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Capricious Selectivity in Electrophilic Deuteration of Methylenedioxy Substituted Aromatic Compounds

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Supporting Information

ABSTRACT: Ring deuteration via the S_EAr mechanism, which is usually problem-free, is found to be troublesome with methylenedioxy substituent aromatics. We report a case where the deuteration not only partially fails at one of the *ortho* positions but also is completely prevented by a conformation dependent effect at the other *o*-position. Such selectivity discrepancies are important due to the widespread occurrence of methylenedioxy substituted natural products. Density functional theory calculations were used to elucidate the exchange reaction mechanism in 1,2-dialkoxybenzenes.

The methylenedioxy group is a common aromatic ring substituent of numerous biologically active compounds that is present for example in the plant lignans podophyllotoxin and cubebin, which are hot lead compounds for the discovery and development of novel anticancer and antimicrobial agents.[1] In studying the biological activity, use is often made of stable isotope labeled analogs (very often containing several deuteriums). The first efforts to deuterate an aromatic compound took place in 1934, when benzene was heated in 3% heavy water in the presence of a nickel catalyst.[2] In the same year, Ingold et al. introduced deuterium atoms into the aromatic nucleus by means of ordinary electrophilic reagents, such as deuterated aqueous sulfuric acid without using any heterogeneous catalysts.[3] They also proposed that the mechanism of the exchange reaction is an ordinary electrophilic aromatic substitution and proved it by comparing the efficiencies of certain acidic and basic deuterating agents and by studying how certain aromatic substituents influence the reaction.[4]

The electrophilic aromatic H/D exchange reactions catalyzed by acids and occasionally by bases are utilized in the deuterium labeling of various polyphenolic compounds.[5,6] In our studies, we found that 35% DCl/D_2O and the use of the ionic liquid 1-butyl-3-methylimidazolium chloride [bmim]Cl as a cosolvent under microwave irradiation constitute an expedient deuteration method for naturally occurring polyphenols, such as lignans.[7] For lignans, the aromatic substituents are usually *ortho*–*para* directing and activating hydroxy and/or methoxy groups.[8] Employing these methods, stable isotopically pure polydeuterated lignans **1**–**6** were synthesized via proton exchange at the activated positions (Figure 1).

However, diverging regioselectivities in the H/D exchange reaction were observed for two closely related analogs, namely dehydroxycubebin (3,3′,4,4′-bismethylenedioxy-9,9′-epoxylignane, **7**, Scheme 1) and brassilignan (3,3′,4,4′-tetramethoxy-9,9′-epoxylignane, **8**), which differ only slightly in the aromatic substituents. In the deuteration reaction, all the aromatic protons of brassilignan were exchanged in over 95% isotopic purity as expected (**8** → **5**, Scheme 1), whereas with dehydroxycubebin (**7**) the degree of deuteration (**7** → **9**, Scheme 1) remained incomplete despite recycled labeling attempts. According to electron ionization mass spectrometry (EI-MS) measurements, a mixture of di-, tri-, and tetradeuterated products was formed from **7** at best. Quantitative ^{13}C NMR studies demonstrated that the 6- and 6′-sites of **7** were fully deuterated, while only one-third of the 2- and 2′-sites (R^2 = H/D = 2:1; see **9** in Scheme 1) carried deuteriums and remarkably the positions 5 and 5′ remained intact.

The reaction was repeated several times with fresh deuteration reagents by recycling the isolated crude product **9**, but the level of deuteration did not improve. Our earlier

Figure 1. Deuterium labeled lignans: lignanolactones **1**–**3** and tetrahydrofuran lignans **4**–**6**.

