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HELIOCIDE H_2: AN INSECTICIDAL SESTERTERPENOID FROM COTTON (GOSSYPIUM)‡

R. D. Stipanovic†, A. A. Bell, D. H. O'Brien, and M. J. Lukefahr
Agricultural Research Service, U. S. Dept. of Agriculture, and Texas A&M University,
P. O. Drawer JF, College Station, Texas.

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Subepidermal pigment glands of Upland cotton (Gossypium hirsutum L.) contain compounds that reduce growth rates of bollworm (Heliothis zea) and tobacco budworm (H. virescens).[1] The structure of one of these compounds, hemigossypolone (1), has been reported.[2] Three compounds structurally related to 1 have now been isolated from G. hirsutum.[3] Because these are toxic to Heliothis spp. and isolated from G. hirsutum, we have named them heliocides H_1, H_2, and H_3;[4] three related compounds from G. barbadense are called heliocides B_1, B_2, and B_3. We now report the structure of heliocide H_2 (2), a new sesterterpenoid (m.p. 123-126°) obtained from 1- to 3-day-old freeze-dried cotton bolls.

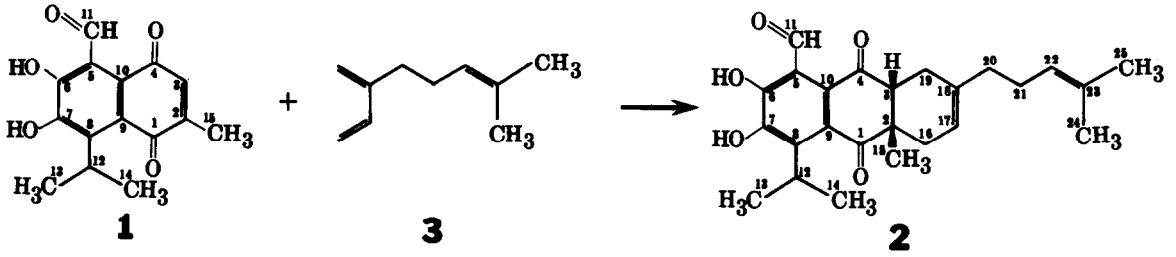
Structure 2 is assigned to heliocide H_2 based on the following information. High resolution mass measurements provided the molecular formula $C_{25}H_{30}O_5$(Calcd.: 410.209300; Found: 410.209910, 31%). The compound readily lost CO in the mass spectrometer to give the fragment $C_{24}H_{30}O_4$ (m/e 382*, 69%), an expected fragment for structure 2.[5] Ions at m/e 341* (16%, $C_{20}H_{21}O_5$) and m/e 313* (55%, $C_{19}H_{21}O_4$), formed by the loss of C_5H_9 and C_5H_9 + CO, respectively, and the ion m/e 69* (100%, C_5H_9) all agree with structure 2 because loss of C_5H_9 from the side chain is an expected mode of cleavage.

Three other diagnostic ions, formed at m/e 274* (36%, $C_{15}H_{14}O_5$), m/e 276 (23%), and m/e 135 (83%, $C_{10}H_{15}$), show that heliocide H_2 breaks into two parts, an oxygen containing part and a hydrocarbon part. The m/e 274 ion is the radical ion of hemigossypolone formed by a reverse Diels-Alder fragmentation of heliocide H_2. Naphthoquinones give large M + 2 ions;[6] thus hemigossypolone should give an ion at m/e 276.

Other spectral data support the structural relationships between the oxygenated part of heliocide H_2 and hemigossypolone. The IR spectrum of heliocide H_2, like that of hemigossypolone, indicates two types of carbonyls (1683 and 1645 cm^{-1}). However, the UV spectrum shows that the ketone carbonyls (C1 and C4) in heliocide H_2 are not conjugated in a quinone ring as found in hemigossypolone.[7] The ^{13}C-NMR spectrum confirms that the ketone carbonyl groups in heliocide H_2 (Table 1) are different from the quinoid ketones in hemigossypolone (Table 2). All other carbons (and the corresponding protons) in the oxygenated part of heliocide H_2, except for carbons 2, 3 and 15, are readily assigned by comparison of their chemical shifts with those of hemigossypolone. For heliocide H_2, a doublet at 54.8δ, a singlet at 49.3δ and a quartet at 22.3δ are assigned to carbons 3, 2 and 15, respectively (Table 1). As expected for structure 2, the protons on C15, appeared as a singlet at 1.35δ (3H) in the ^1H-NMR spectrum.

