Glyceride Studies: Part IX: Intraglyceride Distribution of Vernolic Acid and of Five Conjugated Octadecatrienoic Acids in Seed Glycerides

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ABSTRACT

Vernolic (epoxyoleic) acid, present in six seed oils over the range 19-72%, appears to be preferentially attached to the secondary glycerol hydroxyl group. The distribution of five isomeric conjugated octadecatrienoic acids (8,10,12 and 9,11,13) in 18 seed oils has been examined by lipase-catalyzed deacylation. The results are not entirely consistent and more species must be examined before a rational distribution pattern becomes apparent.

INTRODUCTION

Recent studies of intraglyceride distribution by lipase-catalyzed deacylation have revealed a selective distribution of fatty acids in the lipid reserves laid down as glycerides in seeds. Such oils most commonly contain palmitic, stearic, oleic, linoleic and linolenic acids and it seems that the secondary hydroxyl group is acylated by the unsaturated C_{18} seeds in preference to the saturated acids. The higher unsaturated acids, eicosenoic and docosenoic, however, generally accompany the saturated acids in the 1 and 3 positions (1). Oleic, linoleic and linolenic acids do not, in fact, compete equally for the 2 position and linoleic acid is usually enriched at this position slightly more than oleic acid or linolenic acid (2,3). These widely accepted generalizations are based on results from many plant species and from investigations conducted in several laboratories.

Many unusual acids also occur in seed fats but the glyceride distribution of these acids has been studied less extensively because it is difficult to obtain enough species containing these acids in widely varying amount. It is, therefore, more difficult to arrive at satisfactory generalizations about their intraglyceride distribution. Attempts to do this have been made for hexadec-9 and 11-enoic acids (3), octadec-6-enoic (petroselinic) acid (3), octadeca-6,9,12-trienoic (γ-linolenic) acid (3), octadeca-6,9,12,15-tetraenoic acid (3), some conjugated octadecatrienoic acids (3,4), vernolic acid (4-6), dimorphecolic acid (4), coriolic acid (7), lesquerolic acid (6), ricinoleic acid (6), and 9-hydroxyoctadec-12-enoic acid (8). We report here further experiments with six seed oils containing vernolic acid and 18 seed oils (25 samples) containing five octadeca-9,11,13 and 8,10,12-trienoic acids (eleostearic, punicic, catalpic, calendic, jacaric).

EXPERIMENTAL PROCEDURES

All solvents were distilled before use. Petroleum refers to the fraction boiling between 40 C and 60 C.

Thin layer chromatography (TLC) was carried out on thin layers of silica (0.3 mm for analytical purposes, 1.0 mm for preparative

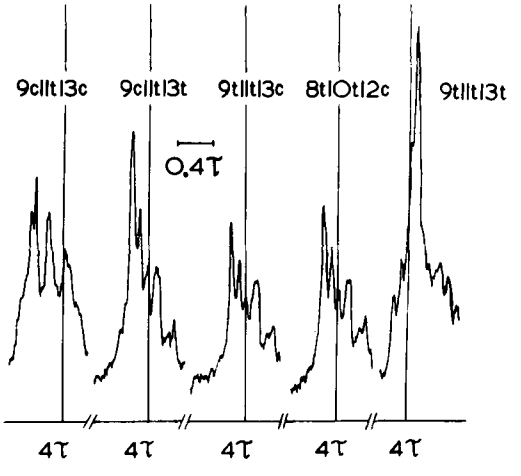

FIG. 1. NMR spectra of *M. balsamina* seed oil (9*c*11*t*13*c*), *M. charantia* seed oil (9*c*11*t*13*t*), *C. speciosa* seed oil (9*t*11*t*13*c*), *C. officinalis* (8*t*10*t*12*c*), and β-eleostearic acid (9*t*11*t*13*t*).

