Influence of Avocado Oil Processing on the Nature of Some Unsaponifiable Constituents

M. Farines[a], J. Soulier[a], A. Rancurel[b,*], M.G. Montaudoin[b] and L. Leborgne[b]

[a]Laboratoire de Chimie Organique des Substances Naturelles, Université de Perpignan, F-66860 Perpignan Cedex, France and [b]Pharmascience, Laboratoire de Recherche, Epernon, France

ABSTRACT: The unsaponifiable part of avocado pear oil, after heating during extraction and processing, contains an abundant fraction that has never been found in the unsaponifiable part of usual vegetable oils nor in cold-pressed avocado oil. Nine components were detected. Eight of them could be identified through chromatographic fractionations and spectroscopic analysis, mainly by mass spectrometry and proton nuclear magnetic resonance. These products are characterized by a homogeneous structure. A furyl nucleus is substituted in position 2 by an aliphatic, mono- or polyunsaturated chain. The chainlength varies from 13 to 17 carbon atoms. The carbon number is always odd. The significance of these components in the biological effects of avocado oil unsaponifiables is still being studied. *JAOCS 72,* 473–476 (1995).

KEY WORDS: Avocado oil, furyl derivatives, H NMR, Lauraceae, MS, *Persea* sp., unsaponifiable.

The pulp of the fruit of avocado tree (*Persea* sp., Mill, Lauraceae) is particularly rich in lipids. The fresh pulp contains between 4–40% fat (1–3), depending on the variety, with the average around 15%. Various authors have extracted the oil from either fresh fruit or after desiccation (4). The unsaponifiable part, currently used in various pharmaceutical (such as Piascledine; Pharmascience Laboratory, Epernon, France) and cosmetic preparations, has also been extracted. Other authors (5–13) have isolated certain lipid fractions directly from fresh pulp. These lipid fractions are mainly composed of triols or hydroxyketones, either free or in the form of monoacetates, based on saturated, unsaturated, or polyunsaturated linear aliphatic chains. In methanol-extracted avocado stones, Alves *et al.* (7) noted the presence of four components with a furan nucleus, which they called "avocatin." We have observed considerable quantitative and qualitative differences in the unsaponifiables of the oil, depending on whether it was extracted from fresh fruit or was extracted after drying. In fresh fruit, the unsaponifiables amount to 1–2%, compared with 3–7% in the dried fruit oil. The differences were associated with the appearance of a new class of compounds, in addition to the alcohols mentioned previously, which accounted for up to 50% of the unsaponifiable part (4). The presence of these components could be linked with pharmacological activity of the unsaponifiable part of avocado lipids; this subject remains under study.

The aim of this paper is to describe the isolation of this class of components, the fractionation of its constituents in their pure form by chromatography, and their identification by various spectroscopic techniques [ultraviolet (UV), infrared (IR), proton nuclear magnetic resonance (H NMR), and mass spectrometry (MS)]. The mechanisms by which these components appear during drying will be described in a later publication.

MATERIALS AND METHODS

Avocado pear (200 g) was sliced (5-mm thickness) and dried in an oven at 80°C for 24 h. The dried fruits were ground, and the powder (35 g) was extracted with boiling hexane (Carlo Erba, Milan, Italy) in a Soxhlet apparatus for 18 h. The solvent was evaporated under reduced pressure, and the crude oil was obtained at a 45% yield based on the dried fruit.

A 15-g portion of oil was saponified by refluxing for 4 h with an alcoholic solution of potassium hydroxide (7.5 mL of 12 N potash in 30 mL ethanol). After addition of water (50 mL), the aqueous layer was extracted six times with 50-mL portions of 1,2-dichloroethane (Atochem, Nanterre, France). The organic phase was washed with water, dried over anhydrous sodium sulfate, and evaporated under reduced pressure (yield 5%).

