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DAMMARANE DERIVATIVES IN THE FRUIT LIPIDS OF *OLEA MADAGASCARIENSIS*

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Key Word Index—*Olea madagascariensis*; Oleaceae; dammarenediol; dammaradienol; sterols; fatty acids.

Abstract—The neutral lipids of the fruits (pulp and kernel) of *Olea madagascariensis* contain about 10% of unsaponifiable matter. Twenty-six 4-demethylsterols and 4,4-dimethylsterols were identified. Among them two compounds, (20*S*)-5α-dammar-24-en-3β, 20-diol and 5α-dammara-20(21), 24-dien-3β-ol, represent 3.3 and 3.2% of the neutral lipids, respectively. The assigned structures of the two main components are based upon GC retention times, ^{1}H and ^{13}C NMR, and mass spectrometry.

INTRODUCTION

The genus *Olea* of the Oleaceae family, is well represented in Madagascar since four species are indigenous [1]. Continuing with our work on the chemistry of oleaginous plants [2–5], an extensive examination of the lipid fraction of *O. madagascariensis* Boivin has been undertaken.

In this study, we describe the composition of the 4-demethylsterol and the 4,4-dimethylsterol fractions which represent 80% of the unsaponifiable lipids of *O. madagascariensis*. Among them, two compounds belonging to the dammarane-type were found in higher amount. Dammarenediol (**1a**) has been reported in resins from certain Dipterocarpaceae [6–9], the fruit of *Pouteria caimato* [10] and *Cacalia atriplicifolia* seed oil [11]. The occurrence of dammaradienol (**2a**) in plants is not widespread [12–17] and a small amount of this component was reported in the unsaponifiable matter of *O. europea* [18].

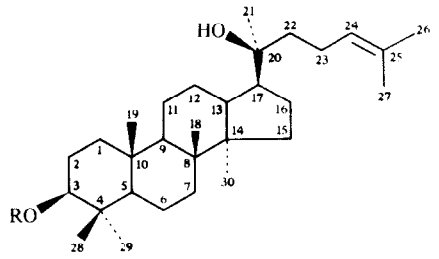
RESULTS AND DISCUSSION

The pulp and kernel lipids of fruits of *Olea madagascariensis* were obtained by petrol extraction. The lipid content ranges from 2% in pulp to 24% in kernel (Table 1). Among the methods described for the unsaponifiable lipid extraction, we have used those of Pelloquin *et al.* [19] with hexane and diethyl ether. The content of unsaponifiable lipids obtained by using the diethyl ether method is slightly higher (Table 1) and represent *ca* 12–15% of the weight of the neutral lipids. TLC examination of the unsaponifiables of *O. madagascariensis* showed many spots and the relative content of each group of compounds (hydrocarbons, tocopherols, 4,4-dimethylsterols, 4α-methylsterols, 4-demethylsterols and triterpenediols) was determined by column chromatography on alumina [2]. The results obtained are given in Table 1 and show that two compound families, the 4,4-dimethylsterols and the 4-demethylsterol plus triterpenediols, represent 80% of the unsaponifiable lipids. These two compound families were analysed by GC as their acetates [20–21].

The capillary gas chromatogram of the 4-demethylsteryl acetates plus triterpenediol acetates fraction showed the presence of 13 peaks and the identity of sterols was determined by comparison of RR_ts with previously published retention data of steryl acetyl standards [20, 21] and by gas chromatography–mass spectrometry (GC/MS).

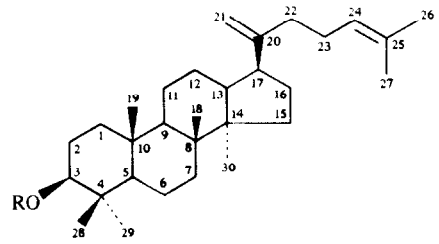
The configuration at C-24 of 24-methyl and 24-ethylsterols was left unidentified as shown in Table 2. This 4-demethylsterol fraction consisted mainly of Δ^5-sterols while Δ^7-sterols were present in smaller proportions. 24ξ-Ethylcholest-5-en-3β-ol (presumably sitosterol, 24α-ethyl) is the major 4-demethylsterol and represents 70% of this compound family. 24ξ-Ethylcholest-5, *trans*-22-dien-3β-ol (presumably stigmasterol, 24α-ethyl) represents 12% of the 4-demethylsterols. The two last peaks on the chromatogram having a RR_t of 3.16 and 4.46 on an OV-17 WCOT glass capillary column was identified as (20*S*)-5α-dammar-24-en-3β,20-diol 3-acetate (**1b**) (dammarenediol monoacetate) and 5α-olean-12-en-3β,28-diol 3-acetate (erythrodiol acetate), respectively, on the basis of their mass spectra by comparison with published results [9, 22].

