Notes

Gas–liquid chromatography of coumarins

Gas–liquid chromatography is a powerful means of demonstrating the homogeneity of known and unknown coumarins and frequently serves for the purpose of identification of plant origins containing coumarins.

The applications[1] of gas–liquid chromatography to coumarins have not yet been widely explored, until we studied the gas–liquid separation of seventeen standard samples, either in the free form or as their trimethylsilyl ethers, and a few plant extracts using two kinds of column with SE-30 and HI-EFF-1BP (diethylene glycol succinate) as stationary phases.

Experimental

Materials. All coumarins used were available in this laboratory. Hexamethyldisilazane and trimethylchlorosilane were purchased from Kokusan Chemical Works, Ltd. Solvents of reagent grade were used.

Gas chromatography. A Shimadzu Model GC-1C gas chromatograph equipped with a hydrogen flame ionization detector was used in this work.

The columns, containing 1.5 % SE-30 on Chromosorb W (60–80 mesh) and 12 % HI-EFF-1BP on Gas Chrom P (80–100 mesh), respectively, were connected to the gas chromatograph, and acetone solutions of the sample in the free form or as its trimethylsilyl ether[2] were injected into the gas chromatograph with a Hamilton microsyringe. The detailed gas chromatographic conditions are shown in Table I.

Extraction and quantitative methods for furanocoumarins. Thirty grams of powdered root, 80 g of powdered leaves of *Heracleum lanatum* Michaux var. *nipponicum* Hara (Umbelliferae) and 15 g of Pimpinellae Radix were extracted according to SVENDSEN's method[3]. These neutral fractions were injected into the gas chromatograph as their acetone solutions.

The relative quantitative analysis for furanocoumarins as shown in Table II was carried out by the use of the half-width method.

Results and discussion

The results of the gas chromatographic separation of coumarins, using 1.5 % SE-30 on Chromosorb W and 12 % HI-EFF-1BP on Gas Chrom P, are shown in Table I. Three per cent XE-60 (nitrile silicone rubber) on Chromosorb W (60–80 mesh) and 3 % SE-30 on Chromosorb W (60–80 mesh) were also tried as stationary phases, but sharp peaks were not obtained.

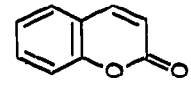
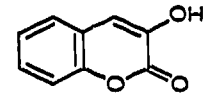
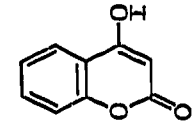
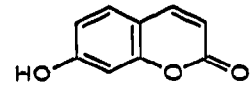
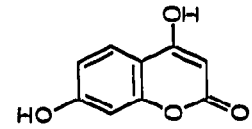
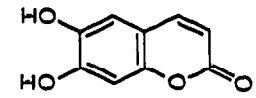
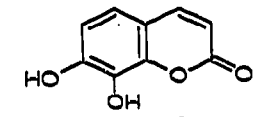
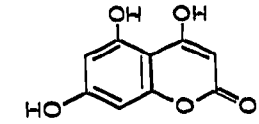
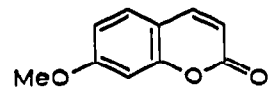
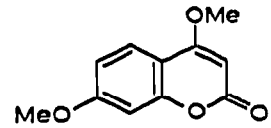
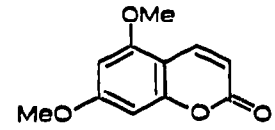
Coumarin itself, having no hydroxyl group, gave a single sharp peak, which moved very fast.

Free hydroxycoumarins did not give good results, but trimethylsilylated hydroxycoumarins gave sharp peaks which separated nicely from each other.

Amongst the monohydroxycoumarins, 3-hydroxycoumarin (t_R = 1.2 min)

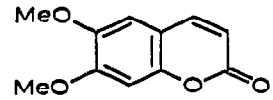
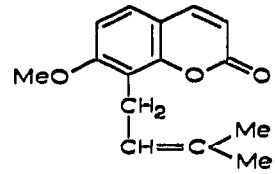
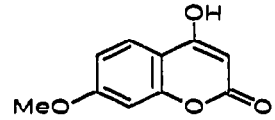
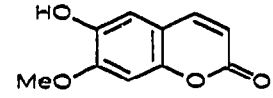
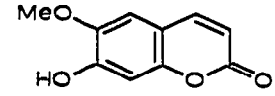
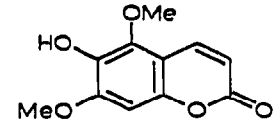
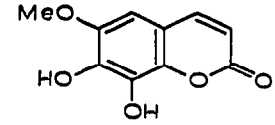
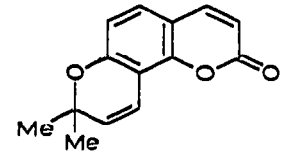
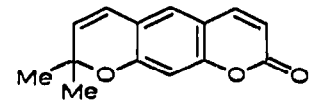
TABLE I

