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NITROXYL DERIVATIVES OF PYRAZOLO[3,4-d]PYRIMIDINE

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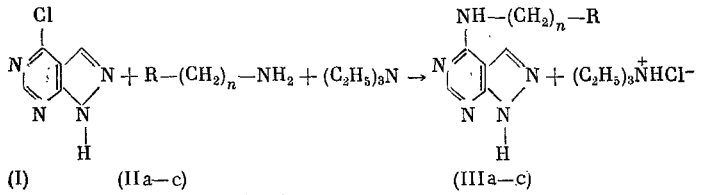
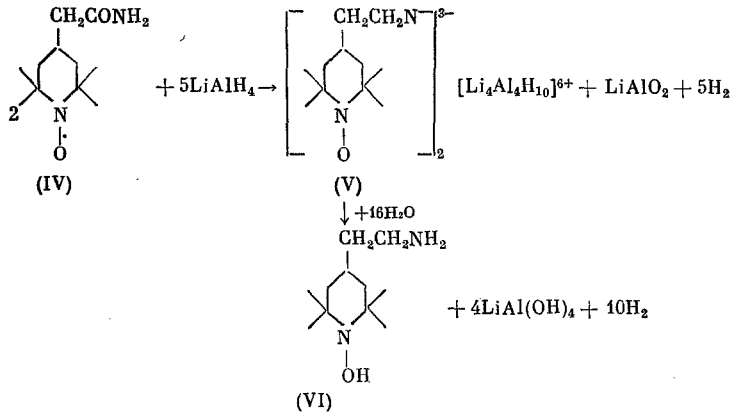
Pyrazolo[3,4-d]pyrimidines are "irreversible" xanthine oxidase inhitibors and are widely used in research into this enzyme [1-3]. Paramagnetic pyrazolopyrimidine derivatives which are capable of binding strongly with xanthine oxidase may be a useful tool for elucidating the topography of the active center of the enzyme.

We have prepared pyrazolopyrimidine derivatives of formula (IIIa-c), which are the first of their kind, by reacting 4-chloropyrazolopyrimidine (I) with amino radicals (IIa-c):



The reaction was performed in boiling ethanol (1-1.5 h), the HCl formed in the reaction being bound with triethylamine. The consumption of the bases was monitored by TLC and by potentiometric titration with 0.1 N HCl. The yield of (IIIa-c) under these conditions is 95-97% (Table 1).

Amino radical (IIc) was prepared by reducing the amide (IV) in THF and then oxidizing the resulting hydroxypiperidine (VI). The reduction of (IV) was monitored on the basis of H_2 evolution and $LiAlH_4$ consumption, indicating that the reaction is practically complete by the time (IV) is extracted into the solution, and that 5 moles of $LiAlH_4$ are consumed and 5 moles of H_2 are released for every 2 moles of (IV). The resulting colorless, THF-insoluble complex (V) releases H_2 and is converted to (VI) when decomposed with water:



The oxidation of (VI) to (II) is effected in an alkaline aqueous methanol medium in the presence of Cu^{2+}. This method of preparing (IIc), which gives a yield of ~60%, is considerably simpler than the methods of [4, 5] since it avoids protection of the amino group during oxidation.

Pyrazolopyrimidines (IIIa-c) are high-melting substances colored in different shades of red. They are readily soluble in alcohols, sparingly soluble in alkanes and water, and moderately soluble in medium-polarity solvents.

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TABLE 1. Pyrazolopyrimidines (IIIa-c)

Compound	Yield, %	mp., °C	Found / Calculated, % C	H	N	M*	Empirical formula
(IIIa)	97	245–248 (from MeCN)	58,24 / 58,11	7,36 / 7,32	29,12 / 29,04	290±3 / 289,36	$C_{14}H_{21}N_6O$
(IIIb)	95	226 (from MeCN)	59,25 / 59,39	7,75 / 7,64	28,08 / 27,70	302±3 / 303,39	$C_{15}H_{23}N_6O$
(IIIc)	95	207–209 (from DCE)	60,50 / 60,54	7,85 / 7,94	27,03 / 26,48	320±10 / 317,41	$C_{16}H_{25}N_6O$

* Molecular weights were determined by ESR in acetone solution, using acetone solutions of amino radical (IIa) as standard.

