SYNTHESIS OF SPECIFICALLY-LABELLED TRITIATED BENZIMIDAZOLE CARBAMATES

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SUMMARY

Benzimidazole carbamates are thought to exert their biological activity as anthelmintics by binding to helminth tubulin. To investigate this hypothesis, the synthesis of two specifically-labelled tritiated benzimidazoles, parbendazole and oxibendazole, has been accomplished. These labels were prepared by debromination of the appropriate 5-bromo analogue using sodium borotritide in the presence of palladium chloride. The brominated derivatives were synthesised by the bromination of the benzimidazole using bromine in glacial acetic acid.

Key words: Benzimidazole, tritium, parbendazole, oxibendazole, bromination-debromination

INTRODUCTION

Benzimidazole carbamates (I) comprise a family of heterocyclic compounds which have been shown to possess a broad spectrum of biological activity (1). They are currently used commercially or experimentally as antifungal (2), antitumor (3)

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and anthelmintic (4-9) agents. The postulated reason for this broad spectrum of activity is their ability to bind to tubulin, inhibiting the formation of microtubules which are essential components of eukaryotic cells (1,10). The therapeutic action of individual benzimidazole carbamates is thought to be due to their selectivity of binding to the tubulin of target species (10).

The general method of radioactive labelling of these compounds for metabolic (4), pharmacokinetic (11) and biochemical (12) studies has principally involved the use of ^{14}C, incorporated in the 2-position of the benzimidazole, utilising thiourea as the ^{14}C source. Although adequate for most metabolic and pharmacokinetic studies, the low specific activity obtainable by this route has limited the investigation of the mode of action of these compounds.

Tritiation of only two benzimidazole carbamates has been noted in the literature, although full synthetic details were not specified. [^{3}H]Mebendazole (I, R = COC_6H_5) has been prepared by acid catalysed tritium exchange(13), while [^{3}H]parbendazole (PBZ, I, R=$(CH_2)_3CH_3$) has been labelled in the benzylic position (14).

A general method of synthesis of tritiated benzimidazole carbamates would thus be very useful for studies of binding and mode of action. Investigation of a number of potential routes for the preparation of specifically tritiated benzimidazole carbamates demonstrated that a bromination-debromination method could be used to produce high yields of compound.

Using this procedure, the preparation of two tritiated anthelmintics, parbendazole and oxibendazole (OBZ, R=$OCH_2CH_2CH_3$) has been achieved. These labels have been used to investigate

the selectivity of binding to helminth tubulin (Sangster, Prichard and Lacey - unpublished results).

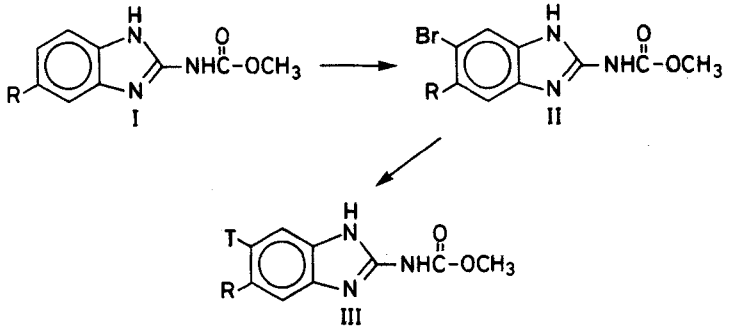

Figure 1. Synthetic route for the preparation of specifically labelled benzimidazole carbamates.

RESULTS AND DISCUSSION

The incorporation of a bromine atom into the benzimidazole at an intermediate step can be readily accomplished in a typical benzimidazole carbamate synthesis by the treatment of a 4-substituted-2-nitroaniline with bromine in acetic acid (Lacey - unpublished results). However, the observation that benzimidazole carbamates could be brominated directly in good yield (Table I) with only minimal work-up negated this approach. The advantages of direct bromination are the ease of label preparation and the higher overall yield.