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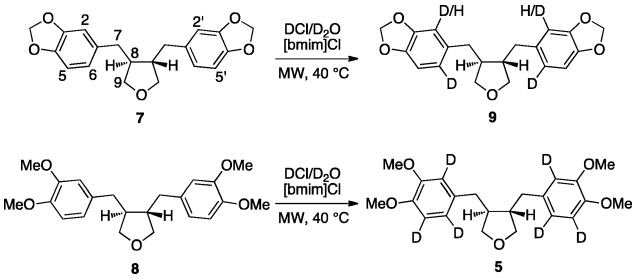
Scheme 1. Deuteration of the Plant Lignans Dehydroxycubebin 7 and Brassilignan 8[a]

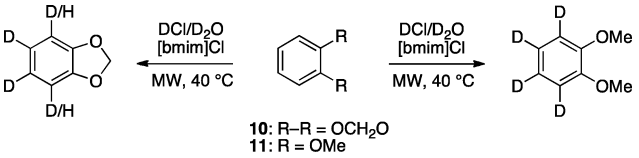


[a]The treatment of **8** gives a hexadeuterated analog **5** in over 95% isotopic purity.

experiments have shown that certain aromatic sites, e.g. structures having two *meta* substituted hydroxy or methoxy groups, are so highly activated that the deuterium atoms are to some extent exchanged back to hydrogens during workup.[6] For such compounds a selective dedeuteration of the labile deuterium labels is performed to obtain isotopically stable products. However, the deuterium labels at C-2 and C-2′ in **9** are not labile, as they could not be replaced using the dedeuteration procedure,[6] i.e. by treating with methanolic HCl. Our initial hypothesis was that the strained methylenedioxy ring may somewhat deform the planar aromatic ring and thus prevent the 5- and 2-positions from being deuterated. Computational studies and additional experiments with model compounds were performed to test the hypothesis and to gain a deeper understanding of the observed discrepancy in the reactivity of dehydroxycubebin **7**.

Deuteration experiments with model compounds gave similar but not identical results as obtained with the lignans. In 1,3-benzodioxole (1,2-methylenedioxybenzene **10**, Scheme 2), the *para*/*meta* positions to the methylenedioxy substituent

Scheme 2. Deuteration of Model Compounds 1,3-Benzodioxole 10 and Veratrole 11



were fully deuterated, while only ca. 30% of the *ortho* positions were deuterated. With veratrole (1,2-dimethoxybenzene **11**), all aromatic positions were successfully deuterated. Acid catalyzed detritiation experiments[9] with aryl tritiated 1,3-benzodioxoles, and deuteration experiments[10] with 4-hydroxy-1,3-benzodioxole (sesamol), have shown that the *para* ring positions are always more reactive than the *ortho* position. This has been rationalized by suggesting a deformation of the aromatic ring (the Mills–Nixon effect) and by the quasi-aromatic nature of the heterocyclic ring of **10**.[11] The deviant behavior of **10** has recently been reported in other types of reactions[12] as well.

The model compound **10** thus serves to demonstrate that diminished reactivity at the *ortho* sites is characteristic of methylenedioxy substituted systems. However, it is not clear why just one *ortho* site in each aryl ring of **7** is H/D exchanged at ca. 30% yield and the other two *ortho* sites remain untouched, whereas in **10** both *ortho* sites participate fully in the exchange.

The 9,9′-epoxylignanes **7** and **8** can adopt three main conformations, the extended **12** and two π-stacked conformations **13** and **14** (depicted for **7** in Figure 2). The calculations

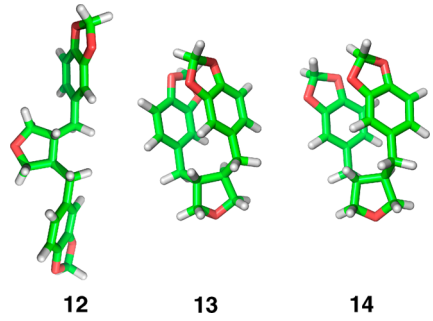


Figure 2. Optimized structures for the three different conformations of dehydroxycubebin **7**: the extended **12** and the two π-stacked conformations **13** and **14**.

imply that the stacked sandwich conformations **13** and **14** are preferred under the reaction conditions (Figure 2 and Table 1).

