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Gas-Liquid Chromatography of Flavone Ethers

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Gas-liquid chromatography of methyl- and trimethylsilyl ethers of hydroxyflavones on single and mixed liquid phases of low polarity has been studied and relative retention times have been determined. The rate of silylation as a function of structure has been studied. The application of GLC analysis to mixed methyl-trimethylsilyl ethers as an aid in the determination of flavone glycoside structure has been evaluated.

Separation of flavonoids by paper and thin-layer chromatography are common and important procedures. Gas-liquid chromatography (GLC), however, has received less attention probably because of the difficulties caused by the low volatility of the hydroxylated flavonoids. Narasimhachari and von Rudloff [1] carried out GLC of derivatives of mono-, di- and trihydroxyflavones, isoflavanones, and flavanones using the silicone polymer SE-30 as liquid phase. Some partially and completely methylated polyhydroxyflavonols were also separated on special columns. Furuya [2] has determined the retention times of a number of trimethylsilyl (TMS) ethers of flavonoids using the same liquid phase. Anthocyanidins have been separated by Keith and Powers [3] and Al-Shakir,[4] and GLC based methods for the analysis of flavanols in tea have been developed.[5,6]

We have been mainly interested in the possibility of using GLC as an aid in the determination of the structure of flavone glycosides and therefore studied the behaviour of flavone derivatives in a number of GLC-systems.

Of the derivatives tried in order to increase the stability and volatility of the hydroxyflavones, acetates were found unsuitable. The methyl and TMS ethers of the flavones studied were found equally suitable for GLC. The preparation of methyl ethers is rather laborious and time-consuming and is best carried out with dimethyl sulphate,[7] since diazomethane does not react with hydroxyls attached to carbon atoms 3 and 5 and involved in hydrogen bonding with the adjacent carbonyl group. The products, on the other hand, are stable and easy to handle. Trimethylsilyl ethers are easily prepared but to some degree sensitive to moisture. As expected the rate of silylation is dependent on the position of attachment of the hydroxyl groups. By following the increase in GLC peak area as a function of time after the addition of

silylating agent to pyridine solutions of hydroxyflavones, almost instantaneous silylation in the absence of hydroxyl groups in positions 3 and 5 was observed with both agents used (*N*,*O*-bis(trimethylsilyl)acetamide (BSA) and hexamethyldisilazane-trimethylchlorosilane (HMDS-TMCS)). Flavonols (3-hydroxyflavones) both with and without a 5-hydroxyl were found to require 15 – 20 min for complete silylation, whereas 5-hydroxyflavones reacted slowly requiring overnight silylation with BSA and even then giving a lower yield with the HMDS-TMCS mixture.

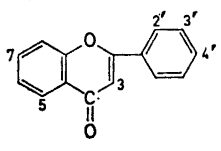
The very low volatility of the flavones restricts the possible stationary phases to some types of temperature tolerant silicones of fairly low polarity on thin film columns.[8] We have used two such compounds, OV-1, a metyl silicone, and OV-17, a methyl-phenyl silicone. The last mentioned should be selective for aromatic compounds due to interaction between aromatic rings in solute and liquid phase. The chromatographic conditions were, if possible, chosen so that both series of ethers could be run under identical isothermal conditions in order to simplify retention time comparisons.

Relative retention times of a number of TMS and methyl ethers of hydroxyflavones are given in Table 1.

Table 1. Retention times of TMS and methyl ethers of hydroxyflavones in relation to flavone and the corresponding ethers of 7-hydroxyflavone or 4,'7-dihydroxyflavone.

