of liberation of the mediator from the sympathetic nerve extremities, and on α-adrenoreceptors. Hence, hydrochlorides VIa-j are interesting as anti-adrenergic compounds with a considerable blocking action on several adrenergic processes, simultaneously.

LITERATURE CITED

1. O. M. Avakyan, Biol. Zh. Armenii, 21, 8-17 (1968).
2. O. M. Avakyan and A. A. Kaltrikyan, Biol. Zh. Aremenii, 32, 142-149 (1979).
3. R. S. Balayan, M. G. Akopyan, O. M. Avakyan, et al., Arm. Khim. Zh., 36, 451-456 (1983).
4. R. S. Balayan, M. G. Akopyan, A. A. Kaltrikyan, and E. A. Markaryan, Arm. Khim. Zh., 36, 653-657 (1983).
5. M. L. Belen'kii, Elements of Quantitative Evaluation of Pharmacological Effect [in Russian], Riga (1959).
6. L. J. Ballamy, Infrared Spectra of Complex Molecules, Wiley (1958).
7. E. S. Markaryan and R. S. Balayan, Arm. Khim. Zh., 29, 704-707 (1976).
8. E. A. Markaryan and R. S. Balayan, Inventor's Certificate No. 572045 (USSR); Otkrytiya, No. 4 (1979).
9. E. A. Markaryan, R. S. Balayan, O. M. Avakyan, and A. A. Kaltrikyan, Inventor's Certificate No. 696009 (USSR); Otkrytiya, No. 41 (1979).
10. P. O. Manolov, Farmatsiya (Sofia), 21, No. 2, 60-64 (1971).
11. L. L. Iversen, The Uptake and Storage of Noradrenaline in Sympathetic Nerves, Cambridge (1967), pp. 147-198.

ANTIHYPOXIC ACTION OF DIOXINDOLE DERIVATIVES

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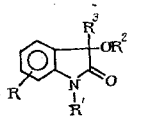
In a study of the antihypoxic activity of isatin and other compounds of the indolinone series [2, 5, 6], we and other authors have noted the presence of this activity under the condition that the 2-carbonyl group is retained. In particular, acetyloxindole, a compound in which the 3-keto group is completely reduced in comparison with isatin, exhibits pronounced protective action during hypobaric hypoxia [2]; this structural fragment is contained also in pyracetam, in which an antihypoxic activity has also been observed [4].

It was interesting to study the antihypoxic properties of derivatives of dioxindole, which occupies an intermediate position between compounds of the oxindole and isatin series.

We therefore synthesized 13 dioxindole derivatives of the general formula



I-XIII

I: $R = R^1 = R^2 = R^3 = H$; II: $R = Me$-5, $R^1 = R^1 = R^2 = R^3 = H$;
III: $R = Cl$-7, $R^1 = R^2 = R^3 = H$; IV: $R = R^2 = R^3 = H$,
$R^1 = Ac$; V: $R = R^1 = R^2 = H$, $R^3 = CH_2Ac$; VI: $R = Br$-5, $R^1 = R^2 = H$, $R^3 = CH_2Ac$;
VII: $R = Br$-5, $R^1 = R^2 = H$, $R^3 =$ pyridyl-2-methyl; VIII: $R = Br$-5,
$R^1 = R^3 = H$; $R^2 = Ac$; IX: $R = Cl$-7, $R^1 = R^3 = H$, $R^2 = Ac$; X: $R = R^3 = H$,
$R^1 = R^2 = COEt$; XI: $R = Me$-5, $R^1 = R^2 = COEt$, $R^3 = H$; XII: $R = Br$-5,
$R^1 = R^2 = COEt$, $R^3 = H$; XIII: $R = Br$-5, $R^1 = R^2 = COPr$, $R^3 = H$.

Dioxindoles containing substituents in the benzene ring and at the nitrogen atom only (II-IV), were obtained by reducing the corresponding isatins by sodium dithionite [8], and 3H-substituted compounds (V-VII) were obtained by condensing CH_2-active compounds with isatins [3].

