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Note

High-performance liquid chromatography of naturally occurring xanthone glycosides

Separation of gentisin and isogentisin

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Xanthone glycosides occur in nature as C-glycosides or O-glycosides[1]. In the former, the sugar moiety is attached to the xanthone skeleton by a C–C bond, resulting in resistance to acidic and enzymatic hydrolysis. Only a few of these C-glycosides have been reported so far, and all possess closely related structures. O-glycosides, which contain the classic glycosidic linkage, are more complex and differ from each other in their oxidation pattern, the relative number of free hydroxyl and methoxyl group, the nature of the sugar moiety and the site of the glycosidic linkage.

The interest in xanthone glycosides is growing since it has been reported that they possess interesting pharmacological activities[2]. These compounds are usually separated by thin-layer chromatography (TLC) or classical column chromatography on polyamide; however, these techniques give poor resolution of isomeric glycosides. This paper describes the high-performance liquid chromatographic (HPLC) separation of naturally occurring glycosides, especially isomers which differ only in the position of the hydrolysable sugar on the xanthone nucleus or in the nature of the oxidation pattern.

We also report the separation of the two isomeric xanthone aglycones gentisin (1,7-dihydroxy-3-methoxyxanthone) and isogentisin (1,3-dihydroxy-7-methoxyxanthone)[3,4].

EXPERIMENTAL

The separations were carried out on a Jasco FLC-A700 instrument. The column used was made of stainless steel, 30 cm long × 4.0 mm I.D., and was slurry packed with microporous chemically bonded silica gel (μBondapak C_{18} from Waters Assoc., Milford, Mass., U.S.A.). Detection was effected by UV absorption at 260 nm with a Jasco UVIDEC-100 variable-wavelength detector. Methanol solutions (5–20 μl) were injected using a Pierce Valveseal septumless injector.

RESULTS AND DISCUSSION

In previous papers[5,6], we reported some applications of xanthone separation

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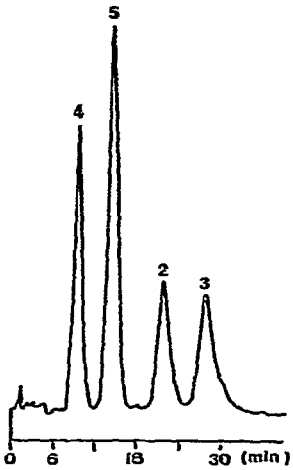
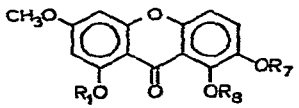


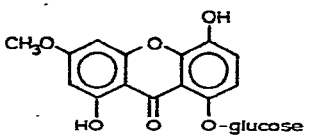
Fig. 1. Separation of various xanthone glycosides (2–5 on μBondapak C_{18}. Mobile phase, methanol–water (2:3); flow-rate, 1.5 ml/min; pressure, 2200 p.s.i.; detection, UV at 260 nm.

by HPLC. Tetramethoxyxanthone isomers and xanthone aglycones possessing one or two free hydroxyl groups could be resolved on NH_2- or CN-chemically bonded phases using *n*-hexane–chloroform mixtures as the solvent. However, attempts to separate more polar positional aglycone isomers or glycosides were unsuccessful. As reversed-phase columns have been used for the separation of flavonoid glycosides[7–9], which are structurally related to xanthone glycosides, we attempted the use of a μBondapak C_{18} column coupled with various solvent systems.

The best separations were obtained using methanol–water mixtures as the solvent. Fig. 1 shows the results obtained with four tetraoxygenated xanthone glycosides of various structures. This separation is easy and could also be achieved by polyamide TLC or open-column chromatography[1]. However, with the latter techniques, no satisfactory resolution of isomeric glycosides could be obtained. By using methanol–water (45:55), baseline separation of the two disaccharide isomers swertianin-1-O-primeveroside (1) and swertianin-8-O-primeveroside (2) which differ only in the positions of the hydrolysable sugars on the xanthone nucleus, was achieved as shown in Fig. 2. Another example of the separation of isomers is shown in Fig. 3. The separated compounds swertianin-8-glucoside (3) and bellidifolin-8-glucoside (6) carry the sugar at the same position, but differ in their oxidation pattern.



1: R_1 = primeverosyl, $R_7 = R_8$ = H; swertianin-1-O-primeveroside.
2: $R_1 = R_7$ = H, R_8 = primeverosyl; swertianin-8-O-primeveroside.
3: $R_1 = R_7$ = H, R_8 = glucosyl; swertianin-8-O-glucoside.
4: R_1 = primeverosyl, R_7 = H, $R_8 = CH_3$; gentiacaulein-1-O-primeveroside.
5: R_1 = primeverosyl, $R_7 = R_8 = CH_3$; decussatin-1-O-primeveroside.
[primeverose = 6-O-(β-D-xylopyranosyl)-β-D-glucopyranose]



6: bellidifolin-8-O-glucoside.

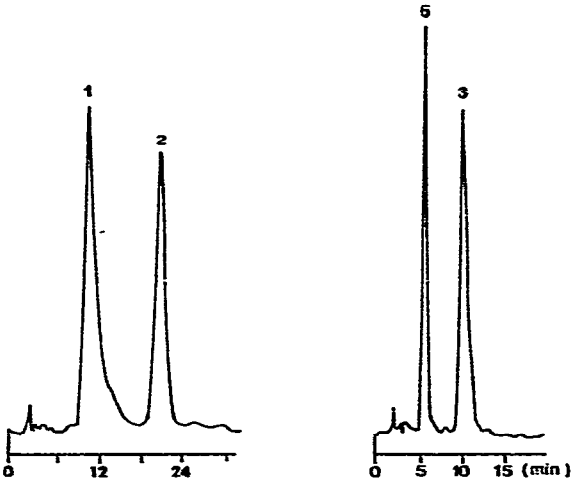


Fig. 2. Separation of the isomeric disaccharides swertianin-1-O-primeveroside (1) and swertianin-8-O-primeveroside (2). Mobile phase, methanol–water (45:55); flow-rate, 0.8 ml/min; pressure, 1500 p.s.i.

