## Gas–liquid chromatography of anthraquinones

Previously we have reported successful applications of gas–liquid chromatography to the separation of opium alkaloids[1], plant glycosides[2], flavonoids[3] and lichen triterpenoids[4]. The present paper deals chiefly with the gas–liquid chromatography of naturally occurring and synthetic anthraquinones using 26 samples which were applied for the process in their free forms or as trimethylsilyl ethers. The relationships between the chemical structures and the retention times are discussed.

*Experimental*

*Materials.* Almost all the anthraquinones used in this study were available in our laboratory. "Reagent grade" solvents were employed for running the chromatography.

*Trimethylsilylation*[3]. One to two mg of sample was dissolved in 0.1 ml of anhydrous pyridine. To the solution 0.1 ml of hexamethyldisilazane and 0.05 ml of trimethylchlorosilane were added. The mixture was shaken vigorously in a glass-stoppered vial for about 30 sec and allowed to stand for 10 min. The reaction mixture was centrifuged, and 0.5 to 2 $\mu$l of the supernatant solution was applied to the gas chromatograph by injection using a Hamilton microsyringe.

*Apparatus.* A Shimadzu Model GC-1B Gas Chromatograph equipped with a hydrogen flame detector was used in this study.

A stainless steel column (U-shape, 2.25 m × 4 mm I.D.) was packed with 1.5 % SE 30 on Chromosorb W (60–80 mesh). $N_2$ flow rate: 110.5 ml/min; column temperature: 240°; detector temperature: 240°; flash heater temperature: 312°.

*Results and discussion*

The gas chromatographic data of the anthraquinones are given in Table I.

The retention time increases with increasing number of hydroxyls in the anthraquinone nucleus. Thus, anthraquinone itself, which has no hydroxyl group, has the shortest retention time. It is of interest to note that 1-hydroxyanthraquinone has a shorter retention time than 2-hydroxyanthraquinone. This may be caused by intramolecular hydrogen bonding in the former.

Similar results were observed on comparing the dihydroxyanthraquinones. Thus, the $\alpha,\alpha$-dihydroxyanthraquinones such as quinizarin, chrysazin, and anthrarufin, have the shortest retention times, while those of the $\alpha,\beta$-disubstituted anthraquinones, such as alizarin and purpuroxanthin, are medium, and those of the $\beta,\beta$-disubstituted anthraquinones, such as histazarin, are the longest.

The effect of intramolecular hydrogen bonding in decreasing the retention time can be demonstrated by the reversed effect which occurs when the $\alpha$-hydroxyl is blocked by methylation or trimethylsilylation. However, such a blocking of hydroxyl groups revealed that the retention time of non-chelated $\beta$-hydroxyanthraquinones, such as histazarin, is also shortened.

An increase of retention time in the sequence $CH_3$, $CH_2OH$ and COOH was observed in a series of $\beta$-, C-substituted naturally occurring anthraquinones, such as chrysophanol, aloe-emodin and rhein. This was demonstrated using a chloroform extract 20 mg of hydrolysates from Chinese rhubarb 5 g which was gas chromatographed after trimethylsilylation (Fig. 1).

