Two New Sesquiterpene Lactones from Ceiba pentandra

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TWO NEW SESQUITERPENE LACTONES FROM *CEIBA PENTANDRA*

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ABSTRACT.—Two new sesquiterpene lactones showing moderate antimicrobial activity have been isolated from the root bark of *Ceiba pentandra* (Bombacaceae) in addition to the known compounds 8-formyl-7-hydroxy-5-isopropyl-2-methoxy-3-methyl-1,4-naphthaquinone [**1**], and 7-hydroxycadalene [**2**]. The new compounds were characterized as 2,7-dimethoxy-5-isopropyl-3-methyl-8,1-naphthalene carbolactone [**3**] and 2-hydroxy-5-isopropyl-7-methoxy-3-methyl-8,1-naphthalene carbolactone [**4**] by chemical and spectroscopic studies.

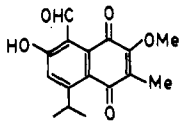
Ceiba pentandra Gaertn. (Bombacaceae) [syn. *Bombax pentandrum* L.] is a medium-sized deciduous tree found throughout the hotter regions of western and southern India and is used in folk medicine as a diuretic (1). Investigation of root bark of this hitherto uninvestigated species has led to the isolation of known compounds **1** and **2** and characterization of two new sesquiterpene lactones **3** and **4**.

RESULTS AND DISCUSSION

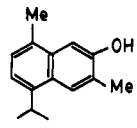
Cc of a $CHCl_3$ extract of *C. pentandra* afforded two new sesquiterpene lactones **3** and **4** along with known compounds 8-formyl-7-hydroxy-5-isopropyl-2-methoxy-3-methyl-1,4-naphthaquinone [**1**] and 7-hydroxycadalene [**2**] previously reported from *Bombax malabaricum* (2).

The uv absorption maxima of **3** and **4** were very similar to those of 6-hydroxy-5-isopropyl-7-methoxy-3-methyl-8,1-naphthalene carbolactone [**4c**] (3) which suggested that both compounds possessed an identical naphthalene system. The molecular ion at *m/z* 286 ($C_{17}H_{18}O_4$) for **3** indicated an additional-CH_2 group in this molecule in comparison with **4** (*m/z* 272, $C_{16}H_{16}O_4$).

Compound **3** showed an ir absorption band at 1770 cm^{-1}, indicating a five-



1



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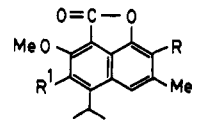
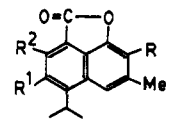
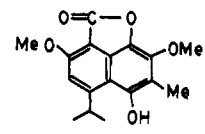


3 R=OMe, R^1=H
3a R=H, R^1=OMe



4 R=OH, R^1=H, R^2=OMe
4a R=H, R^1=OMe, R^2=OH
4b R=OMe, R^1=H, R^2=OH
4c R=H, R^1=OH, R^2=OMe



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membered lactone carbonyl, which was confirmed by the presence of a carbon signal at δ 165.40 in its ^{13}C-nmr spectrum. Other absorptions were observed at 1630 (C=C) and 1165 (-CH(Me)$_2$) cm^{-1}. The ^{1}H-nmr spectrum indicated an aromatic isopropyl group with a six-proton doublet at δ 1.41 (J=7.0 Hz) and a methine septet at δ 3.67 (J=7.0 Hz). A doublet at δ 2.38 with a fine splitting (J=0.7 Hz) integrating for three protons indicated the presence of an aromatic methyl with a free ortho position. This is in accordance with earlier observations on the double bond position in methylnaphthalene derivatives (4). Two sharp singlets at δ 4.31 and 4.24 showed the presence of two methoxyl groups. A sharp singlet at δ 7.02 and a broad singlet at δ 7.42, each integrating for one proton, suggested the presence of two aromatic protons on the naphthalene moiety. The ^{13}C-nmr spectrum showed signals for all the 17 carbons of the molecule, which includes six sp^3 carbons, ten sp^2 carbons, and a lactonic carbon. Based on spectral and biogenetic studies, two possible structures **3** and **3a** can be visualized for **3**.

