CH_3COOH·Asn-Asn-Ile-Ala-O-*t*-Bu (3.52 g, 6.44 mmol), and HOBt (1.23 g, 8 mmol) were dissolved in dimethylformamide. After cooling, *N,N*-diisopropylethylamine (2.75 mL, 16 mmol) was added. The reaction mixture was treated according to the usual procedure (see compound **2**): yield 3.82 g (70%); mp 235 °C dec; $[\alpha]^{25}_D$ −26° (*c* 1, DMF); R_f(E) 0.14. Anal. ($C_{40}H_{64}N_8O_{12}$) C, H, N.

Z-Asn-Lys(Boc)-Asn-Asn-Ile-Ala-O-*t*-Bu (5). Compound **4** (3.82 g, 4.5 mmol) was hydrogenated as previously described for compound **3**. A 3.21-g (4.14 mmol) portion of CH_3COOH·Lys(Boc)-Asn-Asn-Ile-Ala-O-*t*-Bu was recovered; R_f(C) 0.42. Z-Asn-ONp[18] (2.2 g, 5.68 mmol), CH_3COOH·Lys(Boc)-Asn-Asn-Ile-Ala-O-*t*-Bu (3.21 g, 4.14 mmol), and HOBt (0.87 g, 5.68 mmol) were dissolved in dimethylformamide. After cooling, *N,N*-diisopropylethylamine (0.42 g, 10 mmol) was added. The reaction mixture was treated according to the usual procedure (see compound **2**): yield 3.82 g (70%); mp 230 °C dec; $[\alpha]^{25}_D$ −27° (*c* 1, DMF); R_f(F) 0.73. Anal. ($C_{44}H_{70}N_{10}O_{14}$) C, H, N.

Z_3-Arg-Asn-Lys(Boc)-Asn-Asn-Ile-Ala-O-*t*-Bu (6). Compound **5** (3 g, 3.12 mmol) was hydrogenated as previously described for compound **2**. A 2.5-g (2.89 mmol) portion of HCl·Asn-Lys(Boc)-Asn-Asn-Ile-Ala-O-*t*-Bu was recovered; R_f(C) 0.32. Z_3-Arg-OH (0.75 g, 1.3 mmol),[20] HCl·Asn-Lys(Boc)-Asn-Asn-Ile-Ala-O-*t*-Bu (1 g, 1.16 mmol), and BOP (0.57 g, 1.3 mmol) were dissolved in dimethylformamide. After cooling, *N,N*-diisopropylethylamine (0.42 mL, 2.47 mmol) was added. The reaction mixture was treated as already described (see compound **2**): yield 667 mg (40%); mp 225 °C dec; $[\alpha]^{25}_D$ −10° (*c* 1, DMF); R_f(G) 0.28. Anal. ($C_{66}H_{94}N_{14}O_{19}$) C, H, N.

Bis-Boc-Lys-Arg-Asn-Lys(Boc)-Asn-Asn-Ile-Ala-O-*t*-Bu (7). Compound **6** (600 mg, 0.43 mmol) was hydrogenated as previously described for compound **3**. $(CH_3COOH)_3$·Arg-Asn-Lys(Boc)-Asn-Asn-Ile-Ala-O-*t*-Bu (356 mg, 0.31 mmol) was recovered; R_f(C) 0.20. Bis-Boc-Lys-OSu[12] (145 mg, 0.35 mmol) and $(CH_3COOH)_3$·Arg-Lys(Boc)-Asn-Asn-Ile-Ala-O-*t*-Bu (346 mg, 0.3 mmol) were dissolved in dimethylformamide. After cooling, *N,N*-diisopropylethylamine (0.06 mL, 0.35 mmol) was added. The reaction mixture was treated according to the usual procedure (see compound **2**) except that citric acid washings were deleted. Compound **7** was purified by chromatography on a silica gel column using G as the solvent system; yield 70 mg (12.5%) of pure compound. More fractions slightly impure were also recovered (280 mg, 50%): mp 220 °C dec; $[\alpha]^{25}_D$ −24° (*c* 1, DMF); R_f(G) 0.12.

