The Hydrocarbon Fraction of Virgin Olive Oil and Changes Resulting from Refining

Augusto Lanzón[a,*], Tomás Albi[a], Arturo Cert[a] and Jaime Gracián[b]

[a]Instituto de la Grasa y sus Derivados (C.S.I.C.), 41012 Sevilla, Spain and [b]Real Academia Sevillana de Ciencias, 41004 Sevilla, Spain

In numerous Spanish virgin olive oils, 6,10-dimethyl-1-undecene, various sesquiterpenes, the series of *n*-alkanes from C14 to C35, n-8-heptadecene and squalene are the only less volatile components detected by gas chromatography in the hydrocarbon fraction. In oils from olives of the Arbequine variety, a series of *n*-9-alkenes has also been found. In refined oils, notable features are the absence of the most volatile compounds and the appearance of other hydrocarbons produced during the refining process. Among these, *n*-alkanes, alkadienes (mainly *n*-hexacosadiene), stigmasta-3,5-diene, isomerization products of squalene, isoprenoidal polyolefins coming from hydroxy derivatives of squalene and steroidal hydrocarbons derived from 24-methylene cycloartanol were identified. Physical refining produces larger amounts of degradation products and greater losses of *n*-alkanes than chemical processing. Squalene is the major hydrocarbon component in all oils, both virgin and refined. The ranges of concentration for the different hydrocarbons found in Spanish virgin olive oils are presented.

KEY WORDS: Hydrocarbons, refining, virgin olive oil.

The hydrocarbons are insufficiently studied components of the unsaponifiable fraction of olive oil; and moreover, their identification has been unsatisfactory. Eisner *et al.* (1) stated that squalene makes up around 85–90% of the hydrocarbon fraction, and established the presence of various homologous series of normal, *iso*- and/or ante*iso*-hydrocarbons, and multiple branched chains in the range C16 to C36. Capella *et al.* (2) detected only one series of *n*-paraffins in the range C13 to C30, in which alkanes with an odd number of carbon atoms predominated over those of even numbers. Jacini and Fedeli (3) concluded that this fraction is formed of normal-chain paraffins, together with small amounts of *iso* and probably ante*iso* isomers and with olefins. Bastic *et al.* (4), studying a sample of unrefined olive oil by gas chromatography with a capillary column, concluded that squalene makes up more than 90% of the hydrocarbon fraction, the rest being mainly isoprenoidal polyolefins and *n*-paraffins.

We have undertaken a detailed examination of the hydrocarbon components of olive oil. We used an analytical method based on fractionation for the identification and quantitation of its components in both virgin and refined olive oil, with the aim of determining the changes resulting from the refining process.

EXPERIMENTAL PROCEDURES

Oil samples. About 250 virgin olive oils, produced from the 1987–1989 crops from various Spanish regions (Badajoz, Cáceres, Castellón, Córdoba, Granada, Huelva, Jaén, Lérida, Málaga, Seville, Tarragona, Teruel and Toledo), were examined. These samples included oils from the most important olive varieties grown in Spain (Arbequina, Cornicabra, Empeltre, Farga, Hojiblanca, Lechín, Picual, Picudo and Verdial).

In addition, two virgin olive oils (designated A and B) were refined in an industrial plant. Oil A was refined by conventional alkaline neutralization and steam deodorization to give refined oil AR. Oil B was refined by physical refining to give refined oil BR. In both cases, the refining conditions were those usually encountered in industry. In alkaline refining, the oil was neutralized with aqueous NaOH, then it was washed with water and finally bleached with 1.5% Tonsil earth (Süd-Chemie, Munich, Germany) at a temperature of 110–120°C; deodorization was by passage of steam through the oil at 20×10^{-3} bar and 150°C. In physical refining, the oil was submitted to the same purification with bleaching earth (Tonsil, 1–1.5%; temp. 110–120°C), followed by continuous steam distillation at 260°C and $5{,}33 \times 10^{-3}$ bar.

Reagents. Hexane (alkane mixture), A.C.S. reagent, b.p. 65–75°C and ethanol (96%, vol/vol) were distilled through a fractionation column. Silica Gel 60 was used for column chromatography (90–230 mesh), Merck Ref. 7754 (Darmstadt, Germany). A standard solution of *n*-eicosane [Sigma (St. Louis, MO), ≥ 99% tested by gas chromatography] in hexane, 0.125 mg/mL, was utilized. All other materials were analytical grade.

Equipment. A gas chromatograph HP-5890, (Hewlett-Packard, Avondale, PA) was used with a flame-ionization detector and a wide bore capillary column of borosilicate glass [0.75 mm i.d. × 30 m long, coated with a 1-μm film thickness of liquid phase SPB-1 (chemically bonded methylsilicone)].

An injector for packed columns was used with an adaptor for wide bore capillary columns. Data were collected and processed with an integrating recorder (HP-3390A).

