HELSINGIN YLIOPISTO
HELSINGFORS UNIVERSITET
UNIVERSITY OF HELSINKI

Synthesis of isotopically labelled phytoestrogen standards

Kristiina Wähälä

Laboratory of Organic Chemistry, Department of Chemistry

P.O. Box 55, FIN-00014 University of Helsinki, Finland

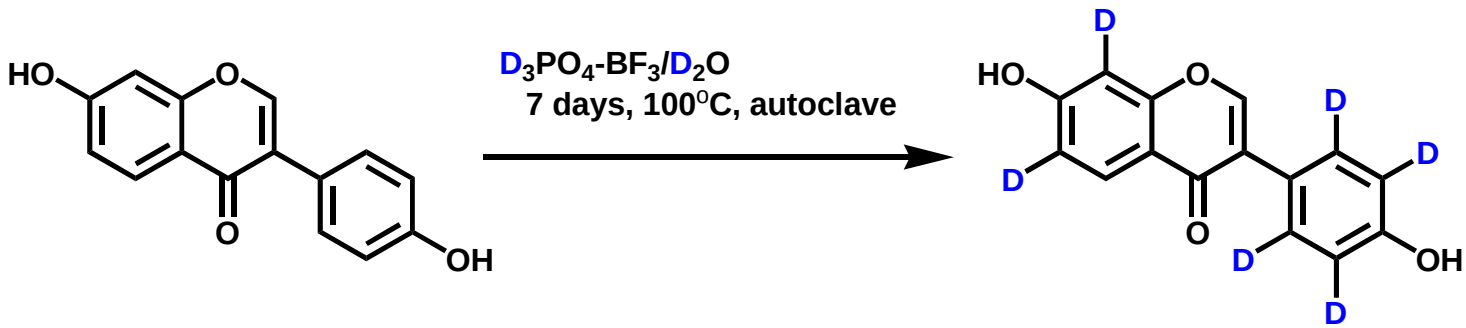
Why label ?

- analytical reference standards in mass spectral quantitation
- elucidation of metabolic pathways
- clarification of NMR spectra and mass spectral fragmentation pathways

Which labels?

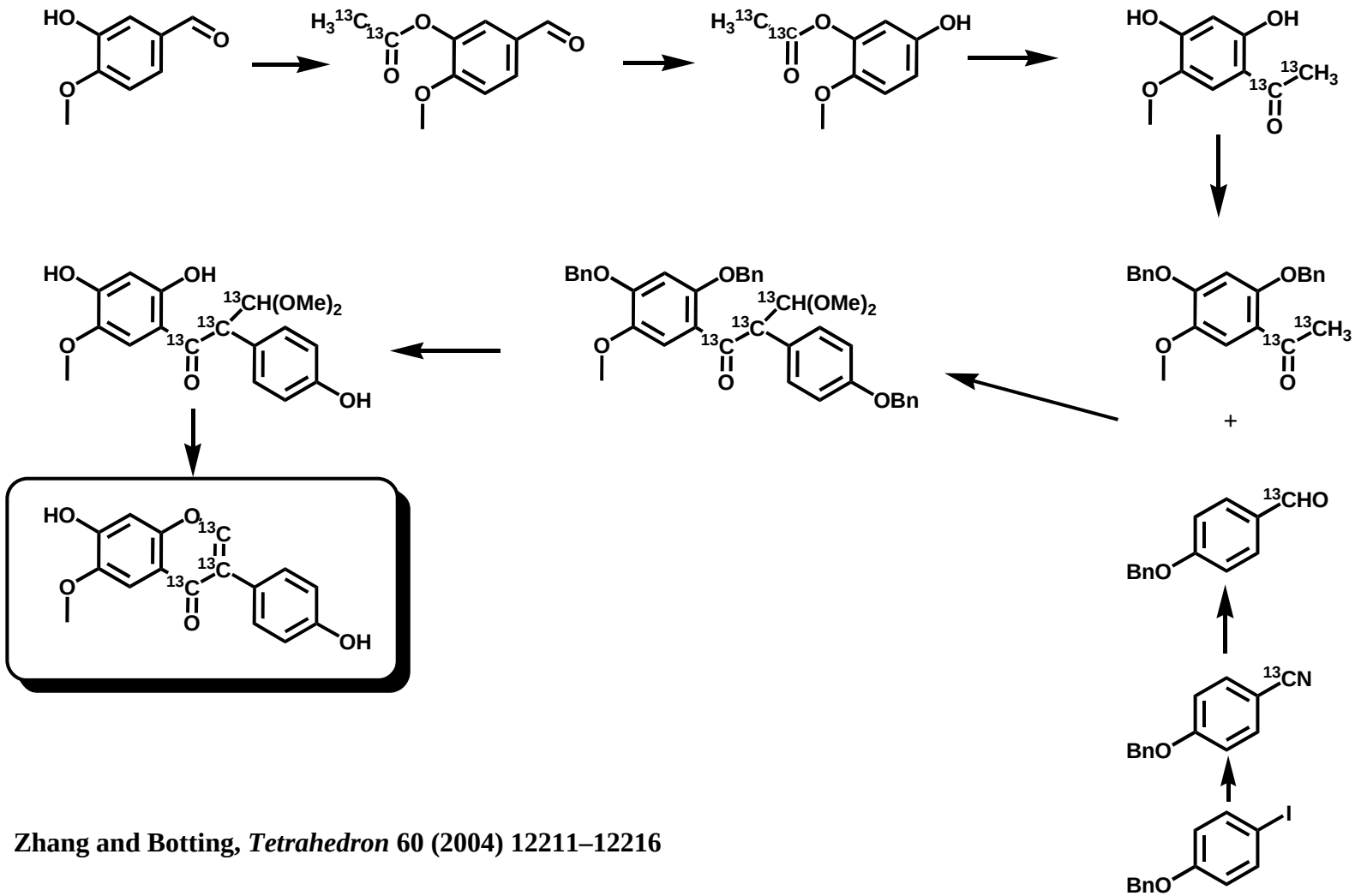
Cold	Stable	Radioactive
H	D	T
^{12}C	^{13}C	^{14}C
^{16}O	^{18}O	
^{14}N	^{15}N	

- certain hydrogens may be changed into deuteriums **within the finished molecular framework**



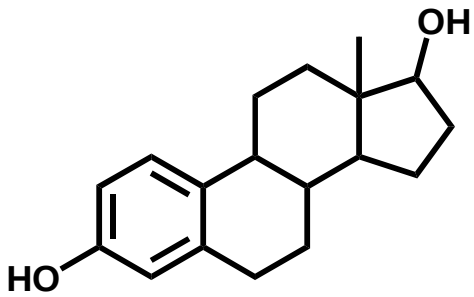
- A ^{13}C-label be must be carried over all the way from a ^{13}C-label containing starting material in a total synthesis sequence

Synthesis of triply labelled glycitein

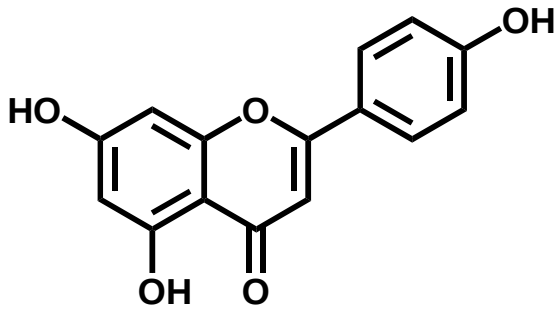


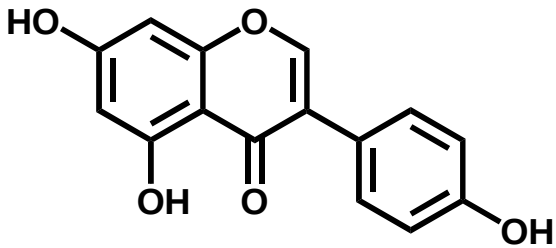
Zhang and Botting, *Tetrahedron* 60 (2004) 12211–12216

- 99 % pure ^{13}C-labeled phenol costs ca. 600 US $ for 100 mg
- Doubly ^{13}C-labeled acetyl chloride 1000 US $ for 1 gram
- 99 % pure D_2O 1 US $ for 1 gram

