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PHYTOCHEMICAL INVESTIGATION OF *Tabebuia palmeri*

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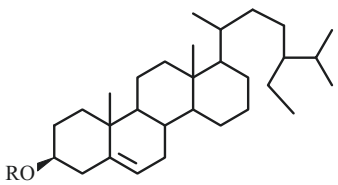
Phytochemical investigation of the stems, flowers, and leaves of Tabebuia palmeri *has led to the isolation of a novel compound, 6-(1-hydroxyundec-3-enyl)-tetrahydropyran-2-one, and 16 known compounds, viz. 1-hexadecanol, 1-triacontanol, stigmast-5-en-3*β*-ol, 2-hydroxy-3-(3-methylbut-2-enyl)-1,4-naphthoquinone (lapachol), methyl 3,4-dimethoxybenzoate, 2-acetyl-4*H*,9*H*-naphtho[2,3-b]furan-4,9-dione, 3,4-dimethoxybenzoic acid, 4-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, 3*β*-hydroxy-12-ursen-28-oic acid, 5,7,4′-trihydroxyflavone,* β*-sitosteryl-*β*-D-galactoside, 6-*O*-(*p*-hydroxybenzoyl)-epiaucubin, 4-*O*-*β*-D-glucosylbenzoic acid, 9Z,12Z-octadecadienoic acid, and hexadecanoic acid. Except lapachol, all these phytoconstituents were isolated from the* Tabebuia palmeri *for the first time, and their structures were established on the basis of spectral data analysis and chemical reactions.*

Keywords: *Tabebuia palmeri,* Bignoniaceae, 6-(1-hydroxyundec-3-enyl)-tetrahydropyran-2-one.

Tabebuia palmeri (Bignoniaceae) is a medium sized tree found mostly in tropical America. The bark decoction is reported to be highly effective against cancer [1]. Plants belonging to the family Bignoniaceae, particularly to the genus *Tabebuia* (Tecoma), are major sources of naphthoquinones possessing several biological and pharmacological activities [2–4]. Only one report has appeared in the literature earlier on the isolation of quinones and 4-aryltetralin derivatives [1] from the bark of *Tabebuia palmeri.* This paper describes for the first time the isolation and structural elucidation of the phytoconstituents of the flowers, stems, and leaves of *Tabebuai palmeri.*

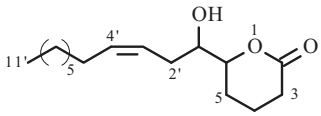
The air-dried stems of *Tabebuia palmeri* were extracted by methanol, and the viscous dark brown mass was adsorbed on silica gel (60–120 mesh) for the preparation of slurry. The slurry was chromatographed over a silica gel column, packed in petroleum ether and eluted successively with mixtures of $CHCl_3$–petroleum ether, followed by mixtures of $CHCl_3$–MeOH, yielding in all nine pure compounds **1–9**.

The air-dried flowers of *T. palmeri* were extracted with methanol, and the pale yellow mass was adsorbed on silica gel (60–120 mesh) for the preparation of slurry. The slurry was chromatographed over a silica gel column, packed in $CHCl_3$ and eluted successively with mixtures of $CHCl_3$–MeOH, yielding nine pure compounds **3**, **8**, **9**, and **10–15**.



3, 13

3: R = H; **13:** R = Gal



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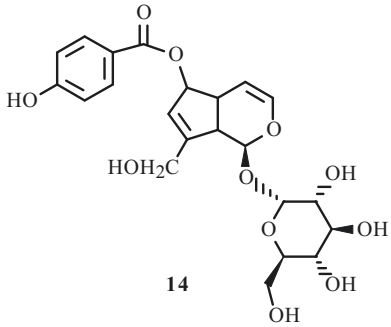

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TABLE 1. ^{1}H (300 MHz) and ^{13}C NMR (75 MHz) Data of Compound **10** ($CDCl_3$, δ, ppm, J/Hz)

C atom	δ_C	δ_H	C atom	δ_C	δ_H
2	176.2	–	5′	31.9	2.03 (2H, m)
3	32.2	2.30 (2H, t, J = 7.3)	6′	28.4	
4	30.0	1.60 (4H, m)	7′	25.6	
5	29.5		8′	25.2	1.25 (10H, br.s)
6	70.1	4.26 (1H, dd, J = 6.2, 12.0)	9′	23.9	
1′	63.3	4.16 (1H, dd, J = 4.3, 12.0)	10′	23.0	
2′	34.4	2.78 (2H, m)	11′	14.4	0.88 (3H, t, J = 6.6)
3′ 4′	131.5, 129.3	5.36 (2H, m)			

The air-dried leaves of *T. palmeri* were extracted with CH_2Cl_2–MeOH mixture, and the green mass was adsorbed on silica gel (60–120 mesh) for the preparation of slurry. The slurry was chromatographed over a silica gel column, packed in $CHCl_3$ and eluted successively with mixtures of $CHCl_3$–MeOH, yielding three pure compounds **1**, **16**, and **17**.

