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Note

High-pressure liquid chromatography of naturally occurring xanthones

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Xanthones occupy an important position in the chemistry of natural compounds. Their structure is related to that of flavonoids and their chromatographic behaviour is also similar. Whereas flavonoids are frequently encountered in nature, xanthones have been found in only a few families. They always occur in Guttiferae and Gentianaceae and can be considered characteristic of these plants. In the last few years, a large number of new naturally occurring xanthones have been identified in the genera Canscora[1], Swertia[2] and Gentiana[3–5] (Gentianaceae). The growing interest in these compounds is easily explained by their pharmacological activity[6] (anti-psychotic action, monoamine oxidase inhibition, anti-tubercular activity) as well as their importance in chemotaxonomy.

Xanthones are usually separated by thin-layer chromatography (TLC)[7,8], classical column chromatography[3] or by gas–liquid chromatography as the trimethylsilyl ethers[9]. The work reported here deals with the separation of naturally occurring xanthones isolated from the genus Gentiana and of their methyl ethers by high-pressure liquid chromatography (HPLC). Emphasis is given to the separation of xanthones which are poorly separated by the other chromatographic methods.

EXPERIMENTAL

Materials

All the naturally occurring xanthone samples were available from our previous work on extracts from Gentiana. The completely methylated xanthones have been obtained by treatment of the natural products by an excess of diazomethane. One exception was the 1,3,6,7-tetramethoxy xanthone which has been synthesized in our laboratory. The samples have been dissolved in chloroform to a concentration of 1 μg/μl. Since these compounds absorb well in the ultraviolet (UV), injections of microgram quantities gave good chromatographic results.

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High-pressure liquid chromatography

The separations were made on a Varian Model 8500 instrument with gradient capability. The columns used were stainless-steel, 25 cm × 2.2 mm I.D., slurry-packed with microporous chemically bonded silica gel (Micropak CN or Micropak NH_2 from Varian with 10 μm average particle size). Detection was made by UV absorption at 254 nm with a Variscan multiple wavelength detector. Solutions (1–10 μl) were injected with an SGE 10-μl syringe and the Varian stop-flow injector.

RESULTS AND DISCUSSION

We have begun our work by investigating the separation of tetramethoxy xanthones because they are not separated well by TLC on either silica gel or polyamide.