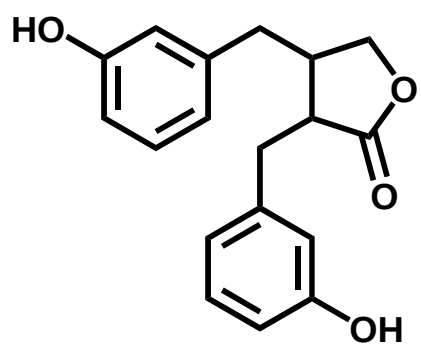


steroid estrogens

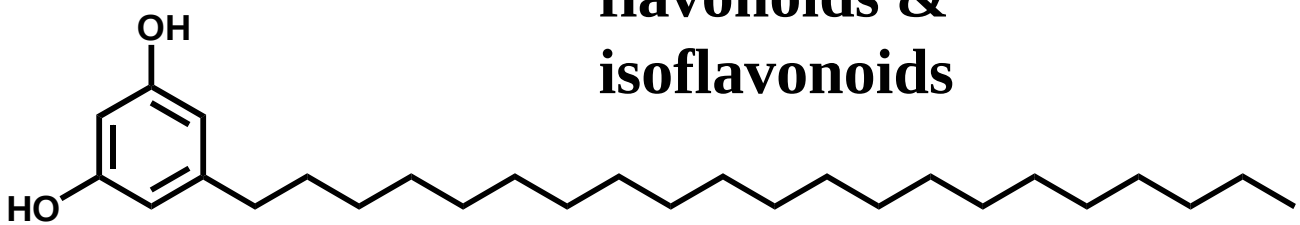




flavonoids & isoflavonoids



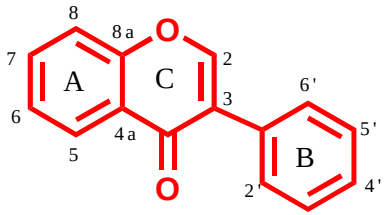
lignans



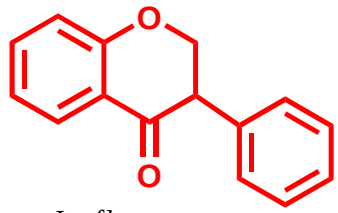
alkylresorcinols

Skeletal structures for the isoflavonoid classes

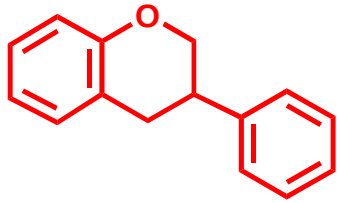
phytoestrogenic isoflavonoids



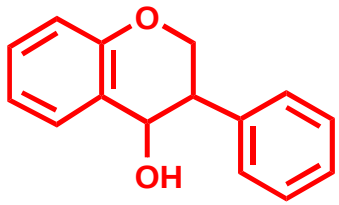
Isoflavones



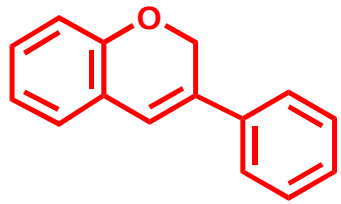
Isoflavanones



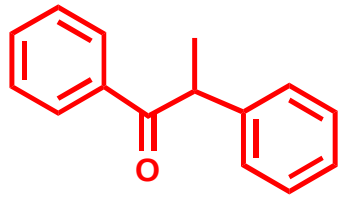
Isoflavans



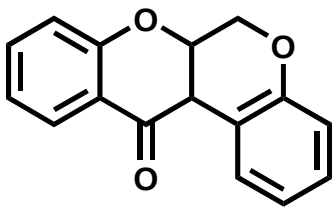
Isoflavanols



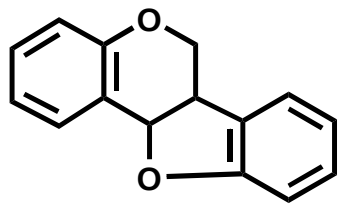
Isoflav-3-enes



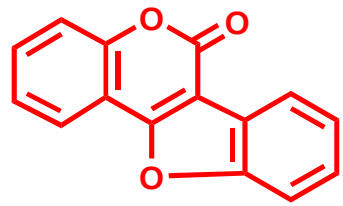
α-Methyldeoxybenzoins



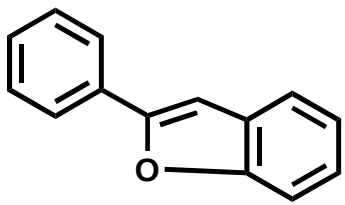
Rotenoids



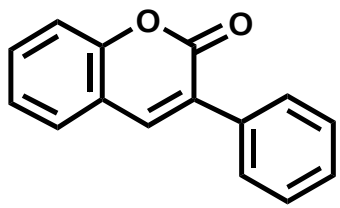
Pterocarpans



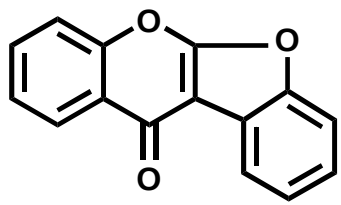
Coumestans



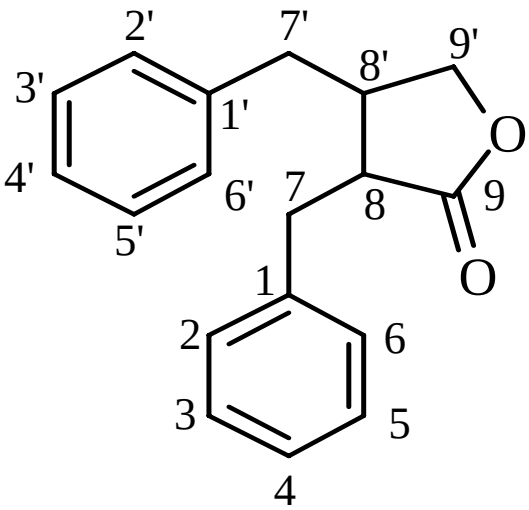
2-Arylbenzofurans



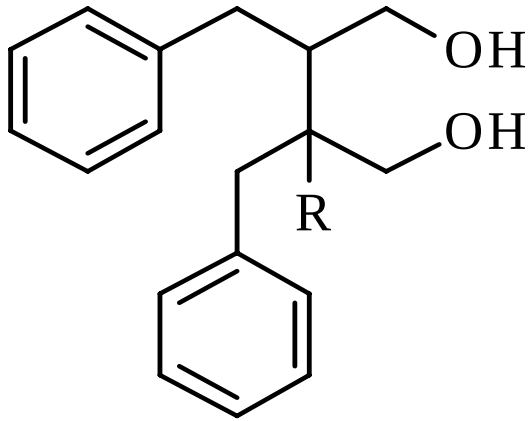
3-Arylcoumarins



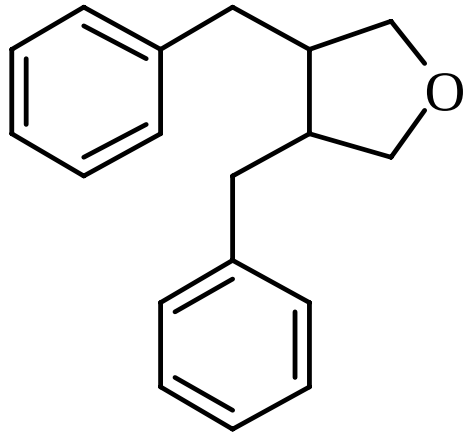
Coumaronochromones



Type 1
Dibenzylbutyrolactones

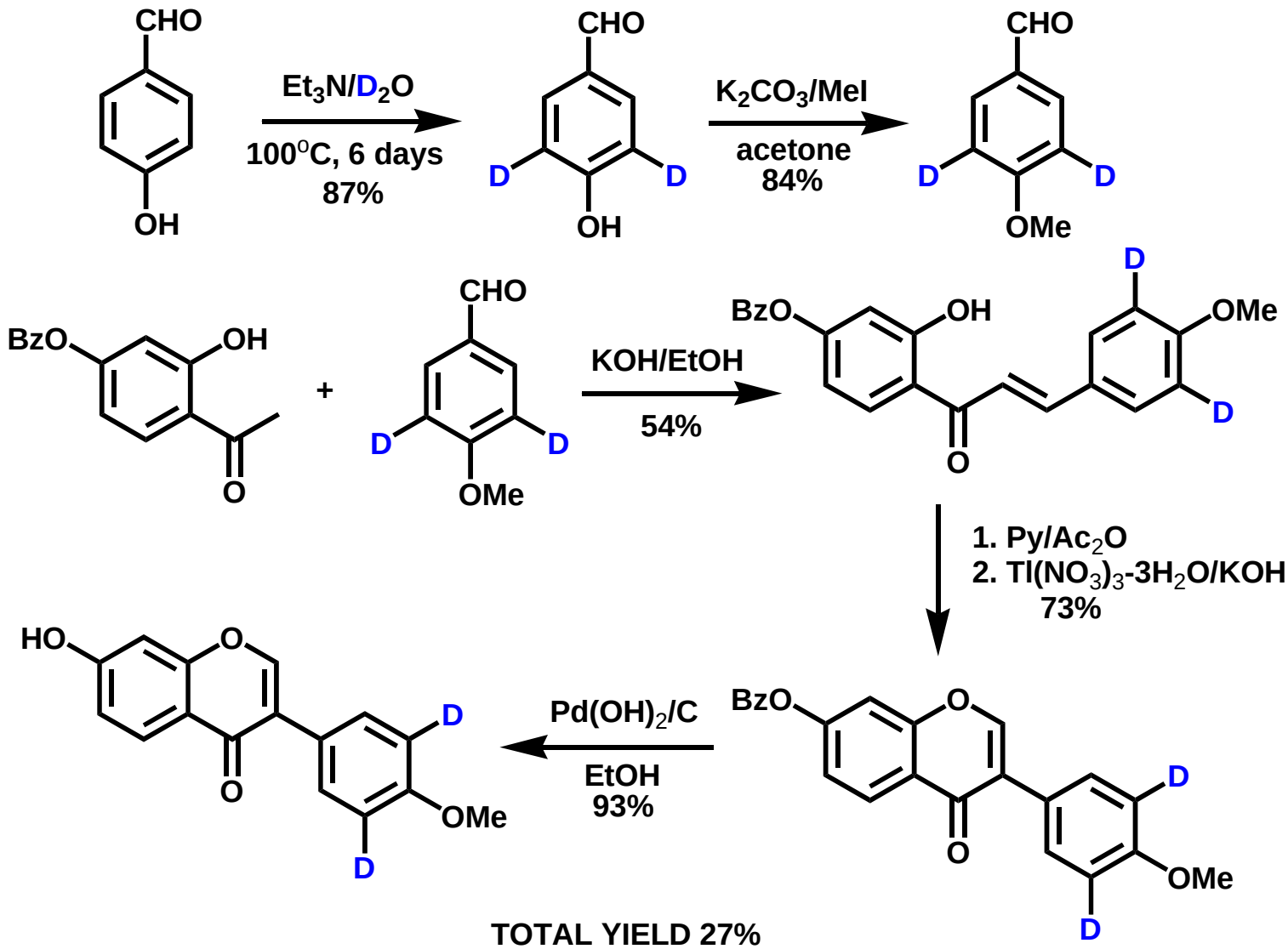


Type 2
Dibenzylbutanediols

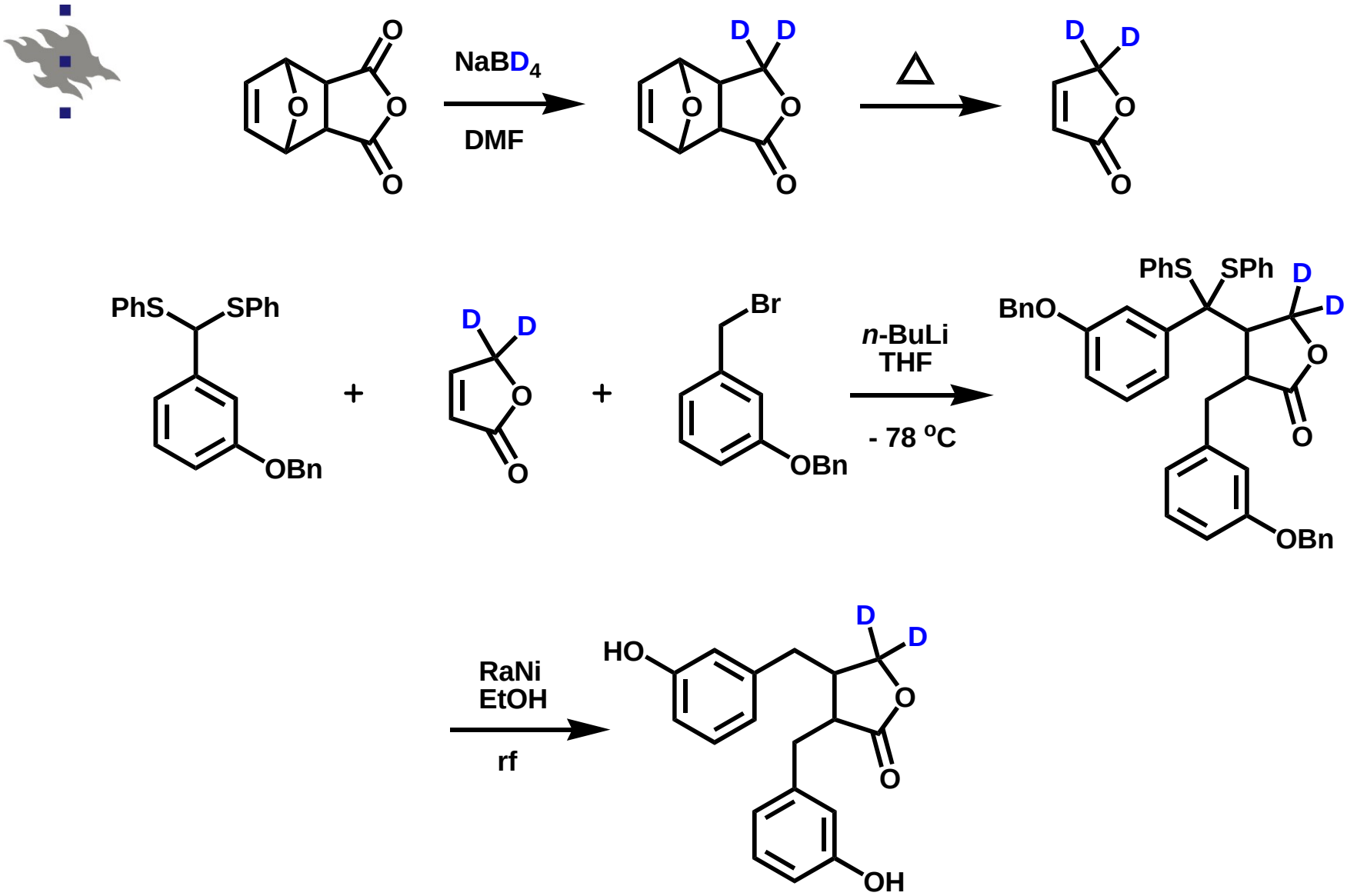


Type 3
Dibenzyltetrahydrofurans

