SYNTHESIS OF 5-SUBSTITUTED 1-METHYL-2-NITRO-1H-IMIDAZOLES (2-^{14}C)

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S U M M A R Y

1-Methyl-2-nitro-1H-imidazoles carrying different 5-side chains (isopropyl, hydroxymethylethyl, ethenyl) have been synthesised labelled with ^{14}C at C_2 of the imidazole nucleus.

I N T R O D U C T I O N

The nitroimidazoles have aroused considerable interest since they were found to be active not only as systemic anti-Trichomoniasis drugs but also against intestinal and skin infections with protozoa (leishmaniases, amoebiases, giardiases) and, recently, as being useful also for treatment of anaerobic bacterial infections and as radiosensitizers of hypoxic cells in radiotherapy of tumors. During the same period, there have been a large number of studies aimed at clarifying the metabolism and the mechanism of action of these compounds.

The research program of our laboratories developed some 2-nitroimidazoles with interesting pharmacological properties. These include 1-methyl-5-(1-methylethyl)-2-nitro-1H-imidazole (VIa), which is very active as a systemic antitrichomonas agent[1], 5-(1-hydroxy-1-methylethyl)-1-methyl-2-nitro-1H-imidazole (VId), which was first isolated in the study of the metabolism of VIa[2]

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in various animal species, and after synthesis was found to have a more favorable therapeutic index than the parent compound[3], and finally, 5-ethenyl-1-methyl-2-nitro-1H-imidazole (VIf), which has a wide spectrum of antimicrobial and antifungal activity which makes it of interest as a possible topical agent[4].

In order to study the metabolism of VIa and VId and the mechanism of action of VIf[5,6], it was necessary to synthesize the compounds labelled with ^{14}C in the 2 position of the imidazole ring. This we have done by suitably adapting the procedures previously employed, as shown in Scheme 1.

The intermediate N-methylaminoaldehydes were prepared as follows: IVa, by hydrolysis of the N-methylaminoacetal I[1]; IVb, by controlled catalytic reduction and hydrolysis of the N-methylaminobutyronitrile II[3]; IVc, by reduction with NaHg of 3-methylaminodihydro-2-(3H)-furanone hydrochloride III[1].

The N-methylaminoaldehydes IVa,b and c, obtained in aqueous solution, were not isolated. On the basis of the yields calculated from unlabelled experiments, they were treated directly with cyanamide-^{14}C under controlled conditions of pH to give the corresponding 2-aminoimidazoles-(2-^{14}C) Va,b and c. After diazotization and treatment with $NaNO_2$ in the presence of Cu, Va gave the corresponding 2-nitroimidazole-(2-^{14}C), VIa.

The same reaction carried out with Vb led to a mixture of the 2-nitroderivative VIb, and the corresponding hydroxy derivative, VId. The reaction mixture was hydrolyzed with HCl to give VId in fairly good yield.

Previous experiments indicated that treatment of 2-nitro imidazoles with thionyl chloride, aimed at exchanging a hydroxyl group on the alkyl chains with a chloro group, caused a loss of the nitro group. Therefore, by treatment with thionyl chloride in benzene, we converted the 5-(2-hydroxyethyl)-2-amino imidazole-(2-^{14}C) Vc into the corresponding 5-(2-chloroethyl) derivative, Ve, which was transformed into the 2-nitroimidazole-(2-^{14}C), VIe, as described above. This latter gave the 5-ethenyl-2-nitroimidazole-(2-^{14}C), VIf, when reacted with potassium t-butoxide.

E X P E R I M E N T A L

Cyanamide-^{14}C was obtained from ICN, Chemical and Radioisotope Division, Irvine, California (USA). 1-5 mCi (0.086 mmole) were used, diluted with cold cyanamide. Yields reported in Table I are based on the initial cyanamide-^{14}C.

The radioactivity of the samples, prepared by adding a solution of 10-20 µg of the compound in 0.1 ml of CH_3OH to 10 ml of Insta-gel Packard, was measured with a Intertechnique Scintillation Counter model SL/30. The specific radioactivity was determined by an internal standard method. The radiochemical purity was established by TLC on Silica-gel F_{254} plates (Merck) followed by scanning with a Packard Scanner mod. 7201. IR and UV spectra and R_f (TLC, spots visualized under UV light at 254 nm) were in accordance with those already determined for unlabelled standards[1,3,7].

