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Labelled Compounds of Interest as Antitumour Agents - VII[1]. [^{2}H]- and [^{14}C]-Curcumin

Ifat Parveen and Michael D. Threadgill*

Department of Pharmacy & Pharmacology, University of Bath,
Claverton Down, Bath BA2 7AY, U. K.
e-mail: m.d.threadgill@bath.ac.uk

SUMMARY

Curcumin (*E,E*-1,7-di(4-hydroxy-3-methoxyphenyl)-3-hydroxyhepta-1,3,6-trien-5-one) is an investigational cancer chemopreventive agent. Lithium-bromine exchange of 1-bromo-4-(1-ethoxyethyl)-3-methoxybenzene with butyl lithium, quench with O^2HCNMe_2 and acidic work-up gave 4-hydroxy-3-methoxybenz-[^{2}H]-aldehyde ([^{2}H]-vanillin) in 27% yield (based on O^2HCNMe_2). Condensation with pentane-2,4-dione then gave *E,E*-1,7-di(4-hydroxy-3-methoxyphenyl)-3-hydroxy-1,7-di-[^{2}H]-hepta-1,3,6-trien-5-one ([$^2H_2$]-curcumin). Adaptation of the procedures and use of $OH^{14}CNMe_2$ provided [^{14}C]-curcumin (specific radioactivity 12.7 MBq mmol^{-1}) *via* [^{14}C]-vanillin.

Keywords: Curcumin, Vanillin, ^{14}C, ^{2}H, Lithiation, Dimethylformamide

INTRODUCTION

Curcumin **1** is a major constituent and pigment of turmeric, a spice prepared from the dried rhizomes of *Curcuma longa*. It is of great current interest as a chemopreventive agent for cancer[2-5], particularly carcinoma of the colon. Several other pharmacological actions have also been described, including immunomodulatory activity (by inhibition of production of interleukin 12)[6,7], anti-inflammatory activity[8] and inhibition of the integration step of HIV infection[9,10]. At the biochemical level, curcumin inhibits cyclooxygenase 2 (COX2) activity indirectly, though inhibition of phosphorylation of IκB, inhibition of NFκB activation and inhibition of COX2 expression[5,11-14]. The concentrations that elicit these effects *in vitro* are in the range 10-100 μM but there is good evidence to suggest that the bioavailability of curcumin in rodents after oral administration is poor[8,15-17]. Thus it is conceivable that one or more of the metabolites of curcumin are involved in its

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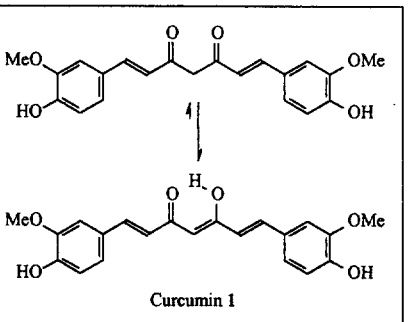
pharmacological activity. The metabolism of curcumin in humans is unknown but, in mice, it undergoes reduction of the heptatriene to give dihydro-, tetrahydro- and hexahydro-curcumin. Parent drug and the reduced species are rapidly glucuronidated prior to excretion[17,18]. In an ongoing collaborative study on the biodistribution and pharmacology of this compound, we required a curcumin labelled with a stable isotope and a sample labelled with a radioisotope. Here we describe our developments of syntheses of a [$^2H_2$] -curcumin and a [^{14}C]-curcumin.



