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Synthetic Intermediates and Byproducts as Inhibitors of Boll Weevil Pheromone Attractancy

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In 1970, the isolation, identification, and synthesis of the male boll weevil pheromone was announced as a mixture of I [*cis*-2-isopropenyl-1-methylcyclobutaneethanol], II [(*Z*)-3,3-dimethylcyclohexane-$\Delta^{1,\beta}$-ethanol], III [(*Z*)-3,3-dimethylcyclohexane-$\Delta^{1,\alpha}$-acetaldehyde], and IV [(*E*)-3,3-dimethylcyclohexane-$\Delta^{1,\alpha}$-acetaldehyde]. Some recent synthetic preparations were found to possess less potency. An investigation was undertaken to identify the inhibitor(s), if present, and to determine their relative importance. As a result, the aldehyde synthesis procedures were identified as responsible for the loss of potency. A total of 17 synthetic intermediates and byproducts were found present in aldehyde preparations. At least four of them were found to be inhibitory in laboratory bioassays at levels at which they were present as impurities. They are [(*E*)-3,3-dimethylcyclohexylidine]methyl formate, (*Z*)-3,3-dimethylcyclohexane-$\Delta^{1,\beta}$-ethyl acetate, and (*Z*)- and (*E*)-dimethylcyclohexane-$\Delta^{1,\alpha}$-acetic acid. I acetate and II formate, though not present, were found to be inhibitory. Several others of the 17 may also be inhibitory but could not be tested in this study.

In 1970, we announced the isolation, identification, and synthesis of the male boll weevil *Anthonomus grandis* (Boh.) pheromone grandlure, (Tumlinson et al., 1970), which is comprised of the compounds I [*cis*-2-isopropenyl-1-methylcyclobutaneethanol], II [(*Z*)-3,3-dimethylcyclohexane-$\Delta^{1,\beta}$-ethanol], III [(*Z*)-3,3-dimethylcyclohexane-$\Delta^{1,\alpha}$-acetaldehyde], and IV [(*E*)-3,3-dimethylcyclohexane-$\Delta^{1,\alpha}$-acetaldehyde].

The pheromone has been used from 1973 to 1984 for monitoring and suppression as part of a series of area-wide cotton *Gossypium spp.* pest management and elimination programs throughout the South. The pheromone has also been used extensively by pest management specialists working with individual growers in the U.S. and in Central and South America. During that period, the USDA has purchased approximately 80 kg, with 25 kg in 1984. It is estimated that perhaps another 25 kg has been synthesized for private uses. It is expected that the use of the pheromone will continue and probably will increase in the future. Because the current (1984) contract price for grandlure was approximately $20 000/kg, the financial considerations and opportunities associated with its continued production and uses are significant.

Recently, several commercial preparations were found to be relatively low in potency in laboratory and field bioassays. Although subsequent preparations had satisfactory potency, an investigation of a series of pheromone preparations to identify the inhibitor(s) if present and to determine their relative importance seemed necessary to ensure the performance of the pheromone in future field programs.

In this present investigation, GLC and GLC–MS indicated that the preparations contained about 90% of the four pheromone components. Subsequently, a number of other extraneous components were isolated from the synthetic mixtures. Several were found to decrease potency at levels at which they were present as impurities in the pheromone preparations. These results provide a basis for exerting better quality control during the commercial synthesis work. The compounds themselves may also be of interest for future investigations. In this report, the structures, their preparations, and their biological potencies will be presented.

MATERIALS AND METHODS

Pheromone Synthesis and Procurement. Structures and nomenclature are given in Figure 1 and Table I. Several synthetic mixtures of the pheromone compounds (preparations A–H) and also several intermediates and