TABLE 2. Characteristics of IR Bands and Electronic Spectra of Pyrazolopyrimidines (IIIa-c)

Compound	IR spectrum* medium	ν, cm^{-1}	group	Electronic spectrum† ν, cm^{-1}	ε, liter/mole·cm
(IIIa)	Mineral oil	1530 m, 1593 s & 1613 s	C_5N_4 ring	22230	10,8±0,1
		3124 m, 3200 m, 3306 m	Bonded NH	34690 sh	(12,6±0,2)·10^3
	$CHCl_3$	1516 m, 1586 s & 1610 s	C_5N_4 ring	35620	(16,8±0,4)·10^3
		3140 w & 3220 w	Bonded NH	37770 sh	(12,6±0,2)·10^3
		3408 w	Exocyclic NH	42150 min	(5,4±0,01)·10^3
		3448 m	Cyclic NH	44390 sh	(8,8±0,04)·10^3
				48770	(20,0±0,4)·10^3
(IIIb)	KBr	1539 m, 1595 s & 1616 s	C_5N_4 ring	22240	9,7±0,1
		3135 s, 3206 s & 3317 s	Bonded NH	34850 sh	(11,2±0,2)·10^3
	$CHCl_3$	1525 m, 1588 s & 1607 s	C_5N_4 ring	35680	(14,4±0,4)·10^3
		3127 w & 3210 w	Bonded NH	37770 sh	(10,9±0,3)·10^3
		3425 w	Exocyclic NH	38840 sh	(9,0±0,2)·10^3
		3455 m	Cyclic NH	41780 min	(4,7±0,1)·10^3
				45260 sh	(9,1±0,3)·10^3
(IIIc)	KBr	1537 s, 1599 s & 1618 s	C_5N_4 ring	22300	10,0±0,05
		3138 s, 3212 s & 3322 s	Bonded NH	34700 sh	(11,8±0,2)·10^3
	$CHCl_3$	1536 m, 1592 s & 1611 s	C_5N_4 ring	35620	(15,3±0,1)·10^3
		3137 w, 3209 w	Bonded NH	37680 sh	(11,6±0,2)·10^3
		3423 w	Exocyclic NH	38740 sh	(9,8±0,2)·10^3
		3449 m	Cyclic NH	41680 min	(5,5±0,1)·10^3
				45380 sh	(10,9±0,1)·10^3

* Band intensity: s = strong; m = medium; w = weak.
† In 95% EtOH; sh = shoulder; min = minimum.

The ESR spectra of dilute solutions of (IIIa-c) consists of three lines with $a_{^{14}N} = 1.57 \pm 0.02$ mT. The line widths of all three radicals are the same and equal to the line widths of (IIa). The molecular weights determined for (IIIa-c) by ESR agree with the calculated values within the limits of experimental error.

The electronic spectra of (IIIa-c) are analogous and comprise bands of the nitroxyl chromophore superposed on absorption of the pyrazolopyrimidine ring (Table 2). The red color of the substances is due to $\rangle N \dashv O$ absorption with $\nu_{max} \approx 22{,}250$ cm^{-1} and $\varepsilon \approx 10$ liters/mole·cm. The second $\rangle N \dashv O$ band is masked by the stronger absorption of the pyrazolopyrimidine ring and may be detected with difficulty as a shoulder at ~ 39,000 cm^{-1}. The pyrazolopyrimidine ring has two bands in the 30,000-50,000 cm^{-1} region. The first, at ~35,650 cm^{-1}, has a vibrational fine structure.

The IR spectra of crystalline samples of (IIIa-c) exhibit three strong bands at ~3140, 3210, and 3320 cm^{-1} due to stretching vibrations of the bonded NH groups. These bands disappear in dilute $CHCl_3$ solution and are replaced by bands at ~3450 and 3415 cm^{-1}. The former is ~5 times stronger than the latter and corresponds to the NH band in (I). The latter has a frequency characteristic of alkylaromatic amines [6] and is evidently due to vibrations of exocyclic NH groups. The bands at ~1530, 1590, and 1610 are connected with stretching vibrations of the pyrazolopyrimidine ring. The spectra of (IIIa-c) contains no bands with frequencies of ~ 1650 cm^{-1} characteristic of C ≡ N groups. Thus, (IIIa-c) exist primarily in the amine form both in solution and in the solid state.

