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Research Article

A convenient synthesis of ^{14}C-labelled resveratrol

Dongli Zeng, Qixi Mi, Hongfang Sun and Haifang Wang*
Department of Chemical Biology, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China

Summary

Resveratrol (*trans*-3,4′,5-trihydroxystilbene) is a naturally occurring phytoalexin and polyphenol existing in grapes and various plants. It shows remarkable beneficial bioactivities in the prevention of cancer, inflammation and platelet aggregation, etc. This paper reports the synthesis of [β-^{14}C]-*trans*-resveratrol using ^{14}C-formic acid (exchanged with sodium ^{14}C-formate) and 3,5-dihydroxybenzoic acid as the starting materials. [^{14}C-formyl]-4-methoxybenzaldehyde and diethyl 3,5-dimethoxy benzylphosphonate reacted following the Wittig–Horner reaction to give *trans*-3,4′,5-[β-^{14}C]-trimethoxystilbene. The final product was obtained through the demethylation of *trans*-3,4′,5-[β-^{14}C]-trimethoxystilbene and identified by TLC and UV spectroscopy. Adoption of the whole procedure provided ^{14}C-resveratrol with a specific radioactivity of 40.8 μCi/mmol, chemical yield of 15.3% and radiochemical yield of 12.5%. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: ^{14}C-labelling; anisaldehyde; *trans*-resveratrol; natural product synthesis

Introduction

Resveratrol (*trans*-3,4′,5-trihydroxystilbene) **1**, a naturally occurring phenolic antioxidant and phytoalexin, has become a compound of intense interest because of its suggested health promoting roles. It can inhibit the low-density lipoprotein (LDL) oxidation and platelet aggregation and is a factor associated with lowering the risk of coronary heart disease (CHD).[1,2] Recently, its potential cancer chemopreventive properties have been recognized owing to its capacity in inhibiting diverse cellular events associated with the three major stages of carcinogenesis: initiation, promotion and proliferation.[3] It also has a direct antiproliferative effect on human breast epithelial cells and consequently is a potential chemopreventive agent for breast cancer.[4] Although resveratrol was reported to suppress the growth of cancer

*Correspondence to: H. Wang, Department of Chemical Biology, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China. E-mail: raindrop@pku.edu.cn

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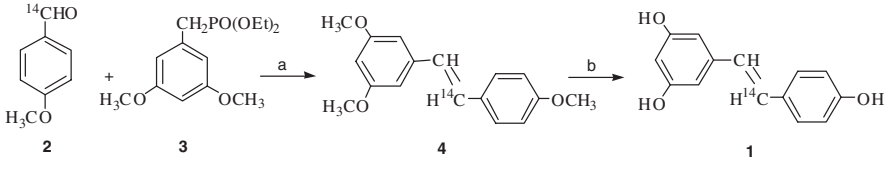
cell lines and decrease the tumor growth in animal models, the mechanism of its antiproliferative activity has not been clearly established.

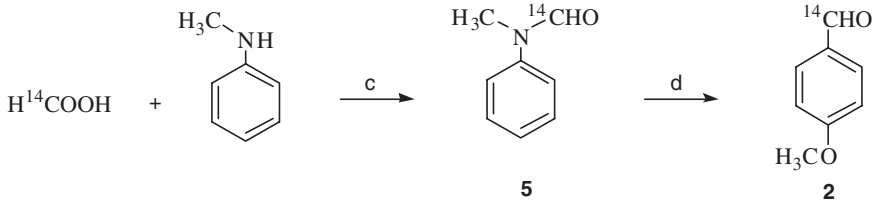
In view of these effects, resveratrol may have a wide range of target organs. Thus, its absorption, distribution, metabolism and excretion *in vivo* are considered to be primarily selective issues. Soleas *et al*. reported that oral administration of resveratrol led to an important absorption resulting in a significant plasma bioavailability in rat.[5] Whether resveratrol is metabolized to other compounds is not however known. In order to carry out our accelerator mass spectrometry (AMS) study on the biodistribution and pharmacokinetics of resveratrol, a ^{14}C-labelled compound is required. In 2003, Vitrac *et al*. reported a biosynthesis of ^{14}C labelled resveratrol.[6] However, this method required professional skills in biology cell culture and some specific equipment. It consumed a large amount of radioactive material and the radiochemical yield was very low due to the early introduction of the labelled atom and multitudinous steps. Here we present a total chemical synthesis of ^{14}C-resveratrol that is convenient and easy to perform. Only a small amount of labelled agent is required and the product was obtained in fairly good radiochemical yield.

