The excitatory amino acids were administered for times sufficient to obtain maximal effects at the particular rate of ejection. The relative potencies of the compounds were determined from a comparison of electrophoretic currents required to produce equal and submaximal excitation of the cells, making allowance for the dilution of some amino acids in 0.15 M NaCl. Antagonism was apparent from a slower onset and reduced degree of excitation.

Kainic Acid Binding Studies. The effects of the compounds on kainic acid binding were studied as described earlier[13] on the basis of a published procedure.[18] The membrane preparation was frozen rapidly at −70 °C and kept at −20 °C for at least 18 h before use in the receptor-binding assay. For the [^{3}H]kainic acid binding assay procedures, aliquots of synaptic membranes (0.8–1.2 mg of protein) were incubated in triplicate at 4 °C for 5 min in 2 mL of 0.05 M Tris-citrate buffer (pH 7.1) containing 0.005 μM [^{3}H]kainic acid, and the IC_{50} values of the agents tested were determined by using conventional procedures.

Acknowledgment. This work was supported by grants from the Danish Medical Research Council and the Australian National University and Grants 11-1837 (Enraf-Nonius CAD-4 diffractometer), 11-2360 (Enraf-Nonius Structure Determination Package), and 11-3531 (an X-ray generator) from the Danish Natural Sciences Research Council. The collaboration and valuable discussions with Drs. J. D. Leah and M. J. Peet, Canberra, Australia, and M. Gajhede, J. J. Hansen, and S. Larsen, Copenhagen, the technical assistance of F. Hansen, P. Searle, and S. Stilling, and the secretarial assistance of B. Hare are gratefully acknowledged. Dr. J. C. Watkin kindly supplied 2APV.

Registry No. 1, 89017-61-8; 2, 95407-17-3; 3, 89017-62-9; 4, 95407-18-4; 5, 75989-23-0; 6, 95407-19-5; 7, 81238-04-2; 8, 95407-20-8; 9, 95407-21-9; 10, 95407-22-0; 11, 95420-13-6; 7-HPCA, 95407-23-1; ABPA, 83643-89-4; AMPA, 74341-63-2; NMDA, 6384-92-5; 5-HPCA, 95407-23-1.

Supplementary Material Available: Lists of structure factors and anisotropic thermal parameters of the non-hydrogen atoms (49 pages). Ordering information is given on any current masthead page.

Notes

Resolution of 5,6-Dihydroxy-2-(*N*,*N*-di-*n*-propylamino)tetralin in Relation to the Structural and Stereochemical Requirements for Centrally Acting Dopamine Agonists

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Received July 5, 1984

The enantiomers of 5,6-dimethoxy-2-(*N*,*N*-dipropylamino)tetralin were prepared with use of (+)- and (−)-dibenzoyltartaric acid as the resolving agent. Ether cleavage with BBr_3 gave the enantiomers of the dihydroxy compound 5,6-dihydroxy-2-(*N*,*N*-dipropylamino)tetralin (5,6-$(OH)_2$-DPATN). The in vitro activities of (+)- and (−)-5,6-$(OH)_2$-DPATN were evaluated in binding studies with rat striatal tissue with use of [^{3}H]-*N*-*n*-propylnorapomorphine (NPA) as the ligand. IC_{50} (nM) values for (−)- and (+)-5,6-$(OH)_2$-DPATN were 2.5 and 400, respectively. The in vivo efficacy of the enantiomers was evaluated by examining their effects on the metabolism of dopamine in rat striatum. After a 0.5 μmol/kg ip injection of the (−) enantiomer, the concentrations of the metabolites HVA and DOPAC were reduced to 50% of control values, whereas at this dose the (+) isomer was inactive. On the basis of these findings together with the stereochemical data of previously described DA agonists, a dopamine-receptor model has been developed which consists of two binding sites for the amine nitrogen of DA agonists in addition to a major binding site for the *m*-hydroxy group. The relevance of this model with its accessory features is discussed in relation to the structure and pharmacological data of different DA agonists.