‡ Mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

* Molecular formulas of ions marked with an asterisk were determined by high resolution mass measurement.



1 3 2

TABLE 1 ^{13}C-NMR and ^{1}H-NMR Chemical Shifts of heliocide H_2[a]

Type	Carbon No.	^{13}C δ[b]	^{1}H δ	Inten.	Type	Carbon No.	^{13}C δ	^{1}H δ	Inten.
$-CH_3$	25	17.6 (q)	1.58[c](bs)	3H	>C=O	1	197.9[c](s)		
	13,14	19.8 (q)	1.45[f](d)	6H		4	198.4[c](s)		
	15	22.3 (q)	1.35 (s)	3H					
	24	25.6 (q)	1.66[c](bs)	3H	=CH-	17	117.6 (d)	5.36[g](m)	1H
						22	123.7 (d)	5.06 (m)	1H
$-CH_2-$	19	26.0 (t)	2.0-2.8[h](m)	2H					
	21	26.6 (t)	2.05 (m)	2H	=C<	23	131.4[c](s)		
	16	32.1 (t)	2.0-2.8[g](m)	2H		18	131.5[c](s)		
	20	37.1 (t)	2.05 (m)	2H					
					Aryl	5	115.2 (s)		
-CH-	12	29.0 (d)	3.64[f](sp)	1H		9	129.1[c](s)		
	3	54.8 (d)	3.03[h](t)	1H		10	134.4[c](s)		
						8	140.0 (s)		
-C-	2	49.3 (s)				7	148.9[d](s)	6.60[e](s)	1H
						6	152.2[d](s)	12.86[e](s)	1H
-HC=O	11	197.1 (d)	10.40 (s)	1H					

[a]Carbon-13 shifts in ppm downfield from TMS using central resonance of $CDCl_3$ as reference (δ=76.9 ppm); proton shifts ($CDCl_3$) in ppm downfield from TMS; numbering of carbon atoms in Tables 1 and 2 are the same for comparison purposes.

[b]Letters after δ's represent the multiplicity of coupled ^{13}C-NMR and ^{1}H-NMR resonances: s=singlet; d=doublet; t=triplet; q=quartet; sp=septet; bs=broad singlet; m=broad multiplet.

[c,d]Shift assignments of structurally similar types of carbons may be interchanged.

[e]Proton (OH) exchanged with D_2O.

[f,g,h]Proton-proton coupling confirmed by spin decoupling techniques.

TABLE 2 ^{13}C-NMR and ^{1}H-NMR Chemical Shifts of Hemigossypolone[a]

Type	Carbon No.	^{13}C δ[b]	^{1}H δ	^{1}H Inten.[b]	Type	Carbon No.	^{13}C δ	^{1}H δ	^{1}H Inten.
$-CH_3$	15	16.6 (q)	2.14 (s)	3H	=CH-	3	134.1 (d)	6.62 (m)	1H
	13,14	19.9 (q)	1.42[f](d)	6H					
					=C<	2	149.4[d](s)		
-CH-	12	28.7 (d)	4.13[f](sp)	1H					
					Aryl	5	115.9 (s)		
-HC=O	11	199.0 (d)	10.70 (s)	1H		9	127.4[e](s)		
						10	127.8[e](s)		
>C=O	1	186.5[e](s)				8	141.8 (s)		
	4	187.6[e](s)				7	149.2[d](s)	6.50[e](s)	1H
						6	152.3[d](s)	12.95[e](s)	1H

[a]See Table 1 for explanation of footnotes *a-f*.