Compound	Relative retention time on							
Liquid phase:	OV-1 2.5 %		OV-17 2 %		OV-17 2 %		OV-1 + OV-17 1.5 % 0.5 %	
Temp:	230°		250°		270°		230 – 275° 5°/min	
Flow:	160 ml/min		120		120		150	
Ether:	TMS	Me	TMS	Me	TMS	Me	TMS	Me
Flavone [a]	*1.0*	*1.0*[b]	*1.0*	*1.0*[c]	–	–	–	–
7-Hydroxy	2.5	2.5	2.5	2.6	–	–	*1.0*[f]	*1.0*[g]
2',3-Dihydroxy-	1.4	1.5	0.8	1.6	–	–	–	–
3,7-Dihydroxy-	2.9	2.8	1.8	2.6	–	–	–	–
5,7-Dihydroxy-	4.9	4.3	2.8	5.7	–	–	1.3	1.9
4',7-Dihydroxy-	9.1	5.1	6.2	6.2	*1.0*[d]	*1.0*[e]	2.8	2.3
3,4'-Dihydroxy-	3.6	2.4	1.8	1.8	–	–	1.0	1.0
3,3',4'-Trihydroxy-	4.8	4.0	3.0	3.0	–	–	–	–
4',5,7-Trihydroxy-	10.0	11.0	8.6	–	1.2	2.2	3.6	4.8
3',4',5,7-Tetrahydroxy-	21.9	19.6	9.9	–	1.3	3.9	–	–
3,4',5,7-Tetrahydroxy-	11.2	11.1	5.1	–	0.7	2.2	3.2	–
2',3,3',4',7-Pentahydroxy-	10.2	13.5	3.7	–	0.5	3.0	–	–
3,3',4',5,7-Pentahydroxy-	20.4	17.1	7.9	–	1.0	3.6	–	–

[a] Flavone numbering:



[b] Retention time 0.8 min.
[c] Retention time 1.7 min.
[d] Retention time 5.8 min.
[e] Retention time 5.8 min.
[f] Retention time 2.4 min.
[g] Retention time 2.4 min.

On the OV-1 phase both absolute and relative retention times of both ether types, are fairly similar. As a rule the retention time increases with the number of ether groups, but the contribution of each group is highly dependent on its position in the flavone molecule. Thus, ether groups at carbon atoms 3′, 4′, and 7 are very efficient in increasing retention time whereas such groups in positions 2′, 3, and 5 in comparison are inefficient. As a result, *e.g.*, flavones and the corresponding flavonols (3-hydroxyflavones) with one more hydroxyl group show very similar retention times (*cf.* the pairs 4′,5,7-trihydroxyflavone – 3,4′,5,7-tetrahydroxyflavone and 3′,4′,5,7-tetrahedroxyflavone – 3,3′,4′,5,7-pentahydroxyflavone). The possibility of incomplete derivatization was considered as a possible explanation, especially since Heglein and Krämer [9] have shown that under some conditions of silylation, 3-hydroxyflavones react as diketones. However, combined GLC-mass spectrometry of the TMS ethers of some of the flavonols showed that normal and complete silylation had taken place. It is also of some interest to note that the free hydroxyflavones in which these hydroxyls are hindered either sterically or by intramolecular hydrogen bonding behave anomalously in some liquid-liquid partition systems.[10]

The overall behaviour of both types of ethers on the OV-17 stationary phase resembles that on OV-1. However, two differences are worth noting. Firstly, separation of the TMS ethers of the flavone-flavonol pairs mentioned above is better. Secondly, the methyl ethers as a rule show longer and in case of the highly substituted ethers much longer retention times than the corresponding TMS ethers. This has been interpreted as due to a more efficient prevention by the bulky TMS groups, especially when in positions 2′, 3, and 5, of interaction between aromatic rings in solute and liquid phase, since the effect is absent or even reversed on OV-1.

Table 2. Retention times of mixed methyl-TMS ethers of some hydroxyflavones.

Flavone	Retention time (min) on					
	Liquid phase:	OV-1 2.5 %	OV-1 2.5 %	OV-17 2 %	OV-17 2 %	OV-1 + OV-17 1.5 % 0.5 %
	Temp:	230°	260°	250°	270°	230 – 275° 5°/min
	Flow:	80 ml/min	80	65	65	150
4′-TMS-7-OMe-		11.3		10.7		6.2
7-TMS-4′-OMe-		10.6		10.2		5.8
4′ TMS-5,7-diOMe-			6.9		12.2	13.0
5-TMS-4′,7-diOMe-			4.7		7.1	8.1
7-TMS-4′,5-diOMe-			6.2		10.3	11.4
3′-TMS-4′,5,7-triOMe-			10.3		15.9	
4′-TMS-3′,5,7-triOMe-			10.5		16.2	
5-TMS-3′,4′,7-tri-OMe-			6.8		10.8	
7-TMS-3′,4′,5-triOMe-			10.1		15.1	

Attempts to improve the separation of both types of ethers by temperature programming and the use of a mixed stationary phase (1.5 % OV-1 and 0.5 % OV-17) were not very successful (examples in Table 1).

