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Analysis of saponins in oat kernels

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A method for analysis of oat kernel saponins is presented. The saponins were extracted from defatted oatmeal with methanol for 24 h in a Soxhlet apparatus. Separation of the saponins was accomplished by high-performance liquid chromatography (HPLC) using an octyl-silica column and gradient elution with acetonitrile in water. Two main peaks, avenacoside A and B, were detected. The identification was achieved by comparing retention times and photodiode array UV spectra with those of an avenacoside A and B standard preparation from oat leaves. The identity of the avenacoside A and B peaks was further supported by incubation of oatmeal suspensions in water, after which the retention times were shifted to those obtained with the corresponding desglucoavenacoside standard. The saponin content in oatmeal containing a mixture of Swedish commercial oat varieties was 0·040% (dry matter basis).

INTRODUCTION

Saponins are glycosides with terpenoid or steroid aglycones (Price *et al.*, 1987). Monodesmosidic saponins have one sugar residue bound to the aglycone and bisdesmosidic saponins have two sugar residues bound at different ends of the aglycone. Saponins occur in a wide variety of plants, where they are supposed to have antibiotic effects.

Two bisdesmosidic steroid saponins, avenacoside A and B, have been isolated from oat kernel and their structures elucidated as 3-*O*-{[α-L-rhamnopyranosyl (1–4)][β-D-glucopyranosyl(l–2)]-β -D-glucopyranosyl}26-*O*-(β-D-glucopyranosyl)furost-5-en-(22*S*:25*S*)epoxy-3β, 26-diol and 3-*O*-{[α-L-rhamnopyranosyl(1–4)][(β-D-glucopyranosyl(1–3))-β-D-glucopyranosyl(1–2)]-β-D-glucopyranosyl}-26-*O*-(β-D-glucopyranosyl)furost-5-en-(22*S*:25*S*)epoxy-3β,26-diol (Figure 1) (Tschesche *et al.*, 1969; Tschesche & Lauren, 1971).

Oat leaves also contain avenacosides. A specific β-glucosidase, occurring naturally in oat leaves, is able to remove the C-26 bound glucose moiety (Grünweller & Kesselmeier, 1985). The desglucoavenacosides formed are monodesmosidic with less bitterness, but with increased antibiotic and haemolytic activities (Tschesche & Wiemann, 1977).

Quantitative determination of saponins has for long been difficult. When using gravimetric methods, additional compounds may be precipitated with the saponins (Price *et al.*, 1987). Methods based on the haemolytic activity of saponins also have limitations. Other compounds could be haemolytic and the haemolytic activity differs for various saponins (Jones & Elliot, 1969). Bioassay methods, using *Trichoderma viride*, are very sensitive for alfalfa saponins, but values obtained for other saponins are low (Livingstone *et al.*, 1984). Spectrophotometric methods (Baccou *et al.*, 1977) have also been shown to be unspecific (Price *et al.*, 1987). Chromatographic methods are widely used for saponin analysis and in the 1980s more specific thin-layer chromatography (TLC) reagents (Curl *et al.*, 1985) and high-performance liquid chromatography (HPLC) methods (Ireland & Dziedzic, 1986) were developed. By these methods, values reported for saponin content in soyabeans decreased from 5·6% to 0·5% (Price *et al.*, 1987).

In the present study, an HPLC method, originally developed for analysis of oat leaves by Kesselmeier and Strack (1981), was used and further developed for oat kernel saponins, especially as regards sample extraction. Identification of the avenacosides was carried out by comparing retention times and UV spectral data with those of saponin standards prepared from oat leaves.

MATERIALS AND METHODS

Materials

Oatmeal containing a mixture of Swedish commercial oat varieties obtained from Kungsörnen, Järna, was used. Particle size distribution was as follows: 14% of the particles were larger than 0·23 mm, 69% were

