MOLECULAR BIOLOGICAL PROBLEMS OF THE CREATION OF DRUGS AND STUDY OF THE MECHANISM OF THEIR ACTION

SYNTHESIS AND PHARMACOLOGICAL PROPERTIES OF PYRAZIDOLE ENANTIOMERS

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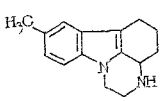
The antidepressant pyrazidole (I·HCl) has been employed in medical practice since 1975 [3, 4, 8]. This preparation belongs to the reversible group of type A MAO inhibitors. It was the first of this type of antidepressent to be used globally. The other foreign MAO inhibitor antidepressants such as oxazolidinone derivatives (cymoxathone and tolaxotone), ethylamine (amyphalmine and mecsiletine), benzamide (moclobemide), and others were placed into clinical practice only within the last decade [1].

Pyrazidole (Pirlindole) has been widely used for the treatment of various type of depression. It is also used for auxiliary therapy in a number of somatic illnesses [9]. Pyrazidole (I·HCl) is industrially produced in the form of a racemic mixture of enantiomers. We have presently obtained pyrazidole enantiomers in an individual form: (−)-I was isolated from a racemic base by the fractional crystallization of I salts with (+)-camphor-10-sulfonic acid, and (+)-I was obtained by asymmetric synthesis from ketocarbazole (II).



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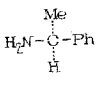
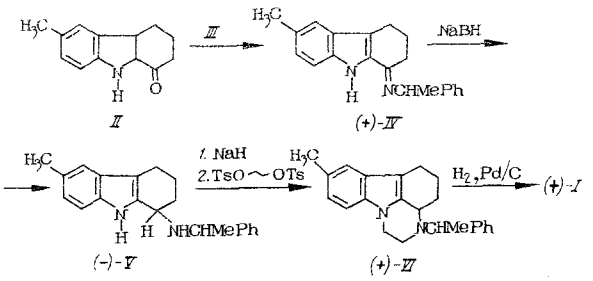


III

The nitrogen-bound carbon atom in position 3a is asymmetrical in molecule I. Frequent use is made of (−)-phenylethylamine (III) [6] as a source of amines to generate asymmetric a ms from ketones. We applied this approach in the following asymmetric synthesis of (+)-I:



The synthesis of racemic imine IV (mp 83°C) from ketone II and racemic 1-phenylethylamine has been described previously [7]. The reaction between ketone II and the optically active amine III results in the enantiomer (+)-IV (mp 113°C).

When reduced by sodium borohydride in ethanol the racemic imine IV yields racemic amine V, mp 95-97°C [7]. Under the same conditions the enantiomer (+)-IV yields the enantiomer (−)-V, mp 104-105°C. The diastereoisomer purity of the product was confirmed by MNR data: The (−)-V spectrum had one set of signals (Table 1). The chemical yield of optically pure (−)-V exceeded 80%. The high optical yield upon reduction allows us to presume that $NaBH_4$ initially yields a complex with molecule IV on the carbazole nitrogen. This is followed by intramolecular reduction which accounts for its stereospecificity.

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TABLE 1. Chemical Shifts in PMR Spectra: δ, ppm (J, Hz)

Compound	NCH-CH_3	N-$CHCH_3$	6-CH_3	N-CH_2COMe	
(+)-IV	1,54(6,5) d	4,88(6,5) q	2,43, s	—	—
(−)-V	1,40(6,5) d	4,09(6,5) q	2,43, s	—	—
(+)-VI	1,40(6,8) d	4,44(6,8) q	2,43, s	—	—
VIII	1,48(6,6) d	4,86(6,6) q	2,43, s	5,47 s	
IXa	1,40(6,4) d	4,05(6,4) q	2,43, s	5,26, d	4,95, d
IXb*	1,32(6,5) d	**	2,41, s	4,98, d	4,68, d

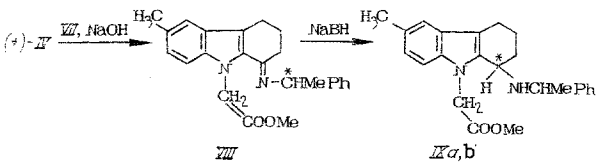
Notes. S) singlet; D) doublet; Q) quartet. Asterisk signifies signals identified from the spectrum of mixture IXa/IXb, 2:1. Two asterisks indicate overlapping signal.

