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The Chromatographic Co-elution of Dihydrodehydrodiconiferyl Alcohol Monopyranose with Eleutheroside E in *Eleutherococcus senticosus*: Implications for Eleutheroside E Assays

B. T. Gaffney,[1] H. M. Hügel[1] and P. A. Rich[2]

[1]Department of Applied Chemistry, Faculty of Applied Science, RMIT University, GPO Box 2476V, Melbourne, Victoria 3001, Australia
[2]Division of Biosciences, School of Medical Sciences, Faculty of Life Sciences, RMIT University Bundoora campus, Bundoora, Victoria 3083, Australia

During a quantification assay of the constituents of *Eleutherococcus senticosus* by reverse-phase HPLC using acetonitrile:water gradient elution, it was observed that a recently reported component, dihydrodehydrodiconiferyl alcohol monopyranose, co-eluted with eleutheroside E. The implications of this finding for researchers and the herbal medicine industry are discussed. Copyright © 2004 John Wiley & Sons, Ltd.

Keywords: HPLC; eleutheroside E; dihydrodehydrodiconiferyl alcohol monopyranose; *Eleutherococcus senticosus*.

INTRODUCTION

The root extract of *Eleutherococcus senticosus* Maxim. (Araliaceae) (*syn*: Siberian Ginseng, *Acanthopanax ginseng*) has been observed in animal trials to extend time until exhaustion in forced exercise and to attenuate organ damage induced by various stressors, and has hence been classified as an 'adaptogen' (Brekhman and Dardymov, 1969). In humans it is used to counter fatigue during severe stress and to increase 'vitality' in diseases where a lack of vitality is considered a predisposing factor (Fulder, 1980; Wagner *et al*., 1994). As part of a clinical trial designed to assess the effects of *E. senticosus* on selected indices of stress (lymphocyte subset numbers and testosterone to cortisol ratio; Gaffney *et al*., 2001), an attempt was made to determine the concentration of its most potent active ingredient, eleutheroside E, using quantitative HPLC. During the process, a compound isolated for use as an external standard under the assumption that it was eleutheroside E was found instead to be a pyranoside derivative of dihydrodehydrodiconiferyl alcohol (see Fig. 1), a compound isolated from *E. senticosus* only once previously (Makarieva *et al*., 1997). Furthermore, it was demonstrated that this compound co-eluted with an authentic eleutheroside E (Fig. 2) sample on HPLC, a finding reported here for the first time.

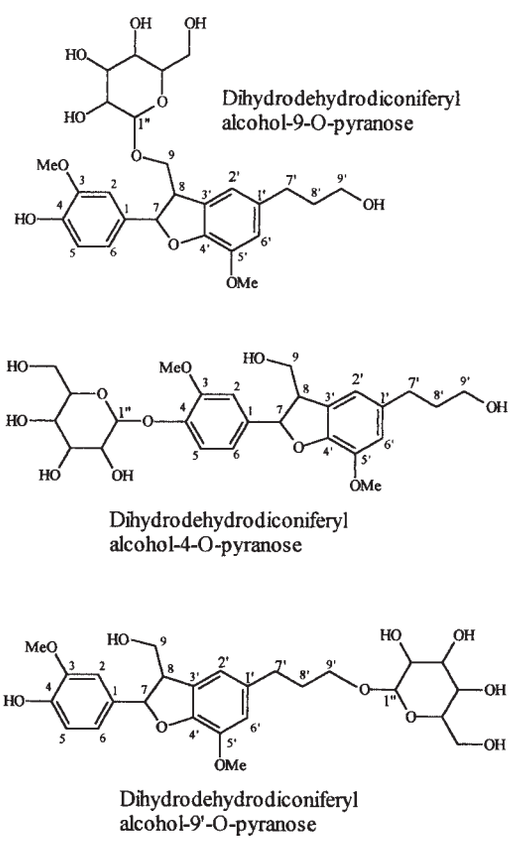

Figure 1. The three possible structural isomers of dihydrodehydrodiconiferyl alcohol monopyranose.