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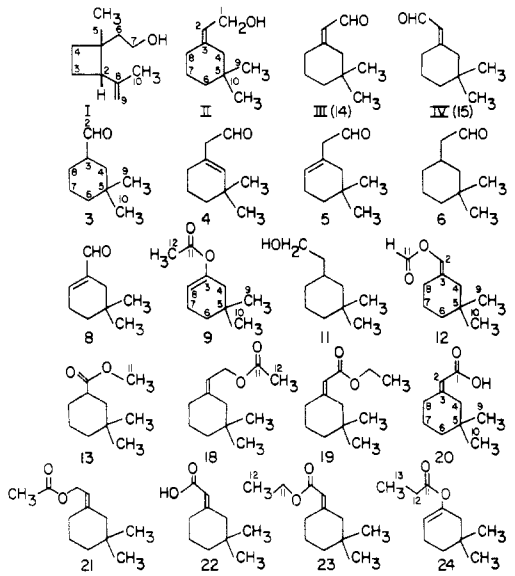


Figure 1. Structures of the boll weevil pheromones, synthetic intermediates, and byproducts isolated from several Grandlure aldehyde synthetic preparations.

Table I. Nomenclature for Proposed Structures of Compounds Isolated from Grandlure Aldehyde Synthetic Preparations (See Figure 1)

I	(+)-*cis*-2-isopropenyl-1-methylcyclobutaneethanol
II	(*Z*)-3,3-dimethylcyclohexane-$\Delta^{1,\beta}$-ethanol
III (14)	(*Z*)-3,3-dimethylcyclohexane-$\Delta^{1,\alpha}$-acetaldehyde
IV (15)	(*E*)-3,3-dimethylcyclohexane-$\Delta^{1,\alpha}$-acetaldehyde
1	3,3-dimethylcyclohexanone
3	3,3-dimethylcyclohexanecarboxaldehyde
4	3,3-dimethylcyclohex-1-eneacetaldehyde
5	3,3-dimethylcyclohex-6-eneacetaldehyde
6	3,3-dimethylcyclohexaneacetaldehyde
8	3,3-dimethylcyclohex-6-enecarboxaldehyde
9	3,3-dimethylcyclohex-6-en-1-yl acetate
11	3,3-dimethylcyclohexaneethanol
12	[(*E*)-3,3-dimethylcyclohexylidine]methyl formate
13	methyl 3,3-dimethylcyclohexanecarboxylate
18	(*Z*)-3,3-dimethylcyclohexane-$\Delta^{1,\beta}$-ethyl acetate
19	ethyl (*Z*)-3,3-dimethylcyclohexane-$\Delta^{1,\alpha}$-acetate
20	(*Z*)-3,3-dimethylcyclohexane-$\Delta^{1,\alpha}$acetic acid
21	(*E*)-3,3-dimethylcyclohexane-$\Delta^{1,\beta}$-ethyl acetate
22	(*E*)-3,3-dimethylcyclohexane-$\Delta^{1,\alpha}$-acetic acid
23	ethyl (*E*)-3,3-dimethylcyclohexane-$\Delta^{1,\alpha}$-acetate
24	3,3-dimethylcyclohex-6-en-1-yl propionate

byproducts (structures **4**, **5**, **19**, **20**, **22**, **23**, and I acetate; see Figure 1 and Table I) were supplied by Frank Enterprises, Inc., Columbus, OH. The synthetic pheromone compounds were also obtained from Albany International, Columbus, OH.

The commercial boll weevil pheromone compounds were obtained as a 3/4/1.5/1.5 I/II/III/IV neat mixture [I = *cis*-2-isopropenyl-1-methylcyclobutaneethanol, II = (*Z*)-3,3-dimethylcyclohexane-$\Delta^{1,\beta}$-ethanol, III = (*Z*)-3,3-dimethylcyclohexane-$\Delta^{1,\alpha}$-acetaldehyde, IV = (*E*)-3,3-dimethylcyclohexane-$\Delta^{1,\alpha}$-acetaldehyde] or as a 1/1 mixture of III and IV. These and other compounds were stored in the freezer until used. Compound I was synthesized by the general procedures of Billups et al. (1973). Compounds II–IV were synthesized by the general procedures of Tumlinson et al. (1971), and the scheme is given in Figure 2. Alternatively, the aldehydes III and IV were supplied as a 1/1 mixture and then mixed with I and II for biological work.

