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Spirotetrahydronaphthalene analogues of sympathomimetic catecholamines. Synthesis and adrenergic activity of 5,6- and 6,7-dihydroxy-3,4-dihydrospiro[naphthalen-1(2*H*)-3′-piperidines]

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Abstract

The 5,6- (**5a**) and 6,7-dihydroxy-3,4-dihydrospiro[naphthalen-1(2*H*)-3′-piperidine] (**6a**) and their *N*-isopropyl derivatives (**5b** and **6b**), DDSNPs, were synthesized. These compounds can be viewed as the result of the combination of the structure of the 3-(3,4-dihydroxyphenyl)-piperidine **2a** or **2b**, with the structure of the corresponding 1-(aminomethyl)-5,6-dihydroxy- (**3a** or **3b**) or 1-(aminomethyl)-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (**4a** or **4b**), 1-AMDTNs. The new compounds (**5a**, **b** and **6a**, **b**) were assayed for their α and β adrenergic properties by means of binding experiments and functional tests and the results were compared with those obtained for catecholamines **1a**, **b** and the previously described 3-(3,4-dihydroxyphenyl)piperidine (3-DPP; **2**) and 1-AMDTNs (**3**, **4**). Comparison of the affinity and activity data of novel derivatives with those of reference compounds **2**, **3** and **4** shows a general low ability of DDSNPs **5** and **6** to interact with both α and β- adrenoceptors.

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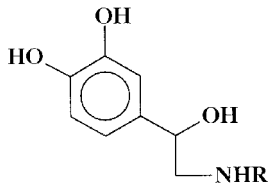
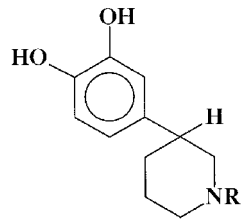
Introduction

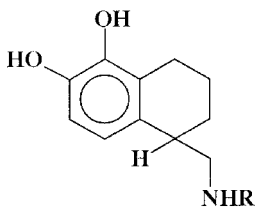
The synthesis and the study of pharmacological properties of rigid or semi-rigid analogues of drugs possessing a conformational freedom represent one of the most effective methods to obtain information on the spatial arrangement of pharmacophores (Smissman et al 1971; Nishikawa et al 1975; De Marinis et al 1982; Ruffolo et al 1982; Hicks et al 1983; De Bernardis et al 1985, 1986, 1987; Kumar et al 1987; Balsamo et al 1989; Macchia et al 1992, 1993, 1995, 1997; Cannon 1995). The adrenergic catecholamine aminoalcohols of type 1 and drugs structurally related to them are molecules which, with a simple rotation around the single bonds C_α—C_1, C_1—C_2, C_2—N, may assume different low-energy conformations, in which the moieties presumed to be responsible for biological activity (alcoholic hydroxy group, amino nitrogen, catecholic group) can interact with the receptor (Easson & Stedman 1933; Ariens 1967; Brittain et al 1970; Petrongolo et al 1974; Triggle 1981). In a previous study we synthesized semi-rigid analogues of noradrenaline (norepinephrine) (**1a**) and isoproterenol (**1b**) (Figure 1), with the aim of verifying the role played by their alcoholic hydroxy group in the interaction with the receptor. The piperidine derivative **2a** (Figure 1) showed affinity and activity values



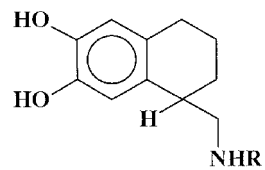
NE and ISO (**1a, b**)



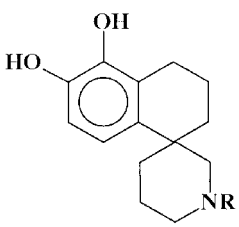
3-DPP (**2a, b**)



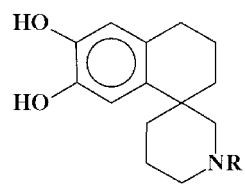
1-AMDTN (**3a, b**)



1-AMDTN (**4a, b**)



DDSNP (**5a, b**)



DDSNP (**6a, b**)

a, R = H; b, R = *i*-Pr

Figure 1 Structures of catecholamines noradrenaline and isoproterenol and some of their semi-rigid analogues.

at the α_2-adrenoceptor that were very close to those of noradrenaline (**1a**) (Macchia et al 1995). The 3-(3,4-dihydroxyphenyl)piperidine (3-DPP) **2a** can be seen as a noradrenaline analogue without the alcoholic function, in which the remaining two pharmacophoric groups (amino nitrogen and catecholic group) are in a steric relationship that mimics that of noradrenaline in its preferred conformation, which would appear also to be the pharmacophoric one. Moreover compound **3a**, which is a desoxy analogue of noradrenaline in which the rotational freedom of the catecholic group is reduced to some extent, revealed high α_2-adrenergic properties (De Bernardis et al 1985; Macchia et al 1988).

5,6-Dihydroxy-dihydrospiro[naphthalen-1-(2*H*)-3′-piperidine] (DDSNP, **5a**) (Figure 1) may be viewed as a structural hybrid between 3-DPP **2a**, which has a high conformational freedom around the C_α—C_1 bond, and the 1-aminomethyldihydroxytetrahydronaphthalene (1-AMDTN) **3a**, which is conformationally restricted around this same bond, but is conformationally free around the C_1—C_2 bond (Macchia 1989). Quite similarly, **6a** is a structural analogue of the previously described **4a**, which is characterized by a different position of the catecholic group.

This study deals with the synthesis and the evaluation of adrenergic properties of regioisomeric DDSNP **5a** and **6a**. Compounds **5** and **6**, together with the corresponding 1-AMDTNs **3a** and **4a**, may be considered as conformationally restricted analogues of two opposite rotameric forms of the catecholic nucleus of noradrenaline (**1a**) and its 3-DPP derivative **2a**. To obtain a wider comparison, the corresponding *N*-iso-

propyl derivatives (**5b** and **6b**) (Figure 1) were also prepared and tested.

Materials and Methods

Chemistry

Melting points were determined on a Köfler hot stage apparatus and are uncorrected. IR spectra were recorded with a Perkin-Elmer model 1310, as Nujol mulls, or liquid films. ^{1}H NMR spectra were routinely recorded with a Varian EM 360 A instrument as ca 5% solutions in $CDCl_3$ (for neutral compounds or free bases) or D_2O (for salts), using Me_4Si or $Me_3Si(CH_2)_3SO_3Na$ as the internal standard, respectively. The ^{1}H NMR for compounds **15** and **16** were recorded using a Varian VXR-300 spectrometer in $CDCl_3$ (0.08 M) solution, and the temperature was controlled to ± 0.1°C. All the solutions were accurately degassed by freeze–pump–thaw cycles. Evaporation was performed in-vacuo (rotating evaporator). $MgSO_4$ was always used as the drying agent.