The ^{1}H-coupled ^{13}C-nmr spectrum of compound **3** is consistent with structure **3** in which the C-6 signal at 110.42 ppm was observed as a doublet of doublets due to ^3J coupling (4.88 Hz) with isopropyl methine proton and ^1J coupling (156.25 Hz) with H-6, and the C-4 signal at 118.87 ppm was observed as a doublet of quartets indicating ^3J coupling (4.88 Hz) with the methyl protons at C-3 and ^1J coupling (158.89 Hz) with H-4.

A comparison of C_6H_6-induced chemical shifts of **3** with 2,7-dimethoxy-4-hydroxy-5-isopropyl-3-methyl-8,1-naphthalene carbolactone [**5**] (synthesized from **1**), and α- and β-naphthyl methyl ethers (5) (Table 1) confirmed the presence of a C-7 methoxyl with an unsubstituted ortho position, which underwent a larger upfield shift (δ +0.29), and a C-2 methoxyl with lesser upfield shift (δ +0.17), which has both ortho positions substituted.

The ^{1}H-nmr and ^{13}C-nmr spectra of **3** and **4** (Table 2) indicated that the two compounds were very similar. The only notable difference was that the ^{1}H-nmr spectrum of **3** exhibited two methoxy singlets at δ 4.31 and 4.24 while **4** had only one methoxy singlet at δ 4.31 and a broad singlet at δ 7.31 assigned to an OH proton (exchangeable with D_2O). The presence of an OH group in **4** was further supported by a strong ir absorption band at 3480 cm^{-1}.

Based on spectral data and visualizing either hemigossypol or isohemigossypol as the precursor of **4**, four possible structures (**4**, **4a**, **4b**, and **4c**) could be assigned for **4**. Absence of a downfield signal around 12.00 ppm due to chelation of OH with the lactonic carbonyl (2) eliminated the possibility of structures **4a** and **4b**.

TABLE 1. C_6H_6-Induced Chemical Shifts (Δ, in ppm) of **3**, **5**, and α- and β-Naphthyl Methyl Ethers.

Compound	Chemical shift of OMe/or OMe's in		Δ
	$CDCl_3/CCl_4$	C_6D_6	
3	4.24	4.07	+0.17
	and	and	and
	4.31	4.02	+0.29
5	4.21	4.04	+0.17
	and	and	and
	4.30	3.96	+0.34
α-Naphthyl methyl ether	3.99	3.54	+0.45
β-Naphthyl methyl ether	3.86	3.41	+0.45

TABLE 2. ^{1}H- and ^{13}C-nmr Assignments for Compounds **3** and **4**.

Proton	^{1}H-nmr ($CDCl_3$, 300.13 MHz)				^{13}C-nmr ($CDCl_3$, 22.5 MHz)		
	Compound				Carbon	Compound	
	3		**4**			**3**	**4**
	ppm	*J* (Hz)	ppm	*J* (Hz)		ppm	ppm
2-OH	—	—	7.31, br s	—	C-1	132.04	131.53
					C-2	159.23	156.96
2-OMe	4.24, s	—	—	—	C-3	129.93	130.06
					C-4	118.87	119.72
3-Me	2.38, d	0.7	2.40, d	0.7	C-5	139.46	140.27
					C-6	110.42	113.80
H-4	7.42, br s	—	7.50, s	—	C-7	156.04	156.70
					C-8	100.24	98.86
5-C*H*Me$_2$	3.67, sept	7.0	3.63, sept	7.0	C-9	132.53	132.05
					C-10	118.07	118.20
5-CH*Me*$_2$	1.41, d	7.0	1.39, d	7.0	C-11	165.40	167.51
					C-12	29.75	29.72
H-6	7.02, s	—	6.99, s	—	C-13	23.41	23.45
					C-14	23.41	23.45
7-OMe	4.31, s	—	4.31, s	—	C-15	18.20	18.24
					C-16	59.76	59.84
					C-17	57.01	—

A distinction between two other possible structures was made by decoupling studies. Irradiation of the isopropyl methine proton at δ 3.63 collapsed the isopropyl methyl doublet at δ 1.39 to a sharp singlet and also increased the intensities of signals at δ 6.99 and 7.50, which favored structure **4** and ruled out **4c** (3). This is further confirmed by irradiation of aromatic methyl at δ 2.40 which increased the intensity of H-4 at δ 7.50. Methylation of **4** yielded a dimethoxy lactone that agreed with **3** in all respects, thus confirming the assigned structure for **4**.

