

Neolignan Glucosides from *Phlomis chimerae* Boiss

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From the aerial parts of the plant *Phlomis chimerae*, a new neolignan glucoside, (–)-4-*O*-methyldehydrodiconiferyl alcohol-9'-*O*-β-D-glucopyranoside (**1**) was characterized along with the known neolignan glucosides, (–)-4-*O*-methyldehydrodiconiferyl alcohol-9'-*O*-β-D-glucopyranoside (= longifloroside A) (**2**) and (–)-dihydrodehydrodiconiferyl alcohol-9'-*O*-β-D-glucopyranoside (**3**). The structure of the new compound was established on the basis of spectroscopic evidence.

Introduction

Lignans and neolignans belong to an important group of natural products, consisting of two phenylpropane monomers linked through carbon-carbon or carbon-oxygen bonds (Whiting, 1985). So far, only two *Phlomis* species, *P. lycia* (Saracoğlu *et al.*, 2002) and *P. fruticosa* (Ersöz *et al.*, 2001a) have been reported to contain neolignan and lignan glucosides. As a part of a project directed at the chemical characterization of *Phlomis* species growing in Turkey, we have isolated an iridoid glucoside, lamiide, and four phenylethanoid glycosides, verbascoside (= acteoside), forsythoside B, alyssonoside and leucosceptoside B, together with a phenolic glucoside, syringin, from *Phlomis chimerae* Boiss. (Ersöz *et al.*, 2001b). Further investigation on the overground parts of this plant yielded a new neolignan glucoside, (–)-4-*O*-methyldehydrodiconiferyl alcohol-9'-*O*-β-D-glucopyranoside (**1**), along with the known neolignan glucosides, (–)-4-*O*-methyldehydrodiconiferyl alcohol-9'-*O*-β-D-glucopyranoside (= longifloroside A) (**2**) and (–)-dihydrodehydrodiconiferyl alcohol-9'-*O*-β-D-glucopyranoside (**3**). The current study describes the structural elucidation of the new glucoside (**1**).

Experimental

General experimental procedures

NMR measurements in CD₃OD were performed on a Varian spectrometer (500 MHz for ¹H and 125 MHz for ¹³C) with a Nalorac MDBG 3 mm probe. ESIMS were recorded in the positive and negative ion modes on a Finnigan TSQ 7000 spectrometer. For open-column chromatography (CC), polyamide (Polyamid-MN-Polyamid SC-6, Machery-Nagel, Düren), and Kieselgel 60 (0.063–0.200 mm, Merck) were used. Medium-pressure liquid chromatography (MPLC) was performed on a Labomatic glass column (26×460 mm, i.d.), packed with LiChroprep RP-18, using a Lewa M5 peristaltic pump. For TLC, pre-coated Kieselgel 60 F₂₅₄ aluminum sheets (Merck) were used. Compounds were detected by UV and 1% vanillin/H₂SO₄.

Plant material

Phlomis chimerae Boiss. (Lamiaceae) was collected from Antalya, Çıralı, Turkey, in July 2000. Voucher specimens have been deposited in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey (HUEF 00–031).

Extraction and isolation

The air-dried and powdered aerial parts of *P. chimerae* (480 g) were extracted twice with MeOH (2×2500 ml) at 40 °C. After evaporation of the combined extracts *in vacuo*, 63 g MeOH extract was obtained. An aliquot (38 g) of the crude extract was dissolved in water and the water-insoluble material was removed by filtration. The filtrate was then extracted successively with CH₂Cl₂ (4×100 ml) and *n*-BuOH (4×100 ml), respectively. The *n*-BuOH extract (14 g) was fractionated over a polyamide column (150 g), eluting with H₂O (500 ml) and gradient MeOH-H₂O mixtures (25–100%) to afford 12 main fractions (A–L). Fr. B (1.37 g) was subjected to RP-18 MPLC using H₂O (200 ml) and MeOH-H₂O gradients (10–100% MeOH) to yield 21 fractions (B₁–B₂₁). Fraction B₁₈ (74 mg) was applied to a silica gel column. Elution with CH₂Cl₂-MeOH-H₂O mixtures (90:10:1 and 80:20:1 v/v) yielded **3** (10 mg). Fraction B₂₀ (39 mg) was also chromatographed on a silica gel column eluting with CH₂Cl₂-MeOH-H₂O mixtures (90:10:1 and 80:20:1 v/v) to afford a mixture of **1** and **2** (4 mg). Despite all efforts, this mixture could not be separated by normal or reversed phase TLC/CC.