Synthesis, Procurement, or Isolation of Intermediates and Byproducts. The following compounds were procured as indicated above for evaluation: ethyl (*Z*)- and (*E*)-3,3-dimethyl-$\Delta^{1,\alpha}$-cyclohexaneacetate (**19** and **23**) (see

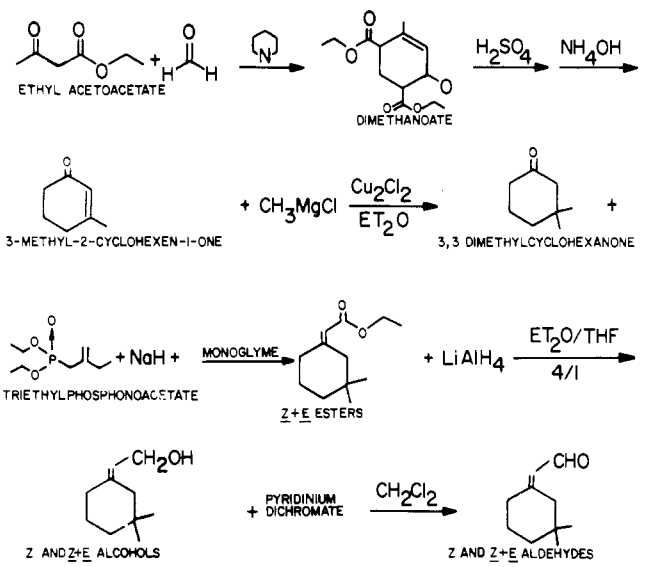


Figure 2. Synthetic scheme for synthesis of the boll weevil pheromone aldehydes.

Table I, Figure 1), a 1/1 mixture of ethyl 3,3-dimethylcyclohex-1-eneacetate and ethyl 3,3-dimethylcyclohex-6-eneacetate, 97% ethyl 3,3-dimethyl-cyclohex-6-eneacetate, a 1/1 mixture of (*Z*)- and (*E*)-3,3-dimethylcyclohexane-$\Delta^{1,\alpha}$-acetic acid (**20** and **22**) and I acetate.

The following intermediates and byproducts were obtained by synthesis and/or isolation:

(1) (Z)- and (E)-3,3-Dimethylcyclohexane-$\Delta^{1,\beta}$-ethanol (II and E-II). These alcohols were obtained by reduction of the respective ethyl (*Z*)- and (*E*)-3,3-dimethylcyclohexane-$\Delta^{1,\alpha}$-acetates with $LiAlH_4$ according to the scheme in Figure 2.

(2) (Z)- and (E)-3,3-Dimethylcyclohexane-$\Delta^{1,\alpha}$-acetaldehydes III, IV, 14, and 15. These aldehydes were obtained by oxidation of the respective alcohols in (1) with pyridinium dichromate in CH_2Cl_2 according to the scheme in Figure 2. Purities: III; 90%, IV, 93%.

(3) 3,3-Dimethylcyclohex-1- and -6-eneacetaldehydes 4 and 5. These aldehydes were obtained by reduction and subsequent oxidation as detailed above of the related byproduct "endo" ethyl esters (ethyl 3,3-dimethylcyclohex-1-eneacetate and ethyl 3,3-dimethylcyclohex-6-eneacetate) that had been separated from the desired esters **19** and **23** by distillation according to the scheme in Figure 2.

(4) 3,3-Dimethylcyclohexanecarboxaldehyde (3). This aldehyde was isolated from the 5% aqueous KOH reflux, 1 h, of [(*E*)-3,3-dimethylcyclohexylidine]methyl formate (**12**) by extraction of the reflux with CH_2Cl_2. No further cleanup was carried out when GLC–MS indicated above 90% purity of an oil, yield 62 mg.

(5) [(E)-3,3-Dimethylcyclohexylidine]methyl Formate (12). A 4-year-old sample of IV (3.0 g), found by GLC–MS to be degraded to approximately 50% of a compound with M^+ 168, was chromatographed on a 3 × 32 cm Biosil A column. The fraction eluting with CH_2Cl_2/hexane (1/9) was shown by GLC–MS to consist almost entirely of one compound, M^+ 168. Yield of the oil: 0.94 g.

(6) (Z)-3,3-Dimethylcyclohexane-$\Delta^{1,\beta}$-ethyl Acetate (18). The acetate was prepared from its alcohol (II) by its reaction with acetic anhydride in pyridine; yield of 18 2.50 g.

