The use of countercurrent chromatography (CCC) in the isolation of bioactive neolignans from *Nectandra leucanta* Ness & Mart (Lauraceae).

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Abstract

The acetonitrile phase of n-hexane extract from leaves of *Nectandra leucantha* was fractionated through spiral coil countercurrent chromatography (CCC), with off-line injection of fractions to ESI-MS/MS in sequence of recovery. Five bioactive neolignans (**1** - **5**), where identified where two of them were obtained on larger lab-scale (**2** and **3**).

Introduction

The CCC as all-liquid method with biphasic solvent systems has several advantages in comparison to traditional chromatographic approaches such as the fractionation of chemical compounds of any polarity range and the easy increase of sample injection amounts Loss of bioactive compounds due to irreversible adsorption is neglectable. Among the various application areas of CCC, the main and growing interest is the area of natural product isolation[1]. Studies from our research group with *Nectandra leucantha* (Lauraceae) reported the presence of neolignans with significant anti-parasitic and anti-tumoral properties[2]. Aiming the isolation of larger amounts, we describe the isolation of bioactive neolignans on extended lab-scale by CCC monitored by direct injection analysis to electrospray mass spectrometry.

Results and Discussion

Dried leaves of *N. leucantha* leaves (2.45 kg) were exhaustively extracted with n-hexane (amb. temp.). After solvent evaporation under reduced pressure, 55 g crude extract were obtained. This extract was resuspended in hexane and partitioned with ACN to afford 34 g of the ACN phase. After solvent system evaluation for CCC, the most suitable biphasic mixture for fractionation was determined as n-hexane:EtOAc:MeOH:H_2O (7/3/7/3; v/v/v/v). Thus, part of ACN phase (16 g) was subjected to fractionation using a spiral coil CCC prototype (5.5 L tube column), and target ions were observed by off-line ESI-MS/MS sequential injections of recovered fractions [3]. The upper phase was used as stationary with 75% of retention (“head-to-tail” mode; rotation speed: 270 rpm; flow: 15.0 mL/min, detection: λ 210 nm). This procedure afforded 194 fraction during *elution* and 136 in *extrusion* pooled together in 16 groups (A-P). Using the ESI-MS/MS injection profile, it was possible to identify signals at *m/z* 365 and 379, corresponding to pseudo-molecular $[M+Na]^+$ ion signals of compound **4** and **5**, respectively, while signals at *m/z* 327 and 341, correspond to pseudo-molecular $[M+H]^+$ ion peaks to compounds **1** + **2** and **3**, respectively. After fractionation, compounds **1 - 5** were subjected to NMR spectroscopy and after comparison with data described in literature[2] was possible to identify dehydrodieugenol (**1 –** mixture 727 mg) and dehydrodieugenol B (**2**, pure 3.5 g), methyl-dehydrodieugenol B (**3**, pure 2.5 g), 7-hydroxy-dehydrodieugenol (**4,** mixture 378 mg) and 7-hydroxy-methyl-dehydrodieugenol (**5,** mixture 1.0 g).

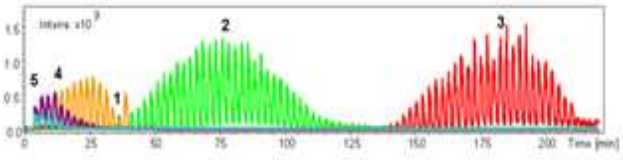

Figure 1. ESI-MS/MS injection profile from spiral coil CCC fractionation

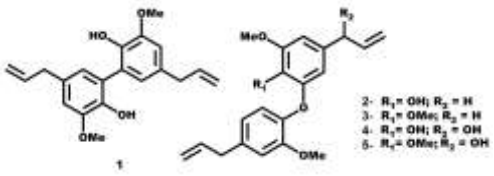

Figure 2. Structural formula of identified compounds (**1**-**5**).

Conclusion

Spiral coil CCC using an appropriate solvent system is advantageous to fractionate natural products compared to classical solid phase chromatography, in view of reduced loss, isolation and identification of ‘target’ compounds in a single processing step.

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