Expedient Deuterolabeling of Polyphenols in Ionic Liquids−DCl/D$_2$O under Microwave Irradiation

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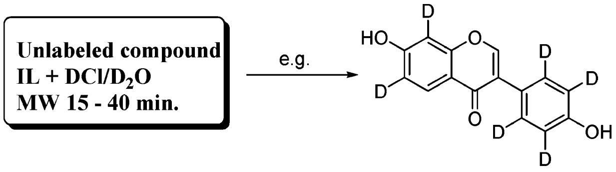

Postsynthetic regioselective aromatic ring H/D exchanges in polyphenolic compounds are rapidly performed in high yields and isotopic purities in ionic liquid−DCl/D$_2$O under microwave irradiation. Other C−H bonds, including benzylic and lactone α-carbonyl sites, are not affected.

Introduction

Biologically active polyphenolic compounds are widely found in plants and food products. In the past years, the possible role of certain naturally occurring polyphenols, e.g., isoflavonoids and lignans (Figure 1), in preventing hormone-dependent diseases has been recognized.[1] Synthetic methods for isotopically labeled analogues of these compounds are therefore required. The labeled analogues are used as internal standards for quantitation from biological samples, screening of biological activities, determining the biosynthesis and metabolic pathways, as well as elucidation of mass spectral fragmentation.[2,3]

In general, deuterium-labeling procedures of polyphenols rely on electrophilic aromatic H/D exchange reactions catalyzed by acids or occasionally by bases. Examples include matairesinol-d_6, which has been prepared with $D_3PO_4{\cdot}BF_3$ in 1 day[4] or D_3PO_4 in 3 days.[5] Similarly, daidzein-d_4 has been produced in $D_3PO_4{\cdot}BF_3/D_2O$ in 3 days[6] or in CF_3COOD in 9 days,[7] while production of daidzein-d_6 required autoclave conditions for 7 days in $D_3PO_4{\cdot}BF_3/D_2O$.[8] Recently, CF_3COOD was reported to allow deuteration of isoflavones in 15 h under microwave irradiation.[9] Thus, although these methods mostly offer good yields and high isotopic purity, very long reaction times are often required, partly due to the poor solubility of polyphenolics in water and other ordinary polar solvents. In contrast to these regioselective polydeuterations of aryl rings of phenolic compounds, a recent paper reports the perdeuteration of alkyl-substituted aromatics.[10] For example, all C−H sites in 2-*n*-propylphenol were indiscriminately deuterated in 24 h by H_2 and D_2O at 180 °C with a Pd/Pt catalyst to give the d_{11} isotopologue.

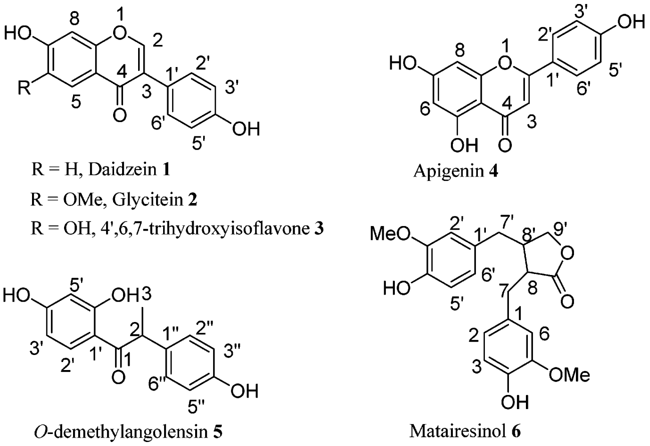
R = H, Daidzein **1**
R = OMe, Glycitein **2**
R = OH, 4′,6,7-trihydroxyisoflavone **3**
Apigenin **4**
O-demethylangolensin **5**
Matairesinol **6**

FIGURE 1. Naturally occurring polyphenols.

