NOOTROPIC ACTIVITY OF UREIDOACIDS AND THIOHYDANTOINS

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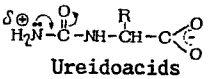
We have recently described a new approach to the identification of compounds having antihypoxic and nootropic activity, based on the definite similarity (with respect to interactions with the appropriate receptor system) between the terminal ammonium grouping of γ-aminobutyric acid (in the zwitterionic form) and the primary amino-group of the amide fragment of pyracetam [1].

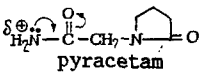
In these terms, it appears logical to examine the biological activity of ureidoacids, which have a clear structural similarity to both GABA and pyracetam. It is necessary to bear in mind, in this connection, that since ureidoacids are fairly strong organic acids they, like GABA, are ionized in neutral media.

The subjects chosen for study were the ureidoacids (Ia-e) and carbamoylproline (II) [5-7], obtained by reacting the appropriate aminoacids with sodium cyanate as described in [7].

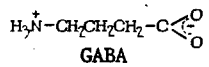


Ureidoacids

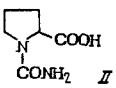
Ia-e



pyracetam



GABA

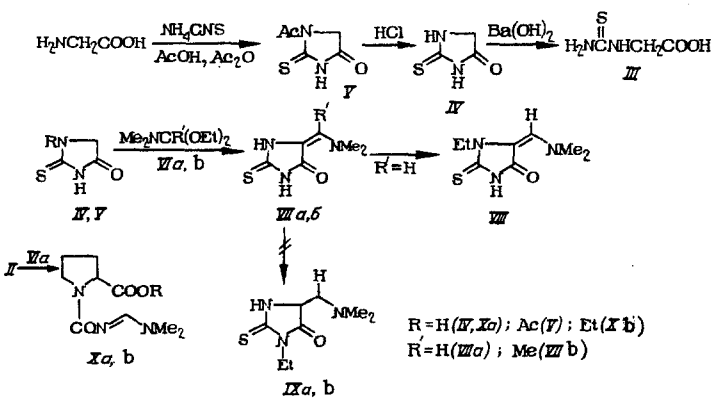


II

R=H(a), CH_2COOH(b), $(CH_2)_2COOH$(c), indolyl-3-methyl(d), p-hydroxylbenzyl (e)

In order to compare the activity of ureido- and thioureidoacids, thiocarbamoylglycine (III) was prepared [4, 9].

Nootropic activity was also tested for in cyclic thioureidoacids, namely thiohydantoins, which are intermediates in the synthesis of the thioureide (III), namely 2-thiohydantoin (IV) and its 3-acetyl derivative (V). It is noteworthy



that the thioureide (III) can also be obtained directly from the acetyl derivative (V) by hydrolysis with barium hydroxide. Compounds (IV) and (V) react with dimethylformamide diethyl acetal (VIa) to give the same product, 4-dimethylaminomethylenethiohydantoin (VIIa), i.e., the condensation of the 3-acetyl derivative (V) with the acetal (VIa) is attended by loss of the N-acetyl group. Similarly, (IV) and (V) react with N,N-dimethylacetamide diethyl acetal (VIb)

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TABLE 1. Physicochemical Properties of Compounds Obtained

Compound	Yield, %	mp, °C, or bp, °C/mm Hg	Empirical formula
VIIa	72	>300	$C_6H_9N_3OS$
VIIb	52	244—245	$C_7H_{11}N_3OS$
VIII	97	174—175	$C_8H_{13}N_3OS$
Xb	38	160—180/2	$C_{11}H_{19}N_3O_3$

Note. Compound (VIIa) was crystallized from DMF, (VIIb) from methanol, and (VIII) from water.

to give (VIIb). Alkylation of (VIIa) with ethyl iodide offords the monoalkyl derivative (VIII), although the site of the ethyl residue is not obvious, since the reaction could give either the 1- or the 3-ethyl derivative, or a mixture of the two. Spectral and chromatoraphic data indicate that only one alkyl derivative is formed, i.e., the reaction is regioselective.

