

Triterpene Alcohols and Sterols of Vegetable Oils

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Abstract

Triterpene alcohols and sterols were separated by thin-layer chromatography and gas-liquid chromatography from the unsaponifiable fractions of the following 18 vegetable oils: linseed, peanut, olive, rice bran, palm kernel, corn, sesame, oiticica, palm, coconut, rapeseed, grape seed, sunflower, poppy seed, castor, tea seed, cocoa butter and soybean. Two triterpene alcohols, cycloartenol and 24-methylene cycloartanol, were found in all of the oils except soybean oil, which contained only cycloartenol. Triterpene alcohols such as α - and β -amyrin, euphorbol, butyrospermol and cyclolaudenol also were encountered occasionally. Three sterols, β -sitosterol, stigmasterol and campesterol were present in all of the oils. In addition a fourth sterol, not yet identified, was found in oils of palm, palm kernel and sunflower in varying amounts. This unknown sterol and brassicasterol were found in rapeseed oil in addition to the three sterols that were common to all of the oils studied.

Introduction

PREVIOUS STUDIES at this Laboratory (1-3) showed that, with very few exceptions, vegetable oil unsaponifiables contained saturated hydrocarbons, squalene, aliphatic alcohols, terpene alcohols, and sterols. However, individual compounds in these classes varied with different oils. This variability was most pronounced among the terpene alcohols.

Previous reports from this Laboratory discussed terpene alcohols and sterols of linseed (4,5), olive (6), tea seed (7), cocoa butter (8) and rapeseed (9) oils. The present paper deals with terpene alcohols and sterols of 13 additional vegetable oils: peanut, rice bran, palm kernel, corn, sesame, oiticica, coconut, palm, grape seed, sunflower, poppy seed, castor and soybean. For clarity, the five oils discussed earlier are considered again.

The following triterpene alcohols were isolated and identified: cycloartenol from linseed, olive and peanut oils; 24-methylcycloartanol from olive and linseed oils; butyrospermol from olive and tea seed oils; α -amyrin from olive oil; and β -amyrin from tea seed and peanut oils. Among the sterols, β -sitosterol, stigmasterol and campesterol in linseed and cocoa butter oils, and brassicasterol in rapeseed oil were identified.

Identification of these substances is of practical interest because, when taken as a group, they furnish a "fingerprint" for the identification of vegetable oils. Compounds were isolated by thin-layer chromatography (TLC) and gas-liquid chromatography (GLC), and identifications were based on comparisons with the chromatographic behavior of pure substances.

Experimental

Materials

Oils were obtained by extraction with hexane, except olive oil, which was obtained by pressing. All oils were degummed. Flaxseed, olives, rice, corn, rape,

grape, sunflower, castor, sesame, and soybeans were grown in Italy; peanuts, coconuts, oiticica, palm, and palm kernel were obtained from Brazil; poppy seed, from Poland; and tea seed, from China.

Saponification

One hundred grams of oil in 1000 ml of alcoholic 0.5N potassium hydroxide were refluxed on a water bath for 1 hr. The reaction mixture was diluted with 2000 ml distilled water, and unsaponifiable material was extracted with three 2000-ml portions of diethyl ether. Ether extracts were combined, washed 10 times with 800-ml portions of distilled water and dried with sodium sulfate. The unsaponifiable fraction was neutralized by elution of the ether solution through alumina.

Thin-Layer Chromatography

Neutralized unsaponifiable material was fractionated on 20 × 20 mm plates spread with a 1 mm layer of Silica Gel G (10). One hundred milligrams of sample was applied uniformly along a line 1 cm from one edge of the plate, eluted with a 1:1 mixture of hexane-ether, developed with the sodium salt of dichlorofluorescein and observed under ultraviolet light. Strips containing terpenes and sterols were removed and extracted with ether in a microextractor. Ether extracts were desiccated for subsequent GLC analysis.

Gas-Liquid Chromatography

Terpene and sterol fractions were analyzed with a C. Erba, model C, chromatograph equipped with a flame ionization detector. The chromatograph was fitted with a 2 m glass column, 2 mm I.D., packed with silanized Gas Chrom P, 100-120 mesh and coated with 1% SE-30. The column was operated at 230C with nitrogen at 20 cc/min as carrier gas. Evaporator temperature was 280C.

Results

Terpenes and sterols were fractionated from neutralized unsaponifiable material by preparative TLC and were obtained in the amounts shown in Table I.