(–)-4-*O*-methyl dihydrodehydrodiconiferyl alcohol-9'-*O*-β-D-glucopyranoside (**1**): ¹H NMR (CD₃OD, 500 MHz): Table I; ¹³C NMR (CD₃OD, 125 MHz): Table I; positive-ion ESIMS *m/z* 559 [M+Na]⁺; negative-ion ESIMS *m/z* 535 [M-H][–].

(–)-4-*O*-methyl dehydrodiconiferyl alcohol-9'-*O*-β-D-glucopyranoside (**2**): ¹H NMR (CD₃OD, 500 MHz): aglycon: δ 6.92 (1H, *d*, *J* = 2.0 Hz, H-2), 6.93 (1H, *d*, *J* = 8.0 Hz, H-5), 5.49 (1H, *d*, *J* = 6.5 Hz, H-7), 3.44 (1H, overlapped, H-8), 6.88 (3H, *br s*, overlapped, H-6, H-2' and H-6'), 3.80 (1H, H-9_b, partly merged with the 3'-OMe signal), 3.73 (1H, *d*, *J* = 8.0 Hz, H-9_a), 6.65 (1H, *d*, *J* = 11.0 Hz, H-7'), 6.21 (1H, *dd*, *J* = 11.0/7.3 Hz, H-8'), 4.27 (1H, *t*, *J* = 7.3 Hz, H-9'_a), 4.48 (1H, *t*, *J* = 7.3 Hz, H-9'_b), 3.84 (3H, *s*, 3'-OMe), 3.75 (3H, *s*, 3-OMe), 3.72 (3H, *s*, 4-OMe); glucose moiety: 4.32 (1H, *d*, *J* = 7.3 Hz, H-1''), 3.22 (1H, *dd*, *J* = 7.3/8.5 Hz, H-2''), 3.32 (1H, *t*, *J* = 8.5 Hz, H-3''), 3.25 (1H, *t*, *J* = 9.4 Hz, H-4''), 3.31 (1H, *m*, H-5''), 3.82 (1H, H-6''_b, partly merged with the 3'-OMe signal), 3.63 (1H, *dd*, *J* = 11.9/6.0 Hz, H-6''_a); ¹³C NMR (CD₃OD, 125 MHz): aglycon: δ 136.94 (*s*, C-1), 110.75 (*d*,

C-2), 150.40 (*s*, C-3), 150.65 (*s*, C-4), 116.70 (*d*, C-5), 119.48 (*d*, C-6), 89.09 (*d*, C-7), 55.55 (*d*, C-8), 65.94 (*t*, C-9), 56.51 (*q*, OCH₃), 56.45 (*q*, OCH₃), 132.46 (*s*, C-1'), 112.94 (*d*, C-2'), 145.56 (*s*, C-3'), 150.40 (*s*, C-4'), 130.22 (*s*, C-5'), 119.48 (*d*, C-6'), 134.22 (*d*, C-7'), 124.32 (*d*, C-8'), 70.94 (*t*, C-9'), 56.78 (*q*, OCH₃); glucose moiety: 103.18 (*d*, C-1''), 75.20 (*d*, C-2''), 78.17 (*d*, C-3''), 71.70 (*d*, C-4''), 78.01 (*d*, C-5''), 62.85 (*t*, C-6''); positive-ion ESIMS *m/z* 557 [M+Na]⁺; negative-ion ESIMS *m/z* 533 [M-H][–].