TABLE I

RETENTION TIMES OF ANTHRAQUINONES

| *Original compounds* | *Derivatives** | $t_R$ (*min*) |
|---|---|---|
| Anthraquinone | | 1.3 |
| 1-Hydroxyanthraquinone | | 1.7 |
| | Me | 2.3 |
| | TMSi | 2.3 |
| 2-Hydroxyanthraquinone | | 2.9 |
| | Me | 2.3 |
| | TMSi | 2.8 |
| Alizarin (1,2-dihydroxyanthraquinone) | | 2.3 |
| | Me (2) | 3.2 |
| | Me (1, 2) | 3.4 |
| | TMSi (1, 2) | 4.5 |
| Purpuroxanthin (1,3-dihydroxyanthraquinone) | | 3.7 |
| | TMSi (1, 3) | 4.3 |
| | Me (1), TMSi (3) | 5.8 |
| Quinizarin (1,4-dihydroxyanthraquinone) | | 2.1 |
| | Me (1) | 2.7 |
| | TMSi (1, 4) | 3.7 |
| Anthrarufin (1,5-dihydroxyanthraquinone) | | 2.1 |
| | TMSi (1, 5) | 4.1 |
| Chrysazin (1,8-dihydroxyanthraquinone) | | 2.2 |
| | Me (1) | 3.9 |
| | TMSi (1, 8) | 4.3 |
| Histazarin (2,3-dihydroxyanthraquinone) | | 8.4 |
| | Ac (2, 3) | 5.7 |
| | TMSi (2, 3) | 5.2 |
| Anthragallol (1,2,3-trihydroxyanthraquinone) | | 5.2 |
| | TMSi (1, 2, 3) | 6.4 |
| Purpurin (1,2,4-trihydroxyanthraquinone) | | 4.7 |
| | TMSi (1, 2, 4) | 6.4 |
| Chrysophanol (1,8-dihydroxy-3-methylanthraquinone) | | 2.7 |
| | TMSi (1, 8) | 5.4 |
| Aloe-emodin (1,8-dihydroxy-3-hydroxymethyl-anthraquinone) | | 7.9 |
| | TMSi (1, 8) | 11.4 |
| Rhein (1,8-dihydroxyanthraquinone-3-carboxylic acid) | TMSi (1, 3, 8) | 14.0 |
| Emodin (1,6,8-trihydroxy-3-methylanthraquinone) | | 6.9 |
| | TMSi (1, 6, 8) | 10.7 |
| Physcion (1,8-dihydroxy-6-methoxy-3-methylanthraquinone) | | 5.3 |
| | TMSi (1, 8) | 9.2 |
| Islandicin (1,4,8-trihydroxy-3-methylanthraquinone) | | 3.8 |
| | TMSi (1, 4, 8) | 8.2 |
| Catenarin (1,4,6,8-tetrahydroxy-3-methylanthraquinone) | TMSi (1, 4, 6, 8) | 14.3 |

* Me = methyl ether; TMSi = trimethylsilyl ether; Ac = acetate. Figures in parentheses indicate the positions of substitution.

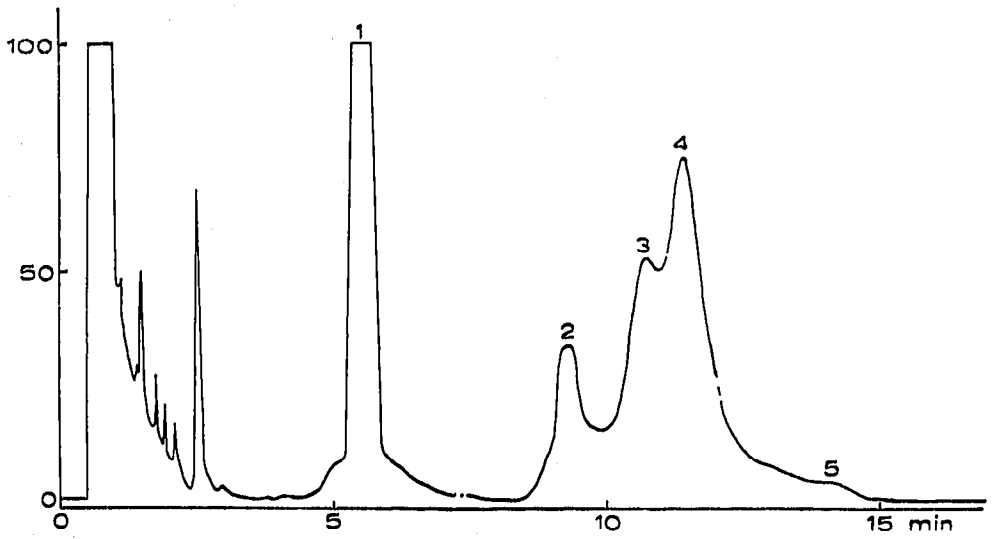


Fig. 1. Gas chromatogram of the trimethylsilylated anthraquinone fraction from Chinese rhubarb. 1 = Chrysophanol; 2 = physcion; 3 = emodin; 4 = aloe-emodin; 5 = rhein.

As the trimethylsilyl ethers of anthraquinones generally give fine sharp peaks in the gas chromatograms, these derivatives can be used for quantitative purposes.

*Acknowledgements*

We thank Miss K. KATSUMOTO for her assistance in carrying out the gas chromatography.

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Received July 12th, 1965