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DIHYDROBENZOFURAN LIGNANS FROM *BOREAVA ORIENTALIS*

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Key Word Index—*Boreava orientalis*; Cruciferae; fruits; dihydrobenzofuran lignan; dihydrobenzofuran lignanamide; dihydrobenzofuran glucoside; glutamic acid derivatives; lignan–amino acid complex.

Abstract—Two new dihydrobenzofuran lignanamides and three known dihydrobenzofuran lignans have been isolated from fruits of *Boreava orientalis*. The structures of the two new compounds were established as *cis*- and *trans*- (2R,3S)-2,3-dihydro-2-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-7-methoxybenzofuran-5-propen-1-ic(1,5-dimethyl)glutamate amide on the bases of chemical and spectral evidence, including NOE, 2D shift-correlation and HMBC NMR experiments. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

In a previous paper, we reported the isolation and identification of a new glucosinolate salt, vanillic and 2,3-dihydroxybenzoic acids and glucosides of these from fruits of *Boreava orientalis*; the 2,3-dihydroxybenzoic acids had radical-scavenging activity [1–4]. As a continuation of our investigation of the constituents of this species we have isolated three known dihydroxybenzofuran lignans (**1–3**) and two new dihydrobenzofuran lignanamides (**4** and **5**), designated as *trans*- and *cis*-boreavan B. In this chapter, we report the structural elucidation of **1–5** obtained from a methanol extract of the fruits of *B. orientalis*, on the bases of UV, CD, mass, ^{1}H and ^{13}C NMR spectral data, including NOE, 2D shift correlation and HMBC experiments.

RESULTS AND DISCUSSION

Extraction was carried out as described in Experimental. The chloroform-soluble fraction of the extract was chromatographed on silica gel to give **1–3**, and the ethyl acetate soluble fraction was subjected to Sephadex LH-20 column chromatography to give **4** and **5**.

Compound **1**, $C_{18}H_{18}O_7$ (HR EI mass spectrum) gave a positive $FeCl_3$ reaction. The IR spectrum showed absorption bands at 3396 (OH), 2920 (CH), 1686 (COOH), 1598 (C=C), 1540, 1518 (aromatic C=C), 1328 (OCH_3) and 1114 (C–O) cm^{-1}. The UV spectrum of **1** showed a maximum at 280 nm. Bathochromic shifts to 263, 301 (sh) and 365 (sh) nm from the addition of sodium hydroxide indicated the presence of a hydroxylated aromatic ring. The EI mass spectrum of **1** showed the presence of a $[M]^+$ at *m/z* 346 and ions due to fragmentation of the aglycone at *m/z* 328, 316, 313, 296, 268, 151 and 137. The *m/z* 151 and 137 fragments are characteristic of a coniferyl residue in lignans [5].

The ^{1}H NMR spectrum of **1** (Table 1) showed the presence of five aromatic protons enclosed in two aromatic systems. The three signals at δ 5.61, 3.55 and 3.82 were attributed to an aliphatic CH–CH–CH_2 link on the basis of ^{1}H NMR and ^{1}H–^{1}H COSY spectral data; two methyl protons on aromatic methoxyls were observed at δ 3.89 and 3.81. Thus, **1** was identified as 2,3-dihydro-2-(4-hydroxy-3-methoxyphenyl)-3-hydroxy-methyl-7-methoxybenzofuran-5-carboxylic acid.

Compound **2** was rechromatographed by preparative HPLC (ODS-C_{18}) to give an amorphous powder, $C_{20}H_{20}O_7$ ($[M]^+$ from HR EI mass spectrum), which gave a positive $FeCl_3$ reaction. Its IR spectrum suggested the presence of hydroxyl, carbonyl and an aromatic ring. The UV spectrum showed absorption maxima at 292 and 323 (sh) nm. The bathochromic shifts of the absorption maximum after the addition of sodium methoxide were similar to those of **1**. The EI mass spectrum of **2** showed peaks at *m/z* 372, 354, 342, 339, 322, 298, 151 and 137. This fragmentation pattern was similar to that of **1**. The ^{1}H NMR spectrum (Table

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Table 1. ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectral data for compounds **1** and **2** in CD_3OD

