Notes

CHROM. 5763

Gas–liquid chromatography of coumarin anticoagulants and their trimethylsilyl ethers

The use of gas–liquid chromatography for the separation of plant coumarins was first reported by BROWN AND SHYLUK[1] in 1962, and this application has been extended by other workers[2,3] concerned with the chemistry of natural products. Our interest in the coumarin anticoagulants led to the adaptation of this analytical method to the detection of these drugs.

The present paper describes the results obtained with coumarin, 4-hydroxycoumarin, five anticoagulants derived from 4-hydroxycoumarin, and the trimethylsilyl ethers of some of these compounds.

Experimental

Materials. Except for coumarin, which was obtained commercially (Merck), all the compounds used in this study were generously donated: 4-hydroxycoumarin, warfarin and bishydroxycoumarin (Wisconsin Alumni Research Foundation, Madison, Wisc.); acenocoumarin (Ciba-Geigy, Basel); phenprocoumon (Hoffman-La Roche, Grenzach/Baden); and ethyl biscoumacetate (Geigy and Roche).

Trimethylsilation[4]. One to two mg of a compound was dissolved in 100 μl of anhydrous pyridine (dimethylformamide, dimethylsulphoxide and chloroform were also used successfully), and 100 μl of hexamethyldisilazane and 50 μl of trimethylchlorosilane were added to this solution. The mixture was agitated mechanically in a stoppered conical test-tube for about 1 min and centrifuged after 10 min. A Hamilton microsyringe was used to inject 0.5–3 μl of the supernatant solutions.

Apparatus. A Varian Aerograph Model 1200 gas chromatograph with a flame ionization detector was used. The 5 ft. × 1/8 in. copper column was packed with 5% SE-30 on Chromosorb W (60–80 mesh)/DMCS. Unless otherwise indicated, the flow-rate of the nitrogen carrier gas was 30 ml/min, and the temperatures of the injector, column oven, and detector were 245°, 205° and 270°, respectively.

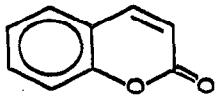
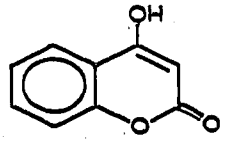
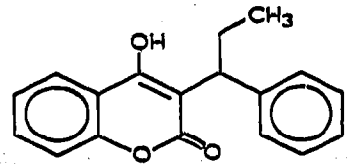
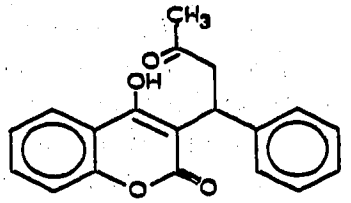
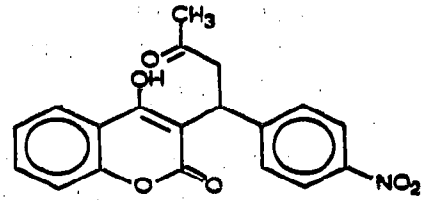
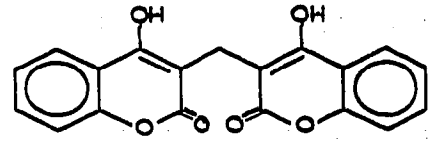
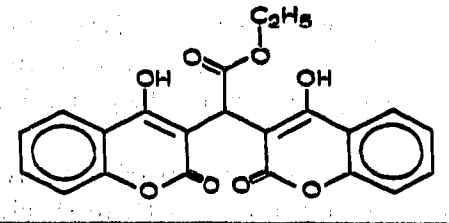
Results and discussion

The retention times observed for the above system are reported in Table I. The monohydroxycoumarins, as well as their trimethylsilyl derivatives, generally gave single sharp peaks in this system, although FURUYA AND KOJIMA[2] reported that free hydroxycoumarins did not give good results. Perhaps this is due to the use of the DMCS-treated support in the present study, whereas they used untreated Chromosorb W.

The retention times of the four monohydroxycoumarins increase with molecular weight for either the free or the derwatized series, although the presence of other functional groups in the side chains obviously also adds to the retention. The anomalously short retention time of free acenocoumarin (1.1 min) may be due to interaction of its nitro and hydroxyl groups. When this interaction was blocked by trimethyl-

TABLE I

RETENTION TIMES OF COUMARIN ANTICOAGULANTS

Compound (mol. wt.)	*Structure*	t_R *(min)*	
		Free	*Trimethylsilyl derivative*
Coumarin (146)		0.5	—
4-Hydroxycoumarin (162)		1.0	1.2 (1.8, 190°) (2.0, 182°) (4.0, 162°)
Phenprocoumon (Marcumar®) (280)		4.4 (8.8, 190°) (13, 180°) (20, 170°)	6.4 (7.2, 202°) (3.4, 220°)
Warfarin (Coumadin®) (308)		8.8 (6.9, 210°) (5.7, 215°) (4.8, 220°) (4.1, 225°)	9.7 (8.2, 210°) (7.1, 215°) (6.0, 220°) (5.0, 225°)
Acenocoumarin (Sintrom®) (353)		1.1 (1.6, 180°) (2.8, 160°)	>30 (16.7, 220°)
Bishydroxycoumarin (Dicumarol®) (336)		32 (22, 215°, decomp.) (14, 225°, decomp.)	—
Ethyl biscoumacetate (Tromexan®) (408)		56 (44, 220°, decomp.) (30, 225°, decomp.)	—

silylation of the hydroxyl group, the retention time was greatly increased (to >30 min).

The retention times observed for bishydroxycoumarin and ethyl biscoumacetate were much longer, due to the presence of two hydroxyl groups per molecule; consequently, the peaks observed for these compounds showed considerable broadening. Injection of the trimethylsilyl preparations of these two compounds did not yield single characteristic peaks.

Retention times for the compounds at other than the standard temperature (205°) are given in parentheses in Table I. Lower retention times usually resulted in sharper peaks, although there was little advantage in operating above 205° for the monohydroxycoumarins. Again, acenocoumarin was an exception: Because of the long retention time of its trimethylsilyl derivative, elevated temperatures greatly sharpened the peak. Bishydroxycoumarin and ethyl biscoumacetate partly decomposed at the elevated temperatures.

Higher flow-rate of the carrier gas also improved the peak quality in some instances by reducing the retention time. For instance, the retention time for the trimethylsilyl derivative of phenprocoumon at 202° could be reduced from 7.2 min at a flow-rate of 30 ml/min to 3.7 min at 90 ml/min. For the trimethylsilyl derivative of acenocoumarin, the use of both elevated temperature and flow-rates resulted in greatly improved peaks: *e.g.* at a flow-rate of 70 ml/min the retention time is 10.7 min at 220° and 7.2 min at 230°; at a flow-rate of 80 ml/min the retention time is 8.8 min at 220° and 6.7 min at 230°.

The system described here has been successfully applied to separate mixtures of some of these compounds (*e.g.*, warfarin and phenprocoumon, two widely used anticoagulants) and to detect these compounds after extraction from plasma containing relatively high levels of anticoagulants. Thus this method might be useful for direct chemical or *in vitro* metabolism studies, as in such cases the concentrations are sufficiently high. Application of this method to pharmacokinetic studies and clinical analyses is under investigation.

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