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Coumarins, Fatty Acids, Volatile and Non-volatile Terpenoids from the Leaves of *Citrus aurantium* L. (Sour Orange) and *Citrus sinensis* (L.) Osbeck (Sweet Orange)

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Coumarins including psoralens were isolated and identified from the leaves of *Citrus aurantium var.* amara L. (sour orange) and *Citrus sinensis* L. Osbeck (sweet orange). Five coumarins (aurapten, auraptene, limettin, umbelliferone and osthol) and two coumarins of the psoralen type (bergapten and bergaptol) were obtained from the leaf extract of sour orange while only bergaptol was extracted from the leaf extract of the sweet orange. Although these compounds were previously isolated from roots and fruit peels, this is the first report of these coumarins from the leaves of either species. Twenty six terpenoids plus apiol were obtained from the volatile and non-volatile residues of the extracts, and four of these constituents, apiole, stigmasterol, β-sitosterol and friedelin are reported here to be isolated for the first time from the leaves of these citrus species. Five fatty acids were obtained from the leaves, linoleic, oleic, linolenic, palmitic and palmitoleic and the first two are the major constituents.

INTRODUCTION

Sweet and sour oranges** are important fruit crops in Egypt, and in a previous study we described the flavonoids in their leaves[1]. We now extend the study to include coumarins, including psoralens, and fatty acids terpenoids in the leaves of both species. Most previous reports of citrus coumarins and terpenoids were for fruit peel oils[2–6] and for roots[7–11].

EXPERIMENTAL

Plant Material: Leaf samples of *Citrus aurantium* L. (sour orange) and *Citrus sinensis* (L.) Osbeck (sweet orange) were obtained from fruiting trees cultivated at the Agricultural Research Institute, Giza, Egypt in October 1996. The plants were identified by Dr. M. El-Gabaily and herbarium specimens are deposited in the herbarium of the Pharmacognosy Department of the Faculty of Pharmacy, Helwan University, Cairo, Egypt.

Authentic reference compounds: Aurapten, auraptene (meranzin), limettin, umbelliferone, osthol, bergapten, bergaptol, α-pinene, β-pinene, δ^3-carene, limonene, *p*-cymene, *m*-cymene, myrecene, γ-terpinene, α-caryophyllene (humulene), β-caryophyllene, neryl acetate, geranyl acetate, citral (neral or geranial), citronellal, thymol, carvacrol, eugenol, linalool, nerol, geraniol, α-terpineol, citronellol, apiole, stigmasterol, β-sitosterol, friedelin, myristic, myrisoleic,

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**In this paper the term "orange" is used for "orange tree".

palmitic, palmitoleic, stearic, oleic, linoleic and linolneic acids were available in the Department of Botany, The University of Texas at Austin, U.S.A.

Special reagents: Iodine-potassium iodide spray reagent[12]: 0.2 g I_2 and 4 g KI were dissolved in 100 mL H_2O. Chlorosulfonic acid reagent[13]: 5 mL chlorosulphonic acid were added to 10 mL glacial acid with cooling.

UV-visible spectrohotometer Shimadzu 1601; Varian INOVA 500 NMR; GC Varian 3400 Gas Chromatograph and Finnigan MAT TSQ 700 CI, VG ZAB ZE High Resolution CI, and Clevenger distillation apparatus.

Extraction, isolation and identification: Dried and powdered leaves (2 kg) of both sweet and sour orange trees were extracted with 95% aqueous ethanol followed by 70% aqueous ethanol. The combined two extracts for each species were evaporated to a small volume *in vacuo*. The two aqueous syrups obtained from leaves were each extracted with *n*-hexane to yield 9.3 and 12.7 g oily residues, respectively[1]. Silica gel (Merck 60A) columns were used for the separation of fatty acids, sterols, psoralens and coumarins by eluting the columns with petroleum ether and benzene in the proportion of (4 : 1), (1 : 1), (1 : 4) benzene and 0.5%, 1%, 2% methanol in benzene with a flow rate of 4 mL/min. Fractions with similar TLC patterns were combined; chromatoplates were visualized under UV at 283 nm using benzene : ethyl acetate (8 : 2) as a solvent system, chlorosulphonic acid reagent[13]; heating at 110°C for a few minutes; as a spray reagent for identification of terpenes and sterols, and I_2/KI as a spray reagent[12] for identifcation of psoralens and other coumarins, and by direct TLC comparison with authentic samples, melting points and undepressed mixed melting points. When necessary further confirmation of the structures of the compounds was carried out by MS, NMR, and UV. The percentage yield of sterols, coumarins, and psoralens are recorded in Table-1.