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TABLE I

Vernolic Acid-Containing Glycerides

Species	Family	V[a]	X_3[b]	X_2V[b]	XV_2[b]	V_3[b]	X_2V[c]	XV_2[c]
Cephalocroton peuschelli (Cp)	Euphorbiaceae	72	3	14	43	40	35	79
Cephalocroton cordofanus (Cc)	Euphorbiaceae	67	4	18	44	34	39	77
Crepis aurea (Ca)	Compositae	60	9	18	59	14	56	96
Euphorbia lagascae[d]	Euphorbiaceae	58	10	15	56	19	20	92
Crepis aurea[d]	Compositae	54	13	18	59	10	57	94
Crepis vesicaria (Cv)	Compositae	52	14	15	60	11	54	96
Cephalaria joppica (Cj)	Dipsacaceae	36	31	38	25	6[e]	50	81
Cephalaria joppica[d]	Dipsacaceae	27	37	40	22	0	57	94
Cephalaria leucantha (Cl)	Dipsacaceae	19	55	32	10	3[e]	51	75

[a]Content (mole %) of vernolic acid in seed oil.

[b]Content (mole %) of glycerides containing 0,1,2 and 3 epoxyacyl chains (V, vernolic acid; X, all other acids).

[c]Content (mole %) of 2-monovernolin in the 2-monoglycerides resulting from lipolysis of these fractions.

[d]Result taken from Reference 5.

[e]These values are too high since the extracted glycerides contain less than 100% of vernolic acid (see Table III).

purposes) containing, where necessary, 15% of silver nitrate. Compounds were made visible by spraying with an ethanolic solution (0.2%) of 2,7-dichlorofluoroscein and viewing under UV light.

A Pye 104 was used for most of the gas liquid chromatography (GLC). Columns were packed with Gas Chrom Z (70-80 mesh) coated with 20% diethylene glycol succinate and operated at 190 C.

UV spectra were recorded in methanol solution on a Unicam SP 700 and NMR spectra were recorded in carbon tetrachloride solution using a Perkin-Elmer R10 spectrometer (60 Mc/sec).

Examination of Seed Oils Containing Vernolic Acid

Triglyceride Isolation and Separation. Seeds were ground in a mortar under petroleum and extracted with this solvent in a Soxhlet.

Triglycerides (~200mg) were separated into five fractions by preparative TLC (10 plates) using petroleum-ether (3:1) and the separated fractions recovered from the silica by extraction with ether in a Soxhlet.

The nonepoxy and monoepoxy glycerides (~6 mg) were each separated further by preparative silver ion chromatography using benzene-ether (9:1 and 3:1, respectively). The separated glycerides were recovered from the silica by stirring with methanol-ether-water (5:5:1) and methyl heptadecanoate was then added as internal standard (9). The mixture was extracted with ether and the product transesterified for GLC examination. A correction factor (1.26) was used with the peak due to methyl vernolate.

Methyl esters were prepared from the whole oil or from separated glycerides (~5 mg) by reaction at room temperature overnight with

TABLE II

Enrichment Factors of Oleic, Linoleic and Vernolic Acids in the X_3, X_2V and XV_2 Glyceride Fractions[a]

	X_3		X_2V			XV_2		
Species	O1	Lin	O1	Lin	V	O1	Lin	V
Cp[b]	1.2	1.4	1.3	1.1	1.1	0.9	0.8	1.2
Cc	1.2	1.4	1.2	1.0	1.2	0.9	0.9	1.1
Ca	1.1	1.6	0.5	1.1	1.7	0.1	0.2	1.5
Cv	1.0	1.4	0.6	0.9	1.6	0.2	0.1	1.4
Cj	1.2	1.6	1.2	1.4	1.5	1.0	1.3	1.3
Cl	1.5	1.7	1.1	1.4	1.6	1.1	1.4	1.3

[a]Enrichment factor: Content of acid in the 2-monoglycerides/Content of the same acid in the triglycerides.