EXPERIMENTAL

Plant material, solvent extraction, and preparative HPLC. The preparation of *E. senticosus* was provided by Mediherb (Warwick, Australia) and was a (1:2) 35% ethanolic extract containing 12.6 mg/mL of dry material. The aim of the isolation procedure was to isolate eleutheroside B and E, presumed to correspond to peaks 2 and 3 (Fig. 3) on analytical HPLC on the basis of published chromatograms (Wagner *et al*., 1982; Slacanin

* Correspondence to: B. Gaffney, Care of Helmut Hügel, Department of Analytical Chemistry, Faculty of Applied Science, RMIT University, GPO Box 2476V, Melbourne, Victoria 3001, Australia.
Email: Bengaffney68@hotmail.com

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(+/-)-Syringaresinol-4',4'-O-bis-β-D-glucose
(eleutheroside E)

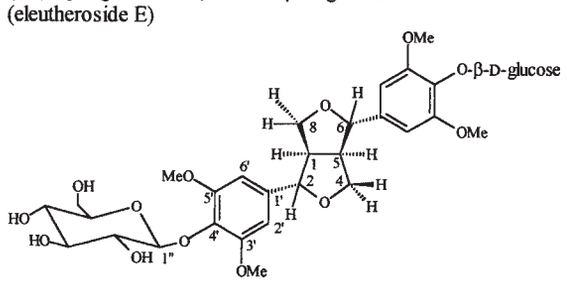

Figure 2. The structure of eleutheroside E.

et al., 1991). After performing solvent extraction and flash chromatographic procedures based on those used by Wagner *et al.* (1982), the butanolic phase was subjected to preparative HPLC using three preparative Nova-Pak® (Waters, Milford, MA, USA) reverse-phase C_{18} columns (100 × 40 mm i.d.; 125 Å; 15–20 μm) connected in series, and an acetonitrile:water mobile phase maintained at 10% acetonitrile for 10 min after sample injection and then rising to 27% acetonitrile over the next 30 min. Detection was carried out with a spectrophotometer set at 220 nm and fractions corresponding to peaks 2 and 3 (Fig. 3) were collected manually upon exit from the detector and evaporated to dryness using rotary evaporation at 36°C.

Semi-preparative HPLC. The incompletely resolved fraction corresponding to peak 3 (Fig. 3) was evaporated to dryness and dissolved in 1% methanol solution for separation by semi-preparative HPLC. An aliquot (50 μL) of the sample was injected onto an Alltech (Deerfield, IL, USA) Allsphere ODS-2 C_{18} column (250 × 7.8 mm i.d.; 5 μm) by an ICI Instruments (Rydalmere, Australia) model DP800 auto sampler in conjunction with an ICI Instruments model DP800 integrated dual pump and reservoir system. The mobile phase of acetonitrile:water was initially passed through the column at 18% acetonitrile and then increased upon sample injection to 24% acetonitrile over 16 min. Detection was carried out with a spectrophotometer set at 220 nm and fractions corresponding to the largest peak (peak 3; Fig. 3) were collected manually upon exit from the detector and evaporated to dryness.