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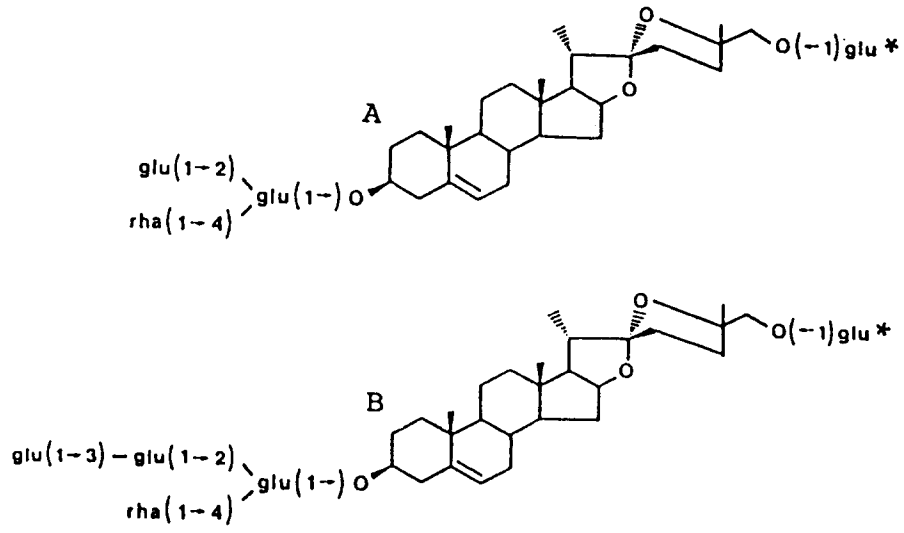

Fig. 1. Structures of Avenacoside A and B. A 26-desglucoavenacoside is formed when the glucose marked * is removed.

between 0·23 and 0·15 mm and 17% were smaller than 0·15 mm. In some experiments a suspension of flour in water (0·5 g/ml) was incubated for 0 min, 5 min and for 3 h at room temperature with continuous shaking. The flour was, in this case, milled oat kernels (Cyclotec sample mill, Tecator, particle size less than 0·5 mm) of the variety Selma and was not heat-treated. After the incubation, the sample was freeze-dried.

An oat leaf saponin extract containing avenacoside A and B and 26-desglucoavenacoside A and B was used as standard. For quantitative analysis a desglucoavenacoside standard prepared from oat leaf was used. These standards were kindly donated by J Kesselmeier, Max-Planck-Institute for Chemistry, Mainz.

Extraction

Fourteen grams of oatmeal were defatted with 150 ml of light petroleum (b.p. 60–80°C) for 16 h in a Soxhlet apparatus. The saponins were then extracted with 150 ml of methanol. To investigate the influence of the extraction time on saponin recovery, the samples were extracted with 150 ml of methanol for 6, 12, 20, 24, 30 and 36 h. Experiments were also done with renewal of the extraction fluid, i.e. three 3-h extractions, two 12-h extractions and three 12-h extractions.

The saponin extracts were evaporated to dryness and dissolved in 5 ml of methanol.

Samples from the incubation experiment were further purified: 0·5 ml of extract was evaporated to dryness and dissolved in distilled water. The solution was applied to a Bond Elut C18 column (100 mg, Analytichem International, Varian, USA) preconditioned first with 1 ml of methanol and thereafter with 2 ml of water. The column was washed with 2 ml of water, 2 ml of 40% methanol and 2 ml of 50% methanol. The saponins were then eluted with 2 ml of 60% methanol and the eluate was evaporated to dryness and dissolved in 0·5 ml of methanol. Recovery for this purification step was checked and found to be 98–101% for the avenacosides and the desglucoavenacosides.

Chromatography

HPLC separations were performed as described by Kesselmeier and Strack (1981). The instrument used was a Varian 5000 Liquid Chromatograph with UV detection at 200 nm. A 20-μl methanol extract were injected via a Rheodyne injection valve. Commercially packed columns (Merck, Darmstadt, 125 mm × 4 mm) of octyl-silica (LiChrosorb 100 CH-8/2, 5 μm) were used. Gradient elution was performed with 25–40% acetonitrile in water for 15 min using a flow-rate of 2 ml/min. The quantities of avenacoside A and B were estimated from peak areas, injecting known amounts of desglucoavenacosides. All analyses were performed at least in duplicate and the values are reported on a dry matter basis.

Photodiode-array UV spectra

A Hewlett-Packard 1090 Liquid Chromatograph with a 9153C Photodiode-array detector was used. HPLC chromatographic conditions were as listed in the preceding section. Spectroscopic data were collected from 190 to 450 nm.

Saponin test (foaming method)

The method of Yoon and Wrolstad (1984) was used. A 1-ml sample (collected HPLC fractions that had been evaporated to dryness and dissolved in water) was mixed with 4 ml of distilled water, heated for 15 min at 80°C and shaken by hand for 1 min. Persistence of foam for 10 min or longer is regarded as a positive test.

RESULTS AND DISCUSSION

HPLC separations

The HPLC method used in this study had been used earlier for saponin analysis in oat leaves. When

analysing oatmeals in the same way, we found many interfering peaks in the chromatogram. A fat extraction with light petroleum, as described above, had to be performed to avoid this problem.