[b]Abbreviations detailed in Table I.

TABLE III

Component Acids (Mole %) of Triglycerides and 2-Monoglycerides Resulting From Lipase Hydrolysis of X_3, X_2V, XV_2 and V_3 Glyceride Fractions

	Triglycerides										2-Monoglycerides						
	12:0	14:0	16:0	18:0	18:1	18:2	18:3	20:0	V[a]	C[b]	14:0	16:0	18:0	18:1	18:2	18:3	V[a]
Cp[c] (29% oil)[d]																	
Whole oil			3.7	2.9	7.4	13.1	0.9		72.0								
X_3 (2.8)			16.0	9.7	30.4	40.7	3.2					1.9		36.5	57.4	4.2	
X_2V (13.6)			7.3	5.2	18.5	32.1	2.1		34.6			1.3		24.1	37.8	1.6	35.2
XV_2 (41.2)			4.4	3.3	8.7	15.2	1.0		67.4			0.5		7.8	13.0		78.7
V_3 (38.8)[e1]									100.0								
Cc (30% oil)																	
Whole oil			4.5	3.2	8.3	16.4	0.9		66.7			2.7		37.5	57.0	2.8	
X_3 (3.8)			13.5	10.5	31.1	41.8	3.1					1.4	1.2	20.3	36.1	1.8	39.2
X_2V (16.7)			6.6	5.4	17.0	35.6	1.8		33.6			0.8	0.4	6.3	15.5	0.5	76.5
XV_2 (40.6)			3.5	3.1	7.4	17.7	0.9		67.4								
V_3 (31.8)[e2]			0.3	0.1	0.4	0.7			98.5								
Ca (30% oil)																	
Whole oil			3.8	2.3	10.8	20.5			59.6	3.0							
X_3 (8.7)			11.4	6.7	22.0	47.1	1.7	1.1		10.0				24.8	73.1	2.1	
X_2V (17.2)			5.1	2.4	16.6	33.4			32.5	10.0				9.0	35.0		56.0
XV_2 (55.2)			3.9	2.0	8.8	18.6			66.2	0.5				1.2	2.8		96.0
V_3 (12.8)[e3]			1.1	0.5	1.6	3.0			93.3	0.5							
Cv (12% oil)																	
Whole oil			5.5	2.1	7.4	31.6	1.2		52.3	1.1							
X_3 (13.5)			13.1	5.5	16.6	59.1				4.5				15.8	82.5	1.2	
X_2V (14.6)			6.1	2.5	12.3	43.1			33.8	2.2				6.7	39.5		53.8
XV_2 (57.5)			3.8	1.6	4.5	22.4			67.7					1.0	2.9		96.1
V_3 (9.9)[e4]			1.3	0.6	1.1	5.8			91.2								
Cj (18% oil)																	
Whole oil		9.2	14.4	2.8	15.3	22.7			35.6								
X_3 (28.8)		10.1	18.2	3.5	28.6	39.6					0.6	0.9	0.4	35.0	63.1		
X_2V (35.4)		9.8	16.5	3.1	16.0	21.4			33.2		0.7	0.8	0.5	18.5	29.3		50.2
XV_2 (23.7)		7.3	12.2	2.2	6.9	7.5			63.9		0.6	1.0	0.4	7.1	9.9		81.0
V_3 (6.7)[e5]		3.1	6.2	1.2	6.6	7.6			75.3								
Cl (15% oil)																	
Whole oil	10.0	9.8	8.1	1.5	19.5	31.7			19.4								
X_3 (53.5)	13.5	11.4	9.2	1.6	23.0	41.3								34.1	65.9		
X_2V (30.7)	10.7	9.5	7.7	1.5	15.9	21.6			33.1					17.7	31.0		51.3
XV_2 (9.9)	6.8	7.1	5.3	1.0	9.1	10.9			59.8					10.1	15.4		74.5
V_3 (2.9)[e6]	5.5	6.2	5.5	1.0	10.3	13.4			58.1								

[a]V, vernolic acid.