In the case of dammarenediol, the two C-20 epimers exhibited similar R_f and RR_t values on TLC and GC, respectively, and mass spectra. Therefore, the occurrence of only one enantiomer (20*S*-configuration at the C-20 hydroxyl groups in **1a** and **1b**) was confirmed by comparison of the ^{13}C NMR spectra of these components with an authentic mixture of (20*S*)-and (20*R*)-dammarenediols obtained from dammar resin [6, 7]. As shown by Asakawa *et al.* [23], the chemical shift differences of the carbons around the C-20 epimers such as C-21 and C-22 may be used for the determination of the C-20 configuration. The resonance of the (20*S*)-epimer for C-21 is more deshielded while C-22 is more shielded than that of the (20*R*)-epimer. Since the ^{13}C NMR spectra of



1a R = H

1b R = Me—C(=O)—



2a R = H

2b R = Me—C(=O)—

1b has become available from the present study, results of **1a** and **1b** are given in Table 3.

The analysis by GC of the 4,4-dimethylsterols as their acetates showed the presence of 15 peaks. Thirteen of them were tentatively identified on the basis of their RR_t with 4,4-dimethylsterol standards [20] and by comparison with published GC/MS data (Table 4). Among them, one component was prominent in pulp and kernel lipids (65–70%) and was isolated in pure form using preparative TLC. This component was identified as 5α-dammara-20(21),24-dien-3β-ol (**2a**, dammaradienol) on the basis of their RR_t [20], mass spectrum [14] and ^{1}H NMR spectrum [24]. The ^{13}C NMR study of **2a** and its acetate **2b** is given in Table 3 since some assignment differences of several carbons was observed with pre-

Table 1. Content of neutral lipids of *O. madagascariensis*, percentage of unsaponifiable lipids and yield of the various fractions from the unsaponifiable lipids by column chromatography

	Fruit part	
Composition	Pulp	Kernel
Neutral lipids [(%) fr. wt]	1.80	23.9
Unsaponifiable ‡		
Hexane‡	12.0 ± 0.8	9.5 ± 0.7
Et_2O†	15.1 ± 1.4	12.0 ± 1.3
Fraction from unsaponifiable*		
Hydrocarbons		0.16
Tocopherols		0.14
4,4-Dimethylsterols		4.64
4-Methylsterols		0.11
4-Demethylsterols + triterpene-diols		3.40
Unknown		0.58

*Determined from the kernel hexane extract, mean of four analyses.

†Mean of four analyses.

‡% in neutral lipids.

Table 2. Composition of the 4-demethylsterol fraction and triterpene diol fraction of *O. madagascariensis* lipids

		Fruit part*	
4-Demethylsterol	RR_t†	Pulp	Kernel
Cholest-5-en-3 β-ol	1.00	tr	tr
24ξ-Methylcholest-5,*trans*-22-dien-3β-ol	1.15	0.7 ± 0.1	0.4 ± 0.1
Cholest-7-en-3β-ol	1.17	0.8 ± 0.2	0.4 ± 0.1
24ξ-Methylcholest-5-en-3β-ol	1.31	1.1 ± 0.2	0.1 ± 0.0
24-Methylenecholest-5-en-3β-ol	1.35	0.4 ± 0.1	—
24ξ-Ethylcholest-5,*trans*-22-dien-3β-ol	1.43	3.5 ± 0.8	0.1 ± 0.0
24ξ-Ethylcholest-5-en-3β-ol	1.65	20.1 ± 2.5	4.7 ± 0.1
24Z-Ethylidenecholest-5-en-3β-ol(Δ^5-avenasterol)	1.83	0.8 ± 0.3	—
24ξ-Ethylcholest-7-en-3β-ol	1.95	0.5 ± 0.2	0.6 ± 0.0
24E-Ethylidenecholest-7-en-3β-ol(28-isoavenasterol)	2.06	tr	—
24Z-Ethylidenecholest-7-en-3β-ol(Δ^7-avenasterol)	2.15	0.8 ± 0.2	—
20(*S*)-5α-Dammar-24-en-3β,20-diol	3.16†	64.2	93.7 ± 0.7
5α-Olean-12-en-3β,28-diol (Erythrodiol)	4.46§	7.1 ± 2.1	—

tr, Denotes that component was detected in a too small amount to quantitative.