A mass spectrometer MS-30 (AEI, Manchester, United Kingdom), VG-updated with computerized data system PDP-11/250, was coupled directly to a gas chromatograph KNK-200°C (Konik, Barcelona, Spain) fitted with a fused silica column (0.25 mm i.d. × 30 m long) coated with the liquid phase OV-1 (chemically bonded methylsilicone).

Extraction of the unsaponifiable fraction. To 20 g of oil was added 1 mL of the standard solution of *n*-eicosane. The mixture was saponified for half an hour with 75 mL of 10% ethanolic potassium hydroxide. The solution was passed to a 500-mL decanting funnel, 100 mL distilled water was added and the mixture was extracted twice with two 100-mL portions of hexane. If any emulsion appeared, the solution was left for a short time for it to break. The hexane extracts were combined in another funnel and were washed several times with 100-mL portions of a mixture of ethanol–water (1:1), until the wash was at neutral pH. The hexane solution was dried over anhydrous sodium sulfate and evaporated to dryness in a rotary evaporator at 30°C under reduced pressure.

Fractionation of the unsaponifiable matter. Fractionation was carried out in a glass chromatography column (1.5 cm i.d. and 50 cm long) with a Teflon stopcock, filled

*To whom correspondence should be addressed at Instituto de la Grasa y sus Derivados (C.S.I.C.), Avda, Padre García Tejero, 4, P.O. Box 1.078, 41012 Sevilla, Spain.

with 15 g silica gel slurried in hexane. The complete unsaponifiable fraction (0.2 g approximately) was dissolved in approximately 1 mL hexane, and the solution was introduced to the column head. The flask was rinsed two or three times with hexane, approximately 1 mL each time. The washes were added to the column head, waiting for the previous addition to pass completely into the column. The sample was then eluted with hexane at a rate of about 1 mL/min. Fractions of 25 mL each were collected and concentrated in the rotary evaporator at 30°C under reduced pressure to approximately 1 mL. Each was analyzed separately by gas chromatography.

Gas chromatography. The chromatographic conditions were: oven temperature, 110°C for 6 min, then programmed at 5°C/min to 300°C; injector temperature, 300°C; detector temperature, 320°C. Nitrogen, with a flow of 14 mL/min, was used as carrier gas.

Mass spectrometry. Electron impact ionization at 70 eV was used. The ionization current was 125 μA and the source temperature was 200°C. The operating conditions were: resolution, 1000; and scan rate, 3 s/decade. The chromatographic conditions of the gas chromatograph coupled to the mass spectrometer were the following: initial temperature, 80°C, held for 2 min, then programmed at 4°C/min to 250°C. The injector and interphase temperatures were 250°C.

Qualitative analysis. The components were identified by means of gas chromatography–mass spectrometry analysis. So were their derivatives, obtained by hydrogenation with Adams-Shriner catalyst (Platinum IV monohydrate; Fluka, Buchs, Switzerland) by oxidation with potassium permanganate according to the method proposed by Urbach and Stark (5), or by *bis*-methylthiolation according the method of Buser *et al.* (6).

Quantitative analysis. Each component in the chromatogram of the combined first two fractions eluted from the silica gel column was quantitated with *n*-eicosane as internal standard. Its response factor relative to *n*-alkanes is close to unity. This standard was used to quantitate the terpenic hydrocarbons by assuming the same response factor. Although this is not absolutely certain, it is acceptable enough for the aims of this work. To quantitate the components of the other fractions, 1 mL of the *n*-eicosane solution was added to each. This hydrocarbon was chosen as standard after checking that its amount in the original chromatogram was negligible.

RESULTS AND DISCUSSION

Virgin olive oils. The results obtained from the samples of virgin olive oils were similar, although quantitative differences were noticeable in some components. Figure 1 shows the chromatogram of the combined first and second fractions eluted from the silica gel column from virgin oil A. Peak 1 showed the following mass spectrum (except for molecular ion, only fragments with relative abundances greater than 10% are given): *m/z* 182 (3%, M^+), 126 (10%), 112 (17%), 111 (13%), 98 (12%), 97 (53%), 96 (11%), 84 (18%), 83 (29%), 71 (32%), 70 (42%), 69 (56%), 57 (85%), 56 (75%), 55 (100%), 43 (81%), 42 (11%), 41 (62%), 39 (14%). It suggests that the compound is a tridecene with branched structure. On gas chromatography–mass spectrometry, the hydrogenated fraction shows displacement of the peak with a retention time slightly higher than that of the nonhydrogenated fraction, and it has a mass spectrum coinciding with that of 2,6-dimethylundecane (U.S. National Bureau of Standards, Washington, D.C.). To find the position of the double bond, a derivative was formed by reaction with dimethyl disulfide. The mass spectrum of the *α-β-bis*(methylthio)alkane formed shows clearly that the double bond is located at a terminal carbon atom. However, we did not know if it was situated at the "head" [CH_2=C(CH_3)−] or "tail" (−CH=CH_2) (in terpene terminology) because in both cases the fragments at *m/z* 61 (CH_2=$\overset{+}{S}CH_3$) and 215 [C_9H_{19}−CH=$\overset{+}{S}$−CH_3] would appear. To clarify this structure, oxidation with $KMnO_4$ was carried out, and the reaction product was studied by gas chromatography and mass spectrometry. The peak corresponding to the hydrocarbon disappeared,