Gas liquid chromatography (GLC) analyses were run on a C. Erba model 4200 apparatus with a flame ionization detector using a column (1.8 m × 3.5 mm) packed with OV1 3% on 80/100 mesh silanized. Chromosorb W was used in the following conditions: oven temperature 230°C, injector and detector temperatures 260°C, carrier flow 30 mL min^{-1}.

Elemental analyses were performed by our analytical laboratory and agreed with theoretical values to within 0.4%.

*1-Cyano-5,6-dimethoxy-1,2,3,4-tetrahydro-1-naphthalene (**9**)*

A suspension of **7** (0.550 g, 2.55 mmol) in EtOH (10 mL) was treated portionwise with solid $NaBH_4$ (0.22 g, 5.82 mmol). The resulting mixture was refluxed for 30 min, then the solvent was removed and the residue was treated with a solution of Et_2O/CH_2Cl_2 (1:1) and then washed with H_2O. The organic layer, washed with HCl 10% and brine, was dried and evaporated to give **9** as a pure solid (GLC) (82%); mp 50–52°C (DeBernardis et al 1985: 51–53°C).

*1-Cyano-6,7-dimethoxy-1,2,3,4-tetrahydro-1-naphthalene (**10**)*

Compound **10** was prepared from **8** following the same procedure as for the preparation of **9**. The product was a pure oil (GLC) **10** (97%) IR ν 2119 cm^{-1} (CN); ^{1}H NMR δ 1.76–2.28 (m, 4H), 2.56–2.93 (m, 2H), 3.93 (s, 7H), 6.66 (s, 1H) and 6.88 (s, 1H).

*1-Cyano-1-ethylpropionyl-5,6-dimethoxy-1,2,3,4-tetrahydronaphthalene (**11**)*

The cyano derivative **9** (0.90 g, 4.14 mmol) was added to a solution of EtONa in EtOH (25 mL) (prepared from 0.033 g, 0.0014 g atoms of sodium). The resulting solution was stirred at room temperature for 30 min, and then ethyl acrylate (0.60 mL, 5.52 mmol) was added and the mixture was maintained for 20 h at room temperature under stirring. The solvent was evaporated and the residue was taken up with $CHCl_3$, and washed with H_2O; the organic layer was dried and evaporated to give **11** as a pure oil (GLC) (76%); IR ν 2125 cm^{-1}(CN); 1730 cm^{-1}(CO); ^{1}H NMR δ 1.23 (t, 3H, J = 7.0 Hz) 1.83–2.88 (m, 10H), 3.80 (s, 3H), 3.85 (s, 3H), 4.16 (q, 2H, J = 7.0 Hz), 6.85 (d, 1H, J = 9.0 Hz) and 7.20 (d, 2H, J = 9.0 Hz).

*1-Cyano-1-ethylpropionyl-6,7-dimethoxy-1,2,3,4-tetrahydronaphthalene (**12**)*

Compound **12** was prepared from **8** following the same procedure for the preparation of **10**. The product was a pure oil (GLC) (68%) IR 2110 cm^{-1} (CN); 1720 cm^{-1} (CO); ^{1}H NMR δ 1.27 (t, 3H, J = 7.0 Hz), 1.76–2.83 (m, 10H), 3.83 (s, 3H), 3.86 (s, 3H), 4.13 (q, 2H, J = 7.0 Hz), 6.58 (s, 1H) and 6.90 (s, 1H).

*5,6-Dimethoxy-3,4-dihydrospiro[naphthalen-3′-piperidin-6′-one] (**13**)*

Ni-Raney (1.068 g) was added to a solution of **11** (5.00 g, 15.7 mmol) in EtOH, and the suspension was hydrogenated at 110 atm at a temperature of 140–150°C for 3 h. After this period, the catalyst was filtered off and the solution was evaporated to yield an oil, that was purified by crystallization to afford **13** as a solid (51%); mp 171–173°C (benzene–hexane); IR ν 1675 cm^{-1} (CONH); ^{1}H NMR δ 1.58–1.95 (bm, 6H), 2.80–3.43 (m, 6H), 3.83 (s, 3H), 3.86 (s, 3H), 6.66 (b, 1H, NH), 6.75 (d, 1H, J = 9.0 Hz) and 7.10 (d, 1H, J = 9.0 Hz) Anal. $C_{16}H_{21}NO_3$.

*6,7-Dimethoxy-3,4-dihydrospiro[naphthalen-3′-piperidin-6′-one] (**14**)*

Compound **14** was prepared from **12** as described for the preparation of **13**. Compound **14** (47%); mp 151–152°C (benzene–petroleum ether 80–100° 1:4); IR ν 1680 cm^{-1} (CONH); ^{1}H NMR δ 1.50–2.02 (m, 6H), 2.66–3.10 (m, 6H), 3.58 (s, 6H), 6.11 (s, 1H), 6.33 (s, 1H) 6.80 (brs, 1H, NH) Anal. $C_{16}H_{21}NO_3$.

*5,6-Dimethoxy-3,4-dihydrospiro[naphthalen-3′-piperidine] hydrochloride (**15**.HCl)*

A solution of **13** (0.5 g, 1.82 mmol) in tetrahydrofuran

(THF; 15.0 mL) was added dropwise to a suspension of $NaBH_4$ (0.389 g, 10.28 mmol) in anhydrous THF (8.80 mL) and $BF_3.Et_2O$ (1.45 g, 1.30 mL, 10.28 mmol) in anhydrous THF cooled to 0°C and stirring after the end of dropping to reflux for 2 h. The solvent was evaporated and the residue was treated with a solution of HCl (10%) and stirred for 1 h at 70°C. The aqueous phase was basified and extracted with Et_2O, and the solvent was evaporated to give a crude oil, which was dissolved in MeOH and treated with an excess of Et_2O.HCl to yield a solid, which was then crystallized from MeOH–Et_2O to give pure **15** HCl as a white solid (55%) mp 232–234°C dec.; ^{1}H NMR δ 1.38–1.80 (m, 8H), 2.46 (m, 2H), 2.94–3.25 (m, 4H), 3.49 (s, 3H), 3.61 (s, 3H), 6.78 (d, 1H, J = 9.0 Hz), 7.06 (d, 1H, J = 9.0 Hz) Anal. $C_{16}H_{23}NO_2$ HCl.

