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PHLOMOSIDE B — AN IRIDOID GLYCOSIDE FROM *Phlomis regelii*

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A new iridoid — phlomoside B — has been isolated from the epigeal part of the plant Phlomis regelii (*M. Pop.*). *Its structure has been established on the basis of spectral investigations and chemical transformations.*

The isolation of an iridoid glycoside — phlomoside A — from the epigeal part of *Phlomis thapsoides* (fam. Lamiaceae) has been reported previously [1]. In an investigation of the epigeal part of another species of this genus — *P. regelii* — in addition to the known compounds ipolamiide (**1**), ipolamiidoside (**2**), phlomoside A (**4**), and lamiide (**5**), we have isolated a new iridoid — phlomoside B (**6**).

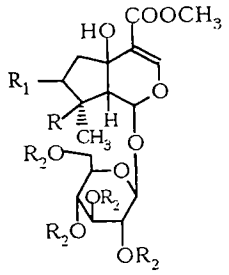
According to its PMR spectrum, iridoid (**6**) contained ester groupings. This was shown by the presence in its PMR spectrum of a six-proton singlet at 2.04 ppm from the methyls of two acetyl groups.

The alkaline hydrolysis of compound (**6**) formed lamiide [2]. The acid hydrolysis of (**6**) led to *D*-glucose and a black decomposition product of the aglycon moiety of the iridoid. On comparing the PMR spectra of (**5**) and of the iridoid (**6**), a considerable paramagnetic shift (0.30 ppm) of the signals of the CH_3-10 methyl group was observed in the latter. This fact permitted the assumption that in iridoid (**6**) one of the acetyl groups was present at the C-8 hydroxyl. A paramagnetic shift of the signals of the H-9 and H-1 protons also confirmed the addition of an acetyl group to the hydroxyl at C-8.

In addition, on the acetylation of OH-7 the signals of the H-7 proton underwent a downfield shift (see Table 1). This could have taken place under the influence of an acetyl group located on the same carbon atom.

Acetylation of the iridoid (**6**) led to a hexaacetate identical, according to its IR and PMR spectra and melting point, with hexa-O-acetyllamiide (**8**). In the PMR spectrum of the hexaacetate the chemical shifts of the signals of the proton at C-7 and of the CH_3-10 methyl group had undergone practically no changes. This additionally confirmed that iridoid (**6**) contained two acetyl groups.

The anomeric proton of iridoid (**6**) resonated at 4.61 ppm in the form of a doublet with J = 7.5 Hz (see Table 1), which showed the β-configuration of the glycosidic center. Consequently, phlomoside B is 7,8-di-O-acetyllamiide.



1. R=OH; R_1=R_2=H
2. R=OAc; R_1=R_2=H
3. R=OAc; R_1=H; R_2=Ac
4. R=R_2=H; R_1=OH
5. R=R_1=OH; R_2=H
6. R=R_1=OAc; R_2=H
7. R=OH; R_1=OAc; R_2=Ac
8. R=R_1=OAc; R_2=Ac

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TABLE 1. Chemical Shifts (ppm), Multiplicities, and SSCCs (J, Hz) of the Signals in the PMR Spectra of Lamiide (**5**) in CD_3OD, of its Acetyl Derivatives (**7**, **8**) in $CDCl_3$, and of Phlomoside B (**6**) in CD_3OD relative to TMS

Protons	Compound:			
	5	6	7	8
H-1	5.81, s	5.93, s	5.67, d J≅1 Hz	5.78, d J≅1 Hz
H-3	7.42, s	7.44, s	7.32, s	7.34, s
H-6	2.37, d.d J=15.5; 5 Hz	2.44, d J=3.5 Hz	2.40, d J≅3.5 Hz	2.44, d J≅3.8 Hz
H-6	2.22, d.d J=15.5; 4.5 Hz	2.44, d J≅3.5 Hz	2.40, d J≅3.5 Hz	2.44, d J≅3.8 Hz
H-7	3.52, d.d J≅5; 3.4 Hz	5.26 d.d ΣJ=6.5 Hz	4.70–5.30	4.70–5.20
H-9	2.78, br.s	3.04, br.s	2.89, br.s	3.18, br.s
CH_3-10	1.08, s	1.38, s	1.16, s	1.38, s
$COOCH_3$	3.72, s	3.73, s	3.77, s	3.77, s
H-1′	4.59, d J=7.9 Hz	4.61, d J=7.5 Hz	4.82, d J=7.5 Hz	4.82, d J=7.5 Hz
$OCOCH_3$	–	2.04, 2.04, s	1.95; 2.01; 2.04 2.12; 2.14	1.95; 2.01; 2.04 2.05; 2.06; 2.12

EXPERIMENTAL

For **general observations**, see [1, 3].