A portion (0.5 g) of the unsaponifiable part obtained was separated on a 10-mm i.d. chromatographic column filled with 20 g of 37–70-μm silica (Amicon, Danvers, MA). The solvent was hexane. Each 50-mL fraction was checked by thin-layer chromatography on 0.25-mm thick silica plates (E. Merck, Darmstadt, Germany). Elution was performed with heptane (Carlo Erba). The fractions with R_f values between 0.15 and 0.5 were recovered.

Gas chromatography (GC) of these fractions was performed on a Fractovap apparatus Mod. 2900 (Carlo Erba), equipped with a Ross injector, a flame-ionization detector, and a 25-m long, 0.32-mm i.d., 0.2-μm film thickness CP Sil-19 CB capillary column (Chrompack, Middelburg, The Netherlands). The injector and detector temperature was

*To whom correspondence should be addressed at Laboratoire de Chimie Organique des Substances Naturelles, Université de Perpignan, avenue de Villeneuve, F-66860 Perpignan Cedex, France.

275°C, and the oven was programmed from 200°C (10 min) to 275°C at a rate of 3.5°C/min. The vector gas was helium at a pressure of 0.5 bar.

High-performance liquid chromatography (HPLC) analysis of the same fractions was done on a Dupont chromatograph (Dupont Instruments, Wilmington, DE) equipped with an automatic injector (Gilson Medical Electronics, Villiers-le-Bel, France), a diode array UV detector (Varian, Palo Alto, CA) and a 250-mm long, 4-mm i.d. Lichrospher RP18 5 µm column (E. Merck). The temperature of the column was maintained at 35°C with a Dupont 850 thermostatic oven. The mobile phase was anhydrous methanol (Carlo Erba), and the flow rate was 1 mL/min. Fractions (20 µL) of a 0.2 mg/mL ethanolic solution were injected.

Semi-preparative separations were performed by HPLC on a Gilson instrument equipped with a 500-mm long, 9.4-mm i.d., RP18 10 µm column (E. Whatman). The mobile phase was a methanol/water (98:2) mixture, the flow rate was 3 mL/min, and the detection was done by differential refractometry (Gilson). The purity of each fraction was checked by GC according to previously described conditions.

H NMR spectra were recorded on a Bruker 360 spectrometer (Brucker Spectrospin Instrument S.A., Wissembourg, France). Samples were dissolved in deuteriochloroform (E. Merck), and tetramethylsilane was used as internal standard.

Mass spectra were obtained by GC/MS. The spectrometer was a Nermag 10-10c (Delsi-Nermag, Argenteuil, France) working in electron impact (70 eV) mode, and it was equipped with a quadrupolar filter. The source temperature was 150°C, and the residual pressure in the apparatus was 3.10^{-6} torr. The gas chromatograph was a Delsi DI200 chromatograph equipped with a split/splitless injector (280°C), an interface at 280°C, and a 25-m long OV1 column of 0.25-mm i.d. with 0.2-µm film thickness (Chrompack). The other analytical conditions were as previously described.

Some of the obtained products were submitted to ozonation. Samples (10 mg) dissolved in chloroform (Carlo Erba) were treated by excess ozone, oxidized with hydrogen peroxide, then esterified by BF_3/methanol complex (E. Merck) in the usual manner.

RESULTS AND DISCUSSION

Analysis of the unsaponifiable part of avocado oil revealed two classes of components (apart from those normally found in vegetable oils) which we have called A and B. Fraction B includes the various triols previously described in the relevant literature (3–13). Fraction A, however, has never been studied systematically and represents a considerable unsaponifiable component (35–50%). The aim of this study was to isolate the individual constituents of this fraction and to identify them by various spectroscopic techniques (UV, IR, GC/MS, H NMR).