Results and discussion

To ensure the retention of resveratrol during the metabolism processes, the radioisotope ^{14}C should be located in the alkene carbon. The majority of previously published syntheses of unlabelled resveratrol involved the condensation of aldehyde and phosphonate via the Wittig–Horner reaction. We planned to synthesize the hydroxystilbene by demethylation of the corresponding methoxystilbene **4**, which was prepared by the Witting–Horner reaction of methoxybenzaldehyde with methoxybenzylphosphonate (Scheme 1). Incorporation of the radioisotope in the aldehyde component rather than in the phosphonate was selected since (a) the appropriate intermediate could be synthesized from a one-carbon unit in very high yield, (b) the labelled intermediate *p*-anisaldehyde may also be of use in other biochemical studies and (c) the starting labelled material, formic acid, could be obtained by exchanging the formic anion between unlabelled anhydrous formic acid and



Scheme 1. (a) NaH, then 3, in dry THF, 94%; (b) BBr_3, dry CH_2Cl_2, 98% (crude), 37% (recrystalized)



Scheme 2. (c) DCC, dry CH_2Cl_2, 100%; (d) *p*-$MeOC_6H_4MgBr$, then saturated NH_4Cl solution, in dry THF, 44%

labelled sodium formate, the latter being one of the most common labelled organics and readily available.

p-Anisaldehyde (4-methoxybenzaldehyde) **2** was chosen as an important intermediate reagent (Scheme 2). Synthesis of ^{14}C-labelled anisaldehyde has not been previously reported. Several methods can be adopted to synthesize anisaldehyde, however, most of them preclude the incorporation of ^{14}C from a one-carbon precursor. Besides the Blanc & Sommelet reactions and Vilsmeier–Haack reaction, the Bouveault reaction provides an alternative route to introduce a labelled carbon atom into the molecular skeleton. In preliminary optimization experiments, we have tried all three mentioned routes. In the Blanc chloromethylation reaction and successive Sommelet reaction, a chloromethyl group was first introduced into the aromatic ring of anisole by treatment with formaldehyde and hydrogen chloride, then anisaldehyde was prepared from the above alkyl halide by treatment with hexamethylenetetramine to yield a quaternary salt and followed by mild hydrolysis. When the procedure was optimized for yield based on formaldehyde, 1.0 to 2.0 molar equivalents of anisole in benzene at various reaction temperatures (−15–70°C) by varying the bubbling time of dry hydrogen chloride gas (30 min to 6 h), under the catalysis of $ZnCl_2$ or without the catalyst, followed by appropriate work-up, gave a maximum yield of benzyl chloride of 25%. And after the Sommelet reaction (with an average yield of 80–90%), the total yield of anisaldehyde is less than 20%. The inefficiency of this process, coupled with difficulties in purification of the unstable benzyl chloride, indicated that another method should be used.

Besides formaldehyde, another compound, *N*-methylformanilide (MFA) **5** was taken into consideration. [^{14}C-formyl]-*N*-methylformanilide could be used in the Vilsmeier–Haack reaction and Bouveault reaction as an intermediate for introducing the labelled aldehyde group into reactive aromatics. MFA was available by simple synthesis from formic acid in very high yield. In the Vilsmeier–Haack reaction, anisole was formylated by MFA and phosphorous oxychloride. In the preliminary investigations, 1 mmol of MFA was used. However, we still could not obtain a satisfactory yield by varying the

experimental conditions including the quantity of phosphorous oxychloride (from 1.0 to 2.0 equivalent moles) and anisole (equivalent or as solvent), reaction temperatures (−20–80°C), holding periods (30 min to 18 h) and reaction modes (by thermal heating or ultrasonic irradiation).[7] One of the by-products, *o*-anisaldehyde (2-methoxysaldehyde) also caused difficulties and this route was therefore given up.