TABLE-1
PERCENTAGE YIELD OF COUMARINS, PSORALENS AND STEROLS FROM DRY LEAVES OF SWEET AND SOUR ORANGE TREES*

R_f	Flouresence under UV	Color with spray reagent		Compound	Sweet orange %	Sour orange %
		I_2/KI	Chlorosulphonic acid			
				Coumarins:		
0.50	sky blue	colourless	—	aurapten	—	0.22
0.47	blue	colourless	—	auraptene	—	0.18
0.53	sky blue	sky blue	—	limettin	—	0.24
0.33	bluish green	colourless	—	umbelliferone	—	0.10
0.68	blue	colourless	—	osthol	—	0.06
				Psoralens:		
0.40	yellow	colourless	—	bergaptol	0.15	0.10
0.65	yellow green	graysih brown	—	bergapten	—	0.08
				Sterols:		
0.70	—	—	reddish violet	stigmasterol	0.26	0.16
0.89	—	—	reddish violet	β-sitosterol	0.62	0.25
0.90	—	—	reddish violet	friedelin	6.25	3.28

*These compounds were isolated by column chromatography.

TABLE-2
FATTY ACIDS IN THE LEAVES OF SWEET AND SOUR ORANGE TREES*

R_t	Mass	m.f.	Fatty acids	Yield in the *n*-hexane extract	
				Sweet orange %	Sour orange %
8.17	228.3	$C_{14}H_{28}O_2$	myristic	0.3	0.3
8.75	226.2	$C_{14}H_{26}O_2$	myristoleic	0.5	0.4
9.35	256.4	$C_{16}H_{32}O_2$	palmitic	6.7	4.3
9.43	254.4	$C_{16}H_{30}O_2$	palmitoleic	5.7	4.8
10.27	284.4	$C_{18}H_{36}O_2$	stearic	1.0	0.8
11.59	282.4	$C_{18}H_{34}O_2$	oleic	10.0	10.8
11.85	280.4	$C_{18}H_{32}O_2$	linoleic	12.4	12.4
12.04	278.4	$C_{18}H_{30}O_2$	linolenic	7.7	9.4

*These compoounds were isolated by column chromatography and identified by GC.

TABLE-3
OIL COMPONENTS OF LEAVES OF SWEET AND SOUR ORANGE TREES
(DETECTED ONLY BY GC).

R_t	Mass	m.f.	Component	Yield in the leaf oil of	
				Sweet orange %	Sour orange %
			Hydrocarbons:		
1.89	136.23	$C_{10}H_{16}$	α-pinene	1.71	1.65
2.23	136.23	$C_{10}H_{16}$	β-pinene	2.90	2.98
2.45	136.23	$C_{10}H_{16}$	δ^3-carene	2.39	2.55
2.65	136.23	$C_{10}H_{16}$	limonene	1.36	2.32
2.85	134.22	$C_{10}H_{14}$	*p*-cymene	1.45	1.50
3.00	134.22	$C_{10}H_{14}$	*m*-cymene	3.42	4.57
3.40	136.23	$C_{10}H_{16}$	myrcene	8.03	8.25
3.99	136.23	$C_{10}H_{16}$	γ-terpinene	4.61	4.05
4.65	204.36	$C_{15}H_{24}$	β-caryophyllene	4.61	4.42
4.87	204.36	$C_{15}H_{24}$	α-caryophyllene	3.93	4.65
			Esters		
5.61	196.29	$C_{12}H_{20}O_2$	neryl acetate	10.51	12.45
5.85	196.29	$C_{12}H_{20}O_2$	geranyl acetate	11.97	12.45
			Aldehydes		
4.41	152.24	$C_{10}H_{16}O$	neral	5.47	5.10
4.45	154.24	$C_{10}H_{18}O$	citronellal	3.42	3.52
			Alcohols		
5.20	154.24	$C_{10}H_{18}O$	nerol	3.76	3.30
5.80	154.24	$C_{10}H_{18}O$	α-terpineol	13.85	16.80
6.22	154.24	$C_{10}H_{18}O$	linalool	21.37	18.90
7.65	154.24	$C_{10}H_{18}O$	geraniol	0.40	3.30
7.85	156.26	$C_{10}H_{20}O$	citronellol	1.53	4.65
			Phenols		
9.01	164.20	$C_{10}H_{12}O_2$	eugenol	0.68	1.20
9.21	150.21	$C_{10}H_{14}O$	thymol	0.17	0.45
9.40	150.21	$C_{10}H_{14}O$	carvacrol	0.76	0.90
			Phenol ether		
8.03	222.24	$C_{12}H_{14}O_4$	apiol	1.16	4.05