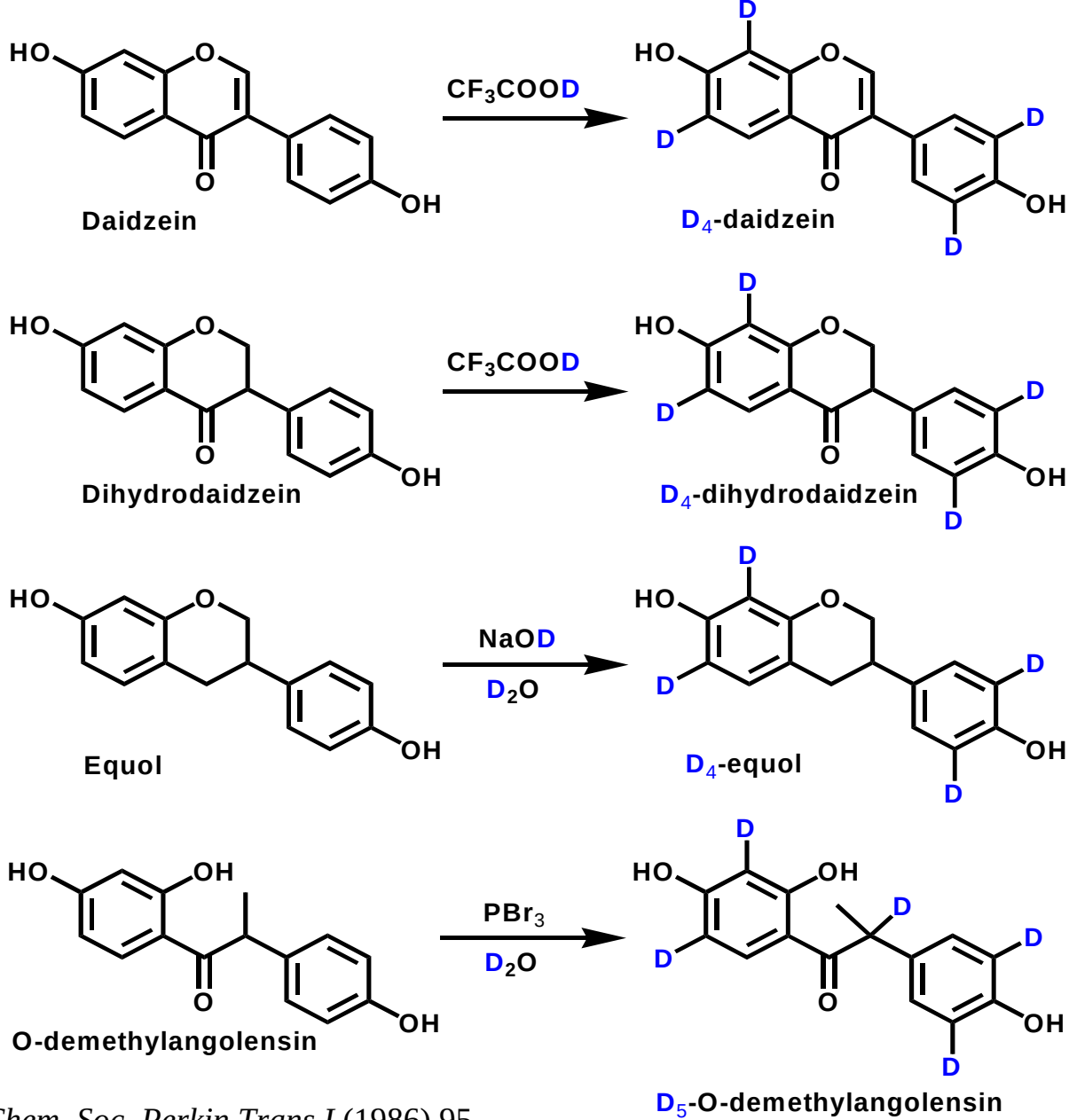
Raffaelli, Hoikkala, Leppälä, Wähälä *J. Chromatogr. B*, 2003.



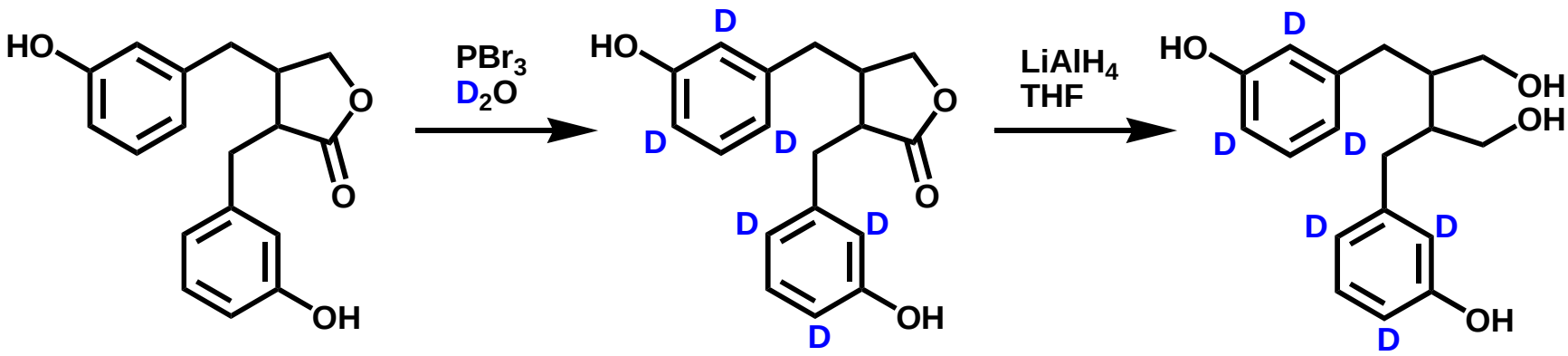
H. A. M. Al-Ani and P. M. Dewick, *J. Chem. Soc. Perkin Trans I* (1984) 2831



Kirk *et al. J. Chem. Soc. Perkin Trans. I* (1985) 35.



K. Wähälä *et al. J. Chem. Soc. Perkin Trans I* (1986) 95
H. Adlercreutz *et al. Clin. Chim. Acta* 199 (1991) 263



ENL

ISOTOPIC PURITY > 90%

END

K. Wähälä *et al. J. Chem. Soc. Perkin Trans I* (1986) 95

Requirements for the deuterium labeled standard

- **No unlabeled species can be present and isotopic purity of the deuterated product should be higher than 90%**

- **D labels must be stable during the analytical procedure**

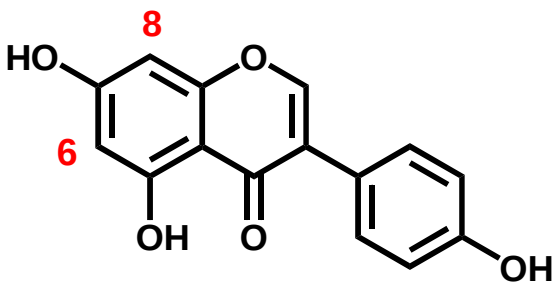
- **A standard should be**

  - **chemically,**
  - **isomerically,**
  - **isotopically pure**

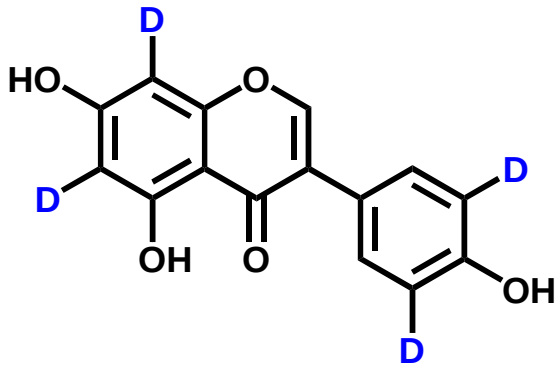
At least three stable D atoms

- **Working with polyhydroxy aromatics, it is desirable that the reference compound contains at least three stable D atoms, since the unlabeled compound, TMS derivatised for GC, will show fairly intense m+1 and m+2 ions in its mass spectrum owing to the heavier natural isotopes of the high number of carbon and silicon atoms in the molecule.**

