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RAIMONDAL, A NEW SESQUITERPENOID FROM PIGMENT GLANDS OF *GOSSYPIUM RAIMONDII*

ROBERT D. STIPANOVIC, ALOIS A. BELL and DANIEL H. O'BRIEN

U.S. Department of Agriculture, SEA, National Cotton Pathology Research Laboratory, P.O. Drawer JF, and Department of Chemistry, Texas A & M University, College Station, TX 77841, U.S.A.

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Key Word Index—*Gossypium raimondii*; Malvaceae; cotton; sesquiterpenoid; naphthaldehyde; *o*-naphthoquinone; *o*-hemigossypolone; 2-hydroxyhemigossypol; 7-methoxyhemigossypol.

Abstract—A new sesquiterpenoid isolated from pigment glands of leaves and immature bolls of *Gossypium raimondii* has been named raimondal and identified as 5-*iso*-propyl-2-methoxy-3-methyl-1,6,7-trihydroxy-8-naphthaldehyde. Raimondal was oxidized by ferric chloride to *o*-hemigossypolone (8-formyl-5-*iso*-propyl-3-methyl-6,7-dihydroxy-1,2-naphthoquinone) which was readily reduced to its hydroquinone, 2-hydroxyhemigossypol. Neither of the two oxidation products were detected in extracts from *G. raimondii*.

INTRODUCTION

Gossypium species produce a myriad of terpenoids with the cadinene skeleton, such as hemigossypol (**1**) and its methyl ether (**2**) [1]. The concentration of these compounds appears to be directly related to disease and insect resistance [2–4]. In an effort to locate new sources of pest resistance, a survey of terpenoids found in wild species of *Gossypium* has been conducted [1]. As a result of this survey, a new sesquiterpenoid aldehyde, which we call raimondal (**4**), was isolated from *G. raimondii*. The identification and some of the chemical reactions of raimondal (**4**) are reported in this paper.

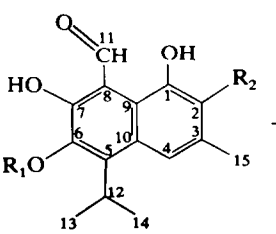
RESULTS AND DISCUSSION

Raimondal gave a parent peak in its MS at *m/e* 290 (100%). High resolution mass measurement indicated the formula $C_{16}H_{18}O_5$. Fragment ions from the loss of a methyl group and a water molecule (*m/e* 275, M−15, 77%; 272, M−18, 35%; 257, M−15,18, 49%) were also produced. The identity of these ions was confirmed by high resolution mass measurement. This fragmentation pattern is characteristic of other sesquiterpenoid aldehydes from *Gossypium* in which C-11 is an aldehyde and −OH groups are located at C-1 and C-7 [5, 6]. Raimondal spontaneously lost water when chromatographed on Si gel plates giving an anhydro derivative **5d**. Hemigossypol (**1**), its methyl ether (**2**) and gossypol (**3**) give similar dehydration products (**5a–c**).

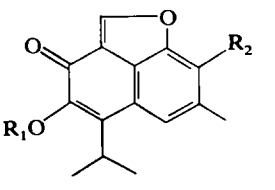
In the ^{1}H NMR spectrum, the low-field chemical shift of the aldehyde proton in raimondal (δ 11.14) indicated chelation to an *ortho* hydroxyl (δ 15.00). Two other −OH groups (δ 6.20 and 6.62) were demonstrated by rapid exchange with D_2O. Aromatic methyl, *iso*-propyl, and methoxyl groups were also present (see Experimental), A broad, one proton-singlet was located at 7.45.