Analytical HPLC. Analyses were performed using an Alltech Allsphere ODS-2 C_{18} column (150 × 4.0 mm i.d.; 5 μm) with the same pump, reservoir, autosampler and detection system as employed for semi-preparative HPLC. The mobile phase of acetonitrile:water, pumped at a rate of 1 mL/min, was 10% acetonitrile until sample injection and then increased to 34% acetonitrile over 16 min. A sample (8.8 mL) of an ethanolic extract of *E. senticosus* was evaporated to dryness, the residue (1.1104 ***g***) dissolved in 60 mL of 67% methanol (AR grade; Merck, Darmstadt, Germany) and shaken with 3 × 30 mL of diethyl ether (AR grade; Merck). The methanol phase was filtered through a 0.45 μm HPLC filtration disk and evaporated to dryness yielding 0.9876 ***g*** of residue. In order to remove any remaining unwanted constituents which might adversely affect the HPLC column, the residue was dissolved in 2 mL of 67% methanol solution and flash chromatographed in a glass column (60 × 3.5 cm i.d.) containing 70 ***g*** of silica gel (230–400 mesh; 60 Å particle size; Merck) with 3 × 1 L of a solution of chloroform:methanol:water (75:25:4) at 1.5 atm pressure. After flashing, the eluent was evaporated to dryness yielding 0.5660 ***g*** of residue which was dissolved in a 1% methanol solution and made up to 250 mL. An aliquot (8 mL) of this solution was made up to 10 mL after mixing with a 1 mL of a solution of internal standard (3,5-dihydroxybenzyl alcohol) and 0.2 mL aliquots of external standard solution (eleutheroside E) in a range of concentrations. The internal and external standards were included in order to construct a plot of external standard concentration with the ratio of external to internal standard peak areas as part of a procedure for determining eleutheroside E concentration. The discovery that peak 3 (Fig. 3) in the *E. senticosus* extract was not eleutheroside E and that no eleutheroside E was present in the sample made this aspect of the work redundant. In the chromatogram depicted in Fig. 3, the concentrations of injected constituents were: *E. senticosus* (1.811 mg/mL), 3,5-dihydroxybenzyl alcohol (0.00491 mg/mL), and eleutheroside E (0 mg/mL). In Fig. 4, which had an external source of eleutheroside E added, the concentration of injected constituents were: *E. senticosus* (1.811 mg/mL), 3,5-dihydroxybenzyl alcohol (0.00491 mg/mL), and standard eleutheroside E (0.0052 mg/mL). HPLC sample injection volume was set at 5 μL.

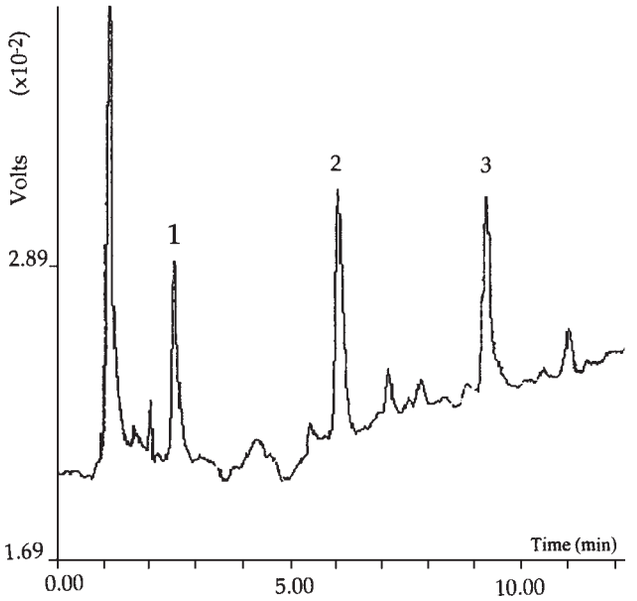

Figure 3. Analytical HPLC chromatogram of a root extract of *Eleutherococcus senticosus* (1.811 mg/mL). Key to peak identity: **1**, internal standard (3,5-dihydroxybenzyl alcohol) (0.00491 mg/mL); **2**, eleutheroside B; **3**, dihydrodehydrodiconiferyl alcohol monopyranose. (For chromatographic protocol see Experimental section.)

