# FURTHER DRIMANE SESQUITERPENES FROM THE STEM BARK OF *CANELLA WINTERANA*

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Canella winterana L. (Canellaceae) is a tree with an aromatic and pungent bark found in southern Florida, through the Caribbean, and in Colombia (1). Extracts of various parts of the plant have been widely used as a spice ("pepper cinnamon") and as an emmenagogue (2). Previous investigations have reported the occurrence of monoterpenes (3), eugenol and mannitol (4), the drimane sesquiterpene canellal [1] (=muzigadial) (5), and 3-methoxy-4,5-methylenedioxycinnamaldehyde (6). We now report the results of a reinvestigation of the stem bark of *C. winterana* collected from the island of Grand Cayman.

Extraction of compounds was achieved by sequential maceration with solvents of increasing polarity. Individual compounds were subsequently separated by column and/or vacuum chromatography. From the petroleum ether extract two phenylpropenes and two sesquiterpenes were isolated, and a third sesquiterpene was identified as **1** by tlc comparison with authentic material. Three of the isolated compounds were identified as myristicin, eugenol, and warburganal [2] and the fourth characterized as a novel drimane sesquiterpene, 9α-hydroxycinnamolide [3]. The combined EtOAc and MeOH extracts yielded another sesquiterpene, identified as mukaadial [4].

9α-Hydroxycinnamolide [3] analyzed for $C_{15}H_{22}O_3$ (hrms). The ir spectrum indicated the presence of a hydroxyl (3425 cm^{-1}) and a γ-lactone (1750 cm^{-1}).

The ^{1}H-nmr spectrum (Table 1, with spectra of **2** and **4** for comparison) revealed the presence of all 22 protons and included resonances for three methyl substituents, an isolated methylene, and an olefinic proton but no aldehyde substituents. Decoupling experiments showed the olefinic proton to be part of an isolated $C=CH-CH_2-CH-R(R_1)$ system analogous to the C-5 to C-7 region in **2**. A singlet at δ 2.30 (replaceable) could be assigned to a hydroxyl which, because of the absence of any oxymethine

1

2 R=H
4 R=OH

3 R=OH
5 R=H
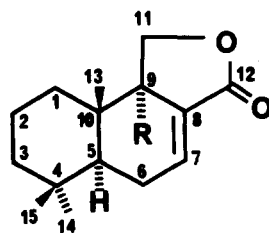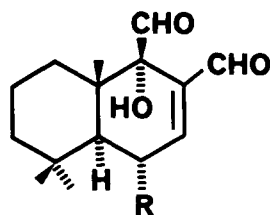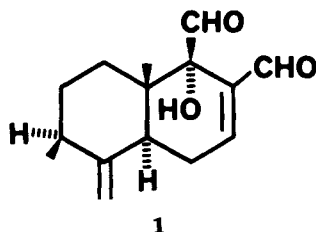
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TABLE 1. ^{1}H-nmr Chemical Shifts for Sesquiterpenes **2**, **3**, and **4** (coupling constants in parentheses).[a]

Proton	Compound		
	2	**3**	**4**
1_{eq}	1.00–1.80 m	1.20 dq (12.3, 1.7[b])	1.24–1.55 m
1_{ax}	1.00–1.80 m	1.94 dt (5.5, 12.3)	2.18 ddd
			(13.1, 13.1, 3.2)
$2_{eq/ax}$	1.10–1.80 m	1.56 m/1.53 m	1.24–1.55 m
$3_{eq/ax}$	1.10–1.80 m	1.46 m/1.27 m	1.24–1.55 m
	1.24–1.35 m		
4-Me$_{ax}$	1.09 s[c]	0.97 s	1.22 s
4-Me$_{eq}$	0.99 s[c]	0.94 s	1.47 s
5_{ax}	1.89 dd (11.7, 5.0)	1.86 dd (11.3, 5.0)	2.51 d (10.4)
6_{eq}	2.58 dt (21.0, 5.0)	2.45 ddd (20.8, 5.0, 4.5)	6.94 d (8.8) (OH)
6_{ax}	2.34 ddd	2.11 ddd	4.80 ddd
	(21.0, 11.7, 2.6)	(20.8, 11.3, 3.2)	(10.4, 8.8, 2.3)
7	7.27 dd (5.0, 2.6)	7.01 dd (4.5, 3.2)	7.33 d (2.3)
9	4.09 s (OH)	2.30 s (OH)	7.04 s (OH)
10-Me	0.95 s[c]	0.88 d (0.6[b])	1.18 s
11	9.73 s	4.21/4.35 ABq (10.0)	10.13 s
12	9.41 s	—	9.57 s

