Phytochemistry, 1974, Vol. 13, pp. 443 to 452. Pergamon Press. Printed in England.

FATTY ACID COMPOSITION OF STEROL ESTERS FROM *CITRUS SINENSIS, C. PARADISI, C. LIMON AURANTIFOLIA* AND *C. LIMETTIOIDES* SACS

HAROLD E. NORDBY and STEVEN NAGY

Citrus and Subtropical Products Laboratory,* Winter Haven, FL 33880, U.S.A.

(*Revised received* 11 *June* 1973. *Accepted* 3 *September* 1973)

Key Word Index—*Citrus sinensis*; *Citrus paradisi*; *Citrus limon aurantifolia*; *Citrus limettioides*; Rutaceae; orange; grapefruit; lemon; lime; fatty acid profiles; sterol; sterol esters; chemotaxonomy.

Abstract—The fatty acid compositions of sterol esters from 4 citrus species, viz. orange, grapefruit, lemon and lime, were determined by GLC. Each species possessed its own intrinsic fatty acid pattern which could be used to differentiate it from the other species. In most cases varieties within a species had fatty acid patterns which could be used for varietal differentiation. In all citrus tested except Columbia lime, the major acid was linoleic acid; this acid varied from 10 to 56% of the total acid content. The ratios of 16/16:1 were distinct for each citrus species. The C_{22}–C_{29} fatty acids were prevalent in citrus sterol esters ranging from 6·5% for some orange and grapefruit varieties to over 41% for two lime varieties. In all varieties C_{24} was the most prominent of these longer chain fatty acids. Argentation TLC indicated that these longer chain fatty acids primarily were esterified to dimethyl sterols.

INTRODUCTION

IN RECENT years, studies on citrus chemotaxonomy have concentrated mainly upon compositions of the essential oils,[1–3] limonoids[4] and flavanones.[5] We first showed the importance of lipid profiles in citrus chemotaxonomy through a study of the fatty acid composition of citrus juice and seeds from several citrus species.[6] Further examination of the fatty acids,[7,8] sterols[9] and hydrocarbons[10] of one common species, *Citrus sinensis* (sweet orange), indicated that lipid profiles could be employed for the differentiation of varieties within a species. Examination of the long-chain hydrocarbon composition of grape-

* One of the laboratories of the Southern Region, Agricultural Research Service, U.S. Department of Agriculture.

[1] SCORA, R. W., ENGLAND, A. B. and BITTERS, W. P. (1966) *Phytochemistry* **5**, 1139.
[2] PIERINGER, A. P., EDWARDS, G. J. and WOLFORD, R. W. (1964) *Proc. Am. Soc. Hort. Sci.* **84**, 204.
[3] KESTERSON, J. W., PIERINGER, A. P., EDWARDS, G. J. and HENDRICKSON, R. (1964) *Proc. Am. Soc. Hort. Sci.* **84**, 199.
[4] DREYER, D. L. (1966) *Phytochemistry* **5**, 367.
[5] ALBACH, R. F. and REDMAN, G. H. (1969) *Phytochemistry* **8**, 127.
[6] NORDBY, H. E. and NAGY, S. (1969) *Phytochemistry* **8**, 2027.
[7] NORDBY, H. E. and NAGY, S. (1971) *Phytochemistry* **10**, 615.
[8] NORDBY, H. E. and NAGY, S. (1971) *Lipids* **6**, 554.
[9] NAGY, S. and NORDBY, H. E. (1971) *Lipids* **6**, 826.
[10] NAGY, S. and NORDBY, H. E. (1971) *Phytochemistry* **10**, 2763.

fruit,[11,12] limes,[13] grapefruit-mandarin hybrids[14] and lemons[15] demonstrated that hydrocarbons were one of the most important chemotaxonomic markers for species and hybrid differentiation.

A recent study conducted on the fatty acid composition of triglycerides from four citrus species showed that these fatty acids differ markedly among species.[16] Because of the differences observed in species triglycerides, the following study was undertaken to determine if the fatty acid composition of sterol esters also differed among and within species (varietal differences).

RESULTS AND DISCUSSION

For clarity of presentation sterol fatty acids are divided into four groups: (a) the major C_{16} and C_{18} acids, (b) the seven major acids in the range C_{22}–C_{26}, (c) the minor even-numbered acids, and (d) the minor odd-numbered acids. These four groups are presented according to their importance in differentiating species and varieties within a species.

Major C_{16} and C_{18} fatty acids

Table 1 shows the percentages of the major C_{16} and C_{18} fatty acids in 15 citrus varieties. In addition, the collective percentage of these five major acids (C_{16}, $C_{16:1}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$) to the total acid content, the approximate ratios of these five acids, and the 16/16:1 ratio also are reported. Total percentages of these acids vary from 37·80% (Columbia lime) to 87·85% (Marsh grapefruit); within a particular species, however, variation is not great except for limes. Oranges show a range 76·84–87·52% with an average of 83·62%, which correlates closely with 77·20% previously determined for several other orange varieties.[8] Homosassa has a noticeably lower $C_{18:2}$ content than the other orange varieties and this accounts for its lower total percentage of C_{16} and C_{18} acids. The ratios of these five acids in the four orange varieties are similar but have minor differences in the $C_{18:2}$ content.