*6,7-Dimethoxy-3,4-dihydrospiro[naphthalen-3′-piperidine] hydrochloride (**16** HCl)*
Compound **16** was prepared from **14** following the same procedure for the preparation of **15**.HCl. Compound **16**.HCl (81%) mp 210–213°C dec.; ^{1}H NMR δ 1.42–1.80 (m, 8H), 2.47 (m, 2H), 2.83–3.36 (m, 4H), 3.58 (s, 3H), 3.63 (s, 3H), 6.58 (s, 1H), and 6.80 (s, 1H). Anal. $C_{16}H_{23}NO_2$.HCl.

*5,6-Dihydroxy-3,4-dihydrospiro[naphthalen-3′-piperidine] hydrobromide (**5a** HBr) and 6,7-dihydroxy-3,4-dihydrospiro[naphthalen-3′-piperidine] hydrobromide (**6a** HBr)*
A mixture of **15**.HCl or **16**.HCl (0.250 g, 0.95 mmol) and 48% aqueous HBr (9.00 mL) was stirred and refluxed under nitrogen for 1.5 h, then evaporated to dryness. The solid residues were crystallized to give the pure solids **5a**.HBr and **6a**.HBr. Compound **5a**.HBr (EtOH–Et_2O) (44%) mp 280°C dec.; ^{1}H NMR δ 1.66–2.06 (m, 8H), 2.65–3.23 (m, 6H), 6.88 (d, 1H, J = 9.0 Hz) and 7.11 (d, 1H, J = 9.0 Hz). Anal. $C_{14}H_{19}NO_2$.HBr. Compound **6a**.HBr (EtOH–Et_2O) (40%) mp 145°C dec.; ^{1}H NMR δ 1.30–1.78 (m, 8H), 2.50–3.10 (m, 6H), 5.73 (s, 1H) and 6.00 (s, 1H). Anal. $C_{14}H_{19}NO_2$.HBr.

5,6-Dimethoxy-3,4-dihydro-N-*isopropylspiro[naphthalen-3′-piperidine] (**17**)*
Anhydrous K_2CO_3 (0.264 g, 1.92 mmol) and 2-bromopropane (0.176 g, 1.43 mmol) were added to a solution of **15** (0.250 g, 0.96 mmol) in acetonitrile (5.0 mL). The resulting suspension was warmed at 80°C for 24 h. After this period, the suspension was treated with Et_2O and filtered. The organic layer was evaporated to give **17** as a pure oil (GLC, 98%) ^{1}H NMR δ 0.90 (d, 3H, J = 6.0 Hz), 1.03 (d, 3H, J = 6.0 Hz), 1.43–1.92 (m, 8H), 2.10–2.93 (m, 7H), 3.79 (s, 3H), 3.83 (s, 3H), 6.76 (d, 1H, J = 9.0 Hz) and 7.23 (d, 1H, J = 9.0 Hz). Anal. $C_{19}H_{29}NO_2$.

6,7-Dimethoxy-3,4-dihydro-N-*isopropylspiro[naphthalen-3′-piperidine] (**18**)*
Compound **18** was prepared from **16** following the same procedure for the preparation of compound **17**. Compound **18** (pure oil GLC 97%) ^{1}H NMR δ 0.87 (d, 3H, J = 6.0 Hz), 0.98 (d, 3H, J = 6.0 Hz), 1.23–1.89 (m, 8H), 2.35–2.75 (m, 7H), 3.66 (s, 3H), 3.73 (s, 3H), 6.18 (s, 1H) and 6.77 (s, 1H). Anal. $C_{19}H_{29}NO_2$.

5,6-dihydroxy-3,4-dihydro-N-*isopropylspiro[naphthalen-3′-piperidine] hydrobromide (**5b**.HBr) and 6,7-dihydroxy-3,4-dihydro*-N-*isopropylspiro[naphthalen-3′-piperidine] hydrobromide (**6b**.HBr)*
A mixture of **17** or **18** (0.350 g, 1.15 mmol) and 48% aqueous HBr (9.00 mL) was stirred and refluxed under nitrogen for 1.5 h, and then evaporated to dryness. The solid residues were crystallized to give the pure solids **5b**.HBr and **6b**.HBr. Compound **5b**.HBr (MeOH–Et_2O) (43%) mp 170°C dec.; ^{1}H NMR δ 1.33 (d, 6H, J = 6.0 Hz), 1.66–2.10 (m, 8H), 2.70–3.53 (m, 7H), 6.75 (d, 1H, J = 9.0 Hz), 7.16 (d, 1H, J = 9.0 Hz) Anal. $C_{17}H_{25}NO_2$.HBr. Compound **6b**.HBr (isopropanol–isopropylic ether) (82%) mp 133–135°C; ^{1}H NMR δ 1.20 (d, 6H, J = 6.0 Hz), 1.43–1.88 (m, 8H), 2.06–3.35 (m, 7H), 6.13 (s, 1H), 6.37 (s, 1H). Anal. $C_{17}H_{25}NO_2$.HBr.

Biological methods

Radioligand binding
Rat brain α_1- and α_2-receptors α_1- and α_2-receptor binding was determined in rat cerebral cortex membranes essentially as previously reported (DeBernardis et al 1985). Rat cerebral cortex was homogenized in 20 volumes of ice-cold 50 mM Tris-HCl buffer at pH 7.7 containing 5 mM EDTA and centrifuged at 48000 *g* for 15 min at 4°C. The resulting pellet was diluted in 20 volumes of 50 mM Tris-HCl buffer, homogenized and re-centrifuged at 48000 *g* for 15 min at 4°C. The resulting pellet was diluted in 20 volumes of the buffer and used in the binding assays. Binding was performed in triplicate by incubating portions of membrane fraction (0.2–0.3 mg protein) in Tris-HCl buffer at pH 7.7 with ^{3}H-prazosin for α_1-assay or ^{3}H-rauwolscine for α_2-assay at 25°C for 60 min. Non-specific binding was defined in the presence of unlabelled 1 μM prazosin and

10 μM rauwolscine, respectively. The binding reaction was terminated by filtration through Whatman GF/C glass-fibre filters under reduced pressure. Filters were washed with 4×5 mL of ice-cold buffer and placed in scintillation vials. Specific binding was obtained by subtracting non-specific binding from total binding and was approximately 80–90% of total binding.