	1		**2**	
C	^{13}C	^1H	^{13}C	^1H
1	134.0		134.2	
2	110.7	6.77 (*d*, *J* = 8.3)	110.7	6.94 (*d*, *J* = 7.8)
3	147.9		147.8	
4	149.2		149.2	
5	116.3	6.94 (*d*, *J* = 2.0)	116.3	6.77 (*d*, *J* = 2.0)
6	120.8	6.85 (*dd*, *J* = 2.0, 8.3)	119.8	683(*dd*, *J* = 2.0, 78)
7	90.2	5.61 (*d*, *J* = 6.8)	89.9	5.57 (*d*, *J* = 6.4)
8	54.7	3.55 (*d*, *t*, *J* = 5.9, 6.4)	54.9	3.53 (*d*, *t*, *J* = 5.9, 6.4)
9	64.7	3.82 (*d*, *J* = 6.4*)	64.7	3.83 (*d*, *J* = 6.4*)
1′	125.6		129.9	
2′	115.4	7.62 (*d*, *J* = 1.9)	113.8	7.18 (*d*, *J* = 1.9)
3′	145.2		145.9	
4′	153.8		151.9	
5′	130.6		131.0	
6′	119.9	7.56 (*d*, *J* = 1.9)	119.0	7.13 (*d*, *J* = 1.9)
7′	170.1		146.6	7.61 (*d*, *J* = 16.1)
8′			116.9	7.34 (*d*, *J* = 16.1)
9′			171.2	
OMe	56.7	3.89	56.9	3.90
	56.4	3.81	56.4	3.81

*Overlapped other peaks.

1) confirmed the presence of typical proton signals of a dihydrobenzofuran lignan similar to **1**. There were also two aromatic methoxyls, five aromatic protons at δ 3.90, 3.81 and 7.18–6.77 and an olefin proton attributable to one *trans* double bond at δ 7.61 and 7.34 with a coupling constant of 16.1 Hz. The chemical shift values of the remaining signals were similar to those reported for the lignan moiety of the lignan–iridoid complex isolated from an extract of the roots of *Buddleja davidii* [6], suggesting the presence of a dihydrobenzofuran moiety in **2**.

The ^{13}C NMR spectrum of **2** (Table 1) gave further supporting evidence for the presence of methoxyl, methylenes and an aromatic ring. In addition, the chemical shift values exhibited characteristic features at δ 89.9, 54.9 and 64.7 due to the aliphatic CH–CH–CH_2 link, in agreement with ^1H NMR data. These results are similar to those reported for other dihydrobenzofuran lignans. From these data, it was apparent that the structure of **2** was 2,3-dihydro-2-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-7-methoxybenzofuran-5-(*trans*)propenic acid.

Compound **3** corresponded with a molecular formula of $C_{26}H_{32}O_{11}$ from an HR positive ion FAB mass spectrum. Enzymic hydrolysis yielded an aglycone (**3a**) and D-glucose. The IR spectrum showed absorption bands at 3412 (OH), 2920 (CH), 1600 (C=C), 1464, 1454 (aromatic C=C), 1284 (OCH_3) and 1080 C–O) cm^{-1}. The UV spectrum of **3** showed maxima at 278 and 335 (sh) nm. The bathochromic shifts to 274 and 406 nm from the addition of sodium hydroxide indicated the presence of a hydroxylated aromatic ring. The FAB mass spectrum of **3** indicated the presence of a quasimolecular ion at *m/z* 543 ($[M + Na]^+$) and ions due to fragmentation of the aglycone at *m/z* 329 and 176. The EI mass spectrum showed characteristic peaks of a coniferyl residue in lignans at *m/z* 338, 323, 151 and 137.

The ^1H NMR spectrum of **3** (Table 2) showed the presence of five aromatic protons enclosed in two aromatic systems. In the same area of this spectrum, the typical pattern of a *trans*-ethylene system was observed at δ 6.21 and 6.52 with a coupling constant of 15.6 Hz; ^1H–^1H correlation was seen between H-7′ and H-8′ in the ^1H–^1H COSY spectrum. In addition, three signals at δ 5.61, 3.66 and 3.86 were attributed to an aliphatic CH–CH–CH_2 link, as found in **1** and **2**, and two methyl protons on aromatic methoxyls were observed at δ 3.86 and 3.81. Compound **3** was a monoglucoside showing an anomeric proton peak at δ 4.35 and carbons signals at δ 104.9, 78.7, 78.5, 75.6, 71.2 and 63.2 in the ^{13}C NMR spectrum (Table 3). In the HMBC spectrum of **3**, long-range coupling of an anomeric proton was observed at C-9, indicating that the glucosyl linkage was at the C-9 hydroxyl group in the aglycone. The negative cotton effect at 270–285 nm in the CD spectrum suggested that **3** may have the same stereostructure as DCG-D reported by Binns *et al.* [7]. Therefore, **3** was identified as (2S, 3R)-2,3-dihydro-2-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-7-methoxybenzofuran-5-(*trans*)propen-1-ol-3-*O*-β-glucoside.