(7) (Z)- and (E)-3,3-Dimethylcyclohexane-$\Delta^{1,\alpha}$-acetic Acids 20 and 22. The mixture of acids was chromatographed on a 3 × 32 cm Biosil A column. The fraction eluting with 100% CH_2Cl_2 was shown by GLC–MS to consist of a 1/1 mixture of the two acids, each with M^+

168. On concentration, a white wax was formed; yield 3.6 g.

(8) (Z)-3,3-Dimethylcyclohexane-$\Delta^{1,8}$-ethyl Formate (II formate). An equal molar ratio of II and formic acid was placed in a sealed tube and agitated by shaking 24 h. After addition of aqueous sodium bicarbonate (5%), the reaction mixture was extracted with CH_2Cl_2. The CH_2Cl_2 extract was chromatographed on a 2 × 10 cm Biosil A column, and the fraction eluting in CH_2Cl_2/hexane (1/1) was found to contain approximately 95% of the formate, yield 0.24 g.

GLC–EI–MS. A DB-1 column (60 m × 0.322 mm) was interfaced to a Hewlett-Packard 5985-B quadrupole mass spectrometer through an open-split interface for acquisition of mass spectra. Alternatively, the column output was directed to an FID detector.

^{13}C NMR. Spectra were obtained with a Varian (T-20) spectrometer at 20 MHz and 37 °C, using an 8K data table. The spectra were obtained from 50 mg or more of the compound in 1.5–2.0 mL of $CDCl_3$. The spectra were obtained during continuous proton decoupling. Chemical shifts were recorded in ppm downfield from Me_4Si or from the central resonance of $CDCl_3$ (δ 76.9) as an internal reference.

IR. Spectra were obtained with a Nicolet 7199 Fourier transform interferometer using thin films prepared from $CHCl_3$ evaporation on KBr disks.

Laboratory Bioassays. The bioassays were conducted according to the original procedures of Tumlinson et al. (1968). The bioassay unit was an arena consisting of an inverted 15-cm funnel glued onto a 4 cm (height) × 15 cm cylinder, in turn glued onto a glass plate floor. Short tubes were attached at each end allowing for the exit of weevils in response to the pheromone or other odorant, or to the blank. Small filter flasks (50 mL) were attached to the tubes to trap responding weevils. Air was constantly drawn from the side exits upward through the inverted funnel. The response was expressed as the index of attraction (IA):

$$\text{IA} = \frac{\text{no. of weevils resp to attractant} - \text{no. resp to control}}{\text{no. of weevils released} - \text{no. resp to control}}$$

Typically, 25 female weevils were placed in the lighted arena (25 °C), and data were recorded after 1 h. The standard pheromone consisted of 8 μg of a 3/4/1.5/1.5 I/II/III/IV mixture in 0.1 mL of CH_2Cl_2 deposited on 1 g of firebrick. Other test compounds were formulated as specified in the text. Standard errors of the mean were calculated.

RESULTS AND DISCUSSION

Work to identify any synthetic intermediates and byproducts present that may have been inhibiting female responses to the boll weevil pheromone was initiated when a complete pheromone formulation (STD 1983 = batch A) was reported to be considerably less attractive than a standard (STD 1980) in several laboratory and field bioassays. To statistically confirm this report, the two preparations were bioassayed 19 and 27 times, respectively, over a 5-month period. The IA was 0.49 ± 0.03 and 0.25 ± 0.04 for STD 1980 and STD 1983, indicating that the difference was statistically significant.

When STD 1983 (batch A in Table IV) was analyzed by GLC–EI–MS (Figure 3), the four pheromone compounds accounted for about 90% of the total GLC integration. Enhancement of the chromatogram (Figure 3) revealed about 10 other maxima. It was considered that the syntheses may have provided one or more incorrect pheromone isomers. However, a comparison of the mass spectra, ^{13}C NMR spectra, and the GLC retention indices with those of the standards and with our previous data (Tumlinson et al., 1969, 1971; Gueldner et al., 1972) indicated no discernible differences (Tables II–IV).