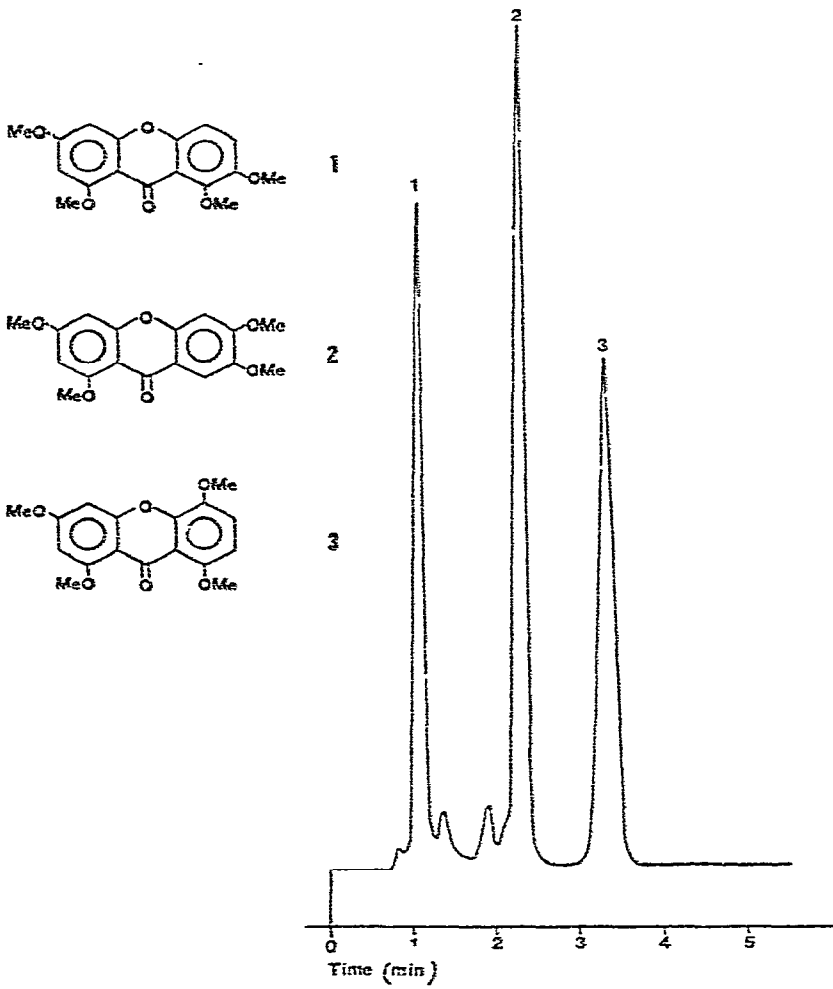


Fig. 1. Tetramethoxy xanthone isomers. Column, 25 cm × 2.2 mm Micropak CN; solvent, *n*-hexane–chloroform (13:7); flow-rate, 60 ml/h; pressure, 70 atm; sample, 5 μl (approx. 1 μg) of each compound; detector, 254 nm. Me = Methyl.

These TLC techniques have been well developed in our laboratory for xanthones and reported elsewhere[3–5].

We initially tried both 5- and 10-μm porous silica gel columns. Even with a variety of solvents and gradient systems no separation of isomers was possible. A Varian Micropak CN column gave the excellent separation of three tetramethoxy xanthone isomers shown in Fig. 1. Both the speed and high resolution confirm the advantages of HPLC for difficult isomer separations such as shown here. Fig. 2 shows the separation of a more complex mixture. This sample contains three tetramethoxy xanthone isomers as well as a trimethoxy xanthone and a tetramethoxy flavone. This mixture is typical of plant extracts which have been methylated.

Methylation of xanthones is a useful technique for structural determinations[8]. HPLC using chemically bonded phases offers a rapid separation of these compounds.

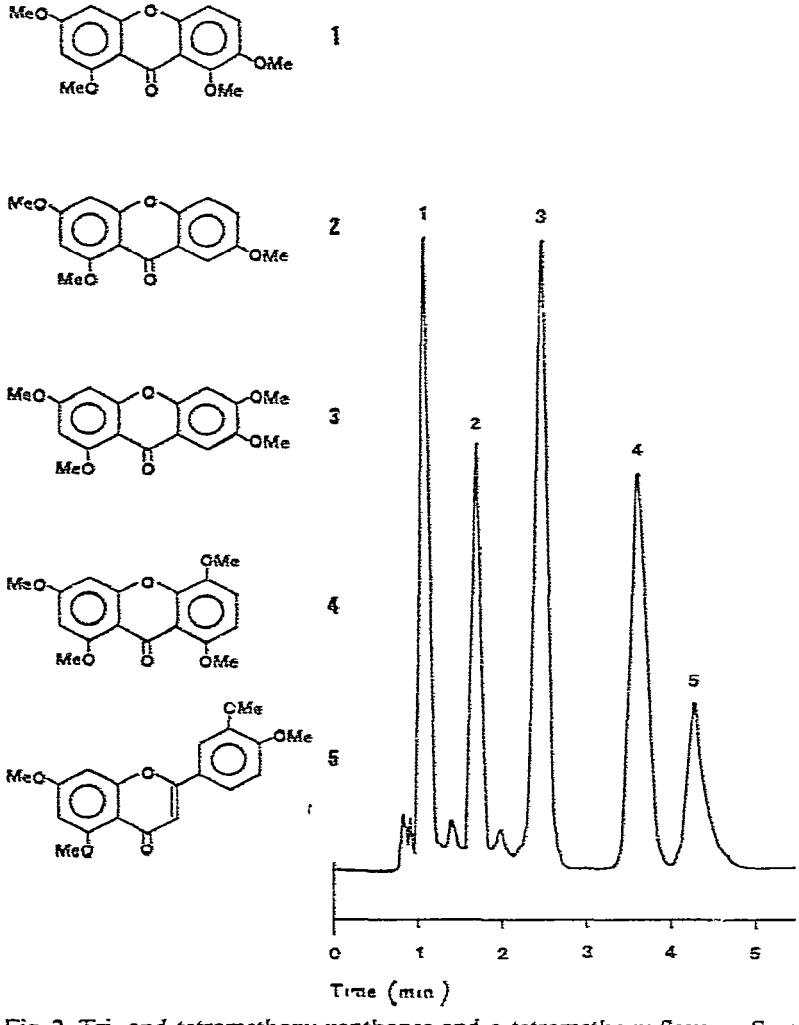


Fig. 2. Tri- and tetramethoxy xanthones and a tetramethoxy flavone. Same conditions as Fig. 1.