Compound **4** was rechromatographed by preparative HPLC (ODS-C_{18}) to give an amorphous powder, $C_{27}H_{33}O_{10}N$ ($[M + H]^+$ from HR FAB mass spectrum), exhibiting positive reactions with both $FeCl_3$ and Dragendorff reagents. Its IR spectrum suggested the presence of hydroxyl, esterified carbonyl, acid amide and an aromatic ring. The UV spectrum showed absorption maxima at 288 and 320 nm. The bathochromic

Table 2. ^{1}H NMR and ^{1}H–^{1}H COSY spectral data for compounds **3–5** in CD_3OD

C	**3**	^{1}H–^{1}H COSY correlated H	**4**	**5**	^{1}H–^{1}H COSY correlated H
1					
2	6.98 (1H, *d*, *J* = 1.7)		6.94 (1H, *d*, *J* = 2.0)	6.94 (1H, *d*, *J* = 2.0)	
3					
4					
5	6.75 (1H, *d*, *J* = 8.3)	6-H	6.77 (1H, *d*, *J* = 8.3)	6.73 (1H, *d*, *J* = 8.3)	6-H
6	6.85 (1H, *dd*, *J* = 1.7, 8.3)	5-H	6.83 (1H, *dd*, *J* = 2.0, 8.3)	6.82 (1H, *dd*, *J* = 2.0, 8.3)	5-H
7	5.61 (1H, *d*, *J* = 5.9)	8-H	5.57 (1H, *d*, *J* = 6.3)	5.57 (1H, *d*, *J* = 6.3)	8-H
8	3.66 (1H, *dd*, *J* = 5.9, 11.7)	7-H, 9-H	3.54 (1H, *dd*, *J* = 6.3, 10.7)	3.54 (1H, *dd*, *J* = 6.3, 10.7)	7-H, 9-H
9	3.86 (2H, *d*, *J* = 11.7)	8-H	3.84 (2H, *d**)	3.84(*)	8-H
1′					
2′	7.01 (1H, *br s*)		7.16 (1H, *br s*)	7.24 (1H, *br s*)	
3′					
4′					
5′					
6′	6.92 (1H, *br s*)		7.11 (1H, *br s*)	7.11 (1H, *br s*)	
7′	6.52 (1H, *d*, *J* = 15.6)	8′-H	7.50 (1H, *d*, *J* = 15.6)	6.73 (1H, *d*, *J* = 12.5)	8′-H
8′	6.21 (1H, *dt*, *J* = 5.9, 15.6)	7′-H, 9′-H	6.52 (1H, *d*, *J* = 15.6)	5.90 (1H, *d*, *J* = 12.5)	7′-H
9′	4.19 (2H, *d*, *J* = 5.9)	8′-H			
OMe	3.86, 3.81 (3HX2, each *s*)		3.66, 3.73, 3.81, 3.90 (3HX4, each *s*)	3.58, 3.81, 3.69, 3.63 (3HX4, each *s*)	
1″	4.35 (1H, *d*, *J* = 7.8)				
2″	3.18–3.35*		4.57 (2H, *dd*, *J* = 5.4, 8.8)	4.57 (2H, *dd*, *J* = 5.4, 8.8)	
3″	3.64–3.90*		1.99, 2.19 (2H, each *m*)	1.99, 2.19 (2H, each *m*)	
4″	*		2.45 (2H, *t*, *J* = 7.3)	2.45 (2H, *t*, *J* = 7.3)	
5″	*				
6″	*				

*Overlapped other peaks.