In a previous report on dopamine (DA) receptor topography[1] we proposed a model of the DA receptor with the nitrogen and the catechol group of DA as essential sites of interaction with the receptor. From MO calculations of DA and comparison of N–OH distances in active and inactive rigid DA analogues, it was found that in order to exhibit activity, these key distances should be about 7.8 Å for the $N–OH_{para}$ and about 6.4 Å for the $N–OH_{meta}$ distances, respectively. In addition, we suggested the presence of a steric barrier at the receptor site to account for the inactivity of isoapomorphine as a DA agonist. Independently a comparable model was developed by McDermed[2] based upon the absolute configuration of 5-hydroxy-2-(*N*,*N*-dipropylamino)tetralin (5-OH-DPATN) and 6,7-dihydroxy-2-(*N*,*N*-dipropylamino)tetralin (6,7-OH-DPATN). As 5,6-$(OH)_2$-DPATN is a closer analogue of dopamine than the 5-monohydroxy analogue, we resolved 5,6-$(OH)_2$-DPATN into its optical stereoisomers and tested their activities in vitro as well as in vivo as DA agonists.

The availability of the stereoisomers of one of the most potent DA agonists can be extremely useful for receptor-binding assays of DA agonists, which have become a powerful tool in investigations of DA receptors. In contrast to the ^{3}H-labeled antagonist binding results, data from the ^{3}H-labeled agonist studies are, however, still controversial. Despite the introduction of several binding methods, optimal assay conditions are still being investigated.[3-5] One of the reasons stems from the different definitions of specific binding that have been used. In our opinion, one

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(2) McDermed, J. D.; Freeman, H. S.; Ferris, R. M. In "Catecholamines: Basic and Clinical Frontiers"; Usin, E.; Pergamon Press: New York, 1979; p 5568.
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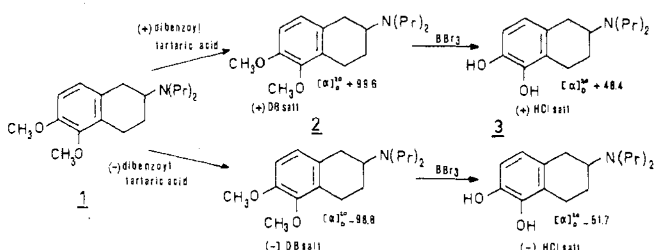
Table I. Effects of 5,6-$(OH)_2$-DPAT on Dopamine Metabolism and Behavior in the Rat

5,6-$(OH)_2$-DPAT	dose, μmol/kg	striatal concn ± SEM as % of control[a] (n = 4) HVA	DOPAC	DA	stereotypy score (n = 4)
	0.25	36.2 ± 2.0	61.0 ± 7.0	103.0 ± 6.0	
(±)	0.5	47.6 ± 3.8*[b]	58.5 ± 2.8*	114.9 ± 3.8	3–4
(−)	0.25	42.5 ± 3.1*	53.7 ± 2.1*	98.4 ± 6.2	3–4
	0.5	42.6 ± 2.6*	58.0 ± 2.3*	114.2 ± 5.3	4
(+)	0.5	86.5 ± 3.6	93.3 ± 2.4	109.4 ± 3.5	0
	5	48.6 ± 2.9*	68.9 ± 6.8	115.0 ± 3.3	2–3
	10	41.2 ± 4.0*	60.1 ± 3.4*	125.8 ± 5.5*	3

[a] Control values were as follows: HVA, 0.83 μg/g; DOPAC, 0.91 μg/g; DA, 8.60 μg/g. [b] Asterisk indicates $P < 0.01$ vs. control.