TFA·Lys-Arg-Asn-Lys-Asn-Asn-Ile-Ala (8). Deprotection of compound **7** (50 mg, 0.036 mmol) in TFA was carried out during 3 h at room temperature, in an anhydrous medium. Then anhydrous ether (300 mL) was added, and compound **8** precipitated out. The residue was filtered and washed with ether: yield 33 mg (60%); R_f(D) 0.1; HPLC (C_{18} column, 0.1% TFA in a mixture of water/acetonitrile) showed single peak; amino acid anal. Asn 3.01, Ala 1.00, Ile 1.01, Lys 2.02, Arg 1.07. Anal. Calcd for $C_{39}H_{71}N_{15}O_{12}$·4CF_3COOH·4H_2O (mol wt 1445): C, 39.22; H, 5.91. Found: C, 39.03; H, 5.74.

Registry No. **1**, 92779-28-7; **2**, 97391-52-1; **3**, 97391-53-2; **4**, 97391-54-3; **5**, 97391-55-4; **6**, 97391-56-5; **7**, 97403-51-5; **8**, 97391-57-6; H-Lys-Arg-Asn-Lys-Asn-Asn-Ile-Ala-Oh, 81117-26-2; Z-Ile-ONp, 2130-99-6; H-Ala-O-*t*-Bu·HCl, 13404-22-3; H-Ile-Ala-O-*t*-Bu·HCl, 97391-58-7; Z-Asn-ONp, 3256-57-3; H-Asn-Ile-Ala-O-*t*-Bu·Hchl, 97391-59-8; H-Asn-Asn-Ile-Ala-O-*t*-Bu·CH_3COOH, 97391-61-2; Z-Lys(Boc)-ONp, 2212-69-3; H-Lys(Boc)-Asn-Asn-Ile-Ala-O-*t*-Bu·CH_3COOH, 97391-63-4; H-Asn-Lys(Boc)-Asn-Asn-Ile-Ala-O-*t*-Bu·HCl, 97391-64-5; Z_3-Arg-OH, 14611-34-8; H-Arg-Asn-Lys(Boc)-Asn-Asn-Ile-Ala-O-*t*-Bu·$(CH_3COOH)_3$, 97391-66-7; $(Boc\text{-}Lys\text{-}OSu)_2$, 30189-36-7; oxyntomodulin, 62340-29-8; pentagastrin, 5534-95-2.

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4-[2-(Di-*n*-propylamino)ethyl]-2(3*H*)-indolone: A Prejunctional Dopamine Receptor Agonist

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4-[2-(Di-*n*-propylamino)ethyl]-2(3*H*)-indolone (**1c**) (SK&F 101468) is a potent and selective prejunctional dopamine receptor agonist. It caused a dose-related inhibition of the constrictor response to electrical stimulation in the isolated perfused rabbit ear artery (EC_{50} = 100 nM), and this response was antagonized by (*S*)-sulpiride (K_B = 7 nM). Compound **1c** did not stimulate or block dopamine-sensitive adenylate cyclase and did not produce stimulation of the central nervous system in rats. It was prepared from (2-methyl-3-nitrophenyl)acetic acid in a multistep sequence based on the Reissert indole synthesis.

Goldberg and Kohli[1] have demonstrated that the peripheral dopamine receptors that modulate the release of norepinephrine from postganglionic sympathetic nerves are activated by a structurally diverse range of compounds. These include ergot and ergoline derivatives, 4-(aminoethyl)indoles, and apomorphine as well as phenethylamine, aminotetralin, and tetrahydro-3-benzazepine derivatives. Within these series, optimal prejunctional potency frequently occurs when the compound contains a tertiary amine and at least one *N*-*n*-propyl substituent. In contrast, only a few agonists are known that activate postjunctional dopamine receptors mediating relaxation of smooth muscle, and almost without exception these are catechol derivatives.