The physical and spectroscopic data of the known compounds isolated agreed with those reported in the literature for naphthoquinones: lapachol (**4**) [5], 2-acetyl-4*H*,9*H*-naphtho[2,3-*b*]furan-4,9-dione (**6**) [6]; flavonoid: 5,7,4′-trihydroxyflavone (**12**) [7]; steroids: stigmast-5-en-3β-ol (**3**) [8], β-sitosteryl-β-D-galactoside (**13**) [9]; aromatic acids: methyl 3,4-dimethoxybenzoate (**5**) [10], 3,4-dimethoxybenzoic acid (**7**) [11], 3,4-dihydroxybenzoic acid (**8**) [11], 4-hydroxybenzoic acid (**9**) [11], 4-*O*-β-glucosylbenzoic acid (**15**) [12]; iridoid: 6-*O*-(*p*-hydroxybenzoyl)-epiaucubin (**14**) [13]; aliphatic compounds: 1-hexadecanol (**1**) [14], 1-triacontanol (**2**) [15], linoleic acid (**16**), and palmitic acid (**17**).

Compound **10** is a new natural product isolated from the methanol extract of flowers as a colorless oil. Its molecular formula was determined to be $C_{16}H_{28}O_3$ on the basis of its EI-MS (*m/z* 268), indicating three degrees of unsaturation. Its IR spectrum showed a characteristic absorption band of a six-membered lactone at 1742 cm^{-1} along with a peak of a hydroxyl group at 3427 cm^{-1}. The presence of these functional groups became more evident by the chemical shift value of lactone carbonyl at δ 176.2 and two other peaks at δ 63.3, 70.1 in its ^{13}C NMR spectrum. However, **10** did not form any acetate under normal conditions (Ac_2O–pyridine), indicating that the hydroxyl group was either secondary or tertiary. A two-proton multiplet at δ 5.36 in its ^{1}H NMR spectrum and their corresponding carbons at δ 129.3 and 131.5 in its ^{13}C NMR spectrum suggested the presence of two olefinic protons in the molecule. A two-proton multiplet at δ 2.03 and another two-proton triplet at δ 2.30 were assigned for the allylic methylene and methylene α to the carbonyl group. Two double doublets each integrating for one proton at δ 4.16, 4.26 (J = 12 Hz each) were assigned to two methines adjacent to oxygen and the carbon bearing the hydroxyl group in the molecule. A two-proton multiplet at δ 2.78 was assigned for the methylene in between the olefinic carbon and the carbon bearing a hydroxyl group. The rest of the methylenes of the long chain and that of the lactone ring were observed at δ 1.25 and 1.60 as ten and four protons, respectively. Two characteristic peaks at *m/z* 256 and 139 were observed in its EI-MS as a result of fragmentation of δ-lactones and allylic cleavage, respectively. On the basis of the above discussion, compound **10** was characterized as 6-(1-hydroxyundec-3-enyl)-tetrahydropyran-2-one, which happened to be a novel natural product.

The insecticidal activity of compound **10** was determined against *Bruchus chinensis* (*Coleoptera: Bruchiae*) by studying the oviposition inhibition assay following our earlier reported procedure [16]. Preliminary data suggested that compound **10** showed oviposition inhibition in *B. chinensis.* Thus, insects have susceptibility and oviposition deterrency to compound **10**, which could be used for the disruption of egg laying in the field and stored grain godowns to reduce the pest population. However, since the compound was isolated in very low amounts, its detailed toxicology studies against *B. chinensis* and other insects could not be carried out.

Compound **11** was characterized as 3β-hydroxy-12-ursen-28-oic acid, commonly known as ursolic acid on the basis of spectral data and comparison of the observed melting point with that reported in the literature [17]. The assigned structure of **11** was further confirmed by carrying out its esterification with diazomethane and acetylation with acetic anhydride in pyridine. The ^{1}H NMR spectra of the ester and acetate of **11** confirmed the formation of a methyl ester and monoacetate, thus further supporting the assigned structure.

The presence of **11** in the flowers makes them more useful as **11** was known to show antioxidative [18, 19], anti-inflammatory [20], and anticancer activity against several human cell lines [21–24], and also pro-apoptotic [25] activities.