Fatty acids, which were eluted with petroleum ether from the silica gel columns, were analyzed by GC-MS. Compared to the available authentic samples, percentage yields are recorded in Table-2. Volatile oils were prepared by steam distillation[14] from the dried leaves (1 kg) of both sweet and sour oranges; the percentage yields determined were 1.71 and 1.50%, respectively. Twenty-three compounds were identified by GC-MS high resolution when compared with standards; the percentage yield of each component is recorded in Table-3.

RESULTS AND DISCUSSION

Five known coumarins, aurapten, auraptene (meranzin), limettin, umbelliferone and osthol, and two known psoralen-type coumarins, bergapten and bergaptol, were isolated from *C. aurantium* (sour orange) and are reported here for the first time from the leaf extract of the sour orange. Although some coumarins were previously reported from the fruit peel oil and root of *C. sinensis*[2, 3, 4, 11], coumarins were not previously isolated from leaves of this species; however, we obtained the coumarin bergaptol (a psoralen-type). Three sterols, stigmasterol, β-sitosterol and friedelin are reported here for the first time from the leaves of both sweet and sour oranges and in a higher concentration from the sweet than from the sour oranges. Friedelin has the highest concentration of all sterols in both oranges.

Table-2 represents the concentrations of the fatty acids in the leaf extracts and their GC retention times. Linoleic and oleic acids were the major fatty acids for both citrus species, while myristic and myristolic were considered as minor constituents. The percentage yields of the volatile oils from the leaves of sweet and sour oranges were 1.71 and 1.50%, respectively. Out of the twenty-three

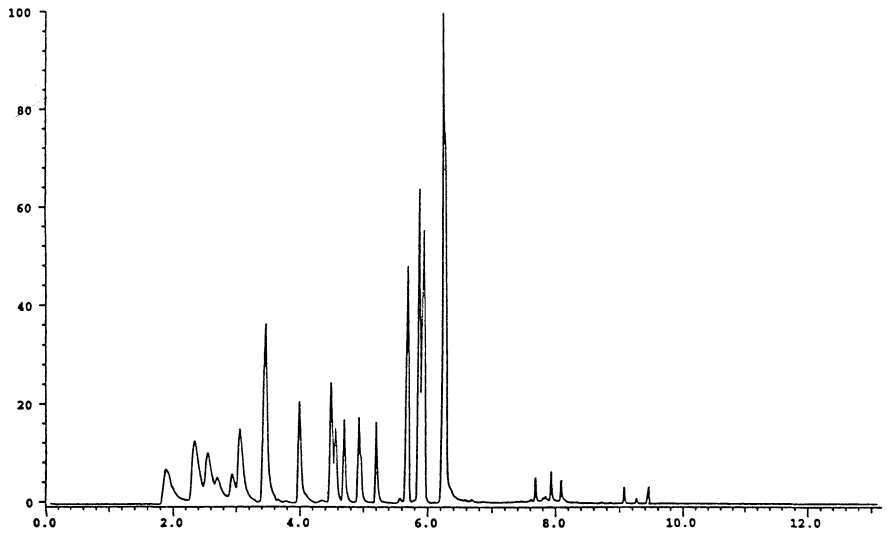

Fig. 1 Gas chromatogram of the leaf of constitutents of *Citrus sinensis*.

