SYNTHESIS AND ANTITUBERCULAR ACTIVITY *IN VITRO* OF TETRAHYDROCARBAZOLE DERIVATIVES. I. N-SUBSTITUTED HEXAHYDRO-1H-PYRAZINO [3,2,1-j,k] CARBAZOLES

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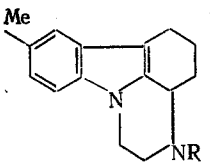
8-Methyl-2,3,3a,4,5,6-hexahydro-1H-pyrazino[3,2,1-j,k]carbazole hydrochloride (Ia), known in the medical practice under the name "pyrazidol" is an effective antidepressant [2, 4]. We found in *in vitro* experiments that pyrazidol inhibits the growth of tuberculosis mycobacteria (*M. tuberculosis* of the human type $H_{37}R_V$). We studied a series of N-substituted pyrazinocarbazoles (II-XVII) to discover possible new antitubercular agents. Compounds with pronounced basic properties were studied in the form of their hydrochlorides (VIIIa-XVIIIa).*



I—XIX

I: R = H; II: R = CHO; III: R = $CO(CH_2)_2COONa$;
IV: R = $COCH=CHCOONa$; V: R = COPh; VI: R = CSNHPh;
VII: R = $SO_2C_6H_4NHCOOMe$ p; VIII: R = CH_2Ac; IX and
X: R = $CH_2CH(OH)CH_2Ph$; XI: R = $CH_2CH(OH)Me$;
XII: R = $CH_2CH(OH)CH_2NH_2$;
XIII: R = $CH_2CH(OH)CH_2NHPr$-iso;
XIV: R = $CH_2CH(OH)CH_2NHCH_2Ph$;
XV: R = $CH_2CH(OH)CH_2NH(CH_2)_3NMe_2$;
XVI: R = $CH_2CH(OH)CH_2N(CH_2)_4$;
XVII: R = $CH_2CH(OH)CH_2N(CH_2CH_2)_2NMe$;

XVIII: R = $CH_2CH(OH)CH_2N(CH_2)_6$; XIX: R = $CH_2\overline{CHCH_2}O$.

The acylation of the pyrazidol base I with formic acid, succinic anhydride, maleic anhydride, benzoyl chloride, phenyl isothiocyanate, and N-carbomethoxysulfanil chloride gives the corresponding derivatives II-VII. The synthesis of the benzoyl compound V has already been described in [3].

Alkylation of pyrazidol base I with bromoacetone, benzyloxirane or epichlorohydrin gives compounds VIII, IX. X and XIX. The melting point of the epoxy compound XIX (85-86°C) differs considerably from that given in [1], 180-182°C; clearly, a misprint occurred in [1]. Amino alcohols XII-XVIII were obtained by the reaction of epoxide XIX with ammonia and amines; reduction of ketone VIII gave alcohol XI.

There are two asymmetric carbon atoms in the molecules of compounds IX-XIX. As the result of this structural feature, these compounds form a mixture of diastereomers. In one case (compounds IX and X), the diastereomers were separated by crystallization. Since it was found that hydrochloride IX and Xa do not differ in their biological activity, we did not try to isolate the individual diastereomers of other compounds.

EXPERIMENTAL (CHEMICAL)

The mass spectra were run on the MAT-112 apparatus (Switzerland)†

3-Formyl-8-methyl-2,3,3a,4,5,6-hexahydro-1H-pyrazino[3,2,i-j,k]carbazole (II). A mixture of 11.45 g (44 mmoles) of Ia, 20 ml of water, 50 ml of dioxane, and 10 ml of Et_3N is heated to dissolution of the precipitate, then it is cooled, and 14 ml of 99% HCOOH are added. The

*In the text of the article, the bases are designated by Roman letters, and their hydrochlorides by the same letters with the suffix "a".

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TABLE 1. 3-R-8-Methyl-2,3,3a,4,5,6-hexahydro-1H-pyrazino [3, 2, 1-j,k]carbazoles II-X

Compound	Yield, %	mp (dec. temp.), °C	Found, %			Empirical formula	Calculated, %		
			C	H	N		C	H	N
II	62	156—7	75,43	7,24	11,08	$C_{16}H_{18}N_2O$	75,56	7,13	11,02
III	86	(110)	62,77	6,44	7,40	$C_{19}H_{21}N_2NaO_3 \cdot H_2O$	62,28	6,05	7,65
IV	85	(140)	62,90	5,70	7,46	$C_{19}H_{19}N_2NaO_3 \cdot H_2O$	62,63	5,81	7,69
V	90	137—8 [3]	...	...	...	$C_{22}H_{22}N_2O$	...	...	...
VI	100	136—8	73,10	6,30	11,32	$C_{22}H_{23}N_3S$	73,09	6,41	11,62
VII	27	213—5	63,02	5,92	9,46	$C_{23}H_{25}N_3O_4S$	62,85	5,73	9,56
VIII	66	110—2	76,56	8,02	9,82	$C_{18}H_{22}N_2O$	76,56	7,85	9,92
IX	21	146—8	79,61	7,64	7,93	$C_{24}H_{28}N_2O$	79,96	7,83	7,77
X	4	115—7	79,68	7,63	7,88	$C_{24}H_{28}N_2O$	79,96	7,83	7,77