RETENTION TIMES OF COUMARINS

Group	*Compound*	*Structure*	t_R (*min*)		
			1.5% SE-30 on Chromosorb W (60–80 mesh)		*12% HI-EFF-1BP on Gas Chrom P (80–100 mesh)*
			Free	*TMSi**	*Free*
Coumarins	Coumarin		2.6		6.0
	3-Hydroxycoumarin			5.5	
	4-Hydroxycoumarin			9.0	
	7-Hydroxycoumarin (Umbelliferone)			8.9	
	4,7-Dihydroxycoumarin			34.5	
	6,7-Dihydroxycoumarin (Aesculetin)			23.4	
	7,8-Dihydroxycoumarin (Daphnetin)			15.5	
	4,5,7-Trihydroxycoumarin			49.5	
	7-Methoxycoumarin (Herniarin)		6.2		21.6
	4,7-Dimethoxycoumarin		17.1		74.4
	5,7-Dimethoxycoumarin (Citropten)		14.3		50.3

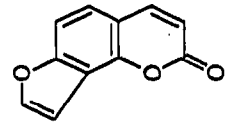
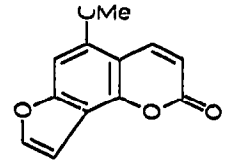
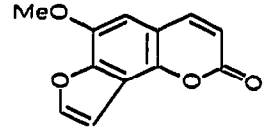
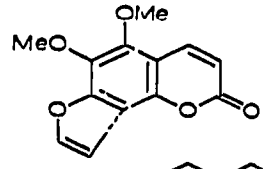
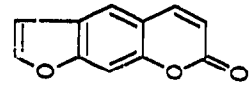
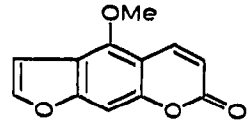
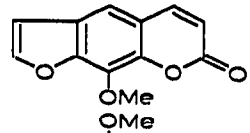
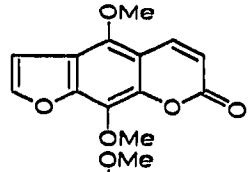
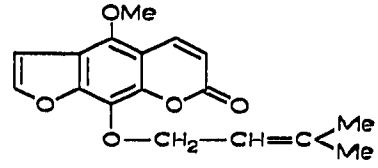
(*continued on p. 384*)

TABLE I (*continued*)

Group	*Compound*	*Structure*	t_R (*min*)		
			1.5% SE-30 on Chromosorb W (60–80 mesh)		*12% HI-EFF-1BP on Gas Chrom P (80–100 mesh)*
			Free	*TMSi**	*Free*
	6,7-Dimethoxycoumarin (Dimethylaesculetin)		13.1		55.1
	Osthol		8.8		37.6
	4-Hydroxy-7-methoxy-coumarin			23.3	
	6-Hydroxy-7-methoxy-coumarin (7-Methylaesculetin)			17.7	
	6-Methoxy-7-hydroxy-coumarin (Scopoletin)			17.9	
	5,7-Dimethoxy-6-hydroxycoumarin (Fraxinol)			25.5	
	6-Methoxy-7,8-dihydroxy-coumarin (Fraxetin)			29.0	
Pyrano-coumarins	Seselin		15.1		28.0
	Xanthyletin		19.4		47.2

(*continued on p. 385*)

TABLE I (*continued*)

Group	*Compound*	*Structure*	t_R (*min*)		
			1.5% SE-30 on Chromosorb W (60–80 mesh)		*12% HI-EFFIBP on Gas Chrom P (80–100 mesh)*
			Free	*TMSi**	*Free*
Furanocoumarins	Angelicin		7.0		24.0
	Isobergapten		15.6		54.4
	Sphondin		17.0		86.9
	Pimpinellin		22.3		69.0
	Psoralen		8.4		35.4
	Bergapten		17.4		74.8
	Xanthotoxin		15.8		80.1
	Isopimpinellin		32.2		151.1
	Phellopterin		12.8**		
Conditions:	Column temp.		180°		210°
	Detector temp.		230°		230°
	Flash heater temp.		250°		250°
	N_2 flow rate		70.4 ml/min		69.1 ml/min

* TMSi = Trimethylsilyl ether.
** Broad peak.

showed the lowest retention time, 4-hydroxycoumarin (t_R = 2.3 min) a medium retention time, and umbelliferone (t_R = 3.9 min) the highest retention time using 3 % SE-30. Comparing these results with those obtained with trimethylsilylated monohydroxycoumarins, 3-hydroxycoumarin (t_R = 5.5 min) also moved the fastest, but umbelliferone (t_R = 8.9 min) showed a slightly shorter retention time than 4-hydroxycoumarin (t_R = 9.0 min) using 1.5 % SE-30. This seems likely to be due to the disappearance of intermolecular hydrogen bonding.

