Unsaponifiable Lipid Constituents of Some Underutilized Tropical Seed Oils

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Sterols, triterpene alcohols, and hydrocarbons present in the unsaponifiable fraction of some underutilized tropical seed oils have been examined. The seeds include *Telfairia occidentalis* (TLO), *Andenopus breviflorus* (ADB), *Cucumeropsis edulis* (CME), *Antiaris africana* (ATF), and *Monodora tenuifolia* (MNT). The oil content of the seeds was high (34.7–68.8%), whereas triacylglycerols comprised the dominant lipid group in the oils (65.4–73.9%). The percentage of unsaponifiables ranged from 1.1 to 7.9%. Ten sterols were identified in the fractions. In the Cucurbitaceae oils (TLO, CME, and ADB), Δ^7-sterols constituted the dominant sterols. These include 24-ethylcholesta-7,-22*E*,25-trienol (**7**), 24-ethylcholesta-7,25-dienol (**9**), 24*Z*-ethylidenecholes-7-enol (**10**), and 24-ethylcholesta-7,24-dienol (**11**). However Δ^5-sterols (**1**–**5**) occurred at the highest concentration in the other two samples (ATF and MNT). Fifteeen triterpene alcohols were detected in the fractions. Olean-12-enol (**16**), isomultiflorenol (**8**), and lupeol (**23**) were the dominant alcohols in the Cucurbitaceae family, whereas α-amyrin (urs-12-enol) (**20**) was the dominant triterpene alcohol in ATF and MNT. A mixture of C_{18}–C_{34} *n*-alkanes, squalene, and some monoterpenes was detected in the hydrocarbon fraction.

INTRODUCTION

Unsaponifiable lipid constituents of seed oils contain a variety of bioactive substances, which includes hydrocarbons, tocopherols, sterols, and terpene alcohols. Terpene alcohols have shown both cytostatic and cytotoxic properties (Jalad et al., 1977; Kupchan et al., 1978). These compounds are also known to possess herbicidal and antimicrobial properties (Nakatani et al., 1981; Dreyer and Trousdale, 1978). A large variety of sterols have also shown insecticidal properties (Heftmann, 1970). To date, hydrocarbons represent the least investigated fraction of the unsaponifiable matter of the fixed oils. There have been limited studies on the nature of hydrocarbons in vegetable oils. Early studies by Capela et al. (1963) revealed the presence of three groups of hydrocarbons in vegetable oils. The authors identified *n*-paraffins, homologues of C_{10}–C_{35}, and also made a presumptive identification of alkenes based on their results of iodine values. Recent studies on the distribution and occurrence of sterols, triterpene alcohols, and other unsaponifiable constituents of vegetable oils have been reported (Andriamanantena et al., 1983; Akihisa et al., 1986, 1988). Although many other studies have been published on the analysis of unsaponifiables of vegetable oils, much remains obscure and needs further investigation.

The high fixed oil content and the edibility characteristics exhibited by some lesser known and underutilized seed oils have been studied by Esuoso and co-workers (Esuoso and Odetokun, 1995; Esuoso, 1996; Esuoso et al., 1998; Esuoso and Bayer, 1998). The results from these studies have prompted us to examine the hydrocarbons and other weakly polar unsaponifiables in the oils. In addition, the roles of these groups of compounds on the biosynthesis of major compounds in the oils are not well understood, and it is not yet clear whether the compounds could serve as chemotaxonomic markers for plant families. These factors coupled with the need to develop environmentally friendly natural insecticides are important considerations that stimulated our interest in examining the nature of these unsaponifiables.

This paper reports, for the first time, the unsaponifiables of five lesser known and underutilized tropical seed oils: *Telfairia occidentalis* (TLO); *Andenopus breviflorus* (ADB); *Cucumeropsis edulis* (CME); *Antiaris africana* (ATF); and *Monodora tenuifolia* (MNT).