The alkylation of the carbazole nitrogen excludes any possible complexing of tetrahydrocarbazoles with $NaBH_4$. According to our data the alkylated iminocarbazole VIII, even upon reduction in methanol, yields a mixture of diastereoisomers IXa, b. However, $NaBH_4$ in methanol is converted into a spatially inconvenient reagent NaHB $(OMe)_3$ which is particularly recommended for the stereoselective reduction of ketones [5]. This result confirms our hypothesis about the intermediate complexing of iminocarbazole IV with $NaBH_4$.

Cycliation with the formation of a pyrazine ring allowed us subsequently to react sodium hydride and ethylene glycol ditosylate with the aminocarbozoles V. This reaction can proceed in dimethylformamide and in dimethyl sulfoxide (DMSO). The yield of pyrazinocarbozole VI in both solvents is low, but the use of DMSO is more convenient because the end product is not dissolved in it so that it is more easily separated. Racemic pyrazinocarbazole VI (mp 193-195°C) was obtained from racemic amine V, and the enantiomer (+)-VI (mp 180-181°C) was synthesized from the enantiomer (−)-V. In both cases we obtained diastereomerically pure products, although the chemical yield did not exceed 30%.

The enantiomer (+)-VI was hydrogenolyzed in the presence of palladium in order to remove the phenylethyl group. This catalyst is known [10] to enable the retention of the nitrogen-bound carbon atom configuration. Consequently, we obtained enantiomer (+)-I. Obviously, this procedure could also be used to obtain its antipode (−)-I if one starts from ketone II and (+)-I-phenylethylamine. However, we were able to separate enantiomer (−)-I by splitting racemic I (see experimental part). The enantiomers were converted to the hydrochlorides for the biological tests so that they could be correctly compared to pyrazidole.

We also attempted an earlier proposed procedure [2] for obtaining pyrazinocarbazoles to accomplish an asymmetric synthesis, i.e., we alkylated enantiomer (+)-IV with methyl bromoacetate (VII) under interphase catalysis conditions. The resultant enantiomer VIII was reduced by sodium borohydride in methanol. Reduction results in the formation of a mixture of diastereoisomers IXa, b from which the individual diastereoisomer IXa was separated by fractional crystallization. The chemical yield of IXa was 16% as calculated per (+)-IV. In view of this time-consuming method for obtaining IXa, we did not pursue this approach for pyrazidole enantiomers.



VIII IXa,b

In analyzing the PMR data (Table 1) one should note that one can detect the formation of the mixture of diasteroisomers IXa, even by doubling the signal of the 6-CH_3 group removed from the chirality centers. The difference in the chemical shifts in the diastereomers in the $NCCH_3$ group is 0.08 ppm. Even more sensitive are the signals of the CH_2CCO diastereotope protons in which the difference in chemical shifts between IXa and IXb is 0.27 ppm. The CH_2COO proton signals in the spectra of compounds IXa and IXb are seen in the form of 4 lines: 2 doublets distorted by AB-reciprocal action, ^2J = 17.7 Hz.

EXPERIMENTAL (CHEMICAL)

The PMR spectra were recorded on a Varian XL-200 spectrometer. Solvent was $CDCl_3$. TMS was the internal standard. Mass spectra was recorded on a Varian MAT-112 instrument with a direct lead of specimens to the ion source. Ionizing electron energy was 70 eV, ionization chamber temperature 180-200°C. Polarimetric measurements made on a Al-EPO photoelectron polarimeter (USSR). Element analysis data correspond to the empirical formulas.

(+)-6-Methyl-1-[(S)-1-phenylethylimino]-1,2,3,4-tetrahydrocarbazole ((+)-IV). A mixture of 200 ml of xylene, 100 g (0.50 mol) of ketocarbazole II, 76 g (0.62 mol) of amine III, 2.2 g (0.01 mol) of n-toluosulfonic dihydrate was boiled for 5 h until all the water evolved. The mass was cooled, and the TsOH·III salt was filtered. The filtrate was vacuum-evaporated by distilling off 130-150 ml of xylene. The residue was kept at room temperature for 20 h. The separated imine (+)-IV was filtered and then washed on a filter with 100 ml of hexane. The yield was 85.2 g of imine, mp 111-112°C. The mixture of the filtrate and hexane was kept 24 h in a refrigerator and 17.5 more g of imine (+)-IV was filtered off, mp 112-114°C. The total yield was 102.7 g (68%). $C_{21}H_{22}N_2$. Mp 113-115°C (from heptane). $[\alpha]_D^{20}$ + 177°C (Benzene, c = 1%). Mass spectrum, m/z (relative intensity, %): 302 (100) M^+, 287 (60) $[M - Me]^+$, 198 (55), $[M - CMePh]^+$, 197 (30) $[M - CHMePh]^+$.

