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Triterpene Alcohols and Sterols of Spanish Olive Oil

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ABSTRACT

Nine Spanish olive oils, including three each of virgin (pressed oil), refined virgin, and B-residue (solvent-extracted pomace oil) oils from different commercial sources, have been analyzed for their unsaponifiable matter (USM). Four sterolic fractions separated from the oils have been analyzed by preparative thin-layer chromatography (TLC); these fractions are triterpene alcohols, 4-methylsterols, sterols and triterpene dialcohols. The compositions of the four sterolic fractions were determined as their acetates by gas-liquid chromatography (GLC) on an OV-17 glass capillary column. Identification of each component was carried out by argentation TLC, GLC and combined gas chromatography-mass spectrometry (GC-MS); 44 components were identified, of which four: 24-methylene-31-nor-9(11)-lanostenol, 24-methyl-31-nor-E-23-dehydrocycloartanol, 24-ethyl-E-23-dehydrolophenol and 5,E-23-stigmastadienol, were considered to be new sterols from natural sources. Several characteristics, including the content of triterpene dialcohols in the USM and that of C-24(28) unsaturated sterols in each of the four sterolic fractions, which can be used to distinguish between virgin and B-residue olive oils, were observed.

INTRODUCTION

Olive oils of different qualities from various origins have been much investigated for their sterolic constituents: sterols (4-desmethylsterols) (1-16); triterpene alcohols (4,4-dimethylsterols) (2,4-6,10,16-21); 4-methyl sterols (4α-monomethylsterols) (16,18,20,22-24); and triterpene dialcohols (9,10,13,16,25). Because of a high complexity of the sterolic compositions, however, especially those of triterpene alcohols and 4-methylsterols, a number of components remained unidentified. It has been proposed or established that the triterpene alcohol composition (2,21) or the content of triterpene dialcohols (26) could be used as tools for the detection and identification of B-residue oil in commercial olive oils. An extensive investigation of the sterolic constituents of virgin, refined virgin and B-residue olive oils coming from Spanish commercial sources was therefore undertaken in this study by the combined use of thin layer chromatography (TLC), gas liquid chromatography (GLC) on an OV-17 glass capillary column, argentation TLC, and gas chromatography-mass spectrometry (GC-MS) as the analysis and identification aids.

EXPERIMENTAL

Saponification of the oils, fractionation by preparative TLC on silica gel (20 × 20 cm, 0.5 mm thick) of the unsaponifiable matter (USM) with three developments using *n*-hexane/ethyl ether (7:3, v/v) as developer, and combined GC-MS (70 eV, >m/e 100; 2% OV-17, 2 m × 3 mm id glass column) were performed. These methods are similar to methods described in earlier reports (8). Preparative argentation (silver nitrate/silica gel, 1:4, w/w) TLC (0.5-mm-thick) was developed 3-5 times with methylene chloride/carbon tetrachloride (1:5, v/v). Upon TLC and argentation TLC, the separated bands and the fractions recovered therefrom were numbered in the order of polarity, beginning with the least polar. GLC on an OV-17 SCOT glass capillary column (30 m × 0.3 mm id, column 260 C) was carried out as previously described (27), and relative retention time (RRT) was given relative to cholesterol acetate. ^{13}C FT NMR spectrum was recorded on a JNM FX-100 spectrometer (Japan Electron Optics Lab. Co., Tokyo) operating at 25.05 MHz in deuteriochloroform. Other techniques used in this work have also been described previously (27). The configuration at C-24 of the sterols possessing an asymmetric carbon atom at the 24 position was undetermined in this study, with the exception of sitosterol.

The USM was separated by preparative TLC into six fractions: fraction 1 (R_f ca. 0.4-1.0), less polar compounds (e.g., hydrocarbons); fraction 2 (R_f 0.37), triterpene alcohols (containing aliphatic alcohols); fraction 3 (R_f 0.29), 4-methylsterols (containing aliphatic alcohols); fraction 4 (R_f 0.20), sterols; fraction 5 (R_f 0.12), triterpene dialcohols; and fraction 6 (R_f 0.0-ca. 0.1), highly polar compounds. The percentage yield of the six fractions was determined from the recovery figure. To determine the approximate relative contents of the four sterolic constituents—triterpene alcohols, 4-methylsterols, sterols and triterpene dialcohols—the acetylated USM was analyzed by GLC (a number of GLC peaks that eluted with small RRT and were attributable to hydrocarbons and aliphatic alcohols were excluded from the determination of sterolic compositions) and the percentages of the component peaks with RRT 2.07 (mainly 24-methylenecycloartanol acetate), 1.49 (obtusifoliol acetate), 1.63 (mainly sitosterol acetate) and 4.46 (erythrodiol diacetate) (which apparently represent characteristic components for each of the four sterolic constituents in the USM) were determined. Since the percentages of these components in each of the fractions were separately determined, calculation of ratios between the percentages in the USM and those in each fraction for the characteristic components enabled the determination of the relative contents of the four sterolic fractions.