Thus several D atoms are required to shift the peaks of the reference compound to higher *m/z* values of interference from the peaks of the analyte



CF_3COOD



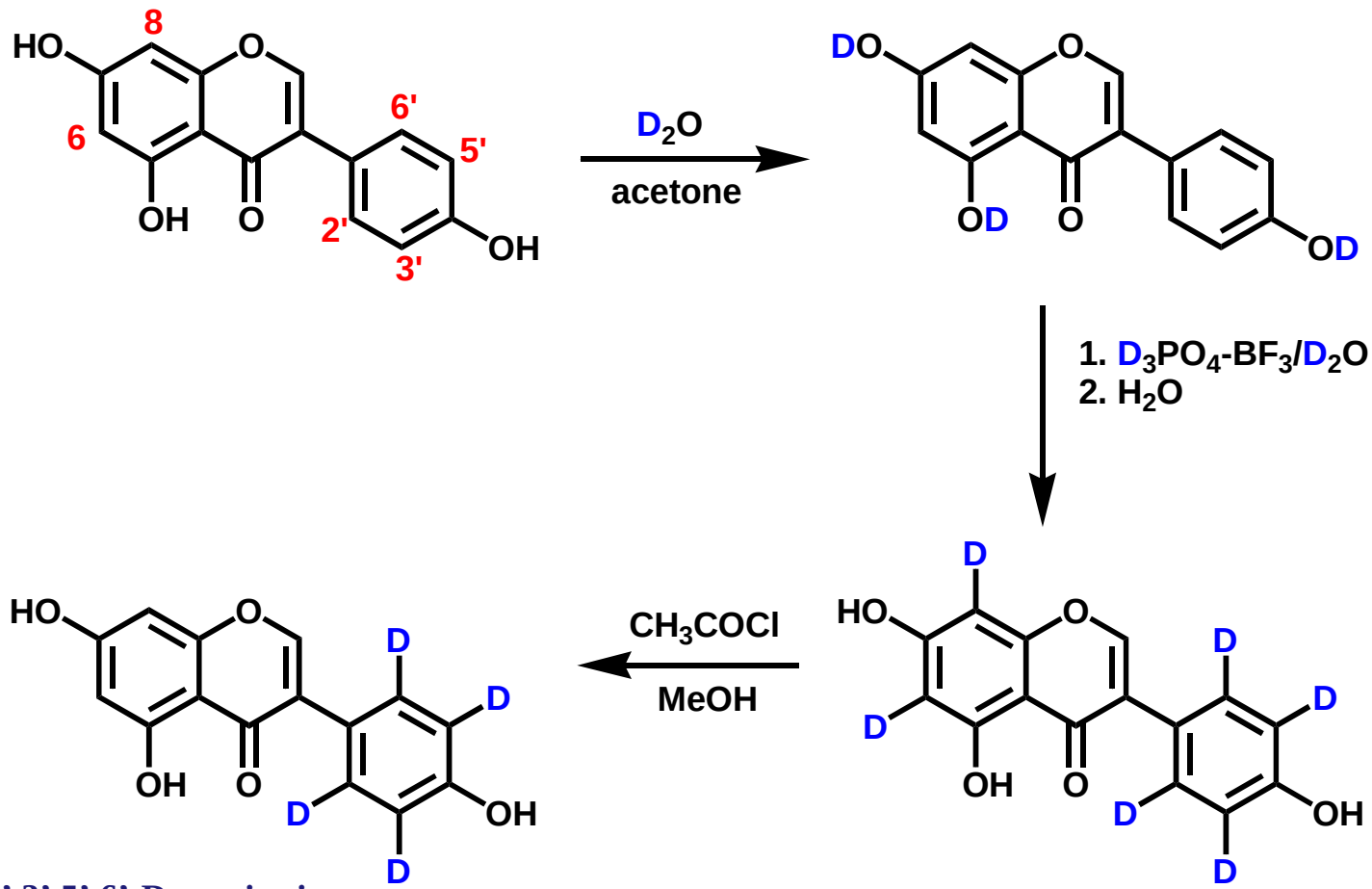
Genistein

6,8,3',4'-D_4-genistein

- **D_3PO_4**
- **$BF_3 \bullet Et_2O/D_2O$**
- **$D_2O \bullet BF_3$**
- **$CH_3COOD/D_2O/K_2PtCl_4$**

Adlercreutz et al. *Clin. Chim. Acta*, **199**, 1991, 263-278

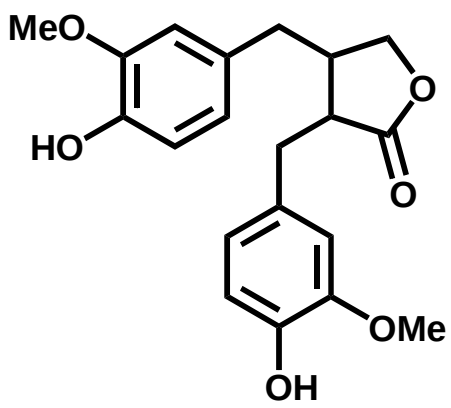
Synthesis of stable 2',3',5',6'-D_4-genistein



stable 2',3',5',6'-D_4-genistein

Yield 91%
Isotopic purity 94%

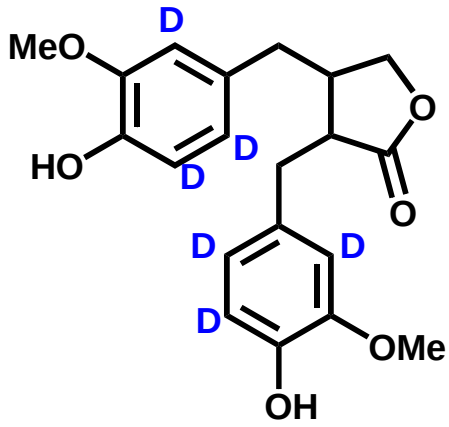
Wähälä & Rasku *THL*, **38** 1997, 7287-7290



Matairesinol

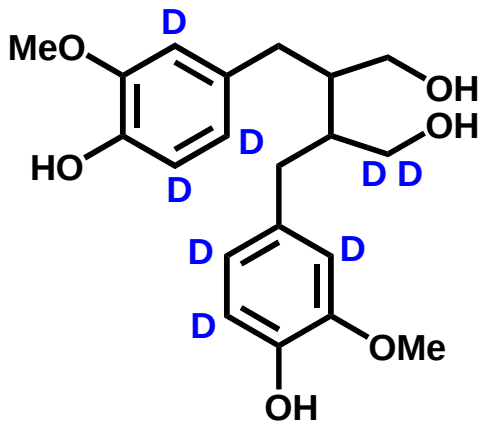
D_3PO_4-BF_3/D_2O

20h



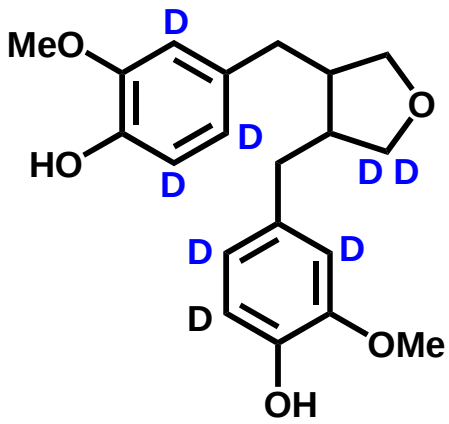
D_6-Matairesinol

$LiAlD_4$, THF



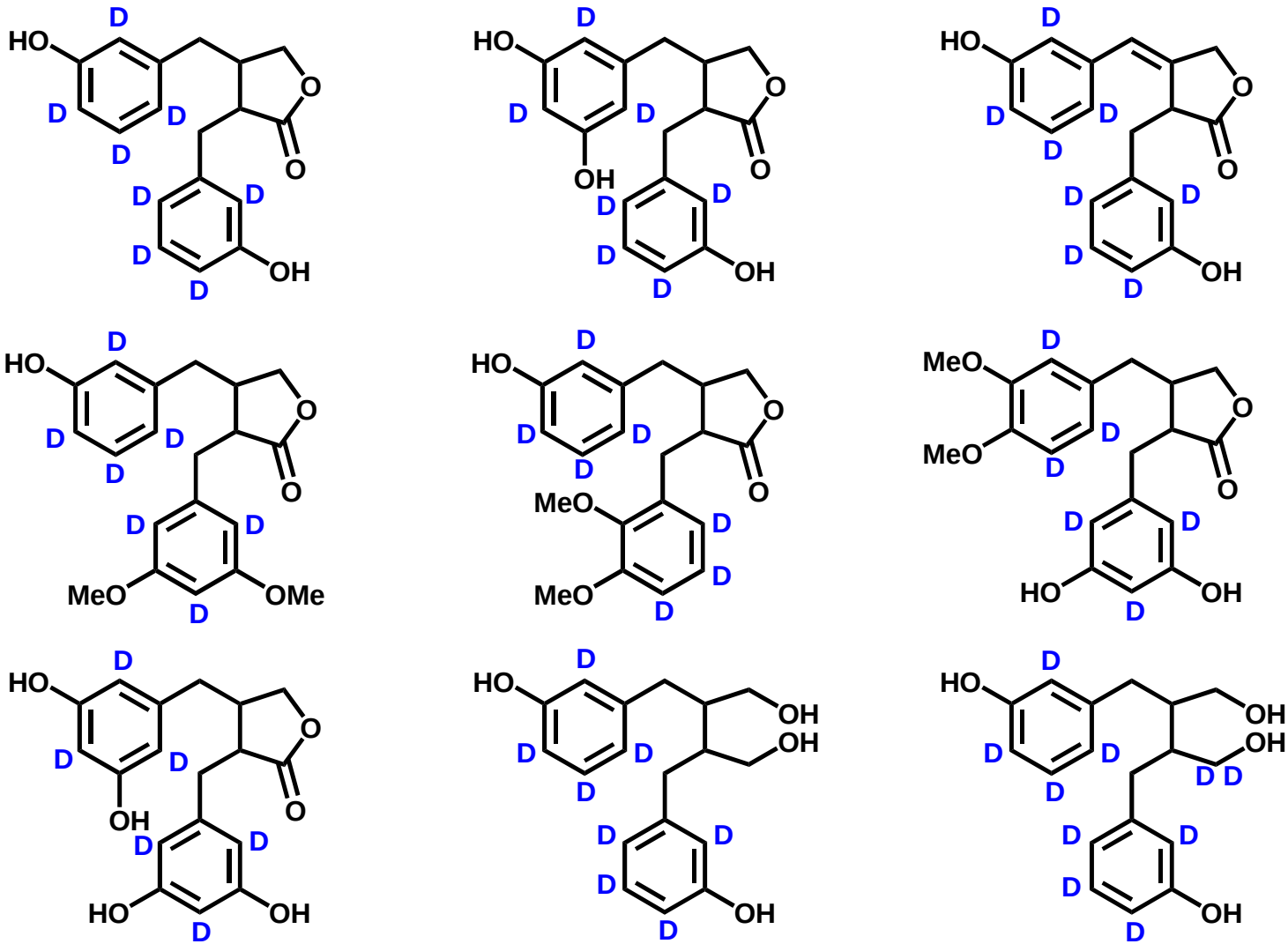
D_8-secoisolariciresinol

$HClO_4$, acetone



D_8-anhydrosecoisolariciresinol

Deuterated mammalian lignans metabolites



Leppälä & Wähälä *JLCR*, 47, 2004, 25.