The mass spectrum of (VIII) shows that initial breakdown involves elimination of Et$^{\cdot}$ and SH$^{\cdot}$ groups. According to the DAD1 spectrum this is followed by loss of 29 and 17 atomic units with the formation of ions with m/z 141 and 153. Examination of the spectrum of the deutero-analog of (VIII), obtained by deuterium exchange on heating with CD_3OD in the direct inlet system of the mass spectrometer, showed that the ion 141 contains some of the label, i.e., it is formed by elimination of either an NMe group or a CDO group. The ion with mass number 153 is formed by elimination of an OD group. This mode of breakdown suggests that the CONH fragment is retained in the ethyl derivative, in accordance with structure (VIII) (rather than (IX), in which this fragment is absent).

Unambiguous information on the structure of the alkylated product was obtained by ^1H NMR spectroscopy. Proof of the structure of the compound was obtained by using the nuclear Oberhauser effect. Irradiation of the signal for the NCH_2 group with a high-frequency field resulted in an increase in the intensity of the singlet for the CH proton of the dimethylaminomethylene fragment by 24%, which could only occur if the vinyl proton is adjacent to the N-ethyl group. This provides unambiguous proof that the N-ethyl derivative is 3-ethyl-4-dimethylaminomethylene-2-thiohydantoin (VIII).

In conclusion, an attempt was made to obtain the N-dimethylaminomethylene derivative of N-carbamoylproline (Xa) from the ureide (II). Reaction of (II) with the acetal (VIa), however, led to the formation of the ureidoester (Xb), i.e., in addition to condensation at the NH_2 group, esterification of the 2-carboxy group occurred. The structure of the product follows from its elemental analysis, and IR and mass spectra.

EXPERIMENTAL (CHEMISTRY)

IR spectra were obtained on a Perkin-Elmer 457 (Sweden), as pastes in Vaseline grease, and UV spectra on a Specord M-40 spectrometer in methanol. The PMR spectrum of (X) was obtained on a JMN-4H-100 spectrometer, standard tetramethylsilane, with $CDCl_3$ as solvent. Mass spectra were obtained on a Varian MAT-112 GC-MS (Switzerland). The ionizing electron energy was 70 eV, and ionization chamber temperature 180°C. The properties of the products are shown in Table 1.

The elemental analyses were in agreement with the calculated values.

Preparation of N-Thiocarbamoylglycine (III) from 3-Acetyl-2-thiohydantoin (V). A mixture of 1.4 g (0.01 mole) of 3-acetylhydantoin and 6.84 g (0.04 mole) of $Ba(OH)_2$ was boiled for 1 h in 80 ml of water. The mixture was cooled, treated dropwise with 25% sulfuric acid to pH ~2, and the precipitated $BaSO_4$ filtered off. The filtrate was evaporated, and the residue triturated with 2-propanol to give (III).

4-(N,N-Dimethylaminomethylene)-2-thiohydantoin (VIIa). Method A. A mixture of 1.16 g (0.01 mole) of the 2-thiohydantoin (IV) and 1.9 g (0.013 mole) of N,N-dimethylformamide diethyl acetal (VIa) in 20 ml DMF was heated at 80-85°C for 40 min, then 1 g of (VIa) was added, and heating continued for a further 40 min. The mixture was then cooled, and the precipitated (VIIa) filtered off. IR spectra, ν_{max}, cm^{-1}: 3130 br (NH), 1700 (C=O).

Method B. A mixture of 1.38 g (0.01 mole) of (V) and 1.9 g (0.013 mole) of (VIa) was stirred at room temperature in 20 ml of abs. alcohol, then 1 ml of the acetal (VIa) was added, and boiling continued for another 30 min. The mixture was then cooled, and the (VIIa) filtered off.

4-(β-Methyl-N,N-dimethyl)aminomethylene-2-thiohydantoin (VIIb). Method A. A mixture of 1.16 g (0.01 mole) of the 2-thiohydantoin (IV) and 2.3 g (0.014 mole) of N,N-dimethylacetamide diethyl acetal (VIIb) was stirred at room temperature in 20 ml of alcohol for 1 h, then 2 ml of the acetal (VIIb) was added, stirred at 50-55°C for 1.5 h, a further 2 ml of (VIIb) added, and boiled for 20 min. The mixture was then cooled, and the precipitated (VIIb) filtered off. Mass spectrum*: M^+ 185 (100), 170 (7), 168 (3), 156 (2), 153 (3), 141 (6), 97 (20), 71 (10), 56 (43). IR spectrum, ν_{max}, cm^{-1}: 3100-3230 (NH), 1660 (C=O).