(−)-6-Methyl-1-[(S)-1-phenylethylamino]-1,2,3,4-tetrahydrocarbazole ((−)-V). At 30°C 30.2 g (0.1 mole) of imine (+)-IV was dissolved in 0.5 liter of abs. ethanol. The solution was cooled to 18°C. A 3.8 g (0.1 mol) portion of $NABH_4$ was introduced into the solution for a period of 15 min, and the temperature was kept within the range of 18-23°C by external cooling. The resultant suspension was stirred for 4 h at 20°C, then diluted with 400 ml of water and filtered. The moist precipitate was crystallized without drying from methanol. The yield was 25 g (82%) of aminocarbazole (−)-V. $C_{221}H_{24}N_2$. Mp 104-105°C, $[\alpha]_D^{20}$ − 66°C (ethanol, c = 1%).

(+)-8-Methyl-3-[(S)-1-phenylethyl]-2,3,3a,4,5,6,-hexahydro-1N-pyrazino[3,2,1-jk]carbazole ((+)-VI). A 12.1 g (40 mmol) mix of aminocarbazole (−)-V was dissolved in 100 ml of dry DMSO at room temperature and 1.14 g (47 mmol) of NaH was introduced in a single operation. The mixture was then stirred for 2 h after which 15 g (40.5 mmol) of ethylene glycol ditosilate [11] was added. The mass was stirred for 2.5 h during which the product precipitated out. It was filtered, washed on a filter successively with acetone, water, and acetone, then dried in a dessicator to yield 4 g of (+)-VI, mp 177-180°C.

The product from two parallel experiments (8 g) was combined and crystallized from 80 ml of methylethyl ketone. The yield was 6.3 g (23.8%) of pyrazinocarbazole (+)-VI. $C_{23}H_{26}N_2$. Mp 180-181°C. $[\alpha]_D^{20}$ + 63° (chloroform, c = 1%). Mass spectrum, m/z (%): 330 (40) M^+, 302 (70) $[M - C_2H_4]^+$, 225 (20)] $[M - C_2H_4 - CHMePh]^+$.

Racemic pyrazinocarbazole VI was obtained in a similar manner from racemic aminocarbazole V. Yield was 30%, mp 193-195°C (from methylethylketone). $C_{23}H_{26}N_2$.

(+)-8-Methyl-2,3,3a,4,5,6-hexahydro-1N-pyrazino[3,2,-1-jk]carbazole ((+)-I). An excess of methanol HCl was added to a suspension of 3.4 g (10.3 mmol) of enantiomer (+)-VI in 40 ml of methanol. The resultant solution was vacuum evaporated to dryness. The residue was dissolved in 50 ml of methanol and transferred to an autoclave containing 30 ml of methanol and 0.52 g of palladium on charcoal (Pd content 0.1 g). The mixture was hydrogenated for 17 h at 22°C. Hydrogen pressure was 1.8-2.0 MPa. The autoclave was then opened. The mass was filtered and the precipitate was thoroughly washed on a filter with warm ethanol. The combined filtrate was vacuum-evaporated and the residue was dissolved in 160 ml of benzene and treated with an ammonia solution. The benzene extract was dried with $MgSO_4$ and evaporated. The residue was crystallized from 7 ml of benzene. The yield was 1.0 g (42%) of the enantiomer (+)-I. $C_{15}H_{18}N_2$. Mp 121.5-122.5°C. $[\alpha]_D^{20}$ + 48° (chloroform, c = 0.5%).

A 1.5 ml of conc. HCl was added to a solution of 2.3 g of (+)-I in 30 ml of ethanol to ethanol to yield (+)-I·HCl. $C_{15}H_{18}N_2$·HCl. Decomposition temperature 247-249°C, $[\alpha]_D^{20}$ + 102° (methanol, c = 0.5%).

(−)-8-Methyl-2,3, 3a,4,5,6-hexahydro-1N-pyrazino[3,2,1-jk]carbazole ((−)-I). A mixture of 11.3 g (50 mmol) of the base (±)-I and 6.25 (25 mmol) of (+)-camphor-10-sulfonic monohydrate was dissolved by heating in 200 ml of methanol. The resultant solution was cooled for 3 h (initially at room temperature, and then in a refrigerator) to 10°C and a seeder of camphor sulfonate (−)-I was introduced. On the next day the flask was transferred to another refrig-

erator and was kept there for another 24 h at −5°C. After the crystallization was completed the solution was decanted. The crystals were suspended in 30 ml of ethanol and filtered off. The yield was 2.96 g, $[\alpha]_D^{20}$ − 37.5°C. After recrystallization from 30 ml of methanol the yield was 2.3 g (20%) of camphorsulfonate (−)-I. $[\alpha]_D^{20}$ − 41.1° (chloroform, c = 1%).

