Journal of Chromatography, 176 (1979) 270–273

CHROM. 11,906

Note

High-performance liquid chromatographic analysis of oat (*Avena sativa*) flavone derivatives

DIETER STRACK, KLAUS FUISTING and GHEORGHE POPOVICI
Botanisches Institut der Universität Köln, Gyrhofstr. 15, D-5000 Köln 41 (G.F.R.)
(First received January 29th, 1979; revised manuscript received April 5th, 1979)

High-performance liquid chromatography (HPLC) has been found to be applicable to the analysis of complex natural mixtures of flavonoids for rapid screening and fingerprinting purposes in investigations of taxonomic problems[1–3]. The HPLC of these mixtures often precludes the quantitative analyses of the individual constituents and therefore this technique seemed not to be useful in biochemical and physiological studies on the metabolism of complex mixtures of flavonoids.

The organs of oat plants contain a complex mixture of flavone derivatives and column or thin-layer chromatographic (TLC) analysis of the various derivatives is complex and slow. We have been able to resolve this flavone mixture by HPLC and it is now possible to employ this method in routine studies of the metabolism of flavone derivatives in oat plants.

EXPERIMENTAL

Extraction

The plant material, grown in a phytotron or under field conditions, was extracted with boiling water and subsequently homogenized with an Ultra Turrax blender. The filtered extract was evaporated to dryness under reduced pressure at 40° and the residue was dissolved in a known volume of 50% methanol.

Preparation for HPLC

Before application of HPLC, the extracts were filtered through a 1-μm Millipore filter.

Chromatographic system

The liquid chromatograph used and the chromatographic columns were as described previously[4].

Analytical method

In order to identify the peaks on the chromatograms obtained from crude oat extracts, each flavone derivative was isolated by thin-layer chromatography[5] and examined separately by HPLC.

Separation was accomplished by gradient elution: solvent A, water–methanol–

acetic acid (90:5:5); solvent B, water–methanol–acetic acid (5:90:5); gradient profile, linear from 0 to 50% B in 65 min; flow-rate, 2.0 ml/min; detection, 365 nm; sample size, 25 μl.

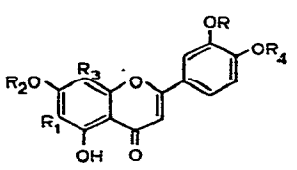
RESULTS AND DISCUSSION

The flavone derivatives which were resolved by HPLC are listed in Table I. These derivatives occur naturally in various organs of oat plants[5,6] and extracts of the intact plant were analysed. Without cleaning or pre-fractionation, it was possible to resolve crude extracts into 11 flavone derivatives within 60 min (Fig. 1) using a linear water–methanol gradient on LiChrosorb RP-8.

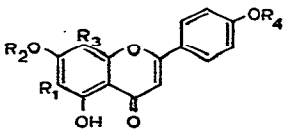
TABLE I

STRUCTURES OF OAT FLAVONE DERIVATIVES, RETENTION TIMES AND CAPACITY FACTORS (k') ON LICHROSORB RP-8 USING A WATER-METHANOL GRADIENT

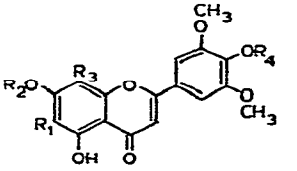
glc = Glucose; ara = arabinose; rha = rhamnose.



A



B



C

Peak No.	t_R *(min:sec)*	k'	R_1	R_2	R_3	R_4
A: Luteolin type						
1	15:12	15.58	C-glc-O-ara	H	H	H
2	17:00	17.55	C-glc	H	H	H
B: Apigenin type						
3	20:00	20.82	H	H	C-glc-O-rha	H
4	22:30	23.55	C-glc-O-ara	H	H	H
5	23:48	24.09	H	CH_3	C-glc-O-rha	H
6	24:54	26.16	C-glc	H	H	H
C: Tricin type						
7	38:12	40.67	H	glc (or ara)	H	H
8	39:28	42.05	H	glc (or rha)	H	rha (or glc)
9	55:00	59.00	H	H	H	H
10	56:06	60.20	H	H	H	glc
11	58:59	63.35	H	H	H	ara

In the resolution of such complex mixtures of flavone derivatives as shown in Fig. 1, all k' values (see Table I) are outside the optimal range. Attempts to improve the chromatographic system to obtain smaller k' values resulted in poor resolutions. Also, with the application of other column packings we could not obtain better results. On RP-18 we observed smaller α values[4] and two components (peaks 3 and 4 in Fig. 1) eluted with the same capacity factor. In general, on RP-18 early peaks showed higher and later peaks smaller k' values compared with those obtained on RP-8.

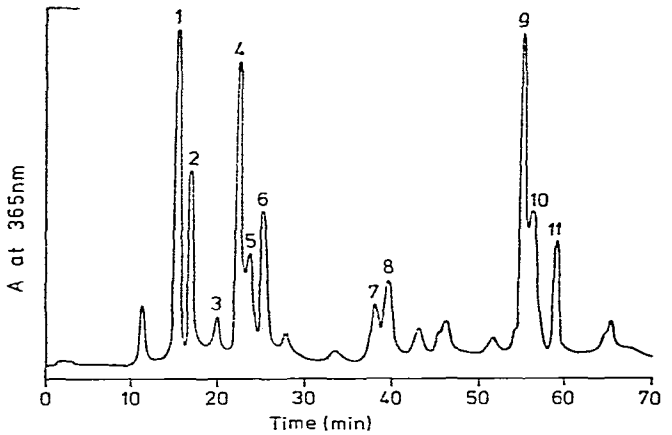


Fig. 1. Resolution of a crude extract of oat flavone derivatives on LiChrosorb RP-8. Peak identifications as in Table I.