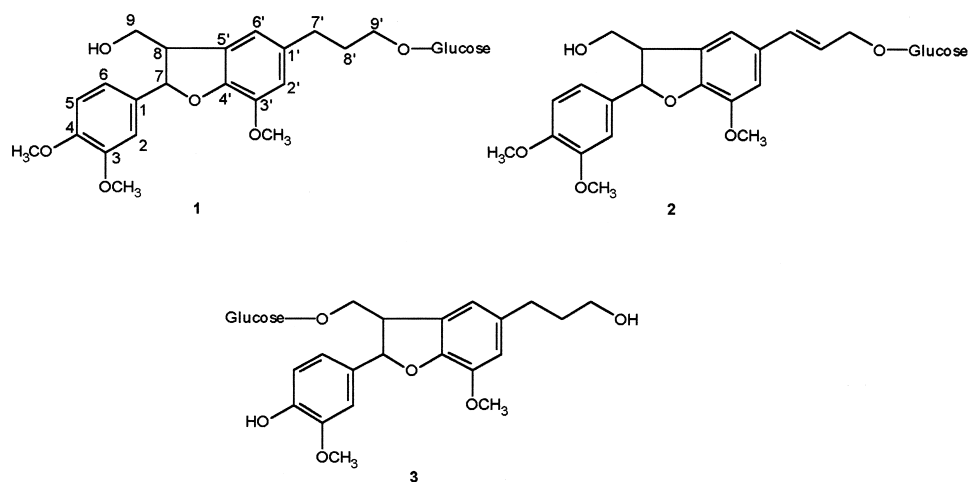
(–)-Dihydrodehydrodiconiferyl alcohol-9-*O*-β-D-glucopyranoside (**3**): [α]_D²⁰ –18° (*c* 0.1, MeOH). The ¹H (CD₃OD, 500 MHz), ¹³C NMR (CD₃OD, 125 MHz) data were identical with those reported in the literature (Abe and Yamauchi, 1986; Wang and Jia, 1997a; Saracoğlu *et al.*, 2002).

Results and Discussion

The methanolic extract of the aerial parts of *P. chimerae* was suspended in water and partitioned successively between CH₂Cl₂ and *n*-BuOH. Chromatographic separations of the *n*-BuOH extract yielded a mixture of compounds **1** and **2** as well as pure **3** (Fig. 1).

Compounds **1** and **2** were obtained as an inseparable (1:1) mixture, [α]_D²⁰ –26° (*c* 0.1, MeOH). The positive ESIMS of this mixture exhibited the pseudomolecular ions [M+Na]⁺ at *m/z* 559 and *m/z* 557, while the negative ESIMS showed the ions [M-H][–] at *m/z* 535 and *m/z* 533 for **1** and **2**. These data were compatible with the molecular formulae C₂₇H₃₆O₁₁ (**1**) and C₂₇H₃₄O₁₁ (**2**). Although most NMR signals appeared double, indicating a close structural similarity between **1** and **2**, ¹H-¹H gCOSY, ¹H-¹³C gHSQC and ¹H-¹³C gHMBC experiments allowed to pick the signals for **1** and **2** individually.

In the ¹H NMR spectrum, five aromatic proton signals were recognized for **1** (Table I). Of these, the proton resonances at δ 6.92 (*d*, *J* = 2.0 Hz, H-2), 6.93 (*d*, *J* = 8.0 Hz, H-5) and 6.88 (*br s*, H-6, overlapped) were observed as an ABX system, suggesting **1** to contain a trisubstituted aromatic moiety. The 2H resonance at δ 6.70 (*br s*) was indicative of the presence of an additional tetrasubstituted aromatic moiety in the structure of **1**. Moreover, the ¹H NMR spectrum of **1** displayed three methoxy singlets at δ 3.81, 3.77 and 3.74 and an anomeric proton signal at δ 4.20 (*d*, *J* = 7.3 Hz)

Fig. 1. Neolignan glucosides from *P. chimerae*.Table I. ^{13}C NMR (CD_3OD , 125 MHz) and ^1H NMR (CD_3OD , 500 MHz) data and HMBC correlations for **1**.*