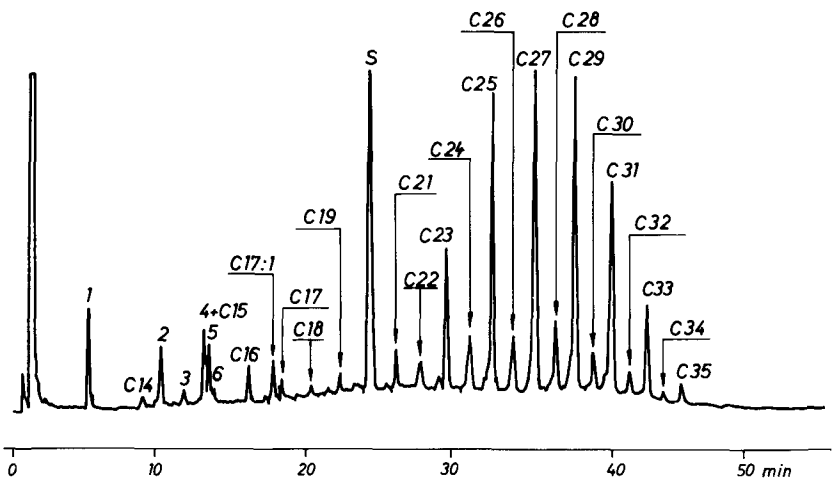


FIG. 1. Gas chromatogram of the combined first and second hydrocarbon fractions from a virgin olive oil: (1) 6,10-dimethyl-1-undecene; (2) α-copaene; (3, 4 and 5) bicyclic sesquiterpenes; (6) α-farnesene; (S) *n*-eicosane (internal standard); (C17:1) 8-heptadecene; (C14–C35) *n*-paraffins.

and another peak appeared with a much higher retention time than that of the original compound. This new peak showed the following mass spectrum (above *m/z* 80, ions with relative abundances greater than 10% are given; under *m/z* 80, only fragments with abundances greater than 25% are given): *m/z* 157 (15%), 138 (11%), 115 (32%), 111 (11%), 98 (12%), 97 (23%), 95 (15%), 88 (10%), 87 (14%), 85 (28%), 83 (23%), 81 (15%), 73 (25%), 71 (67%), 69 (60%), 60 (38%), 57 (100%), 55 (51%), 43 (86%), 41 (64%). This fragmentation corresponds to that of 5,9-dimethyldecanoic acid (not described in the literature), so the presence of a fragment of *m/z* 60 $[(HO)_2C{=}CH_2]^{+\cdot}$ indicates that it is a carboxylic acid, unsubstituted at the α-position, and the ions at *m/z* 73 and 87 indicate no substitution at the β- or γ-position. The ions at *m/z* 157,115, 87 and 85 (the consequence of scissions at the points of maximum branching) and the lack of signals at *m/z* 99 and 101 confirm the terpene structure proposed above for the hydrocarbon. The hydrocarbon is thus identified as 6,10-dimethyl-1-undecene.

Figure 1 also shows four peaks, designated as 2, 3, 4 and 5. These are cyclic sesquiterpenes with a common molecular mass of 204, to which we have tentatively assigned the structures of α-copaene, calarene, eremophyllene and muurolene, respectively. This assignment has been based on information in the library of the mass spectrometer computer (U.S. National Bureau of Standards). The same figure shows an adjacent small component, designated as 6, which also appears as the major peak in the chromatogram of the third fraction eluted from the column (Fig. 2). The mass spectra of this compound and its hydrogenated derivative confirm it as α-farnesene.

In some virgin oils, the fraction containing α-farnesene also contains two compounds 7 and 8 (Fig. 2), whose mass spectra correspond to those of the (*Z*2,*E*4,*E*6) and (*E*2, *E*4,*E*6) allofarnesenes, respectively (7). We have not been able to find any relationship between the presence of these compounds in specific oils and physicochemical characteristics of the oils.

The first compound recorded in the *n*-alkane series is *n*-tetradecane (Fig. 1). The major components of the series are the six with odd numbers of carbon atoms in the chain, between *n*-tricosane and *n*-tritriacontane, comprising more than 75% of the fraction. *n*-Pentadecane is identified only in the chromatogram of the first fraction, because in that obtained from the mixture of the combined two first fractions (an operation necessary for complete quantitation of the linear hydrocarbons), it overlaps with the sesquiterpenes.