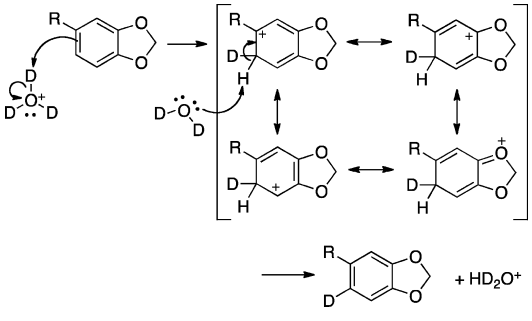
Table 1. Energy Difference (in kcal/mol) between the Stacked and Extended Conformations for 7 and 8

molecule (functional)	$E(13) - E(12)$	$E(14) - E(12)$
7 (B3LYP)	−2.0	−1.2
7 (PWPB95)	−3.3	−2.6
8 (B3LYP)	−3.2	−0.6
8 (PWPB95)	−4.8	−1.4

The energies obtained in the PWPB95 calculations are expected to be more accurate because the molecules are treated at a higher level of theory. The sandwich conformation with the substituents oriented in opposite directions (**13**, Figure 2) is energetically slightly lower than the one having the aryl rings stacked with identical orientation (**14**, Figure 2), probably due to a more favorable multipole interaction between the oxygen containing substituents of **13**.

The H/D exchange reaction is an ordinary electrophilic aromatic substitution taking place in two steps: first the electrophile attacks giving rise to a positively charged resonance stabilized intermediate (an arenium ion, also called a Wheland intermediate or sigma complex), and the leaving group departs in the second step (Scheme 3). Simultaneous attack-and-departure mechanisms are not known.[13] The degree of deuterium incorporation is expected to be governed by

Scheme 3. Mechanism of H/D Exchange[a]



[a]R denotes the rest of the lignan molecule.

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equilibrium factors of the participant D and H species present in D_2O and at the reactive aryl sites.

In the computational studies, the two reaction steps, namely the addition of a proton (deuterium) from one face of the ring plane and abstraction of a proton from the other face were studied separately. The reaction from both faces are illustrated in Figure 3, where the face of the ring plane exposed to the solvent is denoted "up", while the other is "down".

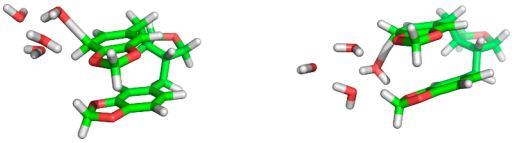

Figure 3. Proton addition from the "up" and "down" faces of structure **13** is illustrated for dehydroxycubebin (**7**).

The calculated differences in the activation energies suggest that the deuterium is added from the "up" face, where the steric pressure is weaker (left of Figure 3), and the proton leaves from the "down" face. A transition state with the water molecules approaching from the "down" face is not feasible for **7**. The calculated energies suggest that the deuteration reaction can proceed normally from the exposed outer "up" face of the molecule, but the proton cannot leave C-5 (see numbering in Scheme 1) as the less flexible **7** does not allow water molecules to approach from the interring "down" face. Thus, the proton departure is impaired and the second step of the electrophilic aromatic substitution reaction cannot occur. Instead, the reaction is reversed and the deuteron is liberated back to the solution. Conversely, both reaction steps are possible for **8**, whose methoxy groups can freely rotate and do not obstruct the approach of the water molecules on the "down" face.

The geometric data show that the interring distance is roughly the same for **7** and **8** in both the neutral and protonated stage (3.5 and 3.3 Å, respectively). The interring distance is always somewhat smaller for the protonated structure, probably due to cation−π interactions between the protonated ring and the other aryl ring.[14] The short distance between the aryl rings may be one reason behind the difficulties of solvating the "down" proton. The standard deviation of the distances of 0.2 Å is also larger for **8**, indicating a greater coplanarity between the rings of **7** with a standard deviation of 0.1 Å. The increased coplanarity is most likely due to interactions with the more rigid methylenedioxy substituent of **7** as compared to the methoxy groups in **8**.