Grapefruit are quite similar to oranges in their total content of these five major acids. In grapefruit, the proportion of palmitic acid (C_{16}) and oleic acid ($C_{18:1}$) are slightly higher and noticeably lower, respectively, than in orange. Linoleic acid ($C_{18:2}$) can be used to differentiate Marsh and Burgundy (55·75 and 54·79%, respectively) from Foster and Thompson (46·06 and 43·01%, respectively).

Average total content for the major acids in lemons is 62·72%, *ca* 20% lower than for oranges or grapefruit. For Kusner, Lisbon and Eureka total acid contents are nearly identical and for Malta only 3–4% lower; Malta, however, shows lower percentages of C_{16}, $C_{16:1}$, $C_{18:1}$ and $C_{18:3}$. The approximate ratios of these five acids in lemons suggest that oleic acid ($C_{18:1}$) content might be capable of differentiating three of the lemon varieties from the fourth. The ratios of these five acids differ noticeably when compared with oranges, grapefruit and limes.

The three limes differ markedly from each other in their total major acid content; varying from 37·8 to 75·1%. This range is caused by differences among the C_{18} unsaturated acids and is manifest in their ratios. The C_{16} and $C_{16:1}$ percentages and the 16/16:1 ratios are, however, similar for all lime varieties.

[11] NAGY, S. and NORDBY, H. E. (1972) *Lipids* **7**, 666.
[12] NAGY, S. and NORDBY, H. E. (1972) *Phytochemistry* **11**, 2789.
[13] NAGY, S. and NORDBY, H. E. (1972) *Phytochemistry* **11**, 2865.
[14] NAGY, S. and NORDBY, H. E. (1972) *Lipids* **7**, 722.
[15] NORDBY, H. E. and NAGY, S. (1972) *Phytochemistry* **11**, 3249.
[16] NAGY, S. and NORDBY, H. E. (1973) *Phytochemistry* **12**, 801.

TABLE 1. MAJOR FATTY ACID COMPOSITION OF STEROL ESTERS FROM CITRUS JUICE SACS (%)

Species–variety	Fatty acid C_{16}	$C_{16:1}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	Total major fatty acids	Ratio 16/16:1	Approximate ratio of five acids
Oranges								
(1) Homosassa	4·46	3·85	13·42	38·15	16·96	76·84	1·16	1 1 3 9 4
(2) Queen	4·06	4·16	14·23	47·84	15·89	86·18	0·98	1 1 3 12 4
(3) Lue Gim Gong	4·18	3·23	13·40	53·92	12·79	87·52	1·29	1 1 3 13 3
(4) Jaffa	4·52	3·73	13·34	46·73	15·63	83·95	1·12	1 1 3 10 3
Av.	4·31	3·74	13·60	46·66	15·32	83·62	1·16	1 1 3 11 4
Grapefruit								
(1) Marsh	5·19	2·80	12·11	55·75	12·00	87·85	1·85	2 1 5 21 5
(2) Burgundy	6·87	3·24	8·56	54·79	11·56	85·02	2·12	2 1 3 16 3
(3) Thompson	5·10	3·13	9·71	46·06	16·37	80·37	1·63	2 1 4 18 6
(4) Foster	5·31	3·34	9·58	43·01	15·03	76·27	1·59	2 1 4 16 6
Av.	5·62	3·13	9·99	49·90	13·74	82·38	1·80	2 1 4 18 5
Lemons								
(1) Kusner	5·83	1·04	8·61	27·72	20·56	63·76	5·61	6 1 9 28 20
(2) Lisbon	6·21	1·04	13·15	27·04	16·54	63·98	5·97	6 1 13 27 16
(3) Malta	3·95	0·58	4·07	36·38	14·95	59·93	6·81	7 1 7 63 26
(4) Eureka	6·83	1·08	5·02	30·19	20·11	63·23	6·32	6 1 5 28 19
Av.	5·70	0·94	7·71	30·33	18·04	62·72	6·18	6 1 8 32 19
Limes								
(1) Key	6·25	2·04	6·83	46·44	13·50	75·06	3·06	3 1 3 23 7
(2) Persian	5·99	2·34	4·32	22·68	8·98	44·31	2·56	3 1 2 10 4
(3) Columbia	6·40	2·45	14·34	10·30	4·31	37·80	2·61	3 1 6 4 2
Av.	6·21	2·28	8·50	26·47	8·93	52·39	2·74	3 1 4 12 4

Although the 16/16:1 ratio is different for each species, deviation within a species is slight (Table 1). Although the contents of C_{16} and $C_{16:1}$ acids are low in Malta lemons, the 16/16:1 ratio is comparable for all lemon varieties. The value of 1·16 for oranges is identical to the value calculated from data previously presented for six other orange varieties.[8] These ratios can be used to differentiate citrus species.

