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Isolation of three dibenzocyclooctadiene lignans from *in vitro* cultures of *Schisandra chinensis* (Turcz.) Baill. – the first report

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Three dibenzocyclooctadiene lignans: deoxyschizandrin (**1**), gomisin A (**2**) and schizandrin (**3**) were isolated from biomass extracts of *Schisandra chinensis* (Turcz.) Baill. shoot-differentiating callus cultures. The mentioned lignans were not isolated earlier from *in vitro* cultures of this plant species. This is the first report concerning on isolation of dibenzocyclooctadiene lignans from *in vitro* cultures of *Schisandra chinensis*.

1. Introduction

Schisandra chinensis (Turcz.) Baill. (Schisandraceae) is a valuable medicinal plant species that has long been used in traditional Far-Eastern medicine. Fruits of *S. chinensis* are still used in modern Far-Eastern medicine (Panossian and Wikman 2008; Szopa and Ekiert 2014a). This plant raw material has also gained a very important position in modern North American (United States Pharmacopoeia 1999) and European phytotherapy (European Pharmacopoeia 8th 2014). Fruits of this plant species are also monographed in the International Pharmacopoeia edited by WHO (WHO Monographs on Selected Medicinal Plants 2007). *Schisandra* fruits are a rich source of specific lignans, dibenzocyclooctadiene derivatives referred to, even in professional publications, as “*Schisandra* lignans”. These lignans are the main group of compounds that determine the therapeutic properties of the *S. chinensis* fruits. The amount of these compounds in the fruits ranges from 7.2 to 19.2% DW (dry weight).

In 1961, the first dibenzocyclooctadiene lignan, schizandrin, was isolated from the fruit extracts (Kochetkov 1961). Nowadays, according to data contained in the monograph of *Fructus Schisandrae* in the Pharmacopoeia of the WHO (2007), the number of isolated and identified lignans is about thirty. These compounds usually have several synonymous names. The main compounds were: schizandrin (synonyms: schisandrin, schisandrol A, wuweizichun A), gomisin A (synonyms: schisandrol B, wuweizichun B, wuweizi alcohol B), deoxyschizandrin (synonyms: deoxyschisandrin, schisandrin A, wuweizisu A) and γ -schizandrin (synonyms: schisandrin B, γ -schisandrin B, wuweizisu B) (WHO Monographs on Selected Medicinal Plants 2007). These compounds are also found in the leaves of this species, but in much smaller quantities (Xiao et al. 2007).

Schisandra Lignans are highly valued mostly for their adaptogenic, hepatoprotective, antioxidant and anticancer properties (Hancke et al. 1999; Panossian and Wikman 2008; WHO Monographs on Selected Medicinal Plants 2007).

In an earlier study in our laboratory, we analyzed the biomass from *S. chinensis in vitro* cultures with different degrees of differentiation, i.e. shoot-differentiating callus cultures and

undifferentiating callus cultures, for their ability to accumulate schizandrin (max. 70.54 mg/100 g DW) and gomisin A (max. 86.41 mg/100 g DW) (Szopa and Ekiert 2011), deoxyschizandrin (max. 308.51 mg/100 g DW) and γ -schizandrin (max. 22.09 mg/100 g DW) (Szopa and Ekiert 2013) and gomisin G (max. 21.89 mg/100 g DW) and schisantherin A (max. 33.45 mg/100 g DW) (Szopa and Ekiert 2014b). The results unambiguously indicate the greater production capacity of lignans in the shoot-differentiating callus cultures in comparison with undifferentiating callus cultures of *S. chinensis*.

The best ‘production medium’ favoring the accumulation of these metabolites in biomass of shoot-differentiating callus cultures of *S. chinensis* was MS (Murashige and Skoog 1962) medium containing 3 mg/l BA (N⁶-benzyladenine) and 1 mg/l NAA (α -naphthaleneacetic acid) (max. total content of analyzed lignans – 542.12 mg/100 g DW) (Szopa and Ekiert 2014a).

In the present study we isolated the main lignans from shoot-differentiating callus. This work focused on the isolation and purification of lignan fractions and confirmed the chemical structure of three compounds: deoxyschizandrin, schizandrin and gomisin A using ¹H NMR spectral analysis.

2. Investigations, results and discussion

Within the experiments we carried out the isolation, purification and identification of three dibenzocyclooctadiene lignans: deoxyschizandrin (**1**), gomisin A (**2**) and schizandrin (**3**) (Fig.) from shoot-differentiating callus of *Schisandra chinensis* tissue culture with high degree of differentiation. These compounds were isolated from biomass extracts of *S. chinensis in vitro* culture for the first time. These results confirmed that biotechnological solutions could be really applicable.