Spectral analyses. Electrospray (ES)/MS were obtained on a Micromass Platform II instrument with an electrospray ion source (Manchester, UK) using acetonitrile:water (50:50) as the mobile phase. ^{1}H-NMR spectra of peaks 2 and 3 (Fig. 3) were obtained in deuterated-methanol solutions with TMS as internal reference using a Bruker AC-F200 spectrometer (Karlsruhe, Germany) recording at 200.132 MHz. ^{1}H-NMR spectra for eleutheroside E (Addipharma, Hamburg, Germany) were obtained in dimethylsulphoxide

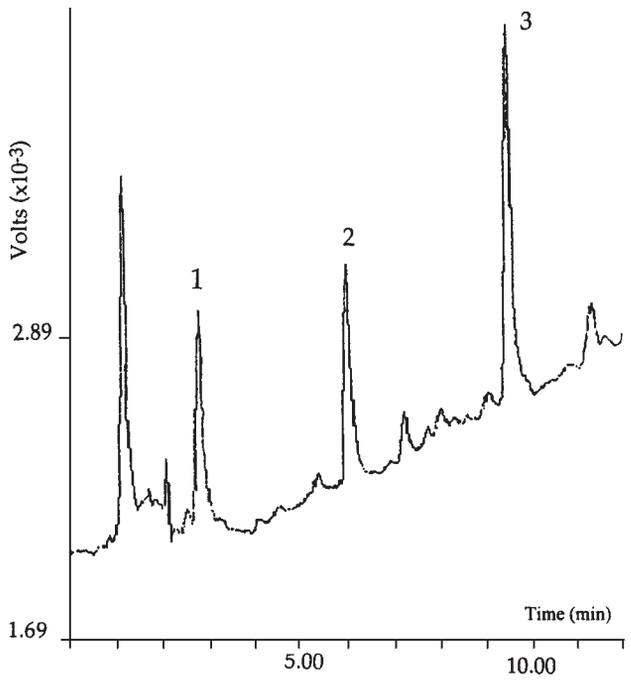

Figure 4. Analytical HPLC chromatogram of a root extract of *Eleutherococcus senticosus* (1.811 mg/mL) mixed with standard eleutheroside E (0.0052 mg/mL; Addipharma). Peaks **1** and **2** are as identified in the legend to Fig. 3: the peak of added eleutheroside E co-eluted precisely with that of dihydrodehydrodiconiferyl alcohol monopyranose as demonstrated by the enlarged area of peak **3** compared with the peak shown at the same retention time in Fig. 3.

(DMSO) with TMS as an internal standard using a Varian Gemini 200 spectrometer (Palo Alto, CA, USA) recording at 199.959 MHz.

RESULTS AND DISCUSSION

Identification of compounds in the preparation of *E. senticosus*

***Eleutheroside B* (peak 2; Fig. 3; 14.3 mg).** ^{1}H-NMR (CD_3OD) δ (ppm); 3.76 (6H, s, 3, 5-OMe), 4.13 (2H, d, J = 5.25 Hz, H-9), 6.20 (1H, dd, J = 10.4, 5.4 Hz, H-8), 6.46 (1H, d, J = 15.5 Hz, H-7), 6.86 (2H, s, H-6, H-2), 3.11–3.71 (7H, m, sugar-H,-OH), 4.85–5.00 (1H, m, 1′-sugar).

***Dihydrodehydrodiconiferyl alcohol monopyranose* (peak 3; Fig. 3; 3.0 mg).** ES/MS (m/z): 521 $[M—H]^-$: ^{1}H-NMR (CD_3OD) δ (ppm); 6.72 (1H, d, J = 2.9 Hz, H-2′) or 6.95 (s, H-2′), 6.72 (1H, d, J = 2.9 Hz, H-6′) or 6.95 (s, H-6′), 2.62 (2H, t, H-7′), 1.80 (2H, t, H-8′), 3.56 (2H, t, H-9′), 3.86 or 3.83 (3H, s, 5′-OMe), 7.03 (1H, d, J = 1.7 Hz, H-2), 7.14 (1H, d, J = 8.2 Hz, H-5), 6.93 (1H, dd, J = 9.4, 1.9 Hz, H-6), 5.55 (1H, d, J = 6.0 Hz, H-7), 3.75 (1H, m, H-8), 3.96 (2H, t, H-9), 3.86 or 3.83 (3H, s, 3-OMe), 4.20 (1H, d, 1″-sugar), 3.29–3.88 (7H, m, sugar-H,-OH).