[b]C, crepenynic acid.

[c]Abbreviations detailed in Table I.

[d]The lipolysis studies of these fractions were carried out on different preparations of the glyceride fractions from those reported here. Differences in triglyceride composition were insignificant.

[e]These oils also contain more polar fractions with a lower concentration of vernolic acid viz. 3.6% (31% vernolic acid)[1], 7.1% (38%)[2], 6.1% (60%)[3], 4.5% (52%)[4], 5.4% (48%)[5] and 3.0% (30%)[6].

TABLE IV

Glyceride Composition of X_3 and X_2V Fractions Determined by Silver Ion Chromatography

X_3 Glycerides	001[a]	011	002	111	012	112	022	122	222
Cp[b] (2.8%)[c]	6	7	11	6	20	13	19	13	5
	8	9	9	4	19	12	20	12	7
Cc (3.8%)	6	9	9	7	21	10	18	12	8
Cj (28.8%)	5	9	9	2	28	10	24	8	5
Cl (53.5%)	9	8	19	3	19	6	21	10	5
X_2V Glycerides	00V[a]	01V	11V	02V	12V	22V			
Cp[b] (13.6%)[c]	1	13	12	26	26	22			
	1	12	10	26	28	23			
Cc (16.7%)	1	10	9	28	26	26			
Cj (35.4%)	19	21	7	30	14	9			
Cl (30.7%)	17	23	8	31	12	9			

[a]These symbols indicate the three acyl chains in the glyceride (0, saturated; 1, monoethenoid; 2, diethenoid; V, vernolic) and include all possible isomers.

[b]Abbreviations detailed in Table I.

[c]Proportion of the total oil contained in this fraction.

anhydrous methanolic sodium methoxide (5 ml, 0.05%) and recovered without acidification of the reaction mixture.

Lipolysis. TRIS buffer was made by dissolving trihydroxymethylaminomethane (12.11 g) in distilled water (20 ml), titrating this with 1 M hydrochloric acid to pH 8.0, and diluting to 100 ml with water. Pancreatic lipase (15 mg) which had been purified by extraction with acetone, was dispersed in TRIS buffer (10 ml) and an aliquot (1 ml) of this was added to the triglyceride (5 mg) in a centrifuge tube. Calcium chloride solution (2.2%, 0.1 ml) and bile salt solution (0.05%, 0.3 ml) were also quickly added. After keeping the mixture at 40 C for 1 min it was stirred at this temperature for 8 min. The reaction mixture was then poured into water, extracted with ether, and the 2-monoglycerides isolated by preparative TLC with chloroform-acetone-0.880 ammonia (80:20:1).

Examination of Seed Oils Containing Conjugated Acids

Triglyceride Isolation. The seeds (full names are given in Tables I and VI) were ground in a mortar under petroleum with six different portions of solvent which was subsequently removed from the filtered solution at room temperature. If TLC (petroleum-ether, 4:1) showed the presence of partial glycerides and free acids, neutral triglycerides were isolated by column chromatography (10) or TLC.

TABLE V

Component Acids (mole %) of Triglycerides Before Lipolysis and of Unreacted Triglycerides Recovered After Lipolysis[a]

	16:0	18:0	18:1	18:2	18:3[b]
C. macrosiphon (9c11t13t)					
Original	5	3	5	35	52
Recovered	4	3	4	34	53
T. anguina (9c11t13c)					
Original	6	5	13	20	56
Recovered	6	5	13	20	55
C. ovata (9t11t13c)					
Original	3	3	8	38	44
Recovered	3	2	8	39	45
T. hyoseroides (8t10t12c)					
Original	5	3	8	41	40
Recovered	5	3	8	42	39

[a]Similar results were obtained with all the oils examined.