MATERIALS AND METHODS

Collection and Pretreatment of Samples. The samples were obtained from selected markets in Ibadan, Warri, Akure, and Benin, all in southern Nigeria. They were identified at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, and thereafter stored for 3–4 weeks in plastic bags at 4 °C prior to extraction and analysis.

Extraction of the Oils. Seed samples were cleaned with water and air-dried in the laboratory. The seeds were ground with a Christy laboratory mill (Comitrol processor model 3600). The powdered samples were extracted using a Soxhlet extractor with petroleum ether (40–60 °C) for 24 h. The extracted

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Table 1. Systematic Names and Codes of the Five Tropical Seeds Studied

code	systematic name	family
TLO	*Telfairia occidentalis*	Cucurbitaceae
CME	*Cucumeropsis edulis*	Cucurbitaceae
ADB	*Andenopus breviflorus*	Cucurbitaceae
ATF	*Antiaris africana*	Moraceae
MNT	*Monodora tenuifolia*	Annonaceae

oil was dried over anhydrous sodium sulfate and the solvent removed under reduced pressure in a rotary evaporator.

Lipid Separation. Lipids were separated into classes on 0.55 mm silica gel plates (20 × 20 cm). The mobile phase used was a solvent mixture of petroleum ether/diethyl ether/acetic acid (80:20:1). It was developed according to the method described earlier (Esuoso et al., 1998).

Isolation of the Unsaponifiables. Oil (10 g) dissolved in 200 mL of ethanolic potassium hydroxide (2 M) was refluxed for 1 h. The reaction mixture was diluted to 400 mL with distilled water and transferred to a separating funnel. The unsaponifiables were extracted three times with 100 mL of diethyl ether. The ether extracts was first washed with 100 mL of aqueous solution of potassium hydroxide (0.5 M) to remove any residual free fatty acids. Further washing and cleaning was carried out five times with 100 mL of distilled water, and the ether layer was dried over anhydrous sodium sulfate. The solution was filtered and the solvent removed in a rotary evaporator. The unsaponifiables fraction in the oil was then expressed in weight percent.

Separation of the Unsaponifiables. A chloroform solution (50%) of the unsaponifiable material (30 mg/plate) was then applied uniformly along a line from the edge of a 20 × 20 cm plate coated with a 0.55 mm layer of silica gel and developed three times with hexane/ethyl acetate (6:1 v/v) as mobile phase. After development, the plates were sprayed with a solution of Rhodamine-6G in ethanol (0.5%) and observed under UV light. Three different zones were marked: R_f 0.9–1.0, hydrocarbons (*n*-alkanes); R_f 0.4–0.5, triterpene alcohols; and R_f 0.02–0.04, sterols. Each zone was carefully scraped from the plates and extracted thoroughly with diethyl ether. Sterols and triterpene alcohols were silanated with 10 μL of bis(trimethylsilane)–trifluoroacetamide (BSTFA) at 60 °C for 1 h. The residue obtained was mixed with water and extracted with diethyl ether, and the solvent was removed at reduced pressure in a rotary film evaporator.

Analysis of Unsaponifiables. Gas chromatograph Chrompack CP 9001 equipped with a flame ionization detector and a mosaic integrator was used for the studies. For the determination of hydrocarbons, the fraction was injected into the GC without derivatization using a capillary column (SE-54, 20 m × 0.27 mm, J&W Scientific, Köln, Germany). The programming was as follows: 35 °C for 3 min, temperature increased at 5 °C/min to 280 °C, and held at 280 °C for 5 min. Further determination was carried out on a GC-MS Varian MAT 112S using an ionization voltage of 60 eV. It was equipped with an AMD Intectra DP-10 data system with a Wiley library. For sterols and triterpene alcohols, the determination was carried out on the GC with an OV-17 glass capillary column (30 m × 0.3 mm i.d.). Relative retention times (RRT) were expressed as silanated cholesterol ether (1.00). The RRT values of authentic samples of sterols and triterpene alcohols are given in Table 3.

