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ISOLATION AND IDENTIFICATION OF 4′,6-DIMETHOXY-7-HYDROXY-ISOFLAVONE FROM ROOTS OF *Hedysarum theinum* CULTIVATED *in vitro*

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Experiments during the last decade have proved that roots of medicinal plants cultivated *in vitro* that retain the capability for biosynthesis of root-specific secondary metabolites can be used to preserve and propagate plant species that require special agricultural growth conditions [1]. Therefore, the compositions of low-molecular-weight compounds in roots of naturally occurring and cultivated *in vitro* plant species must be compared.

We have shown previously that genetic transformation of sterile shoots of *Hedysarum theinum* Krasnob. by the soil bacterium *Agrobacterium rhizogenes* makes it possible to produce stably growing root cultures [2]. A study of the chemical composition of *H. theinum* roots cultivated *in vitro* found that the main secondary metabolites contained isoflavones ononin, formononetin, malonylononin, and 7-glucosyltexasin [2]. In addition, the main HPLC peaks corresponded to the above compounds and another peak that could not be identified. Herein we describe the isolation of the compound corresponding to that peak and elucidate its structure.

Genetically transformed roots were obtained from seeds of *H. theinum* by the literature method [2]. Lyophilized roots (29.93 g) were grown *in vitro* and extracted three times with MeOH using ultrasound (40 min each at 45°C) to produce an extract from which column chromatography over silica gel (Merck 60-200 μm, $CHCl_3$:CH_3OH with a gradient from 0 % to 100:4) and separation of individual fractions by preparative reversed-phase HPLC [Diasorb C16, 7 × 250 mm column, isocratic, CH_3OH:TFA (0.1%) (60:40), flow rate 1 mL/min] isolated the texasin derivative (94% purity by HPLC, 4 mg) 4′,6-dimethoxy-7-hydroxyisoflavone (**1**). Fractions were analyzed by HPLC [Millichrom A-02, RP Prontosil-120-5-C18 AQ, 2 × 75 mm column, CH_3OH:TFA (0.1%)]. UV spectra of individual peaks were recorded in stopped-flow mode. A comparison of spectral ratios enabled the isolated peak for **1** to be identified as the starting peak in the extract.

The structure of **1** was elucidated by spectral analysis using PMR, ^{13}C NMR, and UV spectroscopy in addition to mass spectrometry. The resulting spectra coincided in general with those in the literature [3]. The locations of methoxy and hydroxy groups were established by comparing published ^{13}C NMR spectra for 7-hydroxy-6-methoxy- and 6-hydroxy-7-methoxy- isomers of 4′-methoxyisoflavone [3]. The observed difference in chemical shifts for C-5 and C-8 was 2.05 ppm, which was close to that for the 7-hydroxy-6-methoxy-isomer (literature Δδ 1.9 ppm) whereas the corresponding difference of chemical shifts for the 6-hydroxy-7-methoxy-isomer was 8.1 ppm.

Thus, it was shown that both cultivated *in vitro* and natural roots of *H. theinum* contain the isoflavone formononetin and several of its derivatives [2, 4, 5]. This indicates that the studied samples have common biosynthetic pathways for secondary metabolites. However, roots of *H. theinum* cultivated *in vitro* contained texasin derivatives [2] and did not contain oligomeric proanthocyanidines that are the principal components of natural plant roots in the generative stage [6]. This difference can be explained by the fact that cultivated roots, which do not have secondary thickening, are morphologically closer to roots of juvenile plants instead of roots of plants in the generative growth stage. This should be reflected also in differences in the chemical composition of their components. Apparently additional research is needed to obtain a more accurate answer to the question about the similarities and differences of biosynthetic pathways for forming species-specific compounds in roots of natural and cultivated *in vitro H. theinum*.

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4′,6-Dimethoxy-7-hydroxyisoflavone (1). UV spectrum (MeOH, λ_{max}, nm): 232, 256, 320. HPLC, spectral ratios to channel 220 nm: 1.02 (240 nm), 1.24 (260 nm), 0.60 (280 nm), 0.55 (320 nm), 0.01 (360 nm). Mass spectrum (*m*/*z*, *I*rel, %): 298 (100) $[M]^+$, 268 (19), 166 (14), 132 (20). Found $[M]^+$ 298.0833. $C_{17}H_{14}O_5$. Calc. $[M]^+$ 298.0841.

PMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 3.76 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 6.93 (1H, s, H-8), 697 (2H, d, J = 9, H-3′,5′), 7.42 (1H, s, H-5), 7.49 (2H, d, J = 9, H-2′,6′), 8.27 (1H, s, H-2). ^{13}C NMR spectrum (100 MHz, DMSO-d_6, δ, ppm): 55.64 (OCH_3), 56.38 (OCH_3), 103.35 (C-8), 105.40 (C-5), 114.12 (C-3′,5′), 116.75 (C-4a), 123.15 (C-1′), 125.05 (C-3), 130.50 (C-2′,6′), 147.51 (C-6), 152.30 (C-8a), 153.17 (C-2), 153.53 (C-7), 159.41 (C-4′), 174.72 (C-4).

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