Rat brain β_1-receptors β_1-Receptors were assayed in rat cortical membranes following the procedure previously described (Macchia et al 1992).

Bovine lung β_2-receptors β_2-Receptor binding was studied in bovine lung, using [^{3}H]DHA (dihydroalprenolol) as the ligand (Du Pont de Nemours, New England Nuclear Division, specific activity = 48.1 Ci mmol^{-1}). Membranes were obtained by lung homogenization in 1:20 volumes of 0.32 M sucrose, followed by centrifugation at 800 *g* for 10 min at 5°C. The resulting pellet was suspended in 50 mM phosphate buffer at pH 7.4 containing 0.02% ascorbic acid, and then centrifuged. This step was repeated twice. Crude lung membranes were suspended in $\cong$ 4 mg mL^{-1} proteins and incubated with 1 nM [^{3}H]DHA in the presence of 50 nM CGP 26505 to displace [^{3}H]DHA binding from the β_1-adrenoceptor subpopulation which represents 17% in the bovine lung. After incubation at 25°C for 30 min, the samples were filtered on Whatman GF/B glass-fibre filters, washed with 3×5 mL of phosphate buffer, dried and added to 8 mL of Ready Protein Beckman scintillation fluid. Non-specific binding was measured in the presence of 35 μM L-isoprenaline. For inhibition experiments, a fixed concentration of radioligand and various concentration of unlabelled drugs were added to triplicate tubes containing the membrane suspension. Assays were then performed as described above.

The data of n = 5 experiments carried out in triplicate were analysed by an iterative curve-fitting procedure (program Prism, GraphPad, San Diego, CA), which provided IC50 (molar concentration inhibiting specific binding by 50%), K_i (inhibition constant), and standard deviation values for test compounds, K_i values being calculated from the Cheng & Prusoff equation (Cheng & Prusoff 1973). The ligand affinity (K_d) of [^{3}H] prazosin was 0.24 nM, that of [^{3}H]-rauwolscine was 3.4 nM and that of [^{3}H]DHA was 1 nM.

Functional tests

The assays were conducted on isolated mammalian preparations, in accordance with the legislation of the Italian Authorities (D. L. 27/01/92, no 116) concerning animal experimentation. The rats or guinea-pigs, under light ether anaesthesia, were killed by cervical dislocation and bled. The thoracic and, subsequently, the abdominal cavities were opened by midline incision. The organs were immediately explanted and placed in Tyrode solution (composition (mM): NaCl 136.8, KCl 2.95, $CaCl_2$ 1.80, $MgSO_4.7H_2O$ 1.05, NaH_2PO_4 0.41, $NaHCO_3$ 11.9, glucose 5.5) at room temperature and gassed with carbogen (95% O_2–5% CO_2).

Adrenoceptor activity was assayed for α_1-receptors on isolated rat vas deferens, for α_2-receptors on isolated electrically stimulated guinea-pig ileum, for β_1-receptors on isolated guinea-pig atria and for β_2-receptors on isolated guinea-pig trachea. When not otherwise specified, agonist and antagonist activity was tested on the resting tone of the organ.

Rat vas deferens. Vasa deferentia were taken from Sprague-Dawley male albino rats weighing 200–250 g. Both vasa deferentia were removed, without stretching, from the epididymis to the prostatic urethra, after moving the intestine to one side. The intact duct was carefully separated from extraneous surrounding tissues and placed in a 10-mL organ bath containing Tyrode solution, pH 7.4, at 37°C, bubbled with carbogen. The preparation was suspended longitudinally between the organ holder and a force displacement transducer (Basile Model 7006), loaded with 0.5 g, connected to a unirecord microdynamometer (Basile Model 7050). The organ was left to stabilize for 30 min before beginning the experiment.

Guinea-pig ileum. Dunkin-Hartley male guinea-pigs, 250–300 g, were deprived of food intake for 24 h before the experiments. Portions of ileum 2–3 cm in length, about 10 cm distal to the ileocecal valve, were carefully dissected, freed from the surrounding mesenteric tissue, attached with thread to the organ holder and to the recording system by opposite sides of their open ends and suspended in a 10-mL organ bath containing Tyrode solution, at 37°C, gassed with carbogen. The ileum preparations were placed between two platinum electrodes (4×45 mm) set at a distance of 7 mm in the bath. The tissues were preloaded with a tension of 0.5 g and left to stabilize for 45–60 min before beginning electrical stimulation, which was carried out with a digital stimulator (Biomedica Mangoni Model BM-ST3) using the following parameters: single rectangular pulses, 0.1 Hz frequency, 0.3 ms pulse width, 12 V supramaximal voltage. The activity of the test drugs on α_2-adrenoceptors was evaluated as their ability to inhibit acetylcholine

release evoked by electrical stimulation of nerve fibres. The effect of the released mediator on intestinal smooth muscle was recorded as longitudinal contractions by an isotonic transducer (Basile Model 7006) connected to a unirecord microdynamometer (Basile Model 7050).

Guinea-pig atria. The atria were obtained from the same guinea-pigs used to obtain ileum. After sacrifice, the heart was removed, both the atria were separated from the ventriculi, and a strip was obtained. The ends of the specimens were tied and suspended in an organ bath containing Tyrode solution, at 32°C, aerated with pure O_2. The upper part of the organ was attached to an isometric transducer (Basile Model 7003) connected to a unirecord microdynamometer (Basile Model 7050). The organ was left to stabilize for 30 min before beginning the experiment.

Guinea-pig trachea. Tracheae were obtained from the same guinea-pigs as described above. The trachea was removed, freed from extraneous tissue, and after locating the smooth muscle tissue, the cartilage was cut on the opposite side, obtaining a rectangular piece of tissue with the muscular layer in the middle. The trachea was then cut transversally at equally-spaced intervals up to 1 mm from the left and the right border, resulting in zigzag strips. The strip, tied with threads at the opposite ends, was placed in a 10-mL organ bath as described for previous preparations, filled with Krebs solution (composition (mM): NaCl 118, KCl 4.75, $CaCl_2$ 2.50, $MgSO_4.7H_2O$ 1.19, KH_2PO_4 1.19, $NaHCO_3$ 25, glucose 11.5) at 37°C, and gassed with carbogen. The organs, held at a tension of 0.5 g, were tied to an isotonic transducer (Basile Model 7006) and the last in turn to a unirecord microdynamometer (Basile Model 7050), to record the response of the smooth musculature. After a 1-h stabilizing time interval, the organ was contracted with carbachol (5.5×10^{-6} M) to obtain a marked muscular tone. The agonistic action of the compounds under test was assessed as the ability to inhibit the constant level of tracheal smooth muscle tone induced by carbachol.

