Journal of Chromatography, 205 (1981) 470–472
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,344

Note

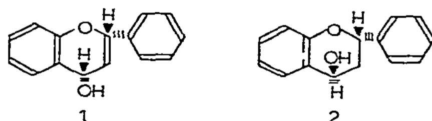
High-performance liquid chromatographic separation of flavan-4α-ol and flavan-4β-ol epimers

S. R. UDUPA* and A. V. PATANKAR

Bio-Organic Division, Bhabha Atomic Research Centre, Modular Laboratories, Trombay, Bombay-85 (India)

(Received September 15th, 1980)

Flavan-4-ol is an important intermediate in the biosynthetic pathways of flavonoids. It exists in two stereoisomeric forms, *viz.*, flavan-4β-ol (1) and flavan-4α-ol (2). In the course of studies[1] on the fermentation of (±)-flavanone by *Gibberella fujikuroi* NCIM 665, optically pure (—)-flavan-4α-ol was isolated. However, a careful study of the nuclear magnetic resonance (NMR) spectrum of a product obtained from the mother liquor revealed that a small amount of flavan-4β-ol was also present. A simple separation technique for these epimers is therefore required.



Recently it has been shown that high-performance liquid chromatography (HPLC) can be employed for the separation of epimers. Thus α- and β-carbinols[2], 16α,17α-acetals of 16α-hydroxyprednisolone[3,4] and *cis*/*trans* isomers of substituted cinnamic acids[5,6] have been successfully separated by reversed-phase HPLC. In the present study this technique was employed for the separation of flavan-4β-ol (1) and flavan-4α-ol (2) on μBondapak C_{18}.

EXPERIMENTAL

Apparatus

A Waters Assoc. (Milford, MA, U.S.A.) Model ALC/GPC-244 liquid chromatograph, equipped with a Model 6000A solvent delivery system and a Model U6K injection system, was used. The reversed-phase column (30 cm × 3.9 mm I.D.) was packed with μBondapak C_{18} [octadecyltrichlorosilane chemically bonded to Porasil (<10 μm) packing; Waters Assoc.]. The mobile phase was prepared from methanol (Uvasol; E. Merck, Bombay, India), double glass-distilled water and double distilled acetic acid. Methanol was filtered through a FHLP 0.5-μm filter (Millipore, Bedford, MA, U.S.A.), and water and acetic acid were filtered through an HAWP 0.45-μm

0021-9673/81/0000–0000/S02.50 © 1981 Elsevier Scientific Publishing Company

filter (Millipore). The column pressure was 2000 p.s.i. at 1.5 ml/min with methanol–water (50:50) as solvent. UV (Kit Model 440) detection was carried out at 254 nm and the sensitivity of the detector was 0.05 absorbance units full scale (a.u.f.s.). The chart speed was 0.1 in./min, the temperature was ambient and the solvent system was used isocratically. Determinations of dead volume were made by using acetone.

Sample preparation

(±)-Flavan-4β-ol (1) was synthesised by $NaBH_4$ reduction[7] of flavanone, while optically pure (—)-flavan-4α-ol (2) was obtained from the fermentation of (±)-flavanone by *Gibberella fujikuroi* NCIM 665 followed by elaborate column chromatography[1]. Sample solution (10 μl) containing flavan-4α-ol and flavan-4β-ol (3 μg each) was chromatographed by HPLC isocratically.

RESULTS AND DISCUSSION

The two flavan-4-ol epimers were separated from each other by HPLC on a reversed-phase μBondapak C_{18} column using methanol–water or methanol–water–acetic acid in different combinations. Fig. 1 shows the HPLC separation of the two epimers in various solvent systems. The epimers were collected and identified by mass spectrometry as well as by co-chromatography. Capacity factors (k') for the isomers (1, 2) in various mobile phases are shown in Table I.

