TABLE VII

The Effect of the State of the Protein and Dry Mixing Under Various Conditions on Complex Formation

Protein	Reaction conditions				Extractable[a] material in product, %
	Water ml, protein g	Temp, C	Time, hr	Lipid	
10 g native egg albumin	0[b]	200	0.5	100 g TOCO A	0
10 g native egg albumin	0[b]	60	2.0	100 g TOCO A	0
10 g native egg albumin	0[b]	60	33.0	100 g TOCO A	4.8
10 g egg albumin denatured by lauric acid[c]	0[b]	60	33.0	100 g TOCO A	6.5
10 g egg albumin denatured by lauric acid[c]	100	60	33.0	30 g TOCO A	0
10 g egg albumin denatured by lauric acid[d]	100	60	24.0	30 g TOCO A	2.6

[a] After alkali hydrolysis and acidification.
[b] Indicates mixing in the dry state.
[c] After removal of lauric acid by Soxhlet extraction.
[d] Before removal of lauric acid.

lier observations of Casselman (13) and Hartroft (2) that the rat red blood cell proteins also complex with oxidized lipids under the present experimental conditions. The ease with which these complexes are formed suggested that lipid-protein complexes may also form *in vivo* under certain pathological conditions. The complexing of oxidized lipid with protein may be responsible for the formation of the brown pigments which have been noted in the uterus of vitamin E deficient rats (5) and for the accumulation of ceroid pigment in the liver of choline deficient rats (3). The observation that complexing can take place at 30C and that optimum complexing was noted at a pH of 7.0 would indicate that *in vivo* lipid-protein complexing could be involved in degenerative or pathological changes in specific organs or tissues (3,4,5,6,7,14).

While it is true that the oxidized lipid-protein complexes are different from the naturally occurring lipoproteins, it seems possible that the interaction between oxidized lipids and the low density lipoprotein of blood serum (8) may be similar to the interaction between the oxidized lipids and other proteins used in this study.

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The Acetylenic Acid in *Comandra pallida* and *Osyris alba* Seed Oils

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Abstract

Gas-liquid chromatographic (GLC) analyses are reported for fatty acid methyl esters from seed oils of two previously unreported species of Santalaceae, *Comandra pallida* A. DC. and *Osyris alba* L. The major component in each (43 and 57%, respectively) is an enynoic acid, probably *trans*-11-octadecen-9-ynoic (ximenynic) acid which has been found in seed oils of other members of this family. Equivalent chain lengths by GLC analysis and infrared and ultraviolet spectra agree with those obtained by our analyses of *Ximenia americana* L., in which ximenynic acid is known to occur. The spectral data also agree with those in literature reports on ximenynic acid. The positions of unsaturation have, however, not been rigorously established for the two species newly reported.

Introduction

POLYUNSATURATED acetylenic oils have been found in plant seeds from only two families, Olacaceae and Santalaceae. Ligthelm and Schwartz (9) proposed four possible structures for an unknown acetylenic acid in seed oil of *Ximenia caffra*, and proposed it be named ximenynic acid. Ligthelm et al. later characterized this acid as *trans*-11-octadecen-9-ynoic acid (10). Ximenynic acid was synthesized by Grigor et al. (2). Simultaneously Gunstone and McGee (3) characterized "santalbic" acid and found it had the same structure as ximenynic acid.

Ximenynic acid has now been reported in seed oil from two species of *Ximenia* (8) in the family Olacaceae and from four species of *Santalum* (3,4), two of *Exocarpus* (5), and one of *Leptomeria* (6) in the Santalaceae.

This paper reports the presence of apparent ximenynic acid in seed oils from two additional genera of Santalaceae. An analysis of seed oil from *Ximenia americana* L., in which ximenynic acid is known to occur (8), is also included.

Procedure

Materials and Methods

Oils were extracted with petroleum ether (30–60C) from the ground seed plus pericarp of *Comandra pallida* A. DC. and of *Osyris alba* L., and from the ground seed of *Ximenia americana* L.

Esters were prepared from the oils of *C. pallida* and *O. alba* by methanolysis with sodium methoxide

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TABLE II

Analysis of Source Materials and Derived Oils

Source	Component analyzed	Source material			Oil	
		Wt/1,000	Oil, dry basis	Protein (N × 6.25), dry basis	Iodine value Wijs, ½ hr	n_D^{40}
		g	%	%		
Comandra pallida A. DC.	Seed and pericarp	140	24	8	112	1.4772
Osyris alba L.	Seed and pericarp	105	36	11	117	1.4780
Ximenia americana L	Seed	834	62	20	85	1.4718

as the catalyst. Oil from *X. americana* was saponified and the saponification mixture was extracted with diethyl ether to remove the unsaponifiables, including the rubbery, acetone-insoluble component previously noted (4). Fatty acids were recovered from the acidified aqueous liquor and converted to methyl esters with hydrogen chloride as the catalyst.