The concentration–response curves were obtained using the method of cumulative concentrations for all organs except the atria. Agonist activity was expressed in terms of pD_2 values ($-\log$ ED50, i.e. the negative logarithm of the drug molar concentration producing 50% of the maximal response) and intrinsic activity (the ratio between the maximal response of a test compound and that of the reference agonist, which was noradrenaline (**1a**) for α-receptors and isoproterenol (**1b**) for β-receptors). Antagonist activity was evaluated as the ability of the compounds under test to reduce the response to a submaximal concentration of isoproterenol after an incubation period of 30 min and expressed as $-\log$ IC50 (i.e. the negative logarithm of the concentration that reduced the agonist response by 50%).

The following drugs were used as salts: **1a** (L-noradrenaline) as bitartrate, **1b** (L-isoproterenol) and compounds **5a**, **b** and **6a**, **b** as a hydrobromides.

Functional tests data analysis. The parameters of potency obtained in the functional tests are expressed as pD_2 ($-\log$ EC50) and represent the mean$\pm$s.d. from 4–6 experiments. Statistical comparison of experimental data sets was performed by analysis of variance. The Bonferroni post test allowed a direct comparison of selected couples of experimental values.

Results and Discussion

Chemistry

The DDSNPs **5a**, **b** and **6a**, **b** were prepared as outlined in Figure 2. The appropriate unsaturated nitriles **7** (DeBernardis et al 1985) and **8** were reduced with $NaBH_4$ in EtOH to the corresponding tetrahydronaphthalene nitriles **9** (DeBernardis et al 1985) and **10**, which, in turn by a Michael reaction with ethyl acrylate in anhydrous EtOH in the presence of EtONa, yielded the corresponding nitrile esters **11** and **12**. Reductive cyclization of **11** and **12** with hydrogen and Ni Raney at high temperature and pressure gave the dimethoxyspiropiperidine derivatives **13** and **14**, which were reduced with diborane in Et_2O to the related spiropiperidines **15** and **16**. Ether cleavage of **15** and **16** with 48% HBr afforded the DDSNPs **5a** and **6a**, respectively. Base-catalysed alkylation of **15** and **16** with Me_2CHBr in MeCN gave the corresponding *N*-isopropyl amines **17** and **18**, which, by treatment with 48% HBr, afforded the *N*-isopropyl-substituted DDSNPs **5b** and **6b** as hydrobromides.

Conformational studies

To obtain information about the conformational profile of DDSNPs **5** and **6** and to compare it with that of the analogues, 1-AMDTNs (**3** and **4**) and 3-DPPs **2**, a conformational study was initially performed by means of proton NMR spectrometry on **15** (a methoxy de-

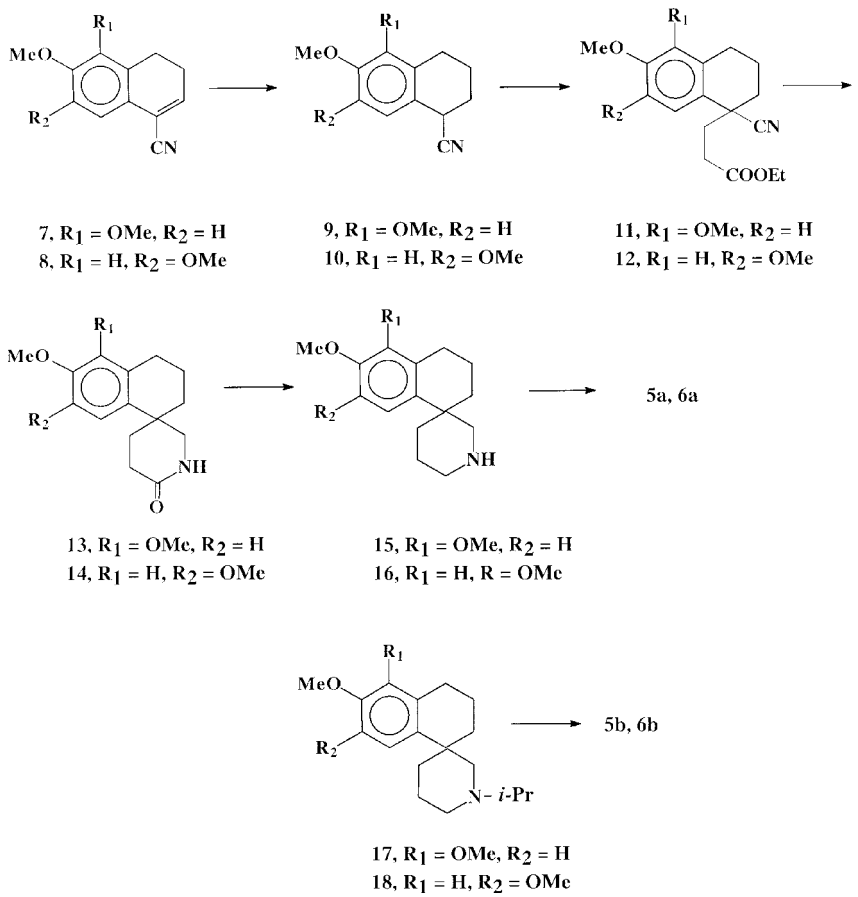


Figure 2 Preparation of the DDSNPs **5a**, **b** and **6a**, **b**.

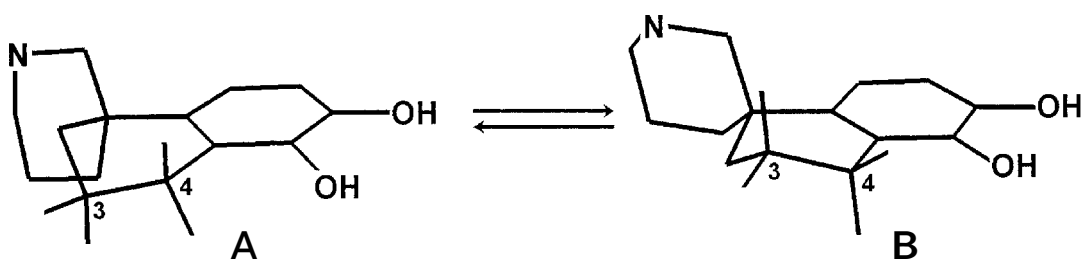


Figure 3 Equilibrium between the two possible half-chair conformations of DDSNP **5a**.

rivative of **5a**), since derivatives like **5** or **6**, are unstable in solution. Subsequently, a theoretical conformational study was carried out on DDSNPs **5a** and **6a** by means of quantum mechanical calculations.