An HPLC chromatogram of an oatmeal extract is shown in Fig. 2(a). Two main peaks (B and A) with retention times 6·5 and 6·9 min, respectively, were seen. The two peaks were collected and a foam test was performed. The test was positive and the taste of the collected material, dissolved in water, was bitter. This indicated that the two peaks were saponins. Injecting a saponin standard (Fig. 2(b)) showed that these two peaks had the same chromatographic behaviour as avenacoside A and B in the standard solution. Mixing the oat kernel extract and the standard gave the expected increments in peak height and area, with no evidence of splitting or broadening of the peaks. The identity of the main peaks was further supported by the incubation experiment (Fig. 3). Incubation of oatmeal in water for 5 min reduced the A and B peaks by 60% and corresponding desglucoavenacosides appeared. The latter peaks also appeared after 3 h, but A and B peaks could no longer be detected at this stage. As in oat leaves, there probably exists a specific β-glucosidase in oat kernels, which can quickly transform the avenacosides to 26-desglucoavenacosides.

The photodiode array UV spectra for the various peaks were also compared. The UV spectra of the peaks containing the avenacosides and the desglucoavenacosides are shown in Fig. 4. The UV spectra were all similar; there was a rapid decrease in UV absorption when moving to higher wavelengths, and at wavelengths over 230 nm the avenacosides and the desglucoavenacosides were not detected at all. Earlier published spectra of bisdesmosidic, terpenoid saponins from *Phytolacca dodecandra* (Phytolaccaceae) (Slacanin *et al.*, 1988) showed the same rapid decrease in UV absorption.

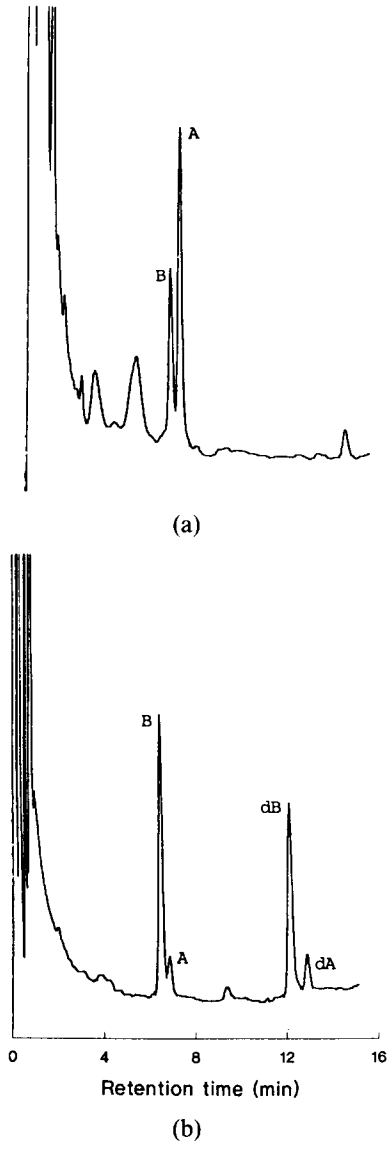

Fig. 2. HPLC analysis of oat saponins. (a) Crude extract of oatmeal. (b) Avenacoside A and B and desglucoavenacoside A and B standard.

Extraction

The amount of avenacoside A and B in oatmeal extracted with different extraction times is presented in Table 1. The saponin content increased with extraction time up to 20 h. Extraction times over 20 h gave the same amount of saponins. There was no difference when the extraction fluid was renewed. Thus, if the defatted oatmeal is extracted for 24 h with a single batch of solvent, all the saponins should be extracted.

