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Journal of Chromatography A, 709 (1995) 345–349

JOURNAL OF
CHROMATOGRAPHY A

Supercritical fluid chromatography of free resin acids on an ODS-silica gel column

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First received 23 November 1994; revised manuscript received 6 April 1995; accepted 6 April 1995

Abstract

The supercritical fluid chromatographic behaviour of resin acids on an inert ODS-silica gel column using supercritical carbon dioxide as the mobile phase was studied. The separation of resin acids could be achieved considerably better by using methanol as a modifier. According to these results, the isomers of abietane-type acids could be separated by SFC without derivatization, particularly esterification.

1. Introduction

Rosin is a natural resin obtained from coniferous trees [1]. The main constituent of this industrially very important material is a mixture of several (usually seven to eight) diterpene monocarboxylic acids known as resin acids, as shown in Fig. 1 [2]. They are closely related to one another in their chemical structures, among which abietic acid and pimaric acid are representative ones for their skeletal features.

In practice, the gas chromatographic analysis of rosin is routinely carried out for quality control in industry following the procedure based on ASTM D3008 [3] using a packed column. This method, however, requires methylation of rosin to improve the volatility of resin acids.

Although a capillary column can easily accomplish a better resolution of resin acids [4], the service life of capillary columns needs considerable improvement, in our experience.

The application of HPLC to the analysis of rosin has had limited success [5,6]. Otsuka and Kubo [6] were able to separate pine oleoresin to its components (resin acids) by HPLC on an ODS-silica gel column only after extensive re-cycling.

In recent years, supercritical fluid chromatography (SFC) has been developed as separation technique that is a bridge between GC and LC. Carbon dioxide is usually used as the mobile phase in SFC because of its properties, safety and cost. However, the addition of a modifier to the mobile phase is often required in order to improve the retention behaviour and the peak shapes [7–13]. An early study of the application

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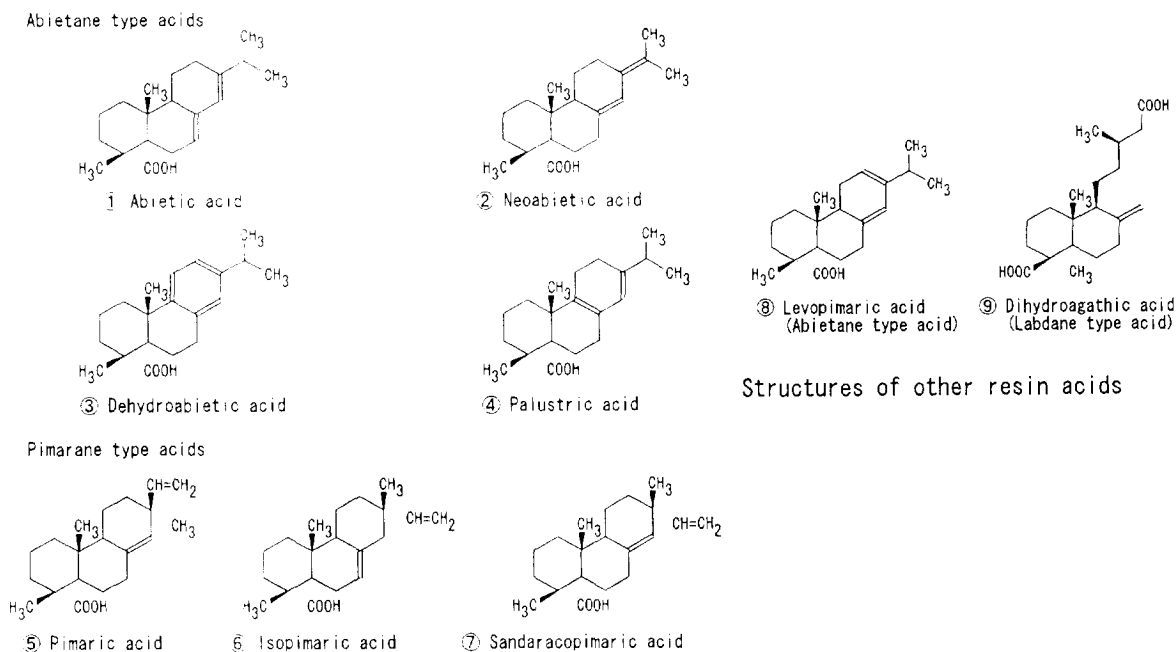