***Eleutheroside E* (Addipharma).** ES/MS (m/z): 741 $[M—H]^-$: ^{1}H-NMR (DMSO) δ (ppm); 3.10 (2H, m, H-1,5), 3.76 (12H, s, OMe), 4.31 (2H, t, J = 5.56 Hz, H-4,8 ax) or 4.20 (2H, m, H-4,8ax), 4.31 (2H, t, J = 5.56 Hz, H-4,8 eq) or 4.20 (2H, m, H-4,8eq), 4.66 (2H, d, J = 3.1 Hz, H-2,6), 6.66 (4H, s, H-2′, 6′), 4.98 (2H, d, J = 3.7 Hz, 1″-sugar), 3.17–3.64 (14H, m, sugar-H, -OH), 4.89 (4H, m, sugar-OH).

The purity of fractions corresponding to peaks 2 and 3 (Fig. 3) that emerged from preparative HPLC was checked using analytical HPLC. The fraction corresponding to peak 2 (Fig. 3) produced one single peak by analytical HPLC, and the ^{1}H-NMR spectrum matched that published for eleutheroside B (Wagner *et al*., 1982). The fraction corresponding to peak 3 (Fig. 3), however, produced several small peaks in addition to one large peak by analytical HPLC, suggesting that the resolving capability of the preparative HPLC procedure was insufficient. The fraction was then subjected to the higher resolving capability afforded by semi-preparative HPLC and the subsequently collected peak 3 fraction did yield a single peak by analytical HPLC. ES/MS analysis of the peak 3 fraction collected from semi-preparative HPLC analysis (henceforth referred to as 'peak 3') confirmed the presence of a single compound but with a mass of 522 amu rather than 742 amu as would be required for eleutheroside E. The ^{1}H-NMR spectrum of the compound associated with peak 3 matched published spectra for mono pyranose derivatives of dihydrodehydrodiconiferyl alcohol (henceforth referred to as dihydrodehydrodiconiferyl alcohol monopyranose; (Fukuyama *et al*., 1996; Matsuda *et al*., 1996; Changzeng and Zhongjian, 1997). The nature of the pyranose moiety and its position (see Fig. 1) could not be ascertained from the ^{1}H-NMR data, and ^{13}C-NMR spectroscopy was not possible owing to the small quantity (3.0 mg) of sample isolated. While a compound of this type has been reported in other plant species (Fukuyama *et al*., 1996; Matsuda *et al*., 1996; Changzeng and Zhongjian, 1997), this appears to be only the second report of its presence in *E. senticosus*, the first being by Makariyeva *et al.* (1997).

Recently, eleutheroside E has become commercially available and the ES/MS and ^{1}H-NMR spectra of a standard purchased from Addipharma conformed with those published for eleutheroside E (Vermes *et al*., 1991). When standard eleutheroside E was added to a sample of *E. senticosus*, its HPLC peak overlapped perfectly with peak 3, identified above as dihydrodehydrodiconiferyl alcohol monopyranose (cf. Figs. 3 and 4). The implications of this finding are two-fold. First, no eleutheroside E could have been present in the *E. senticosus* sample being tested otherwise its presence (along with dihydrodehydrodiconiferyl alcohol monopyranose) would have been detected in peak 3. This appears to be the second report of a sample of *E. senticosus* which did not contain eleutheroside E, the first being by Slacanin *et al*. (1991), who observed that one of the 12 samples of *E. senticosus* tested did not contain the compound. Furthermore, to our knowledge, this is the first report of the chromatographic co-elution of eleutheroside E and dihydrodehydrodiconiferyl alcohol monopyranose. With regard to the possible biological activity of this compound, the only published assay determined its anti-oxidant (rather than its adaptogenic) activity with an *in vitro* assay of lipid peroxidation in rat brain homogenates: no anti-oxidant activity was observed (Fukuyama *et al*., 1996).

