Revised Structure for a Sesquiterpene Lactone from *Bombax malbaricum*

L. S. Puckhaber[†] and R. D. Stipanovic*[,‡]

Department of Soil and Crop Sciences, Texas A & M University, College Station, Texas 77843, and USDA, Agricultural Research Service, Southern Plains Agricultural Research Center, College Station, Texas 77845

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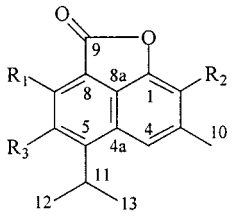
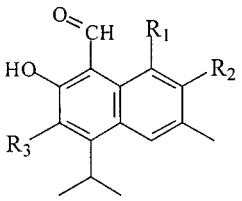
A sesquiterpene lactone isolated from *Salmalia malbaricum* (syn *Bombax malbaricum*) roots was previously identified as hemigossylic acid lactone-7-methyl ether (**1**). 2D NMR experiments have shown this is a new compound, isohemigossylic acid lactone-2-methyl ether (**2**).

Because of our interest in the fungitoxicity of cadalene type sesquiterpenes, we attempted to isolate hemigossylic acid lactone-7-methyl ether (**1**) from roots of *Salmalia malbarica* (*Bombax malbaricum*) by following the procedure of Sood et al.[1] A compound was isolated whose ^{1}H NMR and melting point were in generally good agreement with the earlier findings. However, 2D NMR experiments indicate that the correct structure for this compound is actually isohemigossylic acid lactone 2-methyl ether (**2**).

The ^{1}H NMR spectrum of **2** had peaks characteristic of related compounds (**3**−**7**) and was in generally good agreement with that reported for **1** by Sood et al.[1] The UV spectrum [λ_{max}^{EtOH} (log ϵ) 224 (3.200), 236 (sh), 254 (3.10), 262 (3.13), 342 (2.68), 363 (2.73), 401 (1.58)] was also in close agreement with that reported by Sood et al.[1]





1 R_1=OCH_3; R_2=H; R_3=OH
2 R_1=OH; R_2=OCH_3; R_3=H
5 R_1=OCH_3; R_2=OH; R_3=H
6 R_1,R_2=OCH_3; R_3=H
3 R_1=OCH_3, R_2=OH; R_3=H
4 R_1, R_2=OCH_3; R_3=H
7 R_1, R_3=OH; R_2=OCH_3

^{13}C NMR spectrometers were not widely available, and 2D NMR experiments were unknown in 1982. Results of ^{13}C NMR and 2D NMR experiments show the compound is isohemigossylic acid lactone-2-methyl ether (**2**), a compound not previously reported. An HMQC experiment showed that the protons at δ 7.61, 7.08, 4.26, 3.69, 2.36, and 1.36 are directly attached to the carbons at δ 120.0, 115.2, 60.1, 30.0, 18.2, and 23.7, respectively. The long-range proton−carbon coupling results of the HMBC experiment are shown in Figure 1 of the Supporting Information. The isopropyl methine proton (H-11) showed strong coupling to carbons 5 (δ 156.8), 6 (δ 115.2), and 12 and 13 (δ 23.7). The intense cross-peaks between the proton at δ 7.08 and the quaternary carbons at δ 118.2 and 99.5 and the methine carbon at δ 30.0 establish this proton to be at position 6. The position 6 proton also showed weak coupling to the aromatic carbon to which the hydroxyl group is attached (δ 158.4) and to the carbonyl carbon (δ 165.5). Of particular importance are the strong couplings between the proton at δ 7.61 with the aromatic methyl group (δ 18.2, C-10) and the aromatic carbon to which the methoxy group is attached (δ 157.0). This shows that the methoxy group is at either position 2 or 4 on the naphthyl ring. A NOESY experiment showed a strong interaction between the proton at δ 7.61 and the aromatic methyl protons, and weak interaction with the methine proton and with the isopropyl methyl groups. This establishes the δ 7.61 proton at C-4, and thus the methoxy group is at C-2. The chemical shifts of the protons at positions 4 and 6 are in good agreement with those found in isohemigossypol-1-methyl ether (**3**),[2,3] isohemigossypol-1,2-dimethyl ether (**4**),[2] isohemigossylic acid lactone-7-methyl ether (**5**),[4] and isohemigossylic acid lactone-2,7-dimethyl ether (**6**)[4] (Table 1, see Supporting Information). Note that the downfield shift of the hydroxyl proton (δ 10.14) due to hydrogen bonding to the carbonyl oxygen is not as large as one might expect. The ^{1}H-coupled ^{13}C NMR spectrum agrees with the proposed assignments. The monoacetate of **2** was prepared, and its melting point (170−171.5 °C) was in good agreement with that reported by Sood et al.[1] (165 °C).