The peak marked as C17:1 in Figure 1 showed the following mass spectrum (above *m/z* 200, only ions with relative abundances greater than 15% are given; in the range beween *m/z* 135 and 200, only fragments with abundances greater than 5% are given; under *m/z* 135, only fragments with abundances greater than 30% are given): *m/z* 238 (78%, M^+), 210 (18%), 195 (6%), 182 (6%), 181 (8%), 169 (8%), 168 (9%), 167 (10%), 154 (11%), 153 (8%), 140 (16%), 139 (19%), 138 (8%), 125 (37%), 111 (67%), 98 (33%), 97 (100%), 84 (33%), 83(80%), 71(32%), 70(60%), 69(87%), 67(30%), 57(63%), 56 (47%), 55 (87%), 43 (55%), 41 (62%). It suggests a monounsaturated alkene with 17 carbon atoms. The mass spectrum of its hydrogenated derivative is the same as that of *n*-heptadecane. To verify the position of the double bond, the reaction with dimethyl disulfide was performed. The spectrum of the derivative showed fragments of *m/z* 173 $[CH_3(CH_2)_7CH{=}\overset{+}{S}CH_3)]$ (71%) and 159 $[CH_3\text{-}\overset{+}{S}{=}CH\text{-}(CH_2)_6CH_3]$ (80%), indicating the presence of an ethylene bond at carbon atom 8. The compound was thus identified as 8-heptadecene, possibly formed by biodecarboxylation of oleic acid.

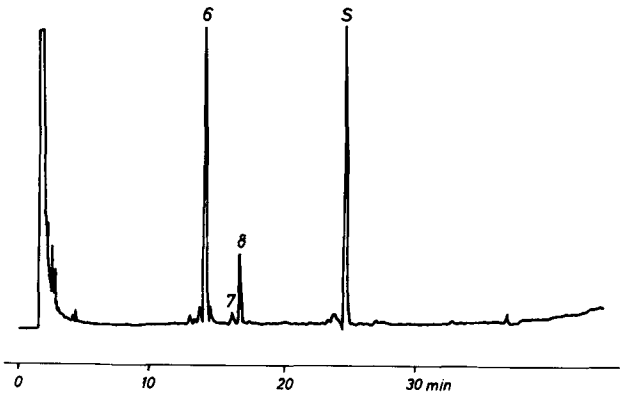


FIG. 2. Gas chromatogram of the third hydrocarbon fraction from a virgin olive oil: (6) α-farnesene; (7 and 8) allofarnesenes; (S) *n*-eicosane (standard).

In the oils obtained from olives of the Arbequina variety, four other hydrocarbons, marked as C22:1, C23:1, C24:1 and C25:1 in Figure 3, were detected. Their mass spectra [the most important ions in the mass spectrum of C25:1 were: *m/z* 350 (2%, M^+), 222 (2%), 195 (3%), 153 (3%), 139 (4%), 125 (6%), 111 (27%), 97 (46%), 85 (32%), 84 (22%), 83 (50%), 82 (23%), 73 (32%), 72 (27%), 71 (43%), 57 (73%), 56 (70%), 43 (100%), 42 (58%)] and those of their hydrogenated derivatives indicate that they are *n*-alkenes. The mass spectra of their reaction products with dimethyl disulfide show peaks at *m/z* 173 that are common for the four derivatives, and peaks at *m/z* 229, 243, 257 and 271 for those of docosene, tricosene, tetracosene and pentacosene, respectively, indicating that the double bond is at position 9 in all.

The only significant component appearing in the chromatogram of the fourth fraction eluted from the silica column is squalene. Traces of others can be observed, among which farnesene could be identified. Further fractions eluted from the column with hexane comprise squalene as sole component. Once this hydrocarbon is eluted completely, the polarity of the solvent must be increased to elute other more polar compounds not included in this work.

Refined olive oils, AR and BR. The hydrocarbon fraction of each refined oil differs considerably from that of the corresponding virgin oil, both qualitatively and quantitatively. The differences undoubtedly derive from refining, and are greater in the oil BR subjected to the more drastic industrial treatment of physical refining. The chromatographic profiles of the corresponding fractions eluted from the silica column are not exactly the same in the two oils AR and BR. This is a result of the quantitative differences recorded in the two samples, although their qualitative compositions are basically the same. To simplify the description, we will refer to the fractionation