The structural analysis combined with the deuteration results indicate that the aryl rings cannot rotate freely in either of the molecules. Thus, there are two atropisomers (**13** and **14**) for both compounds, and the molecules do not switch between the two conformers under the reaction conditions. The small energy difference of 0.7 kcal/mol between the two closed conformers of **7** suggests that the two conformers are present under the experimental conditions, which is the reason for the incomplete deuteration at C-2 and C-2′. The computational studies show that the second step of the reaction is sterically prevented for only one of the conformers, whereas for the other conformer the C-2 and C-2′ sites are more exposed to the solvent.

To conclude, two factors are suggested to be responsible for the deviant deuteration behavior preventing the complete deuteration of dehydroxycubebin **7** at C-2 (2′) and C-5 (5′). One of them is conformation independent, possibly due to a proposed quasi-aromatic nature of the five-membered methylenedioxy substituent ring[11] restricting the complete deuteration at the *ortho* positions, which is observed also for sesamol[10] and the model compound **10**. The other reason seems to be steric interaction, which is conformation dependent and blocks even a partial deuteration at C-5 (C-5′) of **7**.

EXPERIMENTAL SECTION

General Experimental. Lignanolactones were synthesized by the tandem Michael addition−alkylation method followed by Raney nickel desulfurization and debenzylation.[15] To obtain lignanofurans the lignanolactones were further reduced to dibenzylbutanediols with $LiAlH_4$[15] and then cyclized to the corresponding lignanofurans either with concentrated HCl under microwave heating[16] or during the deuteration reaction as described below. The ionic liquid [bmim]Cl was synthesized following the published procedure.[17] DCl solution (35 wt % in D_2O, 99 atom % D) was commercially available from Sigma-Aldrich. ^{1}H NMR and ^{13}C NMR (500 or 300 MHz and 150 or 75 MHz, respectively) spectra were recorded in $CDCl_3$ or $(CD_3)_2CO$. Chemical shifts are given in δ in ppm and J values in Hz, setting the scale relative to the solvent signals.[18] Mass spectra (EI, 70 eV ionization energy, or ESI TOF) were acquired.

Deuteration Procedure. Lignanolactone or lignanodiol (20−90 mg/0.055−0.302 mmol), or the model compounds veratrol or methylenedioxybenzene, and [bmim]Cl (8 mol equiv) were dried under high vacuum (1−0.5 mbar) after which they were placed in a dry 5 mL pressure-proof reaction vial. 35% DCl/D_2O (70−90 mol equiv of DCl) was added, and the vial was sealed. Microwave power of 80 W was used to reach 40 °C. The reaction was held at this temperature with stirring and continuous compressed air cooling (2 bar) for 30 min. After the mixture was allowed to cool to rt, 0.5−1.0 mL of D_2O was added and the mixture was stirred for 10 min and extracted with EtOAc. The organic phase was washed with water and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in a rotary evaporator, and the product was dried under high vacuum. If necessary, the deuteration procedure was repeated until the isotopic purity was more than 90%. The crude product was purified by column chromatography using EtOAc and CH_2Cl_2 as eluent, and the deuterated lignan (74−84%) was obtained as a white waxlike solid or colorless oil.

Characterization of Products. The products were characterized by ^{1}H NMR, ^{13}C NMR, LRMS, and HRMS and comparison with the unlabeled analogues. The sites of deuteration were determined by comparing the ^{1}H NMR and ^{13}C NMR spectra with the spectra of the unlabeled compound assigned with COSY, HSQC, and HMBC. The ^{1}H NMR spectra of deuterium labeled compounds are the same as those for unlabeled compounds, with the exception that signals from the deuterated aromatic sites are lacking and the coupling patterns of the protons attached to the adjacent carbons are simplified accordingly. In ^{13}C NMR, a carbon atom attached to one deuterium produces a low intensity triplet since the spin of deuterium is 1.