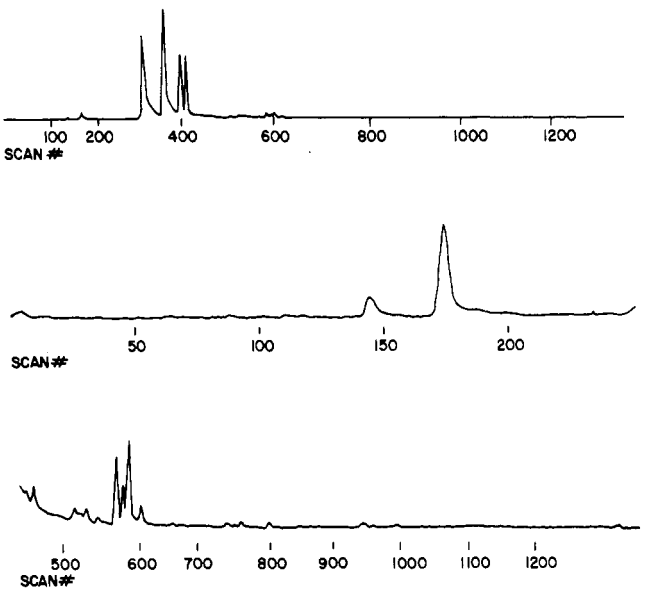


Figure 3. Chromatogram of aldehyde synthesis preparation A with enhancement of pre- and postpheromone maxima.

Table II. Mass Spectra (EI) for the Four Boll Weevil Pheromone Compounds, Synthetic Intermediates, and Byproducts Found by GLC–MS Analysis of Several Grandlure Aldehyde Synthetic Preparations[a]

elut order[b]	M^+	frag (M/e)[c]
I	154	68, 67, 109, 121, 139, 154
II	154	69, 93, 136, 121, 111, 154
III (14)	152	137, 109, 152, 81, 69
IV (15)	152	109, 152, 95, 81, 137
1	126	83, 126, 69, 111, 98
2	150	94, 83, 111, 150, 135, 121
3	140	69, 111, 83, 122, 125, 140
4	152	93, 109, 108, 81, 137, 152
5	152	108, 109, 93, 81, 137, 152
6	154	95, 110, 121, 136, 139, 154
7	127	127, 109, 69, 128, 110
8	138	109, 138, 67, 95, 123
9	168	97, 125, 111, 141, 168, 153
10	152	95, 110, 67, 137, 152, 121
11	156	95, 123, 141, 109, 138
12	168	69, 84, 107, 168, 122, 140
13	168	121, 136, 93, 79, 69, 168
16	169	75, 95, 137, 169
17	169	141, 69, 11, 95, 169
18	196	93, 136, 121, 69, 79, 107
19	196	69, 181, 196, 135, 151
20	168	69, 153, 168, 135, 125, 100
21	196	136, 121, 93, 69, 107, 79
22	168	69, 168, 153, 135, 125, 100
23	196	69, 196, 181, 151, 135
24	182	75, 167, 182, 151, 137
II formate	182	69, 93, 136, 121, 182

[a] See Figure 1 for structural assignments, Table I for nomenclature, Table III for ^{13}C NMR assignments, and Table IV for I_k values and percent distribution. [b] From a 60-m DB-1 GLC column. [c] Major fragments in decreasing order of abundance.

Subsequently, 20 g of A was chromatographed on a 5 × 60 cm Biosil A column and eluted sequentially with hexane, hexane/CH_2Cl_2 mixtures, and CH_2Cl_2 to yield substantial quantities (0.2–0.5 g) of A_1 (mostly acids plus I and II), A_2 (mostly III and IV), A_3 (chiefly aldehydes), and A_4 (aldehydes, esters, hydrocarbons). Analysis of the fractions by GLC–MS revealed the presence of approximately 25 compounds. In conjunction with the calculation of retention indices for each (I_k; Kovats, 1961), the peaks ob-

