SYNTHESIS OF DEUTERIUM LABELED SILYBIN AND ISOSILYBIN

Renata Ferenczi[1], Tibor Kurtán[2], Zoltán Dinya[2], Sándor Antus[2*]
[1]Research Group of Carbohydrates of Hungarian Academy of Sciences Debrecen, P.O.Box 55 H-4010, Hungary
[2]Department of Organic Chemistry, University of Debrecen, P.O.Box 20, H-4010 Debrecen, Hungary

Dedicated to Professor András Lipták on the occasion of this 70th birthday.

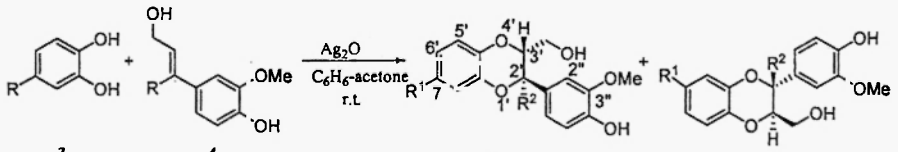
Abstract: A simple synthesis of the deuterium labeled (+)-silybin [(+)-1c,d], (+)-isosilybin [(+)-2c,d] and their 1,4-benzodioxane building block [*rac.*-1f] have been achieved in seven steps starting from vanilline (5).

Introduction

The chemistry of natural polyphenols occuring in fruits of different *Silybum* species has been subject of numerous investigations since the discovery of antihepatotoxic (1) and antioxidant (2-8) activity of flavanolignans. The first and most important representative of this group, (+)-silybin [(+)-1a,b] isolated from the seeds of violet flowered varieties of *Silybum marianum* (9,10) is the most active constituent of Legalon® (Madaus) or Flavobion® (Galenga) used in the liver therapy of liver diseases. Although its chemical and spectroscopic behavior have been investigated in detail (11,12), surprisingly only a few data have appeared about its mass spectrometric properties (11,13). In order to provide suitable products for a detailed study on the fragmentation behaviour of (+)-silybin [(+)-1a,b] its deuterium labeled derivatives (+)-1c,d,-2c,d and *rac.*-1f have been synthetized.

Results and Discussion

The synthetic approach to these compounds [(+)-1c,d,-2c,d, *rac.*-1f] was based on the well-documented oxidative coupling of catechols (3a,b) with coniferyl alcohol (4a) in the presence of silver oxide to afford 2,3-*trans*-1,4-benzodioxans (1a,b, 1e) in acceptable and moderate yield (57/15 %) respectively as depicted in Scheme 1 (14). It has to be noted that this biomimetic transformation starting from (+)-2*R*-3*R*-dihydroquercetin [(+)-3a] did not take place regioselectively [(+)-3a+4a→(+)-1a,b], but the regioisomer of (+)-silybin [(+)-1a,b] called (+)-isosilybin [(+)-2a,b] is also formed in 43 % yield. According to this procedure, oxidation of equimolar amounts of (+)-3a and deuterium labeled coniferyl alcohol (4b) in benzene-acetone was found to give mixture (46 % yield), from which the deuterium labeled (+)-silybin [(+)-1c,d] and (+)-isosilybin [(+)-2c,d] could be isolated, by preparative t.l.c. in 24 % and 22 % yield respectively. The 1,4-benzodioxane building block of (+)-1c,d and (+)-2c,d [*rac.*-1f] was prepared in similar manner in 19 % yield (3b+4b→1f).