3-Methyl-2-methylaminobutanal (IVa).

A solution of 0.94 g (5 mmole) of 3-methyl-2-methylamino butanal diethylacetal (I)[1] in 15 ml of 15% HCl was stirred for 2 hours at 50°C (bath temperature). The solution cooled to -10°C was brought to pH 4.6 by adding 50% NaOH before use for the next step.

3-Methoxy-3-methyl-2-methylaminobutanal (IVb).

3-methoxy-3-methyl-2-methylaminobutyronitrile (II)[3] (1.42 g, 10 mmole) was dissolved into 25 ml of N HCl cooled to 0°C. The solution was hydrogenated over 185 mg of 10% Pd/C at atmospheric pressure and room temperature. After 3 hours the catalyst was filtered and the solution was brought to pH 4.6 with 1N NaOH.

4-Hydroxy-2-methylaminobutanal (IVc).

A solution of 1.89 g (12.5 mmole) of 3-methylaminodihydro-2-(3H)-furanone hydrochloride (III)[1] in 7 ml of water and 3 ml

of EtOH was treated in portions with 48.7 g of 2.5% NaHg with magnetic stirring, while the temperature was kept at 5°C. The pH was maintained at 2.5-3.5 by adding 10% HCl. The Hg was filtered off, the aqueous solution was treated with charcoal, filtered and brought to pH 4.6 by adding 1N NaOH.

5-Substituted 1-Methyl-1-H-imidazol-(2-^{14}C)-2-amine hydrochlorides (Va,b,c).

To the aqueous solution of the corresponding N-methylamino aldehydes (IVa,b,c), obtained as previously described, $NH_2{}^{14}CN$ was added (1 mCi for IVa and 5 mCi for IVb and IVc), and an amount of NH_2CN corresponding to about 0.8 mole/mole of the starting reagents (I, II, III) (see the Table). The reaction mixture was stirred at 50°C for 2 hours (for Vb the pH of the mixture was maintained at 4.6 by addition of 10% HCl). The solvent was evaporated off in vacuo. The unreacted cyanamide and some undefined products were extracted from the residue with boiling Et_2O (3 x 10 ml).

Crude compound IVa and IVc were extracted from the oily residue with boiling absolute EtOH (3 x 20 ml). The ethanol solutions were evaporated to dryness and the 2-aminoimidazoles-(2-^{14}C) were purified by dissolving in a few ml of water and treatment with a solution of 2 g of picric acid in 15 ml of boiling water. On cooling, the picrates crystallized, were collected and dissolved in 20 ml of 15% HCl. The solution was heated at 50°C for 5 min. After cooling, the picric acid was extracted with benzene (3 x 20 ml) and the aqueous solution was evaporated to dryness, giving Va or Vc.

Compound Vb was obtained directly by evaporation of the ethanol extract.

5-(2-Chloroethyl)-1-methyl-1H-imidazol-(2-^{14}C)-2-amine hydrochloride (Vc).

$SOCl_2$ (2 ml, 27 mmole) was added to a solution of 1.35 g (7.6 mmole, 3.6 mCi) of Vc in 40 ml of benzene. The reaction

