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Synthesis of new deuterium-labelled lignanolactones

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Deuteration of several lignanolactones, using $^{2}H_3PO_4 \cdot BF_3/^{2}H_2O$ as the deuteration reagent, is described, affording new stable isotopically pure polydeuterated lignans. All aromatic hydrogens are exchanged, including the less active ones. The order of $^{1}H/^{2}H$ exchange is studied by comparing calculated electrostatic potential values and experimental observations. Labile deuteriums are exchanged back to hydrogens with methanolic HCl to achieve stable isotopically pure compounds.

Keywords: lignan; labelling; deuterium; phytoestrogen; synthesis

Introduction

Dietary lignans are common in the Western diet, good sources being rye and wholegrain products, sesame seeds, flaxseed and various berries for example.[1–5] Lignan contents in various plant foods have recently been compiled to food composition databases[6–8] available for use in epidemiological studies. Recent studies on metabolism have shown that in addition to the known matairesinol and secoisolariciresinol, several other dietary lignans such as 7′-hydroxymatairesinol, pinoresinol, lariciresinol, syringaresinol, arctigenin and sesamin are metabolized to mammalian lignans (enterolignans) by human intestinal bacteria.[4,9–11] Several of these bacteria, taking part in metabolism by converting secolariciresinol diglucoside,[12,13] pinoresinol, lariciresinol and matairesinol to the enterolignans enterolactone and enterodiol,[14] have been identified. A recent *in vitro* finding also indicates that phase II metabolism may occur already in the colon during uptake in colon epithelial cells, forming sulfide or glucuronide derivatives of enterolactone and enterodiol.[15] These most studied mammalian lignans, enterolactone and enterodiol, are of interest due to their biological properties and possible health effects, such as anticancer properties.[16,17]

Recently, in *in vitro*[11,18] and *in vivo*[5,19] studies several new metabolites were found. Some of these compounds were identified by GC-MS or by HPLC-MS(MS), but most of them remained unidentified because of lack of authentic reference compounds. We have published the synthesis of several lignanolactones, potential new metabolites[20] that may be used in biological qualitative analysis. However, labelled compounds are needed as standards for quantitative analysis using GC-MS and HPLC-MS. Such standards must fulfill three important criteria. First, the compounds must remain stable during the entire sample preparation procedure. Second, they should have a difference of at least three units in the M^+ *m/z* values in order to avoid interference from peaks of the labelled reference molecule in the MS spectrum of the analyte. Especially, in GC-MS methods, where trimethylsilyl derivatives are often used, isotopes of carbon and silicon will give relatively strong $M^+ + 1$ and $M^+ + 2$ peaks in the MS spectra. Third, no unlabelled species must be present.

In this paper we present the preparation of seven new stable polydeuterated lignanolactones, (**1c**–**4c** and **5b**–**7b**), which can be used as standards in quantitative analysis. Our deuteration method, using $^{2}H_3PO_4 \cdot BF_3/^{2}H_2O$ as the deuteration reagent, is generally applicable for lignanolactone-type dietary lignans and enterolignans. The deuteration reagent is capable of exchanging all aromatic protons at room temperature, even at the less active positions in the lignan skeleton. We also report the results of computational studies, where calculated electrostatic potential (ESP) values of the aromatic protons are compared with the observed reactivities.

Results and discussion

The labelling method, developed in our laboratory,[21] relies on the relatively inexpensive $^{2}H_2O$ as deuterium source. In order to avoid carrying the labelled starting material through a multistep synthetic sequence, deuteriums were introduced directly into the complete lignan molecule. Lignanolactones (**1a**–**7a**) were prepared according to the previously published method[20] and compounds **4a**–**7a** are reported for the first time here. Lignans **1a**–**7a** were deuterated by treating them with freshly prepared deuteration reagent,[22] $^{2}H_3PO_4 \cdot BF_3/^{2}H_2O$, at room temperature (Scheme 1). The number of introduced deuteriums and their positions were determined subsequently by MS and NMR. This deuteration method has been used earlier for the lignanolactones enterolactone[23] and matairesinol.[24] Now also lignanolactones containing highly activated positions in the aromatic ring have been deuterated, while also the less reactive 5′ positions in **1a**, **3a** and **5a**–**7a** undergo $^{1}H/^{2}H$ exchange as shown by MS and

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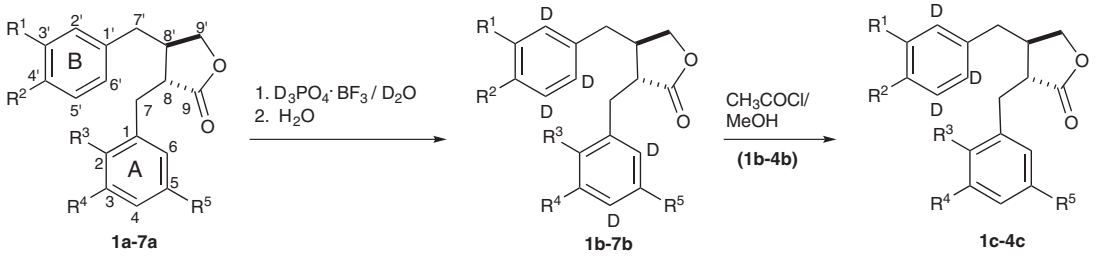
NMR spectroscopy. After the removal of any labile ^{2}H-labels stable deuterated lignanolactones are obtained (Table 1).