RESULTS AND DISCUSSION

The oil content, lipid classes, and percentage of unsaponifiables are presented in Table 2. The oil content of the seeds was high (34.7–68.8%). Triacylglycerols comprised the major component of the oils (65.4–73.9%). Varying concentrations of hydrocarbons, free fatty acids, diacylglycerols, monoacylglycerols, sterols, and polar lipids were also detected in the oils. The lipid classes follow the pattern already reported for similar oils

Table 2. Percentage Oil Content, Unsaponifiables, and Lipid Classes of Five Underutilized Tropical Seed Oils[a]

plant species	% weight								
	oil[b]	UNSP	HC	TG	FFA	DG	ST	MG	PL
TLO	48.6	1.1	1.3	70.0	0.2	2.7	2.0	1.6	3.6
CME	46.1	1.2	1.1	73.9	2.7	1.8	2.6	0.7	1.0
ADB	55.1	1.3	1.0	70.1	1.4	1.1	1.6	0.5	0.6
ATF	68.8	6.5	4.1	65.4	0.8	4.5	2.5	1.1	2.0
MNT	34.7	7.9	3.4	71.2	2.4	1.7	1.6	0.5	0.4

[a] HC, hydrocarbons; TG, triacylglycerols; FFA, free fatty acids; DG, diacylglycerols; MG, monoacylglycerols; PL, polar lipids; UNSP, unsaponifiables. [b] Based on the percent dry weight of the seed.

Table 3. Relative Retention Times of Sterols and Triterpene Alcohols Used as References in Gas Chromatography

code	RRT	compound
sterols		
1	1.00	cholesterol (cholest-5-enol)
2	1.17	24-methylcholesta-5,22E-dienol
3	1.34	campesterol (24-methylcholesterol)
4	1.46	stigmasterol (24-ethylcholesta-5,22E-dienol)
5	1.47	24-ethylcholesta-5,22E,25-trienol
6	1.66	β-sitosterol (24-ethylcholesterol)
7	1.68	24-ethylcholesta-7,22E,25-trienol
8	1.84	isofucosterol (24Z-ethylidenecholesterol)
9	1.95	24-ethylcholesta-7,25-dienol
10	2.17	avenasterol (24Z-ethylidenecholest-7-enol)
11	2.19	peposterol (24-ethylcholesta-7,24-dienol)
terpene alcohols		
12	1.30	euphol (eupha-8,28-dienol)
13	1.33	24-dihydrolanosterol (24-lanoest-8-enol)
14	1.50	tirucallol (tirucalla-8,24-dienol)
15	1.58	taraxerol (D-friedoolean-14-enol)
16	1.66	β-amyrin (olean-12-enol)
17	1.72	butyrospermol (eupha-7,24-dienol)
18	1.77	isomultiflorenol (D:C-friedoolean-8-enol)
19	1.79	24-methylenelanost-8-enol
20	1.87	α-amyrin (urs-12-enol)
21	1.89	24-methylene-24-dihydroparkeol
22	1.90	cycloartenol (9β-; 19-cycloartenol)
23	1.96	lupeol
24	2.10	24-methylenecycloartenol
25	2.40	taraxasterol
26	2.52	ι-taraxasterol

Table 4. Composition of the Sterol Fraction of Five Underutilized Tropical Seed Oils

RRT	plant species[a]				
	TLO	ADB	CME	ATF	MNT
1.17 (**2**)	–	–	–	2.2	4.2
1.34 (**3**)	3.8	5.9	1.2	3.2	5.8
1.46 (**4**)	2.6	3.8	4.2	13.8	12.6
1.47 (**5**)	9.7	8.5	10.2	38.2	35.1
1.66 (**6**)	3.8	4.2	3.7	19.8	20.2
1.68 (**7**)	50.2	55.5	47.6	5.9	3.7
1.84 (**8**)	tr	tr	tr	9.8	8.2
1.95 (**9**)	7.8	6.9	7.5	tr	–
2.17 (**10**)	8.9	5.2	9.8	tr	1.2
2.19 (**11**)	5.6	4.8	7.8	1.5	2.3
unidentified peaks	7.6	5.2	8.0	5.6	6.7

[a] Components add up to 100% in each column. –, below detection limit; tr, trace concentration.