Scheme 1

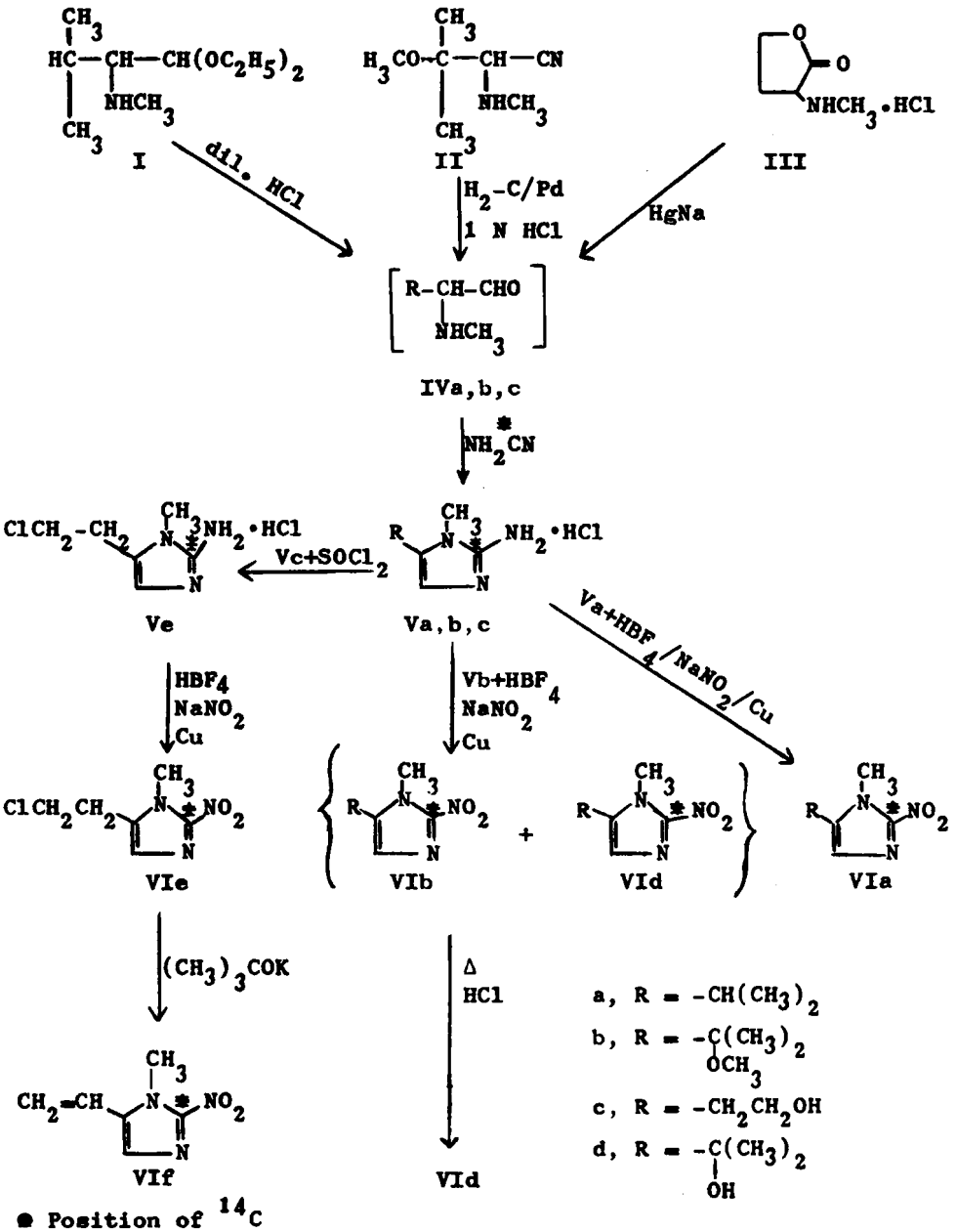
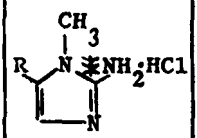
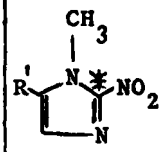

I
II
III
dil. HCl
H_2-C/Pd
1 N HCl
HgNa
IVa,b,c
NH_2CN
Ve
Vc+SOCl$_2$
Va,b,c
Va+HBF$_4$/NaNO$_2$/Cu
HBF$_4$
NaNO$_2$
Cu
Vb+HBF$_4$
NaNO$_2$
Cu
VIe
VIb
VId
VIa
$(CH_3)_3COK$
Δ
HCl
VIf
VId
a, R = -CH(CH$_3$)$_2$
b, R = -C(CH$_3$)$_2$OCH$_3$
c, R = -CH$_2$CH$_2$OH
d, R = -C(CH$_3$)$_2$OH
● Position of ^{14}C