Eija Leppälä and Monika Pohjoispää

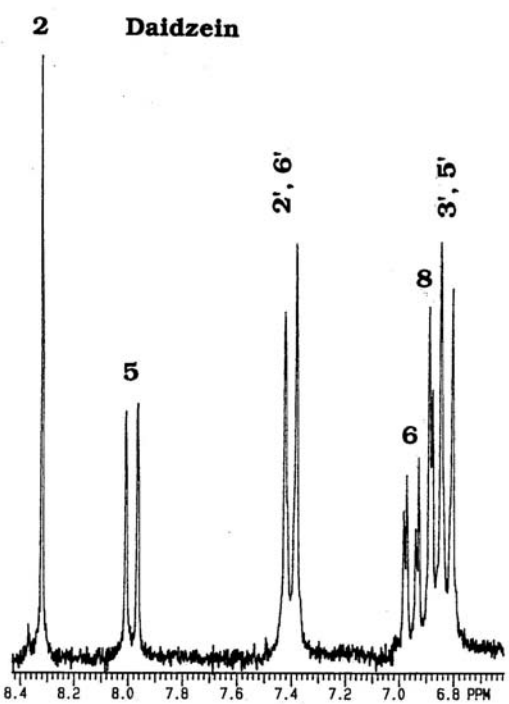
By the combined use of

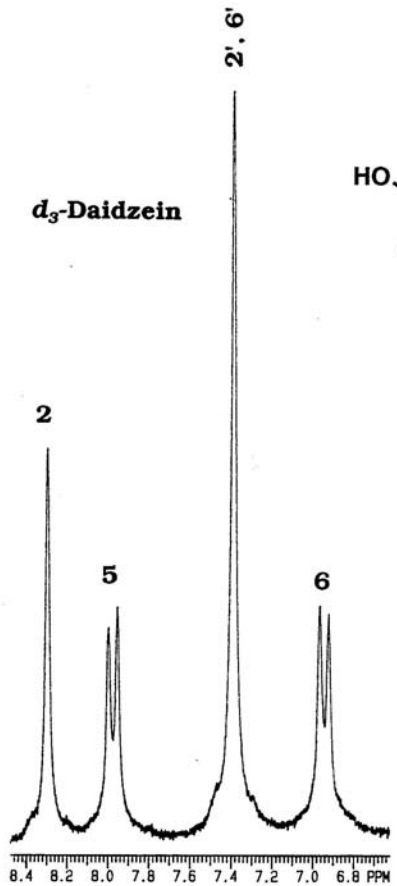
mass spectra and
^{1}H and
^{13}C NMR spectra

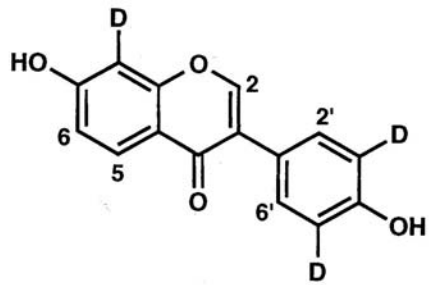
it is easy to establish the degree of

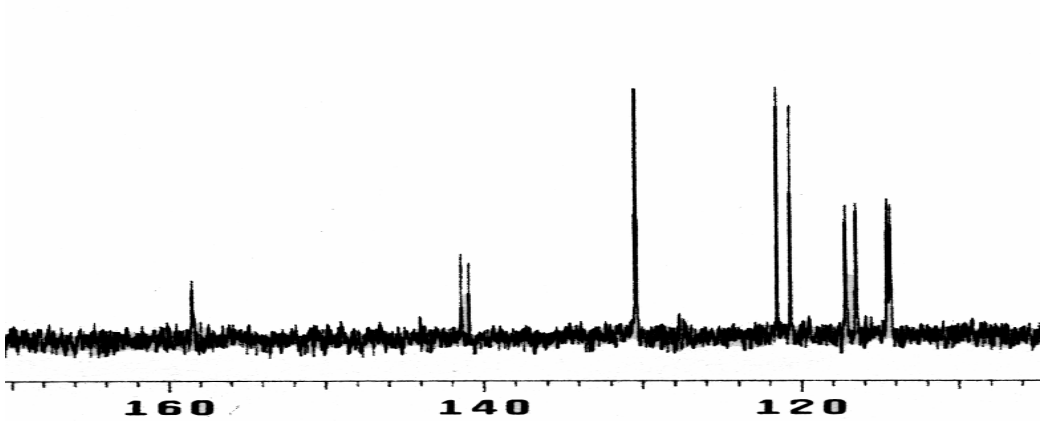
deuteration
purity and
identity

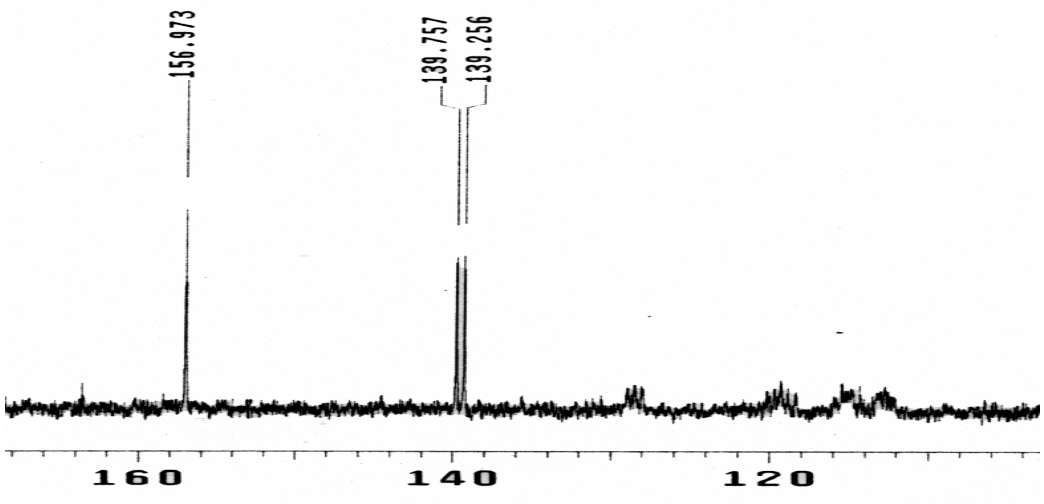
of a polydeuterated product

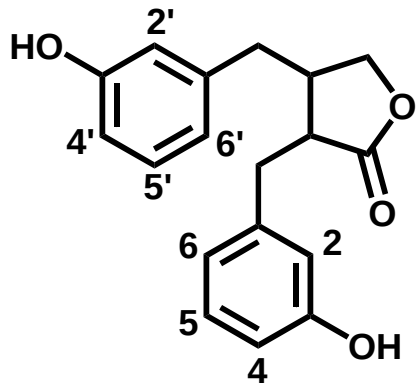

2
Daidzein
5
2', 6'
8
6
3', 5'
8.4 8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 PPM

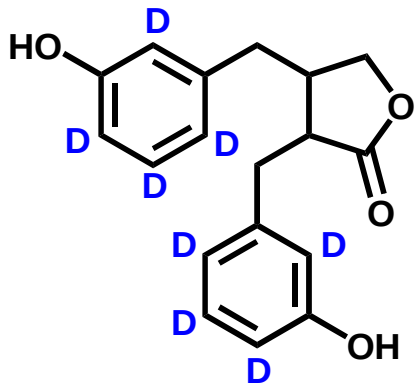

d_3-Daidzein
2', 6'
2
5
6
8.4 8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 PPM