3	R
a	A
b	H

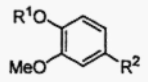
4	R
a	H
b	D

A ≡

1, 2	R¹	R²	Absolute configuration at C-2	C-3	C-2'	C-3'
a	A	H	*R*	*R*	*R*	*R*
b	A	H	*R*	*R*	*S*	*S*
c	A	D	*R*	*R*	*R*	*R*
d	A	D	*R*	*R*	*S*	*S*
e	H	H			*R**	*R**
f	H	D			*R**	*R**

The structure and deuteration degree of (+)-1c,d, (+)-2c,d was supported by their ^{1}H-NMR data measured in acetone-d_6. The comparision of these data with those of (+)-silybin [(+)-1a,b] and (+)-isosilybin [(+)-2a,b] clearly indicated that the deuterium content of our samples [(+)-1c,d, (+)-2c,d] is

higher than 91 %, which corresponds well with that of deuteroconiferyl alcohol (4b) prepared in 5 steps starting from vanilline (5). In the first step its hydroxyl group was protected by methoxymethyl group (MOM) under usual conditions to give 6 aldehyde in 70 % yield, whose reduction by sodium deuteride in methanol at room temperature resulted in a 1:1 mixture of corresponding benzyl alcohol derivatives 7 and 8.



5-12

	R^1	R^2
5	H	CHO
6	MOM	CHO
7	MOM	CH_2OH
8	MOM	CHDOH
9	MOM	CDO
10	H	CDO
11	H	(E)-CD=CH-CO_2Et
12	H	(Z)-CD=CH-CO_2Et

Oxidation of their mixture with MnO_2 in dichloromethane at room temperature took place very smoothly to give the aldehydes 6 and 9 in a 1:12 ratio respectively due to the significant kinetic isotop effect occured in this dehydrogenation process (8→9>>7→6).

The deprotection of 9 by Amberlist-15 resin in toluene at 110°C gave deuterovanilline 10 whose deuterium content was found to be over 92% on the basis its ^{1}H-NMR data. The introduction of (*E*)-olefinic side chain of 4b was performed by a Wittig reaction using Ph_3P=CH-CO_2Et prepared according to the literature (15) to result in a mixture of unsaturated ester 11 and 12 whose separation could be achived by flash-chromatography. Finaly, the (*E*)-ester 11 was reduced with lithium aluminium hydride in ether at room temperature to the corresponding alcohol 4b in 47 % yield, whose structure was comfirmed by its ^{1}H-NMR data and high-resolution mass spectral measurement. The mass spectrometric characteristics and fragmentation pathways of these compounds [(+)-1c,d, *rac.*-1f, (+)-2c,d] with the aid of metastable decomposition (CAD-MIKES) and high resulation measurements are published in a separate paper (16).

Experimental

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. The 200 MHz ^{1}H-NMR spectra were recorded on a Bruker WP 200 SY spectrometres using TMS as internal standard. The high resolution (R=15000) MS spectra were obtained with a VG-7035 spectrometer (70 eV, emission current 200 μA, 150°C, accelerating voltage 4 kV) using perfluorokerosene (PFK) as a reference compound by peak matching technique. Pre-coated silica gel plates (Kieselgel 60 F_{254}, 0.25 mm, Merck) were used for analytical and preparative TLC. Sodium borodeuteride (deuretation degree > 99 %) and other reagents were purchased from Sigma-Aldrich. For workup the solutions were dried ($MgSO_4$) and concentrated in *vacuo*.

3-Methoxy-4-methoxymethoxybenzaldehyde (6)

To the 66 ml dry acetone solution of 6 g (39 mmol) vanilline (5), 4.5 ml methoxymethyl chloride was added under stirring in the presence of 7.39 g (57 mmol) preheated K_2CO_3, and then the mixture was stired for 40 min. at 80°C. K_2CO_3 was filtered off, the solution was concentrated and the residue was taken in water and extracted with CH_2Cl_2. The organic layer was washed with water and dried on $MgSO_4$. The evaporation of solvent resulted in 5.39 g (27.54 mmol) (70 %) product as oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.52 (3H, s, CH_2OMe), 3.97 (3H, s, Ar-OMe), 5.33 (2H, s, OCH_2O), 7.20-7.50 (3H, m, Ar-H), 9.89 (1H, s, CHO)

3-Methoxy-4-methoxymethoxy-(1'-^{2}H)benzaldehyde (9)

