^{3}H—LABELLING OF BELLADONNA ALKALOIDS BY CATALYSED EXCHANGE WITH MICROWAVE EXCITATION*

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ABSTRACT

The ^{3}H-labelled belladonna alkaloids obtained by catalysed exchange method with microwave excitation was investigated. The specific activities of the labelled products were 16— 32 TBq/mol. More than 90% labelled positions of these ^{3}H-tracers were on phenyl rings. The radiochemical purity and chemical purity of crude products were both in 75— 80%.

Keywords: ^{3}H-NMR Microwave catalysed exchange ^{3}H-anisodamine hydrobromide ^{3}H-anisodine hydrobromide ^{3}H-Atropine sulfate ^{3}H-scopolamine hydrobromide ^{3}H-labelling

1 INTRODUCTION

In recent times, numerous tritiated alkaloids are used as radioactive ligands in studies of the neurotransmitter of the central nervous system[1-2]. However, belladonna alkaloids are the heat or light-sensitive compounds which cannot stand the vigorous experiment conditions. This work exhibits the preparation of belladonna alkaloids, such as ^{3}H-anisodine hydrobromide, ^{3}H-anisodamine hydrobromide, ^{3}H-scopolamine hydrobromide and ^{3}H-atropine sulfate by catalysed exchange method combined with microwave excitation. The specific activity of the tracers obtained by this method was 16— 32 TBq/mol, which are three orders higher in comparison with the results from only microwave excitation or only catalysed exchange[3].

The following scheme outlines the process of this labelling:

a. A molecule of belladonna alkaloids $\xrightarrow{Pt}$ Pt.A* unstable ligand

b. Tritium water $\xrightarrow{\text{Microwave excitation}}$ $^{3}H\cdot + OH\cdot$

c. $^{3}H\cdot + OH\cdot + Pt.A^{*} \xrightarrow{^{3}H\text{-}^{1}H\ \text{exchange}} Pt + H_2O + {}^{3}H\text{-}A^{*}$

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The labelled positions of ^{3}H-belladonna alkaloids determined by ^{3}H-NMR were mostly on the phenyl rings of the molecules. ^{3}H-NMR signal integral area comparison on ^{3}H-NMR spectrum exhibited the radiochemical purity was 75— 80%, and ^{1}H-NMR peaks integral area comparison with the impurity peaks on ^{1}H-NMR spectrum showed the chemical purity of the labelled compounds was 75— 80%[4].

2 EXPERIMENTAL

2.1 Preparation of ^{3}H— labelled belladonna alkaloids

The samples under investigation were anisodine hydrobromide, anisodamine hydrobromide, scopolamine hydrobromide and atropine sulfate. Their purities were checked by ^{1}H-NMR spectroscopy.

Isotope exchange procedure: The materials to be labelled (15 mg) together with a known amount of catalyst (40— 50 mg) that had been freshly reduced with $NaBH_4$ (400 — 500 mg) and 50 μl of HTO (0.93 TBq/ml) were placed in a narrow tube, which was then frozen (liquid N_2) and evacuated, after that, the tube was sealed, then the tube was put into the microwave oven (2450 MHz) for 4 min. After being cooled, the tube was opened and the content was mixed with 1 ml of methanol; the catalyst was filtered off and the solution was washed with 1 ml methanol two times for removing any possible labile tritium, after drying over IR lamp, the substrate was right the ^{3}H-labelled belladonna tracers.

2.2 ^{1}H— NMR and ^{3}H— NMR experimental conditions

Triton and proton nuclear magnetic resonance spectra were obtained using a JEOL FX-100 spectrometer, ^{3}H-NMR spectra (with ^{1}H decoupling) were recorded at 106.19 MHz and ^{1}H spectra were recorded at 99.55 MHz. Each sample prepared by above method was redissolved in deuteriated methanol (to provide for field frequency locking) and a trace of tetramethyl silicane (TMS) was added for ^{1}H reference. The solution to be determined by ^{1}H- and ^{3}H-NMR were sealed in a cylindrical microcelles (~40 μl) under lyophilization (liquid N_2) and inserted into standard NMR tubes (5mm) which were capped and mounted in a spinner adapter for a 5 mm probe. ^{1}H-NMR parameters: rf 99.55 MHz, spectral width 1000 Hz, obset 51.95 kHz, pulse width 31.2 μs (90°), dual pulse order; pulse delay 5 s, pulse interval 1.8 s, data points 8 k. ^{3}H-NMR parameters: rf 106.19 MHz, spectral width 1060 Hz, obset 49.40 kHz, pulse width 5 s (5°), pulse interval 1 s, data points 4 k.