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TABLE 1. PMR Spectra and Data of Elemental Analysis of Dioxindoles III, IV, VI-XIII

Compound	Chemical shifts, δ, ppm*				Found, %		Empirical formula	Calc., %	
	aliphatic protons	1-H	3 H	aromatic protons	N	Hal		N	Hal
III	—	10,54 s	6,18 d	6,7—7,3 m (3H)	7,41	18,90	$C_8H_6ClNO_2$	7,62	19,31
IV	2,48 s(3H,CH_3)	—	5,02 s	7,0—7,4 m (3H) 7,9—8,1 m (1H)	7,21	—	$C_{10}H_9NO_3$	7,32	—
VI	1,94 s(3H,CH_3) 2,7—3,4 m 3H,CH_2+OH)	10,24 s	—	6,66 d(1H) 7,2—7,4 m (2H)	4,96	28,00	$C_{11}H_{10}BrNO_3$	4,92	28,12
VII	2,9—3,4 m(3H,CH_2+OH)	10,20 s	—	6,2—7,6 m (6H) 8,22 d (1H)	9,07	25,39	$C_{14}H_{11}BrN_2O_2$	8,77	25,03
VIII	2,13 s (3H)	8,81 s	5,82 s	6,7—7,3 m (3H)	5,06	29,60	$C_{10}H_8BrNO_3$	5,18	29,59
IX	2,10 s(3H)	8,80 s	5,87 s	6,7—7,3 m (3H)	6,45	15,51	$C_{10}H_8ClNO_3$	6,21	15,71
X	1,04 t(6H) 2,30 q(2H) 2,87 q(2H)	—	5,77 s	6,9—7,3 m (3H) 8,08 d(1H)	5,09	—	$C_{14}H_{15}NO_4$	5,36	—
XI	1,10 t(6H) 2,0—2,5 m(5H) 2,88 q	—	5,76 s	6,9—7,1 m (2H) 7,98 d(1H)	5,01	—	$C_{15}H_{17}NO_4$	5,09	—
XII	1,07 t(6H) 2,35 q(2H) 2,85 q(2H)	—	5,73 s	7,2—7,4 m (2H) 7,98 d(1H)	3,98	23,87	$C_{14}H_{14}BrNO_4$	4,12	23,49
XIII	0,7—1,0 m(6H) 1,3—1,7 m(4H) 2,26 t(2H) 2,7—2,9 m (2H)	—	5,73 s	7,2—7,4 m (2H) 7,98 d(1H)	3,64	21,52	$C_{16}H_{18}BrNO_4$	3,80	21,70

*Solvents: d_6-DMSO (III, VI, VII), $CDCl_3$ (IV, VIII, IX), CCl_4 (X-XIII); chemical shifts of 3-OH: 4.83 d (III), 3.85 d (IV).

TABLE 2. Physicochemical Characteristics of Dioxindoles I-XIII

Compound	mp, °C	R_f	R_M^0
I	163—6	0,49	—0,18
II	202—5	0,36	+0,06
III	185—6	0,28	+0,07
IV	124—6	0,40	+0,29
V	163—6	0,37	+0,36
VI	216—8	0,31	+0,47
VII	222—3	0,34	+0,75
VIII	158—9	0,19	+1,15
IX	159—60	0,23	+0,85
X	70—1	0,72	+1,57
XI	76—7	0,79	1,75
XII	102—3	0,79	+1,87
XIII	62	0,85	+2,25

Note. 1) Solvents for crystallization: benzene (I, II, IV), acetone–hexane (reprecipitation) (III), ethanol (V-VIII), CCl_4 (IX), hexane (X, XII, XIII), ether (XI). 2) Eluents: benzene–95% ethanol, 3:1 (I-VII); benzene–95% ethanol, 9:1 (VIII-XIII).

TABLE 3. Data of Biological Testing of Dioxindoles I-XIII

Compound	LD_{50}, mg/kg	Dose, mg/kg	Survival rate	Life duration, min ($X \pm s_X$)*
I	800 (760,45—757,4)	80	20/30	29,85±3,67
II	353 (314,47—396,2)	35,3	3/20	11,78±3,24
III	347 (316,89—380,0)	34,7	4/16	13,37±1,72
IV	447 (400—500,34)	44,7	0/20	5,0±1,49
V	800 (666,67—960)	80	2/20	11,7±2,42
VI	374 (328,2—437,58)	37,4	0/10	17,9±3,48
VII	700 (637,92—776,2)	70	0/10	7,13±1,92
VIII	600 (543,6—639,75)	60	15/20	22,0±3,24
IX	1000	150	0/10	19,75±4,46
X	1000	150	0/10	10,48±2,78
XI	1000	150	0/10	11,54±3,0
XII	1000	150	1/10	11,0±2,22
XIII	1000	150	1/10	15,62±2,11

*The control animals survived 5.19 ± 1.17 min on the average.