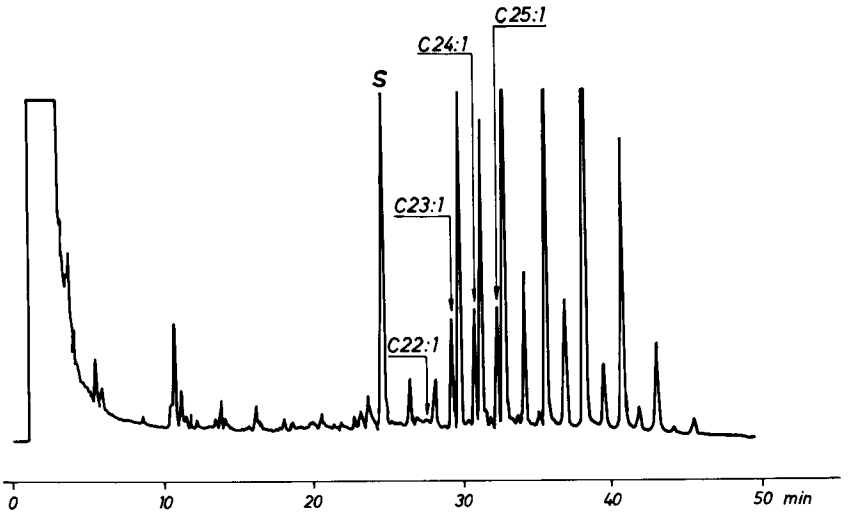


FIG. 3. Gas chromatogram of the combined first and second hydrocarbon fractions from Arbequina virgin olive oil: (S) *n*-eicosane (internal standard); (C22:1) 9-docosene; (C23:1) 9-tricosene; (C24:1) 9-tetracosene; (C25:1) 9-pentacosene.

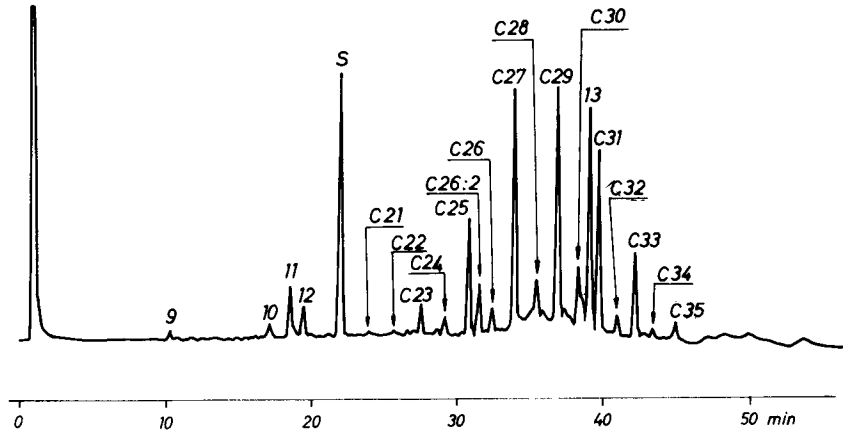


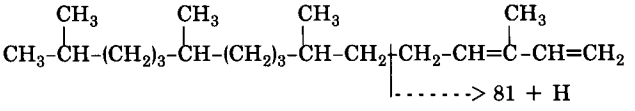
FIG. 4. Gas chromatogram of the combined first and second hydrocarbon fractions from the AR refined olive oil: (9) pentadecene and pentadecadiene; (10) octadecene and octadecadiene; (11 and 12) neophytadienes; (S) *n*-eicosane (internal standard); (13) stigmasta-3,5-diene.

of sample AR, pointing out any differences observed in the results of sample BR.

Figure 4 shows the chromatogram of the first and second fractions, including the standard of *n*-eicosane marked as S in the figure. Tridecene and the sesquiterpenes recorded in the virgin oil do not appear in the refined oil. In the alkane series, *n*-hexadecane is the first compound recorded, followed by all members of the series until pentatriacontane. This loss of the most volatile components is a consequence of the deodorization treatment.

Certain peaks not observed in the virgin oils now appear in the chromatogram. The most representative are those marked as 9 and 10, whose mass spectra indicate that they are binary mixtures of unsaturated straight-chain hydrocarbons. The former comprises pentadecene and pentadecadiene, and the latter octadecene and octadecadiene.

The peak marked as 11 has been identified as neophytadiene by its similarity to the mass spectrum of that product in the library of the recorder. The mass spectrum of the peak marked as 12 shows the same molecular ion as neophytadiene (*m/z* 278) but has some significant differences; the presence of an intense peak at *m/z* 82 could be explained by the following molecular structure:



(McLafferty)

whose base peak at *m/z* 82 would correspond to that of the McLafferty's transposition shown in the formula. The presence of these diterpenes of molecular formula $C_{20}H_{38}$ could be due to the dehydration of phytol, with loss of the primary alcohol group and formation of the corresponding conjugated dienes.

