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Investigación

Major Components from the Epicuticular Wax of *Cocos nucifera*

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Dedicated to Dr. Barbarín Arreguín Lozano

Abstract. The three major components present in the epicuticular wax from leaves of *Cocos nucifera* L. were identified as lupeol-methylether (**1**), skimmiwallin (**2**) [3β-methoxy-25-ethyl-9,19-cyclolanost-24(24[1])-ene] and isoskimmiwallin(**3**) [3β-methoxy-24-ethyl-9,19-cyclolanost-25(25[1])-ene]. Structural elucidation of the metabolites was carried out by analysis of their spectroscopic data and/or by comparison with those reported in the literature.
Keywords: *Cocos nucifera*, epicuticular wax, chemotaxonomy, triterpenes, lupeol-methyl ether, skimmiwallin, isoskimiwallin.

Resumen. Los tres componentes principales presentes en la cera epicuticular de *Cocos nucifera* L. fueron identificados como el éter metílico de lupeol (**1**), skimmiwallina (**2**) [3β-metoxi-25-etil-9,19-ciclolanost-24(24[1])-eno] e isoskimmiwallina (**3**) [3β-metoxi-24-etil-9,19-ciclolanost-25(25[1])-eno]. La elucidación estructural de estos metabolitos se llevó a cabo mediante la interpretación de sus datos espectroscópicos y/o por comparación de los mismos con los reportados en la literatura.
Palabras clave: *Cocos nucifera*, cera epicuticular, quimiotaxonomía, triterpenos, eter metílico de lupeol, esquimiwallina, iso-esquimiwallina.

Introduction

All aerial organs of higher plants are covered by a continuous wax layer on the surface of the cuticle [1]. This layer protects plant cells from various enviromental factors such as drought and UV damage [2], and acts as a first line of defense against insects, bacteria and fungal pathogens [2, 3]. In some higher plants, morphological and chemical studies carried out on epicuticular waxes have been used to correlate the nature and the chemical composition of the wax, with the susceptibility of the plant to insect attack or to chemical agents [4, 5].

The main components of the wax of *Brassica oleracea*, identified as amyrin-type triterpenes, have been recognized as the metabolites responsible for the repellent effect against *Plutella xilostella* aphids [6]. These results have been confirmed by similar studies which have shown that amyrins and other triterpens have repellent or toxic activity against insects [3, 7].

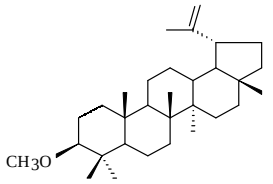
Studies carried out on the chemical composition of the epicuticular waxes from various palm species have resulted in the isolation and identification of a number of triterpenes, including lupeol methylether from *Orbignya speciosa*, *Butia capitata* and *Orbignya phalerata*, 3-β-methoxy-lupane from *Orbignya phalerata*, and cylindrin from *Orbignya cohune* [4]. To date no reports on the chemical composition of the epicuticular wax of *C. nucifera* have been found.

Recently, 18 populations of *Cocos nucifera* L. growing in different regions of Mexico were grouped into five ecotypes according to their similarities in phenotypic characters and isoenzymatic profiles [8]. Since the chemical composition of the epicuticular wax of a number of plant species has been used as a chemotaxonomic marker for classification [4, 5], the main objective of this investigation was to isolate and identify the major components present in the epicuticular wax of *C. nucifera* for future use in chemotaxonomical studies. We wish to report herein the identification of lupeol-methylether (1), skimmiwallin (2) and isoskimmiwallin (3) as the major components of the epicuticular wax of *C. nucifera.*

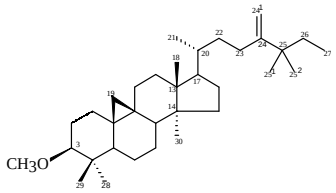
Results and discussion

The hexane extract from pines of leaves of *C. nucifera* showed two main components when analyzed by conventional TLC. Silica gel column chromatography purification of the extract yielded two major fractions, each containing one of the main components in apparent pure form. The fraction containing the more polar component showed a single peak by GC and the pure metabolite was identified as lupeol methylether (**1**) by direct comparison with an authentic sample and by comparing its spectroscopic data with those reported in the literature.