The correlation among carbon numbers, hydroxyl numbers and retention times is illustrated in Fig. 1.

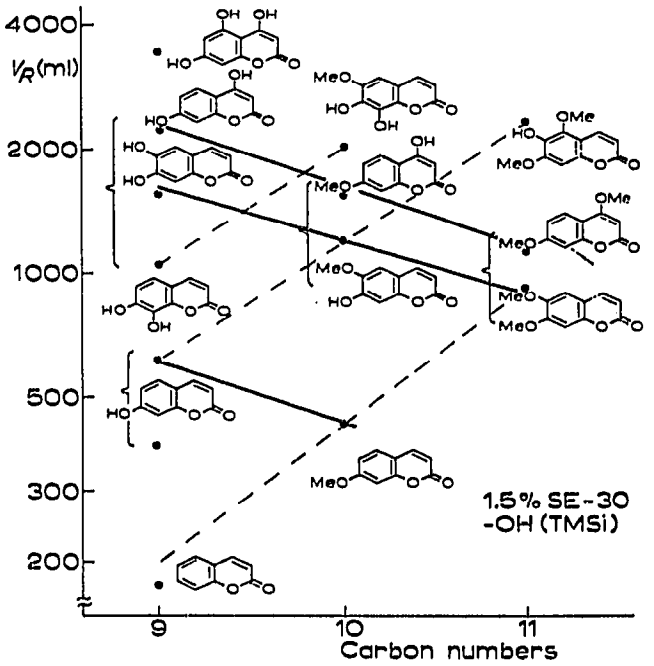


Fig. 1. Relationship between chemical structures and retention volumes.

Especially aesculetin and its methyl ethers showed a good relationship between the hydroxyl numbers and retention times, the order being as follows: t_R = 23.4 min for aesculetin bis-trimethylsilyl ether, 17.9 min for scopoletin trimethylsilyl ether, 17.7 min for 7-methylaesculetin trimethylsilyl ether, and 13.1 min for dimethylaesculetin, using 1.5 % SE-30.

Daphnetin and aesculetin, each having two hydroxyl groups in the *ortho* position, gave lower retention times than 4,7-dihydroxycoumarin.

Trihydroxycoumarin, such as 4,5,7-trihydroxycoumarin, after trimethylsilylation showed the longest retention time. Pyranocoumarins gave sharp peaks; angular types such as seselin (t_R = 15.1 min) had a lower retention time than linear ones such as xanthyletin (t_R = 19.4 min).

Furanocoumarins also gave good gas chromatograms.

Angular furanocoumarins had shorter retention times than linear ones, and the increase in the retention times with increasing number of methoxyl groups was noticeable. In the methoxyfuranocoumarins, different positions of the methoxyl group gave different retention times, as illustrated by isobergapten and sphondin (angular type) and bergapten and xanthotoxin (linear type).

Pimpinellin ran faster than sphondin using 1.5 % SE-30, but was slower when 12 % HI-EFF-1BP was used. It would appear that the presence of the two vicinal methoxyl groups in pimpinellin makes the polarization of the molecule low, and consequently the absorption to the polar liquid phase weak.

The successful separation of some furanocoumarins is illustrated in Fig. 2, and the relative contents of furanocoumarins in the root of *Heracleum lanatum* Michaux var. *nipponicum* Hara and Pimpinellae Radix, which gave almost the same results, are shown in Table II.

