluble material was filtered off and the filtrate was removed under reduced pressure, affording a yellow oil. Chromatography on a silica gel column (20 × 320 mm) with chloroform as eluant gave the *N*-formyl-L-*m*-methoxyphenylalanine methyl ester as a colorless oil (68%) and was carried on to the next step.

The crude *N*-formyl-L-*m*-methoxyphenylalanine methyl ester was taken through the same procedure as described above for L-*m*-hydroxyphenylalanine methyl ester to yield the corresponding ester hydrochloride (97%).

DL-*o*-Methoxyphenylalanine Methyl Ester Hydrochloride. *N*-Acetyl-DL-*o*-methoxyphenylalanine, prepared from *N*-acetyl-DL-2-hydroxyphenylalanine and dimethyl sulfate in aqueous NaOH, was treated in the similar manner as the preparation of L-*m*-hydroxyphenylalanine methyl ester hydrochloride from *N*-formyl-L-*m*-hydroxyphenylalanine, giving the crystalline methyl ester hydrochloride (95%), mp 105–110 °C.

L-*m,p*-Dihydroxyphenylalanine Methyl Ester Hydrochloride. This compound was prepared according to Banerjee and Ressler.[25]

L-*p*-Hydroxy-*m*-methoxyphenylalanine Methyl Ester Hydrochloride. This compound, prepared as described above for L-*m*-hydroxyphenylalanine methyl ester, gave a white crystalline product (69%): mp 173–174 °C; $[\alpha]^{25}_D$ +5.7° (*c* 1, MeOH).

L-*m*-Hydroxy-*p*-methoxyphenylalanine Methyl Ester Hydrochloride. This compound was prepared as described above for L-*m*-hydroxyphenylalanine methyl ester: white needles (83%); mp 194–195 °C.

L-*m,p*-Dimethoxyphenylalanine Methyl Ester Hydrochloride. This compound was prepared according to Schrecker and Hartwell.[26]

General Coupling Procedures. A. β-Benzyl *N*-(benzyloxycarbonyl)-L-aspartate (715 mg, 2 mmol), the amino acid methyl ester hydrochloride (2.1 mmol), and triethylamine (0.24 mL, 2.1 mmol) were dissolved in a solvent system as described for each compound separately. Cooling the solution to −10 °C and stirring were followed by the addition of dicyclohexylcarbodiimide (454 mg, 2.2 mmol) and 1-hydroxybenzotriazole (70 mg, 0.5 mmol). The mixture was stirred for 2 h at 0 °C and then left for 12 h at room temperature. Removal of the solvent under reduced pressure gave a residue, which was taken up in ethyl acetate. The insoluble precipitate was filtered off and the filtrate was washed successively with 0.5 N HCl, water, a 5% aqueous solution of $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The subsequent workup is described for each of the protected dipeptides under the individual titles.

B. To a suspension of amino acid methyl ester hydrochloride (21 mmol) in CH_2Cl_2 (40 mL) was added triethylamine (2.9 mL, 21 mmol). The mixture was stirred for 15 min under N_2 and β-benzyl *N*-(benzyloxycarbonyl)-L-aspartate (6.8 g, 19.1 mmol) was added. A solution of dicyclohexylcarbodiimide (3.94 g, 19.1 mmol) in CH_2Cl_2 (20 mL) was added slowly (5 min) to the stirred, ice-cooled reaction mixture, which was then allowed to warm to

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room temperature and left to stir for 12 h. The addition of a few drops of glacial acetic acid was followed by stirring for 0.5 h and subsequent filtration. The filtrate was washed successively with 1 N HCl, water, a saturated solution of $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The subsequent workup is described for each of the protected dipeptides under the individual titles.

General Hydrogenation Procedures. C. The protected dipeptide (1 mmol) was dissolved in methanol (20 mL) and subjected to hydrogenation in the presence of 10% palladium on charcoal catalyst (50 mg) at room temperature under atmospheric pressure of hydrogen for 12 h. The catalyst was removed by filtration and the filtrate was removed under reduced pressure. The subsequent workup is outlined for each dipeptide methyl ester under the individual titles.

D. The protected dipeptide (1 mmol) was dissolved in glacial acetic acid (20 mL) and subjected to hydrogenation in the presence of 10% Pd/C catalyst (50 mg) at room temperature on a Parr apparatus under hydrogen pressure at 50 psi for 1 h. The catalyst was removed by filtration and the filtrate was removed under reduced pressure. The subsequent workup is outlined for each dipeptide methyl ester under the individual titles.