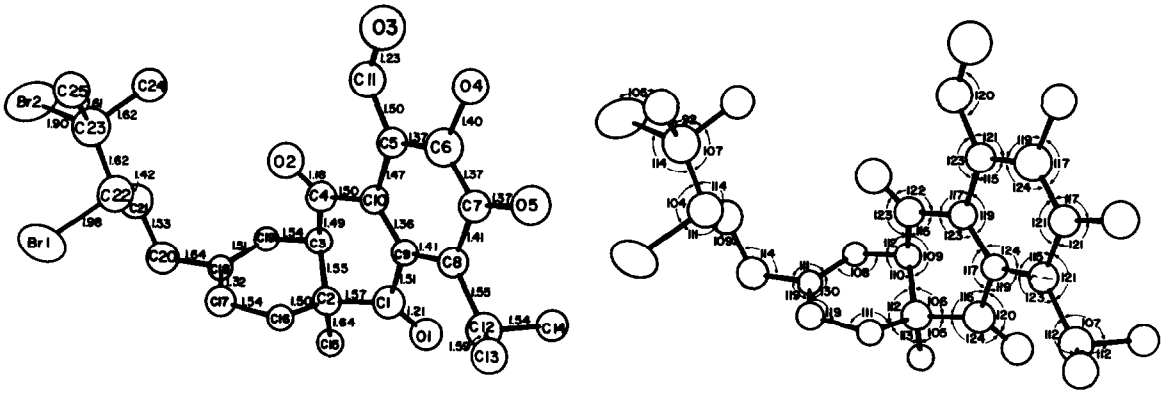
In the hydrocarbon portion of heliocide H_2, the ^{13}C-NMR chemical shifts of carbons 21, 22, 23, 24, and 25 closely resemble those of the isopropylidene end of compounds such as geraniol, farnesol and squalene.[8] This hydrocarbon chain [$-CH_2-CH=C(CH_3)_2$] accounts for the m/e 69 ion observed in the mass spectrum. Three methylene doublets (C16, C19, and C20), an alkenyl doublet (C17), and an alkenyl singlet (C18) in the ^{13}C-NMR spectrum, together with the corresponding proton signals of proper integrated intensity, complete the magnetic resonance characterization of heliocide H_2. Collectively, the above observations indicate heliocide H_2 has structure 2.

Structure 2 was substantiated by the Diels-Alder reaction of myrcene (3) and hemigossypolone (1) at room temperature. The only Diels-Alder product (m.p. 122-126°) detected gave ^{1}H-NMR and mass spectra identical to heliocide H_2, and a mixed m.p. of 121-126.5°. Two isomeric adducts are possible in the Diels-Alder reaction. However, because of the endo-transition state of the Diels-Alder reaction, myrcene would encounter less steric interaction by approaching the quinone ring with the myrcene alkenyl side chain and the quinone isopropyl group as far apart as possible;[9] this would result in the side chain at C18.

Structure 2 was confirmed for heliocide H_2 by X-ray analysis of the dibromide derivative,[10] which also showed the cis-fused ring system resulting from the Diels-Alder reaction. The crystal of the dibromide derivative was monoclinic with four molecules per unit cell and space group P $2_1/c$ [unit cell dimensions: a = 13.557 (4), b = 11.978 (2), c = 15.590 (2) Å; β = 107.86 (2)°; V = 2409.5 (9) Å^3; D_c = 1.569 g/cm^{-3}]. Bond angles and distances are shown in the figure. The structure was solved by direct methods and refined by the full-matrix least-squares method to an R-value of 0.098 without hydrogen atoms.

The X-ray analysis also confirms the structure previously assigned to hemigossypolone.[2]

Both hemigossypolone and the heliocides occur only in the pigment glands of young leaves and flower buds.[3,11] Hemigossypolone is the predominant terpenoid in very young leaves of G. hirsutum, but it is gradually replaced by the heliocides as the leaf ages.[11] Myrcene is a



major terpene (over 8%) in the essential oil of glanded flower buds of cotton.[12] Myrcene and hemigossypolone react in vitro at room temperature giving only structure 2. These observations indicate that heliocide H_2 is formed naturally by a Diels-Alder addition of hemigossypolone and myrcene in the pigment glands of the cotton plant.

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6) J. Heiss, K. P. Zeller and A. Rieker, *Org. Mass Spectr.*, 2, 1325 (1969).

7) Heliocide H_2: λ_{max}^{EtOH} (ε) 385 (4,800), 276 (22,800) nm; $\lambda_{max}^{CHCl_3}$ (ε) 356 (4,000), 305 (sh), 272 (32,300) nm; Hemigossypolone: λ_{max}^{EtOH} (ε) 417 (2,300), 313 (9,900), 269.5 (23,500) nm; $\lambda_{max}^{CHCl_3}$ (ε) 394 (1,900), 312 (8,500), 270 (21,900) nm.

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10) Molecular Structure Corporation, College Station, Tex., performed the x-ray diffraction study. Observed and calculated structural factors and atomic coordinates, which were supplied to the referees, may be obtained from the authors.

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