We also isolated significant quantities of 7-hydroxycadalene in the root extracts. Sankaram et al.[2] and Rao et al.[4] also isolated 7-hydroxycadalene from *B. malbaricum* and *Ceiba pentandra* (syn. *Bombax pentandrum*), respectively.

Rao et al.[4] isolated **5** and **6** from *C. pentandra* (syn. *B. pentandrum*). As one expects, their ^{1}H and ^{13}C NMR spectra are very similar to that of **2** (Tables 1 and 2, respectively, see Supporting Information). Since our 2D NMR experiments firmly establish the ^{13}C NMR chemical shift assignments for **2**, the assignments for carbons 2 and 5 made by Rao et al.[4] should be reversed. In compound **2**, the relative upfield shift for the methoxy-substituted carbon 2 (δ 140.1) is undoubtedly due to the oxygen substituent situated in the *ortho*-position, while the chemical shift for carbon 5 is as expected.

The structure of **2** is in keeping with the findings of Sankaram et al.[2] and Rao et al.[4] and the prediction of Bell et al.[5] That is, the hydroxylation of the cadalene terpenoid aldehydes (TA's) in *Bombax* differ from those in *Gossypium*. In *Bombax*, the TA's are oxygenated in the 2,7-position, while in *Gossypium* the TA's are oxygenated in the 6,7-positions. Also, although *Gossypium* does form 2,7-dihydroxycadalene in foliar tissue, 7-hydroxycadalene has not been identified in *Gossypium*. One species of *Gossypium* does produce a TA, raimondal (**7**),[6] in which the 2 position is oxygenated. However, this oxygenation presumably takes place late in the biosynthetic sequence.

Compound **2** inhibits the growth of *Verticillium dahliae* conidia. Its ED_{50} value is 7.8 ± 2.3 μg/mL.

* To whom inquiries should be addressed. Tel: 979 260 9232. Fax: 979 260 9319. E-mail: rds846a@acs.tamu.edu.
[†] Texas A & M University.
[‡] USDA.

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Experimental Section

General Experimental Procedures. Spectra were recorded as follows: Hewlett/Packard 8453 UV spectrometer, Nicolet Magna-IR 550 (KBr) IR spectrometer, Bruker Avance 300 MHz or ARX 500 MHz NMR spectrometers (acetone-d_6), Hewlett/Packard 5989b mass spectrometer coupled to a 5890 II gas chromatograph [GC/MS conditions as follows: mass range 50−325 amu at 1.1 scans/s, source 280 °C, quadrupole 100 °C, injector 210 °C, transfer line 280 °C, oven 60 °C for 7 min, to 180 °C at 10 °C/min, hold 1 min to 280 °C at 15 °C/min, hold 5 min].