We recently described[2] two phenolic indolone derivatives, **1a** and **1b** that exhibited potent prejunctional dopamine agonist activity as determined in the isolated perfused rabbit ear artery (REA) assay.[3] Compound **1b** was unusually potent in this assay with an EC_{50} of 1.8 ± 0.3 nM (N = 10) compared to an EC_{50} of 110 ± 20 nM for

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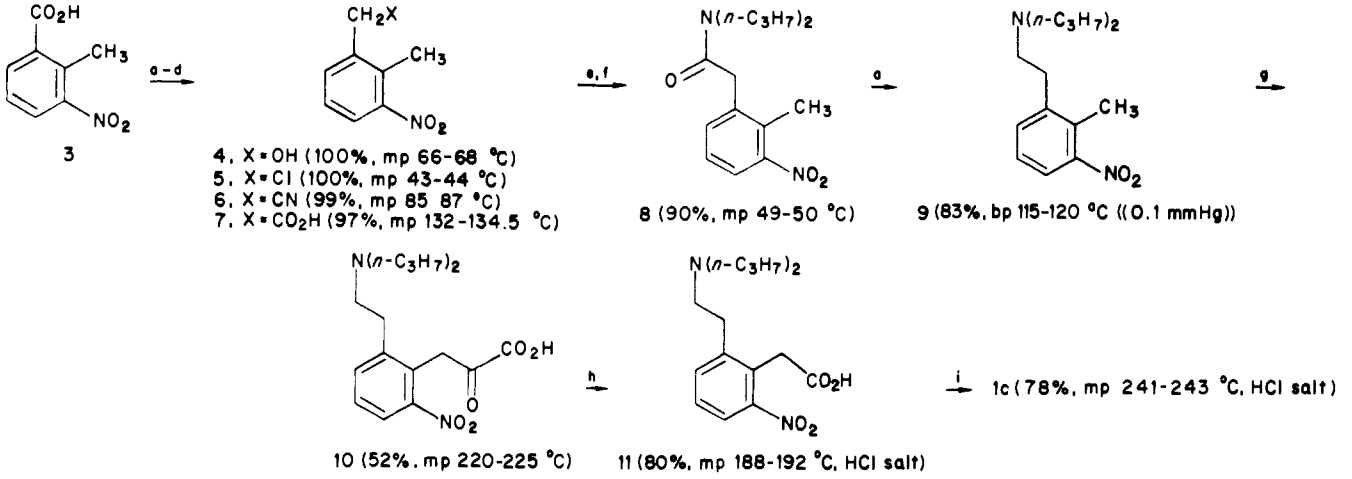
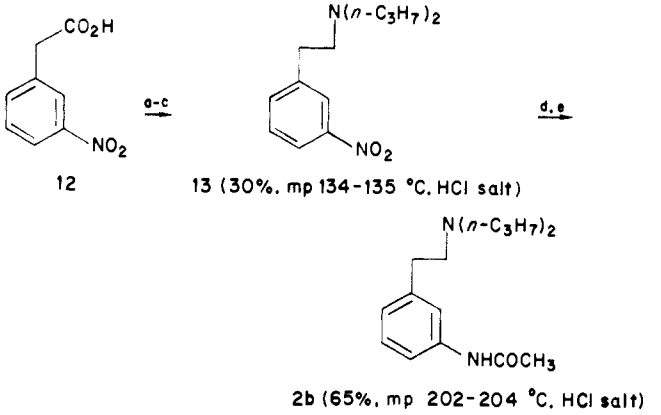
0022-2623/85/1828-1533$01.50/0

Scheme I[a]



[a] Key: a = BH_3/THF; b = concentrated HCl or $SOCl_2$/py; c = KCN/EtOH-H_2O; d = H_2SO_4/HOAc/H_2O; e = $SOCl_2$; f = $HN(n\text{-}C_3H_7)_2$; g = $(C_2H_5OCO)_2/K^+EtO^-$; h = H_2O_2 (2 equiv)/NaOH; i = [H] 5% Pd/C-EtOH.

Scheme II



[a] Key: a = $SOCl_2$; b = $HN(n\text{-}C_3H_7)_2$; c = $BH{\cdot}CH_3SCH_3$; d = H_2, Pd/C; e = Ac_2O, NaOAc.

N,N-di-*n*-propyldopamine.[4] We have continued our investigations of the indolones with the objective of identifying active and selective nonphenolic prejunctional agonists for use in the treatment of cardiovascular disorders. In this paper, we report the synthesis and initial biological evaluation of **1c**, the des-OH derivative of **1b**. For comparison, we have also prepared and tested **2b**, a structurally related ring-opened analogue, and the corresponding hydroxylated cogener **2a**.