Table III. ^{13}C NMR Chemical Shifts (ppm) for the Four Boll Weevil Pheromone Compounds and Several Synthetic Intermediate and Byproducts Isolated from Grandlure Aldehyde Synthetic Preparations[a] (See Figure 1 for Structures)

compd	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11	C-12
I	37.4	42.1	29.8	28.8	23.4	53.3	59.2	145.8	109.9	19.6		
II	58.3	139.6	124.4	42.5	33.2	37.0	24.6	40.3	28.7	28.7		
III (14)	190.1	165.9	127.1	42.5	34.5	37.6	24.0	39.0	28.4	28.4		
IV (15)	190.0	165.8	127.1	28.8	34.5	37.9	24.0	51.1	28.4	28.4		
3		204.7	61.9	46.9	25.2	24.6	21.6	38.8	30.3	30.3		
5	199.9	52.7	129.3	43.3	34.8	29.2	23.4	125.6	28.2	28.2		
6	205.0	51.6	31.3	46.6	33.2	33.1	22.5	39.1	25.0	28.8		
12		126.8	125.3	25.8	32.4	39.4	22.3	43.8	28.3	28.3	158.0	
18	60.5	144.8	116.8	42.0	32.8	36.4	23.8	39.5	28.3	28.3	170.9	20.9
20	172.3	165.1	114.0	42.7	34.6	37.8	23.4	39.0	28.3	28.3		
22	172.3	165.1	114.1	27.0	34.6	37.8	24.3	51.8	28.3	28.3		
23	167.1	162.1	115.1	29.8	34.7	39.7	24.0	51.7	29.1	29.1	59.9	14.9
II formate	60.4	122.0	118.0	42.2	32.6	34.2	23.9	39.8	28.7	28.7	160.9	

[a] Chemical shifts in ppm downfield from Me_4Si or the central resonance of $CDCl_3$ (δ 76.9) as an internal reference.

Table IV. Distribution of Pheromone Compounds, Synthetic Intermediates, and Byproducts, Found by GLC–MS Analysis of Several Grandlure Aldehyde Synthetic Preparations,[a,b] Percent Composition for B–G

no.	I_K	M^+	A_1	A_2	A_3	A_4	B	C	D	E	F	G
1	1036	126			+							
2	1082	150						1.7			2.6	
3	1102	140			+			2.1			0.5	0.5
4	1110	152	+			+	1.2	5.0	1.8		0.3	
5	1125	152		+			4.3	27.5	5.0	2.3	2.6	0.9
6	1130	154						0.6			0.4	
7	1145	127			+		0.6					
8	1152	138			+						0.3	
9	1158	168			+	+	1.2	0.7	0.5	0.4		0.6
10	1174	152			+							
11	1195	156							1.5		0.5	0.5
12	1210	168	+	+		+	2.4	0.4		2.7		
13	1230	168			+	+		8.7				
14	1258	152	+	+	+	+	31.5	6.3	41.0	25.2	38.4	40.4
15	1268	152	+	+	+	+	29.2		38.5	28.2	39.8	44.8
16	1270	168			+		0.8					
17	1285	169	+	+			0.8	1.1				
18	1325	196			+	+	2.4				3.1	1.9
19	1328	196				+	1.5					1.5
20	1330	168	+				0.5	0.3	2.0	9.1		
21	1332	196			+	+	2.8				1.6	3.7
22	1335	168	+				0.5	0.4	1.5	10.8		
23	1345	196				1.2						2.1
24	1360	182			+							
% of total GLC integrn							80.9	54.8	91.8	79.3	90.1	96.9
ratio III + IV/byproducts							3.00	0.38	6.46	2.06	6.57	7.28
lab bioassay: IA[c]							0.25	0.23	0.56	0.28	0.52	0.50

[a] See Figure 1 for structural assignments, Table I for nomenclature, Table II for mass spectral data, and Table III for ^{13}C NMR assignments. [b] Pheromone preparations A–G, see experimental section for identification. [c] When added to the pheromone alcohols and tested according to normal procedures. See Table V for details.

served for A_1, A_2, A_3, and A_4 and also for those of samples B–G were numbered (Figure 1; Table IV) in one sequence.