Fraction A was isolated by chromatography on a silica column and then analyzed by GC. Nine components, A_1 to A_9 (Fig. 1), were observed. Seven of these nine constituents were isolated without difficulty by semi-preparative HPLC on an RP18 column. Each of these seven components was analyzed by GC, thus providing a precise correlation between the GC and HPLC peaks. The amounts of A_5 and A_8 were too low to permit isolation

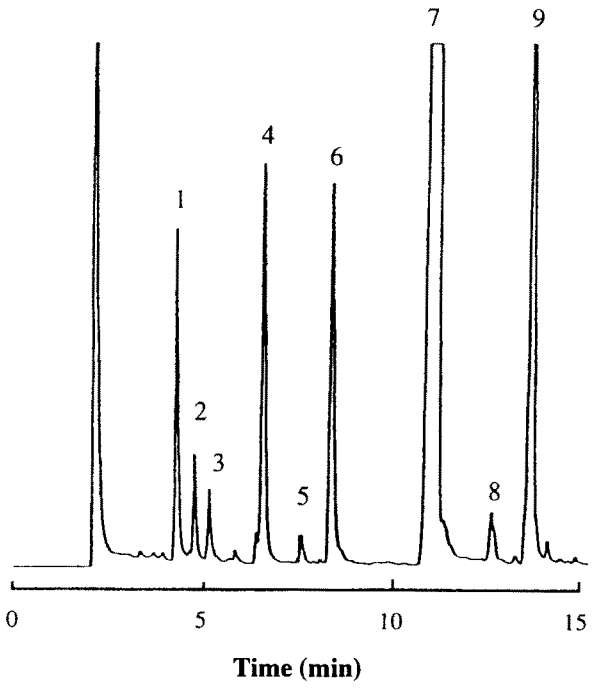

FIG. 1. Gas chromatogram of avocado unsaponifiable fraction A: 1, compound A1; 2, compound A2; 3, compound A3; 4, compound A4; 5, compound A5; 6, compound A6; 7, compound A7; 8, compound A8; and 9, compound A9. Column: CP-Sil-19 CB (Chrompack, Middelburg, The Netherlands), 200°C (10 min) to 275°C at 3.5°C/min rate.

The UV spectrum of total fraction A in solution in cyclohexane showed two absorption regions, one centered at about 220 nm and the other with peaks at 260, 266, and 278 nm (Fig. 2). An HPLC analysis was carried out with a diode array UV detector to establish whether these two absorptions were due to the same components. This method revealed two subclasses, one absorbing at about 220 nm (four constituents: A_1, A_2, A_4, and A_7) and one at 266 nm (three constituents: A_3, A_6, and A_9).

The analysis of fraction A by GC/MS showed that the compounds only contained C, H, and O. Their molecular weights and gross formulae were determined (Table 1). A more detailed study of each of these mass spectra confirmed the differences observed in the UV spectra: A_1, A_2, A_4, and A_7 had two specific splits that produced two peaks, one at *m/z* 81 (100%, C_5H_5O) and one at *m/z* 95 (C_6H_7O, variable intensity). The characteristic ions of A_3, A_6, and A_9 were represented by the peaks at *m/z* 94 (C_6H_6O, 100%) and 107 (C_7H_7O, variable intensity).

IR analysis of each of the isolated compounds failed to reveal characteristic functions, with the exception of A_2 whose spectrum showed the presence of specific vibrations of a true alkyne at 3300 and 2200 cm^{-1}.

The analysis of H NMR spectra of the seven isolated compounds (Table 2) showed structural similarities, with the pres-