Table 3. ^{13}C NMR and ^{13}C–^{1}H COSY spectral data for compound **3** in CD_3OD

C	**3**	Glucose	DEPT	^{13}C–^{1}H COSY correlated H
1	130.55		C	
2	120.95		CH	6.98
3	147.90		C	
4	149.40		C	
5	116.54		CH	6.75
6	111.16		CH	6.85
7	89.72		CH	5.61
8	53.33		CH	3.66
9	72.72		CH_2	3.86
1′	132.98		C	
2′	112.57*		CH	7.01
3′	145.89		C	
4′	149.51		C	
5′	134.90		C	
6′	117.23*		CH	6.92
7′	132.39		CH	6.52
8′	128.02		CH	6.21
9′	63.21		CH	4.19
OMe	57.23		Me	3.86
	57.18		Me	3.81
Glucose moiety				
1″	104.91	103.4	CH	
2″	75.57	75.3	CH	
3″	78.46	78.3	CH	
4″	71.20	71.8	CH	
5″	78.66	78.2	CH	
6″	63.21	6.29	CH_2	

*Overlapped other peaks.

shifts in the absorption maxima from the addition of sodium methoxide were similar to those of **3**. The positive ion FAB mass spectrum of **4** showed quasimolecular ions at *m/z* 552 ($[M + Na]^+$) and ions due to fragmentation of the dihydrobenzofuran lignan moiety at *m/z* 355; the negative ion FAB mass spectrum showed a quasimolecular ion ($[M - H]^-$) at *m/z* 528. The EI mass spectrum showed peaks at *m/z* 529, 511, 499, 479, 336, 324, 151 and 137, and was similar to that of **2**. In the ^1H NMR spectrum (Table 2), there were signals typical of a dihydrobenzofuran lignan similar to **3**. There were also two aromatic methoxyls, five aromatic protons and an olefin proton attributable to one *trans* double bond with a coupling constant of 15.6 Hz. The chemical shifts of the remaining signals were similar to those reported for the lignan moiety of the lignan–iridoid complex described above [6] and glutamic acid dimethyl ester. Thus, **4** had the same dihydrobenzofuran moiety as in **2**. This was confirmed by hydrolysis with 2% HCl; glutamic acid and the dihydrobenzofuran lignan moiety (**2**) were identified by comparing the ^1H NMR and mass spectra with those of authentic samples.

The ^{13}C NMR spectrum of **4** (Table 4) supported the presence of methoxyl, methylenes and an aromatic ring. In addition, the spectrum exhibited characteristic features at δ 90.0, 55.0 and 64.9, similar to **3**. Glutamic acid signals were observed at δ 173.9, 53.2, 27.8, 31.0 and 174.9. These results were similar to those reported for other dihydrobenzofuran lignans and glutamic acid dimethyl esters. From these data, it was apparent that the molecule was a dihydrobenzofuran lignan dimethylglutamate amide.

The amide location in the dihydrobenzofuran lignan moiety was determined by detailed analysis of the ^1H and ^{13}C NMR spectra, including ^1H–^1H COSY, ^{13}C–^1H COSY, DEPT, NOE and HMBC (Table 4) experiments. The assignment of proton and carbon signals was achieved by a combination of ^1H–^1H COSY and ^{13}C–^1H COSY spectral data.

Compound **5** was rechromatographed by preparative HPLC (ODS–C_{18}) to give an amorphous powder, $C_{27}H_{33}O_{10}NNa$ ($[M + Na]^+$ from HR positive ion FAB mass spectrum) exhibiting positive reactions to $FeCl_3$ and Dragendorff reagents similar to **4**. Its IR and FAB mass spectra were also similar to those of **4**. The EI mass spectrum showed the presence of peaks characteristic of a coniferyl residue in lignans. The fragmentation pattern was similar to that of **4**. The UV spectrum showed absorption maxima at 287 and 318 nm. The bathochromics shifts in the absorption maxima from the addition of sodium methoxide were at 295 and 325 nm, again being similar of those of **4**. In the ^1H NMR spectrum (Table 2), **5** showed the presence of typical proton signals due to the aliphatic moiety of the dihydrobenzofuran lignan present in **1**. There were also two aromatic methoxyls and five aromatic protons. An olefin proton due to one *cis* double bond had a coupling constant of 12.5 Hz. The chemical shifts of the remaining signals were similar to

Table 4. ^{13}C NMR spectral data for compounds **4** and **5**, and HMBC data in CD_3OD

