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Tetrahedron Letters 46 (2005) 2973–2977

Tetrahedron Letters

Biotechnological production of two new 8,4′-oxynorneolignans by elicitation of *Echinacea purpurea* cell cultures

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Received 13 January 2005; revised 28 February 2005; accepted 7 March 2005

Abstract—Two new 8,4′-oxynorneolignans were produced by elicitation of *Echinacea purpurea* cell suspension cultures with yeast elicitor. Their structures with an unusual (hydroxy)acetyl group were elucidated by spectroscopic methods including gas chromatography (GC) and electrospray ionization (ESI) mass spectrometry.

Echinacea purpurea is an old well known medicinal plant and is still very popular in North America and Europe. Herbal products containing extracts of this plant are used for the treatment of common cold and as immunostimulants. Extensive phytochemical and pharmacological studies have disclosed their main and bioactive constituents as caffeic acid derivatives, alkylamides, polyacetylenes and polysaccharides.[1]

Plant cell suspension cultures show great advantages for producing secondary and possibly bioactive natural products.[2] Previously *E. purpurea* cell cultures were used to produce immunologically active polysaccharides,[3] however, no further secondary metabolites were investigated. Elicitation of plants and their cell cultures is a method of studying the function and metabolism of phytoalexins.[4] Recently, it has become a popular technique to increase production rates of known compounds or to find new natural products for biotechnological application[5] and drug discovery.[6] In our laboratory *E. purpurea* cell cultures have been established[7] for the production of potential bioactive compounds using elicitation technique.

Cell suspension cultures of *E. purpurea* were grown in modified LS-medium[8] with thiamine, 2,4-diphenoxyacetic acid, 1-naphthylacetic acid and myo-inositol as organic growth factors. Transfer of 3 g cells into the new medium was carried out every 14 days. The suspension cultures were grown in the dark at 25 °C. The 200 mL flasks containing 40 mL medium were shaken at 120 rpm. A yeast elicitor containing polysaccharides prepared as described[9] (1.0 mg/mL) was added to the five-day-old cell suspension cultures in linear growth phase. Equivalent amounts of distilled water were added to the control cultures. In the nonelicited control medium of cell cultures only few phenolics were found by analytical reverse-phase high performance liquid chromatography (HPLC) monitored at 280 nm, however, in yeast elicitor-treated cell cultures a number of phenolics including compounds **1** and **2** were newly formed and accumulated in the medium to a maximum level at 36 h post-elicitation.

In order to identify them, 540 mL of yeast elicitor-treated medium from 30 flasks for cell cultures was collected and centrifuged at 3000*g* for 10 min. The supernatants were adjusted to pH 2 and extracted using ethyl acetate. The further isolation of **1** and **2** was performed by means of semi-preparative HPLC using a Waters chromatography attached to a photodiode array detector for recording UV spectra with a LiChrosorb RP-18 column (Merck, 10 × 250 mm, 7 μm) and a flow rate of 4 mL/min. The solvents were CH_3CN azeotrope containing 15% H_2O (A) and H_2O (B). For elution of the compounds a linear gradient of 5% A–40% A in 55 min was applied. Compound **1** (≈230 μg) and **2** (<100 μg) (Fig. 1) were obtained.

Keywords: *Echinacea purpurea*; Cell suspension cultures; Elicitation; Norneolignan.

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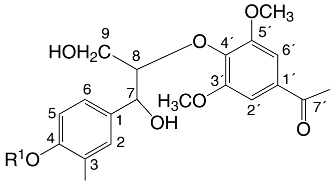
doi:10.1016/j.tetlet.2005.03.036



	R1	R2
1	H	OH
2	H	H
3	Glc	H

Figure 1. Structures of neolignans **1**–**3** from cell suspension cultures of *E. purpurea* (Glc: β-D-glucopyranosyl).