As a result of this process, GLC retention indices (I_k) and mass spectra were obtained for 24 compounds (Tables II and IV). Sufficient quantities of eight compounds were available to obtain ^{13}C NMR spectra (Table III). For five of the GLC peaks (2, 7, 10, 16, 17), no structural assignments were attempted.

With reference to the experimental section (Tables II–IV; Figure 1), the following structural assignments were made:

3,3-Dimethylcyclohexanone (1). 1 is a synthetic intermediate (Figure 2), and the mass spectra are compatible with its assignment.

3,3-Dimethylcyclohexanecarboxaldehyde (3). 3 is an aldehyde based on its carbonyl absorption in the IR of 1705 cm^{-1} and its ^{13}C NMR shifts that are compatible both with the dimethylcyclohexane skeleton and the aldehyde carbon. The mass spectrum is compatible with a saturated aldehyde. This compound was previously found by Henson et al. (1976) as an oxidation product of III and IV stored in pentane solution.

3,3-Dimethylcyclohex-1-eneacetaldehyde (4) and 3,3-Dimethylcyclohex-6-eneacetaldehyde (5). The structures of 4 and 5 were assigned by comparison with the aldehydes prepared by reduction and subsequent oxidation from the synthetic "endo" ethyl ester precursors. A 1/1 mixture of the aldehydes obtained from the ethyl ester precursors gave mass spectra identical with those of 4 and 5. The aldehyde prepared from the higher I_k endo ester gave MS identical with that of 5. The IR carbonyl absorption of 5 at 1728 cm^{-1} was indicative of a nonconjugated aldehyde (as compared to 1675 cm^{-1} for III and IV), thus locating the unsaturation in the cyclohexane ring. The apparent carbons for unsaturation would be either 1 (C-3, C-4) or 6 (C-3, C-8). The I_k of the 6-unsaturation should be higher, being further separated from the *gem*-dimethyl function, and thus assigned to 5. Given this assumption, the remainder of the ^{13}C NMR assignments are predictable.

3,3-Dimethylcyclohexaneacetaldehyde (6). The mass spectrum is compatible with a saturated aldehyde with an M^+ of 154. The IR carbonyl absorption of 6 is at 1705 cm^{-1}, indicative of a nonconjugated aldehyde. The ^{13}C NMR

spectrum revealed the expected carbonyl shift, but no vinyl carbons. The C-2 shift was compatible with that of the carbon adjacent to the carbonyl carbon.

3,3-Dimethylcyclohex-6-enecarboxaldehyde (8). The assignment for this compound is tentative, based only on GLC–MS data and column chromatographic behavior. Alternatively, this maxima could have been assigned to structure 11. However, the structural assignment for 11 is based on the M/e fragments at 138 and 141, and the absence of M/e 156, very characteristic for a saturated alcohol.

3,3-Dimethylcyclohex-6-en-1-yl Acetate (9). This tentative assignment is based on the loss by fission of C_2H_3O from the molecular ion (M^+ 168), yielding $C_8H_{13}O$ (M/e 125) with the subsequent loss of CO to give M/e 97. This sequence of fragmentations has been observed with acetates of other cyclic compounds where rearrangement resulting in a $M^+ - 60$ is hindered.

3,3-Dimethylcyclohexaneethanol (11). This tentative structural assignment is based solely on the GLC–MS data, which feature prominent $M^+ - 15$ and $M^+ - 18$ fragments at M/e 141 and 138, characteristic of an alcohol or aldehyde. Given the probable cyclohexane ring system and the apparent M^+ of 156, the alcohol assignment is presumptive.

[(*E*)-3,3-Dimethylcyclohexylidene]methyl Formate. The IR for this structure indicated a strong absorption at 1735 cm^{-1}, indicative of a carbonyl group, perhaps an aldehyde or ester, and a strong band at 1150 cm^{-1}, characteristic of a prominent C–O function. The mass spectrum gave a M^+ of 168 with prominent M/e fragments of 140 ($M^+ - 28$), 122 ($M^+ - 46$), and 107 ($M^+ - 61$), suggestive of the fragmentation of a hindered acid or ester. ^{13}C NMR gave a shift of 158.0 ppm that is appropriate for a formate and vinyl carbon shifts at 126.8 and 125.3 ppm. The endo unsaturation was excluded because C-2 would have required a shift of 50–60 ppm. As a degradation product of IV, the "trans" configuration would be expected, and C-8 (43.8 ppm) should therefore be higher than C-4 (25.8 ppm). The C-2, being attached to the formate, should be higher than C-3. The structure for this compound along with its cis isomer was previously elucidated by Henson et al. (1976) who isolated it as an oxidation product of III and IV stored in pentane solution. Henson et al. (1976) suggested that the formation of 12 occurred by oxidation of the original aldehyde, first to the intermediate peroxy acid, which in turn reacted with remaining original aldehyde to produce the formate ester.

