SYNTHESIS OF RACEMIC ALKALOID DIPTHALINE AND ITS STRUCTURAL ANALOGS AND THEIR ANTIHYPOXIC ACTIVITY

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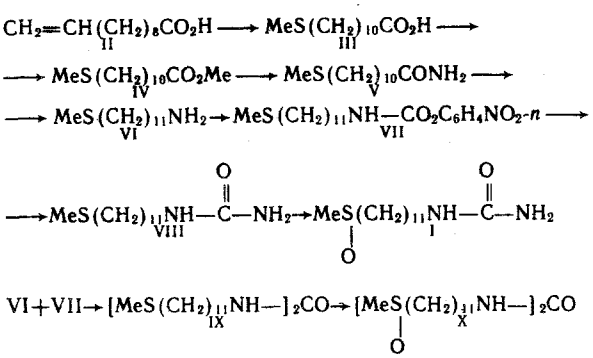
Among the pharmacological agents preventing or decreasing hypoxia, the most widely investigate are derivatives of thiourea and thiabarbituric acid [3, 4, 7]. Preparations based on guitimine have been authorized for clinical use. Despite this, the search for effective antihypoxants, required for clinical and cosmic medicine is still a topical problem. In this connection, the alkaloids from the plant *Dipthychocarpus strictus* with the structure of aliphatic mono- and disulfoxidoureas [12, 13] are of undoubted interest. It was shown on various models of hypoxia that the alkaloid diptocarpidine has antihypoxic activity, and in its activity it is superior to guitimine and isothiobarbamine [1, 2, 5]. Bearing this in mind, and also the difficulties involved in the isolation of the plant material in quantities sufficient for carrying out extensive biotests, we have developed a universal approach to the synthesis of the *Dipthychocarpus strictus* alkaloids in a racemic form [8-11].

In the present work we describe the synthesis of the racemic alkaloid dipthaline (I), its structural analogs (IX, X) and report the results of testing these compounds for the antihypoxic activity.

Thiylation of 10-undecylenic acid (II) by methyl mercaptan with UV-illumination gave 12-thiatridecanoic acid (III) in a 74% yield, which was then converted into a methyl ester IV. Its treatment with ammonia in a methanol solution led to amide V in a 74% yield, which was readily reduced by $LiAlH_4$ into the key compound 12-thiatridecylamine VI. The reaction of amine VI with bis(4-nitrophenyl) carbonate [14] in a CH_2Cl_2 solution gave the intermediate carbamate VII in a 57% yield, whose reaction under the same conditions with ammonia gave the sulfide precursor (VIII) of the alkaloid I in an 85% yield. Oxidation of thiourea VIII by hydrogen peroxide in acetic acid solution concluded the synthesis of the racemic alkaloid dipthaline I, the overall yield of which, based on the starting acid was 14%. Reaction of carbamate VII with amine VI gave the symmetric urea IX in a yield of 82%. Oxidation of the latter led to N,N′-(11-methylsulfinylundecyl)urea X in a yield of 75%.



In the PMR spectrum of the sulfide precursor of I (VIII), the signals of the CH_3 and CH_2 group protons adjacent to the sulfur atom are observed in the form of a singlet (δ 2.08 ppm) and a triplet (δ 2.48 ppm, J = 7.2 Hz), respectively. Similar signals are observed in the spectrum of sulfidourea IX (δ 2.09 and 2.49 ppm). In the case of sulfoxidourea, the proton signals of these groups are shifted to the weaker field because of the descreening effect of the SO group. In the spectrum of compound I, a triproton singlet is observed at 2.56 ppm, while the protons of the CH_2 group attached to the sulfur atom resonate in the

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TABLE 1. Influence of Dipthaline and Its Structural Analogs on the Duration of Life of White Mice under Acute Experimental Hypoxia Conditions

Compound	Increase in duration of life (% with respect to control) during hypoxia	
	gemic	normobaric
I	48±9,9	43±7,1
IX	34±11*	36,2±8,0
X	99±8,0	55±6,3
Guitimine	57±3,7	43,1±4,0
$H_2NC(=S)NHC(=NH)NH_2$		

*$p > 0.01$.

form of a triplet with a chemical shift of 2.72 ppm (J = 6.5 Hz). The proton signals of these groups of the structural analog X are observed at 2.59 and 2.75 ppm (J = 7 Hz).