By acetylating dioxindole and its derivatives substituted in the benzene ring by acyl chlorides in pyridine [7], both O- and N,O-acylated products (VII-XIII) were obtained, since N-acylation can, if necessary, be prevented by keeping to the corresponding temperature regime, as in the preparation of compound VIII. Acylation by carboxylic acid anhydrides at 135°C was found to be suitable for the preparation of N,O-diacylated products. The 7-chloro derivatives, where N-acylation is apparently sterically hindered, are an exception.

The structure of the compounds obtained is confirmed by PMR spectra and elemental analysis data (Table 1).

By evaluating the lipophilicity of the compounds from the parameter R_M^0 (Table 2), it is noted that this is lowest in compounds containing substituents in the benzene ring or at the nitrogen atom only, and increases with substitution of the 3-hydrogen atom. In O-acylated derivatives the R_M^0 values are still higher, and their dependence on the length of the alkyl chain of the acyl group can be traced.

The influence of the structural changes on LD_{50} (Table 3) is complex in character. However, in the series of the compounds studied there is a tendency to decrease in toxicity with increase in lipophilicity. The pronounced protective action under hypobaric hypoxia conditions was observed in the parent compound of this series, dioxindole (I). With the

introduction of substituents into the benzene ring or at the nitrogen atom, no intensification of the action was observed, as in the case of 3-H substitution. O-Acylation appears to be promising: Compound VIII had a strong antihypoxic action on this model. However, compound I should be considered as the optimal structure, since besides pronounced antihypoxic effect, it has a relatively good solubility in water (the remaining compounds of this series, like isatin and oxindole, are very sparingly soluble in water). A drawback of I, as of several other compounds studied, is the tendency to oxidation in air.

Thus, in dioxindoles, which in their degree of reduction are intermediate compounds between isatins and oxindoles, the antihypoxic activity is retained. If we also consider certain other factors (solubility in water, toxicity), their use as antihypoxants may prove to be favorable.

EXPERIMENTAL (CHEMICAL)

The PMR spectra were run on the "Hitachi Perkin-Elmer" spectrometer, with a working frequency of 90 MHz and HMDS as internal standard; the solvents and chemical shifts of protons are given in Table 1.

The TLC was carried out on Silufol UV-254 plates (Czechoslovakia), with development by iodine vapors. The R_M^0 was found by the previously used method of inverted phases chromatography [5]. The R_f and R_M^0 values and also the melting points and solvents for crystallization are given in Table 2.

7-Chlorodioxindole (III). A 3.7-g portion (0.02 mole) of 7-chloroisatin is suspended in 300 ml of water, the mixture is heated to boiling, and 4.35 g (0.025 mole) of technical grade sodium thionite are added. The yellow solution formed is filtered from impurities, cooled and held for 12 h at 0°C. Yield 2.6 g (72%).

3-Acetonyl-5-bromodioxindole (VI). A 15.2-g portion (0.067 mole) of 5-bromoisatin is dissolved, with heating, in 30 ml (24 g, 0.4 mole) of acetone, 3 ml of Et_2NH are added, and the mixture is boiled, with stirring, for 20 min. The solution gradually acquires a green color, and a precipitate separates and is filtered by suction. An additional amount of product is obtained by evaporation of the filtrate. The combined product is crystallized from ethanol. Yield 14.1 g (74%).

3-(Pyridyl-2-methyl)-5-bromodioxindole (VIII). A mixture of 15.2 g (0.067 mole) of 5-bromoisatin with 32 g (0.1 mole) of α-picoline is boiled for 3 h, then cooled and poured into 100 ml of ether. The precipitate is filtered, washed thoroughly with ether, and dried. After twice-repeated recrystallization from ethanol, the yield was 11 g (52%).