(Vasconcellos et al., 1980; Oboh and Oderinde, 1988; Kamal-Eldin et al., 1992; Esuoso and Odetokun, 1995). The percentage of unsaponifiables in the samples ranged from 1.1 to 7.9%.

The result of the analysis of the sterol fraction of the oils is summarized in Table 4. Ten sterols were identified in the fraction. In the Cucurbitaceae oils (TLO, ADB, and CME), Δ^7-sterols were the dominant sterols. They accounted for >72% of the total sterol fraction. The

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Table 5. Composition of the Triterpene Alcohols of Five Underutilized Tropical Seed Oils

	plant species[a]				
RRT	TLO	ADB	CME	ATF	MNT
1.30 (**12**)	1.9	1.2	3.6	8.2	7.4
1.33 (**13**)	3.7	5.2	6.2	tr	tr
1.50 (**14**)	2.7	−	tr	3.7	3.2
1.58 (**15**)	tr	tr	tr	7.5	6.2
1.66 (**16**)	25.1	23.4	20.7	9.6	8.8
1.72 (**17**)	7.7	6.8	5.8	3.0	3.4
1.77 (**18**)	22.5	22.3	23.7	−	−
1.79 (**19**)	2.1	1.2	1.5	1.1	2.5
1.87 (**20**)	5.1	6.1	5.8	36.2	38.6
1.89 (**21**)	3.2	5.8	4.2	9.5	8.2
1.90 (**22**)	1.0	−	−	−	−
1.96 (**23**)	11.5	10.6	12.7	3.2	6.0
2.10 (**24**)	5.8	10.3	8.9	10.4	6.5
2.40 (**25**)	0.2	0.3	0.6	1.5	2.3
2.52 (**26**)	tr	tr	tr	0.2	0.7
unidentified peaks	7.5	6.8	6.3	5.9	6.2

[a] Components add up to 100% in each column; tr, trace concentration; −, not detectable.

Table 6. Hydrocarbon Fractions Identified in Five Underutilized Tropical Seed Oils

| code	hydrocarbon	code	hydrocarbon
1	α-piene	**13**	pentacosane ($C_{25}H_{52}$)
2	camphene	**14**	hexacosane ($C_{26}H_{54}$)
3	myrcene	**15**	octadecanoic acid
4	*p*-cymene		butylamide
5	octadecane ($C_{18}H_{38}$)	**16**	heptacosane ($C_{27}H_{56}$)
6	nonadecane ($C_{19}H_{40}$)	**17**	octacosane ($C_{28}H_{58}$)
7	ethyl ester of palmitic acid	**18**	squalene
8	heneicosane ($C_{21}H_{42}$)	**19**	nonacosane ($C_{29}H_{60}$)
9	docosane ($C_{22}H_{46}$)	**20**	triacotane ($C_{30}H_{62}$)
10	tricosane ($C_{23}H_{48}$)	**21**	hentriacotane ($C_{31}H_{64}$)
11	tetracosane ($C_{24}H_{50}$)	**22**	dotriacotane ($C_{32}H_{66}$)
12	hexadecanoic acid	**23**	tritriacotane ($C_{33}H_{68}$)
	butylamide	**24**	tetraacotane ($C_{34}H_{70}$)

sterols were 24-ethylcholesta-7,22*E*,25-trienol (**7**), 24-ethylcholesta-7,25-dienol (**9**), 24*Z*-ethylidenecholest-7-enol (**10**), and 24-ethylcholesta-7,24-dienol (**11**). Their concentrations were lower in the other samples studied. Δ^7-Sterols accounted for 17.2 and 15.4% in ATF and MNT, respectively. However, Δ^5-sterols (**1**−**5**) were considerably higher in ATF and MNT compared to the members of the Cucurbitaceae. The major Δ^5-sterol in the samples is 24-ethylcholesta-5,22*E*,25-trienol (**5**). The triterpene alcohol composition of the oils is presented in Table 5. Altogether 14 triterpene alcohols were identified by a combination of TLC and GC and, in a few instances, by mass spectrometer. Olean-12-enol (**16**), isomultiflorenol (**18**), and lupeol (**23**) were the dominant triterpene alcohols in the Cucurbitaceae. It is interesting to note that isomultiflorenol (**18**) (see Figure 1), a very rare triterpene alcohol, was detected only in this family.