Scheme I



of the main criteria of specific binding should be based on the stereoselectivity of the receptor for active and inactive enantiomers. Commonly used methods for defining specific binding are the measurement of the displacement of the radiolabel by an excess of the same nonradioactive ligand, by its closest congener or by the active enantiomer of butaclamol, a DA antagonist. In some studies anamolous behavior of binding curves has been obtained, especially when agonists are displaced by antagonists. Part of this behavior was suggested to originate from differences in physicochemical properties between the agonists and the antagonists;[6] therefore, stereoisomers with the same physicochemical properties should be very useful in performing receptor agonist binding studies.

In this paper we describe the preliminary results of the resolution and the biological activity of stereoisomers of 5,6-$(OH)_2$-DPATN. A new DA-receptor model, based on these and stereochemical data of other DA agonists, is discussed.

Chemistry. Racemic 5,6-dimethoxy-2-(*N*,*N*-di-*n*-propylamino)tetralin was synthesized according to standard procedures.[7] The amine was converted to the enantiomeric salts with (−)-dibenzoyl-L-tartaric acid and (+)-dibenzoyl-D-tartaric acid and repeatedly recrystallized from ethanol until they showed constant rotation and melting point (Scheme I). The enantiomeric salts were converted to their amines and demethylated with BBr_3 in methylene chloride at −40 °C. The isolated HBr salts were converted to their HCl salts and recrystallized from MeOH–diethyl ether, giving the (−) isomer with $[\alpha]^{20}_D$ −51.7° (mp 170.5–171 °C) and the (+) isomer with $[\alpha]_{20D}$ + 48.5 (mp 170.5–171 °C).

Pharmacology. The in vivo dopaminergic activities of the enantiomers and racemic 5,6-$(OH)_2$-DPATN were assessed by measuring the decrease of DA metabolite levels[8] in rat brain and by scoring stereotyped behavior in rats. Female Wistar rats (160–180 g) were injected ip with the drugs, dissolved in saline, containing 0.1% sodium bisulfite. After 40 min the rats were decapitated, and striata were dissected, homogenized, and after purification on Sephadex G 10 columns assayed for DA, HVA, and DOPAC by use of reversed-phase HPLC with amperometric detection according to the method of Westerink and Mulder.[8] Stereotypy was scored, also after ip injection of the drugs, during 30 min according to the method of Costall et al.[9] The results of the neurochemical and behavioral tests are shown in Table I.

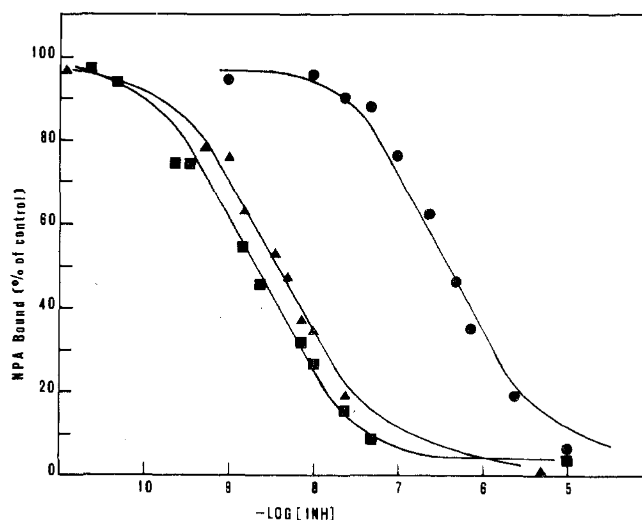


Figure 1. Effect of racemic (▲) 5,6-$(OH)_2$-DPAT and the (+) (●) and the (−) (■) enantiomers on the binding of [^{3}H]NPA to striatal rat homogenate.

Table II. Affinity of Isomers of 5,6-$(OH)_2$-DPAT for Dopamine Receptors

ligand (nM)	IC_{50}, nM (−)-5,6	(+)-5,6	(±)-5,6
[^{3}H]NPA (0.45)	2.5	400	4.0

[a] Striatal tissue was homogenized and incubated at 25 °C with 0.45 nM [^{3}H]NPA in competition with a range of concentrations of the 5,6-DPATN isomers. Values of IC_{50} were computed from log probit analysis of data from at least three determinations. The tabulated values are means, the SD of which were ±5% or less.