The bromination of PBZ and OBZ with bromine in glacial acetic acid gave the crude Br-PBZ (II, R=$(CH_2)_3CH_3$) and Br-OBZ (II, R=$OCH_2CH_2CH_3$) in greater than theoretical yield. The dried crude products possess totally different solubility properties from

Table 1. Physical characteristics of bromobenzimidazole carbamates (II)

II, R	% Yield	% Purity	M.pt(°C)	Retention time(sec)	Proton[a] NMR	Mass Spectra[b]
$OCH_2CH_2CH_3$[c]	117	76	257	360[e]		330(10),328(10),298(2),296(1), 288(10),286(9),250(5),249(40), 83(100,base peak),81(95)
$OCH_2CH_2CH_3$	70	d.	277	360[e]	1.06(3,t,6Hz),1.84(2,m),3.90(3,s), 4.08(2,t,6Hz),7.20(1,s),7.66(1,s)	330(98),328(100 base peak),298 (2),296(1),288(55),286(50),
$CH_2CH_2CH_2CH_3$[c]	106	73	266	918[f]		328(20),326(20),296(5),294(3), 284(7),282(8),248(11),247(12), 83(100,base peak),81(95)
$CH_2CH_2CH_2CH_3$	65	d.	262	918[f]	0.96(3,t,6Hz),1.48(4,m),2.84(2,t, 6Hz),3.92(3,s),7.40(1,s),7.64(1,s)	328(90),326(100,base peak),296 (2),294(1),284(12),282(10),248 (7),217(12)

[a] Chemical shift in ppm (no of protons observed coupling pattern, coupling constant). Coupling pattern abbreviations: s: singlet, t: triplet, m: unresolved multiplet.

[b] Mass/charge ratio of molecular ion and fragments (percentage abundance with respect to base peak)

[c] Crude products

[d] Satisfactory elemental analysis ($\pm$ 0.4% of theoretical for C,H,N.)

[e] Corresponding 4-Bromo isomer eluted at 468 sec

[f] Corresponding 4-Bromo isomer eluted at 1080 sec

those of the recrystallised products. The crude products are freely soluble in methanol and ethanol, while being insoluble in acetic acid and dioxane. Although pure by TLC and HPLC, the crude compounds contain only 75% bromo compound as quantitated by HPLC analysis using the recrystallised product as an external standard. This suggests that the crude products precipitate as molecular complexes with bromine which decompose on heating during recrystallisation. Such complexes have been identified with the bromination of other benzimidazoles (15). The presence of bromine was confirmed by the presence of the m/e 81 and m/e 83 ions (H_2Br^+) in the mass spectra of the crude products, which were absent in the spectra of recrystallised products. In all other respects, the mass spectra of the crude and recrystallised Br-BZs gave identical ions. The major chemical ionisation fragments are due to the loss of methanol, (m/e 298, 296 for Br-OBZ and m/e 296, 294 for Br-PBZ), propene (m/e 288, 286 for Br-OBZ), propane (m/e 284, 282 for Br-PBZ) and hydrogen bromide (m/e 249 for Br-OBZ and m/e 247 for Br-PBZ) correspond to the normal fragmentation losses seen with mono-substituted benzimidazole carbamates (PBZ, OBZ and methyl(5(6)-bromobenzimidazol-2yl)-carbamate) (Lacey - unpublished results).

The position of bromination was observed to be exclusively in the 5-position as shown by the absence of any meta or ortho proton coupling in the ^{1}H-NMR spectra of both Br-PBZ (δ=7.40 and 7.64) and Br-OBZ (δ=7.20 and 7.66). The synthesis of the alternative 4-bromo isomers (4-Br PBZ and 4-Br OBZ) confirmed this identification as both derivatives exhibited meta coupling (1.09 Hz and 2.18 Hz) with aromatic proton resonances occurring at

δ=7.13 and 7.30 for 4-Br PBZ and δ=6.93 and 7.02 for 4-Br PBZ (Lacey, unpublished results). The respective isomers of Br PBZ and Br OBZ could readily identified by HPLC (footnote, Table 1).

Based on the synthetic utility and comparative ease of handling of solid tritium sources such as sodium [^{3}H]borohydride over tritium gas, debromination in the presence of palladium catalyst was examined (16,17). Preliminary investigation, using conditions analogous to those of Satoh et al (17) gave complete debromination using palladium chloride with only partial reduction (<50%, as detected by HPLC, E. Lacey unpublished results) for palladium supported catalysts (Pd/C and Pd/$CaCO_3$). A series of trial reactions using 'cold' borohydride showed that the optimum molar ratio was 5:3:1 for sodium borohydride, palladium chloride and bromo-derivative, respectively.