Chemistry. The preparation of 4-[2-(*N,N*-di-*n*-propylamino)ethyl]-2(3*H*)-indolone (**1c**; SK&F 101468) is outlined in Scheme I.[5] Commercially available **3** was reduced quantitatively to the carbinol **4** by treatment with diborane. Conversion of **4** to the potent lachrymator **5** was accomplished by boiling in concentrated HCl for an extended period or, more efficaciously, by treatment with thionyl chloride in pyridine. Cyanide displacement followed by acid hydrolysis of the nitrile **6** gave a high yield of **7**,[6] which was sequentially treated with thionyl chloride and di-*n*-propylamine. The resulting amide **8** was treated with excess diborane, and the product **9** was homologated with potassium ethoxide and diethyl oxalate to give the intermediate pyruvic acid **10**. This was subsequently cleaved by hydroperoxide anion. Catalytic hydrogenation of **11** in ethanol afforded **1c** directly (mp HCl salt 241–243 °C).

The corresponding open-chain acetamide analogue **2b** was prepared from **12** as shown in Scheme II and characterized as the hydrochloride salt (mp 202–204 °C).

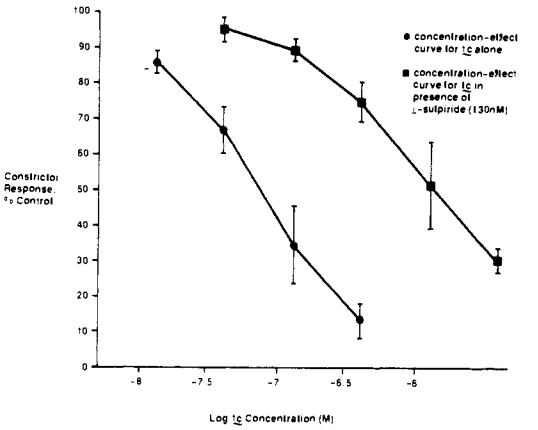


Figure 1. Inhibition of the constrictor response of the isolated perfused rabbit ear artery to field stimulation by **1c** and blockade of this effect by (*S*)-sulpiride. The ear artery was stimulated at 10–15 Hz for 500 ms at 4-min intervals. Each curve represents the mean of four to five experiments ±SEM. The receptor dissociation constant (K_B) for sulpiride was 7 nM.

Biology. Surprisingly, **1c** (EC_{50} = 100 nM) and *N,N*-di-*n*-propyldopamine were essentially equipotent in inhibiting the constrictor response to electrical stimulation in the REA preparation. Figure 1 shows the concentration dependency of this response and the antagonism of the effect by the prejunctional dopamine receptor antagonist (*S*)-sulpiride. It is of interest that **1c** shows substantially greater intrinsic prejunctional dopaminergic potency, as measured in the REA, than the meta-substituted phenolic analogue **2a**[7] or the ring-opened acetylamino analogue **2b** shown in Chart I. In tests carried out in our laboratories,

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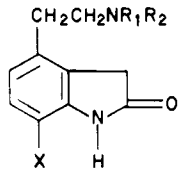
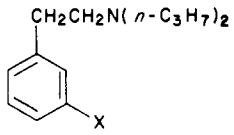
Chart I. Comparative Potencies of Indoloneethylamines and Related Phenethylamines in Inhibiting the Constrictor Response of the Isolated Perfused Rabbit Ear Artery to Field Stimulation[a]



1a: X = OH; R_1 = R_2 = H (EC_{50} = 116 ± 43 nM; N = 8)
b: X = OH; R_1 R_2 = *n*-propyl (EC_{50} = 1.8 ± 0.3 nM; N = 10)
c: X = H; R_1 = R_2 = *n*-propyl (EC_{50} = 100 ± 26 nM; N = 5)



2a: X = OH (EC_{50} = 700 ± 209 nM, N = 5)
b: X = $NHCOCH_3$ (EC_{50} = >1000 nM

[a] See ref 3 and Figure 1 for a description of this test procedure.