Curcumin 1

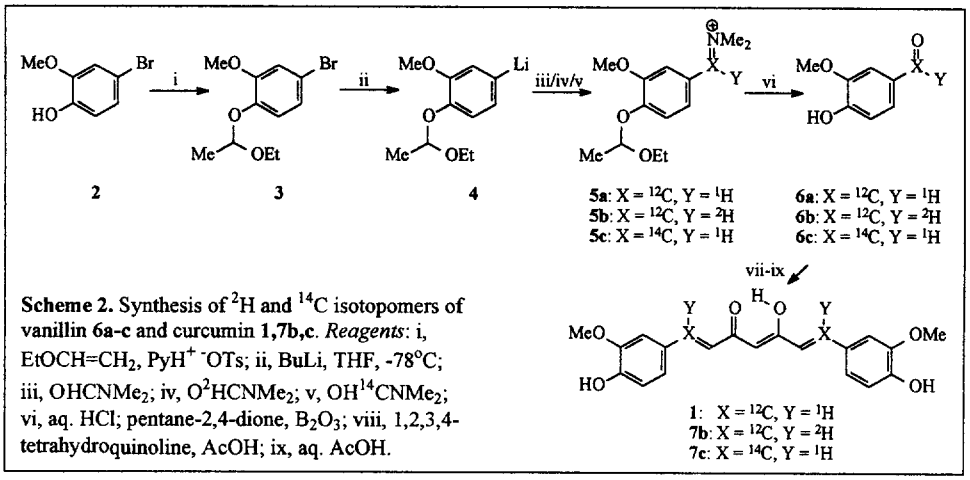
Scheme 1. Tautomeric equilibrium in curcumin 1.

RESULTS AND DISCUSSION

Placement of the isotopic labels in the heptatriene core of curcumin should ensure its retention during metabolic processes. Many of the published syntheses of unlabelled curcumin **1** involve condensation of vanillin **6a** with pentane-2,4-dione under acidic or basic conditions[19-23]. Incorporation of the isotopes in the vanillin component of these condensations, rather than in the pentane-2,4-dione, was selected since *(a)* the appropriate intermediate could be synthesised from a one-carbon unit and *(b)* the intermediate labelled vanillins **6b,c** may also be of use in biochemical studies.

Syntheses of regiospecifically ring-[^2H]-labelled vanillins have been reported[24-26], in which the ^2H was introduced either by direct $^1H \rightarrow {}^2H$ exchange or by replacement of an appropriately located halogen. Ring-[^{14}C]-vanillins are also known[27]. However, adoption of these routes would preclude incorporation of ^{14}C from a one-carbon precursor. More attractive was the prospect of carrying out a halogen $\rightarrow$ lithium exchange on an O-protected 4-bromo-2-methoxyphenol and quench with a formamide isotopomer. In preliminary optimisation experiments, 4-bromo-2-methoxyphenol **2** was protected as its $OSiMe_2Bu^t$ derivative. Br $\rightarrow$ Li exchange with 0.9 to 3.0 molar equivalents of several alkyl lithiums (BuLi, *sec*-BuLi, *tert*-BuLi, MeLi) in dry THF at various reaction temperatures (-78°C to +20°C) for varying periods (10 to 240 min), followed by quench with unlabelled dimethylformamide (DMF) (0.9 to 6.0 equiv.) gave a maximum yield of aldehydes of 21% (based on DMF). The potential isotopic inefficiency of this process, coupled with difficulties in chromatographic purification, indicated that an alternative protecting group should be used.

In their synthesis of 4-hydroxy-3-[$^2H_3$]-methoxybenzaldehyde ([$^2H_3$]-vanillin), Ralph *et al.*[28] protected the OH of 4-bromo-2-[$^2H_3$]-phenol with 1-ethoxyethyl, *i.e.* as an acetal, followed by



Scheme 2. Synthesis of ^{2}H and ^{14}C isotopomers of vanillin **6a-c** and curcumin **1,7b,c**. *Reagents*: i, $EtOCH{=}CH_2$, $PyH^+ {}^-OTs$; ii, BuLi, THF, -78°C; iii, $OHCNMe_2$; iv, O^2HCNMe_2; v, $OH^{14}CNMe_2$; vi, aq. HCl; pentane-2,4-dione, B_2O_3; viii, 1,2,3,4-tetrahydroquinoline, AcOH; ix, aq. AcOH.