The binding assay was carried out with homogenized rat striatal tissue (male Wistar rats, 180–200 g) according to the procedure of Leysen and Gommeren.[6] The ligand was *N*-propylnorapomorphine (^{3}H-NPA; New England Nuclear, 20 Ci-mmol). Blanks were defined by adding an excess (1 μmol) of (+)-butaclamol (a gift from Ayerst Laboratories). The results of the displacement of ^{3}H-NPA by 5,6-$(OH)_2$-DPATN, (−)-5,6-$(OH)_2$-DPATN, and (+)-5,6-$(OH)_2$-DPATN are summarized in Figure 1 and Table II.

Discussion

From the pharmacological results, the pronounced difference between the stereoisomers of 5,6-$(OH)_2$-DPATN

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is evident. The binding experiments showed a 160-fold isomeric difference between (–)- and (+)-5,6-$(OH)_2$-DPATN. Such a preferential effect of (–)-5,6-$(OH)_2$-DAPTN was also found in an in vivo experiment in which the effect on the metabolism of dopamine was measured; i.e., after 0.5 μmol/kg of (–)-5,6-$(OH)_2$-DPATN, the concentrations of HVA and DOPAC were about 50% of the control values while the same dose of the (+) isomer was without effect. However, at higher doses the (+) isomer also decreased the metabolite concentrations. A contamination of (–)-5,6-$(OH)_2$-DPATN by the (+) isomer might be responsible for this effect as there was a small difference in rotation (51 vs. 48).

The stereochemical requirements found in this study and the results from other studies with monohydroxy derivatives[10,11] allow us to define additional topological features of the dopamine receptor. For the enantiomers of 5,6-$(OH)_2$-DPAT and that of 5-OH-DPAT, it was found that the (–) isomer had the *S* and the (+) isomer had the *R* configuration.[12] As in the case of 5-OH-DPAT, the *S* isomer of 5,6-$(OH)_2$-DPAT is found to be the active form in contrast to 6,7-$(OH)_2$-DPAT, where the *R* isomer is the active form.[2] Thus, in order to produce a biological response, a reversal of the configuration from the 5,6- to the 6,7-isomer is necessary. This is in accordance with the work of McDermed et al.[2] and the study of Seiler and Markstein on the monohydroxy derivatives,[10] which led them to suggest the existence of two binding sites: one for the *m*-hydroxy group and one for the amino group of DA, together representing the active site. The *p*-hydroxy group and the aromatic ring would then interact with accessory binding sites. In their view, an opposite configuration of the 5(6) series compared with 6(7) is then necessary to achieve the same orientation toward the major binding sites. Similar conclusions were also reached by Neumeyer,[14] Seeman,[15] and Wikström.[16] However, these studies more or less ignore the difference in distances between the *m*-hydroxy moiety and the nitrogen atom in the 5(6) series compared to that in the 6(7) DPAT series, which are 6.4 and 7.4 Å, respectively. One way to account for this discrepancy is to assume that the receptor is so flexible that the *m*-hydroxy and the nitrogen interaction sites can give an appropriate fit with compounds of both categories. However, considering the fact that an optimal fit is often necessary to evoke a biological response, we do not find this explanation entirely satisfying. We consider the interaction of agonists with the putative OH-binding sites as the most critical factor in determining the position of the aromatic nucleus due to the highly directive nature of H bridges being the most likely binding forces with the catechol group. We therefore wish to propose a modification of the receptor model, which assumes the existence of two separate interaction sites for the nitrogen atom (X and Y), while the interaction sites for the *m*-hydroxy and the accessory sites for the *p*-hydroxy and the aromatic ring are the same (Figure 2). The assumption of these two places does not indicate that there is no flexibility left for the receptor to accept compounds with deviating N–O distances as there are in the dopaminergic active 2-aminoindans with a distance of 5.7 Å (calculated).[13] In our model they should interact with the X site, while in a completely flexible receptor model with one N-interaction site, compounds with distances varying from 5.7 to 7.4 Å should all be adopted by this same site.