Triterpene Alcohols

GLC analysis indicated that the composition of terpene fractions varied markedly from one oil to another. Table II shows the number of components

TABLE I
Terpene and Sterol Fractions from Vegetable Oil Unsaponifiables
(% of oil)

Oil	Neutralized unsaponifiables	Triterpenes	Sterols
Linseed	1.1	0.12	0.5
Peanut	0.9	0.14	0.5
Olive	1.2	0.2	0.6
Rice bran	0.8	0.1	0.4
Palm kernel	0.6	0.03	0.3
Corn	2.3	0.11	1.38
Sesame	0.4	0.03	0.19
Oiticica	0.6	0.02	0.51
Coconut	0.3	0.02	0.14
Palm	0.4	0.02	0.17
Rapeseed	0.8	0.03	0.59
Grape seed	0.3	0.01	0.13
Sunflower	0.6	0.22	0.25
Poppy seed	0.5	0.02	0.25
Castor	0.7	0.05	0.28
Tea seed	0.9	0.04	0.6
Cocoa butter	0.7	0.05	0.3
Soybean	0.7	0.06	0.42

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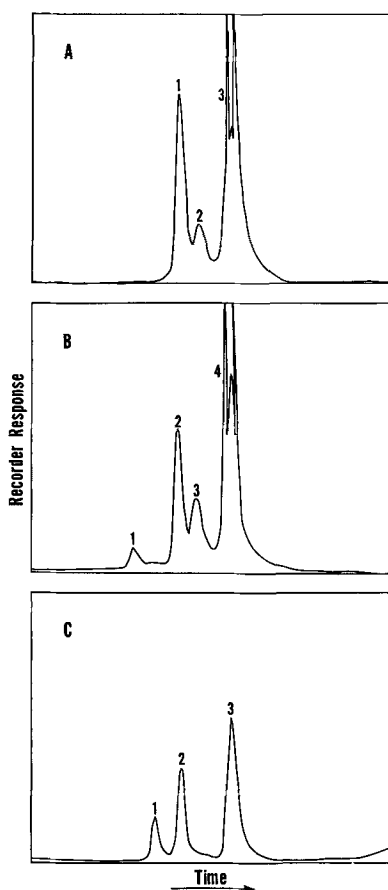


FIG. 2. Gas-liquid chromatograms of sterol fractions from (A) linseed, (B) palm and (C) rapeseed oils.

lowed by campesterol (peak 1) and stigmasterol (peak 2).

A similar ratio of these three components was found in peanut, rice bran, corn, sesame and poppy seed oils. However, in oiticica, coconut, grape seed, castor, tea seed and cocoa butter oils, the decreasing order was β -sitosterol, stigmasterol and campesterol. The more abundant component of olive oil was stigmasterol, followed by β -sitosterol and campesterol.

Palm, palm kernel and sunflower oils, unlike the preceding oils, contained four components. Figure 2B shows that palm oil contained β -sitosterol (peak

4), stigmasterol (peak 3) and campesterol (peak 2) along with a fourth component whose V_R was close to that of cholesterol. Cholesterol has been mentioned by other authors as a component of palm oil (13).

Palm kernel and sunflower oils also contained a fourth component in addition to β -sitosterol, stigmasterol and campesterol. The fourth component was unknown and differed in both of these oils. As in palm oil, β -sitosterol was the most abundant sterol in palm kernel and sunflower oils.

Rapeseed oil was the only oil that did not contain stigmasterol. The GLC curve for this oil is given in Figure 2C. Besides β -sitosterol (peak 3), and campesterol (peak 2), a third sterol has been identified as brassicasterol (peak 1).

Table III gives V_R values for sterols found in this study, expressed as ratios of the retention volume of β -sitosterol. The following V_R values were found for sterols that were identified:

	V_R
β -Sitosterol	1.00
Stigmasterol	0.87
Campesterol	0.80
Brassicasterol	0.68
Cholesterol	0.60

Discussion

Of the two classes of compounds studied, the triterpene alcohols were more varied in composition than the sterols. Marked differences were observed among the oils, either in the number of terpene components or in their chemical structure. No two chromatograms of terpene fractions could be superimposed exactly. Thus, analysis of the terpene fraction appears to afford a rapid and simple laboratory method for differentiating vegetable oils.

Cycloartenol and 24-methylene cycloartanol were found in 17 of the 18 oils studied. The only exception was soybean oil, which contained cycloartenol, but not 24-methylene cycloartanol. Two related mechanisms of biosynthesis, therefore, appear likely for these two terpenes.

The sterol fractions, because of their uniform composition, do not lend themselves well to the characterization of a particular oil.

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TABLE III
Distribution of Sterols in Vegetable Oils

Oil	Number of components	Relative retention volume (V_R of β -sitosterol = 1.00)						
		0.58	0.60	0.68	0.80	0.87	1.00	1.14
Linseed	3				+	+	+	
Peanut	3				+	+	+	
Olive	3				+	+	+	
Rice bran	3				+	+	+	
Palm kernel	4	+			+	+	+	
Corn	3				+	+	+	
Sesame	3				+	+	+	
Oiticica	3				+	+	+	
Coconut	3				+	+	+	
Palm	4		+		+	+	+	
Rapeseed	3			+	+	+	+	
Grape seed	3				+	+	+	
Sunflower	4				+	+	+	+
Poppy seed	3				+	+	+	
Castor	3				+	+	+	
Tea seed	3				+	+	+	
Cocoa butter	3				+	+	+	
Soybean	3				+	+	+	