The two new lactones, **3** and **4**, were assayed for their antimicrobial activity as described by Tomás-Lorente *et al.* (6). The results (Table 3) clearly showed that both compounds were slightly active against *Bacillus subtilis, Staphylococcus aureus, Aspergillus niger,* and *Candida albicans,* while they were inactive against *Escherichia coli* and *Klebsiella pneumoniae* at the 1000 μg/ml level.

TABLE 3. Antimicrobial Activity of Compounds **3** and **4**.

Microorganism	Minimum inhibitory concentration (μg/ml)	
	3	**4**
Bacillus subtilis ATCC 6633 (Gram-positive)	250	350
Staphylococcus aureus ATCC 6538 (Gram-positive)	275	300
Escherichia coli ATCC 10536 (Gram-negative)	>1000	>1000
Klebsiella pneumoniae ATCC 13883 (Gram-negative)	>1000	>1000
Aspergillus niger NRRL 530	200	250
Candida albicans ATCC 10231	175	225

EXPERIMENTAL

INSTRUMENTATION.—The mp's reported were determined on a Mettler FP 51 instrument and are uncorrected. The uv spectra were recorded on a Beckman 25 spectrophotometer. Ir spectra were recorded in KBr discs on a Perkin-Elmer 283 B instrument. Nmr experiments were performed on a Bruker AM-300 or JEOL-FX-90Q spectrometers equipped with 5 mm ^{1}H and ^{13}C probes operating at 300.13 and 75.43, or 90 and 22.5 MHz, respectively. Samples were run in $CDCl_3$, and chemical shifts were referenced to internal TMS (0.00 ppm). Mass spectra were obtained on a VG Instruments VG-70 S instrument in the ei mode at 70 eV.

PLANT MATERIAL.—Root bark of *C. pentandra* was collected at Tirumala Hills in Andhra Pradesh, India, in January 1990. A voucher specimen, DR-786, is on deposit in the Herbarium of the Botany Department, Sri Venkateswara University, Tirupati.

EXTRACTION AND ISOLATION.—Shade-dried, powdered root bark (1.5 kg) was successively extracted with hexane, $CHCl_3$, and MeOH. Concentration of the $CHCl_3$ extract gave 15 g of a brown-colored semisolid which was chromatographed on a Si gel column using a $C_6H_6/CHCl_3$ step gradient. A total of 60 fractions of 100 ml each were collected and combined on the basis of tlc.

Fractions 7–14, eluted with C_6H_6, gave a brownish yellow solid which, on repeated cc, afforded 140 mg of yellow crystals, whose mp (82°) and spectral data (ir, uv, ^{1}H nmr, ms) matched those published (2) for 8-formyl-7-hydroxy-5-isopropyl-2-methoxy-3-methyl-1,4-naphthaquinone [**1**]. Fractions 15–18, eluted with $C_6H_6/CHCl_3$ (8:2), yielded 60 mg of a white crystalline solid whose mp (113–114°) and physical and spectral data were identical with the previously known 7-hydroxycadalene [**2**] (2). The C_6H_6-$CHCl_3$ (1:1) fraction gave 130 mg of yellow crystals of **3** and the C_6H_6-$CHCl_3$ (2:8) fraction gave 120 mg of pale yellow crystals of **4**.

2,7-Dimethoxy-5-isopropyl-3-methyl-8,1-naphthalene carbolactone [**3**].—Mp 110°; uv λ max (MeOH) nm (log ε) 394 (3.63), 357 (4.07), 337 (3.99), 259 (4.50), 255 (4.52), 224 (4.57); ir (KBr) cm^{-1} 2950, 1770, 1630, 1480, 1165, 1030; eims (rel. int.) *m/z* 287 (31), [M]$^+$ 286 (100), 272 (12), 271 (81), 268 (15), 257 (34), 228 (11), 129 (11), 128 (13), 127 (12), 115 (10), 69 (17); hrms found *m/z* 286.1205 (calcd for $C_{17}H_{18}O_4$ *m/z* [M]$^+$ 286.1208); ^{1}H nmr and ^{13}C nmr see Table 2.