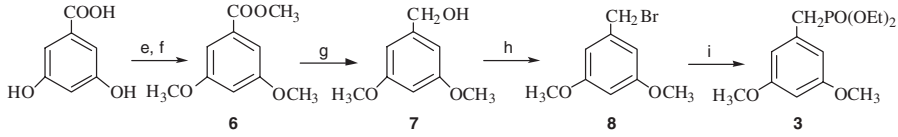
In the Bouveault reaction, substituted formylamine reacted with the Grignard reagent, and following acid hydrolysis afforded anisaldehyde. For incorporation of isotope in the aldehyde from a one-carbon unit, this procedure must be optimized for yield based on the substituted formylamine, which was MFA in our scheme, rather than bromoanisole. The optimization study showed that the MFA would not be used to completion unless excess Grignard reagent was prepared. Further optimization experiments gave the best proportion of these two reagents (shown in Table 1). Reactions in THF gave higher yields than in Et_2O, because the intermediate adduct was only soluble in the former. Entries 1–3 showed that at least 1.5 equiv. Grignard reagent was required. With MFA fixed at 1.0, entries 4–6 confirm the optimum proportion of Bouveault reaction as 1.55:1, giving a maximum yield (45%) of anisaldehyde from MFA.

MFA was prepared from formic acid and *N*-methylaniline. Applying *N*,*N*′-dicyclohexylcarbondiimide (DCC, 1.0 equiv.) as dehydration reagent in CH_2Cl_2 provided a much better yield (>95%) than traditional azeotropic drying methods (< 70%). Exchange between the labelled sodium formate and unlabelled formic acid would introduce the ^{14}C atom into the reaction system. Starting from $Na^{14}COOH$ with these optimized conditions, *p*-anisaldehyde **3** was prepared in good yield (44% from formic acid).

Dimethyl 3,5-dimethoxybenzylphosphonate **3** was prepared from 3,5-dihydroxybenzoic acid as outlined in Scheme 3. This synthesis was as reported in the literature.[8] We used methyl as protecting groups for phenols instead of

Table 1. Optimization of yield of unlabelled *p*-anisaldehyde

				In THF		In Et_2O	
Entry	Mg equiv.	BrPh equiv.	MFA equiv.	Yield from MFA (%)	Residual status (Yes/No)	Yield from MFA (%)	Residual status (Yes/No)
1	1.00	1.00	1.00	<10	Yes	<10	Yes
2	1.20	1.20	1.00	12	Yes	10	Yes
3	1.40	1.40	1.00	25	Yes	20	Yes
4	1.50	1.50	1.00	38	No	25	Yes
5	1.550	1.550	1.00	45	No	25	Yes
6	1.60	1.60	1.00	40	No	25	Yes
7	1.80	1.90	1.00	36	No	25	Yes
8	2.00	2.20	1.00	35	No	25	No



Scheme 3. (e) Me_2SO_4, NaOH; (f) $SOCl_2$, then MeOH, 80%; (g) $LiAlH_4$, dry ethyl ether, 92%; (h) PBr_3, dry CH_2Cl_2, 94%; (i) $P(OEt)_3$, 130°C, 95%

original benzyl groups and obtained a higher total yield (> 60%) than that reported in the literature.

In the condensation of *p*-anisaldehyde **2** with dimethyl 3,5-dimethoxybenzylphosphonate **3**, a slight excess of phosphonate **3** was used. When this Wittig–Horner reaction was performed at relatively low temperature (0°C) and followed by proper work-up, only *trans*-3,4′,5- trimethoxystilbene was obtained (HPLC results). During the final deprotective step, the yield of crude resveratrol was over 95% while the final product yield was much lower (38%). This decrease was attributed to the loss in the micro-recrystalization step.