lithiation and quench with excess unlabelled DMF. For incorporation of isotope in the aldehyde from a one-carbon unit, this procedure must be optimised for yield based on the DMF, rather than on bromoarene. Unlabelled 4-bromo-2-methoxyphenol **2** was protected as the acetal **3** (Scheme 2). Bromine → lithium exchange with butyl lithium at low temperature (giving anion **4**) was followed by quench with unlabelled DMF. Acid hydrolysis then cleaved both the iminium and the protecting acetal in the intermediate **5a** to give vanillin **6a**. The results of this optimisation study with acetal protection are shown in Table 1. Entry 1 shows that an excess of the alkyl lithium is required. With butyl lithium fixed at 3.0 equiv., entries 2 and 3 show the effect of varying the amount of DMF; quench with DMF (3.0 equiv.) gave the higher yield based on **3** but use of only 2.0 equiv. gave the potentially more isotopically efficient yield from DMF. Entries 3-5 confirm the optimum time for bromine-lithium exchange as 2 h at -78°C, giving a maximum yield (32%) of **6a** from DMF.

Application of the optimised litiation conditions, quench with $O^2HCN(C^2H_3)_2$ (2.0 equiv.) and the standard hydrolytic work-up, gave deuteriovanillin **6b** in good yield (55% from **3**). Similar quench of **4** with $OH^{14}CNMe_2$ and hydrolysis of intermediate **5c** provided [^{14}C]-vanillin **6c** in 45% chemical yield (from **3**) and 8.1% radiochemical yield in a one-pot process. This apparently lower efficiency of isotopic incorporation is attributed to mechanical difficulties in delivery of the $OH^{14}CNMe_2$ from the container in which it was supplied.

Table 1. Optimisation of yields of unlabelled vanillin **6a** with variation of the conditions of lithium-halogen exchange in **3** and quench of the intermediate anion **4** with $OHCNMe_2$.

Entry	BuLi equiv.	Lithiation time (min)	DMF equiv.	Yield (from ArBr) (%)	Yield (from DMF) (%)
1	1.5	60	1.5	0	0
2	3.0	60	2.0	55	27
3	3.0	60	3.0	70	23
4	3.0	120	2.0	64	32
5	3.0	240	2.0	45	22

In the condensation of vanillin with pentane-2,4-dione, it is essential that the bis-enol(ate) of the β-diketone is formed, to obviate reaction at the central CH_2. Several groups have complexed the dione with boric acid (H_3BO_3)[20,22] or with borate esters ($B(OR)_3$)[19,29] for this purpose. However, preliminary experiments with unlabelled material showed these methods to be lower yielding in forming curcumin than use of the more reactive boron oxide. Thus pentane-2,4-dione was complexed with B_2O_3 at high temperature. Addition of the vanillin isotopomers **6b,c** and prolonged heating in the presence of acetic acid and the weak secondary amine base 1,2,3,4-tetrahydroquinoline afforded the required isotopomers of curcumin **7b,c** after decomplexation with aqueous acetic acid. Prolonged reaction time (24 h) at 110°C was essential for the high (81%) radiochemical yield of **7c** achieved for this step.

CONCLUSION

A rapid synthetic route to isotopically labelled curcumin isotopomers has been developed. This has enabled preparation of the dideuteriocurcumin **7b** and of the radiolabelled curcumin **7c**, starting from a simple and readily available one-carbon precursor, DMF. The overall radiochemical yield from [^{14}C]-DMF to **7c** was 6.5%, giving material of specific activity 12.7 MBq mmol^{-1}. Applications of these isotopomers of curcumin in gastrointestinal and biodistribution studies of this chemopreventive agent will be published elsewhere.

EXPERIMENTAL

[$^2H_7$]-Dimethylformamide was purchased from Aldrich. Dimethyl-[^{14}C]-formamide was purchased from Sigma. A Jeol EX400 instrument furnished the NMR spectra. Mass spectra were obtained in the fast atom bombardment (FAB) mode. Melting points are uncorrected. Solvents were evaporated under reduced pressure. The chromatographic stationary phase was silica gel.

