CHROM. 5300

GAS–LIQUID CHROMATOGRAPHY OF NATURALLY OCCURRING XANTHONES AND RELATED DERIVATIVES*

A. JEFFERSON AND C. I. STACEY
Western Australian Institute of Technology, Hayman Road, South Bentley, Western Australia 6102 (Australia)

AND

F. SCHEINMANN
University of Salford, Salford M5 4WT, Lancashire (Great Britain)

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SUMMARY

The gas chromatographic analysis of natural and synthetic xanthone derivatives has been achieved using a column containing 2% silicone rubber on silanised Chromosorb W (80–100 mesh). The qualitative analysis of trimethylsilyl ethers of polyhydroxyxanthones is described together with the preparation, quantitative separation and identification of the trimethylsilyl ether (15c) of mangostin, a xanthone metabolite from *Garcinia mangostana (Guttiferae)*.

INTRODUCTION

Most of the naturally occurring xanthones which have so far been characterised were isolated from higher plants[1] although a smaller number of these metabolites have also been isolated from lower plants and fungi[2]. Xanthones from natural sources usually have a hydroxy group at the 1-position. Hydroxy groups may be found at other positions in the nucleus, and the other common substituent groups are methoxyl, methyl, and isoprenoid derived units.

Xanthones are normally purified by column or thin-layer chromatography and their structure is determined by the usual spectroscopic methods, such as ultraviolet[3], infrared[4] and nuclear magnetic resonance spectroscopy[5].

IMUTA AND OUCHI[6] have described the detection of xanthone in depolymerised Japanese brown coal by gas chromatography. The present paper describes the results obtained with a representative variety of natural and synthetic xanthone derivatives which possess hydroxy, methoxy, carboxy and isoprenoid substituents.

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EXPERIMENTAL

Materials

All of the synthetic and naturally occurring xanthone samples were available from previous work on extractives, from *Guttiferae* species and from recent syntheses[7].

Trimethylsilyl chloride, hexamethyldisilazane, dimethylchlorsilazane, silanised Chromosorb W (acid washed) and SE-30 silicone rubber were obtained from Varian Ltd.

Trimethylsililation[8]

(a) Small scale procedure: 1 mg of the xanthone was dissolved in 0.1 ml of dry pyridine. The solution was treated with 0.1 ml of hexamethyldisilazane (HMDS) and 0.05 ml of trimethylchlorsilane (TMCS) with the reaction being carried out in a stoppered centrifuge tube. The mixture was shaken vigorously for about 30 sec, allowed to stand for 10 min, and then centrifuged.

(b) Large scale sililation of mangostin (15a): 0.83 g of mangostin was dissolved in 8.3 ml of pyridine and sililated with 8.3 ml of HMDS and 4.15 ml of TMCS. The mixture was allowed to stand for 20 min, and then it was centrifuged. Excess carbon tetrachloride was added to the supernatant liquid and the volatile material was removed under vacuum at room temperature, the process being repeated at least three times. Twenty 50 μl injections were made into the gas chromatograph.

Gas–liquid chromatography

Qualitative analysis. The instrument used for the analysis was a Varian Model 1400 equipped with a hydrogen flame ionisation detector. An 8 ft. long stainless steel spiral column, external diameter 1/8 in. was used. The column packing was Chromosorb W coated with 2% silicone rubber SE-30. 0.2 μl of the supernatant liquid from the silanisation procedure or 0.2 μl of a dilute acetone solution of the xanthone was used for on-column injection.

Preparative separation. The trimethylsilyl ether of mangostin (15c) was chromatographed on a Varian Model 700, using a thermal conductivity detector. The 7 ft. long column of 3/8 in. external diameter was packed with 3% SE-30 silicone rubber supported on Chromosorb W, and it was maintained at 295°. The helium flow rate was 100 ml/min.