Deuteration percentages of carbons C-2 and C-6 were determined by performing line shape fitting (Lorentzian lines) to measure areas for signals of both deuterated and nondeuterated carbons from the quantitative ^{13}C-spectrum using PERCH software (PERCH Solutions Ltd., Kuopio, Finland). The quantitative ^{13}C-spectrum was recorded using the following: inverse-gated ^{1}H-decoupling sequence, 45° excitation pulse flip angle, 0.4 s acquisition time, 200 s relaxation delay, and 484 repetitions. Carbon C-6 appears to be fully deuterated (clear 1:1:1 triplet), whereas the signal of C-2 consists of a singlet (nondeuterated C-2) and partially overlapping 1:1:1 triplet (deuterated C-2). Location C-5 remained virtually unaffected (Figure S1 in Supporting Information). The deuteration percentage in each location was evaluated from signal areas of deuterated and nondeuterated carbons (Table S1 in Supporting Information). This resulted in 100% and 29% deuteration in locations C-6 and C-2, respectively. The result for C-2 approximately translates into a 2:1 ratio between nondeuterated and deuterated C-2.

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The isotopic purities of deuterated lignans were determined from the ion clusters in the molecular ion region in EI or ESI mass spectra by comparison with those of undeuterated compounds.

The spectroscopic data are given as Supporting Information.

*[2,4,6,2′,4′,6′-$^2H_6$]-3,3′-Dihydroxylignano-9,9′-lactone (d_6-Enterolactone, **1**).* White solid (75 mg, 82% yield). The product was characterized by comparison with the nondeuterated compound.[19] ^{1}H NMR (300 MHz, $(CD_3)_2CO$) δ 2.47−2.72 (4H, m, H-7′, 8, 8′), 2.88 (1H, dd, J = 6.6, 13.8, H-7a), 2.97 (1H, dd, J = 5.7, 13.8, H-7b), 3.88 (1H, t, J = 8.7, H-9′a), 4.04 (1H, dd, J = 6.9, 8.7, H-9′b), 7.09 (1H, s, H-5′), 7.13 (1H, s, H-5), 8.22 (2H, s, 3-OH, 3′-OH). ^{13}C NMR (75 MHz, $(CD_3)_2CO$) δ 35.2 (C-7), 38.6 (C-7′), 42.1 (C-8′), 46.8 (C-8), 71.5 (C-9′), 114.1 (t, C-4′)^D, 114.2 (t, C-4)^D, 116.2 (t, C-2′)^D, 116.8 (t, C-2)^D, 120.3 (t, C-6′)^D, 121.2 (t, C-6)^D, 130.1 (C-5), 130.2 (C-5′), 140.7 (C-1), 141.1 (C-1′), 158.3 (C-3, 3′), 178.8 (C-9). Proportions of isotopologues in EIMS spectrum were 15% $^2H_5$ and 100% $^2H_6$. HRMS (EI) m/z: [M$^+$] calcd for $C_{18}H_{12}D_6O_4$ 304.1582; found 302.1567.

*[2,5,6,2′,5′,6′-$^2H_6$]-4,4′-Dihydroxy-3,3′-dimethoxylignano-9,9′-lactone (d_6-Matairesinol, **2**).* Colorless oil (19 mg, 95% yield). The product was characterized by comparison with the nondeuterated compound.[19,20] ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.42−2.65 (4H, m, H-7′, 8, 8′), 2.84−2.98 (2H, m, H-7), 3.81 (3H, s, OCH_3′), 3.81 (3H, s, OCH_3) 3.88 (1H, dd, J = 6.9, 9.0, H-9′a), 4.15 (1H, dd, J = 6.9, 9.0, H-9′b), 5.5 (br, 4- and 4′-OH). ^{13}C NMR (75 MHz, $CDCl_3$) δ 34.47 (C-7), 38.20 (C-7′), 40.99 (C-8′), 46.60 (C-8), 55.81 (OCH_3), 55.86 (OCH_3), 71.39 (C-9′), 110.69 (t, C-2′)^D, 111.24 (t, C-2)^D, 113.74 (t, C-5′)^D, 114.07 (t, C-5)^D, 120.95 (t, C-6′)^D, 121.70 (t, C-6)^D,129.36 (C-1′), 129.60 (C-1), 144.37 (C-4′), 144.50 (C-4), 146.59 (C-3′), 146.70 (C-3), 178.94 (C-9). Proportions of isotopologues in EIMS spectra were 9% $^2H_5$ and 100% $^2H_6$. EIMS (70 eV) m/z: M$^+$ 364 (55%), 224 (6), 167 (16), 140 (100), 125 (10). HRMS (EI) m/z: [M$^+$] calcd for $C_{20}H_{16}D_6O_6$ 364.1793; found 364.1780.

