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Note

High-performance liquid chromatography of oleanane saponins

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Oleanane saponins, the most important class of naturally occurring triterpene glycosides, are widely distributed in plants[1]. They are of special interest, as they have strong molluscicical activity against the intermediate host of schistosomiasis[2] and could find application in the control of this parasitic disease.

In the course of our investigation of plant extracts for snail-killing compounds, we have observed that only monodesmosidic saponins are active. These glycosides have a single sugar chain attached at C-3 of the aglycone. When the carboxylic group at C-28 is esterified with a second sugar chain (bidesmosidic saponins), there is no significant activity[3–5].

As the triterpene glycosides are not UV-active, their detection in high-performance liquid chromatography (HPLC) is difficult. Detection is usually carried out with a refractive index detector[6] or with a UV detector after derivatization (to dienes[7] or benzoates[8]). Some work on the HPLC of saponins has been reported, namely separations of ginsenosides[7–9] and saikosaponins[6,10]. No reports on the HPLC of oleanane saponins have appeared.

The aim of this work was to develop a suitable method for the analysis of oleane-12-ene triterpene glycosides in order to investigate plant extracts for molluscicidal saponins. Studies using extracts of one of the most promising plants in this field, *Phytolacca dodecandra* l'Hérit (Phytolaccaceae) are described here. The molluscicidal activity of this plant is dependent on its extraction process[4,11]. HPLC would be the method of choice for the study of extracts and for quantification purposes. In addition, it is potentially useful for comparison of different cultivated strains of *Phytolacca dodecandra*.

Triterpene saponins were successfully chromatographed on reversed-phase (RP-8) and polar-bonded supports such as DIOL. Mixtures of methanol and water or acetonitrile and water were used as eluents. Detection was effected at 206 nm. The use of elution gradients was possible with acetonitrile as it has a relatively weak absorption at this wavelength. Under these conditions mono- and bidesmosidic saponins could be separated.

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EXPERIMENTAL

Materials

Acetonitrile and methanol were of LiChrosolv grade (Merck, Darmstadt, F.R.G.). The saponins were previously isolated from *Lonicera nigra* L.[5] and *Phytolacca dodecandra* l'Hérit[4,12]. Samples were dissolved in methanol to give a concentration of 1 mg/ml.

Apparatus

An HPLC system composed of a Spectra-Physics 8700 pump, a Rheodyne injector and an LKB 2151 UV detector was used. Separations were performed on a 7-μm RP-8 column (25 cm × 4.6 mm I.D.) (Knauer) and on a 5-μm DIOL column (25 cm × 4.6 mm I.D.) equipped with 4-cm pre-columns.

RESULTS

Reversed-phase chromatography was used in order to separate mixtures of olean-12-ene saponins. An octyl-bonded phase column was used with methanol–water in preliminary trials with detection at 206 nm. The disadvantage of methanol is that it is limited to isocratic conditions. As saponins may possess large differences in polarity, gradient elution is advisable. Acetonitrile, which has only a week adsorption at 206 nm, is indicated for such separations.

As shown in Fig. 1, a mixture of eight saponins, previously isolated from a

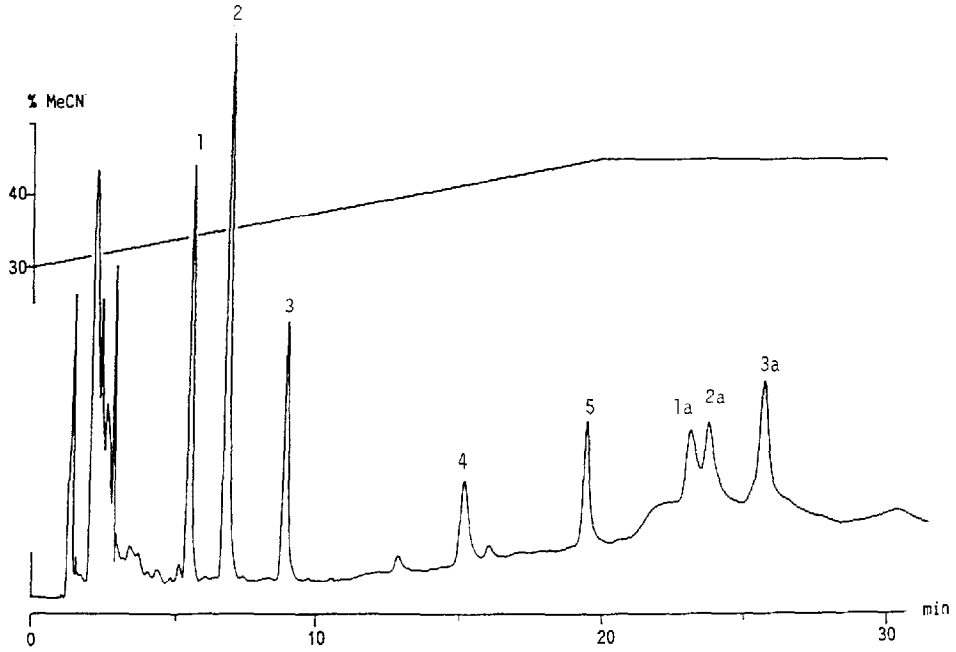
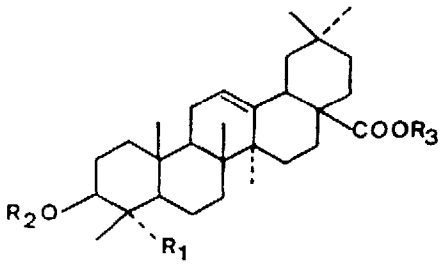