*Area % by GC determined on three samples.

†Relative retention times of 4-demethylsteryl acetates on OV-17 WCOT glass capillary column (cholesteryl acetate: 1.00).

‡Determined as monoacetate.

§Determined as diacetate.

Table 3. ^{13}C NMR chemical shifts of compounds **1a–2b** (50.3 MHz, $CDCl_3$, TMS as int. standard, FT mode)

C	1a	1b	2a	2b
1	39.0	38.1	39.2	37.9
2	27.4	23.8	27.5	23.7
3	78.9	81.2	78.9	80.9
4	39.1	38.9	39.0	38.8
5	55.9	56.2	55.9	56.0
6	18.3	18.3	18.3	18.2
7	35.2	35.3	35.5	35.4
8	40.4	40.6	40.5	40.5
9	50.6	50.8	51.0	50.9
10	37.1	37.2	37.3	37.2
11	21.5	21.7	21.4	21.4
12	25.4	25.5	25.0	25.0
13	42.3	42.5	45.3	45.3
14	50.3	50.5	49.5	49.5
15	31.2	31.4	31.4	31.4
16	27.6	27.7	27.1	27.1
17	49.9	50.0	47.8	47.9
18	16.2	16.5	16.0	16.3
19	15.5	15.8	15.7	15.7
20	75.4	75.6	152.7	152.7
21	24.8	24.9	107.6	107.5
22	40.5	40.7	34.1	34.2
23	22.6	22.7	28.9	28.9
24	124.7	125.2	124.5	124.5
25	131.6	131.9	131.4	131.4
26	25.7	25.8	25.7	25.7
27	17.7	17.8	17.7	17.7
28	28.0	28.1	28.1	28.0
29	15.4	15.6	15.4	15.9
30	16.5	16.5	16.3	16.5
Me–C(O)–		171.4		170.9
Me–C(O)–		21.3		21.3

viously published results [16]. The assignment data reported for 2,6-dimethylhepta-1,5-dien [25] permitted the unambiguous assignments of C-22 and C-23. Assignments of methine carbon signals C-13 and C-17 was achieved by referring to the data for lupane derivatives [26, 27].

The presence of dammarane derivatives in two species of Oleaceae family, *O. madagascariensis* and *O. europea* [18], show that such triterpenes which are common intermediates in the biosynthesis of the lupane, ursane and oleane compounds, are not only characteristic of Dipterocarpaceae and Compositeae families. The high amount of these two triterpene derivatives seems to be interesting from a pharmacological point of view since it was shown that dammarane derivatives have antitumour activities [28].

EXPERIMENTAL

Material. Olea madagascariensis were collected in Ambre mountain (North area of Madagascar) during the years 1980, 1981 and 1982.

General procedure. The neutral lipids were prepared from the corresponding dried pulp and kernel by Soxhlet extraction with petrol (40–60°). Saponification and extraction of the unsaponifiable lipids using hexane or Et_2O was performed as described previously [19]. The unsaponifiable extract (500 mg) was fractionated on a column of alumina, Brockmann grade II–III (120 g) using the following solvent mixtures: 200 ml hexane; 200 ml hexane–C_6H_6(1:1); 200 ml hexane–C_6H_6 (1:4); 500 ml hexane–Et_2O (1:1) 500 ml Et_2O; and 250 ml MeOH. Fractions (20 ml) were collected and each checked using silica gel TLC. The approximate R_f values of compounds on silica gel TLC developed with $CHCl_3$–Et_2O (9:1) were: hydrocarbons, 0.85: tocopherols, 0.72; 4,4-dimethyl sterols, 0.5; 4-methylsterol, 0.45; 4-demethylsterol plus triterpenediol, 0.3–0.4.