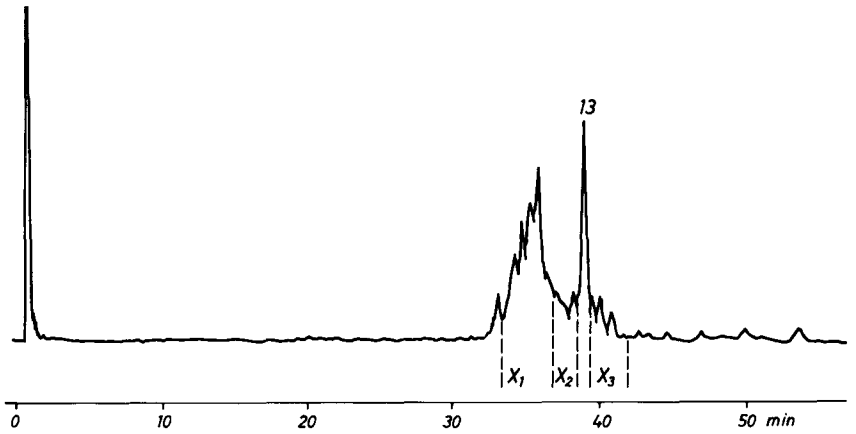


FIG. 5. Gas chromatogram of the third hydrocarbon fraction from the AR refined olive oil: (X_1) squalene isomers [molecular weight (M.W.) 410]; (X_2) isoprenoidal polyolefins (M.W. 408); (X_3) steroidal hydrocarbons (M.W. 422); (13) stigmasta-3,5-diene.

A peak with retention time 31.25 min (Fig. 4) was identified as a *n*-hexacosadiene (C26:2). Its relative proportion is much greater in the oil BR (subjected to physical refining) than in AR (refined by the classical method). Its mass spectrum corresponds to that of a diunsaturated hydrocarbon with 26 carbon atoms, and that of the hydrogenated product is equal to that of *n*-hexacosane. Moreover, the retention time of the latter product is coherent with this assignment. A diene with the same number of carbon atoms has been found by Goh and Gee (8) in refined palm oil, and the 9,17-hexacosadiene was detected by Sen Gupta (9) as a thermal decomposition product of methyl oleate in the absence of oxygen. Although we have not investigated the position of the two double bonds in the molecule, probably in all cases the hydrocarbons are the same.

A major peak, marked as 13 in Figure 4, appears in this fraction close to that of *n*-hentriacontane. Its intensity is also much greater in the oil BR, refined by the physical method. This peak corresponds to stigmasta-3,5-diene, which has been found in refined olive oils by Lanzón *et al.* (10). The presence of hydrocarbons with a sterol structure after the treatment of edible fats with bleaching earths was noted by Niewiadomski (11) and by Roderbourg and Kuzdzal-Savoie (12) in industrially manufactured butter.

The mass spectrum of the product obtained by dehydration of extremely pure β-sitosterol (for which the technique of Montignié (13) to dehydrate cholesterol was used) is identical to that of the stigmasta-3,5-diene of this work. This further confirms the origin of the product in refined oils AR and BR. We have proposed the detection of stigmasta-3,5-diene in olive oil as a characteristic of refined oils to indicate adulteration of those marketed as virgin (10).

The chromatograms of the third and fourth fractions of oils AR and BR eluted from the column are given in Figures 5 and 6. These show a cluster of peaks absent in the virgin oils. They are poorly resolved by gas chromatography, so that obtaining their mass spectra is difficult. Dividing the chromatogram of Figure 5 into three zones, marked X_1, X_2 and X_3, the mass spectra of the maxima of zone X_1 all show a molecular weight of 410, equal to that of squalene but with a different fragmentation

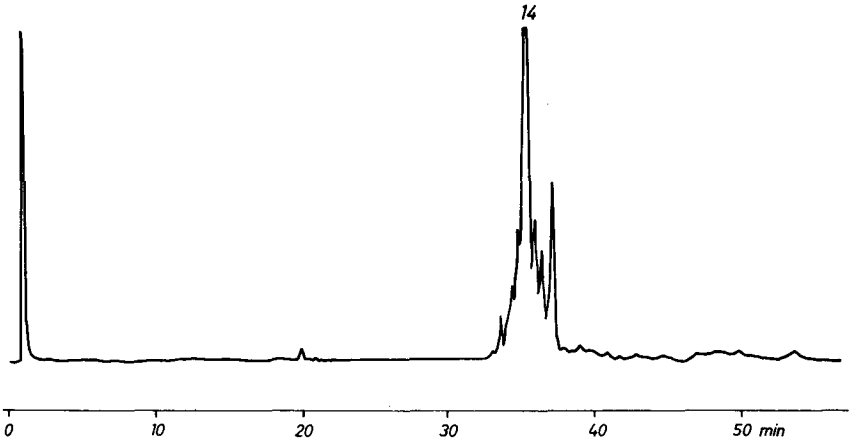


FIG. 6. Gas chromatogram of the fourth hydrocarbon fraction from the AR refined olive oil: (14) squalene.