*[2′,4′,6′-$^2H_3$]-3′-Hydroxylignano-9,9′-lactone (**3**).* Pale yellow viscous oil (21 mg, 78% yield). The product was characterized by comparison with the nondeuterated compound.[6] ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.43 (1H, dd, J = 9.0, 13.0 Hz, H-7′a), 2.47−2.54 (1H, m, H-8′), 2.59 (1H, dd, J = 5.0, 11.0 Hz, H-7′b), 2.59−2.63 (1H, m, H-8), 2.94 (1H, dd, J = 7.0, 14.0, H-7a), 3.09 (1H, dd, J = 5.0, 14.0, H-7b), 3.84 (1H, dd, J = 8.0, 9.0 Hz, H-9′a), 4.08 (1H, dd, J = 7.5, 9.0 Hz, H-9′b), 5.42 (1H, s, OH), 7.11 (1H, s, H-5′), 7.17 (2H, d, J = 7.5 Hz, H-2, 6), 7.23 (1H, t, J = 7.5, H-4), 7.30 (2H, t, J = 7.2, H-3, 5) ^{13}C NMR (75 MHz, $CDCl_3$) δ 35.3 (C-7), 38.4 (C-7′), 41.3 (C-8′), 46.7 (C-8), 71.5 (C-9′), 113.7 (t, C-4′)^D, 115.4 (t, C-2′)^D, 120.8 (t, C-6′)^D, 127.1 (C-4), 129.0 (C-3, 5), 129.5 (C-2, 6), 129.9 (C-5′), 137.9 (C-1), 139.7 (C-1′), 156.1 (C-3′), 179.1 (C-9). Proportions of isotopologues in EIMS spectra were 7% $^2H_2$ and 100% $^2H_3$. EIMS (70 eV) m/z: M$^+$ 285 (100%), 175 (16), 137 (52), 136 (56), 111 (87), 110 (88), 91 (89). HRMS (EI) m/z: [M$^+$] calcd for $C_{18}H_{15}D_3O_3$ 285.1444; found 285.1443.

*[2,5,6,2′,5′,6′-$^2H_6$]-4,4′-Dihydroxy-3,3′-dimethoxy-9,9′-epoxylignan (d_6-Anhydrosecoisolariciresinol, **4**).* White solid (16 mg, 84% yield). The product was characterized by comparison with the nondeuterated compound.[21] ^{1}H NMR (500 MHz, $(CD_3)_2CO$) δ 2.15−2.20 (2H, m, H-8, 8′), 2.49 (2H, dd, J = 8.3, 13.5, H-7a, 7′a), 2.60 (2H, dd, J = 6.3, 13.5, H-7b, 7′b), 3.43 (2H, dd, J = 6.0, 8.5, H-9a, 9′a), 3.79 (2H, dd, J = 6.0, 8.5, H-9b, 9′b), 3.81 (6H, s, OCH_3, OCH_3′), 7.26 (2H, s, OH, OH′). ^{13}C NMR (75 MHz, $(CD_3)_2CO$) δ 39.6 (C-7, 7′), 47.7 (C-8, 8′), 56.3 (3-OCH_3, 3′-OCH_3), 73.8 (C-9, 9′), 112.8 (t, C-2, 2′)^D, 115.3 (t, C-5, 5′)^D, 121.7 (t, C-6, 6′)^D, 133.0 (C-1, 1′), 145.8 (C-4, 4′), 148.3 (C-3, 3′). Proportions of isotopologues in EIMS spectra were 9% $^2H_5$ and 100% $^2H_6$. EIMS (70 eV) m/z: M$^+$ 350 (72%), 141 (62), 140 (100), 126 (9), 125 (10). HRMS (EI) m/z: [M$^+$] calcd for $C_{20}H_{18}D_6O_5$ 350.2000; found 350.1996.