These spectral data indicated that raimondal was similar to hemigossypol (**1**) but with additional oxygenation at either C-2 or C-4. Hemigossypol (**1**) and its methyl ether (**2**) each had two aromatic protons (δ 6.60 and 7.45, and 6.68 and 7.49, respectively) [5]. Gossypol (**3**) has only one aromatic proton at 7.75 [6]. Thus the peaks appearing between 7.4 and 7.7 in gossypol (**3**), hemigossypol (**1**), and its methyl ether (**2**) have been assigned to the proton at C-4 and the peaks between 6.6 and 6.7 have been assigned to the proton at C-2. Because the only aromatic proton in raimondal appeared at 7.45 it must be located at C-4, and C-2, therefore, was oxygenated. Since formation of the anhydro derivative **5d** requires −OH groups at C-1 and C-7, the methyl ether could be located only at C-2 or C-6.

^{13}C NMR studies were used to unequivocally assign the methyl ether to C-2. Shift assignments (Table 1) were made from comparisons of shifts for raimondal with shifts for related compounds, from proton–carbon couplings, and from specific proton decoupling experiments [7]. The aldehyde, aliphatic carbons, and C-4 were assigned based on their chemical shifts and were confirmed by their large one-bond proton–carbon couplings. C-8 was assigned based on its large two-bond coupling to the aldehyde proton. C-3 was a quartet



1 R_1, R_2 = H
2 R_1 = Me, R_2 = H
3 R_1 = H, R_2 = ½
4 R_1 = H, R_2 = OMe



5a R_1, R_2 = H
5b R_1 = Me, R_2 = H
5c R_1 = H, R_2 = ½
5d R_1 = H, R_2 = OMe

Table 1. ^{13}C NMR chemical shifts and long range couplings for raimondal and its derivatives*

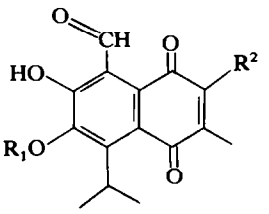
Carbon No.	Raimondal (4) δ	Raimondal (4) Coupling	o-Hemigossypolone (9) δ	o-Hemigossypolone (9) Coupling	2-Hydroxyhemigossypol (11) δ	2-Hydroxyhemigossypol (11) Coupling
1	144.3		181.3		140.7	
2	142.8		182.0		133.0	
3	127.0	^2J_H15: 6.1	133.9		124.2	^2J_H15: 6.1
4	117.3	^2J_H15: 5.5	137.5	^3J_H15: 6.4	116.9	^3J_H15: 5.0
5	134.3		138.3		135.4	
6	141.9		150.3		141.2	
7	156.1	^3J_H11: 4.3	151.0		156.4	^3J_H11: 4.9
8	111.4	^2J_H11: 18.3	119.3	^2J_H11: 20.1	111.6	^2J_H11: 17.7
9	115.0	^3J_H4: 7.3	124.9	^3J_H4: 6.7	116.3	^3J_H4: 7.9
10	125.0	^3J_H12: 4.3	129.2	^3J_H12: 3.7	122.8	^3J_H12: 4.9
11	199.0		198.8		199.1	
12	27.7		27.7		27.7	
13–14	20.1		20.0		19.9	
15	16.5		14.8		16.5	
OMe	60.9					

*Chemical shifts in ppm downfield from TMS using the central resonance of $CDCl_3$ (δ 76.9) or $(CD_3)_2$ CO (29.2) as an internal reference. Solvent for **5** was $CDCl_3$; solvent for **9** and **11** was $(CD_3)_2$ CO. Coupling constants are in Hz.

due to two-bond coupling to the methyl protons. The chemical shifts of related compounds [7] and specific proton-decoupling allowed the assignments of C-9 and C-10. Thus C-9 collapsed to a singlet on selective irradiation of H-4, while the coupling to C-10 was lost on selective decoupling of H-12.

Chemical shifts of the remaining five carbons were located on the region between δ 127 and 156. Since C-7 was expected to appear at the lowest field due to the chelation of its hydroxyl to the aldehyde [7], it was assigned to the resonance at 156.1. Coupling to this carbon also disappeared on selective irradiation of the aldehyde proton. In the deuterium exchanged proton-coupled spectrum, only C-1 has no adjacent protons with which to couple; therefore, the sharp singlet at 144.3 was assigned to this carbon. C-5 appeared as a multiplet at 134.3 in the proton-coupled spectrum. Its shape was characteristic of this carbon in related compounds [7].