D
HO
O
2
2'
D
6
5
O
6'
OH
D



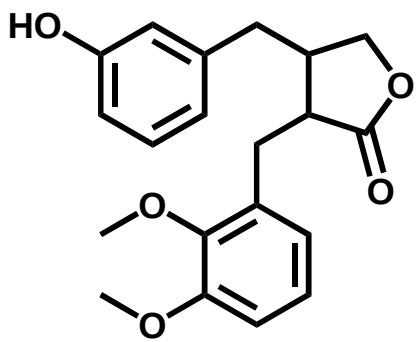


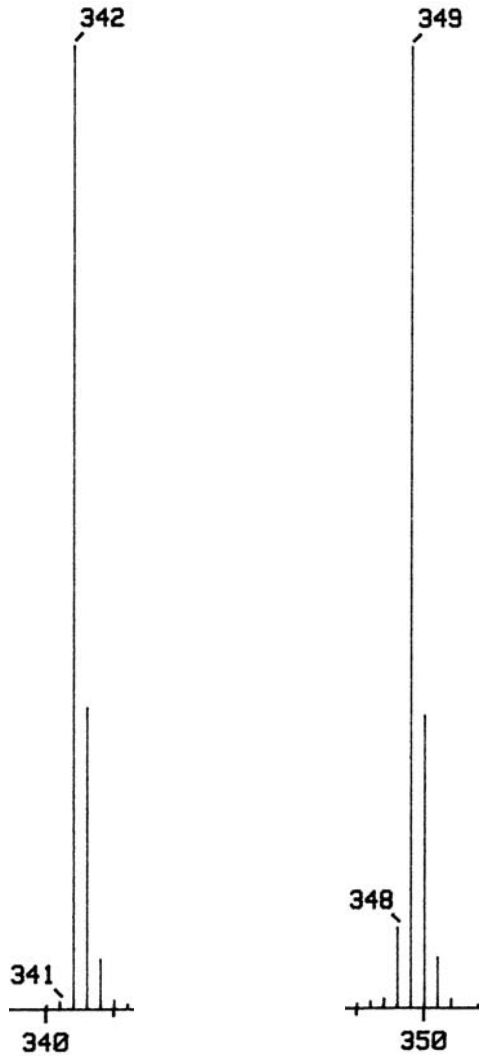


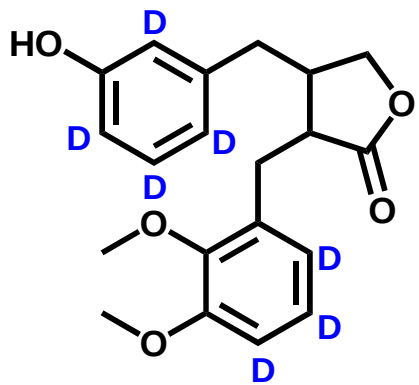


D_8-enterolactone

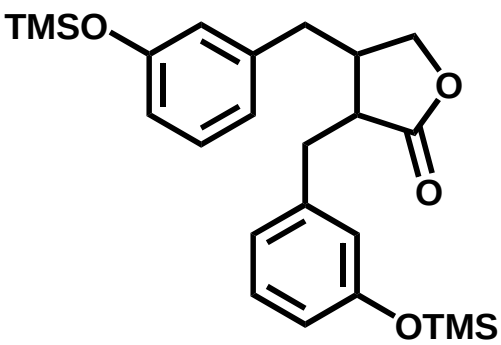
M+ of unlabelled and deuterated 2,3-dimethoxy-3’-hydroxylignano-9,9’-lactone

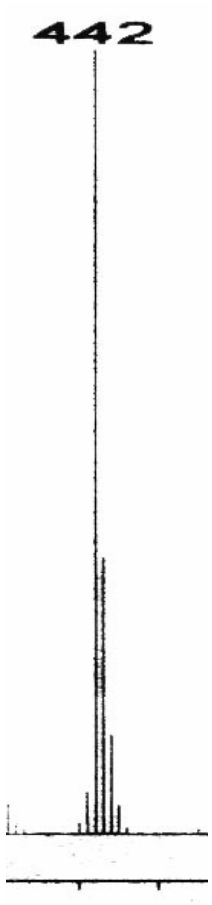


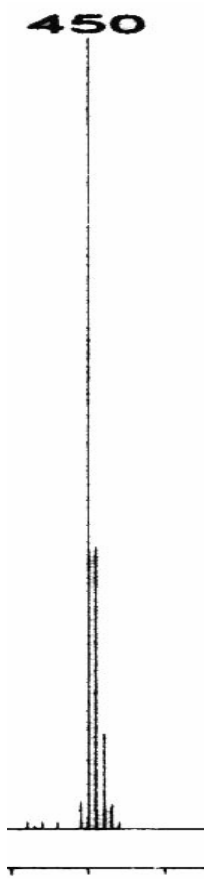


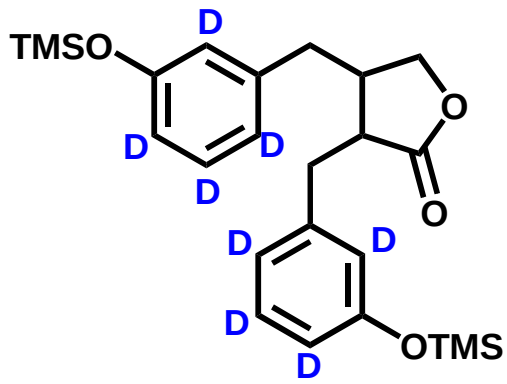


M^+ of unlabelled and deuterated TMS-ENL



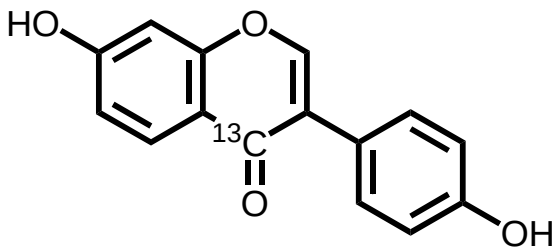




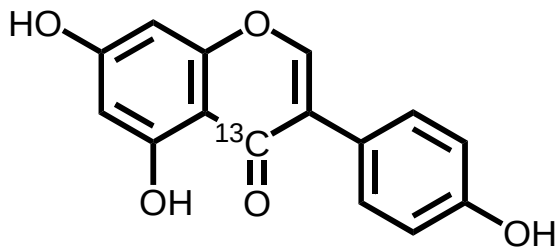


^{13}C-labelled isoflavononoids

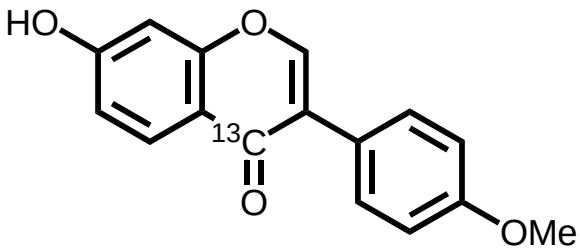
- Single label



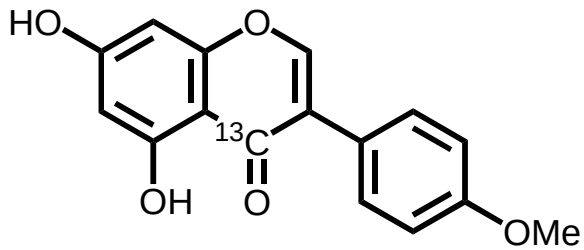
[4-^{13}C]daidzein



[4-^{13}C]genistein



[4-^{13}C]formononetin

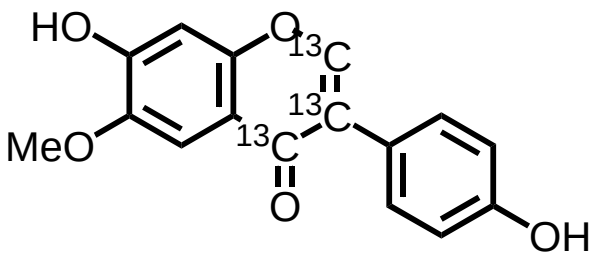


[4-^{13}C]biochanin A

Botting e*t al*. Tetrahedron 56 (2000) 455–460

^{13}C-labelled isoflavonoids

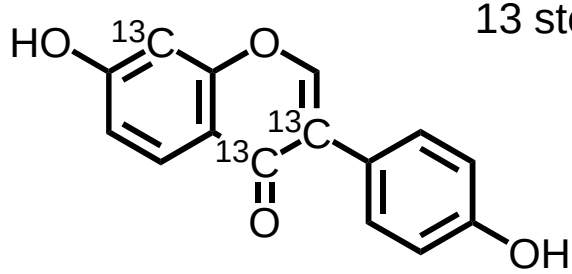
- Triply labelled



[2,3,4-$^{13}C_3$]glycitein

Zhang and Botting, Tetrahedron 60 (2004) 12211–12216

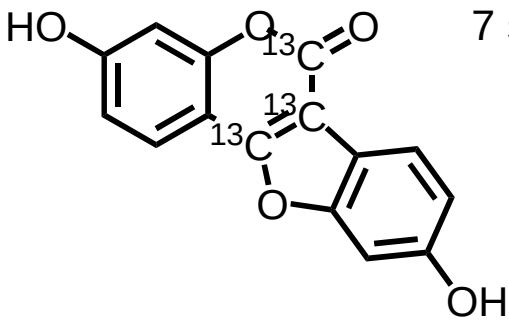
13 steps



[3,4,8-$^{13}C_3$]daidzein

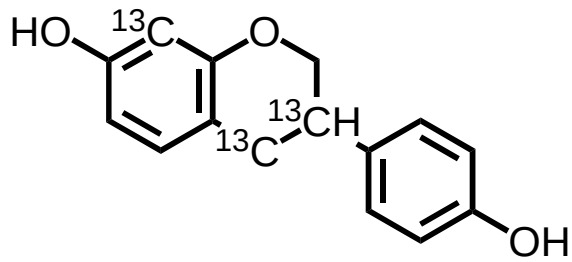
Oldfield *et al.* Tetrahedron 60 (2004) 1887–1893

7 steps



[6,6a,11a-$^{13}C_3$]coumestrol

Al-Maharik *et al.* Tetrahedron 60 (2004) 1637–1642

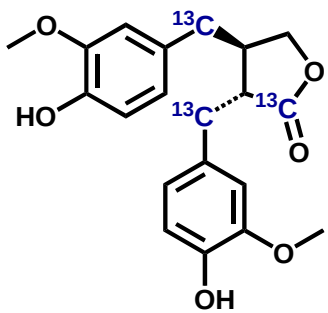


[3,4,8-$^{13}C_3$]equol

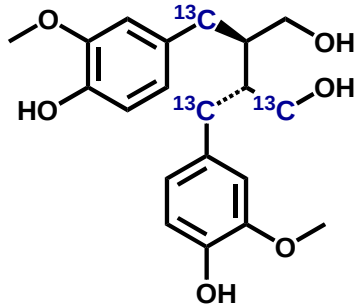
Grace *at al.* Analytical Biochemistry 315 (2003) 114-121

^{13}C-labelled lignans

13 steps

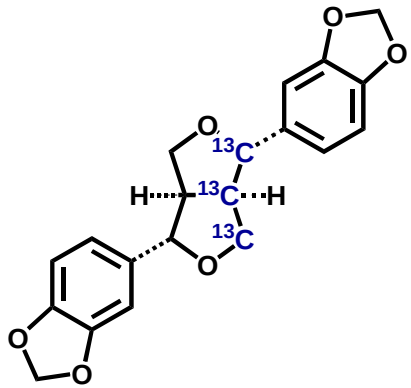


[7,7',9'-^{13}C3]matairesinol

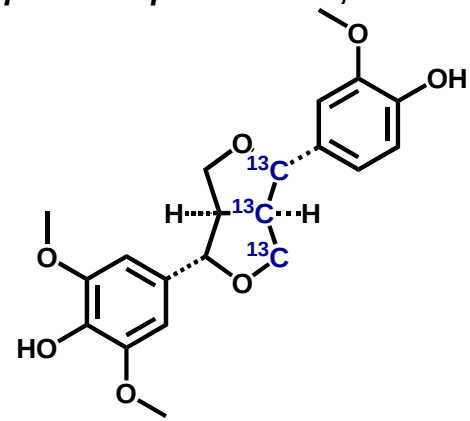


[7,7',9-^{13}C3]secoisolariciresinol

Fryatt, T. and Botting, N. P. *J. Label. Compd. Radiopharm.* 2005; 48: 951-969.