Method B. Obtained as in method A from (V) and (VIIb), by boiling in alcohol for 7 h.

3-Ethyl-4-(N,N-dimethylaminomethylene)-2-thiohydantoin (VIII). A mixture of NaOEt (from 0.08 g of Na and 10 ml of abs. alcohol) and 0.6 g (0.0035 mole) of (VIIa) was stirred at room temperature for 20 min, then boiled for 10 min. The resulting suspension was cooled to 20°C and 0.56 g (0.0036 mole) of EtI added dropwise. The mixture was stirred at room temperature for 1 h 10 min, then boiled for 20 min. It was then cooled and the solid filtered off. The filtrate was evaporated, and the residue triturated with petroleum ether to give (VIII). IR spectrum, ν_{max}, cm^{-1}: 3060-3140 (NH), 1680 (C=O). ^1H NMR spectrum (d_7-DMF): 1.35 (3H, t, CH_3), 3.13 (2H, q, CH_2), 3.19 and 3.51 [6H, br. s, $N(CH_3)_2$], 6.85 (1H, s, CH=), 10.95 (1H, br. s, NH). Mass spectrum: M^+ 199 (100), 170 (47), 166 (11), 153 (3), 141 (18), 111 (18), 83 (61), 61 (13), 58 (11).

N,N-Dimethyl-N'-(2-ethoxycarbonylpyrrolidinyl-1-acetyl)formamidine (Xb). A mixture of 1.58 g (0.01 mole) of (II) and 1.9 g (0.013 mole) of (VIa) in 20 ml of DMF was stirred at 95-100°C for 4.5 h, with the hourly addition of 1.9 g portions of the acetal. The DMF was evaporated, and the residue distilled in vacuo, the fraction bp 160-180°C/2 mm being collected (Xb). M^+ 241. IR spectrum, ν_{max}, cm^{-1}: 1740 (ester CO), 1640 (ureido CO).

EXPERIMENTAL (PHARMACOLOGY)

The compounds were tested in male mice weighing 18-20 g, with 10-12 animals in each dose group. The animals were examined for the effects of the compounds on general condition and behavior, lifespan under conditions of hypoxic hypoxia [2], the convulsant effects of corazole [8] and thiosemicarbazide (TSC), the latent period for the passive flight conditoned reflex (PFCR) in mice [3], and the sedative effects of thiopental sodium (30 mg/kg i.v).

The compounds were all sparingly soluble in water, and were therefore given internally as suspensions in 1% starch mucilage stabilized with Tween-80, in doses of 10-20% of the LD_{50}.

The studies of the effects on behavior and general condition showed that most of the compounds had little effect on group behavior, the orientation and search reaction. Compounds (Ib, c) (carbamides of asparaginic and glutamic acid) had a slight sedative effect, as shown by the increased duration of thiopental sleep from 4.5 ± 1.5 to 7.3 ± 2.0 and 10.6 ± 3.0 min. Subtoxic doses of (IV) and (V) (greater than 500 mg/kg) caused motor excitation and aggression in the animals. Over a period of 3 h, the remaining compounds had no effect on the behavior of the animals. Table 2 shows the pharmacological test results in respect of effects on hypoxic hypoxia, the convulsant effects of corazole and TSC, and the latent period for PFCR.

Antihypoxic Activity. The test results showed that the lifespan of the animals of the control group in a hermetically sealed chamber was 27 ± 2.0 min, and under these conditions pyracetam in a dose of 500 mg/kg increased the lifespan by 18-20%. In an internal dose of 200 mg/kg, (Ic), (Ia), and (III) increased the lifespan of mice in the hermetically sealed chamber by 11.7, 22, and 33% (i.e., to 32.5 ± 4.3, 32.8 ± 2.9, and 35.8 ± 1.8 min). The remaining compounds had no effect on the lifespan of animals under hypoxic conditions (Table 2).