Fig. 1. Structures of resin acids in Chinese gum rosin.

of SFC to rosin analysis was reported by Gere [14], who was able to separate abietic and neoabietic acid on a polystyrene gel column. However, other resin acids were not investigated.

This paper describes the elution behaviour of free resin acids without any derivatization by SFC on an ODS-silica gel column.

2. Experimental

2.1. Apparatus

A Shimadzu (Kyoto, Japan) LC-6A pump was used to deliver carbon dioxide. The pump head was cooled so as to maintain a stable flow. An Isco (Lincoln, NE, USA) Model 100 DM pump was used to deliver modifier. A DKK (Tokyo, Japan) LSA-M mixer was used for mixing carbon dioxide and modifier. A Rheodyne (Cotati, CA, USA) Model 7125 sample injector with a 20- μ l sample loop was used for sample injection. The separation column was kept at 40°C in a column oven from a Shimadzu LC-1 system. A Shimadzu

SPD-6A UV detector was used for detection at 210 nm. The flow-rate of the mobile phase was controlled by a restrictor made of a capillary tube of 300 mm \times 50 μ m I.D. This SFC system is the same as described previously [15].

2.2. Materials

The separation column used in the SFC was an L-column ODS (250 mm \times 4.6 mm I.D., particle size 5 μ m, pore diameter 120 Å) from the Chemicals Inspection and Testing Institute (Tokyo, Japan). An ODS-silica gel column applied to the SFC separation of polar samples using carbon dioxide as the mobile phase without a modifier usually gives considerable tailing of peaks and retardation of sample elution, because polar compounds such as resin acids tend to adsorb to the residual silanol groups of the packing material. In the L-column ODS, the residual silanol groups are minimal.

The rosin sample was a commercial gum rosin, X grade, made in China, which is known to contain seven diterpene resin acids [2]. An aliquot of 0.5 μ l of 5% rosin solution in chloro-

form was used for injection. The authentic pure resin acids, including levopimaric and dihydrogathic acids, used as the reference materials, were conventionally isolated by known methods [2].

3. Results and discussion

The chromatogram of Chinese gum rosin obtained by SFC without a modifier is shown in Fig. 2. The identification of each peak was based on the authentic reference materials.

As can be seen, pimarane-type acids eluted first in one peak followed by abietane-type acids. However, the separation of these acids into individual peaks was not accomplished and all the peaks had considerable tailing, presumably because of adsorption of resin acids on the residual silanol groups still remaining in the ODS-silica gel packing. The peak of neoabietic acid became very broad and could not be identified, for example. The chromatographic separation by SFC could not be improved by varying the carbon dioxide pressure from 12 to 21 MPa.

To improve the separation of resin acids, three types of modifier, water, 1,4-dioxane and methanol, were evaluated. With water, a water content of 0.31% (w/w) was the maximum amount that can be added as a modifier owing to its solubility

in supercritical carbon dioxide. In such a case, the chromatogram was almost the same as that without a modifier (Fig. 2).

Using 2% (w/w) 1,4-dioxane as a modifier, a considerably better separation with less peak tailing compared with the result obtained with water as modifier could be achieved. The use of 1.2% (w/w) methanol instead of dioxane gave an even better result, as shown in Fig. 3. Although the elution pattern of the resin acids using methanol as a modifier was almost the same as that of dioxane modifier, higher chromatographic resolutions calculated for two adjacent peaks were obtained using methanol as a modifier, so further detailed studies were conducted with methanol as a modifier.

