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Note

Gas chromatographic separation of monomethyl ethers of dihydroxycoumarin isomers

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Dihydroxycoumarins and their methyl ethers are natural products that often coexist in the same plant and exert a variety of biological actions[1]. In particular, 6,7-dimethoxycoumarin is the main factor in the cholekinetic activity of *Artemisiae capillaris* Flos, which is used as a crude drug in Chinese medicine[2]. The urinary metabolites of this compound in rabbits, 6-hydroxy-7-methoxycoumarin and 7-hydroxy-6-methoxycoumarin, have been separated and determined by paper chromatography[3].

Gas chromatographic (GC) methods have been applied to the analysis of many neutral and phenolic coumarins[4–7]. Phenolic coumarins have been chromatographed in the free form on an SE-30 column[6], as their acetates on a silicone grease column[4] and as their trimethylsilyl ethers on an SE-30 column[5]. However, no GC study has been reported for the separation of a mixture of two monomethyl ether isomers of a dihydroxycoumarin. In this paper we describe the separation of monomethyl ethers of 6,7-, 7,8- and 5,7-dihydroxycoumarin isomers using a column packed with a phthalate–alkylene glycol polyester phase.

EXPERIMENTAL

Chemicals

The monomethyl ethers of dihydroxycoumarins listed in Table I were synthesized and their structures confirmed as described previously[1].

Gas chromatography

GC analysis was carried out on a Shimadzu GC-9A gas chromatograph equipped with a flame ionization detector. The glass column (1 m × 3 mm I.D.) used was packed with 5% Thermon 3000 on Chromosorb W AW DMCS (80–100 mesh) or 2% Thermon 3000 on Gas-Chrom Q (80–100 mesh), supplied by Shimadzu (Kyoto, Japan). The column temperature was 250°C (isothermal), the injector temperature 280°C and the detector temperature 280°C. Nitrogen was used as the carrier gas at a flow-rate of 60 ml/min.

RESULTS AND DISCUSSION

For the separation of a mixture of 6-hydroxy-7-methoxycoumarin and 7-hydroxy-6-methoxycoumarin we compared seven packed columns containing 5% SE-

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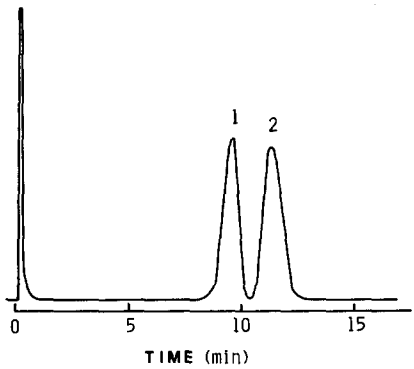

Fig. 1. Gas chromatogram of 6-hydroxy-7-methoxycoumarin (1) and 7-hydroxy-6-methoxycoumarin (2) on a 5% Thermon 3000 column (1 m × 3 mm I.D.). Column temperature, 250°C; carrier gas, nitrogen at a flow-rate of 60 ml/min.

30, 2% OV-1, 2% OV-17, 2% DC-QF-1, 2% XE-60, 2% DEGS + 0.5% H_3PO_4 and 5% Thermon 3000. Although most of the columns tested gave poor separations or no peaks were observed, 5% Thermon 3000, a phthalate–alkylene glycol polyester phase, gave a successful chromatogram, as shown in Fig. 1. Both the peak area responses of the two monomethyl ethers were linearly related to amount in the range 0.2–2 μg.

A 5% Thermon 3000 column also gave a good separation of the two monomethyl ethers of 7,8-dihydroxycoumarin. The retention times of 7-hydroxy-8-methoxycoumarin and 8-hydroxy-7-methoxycoumarin were 4.27 and 9.56 min, respectively, on a 5% Thermon 3000 column under the conditions used. Their responses were similar to those of 6,7-dihydroxycoumarin monomethyl ethers above.

On the other hand, the two monomethyl ethers of 5,7-dihydroxycoumarin were not eluted on a 5% Thermon 3000 column, probably because of decomposition during their longer retention times. We therefore selected 2% Thermon 3000 on Gas-Chrom Q as the stationary phase. Table I shows the relative retention times of the

TABLE I

RELATIVE RETENTION TIMES OF MONOMETHYL ETHERS OF DIHYDROXYCOUMARINS ON A 2% THERMON 3000 COLUMN

Column, 1 m × 3 mm I.D.; column temperature, 250°C; carrier gas, nitrogen at a flow-rate of 60 ml/min. Relative retention time for 6-hydroxy-7-methoxycoumarin taken as 1.00 (absolute retention time 3.79 min).

Compound	*Relative retention time*
6-Hydroxy-7-methoxycoumarin	1.00
7-Hydroxy-6-methoxycoumarin	1.12
7-Hydroxy-8-methoxycoumarin	0.44
8-Hydroxy-7-methoxycoumarin	0.97
5-Hydroxy-7-methoxycoumarin	5.38
7-Hydroxy-5-methoxycoumarin	4.96

six monomethyl ethers on a 2% Thermon 3000 column. 7-Hydroxy-5-methoxycoumarin was eluted at 18.8 min and 5-hydroxy-7-methoxycoumarin at 20.4 min, although partial overlap of the two peaks occurred.

We conclude that a Thermon 3000 column is suitable for the separation of monomethyl ethers of dihydroxycoumarin isomers.

REFERENCES

1 R. D. H. Murray, J. Méndez and S. A. Brown, *The Natural Coumarins*, Wiley, Chichester, 1982.
2 M. Kimura, *Proc. 10th Symposium on WAKAN-YAKU, Toyama, August 28–29, 1976*, Research Institute for WAKAN-YAKU, Toyama, 1977, pp. 121–126.
3 T. Furuya, *Chem. Pharm. Bull.*, 6 (1958) 696.
4 S. A. Brown and J. P. Shyluk, *Anal. Chem.*, 34 (1962) 1058.
5 T. Furuya and H. Kojima, *J. Chromatogr.*, 29 (1967) 382.
6 W. Steck and B. K. Bailey, *Can. J. Chem.*, 47 (1969) 3577.
7 H. E. Nordby and S. Nagy, *J. Chromatogr.*, 207 (1981) 21.