Major C_{22}–C_{26} fatty acids

As observed for the major C_{16} and C_{18} acids, the major C_{22}–C_{26} fatty acid percentages for oranges and grapefruit are very similar (Table 2). The average total contents for these C_{22}–C_{26} acids are 8·48% for oranges and 8·97% for grapefruit. Patterns for Queen, Lue Gim Gong and Jaffa oranges are similar with the following sequence of percentages: $C_{24} > C_{26} > C_{25}$ while the C_{22}, C_{23}, *iso* C_{24}, and *iso* C_{26} acids are found in about equal percentages. For Homosassa, percentages of C_{23}, C_{24}, C_{25} and C_{26} are approximately double those of the other three varieties, which accounts for the higher total percentage (13·51) of these seven C_{22}–C_{26} acids in Homosassa. In grapefruit the total averages for these seven acids vary among varieties. The acid showing the greatest fluctuation is lignoceric acid (C_{24}) which varies from 1·96% (Marsh) to 5·22% (Foster); variability is noticeable also in C_{25}, *iso* C_{26} and C_{26} acids.

For lemons, the average total percentage for these C_{22}–C_{26} acids is 19·45, which is more than twice that found in either oranges or grapefruit. Although the profiles of these seven acids differ only slightly among the four lemon varieties, one exception is the greater percentage of *iso* C_{24} in the Malta. As with oranges and grapefruit, the sequence of these acids in lemons is $C_{24} > C_{26} > C_{25}$, while *iso* $C_{26} >$ *iso* $C_{24} > C_{22}$–C_{23}.

Comparison of these seven acids in limes reveals some interesting similarities and differences. Columbia, a sweet lime of unknown origin, has nearly the same total percentage (32·08) as that found for Persian (32·71), a sour lime. The percentages of the individual

TABLE 2. MAJOR LONG-CHAIN FATTY ACID COMPOSITION OF STEROL ESTERS FROM CITRUS JUICE SACS (%)

Species- variety	Fatty acid C_{22}	C_{23}	*iso* C_{24}*	C_{24}	C_{25}	*iso* C_{26}*	C_{26}	Total major long-chain fatty acids	Approximate ratio of 7 acids						
Oranges															
(1) Homosassa	1·07	0·72	0·57	5·69	1·77	0·57	3·12	13·51	2	1	1	10	3	1	6
(2) Queen	0·74	0·31	0·41	2·44	0·47	0·48	1·29	6·14	2	1	1	8	1	1	4
(3) Lue Gim Gong	0·55	0·36	0·29	2·37	0·86	0·39	1·27	6·09	2	1	1	8	3	1	4
(4) Jaffa	0·78	0·45	0·27	2·93	1·22	0·34	2·17	8·16	3	2	1	11	4	1	8
Av.	0·71	0·46	0·39	3·36	1·08	0·45	1·96	8·48	2	1	1	9	3	1	5
Grapefruit															
(1) Marsh	0·41	0·24	0·30	1·96	0·75	0·55	1·09	5·30	2	1	1	8	3	2	4
(2) Burgundy	0·52	0·33	0·28	2·61	1·16	0·51	1·61	7·02	2	1	1	9	4	2	5
(3) Thompson	0·77	0·61	0·67	3·34	1·52	1·13	2·02	10·06	1	1	1	5	2	2	3
(4) Foster	0·69	0·69	0·69	5·22	2·59	1·02	2·59	13·49	1	1	1	8	4	1	4
Av.	0·60	0·47	0·49	3·28	1·51	0·80	1·83	8·97	1	1	1	7	3	2	4
Lemons															
(1) Kusner	0·82	0·74	1·04	7·44	3·43	2·43	5·29	21·19	1	1	1	10	4	3	7
(2) Lisbon	0·74	0·74	1·44	5·68	2·67	2·79	4·06	18·12	1	1	2	8	4	4	5
(3) Malta	1·07	0·92	2·35	5·89	2·33	3·62	3·27	19·45	1	1	2	6	2	4	3
(4) Eureka	0·77	1·15	1·25	7·04	2·74	2·26	3·80	19·01	1	1	2	7	3	2	4
Av.	0·82	0·89	1·52	6·51	2·79	2·78	4·11	19·45	1	1	2	7	3	3	5
Limes															
(1) Key	0·72	0·32	0·61	2·62	0·92	0·85	1·80	7·84	2	1	2	8	3	3	6
(2) Persian	1·18	1·02	2·39	9·82	4·47	5·61	8·22	32·71	1	1	2	10	4	6	8
(3) Columbia	1·20	1·13	2·64	10·26	4·24	5·71	6·90	32·08	1	1	2	10	4	6	7
Av.	1·03	0·62	1·88	7·57	3·21	4·06	5·64	18·16	2	1	3	12	5	7	9

* *iso*-branched fatty acid.