The high results obtained for the deoxyschizandrin content, especially in shoot-differentiating callus cultures, clearly show that the cells from our cultures of *S. chinensis* cultured under *in vitro* conditions biosynthesize mainly this compound (Szopa and Ekiert 2014a, b). This is in contrast to the parent plant organs where schizandrin and gomisin A quantitatively dom-

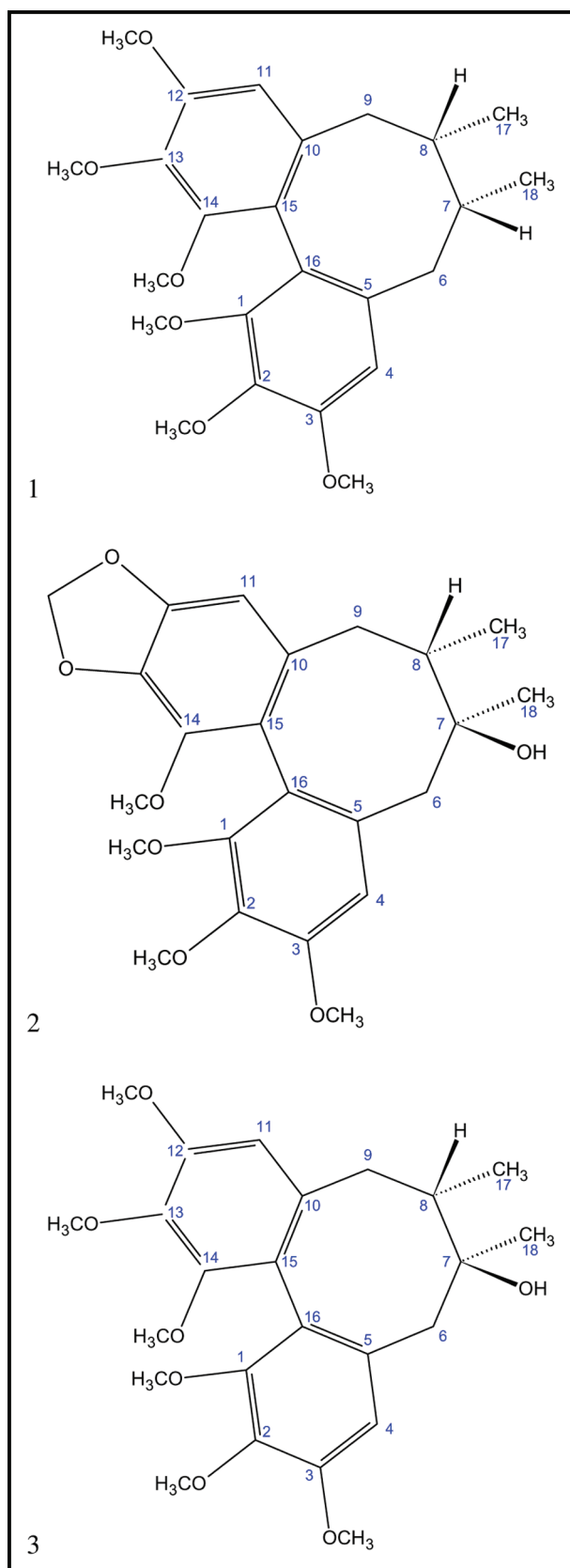


Fig. 1: Chemical structures of the isolated compounds: **1** (deoxyschizandrin), **2** (gomisin A), **3** (schizandrin).

inate (WHO Monographs on Selected Medicinal Plants 2007). Established *in vitro* culture may be a potential alternative source especially of deoxyschizandrin. This compound is outstanding amongst other schisandra lignans by a great biological activity,

e.g. hepatoprotective and anticancer properties (Panossian and Wikman 2008).

3. Experimental

3.1. Establishment of *in vitro* cultures

The *in vitro* cultures were established from leaf buds of *Schisandra chinensis* (Turcz.) Baill. (Schisandraceae) growing in Rogów Arboretum – Warsaw University of Life Sciences, Forest Experimental Station in Rogów (Poland). For details, see Szopa and Ekiert (2011).

3.2. Experimental *in vitro* cultures

Experimental shoot-differentiating callus cultures were maintained on Murashige-Skoog (MS) medium with plant growth regulators, BA (3 mg/l) and NAA (1 mg/l). Cultures were grown under constant artificial light (LF-40 W lamp, 120 $\mu\text{mol m}^{-2} \text{s}^{-1}$, daylight, Piła) at $25 \pm 2^\circ\text{C}$. Material for the isolation constituted biomass collected after 4-weeks of cultivation (three series).

3.3. Extraction and isolation

Isolation and purification were done according to the method described by Yukinobu et al. (1979). The lyophilized biomass from *in vitro* cultures (30 g) was powdered and extracted with boiling methanol under reflux (4×200 ml, 8 h each). The methanolic extract was concentrated to give a brown mass (4.30 g), which was dissolved in methanol and mixed with Celite (No. 535, Ciech-POCH, 3 g). The mixture was dried at room temperature and separated with column chromatography, developing with n-hexane (20 ml), dichloromethane (12 ml) and then methanol (20 ml). The n-hexane eluate was concentrated to give a residue (1.24 g), and was chromatographed on silica gel (Kieselgel 60, 0.2-0.5 mm, 35-70 mesh ASTM, Merck, 20 g), developing with solvent system as following: n-hexane:benzene (1:1, 12 ml, fraction 1), n-hexane:benzene (90:10, 10 ml, fraction 2), benzene (20 ml, fraction 3) and then benzene:acetone (98:2, 15 ml; 96:4, 15 ml; 94:6, 5 ml, fraction 4), benzene:acetone (98:2, 7.5 ml, fraction 5), benzene:acetone (90:10, 28 ml, fraction 6), benzene:acetone (88:12, 15 ml, fraction 7), benzene:acetone (85:15, 20 ml, fraction 7), benzene:acetone (80:20, 30 ml, fraction 9), benzene:acetone (10:90, 30 ml, fraction 10), acetone:methanol (70:30, 30 ml, fraction 11).