As might be expected from the different solubilities of the derivatives considered, the glycosylated C-glucosylflavones were eluted in a shorter time than the corresponding aglycones (see compounds 1 and 2 of the luteolin type and compounds 3 and 6 of the apigenin type). Glycosylation of tricin in R_2 (see peak 7) results in a Δt_R value to the aglycone of 16 min 48 sec.

However, when there is sugar substitution of tricin in R_4 (see peaks 10 and 11), the retention time is enhanced, and even the arabinoside and glucoside derivatives can be resolved (Δt_R = 2 min 53 sec). These two tricin derivatives are very difficult to separate by TLC[5]. A similar qualitative HPLC separation was observed by Niemann and Van Brederode[2] for glycosylated isovitexins.

A marked difference in selectivity between the system employed by Niemann and Van Brederode[2] (Zorbax ODS) and ours (RP-8) is seen on comparing the retention times of isovitexin and its arabinoside; whereas they reported a Δt_R value of 12 sec, we observed a baseline separation of these two compounds with Δt_R = 2 min 24 sec in the reverse order. We also observed another difference in the resolution of isovitexin arabinoside and vitexin rhamnoside. On LiChrosorb RP-18 we were not able to separate these compounds, whereas Niemann and Brederode[2] reported a Δt_R value of 1.5 min on Zorbax ODS. On RP-8 these compounds separated with a Δt_R value of 2.5 min (3 and 4 in Figs. 1 and 2).

In agreement with the HPLC data for flavonol derivatives[1], we observed that the more polar the C_{15} skeleton of the flavone derivative, the closer they are eluted to their corresponding glycosides. For example, isovitexin arabinoside elutes 2 min 24 sec earlier than isovitexin, but isoorientin arabinoside only 1 min 48 sec earlier than isoorientin.

The application of the method is demonstrated in Fig. 2. Crude extracts of primary oat leaves, grown under different environmental conditions, were resolved within 30 min. Tricin derivatives were not detectable; they occur only in oat inflorescence[7]. Comparing the peak patterns in Fig. 2a and b, a marked qualitative and quantitative difference can be observed. Compound 1 is detectable only in primary leaves grown under field conditions. The major compound (4) is reduced in

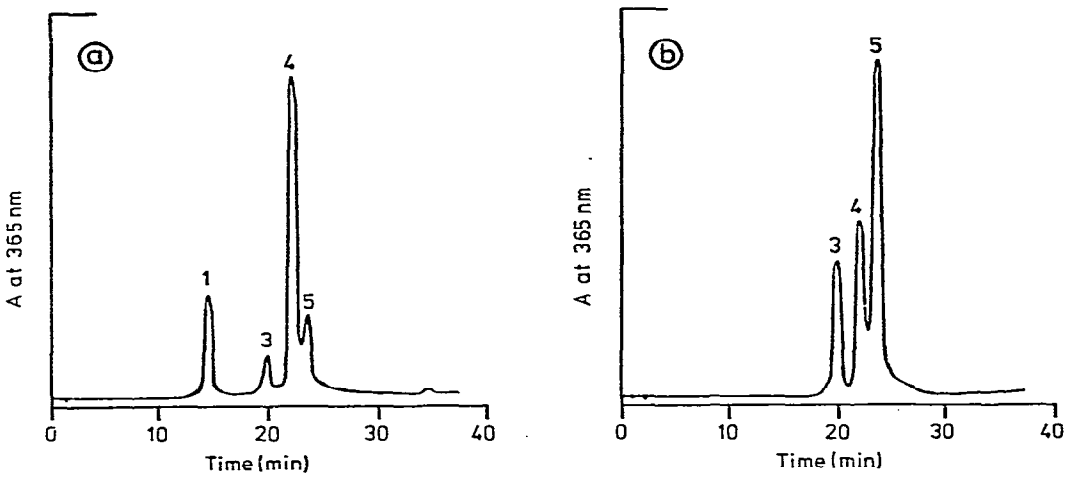


Fig. 2. Resolution of a crude extract of oat flavone derivatives from primary leaves (a) grown under field conditions and (b) grown in a phytotron. Peak identifications as in Table I.

primary leaves of phytotron-grown plants and compound 5 is found as the major constituent.

In biochemical and physiological investigations of the metabolism of flavone derivatives, the following is recommended: octylsilica are preferable to octadecylsilica column packings; a very shallow water–methanol gradient should be used; and the flow-rate should be 2 ml/min or higher.

ACKNOWLEDGEMENT

We are grateful to M. Heinen for carrying out extractions of the primary oat leaves.

REFERENCES

1 G. J. Niemann and J. W. Koerselman-Kooy, *Planta Med.*, 31 (1977) 297.
2 G. J. Niemann and J. van Brederode, *J. Chromatogr.*, 152 (1978) 523.
3 G. J. Niemann, *Z. Naturforsch. C*, 32 (1977) 1015.
4 D. Strack and J. Krause, *J. Chromatogr.*, 156 (1978) 359.
5 G. Popovici, G. Weissenböck, M. L. Bouillant, G. Dellamonica and J. Chopin, *Z. Pflanzenphysiol.*, 85 (1977) 103.
6 J. Chopin, G. Dellamonica, M. L. Bouillant, A. Besset, G. Popovici and G. Weissenböck, *Phytochemistry*, 16 (1977) 2041.
7 G. Popovici and G. Weissenböck, *Ber. Deut. Bot. Ges.*, 89 (1976) 483.