Each of the four sterolic fractions was rechromatographed separately on TLC in order to obtain purified

fractions. After acetylation, the approximate compositions of the four acetylated sterolic fractions were determined by GLC. A rough quantification between each of the overlapped peak components in GLC was carried out by argentation TLC. Components were identified separately in the acetylated sterolic fractions from each of virgin, refined virgin and B-residue oils by GLC and combined GC-MS after the fractionation by preparative argentation TLC, with the exception of the triterpene dialcohol fraction. As for the separated fractions, further argentation TLC was carried out to obtain purified fractions or facilitate the combined GC-MS analysis.

MATERIALS

Nine olive oil samples, including three each of virgin, refined virgin and B-residue oils from different Spanish commercial suppliers (A, B and C), were courteously supplied by an olive oil importer in New Jersey.

The following 40 authentic specimens were used in this study: tirucallol; taraxerol; dammaradienol; α-amyrin; germanicol; butyrospermol; parkeol; α-amyrin; cycloartenol; 7,24-tirucalladienol; 24-methylene-24-dihydroparkeol; 24-methylenecycloartanol (27); cyclobranol (18); obtusifoliol; 24-methyl-31-nor-9(11)-lanostenol; 24-methyllophenol; cycloeucalenol; gramisterol; 24-ethyllophenol; 24-methyl-24(25)-dehydrolophenol; citrostadienol; and 24-ethyl-24(25)-dehydrolophenol (28); 28-isocitrostadienol (29); cholesterol; 7-cholestenol; campesterol; 24-methylenecholesterol; stigmasterol; sitosterol; 28-isofucosterol; 5,24-(25)-stigmastadienol (30); brassicasterol; fucosterol; 7-stigmastenol; avenasterol (8); erythrodiol (27); cyclosadol; 24-methyl-E-23-dehydrolophenol (T. Itoh, N. Shimizu, T. Tamura and T. Matsumoto, *Phytochemistry*, submitted for publication), 5,E-23-stigmastadienol, which was prepared following isomerization of 28-isofucosterol with *N*-lithioethylenediamine (N. Shimizu, T. Itoh, T. Tamura and T. Matsumoto, unpublished results); and 7,E-22-ergostadienol which was generously donated by Dr. H. Yokokawa, Tachikawa College of Tokyo, Tokyo. Rf values upon argentation TLC of the acetates of most of these triterpene alcohols (27), 4-methylsterols (28) and sterols (30) have been described previously.

RESULTS

For brevity, the values given here are averages of the values for the three different oil types. Complete quantitative data is available upon request. Each of the nine oils contained about 1% of USM (0.8-1.7%). Fraction 1 from preparative TLC, which constituted about 70% of the USM from virgin oil, 55% of the USM from refined oil and about 50% of the USM from B-residue oil, was composed of less polar compounds e.g., hydrocarbons) and was not examined further.

Fraction 2-Triterpene Alcohols

GLC-determined composition for the triterpene alcohols are given in Table I. Values for the three samples of each oil type were in good agreement except for the components at RRT 2.07 and 2.50 in the three B-residue oils. The acetylated triterpene alcohol fraction from refined virgin oil was separated into seven major bands upon argentation TLC. The major constituents of band 1 were the acetates of a number of aliphatic alcohols, which eluted with RRT smaller than ca. 0.7. This band contained the acetates of β-amyrin, α-amyrin and an unidentified component (RRT 1.18). Band 2 contained cyclobranol acetate as the major component, accompanied by the acetates of taraxerol and germanicol. Band 3 contained cycloartenol acetate as the major component accompanied by the acetates of cyclosadol, tirucallol and an unidentified component (RRT 2.48). Band 4 was a mixture of the acetates of 7,24-tirucalladienol, butyrospermol and an unidentified component (RRT 2.42). Band 5 gave 24-methylenecycloartanol acetate accompanied by the acetates of parkeol and an unidentified component (RRT 1.26). Band 6 afforded 24-methylene-24-dihydroparkeol acetate, and band 7, the most polar band, gave dammaradienol acetate.