Because of the differing solubility characteristics of the crude and recrystallised brominated compounds, methanol was the solvent of choice for the crude compounds, while 20% DMSO in methanol was used for recrystallised compounds. The use of either crude or recrystallised Br-BZ did not alter the yield of trial 'cold' reactions.

The isolation of the tritiated products was achieved using a silica Sep-Pak (Waters Associates). This method offered the advantage of rapid removal of non-volatile tritium not associated with the required benzimidazole.

Yield and specific activity were determined by dissolving the solids in DMSO (Table 2). The tritiated products were stored as 2mM solutions in DMSO ready for use.

Table 2. Data obtained from tritiated benzimidazole carbamates (III)

	% yield	Mass[a] spect.	Retention time(sec)	% radiochemical purity	Specific activity GBq mmol^{-1}
$OCH_2CH_2CH_3$	55	250(100,Base peak), 218(3),208(22)	252	97.3	25
$CH_2CH_2CH_2CH_3$	66	248(100,Base peak), 216(2),204(12)	576	98.6	17.5

[a] Table 1

CONCLUSION

The use of a direct bromination-debromination procedure for the preparation of specifically-tritiated benzimidazoles has been achieved. This method has considerable advantages with respect to the ease of label preparation, specificity of label incorporation and yield.

EXPERIMENTAL

HPLC analysis of the bromination and debromination reactions was carried out using a Waters Model 6000A solvent delivery system, with a model 440 fixed wavelength detector at 254 nm and a Model U6K manual injection system (Waters Associates). Separation was achieved using a reversed phase C_{18} radial compression cartridge (Radpak B, spherical 10 μm) housed in an RCM-100 radial compression module.

Br-PBZ, Br-OBZ, PBZ and OBZ were eluted with 70% methanol in 0.025M ammonium acetate buffer, pH 6.9 at 1.5 ml/min. Their respective retention times are given in Tables I and II.

Elemental analyses were carried out by Amdel (Melbourne, Australia). Mass spectra were obtained using a Finnegan Quadrupole 3200 Gas Chromatograph/Mass Spectrometer interfaced with a 6110 Data System. Chemical ionisation was achieved using methane gas. NMR spectra were obtained using a Joel FX90Q NMR (90MHz), using a tetramethylsilane (TMS) as reference (δ = 0.00 ppm) in $CDCl_3$. Melting points were obtained using a Mettler FP61 Automatic Melting Point apparatus and are uncorrected. TLC was carried out using Merck silica H60 aluminium plates, with either 20% methanol in chloroform or 10% methanol in chloroform containing 1% formic acid. All solvents were of analytical grade and used without further purification.

General Procedure for Bromination:

The benzimidazole carbamate (0.5gm; 2.5×10^{-3} moles) was dissolved in 10 ml of glacial acetic acid and treated with 1.5 molar equivalents of bromine. The reaction was stirred for 1 hr. The crude product which precipitated was collected, air dried and recrystallised from methoxyethanol.

General Procedure for Tritiation:

The bromobenzimidazole carbamate (10 µmoles) was dissolved in either methanol or 20% DMSO in methanol (400 µl) with 30 µmoles of palladium chloride (as a 0.4% solution in methanol). The solution was treated with 50 µmoles of a 50:50 solution of sodium borohydride with sodium [^{3}H] borohydride in 30 µl of water, stirred for 30 mins, diluted to 2 ml with methanol and filtered through a sintered glass funnel. The solid was washed with a further 10 ml (5x2 ml) of methanol. The filtrate was evaporated to dryness under a stream of nitrogen. The residue was dissolved

in chloroform (10-20 ml) and applied to a Silica Sep-Pak (Waters) prewashed with chloroform. The Sep-Pak was washed with 20 ml of chloroform and the product eluted with 2x10 ml of 10% methanol in chloroform. The eluted compound was evaporated to dryness, weighed and the solid redissolved in 0.5 ml of DMSO to determine the specific activity and yield. The yield was confirmed by quantitation using HPLC against a series of standard solutions of the benzimidazole. Radiochemical purity was determined by TLC using the two systems quoted previously in the text.

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