Fig. 1. Chromatogram of a mixture of monodesmosidic (1a, 2a, 3a, 4, 5) and bidesmosidic (1, 2, 3) saponint. Column, RP-8; eluent, acetonitrile–water, gradient from 30 to 45% acetonitrile (MeCN) in 20 min; flow-rate, 1.5 ml/min; detection, UV (206 nm).

methanolic extract of *Lonicera nigra* L. (Caprifoliaceae), was separated on an RP-8 column using gradient elution. The amount of acetonitrile was increased from 30 to 40% in 20 min. Only a small drift of the baseline was observed at 206 nm. The more polar bidesmosidic saponins 1, 2 and 3 are eluted very quickly. Glucuronides 4 and 5 leave the column before the monodesmosidic saponins 1a, 2a and 3a. Consequently, saponins having a large difference in polarity (one to four sugars attached to the aglycone) can be separated in a single run.



Compound	R_1	R_2	R_3
1	CH_2OH	glc(1→2)ara	glc(1→6)glc
1a	CH_2OH	glc(1→2)ara	H
2	CH_2OH	ara	glc(1→6)glc
2a	CH_2OH	ara	H
3	CH_3	glc(1→2)ara	glc(1→6)glc
3a	CH_3	glc(1→2)ara	H
4	CH_2OH	glur	H
5	CH_3	glur	H

glc = β-D-glucopyranosyl; glur = β-D-glucuronopyranosyl; ara = α-L-arabinopyranosyl

The non-polar support is selective for the lipophilic part of the saponins. Thus the glycosides of hederagenin, which is the most polar aglycone, are eluted before oleanolic acid glycosides. In this way, saponins 1 and 2 are eluted before 3, 4 before 5 and 1a and 2a before 3a.

A mixture containing mono- and bidesmosidic saponins isolated from *Phytolacca dodecandra* l'Hérit was analysed on an RP-8 column as shown in Fig. 2. An elution gradient from 28 to 38% acetonitrile in 30 min was used in order to separate these twelve compounds simultaneously. The more polar bidesmosidic compounds were eluted first. The glycosides of bayogenin (6 and 7) and hederagenin (8) are

