Iridoid Glucosides from *Phlomis tuberosa* L. and *Phlomis herba-ventis* L.

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A new iridoid glucoside, 5-desoxysesamoside, was isolated from *Phlomis tuberosa* L. (Lamiaceae) together with three known iridoid glucosides sesamoside, shanziside methyl ester and lamalbid. Lamiide was found in *P. herba-ventis* ssp. *pungens* in high concentrations.

Introduction

The genus *Phlomis* L. (Lamiaceae) is represented by two species, *P. tuberosa* L. and *P. herba-ventis* ssp. *pungens* (Willd.) Maire ex De Filipps, in the Bulgarian flora (Stojanov *et al.*, 1967). In Asian folk medicine *P. tuberosa* is used as a general roborant, for intoxications, tuberculosis, pulmonary and cardio-vascular diseases and rheumatoid arthritis (Markova *et al.*, 1985). Previous phytochemical analyses of *P. tuberosa* showed the presence of flavonoids (Vavilova, 1973; Glyzin *et al.*, 1972), polyphenolic compounds (Gella *et al.*, 1972), alkaloids (Khokhrina and Peshkova, 1974) and two C_9-type iridoids, harpagide and 8-O-acetylharpagide (Gella *et al.*, 1972). In *P. herba-ventis* ssp. *pungens* flavonoids (Nedonoskova *et al.*, 1974), lamiide and phenylpropanoid glycosides were found (Saracoglu *et al.*, 1997, 1998; Harput *et al.*, 1998). The present paper deals with the study of the iridoid glucosides in these two *Phlomis* species widespread in Bulgaria.

Materials and Methods

General experimental procedures

^{1}H and ^{13}C NMR including distortionless enhancement by polarization transfer (DEPT) and 2D-NMR spectra were recorded on INOVA-500 and Bruker 250 spectrometer in D_2O and CD_3OD and chemical shifts are given in δ (ppm) with 3-(trimethylsilyl) propionic acid-d_4 sodium salt (TSPA-d_4) and tetramethylsylane (TMS) as internal standards. The NOE difference spectra were measured by the use of a standard Bruker software program. FAB mass spectrum was obtained on ZAB-SET mass spectrometer. Optical rotations were measured by a Perkin Elmer 241 polarimeter. Aluminum sheets with silicagel 60 F_{254} were used for TLC. Reverse phase low pressure liquid chromatography (LPLC) was carried out with a Lobar prepacked column Size A LiChroprep RP-18 (Merck Cat. No. 115399) with H_2O–MeOH mixtures as eluent.

Plant material

Phlomis tuberosa and *P. herba-ventis* ssp. *pungens* were collected at florescence in Barmuk bair (Sliven region) and in Cherna Mogila village (Pleven region) (1996) and identified by Dr. L. Evstatieva. The voucher specimens LE 9643 and LE 9644 are deposited in the herbarium of the Institute of Botany, Bulgarian Academy of Sciences (SOM).

Extraction and isolation

P. tuberosa (230 g). Dry aerial parts were extracted twice with EtOH (2×1 l) and the concentrated extract (13.5 g) partitioned between $Cl(CH_2)_2Cl$–H_2O (2×300 ml). The aqueous phase was concentrated and treated with charcoal and eluted with H_2O (1.5 l), MeOH–H_2O (5:95, v/v) (0.5 l), MeOH–H_2O (30:70, v/v) (0.5 l), 50%

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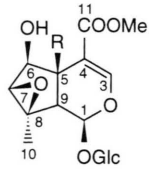
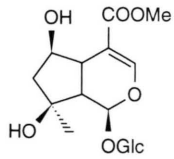
MeOH (1:1, v/v) (0.5 l), MeOH (0.5 l), MeOH–Me_2CO (1:1, v/v) (0.5 l) and MeOH–$Cl(CH_2)_2Cl$ (1:1, v/v) (0.5 l). The combined MeOH (0.3 g) and MeOH–Me_2CO (0.7 g) fractions were separated by ascending droplet counter current chromatography (DCCC) with $CHCl_3$–MeOH–H_2O–*n*PrOH (9:12:8:2). Fractions 33–44 (126 mg) were additionally purified reversed phase LPLC and elution with H_2O–MeOH mixtures to give **1** (fr. 12–15, 55 mg), **2** (fr. 17–22, 26 mg), **3** (fr. 24–32, 74 mg). and **4** (fr. 11–15 (130 mg).