[b]This refers to conjugated octadecatrienoic acids; minor unsaturated acids are omitted.

TABLE VI

Component Acids (Mole %) of Triglycerides and 2-Monoglycerides Resulting From Lipase Hydrolysis of Seed Oils Containing Conjugated Octadecatrienoic Acids

Seed Oil	Triglycerides				2-Monoglycerides				Enrichment factors		
	sat.	18:1	18:2	18:3[a]	sat.	18:1	18:2	18:3[a]	18:1	18:2	18:3[a]
Eleostearic (9c11t13t)											
Tung oil[b]	5	11	15	69	--	9	31	60	0.8	2.1	0.9
Centranthus ruber[c]	7	4	36	52	1	6	91	1	1.5	2.5	0.0
C. ruber	7	3	37	52	1	6	90	3	2.0	2.4	0.1
C. macrosiphon[d]	8	5	35	52	1	10	83	5	2.0	2.4	0.1
Momordica charantia[e]	20	9	10	61	5	5	14	76	0.6	1.4	1.2
M. charantia	22	8	7	63	1	3	9	88	0.4	1.3	1.4
M. charantia	26	6	6	61	3	3	10	79	0.5	1.7	1.3
M. dioica[f]	22	10	10	57	2	7	10	81	0.7	1.0	1.4
Valeriana officinalis[g]	7	3	41	48	3	10	81	5	3.0	2.0	0.1
Punicic (9c11t13c)											
Momordica balsamina[h]	15	7	10	68	1	6	21	72	0.9	2.1	1.1
Punica granatum[i]	4	4	4	86	1	4	12	83	1.0	3.0	1.0
Tricosanthes anguina[j]	11	13	20	56	4	28	49	19	2.2	2.5	0.3
Apodanthera undulata[k]	14	14	38	33	2	17	79	3	1.2	2.1	0.1
Cucurbita palmata[l]	12	20	38	29	2	20	73	4	1.0	1.9	0.1
Catalpic (9t11t13c)											
Catalpa speciosa[m]	7	8	38	45	3	12	70	12	1.5	1.8	0.3
C. speciosa	7	9	47	33	1	15	74	9	1.7	1.6	0.3
C. ovata[n]	5	10	40	41	--	17	67	13	1.7	1.7	0.3
C. ovata	6	8	38	44	--	17	72	8	2.1	1.9	0.2
C. bignonoides[o]	4	10	45	41	--	15	76	9	1.5	1.7	0.2
C. bignonoides	6	9	45	40	--	16	79	5	1.8	1.8	0.1
Chilopsis linearis[p]	8	9	44	26[t]	4	14	67	11[t]	1.6	1.5	0.4
Calendic (8t10t12c)											
Calendula officinalis[q]	3	4	29	64	1	5	14	80	1.3	0.5	1.3
C. officinalis	4	5	31	59	--	3	16	80	0.6	0.5	1.4
Tripteris hyoseroides[r]	8	8	41	40	1	10	41	44	1.3	1.0	1.1
Jacaric (8c10t12c)											
Jacaranda mimosifolia[s]	9	12	38	39	1	17	76	6	1.4	2.0	0.2

[a]This refers to conjugated octadecatrienoic acids; some minor unsaturated acids have been omitted.

[b-s]Plant families: Euphorbiaceae (b), Valerianaceae (c,d,g), Cucurbitaceae (e,f,h,j-l), Punicaceae (i), Bignoniaceae (m-p,s), Compositae (q,r).

[c-s]Structure of octadecatrienoic acids: species c, reference (16), d (17), e (16), f (27), g (20), h (16), j (19), l (20,21), m (16), n (24), p (24), q (18 and 23), r (17), s (25).

[t]Also 18:2 (10*t*12*t*) 12% in triglyceride, 4% in 2-monoglyceride.