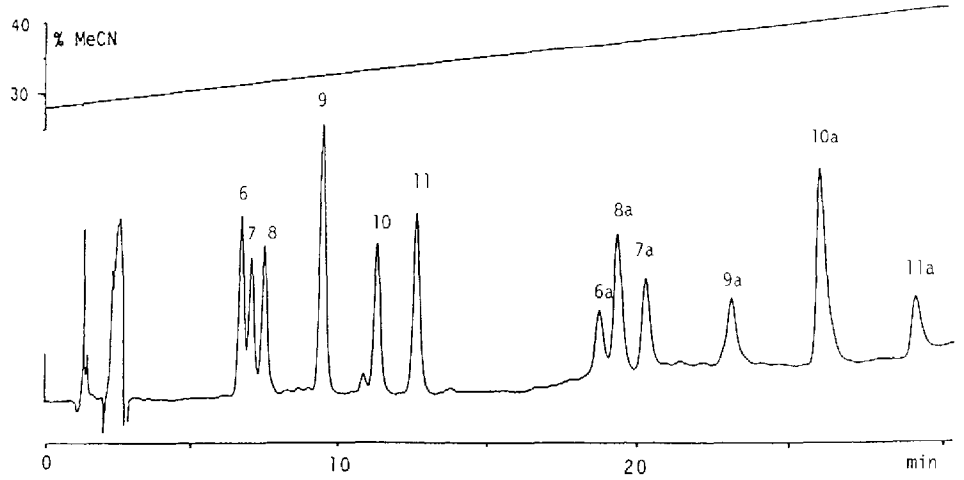
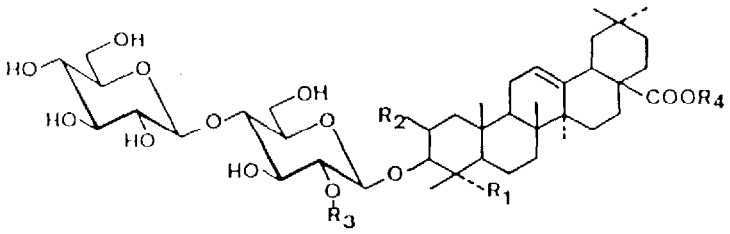


Fig. 2. Chromatogram of a mixture of monodesmosidic (6a–11a) and bidesmosidic (6–11) saponins. Column, RP-8; eluent, acetonitrile–water, gradient from 28 to 38% acetonitrile in 30 min; flow-rate, 1.5 ml/min; detection, UV (206 nm).



Compound	R_1	R_2	R_3	R_4
7	CH_2OH	OH	H	glc
7a	CH_2OH	OH	H	H
8	CH_2OH	H	glc	glc
8a	CH_2OH	H	glc	H
10	CH_3	H	glc	glc
10a	CH_3	H	glc	H

9, 9a, 11, 11a: structure elucidation in progress.

separated before the oleanolic acid derivatives 9, 10 and 11. As previously indicated, this could be explained in terms of selectivity of the non-polar stationary phase for the lipophilic aglycones. Therefore, these glycosides are eluted in order of decreasing polarity of their aglycones. A similar phenomenon was observed for the monodesmosidic saponins. They are eluted later, as the carboxyl group at C-28 is no longer esterified.

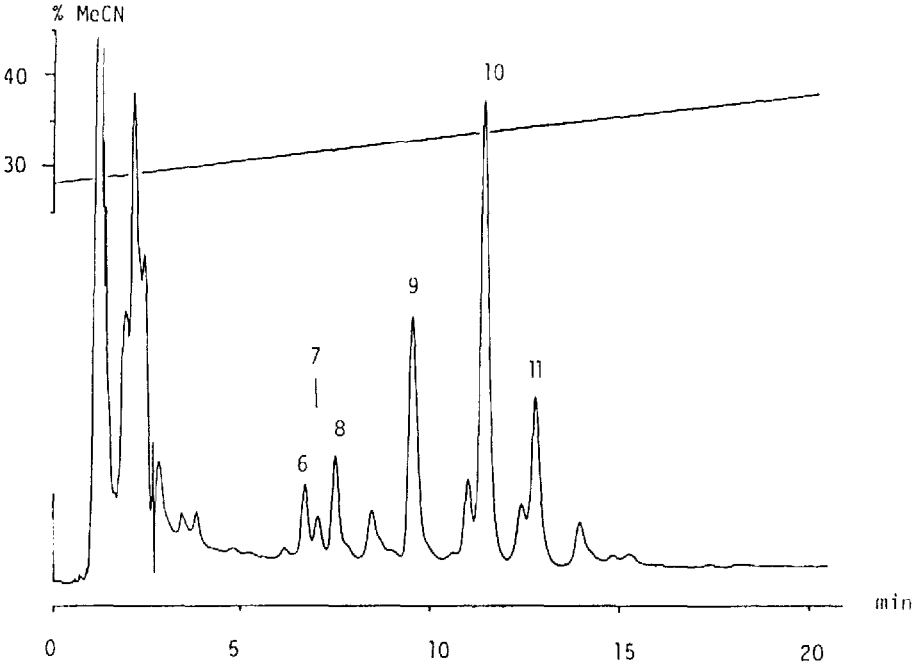


Fig. 3. Chromatogram of a methanolic extract of *Phytolacca dodecandra*. Column, RP-8; eluent, acetonitrile–water, gradient from 28 to 38% acetonitrile in 290 min; flow-rate, 1.5 ml/min; detection, UV (206 nm).