C	4	5	DMG	DEPT	HMBC (C to H)
1	134.31	134.50		C	H-5, H-7, H-8
2	110.81	110.63		CH	H-6, H-7
3	147.88	147.65		C	H-2, H-5, H-6
4	149.31	150.32		C	H-2, H-5, H-6
5	116.41	116.17		CH	
6	120.01	121.11		CH	H-2, H-7
7	89.96	89.58		CH	H-2, H-6
8	54.96	55.05		CH	H-6′
9	64.87	64.80		CH_2	H-7, H-8
1′	130.25	129.89		C	H-8, H-7′, H-8′
2′	113.61	115.62		CH	H-2′, H-7′
3′	145.95	145.03		C	H-2′
4′	151.71	145.82		C	H-2′, H-6′
5′	131.15	130.22		C	H-7, H-8
6′	118.86	119.78*		CH	H-6′, H-7′
7′	143.18	139.73		CH	H-2′, H-6′
8′	118.55	116.19		CH	H-7′
9′	169.24	170.14		C	H-7′, H-8′
OMe	56.95	56.41		Me	
	56.61	55.77		Me	
1″	173.89	173.52	170.51	C	H-2″
2″	53.17	52.77	53.26	CH	H-3″, H-4″
3″	27.82	27.78	26.56	CH_2	H-2″, H-4″
4″	30.99	30.75	30.18	CH_2	H-3″
5″	174.90	174.69	170.05	C	H-3″, H-4″
CO_2Me	53.04	53.13	53.74	Me	
	52.44	52.20	52.40	Me	

DMG = dimethylglutamic acid ester.

those of **4** and glutamic acid dimethyl ester. Thus, **5** suggested the presence of a dihydrobenzofuran moiety and glutamic acid dimethyl ester as present in **4**. Hydrolysis with 2% HCl yielded acid and the dihydrobenzofuran lignan moiety (**2**), which were identified from their IR, ^{1}H NMR and mass spectra.

The ^{13}C NMR spectrum of **5** (Table 4) further supported the presence of methoxyl, methylenes and an aromatic ring. There were also characteristic features due to the aliphatic CH–CH–CH_2 moiety as present in **4**. Glutamic acid signals were observed at δ 173.5, 52.8, 27.8, 30.8 and 174.7, similar to those of **4**. From these data, it was apparent that the molecule was a dihydrobenzofuran lignan dimethylglutamate amide. The amide location in the dihydrobenzofuran lignan moiety was determined by detailed analysis of ^{1}H and ^{13}C NMR spectra including ^{1}H–^{1}H COSY, ^{13}C–^{1}H COSY, DEPT, NOE and HMBC experiments. The assignment of proton and carbon signals was achieved by a combination of ^{1}H–^{1}H COSY and ^{13}C–^{1}H COSY spectral data.

The stereochemistry of H-7 and H-8 of the dihydrobenzofuran lignans **1–5** was confirmed to be *anti* by ^{1}H–^{1}H NOE of ^{1}H NMR and CD experiments. Irradiation of H-7 of **4** gave 9.6% enhancements of H-2 and H-6, and 2.9% enhancement of H-9. Irradiation of H-8 in **3** produced 3.3 and 8.6% enhancements of H-2 and H-9, respectively. These ^{1}H–^{1}H NOE experiments identified dipole coupling between H-7 and two aromatic protons (H-2 and H-6) [8].

The CD curve of **3** exhibited negative Cotton effects at 268, 285 and 344 nm; that of **4** showing positive Cotton effects at 253 and 314 and a negative Cotton effect at 349 nm. The CD curves of **1** and **5** showed similar Cotton effects to those in **4**. In addition, the CD of **2** showed the presence of negative Cotton effects at 253 and 305 nm. Thus, the absolute configuration in **2** and **3** was thought to be a 7S, 8R, indicating that **1**, **4** and **5** must have the 7R,8S configuration [6]. Therefore, **1**, **4** and **5** [*cis*- and *trans*-(2R,3S)-2,3-dihydro-2-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-7-methoxybenzofuran-5-propen-1-ic (1,5-dimethyl)gluta mate amide] were assumed to have the same stereo structure. Compounds **4** and **5** are new natural products and were named *trans*- and *cis*-boreavan B.