TABLE 2. 3-R-8-Methyl-2,3,3a,4,5,6-hexahydro-1H-pyrazino [3, 2, 1-j,k] carbazole Hydrochlorides VIIIa-XVIIIa

Compound	mp (dec. temp), °C	Found, %				Empirical formula	Calculated, %			
		C	H	Cl	N		C	H	Cl	N
VIIIa	(190)	67,14	6,95	11,30	8,73	$C_{18}H_{23}N_2O \cdot HCl$	67,80	7,27	11,12	8,79
IXa	(185)	72,69	7,14	9,29	7,18	$C_{24}H_{29}N_2O \cdot HCl$	72,61	7,36	8,93	7,06
Xa	131—3	72,86	7,59	8,85	6,90	$C_{24}H_{29}N_2O \cdot HCl$	72,61	7,36	8,93	7,06
XIa	(200)	67,24	7,65	10,67	8,56	$C_{18}H_{24}N_2O \cdot HCl$	67,38	7,85	11,05	8,73
XIIa	(225)	58,03	7,44	19,18	11,48	$C_{18}H_{26}N_2O \cdot 2HCl$	58,06	7,31	19,04	11,28
XIIIa	(170)	58,69	8,16	16,42	9,81	$C_{21}H_{32}N_2O \cdot 2HCl \cdot H_2O$	58,33	8,15	16,40	9,72
XIVa	(205)	65,12	7,06	15,34	9,27	$C_{22}H_{31}N_2O \cdot 2HCl$	64,93	7,19	15,33	9,08
XVa	218—20	52,29	8,50	20,07	10,60	$C_{22}H_{34}N_2O \cdot 3HCl \cdot 2H_2O$	52,12	8,18	20,07	10,60
XVIa	208—10	59,76	7,96	15,91	9,86	$C_{22}H_{31}N_2O \cdot 2HCl \cdot H_2O$	59,45	7,94	15,96	9,45
XVIIa	258—60	55,46	7,89	20,84	11,18	$C_{22}H_{34}N_2O \cdot 3HCl \cdot 0,5H_2O$	55,14	7,64	21,22	11,18
XVIIIa	233—5	63,38	8,09	15,52	9,06	$C_{24}H_{32}N_2O \cdot 2HCl$	63,42	8,21	15,60	9,25

mixture is boiled for 15 h, then cooled to ~ 20°C, and the product is filtered. Yield, 9.19 g (83%). After recrystallization from ethanol, 6.93 g (62%) of II are obtained.

Sodium salt of 4-(8-methyl-2,3,3a,4,5,6-hexahydro-1H-pyrazino [3,2,1-j,k]carbazolyl-3)-4-oxobutyric Acid (III). A 2.43 g (24 mmole) portion of succinic anhydride is added to a solution of 5.5 g (24 mmoles) of I in 15 ml of $CHCl_3$, and the mixture is boiled for 30 min. The precipitate is filtered to yield 6.88 g (86%) of 4-(8-methyl-2,3,3a,4,5,6,-hexahydro-1H-pyrazino [3.2.1-j,k]carbazolyl-3)-4-oxobutyric acid, mp 180°C. Found, %: C 69.67; H 6.98; N 8.43. $C_{19}H_{22}N_2O_3$. Calculated, %: C 69.92; H 6.79 N 8.58. A 2.88 g (8.8 mmole) portion of the acid is dissolved in 88 ml of absolute ethanol and 4.75 ml of 2 N NaOH are added. The solution is heated to boiling and then evaporated in vacuo. The residue is dehydrated by distilling water in the form of an azeotrope with benzene,, ground with petroleum ether (b_p 40-70°C;, filtered, and dried. Yield, 2.8 (86%) of III.

In a similar way, 4-(8-methyl-2,3,3a,4,5,6-hexahydro-1H-pyrazino[3,2,1-j,k]carbazolyl-3)-4-oxo-2-butenoic acid, mp (198-200°C, is obtained by the reaction of I with maleic anhydride, Found, %: C 70.20; H 6.30; N 8.73. $C_{19}H_{20}N_2O_3$. Calculated, %: C 70.35; H 6.22; N 8.64. A sodium salt (IV) is obtained from this acid (Table 1).