Use of dielectric microwave heating has been increasingly exploited in organic synthesis[11] including deuteration reactions.[12] Using dipolar or ionic solvents microwave energy can be transferred to the reaction media using two mechanisms, i.e., dipole rotation and ionic conduction. Utilizing ionic liquids as a primary or cosolvent, both mechanisms for energy transfer can operate, thus making ionic liquids a highly suitable medium for microwave-assisted organic synthesis.[13]

We describe here a much improved H/D exchange method for several polyphenols utilizing ionic liquids, namely, 1-butyl-3-methylimidazolium chloride, [bmim]Cl, or [bmim]Br, as a cosolvent in 35% DCl/D$_2$O, under microwave (MW) irradiation. In passing, it may be noted that certain substrates that are poorly

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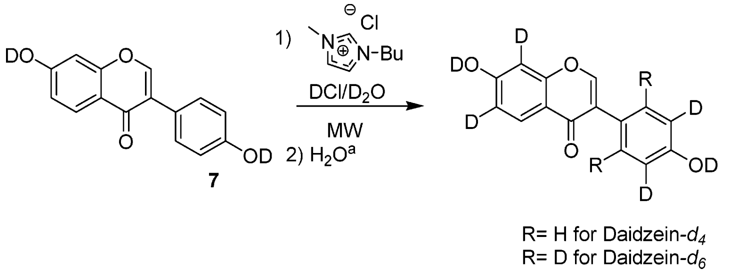
TABLE 1. H/D Exchanges in Pre-*O*-deuterated Daidzein 7 under Microwave/Ionic Liquid Conditions



entry	[bmim]Cl^b/mol equiv	DCl(−D$_2$O)/mol equiv	T^c/°C (P/W)	total time/min	% of H-3 and H-5 exchange^d	% of H-2 and H-6 exchange^d
1	0	40	120 (40)^e	30	45	0
2	4	40	120 (40)^e	30	67	0
3	8	40	120 (40)^e	30	95	0
4	8	40	120 (20)^f	30	65	0
5	8	40	170 (70)^f	10	>95	70
6^g	8 + 8	40 + 40	170 (70)^f	10 + 10	>95	>95
7	8	40	with oil bath heating,^{h} 120 ˚C	30	50	0
8	8	40	with oil bath heating,^{h} 120 °C	120	50	0

[a] After CH/CD exchange, the protic hydroxyls were reinstated by a brief H$_2$O treatment. [b] [bmim]Br may also be used. [c] Temperature recorded by IR sensor. [d] Determined by ^{1}H NMR of the isolated compound. [e] With continuous compressed air cooling (power max). [f] Without continuous cooling (power max off). [g] The reaction and isolation procedure was repeated twice. [h] Preheated.

TABLE 2. Conditions and Results of the Deuteration of Certain Polyphenols

entry	substrate	T^a/°C (P/W)	total time/min	i.p./%^{b} (yield/%)	product^c
1	**1**	120 (40)	30	>85 (94)	[3,5,6,8-d_4]-daidzein
2	**1**	170 (70)	20	>90 (89)	[2,3,5,6,6,8-d_6]-daidzein
3	**2**	120 (40)	30	>85 (95)	[3,55,8-d_4]-4,6,7-trihydroxyisoflavone
4	**2**	170 (70)	40	>78 (80)	[2,3,5,5,6,8-d_6]-4,6,7-trihydroxyisoflavone
5	**4**	120 (50)	20	>90 (93)	[2,3,3,5,6,8-d_5]-apigenin
6	**5**	120 (40)	15	>90 (90)	[2,3,3,5,5-d_5]-*O* - demethylangolensin
7	**6**	70 (20)	40	>90 (91)	[2,2,3,5,6,6-d_6]-matairesinol

[a] Temperature recorded by IR sensor. [b] Isotopic purities were determined by ESI-TOF and ^{1}H NMR comparison of the labeled and unlabeled compounds. [c] Products were characterized by comparison with published data.[3−9,17]

soluble in CH$_2$Cl$_2$, a solvent required for iridium-complex-catalyzed deuteration with D$_2$, undergo partial deuteration under these conditions if an ionic liquid cosolvent is present to allow dissolution of the substrate.[14]