3 RESULTS AND DISCUSSION

Specific activity obtained by the three methods and ^{3}H-NMR results of our products are listed in Table 1. The chemical shift and distribution of labelling on phenyl ring for some ^{3}H-belladonna alkaloids are listed in Table 2. The structure

formulas of belladonna alkaloids are showed in Fig.1.

Table 1

Specific activity and ^{3}H NMR analysis of ^{3}H— belladonna alkaloids

Compound	This method (TBq/mol)	Assignment	Chemical shift (ppm)	^{1}H (%)	Catalysed method (GBq/mol)	Microwave method (GBq/mol)
^{3}H-Anisodine hydrobromide	16.0	phenyl	7.27— 7.36	75	10.8	<1
^{3}H-Anisodamine hydrobromide	18.4	phenyl	7.20— 7.26	75	3.3	<1
^{3}H-Scopolamine hydrobromide	17.5	phenyl	7.17— 7.37	75	3.4	<1
^{3}H-Atropine sulfate	32.8	phenyl	7.23— 7.30	80	0.8	<1

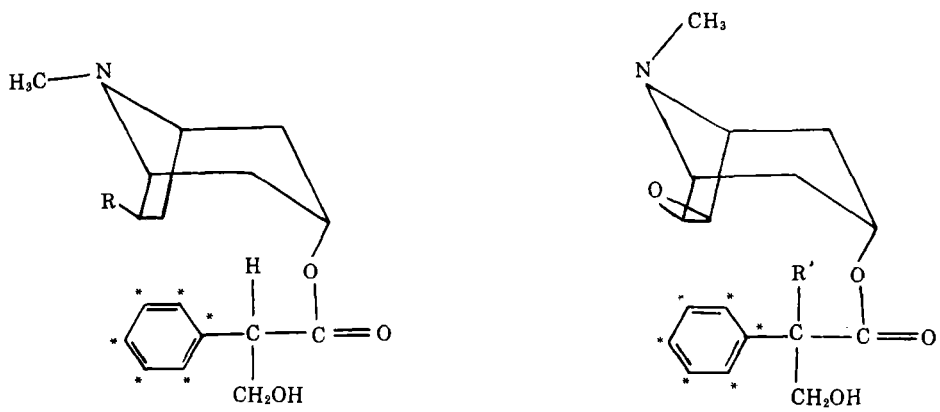
Table 2

Chemical shifts and distribution of labelling on phenyl ring for some ^{3}H— belladonna alkaloids

Compound	Chemical shift (ppm)	Assignment	Distribution of tritium (%)
^{3}H-Anisodine hydrobromide	7.27	Ortho	20
	7.29	Para	20
	7.36	Meta	60
^{3}H-Anisodamine hydrobromide	7.20	Ortho	32
	7.24	Para	48
	7.26	Meta	20
^{3}H-Scopolamine hydrobromide	7.17	Ortho	26
	7.25	Para	51
	7.31	Meta	23
^{3}H-Atropine sulfate	7.23	Ortho	37
	7.28	Para	33
	7.30	Meta	30

^{3}H-NMR analyses: the solutions, which usually contained 0.185— 1.11GBq of radioactivity, were separately loaded into about 3 mm diameter combination tubes. Referencing was to a ghost reference generated from the ^{1}H resonance frequency of the internal standard (measured at 99.55 MHz) by multiplying by the larmor ratio 1.06663974.[5] ^{3}H-^{3}H lines coupling were noticed for the higher radioactivity. Its accurate ^{3}H labelling positions on phenyl ring were assigned by the coupling constants of ortho positions, para positions and meta positions, usually, coupling constants, J Hz, in the monosubstituted benzenes were ortho positions of 7— 10 Hz, para position of 0— 1 Hz and meta positions of 2— 3 Hz, the chemical shifts of ortho positions, para positions and meta positions on ^{3}H-phenyl ring were ortho< para< meta. According to the coupling constants and the chemical shifts and integral area of each chemical shift, the distribution of labelling on phenyl ring were obtained

in Table 2. It can be seen that ring was not uniformly labelled with tritium, the integrated line intensities have given a quantitative tritium distribution on the each site of phenyl ring. All these results showed ^3H NMR spectroscopy is an especially attractive technique for determination of structure and position of H labelled compounds.



I R=OH. ^3H-Anisodamine hydrobromide II R'=OH: ^3H-Anisodine hydrobromide

III R=H: ^3H-Atropine sulfate IV R'=H: ^3H-Scopolamine hydrobromide

* = ^3H

Fig.1 The structure formulas of belladonna alkaloids

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