[7,8,9-13C3]sesamin

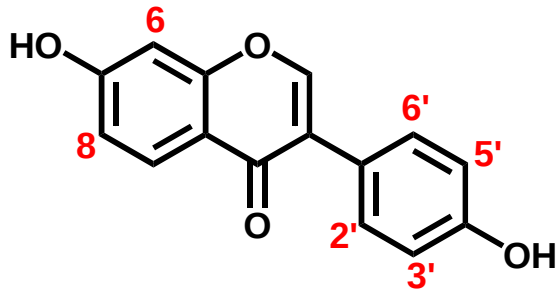


[7,8,9-13C3]medioresinol

12 steps

Haajanen, K. and Botting, N. P. *Steroids* 2006; 71: 231-239.

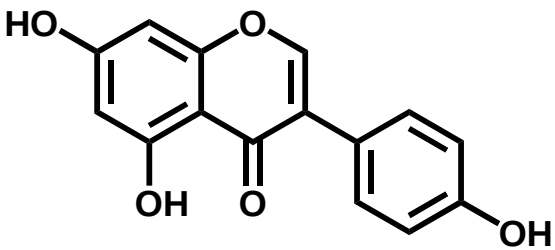
Microwave techniques



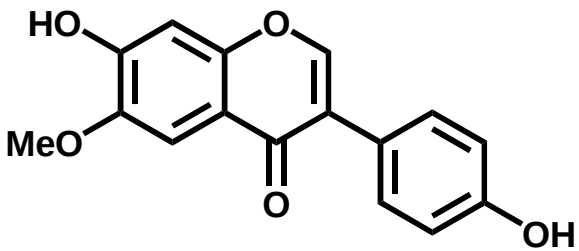
Daidzein

CF_3COO D

Microwave irradiation



Genistein



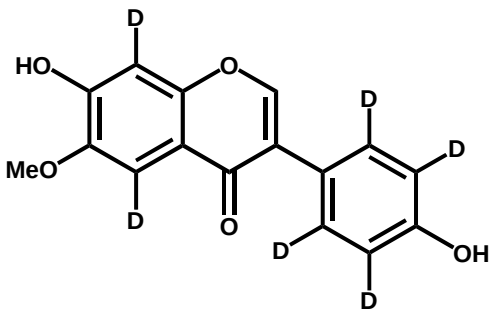
Glycitein

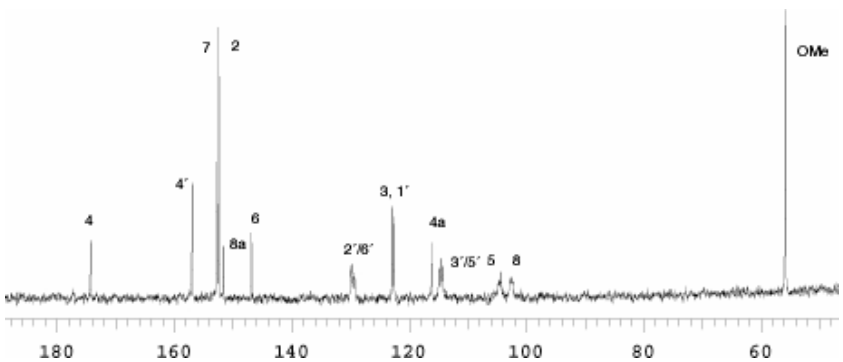
Deuteration of Isoflavones with CF_3COOD

Daidzein	**1) refl. 27h, 2) fresh CF_3COOD refl 8 days**	**Product 6,8,3',5'-D_4- daidzein**	**Yield/ Isotopic purity 75%/ 91%**
Daidzein	**MW 50W, 5h 180°C, 12 bar Repeated 2x**	**6,8,3',5', 2',6'-D_6- daidzein**	**89%/ 90%**
Genistein	**1)refl. 2days 2) fresh CF_3COOD refl 2 days, 2x**	**6,8,3',5'-D_4- genistein**	**75%/ 90 %**
Genistein	**MW 50W, 5h 180°C, 12 bar Repeated 2x**	**6,8,3',5', 2',6'-D_6- genistein**	**90%/ 90%**

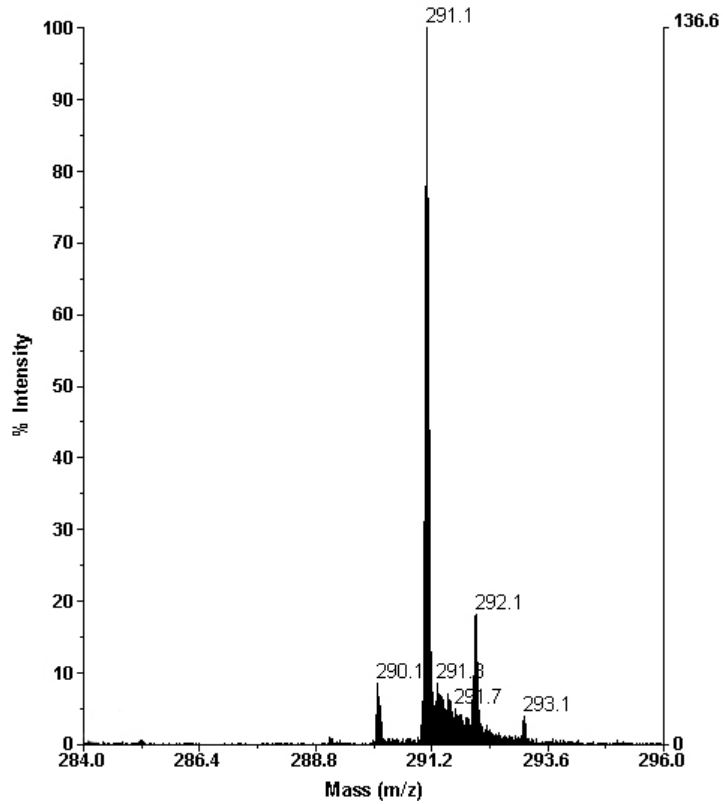
Soidinsalo & Wähälä *JLCR*, 2006, in press.

Synthesis of glycitein-d_6 with CF_3COOD in microwave



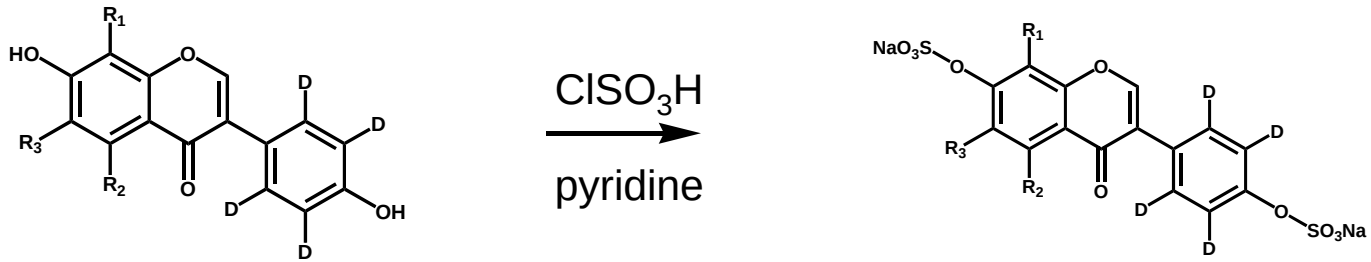


^{13}C-NMR spectrum of glycitein-d_6



ESI+ [M+H] of glycitein-d_6

Sulfates



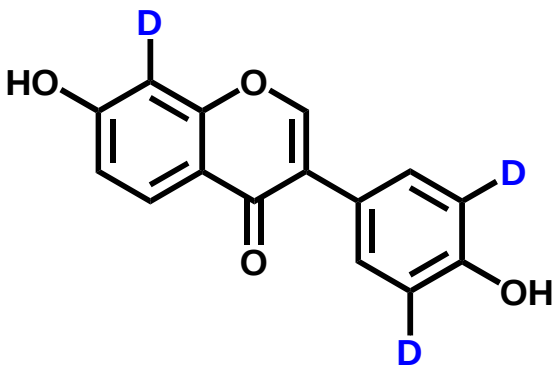
daidzein-d_6 R_1=D, R_2=H, R_3=D
genistein-d_4 R_1=H, R_2=OH, R_3=H
glycitein-d_6 R_1=D, R_2=D, R_3=OMe

Yields 85-90 %
Isotopic purity > 90 %

Isotopic purity > 90 %

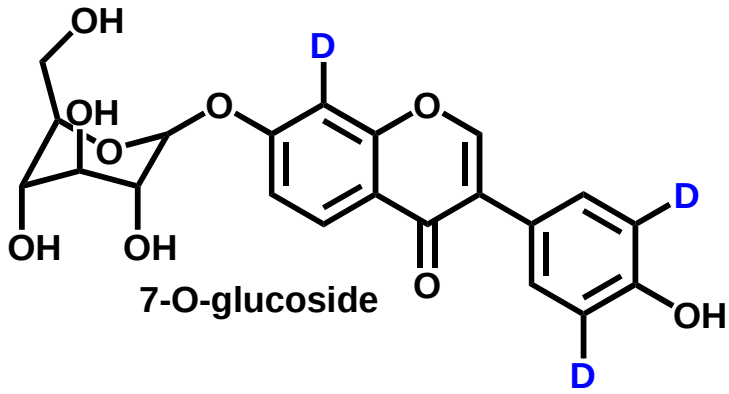
^{1}H and ^{13}C NMR spectra Varian Gemini 2000 nad Bruker Avance 500 spectrometers
LC-MS(ESI+) HP1100 equipped with Mariner ESI-TOF.
High-resolution mass spectra Bruker Mikrotof on the negative mode with Tunemix as the internal standards