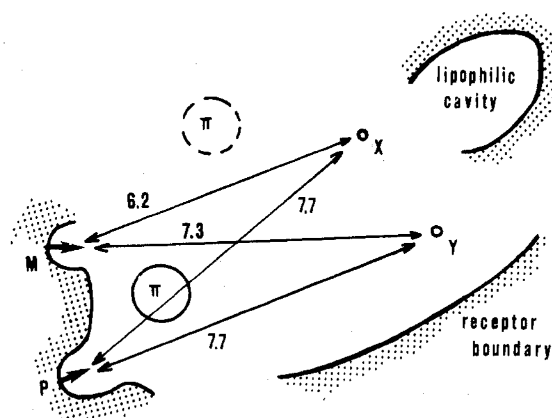

Figure 2. The topography of the dopamine receptor. P and M are putative binding sites on the receptor surface complementary to the *p*-OH and the *m*-OH groups of the agonist molecule; X and Y are putative electronegative binding sites complementary to a nitrogen atom of the agonist. Areas indicated with π are interaction places with an aromatic ring, with π_2 being the accessory binding site as indicated by the activity of the arylbenzazepines[20] and the nomifensine derivative 3,4-DHN.[21]

The interaction sites X and Y can thus be occupied by the nitrogen atom in the α- and β-rotamer analogues, respectively. These two binding sites have the same relative position to the plane of the aromatic ring, so that the opposite configuration of both series, resulting in a similar direction of the nitrogen bond, can be accommodated. To explain the inactivity of the respective "wrong" isomers, it seems unnecessary to assume the presence of an obstacle,[15,16] because the position of the N-binding site, at about 1 Å below the plane of the aromatic ring, excludes the possibility of an interaction with the nitrogen in these inactive isomers.

With regard to the relative importance of the OH-recognition sites, relevant information can be obtained from binding studies where it is found that the *p*-hydroxy group is advantageous but not a prerequisite for dopaminergic activity, while from the relative activities of the 5-OH-DPAT (M), the 6-OH-DPAT (P), and the 7-OHDPAT (M) isomers,[10,17] the M site might be considered as being the most important.

Our model with the two possible nitrogen-interaction sites X and Y might also provide an explanation for the difference in dopaminergic potency of the 5(6)- and 6-(7)-OH-DPAT isomers, independent of the question as to whether a combination of sites M and X or M and Y is the most effective in inducing the biological effects or that these combinations even give qualitatively different responses. In Figure 3 some compounds with an established DA-agonist activity are shown in the appropriate position for interaction with the suggested dopamine receptor. As can be seen from the figure, all compounds exhibit a good fit with the receptor and can be divided into two classes: those interacting with M(P)–X and a category interacting

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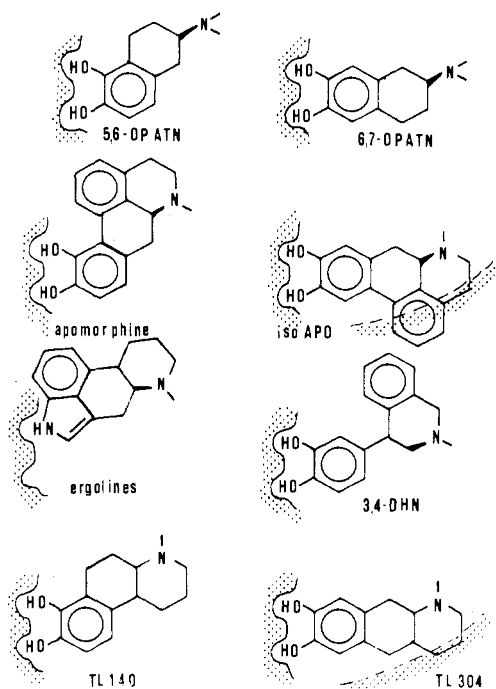