TABLE 3. MINOR EVEN-NUMBERED FATTY ACID COMPOSITION

Species variety	C_{12}	*iso* C_{14}	C_{14}	$C_{14:1}$	*iso* C_{15}	*iso* $C_{15:1}$	*iso* C_{16}	*iso* $C_{16:1}$	Fatty C_{18}
Oranges									
(1) Homosassa	1·01	0·14	2·35	0·41	0·16	0·21	T*	0·48	0·32
(2) Queen	0·81	0·12	2·07	0·19	0·17	0·11	0·11	0·57	0·40
(3) Lue Gim Gong	0·44	T	1·16	0·23	0·12	0·21	T	0·57	0·25
(4) Jaffa	0·68	0·13	1·68	0·23	0·14	0·11	T	0·53	0·44
Av.	0·74	†	1·82	0·27	0·15	0·16		0·54	0·35
Grapefruit									
(1) Marsh	0·15	0·13	1·44	T	0·19	0·13	0·12	0·79	0·42
(2) Burgundy	0·21	T	1·95	0·27	0·18	0·51	T	0·39	0·62
(3) Thompson	0·39	T	1·96	0·13	0·24	0·17	0·10	0·77	0·47
(4) Foster	0·36	0·24	2·15	0·21	0·28	0·29	0·35	0·75	0·53
Av.	0·28		1·88		0·22	0·28		0·68	0·51
Lemons									
(1) Kusner	0·82	0·26	1·71	0·20	0·21	†	0·20	1·91	0·70
(2) Lisbon	0·83	0·41	1·70	0·21	0·22	†	0·22	2·06	0·69
(3) Malta	0·93	0·16	2·25	T	0·24	†	0·27	2·59	0·41
(4) Eureka	1·94	0·37	3·31	T	T		0·33	1·16	1·20
Av.	1·13	0·30	2·24				0·26	1·93	0·75
Limes									
(1) Key	0·77	0·37	4·39	0·25	0·77	0·42	0·43	1·73	0·83
(2) Persian	0·81	0·58	3·70	0·38	0·90	T	0·57	1·07	1·13
(3) Columbia	1·51	0·75	4·21	0·70	1·00	0·25	0·69	1·17	1·40
Av.	1·03	0·57	4·10	0·44	0·89		0·56	1·32	1·12

* Value in trace amount (0·01–0·1%).

† Average not calculable because of trace percentage.

acids are nearly identical except for an additional 1% for C_{26} found in Persian lime. Key lime has about the same amount of these long chain acids as orange and grapefruit.

Minor even-numbered C_{12}–C_{28} fatty acids

Acids in this region are quite similar for oranges and grapefruit, 5·86 and 6·15%, respectively (Table 3). Intervarietal differences for individual acids of orange are small. Linear C_{14} myristic is the major acid and C_{12}, C_{20} and C_{28} occur in decreasing amounts. The content of *iso* $C_{18:1}$ *iso* C_{18} are about 0·5% and trace, respectively; this contrasts with orange juice total lipids[7] where *iso* C_{18} was greater than *iso* $C_{18:1}$. Grapefruit possesses greater proportions of C_{18} and $C_{28:1}$, and less C_{12} than the orange varieties.

Lemons contain an average of 9·76% minor even-numbered fatty acids, and show few varietal differences. However, analysis of the individual acids shows that linear monoenes are almost completely absent. The one exception is $C_{20:1}$, where lemons like the orange and grapefruit species contain *ca* 0·4%. Lemons contain nearly twice the amount of linear saturates and greater than twice the amount of *iso*-saturates and *iso*-monoenes found in oranges and grapefruit. *iso* $C_{18:1}$ is present in three of the four lemon varieties at 2% of the total fatty acids.

Limes contain from two to three times the amount of minor even-numbered fatty acids as the other three citrus species. Linear C_{14} is present in limes at 4% while linear C_{12}, C_{18}, C_{20} and C_{28} are all present at *ca* 1%. *iso* C_{14},-C_{16},-C_{18} and -C_{22} are present at about 0·5% and *iso* C_{28} about 1%. The linear monoenes, viz. C_{14}, C_{20}, C_{22}, C_{24}, C_{26} and C_{28} are all present in the 0·5–1% range. Columbia has a larger percentage of monoenes than the other two limes. This accounts for the higher amount of even-numbered minor acids (24%) than in Key (13%) or in Persian (13%). This contrasts with results in Table 2 where Persian

OF STEROL ESTERS FROM CITRUS JUICE SACS (%)