A 20 ml portion of a conc. ammonia solution was added to a suspension of 7.95 g (17.3 mmol) of camphor sulfonate (−)-I in 80 ml of benzene. This was stirred until the precipitate was dissolved. The benzene solution was separated, washed with water, and dried, and the benzene was vacuum-distilled. The residue was crystallized from a mixture of 5 ml of benzene and 10 ml of ether. Yield was 3.2 g (81%) of (−)-I. Mp 121-123°C, $[\alpha]_D^{20}$ − 48° (chloroform, c = 1%).

A hot solution of 1.1 g (4.9 mmol) of the base (−)-I in 30 ml of ethanol was acidified with 5% HCl to pH 2-3 and the resultant precipitate was filtered off. Yield was 0.94 g (73%) of (−)-I·HCl. $C_{15}H_{18}N_2 \cdot HCl$. $[\alpha]_D^{20}$ − 105° (methanol, c = 0.5%).

(+)-9-Carbomethoxymethyl-6-methyl-1-[(S)-1-phenylethylamino)]-1,2,3,4-tetrahydrocarbazole (VIII). Solutions of 15.1 g (50 mmol) of imine (+)-IB in 100 ml of benzene and 20 g of NaOH in 20 ml of water are mixed to which 2.7 g of tetrabutyl ammonium bromide are added. After 10 min of intensive stirring a solution of 14.7 g (8 ml, 96 mmol) of the ester VII in 40 ml of benzene was added. The temperature of the reaction mass was kept between 4-10°C. Then the mass was stirred for 2.5 h at room temperature and cooled again. A solution of 23 ml of acetic acid in 50 ml of benzene was then gradually added at a temperature of 10-15°C. A 25 ml portion of water was then added. The aqueous layer was then separated. The benzene layer was washed with water, dried with $MgSO_4$ and evaporated. The residue was crystallized from hexane, and then from methanol. Yield was 9.3 g (50%) of iminocarbazole VIII. $C_{24}H_{26}N_2O_2$. $[\alpha]_D^{20}$ + 136° (chloroform, c = 1%). M^+ 374.

(+)-9-Carbomethoxymethyl-6-methyl-1-[(S)-1-phenylethylamino]-1,2,3,4-tetrahydrocarbazole (IXa). A 14.8 g (39.5 mmol) portion of iminocarbazole VIII was dissolved in 600 ml of boiling methanol. The solution was then cooled to 20°C. A 2.6 g (68 mmol) of $NaBH_4$ was added at this temperature over a period of one half hour. The reaction mixture was cooled and 3.9 ml (68 mmol) of glacial acetic acid was added at +5°C. The methanol was evaporated and the residue was treated with 300 ml of benzene and water. The benzene extract was evaporated and the residue was crystallized three times from methanol. Each time the readily soluble needle-like crystals were separated from the less soluble particles. Yield was 4.7 g (31.5%) of the individual diastereoisomer IXa (Table 1). $C_{24}H_{28}N_2O_2$. Needle-like crystals, mp 75.5-77.5°C. $[\alpha]_D^{20}$ + 75° (acetonitrile, c = 1.2%). Mass spectrum, m/z (%): 376 (15) M^+, 256 (40) $[M\text{-}NHCHMePh]^+$, 255 (100) $[M\text{-}NH_2CHMePh]^+$, 196 (30) $[M\text{-}NH_2CHMePh\text{—}COOMe]^+$.

The fraction of the poorly soluble crystals were crystallized from methanol. Yield was 1.3 g of the precipitate, mp 91-95°C, $[\alpha]_D^{20}$ + 64° (acetonitrile, c = 1.2%). According to the PMR data the precipitate constitutes a 1:1 mixture of diastereoisomers IXa and b.