In ring A of structures **1a**–**4a**, two *meta*-substituted hydroxy or methoxy groups strongly activate the aromatic sites at 2, 4 and 6. This means that the $^2H \rightarrow {}^1H$ back exchange may occur to some extent during the sample preparation procedure, where relatively acidic conditions are used. For this reason, to get stable deuterated molecules, the labile ^{2}H atoms at C-2, 4 and 6



1a: $R^1 = R^4 = R^5 = OH$, $R^2 = R^3 = H$
2a: $R^1 = R^2 = OMe$, $R^3 = H$, $R^4 = R^5 = OH$
3a: $R^1 = OH$, $R^2 = R^3 = H$, $R^4 = R^5 = OMe$
4a: $R^1 = R^2 = R^4 = R^5 = OMe$, $R^3 = H$
5a: $R^1 = OH$, $R^2 = R^5 = H$, $R^3 = R^4 = OMe$,
6a: $R^1 = OH$, $R^2 = R^4 = R^5 = H$, $R^3 = OMe$
7a: $R^1 = OH$, $R^2 = R^3 = R^4 = R^5 = H$

1b: $R^1 = R^4 = R^5 = OH$, $R^2 = R^3 = \mathbf{D}$
2b: $R^1 = R^2 = OMe$, $R^3 = \mathbf{D}$, $R^4 = R^5 = OH$
3b: $R^1 = OH$, $R^2 = R^3 = \mathbf{D}$, $R^4 = R^5 = OMe$
4b: $R^1 = R^2 = R^4 = R^5 = OMe$, $R^3 = \mathbf{D}$
5b: $R^1 = OH$, $R^2 = R^5 = \mathbf{D}$, $R^3 = R^4 = OMe$,
6b: $R^1 = OH$, $R^2 = R^4 = R^5 = \mathbf{D}$, $R^3 = OMe$
7b: $R^1 = OH$, $R^2 = R^3 = R^4 = R^5 = \mathbf{D}$

1c: $R^1 = R^4 = R^5 = OH$, $R^2 = \mathbf{D}$, $R^3 = H$
2c: $R^1 = R^2 = OMe$, $R^3 = H$, $R^4 = R^5 = OH$
3c: $R^1 = OH$, $R^2 = \mathbf{D}$, $R^3 = H$, $R^4 = R^5 = OMe$
4c: $R^1 = R^2 = R^4 = R^5 = OMe$, $R^3 = H$

Scheme 1. Deuteration of lignanolactones **1a**–**7a**.

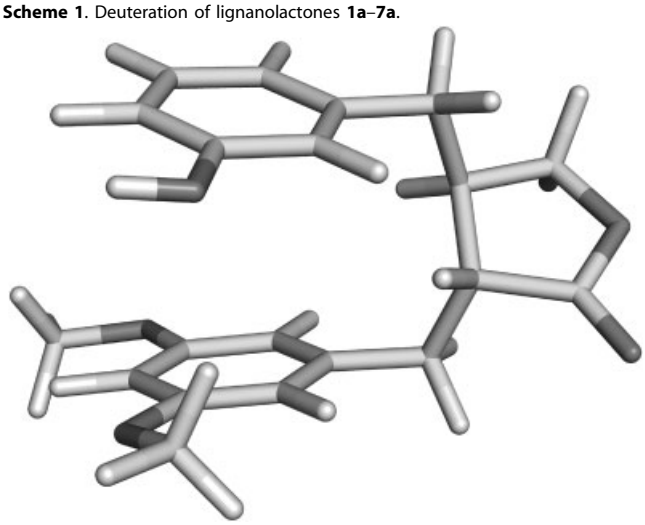


Figure 1. Sandwich conformation of **3a**.

Table 1. Isotopic purities of deuterium-labelled molecules

Molecule	Positions of the deuteriums	Isotopic purity (%)
1c	2′, 4′, 5′, 6′	92[a]
2c	2′, 5′, 6′	95[a]
3c	2′, 4′, 5′, 6′	85[b]
4c	2′, 5′, 6′	92[a]
5b	4, 5, 6, 2′, 4′, 5′, 6′	92[b]
6b	3, 4, 5, 6, 2′, 4′, 5′, 6′	87[a]
7b	2, 3, 4, 5, 6, 2′, 4′, 5′, 6′	87[c]

[a]Determined using TOF-MS.
[b]Determined using GC-MS.
[c]Determined using EIMS.

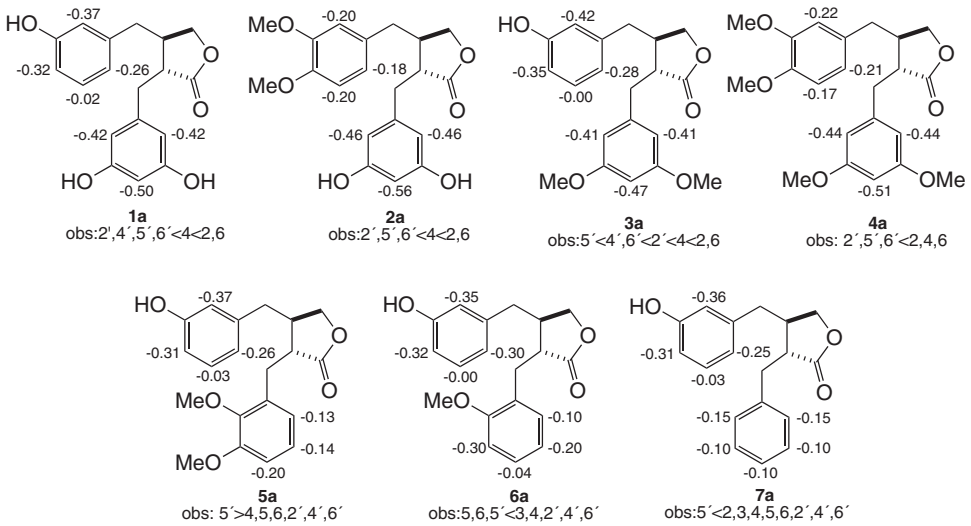


Figure 2. Calculated ESP charges for unsubstituted aromatic positions in molecules **1a**–**7a** and observed reactivity order (obs) during the reaction.

in **1b**–**4b** were replaced by ^{1}H atoms by treating the compounds with methanolic HCl[22] (0.5–1% CH_3COCl in MeOH). This precaution gave the stable tetradeutero **1cx** (92% i.p.) and **3c** (85% i.p.) and the trideutero **2c** (95% i.p.) and **4c** (92% i.p.) derivatives. To confirm that **5b**–**7b** and also the earlier reported $^{2}H_8$-enterolactone[23] were stable deuterated molecules, they were further refluxed for 30 min in 0.5% CH_3COCl/MeOH. No evidence of back exchange was found by mass and NMR spectroscopy. In addition, to ascertain that benzylic or α-carbonyl protons are not exchanged in the reaction, one of the reaction mixtures was quenched with $^{2}H_2O$ instead of water as in the normal workup procedure. No exchange could be seen in the benzylic or α-carbonyl positions.