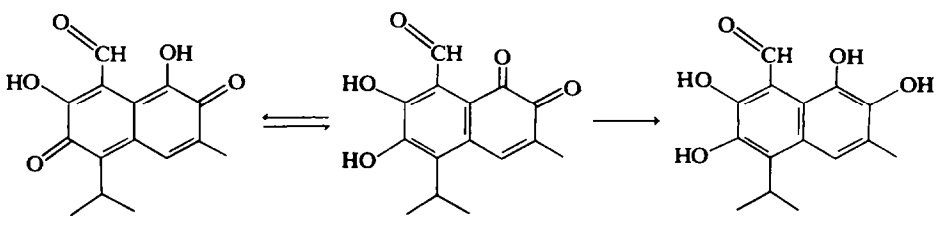
C-2 and C-6 both appeared as multiplets in the proton coupled ^{13}C NMR spectrum. However, the peak at δ 142.8 collapsed to a broad doublet on selective decoupling of the protons on C-15, showing that this resonance was due to C-2. The assignment of a hydroxyl group to C-6 and a methoxyl group to C-2 was determined by a study of the proton-coupled spectra of raimondal before and after deuterium exchange. Only the peak at 141.9 (C-6) was simplified by deuterium exchange, while the peak at 142.8 (C-2) remained unchanged. Previous studies have shown



6 R_1, R_2 = H
7 R_1 = Me, R_2 = H
8 R_1 = H, R_2 = ?

that the hydroxyl protons show coupling to the attached carbons on these molecules [7]. Raimondal must therefore have structure **4**.

Hemigossypol (**1**), its methyl ether (**2**), and gossypol (**3**) react with ferric chloride to give the corresponding quinones **6**, **7**, and **8**, respectively [8–10]. Quinones **6** and **7** are prominent terpenoids in pigment glands of *G. hirsutum* [8] and *G. barbadense* [9], respectively. TLC analysis of *G. raimondii* plant extracts failed to indicate any quinones. When raimondal was oxidized with ferric chloride, the product was not *p*-quinone like those produced from hemigossypol (**1**) and its methyl ether (**2**). Instead, raimondal gave *o*-hemigossypolone (**9**), as deduced from its ^1H NMR and MS (see Experimental). Structure **9**, rather than the tautomeric amphinaphthoquinone structure **10**, was assigned to the product based on its proton-coupled ^{13}C NMR spectrum (Table 1). The two



10 **9** **11**

quinone carbonyls appeared at δ 181.3 and 182.0. In the proton-coupled spectra, C-2 appeared as a multiplet (182.0) due to coupling to protons on C-15 and C-4; the other carbonyl appeared as a singlet (181.3). A carbonyl at C-1 would appear as a singlet, but one at C-6 would show coupling to both the aldehyde proton and the proton on C-12 [7].

o-Hemigossypolone was readily reduced by sodium hydrosulfite to the hydroquinone, 2-hydroxyhemigossypol (**11**), which was rapidly reoxidized in air to *o*-hemigossypolone (**9**). If air was carefully excluded, the compound was quite stable.

Immature and older fresh leaves, immature bolls, root bark, stems and infected roots of *G. raimondii* were examined for the presence of *o*-hemigossypolone (**9**) and its hydroquinone **11**. These compounds were not observed in any of the extracts.