EXPERIMENTAL (PHARMACOLOGICAL)

The pharmacological examination of the enantiomers comprised a testing of antidepressant activity that is characteristic of pyrazidole. We examined: 1) the activating effect of the compounds on mice active avoidance of water in the behavioral emotional-stress swimming test; 2) antireserpine activity (by the reduction of blepharitis in mice induced by reserpine − 2.5 mg/kg ip); 3) the ability to potentiate the effects of L-DOPA (L-DOPA 200 mg/kg ip, hyperthermia) and 5-oxytryptophan (5-OTP, 50 mg/kg ip, head shake), 4) cholinolytic action by the effect on the hypothermic effect of tremorin and its induction of tremor.

The experiments were conducted on white mice of both sexes, weighing 18-22 g. The test compounds were administered orally 1 h before the behavioral test or 1 h before the administration of reserpine, L-DOPA, 5-OTP or tremorin. The tests in these experiments were conducted at times which were maximally effective for neurochemical analyzers, i.e., 4 h after the administration of reserpine, 0.5 h after the administration of L-DOPA or 5-OPT, and 1 h after the administration of tremorin. The acute-toxicity of the compounds was also assayed in white mice via oral administration.

We obtained the following results: 1) the enantiomers of pyrazidole were found to be similar to each other and to pyrazidole with respect to activating effects in the behavioral swimming test. At a dose of 25 mg/kg the (+), (−)-enantiomers and racemic, I·HCl increased the active attempts by mice to get out of the water with 41 ± 0.8 in the control to 55 ± 1.2, 51 ± 0.8,

and 55 ± 1.8 respectively. 2) The (+) enantiomer was found to have a more pronounced antireserpine effect than its antipode. Thus, reserpine blepharoptosis in the control mice was 3.7 ± 0.19 (n = 30) whereas in the mice preliminarily given (+)-I·HCl, (−)-I·HCl and pyrazidole, that figure was 1.7 ± 0.14 (n = 30), 3.0 ± 0.18 (n = 30), and 2.4 ± 0.14 (n = 30) respectively. 3) With respect to L-DOPA and 5-OTP potentiation both enantiomers turned out to have similar effects: At doses of 5 and 10 mg/kg they induced an identical increasing hyperthermic effect of L-DOPA. An increase of 1.5-1.7°C at a dose of 5 mg/kg and an increase of 2.6-3.0°C at a dose of 10 mg/kg. Pyrazidole (racemate) at doses of 5 and 10 mg/kg increased hyperthermia by 1.6 and 3°C respectively. At a dose of 25 mg/kg (+)- and (−)-enantiomers potentiated the tremor action of 5-OTP to the same degree as did pyrazidole: Head trembling was noted in 60-70% of the animals. 4)The enantiomers of pyrazidole, like the racemate, did not exhibit any cholinolytic activity.

(+)-I·HCl turned out to be less toxic than the (−)-enantiomer and pyrazidole (racemate): The LD_{50} upon oral administration to white mice was 500, 370, and 450 mg/kg respectively.

Thus, we did not observe any significant differences in the activity of pyrazidole enantiomers. The differences in toxicity were also insignificant. In that connection the continued manufacture of pyrazidole without separating into its enantiomers would seem to be advisable.

LITERATURE CITED

1. N. I. Andreeva, V. Z. Gorkin, and M. D. Mashkovskii, Khim.-farm. Zh., No. 6, 650-657 (1985).
2. A. I. Bokanov, P. Yu. Ivanov, t. V. Akalaeva, et al., Khim. Geterotsikl. Soedin., No. 12, 1632-1635 (1987).
3. M. D. Mashkovskii, A. N. Grinev, N. I. Andreeva, et al., Khim.-farm. Zh., No. 3, 60-63 (1974).
4. M. D. Mashkovskii, A. N. Grinev, V. I. Shvedov, et al., USSR Patent No. 427713, RZhKhim., No. 18[0]310 (1975).
5. Organicum [in Russian], Moscow (1979), 2, p. 192.
6. V. M. Potapov, Modern Asymmetrical Synthesis [in Russian], Itogi Nauk. Tekh. Org. Khim., Moscow (1984), 4, p. 45.
7. L. N. Filitis, T. V. Akalaeva, O. Yu. Amel'kin, et al., Khim.-farm. Zh., No. 10, 1217-1222 (1988).
8. V. I. Shvedov, L. B. Altukhova, N. I. Andreeva, et al., Khim.-farm. Zh., No. 10, 14-17 (1972).
9. M. I. Shubina and V. P. Zaitsev, Klin. Med., No. 1, 42-45 (1988).
10. G. Knupp and A. W. Frahm, Chem. Ber., 117, No. 6, 2076-2098 (1984).
11. G. H. Searle and R. J. Geue, Aust. J. Chem., 37, No. 5, 959-970 (1984).