SYNTHESIS OF **5**.—Compound **1** (50 mg) was dissolved in $CHCl_3$ (5 ml) and MeI (1 ml), and Ag_2O (150 mg) was added in small portions. The reaction mixture was kept at room temperature for 10 h with constant stirring, when a yellow crystalline compound was obtained which, upon crystallization from CH_2Cl_2, afforded the methyl ether (40 mg) as pale yellow crystals: mp 120°, hrms found *m/z* 302.1153 (calcd for $C_{17}H_{18}O_5$ *m/z* [M]$^+$ 302.1154). The methyl ether of **1** (30 mg) was dissolved in $CHCl_3$ and exposed to sunlight for 6 h to yield a yellow solid which on cc over Si gel and elution with C_6H_6-$CHCl_3$ (1:1) gave **5** as yellow crystals (20 mg) from CH_2Cl_2: mp 185°; ir (KBr) cm^{-1} 3420 (-OH), 1725 (γ-lactone C=O); eims (rel. int.) *m/z* 303 (20), [M]$^+$ 302 (100), 300 (10), 287 (25), 285 (10), 273 (7), 250 (15); hrms found *m/z* 302.1152 (calcd for $C_{17}H_{18}O_5$ *m/z* [M]$^+$ 302.1154); ^{1}H nmr (90 MHz, $CDCl_3$) δ 7.06 (1H, s, H-6), 4.30 (3H, s, 7-OMe), 4.21 (3H, s, 2-OMe), 3.67 [1H, sept, J=7.0 Hz, -C*H* Me_2], 2.25 (3H, s, 3-Me), 1.38 [6H, d, J=7.0 Hz, -CH*Me*$_2$].

2-Hydroxy-5-isopropyl-7-methoxy-3-methyl-8,1-naphthalene carbolactone [**4**].—Mp 157–158°; uv λ max (MeOH) nm (log ε) 392 (3.63), 358 (4.07), 337 (3.59), 259 (4.50), 253 (4.52), 222 (4.57); ir (KBr) cm^{-1} 3480, 2960, 1745, 1635, 1615, 1480, 1165; eims (rel. int.) *m/z* 273 (10), [M]$^+$ 272 (100), 257 (36), 254 (8), 248 (10), 229 (7); hrms found *m/z* 272.1091 (calcd for $C_{16}H_{16}O_4$ *m/z* [M]$^+$ 272.1095); ^{1}H nmr and ^{13}C nmr see Table 2.

The acetate of **4**, prepared by treating with Ac_2O and pyridine at room temperature for 48 h, was isolated as colorless crystals from C_6H_6: mp 164°; ir (KBr) cm^{-1} 2960, 1775, 1630, 1480; eims (rel. int.) *m/z* 315 (18), [M]$^+$ 314 (69), 274 (28), 273 (100), 272 (27), 271 (46), 258 (48), 257 (68), 248 (68), 229 (59), 228 (29), 227 (23), 186 (24), 185 (36), 183 (19), 157 (30), 141 (36), 129 (53), 128 (69), 127 (38), 115 (59); ^{1}H nmr (90 MHz, $CDCl_3$) δ 7.54 (1H, s, H-6), 7.15 (1H, s, H-4), 4.31 (3H, s, 7-OMe), 3.69 [1H, sept, J=7.0 Hz, -C*H*Me$_2$], 2.46 (3H, s, 2-OAc), 2.42 (3H, s, 3-Me), 1.40 [6H, d, J=7.0 Hz, -CH*Me*$_2$].

Methylation of **4** with Me_2SO_4 and anhydrous K_2CO_3 in the presence of dry Me_2CO yielded yellow crystals in CH_2Cl_2, mp 109°. The physical characteristics and spectral data of the methylated product were identical in all respects with compound **3**.

ANTIMICROBIAL ACTIVITY.—Antimicrobial activity was carried out according to the method described by Tomás-Lorente *et al.* (6). The microorganisms used in this study were procured from culture collections of the Department of Microbiology, S.V. Medical College, Tirupati.

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