To study the relative reactivities of the aromatic protons, ESP charges were calculated for these compounds. The global minima (sandwich conformation, Figure 1) obtained by conformational search with MacroModel were optimized with Spartan using AM1 and ESP charges (Figure 2) were obtained for these structures. Smaller ESP values indicate a lower electron charge density and therefore in this case more labile protons in the aromatic ring.

The observed reaction order was monitored with ^{1}H-NMR and results are shown in Figure 1 with calculated ESP values. The aim of this study was to optimize the labelling reactions to achieve stable and isotopically pure compounds. Labelling order of each lignan structure was studied separately and a detailed research of reaction conditions and times for compounds was not the primary importance.

It is interesting that the 2′ position in molecules **1a**, **5a** and **6a** has an ESP value of −0.37 but in **3a** this value is −0.42. This difference can be seen also in exchange reactions. In the dedeuteration of **3b** the 2′ position was more prone to back exchange giving isotopologues $^{2}H_4$ 85% and $^{2}H_3$ 15%.

According to calculated ESP values, the reactivity order for **1a**–**7a** in aromatic electrophilic substitution reaction was generally in agreement with the observations, regardless of the presumed inequality of the conformations in liquid and in vacuum (calculated) surroundings.

Experimental

Materials and methods

All compounds were characterized by ^{1}H-NMR, ^{13}C-NMR, LRMS and HRMS. $^{2}H_2O$ (99.9%) was obtained from Aldrich. NMR data were recorded on a Varian ^{UNITY}INOVA 300, Varian GEMINI 2000 or Bruker Avance 500 spectrometer. Chemical shifts are given in ppm with $SiMe_4$ as an internal standard. In the ^{13}C-NMR spectra the shifts given for the C–^{2}H triplets correspond to the center of the pattern and are marked 'D'. EIMS and HRMS were obtained using a JEOL JMS SX 102 spectrometer and the samples were introduced by a direct inlet probe. GC-MS analysis was obtained using a Micromass UK Autospec spectrometry equipped with DB-5 (HP) column. The oven temperature was programmed to rise from 70 to 280°C in 10 min. The temperature of interface and ion source was 280°C and electron energy of 70 eV was used. TOF-MS analyses were obtained using Bruker MicroTOF in the positive mode. Melting points were determined in open capillary tubes with a Buchi Melting Point B-545 apparatus and are uncorrected.

General procedure for lignanolactones

Lignanolactones were synthesized by the tandem Michael addition–alkylation method followed by Raney Ni desulfurization and debenzylation.[20] Molecules **2a** and **3a** have been characterized earlier.[20] Molecules **1a** and **4a**–**7a** were characterized by 2D NMR methods (heteronuclear multiple bond correlation, heteronuclear single quantum correlation (HSQC) and correlation spectroscopy).

3,5,3′-Trihydroxylignano-9,9′-lactone ***1a***: Prepared as previously reported forming a white amorphous solid.[25]: ^{1}H-NMR (500 MHz, d_6-acetone): δ 2.49 (1H, dd J = 9.8, 13.3 Hz, H-7′a), 2.53–2.61 (1H, m, H-8′), 2.62–2.67 (1H, m, H-8), 2.72 (1H, dd J = 4.3, 13.3 Hz, H-7′b), 2.80 (1H, dd J = 6.8, 13.8 Hz, H-7a), 2.90 (1H, dd J = 5.3, 13.8 Hz, H-7b), 3.88 (1H, t J = 8.8 Hz, H-9′a), 4.03 (1H, dd J = 7.1, 8.8 Hz, H-9′b), 6.24 (1H, t J = 2.0 Hz, H-4), 6.30 (2H, d J = 2.5 Hz, H-2, 6), 6.61 (1H, d J = 7.5 Hz, H-6′), 6.66 (1H, s, H-2′), 6.67 (overlapping H-2′, 1H, dd J = 1.5, 8.2 Hz, H-4′), 7.08 (1H, t J = 7.8 Hz, H-5′). ^{13}C-NMR (126 MHz, d_6-acetone): δ 35.5 (C-7), 38.7 (C-7′), 42.2 (C-8′), 46.7 (C-8), 71.5 (C-9′), 101.9 (C-4), 108.7 (C-2, 6), 114.3 (C-4′), 116.5 (C-2′), 120.6 (C-6′), 130.4 (C-5′), 141.3 and 141.5 (C-1, 1′), 158.5 (C-3′), 159.6 (C-3, 5), 178.8 (C-9). EIMS (70 eV) *m/z*: M^+ 314 (20%), 207 (8), 151 (12), 124 (49), 108 (29), 107 (19).

3,5,3′,4′-Tetramethoxylignano-9,9′-lactone ***4a***: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.50 (1H, dd J = 8.3, 16.8 Hz, H-7′a), 2.46–2.53 (1H, m, H-8′, overlapping 7′a), 2.56–2.63 (1H, m, H-8, overlapping 7′b), 2.60 (1H, dd J = 7.8, 16.3 Hz, H-7′b), 2.85 (1H, dd J = 7.5, 14.0 Hz, H-7a), 3.01 (1H, dd J = 5.0, 13.5 Hz, H-7b), 3.75 (6H, s, 3, 5-OMe), 3.82 (3H, s, 3′-OMe), 3.85 (3H, s, 4′-OMe), 3.88 (1H, dd J = 7.5, 9.5−Hz, H-9′a, overlapping 4′-OMe), 4.15 (1H, dd J = 7.0, 9.0 Hz, H-9′b), 6.32–6.33 (3H, m, H-2, 4, 6), 6.48 (1H, d J = 2.0 Hz, H-2′), 6.54 (1H, dd J = 2.0, 8.0 Hz, H-6′), 6.74 (1H, d J = 8.5 Hz, H-5′). ^{13}C-NMR (126 MHz, $CDCl_3$): δ 34.4 (C-7), 37.2 (C-7′), 40.4 (C-8′), 45.1 (C-8), 54.2 (3, 5-OMe), 54.7 (3′-OMe), 54.9 (4′-OMe), 70.3 (C-9′), 97.6 (C-4), 106.3 (C-2, 6), 110.3 (C-5′), 110.7 (C-2′), 119.5 (C-6′), 129.5 (C-1′), 139.0 (C-1), 146.8 (C-4′), 148.1 (C-3′), 160.0 (C-3, 5), 177.6 (C-9). EIMS (70 eV) *m/z*: M^+ 386 (85%), 235 (11), 221 (14), 189 (11), 177 (23), 152 (100), 151 (63), 91 (11); HRMS (*m/z*): M^+ calcd. for $C_{22}H_{26}O_6$: 386.1729; found: 386.1724.

