Journal of Chromatography, 248 (1982) 469–471
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 15,131

Note

Separation of glycyrrhizinic acid isomers by high-performance liquid chromatography

KAZUYUKI TSUBONE*, SHIGEKI OHNISHI and TOHRU YONEYA
Kanebo, Ltd., Cosmetics Laboratory, 3-28, Kotobuki-cho 5-chome, Odawara City 250 (Japan)
(First received March 10th, 1982; revised manuscript received June 21st, 1982)

Liquorice, the roots of *Glycyrrhizia glaubra*, has been in medical use for over 2000 years. Shabana[1] has demonstrated its anti-ulcerative activity and it has been shown that glycyrrhizinic acid (GA) (Fig. 1) and its aglycone, glycyrrhetinic acid, are the active constituents. GA has two stereoisomers, the 18α-form (*trans*) and the 18β-form (*cis*) (Fig. 1), whose structures were studied by Ružicka and Jeger[2], Steiner[3] and Beaton and Spring[4].

Methods for the determination of GA based on gravimetric analysis[5], polarography[6], colorimetry[7] and thin-layer chromatography (TLC) with flame-ionization detection[8] have been reported. The separation of GA isomers has been effected by

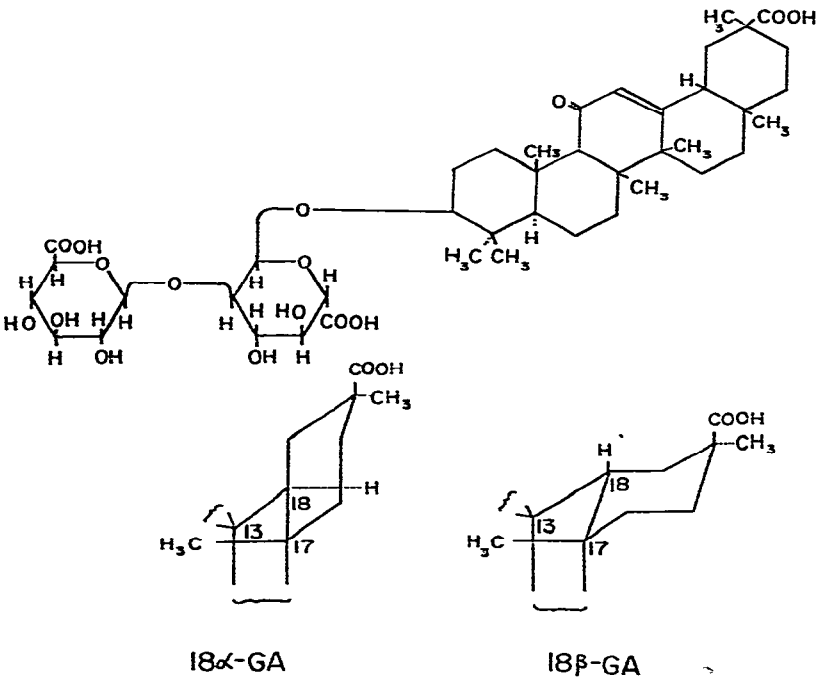

Fig. 1. Structure of glycyrrhizinic acid (GA), 18α-GA and 18β-GA.

0021-9673/82/0000–0000/$02.75

gas–liquid chromatography (GLC), GA being hydrolysed to glycyrrhetinic acid isomers, which are detected as the silyl ether derivatives.

High-performance liquid chromatography (HPLC) has been reported for the determination of 18β-GA. As it is an ionic glucoside, ion-exchange chromatography with an anion-exchange resin[9] as the stationary phase and ion-pair partition chromatography with octadecylsilica gel[10] have been applied to separate many components from liquorice extract. For the separation of GA isomers, a method using a reversed-phase column packing after hydrolysis and chloroform extraction has been studied[11].

This paper describes a direct method for separating GA isomers by HPLC.