As a result of the superimpositions of signals of diagnostic protons, the ^{1}H NMR analysis of **15** did not provide unequivocal information about the conformation of this kind of derivative. However, as regards the conformation of the alicyclic portion of the tetrahydronaphthalene system, the values of the vicinal coupling constants between the methylene protons linked to C4 and for those linked to C3 (J = 5.6 Hz) were intermediate between those of a pure *trans* coupling constant (J = 8–10 Hz) and a pure *gauche* one (J = 2–3 Hz), indicating the possible existence of an equilibrium between two half-chair conformations and like those shown in Figure 3A, B (Jackman & Sternhell 1969).

A conformational analysis was carried out on catechols **5a** and **6a**, using the GMMX method contained in the PCMODEL programme (ver. 7.0, 1999; Serena software, Bloomington, IN), which revealed four possible conformations both for compound **5a** and **6a**. Each conformer obtained was minimized by means of the semi-empirical PM3 method (Gaussian 94, revision E1, 1994; Gaussian Inc., Pittsburgh, PA). Four conformers were found for both compounds **5a** and **6a** which correspond to the two possible chair conformations that both the cyclohexane ring and the piperidine ring can assume. The conformation which proved to be the preferred one is that indicated in Figure 3A.

These results are thus in agreement with the hypothesis based on NMR proton data, in that they support the existence of a conformational equilibrium for the spiropiperidine system.

Pharmacology

The affinity indices obtained for DDSNPs **5** and **6** are shown in Table 1, together with those obtained with the same experimental protocol for noradrenaline and isoproterenol (**1**), 3-DPPs (**2**) and 1-AMDTNs (**3** and **4**).

The α-adrenergic activity of DDSNPs **5** and **6** are shown in Table 2, together with those obtained in the same functional tests for catecholamines **1** and the corresponding semi-rigid analogues 3-DPPs (**2**), and 1-AMDTNs (**3** and **4**).

The DDSNP analogue of **3a** (**5a**) showed an affinity for α_1-receptors which was almost three times lower than that of 1-AMDTN **3a** and almost double that of 3-DPP **2** (Table 1). The regioisomer **6a** was characterized by an affinity for α_1-receptors slightly lower than that of **5a**, while the tetrahydronaphthalene analogue **4b** was practically devoid of any affinity. Both the *N*-isopropyl-substituted DDSNPs **5b** and **6b**, like the 1-AMDTN analogues **3b** and **4b** and the 3-DPP **2b**, showed similar affinity indices for α_1-receptors, in all cases not very different from those of the *N*-unsubstituted analogues, but about 3-fold lower than that of isoproterenol.

As regards α_2-adrenoceptors (Table 1), the DDSNP derivative **5a** showed a certain affinity, with an affinity index about 50 or 28 times that of the corresponding 1-AMDTN (**3a**) or 3-DPP **2a**, respectively. These last compounds, in turn, proved to possess an affinity for these receptors practically identical to that of noradrenaline. The regioisomers of DDSNP derivative **5a** (**6a**) had a K_i value 36 or 66 times that of **5a** and 1-AMDTN **4a**, respectively. The *N*-isopropyl-substituted DDSNP derivative **5b** exhibited an affinity index 15 and 67 times that of the corresponding 1-AMDTN **3b** and the 3-DPP **2b**. The DDSNP **6b** showed a modest affinity, while the 1-AMDTN **4b** showed an affinity similar to that of **5b**.

At the level of β_1-adrenoceptors (Table 1), both DDSNPs **5** and **6** proved to be practically devoid of any affinity. On the same β-adrenoceptor, both 3-DPPs (**2**) and 1-AMDTNs **3** and **4** revealed a very modest affinity,

Table 1 Radioligand binding affinities of compounds 1–6.

Compound	K_i (nM)			
	α-Adrenergic binding affinity		β-Adrenergic binding affinity	
	Rat brain (α_1)	Rat brain (α_2)	Rat brain (β_1)	Bovine lung (β_2)
1a (Noradrenaline)	450 (70)	4.8 (1.3)	126 (18)	6000 (700)
2a (3-DPP)	4250 (225)	10 (5.5)	29700 (3000)	10900 (1200)
3a (1-AMDTN)	19000 (1500)[a]	5.6 (0.6)[a]	20000 (2000)[a]	7000 (700)[a]
4a (1-AMDTN)	64000 (2100)	153 (20)	48000 (2000)	83000 (2600)
5a (DDSNP)	7750 (500)	280 (40)	40500 (1800)	81000 (1800)
6a (DDSNP)	9700 (450)	10100 (900)	>100000	>100000
1b (Isoproterenol)	35500 (3750)	21000 (4000)	80 (24)	110 (15)
2b (3-DPP)	6250 (600)	115 (18)	50300 (3200)	86000 (34000)
3b (1-AMDTN)	12000 (1600)[a]	500 (50)[a]	17000 (3000)[a]	7600 (700)[a]
4b (1-AMDTN)	11000 (1250)	4000 (450)	45000 (7500)	8500 (450)
5b (DDSNP)	9000 (1600)	7700 (900)	>100000	64000 (1400)
6b (DDSNP)	6700 (800)	5500 (800)	>100000	>100000

Means of five separate determinations with standard deviation shown in parentheses. [a]Macchia et al 1988.

Table 2 Adrenergic activities of compounds **1**–**6** on isolated preparations.

