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Heliocide H_1. A New Insecticidal C_{25} Terpenoid from Cotton (*Gossypium hirsutum*)

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Two new C_{25} terpenoids, heliocide H_1 and heliocide H_4, have been isolated from domestic cotton (*Gossypium hirsutum*). They were synthesized by the Diels–Alder reaction of hemigossypolone and *trans*-β-ocimene. The stereochemical configuration of these compounds was deduced from the ^{13}C NMR spectrum. Heliocide H_1 is toxic to *Heliothis virescens* and appears to be involved in the host-plant resistance of glanded cotton to this insect pest. Heliocide H_4 shows a very low level of toxicity to *H. virescens*.

Some primitive and wild strains of cotton (*Gossypium hirsutum* L.) are more resistant to bollworms and tobacco budworms (*Heliothis* spp.) than cultivated cottons (Lukefahr et al., 1969; Shaver and Lukefahr, 1971). This resistance was correlated with high concentrations of five terpenoids isolated from flower bud extracts (Stipanovic et al., 1976; Seaman et al., 1977). One of these compounds was the sesquiterpenoid, hemigossypolone (HGQ, **5**) (Stipanovic et al., 1976; Gray et al., 1976). Two other compounds were identified as C_{25} terpenoid derivatives of hemigossypolone, heliocide H_2 (H_2, **2**) (Stipanovic et al., 1977) and heliocide H_3 (H_3, **3**) (Stipanovic et al., 1978). We now report the identification and synthesis of two new C_{25} terpenoids, heliocide H_1 (H_1, **1**) and heliocide H_4 (H_4, **4**).

EXPERIMENTAL SECTION

Isolation of Heliocide H_1. Young bolls (2–3 days old) and bracts of the *Heliothis* resistant commercial variety, HG-6-1N, were collected in the field, stored over ice, frozen to −20 °C, and lyophilized. This material was ground to a powder in a blender, extracted, and chromatographed as previously reported (Stipanovic et al., 1978). The heliocides gave bright yellow-orange spots when sprayed with concentrated HCl and 5% ethanolic phloroglucinol (1:1). HGQ and gossypol gave magenta spots. Heliocide H_1 was obtained as a dark oil, that eventually crystallized on scratching and cooling (mp 110–112 °C/hexane).

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Isolation of Heliocide H_4. Although heliocide H_4 was present in *G. hirsutum* it was more easily isolated from *G. barbadense* (Seabrook Sea Island). Young bolls (2–3 days old) of Seabrook Sea Island 12B2 were collected and extracted as in the isolation of heliocide H_1. The residue from 100 g of freeze-dried powder was chromatographed over 20 g of Si gel using EtOAc-hexane-HOAc (10:90:0.25, solvent 1) as the developing solvent. The first 20 mL of colored material eluting from the column was rechromatographed on 20 g of Si gel using $CHCl_3$–HCOOH (99.5:0.5) as the eluting solvent. The first 80 mL of colored material was then chromatographed over 30 g of Si gel eluting with Et_2O–hexane–HCOOH (10:90:0.5). Fractions (15 mL) were collected and heliocide H_4 was found in fractions 2–4. These fractions were combined and chromatographed over Si gel plates developing with cyclohexane–acetone–HCOOH (95:5:0.5). Heliocide H_4 (R_f 0.35) gave a yellow color with phloroglucinol reagent. Heliocide H_4 was finally purified by TLC on Si gel with two successive developments in solvent 1. Heliocide H_4 was very unstable and was isolated as a dark oil.

Synthesis of Heliocides H_1 and H_4. HGQ (50 mg) was dissolved in benzene (1 mL) and *trans*-β-ocimene (**6**) (90% trans and 10% cis, provided by International Flavors and Fragrances, Inc.) (10 mL). The reaction mixture was protected from light and stirred under nitrogen for 5 days. The excess ocimene was removed as an azeotrope with methanol (4 × 300 mL) under reduced pressure. The product was chromatographed over Si gel (10 g) using hexane–EtOAc–HOAc (89:10:1). The first yellow band (H_1 and H_4) was clearly separated from the trailing orange

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Table I. ^{13}C NMR Chemical Shifts of Heliocide H_1 (H_1) and Heliocide H_4 (H_4)[a]