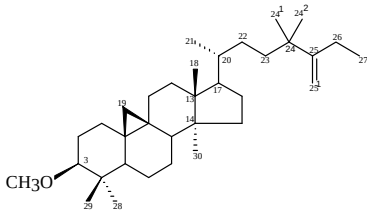
Even though the least polar component appeared as a single spot on TLC, its GC analysis showed that it was in fact a mixture of two metabolites that could only be separated by using $AgNO_3$-impregnated silica gel TLC plates. Successive $AgNO_3$-impregnated silica gel column chromatography and preparative TLC yielded both components in pure form. The EIMS of the less polar metabolite showed a molecular ion



(**1**)



(**2**)



(**3**)

peak at *m/z* 482 indicating a molecular formula of $C_{34}H_{58}O$ and suggesting a triterpenoid structure. While the two signals at 0.32 and 0.56 ppm in its ^{1}H NMR indicated the presence of a cyclopropane ring in the structure and strongly suggested a cycloartane skeleton, the presence of a sharp singlet at 3.36 ppm clearly indicated that the single oxygen in the molecular formula was part of a methoxyl group. Confirmation of the cycloartane skeleton for this metabolite came from its EIMS where the characteristic fragment ion peak at *m/z* 328, originated by loss of the A ring in cycloartanes [9, 10], was observed.

The same fragment ion peak could be explained as resulting from the loss of a $C_{11}H_{21}$ side chain in the molecule; this data, together with four methyl signals at 0.90 (d, 6.5 Hz), 0.95 (s), 0.97 (s), and 1.05 ppm (t, 7.5 Hz), and two vinylic proton signals at 4.77 (bd, 1 Hz) and 4.79 (bs) ppm, was in agreement with an eight-carbon side chain having a *gem*-dimethyl group and a 1,1 disubstituted double bond as substituents. This data proved to be identical to those reported for skimmiwallin (**2**) [3β-methoxy-25-ethyl-9,19-cyclolanost-24(24^1)-ene], a cycloartane isolated from the petrol ether extract of *Skimmia wallichii* [11].

The second most polar component showed identical spectral data to those of **2**, suggesting an isomeric structure. Significant differences could only be observed on comparing the HMBC experiment results for each component; while the HMBC experiment of **2** showed a clear ^{3}J correlation between the C27-methyl signal (1.05 ppm) and the *sp*^{3}-quaternary carbon at 39.51 ppm (C25), the same experiment in the new metabolite showed a definite ^{3}J correlation between the same methyl group (C27, 1.05 ppm) and an *sp*^{2}-quaternary carbon (C25, 157.92 ppm). This data is in agreement with an isomeric structure **3** for the new metabolite, where the only structural difference between **2** and **3** is located at the side chain of the molecule. Because of the isomeric relationship with **2**, we have designated the new metabolite as isoskimmiwallin (**3**).

The purified metabolites **1**-**3** were used as standards in GC analyses to show that the epicuticular wax profiles of the five main ecotypes of *C. nucifera* are qualitatively similar, but quantitatively different [12]. These results also showed that there exists a correlation between the concentrations of **1**, **2**, and **3** in the epicuticular wax and the resistance or susceptibility of a given ecotype to the lethal yellowing disease of coconut palms. Presently, studies are underway in order to establish if the components, pure or combined, show biological activity against the insect vector that transmits the disease. The results of these studies will be published in due course.