EXPERIMENTAL

General Procedures. Melting points were determined on a sulfuric acid bath and are uncorrected. Column chromatography was performed on silica gel (60–120 mesh, Merck), with gradient elution using petroleum ether, $CHCl_3$, and CH_3OH in increasing polarity, and analytical thin layer chromatography (TLC) was performed using silica gel G coated TLC plates (Merck). ^1H (300 MHz), ^{13}C (75 MHz) NMR spectra were recorded on a Bruker AM 300 spectrometer in $CDCl_3$, CD_3OD, and CD_3SOCD_3. IR spectra were recorded on a Shimadzu model 435 spectrophotometers as thin film or KBr discs. Mass spectra were recorded on a varian MAT 311A instrument using electron ionization (EI) at 70 eV and TOF MS on LCT micromass. For insecticidal studies, *B. chinensis*, a pulse beetle, was used to examine the activity of compound **10**. For this purpose, adult insects of *B. chinensis* were collected from the stored grain house, and their cultures were maintained in the laboratory at 28 ± 2°C, 75 ± 5% relative humidity, and a photoperiod of 12:12 (L:D) h. The insects were reared on gram seeds at 10–12% moisture content.

Plant Material. *Tabebuia palmeri* was introduced by the Plant Introduction Division in National Botanical Research Institute (NBRI), Lucknow, India in the late eighties and was brought from the NBRI Nursery. It was grown in Haryana in 2002. The stems, flowers, and leaves were collected from the Gurgaon region located in Haryana, India in January 2007. The plant was identified by Dr. S. C. Sharma, Head of the Department, Garden Division, NBRI, Lucknow, India.

Extraction and Isolation. Dried stems (700 g) were chopped and extracted with MeOH (4 L) for 24 h in a Soxhlet apparatus. Dried flowers (24 g) were crushed and extracted with MeOH (1 L) for 24 h at room temperature. Dried and milled leaves (1200 g) were extracted with CH_2Cl_2–MeOH (1:1) (2 L) for 24 h at room temperature. Liquid extracts were evaporated under reduced pressure to dryness. The resulting extracts of stems (40 g), flowers (4.6 g), and leaves (36 g) were chromatographed over a silica gel column separately, and 17 compounds were isolated by different purification techniques.

Methanol Extract of the Stems. The MeOH extract (40 g) of the stems was packed over a silica gel column chromatograph in petroleum ether. Elutions with chloroform–petroleum ether (10:90, 20:80, 50:50) followed by $CHCl_3$ and then MeOH–$CHCl_3$ (2:98, 5:95, 10:95, 20:80) and MeOH provided a total of 178 fractions (400 mL each), of which similar composition fractions as determined by TLC were pooled to get 12 major fractions. These 12 fractions were subjected to repeated silica gel column chromatography, preparative thin-layer chromatography, and crystallization techniques to yield nine compounds in pure form. The above techniques afforded hexadecanol (**1**, fraction 1–15, 86 mg, R_f 0.6; petroleum ether–EtOAc (98:2)), triacontanol (**2**, fraction 6–31, 8 mg, R_f 0.4; petroleum ether–EtOAc (98:2)), stigmast-5-en-3β-ol (**3**, fraction 32–39, 18 mg, R_f 0.3; petroleum ether–EtOAc (97:3)), lapachol (**4**, fraction 55–65, 990 mg, R_f 0.5; petroleum ether–chloroform (1:1)), methyl 3,4-dimethoxybenzoate (**5**, fraction 66–74, 60 mg, R_f 0.7; $CHCl_3$), 2-acetyl-4*H*,9*H*-naphtho[2,3-b]furan-4,9-dione (**6**, fraction 75–87, 5 mg, R_f 0.2; MeOH–$CHCl_3$ (2:98)), and 3,4-dimethoxybenzoic acid (**7**, fraction 75–87, 36 mg, R_f 0.2; MeOH–$CHCl_3$ (5:95)), 3,4-dihydroxybenzoic acid (**8**, fraction 107–126, 210 mg, R_f 0.2; MeOH–$CHCl_3$ (12:88)), and 4-hydroxybenzoic acid (**9**, fraction 88–106, 258 mg, R_f 0.2; MeOH–$CHCl_3$ (10:90)).