Table I

	Compd.	mmole	Yield %	Total Activity mCi	Radioch. Yield %	Specific activity mCi/mmole	TLC[1] Rf
NH_2-^{14}CN		3.51		1		0.285	
added		8.08		5		0.618	
		10.08		5		0.496	
	Va	1.94	55.3	0.52	52.0	0.268	
	Vb	7.05	87.2	4.3	86.0	0.610	
	Vc	7.6	75.4	3.6	72.0	0.473	
	VIa	0.81	23.0	0.24	24.0	0.296	0.47
	VId	1.96	24.2	1.19	23.8	0.607	0.60
	VIf	1.30	12.8	0.62	12.4	0.476	0.73

R R'

Va = $-CH(CH_3)_2$ VIa = $-CH(CH_3)_2$

Vb = $-C(CH_3)_2$ (OCH_3) VId = $-C(CH_3)_2$ (OH)

Vc = $-CH_2-CH_2OH$ VIf = $-CH=CH_2$

1) Developed with a 4:1 mixture of benzene-MeOH

* Position of ^{14}C

mixture was stirred for 1 hour at room temperature, then refluxed for 1 hour.

After cooling, the desired compound was extracted with water (5 x 10 ml). The aqueous solution was treated with charcoal and evaporated to dryness.

The residue was triturated with Me_2CO and filtered. Yield 1.27 g (6.5 mmole, 85.5%; 3.05 mCi, 84.7%).

1-Methyl-5-(1-methylethyl)-2-nitro-1H-imidazole-(2-^{14}C) (VIa).

A solution of 0.14 g (2 mmole) of $NaNO_2$ in 1 ml of water was added dropwise at -20°C to a stirred solution of 0.33 g (1.9 mmole) of Va in 1.7 ml of 40% HBF_4. Stirring was countinued for 15 min, then the solution was maintained at -10°C and poured in portions into a stirred mixture of 1.33 g (19 mmole) of $NaNO_2$ and 0.4 g of Cu powder in 35 ml of water, while N_2 was bubbled in. After 30 min the insoluble material was filtered off, the solution was brought to pH 2.5 with 10% HCl and allowed to stand for 1 hour under bubbling N_2. The reaction mixture was extracted with AcOEt (5 x 25 ml). The extracts were washed with 10% Na_2CO_3 solution (3 x 20 ml), then with water (2 x 20 ml), dried over Na_2SO_4, and evaporated. The residue was dissolved in a few ml of EtOAc and purified by TLC, developing with a 1:4 mixture of MeOH and benzene.

The 2-nitro-1H-imidazoles-(2-^{14}C), VId and VIe, were prepared as described above, starting with 7.05 mmole of Vb and 6.5 mmole of Ve. The residues obtained from the EtOAc extracts were worked up as follows:

Compound VId was obtained together with a certain amount of VIb (TLC, developed with a 1:9 mixture of MeOH and $CHCl_3$: VId R_f 0.43; VIb R_f 0.70). The crude residue was dissolved in 7 ml of 2 N HCl by heating at 70°C for 5 min. The cooled solution was extracted with EtOAc (5 x 10 ml). The extracts were dried over Na_2SO_4 and evaporated to give a residue which was crystallized from benzene.

Compound VIe was dried in vacuo on P_2O_5. Yield 0.36 g (1.9 mmole, 29.2%; 0.83 mCi, 27.2%; TLC R_f 0.53, developed with a 1:9 mixture of MeOH and $CHCl_3$). It was used directly for the next step.

5-Ethenyl-1-methyl-2-nitro-1H-imidazole-(2-^{14}C) (VIf).

To a cooled solution (10°C) of 0.36 g (1.9 mmole, 0.83 mCi) of VIe in 10 ml of anhydrous benzene, 0.2 g (1.8 mmole) of potassium t-butoxide was added. The reaction mixture was stirred for 45 min at the same temperature and checked by TLC. Since starting materiale was still present, an additional 0.05 g (0.45 mmole) of potassium t-butoxide was added.

Stirring was continued for 1 hour, then the insoluble material was filtered and washed with $CHCl_3$. The soluble material and washing were combined, concentrated to a small volume and purified by preparative TLC (eluting with a 9:1 mixture of $CHCl_3$ and Me_2CO). Obtained 0.2 g (Yield 1.3 mmole, 68.4%; 0.62 mCi, 74.7%). The compound should be protected from light.

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