Fraction 3-Methylsterols

Table II shows the average values for 4-methylsterols in the three oils. Again, agreement was generally good within each type except for 24-ethyl-24(25)-dehydrolophenol in the B-residue oils. The 4-methylsterol fraction from the USM of refined virgin oil, on further TLC (28), gave one band which was then divided into halves to give fraction A from the less polar half and fraction B from the more polar half.

The acetate of A was separated into four major bands by argentation TLC. Band A-1 was a mixture of the acetates of 24-methyl-31-nor-9(11)-lanostenol and three sterols with RRT 1.33, 1.50 and 1.78, accompanied by several aliphatic alcohol acetates. The MS of the sterol acetate at RRT 1.78 showed the following prominent ions: m/e 468 (M^+,

TABLE I

Average Composition of Triterpene Alcohol Fractions of Olive Oils

Acetate RRT compound	Composition (%) Virgin oil	Refined virgin oil	B-Residue oil
1.18 Unidentified	–	0.1	1.6
1.26 Unidentified	0.3	0.4	1.0
1.47 Tirucallol	–	0.1	0.1
1.57 Taraxerol	0.8	0.8	1.2
1.65 Dammaradienol β-Amyrin Germanicol	5.0	6.1	9.0
1.70 Butyrospermol	6.8	5.9	11.1
1.80 Parkeol	0.4	0.3	1.3
1.86 Cycloartenol>>α-Amyrin	10.2	13.6	11.8
1.92 7,24-Tirucalladienol	10.9	8.3	15.9
2.00 24-Methylene-24-dihydroparkeol	0.4	1.9	4.1
2.07 24-Methylenecycloartanol>cyclosadol	63.8	57.8	31.7[a]
2.42 Unidentified	–	1.2	3.2
2.48 Unidentified	1.0	–	–
2.50 Cyclobranol	0.4	3.7	7.9[b]

[a]Values for the 3 B-residue oils were 18.2, 31.4 and 45.5%.

[b]Values for the 3 B-residue oils were 12.1, 8.9 and 2.8%.

TABLE II

Average Composition of 4-Methylsterol Fractions of Olive Oils

RRT compound	Composition (%)[a]		
	Virgin oil	Refined virgin oil	B-Residue oil
1.26 Unidentified	–	–	0.1
1.33 Unidentified	2.5	1.3	4.0
1.49 Obtusifoliol	11.6	9.9	8.7
1.58 Unidentified	0.6	0.6	1.4
1.64 24-Methyl-31-nor-9(11)-lanostenol	0.5	0.6	1.3
1.70 24-Methylene-31-nor-9(11)-lanostenol	0.8	2.0	1.5
1.77 Cycloeucalenol, Gramisterol>> 24-Methyl-31-nor-E-23-dehydrocycloartanol, 24-methyl-E-23-dehydrolophenol	32.8	23.2	13.4
1.94 Unidentified	0.7	0.8	4.1
2.15 24-Ethyllophenol, 24-ethyl-E-23-dehydrolophenol, 24methyl-24(25)-dehydrolophenol	2.5	5.6	9.1
2.29 28-Isocitrostadienol	1.4	3.0	2.8
2.41 Citrostadienol	43.5	46.5	29.0
2.60 24-Ethyl-24(25)-dehydrolophenol	1.8	1.6	4.4[b]
2.95 Unidentified	–	0.4	5.1
3.40 Unidentified	1.5	4.4	14.4

[a]A trace amount of 24-methyllophenol was detected in all of the samples.