acid										
iso C_{20}	C_{20}	$C_{20:0}$	*iso* C_{32}	*iso* $C_{22:1}$	$C_{24:1}$	$C_{26:1}$	*iso* C_{28}	C_{28}	$C_{28:1}$	Total minor even-numbered fatty acids
T	0·38	0·38	0·21	0·26	0·29	0·26	0·10	0·47	0·10	7·53
T	0·16	0·35	0·18	T	0·24	0·26	T	0·16	T	5·90
T	0·34	0·46	T	0·20	0·13	0·25	T	0·17	T	4·53
T	0·27	0·31	0·22	0·19	0·17	0·18	T	0·24	T	5·49
	0·29	0·38			0·21	0·24		0·26		5·86
T	0·28	0·36	T	T	0·26	0·17	0·25	0·18	0·22	5·09
T	0·24	0·28	T	0·23	0·18	0·20	T	0·19	0·17	5·62
T	0·29	0·40	0·17	0·33	0·13	0·49	0·24	0·24	0·54	7·06
T	0·27	0·30	T	T	0·17	0·30	0·13	0·25	0·26	6·84
	0·27	0·34			0·19	0·29		0·22	0·30	6·15
T	0·96	T	T	—	—	—	0·82	0·86	—	8·65
0·28	0·42	0·68	0·31	—	—	—	1·03	0·85	—	9·91
T	0·39	0·43	0·37	—	—	—	0·94	0·65	—	9·63
T	0·42	0·49	0·31	—	—	—	0·68	0·63	—	10·84
	0·55						0·87	0·75		9·76
0·35	0·74	0·39	0·39	0·29	0·16	0·19	0·32	0·56	0·26	13·61
T	0·82	T	0·55	T	T	T	1·26	1·52	0·41	13·70
T	1·11	1·33	0·41	0·94	2·15	2·52	1·51	0·96	1·37	23·97
	0·89		0·45				1·03	1·01	0·68	17·08

‡ Fatty acid not detected under GLC parameters (>0·01%).

TABLE 4. MINOR ODD-NUMBERED FATTY ACID COMPOSITION

Species variety	Fatty								
	Al-C_{15}*	C_{15}	$C_{15:1}$	Al-C_{17}	Al-$C_{17:1}$	C_{17}	$C_{17:1}$	Al-C_{19}	Al-$C_{19:1}$
Oranges									
(1) Homosassa	0·11	0·12	0·25	T	0·24	T	0·24	T	T
(2) Queen	T	T	0·24	T	0·28	T	0·30	T	T
(3) Lue Gim Gong	0·11	0·10	0·19	T	0·29	T	0·23	T	T
(4) Jaffa	0·11	0·11	0·22	T	0·26	T	0·26	T	T
Av.			0·23		0·27		0·26		
Grapefruit									
(1) Marsh	T	0·16	0·22	T	0·15	T	0·31	T	T
(2) Burgundy	0·37	0·23	0·43	T	0·40	T	0·33	T	T
(3) Thompson	T	0·16	0·24	T	0·22	0·10	0·31	T	T
(4) Foster	0·22	0·30	0·57	0·12	0·28	0·16	0·41	T	T
Av.		0·21	0·37		0·26		0·34		
Lemons									
(1) Kusner	0·18	0·20	0·41	0·35	T	0·16	0·40	1·12	0·65
(2) Lisbon	0·17	0·17	0·55	0·16	T	0·12	0·60	1·41	T
(3) Malta	0·11	0·31	0·89	0·51	T	0·16	0·30	1·66	2·03
(4) Eureka	0·56	0·38	1·12	0·12	T	0·14	0·57	0·80	T
Av.	0·26	0·26	0·74	0·29		0·15	0·47	1·24	
Limes									
(1) Key	0·28	0·31	0·39	0·41	0·19	0·18	0·28	0·53	T
(2) Persian	0·43	0·38	0·61	0·43	0·24	0·24	0·15	0·36	0·73
(3) Columbia	0·27	0·17	0·30	0·34	0·10	0·17	0·21	0·19	0·38
Av.	0·33	0·29	0·43	0·39	0·18	0·20	0·21	0·36	

* *Anteiso*-branched fatty acid.

and Columbia limes show similar profiles. As in other species, *iso* $C_{18:1}$ is present to a greater extent than *iso* C_{18} however, in limes the ratio of these two acids is only 2:1.

Minor odd-numbered fatty acids

Minor odd-numbered fatty acids (Table 4) are present collectively in insignificant (2–2·5%) amounts in oranges and grapefruit; only *anteiso* $C_{19:2}$ exceeded 0·5%. This acid was not reported previously in a study of orange sterol esters[8] because of GLC limitations. The use of a highly efficient 3% SP-1000 column enabled us to resolve *anteiso* C_{19} from $C_{18:2}$ and *anteiso* $C_{19:2}$ from $C_{18:3}$; however, *anteiso* $C_{19:1}$ was still unresolved from $C_{18:3}$. Values for *anteiso* $C_{19:1}$ are calculated as the difference between the total *anteiso* C_{19} content (value obtained after hydrogenation) and the sum of *anteiso* C_{19} and *anteiso* $C_{19:2}$. For oranges and grapefruit *anteiso* C_{19} and *anteiso* $C_{19:1}$ fall below 0·1% and, therefore, are not reported.

Lemons contain over 8% minor odd-numbered fatty acids. Saturated *anteiso* fatty acids make up 50% of this group of acids with every fatty acid from C_{15} to C_{29} present at concentrations greater than 0·1%. Major differences in lemon varieties occur in the *anteiso* C_{19}, *anteiso* $C_{19:1}$ and *anteiso* $C_{19:2}$ areas. Totals of these three acids vary from 1·0% for Eureka to 4·2% for Malta. Limes contain 3·5–9·3% minor odd-numbered fatty acids. This variation is due in part to the differences in *anteiso* C_{25} and *anteiso* C_{27} among the three varieties. Generally limes resemble lemons except in the *anteiso* C_{19}, *anteiso* $C_{19:1}$ and *anteiso* $C_{19:2}$ areas where the total percentages for these three acids are found in the narrow range 0·8–1·9%.

