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SYNTHESIS AND PHARMACOLOGICAL EXAMINATION OF HYDROGENATED 8-PHENYL-1H-PYRAZINO-[3,2,1-jk]CARBAZOLES

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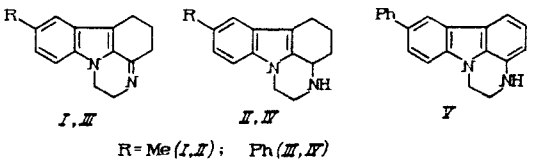
Some pyrazinocarbazoles have been found to possess antidepressant activity. For example, the hydrochloride of (I) has been patented as an antidepressant [3], and its dihydro-derivative (II·HCl) is already in therapeutic use (as pyrazidol [2, 4]).



In the search for active pyrazinocarbazoles, a number of compounds have been examined (R = Me, F, Cl, Br, MeO [3]), but no compounds with aromatic substituents (R = Ph) have yet been reported, although a knowledge of their pharmacological properties would be useful in establishing relationships between the biological activity and the structure of pyrazinocarbazoles.

The aim of the present investigation was to obtain the compound (IV) and its dehydro-derivatives (III and V), and to compare the pharmacological activity of these compounds (III-V) with that of pyrazidol.

The pyrazinocarbazole (IV) was synthesized as follows. Reaction of the diazobiphenyl (VI) with the formylcyclohexanone salt (VII) afforded the cyclohexanedione biphenylylhydrazone (VIII), which was then converted into the ketocarbazole (IX) by the Fischer method. The overall yield of the ketone (IX) (calculated on 4-aminobiphenyl) was 70%. Using a method similar to that used for pyrazidol [1], the ketocarbazole (IX) was converted via the imino-carbazoles (X) and (XI) into the required compound (IV). The overall yield of the pyrazino-carbazole (IV) was 44%, calculated on the ketone (IX).

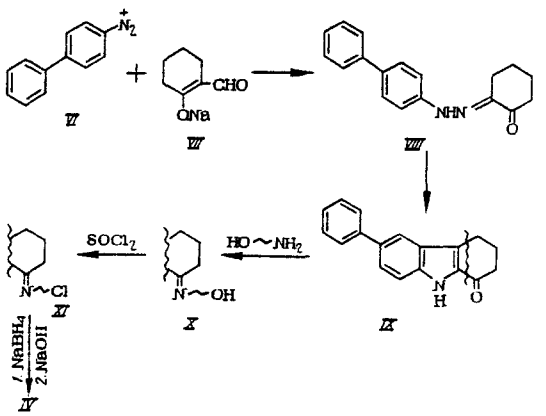


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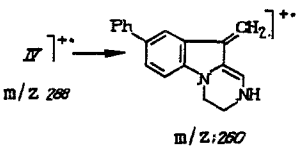
TABLE 1. Properties of (III-V) and (VIII-XI)

Compound	Yield, %	mp, °C (solvent)	Empirical formula
III	47	215—216 (ethanol)	$C_{20}H_{18}N_2$
III·$MeSO_3H$	67	213—216 (MeCN)	$C_{21}H_{22}N_2O_3S$
IV	69	140—142 (MeCN)	$C_{20}H_{20}N_2$
IV·HCl	95	240—242 (aq. ethanol)	$C_{20}H_{20}N_2$·HCl
V·HCl	61	210 (decomp.)	$C_{20}H_{16}N_2$·HCl
VIII	89	186—187 (2-propanol)	$C_{18}H_{18}N_{20}$
IX	78	230—233 (ethanol)	$C_{18}H_{15}NO$
X	96	172—175 (ethanol)	$C_{20}H_{20}N_2O$
XI	67	225 (decomp.) (ethanol)	$C_{20}H_{19}ClN_2$·HCl

On boiling in xylene with a palladium catalyst, (IV) was converted into the dihydropyrazinocarbazole (V), while oxidation with mercury oxide gave the tetrahydropyrazinocarbazole (III). Compound (V) was unstable as the free base (being readily oxidized), so that it was characterized as its hydrochloride only. The base (III), in contrast, was purified and characterized, but it was not possible to obtain a satisfactory elemental analysis for its hydrochloride. For the biological tests, (III) was converted into its methanesulfonate, and (IV) and (V) into their hydrochlorides. The salt (III·$MeSO_3H$) was soluble in water, unlike (IV·HCl) and (V·HCl), which were virtually insoluble in water.

According to mass spectrometry, the fragmentation of (IV) under electron impact is markedly different from that of (III) and (V). The mass spectrum of (IV) consists almost entirely of three peaks, with m/z (intensity, %) of 288 (50) $[M]^{+\cdot}$, 287 (23) $[M-H]^+$, and 260 (100) $[M-C_2H_4]^{+\cdot}$. The ion with m/z 260, which has the greatest intensity, is formed by retrodiene synthesis with elimination of a molecule of ethylene.