Experimental

General

Samples for IR were dissolved in $CHCl_3$ (Merck, uvasol) and spectra were recorded using a Nicolet Magna Protégé 460 FT-IR instrument. ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian Unity-300, Bruker AMX-400, and Varian Unity Plus-500 spectrometers using $CDCl_3$ as solvent and residual solvent signals for reference. EIMS were recorded at 70 eV on a JEOL-JMSAX505HA and JEOL-JMS-SX102A mass spectrometer for low and high resolution, respectively; while GC-MS analyses were carried out in a Hewlett Packard 5890 gas chromatographer coupled to a 5971 mass selective detector (GC conditions: column Hp Ultra 1; flow rate 1mL/min; oven temperature 280 to 300 °C; gradient 5 C/min; injector 290 °C; detector 300°). Analytical TLC was performed on aluminum-backed Silica gel 60 F_{254} plates (E. M. Merck, 0.20 mm thickness), both normal and impregnated with a 5% $AgNO_3$ solution, and preparative TLC (PTLC) was performed on glass-coated (0.25 mm thickness) Silica gel 60 F_{254} (E. M. Merck) plates (20 × 20 cm) impregnated with a 5% $AgNO_3$ solution. Flash column chromatography purifications were run using Silica gel G (200-400 mesh, Aldrich Chemical Co.).

Plant material

Pines of *Cocos nucifera* were collected in September 1999 from plants (Alto del Pacífico and Enano Malayo) growing in the San Crisanto plantation located in Sinanché, Yucatán, México.

Extraction and isolation

Six pines were cut at the base and immersed for 40 seconds in a liter of hexane contained in a measuring cylinder. The solvent was evaporated to dryness under reduced pressure and the crude wax extract was purified by flash column chromatography (H_x / CH_2Cl_2 7:3 as the eluting solvent) to pro-

Table 1. Spectroscopic data of skimiwallin (**2**) and isoskimiwallin (**3**).

Position	**2**				**3**			
	^{1}HNMR	^{13}CNMR[a,b]	HMBC		^{1}HNMR	^{13}CNMR[a,b]	HMBC	
			J2	*J3*			*J2*	*J3*
3	2.72 dd (4.5,11.2)	88.50			2.70 dd (3.9, 10.5)	88.55		
18	1.01 s	17.98			0.95 s	27.79	C-13	C-14, C-17
19	0.32 d (4.5) exo	29.67		C-1,C-11	0.32 d (4.0) exo	29.68		
	0.56 d (4.0) endo			C-1,C-11	0.55 d (4.0) endo			
20		36.43				36.59		
21	0.90 d (6.5)	18.44			0.84 d (6.5)	18.44	C-20	C-17, C-22
22		36.00				37.59		
23		27.67				26.52		
24		156.75				39.15		
24^1	4.77 bd (1.0)	107.45		C-23, C-25	1.01 s	17.93	C-24	C-25, C-23
	4.79 bs							
24^2					1.02 s	25.51	C-24	C-25, C-23
25		39.51				157.92		
25^1	0.97 s	26.93	C-25	C-24, C-26	4.76 d	106.37	C-25	C-26, C-24
					4.78 d			
25^2	0.95	26.93	C-25	C-24, C-26				
26	1.38 d (7.5)	33.26	C-27, C-25	C-24, C-25^1	1.97 d (7.5)	23.33	C-27, C-25	C-251
	1.39 d (7.5)	9.01	C-27		2.00 d (7.5)		C-27, C-25	C-251
27	1.05 t (7.5)	25.52	C-26	C-25	1.05 t (7.5)	13.01	C-26	C-25
28	0.94 s	25.52			0.94 s	27.48	C-4	C-3
29	0.79 s	14.75			0.79 s	14.77	C-4	C-3
30	0.88 s	19.30			0.88 s	19.29	C-14	
OCH_3	3.36 s	57.62				57.63		

[a] Assigments made by comparing chemical shift values with those reported in Ref. 11.
[b] Chemical shift values for carbons 1, 2 and 4 to 17 are listed in the experimental section.

duce two major fractions, each showing a single component on TLC. GC-MS analysis of fraction A showed the presence of a single component at R_t 10.73 having a fragmentation pattern very similar to that of lupeol-methylether (**1**). The identity of **1** (60.3 mg) was confirmed by direct comparison with an authentic sample and by comparing its spectroscopic data with those reported in the literature [11].