1: R = H

2: R = OH



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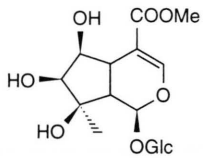

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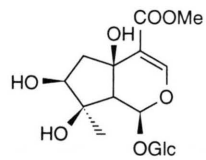

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Desoxysesamoside (**1**), 55 mg. [α] −69.6° (MeOH, c 2.0); UV γ_{max}, nm 235 (MeOH); IR (KBr) cm^{-1}: 3200–3500, 1675, 1630, 1440, 1397, 905, 890, 770. FAB MS: *m/z* 497 (M + glycerol), 443 $(M + K)^+$, 427 $(M + Na)^+$, 405 $(MH)^+$. ^1H NMR (500 MHz, CD_3OD): 7.54 (1H, *s*, H-3); 5.26 (2H, *d*, *J* = 9.4 Hz, H-1); 4.80 (1H, *d*, *J* = 8.1 Hz, H-1′); 3.99 (1H, *dd*, *J* = 1.3/7.7 Hz, H-6); 3.93 (1H, *dd*, *J* = 2.1/12.0 Hz, H-6′a); 3.75 (3H, *s*, OMe); 3.63 (1H, *dd*, *J* = 6.8/12.0 Hz, H-6′b); 3.39 (1H, *t*, *J* = 9.0 Hz, H-3′); 3.34 (1H, *bs*, H-7); 3.31 (1H, *m*, H-5′); 3.26 (1H, *dd*, *J* = 9.4 Hz, H-4′); 3.22 (1H, *dd*, *J* = 9.0/8.1 Hz, H-2′); 2.67 (1H, *dt*, *J* = 1.3/7.7 Hz, H-5); 2.41 (1H, *dd*, *J* = 7.7/9.4 Hz, H-9); 1.54 (3H, *s*, H_3-10); ^{13}C NMR: Table I.

P. herba-ventis ssp. *pungens* (130 g); EtOH extract (14.8 g); the MeOH fraction (1.9 g) consisted of pure **5**, the MeOH–acetone fraction of impure **5** (0.9 g).

Results and Discussion

The ethanol extracts of *P. tuberosa* and *P. herba-ventis* ssp. *pungens* were partitioned between dichloroethane and water. The water soluble parts were treated with charcoal and eluted with water, water–methanol, methanol–acetone and methanol–dichloroethane mixtures to remove the sugars and aromatics. Purification of the methanol and methanol-acetone fraction of *P. tuberosa* by DCCC and reverse phase LPLC yielded four pure compounds **1–4**. They were identified by spectral data as the known iridoid glucosides sesamoside (**2**) (Potterat *et al.*, 1988), shanziside methyl ester (**3**) (Boros and Stermitz, 1990) and lamalbide (**4**) (El-Nagar and Beal, 1980) in addition to the new compound **1**. All four iridoids were present in similar concentrations (total iridoids 0.4% of the dry plant material).

Compound **1** was obtained as an optically active amorphous powder. The UV spectrum of **1** showed an absorption maximum at 235 nm, typical of a conjugated carbonyl function. The FAB mass spectrum suggested a molecular formula of $C_{17}H_{24}O_{11}$ on the basis of the quasimolecular ions at *m/z* 427 $(M + Na)^+$, *m/z* 443 $(M + K)^+$ and *m/z* 405 $(MH)^+$. The ^1H and ^{13}C NMR spectra displayed a carboxyl signal at δ_C 171.1, a trisubstituted double bond at δ_H 7.54 (δ_C 154.3 and 107.7) and an acetal signal at δ_H 5.26 (δ_C

Table I. ^{13}C NMR chemical shifts of **1** and **2** in D_2O.

C-atom	**1**[a]	**2**
1	96.5 *d*	95.7
3	154.3 *d*	156.2
4	107.7 *s*	111.6
5	37.0 *d*	75.2
6	78.5 *d*	75.2
7	65.4 *d*	66.6
8	64.1 *s*	64.8
9	43.9 *d*	53.0
10	17.4 *t*	16.8
11	171.1 *s*	169.2
1′	99.4 *d*	99.4
2′	73.6 *d*	73.3
3′	76.6 *d*	76.3
4′	70.4 *d*	70.4
5′	77.2 *d*	77.2
6′	61.5 *t*	61.5
OMe	52.9 *q*	52.9

[a] Carbon multiplicities, determined by DEPT, C-H connectivities by Heteronuclear Multiple Bond Correlation (HMQC).

96.5), characteristic for a C_{10} iridoid with a 4-carboxy substituent. The C–H connectivities were assigned by HMQC data. The ^{13}C NMR spectrum of **1** (Table I) contained 17 carbon signals, six of which were readily assigned to a β-glucopyranosyl moiety and eleven suggested a similarity to a sesamoside moiety (**2**) (Potterat *et al.*, 1988). The C-5 carbon atom appeared as a methine upfield signal at δ 37, which together with the shifted signals for C-4 (Δδ −3.9), C-6 (Δδ +3.3) and C-9 (Δδ −9.1), confirmed the lack of a 5-OH group when compared with **2**. Moreover, the ^1H NMR spectrum revealed a signal for H-5 at δ 2.67. The lack of a positive NOE between H-5 and H-6 established the *trans*-configuration of these protons and thus, stereochemistry with the β-position of the 6-OH group like in sesamoside. Thus, the only difference with **2** consisted in the lack of the 5-OH group. Accordingly, the structure of **1** was established as 5-desoxysesamoside.

Our study on *P. tuberosa* did not confirm the presence of the C_9 iridoids, harpagide and 8-O-acetyl-harpagide, found by Gella *et al.* (1972). Till now, in fifteen *Phlomis* species only C_{10} iridoids were isolated with the exception of the above-mentioned report. The identification of the reported by Gella *et al* iridoids is not convincing (hydrolysis, chromatography) and makes doubtful their presence in *P. tuberosa.*.