EXPERIMENTAL

General. ^{1}H NMR were measured at 400 MHz, ^{13}C NMR at 100 MHz, in CD_3OD. Chemical shifts are given in δ relative to TMS as int. standard. EIMS was obtained by a direct inlet at 70 eV with an ion source temp. of 200°. Negative ion FABMS was measured using Xe, with the ion gun at 7 kV and glycerol or thioglycerol as matrix. MeOH solns of samples were injected into an HPLC instrument fitted with a 250 × 4 mm i.d. Nucleosil 5 C_{18} column and prep-HPLC (Nomura Chemicals). The UV detector was equipped with a 280 or 330 nm filter. H_2O–MeOH (11:9, A) was used as solvent system at 0.3 ml min^{-1} with a pressure drop of 20 kg cm^{-2}. GC was carried out on an instrument fitted with an FID.

Material. Plants of *B. orientalis* (Jand. and Spach) were collected near Ankara in 1990. A voucher specimen is deposited in the Ankara Üniversitesi Eczacilik Fakültesi Herbaryumu (AEF).

Extraction and isolation. Dried fruits (1 kg) were extracted with petrol and then MeOH. The concd extract plus H_2O was extracted successively with Et_2O, $CHCl_2$, EtOAc and *n*-BuOH. The EtOAc extracts (1.1 g) were subjected to CC over Sephadex LH-20 with a H_2O–MeOH gradient and the $CHCl_3$ extracts (3.7 g) to CC over silica gel with a $CHCl_3$–MeOH gradient. Frs containing **1–5** were rechromatographed on LH-20 or silica gel and prep-HPLC and purified by rechromatography to give **1** (2.3 mg), **2** (1.9 mg), **3** (15.0 mg), **4** (18.0 mg) and **5** (6.0 mg).

2,3-*Dihydro*-2-(4-*hydroxy*-3-*methoxyphenyl*)-3-*hydroxymethyl*-7-*methoxybenzofuran*-5-*carboxylic acid* (**1**). Pale yellow amorphous powder, mp 82.5–85.0°. HRPIFABMS *m/z*: 346.1036 $[M]^+$ $C_{18}H_{18}O_7$, requires 346.1052. Brown colour with $FeCl_3$ reagent. HPLC (A): R_t (min): 24.2. IR ν_{max}^{KBr} cm^{-1}: 3396 (OH), 2920 (CH), 1686 (CO_2H), 1598 (C=C), 1540, 1518 (aromatic C=C), 1464, 1378, 1328, 1276, 1206 (C–O), 1114, 1032 (C–O). UV λ_{max}^{MeOH} nm (log ε): 280 (2.14). +MeONa: 263, 301 (sh), 365 (sh). EIMS *m/z* (rel. int): 346 $[M = C_{18}H_{18}O_7]^+$ (64), 328 $[M - H_2O]^+$ (100), 316 $[M - CH_2O]^+$ (76), 313 $[M - H_2O - Me]^+$ (55), 296 (33), 268 (20), 151 $[C_8H_7O_3]^+$ (23), 137 $[C_8H_9O_2]^+$ (55). ^{1}H and ^{13}C NMR: Tables 1. CD (MeOH, *c* 0.1). $[\theta]_{245}$, 0; $[\theta]_{261}$, 5882; $[\theta]_{280}$, 1730; $[\theta]_{291}$, 4498; $[\theta]_{310}$, 0.

2,3-*Dihydro*-2-(4-*hydroxy*-3-*methoxyphenyl*)-3-*hydroxymethyl*-7-*methoxybenzofuran*-5-*carboxylic acid* (**2**). Pale yellow amorphous powder, mp 94.0–98.0°. HRPIFABMS *m/z*: 372.1205 $[M]^+$ $C_{20}H_{20}O_7$, requires 372.1208. Brown colour with $FeCl_3$ reagent. TLC [silica gel, *n*-BuOH–HOAc–H_2O (3:1:1, B)], R_f: 0.28. HPLC (A): R_t (min): 31.8. IR ν_{max}^{KBr} cm^{-1}: 3424 (OH), 2920 (CH), 1688 (CO_2H), 1596 (C=C), 1516, 1498, 1462 (aromatic C=C), 1426, 1374, 1326, 1270 (C–O), 1146, 1122, 1030 (C–O). UV λ_{max}^{MeOH} nm (log ε): 292 (2.93), 323 (sh). +MeONa: 296, 375 (sh). EIMS *m/z* (rel. int): 372 $[M = C_{20}H_{20}O_7]^+$ (53), 354 $[M - H_2O]^+$ (91), 342 $[M - CH_2O]^+$ (35), 339 $[M - H_2O - Me]^+$ (41), 322 (33), 310 (21), 298 (30), 167 (42), 151 $[C_8H_7O_3]^+$ (48), 137 $[C_8H_9O_2]^+$ (100). ^{1}H and ^{13}C NMR: Tables 1. CD (MeOH, *c* 0.1). $[\theta]_{245}$, 0; $[\theta]_{253}$, −1934; $[\Delta\varepsilon]_{270}$, 0; $[\Delta\varepsilon]_{305}$, −3422; $[\Delta\varepsilon]_{360}$, 0.