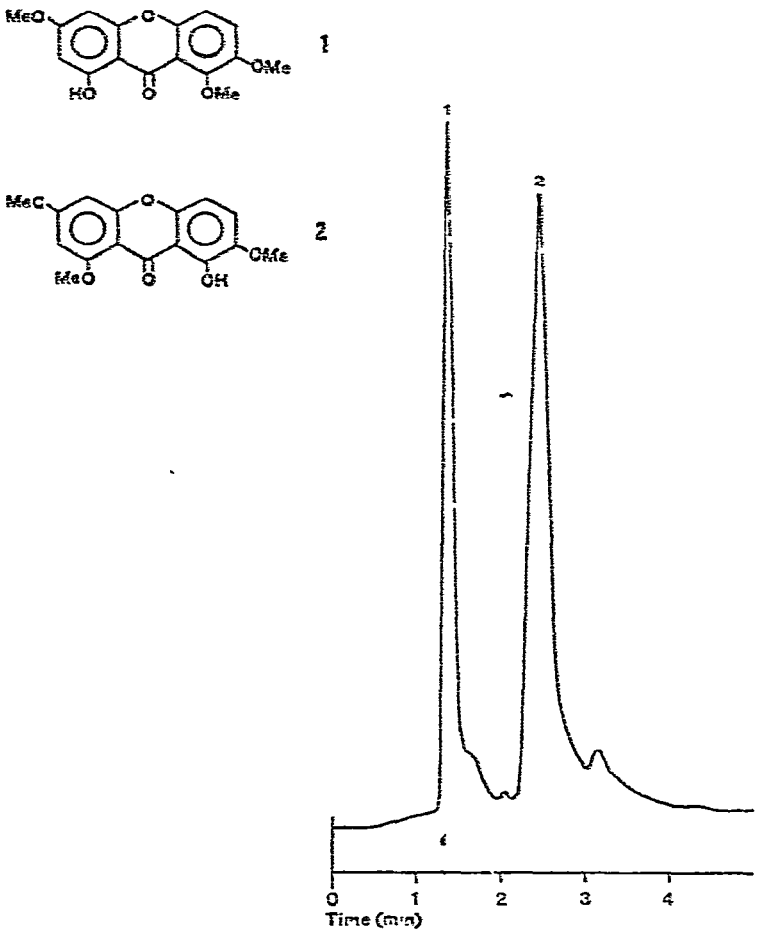
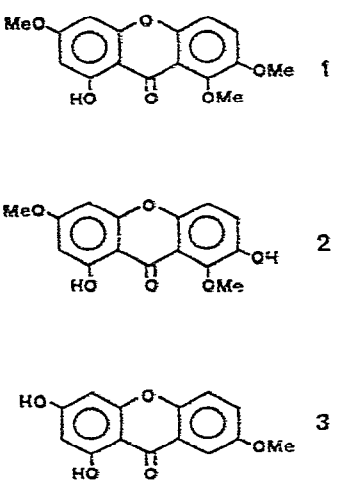


Fig. 3. Monohydroxy xanthone isomers. Conditions are the same as in Fig. 1 except the solvent which is *n*-hexane–chloroform (2:3).

It is necessary however to separate xanthones containing free hydroxyl groups as well. To date we have been only moderately successful in the separation of the more polar xanthones. Fig. 3 shows the separation of two monohydroxy isomers. Note that a more polar solvent is required. Fig. 4 shows the results obtained with dihydroxy xanthones. On the Micropak CN column the third peak tails badly, conditions of solvent polarity and flow-rate are critical. Also shown in Fig. 4 is the same sample on a Micropak NH_2 column. The active functional group is a weaker $-NH_2$ group and we would expect less retention of the polar samples on this column. This conclusion is verified by the result shown in both Figs. 4 and 5. Fig. 5 shows the separation of a tri- and tetrahydroxy xanthone. These are the most polar xanthones separated to date, but in future work we will use reverse-phase columns in an attempt to increase the range of xanthones handled rapidly by HPLC. Attempts to separate positional isomers of either tri- or tetrahydroxy xanthones under conditions similar to Fig. 5 were unsuccessful.