1c was also more potent than the nonphenolic (oxygen-free) *trans*-(±)-pyrazoloquinoline, LY 141865, described by Bach et al.[8] For EC_{50} for LY 141866 in the REA assay is 150 nM.

Compound **1c** did not stimulate or block the dopamine-sensitive adenylate cyclase in homogenates from the rat caudate at concentrations up to 10^{-4} M. Unlike apomorphine it did not increase confinement motor activity (CMA) upon intraperitoneal administration to conscious rats but actually produced some depression of CMA at high doses (>1 mg/kg).[9,10] At doses up to 1 mg/kg (ip) **1c** also did not potentiate hexobarbital-induced sleep time in the rat.[9] These results indicate that **1c** does not produce the central behavioral effects often seen with dopamine agonists.

Compound **1c** has been selected for further characterization, and a full account of its pharmacology and the SAR of a series of indolones related to it will be published at a later date.

Experimental Section

Melting points were taken either in a Mel-Temp hot stage or in open capillary tubes with a Thomas-Hoover Unimelt apparatus and are uncorrected. When analyses are reported by symbols of the elements, results were within 0.4% of calculated values. Melting points, and yields are recorded for new compounds in Schemes I and II. IR spectra were recorded on a Perkin-Elmer Model 683 spectrophotometer, and ^1H NMR spectra were obtained on a Varian EM-390 spectrometer. Spectral data for all compounds were consistent with assigned structures. The C, H, and N analyses were carried out by the Analytical, Physical and Structural Chemistry Department of Smith Kline & French Laboratories.

(2-Methyl-3-nitrophenyl)-*N*,*N*-di-*n*-propylacetamide (8). To 50.0 g (0.256 mol) of (2-methyl-2-nitrophenyl)acetic acid (7)[6] was added dropwise with stirring 95 g (0.80 mol) of $SOCl_2$. When gas evolution ceased, the solution was concentrated in vacuo and several small portions of dry toluene were added and removed in vacuo. The residue was dissolved in 300 mL of toluene and added at 10 °C to 600 mL of a 50:50 H_2O–toluene mixture containing 30 g (0.283 mol) of Na_2CO_3. Di-*n*-propylamine (30.1 g, 0.30 mol) was added with cooling and slow stirring, and after 0.5 h the mixture was brought to room temperature and stirred for an additional 1 h. An additional 1.0 g (0.0094 mol) of Na_2CO_2 was added, and the toluene phase was separated and washed with 5% Na_2CO_3, 1.5 N HCl, and H_2O. After drying ($MgSO_4$), the solvent was removed and the thick residual oil was distilled in a Kugelrohr apparatus to give 64 g of product, bp 130 °C (0.1 mmHg), which crystallized to long needles, mp 49–50 °C. Anal. ($C_{15}H_{22}N_2O_3$) C, H. N.

2-Methyl-3-nitro-*N*,*N*-di-*n*-propylphenethylamine (9). To a solution of 155.74 g (0.560 mol) of **8** in 1250 mL of anhydrous THF was added dropwise 848 mL of 1.0 M borane in THF. The mixture was refluxed for 1 h, an additional 150 mL of 1.0 M borane–THF was added, and this solution was stirred overnight. Anhydrous MeOH was added cautiously, and the solution was concentrated in vacuo. The residual syrup was warmed on a steam bath with 6 N HCl (200 mL) for 1 h and then cooled and made basic with 40% NaOH. The oily product was taken into Et_2O, washed with brine, concentrated in vacuo, and distilled in a Kugelrohr flask to yield 123.94 g (83%) of thick oil, bp 115-120 °C (0.1 mmHg). Anal. ($C_{15}H_{24}N_2O_2$) C, H, N.