TABLE 1

Concentration Ranges of Terpenic Hydrocarbons Found in 250 Samples of Spanish Virgin Olive Oils

Hydrocarbons	Peak number	mg/kg
6,10-Dimetyl-1-undecene	1	n.d.[a]-7.67
α-Copaene[b]	2	0.12-4.77
Calarene[b]	3	n.d.-0.26
Eremophylene[b]	4	n.d.-2.60
Muurolene[b]	5	n.d.-1.51
α-Farnesene	6	n.d.-32.59
Allofarnesene (*Z*2,*E*4,*E*6)	7	n.d.-0.15
Allofarnesene (*E*2,*E*4,*E*6)	8	n.d.-2.44
Squalene	14	800-12,000

[a]n.d., Not detected.
[b]Tentative assignment.

pattern and different one from another. In zone X_2, the spectra of the two maxima give a molecular weight of 408, and in zone X_3 the molecular weight is 422. The presence of stigmasta-3,5-diene is noticed (peak number 13). The mass spectrometry of the fourth fraction (Fig. 6) indicates the presence of squalene (peak number 14), surrounded by a cluster of peaks similar to the X_1 zone in the third fraction. The compounds of molecular weight (M.W.) 410 are isomerization products of squalene, produced by the effect of temperature and bleaching earth, as indicated by Mariani *et al.* (14).

The compounds of M.W. 408 give a mass spectrum similar to that of the hydrocarbon originated as an artifact in gas chromatography analysis of 2,6,10,15,19,23-hexamethyl-tetracos-3,6,10,14,18,22-hexaen-2-ol, an oxidation product of squalene identified by Lanzón *et al.* (15). Isomers of this hydroxylated derivative have been demonstrated in olive oil by Paquot and Kallel (16). These facts suggest that these compounds are dehydrated during refining, yielding the corresponding isoprenoidal polyolefins. It is also possible that there is isomerization of the hydrocarbons formed.

The compounds of M.W. 422 may be due to dehydration of 24-methylenecycloartanol and its isomers, and to subsequent isomerization of the corresponding hydrocarbon as a consequence of the refining treatment.

The chromatogram of the fifth fraction shows an intense peak at retention time 35.3 min whose mass spectrum corresponds to that of squalene.

Quantitative aspects. In the Spanish virgin olive oils, the hydrocarbons show wide range of concentrations (Tables 1 and 2). The chromatographic profiles and the terpenes/alkanes ratios vary depending on the origin of the oil.

In the refined oils, the sesquiterpenic hydrocarbons are absent, and losses of alkanes are noticed, mainly those of low M.W. (Table 3). These losses are greater in oils treated by the physical refining system than by the milder conventional method of alkaline refining. Among the new hydrocarbons appearing after the refining process, a hexacosadiene and stigmasta-3,5-diene are the main degradation products, and they are more abundant in the physically refined oil than in the chemical by refined oil (Table 4).

In virgin and refined olive oils, a cluster of evenly distributed hydrocarbon peaks, ranging from C14 to C20, pristane and phytane were not found (Figs. 1 and 4). According to Tan and Kuntom (17), it indicates absence of contamination with mineral oil from industrial processing.

TABLE 2

Concentration Ranges of *n*-Alkanes and *n*-Alkenes Found in 250 Samples of Spanish Virgin Olive Oils

Hydrocarbon	mg/kg
C14	n.d.[a]-0.05
C16	trace-0.50
C17	trace-0.26
C17:1	n.d.-0.45
C18	trace-0.15
C19	trace-0.51
C21	trace-0.72
C22	0.12-1.04
C22:1[b]	n.d.-0.15
C23	0.65-16.35
C23:1[b]	n.d.-1.25
C24	0.47-14.93
C24:1[b]	n.d.-1.12
C25	2.51-28.80
C25:1[b]	n.d.-1.18
C26	0.74-3.26
C27	3.61-13.69
C28	0.81-2.28
C29	3.07-9.90
C30	0.46-1.95
C31	1.89-8.83
C32	0.16-1.09
C33	0.70-5.52
C34	trace-0.51
C35	0.12-1.33

[a]Not detected.
[b]Found in oils from the Arbequina variety.