Type	Carbon no.	δ H_1	δ H_4	Type	Carbon no.	δ H_1	δ H_4
$-CH_3$	25	17.6	17.6	>C=O	4	198.8	199.3
	24	25.6	25.5		1	202.3	201.4
	20	21.4	21.6				
				-CH=	22	123.3	124.2
	15	23.6	23.7		18		117.7
	14	19.6[b]	19.4[b]		17	118.6	
	13	19.8[b]	20.2[b]				
				>C=	23	133.2[c]	131.5
$-CH_2-$	21	27.6	30.4		18	135.0[c]	
	19		26.1		17		136.2
	16	32.3					
				Aryl	10	131.8[d]	Overlap[d]
-CH-	19	39.5			9	130.0[d]	130.6[d]
	16		49.0[c]		8	140.3	141.2
	12	28.8	29.3		7	148.3	148.3
	3	57.3	51.3[c]		6	152.2	152.4
					5	114.1	113.7
-C-	2	49.0	51.6				
-CH=O	11	197.7	198.2				

[a] ^{13}C NMR shifts are expressed in ppm downfield from Me_4Si using the central resonance of $CDCl_3$ as reference (δ 76.9). [b-d] Shift assignments of carbons so designated may be interchanged.

band (HGQ). The heliocides (5 mg/plate) were then chromatographed on Si gel plates and developed twice with the above solvent system. The top band, H_1, was isolated in twice the yield of the lower band, H_4.

Spectra of Heliocide H_1. UV λ_{max} ($CHCl_3$) (ε) 351 (3900), 272 (31 200), 245 (sh) nm; IR ν_{max} ($CHCl_4$) 1700, 1675, 1650 cm^{-1}; MS (probe 100°) *m/e* (%, molecular formula as determined by high-resolution mass measurement) 410 (8, $C_{25}H_{30}O_5$), 314 (13), 313 (32, $C_{19}H_{21}O_4$), 275 (29, $C_{15}H_{15}O_5$), 136 (13), 135 (100, $C_{10}H_{15}$), 107 (13), 93 (35), 91 (14, C_7H_7), 69 (27, C_5H_9); ^1H NMR δ ($CDCl_3$) 1.33 (3 H, s), 1.35 (3 H, bs), 1.38 (6 H, d), 1.54 (3 H, bs), 1.75 (3 H, bs), 1.6–2.5 (4 H, m), 2.5–2.7 (1 H, m), 3.20 (1 H, bs), 3.75 (1 H, sept), 4.65 (1 H, m) 5.36 (1 H, m), 6.53 (1 H, bs, exchanged with D_2O), 10.24 (1 H, s), 12.85 (1 H, s, exchanged with D_2O).

Spectra of Heliocide H_4. UV λ_{max} ($CHCl_3$) 351 (3500), 272 (28 500), 245 (sh) nm; IR ν_{max} (CCl_4) 1698, 1685, 1648 cm^{-1}; MS (probe 100°) *m/e* (%, molecular formula as determined by high-resolution mass measurement) 410 (4, $C_{25}H_{30}O_5$), 408 (4), 341 (13), 323 (12), 314 (13), 313 (13), 276 (21), 136 (23), 135 (100), 107 (19), 93 (47), 91 (19), 77 (10), 69 (47); ^1H NMR δ ($CDCl_3$) 1.40 (6 H, d), 1.43 (3 H, s), 1.50 (3 H, bs), 1.55 (3 H, bs), 1.74 (3 H, bs), 1.6–2.4 (5 H, m), 3.10 (1 H, m), 3.83 (1 H, sept), 4.94 (1 H, m), 5.40 (1 H, m), 6.50 (1 H, bs, exchanged with D_2O), 10.53 (1 H, s), 13.15 (1 H, bs, exchanged with D_2O).

RESULTS AND DISCUSSION

Heliocide H_1 (**1**, $C_{25}H_{30}O_5$) was isomeric with heliocides H_2 (**2**) and H_3 (**3**). The ^1H NMR of H_1 was similar to H_2 and H_3, but one additional methyl group was indicated.