[2-Nitro-6-[2-(*N*,*N*-di-*n*-propylamino)ethyl]phenyl]pyruvic Acid (10). Absolute EtOH (0.89 g, 0.0193 mol) was added dropwise to freshly cut K metal (0.75 g, 0.192 mol) in anhydrous Et_2O under a nitrogen atmosphere. Diethyl oxalate (2.77 g, 0.0190 mol) was added dropwise with stirring after the metal had dissolved. After 10 min, **9** (5.03 g, 0.019 mol) was added dropwise. The dark purple solution was allowed to stand overnight. It was concentrated with a stream of N_2, and 100 mL of H_2O was added (pH 10). The solution was extracted with Et_2O, and after drying ($MgSO_4$), the ether was removed to provide 2.59 g of crude unreacted starting material **9**. The H_2O layer was diluted with 300 mL of H_2O and acidified to pH 1.5 with 3 N HCl. The tan precipitate was separated and crystallized from HOAc: 3.37 g (52%); mp 220–225 °C. Anal. ($C_{17}H_{24}N_2O_5 \cdot 0.25H_2O$) C, H, N.

[2-Nitro-6-[2-(*N*,*N*-di-*n*-propylamino)ethyl]phenyl]acetic Acid Hydrochloride (11). To a cold (10 °C) mixture of 26.0 (0.0773 mol) of **10** in 400 mL of 2% NaOH (0.20 mol) was added 13.7 mL (0.159 mol) of 30% H_2O_2. After addition was completed, the solution was brought to room temperature and stirred for 1 h. The pH adjusted to 1.5 by careful addition of concentrated HCl. The volume was reduced in vacuo and the solution cooled to room temperature to give 18.5 g. A second crop, 2.77 g, was obtained when the filtrate was cooled overnight at 10 °C: total yield 21.26 g (80%); mp 188–192 °C. Anal. ($C_{16}H_{24}N_2O_4 \cdot HCl$) C, H, N.

4-[2-(*N*,*N*-Di-*n*-propylamino)ethyl]-2 (3*H*)-indolone Hydrochloride (1c). A mixture of 2.0 g (0.0058 mol) of **11** and 0.205 g of 5% Pd/C catalyst in 100 mL of EtOH was hydrogenated at room temperature and 50 psi for 5 h. The catalyst was removed and the solution concentrated in vacuo to a white powder. Crystallization from 400 mL of CH_3CN gave **1c**: 1.34 g (78%); mp 241–243 °C.

3-Nitro-*N*,*N*-dipropylbenzeneethanamine (13). A mixture of 5.0 g (27.6 mmol) of (*m*-nitrophenyl)acetic acid in 25 mL of thionyl chloride was refluxed for 1.5 h. It was cooled and treated with 50 mL of hexane and the resulting precipitate removed by filtration to give 5.2 g (95%) of white solid. This was dissolved in 50 mL of CH_2Cl_2, treated with 2.7 g of dipropylamine and 3.3 g of triethylamine, and refluxed overnight. The mixture was washed with H_2O, 3 N HCl, 5% Na_2CO_3, and H_2O, dried, and evaporated to give 5.0 g (69%) of amber oil which was used without further purification. This was dissolved in 15 mL of dry THF, cooled in ice, and treated dropwise with 35 mL of borane methyl sulfide in THF. After addition was complete, the mixture was refluxed overnight, cooled, treated with 10 mL of MeOH, and stirred until gas evolution ceased. Gaseous HCl was bubbled in to pH 1 and the mixture refluxed for 1 h. The reaction was evaporated to leave a pale yellow oil. This was taken up in Et_2O and layered with 10% NaOH. The ether solution was washed with H_2O, dried over KOH, and treated with excess HCl. An oil precipitated that crystallized upon standing in the cold. The supernatent was decanted and the residue recrystallized from EtOAc–MeOH to give 2.24 g (43%) of white crystals, mp 134–135

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°C. Anal. ($C_{14}H_{22}N_2O_2$·HCl) C, H, N.

N-[3-[2-(Dipropylamino)ethyl]phenyl]acetamide (2b). A solution of 2.29 g of **13** in 150 mL of EtOH was hydrogenated at 60 psi over 200 mg of 10% Pd/C for 1 h. The catalyst was removed by filtration, etherial HCl was added, and the mixture was evaporated to a pale yellow oil. A solution of 848 mg of the amine hydrochloride in 35 mL of H_2O was warmed to 50 °C and treated with 475 mg of acetic anhydride and 576 mg of sodium acetate. It was stirred for 30 min, cooled, adjusted to pH 11 with 10% NaOH, and extracted with CH_2Cl_2. The extracts were washed with water, dried, and evaporated to give a yellow oil. This was taken up in Et_2O and treated with saturated etherial HCl to give 1.03 g of solid. This was recrystallized from MeOH–EtOAc to give 695 mg of white crystals (67%), mp 202–204 °C. Anal. ($C_{16}H_{26}N_2O_2$·HCl) C, H, N.