3′-Hydroxy-2,3-dimethoxylignano-9,9′-lactone ***5a***: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.32 (1H, dd J = 10.2; 14.4 Hz, H-7′a), 2.5–2.6 (2H, m, H-7′b, 8′), 2.68 (1H, dt J = 5.1, 8.6 Hz, H-8), 2.85 (1H, dd J = 13.6, 8.7 Hz, H-7a), 3.27 (1H, dd J = 5.0, 13.6 Hz, H-7b), 3.85 (3H, s, 2-OMe), 3.87 (3H, s, 3-OMe), 3.83 (1H, t J = 9.0 Hz, H-9′a, overlapping 2, 3-OMe), 4.11 (1H, dd J = 7.3, 9.1 Hz, H-9′b), 5.11 (1H, s, OH), 6.40 (1H, s, H-2′), 6.53 (1H, d J = 7.5 Hz, H-6′), 6.65 (1H, dd J = 2.4, 8.1 Hz, H-4′), 6.83 (1H, d J = 7.6 Hz, H-6), 6.85 (1H, d J = 8.0 Hz, H-4), 7.01 (1H, t J = 8.0 Hz, H-5), 7.08 (1H, t J = 7.9 Hz, H-5′). ^{13}C-NMR (126 MHz, $CDCl_3$): δ 30.0 (C-7), 38.3 (C-7′), 41.7 (C-8′), 45.4 (C-8), 55.7 (3-OMe), 60.6 (2-OMe), 71.3 (C-9′), 111.3 (C-4), 113.6 (C-4′), 115.5 (C-2′), 121.0 (C-6′), 122.8 (C-6), 124.3 (C-5′), 129.8 (C-5), 131.9 (C-1), 140.0 (C-1′), 147.5 (C-2), 152.8 (C-3), 155.8 (C-3′), 179.0 (C-9). EIMS (70 eV) *m/z*: M^+ 342 (100%), 235 (38), 189 (25), 177 (27), 151 (77), 136 (74), 107 (63), 91 (66); HRMS (*m/z*): M^+ calcd. for $C_{20}H_{22}O_5$: 342.1467; found: 342.1479.

3′-Hydroxy-2-methoxylignano-9,9′-lactone ***6a***: M.p. 121°C (chloroform–hexane). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.34 (1H, dd J = 9.3, 13.8 Hz, H-7′a), 2.48–2.57 (1H, m, H-8′, overlapping 7′b), 2.58 (1H, dd J = 5.3, 13.3 Hz, H-7′b), 2.69–2.73 (1H, m, H-8), 2.82 (1H, dd J = 8.0, 13.5 Hz, H-7a), 3.30 (1H, dd J = 5.0, 14.0 Hz, H-7b), 3.81 (3H, s, OMe), 3.83 (1H, t J = 8.5 Hz, H-9′a, overlapping OMe),

4.11 (1H, t J = 8.3 Hz, H-9′b), 6.38 (1H, s, H-2′), 6.48 (1H, d J = 7.0 Hz, H-6′), 6.66 (1H, dd J = 2.3, 8.3 Hz, H-4′), 6.86 (1H, d J = 8.0 Hz, H-3), 6.91 (1H, t J = 7.3 Hz, H-4), 7.07 (1H, t J = 8.0 Hz, H-5′), 7.19 (1H, d J = 7.0 Hz, H-6), 7.24 (1H, td J = 1.5, 8.0 Hz, H-5). ^{13}C-NMR (126 MHz, $CDCl_3$): δ 30.3 (C-7), 38.4 (C-7′), 41.7 (C-8′), 45.0 (C-8), 55.3 (OMe), 71.5 (C-9′), 110.5 (C-3), 113.7 (C-4′), 115.6 (C-2′), 120.9 (C-4, 6′), 126.5 (C-1), 128.5 (C-5), 129.9 (C-5′), 131.3 (C-6), 140.1 (C-1′), 156.1 (C-3′), 157.8 (C-2), 179.1 (C-9). EIMS (70 eV) *m/z*: M^+ 312 (96%), 205 (44), 178 (43), 147 (31), 121 (100), 108 (49), 107 (55), 91 (96); HRMS (*m/z*): M^+ calcd. for $C_{19}H_{20}O_4$: 312.1362; found: 312.1350.

3′-Hydroxylignano-9,9′-lactone ***7a***: M.p. 111°C (chloroform–-hexane). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.44 (1H, dd J = 9.0, 13.0 Hz, H-7′a), 2.46–2.55 (1H, m, H-8′, overlapping 7′a), 2.60 (1H, dd J = 5.5, 13.0 Hz, H-7′b), 2.58–2.63 (1H, m, H-8), 2.94 (1H, dd J = 8.0, 14.0 Hz, H-7a), 3.10 (1H, dd J = 5.0, 14.0 Hz, H-7b), 3.85 (1H, dd J = 7.8, 9.3 Hz, H-9′a), 4.10 (1H, dd J = 7.3, 9.3 Hz, H-9′b), 6.43 (1H, t J = 2.0 Hz, H-2′), 6.56 (1H, d J = 7.5 Hz, H-6′), 6.68 (1H, dd J = 2.3, 7.8 Hz, H-4′), 7.12 (1H, t J = 7.8 Hz, H-5′), 7.17–7.19 (2H, m, H-2, 6), 7.24 (1H, t J = 7.5 Hz, H-4), 7.30 (2H, t J = 7.3 Hz, H-3, 5). ^{13}C-NMR (126 MHz, $CDCl_3$): δ 35.1 (C-7), 38.4 (C-7′), 41.1 (C-8′), 46.5 (C-8), 71.2 (C-9′), 113.8 (C-4′), 115.5 (C-2′), 121.0 (C-6′), 126.9 (C-4), 128.8 (C-3, 5), 129.3 (C-2, 6), 129.9 (C-5′), 137.7 (C-1), 139.7 (C-1′), 155.9 (C-3′), 178.6 (C-9). EIMS (70 eV) *m/z*: M^+ 282 (100%), 175 (46), 134 (76), 133 (61), 108 (85), 107 (65), 91 (85); HRMS (*m/z*): M^+ calcd. for $C_{18}H_{18}O_3$: 282.1256; found: 282.1249.

