Formation of a Δ^7 Triterpene Alcohol in Refined Olive Oils

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ABSTRACT: The triterpene alcohol fraction in several virgin olive oils and the corresponding oils refined by alkali and by physical processes was analyzed by gas chromatography. A Δ^7 compound was detected in all refined olive oils but not in virgin olive oils. This compound was tentatively identified by gas chromatography–mass spectrometry as 24-methyl-5α-lanosta-7,24-dien-3β-ol, a 24-methylenecycloartanol isomer produced during the refining process by the opening of the 9,19 cyclopropane ring with formation of a double bond in the Δ^7 position and the translocation of a double bond in the side chain from the 24-28 to the 24-25 position.

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The physical as well as the chemical refining process alters qualitatively and quantitatively the triterpene alcohol fraction of virgin olive oil. As consequences of the temperatures at which the oil is tested and of contact with bleaching earth, a structural alteration of some of the components of this fraction takes place, especially of cycloartenol and 24-methylenecycloartanol, resulting in the formation of chemical species different from the ones naturally occurring in the oil. Until now, cyclobranol is the only compound found with certainty in all of the refined olive oils, resulting from the isomerization of the 24-methylencycloartol (1–3).

The authors observed that, when a virgin olive oil was refined using the physical processes as well as the alkaline neutralization processes, a compound was formed that appeared in the triterpene alcohol fraction of the unsaponifiable matter of the refined oil. This compound was analyzed as the trimethylsilyl ether derivative by gas chromatography–mass spectrometry (GC–MS) and has been tentatively identified as 24-methyl-5α-lanosta-7,24-dien-3β ol. This compound is an isomer of 24-methylenecycloartanol, and it is produced by the opening of the 9,19 cyclopropane ring, with the formation of a double bond in the Δ^7 and the translocation of the double bond in the side chain from the 24–28 to the 24–25 position.

EXPERIMENTAL PROCEDURES

Reagents. The reagents used were of chromatography grade when required and analytical grade otherwise.

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Materials. Five virgin olive oils were refined in an industrial plant by conventional alkaline neutralization and by steam deodorization (physical process) to give 10 refined olive oils. Fifteen samples of these oils were analyzed (5 virgin olive oils and 10 refined olive oils).

Isolation of unsaponifiable matter. The unsaponifiable matter was isolated according to the official method of the European Union Commission (Reglamento CEE 2568/91 Anexo V) (4).

Isolation of the triterpene alcohol fraction. This fraction was separated from the unsaponifiable matter by thin-layer chromatography (TLC). The plates were developed using a hexane/diethyl ether (65:35, vol/vol) mixture. The compounds were visualized with a 0.2% solution of 2,7-dichlorofluorescein. Spots of interest were scraped from the TLC plate into a vial. Isopropyl ether was used to extract the triterpene alcohols from the silica, and then was evaporated.

GC. The triterpene alcohol fraction was silanized by adding a freshly prepared pyridine/hexamethyldisilazane/trimethylchlorosilane (9:3:1, vol/vol/vol) mixture. An aliquot was taken from the clear solution and injected into the gas chromatograph.

Triterpene alcohol analyses were performed on a Hewlett-Packard 5890 series II gas chromatograph (Palo Alto, CA), equipped with a split/splitless injector and fitted with a glass insert filled with stationary phase and silanized glass wool, as well as a flame-ionization detector. The output signal of the detector was processed with a Hewlett-Packard 3396 series II integrator-recorder. A capillary column of fused silica SPB-5 (Supelco, Inc., Bellefonte, PA; 30 m long, 0.25 mm i.d., 0.25 μm film thickness) was also used. The chromatographic conditions employed were: injection in split mode; pressure at column head,120 kPa; carrier gas, hydrogen; injector temperature, 285°C; detector temperature, 325°C; initial oven temperature, 210°C; initial time, 4 min; ramp, 2°C/min; final temperature, 275°C.

MS. MS was performed with a Finnigan MAT95 (Bremen, Germany) high-resolution mass spectrometer interfaced with a Hewlett-Packard 5890 Series II gas chromatograph (Palo Alto, CA) using a capillary column SPB-5 (Supelco; 30 m long, 0.25 mm i.d., 0.25 μm of film thickness). The samples were injected in a split mode. The aliphatic and triterpene alcohols were resolved by temperature programming from 210 to 275°C at 2°C/min. Both transfer line and ion source were maintained at 375°C. The helium carrier gas head pressure was 15 psi. Electron impact (EI) spectra were recorded at 70

eV. Full spectra (50–1000 amu) were recorded at a scan speed of 2 s/decade over the entire elution profile. Data were analyzed by means a ICIS II Data System from Finnigan MAT.

RESULTS AND DISCUSSION

Typical chromatograms of silanized derivatives from aliphatic and triterpene alcohols obtained from virgin and refined olive oils by the physical refining process are shown in Figures 1 A and 1 B, respectively. The chromatogram corresponding to olive oil refined by a chemical process is not shown because it exhibits the same 12 peaks identified in Figure 1B.

The assignment of four peaks designated as 2,3,4, and 6 (Figs. 1A and 1B) is based on information in the library of the mass spectrometer computer at the (U.S. National Bureau of Standards.) The peak marked as 5 (Fig. 1B) has been identified by Lanzón *et al*. (5). The five peaks marked as 7,8,9,10, and 12 (Figs. 1A and 1B) were identified by GC–MS after comparing them with literature values (6–9).

The mass spectrum (Fig. 2) of the peak marked as 11 (Fig. 1B) gave the molecular ion M^+ 512 ($C_{34}H_{60}OSi$, relative intensity 10%), with other principal ions at *m/z* 497 (M – CH_3, 22%), 428 (M – C_6H_{12}, 14%), 407 [M - CH_3 - $(CH_3)_3SiOH$, 100%], 385 [M – C_9H_{17} (side chain) – 2H, 11%].