This terpene and the isomer multiflorenol were identified by Akihisa et al. (1988) in their study of the triterpene alcohols of 16 members of the Cucurbitaceae family, which are different from those considered in this paper. Our results therefore support the view that this terpene alcohol could serve as a chemotaxonomic marker for the Cucurbitaceae family. α-Amyrin (urs-12-enol) (**20**) was the dominant terpene alcohol in the other two samples studied. GC and GC-MS studies of the hydrocarbon fraction revealed a mixture of C_{18}−C_{34} *n*-alkanes (Tables 4 and 5). In addition, some monoterpenes were also detected in this fraction. These include α-piene, camphene, myrcene, and *p*-cymene. Myrcene and cymene were identified in only two members of the Cucurbita-

Table 7. Distribution of Hydrocarbons in Five Underutilized Tropical Seed Oils

	plant species[a]				
peak	TLO	ADB	CME	ATF	MNT
1	−	−	−	2.2	10.3
2	−	−	−	−	1.8
3	1.4	1.7	−	−	2.8
4	0.6	0.7	−	−	2.8
5	−	−	2.3	−	2.2
6	0.6	0.6	−	1.4	0.7
7	−	4.3	7.0	6.8	4.7
8	1.0	1.1	1.4	2.9	1.5
9	2.5	2.6	2.3	3.7	11.9
10	3.5	3.5	4.2	4.1	2.9
11	15.3	16.6	18.0	6.8	5.1
12	−	3.5	4.2	4.1	2.9
13	6.2	5.6	5.6	5.9	4.7
14	17.0	8.5	6.5	23.3	5.1
15	−	5.4	3.6	5.9	6.7
16	7.8	6.9	5.6	5.9	5.9
17	7.4	4.8	4.5	4.1	5.1
18	12.3	16.0	7.9	6.8	6.1
19	6.5	5.8	7.1	5.2	4.7
20	5.8	5.3	6.8	3.7	3.7
21	5.1	3.2	4.9	3.7	3.5
22	3.1	1.7	3.4	2.1	1.9
23	2.5	1.1	3.3	1.4	1.5
24	1.4	1.1	1.4	−	1.0

[a] Components add up to 100% in each column; −, not detectable.
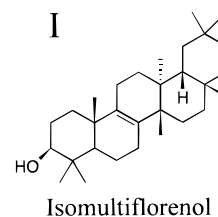
Figure 1. Structure of isomultiflorenol.

ceae family. They constituted <2% of the total fraction. However, all four terpenes were identified in MNT; α-piene accounted for >10% of the total hydrocarbon from this sample. Ekundayo and Hammerschmidt (1988) earlier reported the presence of these compounds in addition to many others in the essential oils of *Monodora myristica*, also an Annonaceae. Squalene was present in all of the samples. The ethyl ester of fatty acids found in some of the hydrocarbon fractions of some of the oils might be due to the transesterification of lipids, which takes place during the saponification of the triacylglycerols. In view of the results obtained from this study, preliminary work is already in progress not only on the investigation of other members of the family but also on the possible insect repellant activity of the oils.

CONCLUSIONS

Our preliminary studies of the unsaponifiables of some tropical underutilized oils have revealed a number of sterols, triterpene alcohols, and hydrocarbons. The identification of a rare triterpene alcohol (isomultiflorenol) in the three members of the Cucurbitaceae family studied suggests it could be used as a chemotaxonomic marker for the family.

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