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Note

High-performance liquid chromatography of flavonoid glycosides from *Dryas octopetala*

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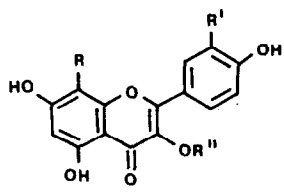
Flavonoids are very widely distributed in plants and the methods for their separation, purification and structure elucidation have been extensively studied[1,2]. In recent years high-performance liquid chromatography (HPLC) has been applied to flavonoids[3].

However, during our research on medicinal plants, we found it difficult to separate the flavonoid glycosides of *Dryas octopetala* L. (Rosaceae) by using standard procedures[1–3]. In fact, their similar mobilities on column chromatography (CC) with different substrates (silica gel, cellulose, polyamide) and eluents led to obtain fractions that seemed homogeneous on thin-layer chromatography (TLC) under the same conditions as for CC, but showed by field-desorption mass spectrometry to be mixtures of two or three compounds. As the system *tert.*-butanol–water on HPTLC RP-18 pre-coated plates (Merck) gave the best results on TLC, these conditions were employed successfully in HPLC for both analytical and preparative purposes.

Six flavonoid glycosides (1–6) were obtained in pure form mainly by semi-preparative HPLC and their structures were elucidated by spectroscopic methods[4]. Analytical HPLC was used for monitoring single CC fractions in order to establish their composition and to record a profile of the total flavonoid glycoside content of the leaves of *Dryas octopetala* collected at different altitudes and in different environments.



	R	R'	R''
1	OCH_3	OH	β – D – galactopyranosyl
2	OCH_3	OH	α – L – arabinofuranosyl
3	OCH_3	H	β – D – galactopyranosyl
4	H	OH	β – D – galactopyranosyl
5	H	OH	β – L – arabinopyranosyl
6	H	OH	α – L – arabinofuranosyl

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EXPERIMENTAL

Apparatus

A minicomputer-controlled Waters Assoc. 6000 liquid chromatograph equipped with a U6K injector and a Beckman 25 recorder was used. The detector was a Beckman 25 spectrophotometer set at 355 nm.

Columns

μBondapak RP-18 (10-μm particles) 30 cm × 3.9 mm I.D. and 30 cm × 7.8 mm I.D. columns (Waters Assoc.) were used as analytical and semi-preparative columns, respectively.

Reagents

Mixtures of glass-distilled water filtered on a 4.5-μm Millipore filter and *tert.*-butanol (analytical-reagent grade; Riedel de Haen) were employed as mobile phase.

Degassing was accomplished under vacuum just before the analysis.

Sep-Pak (silica gel) and Sep-Pak (C_{18}) cartridges (Waters Assoc.) were used for HPLC sample preparation.

Conditions and procedures

Before injection into the HPLC system, samples of the ethanolic extracts of leaves of *D. octopetala* or of the CC fractions were rapidly filtered by means of a syringe on a silica gel cartridge, eluting with chloroform and an increasing proportion of methanol in order to eliminate tannins (more polar than the glycosides). Similarly, any chlorophyll present was then eliminated by elution on a C_{18} cartridge with methanol and an increasing proportion of water.

Analytical HPLC of flavonoid glycosides was performed with μBondapak RP-18, elution with water–*tert.*-butanol (86:14), flow-rate 0.5 ml/min for 70 sec, then increased to 2 ml/min in 4 min; the sample (5 μl) was injected by dilution of 1 mg in 10 μl.

Semi-preparative HPLC was performed on the larger μBondapak RP-18 column by elution with water–*tert.*-butanol (86:14), flow-rate 0.7 ml/min for 1 min, then increased to 2.0 ml/min for 3 min and to 2.5 ml/min for 5 min; the sample (40 μl) was injected by dilution of 10 mg in 100 μl.

RESULTS AND DISCUSSION

The flavonoid glycosides (1–6) of *Dryas octopetala* are galactosides and arabinosides of corniculatusin, quercetin and sexangularetin[4] and show very similar chromatographic properties. The difficulty in separating the mixture of aglycones of *D. octopetala*, obtained by hydrolysis of glycosides, has been noted previously[5].