(±)-1-Bromo-4-(1-ethoxyethyl)-3-methoxybenzene (3). 4-Bromo-2-methoxyphenol **2** (13.63 g, 67 mmol) was stirred with pyridinium 4-methylbenzenesulphonate (60 mg) in ethoxyethene (25 mL) and CH_2Cl_2 (13 mL) for 16 h. The evaporation residue, in EtOAc, was washed with aq. $CuSO_4$ (2 ×), aq. $NaHCO_3$ (2 ×) and aq. NaCl and was dried ($MgSO_4$). Evaporation and chromatography (EtOAc / hexane 1:5) gave **3** (18.23 g, 99%) as a colourless oil (lit.[28] oil (1-bromo-4-(1-ethoxyethyl)-3-[$^2H_3$]-methoxybenzene)): δ_H ($(CD_3)_2SO$) 1.08 (3 H, t, J = 7.0 Hz, CH_2CH_3), 1.36 (3 H, d, J = 5.3 Hz, $CHCH_3$), 3.48 (1 H, dq, J = 9.5, 7.0 Hz) and 3.67 (1 H, dq, J = 9.5, 7.0 Hz) (CH_2), 3.78 (3 H, s, OCH_3), 5.33 (1 H, q, J = 5.3 Hz, CH), 6.99 (1 H, d, J = 8.6 Hz, Ar 5-H), 7.03 (1 H, dd, J = 8.6, 2.0 Hz, Ar 6-H), 7.16 (1 H, d, J = 2.0 Hz, Ar 2-H).

4-Hydroxy-3-methoxybenz-[^{2}H]-aldehyde (6b). Butyl lithium (1.6 M in hexanes; 3.6 mL, 5.6 mmol) was added to **3** (525 mg, 1.9 mmol) in dry THF (3.0 mL) at -78°C under Ar; the mixture was stirred at -78°C for 2 h; di-[$^2H_3$]-methyl-[^{2}H]-formamide (281 mg, 3.5 mmol) was added and the mixture was stirred at -78°C for 2 h and at 20°C for 16 h under Ar. Aq. HCl (3 M, 7.0 mL) was added under Ar and the mixture was stirred for 30 min before being extracted with EtOAc. The organic phase was washed with aq. $NaHCO_3$ (2 ×) and aq. NaCl and was dried ($MgSO_4$). Evaporation and chromatography (EtOAc / hexane 1:4) gave **6b** (161 mg, 55%) as off-white crystals: mp 72-75°C (lit.[29] mp 76.5-78°C (unlabelled material)); δ_H ($CDCl_3$) 3.91 (3 H, s, CH_3), 7.01 (1 H, d, J = 8.6 Hz, 5-H), 7.40 (2 H, m, 2,6-H_2); δ_C ($CDCl_3$) 55.97 (CH_3), 108.95 (2-C), 114.46 (5-C), 127.37 (6-C), 129.59 (1-C), 147.24 (4-C), 151.86 (3-C), 190.64 (t, J = 26.4 Hz, C^2HO).