The absence of ions *m/z* 300 and 353 indicates that there is

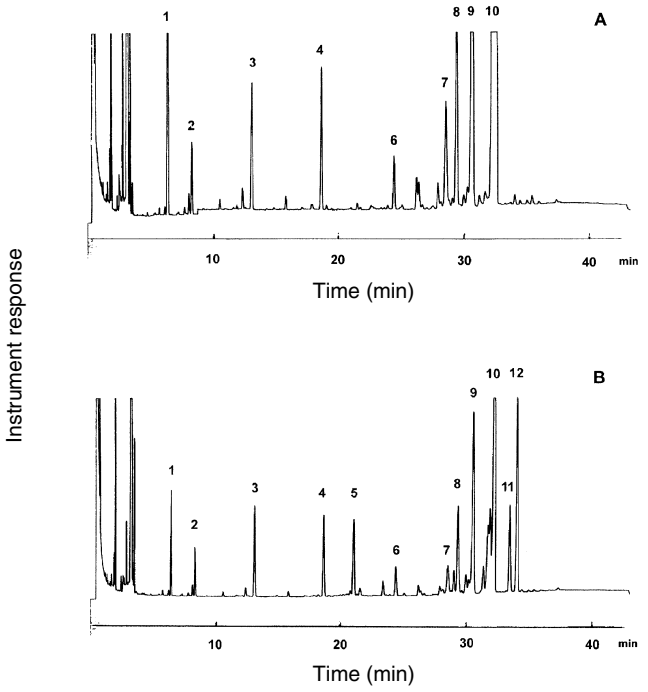

FIG. 1. Gas chromatograms of the trimethylsilyl derivatives of aliphatic and triterpene alcohols from (A) virgin and (B) refined olive oils. (1) Heneicosanol (standard); (2) docosanol; (3) tetracosanol; (4) hexacosanol; (5) 2,6,10,15,19,23-hexamethyl-tetracos-3,6,10,14,18,22-hexaen-2-ol; (6) octacosanol; (7) parkeol; (8) butyrospermol; (9) cycloartenol; (10) 24-methylenecycloartanol; (11) 24-methyl-5α-lanosta-7,24-dien-3β-ol; (12) cyclobranol.

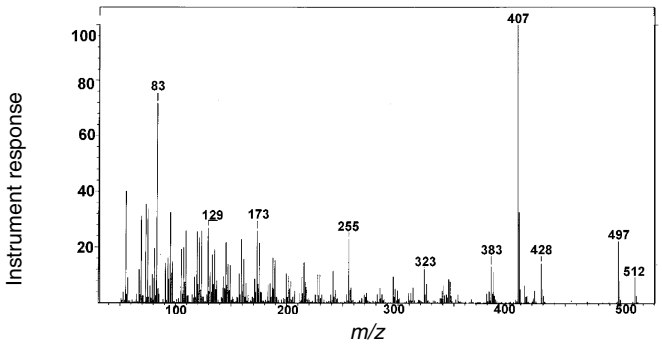

FIG. 2. Mass spectrum of the peak marked 11 in Figure 1B.

no 9,19-cyclopropane ring present in the 24-methylenecycloartanol, so we must assume that this ring opened with the formation of a double bond in the ring system. The peak at *m/z* 385 gives us an ionic mass for the lateral chain of 125 (C_9H_{17}), which implies the presence of a double bond that must be located at carbon 24, since if it were located at carbons 25 or 22, the McLafferty rearrangement mechanism would give us an allylic break with the M – 125 and M – 43 ions (10), which are not observed in this compound.

If there is a double bond at carbon 24, it cannot have occurred by the formation of a terminal methylene ($\Delta^{27,28}$), but by a $\Delta^{24,25}$ double bond instead, since in the first case the retention time would be greater (10,11). Migration of the double bond of the methylene group, existing in 24-methylenecycloartanol, to the secondary contiguous carbon is the same isomerization process that gives rise to the formation of the cyclobranol. This has been studied by Asano *et al.* (12).

According to the position of the double bond in the nucleus, there are three possibilities for carbons 7, 8 or 9. The Δ^8 position is eliminated, as it does not appear in the spectrum of the M – 2H and M – CH_3 – 2H ions that are characteristic of these sterols (13). The Δ^9 position does not fit with the location of this compound in the chromatogram, having a higher retention time than that of 24-methylenecycloartanol. Itoh *et al.* (6) studied the correlation of the gas chromatographic behavior of triterpene alcohols of the lanostan series with the characteristics of the skeleton and the modifications of the side chain. According to the values found in their study, one can calculate that an isomers of the 24-methylene cycloartanol without the 9,19 cyclopropane ring and with a Δ^9 double bond would have a slightly lower retention time than that of the 24-methylene cycloartanol. Consequently, it would not be located in the position of this compound; on the contrary, the change of position to Δ^7 would appreciably increase the retention time.

Another result that confirms the hypothesis that it could be a Δ^7 sterol is the observation by Wyllie and Djerassi (14) concerning the appearance in the spectrum of ionic masses with a *m/z* equal to the M – side chain – 2H, characteristic of the Δ^5 and Δ^7 sterols, beside very low intensity ions (for sterols with the double bond in the carbon 24 of the side chain) to

m/z 299, 300, 301, whose origin is due to the McLafferty rearrangement. In the case of the Δ^7 sterols, the ions with a *m/z* equal to the M − side chain − 2H have a much higher intensity than the ions with *m/z* 299, 300, 301, which is observed in this compound contrary to what occurs with the Δ^5 sterols.

Thus, this compound is tentatively identified as 24-methyl-5α-lanosta-7,24-dien-3β-ol.

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