Compound	α-Adrenergic activity				β-Adrenergic activity			
	Rat isolated vas deferens (α_1)		Guinea-pig isolated ileum (α_2)		Guinea-pig isolated atria (β_1)		Guinea-pig isolated tracheal strip (β_2)	
	pD_2	i.a.	pD_2	i.a.	pD_2	i.a.	pD_2	i.a.
1a (Noradrenaline)	5.12 (±0.22)[a]	1.00[a]	6.56 (±0.25)[a]	1.00[a]	6.32 (±0.16)[a]	1.00[a]	6.03 (±0.15)[a]	1.00[a]
2a (3-DPP)	5.37 (±0.29)[b]	0.92[b]	7.03 (±0.20)[b]	1.00[b]	4.90 (±0.60)	0.90	5.69 (±0.40)	0.82
3a (1-AMDTN)	4.76 (±0.39)[c]	0.90[c]	7.56 (±0.29)	1.00	4.53 (±0.42)[c]	0.70[c]	4.56 (±0.59)[c]	0.70[c]
4a (1-AMDTN)	4.12 (±0.25)[d]	1.00[d]	5.90 (±0.37)	0.70[d]	4.37 (±0.16)[d]	0.55[d]	⩽ 3.50[d]	
5a (DDSNP)	5.15 (±0.11)	0.40	5.63 (±0.32)	1.00	4.87 (±1.36)	0.20	⩽ 3.50	
6a (DDSNP)	—		—		—		—	
1b (Isoproterenol)	3.50 (±0.34)[a]	0.83[a]	4.95 (±0.34)[a]	0.74[a]	8.45 (±0.27)[a]	1.00[a]	8.33 (±0.36)[a]	1.00[a]
2b (3-DPP)	⩽ 3.50		6.09 (±0.25)	0.87	4.37 (±0.07)	0.80	4.83 (±0.20)	0.90
3b (1-AMDTN)	—[a]		4.30 (±0.29)	0.70	—[a]		—[a]	
4b (1-AMDTN)	—[d]		—[d]		—[d]		—[d]	
5b (DDSNP)	⩽ 3.50		⩽ 3.50		—		—	
6b (DDSNP)	⩽ 3.50		—		—		—	

The values represent the mean of 4–6 experiments for each drug (±s.d. in parentheses). Intrinsic activity (i.a.) is the ratio between the maximal response elicited by the compound under test and that elicited by the full agonist, namely noradrenaline and isoproterenol for α- and β-adrenoceptors, respectively. Macchia et al [a]1992, [b]1995, [c]1988, [d]1989.

with respect to corresponding catecholamines **1a** and **1b**.

Also on β_2-adrenoceptors (Table 1), DDSNPs **5** and **6**, the 1-AMDTNs **4a** and the 3-DPP **2b** were devoid of any affinity. The *N*-unsubstituted 3-DPP (**2a**) and the 1-AMDTN (**3a**), together with the *N*-isopropyl-substituted 1-AMDTN **3b** and **4b**, exhibited affinity indices similar to that of noradrenaline, but considerably lower than that of isoproterenol.

As regards the results of the functional tests (Table 2), at the level of α_1-adrenoceptors, the DDSNP derivative **5a** showed a pD_2 value slightly lower than that of the 3-DPP **2a**, and slightly higher than that of the 1-AMDTN **3a** (although these differences were not statistically significant), but with a low intrinsic activity (ratio between the maximal response elicited by the compound under test and that elicited by the full agonist – noradrenaline and isoproterenol for α- and β-adrenoceptors, respectively). The regioisomer of **5a** (**6a**) was found to be completely inactive.

On the same α_1-adrenoceptors, both the *N*-isopropyl-substituted DDSNPs (**5b** and **6b**) were found to be practically devoid of any activity, just as the corresponding 3-DPP (**2b**) and 1-AMDTNs (**3b** and **4b**).

As for the activity on α_2-adrenoceptors (Table 2), the DDSNP **5a** showed a pD_2 value of 5.63 ($P < 0.001$) and an intrinsic activity of 1.00, while the 3-DPP (**2a**) and the corresponding 1-AMDTN (**3a**) exhibited a pD_2 value of 7.03 ($P < 0.001$) and 7.56 ($P < 0.001$) and an intrinsic activity value of 1.00. On the same adrenoceptor, noradrenaline (**1a**) showed a pD_2 of 6.56. The regioisomer of **5a** (**6a**) proved to be completely inactive, while the corresponding 1-AMDTN (**4a**) showed a pD_2 value of 5.90, but with a low intrinsic activity value (0.70).

The *N*-isopropyl-substituted DDSNPs **5b** and **6b**, like the 1-AMDTN **4b**, were completely inactive. The 1-AMDTN **3b** was slightly active with a pD_2 value of 4.30 and an intrinsic activity value of 0.70. As regards β_1-adrenoceptors (Table 2), DDSNP **5a** showed a pD_2 value (4.87, not statistically different) similar to that of 3-DPP **2a** (pD_2 4.90, not statistically different) and the corresponding 1-AMDTN **3a** (pD_2 4.53, not statistically different), but with a very low intrinsic activity value (0.20 vs 0.90 for **2a** and 0.70 for **3a**). The regioisomer of **5a** (**6a**) was completely inactive, while the corresponding 1-AMDTN (**4a**) showed a modest activity (pD_2 4.37, intrinsic activity 0.55). The *N*-isopropyl-substituted DDSNPs **5b** and **6b** proved to be completely inactive, like the corresponding 1-AMDTNs **3b** and **4b**. The *N*-isopropyl-substituted 3-DPP **2b** appeared to possess an activity much lower than that of isoproterenol (**1b**) with pD_2 and intrinsic activity values of 4.37 ($P < 0.001$) and 0.80, respectively, vs pD_2 8.45 ($P < 0.001$) and intrinsic activity 1.00 for **1b**.

On β_2-adrenoceptors (Table 2), both the DDSNPs **5a** and **6a** were practically inactive, while 3-DPP **2a** and 1-

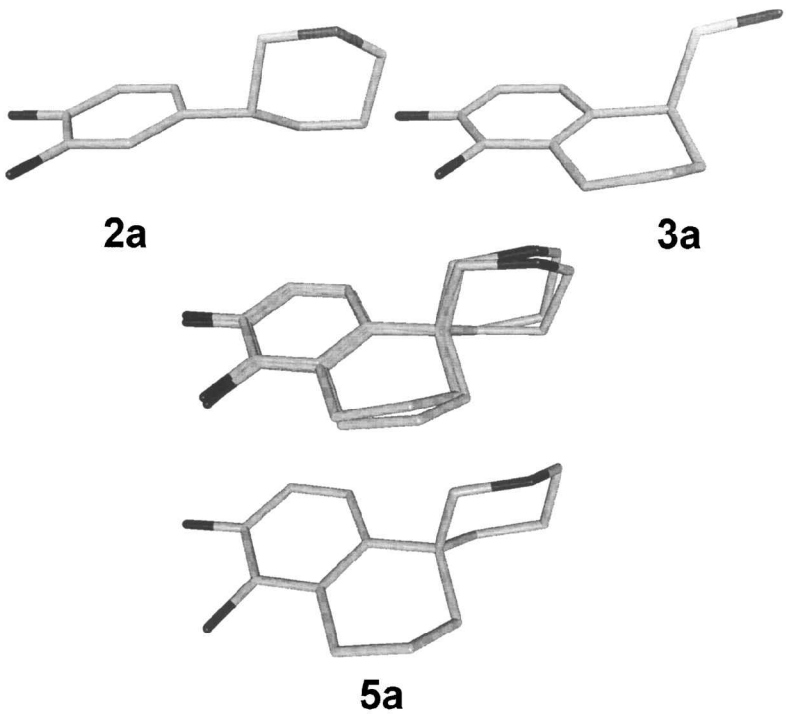


Figure 4 Compounds **2a**, **3a** and **5a** in low energy conformations; in the middle the three conformations are superimposed.