5-Bromo-O-acetyldioxindole (VIII). A 3.6-ml portion (4 g, 0.05 mole) of AcCl is added dropwise to a cold solution of 7 g (0.03 mole) of 5-bromodioxindole in 40 ml of pyridine, while the temperature of the mixture is maintained at 0-5°C. Cooling is discontinued, and when the temperature of the mixture reaches room temperature, 250 ml of water are added. The oily layer is separated, shaken with water until crystals are formed, and allowed to stand for 12 h. It is then washed with water to the disappearance of a pyridine odor, and crystallized from water to yield 5.7 g (70%) of a product.

N,O-Dipropionyldioxindole (X). Method A. A 0.14-mole portion of I is added to 0.7 mole of $(EtCO)_2O$ at 135°C (bath temperature), and the mixture is left to stand at this temperature for 10 min. The mixture is cooled to room temeprature, and 500 ml of ether are added. The mixture is washed thoroughly with water, a 10% solution of Na_2CO_3, and again with water, and dried over Na_2SO_4. Ether is distilled and the residue is ground with a small amount of ethanol to form a thick paste. Ethanol is removed by suction, and the residue is washed slowly in small portions by 40 ml of 80% ethanol.

Method B. A 0.12-mole portion of EtCOCl is added to a cooled solution of 0.03 mole of I in 15-25 ml of pyridine, while the temperature of the mixture is maintained at 20-25°C. The mixture is heated, and held for 25 min at 50-70°C. It is then cooled to room temperature, and 150 ml of water are added. A brown oily layer separates, which is shaken with acidified water, filtered by suction, and washed with 20 ml of 80% ethanol.

5-Methyl-N,O-dipropionyldioxindole (XI) is obtained from II in the same way as X.

5-Bromo-N,O-dipropionyldioxindole (XII) is obtained from 5-bromodioxindole in the same way as X.

5-Bromo-N,O-dibutyryllioxindole (XIII) is obtained from 5-bromodioxindole by reaction with butyric acid anhydride or butyryl chloride in the same way as X.

7-Chloro-O-acetyldioxindole (IX). A 9.2-g portion (0.05 mole) of III is added to 30 ml (0.3 mole) of Ac_2O at 135°C (bath temperture), and the mixture is allowed to stand at this temperature for 10 min. The mixture is cooled to room temperature, and pinkish crystals separate, which are filtered, washed with ether, dried, and crystallized from CCl_4 to yield 6.5 g of the product (yield 57%).

EXPERIMENTAL (BIOLOGICAL)

The acute toxicity was determined on white non-pedigree mice of both sexes with single intraperitoneal administration of 0.5-5% aqueous solutions in the form of a thin suspension, stabilized by Tween-80. The LD_{50} and its confidence boundaries were calculated by the method of probit analysis according to Leachfield and Wilkoxon in the Roth modification [1].

The hypoxic state in male rats was induced by gradual reduction of atmospheric pressure up to 19.4 kPa in a tributary-ventillation type pressure chamber (this pressure corresponds to a "height" of 12000 m) and holding under these conditions for 45 min. The preparations were administered intraperitoneally 45 min before the animals were placed in the pressure chamber. The criteria of the antihypoxic activity were the percent of surviving animals and life duration of the animals that died. The data are shown in Table 3.

LITERATURE CITED

1. M. L. Belen'kii, Elements of Quantitative Evaluation of Pharmacological Effect [in Russian], 2nd ed., Leningrad (1963).
2. V. N. Garalene and L. I. Mazhilis, in: Congress of Pharmaceutists of the Lithuanian SSR. 3rd edn. Summaries of Lectures [in Russian], Kaunas (1982), pp. 29-30.
3. G. I. Zhungietu, Oxindole and Its Derivatives [in Russian], Kishinev (1973).
4. M. D. Mashkovskii, L. F. Roshchina, A. I. Polezhaeva, et al., Khim.-farm. Zh., No. 8, 132-138 (1977).
5. A. P. Stankyavichyus, L. I. Mazhilis, V. N. Garagene, et al., Khim.-farm. Zh., No. 12, 31-34 (1981).
6. A. B. Tomchin, A. E. Aleksandrova, and V. M. Vinogradov, Farmakol. Toksikol., No. 4, 482-490 (1978).
7. H. Hellmann and E. Renz, Chem. Ber., 84, 901-911 (1951).
8. L. Vnino, Handbook of Preparative Chemistry, Vol. 2, The Organic Part [in Russian], Stuttgart (1923).