EXPERIMENTAL

Apparatus

The chromatographic system consisted of a Hitachi Model 635S pumping system (Hitachi, Tokyo, Japan), a Hitachi sampling injection valve (cat. no. 638-0801) and a variable-wavelength detector (UVILOG 5III; Applied Spectroscopic Instruments, Tokyo, Japan). The column temperature (40°C) was controlled with a circulating water-bath controller (41K40A-B; Oriental Motor Co., Tokyo, Japan). A home-made column (250 × 4.0 mm I.D.) was packed with 5-μm Nucleosil 5CN (Machery, Nagel & Co., Düren, G.F.R.). The mobile phase was aqueous phosphate buffer solution.

Reagents

Monoammonium salts of 18α-GA and 18β-GA were purchased from Maruzen Kasei (Tokyo, Japan). Their purities were 94% and 100%, respectively, as determined by GLC. Sample solutions were prepared by dissolving 12 mg of 18α-GA or 22 mg of 18β-GA in 100 ml of water. A 4-μl volume of each solution was injected into the HPLC system.

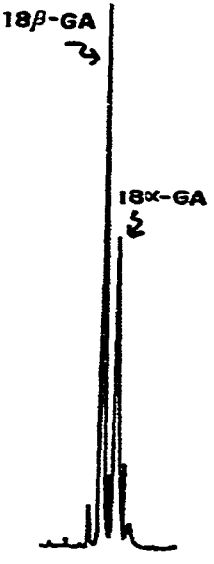

Fig. 2. Separation of glycyrrhizinic acid isomers. Column, Nucleosil 5CN; mobile phase, 0.05 *M* KH_2PO_4 (pH = 7.0); flow-rate, 0.8 ml/min; temperature, 40°C; detection, UV at 257 nm (0.08 a.u.f.s.).

RESULTS AND DISCUSSION

On a cyanopropylsilica gel packing column under optimal conditions with a pH of the mobile phase of 7.0 and a potassium dihydrogen orthophosphate concentration of 0.03–0.05 *M*, GA isomers were efficiently separated ($R_s = 2.2$), as shown in Fig. 2. The limit of detection (twice the signal-to-noise ratio) was 20 ng for 18β-GA.

This HPLC method can be used for the direct quality control of GA in *Glycyrrhizia glaubra*.

ACKNOWLEDGEMENTS

We express our sincere appreciation to Mr. Shoji Hara, Professor of Tokyo College of Pharmacy, for valuable discussion of our manuscript. We also thank Mr. Minoru Yamagishi, Director of Kanebo, Ltd., Cosmetics Laboratory, for his encouragement throughout this work.

REFERENCES

1 M. Shabana, *J. Pharm. Sci.*, 17 (1978) 283.
2 L. Ružicka and O. Jeger, *Helv. Chim. Acta*, 26 (1943) 265.
3 R. Steiner, *Modern Methods Plant Anal.*, 3 (1958) 58.
4 J. M. Beaton and F. S. Spring, *J. Chem. Soc.*, (1955) 3126.
5 R. Eder and A. Sack, *Pharm. Acta Helv.*, 4 (1929) 23.
6 H. Onrust and A. A. Jansen, *Rec. Trav. Chim. Pays-Bas*, 74 (1955) 1515.
7 J. Gooties and W. T. Nauta, *Rec. Trav. Chim. Pays-Bas*, 74 (1954) 886.
8 T. Namba and M. Yoshiyuki, *Yakugaku Zasshi*, 95 (1975) 809.
9 Y. Sakiya and Y. Miyaguchi, *Chem. Pharm. Bull.*, 37 (1979) 1240.
10 T. H. Beasley, Sr., H. W. Ziegler and A. D. Bell, *J. Chromatogr.*, 175 (1979) 350.
11 C. Tisse and G. Artaud, *Ann. Falsif. Expert. Chim.*, 72 (1976) 565.