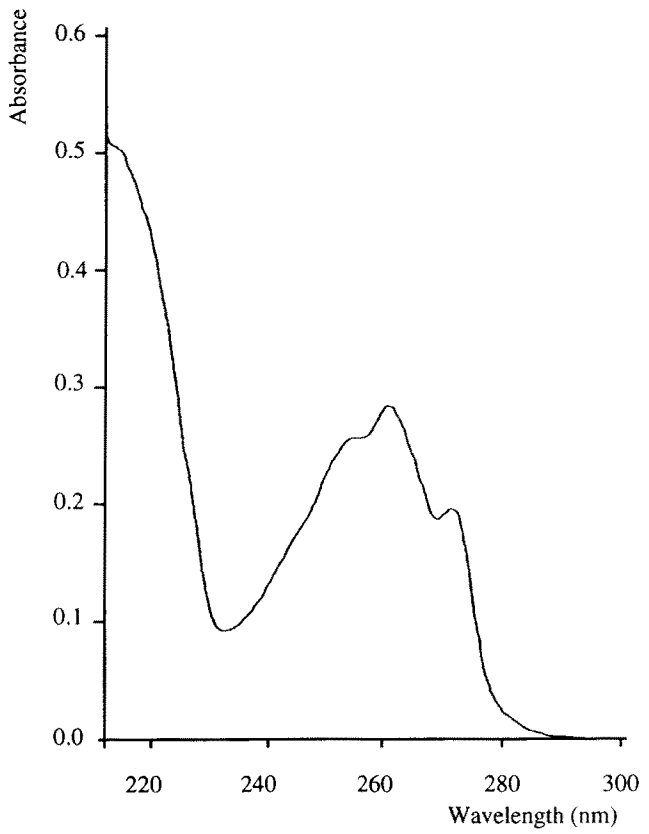

FIG. 2. Ultraviolet spectra of total fraction A in cyclohexane.

ence, at low field, of three signals with an integration ratio of 1:1:1. The chemical shifts and coupling constants of these three signals suggested an α-mono-substituted furan cycle. The presence of a triplet (2 *H*, $J = 7.6$ Hz) at 2.6 ppm in compounds A_1, A_2, A_4, and A_7 was typical of a methylene group α to an aromatic nucleus. A decoupling experiment showed that this signal was coupled with a broad multiplet at 1.6 ppm.

This hypothesis was confirmed by MS observation of the 81 mass ion, which corresponds to cleavage of the chain α of the furan nucleus. The 95 mass peaks thus represented a $(\text{furyl-CH}_2\text{-CH}_2)^+$ ion.

In the H NMR spectra of compounds A_3, A_6, and A_9, the signal of the H_c proton of the furan nucleus was shifted around 0.15 ppm toward low field in the chemical shift of the H_c proton of the furan nucleus, suggesting a specific function in the furan nucleus. The presence of a double bond conjugated with the furan nucleus was suggested by the systematic observation of a doublet and a double triplet. The *E* configuration of this double bond was proven by the value of the coupling constant ($J = 15$ to 16 Hz). This proposal was confirmed by the specifie UV absorption at 266 nm and by MS cleavage corresponding to the formation of $(\text{furyl-CH=CH- + H})^+$ and $(\text{furyl-CH=CH-CH}_2)^+$ ions. Thus, the general formula of A_3, A_6, and A_9 is furyl-CH=CH-R, R being a saturated or unsaturated aliphatic chain.

On the basis of these general observations, the structure of each of the isolated compounds can be determined (Table 2 and Fig. 3). A_1 ($C_{17}H_{28}O$) has a double bond at the end of the aliphatic chain according to the NMR spectrum and is thus tridec-12′-enyl-2-furan. A_2 ($C_{17}H_{26}O$) has a triple bond at the end of the chain according to the IR and NMR spectra and is thus tridec-12′-ynyl-2-furan. A_4 ($C_{19}H_{34}O$) has a saturated chain and is thus pentadecanyl-2-furan. A_5 ($C_{19}H_{30}O$), present in small quantity (<1%), has two unsaturations which could not be localized. A_7 ($C_{21}H_{34}O$) has two unsaturations in its lateral chain. The value of the chemical shift of the ethylenic proton (4 H, *m*) is comprised between 5.3 and 5.4 ppm. A triplet at 2.78 represents a "methylene-interrupted" group similar to the one observed in linoleic acid.