General procedure for the deuteration of lignanolactones

The deuteration reagent $^2H_3PO_4 \cdot BF_3/^2H_2O$ was prepared by dissolving dry P_2O_5 (1.5 g, 11 mmol) in ^2H_2O (1.5 ml, 83 mmol) at 0°C. The resulting deuterated phosphoric acid was then saturated with BF_3 gas at room temperature. The reagent (4 ml/100 mg of lignanolactone) was added to the lignanolactone and the mixture was stirred at room temperature. After deuteration the reaction mixture was poured into ice water and the product was extracted with EtOAc. The organic extract was washed with water until neutral, dried with anhydrous Na_2SO_4 and evaporated. The procedure was repeated if necessary.

[2,4,6,2′,4′,5′,6′-$^2H_7$]-3,5,3′-trihydroxylignano-9,9′-lactone ***1b***: The reaction mixture was stirred for 18 h using **1a** (0.062 g, 0.20 mmol) as the starting material. The crude product, an amorphous solid (0.050 g, 81%), was used directly for the dedeuteration step. ^{1}H-NMR (300 MHz, d_6-acetone): δ 2.46–2.68 (3H, m, H-7a, 8′, 8), 2.74 (1H, dd J = 3.6, 12.0 Hz, H-7′b), 2.80 (1H, dd J = 6.5, 13.5 Hz, H-7a), 2.90 (1H, dd J = 5.2, 13.6 Hz, H-7b), 3.88 (1H, t J = 8.5 Hz, H-9′a), 4.03 (1H, dd J = 7.1, 8.8 Hz, H-9′b), 6.29 (0.3H, s, H-2, 6), 8.15 (1H, s, OH), 8.20 (0.5H, s, OH). ^{13}C-NMR (75 MHz, d_6-acetone): δ 35.3 (C-7), 38.6 (C-7′), 42.1 (C-8′), 46.7 (C-8), 71.5 (C-9′), 101.7 (t, C-4)^D, 108.4 (t, C-2, 6)^D, 113.9 (t, C-4′)^D, 116.2 (t, C-2′)^D, 120.1 (t, C-6′)^D, 129.8 (t, C-5′)^D,141.1 and 141.2 (C-1′, 1), 158.4 (C-3′), 159.5 (C-3, 5), 178.9 (C-9). EIMS (70 eV) *m/z*: M^+ 321 (5%), 320 (15), 319 (15), 318 (13), 208 (5), 126 (24), 125 (40), 124 (33), 112 (16), 111 (18), 110 (12), 91 (13); HRMS (*m/z*): M^+ calcd. for $C_{18}H_{11}{}^2H_7O_5$: 321.1594; found: 321.1596.

[2,4,6,2′,5′,6′-$^2H_6$]-3,5-dihydroxy-3′,4′-dimethoxylignano-9,9′-lactone ***2b***: The reaction mixture was stirred for 22 h using **2a** (0.10 g, 0.28 mmol) as the starting material. The crude product, an amorphous solid (0.98 g, 96%), was used directly for the dedeuteration step. ^{1}H-NMR (300 MHz, d_6-acetone): δ 2.5–2.7 (4H, m, H-7′, 8 ,8′), 2.79 (1H, dd J = 13.8, 6.3 Hz, H-7a), 2.89 (1H, dd J = 5.1, 13.5 Hz, H-7b), 3.76 (3H, s, 4′-OMe), 3.78 (3H, s, 3′-OMe), 3.90 (1H, t J = 8.7 Hz, 9′a), 4.07 (1H, dd J = 7.4, 8.6 Hz, H-9′b), 6.28 (0.2H, s, H-2, 6), 8.20 (1H, s, OH). ^{13}C-NMR (75 MHz, d_6-acetone): δ 35.3 (C-7), 38.1 (C-7′), 42.4 (C-8′), 46.5 (C-8), 56.0 (4′-OMe), 56.1 (3′-OMe), 71.6 (C-9′), 101.9 (t, C-4)^D, 108.5 (t, C-2, 6)^D, 112.8 (t, C-5′)^D, 113.1 (t, C-2′)^D, 121.0 (t, C-6′)^D, 132.2 (C-1′), 141.4 (C-1), 149.0 (C-4′), 150.4 (C-3′), 159.5 (C-3, 5), 178.9 (C-9). EIMS (70 eV) *m/z*: M^+ 364 (32%), 363 (95), 362 (74), 361 (26), 181 (24), 180 (77), 179 (14), 156 (35), 155 (100), 154 (100), 153 (33), 140 (15), 141 (15), 127 (27), 126 (50), 125 (50), 124 (31), 111 (14), 110 (13); HRMS (*m/z*): M^+ calcd. for $C_{20}H_{16}{}^2H_6O_6$: 364.1793; found: 364.1776.