Table 4. Composition of the 4,4-dimethylsterol fraction of *O. madagascariensis* lipids

4,4-Dimethylsterol	*RR*,†	Fruit part*	
		Pulp	Kernel
10α-Cucurbita-5-en-3β-ol	1.17	2.5 ± 0.2	4.6 ± 2.0
Unknown	1.25	0.5 ± 0.2	0.5 ± 0.2
5α-Lanosta-24-en-3β-ol(24-dehydrolanosterol)	1.30	2.2 ± 0.2	1.8 ± 0.5
9β,19-Cyclo-5α-lanosta-7,24-dien-3β-ol(7-dehydrocycloartenol)	1.38	0.5 ± 0.1	0.3 ± 0.2
5α-Tirucalla-8,24-dien-3β-ol(tirucallol)	1.44	1.7 ± 0.1	1.6 ± 0.3
5α-Taraxer-14-en-3β-ol(taraxerol)	1.57	tr	0.2 ± 0.1
5α-Dammara-20,24-dien-3β-ol(dammaradienol)	1.67	76.3 ± 3.0	65.1 ± 10
5α-Eupha-7,24-dien-3β-ol(butyrospermol)	1.71	0.3 ± 0.2	3.7 ± 2.1
9 β,19-Cyclo-5α-lanost-24-en-3β-ol(cycloartenol)	1.87	1.9 ± 1.1	0.3 ± 0.1
5α-Tirucalla-7,24-dien-3β-ol(7,24-tirucalladienol)	1.93	4.7 ± 1.5	3.3 ± 1.1
Unknown	1.99	0.5 0.2	8.0 6.1
24-Methylene-9β,19-cyclo-5α-lanostan-3β-ol(24-methylenecycloartanol	2.08	2.8 ± 0.2	4.5 ± 3.0
5α-Lanostan-3β-ol (lanostanol)	2.24	1.1 ± 0.6	1.1 ± 0.8
5α-Taraxast-20-en-3β-ol(ψ-taraxasterol)	2.43	3.4 ± 0.1	2.0 ± 0.5
24-Methyl-9,19-cyclo-5α-lanost-24-en-3β-ol(cyclobranol)	2.52	1.5 ± 0.1	2.0 ± 0.5

tr, Denotes that component was detected in a too small amount to quantitate.

*Area % by GC determined on three samples.

†Relative retention times of 4,4-dimethylsterol acetates on OV-17 WCOT glass capillary column (cholesterol acetate: 1.00).

GC and GC/MS analysis. 4,4-Dimethylsterol and 4-demethylsterol plus triterpenediol fractions were acetylated as previously described [21]. Relative retention times (RR_1) were expressed against cholesteryl acetate. The WCOT column was a 40m glass capillary column, 0.32mm i.d., coated with OV-17 (0.15 μm). The column temperature was 260° and inlet and detector ovens were 290°. For GC/MS analyses the chromatograph was fitted with a WCOT Si capillary column (25m, 0.32mm i.d.) coated with OV-1701(0.1 μm). Operating conditions were: 250° for column and 270° for inlet, He as carrier gas 0.5 bar, ion source 150° and ionizing voltage 70 eV.

Isolation of pure dammarene diol **1a**, *dammaradienol* **2a** *and their corresponding acetates* **1b** *and* **2b**. Purification of **1a** was obtained by crystallization of the 4-demethylsterol plus triterpenediol fraction in EtOH (mp 133°). Purification of the monoacetate **1b** was achieved using prep. TLC on silica gel plates developed with hexane–Et_2O (7:3). The R_f of **1b** was 0.12. The triterpenol fraction was acetylated and crystallized in EtOH to give 700 mg of dammaradienyl acetate **2b** (mp 146°, 95% purity by GC). Purification of **2b** was achieved using prep. TLC on $AgNO_3$–silica gel plates. After development of the 4,4-dimethylsteryl acetate fraction with hexane–$MeCO_2H$–Et_2O(8:2:1), the **2b**-acetate band (R_f, 0.21) was scraped off the plate and extracted with hexane (purity 99% by GC). Dammaradienol (**2a**) was obtained from pure **2b** by saponification. ^{13}C and ^1H NMR spectra of **1a**–**2b** were taken in $CDCl_3$ and chemical shifts are given in ppm with TMS as int. standard. Spectra were obtained with a Varian XL-200, FT mode at 50.309 MHz.

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