1.9 g (9.7 mmol) 3-methoxy-4-methoxymethoxybenzaldehyde (6) was dissolved in 12.6 ml methanol and 380 mg (89.09 mmol) $NaBD_4$ was added under stirring. The reaction mixture was acidified by one drop of dilute sulfuric acid (4.8 ml 96 % H_2SO_4 in 100 ml H_2O). After addition of water, the product was extracted with CH_2Cl_2. The organic layer was stirred with 3.4 g (44.15 mmol) MnO_2 for 24 h at room temperature. After filtration the solution was concentrated in vacuum to afford 1.56 g yellow oil. Besides the deuterated derivative, 8 % nondeuterated compound was observed and henceforth this mixture was used for the further steps. ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.51 (3H, s, $CH_2\underline{OMe}$), 3.97 (3H, s, Ar-OMe), 5.34 (2H, s, OCH_2O), 7.25-7.50 (3H, m, Ar-H)

4-Hydroxy-3-methoxy-(1'-^{2}H)benzaldehyde (10)

1.44 g 9 was dissolved in 72 ml toluene and was stirred with 1.8 g Amberlyst-15 acidic resin for 30 min at 100°C temperature. After filtration, the evaporation of solvent yielded 895 mg (81 %) product of mp. 70-72°C. ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.98 (3H, s, Ar-OMe), 6.25 (1H, s, OH), 7.13 (1H, d, J=9 Hz, H-5), 7.42 (2H, m, Ar-H)

(*E/Z*)-4-Hydroxy-3-methoxy-(1'-^{2}H)cynnamic acid ethyl ester (11, 12)

850 mg 10 and 2.5 g (7.18 mmol) phosphorous ylide were dissolved and refluxed in 42 ml dry benzene under N_2 atmosphere for 3.5 h. Then benzene was evapoated in vacuum and the mixture of 11 and 12 was separeted by coloumn chromatography (hexane:ethyl acetate 4:1) to afford 12 (148 mg) and 11 (747 mg) as colourless oils.

11: ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.32 (3H, t, J=6 Hz, $CH_2\underline{CH_3}$), 3.93 (3H, s, Ar-OMe), 4.27 (2H, q, J=6 Hz, $\underline{CH_2}CH_3$), 5.91 (1H, s, OH), 6.28 (1H, s, H-2'), 6.91 (1H, d, J=9 Hz, H-5), 6.98-7.11 (2H, m, Ar-H)

12: ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.28 (3H, t, J=6 Hz, $CH_2\underline{CH_3}$), 3.92 (3H, s, Ar-OMe), 4.20 (2H, q, J=6 Hz, $\underline{CH_2}CH_3$), 5.78 (1H, s, OH), 5.82 (1H, s, H-2'), 6.88 (1H, d, J=9 Hz, H-5'), 7.12 (1H, dd, J=9 and 2 Hz, H-6), 7.80 (1H, d, J=2 Hz, H-2)

(*E*)-(1'-^{2}H)-coniferyl alcohol (4b)

439 mg (1.9 mmol) $LiAlH_4$ was suspended on 15 ml dry ether under N_2 at 0°C, and the ether solution (11 ml) of 727 mg 11 was added dropwise to it. The mixture was stirred for 4 h at 0°C and then aqueous solution of NH_4Cl was added and the suspension was extracted with ethyl acetate. The organic layer was washed with water, dried on $MgSO_4$ and concentrated in vacuum. The purification by coloumn chromatography (hexane:ethyl acetate 1:1) resulted in 274 mg yellow crystallins product with mp. 68-69°C. ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.46 (1H, s, $CH_2\underline{OH}$), 3.90 (3H, s, Ar-OMe), 4.30 (2H, m, H-3'), 5.12 (1H, s, Ar-OH), 6.18-6.28 (1H, m, H-2'), 6.83-6.95 (3H, m, Ar-H)

(*2R, *3R**)-2-(4'-Hydroxy-3'-methoxyphenyl)-3-hydroxymethyl-(2-^{2}H)1,4-benzodioxane (1f)**