As described above, none of the usual substrates (silica gel, cellulose, polyamide) nor the usual solvents for reversed-phase substrates (methanol–water, acetonitrile–water, etc.) allowed a good separation of 1–6. No improvements were obtained with other alcohols (ethanol, propanol, etc.) or with gradient elution. Only *tert.*-butanol–water (14:86) solved the problem. To our knowledge this is the first time that *tert.*-butanol–water has been used for the HPLC analysis of flavonoid glycosides.

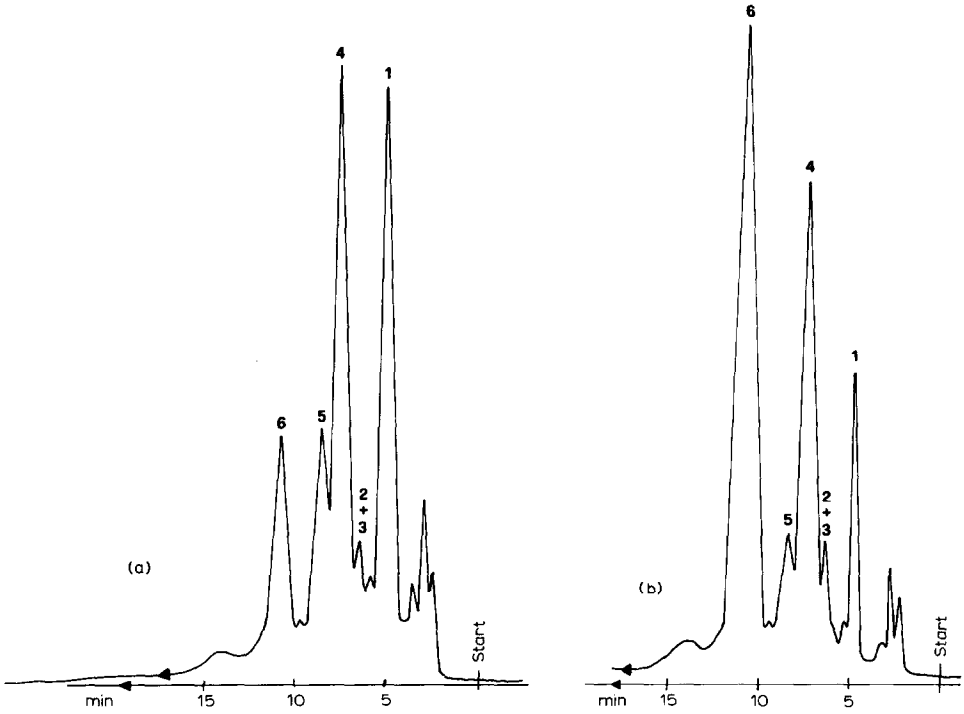

Fig. 1. HPLC traces of the flavonoid fraction from *Dryas octopetala* leaves: (a) "mountain" and (b) "plain" samples. Column: μBondapak RP-18 (10 μm) (300 × 3.9 mm I.D.). Eluent: *tert.*-butanol–water (14:86); flow-rate, 0.5 ml/min for 70 min, then increased to 2 ml/min in 4 min. Detection: 355 nm.

It is interesting that under the conditions used first the 8-methoxyglycosides (1–3) and then the quercetin glycosides (4–6) are eluted, irrespective of the polarity of the compounds. As shown in Fig. 1, only compounds 2 and 3 could not be separated by this method and were eluted with the same retention time. However, by this means we were able to separate not only glycosides having the same molecular weight 2 and 4, but also glycosides differing only in the shape of the sugar moiety (5 and 6).

As an example of an application, Fig. 1 shows the profiles of the total contents of the leaves of *D. octopetala* collected (a) at 1500–2000 m (mountain valleys in the Dolomites) and (b) at 200 m (Val Cellina). The most significant difference between the "mountain" and "plain" populations was only quantitative, owing to the relatively high concentration of 6 in the latter sample.

We should stress that the preliminary purification, especially from tannins, of the flavonoid glycosides by filtration on the cartridges allows one to perform, without loss of material, very clean analysis and to preserve the HPLC columns for long periods.

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