(2S,3R)-2,3-*Dihydro*-2-(4-*hydroxy*-3-*methoxyphenyl*)-3-*hydroxymethyl*-7-*methoxybenzofuran*-5-(trans)*propen*-1-*ol*-3-O-β-*glucoside*(**3**). Pale yellow amorphous powder, mp 119.0–121.5°. HRPIFABMS *m/z*: 520.1946 $[M]^+$ $C_{26}H_{32}O_{11}$, requires 520.1946. Brown colour with $FeCl_3$ reagent. TLC [silica gel, EtOAc–MeCOEt–HCO_2H–C_6H_6–H_2O (4:3:1:1:2, upper layer; A)], R_f: 0.8. HPLC (A): R_t (min): 28.8. IR ν_{max}^{KBr} cm^{-1}: 3412 (OH), 2920 (CH), 1600, 1520 (C=O), 1464 (aromatic C=C), 1454, 1330, 1284, 1276 (C–O),

1216, 1080 (C–O), 1034 (C–O). UV λ_{max}^{MeOH} nm(ε): 278 (2.98), 335 (sh). +MeONa: 274, 300 (sh), 405 (sh). PIFABMS (glycerol + NaI) *m/z*: 543 [M = $C_{26}H_{32}O_{11}Na]^+$, 520 $[M]^+$, 503 $[M-18\ (H_2O)]^+$. 329, 176. EIMS *m/z* (rel. int): 338 (79), 323 (15), 312 (17), 200 (22), 198 (17), 151 (22), 137 (21), 57 (100). ^1H and ^{13}C NMR: Tables 2 and 3. CD (MeOH, *c* 0.1142). $[\theta]_{245}$, 0; $[\theta]_{268}$, 6830; $[\theta]_{285}$, −7443; $[\theta]_{320}$, 0; $[\theta]_{344}$, −2014; $[\theta]_{360}$, 0.

Acidic hydrolysis of **3**. Compound **3** (1 mg) was refluxed with 3% HCl (3 ml) for 2 hr. The reaction mixt. was extracted with EtOAC. The EtOAc extract was washed with H_2O and then evapd to dryness *in vacuo*. The extract was subjected to CC on silica gel and afforded **3a** [8].

Enzymic hydrolysis of **3**. A soln of **3** (*ca* 1 mg) was treated at room temp. with β-glucosidase for 7 days. The reaction mixt. was extracted with *n*-BuOH. The *n*-BuOH layer was washed with H_2O and evapd to dryness *in vacuo* to give **3a**, which was identified by comparison with an authentic sample. The H_2O layer was treated in the usual way and then examined by GC [2]. D-Glucose was identified as its TMSi derivative.