[b]Values for the 3 B-residue oils were 9.3, 2.8 and 1.1%.

relative intensity 9%), 453 (13%), 408 (99%), 393 (80%), 371 (12%), 311 (60%), 300 (15%), 285 (11%), 283 (17%), 281 (15%), 241 (10%), 227 (10%), 203 (34%), 189 (47%), 173 (68%), 147 (84%), 121 (97%) and 107 (100%). The M^+ at m/e 468 ($C_{32}H_{52}O_2$) indicated that it was an acetate of a C_{30} sterol with two double bonds, one of which was located in the C_9 side chain (m/e 281, M-C_9H_{17} [side chain, SC]-HOAc-2H) (31). The ions at m/e 241 (M-SC-HOAc-C_3H_6 [part of ring D] and 227 (m/e 241-CH_2) indicated the presence of an additional C-32 methyl group in the ring system (32), and the fragments at m/e 371 (M-C_7H_{13} [part of SC]) and 311 (m/e 371-HOAc) suggested that the side chain double bond was located at C-23 to facilitate cleavage at the C-20, C-22 bond (33). Moreover, the ions at m/e 300 (M-$C_{10}H_{16}O_2$ [ring A]) and 285 (m/e 300-Me) were probably caused by the presence of a 9β,19-cyclopropyl group rather than a double bond in the ring system (34), and hence the sterol was suggested to possess a 9β,19-cyclo-31-nor-lanostane ring system with a 24-methyl-Δ^{23} side chain. The following GLC correlation provided evidence for the E-configuration of the Δ^{23}-bond of the sterol. The separation factor of this sterol acetate (RRT 1.78) vs cycloeucalenol acetate (RRT 1.77) was calculated to be 1.006, which was consistent with the 24-methyl-E-Δ^{23}/24-methylene side chain separation factor (1.005) calculated from the retention data of cyclosadol acetate (RRT 2.08) vs 24-methylenecycloartanol acetate (RRT 2.07). It could therefore be concluded that the sterol had the structure 24-methyl-31-nor-E-23-hydrocycloartanol. Band A-2 afforded an unidentified sterol acetate (RRT 1.26) accompanied by several components, and band A-3 was a mixture of the acetates of cycloeucalenol and obtusifoliol.

Band A-4 afforded a sterol acetate (RRT 1.70) accompanied by several minor components. The MS of the sterol acetate showed the following prominent ions: m/e 468 (M^+, rel. int. 13%), 453 (57%), 425 (19%), 408 (13%), 393 (58%), 384 (9%), 369 (11%), 365 (6%), 341 (100%), 325 (12%), 309 (14%), 301 (20%), 287 (19%), 281 (14%), 275 (13%), 241 (23%), 227 (17%), 215 (21%), 201 (25%), 189 (24%), 173 (34%), 159 (40%), 119 (58%) and 107 (62%). The M^+ at m/e 468 and the fragments at m/e 341 (M-C_9H_{17}[SC]-2H), 301 (M-SC-C_3H_6), 287 (m/e 301-CH_2), 281 (m/e 341-HOAc), 241 (m/e 301-HOAc) and 227 (m/e 287-HOAc) indicated that it was an acetate of a C_{30} sterol with two double bonds, one of which was located in the C_9 side chain, and with an additional C-32 methyl group in the ring system. The ions at m/e 384 (M-C_6H_{12} [part of SC]), 369 (m/e 384-Me) and 309 (m/e 369-HOAc) showed that the side chain double bond was located either at C-24(28) or C-24(25) (31), and a further occurrence of fragments at m/e 425 (M-C_3H_7) and 365 (m/e 425-HOAc) indicated the C-24(28) location of the double bond (35,36). Moreover, the ring system double bond was shown to be located at C-9(11) since the sterol acetate afforded 24-methyl-31-nor-9(11)-lanostenol acetate upon partial hydrogenation. Thus the sterol was considered to have the structure 24-methylene-31-nor-9(11)-lanstenol.

The acetylated 4-methylsterol fraction B was separated into four major bands upon argentation TLC. Band B-1 was a mixture of the acetates of 24-methyllophenol, 24-ethyllophenol and several aliphatic alcohols. Band B-2 consisted of an unidentified sterol acetate (RRT 3.40), accompanied by the acetates of 24-methyl-24(25)-dehydrolophenol and 24-ethyl-24(25)-dehydrolophenol.