The presence of SO groups in the molecules of compounds I and X is also confirmed by the IR spectra, in which there is an intense absorption band at 1025 cm^{-1}.

On models of acute gemic hypoxia and normobaric hypoxia with hypercapnia [6] it was shown that compounds I, IX, X have a pronounced antihypoxic effect (Table 1). Thereby ureas I and IX exhibit on models of gemic and normobaric hypoxia an activity commensurable with that of guitimine, while the symmetric sulfoxidourea X surpasses by twofold the activity of guitimine on the model of gemic hypoxia. It is clear that the intensification of the antihypoxic action is directly dependent on the presence of sulfoxide groups and the degree of symmetry of the structure of the ureas. A similar supposition was made in the study of the diptocarpidine alkaloid [N,N′-bis(6-methylsulfinylhexyl)urea] and its structural analogs [5].

It should be noted that compound X is notably superior in the protective action to the antihypoxant guitimine (by 74 and 28%, respectively, for the models of gemic and normobaric hypoxia), whereby the ED_{50} of urea X is considerably lower (Table 2). Therefore, despite the fact that compound X has a higher toxicity it is fully commensurable with guitimine with respect to the latitude of the antihypoxic action and is of undoubted interest for further investigation.

EXPERIMENTAL (CHEMICAL)

The IR spectra were run on a UR-20 spectrophotometer in Nujol or in a thin layer. The PMR spectra were obtained on a Tesla BS-567 B spectrometer with a working frequency 100 MHz. The ^{13}C NMR spectra were recorded on a JEOL FX = 90 Q spectrometer with a working frequency of 22.5 MHz, using $CDCl_3$ as solvent and TMS as internal standard. The products of the synthesis were separated by column chromatography on silica gel L 40/100 and L 100/160 (CSFR). The elemental analysis data of all the compounds corresponded to the calculated values.

12-Thiatridecanoic Acid (III). A 9.22-g portion ($5 \cdot 10^{-2}$ mole) of undecylenic acid and 0.01 g of azobis(isobutyronitrile) were placed into a flask made of quartz glass, and the mixture was cooled to 5°C. Methyl mercaptan (3.6 g, $7.5 \cdot 10^{-2}$ moles) was added cautiously, in one portion, and the mixture was stirred with illumination by a UV lamp (PRK-4) (5°C) for 1 h. The excess of methyl mercaptan was removed and acid III was purified via the sodium salt. Yield, 8.6 g (74%) of compound III in the form of colorless crystals, mp 36-38°C (hexane). IR spectrum (ν_{max}, cm^{-1}): 1380 (CS), 1710 (C=O), 2400-3550 (OH). PMR spectrum (δ, ppm): 1.29-1.62 (16H, m, $8CH_2$), 2.09 (3H, s, CH_3S), 2.35 (2H, t, SCH_2, J = 7.5 Hz), 2.49 (2H, t, CH_2CO_2, J = 7.2 Hz), 10.72 (1H, br.s, OH). ^{13}C NMR spectrum (δ, ppm): 15.52 (CH_3S), 24.68, 28.80, 29.05, 29.20, 29.34, 29.43 ($8CH_2$), 34.11 (SCH_2), 34.34 (CH_2CO_2), 180.18 (C=O).