(2S,3R) - 2,3 - *Dihydro* - 2 - (4 - *hydroxy* - 3 - *methoxyphenyl*) - 3 - *hydroxymethyl* - 7 - *methoxybenzofuran* - 5 - (trans)*propen* - 1 - *ic*(1,5-*dimethyl*)*glutamate amide* (**4**). Pale yellow amorphous powder, mp 82.0–84.5°. HRPIFABMS *m/z*: 530.2025 $[M+H]^+$ $C_{27}H_{32}O_{10}N$ requires 530.2041. TLC [silica gel, R_f: 0.51. HPLC (A): R_t (min): 80.7. IR ν_{max}^{KBr} cm^{-1}: 3380 (OH), 2936 (CH), 1730 (C=O), 1606 (CONH), 1518 (CONH), 1462 (aromatic C=C), 1434, 1274, 1210 (C–O), 1178, 1116, 1030 (C–O). UV λ_{max}^{MeOH} nm(ε): 288 (3.30), 320 (3.32). +MeONa; 289, 325, 350 (sh). PIFABMS (glycerol) *m/z*: 552 [M = $C_{27}H_{31}O_{10}N + Na]^+$, 529 $[M]^+$, 355 [tetrahydrobenzofuran lignan moiety = $C_{20}H_{19}O_6]^+$. NIFABMS (thioglycerol) *m/z*: 528 $[M-H]^-$, 353 [tetrahydrobenzofuran lignan moiety $-H]^-$, 175 [glutamic acid dimethyl ester moiety = $C_7H_{13}O_4N]^-$. EIMS *m/z* (rel. int): 529 $[M]^+$ (47), 511 $[M-H_2O]^+$ (34), 499 $[M-CH_2O]^+$ (29), 479 (23), 467 (10), 355 [M+glutamic acid dimethyl ester moiety = $C_7H_{13}O_4N+H]^+$ (17), 336 $[M-C_7H_{13}O_4N-18]^+$ (100), 324 $[M-CH_2O-CH_2O-C_7H_{13}O_4N]^+$ (56), 151 (26), 137 (34). ^1H NMR (in CD_3OD): Table 2; ^{13}C NMR: Table 4. CD (MeOH, *c* 0.0933). $[\theta]_{250}$, 0; $[\theta]_{253}$, 1072; $[\theta]_{270}$, 0; $[\theta]_{314}$, 5895; $[\theta]_{340}$, 0; $[\theta]_{349}$, −2572; $[\theta]_{380}$, 0.

Acidic hydrolysis of **4**. Compound **4** (2 mg) was refluxed with 2% HCl (2 ml) for 4 hr. The reaction mixt. was extracted with EtOAc and the H_2O layer evapd to dryness *in vacuo*. The H_2O layer was subjected to CC on silica gel affording glutamic acid. TLC [silica gel, *n*-BuOH–HOAc–H_2O (3:1:1, B)], R_f: 0.28. ^1H NMR data were in agreement with those for **2**.

(2R,3S) - 2,3 - *Dihydro* - 2 - (4 - *hydroxy* - 3 - *methoxyphenyl*) - 3 - *hydroxymethyl* - 7 - *methoxybenzofuran* - 5 - (cis)*propen* - 1 - *ic*(1,5-*dimethyl*)*glutamate amide* (**5**). Pale yellow amorphous powder, mp 95.0–99.0°. HRPIFABMS *m/z*: 552.1848 $[M+Na]^+$ $C_{27}H_{32}O_{10}NNa$ requires 552.1846. HPLC (A): R_t (min): 50.7. IR ν_{max}^{KBr} cm^{-1}: 3418 (OH), 2924 (CH), 1734 (C=O), 1602 (CONH), 1514 (CONH), 1452 (aromatic C=C), 1274, 1210 (C–O), 1144, 1030 (C–O). UV λ_{max}^{MeOH} nm(ε): 287 (3.07), 318 (1.83). +MeONa; 295, 325, 400 (sh). PIFABMS (glycerol) *m/z*: 552 [M = $C_{27}H_{31}O_{10}N + Na]^+$, 529 $[M]^+$, 355 [tetrahydrobenzofuran lignan moiety = $C_{20}H_{19}O_6]^+$. NIFABMS (thioglycerol) *m/z*: 528 $[M-H]^-$, 353 [tetrahydrobenzofuran lignan moiety $-H]^-$, 175 [glutamic acid dimethyl ester moiety = $C_7H_{13}O_4N]^-$. EIMS *m/z* (rel. int): 529 $[M]^+$ (38), 511 $[M-H_2O]^+$ (33), 499 $[M-CH_2O]^+$ (32), 355 [M − glutamic acid dimethyl ester moiety = $C_7H_{13}O_4N+H]^+$ (17), 336 $[M-C_7H_{13}O_4N-18]^+$ (100), 324 $[M-CH_2O-CH_2O-C_7H_{13}O_4N]^+$ (56), 167 $[C_8H_7O_4]^+$ (38), 151 $[C_8H_7O_3]^+$ (46), 137 $[C_8H_9O_2]^+$ (34). ^1H NMR (in CD_3OD): Table 2; ^{13}C NMR: Table 4. CD (MeOH, *c* 0.1489). $[\theta]_{248}$, 0; $[\theta]_{255}$, 1621; $[\theta]_{280}$, 0; $[\theta]_{305}$, 4324; $[\Delta\varepsilon]_{350}$, 0.

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