L-Aspartyl-D-tyrosine Methyl Ester (5). The coupling reaction followed general procedure A. The solvent mixture used was chloroform/tetrahydrofuran/*N,N*-dimethylformamide (2:1:1). The residue obtained from evaporation was crystallized from isopropyl alcohol to give *N*-(benzyloxycarbonyl)-β-benzyl-L-aspartyl-D-tyrosine methyl ester as colorless needles (82%), mp 115–116 °C. The deprotection followed general procedure C. The dipeptide ester **5** was obtained as white powder (90%): mp 155–156 °C (from methanol); $[\alpha]^{25}_D$ +7.7° (*c* 0.9, H_2O). Anal. ($C_{14}H_{18}N_2O_6 \cdot 0.5CH_3OH \cdot 0.5H_2O$) C, H, N.

L-Aspartyl-L-*m*-hydroxyphenylalanine Methyl Ester (8). The coupling reaction followed general procedure A, and the solvent mixture used was chloroform/tetrahydrofuran (1:1). The residue from the evaporation was a colorless oil, which was carried without further purification to the next step.

The deprotection reaction followed general procedure C. The residue obtained from evaporation was chromatographed on a Sephadex LH-20 column (20 × 320 mm) and eluted with methanol. Analogue **8** was obtained as a colorless powder (42%): mp 146–148 °C; $[\alpha]^{25}_D$ −6.9° (*c* 1, MeOH). Anal. ($C_{14}H_{18}N_2O_6 \cdot 2.5H_2O$) C, H, N.

L-Aspartyl-D-*m*-hydroxyphenylalanine Methyl Ester (9). The coupling reaction followed general procedure A. The solvent used was chloroform. The oily residue obtained from removal of the solvent under reduced pressure was chromatographed on a silica gel column (20 × 320 mm) and eluted with chloroform. Benzyloxycarbonyl-β-benzyl-L-aspartyl-D-*m*-hydroxyphenylalanine methyl ester was obtained as an oil, which solidified on standing (71%), mp 96–98 °C.

The deprotection reaction followed general procedure C. The residue obtained from removal of the solvent under reduced pressure was crystallized from methanol to give **9** as colorless needles (48%): mp 113–116 °C; $[\alpha]^{25}_D$ −0.5° (*c* 1, MeOH). Anal. ($C_{14}H_{18}N_2O_6 \cdot 1.5H_2O$) C, H, N.

L-Aspartyl-L-*m*-methoxyphenylalanine Methyl Ester (11). The coupling reaction followed general procedure A. The solvent used was chloroform. The residue obtained from removal of the solvent under reduced pressure was purified by preparative silica gel layer chromatography (2-mm thickness; solvent system: benzene/ethyl acetate, 3:2). *N*-(Benzyloxycarbonyl)-β-benzyl-L-aspartyl-L-*m*-methoxyphenylalanine methyl ester was obtained as a colorless oil, which solidified on standing (90%), mp 111–112 °C.

The deprotection reaction followed general procedure C. When the solvent was removed under reduced pressure, compound **11** was obtained as a colorless powder (58%): mp 143–144 °C; $[\alpha]^{25}_D$ −11.3° (*c* 0.9, MeOH). Anal. ($C_{15}H_{20}N_2O_6 \cdot 1.5H_2O$) C, H, N.

L-Aspartyl-L-*o*-hydroxyphenylalanine Methyl Ester (13). The coupling followed general procedure A. The solvent used was chloroform. The residue obtained after removal of the solvent under reduced pressure was purified by column chromatography (20 × 320 mm) on silica gel using chloroform as the eluant. *N*-(Benzyloxycarbonyl)-β-benzyl-L-aspartyl-L-*o*-hydroxyphenylalanine methyl ester was obtained as a colorless oil (71%).

The deprotection reaction followed general procedure C. Removal of the solvent under reduced pressure yielded the crude product, which was then chromatographed on a Sephadex LH-20 column (20 × 320 mm) using methanol as eluant. The dipeptide methyl ester **13** was obtained as colorless crystals from methanol (60%): mp 175–176 °C dec; $[\alpha]^{25}_D$ −12.1° (*c* 1, H_2O). Anal. ($C_{14}H_{18}N_2O_6 \cdot 0.5CH_3OH$) C, H, N.

L-Aspartyl-D-*o*-hydroxyphenylalanine Methyl Ester (14). The coupling reaction followed general procedure A. The solvent system used was chloroform/tetrahydrofuran/*N,N*-dimethylformamide (2:1:1). The residue obtained from the removal of the solvent under reduced pressure was purified by column chromatography (20 × 300 mm) on silica gel using chloroform as eluant. *N*-(Benzyloxycarbonyl)-β-benzyl-L-aspartyl-D-*o*-hydroxyphenylalanine methyl ester was obtained as a colorless oil (93%).