Fourier IR spectra were taken on a Perkin-Elmer System 2000 FT-IR spectrometer with KBr.

For column chromatography we used KSK silica gel and the following solvent systems: chloroform—methanol (50:1) (1) and (9:1) (2); and chloroform—methanol—water (5:1:0.1) (3) and (70:23:3) (4).

Isolation of the Main Iridoids. The epigeal part of *Phlomis regelii* (M. Pop.) was gathered in 1993 in the Chimkent province of Kazakhstan, in Alim-Tau (environs of Zhilga). The air-dry cominuted raw material (660 g) was extracted with methanol (5 × 5 liters). The resulting methanolic extract was evaporated to small volume and diluted with water, and the precipitate that deposited was separated off. The methanol was eliminated from the aqueous methanolic mother solution by evaporation, and the aqueous residue was treated successively with chloroform and butanol. After the butanol had been distilled off, 28.9 g of iridoids was obtained in the form of a light brown powder, part (24.7 g) of which was chromatographed on a column of silica gel. Elution was conducted first with system 2, giving 118 mg of a mixture of substances (**1**, **2**, and **6**), and then with system 4. This led to the isolation of 568.8 mg (0.1%) of iridoid (**1**) (here and below the yields are calculated on the air-dry raw material), $C_{17}H_{26}O_{11}$, amorphous, UV spectrum (EtOH, λ_{max}, nm): 228 (log ε 4.00); IR spectrum (KBr, ν, cm^{-1}): 3412 (OH), 1699 (C=O), 1633 (C=C), (M + Na^+) 429.

PMR (100 Mz, CD_3OD, δ, ppm, 0-TMS): 1.14 (3H, s, CH_3-10), 1.32-2.48 (4H, m, H-6 and 7), 2.46 (1H, s, H-9), 3.73 (3H, s, OCH_3), 4.58 (1H, d, H-1′; J = 7.5 Hz), 5.82 (1H, s, H-1), 7.43 (1H, s, H-3).

^{13}C NMR (100 MHz, CD_3OD, 0-TMS, δ, ppm): 94.19 (C-1, d), 152.60 (C-3, d), 115.18 (C-4, s), 71.63 (C-5, s), 38.84 (C-6, t), 40.34 (C-7, t), 78.88 (C-8, s), 61.52 (C-9, d), 23.23 (C-10, q), 168.06 (C-11, s), 51.69 (OCH_3, q), 99.57 (C-1′, d), 74.32 (C-2′, d), 77.32 (C-3′, d), 71.63 (C-4′, d), 78.36 (C-5′, d), 62.82 (C-6′, t).

From its spectral characteristics, iridoid (**1**) was identified as ipolamiide [2, 4].

On continuing the elution of the column with system 4, we isolated 1.3 g (0.23%) of phlomoside A (**4**), $C_{17}H_{26}O_{11}$, $[\alpha]_D$ −115.7±2° (*c* 0.62; methanol) [1]. Further washing of the column with the same system gave 8.2 g (1.45%) of iridoid (**5**), $C_{17}H_{26}O_{12}$, amorphous powder, $[\alpha]_D$ −81.4° (0.57; methanol), IR spectrum (KBr, ν, cm^{-1}): 3393 (OH), 1701 (C=O), 1635 (C=C); UV spectrum (EtOH, λ_{max}, nm): 229 (logε = 4.03); ^{13}C NMR (100 MHz, CD_3OD, 0-TMS, δ, ppm): 94.42 (C-1, d), 152.45 (C-3, d), 115.33 (C-4, s), 69.17 (C-5, s), 46.68 (C-6, t), 77.83 (C-7, d), 79.10 (C-8, s), 57.89 (C-9, d), 21.21 (C-10, q), 167.99 (C-11, s), 51.69 (OCH_3, q), 99.42 (C-1′, d), 74.32 (C-3′, d), 71.56 (C-4′, d), 78.28 (C-5′, d), 62.72 (C-6′, t).