3-(N-Phenylthiocarbamoyl)-8-methyl-2,3,3a,4,5,6-hexahydro-1H-pyrazino[3,2,1-j,k]carbazole (VI). A solution of 1.35 g (10 mmoles) of phenyl-isothiocyanate in 5 ml of dry benzene is added to a solution of 2.26 g (10 mmoles) of I in 15 ml of dry benzene and the mixture is boiled for 30 min with stirring. The fine-crystalline precipitate of VI is filtered; the yield is quantitative.

3-(N-Carbomethoxysulfanil)-8-methyl-2,3,3a,4,5,6-hexahydro-1H-pyrazino [3,2,1-j,k]carbazole (VII). A mixture of 4,52 g (20 mmoles) of I, 5.94 g (25 mmoles) of N-carbomethoxysulfanil chloride, 26 ml of pyridine, and 36 ml of dichloroethane is boiled with stirring for 1 h. Pyridine hydrochloride is filtered, and the filtrate is evaporated in vacuo, The residue is treated by 100 ml of water, filtered, and recrystallized from 250 ml of ethanol. Yield 2.39 g (27%) of VII.

3-(2-Oxopropyl)-8-methyl-2,3,3a,4,5,6-hexahydro-1H-pyrazino[3,2,1-j,k]carbazole (VIII). A solution of 42.8 g (0.3 mole) of bromoacetone in 70 ml of benzene is added at 5-10°C to a mixture of 68.5 g (0.3 mole) of I and 42.8 ml (0.3 mole) of Et_3N in 200 ml of dry benzene. The mixture is held for 12 h at ~ 20°C, washed with water (3 times 250 ml),, benzene is distilled in vacuo, and the residue is recrystallized from 180 ml of absolute ethanol to yield

TABLE 3. Minimal Inhibiting Concentrations (MIC) of Pyrazinocarbazoles in Experiments *in Vitro*

Compound	MIC, μg/ml		Compound	MIC ($H_{37}R_V$), μg/ml	Compound	MIC ($H_{37}R_V$), μg/ml
	$H_{37}R_V$	ATCC				
Ia	8	32	VII	500	XIIIa	16
II	8	64	VIIIa	64	XIVa	4
III	32	250	IXa	4	XVa	64
VI	16	125	Xa	4	XVIa	64
V	1000		XIa	64	XVIIa	16
VI	1000		XIIa	32	XVIIIa	4

57 g (66°C) of VIII. Mass spectrum m/z (rel. I): 280 (50), 254 (50), 239 (85), 235 (20), 225 (20), 224 (85), 211 (100).

A 6 ml portion of concentrated HCl is added to a solution of 14.1 g of VIII in 150 ml of absolute ethanol. The solution is evaporated in vacuo, absolute ethanol is added in two portions of 30 ml to the residue, and ethanol is distilled together with traces of water.

The solidified product is crystallized from 250 ml of absolute ethanol to yield 11.4 g of VIIIa (table 2).

3-(2-Hydroxy-3-phenylpropyl)-8-methyl-2,3,3a,4,5,6-hexahydro-1H-pyrazino[3,2,1-j,k] carbazoles (IX, X). A solution of 11.3 (50 mmoles) of I and 9.4 g (70 mmoles) of benzyloxirane [5] in 50 ml of ethanol is boiled for 90 min, then cooled, and the crystals that separated, are filtered. After two recrystallizations from acetone, 3.86 g (21.3%) of diaseteromer IX are obtained. Mass spectrum m/z (rel. D): 360 (38), 332 (100), 269 (8), 239 (73), 211 (21).

The ethanolic solution of the reaction mixture obtained after the separation of diastereomer IX, is evaporated to dryness, the residue is ground with 20 ml of ether, and filtered. After two recrystallizations from ether and one recrystallizations from hexane, 0.76 g (4.2%) of diastereomer X is obtained. Mass spectrum m/z (rel. I): 360 (40), 332 (100), 269 (5), 239 (24), 211 (29).

A 0.3 ml portion of concentrated HCl is added to a suspension of 1 g of base IX in 10 ml of ethanol. The mixture is heated to dissolution, cooled, and the hydrochloride that separates is filtered and crystallized from ethanol to yield 0.7 g of IXa. Compound Xa is obtained in a similar way from X.