TABLE 1
Alkylfuranes: Gas Chromatographic Data and Typical Cleavages in Mass Spectrometry

Number	RRT[a]	Proportion (%)	Mass spectrometry MM[b]	81(C_5H_5O)	95(C_6H_7O)	94(C_6H_6O)	107(C_7H_7O)
A_1	0.19	3–6	248 (6%) $C_{17}H_{28}O$	100	49	—	—
A_2	0.21	1–2	246 (2%) $C_{17}H_{26}O$	100	25	—	—
A_3	0.23	1–3	246 (21) $C_{17}H_{26}O$	—	—	100	67
A_4	0.29	6–8	278 (8%) $C_{19}H_{34}O$	100	64	—	—
A_5	0.34	<1	274 (12%) $C_{19}H_{30}O$	100	—	—	—
A_6	0.37	8–15	276 (18%) $C_{19}H_{32}O$	—	—	100	65
A_7	0.50	40–65	302 (9%) $C_{21}H_{34}O$	100	42	—	—
A_8	0.56	<1	—				
A_9	0.61	16–25	300 (15%) $C_{21}H_{32}O$	—	—	100	75

[a]Relative to squalane. [b]MM, molecular mass.

TABLE 2
Alkylfurans: Proton Nuclear Magnetic Resonance Data

	H_a (*dd*)[a]	H_b (*dd*)[a]	H_c (*dd*)[a]	H1	H2	H3	H10	H11	H12	H13	Me15	Others
A_1	7.281	6.264	5.959	2.606 (*t*) (7.6 Hz)	1.626 (*tt*)			2.037 (*td*) (6.8 Hz)	5.810 (*dd*) (10.3/17.1 Hz)	4.924/4.986 (*d*) (2.0 Hz)		1.30 (*m*)
A_2	7.280	6.261	5.956	2.603 (*t*) (7.6 Hz)	1.623 (*tt*)		1.509 (*tt*) (7.1 Hz)	2.172 (*td*) (2.9/7.1 Hz)		1.925 (*td*) (2.9 Hz)		1.25 (*m*)
A_3	7.278	6.315	6.099	6.180 (*d*) (15.8 Hz)	6.128 (*dt*) (15.8/5.4 Hz)	2.150 (*td*) (5.4 Hz)		2.020 (*td*) (6.6 Hz)	5.794 (*dd*) (10.3/17.3 Hz)	4.971/4.907 (1.6 Hz)		1.25 (*m*)
A_4	7.269	6.250	5.945	2.591 (*t*) (7.6 Hz)	1.601 (*tt*)						0.867 (*t*) (6.7 Hz)	1.30 (*m*)
A_6	7.291	6.328	6.112	6.189 (*d*) (15.3 Hz)	6.162 (*td*) (15.3/7.0 Hz)	2.169 (*td*) (7.0 Hz)					0.880 (*t*) (6.7 Hz)	1.30 (*m*)
	H_a (*dd*)[a]	H_b (*dd*)[a]	H_c (*dd*)[a]	H1	H2	H3	H10	H8,12	H9,11	H7,13	Me17	Others
A_7	7.271	6.264	5.958	2.608 (*t*) (7.6 Hz)	1.630 (*tt*) (7.6/7.1 Hz)		2.771 (*t*) (6.2 Hz)	5.39 (*td*) (6.8/11 Hz)	5.35 (*td*) (6.2/11 Hz)	2.048 (*td*) (6.8/6.7 Hz)	0.888 (*t*) (6.8 Hz)	1.30 (*m*)
A_9	7.290	6.328	6.116	6.194 (*d*) (15.6 Hz)	6.174 (*td*) (15.6/5.4 Hz)	2.177 (*td*) (5.6/6.3 Hz)	2.782 (*t*) (6.2 Hz)	5.39 (*td*) (10.8/6.2 Hz)	5.35 (*td*) (10.8/6.2 Hz)	2.06 (*m*) (6.2 Hz)	0.899 (*t*) (6.7 Hz)	1.30 (*m*)

[a]*J*ab = 1.9 Hz, *J*ac = 0.9 Hz, *J*bc = 2.9 Hz (see Fig. 3 for positions).