[2,4,6,2′,4′,5′,6′-$^2H_7$]-3′-hydroxy-3,5-dimethoxylignano-9,9′-lactone ***3b***: The reaction mixture was stirred for 20 h using **3a** (0.10 g, 0.29 mmol) as the starting material. The crude product, an amorphous solid (0.09 g, 86%), was used directly for the dedeuteration step. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.52 (4H, m, H-7′, 8, 8′), 2.85 (1H, dd J = 6.6, 14.1 Hz, H-7a), 3.00 (1H, dd J = 4.2, 14.1 Hz, H-7b), 3.76 (6H, s, 2 × OMe), 3,83 (1H, m, H-9′a), 4.11 (1H, dd J = 7.2, 8.7 Hz, H-9′b). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 35.2 (C-7), 38.2 (C-7′), 41.0 (C-8′), 46.3 (C-8), 55.4 (2 × OMe), 71.4 (C-9′), 98.9 (s and t, C-4)^D, 107.4 (s and t, C-2,6)^D, 113.4 (t, C-4′)^D, 115.3 (t, C-2′)^D, 120.2 (t, C-6′)^D, 129.0 (t, C-5′)^D, 139.4 (C-1′), 139.9 (C-1), 156.1 (C-3′), 160.9 (C-3, 5), 179.1 (C-9). EIMS (70 eV) *m/z*: M^+ 349 (100%), 238 (22), 180 (17), 154 (70), 139 (70), 111 (39), 94 (43); HRMS (*m/z*): M^+ calcd. for $C_{20}H_{15}{}^2H_7O_5$: 349.1907; found: 349.1912.

[2,4,6,2′,5′,6′-$^2H_6$]-3,5,3′,4′-tetramethoxylignano-9,9′-lactone ***4b***: The reaction mixture was stirred for 21 h using **4a** (0.115 g, 0.30 mmol) as the starting material. The crude product, an amorphous solid (0.113 g, 97%), was used directly for the dedeuteration step. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.44–2.54 (2H, m, H-7′a, 8′), 2.56–2.63 (2H, m, H-8, 7′b), 2.85 (1H, dd J = 7.3, 13.9 Hz, H-7a), 3.02 (1H, dd J = 4.1, 14.0 Hz, H-7b), 3.75 (6H, s, 3, 5-OMe), 3.82 (3H, s, 3′-OMe), 3.85 (3H, s, 4′-OMe), 3.88 (1H, m, H-9′a, overlapping 4′-OMe), 4.15 (1H, dd J = 6.6, 8.7 Hz, H-9′b), 6.32 (0.1H, s, H-2, 4, 6). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 35.3 (C-7), 38.1 (C-7′), 41.4 (C-8′), 46.2 (C-8), 55.3 (3, 5-OMe), 55.7 (3′-OMe), 55.9 (4′-OMe), 71.3 (C-9′), 98.3 (t, C-4)^D, 107.1 (t, C-2, 6)^D, 110.5 (t, C-5′)^D, 111.5 (t, C-2′)^D, 120.2 (t, C-6′)^D, 130.3 (C-1′), 140.0 (C-1), 147.8 (C-4′), 149.1 (C-3′), 161.0 (C-3, 5), 178.6 (C-9). EIMS (70 eV) *m/z*: M^+ 392 (68%), 391 (31), 237 (18), 223 (18), 181 (19), 180 (37), 156 (62), 155 (81), 154 (100), 153 (73), 110 (27), 109 (21), 94 (26), 93 (21); HRMS (*m/z*): M^+ calcd. for $C_{22}H_{20}{}^2H_6O_6$: 392.2106; found: 392.2105.

[4,5,6,2′,4′,5′,6′-$^2H_7$]-3′-hydroxy-2,3-dimethoxylignano-9,9′-lactone ***5b***: The reaction mixture was stirred for 24 h using **5a** (0.10 g, 0.30 mmol) as the starting material. The crude product, an amorphous solid (0.097 g, 93%), was purified by short flash chromatography (column: 1.5 cm × 5.5 cm) with CH_2Cl_2/EtOAc (1:1) as the eluent affording an off white amorphous solid. Proportions of isotopologues in GC-MS spectra were 8% $^2H_6$ and 100% $^2H_7$. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.30 (1H, dd J = 11.1, 15.0 Hz, H-7′a), 2.53 (1H, dd J = 4.5, 15.0 Hz, H-7′b), 2.40 (1H, m, H-8′, overlapping H-7′) 2.68 (1H, dt J = 5.1, 8.6 Hz, H-8), 2.85 (1H, dd J = 13.5, 8.4 Hz, H-7a), 3.26 (1H, dd J = 5.1, 13.5 Hz, H-7b), 3.84 (3H, s, 2-OMe), 3.86 (3H, s, 3-OMe), 3.83 (1H, m, H-9′a, overlapping 2, 3-OMe), 4.09 (1H, dd J = 7.1, 8.9 Hz, H-9′b). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 29.8 (C-7), 38.1 (C-7′), 41.7 (C-8′), 45.4 (C-8), 55.7 (3-OMe), 60.6 (2-OMe), 71.4 (C-9′), 110.9 (t, C-4)^D, 113.2 (t, C-4′)^D, 115.2 (t, C-2′)^D, 120.3 (t, C-6′)^D, 122.4 (t, C-6)^D, 123.9 (t, C-5′)^D, 129.2 (t, C-5)^D, 131.7 (C-1), 139.7 (C-1′), 147.3 (C-2), 152.6 (C-3), 156.0 (C-3′), 179.3 (C-9). EIMS (70 eV) *m/z*: M^+ 349

(61%), 238 (7), 180 (6), 154 (22), 139 (23), 111 (12), 94 (13); HRMS (*m/z*): M^+ calcd. for $C_{20}H_{15}{}^2H_7O_5$: 349.1907; found: 349.1890.