144 mg 4b and 88 mg (0.8 mmol) pirocatechol (3b) was stirred in the 3:2 mixture of dry benzene and dry acetone (13.7 ml) with 633 mg (2.7 mmol) Ag_2O in dark for 48 h at room temperature. The resultant suspension was filtered and evaporation of solvent afforded yellowish brown oil which was purified by coloumn chromatography (hexane:ethyl acetate 1:1) to give *rac*-1f [46 mg (19 %) mp. 172-174°C]. ^{1}H-NMR (DMSO) δ (ppm): 3.48-3.58 (2H, m, $\underline{CH_2}OH$), 3.78 (1H, s, Ar-OMe), 4.10-4.20 (1H, m, H-3), 4.95 (1H, t, J=5.5 Hz, $CH_2\underline{OH}$), 6.75-7.05 (7H, m, Ar-H), 9.18 (1H, s, Ar-OH). HRMS: calc. for $C_{16}H_{15}DO_5$ (M^+) 289.1305, found: 289.1313

(+)-(2'-^{2}H)-silybin (1c,d) and (+)-(2'-^{2}H)-isosilybin (2c,d)

110 mg (E)-(1'-^{2}H)coniferyl alcohol (4b) and 180 mg (0.6 mmol) (+)-2R,3R-dihydroquercetin (3a) were stirred in the mixture of 3 volume dry benzene and 2 volume dry acetone (230 ml) with 297 mg (1.28 mmol) Ag_2O in dark at 55°C for 40 hours. After filtration and evaporation of the solvent, dark brown crystals were optained where purification by preparative TLC (chloroform:ethyl acetate:acetone:formic acid 8:1:1:0.01) yielded 70 mg (+)-1c,d of mp. 152-157°C and 64 mg (+)-2c,d of mp. 159-165°C.

1c,d: ^{1}H-NMR (Me_2CO-d_6) δ (ppm): 3.45-3.59 [1H, m, CH_2(A)], 3.78 [1H, d, J=12.6 Hz, CH_2(B)], 3.86 (3H, s, Ar-OMe), 4.05 (1H, t, J=5.7 Hz, CH_2OH), 4.15 (1H, t, J=1.8 Hz, 3'-H), 4.70 (1H, s, 3-H), 4.78 (1H, s, OH), 5.10 (1H, d, J=11.4 Hz, 2-H), 5.95 (1H, d, J=1.9 Hz, 6-H), 5.98 (1H, d, J=1.9 Hz, 8-H), 6.89 (1H, d, J=8.1 Hz, 5"-H), 6.95 (1H, d, J=8.1 Hz, 5'-H), 6.98 (1H, dd, J=8.0 and 1.9 Hz, 6"-H), 7.08 (1H, dd, J=8.5 and 1.8 Hz, 6'-H), 7.12-7.15 (2H, m, 8'-H, 2"-H), 7.78 (1H, s, OH), 9.70 (1H, s, 7-OH), 11.69 (1H, s, 5-OH) HRMS: calc. for $C_{25}H_{21}DO_{10}$ (M^+) 483.1753, found: 483.1762

2c,d: ^{1}H-NMR (Me_2CO-d_6) δ (ppm): 3.42-3.60 [1H, m, CH_2(A)], 3.78 [1H, d, J=12.5 Hz, CH_2(B)], 3.85 (3H, s, Ar-OMe), 4.03 (1H, t, J=5.7 Hz, CH_2OH), 4.16 (1H, t, J=2.9 Hz, 3'-H), 4.70 (1H, s, 3-H), 4.78 (1H, s, OH), 5.12 (1H, d, J=11.4 Hz, 2-H), 5.98 (1H, d, J=2.1 Hz, 6-H), 6.0 (1H, d, J=2.1 Hz, 8-H), 6.89 (1H, d, J=8.0 Hz, 5'-H), 6.98 (1H, dd, J=8.5 and 1.8 Hz, 6"-H), 7.08 (1H, dd, J=8.2 and 1.8 Hz, 6'-H), 7.12-7.14 (2H, m, 8'-H, 2"-H), 7.76 (1H, s, OH), 9.7 0(1H, s, 7-OH), 11.69 (1H, s, 5-OH) HRMS: calc. for $C_{25}H_{21}DO_{10}$ (M^+) 483.1753, found: 483.1762

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