Although fraction B showed a single component on normal TLC, its GC analysis clearly indicated the presence of two metabolites (R_t 14.45 and 15.14 min) that could only be separated using $AgNO_3$-impregnated Silica gel TLC plates (CH_2Cl_2). Successive purifications using $AgNO_3$-impregnated Silica gel column chromatography (Hx / CH_2Cl_2 7:3 as the eluting solvent) and PTLC (CH_2Cl_2 as the eluting solvent) resulted in the isolation of **2** (16.9 mg) and **3** (10.9 mg) in pure form. These components were identified as skimmiwallin (**2**) and isoskimmiwallin (**3**), respectively.

Skimmiwallin (2): mp. 156-159º. IR ($CHCl_3$ cm^{-1}) 1097, 1468; HREIMS *m/z* 482.42881 (calcd. for $C_{34}H_{58}O$, 482.448767); LREIMS *m/z* (rel. int.); 482 $[M]^+$ (7), 467 $[M\text{-}Me]^+$ (25), 450 $[M\text{-}MeOH]^+$ (71), 435 $[M\text{-}Me\text{-}MeOH]^+$ (100), 407 $[M\text{-}MeOH\text{-}43]^+$ (46), 381 $[M\text{-}MeOH\text{-}69]^+$ (30), 328 $[M\text{-}C_{11}H_{21}]^+$ (16), 297 $[M\text{-}C_{11}H_{21}\text{-}MeOH]^+$ (20), 175 $[M\text{-}C_{11}H_{21}\text{-side chain}]^+$ (74); ^{1}H NMR (400 MHz, $CDCl_3$) see Table 1, ^{13}C NMR (100 MHz, $CDCl_3$) δ 31.79 (C-1), 25.42 (C-2), 40.46 (C-4), 47.63 (C-5), 20.93 (C-6), 25.94 (C-7), 47.97 (C-8), 19.97 (C-9), 26.29 (C-10), 25.50 (C-11), 45.29 (C-13), 48.83 (C-14), 35.54 (C-15), 28.13 (C-16), 52.27 (C-17). For additional ^{13}C NMR data see Table 1.

Isoskimmiwallin (3): IR ($CHCl_3$ cm^{-1}) 1098, 1470; HREIMS *m/z* 482.35862 (calcd. for $C_{34}H_{58}O$, 482.448767); LREIMS *m/z* (rel. int.); 482 $[M]^+$ (7), 467 $[M\text{-}Me]^+$ (22), 450 $[M\text{-}MeOH]^+$ (60), 435 $[M\text{-}Me\text{-}MeOH]^+$ (100), 407 $[M\text{-}MeOH\text{-}43]^+$ (47), 381 $[M\text{-}MeOH\text{-}69]^+$ (38), 328 $[M\text{-}C_{11}H_{21}]^+$ (22), 297 $[M\text{-}C_{11}H_{21}\text{-}MeOH]^+$ (24), 175 $[M\text{-}C_{11}H_{21}\text{-side chain}]^+$ (78); ^{1}H NMR (400 MHz, $CDCl_3$) see Table 1, ^{13}C NMR (100 MHz, $CDCl_3$) 31.81 (C-1), 30.78 (C-2), 40.48 (C-4), 47.95 (C-5), 20.94 (C-6), 25.43 (C-7), 47.66 (C-8), 19.98 (C-9), 26.29 (C-10), 25.96 (C-11), 32.86 (C-12), 45.2 (C-13), 45.80 (C-14), 35.54 (C-15), 28.87 (C-16), 52.14 (C-17). For additional ^{13}C NMR data see Table 1.

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