TABLE VII

Component Acid (mole %) of Triglycerides and 2-Monoglycerides Resulting From Lipase Hydrolysis of Seed Oils Containing Conjugated Dienoic, Trienoic and Tetraenoic Acids

	16:0	18:0	18:1	18:2	18:2[a]	18:3[a]	18:4[a]
Parinarium laurinum (Chrysobalaneae)							
Triglyceride	3.4	3.9	1.9	1.8	17.8[b]	30.3[c]	40.9[d]
2-Monoglyceride	1.5	0.9	2.6	3.5	28.2	32.8	30.5
Enrichment factor	---	---	---	---	1.6	1.1	0.7
Impatiens gladuligera (Balsaminaceae)							
Triglyceride	8.8	11.8	14.7	29.8	10.3[b]	6.6[b]	17.4[d]
2-Monoglyceride	3.0	9.4	16.5	43.1	10.8	5.4	11.0
Enrichment factor	0.3	0.8	1.1	1.4	1.0	0.8	0.6

[a]These acids have conjugated unsaturation.

[b]Detailed structure not known.

[c]Eleostearic acid (9c11t13t).

[d]Parinaric acid (9*c*11*t*13*t*15*c*).

Extraction and purification was carried out on the same day as the glycerides were required for analysis.

Lipolysis and Separation and Analysis of Glycerides. The glycerides (400 mg) were subject to lipolysis as described by Desnuelle and Savary (11) and Coleman (12). After 4-5 min the lipolysis mixture was acidified and extracted with ether.

Aliquots of the lipolysis product (~30 mg) were placed on a TLC plate and developed with chloroform-acetone-0.880 ammonia (80:20:1) [a mixture of ether-petroleum (15:85 was used as developing solvent to isolate unreacted triglycerides]. The separated components were extracted from the silica with ether (six extractions) and converted to methyl esters by reaction with sodium methoxide (13) in a nitrogen atmosphere at room temperature for 2 hr.

The methyl esters were examined by GLC and, in some cases, by UV spectroscopy also. These two procedures gave similar results for the proportion of conjugated octadecatrienoates and the following examples are typical: tung (*Aleurites montana*), 69.0% by GLC and 70.5% by UV spectroscopy; *M. charantia*, 61.2% and 59.5%; *C. ruber*, 52.3% and 48.0%; *C. ovata*, 40.6% and 40.2%; *C. bignonoides*, 40.1% and 40.0%. The GLC procedure which was necessary for the nonconjugated components of the mixtures was thus used for the conjugated esters also.

NMR Spectra. The examination of a number of the oils available to us and, in some cases, of the isolated conjugated octadecatrienoic acids, along with β-eleostearic acid formed through isomerization of the α-isomer in tung oil, showed that there was a marked difference in the signals produced by the six olefinic protons (Fig. 1). All gave complex signals with the most intense signal at 4.1τ for the all *trans* isomer (9*t*11*t*13*t*), at 3.8τ for *ttc* isomers (9*c*11*t*13*t*; 9*t*11*t*13*c*; and 8*t*10*t*12*c*), and at 3.7τ for a *ctc* isomer (9*c*11*t*13*c*).

RESULTS AND DISCUSSION

Vernolic Acid

The proportion of glycerides containing three, two, one and no vernolic acid chain is summarized in Table I which contains our results along with those previously reported by Tallent et al. (5). Our separations were effected by TLC and theirs by column chromatography but the results are in general agreement. The figures do not, in general, agree with values calculated according to a 1,2,3-random or to a 1,3-random-2-random distribution pattern as already reported by Tallent et al. (5).