2′,3′,4′,6′,7,8-Hexaacetate (8) and 2′,3′,4′,6′,7-Pentaacetate (7) of Iridoid (5). A solution of 500 mg of iridoid (**5**) in 10 ml of pyridine was acetylated with 10 ml of acetic anhydride at room temperature for 6 days. The reaction mixture was diluted with water, and the resulting precipitate (603 mg) was filtered off and chromatographed on a silica gel column.

Elution with system 1 yielded 220 mg of the hexaacetate (**8**), $C_{29}H_{38}O_{18}$, mp 206-208°C (methanol), IR spectrum (KBr, ν, cm^{-1}): 3540 (OH), 1632 (C=C), 1718 (C=O), 1751, 1232 (ester group).

Further elution of the column with the same solvent mixture led to 160 mg of the pentaacetate (**7**), $C_{27}H_{36}O_{17}$, 3506 (OH), 1638 (C=C), 1748 (broad band), 1229 (ester group).

The physicochemical constants and spectral characteristics of iridoid (**5**) and of its acetates (**8**) and (**7**) identified (**5**) as lamiide [2].

Isolation of Phlomoside B (6) and Ipolamiidoside (2). The mixture of iridoids obtained on the chromatography of the butanol fraction (118 mg) (see above) was rechromatographed on a column of silica gel. Elution with system 3 led to the successive isolation of 53 mg (0.009%) of phlomoside B (**6**) and 28 mg (0.005%) of ipolamiidoside (**2**), $C_{19}H_{28}O_{12}$, amorphous, PMR (100 MHz, CD_3OD, δ, 0 — TMS): 1.42 (3H, s, CH_3-10), 1.56-2.55 (4H, m, H-6 and 7), 2.71 (1H, s, H-9), 3.72 (3H, s, OCH_3), 4.56 (1H, d, J = 7.5 Hz, H-1'), 6.06 (1H, s, H-1), 7.54 (1H, s, H-3).

Acetylation of Ipolamiidoside (2). At room temperature, 20 mg of ipolamiidoside in 0.7 ml of pyridine was acetylated with 0.7 ml of acetic anhydride for 5 days. After the solvent had been distilled off, the residue was chromatographed on silica gel. Elution with system 1 gave 15 mg of ipolamiidoside pentaacetate (**3**), $C_{27}H_{36}O_{16}$, mp 142-144°C (methanol).

On the basis of its physicochemical constants and spectral characteristics and those of its acetyl derivative (**3**), iridoid (**2**) was identified as ipolamiidoside [4].

Phlomoside B (6), $C_{21}H_{30}O_{14}$, amorphous, IR spectrum (KBr, ν, cm^{-1}): 3398 (OH), 1634 (C=C), 1743 (broad band), 1260 (ester group).

Acid Hydrolysis of Phlomoside B (6). A solution of 10 mg of the glycoside in 5 ml of 5% sulfuric acid was heated at 100°C for 3 h, and the precipitate that had formed was separated off. The hydrolysate was neutralized with barium carbonate, concentrated in vacuum, and subjected to TLC in the butanol—pyridine—water (6:4:3) system, which revealed the presence of *D*-glucose.

Alkaline Hydrolysis of Phlomoside B (6). To 5 ml of a 0.5% aqueous methanolic solution of $KHCO_3$ was added 10 mg of iridoid (**6**), and the resulting reaction mixture was left at room temperature for 24 h. Then it was diluted with water and neutralized with acetic acid, and the reaction product was extracted with butanol.

After the solvent had been distilled off, chromatography in system 4 yielded 5 mg of iridoid (**5**). In its physicochemical constants, IR and PMR spectral characteristics, and TLC behavior, iridoid was identical with lamiide [2].

Acetylation of Phlomoside B (6). Phlomoside B (**6**) (20 mg) was acetylated with 0.7 ml of acetic anhydride in 0.7 ml of pyridine at room temperature for 3 days. After an appropriate work-up, the reaction mixture was chromatographed. Elution of the column with system 1 yielded 15 mg of the hexaacetate (**8**), $C_{29}H_{38}O_{18}$, mp 206-208°C (methanol). It was identified from its physicochemical characteristics as lamiide hexaacetate (**8**) [2].

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