Summary of fatty acid composition of sterol esters

All four species show patterns characteristic of citrus sterol esters[8] but differ from one

OF STEROL ESTERS FROM CITRUS JUICE SACS (%)

acid									
Al-$C_{19:2}$	Al-C_{23}	*iso*-C_{25}	Al-C_{25}	*iso*-C_{27}	Al-C_{27}	C_{27}	Al-C_{29}	C_{29}	Total minor odd-numbered fatty acids
0·45	T	0·20	0·25	—	T	0·26	T	T	2·12
0·47	T	0·15	0·19	—	T	0·15	T	T	1·78
0·78	T	T	T	—	T	0·16	T	T	1·86
1·22	T	T	T	—	T	0·22	T	T	2·40
0·73						0·20			2·04
0·73	T	T	T	—	T	0·19	—	—	1·76
0·42	T	T	T	—	T	0·16	T	T	2·34
0·71	T	0·28	0·25	—	T	0·24	T	T	2·51
0·44	T	0·31	0·21	T	0·17	0·21	T	—	3·40
0·58						0·20			2·50
T	T	0·32	0·74	0·21	0·83	0·62	0·21	T	6·40
0·68	T	0·40	1·40	0·19	1·22	0·60	0·22	0·10	7·99
0·48	T	0·67	1·42	0·35	1·42	0·47	0·21	T	10·99
0·21	0·22	0·32	0·80	0·27	0·91	0·43	T	T	6·92
		0·43	1·08	0·26	1·09	0·53			8·08
0·44	T	T	0·15	—	0·15	0·18	T		3·49
0·76	0·27	T	2·01	—	1·68	0·99	T		9·28
0·29	T	0·86	1·40		0·63	0·67	0·17	—	6·15
0·50			1·19		0·82	0·61			6·31

another in other ways. The major acid in all varieties (except Columbia lime) is linoleic acid. Contents of fatty acids of chain length higher than C_{20} range from 6·5% for oranges to over 41% for limes. Although the 16/16:1 ratios vary from 1:1 for oranges to 6:1 for lemons, these ratios are remarkably constant for varieties within any one species. In the orange group, Homosassa shows higher contents of C_{24} and C_{26} than other orange varieties. Jaffa shows the highest percentage of *anteiso* $C_{19:2}$ while Lue Gim Gong manifests low percentages for C_{12}, C_{14} and C_{18} acids. In grapefruit Marsh shows a high $C_{18:1}$ content, Foster a high C_{24}, Burgundy a low *iso* $C_{18:1}$ and Thompson a high $C_{28:1}$ percentage when compared with the other varieties. In lemons, Lisbon is noted by its high $C_{18:1}$, Malta by its high *iso* C_{24} Kusner by a high C_{26} and Eureka by high contents of C_{12} and C_{14} acids. The lime varieties show major differences in $C_{18:2}$ percentages.

Argentation TLC of sterol esters

Citrus sterol esters are very difficult to separate from carotenoid esters by conventional TLC. Since citrus sterol esters are the only class of lipids to show a high percentage of long-chain acids,[8] it could be argued that these long-chain acids are, in reality, derived from carotenoid esters. Carotenoids are highly unsaturated structures and would tend to form strong complexes with the silver ion on silver nitrate-impregnated TLC plates. Sterols, on the other hand, would form weak complexes with silver because of the limited number of double bonds in the ring and side chain. Citrus long-chain fatty acids ($>C_{20}$) are primarily saturated structures[6,8] and, therefore, would not complex with silver during TLC separation. Separation of sterol esters from carotenoid esters should be effected because the silver ion would retard the highly unsaturated carotenoid from migrating while allowing the sterol ester group to separate into its component bands.

To test this hypothesis, sterol esters from Key lime and Marsh grapefruit were subjected

to silver nitrate TLC. The sterol ester group upon development was shown to fractionate into three primary bands. The fastest migrating fraction (Band I) had relatively similar R_f values for Key lime (R_f 0·65) and Marsh grapefruit (R_f 0·67). Band II of Key and Band III of Marsh likewise showed a similar R_f of 0·42. Band III of Key (R_f 0·28) and Band II of Marsh (R_f 0·54) exhibited no correlation.