Soidinsalo & Wähälä, JLCRP, 2006, in press



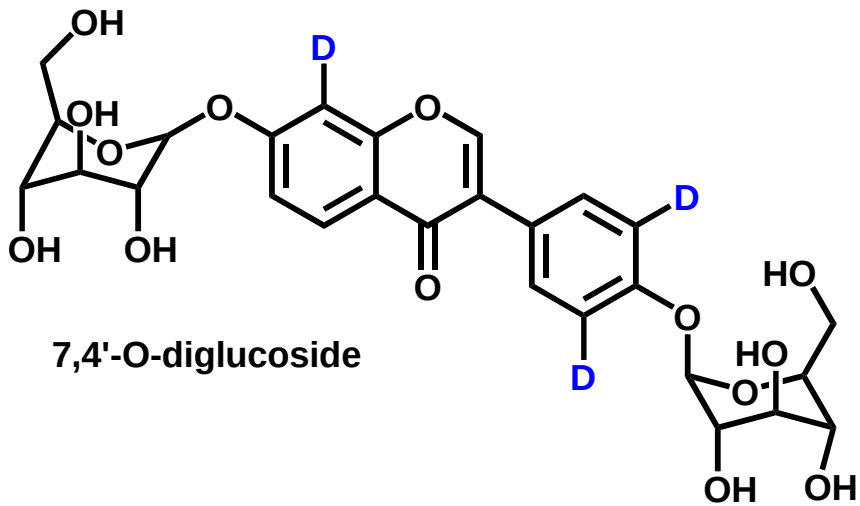
D_3-daidzein

a)



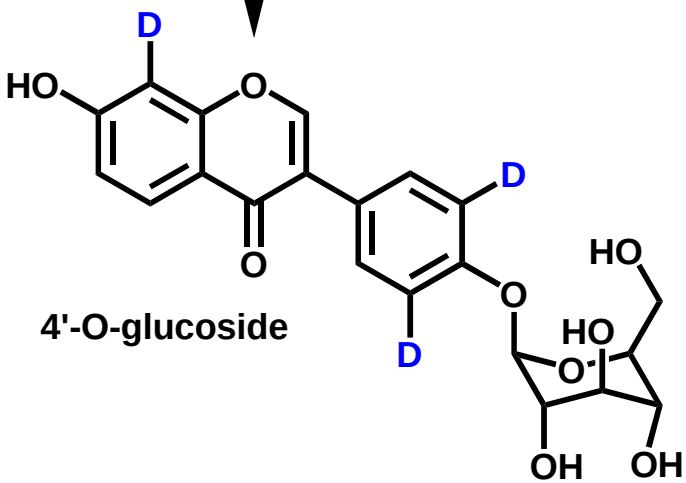
7-O-glucoside

b)



7,4'-O-diglucoside

c)



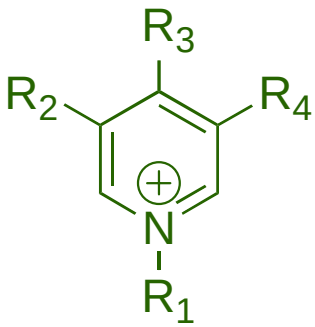
4'-O-glucoside

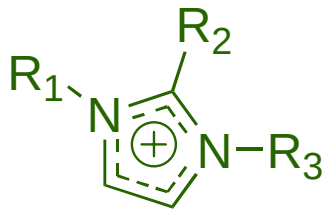
a) 1. K_2CO_3, acetonitrile
2. 18-crown-6, α-acetobromoglucose
3. NaOMe/MeOH

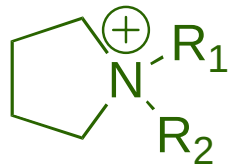
b) 1. *t*-BuOK, acetonitrile
2. 18-crown-6, α-acetobromoglucose
3. NaOMe/MeOH

c) 1. Quinoline, Ag_2CO_3
2. α-acetobromoglucose
3. NaOMe/MeOH

Examples of Ionic Liquids



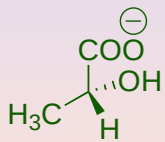




$Cl^-/[AlCl_3]$

Cl^- Br^- I^- $Cl^-/[InCl_3]$

$[NO_3]^-$ $[SO_4]^{2-}$



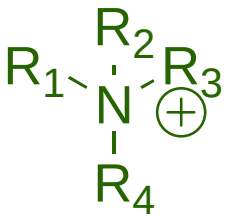
$[CF_3SO_2]^-$

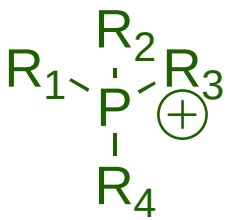
$[CF_3COO]^-$

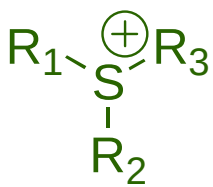
$[BF_4]^-$

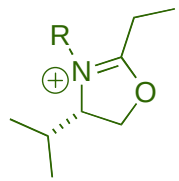
$[PF_6]^-$

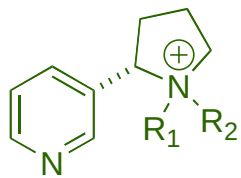
$[(CF_3SO_2)_2N]^-$

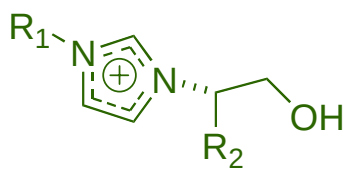












Key Features of Ionic Liquids

- Liquid range of 300 °C (-96 - +200 °C)
- Excellent solvents for organic, inorganic and polymeric materials
- Catalysts as well as solvents
- Highly solvating - low volumes used
- No measurable vapour pressure
- Non-flammable
- Thermally stable under conditions up to 200 °C
- Display Brönsted, Lewis and ‘super’ acidity
- Electric conductivity
- Biphasic systems possible
- Liquid crystalline structures

Ionic Liquids: “Designer” Solvents for Green Synthesis by Kenneth R. Seddon , *ChemFiles, Enabling Technologies, Ionic Liquids*, Vol. 5, 2005

DCl-D_2O and ionic liquid

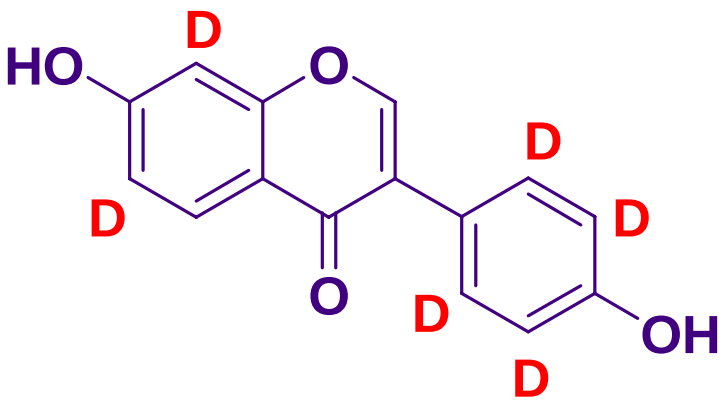
compound	reaction time/min	power/W	yield/%	Isotopic purity
D_4-daidzein	30	50	95	> 90
D_6-daidzein	10 + 10	100	90	>90
D_6-matairesinol	30	40	92	> 92
D_5-ODMA	15	40	90	> 90
D_6-2,4,4'trihydr-oxydeoxybenzoin	30	50	95	> 90
D_3-glycitein	30	50	90	> 90

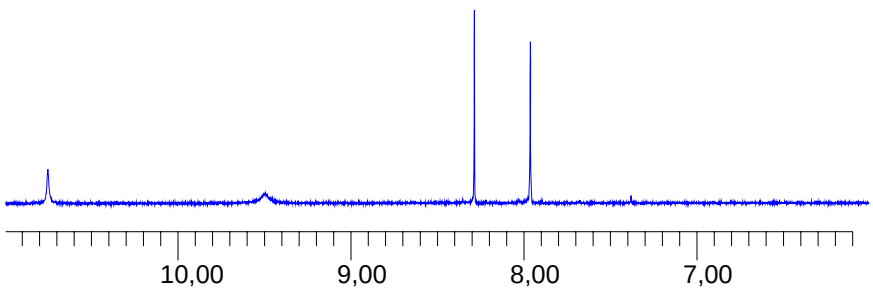
Deuteration with ionic liquid

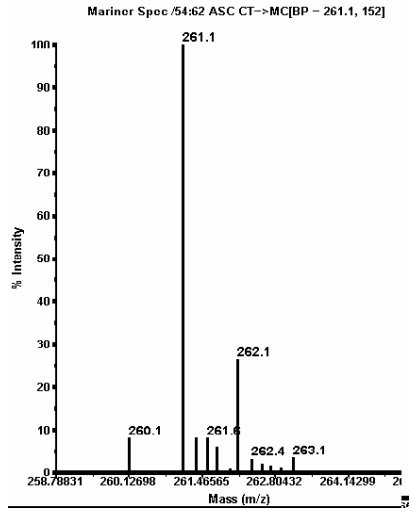
Ullastiina Hakala

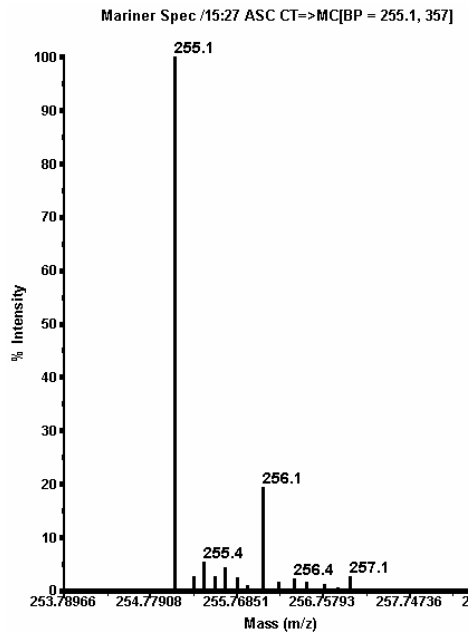
D_6-Daidzein

c:\perch\koe.obs









Conclusion

Deuterated and C-13 labeled isoflavonoids and lignans can be used as internal standads in quatitation of phytoestrogens

Acknowledgements

- Dr. S. Rasku
- Dr. P. Kiuru
- Dr. A. Salakka
- Dr. P. Lewis
- U. Hakala
- E. Leppälä
- M. Pohjoispää
- B. Raffaelli
- O. Soidinsalo
- T. Jokela
- S. Heinonen
- H. Kanerva
- V. Johansson

- Finnish Academy
- University Research Funds
- Emil Aaltonen Foundation
- Antti and Jenny Wihuri Foundation

- PHYTOHEALTH EU-project

Dr Johanna Lampe, Fred Hutchinson Cancer Research Center , US


Helsingin Yliopisto
Kemian Laitos
Jussi Tiainen


QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.