Fig. 3. Separation of the isomeric O-glucosides bellidifolin-8-O-glucoside (6) and swertianin-8-O-glucoside (3). Mobile phase, methanol–water (55:45); flow-rate, 1 ml/min; pressure, 1400 p.s.i.

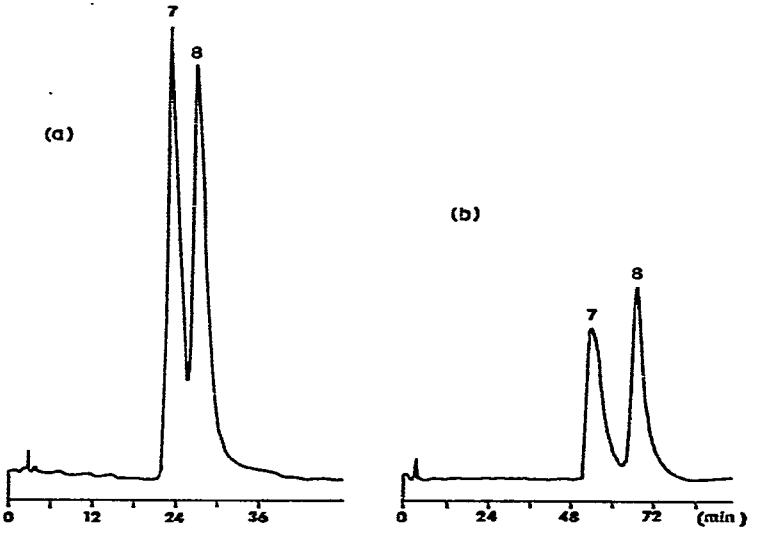
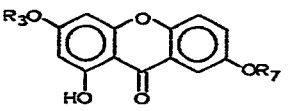


Fig. 4. (a) Separation of isogentisin (7) and gentisin (8). Mobile phase, methanol–water (1:1); flow-rate, 1 ml/min. (b) As (a), except that the solvent is methanol–water (2:3). Pressure, 1600 p.s.i.



7: $R_3 = H$, $R_7 = CH_3$; isogentisin.
8: $R_3 = CH_3$, $R_7 = H$; gentisin.

In addition, the isomeric xanthone aglycones gentisin (8) (1,7-dihydroxy-3-methoxyxanthone) and isogentisin (7) (1,3-dihydroxy-7-methoxyxanthone) have been separated using methanol–water (1:1) (see Fig. 4). As expected on a reversed-phase column, the retention time of the more polar isogentisin is shorter. An increase in the amount of water in the solvent mixture gives a better resolution, but the separation time is much longer, as shown in Fig. 4b.

Gentisin and isogentisin are useful markers in the chemotaxonomy of the *Gentianaceae*[3,4].

Gentisin is found in the roots of *Gentiana lutea* L., a well known European medicinal plant, and isogentisin occurs in the roots of the very closely related species *Gentiana purpurea* L., *Gentiana punctata* L. and *Gentiana pannonica* Scop.[3]. The separation of these isomers may provide an easy means of distinguishing the species *Gentiana lutea* L. from the other giant gentians. Thus HPLC may be useful for the quality control of *Gentiana* roots which are used in prescription drugs as well as in some liquor products.

CONCLUSION

C_{18} chemically bonded silica gel, used with different methanol–water mixtures as the solvent, seems to be an ideal system for the separation of naturally occurring xanthone glycosides. Glycosides differing only in the position of the hydrolysable sugar on the xanthone nucleus as well as isomeric glycosides with different oxidation patterns could be easily resolved. The difficult separation of the aglycones gentisin and isogentisin could also be achieved. Thus, by simply varying the methanol–water mixtures, it has been possible, using the same reversed-phase column, to separate isomeric disaccharides and monosaccharides as well as xanthone aglycones.

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REFERENCES

1 K. Hostettmann and H. Wagner, *Phytochemistry*, 16 (1977) 821.
2 S. Ghosal, P. V. Sharma, R. K. Chaudhuri and S. K. Bhattacharya, *J. Pharm. Sci.*, 64 (1975) 80.
3 A. M. Verney and A. M. Debelmas, *Ann. Pharm. Fr.*, 31 (1973) 415.
4 M. Massias, J. Carbonnier and D. Molho, *Bull. Mus. Nat. Hist. Nat., Sci. Phys.-Chim.*, 10 (1976) 45.
5 K. Hostettmann and H. M. McNair, *J. Chromatogr.*, 116 (1976) 201.
6 K. Hostettmann and A. Jacot-Guillarmod, *J. Chromatogr.*, 124 (1976) 381.
7 L. W. Wulf and C. W. Nagel, *J. Chromatogr.*, 116 (1976) 271.
8 J. F. Fisher and T. A. Wheaton, *J. Agr. Food Chem.*, 24 (1976) 898.
9 H. Becker, G. Wilking and K. Hostettmann, *J. Chromatogr.*, 136 (1977) 174.