Methyl Ester of 12-Thiatridecanoic Acid (IV). A mixture of 13 g ($15.6 \cdot 10^{-2}$ mole) of acid III, 0.25 g of p-toluenesulfonic acid and 250 ml of anhydrous methanol was stirred for 24 h. The solvent was evaporated, the residue was diluted with 100 ml of diethyl ether, washed with a 5% solution of $NaHCO_3$ (2 × 50 ml), the organic layer was dried over $MgSO_4$, evaporated, and the residue was chromatographed (hexane–ethyl acetate, 7:3). Yield, 10 g (80%) of ester IV in the form of oil. IR spectrum (ν_{max}, cm^{-1}): 1375 (CS), 1745 (C=O). PMR spectrum (δ, ppm): 1.28-1.69 (16H, m, $8CH_2$), 2.09 (3H, s,

TABLE 2. Toxic Characteristics of N,N′-Bis(11-methylsulfinylundecyl) Urea X

	Compound X	Guitimine
Mean lethal dose, LD_{50}, mg/kg	225 (204,3—245,7)	1440 (1260—1620)
Mean therapeutic dose, ED_{50}, mg/kg	16 (101—21,9)	80 (54—106)
Therapeutic index, TI-LD_{50}/ED_{50}	14	18

CH_3S), 2.32 (2H, t, SCH_2, J = 7.2 Hz), 2.52 (2H, t, CH_2CO_2, J = 7.2 Hz), 3.67 (3H, s, OCH_3). ^{13}C NMR spectrum (δ, ppm): 15.53 (CH_3S), 25.00, 28.85, 29.24 ($8CH_2$), 34.07 (SCH_2), 34.34 (CH_2CO_2), 51.37 (OCH_3), 174.16 (C=O).

Amide of 12-Thiatridecanoic Acid (V). A mixture of 10 g ($4.1 \cdot 10^{-2}$ mole) of methyl ester IV and 400 ml of a saturated solution of anhydrous ammonia in methanol was allowed to stand at 5°C for 10 days. The solvent and excess of ammonia were evaporated, and the residue was crystallized from a mixture of methanol and ether. Yield, 7 g (74%) of amide V in the form of white crystals, mp 96-98°C. IR spectrum (ν_{max}, cm^{-1}): 1250 (C–N), 1450 (NH), 1650 (C=O), 3420 (NH). PMR spectrum (δ, ppm): 1.29-1.69 (16H, m, $8CH_2$), 2.09 (3H, s, CH_3S), 2.23 (2H, m, SCH_2, J = 7.2 Hz), 2.49 (2H, t, CH_2CO_2, J = 7.2 Hz), 5.76 (2H, m, NH_2). ^{13}C NMR spectrum (δ, ppm): 15.54 (CH_3S), 25.52, 28.85, 29.84 ($8CH_2$), 34.33 (SCH_2), 34.93 (CH_2CO_2), 175.91 (C=O).

12-Thiatridecylamine (VI). A 2.4-g portion ($7 \cdot 10^{-2}$ moles) of $LiAlH_4$ was added in three portions to a stirred suspension of 6.11 g ($2.8 \cdot 10^{-2}$ moles) of amide V in 50 ml of anhydrous ether. The reaction mixture was heated (30°C) for 12 h, then was cooled, and after adding 0.49 of ($1.3 \cdot 10^{-2}$ mole) of $LiAlH_4$ was stirred for 2 h at room temperature. A 100-ml portion of undried ether was added cautiously, and then 10 ml of a saturated aqueous solution of KOH, the precipitate was filtered off, the organic layer in the filtrate was separated, and the aqueous layer was extracted with ether (2 × 10 ml). The combined extracts were dried over Na_2SO_4, evaporated to yield 4.84 g (85%) of amine VI in the form of an oil. IR spectrum (ν_{max}, cm^{-1}): 1350 (CS), 1560-1640, 3342 (NH). PMR spectrum (δ, ppm): 1.28-1.66 (18H, m, $9CH_2$), 2.09 (3H, s, CH_3S), 2.55 (2H, t, SCH_2, J = 7.2 Hz), 2.69 (2H, t, CH_2N, J = 7.2 Hz), 3.62 (2H, m, NH_2).