[3,4,5,6,2′,4′,5′,6′-$^2H_8$]-3′-hydroxy-2-methoxylignano-9,9′-lactone ***6b***: The reaction mixture was stirred for 19 h using **6a** (0.086 g, 0.27 mmol) as the starting material. The brownish crude product (0.083 g, 96%) was purified by reversed phase preparative layer chromatography (PLC) with MeOH/H_2O (4:1) as the eluent to get an off white amorphous solid. Proportions of isotopologues in TOF-MS spectra were 14% $^2H_7$ and 100% $^2H_8$. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.35 (1H, dd *J* = 8.6, 12.8 Hz, H-7′a), 2.46–2.62 (1H, m, H-8′, overlapping H-7′b), 2.59 (1H, dd *J* = 5.1, 12.8 Hz, H-7′b), 2.70 (1H, dt *J* = 5.0, 8.3 Hz, H-8), 2.82 (1H, dd *J* = 8.4, 13.5 Hz, H-7a), 3.30 (1H, dd *J* = 4.8, 13.5 Hz, H-7b), 3.82 (3H, s, OMe), 3.84 (1H, m, H-9′a, overlapping OMe), 4.13 (1H, dd *J* = 7.1, 9.2 Hz, H-9′b). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 30.1 (C-7), 38.2 (C-7′), 41.6 (C-8′), 44.9 (C-8), 55.2 (OMe), 71.4 (C-9′), 110.0 (t, C-3)^D, 113.2 (t, C-4′)^D, 115.1 (t, C-2′)^D, 120.3 (t, C-4, 6′)^D, 126.1 (C-1), 127.8 (t, C-5)^D, 129.3 (t, C-5′)^D, 130.8 (t, C-6)^D, 139.8 (C-1′), 155.9 (C-3′), 157.6 (C-2), 179.6 (C-9). EIMS (70 eV) *m/z*: M^+ 320 (93%), 319 (62), 209 (28), 182 (35), 151 (27), 124 (40), 125 (100), 126 (40), 112 (36), 111 (50), 95 (97), 96 (30); HRMS (*m/z*): M^+ calcd. for $C_{19}H_{12}{}^2H_8O_4$: 320.1864; found: 320.1850.}

[2,3,4,5,6,2′,4′,5′,6′-$^2H_9$]-3′-hydroxylignano-9,9′-lactone ***7b***: The reaction mixture was stirred for 23 h using **7a** (0.039 g, 0.14 mmol) as the starting material. The brownish crude product (0.037 g, 91%) was purified by reversed phase preparative layer chromatography (PLC) with MeOH/H_2O (4:1) as the eluent to get an off white amorphous solid. Proportions of isotopologues in EIMS spectra were 2% $^2H_7$, 15% $^2H_8$ and 100% $^2H_9$. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.41–2.65 (4H, m, H-7′a, 8′, 7′b, 8), 2.94 (1H, dd *J* = 6.9, 14.1 Hz, H-7a), 3.09 (1H, dd *J* = 4.5, 14.1 Hz, H-7b), 3.85 (1H, t *J* = 8.0 Hz, H-9′a), 4.10 (1H, t *J* = 7.8 Hz, H-9′b). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 35.0 (C-7), 38.2 (C-7′), 41.1 (C-8′), 46.5 (C-8), 71.2 (C-9′), 113.4 (t, C-4′)^D, 115.2 (t, C-2′)^D, 120.6 (t, C-6′)^D, 126.4 (t, C-4)^D, 128.2 (t, C-3, 5)^D, 128.9 (t, C-2, 6)^D, 129.5 (t, C-5′)^D, 137.4 (C-1), 139.6 (C-1′), 155.8 (C-3′), 178.7 (C-9). EIMS (70 eV) *m/z*: M^+ 291 (99%), 181 (25), 180 (20), 138 (63), 137 (59), 112 (94), 111 (81), 96 (100); HRMS (*m/z*): M^+ calcd. for $C_{18}H_9{}^2H_9O_3$: 291.1821; found: 291.1831.

General procedure for dedeuteration

The deuterated product was dissolved in HCl/MeOH (from 0.5 to 1% CH_3COCl/MeOH) (0.10–0.25 ml/mg) and stirred in ambient temperature or refluxed for 0.5–4 h. The cooled reaction mixture was poured into ice water (1.5–2.5 ml/mg) and extracted with EtOAc, washed with brine, dried with anhydrous Na_2SO_4 and evaporated.[22]

[2′,4′,5′,6′-$^2H_4$]-3,5,3′-trihydroxylignano-9,9′-lactone ***1c***: **1b** (0.008 g, 0.025 mmol) was stirred in 0.5% CH_3COCl/MeOH (2 ml) for 1 h at room temperature. The brownish product (0.007 g, 89%) was purified by reversed phase preparative layer chromatography (PLC) with MeOH/H_2O (4:1) as the eluent to get an off white amorphous solid. Proportions of isotopologues in TOF-MS spectra were 9% $^2H_3$ and 100% $^2H_4$. ^{1}H-NMR (300 MHz, d_6-acetone): δ 2.49 (1H, dd *J* = 9.3, 12.6 Hz, H-7′a), 2.51–2.68 (2H, m, H-8′, 8), 2.73 (1H, dd *J* = 3.7, 12.1 Hz, H-7′b), 2.80 (1H, dd *J* = 6.2, 13.7 Hz, H-7a), 2.90 (1H, dd *J* = 5.3, 14.0 Hz, H-7b), 3.88 (1H, t *J* = 8.5 Hz, H-9′a), 4.03 (1H, dd *J* = 7.1, 8.9 Hz, H-9′b), 6.25 (1H, t *J* = 2.2 Hz, H-4), 6.29 (2H, d *J* = 2.1 Hz, H-2, 6), 8.19 (2H, s, OH). ^{13}C-NMR (75 MHz, d_6-acetone): δ 35.4 (C-7), 38.6 (C-7′), 42.2 (C-8′), 46.7 (C-8), 71.5 (C-9′), 101.9 (C-4), 108.7 (C-2, 6), 113.8 (C-4′)^D, 116.3 (C-2′)^D, 120.2 (C-6′)^D, 129.9 (C-5′)^D, 141.2 and 141.5 (C-1′, 1), 158.3 (C-3′), 159.6 (C-3, 5), 178.8 (C-9). EIMS (70 eV) *m/z*: M^+ 318 (38%), 317 (31), 207 (20), 136 (21), 126 (33), 125 (85), 124 (100), 123 (27), 112 (24), 111 (39), 110 (38), 109 (22); HRMS (*m/z*): M^+ calcd. for $C_{18}H_{14}{}^2H_4O_5$: 318.1405; found: 318.1391.

