High Performance Liquid Chromatographic Analysis of Steroidal Saponins from *Avena sativa* L.

J. Kesselmeier and D. Strack

Botanisches Institut der Universität zu Köln, Gyrhofstr. 15, D-5000 Köln 41

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High Performance Liquid Chromatography (HPLC), Steroidal Saponins, *Avena sativa,* Etioplasts

Reversed phase high performance liquid chromatography offers an efficient and rapid method for analysis of steroidal saponins. Crude extracts from primary leaves of *Avena sativa* and isolated etioplasts therefrom have been resolved into four saponins (avenacosides) using a water-acetonitrile gradient system on RP-8 and monitoring the column effluent at 200 nm with an UV-detector. Detectability was found to be in the range of 50 ng avenacoside B and the detector response was linear up to 8 μg tested. The described method is applicable to studies on localization and physiology of *Avena* saponins during development of the primary leaf.

Introduction

Steroidal saponins seem to play an important role in building up prolamellar bodies in etioplasts of *Avena sativa* primary leaves [1, 2]. For qualitative and especially quantitative studies on these saponins it is of great interest to develop a rapid and sensitive method. Earlier studies, using densitometric or colorimetric determinations after thin layer chromatography [2, 3] were time-consuming and elaborate.

Since HPLC has turned out to be an essential tool in almost all fields of studies on natural plant products, especially for those with high molecular weights or exhibiting temperature lability, we assumed that this technique could also be applied for analyses of steroidal saponins from *Avena sativa.*

The major problem in using HPLC is the limit of photometrical detection of those compounds, which do not exhibit a specific and strong absorption of light in the visible region or at least in the UV-region of longer wavelenghts, as pigments and simple phenolics do, respectively. In these cases detection can be achieved using wavelenghts around 200 nm, at which the compounds in question, however, cannot be selectively detected out of a highly complex composition of a crude natural extract. In addition the solvent systems applicable are very limited. To overcome these problems, an increase in selectivity and sensitivity of photometrical detection can be achieved by chemical derivatization [4–7], leading to chromophoric moieties.

However, this in not necessary in all cases, which was shown *e.g.* for HPLC of essential oils [8] or sesquiterpene lactones [9]. In these studies it was demonstrated that the combination of reversed phase column packings with mixtures of water and acetonitrile as solvent systems can solve the chromatographic problem. This method turned out to be also applicable to analyses of steroidal saponins from *Avena sativa,* which will be described in the present communication.

Results and Discussion

Alcoholic extracts from etiolated and green primary leaves of *Avena sativa* (oat) contain two groups of steroidal saponins: avenacosides A and B, nearly insoluble in water, and two others, tentatively named avenacosides C and D [10] which are closely related to A and B, but much more water soluble. Avenacosides A and B are present in high amounts in highly purified prolamellar bodies [1, 2]. The localization of C and D is under investigation.

First attempts to analyze these saponins by HPLC were made with benzoyl derivatives according to Besso *et al.* [6]. The results were somewhat disappointing, because we were unable to detect the constituents C and D, probably due to their decomposition or structural alteration during the derivatization procedure. We therefore tried to analyze the native saponins by HPLC, using essentially the same methods as described in ref. [8] and [9].

A water-acetonitrile gradient elution system on octyl-silica stationary phase (RP-8) with the column effluent monitored at 200 nm turned out to be the method of choice. To achieve chromatograms free of interfering contaminants (Fig. 1) it is important, to use the following extraction procedure. Primary leaf pieces were boiled three times in hot methanol. The cooled extracts were concentrated by a rotary evaporator, diluted with water to give 80% methanol (v/v), and centrifuged at 3000 × *g* for 10 min. 20 μl of the clear supernatant were directly injected onto the chromatographic column. Details of HPLC are described in Fig. 1.

Reprint requests to Dr. J. Kesselmeier.

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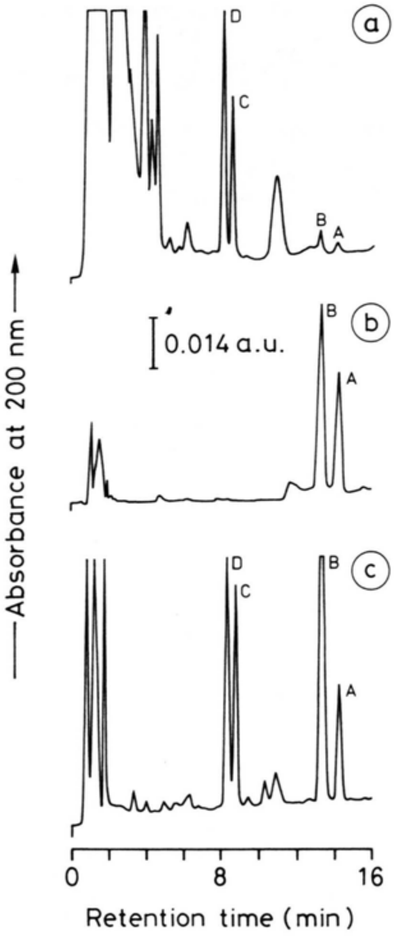