Band B-3 contained citrostadienol acetate as the predominant component, accompanied by the acetates of 28-isocitrostadienol and two sterols at RRT 1.94 and 2.15. The MS of the sterol acetate (RRT 2.15) showed the following prominent ions: m/e 468 (M^+, rel. int. 21%), 453 (16%), 408 (4%), 393 (5%), 357 (11%), 327 (100%), 297 (27%), 287 (4%), 269 (19%), 267 (13%), 241 (15%), 227 (20%), 215 (11%), 197 (19%), 135 (27%), 121 (36%) and 109 (29%). The M^+ at m/e 468 indicated that it was an acetate of a C_{30} sterol with two double bonds. The fragments at m/e 327 (M-$C_{10}H_{19}$[SC]-2H) and 267 (m/e 327-HOAc) showed the presence of a C_{10} side chain with one double bond. The fragments at m/e 357 (M-C_8H_{15} [part of SC]) and 297 (m/e 357-HOAc) suggested that the side chain double bond was located at C-23 (33), and the other double bond in the ring system was considered to be located at C-7, since the ion at m/e 327 was observed as the base peak (35). Hence, this sterol was suggested to possess a lophenol ring system with a 24-ethyl-Δ^{23} side chain. The configuration at C-23 was assumed to be E because the sterol acetate showed an RRT of 2.15 in GLC identical to that of 24-ethyllophenol acetate. This correlation was found to be consistent with that observed between the acetates of 5,E-23-stigmastadienol, (RRT 1.62) and sitos-

terol (RRT 1.63). Thus, the sterol was recognized to have the structure 24-ethyl-E-23-dehydrolophenol. Band B-4 afforded gramisterol acetate accompanied by an unidentified component (RRT 2.95).

Fraction 4-Sterols

The compositions of the sterol fractions are given in Table III. The acetylated sterol fraction of refined virgin oil was separated into five major bands upon argentation TLC. Band 1 gave sitosterol acetate accompanied by the acetates of cholesterol and campesterol. Sitosterol acetate was crystallized from these components (ca. 97% pure by GLC), mp 123-125 C, which on hydrolysis gave free sitosterol, mp 139-141 C (lit. mp 140 C [37]). The ^{13}C NMR spectrum was consistent with that of sitosterol (24R-ethylcholesterol) (38), and hence the 24R-ethyl configuration was confirmed. Band 2 was a mixture of the acetates of brassicasterol, 7-cholestenol, stigmasterol and 5,24(25)-stigmastadienol; stigmasterol acetate was the major one. Band 3 was a mixture of the acetates of 7,E-22-ergostadienol and 5,E-23-stigmastadienol. MS of the latter showed the following prominent ions: m/e 394 (M^+-HOAc, rel. int. 100%), 379 (10%), 313 (M-$C_{10}H_{19}$[SC]-2H, 10%), 288 (10%), 283 (M-C_8H_{15}[part of SC]-HOAc, 50%), 275 (8%), 255 (25%), 253 (m/e 313-HOAc, 17%), 228 (10%), 213 (21%), 159 (32%), 147 (54%), 145 (53%), 133 (48%) and 107 (30%). Band 4 afforded a mixture of the acetates of fucosterol, 28-isofucosterol and avenasterol, with isofucosterol being the most prominent. Band 5 also contained isofucosterol acetate as the major component, accompanied by the acetates of 24-methylenecholesterol and a sterol with RRT 1.61. The MS of this sterol acetate showed the following prominent ions: m/e 440 (M^+, rel. int. 10%), 425 (16%), 380 (6%), 365 (9%), 356 (33%), 351 (7%), 313 (100%), 288 (6%), 273 (8%), 255 (26%), 253 (22%), 227 (18%), 213 (30%), 197 (22%), 145 (19%), 135 (22%), 119 (26%) and 107 (37%). The M^+ at m/e 440 showed that it was an acetate of a C_{28} sterol with two double bonds, one of which was located in the C_9 side chain (m/e 313, M-C_9H_{17}-[SC]-2H). The fragment at m/e 356 (M-C_6H_{12}[part of SC]) suggested that the side chain double bond was located either at C-24(28) or C-24(25), and the fragment at m/e 313 that formed the base peak might be attributed to the presence of a Δ^7-bond in the ring system. Taking into consideration the GLC correlation with the other related sterols, the sterol was regarded to have the structure 7,24-(28)-ergostadienol.