4-Nitrophenyl N-(12-Thiatridecyl)carbamate (VII). A solution of 2.17 g ($1 \cdot 10^{-3}$ mole) of amine VI in 10 ml of CH_2Cl_2 was added dropwise with stirring and cooling (–10°C) in the course of 30 min to 3.04 g ($1 \cdot 10^{-3}$ mole) of bis(4-nitrophenyl)carbonate in 50 ml of anhydrous CH_2Cl_2. The reaction mixture was stirred for another 2 h (–10°C), then was washed with a 10% solution of $NaHCO_3$ (4 × 25 ml), and saturated solution of NaCl (30 ml) and the organic layer was dried over $MgSO_4$. The solution was evaporated and the residue was crystallized from methanol. Yield, 2.19 g (57%) of carbamate VII in the form of light-yellow crystals, mp 80-82°C (methanol). IR spectrum (ν_{max}, cm^{-1}): 1375 (CS), 1680 (C=O), 3325 (NH). PMR spectrum (δ, ppm): 1.26-1.42 (14H, m, $7CH_2$), 1.60 (2H, m, $2CH_2$), 2.09 (3H, s, CH_3S), 2.48 (2H, t, SCH_2, J = 7.2 Hz), 3.26 (2H, m, CH_2N), 5.22 (H, br.s, NH), 7.38 (2H, d, Ar, J = 9.1 Hz), 8.27 (2H, d, Ar, J = 9.1 Hz). ^{13}C NMR spectrum (δ, ppm): 15.56 (CH_3), 26.73, 28.82, 29.23, 29.72 ($9CH_2$), 34.40 (SCH_2), 41.48 (CH_2N), 121.90, 125.90, 125.09, 144.77, 153.12 (Ar), 156.14 (C=O).

N-(12-Thiatridecyl)urea (VIII). A mixture of 2.0 g ($5.2 \cdot 10^{-3}$ moles) of carbamate VII, 0.5 g ($2.5 \cdot 10^{-2}$ moles) of liquid ammonia and 50 ml of anhydrous CH_2Cl_2 was stirred for 4 h at 0°C. The mixture was washed with 5% HCl (2 × 30 ml) and a saturated solution of $NaHCO_3$ (2 × 15 ml), the organic layer was dried over Na_2SO_4, evaporated, and the residue was crystallized from a methanol–ether mixture. Yield 1.1 g (85%) of urea VII in the form of white crystals, mp 98-100°C (ether). IR spectrum (ν_{max}, cm^{-1}): 1375 (CS), 1600 (NH), 1650 (C=O), 3342 (NH). PMR spectrum (δ, ppm): 1.20-1.62 (18H, m, $9CH_2$), 2.08 (3H, s, CH_3S), 2.48 (2H, t, CH_2S, J = 7.2 Hz), 3.14 (2H, m, CH_2N), 4.24-4.70 (3H, m, NH, NH_2).

N-(11-Methylsulfinylundecyl)urea [(±)-Dipthaline I]. A 0.45-g portion ($4.8 \cdot 10^{-3}$ moles) of a 30% H_2O_2 was added at 25°C to a stirred solution of 1.0 g ($3.8 \cdot 10^{-3}$ moles) of sulfidourea VIII in 15 ml of glacial AcOH and 15 ml of acetone. After 2 h, the reaction mixture was diluted with 30 ml of $CHCl_3$, and washed with a saturated solution of $NaHCO_3$ to remove traces of acid. The organic layer was then dried over Na_2SO_4, evaporated, and the residue was chromatographed (chloroform–methanol, 9:1). Yield, 0.8 g (74%) of (±)-dipthaline (I) in the form of white crystals, mp 122-124°C (literature data [13] — oil). IR spectrum (ν_{max}, cm^{-1}): 1024 (S=O), 1375 (CS), 1550 (NH), 1680 (C=O), 3340 (NH). PMR spectrum (δ, ppm): 1.22-1.75 (18H, m, $9CH_2$), 2.56 (3H, s, CH_3SO), 2.72 (2H, t, $OSCH_2$, J = 6.5 Hz), 3.12 (2H, q, CH_2N, J = 6.4