TABLE 5. DISTRIBUTION OF STEROL GROUPS IN STEROL ESTER FRACTIONS SEPARATED BY ARGENTATION TLC

	Marsh grapefruit Sterol group (%)			Key lime Sterol group (%)		
Band	Desmethyl	Monomethyl	Dimethyl	Desmethyl	Monomethyl	Dimethyl
I	32·8	10·6	56·6	24·5	12·9	62·6
II	73·1	5·0	21·9	62·2	20·1	17·7
III	77·3	11·4	11·3	20·6	30·9	48·5

TABLE 6. FATTY ACID COMPOSITION FROM ARGENTATION TLC SEPARATIONS OF STEROL ESTERS (%)

	Key lime				Marsh grapefruit			
Fatty acid	Total S.E.	Band I	Band II	Band III	Total S.E.	Band I	Band II	Band III
12	0·8	6·7	0·3	7·5	0·2	T*	0·3	—†
14	4·4	7·2	1·5	1·5	1·4	3·7	1·8	0·3
15	0·3	0·6	0·5	T	0·2	1·5	0·3	T
iso 16	0·8	0·8	T	1·5	0·2	0·7	T	0·1
16	6·3	11·4	4·3	2·4	5·2	41·9	7·7	0·6
16:1	2·0	3·0	4·8	0·8	2·8	3·9	14·2	1·2
17	0·2	0·3	0·1	T	T	1·3	0·1	T
17:1	0·3	0·3	0·5	0·6	0·3	0·3	1·7	0·1
iso 18	0·4	0·9	T	0·3	0·1	0·9	T	T
iso 18:1	1·7	3·1	1·9	0·5	0·8	0·2	3·0	0·7
18	0·8	1·5	0·8	0·8	0·4	5·4	0·1	—
18:1	6·8	12·5	8·1	1·2	12·1	10·5	63·0	3·3
18:2	46·4	8·1	72·2	75·7	55·8	2·0	T	86·1
18:3	13·5	—	1·4	1·4	12·0	—	T	0·8
AI-19	0·5	1·3	T	T	T	T	0·6	T
AI-19:2	0·4	—	T	0·2	0·7	T	1·4	0·2
20	0·7	1·3	0·3	0·4	0·3	1·8	0·7	0·2
22	0·7	2·7	T	0·5	0·4	2·6	0·3	0·4
23	0·3	1·3	T	—	0·2	1·4	0·3	0·3
iso 24	0·6	2·2	T	T	0·3	1·8	T	0·4
24	2·6	11·6	0·4	1·3	2·0	7·1	1·1	2·7
25	0·9	3·3	0·3	0·6	0·8	2·1	0·4	0·7
iso 26	0·9	3·0	T	0·4	0·6	2·5	T	0·6
26	1·8	6·7	0·4	1·0	1·1	1·8	0·3	0·8
Total 'minor fatty acids'	5·9	10·2	2·2	1·4	2·1	6·6	2·7	0·5
Total saturates	26·2	68·9	9·3	19·6	14·0	80·9	14·1	7·1
Total monoenes	13·4	23·1	16·0	3·1	17·5	16·9	83·9	5·8
Total dienes	46·9	8·0	73·3	75·9	56·5	2·2	2·0	86·3
Total trienes	13·5	—	1·4	1·4	12·0	T	T	0·8
Total (C_{22}–C_{29})‡	10·5	38·8	1·1	3·8	6·6	22·7	2·4	6·1

* Less than 0·1%.
† Not detectable under GLC parameters (>0·01%).
‡ Includes all linear and branched C_{22}–C_{29} acids.

Saponification of these bands, followed by TLC revealed the presence of free fatty acids and 4-desmethyl, 4α-monomethyl and 4,4-dimethyl sterols. No carotenoids were detected in any band. Each band was examined for distributions of sterol groups (Table 5) and of fatty acids (Table 6). Band I of both Marsh grapefruit and Key lime show high relative percentages of dimethyl sterols, viz. 56·6 and 62·6%, respectively. Table 6 shows that the major portion of long-chain acids (C_{22}–C_{29}) occur in band I. Secondly, the data indicate that these long-chain acids are primarily esterified to dimethyl sterols. Band I also contains the highest percentage of saturates, 68·9% in Key and 80·9% in Marsh.

Band II of Marsh contains 73·1% desmethyl sterols (Table 5) and 83·9% monoenoic acids. Band II of Key and Band III of Marsh, possessing similar R_f values, contain 73 and 86% dienes, respectively. These two bands also show high percentages of desmethyl sterols (62·2% Key, 77·3% Marsh). Band III of Key contains 75·9% dienes and only 20·6% desmethyl sterols. The data in Tables 5 and 6 suggest that argentation TLC separates sterol esters on the basis of both fatty acid and sterol moieties.

EXPERIMENTAL

Citrus sources and preparation of juice sac lipids. Oranges. Four orange varieties of *C. sinensis* (Homosassa, Queen, Lue Gim Gong and Jaffa) were obtained at a mature stage from Whitmore Experimental Farm (Plant Science Research Division, USDA, Orlando, Florida). *Grapefruit.* Four varieties of *C. paradisi* were employed. Marsh seedless and Foster were obtained from Whitmore Experimental Farm, Burgundy from IFAS Agricultural Research Center, Ft. Pierce, Florida, and Thompson from Adams Citrus Nursery, Haines City, Florida. *Lemons.* Three varieties of *C. limon* (Lisbon, Malta and Kusner) were obtained from Whitmore Experimental Farm while the fourth variety (Eureka), was obtained from a local market. *Limes.* Two varieties of sour lime, *C. aurantifolia* Swingle cv. Key and *C. latifolia* Tanaka cv. Persian and one variety of sweet lime, *C. limettioides* Tanaka cv. Columbia were obtained from USDA groves in Homestead, Ft. Pierce and Leesburg, Florida, respectively. Juice sac lipids were prepared and purified by a method previously described.[7] 4–6 Extractions were run on each citrus variety.