The GLC equipment and method of analysis were as previously described (11), except that two columns 125 × 0.3 cm I.D. were used, instead of columns 200 × 0.6 cm and 275 × 0.6 cm, to determine the percentages of C_{24} to C_{30} acids in *X. americana* seed oil. Methyl esters from *C. pallida* and *O. alba* were analyzed also on these small columns to determine whether acids longer than C_{18} were present.

The major component in *C. pallida* and *O. alba* methyl esters had the same equivalent chain lengths (12), 18.90 on Apiezon L and 22.10 on LAC-2-R 446, as methyl ximenynate in *X. americana* esters. This component from *C. pallida* esters was trapped in a 0.1 × 15 cm glass U-tube cooled in an acetone-solid carbon dioxide bath at the exit of a preparative-scale GLC column. This column was 200 × 1.25 cm I.D. packed with 12% LAC-2-R 446 on 60–100 mesh Celite 545 and had a working capacity of 100 mg of sample per injection. The collected fraction was subjected to infrared and ultraviolet examination.

Results and Discussion

GLC analyses of the methyl esters from the three oils are in Table I. Analytical data on the source materials and derived oils are shown in Table II.

The ultraviolet analysis of the three seed oils showed the characteristic absorption spectrum of a conjugated enynic system, which has a maximum at 229 mμ and a point of inflection at about 240 mμ. When calculated as methyl ximenynate, using an E value of 549 (10), the absorption is equivalent to 51.6% in *C. pallida*, 59.4% in *O. alba*, and 11.0% in *X. americana* oil. These figures include both methyl ximenynate and methyl hydroxyximenynate and are in good agreement with the percentage obtained by adding the GLC values for these two components.

When *C. pallida* oil was heated at 180C for 25 min in a 6.6% solution of KOH in ethylene glycol, the enynic absorption at 229 mμ decreased and new maxima were observed at 237, 268, and 315 mμ. Continued heating to 60 min nearly eliminated the 229 and 237 mμ peaks, and the peak at 268 mμ increased proportionately. These results duplicate those obtained by Ligthelm (10) showing that ximenynic acid rearranges to a conjugated triene when treated with hot alkali.

The relatively small absorption at 315 mμ is probably due to conjugated tetraene resulting from dehydration and rearrangement of hydroxyximenynic acid as shown to occur by Ligthelm (7). The GLC results for apparent hydroxyximenynic acid may be somewhat questionable because they are based on peaks not adequately identified.

Ultraviolet analysis of the collected fraction of *C. pallida* methyl esters gave an E value (1%, 1 cm) of 537 at 229 mμ, which is in good agreement with the value of 549 obtained by Ligthelm (10) for pure methyl ximenynate. The E value at 268 mμ was only 2.60 and indicated little rearrangement of the ester at a detector temperature of 215C and column temperature of 200C.

Infrared analysis of the trapped fraction showed a slightly stronger 953 cm^{-1} band (*trans* C=C) than appeared in the original oil. Gunstone reported this same absorption band in the infrared spectra of ximenynic acid (3).

The compositions of *Comandra* and *Osyris* oils are very similar to those reported for other oils of the Santalaceae (3–6). The major component is probably ximenynic acid, although the positions of the enynic unsaturation have not been established. The composition of *Ximenia* oil reported here is similar to those reported previously (1,8) in that ximenynic, hydroxyximenynic, and acids longer than C_{18} are present.

TABLE I

Composition of Methyl Esters by GLC (area %)

Source	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:1}$	$C_{24:0}$
Comandra pallida A. DC.	2.3	0.4	0.8	40.8	1.5	5.8	Trace	.....
Osyris alba L.	0.8	0.7	3.4	31.6	1.8	2.2	.....	.....
Ximenia americana L.	1.0	0.2	0.7	48.7	0.3	0.5	.....	1.7

Source	$C_{24:1}$	$C_{26:0}$	$C_{26:1}$	$C_{28:0}$	$C_{28:1}$	$C_{30:1}$	[a]	[b]
Comandra pallida A. DC.	.....	.....	.....	.....	.....	.....	43.0	5.3
Osyris alba L.	.....	.....	.....	.....	.....	.....	57.1	2.4
Ximenia americana L.	3.5	2.7	3.9	1.2	12.8	5.5	6.3	11.0 [c]

[a] Methyl ximenynate (equivalent chain lengths—18.90 on Apiezon L column, 22.10 on LAC-2-R 446 column).

[b] Unidenitfied peaks considered to be decomposition products of methyl hydroxyximenynate.

[c] Contains ca. 5% C_{20} and C_{22} methyl esters.

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