TABLE 3

Changes in *n*-Alkanes and Squalene Concentrations by Refining Processes

	Olive oil A				Olive oil B			
	Virgin		Refined, classical process		Virgin		Refined, physical process	
Hydrocarbon	Saturated fraction (%)	mg/kg	Saturated fraction (%)	mg/kg	Saturated fraction (%)	mg/kg	Saturated fraction (%)	mg/kg
C14	0.20	0.04	—	—	0.4	0.08	—	—
C15	trace	trace	—	—	trace	trace	—	—
C16	1.6	0.47	0.3	0.06	0.5	0.12	0.4	0.06
C17	0.9	0.26	—	—	1.0	0.23	0.6	0.09
C18	0.5	0.14	—	—	0.6	0.14	0.6	0.08
C19	0.7	0.21	—	—	1.3	0.29	0.9	0.13
C21	1.8	0.51	1.1	0.23	1.8	0.41	1.4	0.20
C22	2.0	0.58	1.6	0.34	2.1	0.47	2.0	0.30
C23	5.6	1.61	3.0	0.62	5.5	1.23	3.3	0.49
C24	3.1	0.91	1.8	0.39	3.1	0.71	1.9	0.28
C25	12.8	3.69	13.1	2.74	14.4	3.23	14.6	2.15
C26	3.8	1.10	5.1	1.06	3.2	0.72	3.5	0.51
C27	20.1	5.80	24.0	5.04	16.1	3.72	16.2	2.38
C28	4.6	1.33	4.4	0.92	3.2	0.72	3.1	0.45
C29	17.3	4.99	19.4	4.05	16.8	3.78	17.4	2.56
C30	3.0	0.87	2.8	0.60	2.6	0.58	2.3	0.34
C31	11.4	3.31	14.3	2.98	14.9	3.35	15.1	2.23
C32	1.6	0.46	1.6	0.33	1.7	0.39	1.8	0.26
C33	5.9	1.71	5.2	1.10	7.9	1.79	10.9	1.60
C34	1.4	0.40	0.6	0.13	0.9	0.20	1.2	0.17
C35	1.7	0.51	1.7	0.35	2.4	0.53	2.8	0.41
Squalene		4.23[a]		3.98[a]		3.12[a]		2.51[a]

[a]$\times 10^3$.

TABLE 4

Degradation Hydrocarbons Appearing in Refined Olive Oils

Component	Refined oil AR (mg/kg)	Refined oil BR (mg/kg)
Pentadecene + pentadecadiene	0.24	—
Octadecene + octadecadiene	0.40	0.52
Neophytadiene	1.22	0.58
Neophytadiene isomer	0.68	0.27
n-Hexacosadiene	1.33	9.40
Stigmasta-3,5-diene	8.98	21.53
Squalene isomers (M.W. 410)	—	—
Isoprenic polyolefins of M.W. 408	—	—
Steroidal hydrocarbons of M.W. 422	—	—

ACKNOWLEDGMENTS

The authors express their sincere gratitude to CICYT for supporting this research project, ALI88-0187-C02-01. Thanks are due to Jose L. Rios Marín for performing the mass spectrometry and Rosario G. Cordones for the technical assistance.

REFERENCES

1. Eisner, J., J.L. Iverson, A.K. Mozingo and D. Firestone, *J. of the A.O.A.C. 48*:417 (1965).
2. Capella, P., E. Fedeli, H. Cirimele and G. Jacini, *Riv. Ital. Sostanze Grasse 40*:603 (1963).
3. Jacini, G., and E. Fedeli, *Fette Seifen Anstrichm. 77*:1 (1975).
4. Bastic, M., L.J. Bastic, J.A. Jovanovic and G. Spiteller, *J. Am. Oil. Chem. Soc. 55*:886 (1978).
5. Urbach, G., and W. Stark, *J. Agro. Food Chem. 23*:20 (1975).
6. Buser, H.R., H. Arn, P. Guerin and S. Ranscher, *Anal. Chem. 55*:818 (1983).
7. Williams, H.J., M.R. Straud and S.B. Winson, *Tetrahedron 37*:2763 (1981).
8. Goh, S.H., and P.T. Gee, *J. Am. Oil Chem. Soc. 63*:226 (1986).
9. Sen Gupta, A.K., *Fette Seifen Anstrichm. 68*:475 (1966).
10. Lanzón, A., A. Cert and T. Albi, *Grasas y Aceites 40*:385 (1989).
11. Niewiadomski, H., *Die Nahrung 19*:525 (1975).
12. Roderbourg, H., and S. Kuzdzal-Savoie, *J. Am. Oil Chem. Soc. 56*:485 (1979).
13. Montignié, E., *Soc. Chim. France 41*:524 (1927).
14. Mariani, C., S. Venturini, P. Bondioli, E. Fedeli and K. Grob, *Riv. Ital. Sostanze Grasse 69*:393 (1922).
15. Lanzón, A., T, Albi, A. Cert and J. Gracian, *Grasas y Aceites 43*:271 (1992).
16. Paquot, C., and H. Kallel, *Rev. Fr. Corps Gras 20*:329 (1973).
17. Tan, Y.A., and A. Kuntom, *J.A.O.A.C. Int. 76*:371 (1993).

[Received March 11, 1993; accepted November 15, 1993]