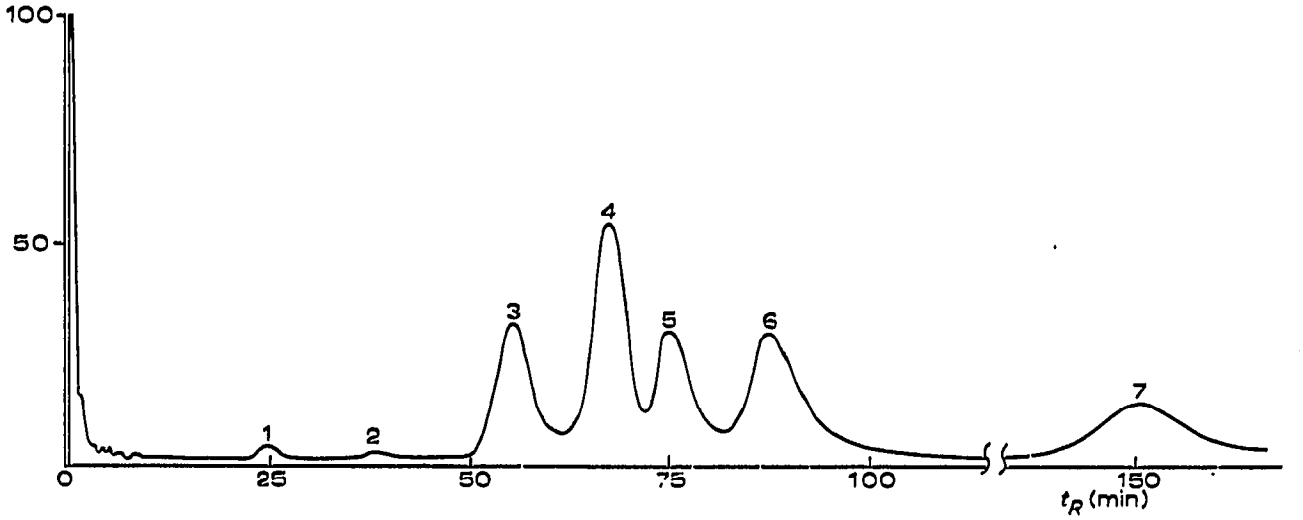


Fig. 2. Gas–liquid chromatogram of furanocoumarins from the root of *Heracleum lantum* Michaux var. *nipponicum* Hara. 1 = Angelicin; 2 = psoralen; 3 = isobergapten; 4 = pimpinellin; 5 = bergapten; 6 = sphondin; 7 = isopimpinellin.

TABLE II

CONTENT OF FURANOCOUMARINS IN CRUDE DRUGS

Compound	*Root of H. lanatum Michaux var. nipponicum Hara*		*Pimpinellae Radix*	
	Peak area (cm^2)	*Relative amount*	*Peak area (cm^2)*	*Relative amount*
Angelicin	1.4	1	0.2	1
Psoralen	trace	trace	—	—
Isobergapten	33.8	24	7.7	39
Bergapten	27.7	20	1.9	10
Sphondin	30.1	22	2.2	11
Pimpinellin	78.3	56	29.0	145
Isopimpinellin	34.4	24	10.0	50

As a result of the gas chromatographic analysis the presence of small amounts of angelicin and traces of psoralen were identified in the root of *Heracleum lanatum* Michaux var. *nipponicum* Hara, but furanocoumarins were not confirmed in the leaves as previously reported[4].

The gas–liquid chromatographic technique described above can be applied extensively to both the identification of small quantities of coumarins and to the quantitative analysis of mixtures from plant extracts.

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Separation of the major alkaloids of *Peganum harmala* by high voltage ionophoresis

In the opinion of CROMWELL[1] none of the existing methods for the determination of the alkaloids of *Peganum harmala* are entirely satisfactory and for this reason he considers a new analytical study of these alkaloids to be necessary. CROMWELL's assertion continues to hold true ten years after it was first enunciated. The key point in all the determinations is the separation of harmine and harmaline. The technique is substantially the same as the fractional precipitation process followed when extracting these alkaloids from the plant. Here we shall deal with—among the different separation methods we have tried—the possibilities offered by ionophoresis on paper as the starting point for a micromethod which permits the evaluation of these tryptophan metabolism compounds in vegetables.

Several authors[2–8] have shown that a considerable number of alkaloids may be separated by ionophoresis on paper as long as they are sufficiently soluble in the buffer and do not remain adsorbed in the carrier. Most of such separations have been carried out at gradients not exceeding 10 V/cm. The advantage of using, in similar cases, fields of an intensity ten or twenty times higher is obvious. The speed of the separation will limit the broadening, distortion and overlapping of the bands due to diffusion.

But this economy of time and, above all, the desired increase of the resolving power will only be attained by either using a device capable of absorbing the heat produced by the Joule effect or adopting the necessary precautions so that the heat released is negligible. Both methods have been used in this work, with different results. We have used a heat exchanging device, cooled by brine circulation, capable of reaching temperatures of —18° for all the ionograms run in aqueous systems. When formamide or dimethylformamide have been used as the solvents the production of