Compound **1** showed UV absorptions at 220 and 284 nm indicating the presence of a benzenoid moiety. The molecular weight of **1** was deduced as 408 from the positive and negative electrospray ionization (ESI) mass spectra. The positive high resolution (HR)-ESIMS (found 431.1324, calcd 431.1318, $C_{20}H_{24}O_9Na$) determined its molecular formula as $C_{20}H_{24}O_9$. The ^1H NMR (600 MHz, CD_3OD) spectrum of **1** showed the presence of 1,3,4-trisubstituted phenyl aromatic protons at δ 6.95 (1H, d, $J = 1.8$ Hz), 6.70 (1H, d, $J = 8.4$ Hz), 6.77 (1H, dd, $J = 8.4$, 1.8 Hz) and one pair of equivalent aromatic protons at 7.22 (2H, s); three methoxy groups at 3.80 (3H, s) and 3.86 (6H, s); a hydroxyacetyl group at 4.58 (2H, s); 1-phenyl-2-aryloxypropane-1,3-diol moiety at 4.86 (1H, overlapped), 4.46 (1H, m), 3.90 (1H, dd, $J = 12.0$, 5.4 Hz) and 3.67 (1H, dd, $J = 12.0$, 4.0 Hz). These structural moieties were further supported by the ^1H–^1H COSY spectrum with the following correlations: 6.95–6.77; 6.70–6.77; 4.86–4.46; 4.46–3.90 and 3.90–3.67. The ^1H NMR data for parts A and B of **1** were similar as those of 1-(4-hydroxy-3-methoxyphenyl)-2-{2-methoxy-4-[1-(*E*)-propen-3-ol]-phenoxy}-propane-1,3-diol[10,11] and 2,4′-dihydroxy-3′,5′-dimethoxyacetophenone (α-hydroxyacetosyringone),[12,13] respectively, which were also isolated as elicitor-induced products.

Trimethylsilyl (TMS) derivative of **1** was prepared with *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA), which converts all hydroxy groups as well as ketones and aldehydes into corresponding TMS ether and enol ether groups.[14,15] Figure 2A shows the gas chromatography (GC) electron impact (EI) mass spectrum and analysis of fragmentations of the TMS derivative of **1**. The molecular ion at *m*/*z* 768 [M+72(TMS)×5] indicated that it contained five TMS groups derived from a ketone and four hydroxy groups. The ion at *m*/*z* 103 ($CH_2{=}O^+TMS$) indicated a trimethylsilyloxymethyl group; the ion at *m*/*z* 209 indicated the presence of 3-methoxy-4-trimethylsilyloxyphenyl ring, and the ion at *m*/*z* 297 indicated the

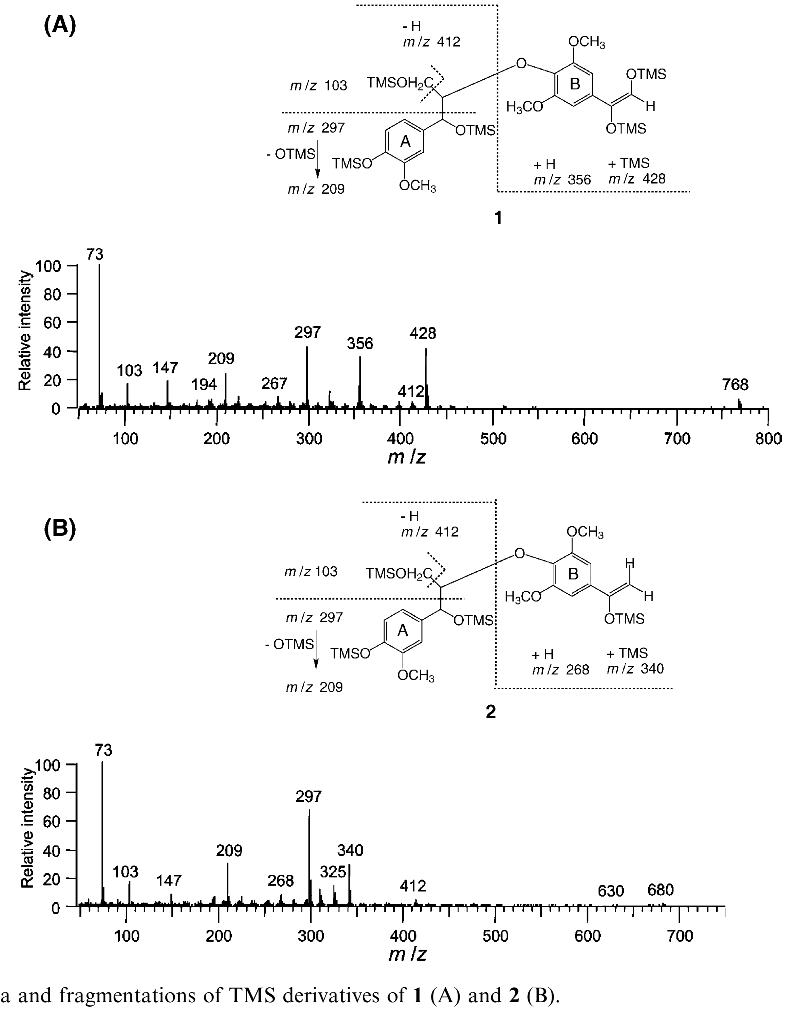


Figure 2. GC–EI mass spectra and fragmentations of TMS derivatives of **1** (A) and **2** (B).