The present lipolysis studies of the mono- and divernolin fractions confirm and extend the earlier results (5). The 2-monoglycerides resulting from lipase-catalyzed deacylation are generally enriched in vernolic acid both in the monovernolins (mainly 50-75% compared with 33% in the triglycerides) and the divernolins (75-96% compared with 67% in the triglycerides). In the divernolins from *E. lagascae, C. aurea, C. vesicaria,* and *C. joppica* the 2 position is acylated almost entirely by vernolic acid (92-96%). Tallent et al. (5) have already drawn attention to the unusually low concentration of vernolic acid (20%) in the 2-monoglycerides from *E. lagascae* monovernolins. Our results with two other species of the Euphorbiaceae family, both with high proportions of vernolic

acid (58-72%), give values for vernolic acid in the 2-monoglycerides from the monovernolins which are a little higher (35% and 39%), but not markedly above the random value (33%).

In Table II we summarize the enrichment factors (14,15) for oleic, linoleic and vernolic acid in the X_3, X_2V and XV_2 glyceride fractions (V, vernolic acid; X, any other acid). The factors for oleic and linoleic acid are generally a little lower in the X_2V and XV_2 glycerides than in those glycerides with no vernolic acid but in the divernolins from *C. aurea* and *C. vesicaria* these values are exceptionally low. These results again indicate that vernolic acid competes successfully with oleic and linoleic acid for the C_2 position. Full results are given in Table III.

Some of the X_3 and X_2V glyceride fractions have also been examined by silver ion thin layer chromatography (9) and the results are given in Table IV. Both fractions from *C. peuschelli* were analyzed twice and all values are quoted to show the reproducibility of the results.

Conjugated Octadecatrienoic Acids

It has been possible to examine 18 species (25 samples) whose seed oils contain, between them, the five known conjugated octadecatrienoic acids. When these oils are hydrolyzed in the presence of pancreatic lipase, reaction occurs as quickly as with oils as typical as cottonseed oil. Since, in addition, unchanged triglycerides had virtually the same composition as the original oils, lipolysis occured with no undesirable selectivity arising from the unusual acid present. Some typical results are given in Table V. The proportion of conjugated ester can be determined by UV spectroscopy or by GLC. In the latter, some isomerization probably occurs on the column but the total area of peaks for conjugated octadecatrienoates is easily measured. These two methods gave similar values in some test cases and we find the GLC method simpler.

It is possible to distinguish *ctt* or *ttc* isomers (eleostearic, catalpic, calendic) from *ctc* isomers (punicic, jacaric) and both of these from *ttt* isomers by NMR spectroscopy (see Fig. 1). We therefore examined each oil and found results consistent with the structures proposed for the acid present in each oil (references are included in Table VI). Isomeric conjugated acids could be detected by this means at a 10% level and we found our sample of *V. officinalis* to contain 10-15% of the all *trans* isomer. This was also apparent in several old samples of seed oils containing conjugated acids (not used in this investigation) and probably arises from degradative changes in storage. It should not affect the results of lipolysis.

The results of this investigation are given in Table VI. Different samples of some species were examined but these gave consistent results and in the following discussion we refer to the number of species examined rather than to the number of samples. The discussion is conducted in terms of the enrichment factors listed in Table VI. Low values indicate that the acid in question is concentrated at C_1 or C_3 or both rather than C_2. A value of 1.0 signifies random distribution of the acid in question, values greater than 1.0 indicate a preference for the C_2 position. In assessing the significance of the enrichment factor, account must be taken of the total proportion of the acid and the nature of the other acids also present.

Catalpic acid ($9t11t13c$) gives uniformly low enrichment factors (0.1-0.4) for the four species examined, all belonging to the family Bignoniaceae. The single jacaric ($8c10t12c$) acid-bearing species belongs to this same family and also has a low enrichment factor (0.2). Calendic acid ($8t10t12c$) could be examined in only two species of Compositae and in both the enrichment factor (1.1-1.4) is comparable with that normally observed for oleic and linoleic acid. This leaves punicic acid ($9c11t13c$) and eleostearic acid ($9c11t13t$) for which the results are somewhat confusing since both low (0-0.3) and high (0.9-1.4) enrichment factors are observed. In three species of Valerianaceae eleostearic acid has a low enrichment factor but the same acid shows a high enrichment factor in one Euphorbiaceae (tung oil) and in two Cucurbitaceae species. Punicic acid shows low factors for three Cucurbitaceae species and high factors for one Cucurbitaceae and for the single Punicaceae species examined. Of the six Cucurbitaceae species which we have been able to examine, three (containing punicic acid) have low enrichment factors. The remainder are members of the *Momordica* genus and have high enrichment factors; two contain eleostearic acid and one contains punicic acid.