RESULTS AND DISCUSSION

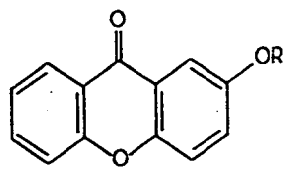
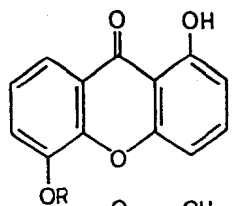
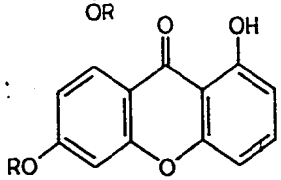
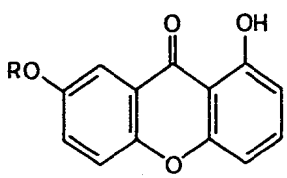
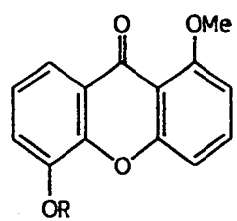
Gas–liquid chromatography of fifteen parent oxygenated xanthones was studied. Wherever possible the corresponding methoxy derivative, which was prepared by the reaction of the non chelated hydroxy groups with diazomethane, was also analysed and compared with the related trimethylsilyl ether. The relative retention times of the parent xanthones and their derivatives are given in Table I.

The column was operated at three different temperatures. Low molecular weight xanthones gave good results when the column was maintained at 200 or 230° and the relative retention times are quoted with respect to pure xanthone which was added as a standard. Derivatives of isoprenylated or high molecular weight xanthones gave

TABLE I

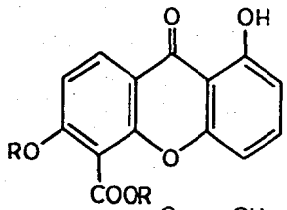
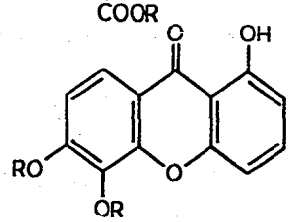
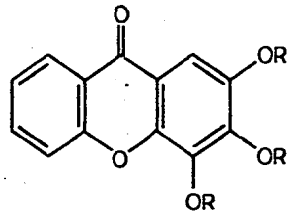
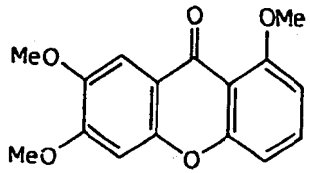
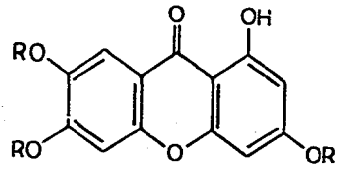
RELATIVE RETENTION TIMES OF SYNTHETIC AND NATURALLY OCCURRING XANTHONES

Conditions: Stainless steel column 8 ft. long, 1/8 in. O.D.; packing 2% SE-30 on DMCS treated Chromosorb W (60–80 mesh); injector temp. 270°; detector temp. 260°; nitrogen flow rate 40 ml/min. Reference standard at 200 and 230° was xanthone and at 260° was jacareubin dimethyl ether (14b). Key: i = impurity; s = shoulder; t = tailing.

No.	*Structure*	*Parent compound R = H*	*Relative retention times at 200°, 230° and 260° (column)*								
			(a) R = H			*(b) R = Me*			*(c) R = Si(Me)$_3$*		
			200°	*230°*	*260°*	*200°*	*230°*	*260°*	*200°*	*230°*	*260°*
1		2-Hydroxyxanthone	3.77	2.85	0.17	2.16	1.77	0.13	3.04	2.54	0.17
2		1,5-Dihydroxyxanthone	2.69t	2.31t	0.18	2.80	2.35	0.17	3.40	2.59	0.19
3		1,6-Dihydroxyxanthone	4.60t	4.40t	—	3.06	2.71	0.18	3.30	3.09	0.30
4		1,7-Dihydroxyxanthone (euxanthone)	4.31t	3.42t	0.27	2.80	2.40	0.18	4.05	3.18	0.26
5		5-Hydroxy-1-methoxy xanthone	5.70t	3.64t	0.28	5.11	3.77	0.24	6.00	4.08	0.27

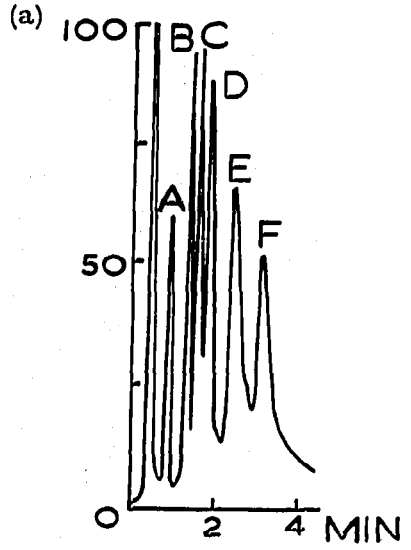
(Continued on p. 250)