Registry No. **1c**, 91374-20-8; **2b**, 97351-98-9; **2b**·HCl, 97351-94-5; **3**, 1975-50-4; **4**, 23876-13-3; **5**, 60468-54-4; **6**, 23876-14-4; **7**, 23876-15-5; **8**, 91374-22-0; **9**, 91374-23-1; **10**, 97351-95-6; **11**, 91374-25-3; **12**, 1877-73-2; **13**, 97351-96-7; **13**·HCl, 97351-99-0; *m*-$ClCOCH_2C_6H_4NO_2$, 38411-41-5; *m*-$NO_2C_6H_4CH_2CONPr_2$, 97351-97-8; di-*n*-propylamine, 142-84-7; diethyl oxalate, 95-92-1.

Conformationally Restricted C-Terminal Peptides of Substance P. Synthesis, Mass Spectral Analysis and Pharmacological Properties

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Four cyclic analogues of the C-terminal hepta- or hexapeptide of substance P were prepared by the solution method. The cyclizations were obtained by substituting with cysteine the residues normally present in positions 5 or 6 or 11 of substance P and by subsequent disulfide bond formation. The final products were identified by ordinary analytical procedures and advanced mass spectroscopy. The biological activities were determined on three bioassays: the guinea pig ileum, the guinea pig trachea and the rabbit mesenteric vein. Results obtained with these assays indicate that all peptides with a disulfide bridgehead in position 11 are inactive and that a cycle between positions 5 and 6 already strongly reduces the biological activity. The acyclic precursors containing thiol protection groups display weak biological activities. These results further underline the importance of the side chain in position 11 of substance P and suggest that optimal biological activities may require a linear peptide sequence.

Conformationally restricted analogues of linear peptide hormones provide a useful approach to obtain hormone derivatives with increased affinities and/or durations of action. Successful cases in recent years are orally active somatostatin analogues,[1] cyclic hyperactive α-MSH analogues,[2] and cyclic enkephalins with increased activity[2] or increased selectivity.[3]

No such studies have been reported on substance P and other tachykinins. Generally, the structure–activity studies carried out on this neuropeptide concentrated rather on single substitution (e.g., the L-alanine series[4]) or on modifications of the C-terminal residue,[5] particularly the methionine side chain[6] or the C-terminal amide, which was methylated to reduce metabolic degradation.[7] Retro-inverso sequences[8] or substitutions of the aromatic residues[9] have also been reported.

Multiple substitutions with D-tryptophan have led to the obtainment of reasonably active competitive antagonists for substance P (for a review see ref 10). The affinities of these compounds are however still somewhat disappointing, because most of them do not exceed pA_2 values of 7.0. Compounds, both agonists and antagonists, with high affinities are urgently needed and could eventually be obtained by preparing cyclic analogues of SP or its active C-terminal fragments.

With this in mind, a series of analogues of the heptapeptide sequence SP,[5-11] containing a cyclic disulfide bridge between positions 5 and 6, 5 and 11, or 6 and 11, were prepared for their biological activities with the corresponding linear sequences.

Syntheses

All compounds were synthesized by the classical solution method by combination of stepwise elongation from the C-terminal end with fragment couplings. The stepwise elongation was performed either with the formation of intermediate active esters of *N*-hydroxybenzotriazole, by direct activation with dicyclohexylcarbodiimide, by preformed *N*-hydroxysuccinimide-active esters, or by the mixed-anhydride method. The fragment couplings were performed with the dicyclohexylcarbodiimide and *N*-hydroxybenzotriazole technique. Generally, the *tert*-butyloxycarbonyl group was used for N protection, while the thiol side chains were protected either with the benzyl thioether or with the *S*-acetamidomethyl (*S*-Acm) group.

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