3-(2-Hydroxypropyl)-8-methyl-2,3,3a,4,5,6-hexahydro-1H-pyrazino[3,2,1-j,k]carbazole Hydrochloride (XIa). A 14. 1 g (50 mmoles) portion of powdered VIII is added to a suspension of 1.1 g (29 mmoles) of $NaBH_4$ in 100 ml of isopropanol. The mixture is heated and VIII passes into the solution at 75°C. The mixture is boiled for 2.5 h, cooled to 3°C, introduced into dilute (1:3) HCl, and 40 ml of water and 25 ml of 15% NH_4OH are added to the solution obtained. After 12 h, 13.2 g (93%) of XI are filtered, mp. 120-140°C (mixture of diastereomers).

A solution of 7.7 g of base XI in 70 ml of absolute ethanol is treated with concentrated HCl to pH 1.0. Ethanol is distilled *in vacuo*, and the residue is suspended in 30 ml of dry ether, filtered, and crystallized from 90 ml of absolute ethanol. Yield 5.7 g of XIa.

3-(2,3-Epoxypropyl)-8-methyl-2,3,3a,4,5,6-hexahydro-1H-pyrazino[3,2,1-j,k]carbazole (XIX). A solution of 32 g (0.8 mole) of NaOH in 48 ml of water and 74 g (0.8 mole) of epichlorohydrin is added to a solution of 22.6 g (0.1 mole) of I in 270 ml of dioxane. The mixture is stirred for 90 min at 95-100°C. The jelly-like precipitate is filtered, the filtrate is transferred into a separatory funnel, diluted with 200 ml of water, and extracted by benzene (3 times 100 ml). The benzene extract is washed with water to neutral reaction, and the solvent is distilled in vacuo. The oily residue is dissolved in 50 ml of boiling absolute ethanol, and on cooling XIX crystallizes slowly. Yield 19.5 g (69%), mp 85-86°C. Mass spectrum, m/z (rel. I): 282 (42), 281 (13), 254 (100), 225 (5), 197 (27). Found, %: C 76.40; H 8.04; N 9.60. $C_{18}H_{22}N_2$. Calculated, %: C 76.56; H 7.85; N 9.92.

3-(3-Amino-2-hydroxypropyl)-8-methyl-2,3,3a,4,5,6-hexahydro-1H-pyrazino[3,2,1-j,k]carbazole Dihydrochloride (XIIa). Solutions of 10 g (35 mmoles; of XIX in 25 ml of dioxane and 32 g of ammonia in 250 ml of absolute ethanol are mixed together. The mixture is held for 3 days

at 20°C. The solvents are then removed in vacuo, and the residue is crystallized from 45 ml of benzene to yield 4.2 g of XII, mp 130-145°C (mixture of diastereomers): Hydrogen chloride gas is passed through a solution of 4.2 g of XII in ethanol, and the hydrochloride is filtered and crystallized from a mixture of 50 ml of dioxane and 12 ml of water. Yield, 1.15 g (9%) of XIIa.

3-(3-Isopropylamino-2-hydroxypropyl)-8-methyl-2,3,3a,4,5,6-hexahydro-1H-pyrazine[3,2,1-j,k] carbazole (XIIIa). A 6,1 g (103.6 mmole) portion of isopropylamine is added to a suspension of 9.7 g (34.5 mmoles) of XIX in 70 ml of absolute ethanol. The mixture is heated to boiling, and the solution obtained is boiled for 10 h. The alcohol is evaporated in vacuo and 40 ml of dry ether and then an ethereal solution of HCl gas are added to the oily residue. Compound XIIIa obtained is filtered, washed with dry ether, and crystallized from absolute ethanol. Yield, 6.9 g (46%) of XIIIa.

Compounds XIVa-XVIIIa are obtained in a similar way; compounds XIVa, XVIa are crystallized from absolute ethanol, XVa and XVIIa from aqueous ethanol, and XVIIIa from MeOH.

EXPERIMENTAL (BIOLOGICAL)

The antitubercular activity of pyrazinocarbazoles was determined by *in vitro* experiments by the standard dilution method, according to Soton. We used as the test cultures the human type mycobacteria ($H_{37}R_V$) and the saprophytic mycobacteria ATCC-607. The time of cultivation at 37°C was 14 and 6 days, respectively. The minimal inhibiting concentration (MIC), expressed in micrograms in 1 ml was established (Table 3).

As the result of the investigation we found that of the 18 pyrazinocarbazoles studied, 6 compounds (Ia, II, IXa, Xa, XIVa, and XVIIIa) have a medium activity *in vitro* against $H_{37}R_V$ (MIC = 4-8 μg/ml). The compounds are practically inactive against ATCC-607. Positive results were not obtained in the study of the therapeutic activity of compounds Ia, VIIIa, and XVIIIa on mice with experimental tuberculosis.

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