Fractions 6-9 were combined, concentrated and purified by TLC (thin layer chromatography) (TLC Silica gel 60 F₂₅₄ Merck), using n-hexane:ethyl acetate (7:3 v/v) and benzene:diethyl ether (7:3 v/v).

The dark prisms were eluted with methanol and all prisms with the same R_f were combined, and then they were subjected to TLC developing in following systems: acetone:n-hexane (3:7 v/v), n-hexane:ethyl acetate (7:3 v/v), benzene: diethyl ether (7:3 v/v) and diethyl ether:n-hexane (2:1 v/v). Separation was carried out until complete separation of the prisms. The darkest prisms from the plates were extracted with methanol, and then dried in an exsiccator (7 days), and subsequently identified as compound **1** (0.9 mg), compound **2** (0.8 mg) and compound **3** (1.0 mg).

Compounds were identified by TLC (Yukinobu et al. 1979) and DAD-HPLC (high-performance liquid chromatograph-diode array detector) (Zhang et al. 2009) method with comparison with standards and by comparison of its ¹H NMR spectroscopy data with those reported (Chu et al. 2013).

Deoxyschizandrin (**1**) (deoxyschisandrin, schisandrin A, wuweizisu A) (Fig.) – in TLC was obtained as colorless prisms. ¹H NMR (CDCl₃, 600 MHz): δ = 6.56 (1H, s, H-4), 2.29 (1H, dd, J = 13.2, 9.6 Hz, H-6 α), 2.07 (1H, br d, J = 13.1 Hz, H-6 β), 1.82 (1H, m, H-7), 1.92 (1H, m, H-8), 2.51 (1H, dd, J = 13.6, 1.8 Hz, H-9 α), 2.59 (1H, dd, J = 13.6, 7.5 Hz, H-9 β), 6.55 (1H, s, H-11), 0.75 (3H, d, J = 7.2 Hz, Me-17), 1.01 (3H, d, J = 6.9 Hz, Me-18), 3.91, 3.90, 3.90, 3.89, 3.60, 3.60 ppm (each 3H, s, 6 \times OMe). The isolated deoxyschizandrin was contaminated with β -sitosterol. The identity of the β -sitosterol was confirmed by TLC analysis by comparing the retention times of the standard substances (TLC, developing system: toluene: chloroform: methanol- 4:4:1 v/v/v, causative agent: 20% H₂SO₄ in methanol, 3 min., temp. 100 °C, R_f of β -sitosterol = 0.5 (Murthy and Mishra 2009).

Gomisin A (**2**) (schisandrol B, wuweizichun B, wuweizi alcohol B) (Fig.) – in TLC was obtained as colorless prisms. ¹H NMR (CDCl₃, 600 MHz): δ = 6.63 (1H, s, H-4), 2.69 (1H, d, J = 13.5 Hz, H-6 α), 2.37 (1H, br d, J = 13.4, H-6 β), 1.89 (1H, m, H-8), 2.36 (1H, br d, J = 14.2 Hz, H- α), 2.60 (1H, dd, J = 14.2, 1.5 Hz, H-9 β), 6.49 (1H, s, H-11), 0.83 (3H, d, J = 7.3 Hz, Me-17), 1.27 (3H, s, Me-18), 5.98, 5.97 (2H, each d, J = 1.5 Hz, -OCH₂O-), 3.92, 3.92, 3.85, 3.53 ppm (each 3H, s, 4 \times OMe). Trace contaminants.

Schizandrin (**3**) (schisandrin, schisandrol A, wuweizichun A) (Fig.) – in TLC was obtained as colorless prisms. ¹H NMR (CDCl₃, 600 MHz): δ = 6.62 (1H, s, H-4), 2.68 (1H, br d, J = 13.5 Hz, H- α), 2.38 (1H, br d, J = 13.5 Hz, H-6 β), 1.89 (1H, m, H-8), 2.39 (1H, br d, J = 14.3, H-9 α), 2.66 (1H, dd, J = 14.3,

1.7 Hz, H-9 β), 6.55 (1H, s, H-11), 0.83 (3H, d, J = 7.3 Hz, Me-17), 1.27 (3H, s, Me-18), 3.92, 3.90, 3.90, 3.89, 3.60, 3.57 ppm (each 3H, s, 6 \times OMe). No contamination.

The identity of schizandrin was also confirmed by testing specific optical rotation (polarimeter Pol AAr31), $[\alpha]^{23,2} \pm +88,1$ (23,2 °C, CHCl₃) - in accordance with the literature data ($[\alpha]^{22} \pm +88,7$ (22 °C, CHCl₃) (Yukinobu et al.1979)).

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