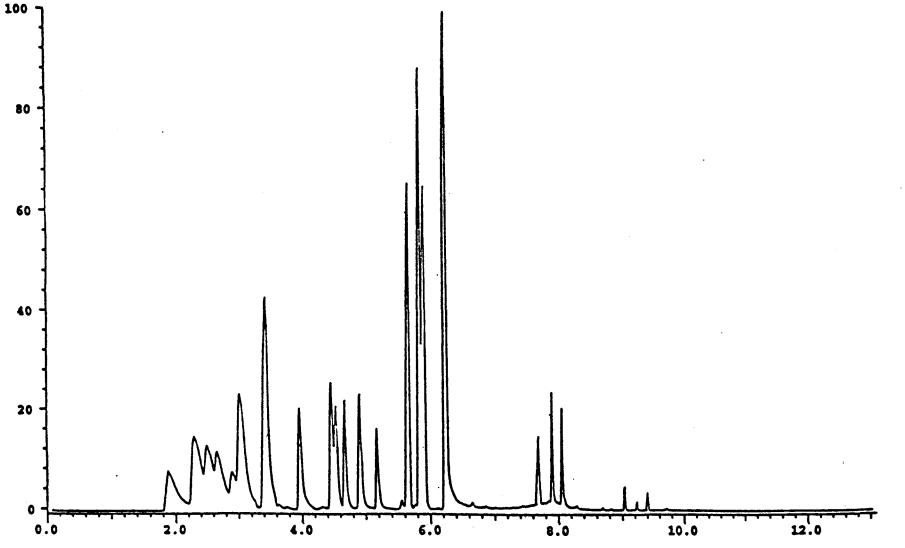

Fig. 2 Gas chromatogram of the leaf of constitutents of *Citrus aurantium.*

known[16, 17] identified components only apiol (a phenol ether) is reported here for the first time as a constituent of either citrus species. However, its concentration in leaves of sour oranges is more than 3 times higher than in leaves of sweet ones. The terpenoids linalool and α-terpineol are the major oil components in leaves of both oranges.

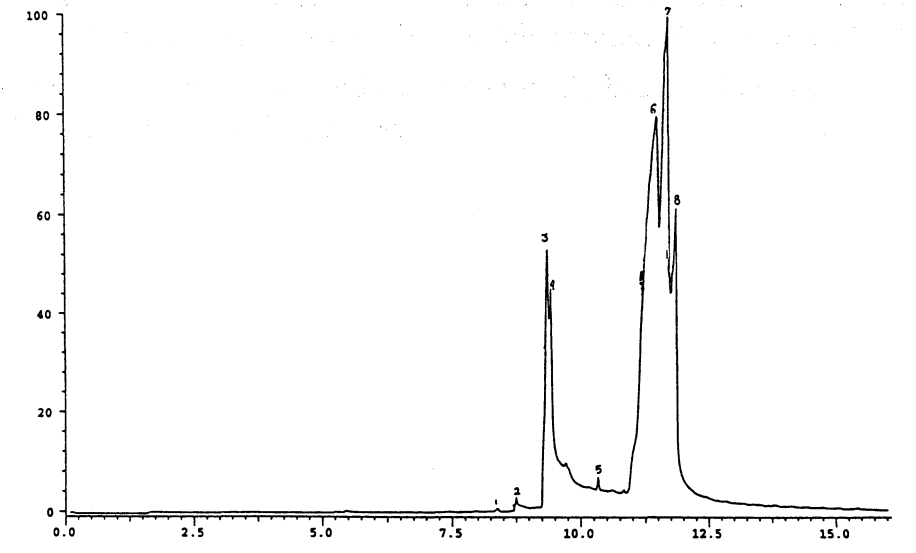

Fig. 3 Gas chromatogram of fatty acids in leaf extract of *Citrus sinensis.*

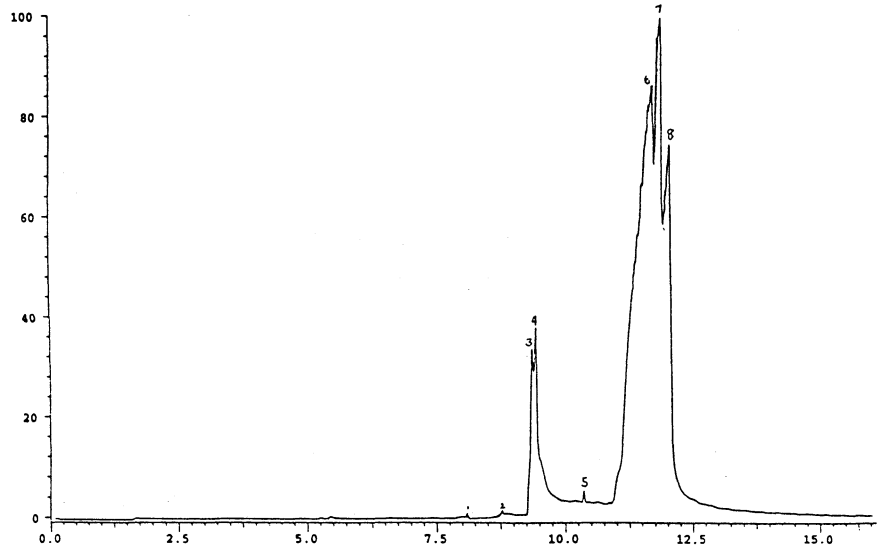

Fig. 4 Gas chromatogram of fatty acids in leaf extract of *Citrus aurantium.*

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