Extraction and Purification. *B. malbaricum* air-dried root powder was provided by Shraddha Exports, Ahmedabad, India. The powder was extracted twice with methanol (1 L per 200 g of powder). The crude extract was concentrated, mixed with an equal volume of 5% Na_2CO_3 solution, and then extracted multiple times with ethyl ether. Compound **2** represented about 10−15% of the total UV absorbing material in the ether extract. This extract was subjected to column chromatography (Baker 40−140 mesh CC silica gel) using a gradient of toluene and ethyl acetate. Compound **2** eluted in the 80:20 and 70:30 toluene/ethyl acetate fractions at 25−30% purity based on total UV absorption. These fractions were subjected to a second chromatography (Baker CC silica gel) using a gradient of hexane and ethyl acetate. Compound **2** eluted in the 75:25 and 70:30 hexane/ethyl acetate fractions (40−65% pure). In a subsequent flash chromatography (Biotage Flash40 system with a KP-Sil column; using 90:10 then 80:20 cyclohexane/acetone), compound **2** eluted with the 80:20 mixture (65−98% pure). FC fractions with <90% compound **2** were purified via TLC using Baker G/HR silica plates (75:25 cyclohexane/acetone or 95:5 toluene/methanol). Compound **2** was finally crystallized from ether/cyclohexane (76 mg/1.8 kg powder; >99.2% pure by HPLC).

Isohemigossylic acid lactone-2-methyl ether (2): mp 209−210 °C with a phase change from cubes to needles at 153−155 °C (ether/cyclohexane); IR (KBr) cm^{-1} 3232, 1724, 1637, 1492, 1453, 1436, 1426, 1393, 1355, 1268, 1246, 1190, 1165, 1142, 1110, 1032, 993, 964, 930, 883, 872, 862, 749, 725; ^{1}H NMR δ 1.36 (6H, d, J = 6.7 Hz); 2.36 (3H, bs); 3.69 (1H, sept, J = 6.7 Hz); 4.26 (3H,s); 7.08 (1H, s); 7.61 (1H, bs); 10.14 (1H, s, exchanged with D_2O); ^{13}C NMR (carbon−proton coupling constants given in Supporting Information) 165.5 (C9), 158.4 (C7), 156.8 (C5), 140.1 (C2), 133.2 (C1), 133.0 (C8a), 130.0 (C3), 120.0 (C4), 118.9 (C4a), 115.2 (C6), 99.5 (C8), 60.1 (OCH_3), 30 (C11), 23.7 (C12), 18.2 (C10); UV λ_{max}^{EtOH} (log ϵ) 224 (3.200), 236 (sh), 254 (3.10), 262 (3.13), 342 (2.68), 363 (2.73), 401 (1.58); EIMS *m/z* (rel int) 272 [M^+] (100), 257 (46), 254 (26), 243 (21), 229 (16) 128 (11) 115 (9), (ret time 26.3 min.).

Monoacetate of 2. The monoacetate was prepared from **2** (13 mg) using dry pyridine (200 μL) and acetic anhydride (200 μL). After 36 h in the dark and normal workup, the solvent was removed and the white precipitate was crystallized from ether/hexane to give 9 mg of the monoacetate: mp 170−171.5 °C; ^{1}H NMR (acetone-d_6) 7.80 (1H, d, J = 0.7 Hz), 7.32 (1H, s), 4.31 (3H, s), 3.81 (1H, sept, J = 6.9 Hz), 2.42 (3H, d, J = 0.9 Hz), 2.39 (3H, s), 1.35 (6H, d, J = 6.9 Hz).

Identification of 7-Hydroxycadalene. 7-Hydroxycadalene was identified on the basis of its HPLC retention time and UV spectrum compared to a synthetic standard and its ^{1}H NMR spectrum[7] (see Supporting Information).

Bioassay of 2. The effect of compound **2** on the growth of *Verticillium dahliae* strain V76 conidia was evaluated using a turbimetric bioassay previously described in detail[8] using a solution of compound **2** with DMSO (4%). Compound **2** had an inhibitory effect on conidia over the 0.5−30.0 μg/mL range tested.

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Supporting Information Available: This information is available free of charge via the Internet at http://pubs.acs.org.

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