Table VII shows some interesting results with oils containing conjugated dienoic, trienoic and tetraenoic acids. In *P. laurinum* where these three acids comprise 89% of the total there is evidence that they compete for attachment at the C_2 position in order of decreasing effectiveness: diene>triene>tetraene. The same order is apparent in *I. glanduligera* (26), but here oleic and linoleic compete even more effectively than the conjugated acids.

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REFERENCES

1. Mattson, F. H., and R. A. Volpenhein, J. Biol. Chem. 236:1891 (1961).
2. Mattson, F. H., and R. A. Volpenhein, J. Lipid Res. 4:392 (1963).
3. Gunstone, F. D., R. J. Hamilton, F. B. Padley and M. Ilyas Qureshi, JAOCS 42:965 (1965).
4. Tallent, W. H., and R. Kleiman, J. Lipid Res. 9:146 (1968).
5. Tallent, W. H., D. G. Cope, J. W. Hagemann, F. R. Earle and I. A. Wolff, Lipids 1:335 (1966).
6. Tallent, W. H., R. Kleiman and D. G. Cope, J. Lipid Res. 7:531 (1966).
7. Tallent, W. H., J. Harris, G. F. Spencer and I. A. Wolff, Lipids 3:425 (1968).
8. Gunstone, F. D., and M. Ilyas Qureshi, J. Sci. Fd. Agric. 19:386 (1968).
9. Gunstone, F. D., and F. B. Padley, JAOCS 42:957 (1965).
10. Quinlin, P., and H. J. Weiser, Jr., Ibid. 35:325 (1958).
11. Desnuelle, P., and P. Savary, J. Lipid Res. 4:369 (1963).
12. Coleman, M. H., JAOCS 38:685 (1961).
13. Luddy, F. E., R. A. Barford and R. W. Riemenschneider, Ibid. 37:447 (1960).
14. Gunstone, F. D., and A. J. Sealy, J. Chem. Soc. 1964:4407.
15. Gunstone, F. D., R. J. Hamilton and M. Ilyas Qureshi, Ibid. 1965:319.
16. Hopkins, C. Y., and M. J. Chisholm, Can. J. Chem. 40:2078 (1962).
17. Hopkins, C. Y., and M. J. Chisholm, Ibid. 43:3160 (1965).
18. Chisholm, M. J., and C. Y. Hopkins, Ibid. 38:2500 (1960).
19. Chisholm, M. J., and C. Y. Hopkins, Ibid. 42:560 (1964).
20. Chisholm, M. J., and C. Y. Hopkins, JAOCS 43:390 (1966).
21. Bemis, W. P., J. W. Berry, M. J. Kennedy, D. Woods, M. Moran and A. J. Deutschman, Jr., JAOCS 44:429 (1969).
22. Hopkins, C. Y., and M. J. Chisholm, J. Chem. Soc. 1962:573.
23. McLean, J., and A. H. Clark, Ibid. 1956:777.
24. Chisholm, M. J., and C. Y. Hopkins, Can. J. Chem. 41:1888 (1963).
25. Hopkins, C. Y., and M. J. Chisholm, J. Org. Chem. 27:3137 (1962).
26. Kaufmann, H. P., and M. Keller, Chem. Ber. 81:152 (1948).
27. Chisholm, M. J., and C. Y. Hopkins, Can. J. Biochem. 45:1081 (1967).

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