Hz), 4.65 and 5.20 (3H, m, NH, NH_2). ^{13}C NMR spectrum (δ, ppm): 22.54, 26.84, 28.69, 29.09, 29.12, 29.21, 29.35, 29.73, 30.16 ($9CH_2$), 38.50 (CH_3SO), 40.65 (CH_2N), 54.88 ($OSCH_2$), 159.18 (C=O).

N,N'-Bis(12-thiatridecyl)urea (IX). A solution of 4.43 g ($2 \cdot 10^{-3}$ moles) of amine VI in 20 ml of CH_2Cl_2 was added dropwise with stirring, in the course of 15 min to 3.04 g ($1 \cdot 10^{-3}$ mole) of bis(4-nitrophenyl) carbonate in 50 ml of anhydrous CH_2Cl_2. The reaction mixture was stirred for another 2 h at room temperature and was treated similarly as the sulfide precursor of dipthaline VIII. Yield 3.77 g (82%) of urea IX in the form of white crystals, mp 79-80°C (ether). IR spectrum (ν_{max}, cm^{-1}): 1380 (CS), 1600, 1645 (NHCO), 3342 (NH). PMR spectrum (δ, ppm): 1.29-1.58 (36H, m, $18CH_2$), 2.09 (6H, s, $2CH_3S$), 2.49 (4H, t, $2CH_2S$, J = 7 Hz), 3.17-3.30 (4H, m, $2CH_2N$), 4.60-5.08 (2H, m, 2NH).

N,N'-Bis(11-methylsulfinylundecyl)urea (X). A 1.9-g portion ($1.5 \cdot 10^{-2}$ mole) of a 30% H_2O_2 was added at 25°C to a stirred solution of 3.5 g ($7.6 \cdot 10^{-3}$ moles) of sulfidourea IX in 15 ml of glacial AcOH and 15 ml of acetone. After 2 h, the treatment of the reaction mixture was carried out by the same procedure as that used in the synthesis of dipthaline I. Yield, 2.8 g (75%) of urea X in the form of white crystals, mp 116-118°C. IR spectrum (ν_{max}, cm^{-1}): 1020 (SO), 1375 (CS), 1580, 1635 (NHCO), 3220, 3340 (NH). PMR spectrum (δ, ppm): 1.27-1.52 (36H, m, $18CH_2$), 2.59 (6H, s, $2CH_3SO$), 2.75 (4H, t, $2CH_2SO$, J = 6.4 Hz), 3.17 (4H, t, $2CH_2N$, J = 6.2 Hz), 5.17 m (2H, 2NH). ^{13}C NMR spectrum (δ, ppm): 22.42, 26.79, 28.63, 29.06, 29.19, 29.76, 30.08 ($18CH_2$), 38.50 ($2CH_3SO$), 40.74 ($2CH_2N$), 54.84 ($2CH_2SO$), 159.39 (CO).

EXPERIMENTAL (PHARMACOLOGICAL)

The antihypoxic activity of the compounds was studied on white nonpedigree male mice, each weighing 16-20 g. The compounds were administered intraperitoneally in the form of aqueous solutions or suspensions (Tween-80, a 5% solution) in a dose of 100 mg/kg, 30 min before the hypoxic effect. The activity of the compounds studied was compared with the activity of the antihypoxant guitimine. The control animals received an equal amount of the solvent. The acute gemic hypoxia was induced by a subcutaneous administration of methemoglobin forming agent — a 0.2% solution of sodium nitrite in a dose of 200 mg/kg. The hypoxic normobaric hypoxia with hypercapnia was produced in a 250 cm^3 germochamber. The criterion for the evaluation of the antihypoxic activity of chemical compounds was a change in duration of life of the animals under acute hypoxia conditions.

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