We have synthesized H_2 and H_3 from myrcene (**7**) and HGQ (**5**). Myrcene is one of the major monoterpenes (8%) found in glanded cotton flower buds (Minyard et al., 1965). Another monoterpene, *trans*-β-ocimene (**6**) is present in 14%. The Diels-Alder reaction of *trans*-β-ocimene with HGQ provided two products, which were isomeric with H_2 and H_3 but with one additional methyl group. The major product had IR, UV, ^1H NMR, and mass spectra that agreed with those of the naturally occurring H_1. R_f values of synthetic H_1 also coincided with natural H_1 in several solvent systems. Adducts of HGQ with alloocimene, and α-phellandrene had different spectra and R_f values than those of H_1. Therefore, we conclude that H_1 is a Diels-Alder adduct of *trans*-β-ocimene (**6**) and HGQ (**5**); the minor product of this reaction was designated H_4. The minor product had IR, UV, ^1H NMR, and mass spectra that agreed with those of the naturally occurring heliocide H_4.

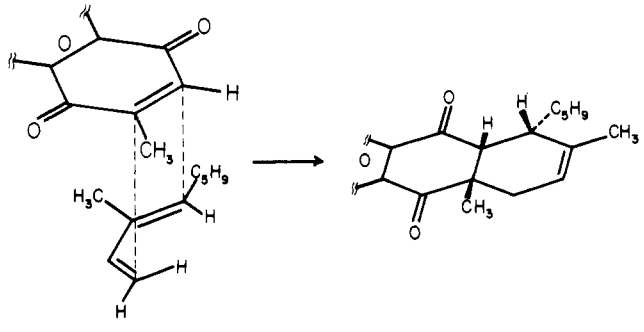

Figure 1. The endo transition of the reaction of HGQ with *trans*-β-ocimene.

Cis substituted dienes do not compete effectively with trans substituted dienes in the Diels–Alder reaction (Craig, 1943). Therefore the adducts isolated in the Diels–Alder reaction of HGQ with β-ocimene (90% trans, 10% cis) must be the two possible products from the trans isomer of β-ocimene.

Heliocide H_1 and H_4 do not revert to hemigossypolone and β-ocimene at room temperature, but they are synthesized in the Diels–Alder reaction at room temperature. Therefore, H_1 and H_4 are the products of the kinetically controlled reaction. A kinetically controlled reaction proceeds through an endo transition state giving a cis-fused product (Criegee and Becher, 1957) (Figure 1). Furthermore, trans 1-substituted butadienes and quinones form adducts in which the diene substituent is trans to the substituents on the quinone ring (Bloom, 1959; Martin and Hill, 1961). Thus, H_1 and H_4 have cis-fused ring systems with transoid isopentenyl side chains as shown for structures **1** and **4**.

The assignments of structures **1** and **4** to H_1 and H_4, respectively, were made from a careful study of the ^{13}C NMR spectra of the compounds (Table I) and particularly from comparisons of the chemical shift changes (Δδ) of the four critical carbons (C19, C16, C3, and C2) in heliocides H_1 and H_4 with those in heliocides H_2 and H_3, respectively (Table II). The x-ray crystallographic study established that H_2 has the alkyl chain in the cyclohexenyl ring at-