Fraction 5-Triterpene Dialcohols

The acetylated triterpene dialcohol fraction of refined virgin oil afforded two GLC peaks at RRT 4.46 and 5.10, and the faster eluted component was identified as erythrodiol diacetate. The slower eluted component showed the following ions in MS: m/e 526 (M^+, $C_{34}H_{54}O_4$, rel. int. 1%), 466 (15%), 453 (2%), 451 (3%), 423 (2%), 406 (2%), 393 (3%), 276 (5%), 249 (6%), 216 (100%), 203 (89%), 189 (37%), 187 (22%), 133 (48%) and 119 (31%). The fragments at m/e 276 and 216 (m/e 276-HOAc) indicated a retro-Diels-Alder fragmentation in ring C, which is characteristic for the triterpenes of Δ^{12}-oleanenes and ursenes (39). The prominent ion at m/e 203, which was evolved by the loss of 73 mass units (CH_2OAc) from the ion at m/e 276, indicated that the angular C-17 position was substituted by an acetoxy methyl group (39). The overall fragmentation pattern was found to be similar to that of Δ^{12}-oleanen-3β,28-diol diacetate; the triterpene was, therefore, considered to be Δ^{12}-ursen-3β,28-diol, i.e., uvaol. The relative concentrations of erythrodiol and uvaol were: virgin oils, 83.0 and 17.0; refined virgin, 83.2 and 16.8; and B-residue, 87.2 and 12.8, respectively.

DISCUSSION

Forty-four sterolic components were identified or their structures tentatively determined in this study. Four sterols among them, i.e., 24-methylene-31-nor-9(11)-lanostenol, 24-methyl-31-nor-E-23-dehydrocycloartanol, 24-ethyl-E-23-dehydrolophenol and 5,E-23-stigmastadienol, are considered to be new compounds from natural sources. 7,E-22-Ergostadienol has previously been identified in fungi (40), but this study seems to be the first instance of its identification in higher plants. Among the other sterolic components described here, eight triterpene alcohols: tirucallol, taraxerol, dammaradienol, germanicol, parkeol, 7,24-tirucalladienol, 24-methylene-24-dihydroparkeol and cyclosadol; seven 4-methylsterols: 24-methyl-31-nor-9(11)-lanostenol, 24-methyllophenol, 24-methyl-E-23-dehydrolophenol, 24-ethyllophenol, 24-methyl-24(25)-dehydrolophenol, 28-isocitrostadienol and 24-ethyl-24(25)-dehydrolophenol; and five sterols: 7-cholestenol, 24-methylenecholesterol, 7,24-(28)-ergostadienol, fucosterol and 5,24(25)-stigmastadienol, are considered to be identified for the first time in olive oil. Though previous studies have reported the presence of 7-stigmastenol in the sterol fraction from various olive oils (8-16), that sterol could not be identified in this study.

Four 24-alkylated $\Delta^{24(25)}$-sterols and five 24-alkylated E-Δ^{23}-sterols were detected in this study as the minor sterols. The biogenetic significance of these sterols has been discussed elsewhere. Some evidence has been given (41-46) that the major biosynthetic pathway leading to 24α-alkylsterols such as sitosterol probably involves the intermediacy of $\Delta^{24(25)}$-sterols. Other possible routes involve Δ^{23}-sterols (33) as intermediates in the biosynthesis of 24-alkylsterols (47). On the other hand, 24-alkylated $\Delta^{24(25)}$- and/or Δ^{23}-sterols have been shown to be produced from $\Delta^{24(25)}$.

TABLE III

Average Composition of Sterol Fractions of Olive Oils

RRT compound	Composition (%)[a]		
	Virgin oil	Refined virgin oil	B-Residue oil
1.31 Campesterol	3.0	3.3	3.7
1.35 24-Methylenecholesterol>7,E-22-ergostadienol	0.4	0.5	0.3
1.43 Stigmasterol	0.7	3.0	2.3
1.63 Sitosterol>>5,E-23-stigmastadienol	89.3	87.2	91.0
1.72 Fucosterol	0.3	0.3	0.5
1.81 28-Isofucosterol	5.6	5.2	1.7
1.95 5,24(25)-Stigmadienol	0.1	0.4	0.5

[a]Trace amounts of cholesterol, brassicasterol, 7-cholestenol, 7,24(28)-ergostadienol and avenasterol also were detected in all of the oils.