The molecules of (III) and (V) are stable to electron impact, the molecular ions having the greatest intensity, and breakdown of the molecule with elimination of ethylene being suppressed. The most intense ions were, for (III); 286 (100) and 258 (12), and for (V); 284 (100), 269 (13), and 142 (20). The intensities of the other peaks did not exceed 10%.

EXPERIMENTAL (CHEMISTRY)

Electron impact mass spectra were obtained on a Varian MAT-112 (West Germany), ionizing electron energy 70 eV, ionization chamber temperature 180°C, with direct introduction of the sample into the ion source. The elemental analyses agreed with the empirical formulae given in Table 1.

Cyclohexane-1,2-dione p-Vinylphenylhydrazone (VIII). A suspension of 16.92 g (0.10 mole) of 4-aminobiphenyl in 150 ml of water was heated until the aminobiphenyl melted (50-55°C), and to the resulting emulsion was added 25.7 ml of 36% HCl. The resulting suspension was cooled to −5°C, and a solution of 7.6 g (0.11 mole) of sodium nitrite in 25 ml of water added gradually. The resulting solution of the diazo-compound (VI) was stirred for one hour at 5°C.

At the same time, the salt (VII) was prepared from 19.6 g (0.2 mole) of cyclohexanone, 14.8 g (0.2 mole) of ethyl formate, and 0.22 mole (of sodium methoxide? — omitted from original (translator)), dissolved in 40 ml of methanol, as described in [2]. The salt (VII), which solidified, was dissolved in 35 ml of water before use.

A few crystals of the hydrazone (VIII) were added to the solution of (VI) in order to facilitate crystallization of the product, and the solution of salt (VII) added slowly over 0.7-0.8 h to the cooled solution of salt (VII), keeping the temperature of the solution below 5°C, following which the mixture was stirred for a further hour at 5°C. The product was filtered off, and washed on the filter with warm water followed by 80 ml of methanol to 24.95 g of the hydrazone (VIII).

6-Phenyl-1-keto-1,2,3,4-tetrahydrocarbazole (IX). A suspension of 70.1 g (0.25 mole) of the hydrazone (VIII) in a mixture of 165 ml of 85% formic acid and 50 ml of DMF was heated slowly. At 85°C, an exothermic reaction set in, following which the mixture was boiled for a further half hour at 114-116°C, cooled to 0°C, and kept at this temperature for 1.5 h. The product was filtered off, and washed with water and 150 ml of methanol to give 51.5 g of the ketone (IX).

1-(2-Hydroxyethylimino)-6-phenyl-1,2,3,4-tetrahydrocarbazole (X). A mixture of 13.07 g (50 mmole) of the ketone (IX), 6.1 g (0.1 mole) of ethanolamine, and 25 ml of xylene was boiled for 4 h in a flask fitted with a Dean and Stark apparatus. The mixture was cooled, and the product filtered off and washed with 35 ml of methanol to give 14.7 g of the imine (X).

1-(2-Chloroethylimino)-6-phenyl-1,2,3,4-tetrahydrocarbazole Hydrochloride (XI). To a suspension of 30.44 g (0.10 mole) of the finely ground imine (X) in a mixture of 150 ml of benzene and 75 ml of DMF was added 9 ml (0.12 mole) of thionyl chloride at such a rate that the temperature did not rise above 30°C. The mixture was then slowly heated, and the resulting solution kept at 70-76°C for one hour, then cooled to 0°C. The product was filtered off and washed with 35 ml of benzene to give 24 g of the salt (XI).

8-Phenyl-2,3,3a,4,5,6-hexahydro-1H-pyrazino[3,2,1-jk]carbazole (IV). To a suspension of 24 g (66.8 mmole) of the salt (XI) in 120 ml of absolute ethanol was added gradually at 0-5°C 2.53 g (66.8 mmole) of $NaBH_4$. The mixture was stirred for 1 h at 0°C, and dilute HCl (12.5 ml of conc. Hcl in 12.5 ml of water) added. The solid which separated was filtered off, and washed successively with 50 ml of ethanol and 80 ml of ether.

The reduction product (26.6 g) was suspended in 160 ml of benzene, and 2.38 g (7.3 mmole) of tetrabutylammonium bromide and 50% NaOH (from 37 g of NaOH) in water added. The mixture was stirred at room temperature under nitrogen for 5 h. After this time, the mixture was treated with 80 ml of benzene and 80 ml of water, and the benzene layer separated, washed with water, and evaporated. The residue was crystallized from acetonitrile to give 13.3 g (69%) of pyrazinoacarbazole (IV), mp 139-142°C. A further recrystallization from acetonitrile gave 10.9g material mp 140-142°C.

(IV). Hydrochloride. To a solution of 10.9 g of the free base (IV) in 100 ml of absolute ethanol was added with cooling 25 ml of ethanolic HCl, the mixture kept for 1 h at 0-5°C, and the (IV)·HCl filtered off. Yield 11.7 g, $M^{+\cdot}$ 288.