[a]Spectra for **3** and **4** were run at 360 MHz and for **2** at 250 MHz. CDCl$_3$ was used for **2** and **3** and pyridine-d_5 for **4**.

[b]Resonances showing long-range 'W-bond' coupling; H-1$_{eq}$ to H-3$_{eq}$ and 10-Me to H-1$_{ax}$.

[c]Assignments interchangeable.

resonance, must be tertiary. These data suggested a drim-7-ene sesquiterpene in which the 11 and 12 methyls were involved in the formation of a lactone ring and with the hydroxyl substituent placed at C-9.

On this basis the structure can be deduced as either **3** or the isomer in which C-11 forms the lactone carbonyl. Structure **3** was confirmed by a series of nOe experiments (Figure 1). The C-4 equatorial methyl (δ 0.94) was identified by its strong interaction with the ring junction proton H-5 and with the equatorial H-6. The other two methyls (δ 0.97 and 0.88) showed mutual enhancement which indicated that they were axial at C-4 and C-10, and the latter also interacted strongly with one of the H-11 protons. This required that the methylene be derived from the 11-methyl and not the 12-methyl and that the lactone ring be equatorial. These observations also confirmed a *trans* ring junction. Com-
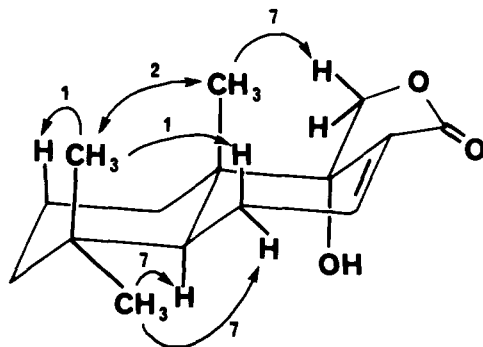
FIGURE 1.   Major nOe (% enhancement) caused by irradiation of the methyl substituents of **3**.

pound **3** must, therefore, be the novel 9α-hydroxy derivative of cinnamolide [**5**], the latter having previously been isolated from *Warburgia* (7) and *Cinnamosma* (8).

In the previous report of **4** (9) cims using isobutane appears to have given the quasi-molecular ion but in this study cims using methane failed to give a quasi-molecular ion. Consequently, we examined the use of derivatization followed by gc-ms as a method for obtaining the molecular ion. Derivatization with *N*O-bistrimethylsilyl-trifluoroacetamide followed by gc-ms still failed to give a molecular ion, the highest fragment at m/z 371 ($C_{20}H_{37}O_3Si_2$) indicating the di-trimethylsilyl ether but with facile loss of one aldehyde group. Reaction of **4** with methoxylamine hydrochloride and then with pentafluoropropionyl (PFP) anhydride was anticipated to give the di-oxime (at aldehydes) di-PFP ester (at hydroxyls). Gc-ms analysis revealed four products, negative ion cims of which gave a highest fragment m/z 452 ($C_{20}H_{25}N_2O_4F_5$) for the di-oxime mono-PFP ester. Thus, these data collectively establish the functionality of **4** without showing a molecular ion.

Previously only one drimane sesquiterpene derivative had been recorded from *C. winterana*. This investigation, performed on only a small bark sample, confirms that this species contains a wide range of these compounds which are well known for their broad antifeedant, antifungal, molluscicidal, and cytotoxic activities (7). One feature of all the sesquiterpenes isolated from *C. winterana* to date is the presence of the tertiary 9α-hydroxyl group. This substituent occurs in some drimane sesquiterpenes isolated from all Canellaceae studied to date but only in *C. winterana* is it a constant feature among all of the isolated sesquiterpenes.