EXPERIMENTAL

Extraction and purification. G. raimondii was grown at College Station, Texas. Leaves were collected, freeze-dried and ground to a powder. The freeze-dried leaves (100 g) were extracted successively with EtOAc–hexane (1:3, 1600 ml) containing H_2O (5 ml), and with Et_2O (1200 ml). The combined crude extract was fractionated on Si gel column eluted with cyclohexane–Me_2CO (93:7). Individual fractions were examined by TLC (Si gel; cyclohexane–Me_2CO, 4:1). Those fractions which showed a purple spot (raimondal) at R_f 0.55 when sprayed with phloroglucinol reagent [8] were combined and fractionated on a Si gel column eluted with hexane–Et_2O–HOAc (84.8:15:0.2). Fractions were examined by TLC. The raimondal fractions were combined and fractionated on a third Si gel column (C_6H_6–Et_2O–HOAc, 96.8:3:0.2). Fractions containing raimondal were combined, and raimondal was crystallized from C_6H_6–hexane (900 mg; yellow crystals, mp 128–130°).

Raimondal (**4**). UV λ_{max}^{EtOH} nm (ε): 387 (9200), 298 (sh), 288 (sh), 276 (13000), 236 (41900); $\lambda_{max}^{CHCl_3}$ nm (ε): 376 (10000), 287 (sh), 278 (12600) 270 (sh). IR ν_{max}^{KBr} cm^{-1}: 3530, 1618, 1578. ^{1}H NMR (100 MHz, $CDCl_3$): δ 1.45 (6 H, *d*, *J* = 8.0 Hz, C-13, C-14), 2.43 (3H, *br s* C-15), 3.80 (1H, *septet*, *J* = 8.0 Hz, C – 12), 3.83 (3H, *s*, OMe), 6.20 (1H, *s* D_2O exchanged, OH at C-6), 6.62 (1H, *s*, D_2O exchanged, OH at C-1), 7.45 (1H, *s*, C-4), 11.14 (1H, *s*, C-11), 1500 (1H, *s*, D_2O exchanged, OH at C-7). MS (Probe 50°) *m/e* (rel. int.): 290.116341 (calc. for $C_{16}H_{18}O_5$: 290.115414, 100), 275.090781 (calc. for $C_{15}H_{15}O_5$: 275.091925, 77), 272.10367 (calc. for $C_{16}H_{16}O_4$: 272.104840, 35), 257.080307 (calc. For $C_{15}H_{13}O_4$: 257.081365, 49), 244.071989 (calc. for $C_{14}H_{12}O_4$: 244.073540, 23) 243 (16), 241 (16), 229 (26), 215 (16), 201 (32), 173 (17), 129 (18), 128 (23), 115 (24).

o-Hemigossypolone (**9**). Raimondal (29.0 mg) was dissolved in Me_2CO (3 ml) and HOAc (6 ml). $FeCl_3$ (4.5 ml of 10% aq.) was added, dropwise. Stirring was continued for 6 min at 25°. Et_2O was added, and the organic phase was washed successively with dil H_2SO_4 and H_2O. The soln was filtered through a short column of Si gel, and crystallization from Me_2CO–cyclohexane gave dark red crystals (mp 199–203°, 18 mg.). UV $\lambda_{max}^{CHCl_3}$ nm (ε): 488 (1500), 400 (2900), 334 (12200), 276 (13100). IR ν_{max}^{KBr} cm^{-1}: 1660, 1642. ^{1}H NMR (90 MHz, $CDCl_3$): δ 1.47 (6H, *d*, *J* = 7.0 Hz, C-13, C-14), 2.08 (3H, *d*, *J* = 1.2 Hz, C-15), 3.59 (1H, *septet*, *J* = 7.0 Hz, C-12), 6.71 (1H, *s*, D_2O exchanged, OH at C-6), 7.60 (1H, *q*, *J* = 1.2 Hz, C-4), 10.75 (1H, *s*, C-11), 12.70 (1H, *s*, D_2O exchanged, OH at C-7). MS (Probe 30°) *m/e* (rel. int.): 276.099036 (calc. for $C_{15}H_{16}O_5$: 276.099750, 100), 275 (26), 274.083178 (calc. for $C_{15}H_{14}O_5$: 274.084100, 93), 259.061290 (calc. for $C_{14}H_{11}O_5$: 259.060625, 36), 258 (16), 247 (19), 246.089255 (calc. for $C_{14}H_{14}O_4$: 246.089190, 74), 243.064813 (calc. for $C_{14}H_{11}O_4$: 243.065715, 33), 231.029325 – major peak and 231.065983 – minor peak (calc. for $C_{12}H_7O_5$: 231.029325; calc. for $C_{13}H_{11}O_4$: 231.065715, 42), 218.093597 (calc. for $C_{13}H_{14}O_3$; 218.094280, 16), 203.070394 (calc. for $C_{12}H_{11}O_3$: 203.070805, 67, 149 (31), 129 (25), 128 (18), 115 (25), 111 (20), 109 (15).