A limitation of the current study was that isolated dihydrodehydrodiconiferyl alcohol monopyranose was

not mixed with isolated eleutheroside E and analysed by HPLC for retention time comparison. Hence, the possibility could not be ruled out that in the *E. senticosus* extract the compound associated with peak 3 was originally eleutheroside E but was later completely converted into dihydrodehydrodiconiferyl alcohol monopyranose, perhaps by thermally driven rearrangement, prior to characterisation. Such a conversion would explain the observed HPLC co-elution of peak 3 with authentic eleutheroside E. However, the possibility of such a conversion proceeding without a trace of starting material seems unlikely especially given the relatively low rotary evaporation temperature, 36°C, and the lack of any report of such a conversion of eleutheroside E in the literature (Wagner *et al*., 1982; Slacanin *et al*., 1991).

The HPLC procedure employed in the present study is the most commonly reported chromatographic procedure used to determine the concentration of eleutheroside E (Slacanin *et al*., 1991). Therefore, the finding that dihydrodehydrodiconiferyl alcohol monopyranose, a compound with no documented adaptogenic activity, co-elutes with eleutheroside E in such a procedure has implications for eleutheroside E assays conducted by researchers and the herbal medicine industry alike. Specifically, if no post column analysis is undertaken on a sample of *E. senticosus* containing dihydrodehydrodiconiferyl alcohol monopyranose, then an invalid eletheroside E assay may result. Therefore, it is recommended that compounds eluting from HPLC be suitably analysed, e.g. with ES/MS or photodiode array analysis, and not assumed to be identical to a reference compound on the basis of identical chromatographic retention time alone.

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REFERENCES

Brekhman II, Dardymov IV. 1969. New substances of plant origin which increase non-specific resistance. *A Rev Pharmac* **9**: 419–430.

Changzeng W, Zhongjian J. 1997. Lignan, phenylpropanoid and iridoid glycosides from *Pedicularis torta*. *Phytochemistry* **45**: 159–166.

Fukuyama Y, Nakahara M, Minami H, Kodama M. 1996. Two new benzofuran-type lignans from the wood of *Viburnum awabuki*. *Chem Pharm Bull* **44**: 1418–1420.

Fulder SJ. 1980. *The Root of Being: Ginseng and the Pharmacology of Harmony*. Hutchinson: London.

Gaffney BT, Hügel HM, Rich PA. 2001. The effects of *Eleutherococcus senticosus* and *Panax ginseng* on steroidal hormone indices of stress and lymphocyte subset numbers in endurance athletes. *Life Sci* **70**: 431–442.

Makarieva TN, Dmitrenok AS, Stonik VA, Patel AV, Canfield LM. 1997. Lignans from *Eleutherococcus senticosus* (Siberian Ginseng). *Pharm Sci* **3**: 525–527.

Matsuda N, Sato H, Yaoita Y, Kikuchi M. 1996. Isolation and absolute structures of the neolignan glycosides with the enantiometric aglycones from the leaves of *Viburnum awabuki* K. Koch. *Chem Pharm Bull* **44**: 1122–1123.

Slacanin I, Marston A, Hostettmann K. 1991. The isolation of *Eleutherococcus senticosus* constituents by centrifugal partition chromatography and their quantitative determination by high performance liquid chromatography. *Phytochem Anal* **2**: 137–142.

Vermes B, Seligmann O, Wagner H. 1991. Synthesis of biologically active tetrahydro-furofuranlignan-(syringin, pinoresional) mono- and bis-glucosides. *Phytochemistry* **30**: 3087–3089.

Wagner H, Heur YH, Obermeier A, Tittel G, Bladt S. 1982. DC and HPLC analysis of *Eleutherococcus*. *Planta Med* 44: 193–198.

Wagner H, Nörr H, Winterhoff H. 1994. Plant adaptogens. *Phytomedicine* **1**: 63–76.