The elution behaviour of resin acids is affected by the addition of a methanol modifier, hence addition of less than 1% (w/w) of methanol made the resolution poorer because of peak tailing whereas too much methanol gave poorer resolution. As a result, the addition of 1.2% (w/w) methanol was adopted as the most suitable in further detailed studies. Fig. 4 shows the relationship between $\log k'$ of resin acids and the methanol concentration in the mobile phase. Chloroform was used to obtain t_0 for calculating k' . A linear relationship was obtained above a concentration of about 0.5% (w/w) of methanol,

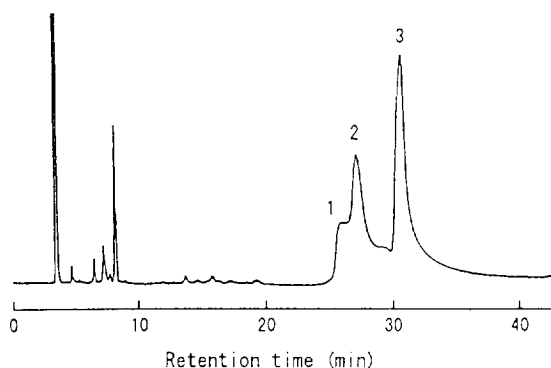


Fig. 2. Chromatogram of Chinese gum rosin. Peaks: 1 = pimaric acid, isopimaric acid; 2 = dehydroabietic acid, palustric acid; 3 = abietic acid. Conditions: CO₂ pressure, 12 MPa; CO₂ flow-rate, 430 ml/min at room temperature under 760 mmHg; other conditions as in text.

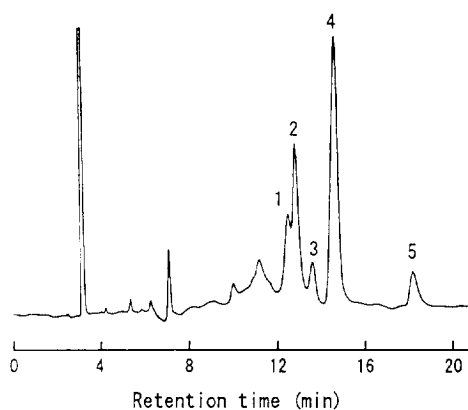


Fig. 3. Chromatogram of Chinese gum rosin [modifier: 1.2% (w/w) methanol]. Peaks: 1 = pimaric acid; 2 = sandaracopimaric acid, isopimaric acid, dehydroabietic acid; 3 = palustric acid; 4 = abietic acid; 5 = neoabietic acid. Other conditions as in Fig. 2.

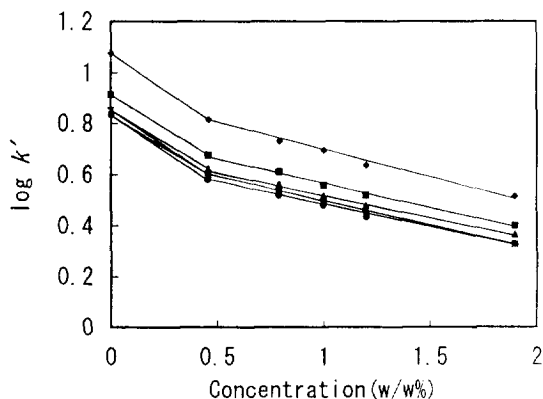


Fig. 4. Effect of methanol concentration in the mobile phase on k' . \blacklozenge = Neoabietic acid; \blacksquare = abietic acid; \blacktriangle = palustric acid; \times = dehydroabietic acid; \diamond = sandaracopimaric acid, isopimaric acid; \bullet = pimaric acid.

although the elution order of the resin acids remained unaltered. The difference between the values obtained by extrapolating to the ordinate and those observed seems to be caused by adsorption of the resin acids on the residual silanol groups.