All three phases were also tested for their capability to separate mixed methyl-TMS ethers of di-, tri-, and tetrahydroxyflavones (Table 2). These were chosen so as to simulate the products of methylation, hydrolysis and silylation of flavone monoglycosides in order to study the use of GLC in structure analysis. Although only the three isomers of mono-TMS-dimethylflavone could be separated satisfactorily from each other, overlapping occurring in some cases in the other series, the overall degree of separation achieved is such that GLC, especially if combined with mass spectrometry, could be a useful tool in structure analysis of flavone glycosides.

EXPERIMENTAL

Material. Flavones were partly obtained from commercial sources, partly synthesized by known recorded methods. The preparation of some partially methylated flavones has been described elsewhere.[11] The purity of the samples was ascertained by paper chromatography.

Preparation of methyl ethers. The methoxyflavones were mainly obtained as intermediates in synthesis. Complete methylation was performed in acetone solution with dimethyl sulphate in the presence of potassium carbonate.[7]

Preparation of TMS ethers. The samples (0.1 – 1.0 mg) were dissolved in pyridine and silylated by either of two methods: a. Addition of 0.1 ml of HMDS and 0.05 ml of TMCS followed by incubation at room temperature overnight. b. Addition of 0.1 ml of BSA followed by incubation at room temperature overnight.

Preparation of acetates. The sample was dissolved in a suitable amount of pyridine. After the addition of an equal volume of acetic anhydride and standing overnight the reagents were removed *in vacuo*.

Gas-liquid chromatography. GLC was carried out on a Barber-Coleman 5000 chromatograph equipped with U-column oven and a hydrogen ionization detector. Glass columns 6 ft × ¼ in. i.d. were used. Packings were prepared using Gas-Chrom Q as support. Injector and detector temperatures were held 20°C above column temperature.

Combined gas-liquid chromatography-mass spectrometry. An LKB 9000 instrument fitted with a 12 ft × ¼ in. o.d. spiral glass column packed with 1 % Se-30 on Gas-Chrom Q (100 – 120 mesh) was used. Spectra were obtained at 70 eV. Other parameters were: column temp. 220°, He-flow 20 ml/min, injector temp. 270°, ion source temp. 300°, accelerating voltage 3.5 kV, and ionizing current 60 μA.

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REFERENCES

1. Narasimhachari, N. and von Rudloff, E. *Can. J. Chem.* **40** (1962) 1123.
2. Furuya, T. *J. Chromatog.* **19** (1965) 607.
3. Keith, E. S. and Powers, J. J. *J. Food Sci.* **31** (1966) 971.
4. Al-Shakir, S. H. *Dissertation Abstr.* **B 28** (1968) 2892.
5. Pierce, A. R., Graham, H. N., Glassner, S., Madlin, H. and Gonzales, J. G. *Anal. Chem.* **41** (1969) 298.
6. Collier, P. D. and Mallows, R. *J. Chromatog.* **57** (1971) 29.
7. Baker, W. and Robinson, R. *J. Chem. Soc.* **1928** 3115.
8. Horning, E. C., VandenHeuvel, J. A. and Creech, B. G. *Methods Biochem. Anal.* **11** (1963) 69.
9. Heglein, F. A. and Krämer, J. *Chem. Ber.* **92** (1959) 2585.
10. Bate-Smith, E. C. and Westall, R. G. *Biochim. Biophys. Acta* **4** (1950) 427.
11. Nordström, C. G. and Swain, T. *J. Chem. Soc.* **1953** 2764.

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