Figure 3. Interaction of α-rotamer analogues and β-rotamer analogues at the dopamine receptor.

with M(P)–Y. (It is noteworthy that the DA agonists with a more or less selective presynaptic action (7-OH-DPAT, 3-PPP) all belong to the same series.) The position of the ergoline derivatives indicates that the NH pyrrole moiety as such is comparable to the *m*-OH groups and that the benzene ring in the ergolines is not necessary, as confirmed by the activity of ergolines lacking this ring.[18] Whether the pyrrole ethylamine or the phenylethylamine moiety is the dopaminergic pharmacophore in the ergolines, which is still controversial,[19] is irrelevant in this respect.

A further feature of our original receptor model was the introduction of a receptor boundary in order to explain the inactivity of isoapomorphine, a concept confirmed by McDermed,[2], Neumeyer,[14] and Wilkström.[16] When this receptor boundary is correctly positioned with regard to the binding sites as indicated in Figure 3, it is obvious that it hinders the attachment of all compounds which have steric bulk on that side of the molecule opposite the *m*-hydroxy group after the molecule is positioned along the P, M and X or Y interaction sites. Additional support for our model with the two N-interaction sites comes from the interesting effect on dopaminergic activity upon N,N-dialkylation of the aminotetralins.[10,17] From in vitro and in vivo data, it has been concluded that the β-rotamer analogue as represented by (6),7-(di)hydroxy-2-aminotetralin is more active as a DA agonist than the 5-mono or the 5,6-dihydroxy-2-aminotetralin, which are α-rotamer representatives. Upon N,N-dialkylation, especially with *n*-propyl substituents, the potencies of the α-rotamers are increased whereas those of the β-series do not change significantly or even decrease. This phenomenon has been explained by Seiler et al., who made the assumption of the occurrence of an accessory lipophilic binding site which is more accessible for alkyl groups in α-rotamers than in β-rotamers. A similar conceptual hypothesis about a difference in accessibility for agonists to the nitrogen binding site in relation with the length and direction of the *N*-alkyl substituents was put forward by Wikström in explaining the post- and presynaptic activities of the rotamers of 3-PPP.[16] When the two different positions of the binding sites X and Y, with respect to such a lipophilic site, are taken into account, the relation between the accessibility of the lipophilic site and the geometry of the agonists (α or β) becomes understandable. Firstly, the lipophilic site might be situated more conveniently with respect to the binding site X (the interaction site for α-rotamers) while, secondly, N,N-dialkylated compounds interacting with site Y, situated closer to the boundary, might in addition experience steric hindrance by this boundary. It is tempting to speculate about the nature of the two nitrogen interaction sites X and Y. As both α and β rotamers can evoke a comparable dopaminergic effect, the origin of X and Y should be comparable. Bearing in mind the fact that from molecular models a distance of 2.4 Å is found between both sites, we suggest that X and Y are possibly two oxygen atoms with a delocalized negative charge, e.g., a phosphate group.

Additional experiments with newly synthesized model compounds are clearly indicated to probe the validity of the above-hypothesized DA receptor model.

Experimental Section

General Methods. All melting points were determined on a Reichert melting point microscope and are uncorrected. Optical rotations were obtained on a Perkin-Elmer polarimeter (Model 241).

Resolution of 5,6-Dimethoxy-2-(*N*,*N*-di-*n*-propylamino)-tetralin ((+)- and (−)-2). To a solution of 7.6 g (26 mmol) of (+)-5,6-dimethoxy-DPAT in 70 mL of dry ether was added a solution of 4.1 g (11 mmol) of (+)-dibenzoyl-D-tartaric acid (Aldrich; $[\alpha]^{24}_D$ +111.7° (c = 9, EtOH) in 5 mL of dry methanol. After the mixture was left standing for 1 day at 20 °C, a voluminous precipitate was formed, which was collected and dried, giving 3.3 g of a light green powder. This (+)-dibenzoyl-D-tartrate salt was recrystallized six times from ethanol, giving white crystals: mp 141.5 °C; yield 0.95 g. Further recrystallization did not change the specific rotation: $[\alpha]_{20D}$ +99.6° (c = 0.15, MeOH).