Scheme I

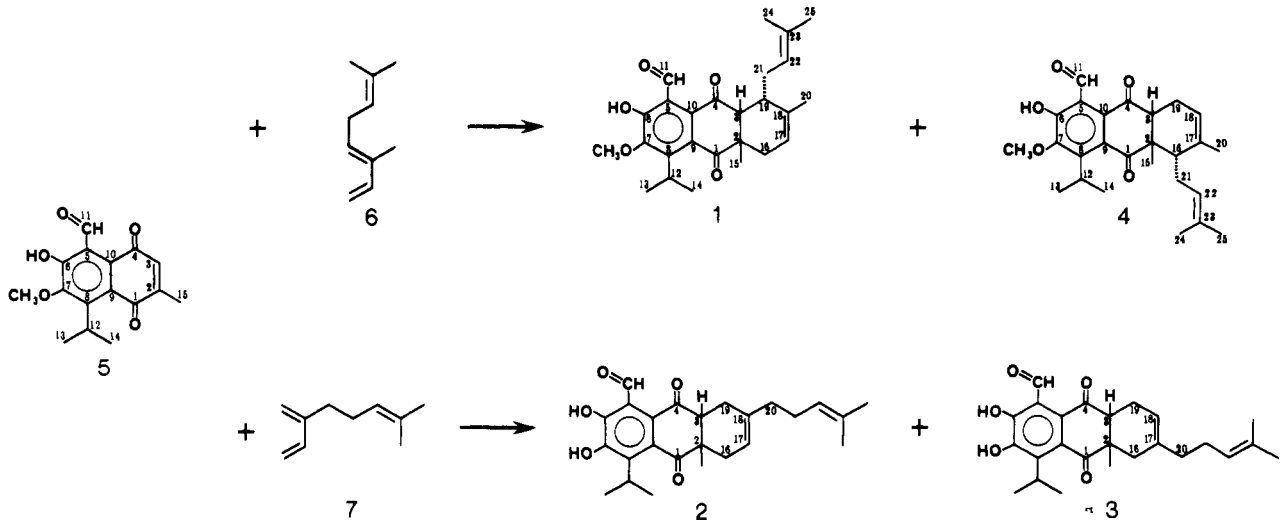

Table II. Carbon-13 Chemical Shift Comparisons for the Cyclohexenyl Ring[a]

Carbon	H_2	H_1	$\Delta\delta$	H_3	H_4	$\Delta\delta$
C19	26.6	39.5	+12.9	24.1	26.1	+2.0
C16	32.1	32.3	+0.2	35.0	49.0	+14.0
C3	54.8	57.3	+2.5	54.4	51.3	-3.1
C2	49.3	49.0	-0.3	49.9	51.6	+1.7

[a] Carbon 13 shifts in ppm downfield from Me_4Si using central resonance of $CDCl_3$ as reference (δ 76.9); downfield chemical shift changes ($\Delta\delta$) are positive.

tached at C18. Because H_3 is the isomeric Diels–Alder adduct of H_2, the alkyl side chain in H_3 must be attached at C17 (Stipanovic et al., 1978). Since structure 1 has alkyl substituents at C18 and C19, the ^{13}C NMR of the compound (H_1 or H_4) which best correlated with H_2 at C2, C3, C16, and C19 was assigned structure 1; similarly the ^{13}C NMR chemical shifts of C2, C3, C16, and C19 in the other compounds, with alkyl substituents at C16 and C17, must correlate with H_3. It was found that the only reasonable correlation of C16, C19, C3, and C2 was between H_2 and H_1 and between H_3 and H_4 (Table II). Therefore H_1 is assigned structure 1 with alkyl substituents at C18 and C19, and H_4 is assigned structure 4 with alkyl substituents at C16 and C17.

The correlation of chemical shifts of C2, C3, C16, and C19 and structure assignment of H_1 and H_4 is explained as follows. The proton couple ^{13}C NMR of H_2 has two triplets at δ 26.6 and 32.1, assigned to the methylene carbons C19 and C16, respectively. Carbon 16 appears at lower field than C19 (+5.5 ppm) due to the β-substituent effect of the bridgehead methyl (C15) (Wehrli and Wirthlin, 1976). H_1 differs from H_2 by an additional alkyl group at C19, thus a large downfield shift should be observed for this carbon (Table II). In the proton coupled spectrum of H_1, two doublets at δ 57.3 and 39.5 are assigned to C3 and C19, respectively. This gives a downfield shift upon alkyl substitution at C19 of +12.9 ppm when C19 in H_1 is compared to H_2 (Table II). This shift is similar to that observed for C3 in 3-*n*-butylcyclohexene compared to C3 in cyclohexene (37.7 - 25.5 = +12.2 ppm) (Stothers, 1972). Also, a small downfield β-substituent effect is observed at C3 of H_1 compared to H_2 (+2.5 ppm, Table II). Only a very small shift at C2 is expected in H_1 compared to H_2 because C2 is a quaternary carbon and the alkyl substituent at C19 is in a nearly anti conformation to C2 (Wehrli and Wirthlin, 1976). The small shift (-0.3 ppm, Table II) that occurred was upfield as expected.

The resonance changes in H_3 compared to H_4 (Table II), particularly the downfield shift at C16 (+14.0 ppm, Table II) are equally plausible for assigning structure 4 to H_4. However, the ^{13}C NMR chemical shift assignments were based on a decoupled spectrum because of the limited quantity of material.

If structure 4 were assigned to H_1 and structure 1 to H_4, the shift changes would no longer be reasonable. For example, a large shift of +22.4 ppm (49.0 - 26.6) would be obtained for conversion of the C19 methylene to a methine and a shift of only +4.5 ppm (39.5 - 35.0) would be obtained for conversion of the C16 methylene to a methine.