Methanol Extract of the Flowers. The MeOH extract (4.6 g) of the flowers was chromatographed using silica gel column chromatography in $CHCl_3$, followed by elutions with MeOH–$CHCl_3$ (2:98, 5:95, 10:90, 20:80, 50:50) and finally with 100% MeOH of 120 mL each to collectively get 89 fractions. Similar fractions were mixed as per their TLC behavior to yield eight major fractions. From these fractions nine compounds were isolated in pure form, of which stigmast-5-en-3β-ol (**3**), 3,4-dihydroxybenzoic acid (**8**), and 4-hydroxybenzoic acid (**9**) were also isolated from the stems. The remaining six compounds were characterized as 6-(1-hydroxyundec-3-enyl)-tetrahydropyran-2-one (**10**, 3 mg, fraction 1–8, R_f 0.4, petroleum ether) 3β-hydroxy-12-ursen-28-oic acid (**11**, fraction 9–19, 37 mg, R_f 0.4, EtOAc–petroleum ether (30:70)), 5,7,4′-trihydroxyflavone (**12**, fraction 20–27, 4 mg, R_f 0.3, MeOH–$CHCl_3$ (8:92)), β-sitosteryl-β-D-galactoside (**13**, fraction 28–32, 5 mg, R_f 0.6, MeOH–$CHCl_3$ (12:83)), 6-*O*-(*p*-hydroxybenzoyl)-epiaucubin (**14**, fraction 33–38, 400 mg, R_f 0.2, MeOH–$CHCl_3$ (15:85)), and 4-*O*-glucosylbenzoic acid (**15**, fraction 68–89, 20 mg, R_f 0.5, MeOH–$CHCl_3$ (20:80)).

6-(1-Hydroxyundec-3-enyl)-tetrahydropyran-2-one (10). Colorless oil. IR (KBr, ν_{max}, cm^{-1}): 3427, 2920, 2852, 1742, 1463, 1377, 1164, 1377, 1164, 1099, 721. For PMR and ^{13}C NMR spectral data, see Table 1. Mass spectrum (EI, 70 eV) (*m/z*, $I_{rel.}$, %): 268 (M^+, 93), 256 (22), 244 (12), 238 (58), 224 (25), 210 (31), 194 (37), 181 (37), 168 (48), 153 (58), 139 (80), 125 (100). HR-TOF-MS *m/z* 269.2122 $[M + H]^+$ (calcd for $C_{16}H_{29}O_3$, 269.2107).

3β-Hydroxy-12-ursen-28-oic Acid (11). Colorless needles, mp 288–290°C (lit. [17] mp 289–292°C). It gave a positive Liebermann–Burchard test for triterpenes. It also gave a pale yellow color with tetranitromethane.

Esterification of 11. Compound **11** (10 mg) was subjected to esterification with excess diazomethane. The solvent was evaporated to get a pure esterified product. PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz, 0 = TMS): 5.24 (1H, m, H-12),

3.60 (3H, s, $COOCH_3$), 3.20 (1H, m, H-3α), 2.22 (1H, d, J = 11.0, H-18), 1.99–1.21 (m, cyclic methylenes and methines), 1.07 (3H, s), 0.98 (3H, s), 0.94 (3H, s), 0.92 (3H, d, J = 5.9), 0.85 (3H, d, J = 6.0), 0.78 (3H, s), 0.74 (3H, s).

Acetylation of 11. Compound **11** (15 mg) was refluxed with acetic anhydride (0.5 mL) and dry pyridine (0.2 mL) for about 2 h. The contents were kept overnight at room temperature. Crushed ice was subsequently added to it, and the white precipitate that separated was filtered, washed with water, and dried. It was recrystallized from petroleum ether–chloroform to give pure acetate (mp 190–192°C, R_f 0.43 in $CHCl_3$ as developing solvent). PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz, 0 = TMS): 5.25 (1H, m, H-12), 4.99 (1H, m, H-3α), 2.18 (1H, d, J = 1.2, H-18), 2.04 (3H, s, $OCOCH_3$), 1.98–1.29 (m, cyclic methylenes and methines), 1.08 (3H, s), 0.95 (6H, s), 0.85 (9H, br.s), 0.79 (3H, s).

Dichloromethane/Methanol Extract of the Leaves. The CH_2Cl_2–MeOH extract (36 g) was subjected to silica gel column chromatography in chloroform. Elutions with increasing polarities with MeOH–$CHCl_3$ (2:98, 5:95, 10:90, 20:80) led to the collection of 92 fractions. These fractions were separately screened on TLC to reduce the number of fractions to 10 fractions. However, most of the above fractions contained major amounts of chlorophyll and other green components in them. Thus, only 3 long-chain aliphatic compounds were isolated, viz. 9*Z*,12*Z*-octadecadienoic acid (**16**, 20 mg, R_f 0.2, EtOAc–$CHCl_3$ (3:97)), hexadecanol (**1**, 36 mg R_f 0.7, petroleum ether), and hexadecanoic acid (**17**, 40 mg, R_f 0.1, $CHCl_3$).

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