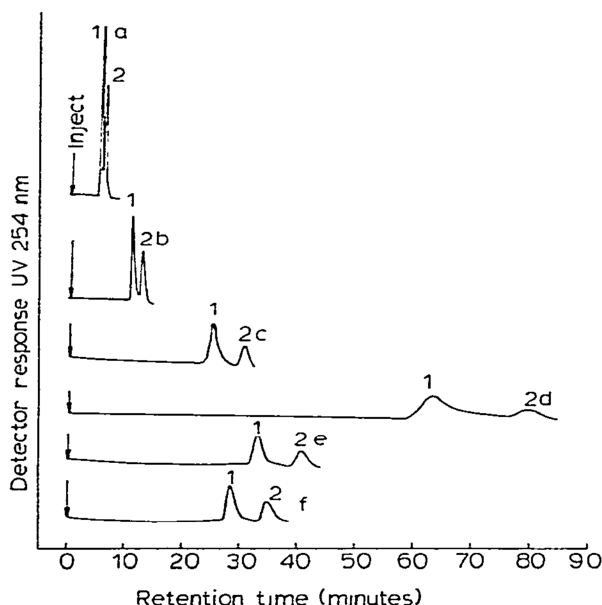


Fig. 1. HPLC separation of flavan-4β-ol (1) and flavan-4α-ol (2) isomers in various solvent systems: methanol–water (6:4) (a), (1:1) (b), (4:6) (c), (3:7) (d); methanol–water–acetic acid (33:65:2) (e), (30:65:5) (f). Other conditions: column, μBondapak C_{18} (30 cm × 3.9 mm I.D.); flow-rate, 1.5 ml/min; detector, UV 254 nm; sensitivity, 0.05 a.u.f.s.; chart speed, 0.1 in./min; sample injection, 10 μl containing 3 μg each of 1 and 2.

TABLE I

CAPACITY FACTORS (k') FOR FLAVAN-4α-OL AND FLAVAN-4β-OL ON REVERSED-PHASE μBONDAPAK C_{18} IN VARIOUS SOLVENT SYSTEMS

$k' = (t_R - t_0)/t_0$; t_0 = retention time of one column void volume, t_R = retention time of the compound. t_0 for solvent systems 1–3, 2 min 36 sec; t_0 for solvent systems 4–6, 2 min 56 sec.

Solvent system	*k'*	
	Flavan-4β-ol	*Flavan-4α-ol*
1 Methanol–water (60:40)	1.30	1.50
2 Methanol–water (50:50)	3.50	4.33
3 Methanol–water (40:60)	8.62	10.69
4 Methanol–water (30:70)	23.62	29.92
5 Methanol–water–acetic acid (33:65:2)	11.69	14.69
6 Methanol–water–acetic acid (30:65:5)	9.77	12.31

The results indicate that an increase in the proportion of water improves the epimer separation due to its effect[3] on k'. It was also observed that addition of acetic acid (2–5%) to the methanol–water solvent system considerably reduced the retention time of the two epimers (Fig. 1), giving better resolution as compared to the water–methanol solvent system. This is in good agreement with an earlier observation[8] where the addition of acetic acid (*ca.* 5%) to the methanol–water solvent system improved the separation of flavonoids.

The qualitative determination of these epimers in a given sample can be achieved by using methanol–water (50:50) as solvent, while methanol–water–acetic acid (30:65:5) could be used for the quantitative separation of the epimers. The above results showed that HPLC separation on μBondapak C_{18} is an effective and rapid method for the separation of isomeric flavan-4-ols.

REFERENCES

1 S. R. Udupa and M. S. Chadha, *Indian J. Biochem. Biophys.*, 15 (1978) 328.
2 R. W. Souter, *J. Chromatogr.*, 134 (1977) 187.
3 A. Wikby, L. Nilsson and G. Hällsas, *J. Chromatogr.*, 157 (1978) 51.
4 A. Wikby, A. Thalén and G. Oresten, *J. Chromatogr.*, 157 (1978) 65.
5 S. Caccamese, R. Azzolina and M. Davino, *Chromatographia*, 12 (1979) 545.
6 R. D. Hartley and H. Buchan, *J. Chromatogr.*, 180 (1979) 139.
7 H. G. Krishnamurty, V. Krishnamoorthy and T. R. Seshadri, *Phytochemistry*, 2 (1963) 47.
8 G. J. Niemann and J. W. Koerselman-kooy, *Planta Med.*, 31 (1977) 297.