EXPERIMENTAL

PLANT MATERIAL.—Stem bark of *C. winterana* was collected from the coastal bluffs at East End, Grand Cayman in August 1981. A voucher specimen, A.I. Gray s.n., has been deposited at the Herbarium of the Botanical Garden, Edinburgh.

EXTRACTION AND ISOLATION OF COMPOUNDS.—Ground stem bark (85 g) was macerated with, in succession, petroleum ether (bp 40–60°), EtOAc, and MeOH. Each extraction was carried out for 5 days. On concentration the petroleum ether extract yielded 6.14 g of crude extract which was chromatographed over Si gel eluting with petroleum ether containing increasing amounts of EtOAc to give myristicin (10 mg), eugenol (60 mg), **2** (54 mg), and finally **3** (25 mg). EtOAc and MeOH extracts of the stem bark were bulked, concentrated, and subjected to vacuum chromatography over Si gel using $CHCl_3$ followed by $CHCl_3$ containing increasing amounts of MeOH. Initial elution with $CHCl_3$ gave **4** (82 mg).

WARBURGANAL [**2**].—Needles from petroleum ether/EtOAc, mp 112–113° [lit. (7) 134–136°]; [α]D −216° (c = 0.1, $CHCl_3$) [lit. (10) −260°]; ^1H nmr see Table 1; calcd for $C_{15}H_{22}O_3$, 250.1569, found (ei) 250.1569.

9α-HYDROXYCINNAMOLIDE [**3**].—Needles from petroleum ether/EtOAc {R_f 0.34 on Si gel, solvent petroleum ether-EtOAc (7:3)}, mp 150–155°; [α]D − 10° (c = 0.44, $CHCl_3$); ir max 3425 (OH), 1750 (CO lactone) cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr (90.56 MHz, $CDCl_3$) ppm 169.3 (s, C-12), 141.1 (d, C-7), 130.0 (s, C-8), 77.3 (s, C-9), 74.3 (t, C-11), 41.7 (d, C-5), 41.6 (t, C-1), 38.5 (s, C-10), 33.3 (q, C-15), 32.7 (s, C-4), 31.3 (t, C-3), 25.3 (t, C-6), 21.5 (q, C-14), 17.9 (t, C-2), 17.0 (q, C-13); eims m/z (rel. int.) $[M]^+$ 250 (2), 232 (12), 147 (21), 127 (80), 124 (61), 109 (100); calcd for $C_{15}H_{22}O_3$, 250.1569, found (hreims) 250.1569.

MUKAADIAL [**4**].—Needles from EtOAc/MeOH, subliming at 230° [lit. (9) mp 173°]; [α]D −30° (c = 0.05, MeOH); ir max 3350, 1720, 1650 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr (90.56 MHz, pyridine-d_5) ppm 202.5 (d, C-11), 192.3 (d, C-12), 158.7 (d, C-7), 139.3 (s, C-8), 77.6 (s, C-9), 67.2 (d, C-6), 47.9 (d, C-5), 43.1 (s, C-10), 42.4 (t, C-1), 35.8 (q, C-15), 32.6 (t, C-2), 32.6 (s, C-4), 21.9 (q, C-14), 17.3 (t, C-3), 17.1 (q, C-13) [although the running of a ^{13}C nmr was mentioned in the original report of **4** (9) no details were published]; eims m/z (rel. int.) $[M − H_2O]^+$ 248 (12), $[M − CHO]^+$ 237 (100), 109 (13). Gc-ms was performed using an SE-30 (25 m × 0.2 mm) column, carrier gas He, column temperature rising from 80° to 280° at 10° per min. The trimethylsilyl ether was prepared in the normal manner using NO-bis-trimethylsilyl-trifluoroacetamide, and eims was performed using ei at 70 eV. To prepare the oxime ether, **4**

was dissolved in pyridine and methoxylamine hydrochloride added. This was followed by addition of PFP anhydride, and the reaction mixture was subjected to gc-ms using negative ion cims (70 eV) as the detection method.

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