*[2,5,6,2′,5′,6′-$^2H_6$]-3,3′,4,4′-Tetramethoxy-9,9′-epoxylignan (d_6-Brassilignan, **5**).* White solid (17 mg, 77% yield). The product was characterized by comparison with the nondeuterated compound **8**.[22] ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.13−2.25 (2H, m, H-8, 8′), 2.53 (2H, dd, J = 8.1, 13.8, H-7a, 7′a), 2.64 (2H, dd, J = 6.0, 13.8, H-7b, 7′b), 3.53 (2H, dd, J = 6.2, 8.9, H-9a, 9′a), 3.84 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 3.91 (2H, dd, J = 6.8, 8.9, H-9b, 9′b). ^{13}C NMR (75 MHz, $CDCl_3$) δ 39.1 (C-7, 7′), 46.7 (C-8, 8′), 55.9 (OCH_3), 56.0 (OCH_3), 73.4 (C-9, 9′), 111.0 (t, C-5, 5′)^D, 111.8 (t, C-2, 2′)^D, 120.3 (C-6, 6′)^D, 133.0 (C-1, 1′), 147.5 (C-4, 4′), 149.0 (C-3, 3′). Proportions of isotopologues in EIMS spectra were 3% $^2H_5$ and 100% $^2H_6$. EIMS (70 eV) m/z: M$^+$ 378 (93%), 180 (4), 155 (73), 154 (100), 140 (10), 124 (15). HRMS (EI) m/z: [M$^+$] calcd for $C_{22}H_{22}D_6O_5$ 378.2313, found 378.2308.

*[2,4,6,2′,4′,6′-$^2H_6$]-3,3′-Dihydroxy-9,9′-epoxylignan (d_6-Enterofuran, **6**).* White solid (14 mg, 74% yield). The product was characterized by comparison with the nondeuterated compound.[22] ^{1}H NMR (300 MHz, $(CD_3)_2CO$) δ 2.13−2.26 (2H, m, H-8, 8′), 2.50 (2H, dd, J = 8.7, 13.5, H-7a, 7′a), 2.67 (2H, dd, J = 5.7, 13.5, H-7b, 7′b), 3.43 (2H, dd, J = 6.6, 8.7, H-9a, 9′a), 3.79 (2H, dd, J = 7.2, 8.7, H-9b, 9′b), 7.08 (2H, s, H-5, 5′), 8.13 (2H, s, 3-OH, 3′-OH). ^{13}C NMR (75 MHz, $(CD_3)_2CO$) δ 39.7 (C-7, 7′), 47.5 (C-8, 8′), 73.7 (C-9, 9′), 113.6 (t, C-4, 4′)^D, 116.2 (t, C-2, 2′)^D, 120.4 (t, C-6, 6′), 130.0 (C-5, 5′), 143.1 (C-1, 1′), 158.3 (C-3, 3′). Proportions of isotopologues in ESI-TOF spectra were 4% $^2H_5$ and 100% $^2H_6$. HRMS (EI) m/z: [M$^+$] calcd for $C_{18}H_{14}D_6O_3$ 290.1789; found 290.1793.