From *P. herba-ventis* ssp. *pungens* we isolated only one iridoid in high concentrations (1%), identified by spectral data as the known iridoid glucoside lamiide (**5**) (Junior, 1985). Thus, our data completely confirmed the results of Saracoglu *et al.* (1997, 1998) for *P. pungens* (taxon synonym) from Turkish origin.

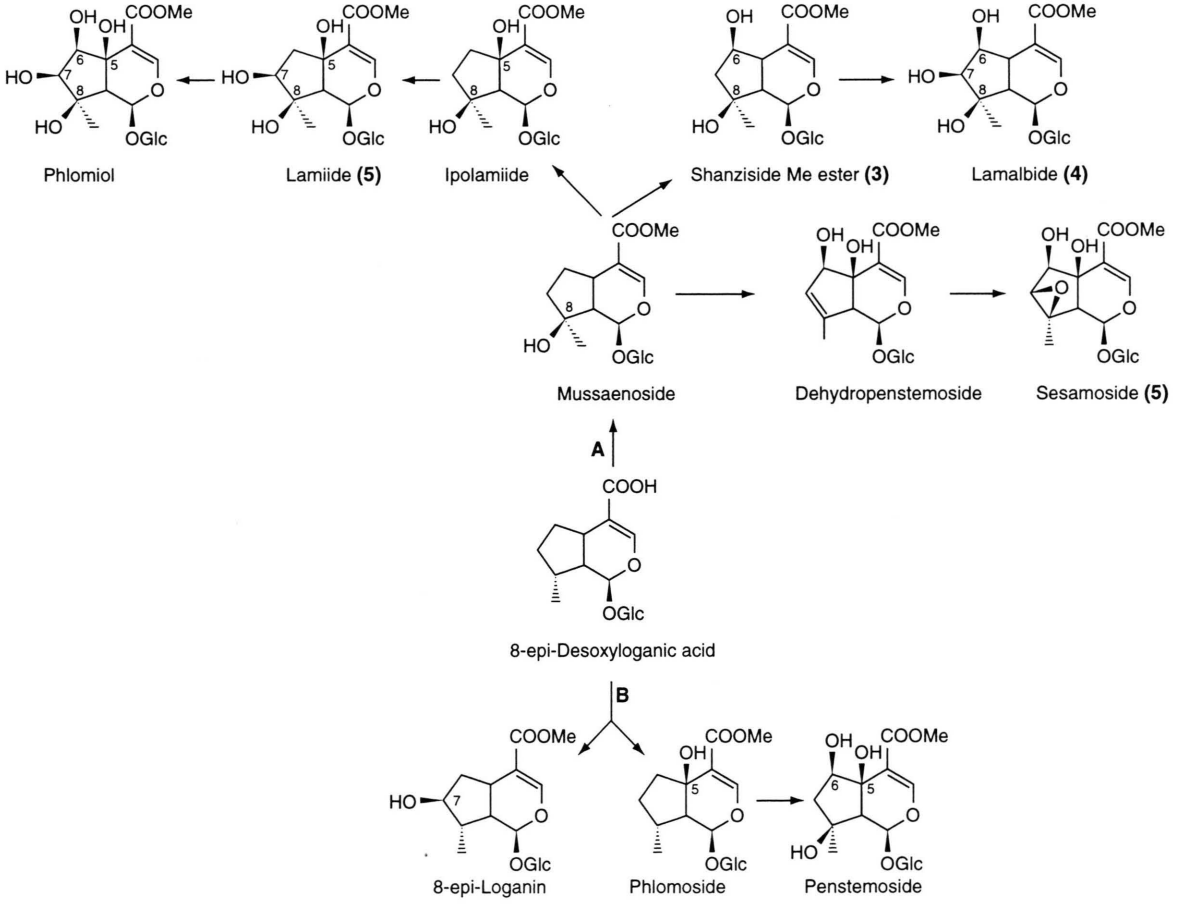

Fig. 1. Possible routes of some of the *Phlomis* iridoids.

The completely different iridoid composition of *P. tuberosa* (sect. Phlomoides; subsect. Anisostyleae) and *P. herba-ventis* (sect. Phlomis; subsect. Oxyphlomis), gives a possibility for their chemical distinction.

A probable biogenetic scheme of *Phlomis* iridoids is given on Fig. 1. Untill now, all 23 iridoids isolated from 15 *Phlomis* species, belong to the 8-*epi*-series. The formation of the 8-*epi*-series *via* 8-*epi*-desoxyloganic acid involves iridoids with (route A) and without 8-O-substitution (route B). The 8-O-substituted iridoids arise via mussaenoside and involve hydroxylation at positions 5, 6 and 7 and further hydroxylation and esterification or formation of iridoids with a 7,8- double bond and a 7,8-epoxy group. The modification in the oxydation level of the iridoids in the different *Phlomis* species seems to be a promising marker for chemotaxonomic purposes.

Acknowledgements

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