sterols by chemical isomerization under reflux with iodine in benzene (33,48), sulfuric acid in isopropyl alcohol (49), and N-lithioethylenediamine in ethylenediamine (T. Itoh, N. Shimizu, T. Tamura and T. Matsumoto, *Phytochemistry*, submitted for publication). Whether the 24-alkylated $\Delta^{24(25)}$- and E-Δ^{23}-sterols found in olive oil are true natural products or are artifacts produced from $\Delta^{24(28)}$-sterols during oil processing, extraction and the extensive separation procedures of sterols from the oil remains an open question. The 24E-ethylidene sterols—28-isocitrostadienol and fucosterol—detected in this study to be minor components accompanied by larger amounts of their 24Z-isomers, citrostadienol and 28-isofucosterol, have been found just recently (in higher plants) in two Solanaceae seed oils and in rice bran oil, accompanied also by their 24Z-isomers (29). Finding the two 24E-ethylidene sterols in higher plants is of interest in sterol biogenesis. However, further experimental proof might be needed to determine whether the 24E-ethylidene sterols in olive oil participated in the sterol biogenesis, since 24Z-ethylidene sterols are less stable (50) and are considered to be susceptible to chemical isomerization to the more stable 24E-isomers.

Several characteristics by which each of the Spanish virgin, refined virgin and B-residue commercial olive oils could be distinguished were observed in this study. The content of fraction 1 (e.g., hydrocarbons) from USM was found to be higher in virgin oils (65-76%) than in B-residue oils (44-54%). On the other hand, B-residue oils contained (20-24%) two- to threefold as much of fraction 2 (triterpene alcohols and aliphatic alcohols) as did virgin oils (7-12%). One of the most striking differences observed between virgin and B-residue oils was the content of triterpene dialcohols. The relative content of triterpene dialcohols in the total sterolic constituents was trace-1% for virgin oils, 2% for refined virgin oils and 8-10% for B-residue oils. This value might be a reliable indicator for distinguishing virgin and B-residue oils. Similar results have been observed for Iranian olive oils (16), and the content of triterpene dialcohols has already been established as a tool for identifying Italian pressed oils (26).

The most outstanding difference in the triterpene alcohol composition (Table I) between virgin and B-residue oils was the content of 24-methylene-cycloartanol (accompanied by a minor amount of cyclosadol. The highest proportion of this component was observed for virgin oils (56.7-67.5%), followed by refined oils (53.1-60.3%), and B-residue oils (18.2-45.5%). This compound may be an indicator with which virgin and B-residue oils can be differentiated, but as observed for one B-residue oil, some commercial B-residue oils show values close to those for virgin oils. The content of an unidentified component (acetate, RRT 2.42) and cyclobranol can be used as indicators for identifying virgin oils, since the unidentified component was not detected and cyclobranol was found as only a very minor component (0.3-0.5%). On the other hand, refined virgin and B-residue oils contained trace-4.2% of the unidentified component and 1.7-12.1% of cyclobranol.

As was recognized in the triterpene alcohol fraction, a lower content of C-24(28) unsaturated sterols cycloeucalenol, gramisterol and citrostadienol, was found in the 4-methylsterol fraction for B-residue oils, compared to virgin and refined virgin oils (Table II). If the sum of cycloeucalenol and gramisterol is taken, two- to threefold as much was accounted for in virgin oils (30.5-33.9%) as was in B-residue oils (11.4-16.2%).

An appreciable difference was observed for the contents of a C-24(28) unsaturated sterol-28-isofucosterol-in virgin and B-residue oils (Table III), as has been recognized for Iranian olive oils (16). Virgin and refined virgin oils contained 3.7-7.3% of this sterol, whereas a much lower level, 1.5-1.9%, was in B-residue oils. Moreover, the stigmasterol content can be used to identify virgin oil: virgin oils contained 0.5-0.9%, whereas the level for refined virgin (2.6-3.4%) and B-residue (1.9-2.7%) oils was distinctly higher. Spanish virgin olive oils have been reported to be divided into two types according to their content of 28-isofucosterol. One type contains a high level (mean 22.6%) of the sterol and the other contains a lower level (mean 8.5%) (14). Taking into account this classification, all of the Spanish oils investigated in this study fell into the low-level type. Though a significant difference was observed in the levels of triterpene dialcohols in virgin and B-residue oils (26), no notable difference was observed in the composition of triterpene dialcohols in these oils.

Although several characteristics by which the Spanish virgin and B-residue olive oils might be distinguished were found, they seem to have limited value for quantifying the degree of adulteration of commercial olive oils. A further systematic study might be needed, therefore, to more reliably quantitate the degree of adulteration of olive oils.