Results and Discussion

Pre-*O*-deuterated daidzein **7** was used as a model substrate for screening expedient DCl/D$_2$O−ionic liquid−microwave conditions (Table 1). In the absence of an ionic liquid cosolvent (entry 1), low deuterium efficiency resulted due to the low substrate solubility, while use of 8 mol equiv of [bmim]Cl gave a good substrate solubility and hence H/D exchange in DCl/D$_2$O at 120 °C with an initial MW power input of 40 W (entry 3). Under these conditions over 90% exchange took place at the more active aromatic sites of daidzein, i.e., C-8, -6, -3′, and -5′, while the less active 2′ and 6′ positions remained unchanged. Interestingly, when a MW power of 20 W was used to reach a temperature of 120 °C (entry 4), less H/D exchange reaction took place. Thus, presumably increased microwave thermal effects are available with higher energy input, i.e., rapid heating, volumetric heating, superheating, and hotspots. The less active aromatic 2′ and 6′ positions started to be slowly exchanged when the reaction temperature rose over 140 °C, and at 170 °C (70 W of MW power, entry 5 and 6), the 2′ and 6′ hydrogens also underwent H/D exchange to an extent of more than 90%. As expected, the pyrone ring 2-H remained completely inactive under these conditions. For comparison, a test reaction was also conducted with conventional heating (entry 7 and 8). Under these conditions, the exchange reaction occurred sluggishly not only at C-3′ and -5′ but also at C-6 and -8. Without an ionic liquid cosolvent, only marginal deuteration occurred under refluxing conditions.

The results of this deuterolabeling procedure applied to a number of polyphenolic compounds are summarized in Table 2. In glycitein **2**, the C-6 methoxy group underwent ether cleavage resulting in polydeuterated analogues of compound **3**. The 4′,6,7-trihydroxyisoflavone-d_4 (entry 3) and 4′,6,7-trihydroxyisoflavone-d_6 (entry 4) obtained are of interest in metabolism studies of daidzein and other nutritional isoflavones. A high yield and isotopic purity were also gained in the H/D exchange reaction of apigenin **4** in 20 min and *O*-demethylangolensin **5** in 30 min to obtain the d_5 analogues (entries 5 and 6). Furthermore, our deuterolabeling method was very suitable for matairesinol **6**, in which all six aromatic protons underwent H/D exchange at 70 °C (entry 7), but interestingly no benzylic or carbonyl α-H sites were exchanged. When the same reaction conditions were used without an ionic liquid, H/D exchange occurred at a level of only about 50%, showing that the presence of ionic cosolvent was crucial. As expected, [bmim]Cl is completely resistant to the acidic conditions employed. It is interesting to note that 2-mono[15] and 2,4,5-tri[16]

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deuterated imidazolium ionic liquids are obtained by D_2O/DO^- treatment of the undeuterated materials.

The sites and preferred order of exchange in polyphenolic compounds are governed by the relative electronegativities at the various positions. We reported[6] earlier how Mulliken charges calculated with the semiempirical AM1 method can be used to rationalize the electrophilic aromatic deuteration results.

In conclusion, we developed a fast, high-yielding, and aryl-ring-selective acid-catalyzed deuteration method for representative polyphenols in ionic liquids using 35% DCl/D_2O as a cheap deuterium source under microwave irradiation. This postsynthetic methodology is expected to be generally applicable and simple to carry out with reaction times shortened from several days or 15 h to 20 − 40 min and the ionic solvent easily recycled.

Experimental Section

General Procedure. A mixture of *O*-predeuterated (D_2O/acetone) polyphenol (0.39 mmol), [bmim]Cl (0.55 g, 3.14 mmol), and DCl/D_2O (1.5 mL) was heated for the appropriate time and at the temperature indicated. The product was isolated either by filtration or by extraction from the reaction mixture. To recover the remaining ionic liquid, the water phase or filtrate was concentrated in a rotary evaporator and ionic liquid collected in 90% yield.

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Supporting Information Available: Experimental details, ^1H NMR spectra, and MS(ESI+ or ESI-) data of all products listed in Table 2. This material is available free of charge via the Internet at http://pubs.acs.org.

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