The deprotection reaction followed general procedure C. Removal of the solvent under reduced pressure yielded the crude product, which was then chromatographed on an LH-20 column (20 × 320 mm) using methanol as eluant. The dipeptide methyl ester **14** was obtained as colorless crystals from methanol (45%): mp 109–112 °C; $[\alpha]^{25}_D$ +24.0° (*c* 0.9, H_2O). Anal. ($C_{14}H_{18}N_2O_6 \cdot CH_3OH \cdot H_2O$) C, H, N.

L-Aspartyl-DL-*o*-methoxyphenylalanine Methyl Ester (16). The coupling reaction followed general procedure A. The solvent used was chloroform. The residue obtained from the removal of the solvent under reduced pressure was purified by column chromatography (20 × 320 mm) on silica gel using chloroform as eluant. *N*-(Benzyloxycarbonyl)-β-benzyl-L-aspartyl-DL-*o*-methoxyphenylalanine methyl ester was obtained as a colorless oil (62%).

The deprotection reaction followed general procedure C. The residue from the removal of the solvent under reduced pressure was crystallized from water to give **16** as colorless needles (85%): mp 157–158 °C; $[\alpha]^{25}_D$ +5.0° (*c* 1, MeOH). Anal. ($C_{15}H_{20}N_2O_6 \cdot 1.5H_2O$) C, H, N.

L-Aspartyl-L-*m,p*-dihydroxyphenylalanine Methyl Ester (17). The coupling reaction followed general procedure B. The residue obtained from the removal of the solvent under reduced pressure was crystallized from ethyl acetate/hexane to give *N*-(benzyloxycarbonyl)-β-benzyl-L-aspartyl-L-*m,p*-dihydroxyphenylalanine methyl ester as a white powder (48%): mp 108–109 °C; $[\alpha]^{25}_D$ +35.65° (*c* 2.1, $CHCl_3$). Anal. ($C_{29}H_{30}N_2O_9$) C, H, N.

The deprotection followed general procedure D. The residue was lyophilized from water (2 × 10 mL) to give **17** as a light blue powder (69%); mp 130–131 °C. Further attempts to remove minor impurities caused complete degradation to highly colored material. Anal. ($C_{14}H_{18}N_2O_7 \cdot 2H_2O$) H, N; C: calcd, 46.41; found, 49.59.

L-Aspartyl-L-*m*-hydroxy-*p*-methoxyphenylalanine Methyl Ester (18). The coupling reaction followed general procedure B. The residue obtained from removal of the solvent under reduced pressure was passed through a preparative high-performance liquid chromatography column using a prepacked silica gel cartridge and 1% methanol in chloroform as eluent. The recovered clear oily dipeptide was taken directly to the next step.

The deprotection reaction followed general procedure D. The residue obtained from removal of the solvent under reduced pressure was lyophilized from water (2 × 10 mL) to give a material which was passed through a partition chromatography column (15 × 1000 mm) on Sephadex G-25 using a butanol/acetic acid/water (4:1:5) system. The dipeptide ester recovered from the chromatography by removal of the solvent under reduced pressure was lyophilized from water (2 × 10 mL) to give **18** as a white solid (80%): mp 140–141 °C; $[\alpha]^{25}_D$ +1.45° (*c* 1.1, CH_3OH). Anal. ($C_{15}H_{20}N_2O_7 \cdot H_2O$) C, H, N.

L-Aspartyl-L-*m*-methoxy-*p*-hydroxyphenylalanine Methyl Ester (19). The coupling reaction followed general procedure B. The residue obtained from evaporation was chromatographed on a silica gel column (20 × 320 mL) and eluted with ethyl acetate/hexane (70:30) to give the protected dipeptide as a white powder (63%): mp 130–131 °C; $[\alpha]^{25}_D$ +37.7° (*c* 2, $CHCl_3$). Anal. ($C_{30}H_{32}N_2O_9$) C, H, N.

The deprotection reaction followed general procedure D. The residue obtained from removal of the solvent under reduced pressure was crystallized from ethanol/water to give **19** as a gray powder (89%): mp 149–150 °C; $[\alpha]^{25}_D$ −32.6° (*c* 2.2, CH_3OH). Anal. ($C_{15}H_{20}N_2O_7 \cdot 2H_2O$) C, H, N.

L-Aspartyl-L-*m,p*-dimethoxyphenylalanine Methyl Ester (20). The coupling reaction followed general procedure B. The residue obtained from removal of the solvent under reduced pressure was crystallized from isopropyl alcohol to give *N*-(benzyloxycarbonyl)-β-benzyl-L-aspartyl-L-*m,p*-dimethoxyphenylalanine methyl ester as a white solid (61%): mp 154–155 °C; $[\alpha]^{25}_D$ +41.4° (*c* 1.0, $CHCl_3$). Anal. ($C_{31}H_{34}N_2O_9$) C, H, N.