*[6,6′-$^2H_2$]-(3,4),(3′,4′)-Bismethylenedioxy-9,9′-epoxylignan (d_2-Dehydroxycubebin, **9**).* Pale yellow solid (18 mg, 75% yield). The product was characterized by comparison with the nondeuterated compound **7**.[23,24] ^{1}H NMR (500 MHz, $(CD_3)_2CO$) δ 2.13−2.21 (2H, m, H-8, 8′), 2.51 (2H, dd, J = 8.0, 14.0, H-7a, 7′a), 2.60 (2H, dd, J = 6.5, 14.0, H-7b, 7′b), 3.42 (2H, dd, J = 6.0, 8.5, H-9a, 9′a), 3.80 (2H, dd, J = 6.5, 8.5, H-9b, 9′b), 5.94 (4H, s, O−CH_2−O, O−CH_2−O′), 6.65 (2H, s, H-2, 2′), 6.72 (2H, s, H-5, 5′). ^{13}C NMR (75 MHz, $(CD_3)_2CO$) δ 39.6 (C-7, 7′), 47.4 (C-8, 8′), 73.6 (C-9, 9′), 101.7 (C-10, 10′), 108.6 (C-5, 5′), 109.8 (C-2, 2′), 122.1 (t, C-6, 6′)^D, 135.4 (C-1, 1′), 146.8 (C-4, 4′), 148.6 (C-3, 3′). Proportions of isotopologues in EIMS were 100% $^2H_2$, 68% $^2H_3$, and 43% $^2H_4$. When the natural abundance of ^{13}C is taken into account, the ratio of species d_2:d_3:d_4 is about 6:3:2. EIMS (70 eV) m/z: 345 (9%), 344 (18), 343 (28), [$^2H_2$]-M$^+$ 342 (41), 206 (4), 163 (5), 162 (4), 138 (53), 137 (100), 136 (76). HRMS (EI) m/z: [M$^+$] calcd for $C_{20}H_{18}D_2O_5$ 342.1436; found 342.1452.

■ COMPUTATIONAL DETAILS

The initial molecular structures were constructed manually and preoptimized at the semiempirical level[25,26] as described below. The structures were then optimized at the density functional theory (DFT) level using the Becke−Perdew (BP86) functional[27,28] and split-valence basis sets augmented with polarization functions[29] employing the resolution of the identity approximation to speed up the calculations.[30−32] The Cartesian coordinates of the optimized molecular structures of **12**, **13**, **14** are given as Supporting Information. The relative energies were calculated at the DFT level using the B3LYP[33−36] functional in combination with triple-ζ polarization basis sets.[37] The energies were also assessed using the PWPB95 double-hybrid functional in combination with quadruple-ζ polarization basis sets.[37] The D3 dispersion correction was used in all calculations.[38] The conductor-like screening model with a dielectric constant of 80 was used to model solvent effects.[39,40] The chain of spheres algorithm was used to speed up the hybrid and double-hybrid DFT calculations.[41] The calculations were performed with the ORCA software.[42,43]

Reaction Path Calculations. Solvent molecules were included to allow for a more accurate description of the mechanism of the deuteration reaction. For each system, four water molecules were explicitly considered to hydrate the proton in addition to the dielectric continuum. For each reaction path, a proton was initially placed 2.2 Å farther away from the carbon atom where the reaction occurs than in the corresponding protonated optimized geometry. The four water molecules were manually added close to the proton to avoid clashes among them and with the organic molecule. The structure was preoptimized at the semiempirical PM6-D3H4 level of theory[25,26] while keeping the proton−carbon distance fixed. The obtained conformation was taken as the starting point for the next step, in

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which the proton was moved 0.1 Å closer to carbon. The procedure was repeated until a structure with a carbon−proton distance equal to the equilibrium geometry for the protonated molecules was reached. After the procedure was completed, all the semiempirical structures were refined by geometry optimizations at the BP86/def2-SVP level of theory with a fixed proton−carbon distance. Single-point B3LYP/def2-TZVP energies were calculated using the BP86-optimized geometries.

■ ASSOCIATED CONTENT

Supporting Information

Spectroscopic data for deuterated compounds (**1**−**6**, **9**) and the Cartesian coordinates of **12**−**14**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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