Anticonvulsant Activity. The test showed that the most active compound with respect to the convulsant effects of corazole (130 mg/kg, s/c) was (IV), which in a dose of 40 mg/kg increased the latent period of onset of death by 50%. The remaining compounds had low anticonvulsant activity towards both corazole and TSC. For example, (VIIb), (III), and (Ib) increased the latent period of onset of death by 10, 17, and 23%.

*The m/z values for the molecular and fragment ions are given, with intensities as a % of the main peak in brackets.

TABLE 2. Pharmacological Activity of (I-XIII)

Compound	Dose, mg/kg (internal)	Effect on sedative effects of thiopental, min	Hypoxic hypoxia		Latent period of onset of death				PFCR		Acute toxicity, LD_{50}
			lifespan		TSC (20 mg/kg)		Corazole (130 mg/kg		latent period of entry into a dark chamber		
			min	%	min	a%	min	%	min	%	
Control	—	4,5±1,5	27±2,0	—	67,4±8,8	—	10,4±5,5	—	77,8±4,2	—	—
Pyracetam	500	—	32±3,2	12,5	79,3±15*	17	12,3±1,5	17	100±9,3	25,0	2000
Ia	200	9±2,5*	32,8±2,9	22	78,4±1,2	16	9,6±6,8	—	76,6±13,2	—	>1000
Ib	200	73±2,0	28,4±4,8	—	68±5,9	—	12,7±4,4	23	116±3*	48,7	>1000
Ic	200	10,6±3*	32,5±4,3	11,7	76,6±11	13,6	10,5±5,3	—	100±7,2*	25,5	>1000
Id	200	8,3±2,0	30±4,6	11	84,6±1,2*	25	10,5±3,8	—	75±11	—	>1000
Ie	200	8,0±2,5	28,2±2,2	—	84±9,6*	27	10±3,4	—	73±8,5	—	>1000
II	200	—	26,6±1,8	—	64,5±5,4	—	10±4	—	88±11,7	12,8	>1000
III	200	9,6±2*	35,8±1,8*	33*	61,6±7,7	8	12±4,5	17	68±8	—	>1000
IV	40	10,8±3,5*	31±4,4	15,5	63,4±7	—	15,5±5*	50	75±18	—	417
V	60	—	26,1±2,0	—	74,6±6,8	10	12±9	17	89,5±11	14	600
VIIa	200	—	30±2,4	11	63,8±9	—	12±7,7	17	70±5,3	—	>1000
VIIb	200	—	30,6±3,5	11	64±9,2	—	9±4,8	10	66,5±12	—	>1000

*Difference from controls significant at $P < 0.05$.

The most active compounds in respect of TSC (20 mg/kg, s/c) were (Id) and (Ie), which in a dose of 200 mg/kg increased the latent period to death by 25 and 27% (i.e., from 67.4 ± 8.8 to 84.6 ± 1.2 and 84 ± 9.6 min). The other compounds reduced the convulsant effects of TSC to varying extents, increasing lifespan by 10-13% (Table 2).

Effects on Latent Period for PFCR. The test results showed that in the animals of the control group the latent period for PFCR was 77.8 ± 4.2 sec. In a dose of 500 mg/kg, pyracetam increased the latent period by 25%. Compounds (Ic) and (Ib) in a dose of 200 mg/kg increased this period by 25.5 and 48.7% (Table 2). In doses of 200 mg/kg and 60 mg/kg, (II) and (V) slightly increased (by 12-14%) the duration of the latent period for PFCR, but the remaining compounds were virtually inactive.

These pharmacological tests of (I-V) and (VII) for effects on the hypoxic state, the convulsant effects of TSC and corazole, and the latent period for PFCR in mice have shown that (III) has relatively high antihypoxic activity, while (Id) and (Ie) show anticonvulsant activity in model GABA-deficient convulsions, (IV) shows high antoganism to corazole, and (Ib) has a positive effect on the latent period for PFCR. None of the test compounds were superior to pyracetam in any of these tests.

The results show that the test compounds possess nootropic activity.

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