***E,E*-1,7-Di(4-hydroxy-3-methoxyphenyl)-3-hydroxy-1,7-di-[^{2}H]-hepta-1,3,6-trien-5-one ([$^2H_2$]-curcumin) (7b).** Pentane-2,4-dione (43 mg, 430 μmol) was heated with B_2O_3 (117 mg, 1.7 mmol) and dry DMF (90 μL) at 120°C for 15 min. [^{2}H]-Vanillin **6b** (134 mg, 876 μmol) in dry DMF (300 μL) was added and the mixture was stirred at 120°C for 15 min. 1,2,3,4-Tetrahydroquinoline (10.6 mg, 80 μmol) and acetic acid (26 mg, 437 μmol) were added and the mixture was stirred at 110°C for 8.5 h. Aq. acetic acid (20%, 5 mL) was added and the mixture was stirred at 20°C for 16 h. Evaporation and chromatography (EtOAc / hexane 1:1) gave [$^2H_2$]-curcumin **7b** (70 mg, 43%) as an orange-brown solid: mp 183-185°C (lit.[31] mp 185-186°C (unlabelled material)); δ_H ($(CD_3)_2SO$) 3.84 (6 H, 2 × Me), 6.06 (1 H, s, 4-H), 6.75 (2 H, s, 2,6-H_2), 6.82 (2 H, d, J = 8.1 Hz, 2 × Ar 5-H), 7.16 (2 H, dd, J = 8.1, 1.5 Hz, 2 × Ar 6-H), 7.33 (2 H, d, J = 1.5 Hz, 2 × Ar 2-H), 9.67 (2 H, br, 2 × ArOH); δ_C 56.46 (2 × Me), 101.46 (4-C), 111.97, 116.33, 121.60, 123.71, 126.88, 140.92 (br, 1,7-C_2), 148.54, 149.89, 183.67 (3,5-C_2); MS (FAB +ve ion) *m/z* 372.1544 (M + H) ($^{13}C_1{}^{12}C_{20}{}^2H_2{}^1H_{19}{}^{16}O_6$ requires 372.1464), 371.1489 (M + H) ($^{12}C_{21}{}^2H_2{}^1H_{19}{}^{16}O_6$ requires 371.1464), 370.1406 (M) ($^{12}C_{21}{}^2H_2{}^1H_{18}{}^{16}O_6$ requires 370.1386).

4-Hydroxy-3-methoxybenz-[^{14}C]-aldehyde (6c). Butyl lithium (1.6 M in hexanes; 1.0 mL, 1.6 mmol) was added to **3** (141.2 mg, 0.52 mmol) in dry THF (1.5 mL) at -78°C; the mixture was stirred at -78°C for 2.5 h under Ar; dimethyl-[^{14}C]-formamide (3.0 mg, 41 μmol, 18.5 MBq) and dry DMF (57 mg, 776 μmol) in dry THF (750 μL) were added and the mixture was stirred at -78°C for a further 2 h and at 20°C for 12 h under Ar. Aq. HCl (2.0 M, 2 mL) was added and the mixture was stirred for 30 min before being extracted with EtOAc (2 ×). The combined extracts were washed with aq. $NaHCO_3$ and with aq. NaCl and were dried ($MgSO_4$). Evaporation and chromatography (EtOAc / hexane 1:1) gave **6c** (35.6 mg, 45% chemical yield; 1.49 MBq, 8.1% radiochemical yield, specific activity 6.36 MBq mmol^{-1}) as a pale buff solid chromatographically identical to **6a**.

***E,E*-1,7-Di(4-hydroxy-3-methoxyphenyl)-3-hydroxy-1-[^{14}C]-hepta-1,3,6-trien-5-one ([^{14}C]-curcumin) (7c).** Pentane-2,4-dione (11.7 mg, 117 μmol)) was heated with B_2O_3 (28.0 mg, 402 μmol) and dry DMF (223 μL) at 120°C for 15 min. [^{14}C]-Vanillin **6c** (35.4 mg, 233 μmol, 1.48 MBq) in dry DMF (500 μL) was added and the mixture was stirred at 120°C for a further 15 min. 1,2,3,4-Tetrahydroquinoline (2.7 mg, 20 μmol) and AcOH (7.9 mg, 131 μmol) were added and the mixture was stirred at 110°C for 24 h. Aq. AcOH (20%, 1.0 ml) was added and the mixture was stirred at 20°C for 24 h. Evaporation and chromatography (EtOAc / hexane 1:4 → EtOAc → EtOAc / MeOH 19:1) gave **7c** (34.7 mg, 81% chemical yield; 1.20 MBq, 81% radiochemical yield, specific radioactivity 12.7 MBq mmol^{-1}) as an orange-brown solid chromatographically identical to **1**.

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