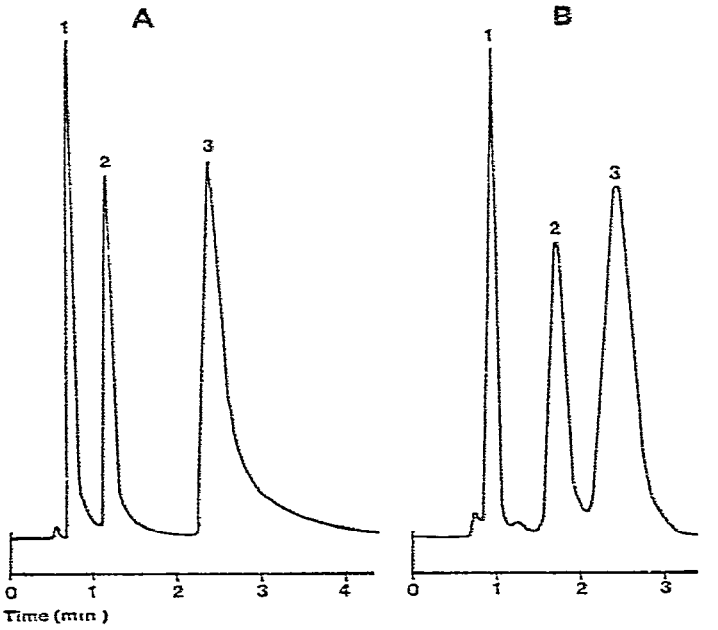
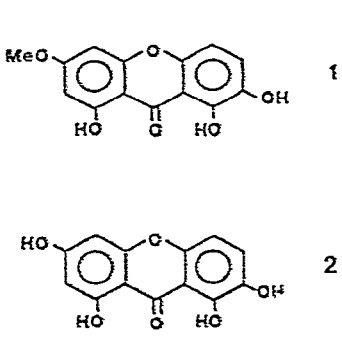


Fig. 4. Dimethoxy xanthones on Micropak CN and Micropak NH_2 columns. A: Column, 25 cm × 2.2 mm Micropak CN, solvent, cyclohexane–chloroform (1:4); flow-rate, 80 ml/h, pressure, 70 atm. B: Column, 25 cm × 2.2 mm Micropak NH_2; solvent, isooctane–chloroform (3:17); flow-rate, 70 ml/h; pressure, 60 atm.



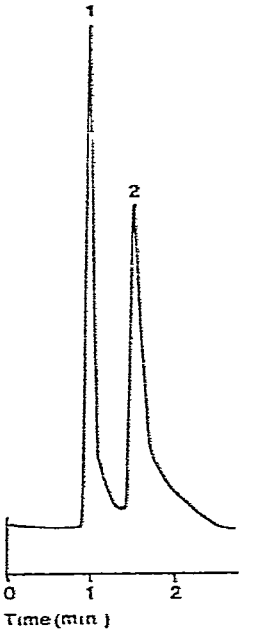


Fig. 5. Tri- and tetrahydroxy xanthones. Column, Micropak NH_2; solvent, dioxane–dichloromethane (1:9); flow-rate, 70 ml h: pressure, 60 atm.

CONCLUSION

HPLC is used to separate naturally occurring xanthones isolated from the genus Gentiana and their methyl ethers. Positional isomers, as well as mono-, di-, tri- and tetrahydroxy xanthones, are resolved on two chemically bonded phases: Micropak NH_2 and Micropak CN.

HPLC offers the separation of xanthone isomers not possible by TLC or column chromatography. Results in our laboratory with microporous silica gel have shown essentially no selectivity for positional isomers of xanthones. More polar xanthones, with three and four free hydroxyl groups, require more polar solvents and the less polar Micropak NH_2 column. Reversed-phase columns may prove even more useful.

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