Fig. 1 a–c. HPLC analyses of steroidal saponins. The liquid chromatograph used was Kontron (Zürich, Switzerland) and included two Model LC 410 pumps with Model 200 solvent programmer. Injection was done *via* a Rheodyne rotary valve with a 20 µl loop. Detection and quantification was achieved with a Kontron Uvikon 720 LC detector coupled with a Hewlett and Packard 3380 A computing integrator. Separation was accomplished on 100 × 4.6 chromatographic column prepacked with Lichrosorb RP 8 (5 µm, Kontron) by a 15 min gradient elution from 25–40% acetonitrile in water. A, B, C, D: Avenacosides A–D. a) Analysis of a crude methanol extract of light grown *Avena* primary leaves. (4.5 µg D; 2.4 µg C; 0.28 µg B; 0.2 µg A.) b) Analysis of a crude methanol extract of isolated etioplasts [11] of dark grown *Avena* primary leaves. (3.5 µg B; 2.3 µg A.) c) Analysis of an artifical mixture, pre-purified by gel-filtration on Sephadex LH-20 in Methanol. (D: 3.6 µg; 2.9 µg C; 8.6 µg B; 1.7 µg A.)

Identification of the individual saponins was achieved by mixing isolated known samples with the extracts. In order to obtain quantitative values the detection response was calibrated by standards of avenacoside B (for isolation see ref. [1]). Detectability was found to be in the range of 50 ng and linearity up to 8 µg tested. The calculated quantities of the saponins from crude extracts coincided with the results obtained by densitometric determinations after TLC. Thus this HPLC offers an efficient and rapid method for analyses of these steroidal saponins and will be the method of choice in studies on the localization and physiology of these compounds.

In contrast to extracts of green leaves, which show essentially only the two more polar saponins C and D (Fig. 1 a), extracts of etioplasts, derived from dark-grown plants, exhibit only avenacosides A and B (Fig. 1 b), which is in accordance with ref. [1] and [2]. Fig. 1 c shows a resolution of an artifical mixture of all the four saponins after enrichment on a Sephadex LH-20 column (20 × 2,5 cm eluted with methanol).

We applied the described technique to a preparative scale in order to rapidly obtain sufficient amounts of the still unknown saponins C and D for elucidation of their structures. For this purpose we used a 250 × 9 mm column prepacked with Partisil-10 ODS (10 µm; Whatman, Clifton, New Jersey). The sample load was 100 µl of a 20–30 times concentrated extract as used in the analytical chromatography but purified by Sephadex LH-20 chromatography. Elution was started with 30% acetonitrile in water for 10 min and was subsequently raised to 40% within another 10 min at a flow rate of 3 ml/min. By this procedure we collected saponin D from 10 chromatographic runs and obtained ca. 1.5 mg with ca. 95% purity.

Acknowledgements

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[1] J. Kesselmeier and H. Budzikiewicz, Z. Pflanzenphysiol. **91,** 333 (1979).

[2] J. Kesselmeier and H. G. Ruppel, Z. Pflanzenphysiol. **93,** 171 (1979).

[3] C. Lütz, Z. Naturforsch. **35 c,** 519 (1980).

[4] F. Nachtmann, H. Spitzy, and R. W. Frei, Anal. Chem. **48,** 1576 (1976).

[5] F. Nachtmann, H. Spitzy, and R. W. Frei, J. Chromatogr. **122,** 293 (1976).

[6] H. Besso, Y. Saruwatari, K. Futamura, K. Kunihiro, T. Fuwa, and O. Tanaka, Planta Med. **37,** 226 (1979).

[7] R. W. Frei, Planta Med. **38,** 1 (1980).

[8] D. Strack, P. Proksch, and P.-G. Gülz, Z. Naturforsch. **35 c,** 675 (1980).

[9] D. Strack, P. Proksch, and P.-G. Gülz, Z. Naturforsch. **35 c,** 915 (1980).

[10] J. Kesselmeier, in preparation.

[11] J. Kesselmeier, Protoplasma **104,** 295 (1980).