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HELIOCIDE H_3 AN INSECTICIDAL TERPENOID FROM *GOSSYPIUM HIRSUTUM*

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INTRODUCTION

Gossypol is the predominant sesquiterpenoid in lysigenous glands in seeds of most *Gossypium* spp. [1]. However, it is a minor terpenoid constituent in glands in foliar plant parts [2]. We previously isolated and identified the terpenoids, hemigossypolone (**1**) [2–4], and heliocide H_2 (**2**) [5], from the glands of leaves and bolls. We have now isolated a third terpenoid from young bolls, which we call heliocide H_3 (**3**). All of these compounds are toxic to *Heliothis* and appear to be involved in the resistance of certain varieties of cotton to this insect [6,7].

RESULTS AND DISCUSSION

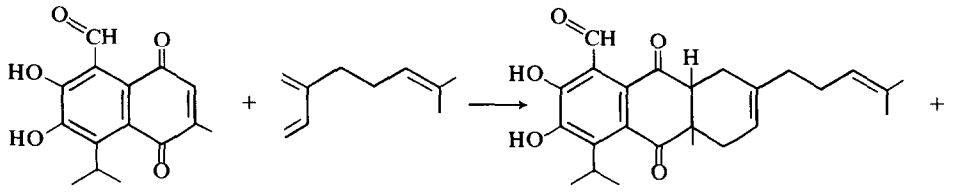
Heliocide H_3 (**3**), $C_{25}H_{30}O_5$ (high resolution MS), mp 128–134° (hexane), was isomeric with heliocide H_2, and it had UV, IR, and mass spectra similar to those of heliocide H_2 [5]. The R_f values of heliocide H_3 also were similar to those of heliocide H_2 in various solvent systems, but the two were separated by repeated development in solvent 1 (see Experimental).

Heliocide H_2 (**2**) was synthesized by the Diels–Alder reaction of myrcene (**4**) with hemigossypolone (**1**), and its structure was confirmed by X-ray crystal analysis. We reported that the only Diels–Alder adduct isolated was heliocide H_2, which was crystallized on addition of hexane [5]. However, repeated chromatography of the mother liquors from the Diels–Alder reaction yielded a second minor adduct. This adduct and the naturally occuring heliocide H_3 had identical ^{1}H-NMR, IR, UV and mass spectra. The synthetic product had

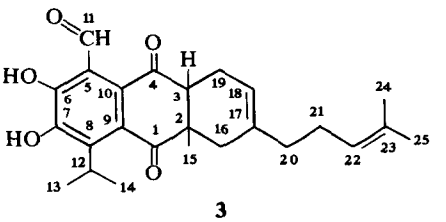
Table 1. ^{13}C-NMR chemical shifts of heliocide H_3*

Carbon type	Carbon no.	δ	Carbon type	Carbon no.	δ
Me	25	17.6	>C=O	4	198.7
	13,14	19.8		1	202.1
	15	22.6			
	24	25.5	=C<	23	131.2†
				17	131.5†
$-CH_2-$	21	26.0			
	19	24.1	=CH—	18	117.0
	16	35.0		22	123.7
	20	37.1			
			Aryl	5	115.3
—CH—	12	29.0		10	135.0‡
	3	54.4		9	129.2‡
				8	140.0
—C—	2	49.9		7	148.9§
				6	152.2§
—HC=O	11	197.9			

*Carbon-13 shift in ppm downfield from TMS using central resonance of $CDCl_3$ as reference (δ = 76.9 ppm).
†,‡,§ Shift assignments of similar types of carbons may be interchanged.



1 **4** **2**



3

mp 131.5–135° and a mixed mp with heliocide H_3 of 130–135°.

In the ^{13}C-NMR spectrum (Table 1), the resonances in heliocide H_3 at C16 and C19 (δ 35.0 and δ 24.1, respectively) are significantly different from those in heliocide H_2 (δ 32.1 and δ 26.6, respectively [5, 8]). The resonances of all other carbons are essentially unchanged ($\Delta\delta < 0.6$ ppm). The upfield shift for C16 and downfield for C19 agree with the structure **3** based on empirical correlations [9].