AMDTN **3a** exhibited a certain degree of activity, with pD_2 values of 5.69 (intrinsic activity 0.82) and 4.56 (intrinsic activity 0.70), respectively. Also the *N*-isopropyl-substituted DDSNPs **5b** and **6b**, like the 1-AMDTN analogues **3b** and **4b**, were found to be completely inactive on this adrenoceptor. The *N*-isopropyl-substituted 3-DPP (**2b**) exhibited pD_2 and intrinsic activity values of 4.83 and 0.90, respectively.

Binding assays showed that the DDSNP **5a** possessed an appreciable affinity for the α-adrenoceptor, even if it was more than one order of magnitude lower than that of the corresponding 3-DPP **2a** and 1-AMDTN **3a**. The *N*-isopropyl derivative **5b** appeared to possess a much lower affinity.

In the functional tests, the activity index trend appeared to be in agreement with that of the affinity indices. The DDSNP **5a** possessed a discrete activity on the α_2-adrenoceptor, even if about 1.5 to 2 orders of magnitude lower than that of the 3-DPP **2a** ($P < 0.001$) and the corresponding 1-AMDTN **3a** ($P < 0.001$), respectively. The different position of the catecholic hydroxyl of **6a** compared with that of **5a** involves the loss of the α_2-adrenergic activity, as happens with the insertion of the isopropyl substituent on the nitrogen of the DDSNP **5a** (**5b**).

On the remaining adrenoceptors (α_1, β_1, β_2) the DDSNPs **5** and **6** appeared to have little or no adrenergic properties, as had already been found for the reference analogues, the 3-DPPs (**2**) and the 1-AMDTNs (**3** and **4**).

Conclusions

The DDSNP **5a** was synthesized to evaluate the influence exerted on the α_2-adrenergic properties by the combination in a single molecular structure of the two semi-rigid analogues of noradrenaline (**1a**) (i.e. the 3-DPP (**2a**) and 1-AMDTN (**3a**)), which possess a high affinity and α_2-adrenergic activity. The overall results (Tables 1 and 2) for **2a**, **3a** and **5a** indicate that the DDSNP **5a** only partly maintains the good α_2-adrenergic properties of the 3-DPP **2a** and 1-AMDTN **3a**. As it was possible that the difference between the adrenergic properties of DDSNP **5a** and those of the conformationally more mobile analogues, **2a** and **3a**, might lie in differences in the spatial arrangement of the groups that are active in these compounds, we compared their stereostructures in their minimum-energy conformations, which were established by means of theoretical and experimental studies (Figure 4). However, the ap-

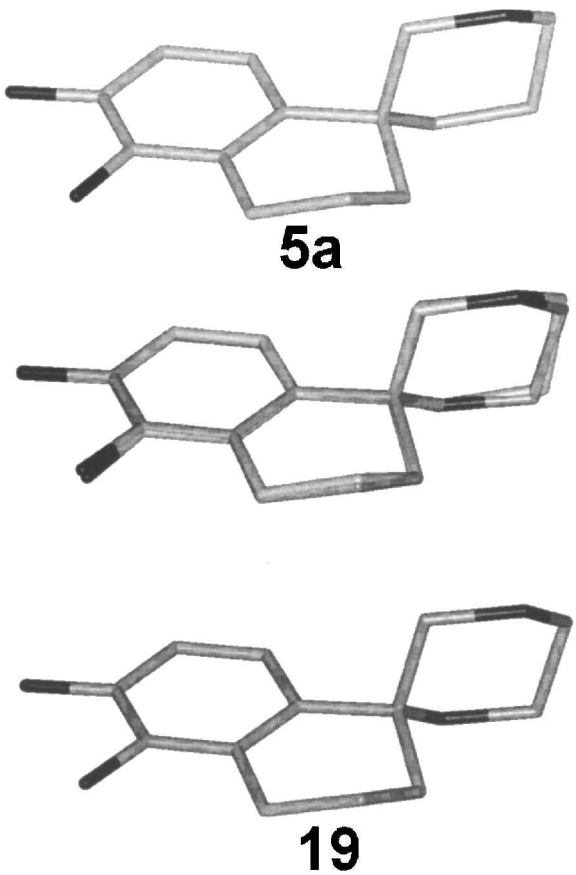


Figure 5 Compounds **5a** and **19** in their preferred conformations; in the middle the two conformations are superimposed.

preciable overlapping of the active groups (catecholic group and amino nitrogen), as seen in Figure 4, allowed us to exclude this hypothesis.

A more probable hypothesis might be that a partial obstacle for an optimal interaction with the α_2-adrenergic receptor may be created by the greater rigidity of the spiropiperidine system of **5a** compared with the piperidine system of **2a** or the tetrahydronaphthalene system of **3a**, or the presence in **5a** of further steric hindrances due to the additional atoms needed to make the piperidine derivative **2a** or the tetrahydronaphthalene derivative **3a** more rigid. A comparison of the structure of the DDSNP **5a** with that of dihydroxy-3,4-dihydrospironaphthalen-1-(2*H*)-2′,5′-morpholine (DDSNM) **19**, previously studied (Balsamo et al 1990) for its α_2-adrenergic properties, shows that **5a** can be seen as the carba-analogue of **19**.

A comparison of the indices of affi nity and activity for the α_2-adrenergic receptor shown by the two compounds (K_i 280 nM and pD_2 5.63 for **5a** vs K_i 2900 nM and pD_2 5.01 for **19**) indicates that the two types of molecules present a fairly similar capacity to interact with the α_2-adrenergic receptor. This finding appears to be justified if we consider that the two types of spiroderivatives, for which Figure 5 shows a superimposition of their respective structures in their minimum-energy conformations, appear practically to coincide, both as regards the phenol system and the amino nitrogen, the only difference being the presence or absence of the ethereal oxygen.

It would, therefore, seem that the ethereal oxygen present in DDSNM **19**, but lacking in DDSNP **5a**, does not play any decisive role in the weak interaction of these spiroderivatives with the α_2-adrenergic receptor.

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