8-Phenyl-2,4,5,6-tetrahydro-1H-pyrazino[3,2,1-jk]carbazole (III). The base (IV) (5.76 g, 20 mmole) was dissolved in a mixture of 40 ml of glacial acetic acid and 40 ml of water, and to the resulting solution was added 8.89 g (41 mmole) of mercuric oxide. The mixture was boiled for 0.5 h, cooled to room temperature, diluted with 80 ml of absolute ethanol, and the mercurous acetate filtered off. The filtrate was evaporated, the residue dissolved in 100 ml of water, and the solution basifed with aqueous ammonia. The solid which separated was extracted with benzene, and the extract dried over $MgSO_4$ and evaporated. The dry residue (4.2 g) was crystallized from ethanol to give 2.7 g of (III), $M^{+\cdot}$ 286.

(III) Methanesulfonate. A solution of 2.7 g of the base (III) in 60 ml of 2-propanol was acidified with a solution of methanesulfonic acid in 2-propanol. The precipitated solid was filtered off and washed successively with 2-propanol and ether to give 2.44 g (67%) of III·$MeSO_3H$, mp 209-214°C. Recrystallization from 7 ml of acetonitrile gave material mp 213-216°C.

8-Phenyl-2,3-dihydro-1H-pyrazino[3,2,1-jk]carbazole Hydrochloride (V·HCl). In 30 ml of ethanol was suspended 3.5 g of 20% palladium hydroxide on charcoal [6], and hydrogenation carried out at room temperature until uptake of hydrogen ceased. The catalyst obtained was filtered off, washed with xylene, and added to a solution of 5 g (173 mmole) of (IV) in 95 ml of xylene. The mixture was boiled for one hour under nitrogen, then the catalyst was filtered off and the xylene evaporated. The dry residue was dissolved in 15 ml of chloro-

form, and the solution passed through a layer of silica gel (40 × 100 μm) and eluted with chloroform. The eluate was evaporated, the residue dissolved in 750 ml of dry ether, the solution filtered, and the filtrate acidified with alcoholic HCl to give 3.4 g of (V·HCl), $M^{+\cdot}$ 284.

EXPERIMENTAL (PHARMACOLOGY)

Bearing in mind the structural similarity of (IV) and its dehydro-derivatives (III) and (V) to pyrazidol, we carried out the pharmacological examination of these compounds in respect of antidepressant activity as for pyrazidol [2].

Using mice as the test subjects, the effects of the compounds on 'escape from water' behavior in an emotional-stressor behavioral test [5], the depressant effects of reserpine (blepharoptosis), the hypothermic activity of tremorine, and the excitatory effects of 5-hydroxytryptophan (5-HTP) and L-dopa were examined. The tests were carried out on mice of both sexes weighing 18-20 g.

The acute toxicities of the compounds by the oral route were also determined in white mice.

The tests results showed that (IV) and (III) were more pharmacologically active than (V). Antagonism of reserpine and enhancement of the effects of L-dopa and 5-HTP with (IV) and (III) were seen at doses of 1, 2.5, and 5 mg/kg, but in the case of (V) only at doses of 10 and 25 mg/kg. For example, in doses of 2.5 mg/kg (IV) and (III), given internally, reduced the blepharoptosis induced by reserpine (2.5 mg/kg i/p) by 40-60%, whereas (V) reduced the effect of reserpine by 30% only in a dose of 25 mg/kg. The activating effects of (III), (IV), and (V) in the 'escape from water' behavioral test were more similar. In doses of 10 and 25 mg/kg, they reduced by 30-60% the number of active attempts by the animals to get out of the water.

In their spectrum of activity, the test compounds resembled pyrazidol. Compounds (IV) and (III) were more active than pyrazidol, since their antireserpine and 5-HTP- and L-dopa-potentiating effects were apparent at lower doses than with pyrazidol [2].

The acute toxicities of (IV) and (V) were lower than that of pyrazidol, but that of (III) was much higher. The LD_{50} values in white mice were (mg/kg): for (V) 750, (IV) 550, pyrazidol 450, and (III) 180.

Comparison of the pharmacological properties of pyrazidol with those of (II·HCl) and (IV·HCl) showed that the introduction of a phenyl substituent into the 8-position of the heterocycle in place of methyl increased the activity of the compound in respect of several indicators of antidepressant activity, while slighly reducing the toxicity. Dehydrogenation of the pyrazine moiety of the molecule (III·$MeSO_3H$) did not reduce activity, but it did increase toxicity substantially.

Aromatization of the carbazole moiety (V·HCl) decreased both activity and toxicity as compared with (IV·HCl). It should be stressed that this structural change reduced the favorable effects of the aromatic substituent in the 8-position, so that the activity of (V·HCl) was no greater than that of pyrazidol.

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