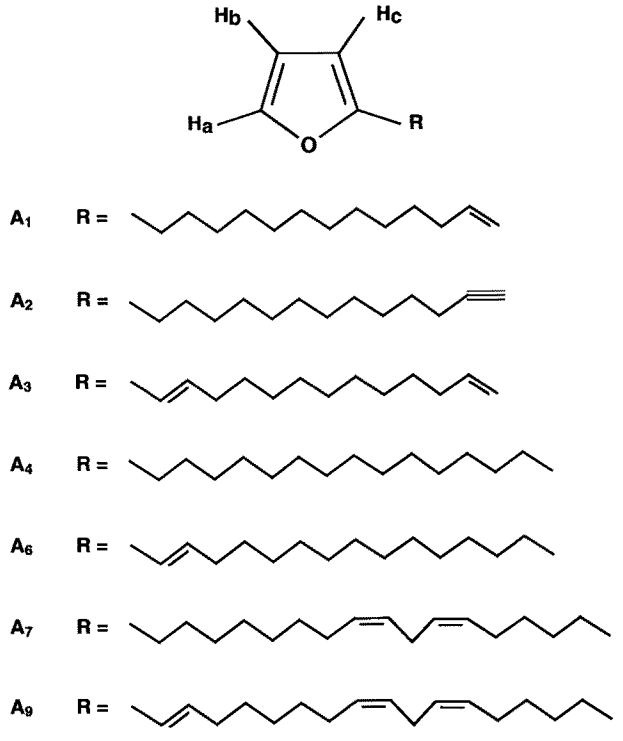

FIG. 3. Structures of the different compounds of avocado unsaponifiable fraction A.

To localize these double bonds with accuracy, the compound was subjected to ozonation followed by oxidative hydrolysis and esterification, which revealed the presence of methyl hexanoate. The compound is thus heptadeca-8′,11′-dienyl-2-furan.

A_3 ($C_{17}H_{26}O$), apart from the double 1′-2′ double bond conjugated with the furan nucleus, is unsaturated at the end of the chain, according to its NMR spectrum, and is thus trideca-1′,12′-dienyl-2-furan. A_6 ($C_{19}H_{32}O$) has only one saturated chain after C2′. Its structure is thus that of pentadec-1′-enyl-2-furan. A_9 ($C_{21}H_{32}O$) has a triunsaturated chain. Its NMR spectrum is similar to that of A_7. A similar ozonation test confirmed that the compound is heptadeca-1′,8′,11′-trienyl-2-furan.

Fraction A of the unsaponifiable part of avocado oil is thus made up of a homogeneous series of compounds with a long aliphatic chain fixed α to a furyl nucleus. In three instances, this chain is unsaturated in 1′2′.

REFERENCES

1. Mazliak, P., *Fruits 20*:49 (1965).
2. Heller, H., and W. Asche, *Seifen Ole Fette Wachse 111*:164 (1985).
3. Gutfinger, T., and A. Letan, *Lipids 9*:658 (1974).
4. Rancurel, A., *Parfums, Cosmétiques, Arômes 61*:91 (1985).
5. Kashman, Y., I. Neeman and A. Lifshitz, *Israel J. Chem 7*:173 (1969).
6. Kashman, Y., I. Neeman and A. Lifshitz, *Tetrahedron 25*:461 (1969).
7. Alves, M., D.T. Coxon, C.P. Falshaw, W.O. Godtfredsen and W.D. Ollis, *An. Acad. Brasil. Ciénc. 42*:45 (1970).
8. Neeman, I., A. Lifshitz and Y. Kashman, *Appl. Microbiol. 19*:470 (1970).
9. Bittner, S., S. Gazit and A. Blumenfeld, *Phytochem.10*:1417 (1971).
10. Brown, B.I., *J. Agric. Food Chem. 20*:753 (1972).
11. Joseph, D., and I. Neeman, *Riv. Ital. Sostanze Grasse 59*:269 (1982).
12. Werman, M.J., S. Mokady and I. Neeman, *J. Agric. Food Chem. 38*:2164 (1990).
13. Scora, R.W., and M. Ahmed, *Ibid. 41*:1971 (1993).

[Received July 1, 1994; accepted December 21, 1994]