EXPERIMENTAL

The IR spectra were obtained using a Specord 75-IR spectrometer (German Democratic Republic), the electronic spectra using a Specord UV-VIS spectrometer, and the ESR spectra using an ÉPA-2M 3-cm radiospectrometer. The melting points were determined using a RNMK apparatus (German Democratic Republic).

4-Chloropyrazolo[3,4-d]pyrimidine (I) was prepared as in [7]; mp 150-155°C. IR spectrum (in KBr, ν, cm^{-1}): 1575, 1590, and 1600 (C_5N_4), 2800-3200 (CH and NH). The 1700 cm^{-1} absorption of the starting 4-hydroxypyrazolopyrimidine was absent.

4-Amino-2,2,6,6-tetramethylpiperidin-1-oxyl (IIa) was prepared as in [8]; mp 34-35°C. IR spectrum (in CCl_4, ν, cm^{-1}): 3303 (ε = 6.1 liter/mole · cm) and 3383 (ε = 9.3 liters/mole · cm) [NH_2]. Electronic spectrum (in EtOH): ν_{max} = 22,200 cm^{-1} (ε = 10.6 liters/mole · cm).

4-Aminomethyl-2,2,6,6-tetramethylpiperidin-1-oxyl (IIb) was prepared as in [4], mp 56-57°C. IR spectrum (in CCl_4, ν, cm^{-1}): 3334 (ε = 12 liters/mole · cm) and 3401 (ε = 16 liters/mole · cm) (NH_2).

4-(β-Aminoethyl)-2,2,6,6-tetramethyl-1-hydroxypiperidine (VI). By boiling in an extractor, 3.21 g of 4-carbamoylmethyl-2,2,6,6-tetramethylpiperidin-1-oxyl (IV) was extracted with 50 ml of a 0.7 M solution of $LiAlH_4$ in THF. When extraction was complete and H_2 evolution had ceased (37.7 mmole in ~2.5 h), the precipitated colorless complex was decomposed with 2.6 ml of water. The resulting $LiAl(OH)_4$ was filtered off and the solution was evaporated under vacuum. The viscous residue (2.3 g) was triturated with 5 ml of ether. The ether-insoluble colorless crystals of 4-carbamoylmethyl-2,2,6,6-tetramethyl-1-hydroxypiperidine (0.262 g) were filtered off and the ether solution was evaporated. The residue (1.98 g of a rose-colored oil) consisted mainly of hydroxypiperidine (VI) according to TLC (Silufol UV-254; $CHCl_3 \cdot Me_2CO$: MeOH = 5.5 : 1 : 1) and IR spectra. IR spectrum (liquid film, ν, cm^{-1}): 3595 (OH), 3363 and 3295 (NH_2), 3180 (OH and NH_2); (in CCl_4): 3220 and 3595 (OH), 3310 and 3383 (NH_2). The rose color was due to the presence of (IIc) as a minor impurity.

4-(β-Aminoethyl)-2,2,6,6-tetramethylpiperidin-1-oxyl (IIc). A portion (1.95 g) of (VI) was oxidized with oxygen in a mixture of 10 ml methanol, 10 ml of 10^{-4} M $CuSO_4$ and 10 ml of 0.1 N NaOH in water. When O_2 absorption ceased (61 ml in ~2.5 h), the reaction mixture was filtered, treated with 10 g of K_2CO_3, and extracted with 60 ml of ether. The ether extract was dried with Na_2SO_4, evaporated under vacuum, and the residue (1.84 g of a red oil) was distilled to give (IIc), bp 51-59°C/2-3 Pa. IR spectrum (liquid film, ν, cm^{-1}): 1595, 3300, and 3368 (NH_2).

Piperidinoxyl Derivatives of 4-Aminopyrazolo[3,4-d]pyrimidine (IIIa-c). A mixture of 5.5 mmole (IIa-c), 5.5 ml ethanol, 5 mmole triethylamine, and 5 mmole (I) was boiled until the reaction was complete (1-1.5 h), and then cooled, treated with 25 ml of water, and the precipitated (IIIa-c) separated. The yields of (III) and their constants are given in Tables 1 and 2.

CONCLUSIONS

Pyrazolo[3,4-d]pyrimidine nitroxyl derivatives having different distances between the paramagnetic center and the pyrazolopyrimidine ring have been prepared for the first time.

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