As shown by these two examples, the above methods allow the determination of both mono- and bidesmosidic saponins.

Fig. 3 exemplifies the analysis of the triterpene glycosides contained in the crude methanol extract of *Phytolacca dodecandra* (strain 1733). Similar chromatographic conditions were used, *viz.* an elution gradient from 28 to 38% acetonitrile in 20 min. A complex mixture of saponins was observed. Comparison of the retention times with those of standards readily allowed the identification of the peaks. Only bidesmosidic saponins are present in this extract, which is in agreement with the bioassays, as no molluscicidal activity is observed.

These examples clearly show that reversed-phase chromatography is suitable for the separation of mono- and bidesmosidic saponins.

In addition to reversed-phase columns, other chromatographic systems using alternative polar-bonded phases were developed. By this means olean-12-ene triterpene glycosides could be separated on a DIOL column with acetonitrile–water mixtures as eluents. Thus, the crude methanolic extract of *Phytolacca dodecandra* was chromatographed on a DIOL column using acetonitrile–water (85:15). Under these conditions the saponins were eluted in order of increasing polarity (Fig. 4). The less polar triglucoside of bayogenin (7) was eluted before the tetraglycosides of oleanolic acid (11 and 10) and before those of hederagenin (8) and bayogenin (6). The pentaglycoside of oleanolic acid (9) was the last saponin eluted. This example shows that DIOL supports can be used for the separation of saponins. Elution mixtures of acetonitrile and water are compatible with the gradients necessary for the separation of mono- and bidesmosidic saponins.

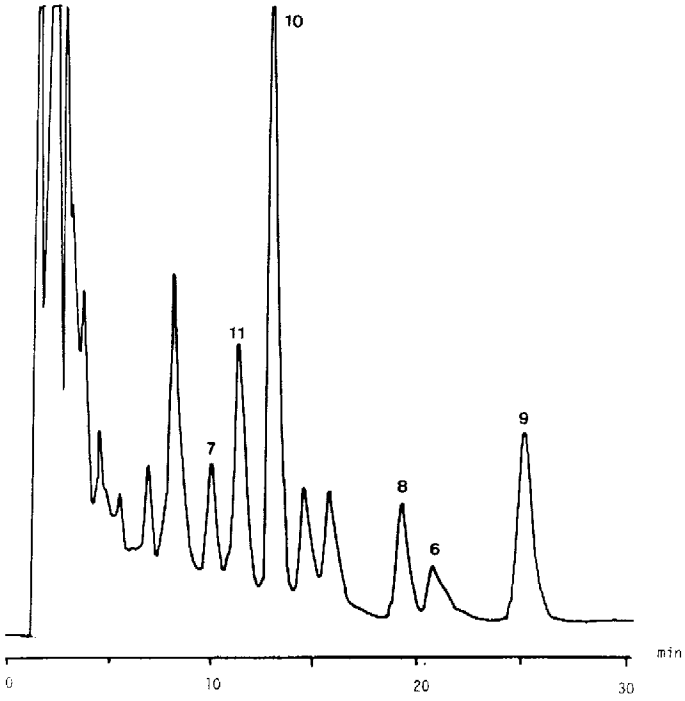


Fig. 4. Chromatogram of a methanolic extract of *Phytolacca dodecandra*. Column, DIOL; eluent, acetonitrile–water (85:15); flow-rate, 1.5 ml/min; detection, UV (206 nm).

CONCLUSION

HPLC is a powerful method for the analysis of olean-12-ene saponins. Either reversed-phase or polar bonded-phase supports can be used. Elution is effected in both instances with mixtures of acetonitrile and water. This eluent is compatible with detection at 206 nm and with the use of gradients in order to perform separations of saponins having large differences in polarity. Mono- and bidesmosidic saponins can be easily chromatographed in a single run. Non-polar and polar supports are complementary for the analyses of triterpene glycosides; the former show selectivity for the non-polar aglycones and the latter interact with the glycosidic parts of the saponins.

The present method could find application in the study of plant extracts for their saponin content. For example, HPLC has been applied to the investigation of methanol and water extracts of *Phytolycca dodecandra*. As quantification of the molluscicidal compounds is possible, determination of the content of active saponins in different strains of cultivated *Phytolacca dodecandra* can be carried out by this means, thus permitting an efficient screening of the plant material. Further, HPLC can be used for selecting conditions transposable to the preparative scale.

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