The filtrate of the first precipitate was collected and a solution of 4.1 g (11 mmol) of (−)-dibenzoyl-L-tartaric acid (Aldrich; $[\alpha^{20}_D$ −105° (c = 1.5, EtOH)) in 5 mL of methanol was added. The precipitate was collected, dried, and recrystallized four times from ethanol to give 1.1 g of (−)-2 salt: mp 141.5 °C; $[\alpha]_{20D}$ −98.8° (c = 0.15, MeOH).

Demethylation of (+)- and (−)-5,6-$(MeO)_2$-DPAT. To 0.25 g (410 μmol) of (−)-2 salt in water was added a solution of 2.5 M KOH and the free base was extracted into ether. After drying ($MgSO_4$) and evaporation, the yellow oil was dissolved in dry CH_2Cl_2. The solution was cooled at −54 °C and 1 mL of dry BBr_3 was added with stirring. The solution was stirred for a further 10 h, during which time the temperature reached 18 °C. A few milliliters of dry methanol was added and the solution was evaporated, yielding a gray powder which was converted to the HCl salt by adding a solution of HCl-saturated methanol and evaporating. The final HCl salt was recrystallized from methanol–ether, giving 90 mg (73%) of white crystals: mp 170.5–171 °C; $[\alpha]^{20}_D$ −51.7° (c = 0.15, MeOH). Following the same procedure, the (+)-HCl salt was obtained (45%): mp 170–5–171 °C; $[\alpha]^{20}_D$ + 48.8° (c = 0.15, MeOH).

Pharmacology. Determination of Metabolites of Dopamine. Female albino rats of a Wistar-derived strain (C.D.L., Groningen, The Netherlands) were used. The body weights of the rats varied from 180 to 220 g. The compounds were admin-

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istered by intraperitoneal (ip) injection in a volume of 2.0 mL kg^{-1}, dissolved in saline containing 0.1% sodium bisulfite. After 40 min, during which time the behavioral effect was scored according to the method of Costall et al.,[9] the rats were killed by cervical dislocation.

The corpora striata were rapidly dissected, frozen on dry ice, and stored at -80 °C. Following weighing of the frozen samples, homogenization in 0.1 M perchloric acid, and centrifugation (3000 g, 7 °C, 15 min), the amount of dopamine and its metabolites HVA and DOPAC in the supernatants was determined according the method of Westerink and Mulder[8] by use of purification on Sephadex G 10, separation on a reverse-phase (RP 18) high-performance liquid chromatographic column, and amperometric detection.

Binding Experiments. The binding studies were carried out according to the procedure of Leysen and Gommeren.[6] Striata of female Wistar rats (C.D.L., Groningen) were dissected and homogenized in 40 volumes of ice cold 15 mM Tris-chloride buffer (1 mM EDTA and 0.01% ascorbic acid added, pH 7.5 at 5 °C) with an ultra-turrax (IKA, 165 rpm, 45 s). The homogenate was centrifuged at 35000*g* for 10 min, washed with buffer, and recentrifuged at the same speed two times. The final pellet was rehomogenized at a final concentration of 50 mg of original wet weight per millimeter in cold fresh 15 mM Tris buffer containing 1 mM EDTA and 0.01% ascorbic acid. In the competition experiments, to incubation tubes in triplicate were added 100 μL of [^{3}H]NPA (final concentration 0.46 nM), increasing concentrations of competing drugs, soluted in the buffer (10^{-11} to 10^{-5} M) or 200 μl of (+)-butaclamol (final concentration 10^{-6} M), and 100 μL of the tissue suspension, giving a final volume of 1 mL.