Separation of sterol esters and preparation of fatty acid methyl esters. The purified juice sac lipid (*ca* 200 mg) was dissolved in $CHCl_3$ and percolated onto an 0·9 × 30 cm column containing 9 g Baker, 60–200 mesh silica gel (Baker Chemical Co., Phillipsburg, New Jersey). The neutral lipids, which contained the sterol esters, were eluted with 200 ml $CHCl_3$, concentrated, taken up in hexane and streaked on precoated silica gel G plates (20 × 20 cm, 500 μ, Analtech, Inc., Wilmington, Delaware). The TLC plate was placed in a chamber lined with filter paper and developed with hexane-ethyl ether (9:1) containing 0·1% di-tert-butyl-cresol. The sterol ester band was visualized with Rhodamine 6G and eluted with ethyl ether. The sterol esters were concentrated to dryness under nitrogen and hydrolyzed with 3 ml 6% KOH in 95% EtOH in 10-ml sealed acetylation tubes (Regis Chemical Co., Chicago, Illinois) for 1 hr at 75°. After neutralization the products were extracted into ethyl ether, dried, reduced to dryness and chromatographed on TLC plates. The plates were developed in $CHCl_3$. This solvent separates free fatty acids from sterols. The free fatty acid band was scraped from the plate and methylated directly with BF_3–MeOH.[6] Representative fatty acid methyl ester samples from each variety were hydrogenated under 68 kg/cm^2 at room temp. for 1 hr with 10 mg of 10% Pd–C catalyst in a Parr apparatus.

Argentation TLC separation of sterol esters. $AgNO_3$ plates were prepared and activated as described previously.[7] Sterol esters from Key lime and Marsh grapefruit were separately streaked on $AgNO_3$–TLC plates and developed in hexane–Et_2O (9:1). The sterol ester group from each of these two varieties fractionated into three primary bands. The bands were visualized with Rhodamine 6G, recovered with ether and saponified by the above described procedure. The saponified products were spotted on plain TLC plates and developed in $CHCl_3$. The free sterol band was scraped from these plates, eluted with Et_2O and respotted on prewashed plain silica gel G plates along with cholesterol and lanosterol standards. Upon development in C_6H_6–EtOAc (4:1) the plate was sprayed with 50% H_2SO_4, charred in a muffle furnace at 210° for 20 min and density values of 4-desmethyl, 4-monomethyl and 4,4-dimethyl sterols determined by densitometry.[17] Density values, after normalization to the two sterol standards, were converted to relative per cents.

GLC. Fatty acid methyl esters were analyzed by two methods. (1) The lower fatty acids (C_{12}–$C_{18:3}$) were determined on a polar column and the results combined with those obtained for the longer chain fatty acids on a non-polar column. The lower fatty acids were determined with an F & M Model 5750 gas chromatograph equipped with flame ionization detectors. The column was 3·05 m long, 4 mm i.d., packed with 10% SP-1000 (Supelco, Inc., Bellefonte, Pennsylvania) coated on 100/120 mesh Gas Chrom Q (Applied Science, State College, Pennsylvania).

[17] NAGY, S. and NORDBY, H. E. (1970) *J. Agric. Food Chem.* **18,** 593.

The injection port and detector were at 245°. The samples were injected on-column with helium flow rate 55 ml/min and oven at 210°. When the 18:3 methyl ester had eluted the temp. was raised to 225° and held at this temp. until the longer chain fatty acid methyl esters had eluted. The peak areas were measured with the aid of a disc integrator. The non-polar column consisted of a 1·22-m glass U-tube filled with 10% OV101 (Western Analytical Service, Grinda, California) coated on 100/200 mesh Gas Chrom Q. Samples were injected on-column into an F & M Model 7610A research gas chromatograph equipped with a FID. Injection port and detector were at 245° with a helium flow rate of 55 ml/min. Optimum temp. program was: run isothermal at 150° for 4 min then 150–170° at 2°/min, then 170–250° at 4°/min and finally hold until C_{29} had eluted from column. The percentages were calculated with the aid of an Autolab System IV computing integrator for chromatography (Autolab Inc., Mountain View, California). (2) Methyl esters were analyzed on a 3·05 m long, 4 mm i.d. glass U-tube packed with 3% SP-1000 coated on 100/120 mesh Gas Chrom Q. Injection port and detector were at 245° with a helium flow rate of 55 ml/min. Optimum temp. program was: 140–170° at 2°/min then 170–195° at 4°/min then 195–230° at 30°/min and finally hold at 230° until C_{29} methyl esters had eluted from column. Results were quantified by measurement of peak areas with the aid of a disc integrator. Four or more analyses were run on each variety by both GLC methods and results of the combined eight or more analysis were averaged.