Experimental

[^{14}C] Sodium formate, specific activity 53 mCi/mmol, ethanol–water (7:3) solution (0.1 mCi/ml), was purchased from Movarek Products Inc. and was used without further purification. Tetrahydrofuran (THF), dichloromethane (CH_2Cl_2) and diethyl ether (Et_2O) were dried and redistilled before use. Anhydrous formic acid and *N*-methylaniline were purified before use. ^{1}H-NMR spectra were measured in $CDCl_3$ or d_6-DMSO solutions. Mass spectra (MS) and high resolution mass spectra (HRMS) were obtained in the electronic impact (EI) mode. The stationary phase of the thin layer chromatograph (TLC) was silica gel 254. Some spectral data of the intermediates were obtained by using unlabelled compounds in the cold experiments. The final labelled resveratrol was characterized by TLC and UV spectroscopy.

[^{14}C-formyl]-N-methylformanilide **5**.

500 µl of ethanol–water (7:3) solution of [^{14}C] sodium formate (0.1 mCi/ml) was placed in a 5-ml flask and evaporated to dryness by N_2 bubbling leaving solute on the wall of the flask. Dry CH_2Cl_2 (1 ml) and anhydrous formic acid (as carrier, 38 µl, 1.0 mmol) were added. The mixture was sonicated for 20 min in an ultrasonic cleaner at room temperature to dissolve the labelled formate and promote the isotope exchange. Thereafter, freshly distilled *N*-methylaniline (110 µl, 1.0 mmol) was added into the flask. DCC (*N*,*N*'-dicyclohexylcarbondiimide) (206 mg, 1.0 mmol) in dry CH_2Cl_2 (1 ml)

was added dropwise while stirring in an ice bath to prevent the decomposition of formic acid. Then the isolated mixture was stirred at room temperature for 2 h, resulting in a milky suspension. The resulting mixture was filtered through a fritted glass funnel and the precipitate washed with a little CH_2Cl_2; the combined filtrates were first washed with 10% HCl (1 ml × 2) and then 1% $NaHCO_3$ (1 ml × 1), dried over anhydrous Na_2SO_4 and evaporated, giving 142 mg (100%) of a pale yellow oil, a crude product of [^{14}C-formyl]-*N*-methylformanilide (MFA).

^{1}H NMR(BRUKER ARX400, $CDCl_3$, δ_{ppm}) 8.48 (s, 1 H, -CHO); 7.42 (t, 2 H, Ph–H); 7.29 (d, 1 H, Ph–H); 7.18 (d, 2 H, Ph–H); 3.33(s, 3 H, -CH_3)

MS (EIMS): *m*/*e* calculated: 135, 106, 94 found: 135, 106, 94.

[^{14}C-formyl]-4-methoxybenzaldehyde **2**

Magnesium (Mg) turnings (37.2 mg, 1.55 mmol), *p*-methoxy bromobenzene (195 µl, 1.55 mmol) and freshly dried THF (1.5 ml, as solvent) were placed into a dry, 5 ml flask equipped with a condenser connected to a calcium chloride tube and an additional funnel. The contents were stirred at room temperature and the Grignard reaction commenced after ca 10 min. When all of magnesium was dissolved, the mixture was cooled to 0°C and MFA **5** (product of last step, 142 mg) in dry THF (1 ml) was added dropwise into the flask via the funnel. Then the reaction mixture was stirred at room temperature for another hour, and the reaction was quenched with 1.5 ml of saturated ammonium chloride solution. The two phases were separated, and the aqueous phase was extracted with ethyl ether (2 ml × 2). The organic phases were combined and condensed to give a heavy yellow oil. After dissolving the oil in 0.5 ml of 95% ethanol, 0.2 g $NaHSO_3$ was added in 6 drops of water into this solution and a slightly white glossy precipitate formed. The precipitate was filtered, washed with ice-cold ethanol, and then hydrolyzed using saturated $NaHCO_3$ solution (3 ml). The mixture was extracted with Et_2O (2 ml × 3), then the combined organic layers were dried over $MgSO_4$ and the solvent evaporated off to give 59 mg (44%) of *p*-anisaldehyde **2** as a light yellow oil with fragrant odour.