C/H Atom		δ_{C}	δ_{H} J (Hz)	HMBC
1	C	136.0		
2	CH	110.8	6.92 <i>d</i> (2.0)	C-3, C-6
3	C	150.7		
4	C	150.3		
5	CH	116.7	6.93 <i>d</i> (8.0)	C-4, C-6
6	CH	119.5	6.88 <i>dd</i> (8.0/2.0)	C-1, C-5
7	CH	88.7	5.52 <i>d</i> (6.5)	C-1, C-2, C-6
8	CH	55.3	3.42 ^a	C-9
9	CH ₂	65.1	3.80 ^b	C-7
3'-OMe	CH ₃	56.5	3.72 <i>d</i> (8.0)	
4'-OMe	CH ₃	56.5	3.77 <i>s</i>	C-3
1'	C	136.3	3.74 <i>s</i>	C-4
2'	CH	114.3		
3'	C	145.2	6.70 <i>br s</i>	C-4'
4'	C	147.5		
5'	C	129.7		
6'	CH	118.1	6.70 <i>br s</i>	C-3', C-4'
7'	CH ₂	32.9	2.62 <i>t</i> (7.3)	C-1', C-2', C-6', C-8'
8'	CH ₂	32.9	1.85 <i>t</i> (7.3)	
9'	CH ₂	69.9	3.90 <i>t</i> (7.3)	C-8'
3'-OMe	CH ₃	56.8	3.52 <i>t</i> (7.3)	
1''	CH	104.5	3.81 <i>s</i>	
2''	CH	75.2	4.20 <i>d</i> (7.3)	C-9'
3''	CH	78.2	3.20 <i>dd</i> (7.3/8.5)	
4''	CH	71.7	3.32 <i>t</i> (8.5)	
5''	CH	78.0	3.25 <i>t</i> (9.4)	
6''	CH ₂	62.8	3.31 <i>m</i>	
			3.82 ^b	
			3.63 <i>dd</i> (11.9/6.0)	

* The ^{13}C and ^1H assignments were based on HSQC, HMBC and COSY experiments.^a Unclear due to signal overlapping.^b Partly merged with the 3'-OMe signal.

suggesting the presence of a β -glucopyranose unit within **1**. Apart from six signals due to a β -glucopyranoside unit and three OMe signals, the ^{13}C NMR spectrum of **1** (Table I) contained 18 skeletal carbon resonances, which were classified as four methylenes, seven methines and seven quaternary carbon atoms by a DEPT-135 experiment. From the detailed inspection of these data, associated with the interpretation of the 2D NMR data, compound **1** was predicted to be 4-*O*-methyldehydrodiconiferyl alcohol-9'-*O*- β -glucoside. Accordingly, methine protons at δ 3.42 (overlapped), which coupled to the oxymethine proton at δ 5.52 (*d*, $J = 6.5$ Hz) in the gCOSY spectrum, were ascribed to H-8 and H-7 of the benzofuran ring, respectively. H-8 showed additional coupling with the hydroxymethyl protons (δ 3.80, partly merged with OMe signal; 3.72, *d*, $J = 8.0$ Hz, H₂-9). Two methoxy functions were placed at C-3 and C-4 of the tetrasubstituted aromatic ring on the basis of prominent HMBC correlations shown in Fig. 2. Additional long-range couplings between H-8/C-9, H₂-9/C-7, H-7/C-1, H-7/C-2 and H-7/C-6 secured the aromatic substitution to take place at C-7. The assignments of the third methoxy group (C-3') as well as H-2' and H-6' of the benzofuran ring were also possible by the cross-peaks observed in the gHMBC spectrum (Fig. 2). Thus the

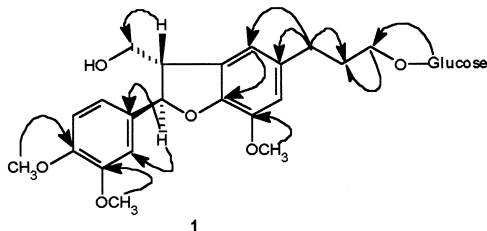


Fig. 2. Selected HMBC correlations for **1**.