All tubes were incubated at 25 °C for 30 min and the contents rapidly filtered under vacuum through Whatman glass fiber GF/B filters and washed three times with 5 mL of ice-cold buffer. The filters were collected and placed in glass vials with 6 mL of scintillation cocktail, left overnight, shaken for 20 min, and counted by liquid scintillation spectrometry (Beckman LS 18) at a counting efficiency of 45-50%. Saturable or specific binding was defined as the difference between the binding in the absence and in the presence of 10^{-6} M *d*-butaclamol (total binding 200 dps, nonspecific binding 40 dps as typical values were found).

Registry No. (+)-**2**·DB, 94844-52-7; (-)-**2**·DB, 94844-54-9; (-)-**3**·HCl, 94903-51-2; (-)-**3**, 82730-71-0; (+)-**3**·HCl, 94903-52-3; (+)-**3**, 82730-70-9; (±)-**3**, 72189-85-6; (+)-2-amino-5,6-dimethoxytetralin, 94903-49-8; (-)-2-amino-5,6-dimethoxytetralin, 94903-50-1.

Synthesis and Interaction of 5-(Substituted-phenyl)-3-methyl-6,7-dihydropyrazolo[4,3-*e*][1,4]diazepin-8(7*H*)-ones with Benzodiazepine Receptors in Rat Cerebral Cortex

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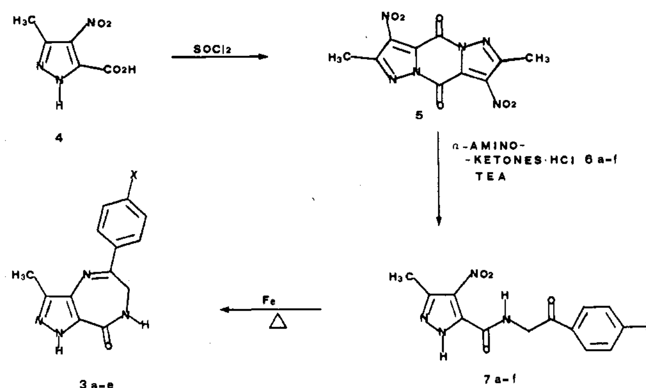
On the basis of the anxiolytic property of ripazepam, 1-ethyl-4,6-dihydro-3-methyl-8-phenylpyrazolo[4,3-*e*][1,4]-diazepin-5(1*H*)-one (1), a series of isomeric 5-(phenyl-substituted)pyrazolo[4,3-*e*][1,4]diazepin-8-ones **3a-f** were prepared and tested for their ability to bind to the benzodiazepine receptor. All compounds **3a-f** display affinities for the benzodiazepine receptor in the μM range of concentration; in particular 5-phenyl-3-methyl-6,7-dihydropyrazolo-[4,3-*e*][1,4]diazepin-8(7*H*)-one (**3a**) is 2 orders of magnitude less potent in inhibiting [^{3}H]flunitrazepam binding than diazepam and displays an affinity for the benzodiazepine receptor practically comparable to that of its structural isomer, ripazepam, and to that of chlordiazepoxide.

Since the discovery of benzodiazepines (BZ) in 1960, extensive research efforts in the field led to the development of a variety of modified derivatives.[1] Since almost all BZs display a wide pharmacological spectrum, e.g., anxiolytic, sedative-hypnotic, anticonvulsive, and muscle relaxant properties, an important goal of these investigations is to identify analogues that possess a more selective action.[2] Much attention has been paid to the role of the replacement of the fused benzo ring by an heterocyclic ring, so 1,4-diazepines fused to thiophenes,[3a] imidazoles,[3b] pyrroles,[3c] isoxazoles,[3d] pyrazines,[3e] and pyrazoles[3f] have been reported. However, of all these systems, pyrazolodiazepines have proven to be the most important ones since two derivatives, ripazepam (1) and zometapine (2) are in an advanced phase of clinical investigation.[3f]

Taking these results into consideration, we decided to synthesize a new series of pyrazolo[4,3-*e*][1,4]diazepin-8-ones (**3a-f**) isomeric with respect to the ring system of

Scheme I



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