NOMENCLATURE

For the systematic nomenclature of the compounds omitted below, refer to the previous papers: triterpene alcohols (18,27), 4-methylsterols (28,29), and sterols (8,30). Cyclosadol = 24-methyl-9β,19-cyclo-5α-lanost-E-23-en-3β-ol, 24-methylene-31-nor-9(11)-lanostenol = 4α,14α-dimethyl-5α-ergosta-9(11),24(28)-dien-3β-ol, 24-methyl-31-nor-E-23-dehydrocycloartanol = 4α,14α-dimethyl-9β,19-cyclo-5α-ergost-E-23-en-3β-ol, 24-methyl-E-23-dehydrolophenol = 4α-methyl-5α-ergosta-7,E-23-dien-3β-ol, 24-ethyl-E-23-dehydrolophenol = 4α-methyl-5α-stigmasta-7,E-23-dien-3β-ol, 7,E-22-ergostadienol = 5α-ergosta-7,E-22-dien-3β-ol, 7,24(28)-ergostadienol = 5α-ergosta-7,24(28)-dien-3β-ol, 5,E-23-stigmastadienol = stigmasta-5,E-23-dien-3β-ol, erythrodiol = 5α-olean-12-ene-3β,28-diol, uvaol = 5α-urs-12-ene-3β,28-diol.

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⁂Quantitative Isolation of Lipids of Partially Defatted and Whole Peanuts by a Dry Column Method

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ABSTRACT

A dry column method of lipid extraction was found to be applicable to peanuts—partially defatted as well as whole. Total lipid was obtained from the peanut/sodium sulfate/Celite 545 columns by isocratic elution with dichloromethane/methanol 9:1. Moreover, neutral lipids were obtained by sequential elution that were completely free from polar lipids. First, dichloromethane eluted the neutral lipids, then the 9:1 solvent eluted the polar lipids—at times containing small quantities of neutral lipids. Total lipid values obtained by the column extraction method were slightly higher than those obtained by the standard Soxhlet extraction procedure. This was due in part to the more complete polar lipid isolation produced by the column method. In partially defatted peanuts produced by mechanical pressing, 99% of the polar lipids remained in the retained oil, and these were shown to be slightly less unsaturated than the neutral lipids.

INTRODUCTION

While studying the oxidative stability and related phenomena in partially defatted peanuts (1), the fate of the polar lipids during mechanical oil separation was investigated. To isolate the peanut lipids and concomitantly separate the polar from the neutral lipids, a dry column method recently developed for meat tissues was used (2,3). The efficacy of this method for lipid quantitation and separation in whole and partially defatted peanuts was evaluated, and the results were compared with those obtained by traditional lipid isolation techniques.

EXPERIMENTAL PROCEDURES

Partially defatted peanuts (PDP) were prepared by pressing 100 g unroasted, seed quality Comet type Spanish variety peanuts in a 2.5-in. id test cylinder outfit in a Carver hydraulic press at 3,000 psi for 30 min. Oil contents were determined either by AOCS official method Ab 3-49 (4), the method of Folch et al. (5), or by the dry column method, as indicated below.

The column had the dimensions shown in Fig. 1. Glass wool was inserted into the column's tip. The column was then charged with 10 g of a 9:1 well-blended mixture of Celite 545 and $CaHPO_4 \cdot 2H_2O$; this was tightly compressed. Either whole peanuts (WP) or PDP were ground in a CRC micro-mill (Chemical Rubber Company, Cleveland, OH) for 3-15 sec for uniformity in sampling. Ground PDP (3.5 g) or ground WP (2 g) were mixed with 5 times their weight of anhydrous Na_2SO_4 in a 750-ml mortar, and ground thoroughly. Celite 545 (15 g) (non-acid washed, Johns Manville Co., Maryland Heights, MO) was added and grinding was continued until the mixture was thoroughly blended. The mixture was transferred with the aid of a metal spoon to the column, on top of the Celite 545 and $CaHPO_4 \cdot 2H_2O$ mixture previously placed there. The column packing was tamped down to a height of 17-18 cm. The mortar, pestle and spoon then were washed with the solvent used in the extraction, which subsequently was transferred to the column using a disposable pipette. When room temperature was above 27 C, the column was cooled with running tap