saturated side chain and the β -glucopyranoside unit were remained to be positioned. In the gCOSY spectrum, the benzylic methylene protons (δ 2.62, 2H, *t*, $J = 7.3$ Hz, H₂-7') coupled with the C-8' protons (δ 1.85 2H, *t*, $J = 7.3$ Hz). The latter showed further couplings with the oxymethylene protons (δ 3.90 1H, *t*, $J = 7.3$ Hz, H-9'_b and 3.52 1H, *t*, $J = 7.3$ Hz, H-9'_a). Heteronuclear HMBC cross couplings between H₂-7'/C-1', H₂-7'/C-2', H₂-7'/C-6', H₂-7'/C-8' and H₂-9'/C-8' indicated the attachment of the side chain at C-1', as expected. The glycosidic linkage was determined to

be at C-9' due to the downfield shift of the C-9' (δ 69.9), in addition to the HMBC correlation observed from H-1'' (δ 4.20) of the glucose moiety to C-9' atom. Although, the stereochemistry at C-7 and C-8 could not be established from the available data, but the negative optical rotation value of the mixture ($[\alpha]_{\text{D}}^{20} - 26^\circ$) suggested that **1** may have the (–) stereoisomeric structure (Abe and Yamauchi, 1986; Wang and Jia, 1997a). Consequently, compound **1** was identified as (–)-4-*O*-methyldehydrodiconiferyl alcohol-9'-*O*- β -D-glucopyranoside. To the best of our knowledge, **1** is being reported for the first time in nature.

The complete analysis of the remaining ^1H and ^{13}C NMR signals, assigned by 2D NMR experiments (DQF-COSY, HSQC and HMBC) again, revealed that **2** was almost identical with **1**. However, the presence of two extra proton signals at δ 6.65 (1H, *d*, $J = 11.0$ Hz) and δ 6.21 (1H, *dd*, $J = 11.0/7.3$ Hz) in **2** instead of the two methylene groups in **1**, was indicative of an unsaturated side-chain. The olefinic bond was determined to be at $\Delta^{7',8'}$, based on the results of the gCOSY and gHMBC experiments. The stereochemistry of **2** was also assumed to be (–) as in the case of **1**. Therefore, the structure of **2** was established as (–)-4-*O*-methyldehydrodiconiferyl alcohol-9'-*O*- β -D-glucopyranoside, which is identical to that of longifloroside A, previously isolated from *Pedicularis longiflora* (Scrophulariaceae) (Wang and Jia, 1997b).

(–)-Dihydrodehydrodiconiferyl alcohol-9-*O*- β -D-glucopyranoside (**3**) was identified by comparison of its physical and spectroscopic (^1H NMR, ^{13}C NMR and ESIMS) data with those published in the literature (Abe and Yamauchi, 1986; Wang and Jia, 1997a; Saracoğlu *et al.*, 2002).

Reports for the isolation of the lignan glucosides from the genus *Phlomis* are in limited number. Up to now, only the neolignan glucoside, (–)-dihydrodehydrodiconiferyl alcohol-9-*O*- β -D-glucopyranoside (**3**) from *P. lycia* (Saracoğlu *et al.*, 2002) and the lignan glucoside, syringaresinol-4'-*O*- β -D-glucoside from *P. fruticosa* (Ersöz *et al.*, 2001a) have been reported. Of the three lignan glucosides characterized from *P. chimerae*, compounds **1** and **3** were identified as dihydrodehydrodiconiferyl alcohol-type lignan glucosides, whereas **2** was a dehydrodiconiferyl alcohol-type glucoside. In addition to the new neolignan glucoside **1**, this is the

first demonstration of the occurrence of (–)-4-*O*-methyldehydrodiconiferyl alcohol-9'-*O*-β-D-glucopyranoside (= longifloroside A) (**2**) and the second report for the isolation of (–)-dihydrodehydrodiconiferyl alcohol-9-*O*-β-D-glucopyranoside (**3**) in the genus *Phlomis*.

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