The deprotection reaction followed general procedure D. Removal of the solvent under reduced pressure yielded **20** as a white solid (98%): mp 139–140 °C; $[\alpha]^{25}_D$ −1.2° (*c* 1.2, CH_3OH). Anal. ($C_{16}H_{22}N_2O_7$) C, H, N.

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4-Amino-4-arylcyclohexanones and Their Derivatives, a Novel Class of Analgesics. 1. Modification of the Aryl Ring

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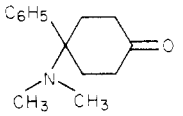
Investigation of central nervous system activity of phenylcyclohexylamines was continued by preparation of "reversed" analogues. Following the unexpected finding of analgesic activity with 1-(dimethylamino)-1-phenylcyclohexylamine, the SAR of the series was investigated. Synthesis starts by double Michael reaction of acrylate on arylacetonitriles. Following cyclization, decarboxylation, ketalization, and saponification, the geminally substituted acid is rearranged to the isocyanate by means of $(C_6H_5O)_2PON_3$. Isocyanates were then converted to the title compounds. Analgesic activity is very sensitive to the nature and position of the substitutent on the aromatic ring. The most potent compounds in this series (*p*-CH_3, *p*-Br) showed 50% the potency of morphine. Deletion of the ring oxygen abolishes activity.

Compounds related to 4-phenylcyclohexylamine have proven a fruitful nucleus for the preparation of biologically active compounds. Suitable modifications of this moiety have provided several series of compounds which show neuroleptic activity;[2] substitution of a carbon onto the ring atom bearing aryl leads to hypotensive agents.[3] One of the more interesting of the earlier series was that in which that same carbon bore an oxygen substituent.[2] It was thus of some interest to ascertain the effect on biological activity of placing a nitrogen atom on that apparently important position. Specifically, we undertook the preparation of 4-phenyl-4-(dimethylamino)cyclohexanone (1). Random

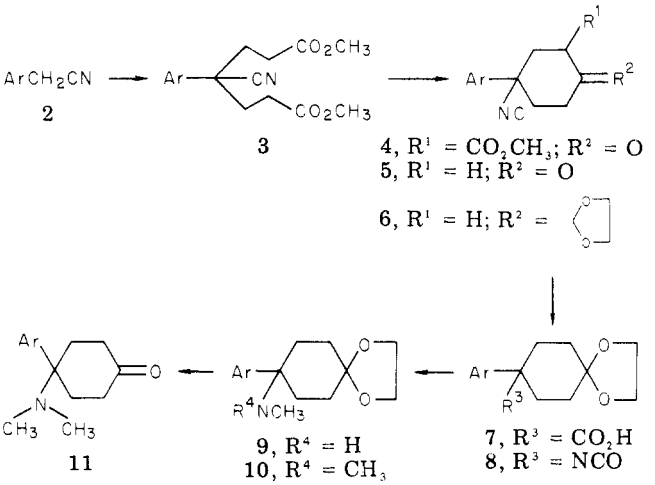


1

screening surprisingly showed this compound to exhibit narcotic-like analgesic activity. This was particularly unanticipated because the molecule departs so radically from the various SAR correlations proposed for centrally acting analgesics.[4] We thus undertook the systematic investigation of the SAR in this series. The present report deals with the effect on activity of modification of the aromatic moiety.

Chemistry. Our initial approach to these deceptively simple compounds relied heavily on the scheme we had devised in connection with the earlier work for construction of the substituted carboxylic acids (**7**) (Scheme I).[3] This route offered the advantage that most of the required arylacetonitriles are commercially available (the *p*-*tert*-butylnitrile was obtained in a straightforward manner from the benzyl alcohol). The key to the sequence was the

Scheme I



recently developed modification of the Curtius reaction which allows this transformation to be carried out in the presence of acid-labile groups.[5] We modified this procedure yet further in that we substituted an inert high-boiling solvent (anisole) for the alcohols used in the original work. It is a tribute to the extreme steric hindrance about the quaternary carbon that the isocyanates (**8**) obtained by this reaction sequence are usually stable to chromatography on silica gel—the routine isolation procedure. For reasons which are not immediately apparent, the product from the acid containing the 2-thienyl group as the aromatic substituent showed the expected isocyanate reactivity; in this case, the reaction was run in ethanol to afford the corresponding carbamate. Reduction of **8** by means of $LiAlH_4$ afforded the secondary amine (**9**). This was then methylated by means of CH_2O and $NaBH_4$[6] (the hindered nature of the amine again manifested itself in the observation that at least one recycle was required to assure complete

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(2) See, for example, D. Lednicer, D. E. Emmert, R. A. Lahti, and A. D. Rudzik, *J. Med. Chem.*, **16**, 1251 (1973).
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