[2′,5′,6′-$^2H_3$]-3,5-dihydroxy-3′,4′-dimethoxylignano-9,9′-lactone ***2c***: **2b** (0.088 g, 0.24 mmol) was refluxed in 1% CH_3COCl/MeOH (15 ml) for 2 h. The brownish crude product (0.077 g, 88%) was purified with Sephadex LH-20 (eluent: first 20:0.5 (v/v) CH_2Cl_2–MeOH then 20:1 (v/v) CH_2Cl_2–MeOH and finally 20:1.5 (v/v) CH_2Cl_2–MeOH). Crystallization (water, isopropanol) gave an off white solid, m.p. 106°C. Proportions of isotopologues in TOF-MS spectra were 5% $^2H_2$ and 100% $^2H_3$. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.48–2.72 (4H, m, H-8, 8′, 7′), 2.84 (1H, dd *J* = 6.3, 13.8 Hz, H-7a), 2.89 (1H, dd *J* = 5.2, 13.6 Hz, H-7b), 3.76 (3H, s, 4′-OMe), 3.78 (3H, s, 3′-OMe), 3.90 (1H, t *J* = 8.7 Hz, H-9′a), 4.07 (1H, dd *J* = 6.9, 8.7 Hz, H-9′b), 6.26 (1H t *J* = 1.8 Hz, H-4), 6.29 (2H, d *J* = 2.1 Hz, H-2, 6), 8.22 (1H, br s, OH). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 35.4 (C-7), 38.1 (C-7′), 42.4 (C-8′), 46.4 (C-8), 55.9 (4′-OMe), 56.0 (3′-OMe), 71.6 (C-9′), 101.6 (C-4), 108.7 (C-2, 6), 113.1 (t, C-2′, 5′)^D, 121.0 (t, C-6′)^D, 132.1 (C-1′), 141.6 (C-1), 148.9 (t, C-4′), 150.3 (C-3′), 159.6 (C-3, 5), 178.9 (C-9). EIMS (70 eV) *m/z*: M^+ 361 (100%), 238 (5), 180 (32), 154 (97), 140 (11), 124 (46), 110 (7); HRMS (*m/z*): M^+ calcd. for $C_{20}H_{19}{}^2H_3O_6$: 361.1591; found: 361.1605.

[2′,4′,5′,6′-$^2H_4$]-3′-hydroxy-3,5-dimethoxylignano-9,9′-lactone ***3c***: The following dedeuteration procedure was performed two times: **3b** (0.058 g, 0.17 mmol) was refluxed in 0.5% CH_3COCl/MeOH (6 ml) for 2 h. The brownish crude product (0.047 g, 81%) was purified by reversed phase column chromatography with MeOH/H_2O (5:1) as the eluent to get an off white amorphous solid. Proportions of isotopologues in GC-MS spectra were 17% $^2H_3$ and 100% $^2H_4$. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.41–2.63 (4H, m, H-7′, 8, 8′), 2.85 (1H, dd *J* = 14.0, 7.1 Hz, H-7a), 3.00 (1H, dd *J* = 13.7, 4.7 Hz, H-7b), 3.76 (6H, s, 2 × OMe), 3,84 (1H, dd *J* = 7.2, 9.0 Hz, H-9′a), 4.13 (1H, dd *J* = 8.7, 6.9 Hz, H-9′b), 6.34 (3H, s, H-2, 4, 6). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 35.3 (C-7), 38.2 (C-7′), 41.1 (C-8′), 46.3 (C-8), 55.4 (OMe), 71.4 (C-9′), 98.9 (C-4), 107.3 (C-2, 6), 113.4 (t, C-4′)^D, 115.3 (C-2′)^D, 120.2 (t, C-6′)^D, 129.0 (t C-5′)^D, 139.5 (C-1′), 140.0 (C-1), 156.1 (C-3′), 161.1 (C-3, 5), 179.0 (C-9). EIMS (70 eV) *m/z*: M^+ 346 (37%), 235 (7), 189 (7), 152 (100), 137 (12), 111 (9); HRMS (*m/z*): M^+ calcd. for $C_{20}H_{18}{}^2H_4O_5$: 346.1718; found: 346.1733.

[2′,5′,6′-$^2H_3$]-3,5,3′,4′-tetramethoxylignano-9,9′-lactone ***4c***: **4b** (0.076 g, 0.19 mmol) was refluxed in 1% CH_3COCl/MeOH (16 ml) for 3 h. The brownish product (0.071 g, 96%) was purified by reversed phase column chromatography with MeOH/H_2O (4:1) as the eluent to get an off white amorphous solid. Proportions of isotopologues in TOF-MS spectra were 8% $^2H_2$ and 100% $^2H_3$. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.44–2.54 (2H, m, H-7′a, 8′), 2.55–2.63 (2H, m, H-8, 7′b), 2.86 (1H, dd *J* = 7.2, 13.8 Hz, H-7a), 3.02 (1H, dd *J* = 4.9, 13.9 Hz, H-7b), 3.76 (6H, s, 3, 5-OMe), 3.82 (3H, s, 3′-OMe), 3.85 (3H, s, 4′-OMe), 3.88 (1H, dd *J* = 6.9, 9.3 Hz, H-9′a, overlapping 4′-OMe), 4.15 (1H, dd *J* = 7.1, 9.2 Hz, H-9′b), 6.33 (3H, s, H-2, 4, 6). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 35.4 (C-7), 38.1 (C-7′), 41.4 (C-8′), 46.2 (C-8), 55.3 (3, 5-OMe), 55.6 (3′-OMe), 55.9 (4′-OMe), 71.3 (C-9′), 98.6 (C-4), 107.3 (C-2, 6), 111.0 (C-5′)^D, 111.5 (C-2′)^D, 120.2 (C-6′)^D, 130.3 (C-1′), 140.1 (C-1), 147.8 (C-4′), 149.1 (C-3′), 161.0 (C-3, 5), 178.6 (C-9). EIMS (70 eV) *m/z*: M^+ 389 (82%), 221 (16), 180 (22), 155 (34), 154 (79), 153 (36), 152 (100), 151 (28), 108 (13), 109 (13), 91 (113); HRMS (*m/z*): M^+ calcd. for $C_{22}H_{23}{}^2H_3O_6$: 389.1915; found: 389.1923.

Conclusions

An efficient deuteration method forming seven new labelled lignanolactones is described. The $^{1}H/^{2}H$ exchange order was predicted using ESP calculations and was found to be in agreement with the observations.

The labelled lignans are isomerically and isotopically pure molecules possessing three to nine deuteriums in the aromatic rings. These stable compounds can be reliably used as reference materials in GC-MS and LC-MS methods of quantitative analysis and also in metabolic studies.

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