Comparison of the ^{13}C-NMR data of heliocides H_2 and H_3 and their synthesis from hemigossypolone (**1**) and myrcene (**4**) show that heliocide H_3 is the isomeric Diels–Alder adduct of heliocide H_2. The location of the side chain at C18 in heliocide H_2 has been proven by X-ray crystal analysis [5]. The side chain must therefore be located at C17 in heliocide H_3.

The *endo*-transition state of the Diels–Alder reaction produces a *cis*-fused ring product. The *cis*-fused ring was proven for heliocide H_2 [5]. Heliocide H_3 apparently retains this *cis*-fused ring as shown in **3** because it was synthesized at room temperature and did not revert to hemigossypolone and myrcene. It is therefore the product of the kinetically controlled reaction. Also, larger differences between the ^{13}C-NMR spectra of heliocides H_2 and H_3 would be expected if their ring fusions were different.

EXPERIMENTAL

Extraction and purification. Young bolls with bracts were collected in the field, frozen at −15°, lyophilized, and ground to a powder in a blender. The powder (100 g) was extracted successively with hexane–EtOAc–H_2O (1.3.0.4) (500 ml), hexane–EtOAc (1:3) (1 l.), and Et_2O (1 l.). The extracts were filtered through a short column of Si gel and the solvent evapd. The residue was dissolved in EtOAc–hexane (0.1.1; 500 ml) and extracted with MeOH–2% aq. Na_2CO_3 (19.1; 4 × 200 ml). A 50% satd soln of NaCl (1.81.) was added to the MeOH extract, the pH was adjusted to 5, EtOH (50 ml) was added, and the soln extracted with EtOAc (3 × 200 ml). The EtOAc fraction was evapd and the MeOH–2% aq. Na_2CO_3 extraction procedure was repeated. The resulting dark oil was dissolved in C_6H_6, adsorbed onto Si gel (30 g, Mallinckrodt CC-4), and chromatographed on Si gel (20 g). The product was eluted with hexane–EtOAc–HOAc (90:10:0.5, solvent 1), and 5 ml fractions were collected. The fractions (numbers 10–13) containing the heliocides were chromatographed on Si gel (22 g) eluting with $CHCl_3$–HCO_2H (95.5 0.5; solvent 2). The first eluate (25 ml) was discarded and the next 110 ml contained 500 mg of crude product. The product was further purified by TLC (solvent 2). The upper light yellow bands (R_f 0.5–0.7) were scraped off together and rechromatographed (solvent 1) The top band (R_f 0.43) was heliocide H_1, and the next band (R_f 0.34) was heliocides H_2 and H_3. Heliocides H_2 and H_3 were collected as one band, and heliocide H_2 was purified by crystallization from hexane. Heliocide H_3 was then purified by TLC of the mother liquors in solvent 1. Finally, heliocide H_3 was crystallized from hexane.

Spectra of heliocide H_3. UV $\lambda_{max}^{CHCl_3}$ (ε) 356 (4700), 308 (*sh*), 272 (37700) nm. IR ν_{max}^{KBr} 3300, 1695, 1682, 1643 cm^{-1}. MS (Probe = 90°) *m/e* (%) 410 (28, $C_{25}H_{30}O_5$), 383 (15), 382 (55), 314 (23), 313 (91, $C_{19}H_{21}O_4$), 297 (19), 275 (15), 274 (17), 259 (19), 135 (19), 105 (25), 93 (23), 91 (28), 79 (18), 77 (20), 69 (100). ^{1}H-NMR ($CDCl_3$). δ 1.35 (3H, *s*), 1.40 (6H, *d*), 1.55 (3H, *bs*), 1.63 (3H, *bs*), 2.0–2.8 (4H, *m*), 2.02 (4H, *m*), 2.96 (1H, *t*), 3.66 (1H, sept), 5.05 (1H, *m*), 5.42 (1H, *m*), 6.50 (1H, *s*, exchanged with D_2O), 10.42 (1H, *s*), 12.92 (1H, *s*, exchanged with D_2O)

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