TABLE I *(continued)*

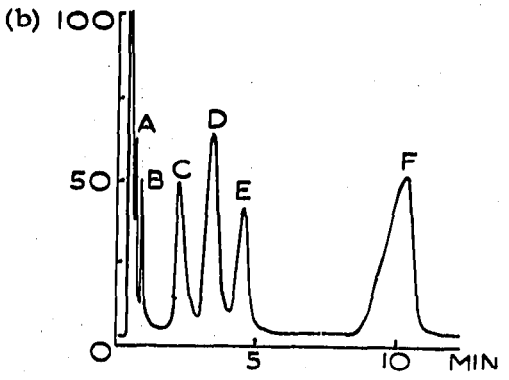
No.	Structure	Parent compound R = H	Relative retention times at 200°, 230° and 260° (column)								
			(a) R = H			(b) R = Me			(c) R = Si(Me)$_3$		
			200°	230°	260°	200°	230°	260°	200°	230°	260°
6		1,6-Dihydroxyxanthone-5-carboxylic acid	—	—	—	—	5.93	0.37	—	12.27	0.43S 0.59
7		1,5,6-Trihydroxyxanthone	—	5.69t	0.37	5.37	4.08	0.24	8.50	6.00	0.35
8		2,3,4-Trihydroxyxanthone	—	—	—	5.82	4.31	0.26	10.5	7.25	0.36
9		1,6,7-Trimethoxyxanthone	11.43	6.73	0.44	—	—	—	—	—	—
10		1,3,6,7-Tetrahydroxy-xanthone	—	—	—	—	6.70	0.61	—	17.39	0.93

11	1,5,6,7-Tetrahydroxy-xanthone	—	—	—	7.60	5.53	0.35	14.50	9.37	0.44
12	1,3,7-Trihydroxy-2-(γ,γ-dimethylallyl)xanthone	—	—	—	—	23.3	0.84	—	21.08 25.39[i]	1.20
13	1,3,5,6-Tetrahydroxy-2-(γ,γ-dimethylallyl)xanthone	—	—	—	—	28.31	1.35	—	—	1.76
14	Jacareubin	—	—	—	—	26.0	1.00	—	27.23	1.30
15	Mangostin	—	—	—	—	—	3.20	—	—	3.40

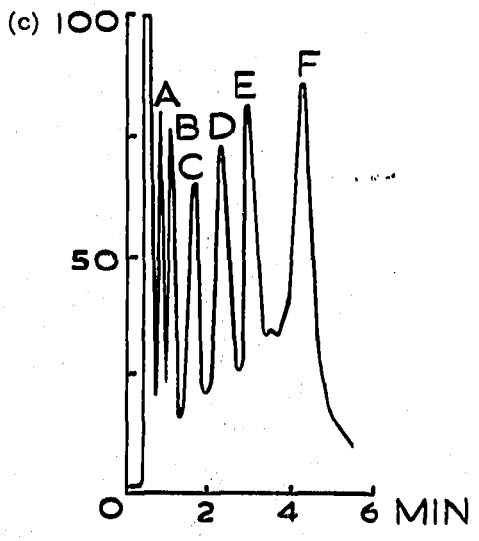
[i]



A = xanthone
B = 1b
C = 4b
D = 3b
E = 7b
F = 11b



A = xanthone
B = 4b
C = 10b
D = 14b
E = 13b
F = 15b



A = 2c
B = 7c
C = 10c
D = 14c
E = 13c
F = 15c

Fig. 1. Gas chromatograms of natural and synthetic xanthone derivatives. Column temperatures as follows: (a) methyl ethers of simple hydroxyxanthones at 230°; (b) methyl ethers of naturally occurring xanthones at 260°; (c) trimethylsilyl ethers at 280°.

better results when the column temperature was raised to 260°. The 5,6-dimethyl ether of jacareubin (14b) was a satisfactory reference standard at 260°.

Simple hydroxyxanthones and their methylation products could be injected directly onto the column, but compounds with a hydroxy unit at any other than the 1-position tailed at lower temperatures. The peaks tailed badly as the number of hydroxy units was increased and these compounds gave better results when they were methylated or silanised. As predicted from previous work in the flavone field[9], a fairly regular increase in retention time was generally observed as the molecular weight of the substituted xanthone was increased, and this is readily observed in Table I.