Although the chromatogram shown in Fig. 3 was better, some efforts were necessary to improve the separation of peaks 1-3 by connecting the same type of columns. The chromatogram obtained under the same conditions as in Fig. 3 except that three of the same ODS-silica gel columns were connected in series is shown in Fig. 5. The pressure of the mobile phase was adjusted so that the same mean pressure calculated from the inlet and outlet pressures of the columns could be established among three types of column connection, that is, single, double and triple connections. A three times longer analysis time was necessary, as in LC analysis, but considerably better resolution was obtained by tripling the column length, as expected.

Using a 50 cm (2×25 cm) column length, the elution behaviour of two important resin acids, levopimaric and dihydroagathic acids, both absent from Chinese gum rosin, was studied. As indicated before, levopimaric acid cannot be separated from palustric acid by GC under the current ASTM packed column standard conditions [3], but this resolution is readily accomplished with capillary columns [4].

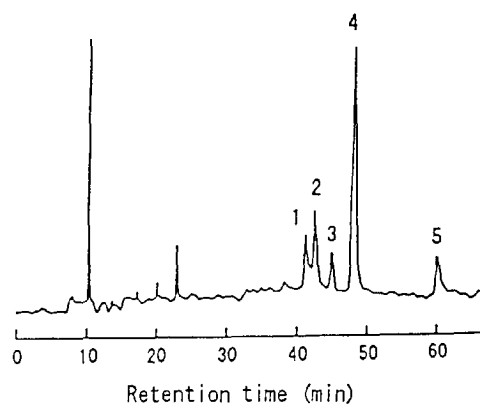


Fig. 5. Chromatogram of Chinese gum rosin on three columns [modifier: 1.2% (w/w) methanol]. Other conditions as in Fig. 2 and peak identifications as in Fig. 3.

Dihydroagathic acid, specifically contained in S. East Asian pine resin (*Pinus merkusii* Jungh. & de Vriese exudate) and easily separable by GC, has a characteristic structure with two carboxyl groups in the molecule. These two acids were mixed with Chinese gum rosin and subjected to SFC, giving the chromatogram shown in Fig. 6.

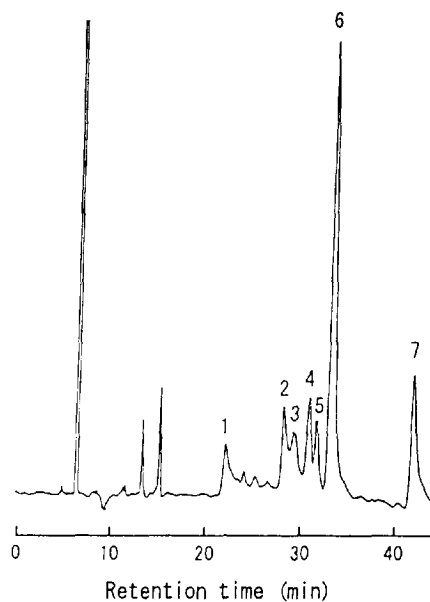


Fig. 6. Chromatogram of nine resin acids on two columns. Peaks: 1 = dihydroagathic acid; 2 = pimaric acid; 3 = sandaracopimaric acid, isopimaric acid, dehydroabietic acid; 4 = palustric acid; 5 = levopimaric acid; 6 = abietic acid; 7 = neoabietic acid. Other conditions as in Fig. 2.

Interestingly, dihydroagathic acid was eluted far ahead of others and levopimaric acid was eluted immediately after palustric acid.

4. Conclusion

The elution behaviour of resin acids by SFC on an inert ODS-silica gel column using supercritical carbon dioxide as the mobile phase was studied. The separation of resin acids could be considerably improved by using methanol as a modifier. According to the results, the isomers of abietane-type acids could be separated by SFC without derivatization, particularly esterification. Therefore, the development of a perfectly inert column is still required for the SFC separation of free resin acids (rosin per se without esterification, for example) without a modifier.

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