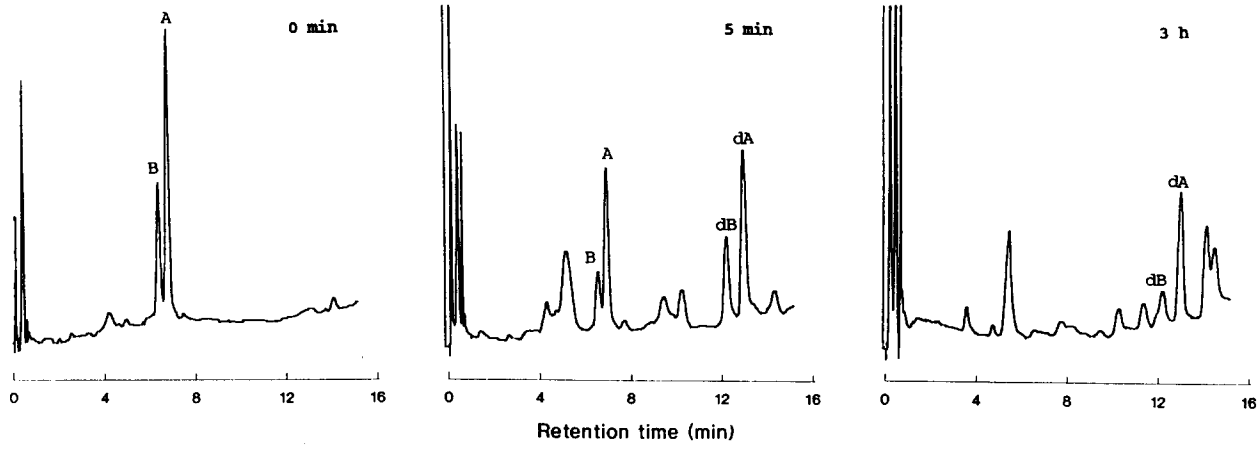

Fig. 3. HPLC analysis of oatmeal incubated for various times. (For identity of components, see Fig. 2.)

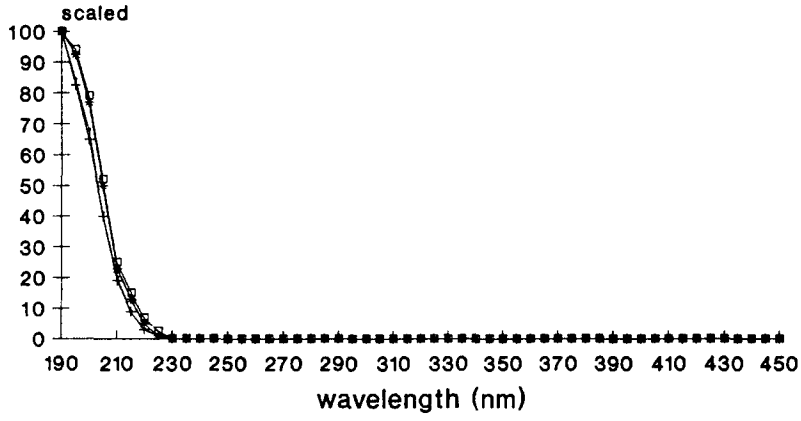

Fig. 4. Photodiode array UV spectra of oat saponins (■, Avenacoside A; + avenacoside B; *, desglucoavenacoside A; □, desglucoavenacoside B)

Different extraction times have been used in different saponin studies. For example, defatted soyabeans were extracted with methanol for 30 h in one study (Curl *et al.*, 1985) and for 36 h in another (Ireland & Dziedzic, 1986). There is a tendency in later publications to increase extraction times. The oat saponins seem to survive a long heat treatment with methanol without degradation.

Avenacoside A and B content

Table 2 compares the saponin content of oat kernel meal, obtained in the present study (0·040%, dry matter basis), with those published previously. Our saponin level is in close agreement with the value reported by Tshesche *et al.* (1969), although Fenwick and Oakenfull (1983) reported a higher value. One explanation for this difference could be that we did not determine all the oat saponins. On the other hand, the method used by Fenwick and Oakenfull has been criticised for giving a too high saponin value as a result of unspecific TLC reagents (Curl *et al.*, 1985).

Table 1. Influence of the extraction time on the amount of avenacoside A and B in oats (%, dry matter basis)

Extraction time (h)	Avenacoside A	Avenacoside B	Sum
6	0·008	0·003	0·011
3×3	0·024	0·009	0·033
12	0·022	0·008	0·030
20	0·029	0·011	0·040
24, 2×12	0·030	0·010	0·040
30	0·029	0·010	0·039
36, 3×12	0·028	0·011	0·039

Table 2. Saponin content in oat kernel (%, dry matter basis)

Reference	Method	Saponin content
This study		
24 h MeOH extraction, $n = 2$	HPLC	0·040[a]
36 h MeOH extraction, $n = 5$	HPLC	0·039 ± 0·003[a] (mean ± SD)
Tschesche *et al.* (1969)	TLC	0·040[a]
Fenwick & Oakenfull (1983)	TLC	0·1

[a] Avenacoside A + B.

CONCLUSION

The HPLC method presented here seems suitable for analysis of the saponins avenacoside A and B and 26-desglucoavenacoside A and B in oat kernels. Further work is needed to identify additional saponins possibly present in oat kernels.

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