The relative in vitro synthetic yields of H_1 and H_4 further substantiates their structures as 1 and 4, respectively. In the Diels–Alder reaction of HGQ and β-ocimene, H_1 is the major adduct (66%). Likewise in the synthesis of H_2 and H_3 from HGQ and myrcene, H_2 was the major adduct (60%) (Stipanovic et al., 1977). The Diels–Alder reaction is very sensitive to steric interaction between substituents on the diene and the dienophile because of the endo transition state (Ansell et al., 1963). In the synthesis of H_2 and H_3, apparently the HGQ isopropyl group sterically interacted with the myrcene side chain in the transition state leading to H_3 and thus decreased the yield of H_3. This steric interaction, and thus the energy of activation, would be minimized in the transition state leading to H_2 because the side chain from myrcene is as far removed from the HGQ isopropyl group as possible. The HGQ aldehyde group offers minimal obstruction because it is held in the plane of the aromatic ring by hydrogen bonding to the *o*-hydroxyl group. In the synthesis of H_1 and H_4 an even larger steric interaction would occur between the HGQ isopropyl group and β-ocimene because β-ocimene is a 1,2-disubstituted diene. Thus, the major product (H_1) should have structure 1, which is formed with the least steric interaction.

We have reported that H_1 is four to five times as toxic as HGQ or H_2 to the tobacco budworm (Lukefahr et al., 1977). Heliocide H_4 shows a very low level of toxicity to the tobacco budworm. The toxicity of the heliocides therefore depends not only on the structure of the monoterpene diene involved in their synthesis but also on the stereochemistry of the product. Different lines of cotton have been analyzed for their individual heliocide concentrations. The race stocks which have been examined shows variations in the relative concentrations of the heliocides. This indicates that the synthesis of the heliocides is under some enzymatic control. Therefore, it

should be possible to specifically breed cotton plants that contain the most effective insecticidal mixture of heliocides.

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A New Metabolite of Chlorpyrifos: Isolation and Identification

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A new metabolite of chlorpyrifos was discovered in a human poisoning case in which a lethal quantity of the pesticide was ingested. The metabolite was isolated from a human liver extract. After extensive cleanup, the metabolite was subjected to various instrumental analyses such as gas chromatography, mass spectrometry, and nuclear magnetic resonance. The metabolite was identified as a compound similar to chlorpyrifos with a methylthio ($-SCH_3$) group substituted for a chlorine on the pyridinol ring. The method of isolation and the data obtained from the instrumental analyses are presented.

Pesticide residue analyses were performed on autopsy samples taken from an individual suspected of having accidentally ingested a pesticide formulation (Lores et al., 1978). The results of these analyses indicated that a mixture of chlorpyrifos [*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate] and malathion (diethyl mercaptosuccinate, S ester with *O,O*-dimethyl phosphorodithioate) had been ingested. During the analysis, a previously unreported metabolite of chlorpyrifos was detected in the liver.

This new metabolite was isolated from human liver. Gas chromatography (GC) with sulfur, phosphorus, and nitrogen specific detectors; combined gas chromatography–mass spectrometry (GC–MS); high-resolution mass spectrometry (HRMS); and nuclear magnetic resonance spectroscopy (NMR) were used to characterize the metabolite. It was identified as the *O,O*-diethyl phosphorothioate ester of a dichloro, methylthio, 2-pyridinol. The exact position of the methylthio ($-SCH_3$) group on the pyridine ring could not be determined; but one of the three possible positions was ruled out by proton NMR.

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EXPERIMENTAL SECTION

Gas Chromatography. All gas chromatographic analyses were carried out on a Tracor Model 222 GC. Two types of detectors were used. A Flame Photometric Detector (FPD) equipped with a Spectrum 1020 noise filter and a variable voltage power supply (Power Designs) was used to detect phosphorus or sulfur. The selectivity of the detector could be changed simply by changing the light filter between the flame and the phototube. A Hall electrolytic conductivity detector was used to selectively detect nitrogen.

Three columns were used in the gas chromatographic analyses: 5% OV-210 on Gas-Chrom Q 80–100 mesh; 4% SE-30/6% OV-210 on Gas-Chrom Q 80–100 mesh; and 1.5% OV-17/1.95% OV-210 on Gas-Chrom Q 80–100 mesh. All columns were operated at 200 °C with 40 mL/min N_2 carrier gas. The inlet was kept at 225 °C. The flame photometric detector was operated at 175 °C. The Hall detector furnace was operated at 860 °C.

Gas Chromatography–Mass Spectrometry. Initial 70-eV electron impact mass spectra were obtained from a Hewlett-Packard 5930A mass spectrometer equipped