The chelated 1-hydroxy group caused less retention on the column than a methoxy situated at the same position. Similarly methoxy units substituted at other positions in the xanthone nucleus caused less retention than the corresponding trimethylsilyl units.

Another common pattern which emerged was the lower retention times of compounds possessing adjacent substituent groupings compared with the corresponding isomeric structures where the groups are scattered throughout the ring system. This point is illustrated by a comparison of the relative retention times of 1-hydroxy-3,6,7-trimethoxyxanthone (10b) and 1-hydroxy-5,6,7-trimethoxyxanthone (11b) or 1,6,7-trimethoxyxanthone (9) and 2,3,4-trimethoxyxanthone (8b), respectively.

Fig. 1a illustrates a typical chromatogram obtained from the analysis of an aliquot of a mixture derived from the diazomethane methylation of several low-molecular-weight xanthones. Fig. 1b shows the results obtained from the analysis of the methyl ether derivatives of some common naturally occurring metabolites. Since some naturally occurring xanthones may already possess methoxy substituents it may be more satisfactory to silanise the sample prior to analysis, and Fig. 1c shows the results obtained from the analysis of a mixture of trimethylsilyl ethers.

Isoprenylated xanthones possessing an *o*-hydroxy group are susceptible to ring closure giving a dihydropyran on heating in the presence of acidic reagents[10]. In order to test whether the trimethylsilyl ether of mangostin (15c) could be recovered

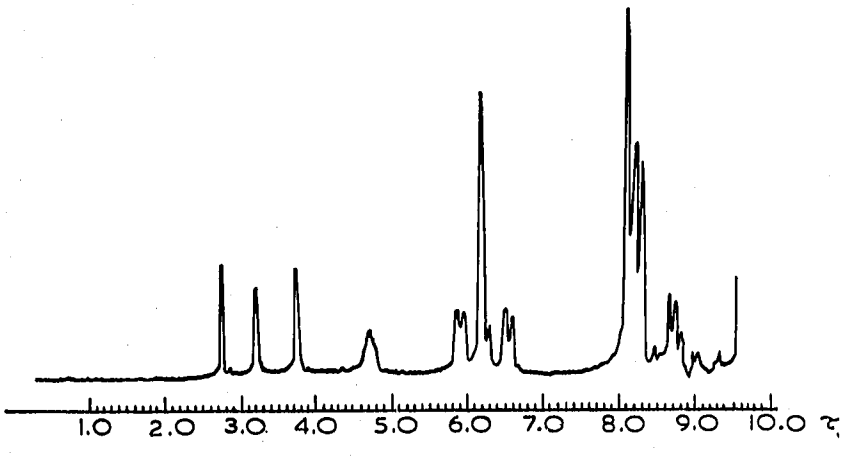


Fig. 2. 100 Mc (Varian HA-100 spectrometer) NMR spectrum of the trimethyl silyl ether (15c) of mangostin in deuterochloroform solvent.

unchanged after passage through the hot column, a sample was collected and its nuclear magnetic resonance (NMR) spectrum was determined. The NMR spectrum (Fig. 2) showed that the silanised mangostin could be recovered essentially unchanged Thus the two isolated aromatic protons (H4 and H5) appeared as singlets at τ3.73 and 3.27 in good agreement with the expected values for this compound[11]. The two 3-methylbut-2-enyl side chains were attached to magnetically dissimilar sites. The two benzylic CH_2 groups gave doublets at τ5.86 (2H) and 6.67 (2H), and the allyl –CH= groups gave a complex resonance at τ4.73 (2H), whilst the gem-dimethyl group appeared as peaks at τ8.07, 8.15 and 8.22 (12H). A singlet at τ9.63 (18H) was assigned to the two trimethylsilyl units with the hydrogen-bonded hydroxy proton appearing as a sharp singlet at -3.60τ. There was a trace amount of an impurity which gave weak signals around 8.8τ.

Gas chromatography promises to be a useful method for the screening of timbers in order to detect the presence of common metabolites such as euxanthone and jacareubin.

Xanthones are often difficult to analyse by the normal method using thin-layer chromatography. Gas–liquid chromatography is an alternative method of analysis which has the added advantage of high sensitivity.

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