^{1}H NMR (Varian 300 MHz, 25°C, $CDCl_3$, δ_{ppm}): 9.89 (s, 1 H, -CHO); 7.85 (d, 2 H, Ph–H); 7.01 (d, 2 H, Ph–H); 3.90 (s, 3 H, -OCH_3)

MS (EIMS): *m*/*e* calculated: 135, 111, 77 found: 135, 111, 77.

[β-^{14}C]-3,4′,5-trimethoxystilbene **4**

Sodium hydride (0.2 g) was added to a well-stirred solution of phosphonate **3** (130 mg, 0.45 mmol) in dry THF (1 ml) at –10°C. After 30 min, *p*-anisaldehyde **2** (0.44 mmol) in THF (1 ml) was added dropwise, and the reaction mixture was stirred at 0°C for over 10 h. The excess of sodium hydride was quenched with several drops of ice cool water, followed by addition of 10% HCl to

adjust the pH to about 7. The product was extracted with ethyl acetate (2 ml × 2) and the organic layer washed with a saturated solution of sodium chloride (2 ml × 1). The ethyl acetate layer was concentrated to a yellow oil. On adding methanol an indissolvable white oil was formed and after discarding it the residue was crystallized in methanol–water and a white solid precipitated at low temperature. On separation 110 mg (0.41 mmol, 94%) of 3,4′,5-trimethoxystilbene **4** was obtained.

m.p.–55–57°C, lit. 56–57°C.

^{1}H NMR (BRUKER ARX400, $CDCl_3$, δ_{ppm}) 7.46 (d, 2 H, Ph–H); 7.02 (s, 1 H, -CH=); 6.92 (s, 1 H, Ph–H); 6.90 (d, 2 H, Ph–H); 6.65 (s, 2 H, Ph–H), 6.37 (s, 1 H, -CH=); 3.83 (s, 9 H, $-OCH_3$)

MS (EIMS): *m*/*e* calculated: 270 found: 270.

[β-^{14}C]-3,4′,5-trihydroxystilbene **1**

Boron tribromide (3 mmol, 3 ml of 1 M solution in CH_2Cl_2) was slowly added to a well stirred solution of [*β*-^{14}C]-3,4′,5-trimethoxystilbene **4** (110 mg, 0.41 mmol) in CH_2Cl_2 (7 ml) in an ice bath. The mixture was stirred at 0°C for 30 min and left to stand at room temperature for another 30 min, and then poured into 10 ml of ice-water, and the mixture stirred until the red color disappeared. The mixture was centrifuged at 5000 rpm at 4°C for 5 min. The solid was separated and recrystallized in ethanol–water to give 35 mg of resveratrol **1** (0.15 mmol, 38%).

m.p.– 254–257 °C, lit. 256°C, 260–261°C

^{1}H NMR (Varian 300 MHz, d_6-DMSO, δ_{ppm}) 9.55 (s, 1 H); 9.19 (s, 2 H); 7.39 (d, 2 H, 2′,6′-H); 6.93 (d, 1 H, -CH=); 6.80 (d, 1 H, -CH=); 6.75 (d, 2 H, 3′,5′-H); 6.38 (s, 2 H, 2,6-H), 6.11 (s, 1 H, 4-H)

MS (EIMS): *m*/*e* calculated: 288.1 found: 288; (HRMS): *m*/*e* calculated: 228.07864 found: 228.07776.

Conclusion

A convenient and easily performed method for the chemical synthesis of ^{14}C-labelled resveratrol **1** has been developed. This has enabled the preparation of resveratrol **1** starting from a simple and readily available one-carbon precursor, H^{14}COONa. The overall chemical yield from formic acid was 15.3%; the radiochemical yield from [^{14}C] sodium formate was 12.5%. 6.3 μCi (0.23 MBq) of ^{14}C-labelled resveratrol was obtained with a specific activity of 40.8 μCi/mmol (1.5 MBq/mmol).

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