2-Hydroxyhemigossypol (**11**). *o*-Hemigossypolone (20 mg) was dissolved in Et_2O (50 ml) and placed in a separatory funnel and 10 ml 10% aq. $Na_2S_2O_4$ was added. After purging with N_2, it was shaken for *ca* 2 min. The dark red soln turned yellow, and the Et_2O layer was washed with H_2O, excluding air before shaking. After drying (Na_2SO_4), the Et_2O soln was filtered through a short column of Si gel. The yellow crystalline product (mp 163–169°, C_6H_6–Me_2CO) was analysed without additional purification. λ_{max}^{EtOH} nm (ε): 399 (4300), 304 (sh), 295 (sh), 274 (6100), 238 (18500); $\lambda_{max}^{EtOH/NaOH}$ nm (ε): 450 (2600), 384 (3200), 290 (5600); $\lambda_{max}^{EtOH/HCl}$ nm (ε): 389 (3100), 348 (sh), 279 (5100), 232 (20900). IR ν_{max}^{KBr} cm^{-1}: 1612. ^{1}H NMR (90 MHz, $CDCl_3$): δ 1.52 (6H, *d*, *J* = 7.1 Hz, C-13, C-14), 2.47 (3H, *br s*, C-15), 3.83 (1H, *septet*, *J* = 7.1 Hz, C-12), 4.83 (1H, *s*, D_2O exchanged, OH at C-2), 6.21 (1H, *s*, D_2O exchanged, OH at C-6), 6.41 (1H, *s*, D_2O exchanged, OH at C-1), 7.51 (1H, *br s*, C-4), 11.24 (1H, *s*, C-11), 15.22 (1H, *s*, D_2O exchanged, OH at C-7). MS (Probe 145°) *m/e* (rel. int.): 276.099036 (calc. for $C_{15}H_{16}O_5$: 276.099765, 100), 259 (16), 258 (63), 257 (12), 244 (16), 243 (98), 230 (18), 217 (16), 215 (17), 115 (14).

Anhydroraimondal (**5d**). Anhydroraimondal (mp 176–180°) appeared as a light orange spot with slightly lower R_f when raimondal was chromatographed on Si gel plates. UV $\lambda_{max}^{CHCl_3}$ nm (ε): 465 (5000) 340 (3600), 312 (3700), 270 (17200), 252 (sh). IR ν_{max}^{KBr} cm^{-1}: 1648, 1625. ^{1}H NMR (90 MHz, $CDCl_3$): δ 1.47 (6H, *d*, *J* = 7.0 Hz, C-13, C-14), 2.39 (3H, *br s*, C-15), 3.51 (1H, *septet*, *J* = 7.0 Hz, C-12), 4.36 (3H, *s*, OMe), 7.13 (1H, *s*, D_2O exchanged, OH at C-6), 7.49 (1H, *br s*, C-4), 8.53 (1H, *s*, C-11). MS (Probe 125°) *m/e* (rel. int.): 272.104479 (calc. for $C_{16}H_{16}O_4$: 272.104840, 82), 258 (19), 257.082602 (calc. for $C_{15}H_{13}O_4$: 257.081365, 100), 244.072718 (calc. for $C_{14}H_{12}O_4$: 244.073540, 41), 242 (19), 241 (24), 231 (14), 229 (18), 129 (12), 128 (17), 127 (10), 115 (14).

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