Methyl 3,3-Dimethylcyclohexanecarboxylate (13). This presumptive structural assignment is based primarily on the rather low intensity of the M^+ (168), coupled with a strong $M^+ - 32$ (140) and a weak $M^+ - 15$ (153). These features appear to be common to hindered methyl esters including methyl cyclohexanecarboxylate, and methyl *trans*-3-, *trans*-4-, *trans*-5-, and *trans*-6-heptenoate (Stenhagen et al., 1974a).

(*Z*)-3,3-Dimethylcyclohexane-$\Delta^{1,\beta}$-ethyl Acetate (18). The structure for this component was assigned on the basis of its congruence with an authentic synthetic sample, both examined by GLC–MS. The M^+ of 196 is absent as might be expected with an acetate. The M/e 136 is the strongest fragment, followed by M/e 121, nearly identical with that of the trans isomer 21. ^{13}C NMR of the synthesized sample revealed a typical shift of 170.9 ppm for the acetate carbon with the acetate methyl carbon at 20.9 ppm. The vinyl carbons were at 144.8 and 116.8 ppm, similar to those of II and III.

Ethyl (*Z*)-3,3-Dimethylcyclohexane-$\Delta^{1,\alpha}$-acetate (19). The structure for this compound, a synthetic intermediate (Figure 2), was assigned on the basis of its congruence with an authentic synthetic sample both examined by GLC–MS. The molecular ion (M^+ 196) is prominent, and the $M^+ - 15$ (181) is the base peak. Interestingly, with the *E* isomer, the molecular ion is more intense than the $M^+ - 15$ isomer. This same relationship occurs with the pheromone aldehydes III and IV.

(*Z*)-3,3-Dimethylcyclohexane-$\Delta^{1,\alpha}$-acetic Acid (20) and (*E*)-3,3-Dimethylcyclohexane-$\Delta^{1,\alpha}$-acetic Acid (22). The *Z* and *E* acids were identified by comparison of the GLC–MS with the commercially supplied acids (subsequently chromatographed on a Biosil A column). The acids exhibited expected chromatographic behavior and gave a characteristic acid absorption by IR at 1720 cm^{-1} and a strong band at 1150 cm^{-1}, characteristic of a prominent C–O function. The MS of the *Z* isomer showed a strong M^+ at 168 with the base peak at M/e 153. As with the related aldehydes and ethyl esters, the molecular ion (M/e 168) of the *E* isomer was stronger than the M/e 153. ^{13}C NMR was performed on the mixed isomers. In the spectra, 12 shifts were observed. The shifts for eight of the 10 carbons of the two acids were identical. The shifts for C-4 were 42.7 and 27.0 ppm for the *Z* and *E* isomers, respectively, while the shifts for C-8 were 39.0 and 51.8 ppm for the *Z* and *E* isomers. The shift for the acid carbon was 172.3 ppm, and the vinyl carbon shifts were at 165.1 and 114.0 ppm, consistent with aldehydes III and IV.

(*E*)-3,3-Dimethylcyclohexane-$\Delta^{1,\beta}$-ethyl Acetate (21). The structure for this compound was assigned from its GLC–MS and its relationship to the corresponding *Z* isomer (18) where ^{13}C NMR spectra were obtained from the synthetic species. As with 18, the molecular ion (M^+ 196) is not present and M/e 136 is the base peak, supported by a strong M/e 121 fragment. In contrast to the aldehydes, esters, and acids where there is a reversal in intensity of the M^+ and $M^+ - 15$ species when considering the *Z* and *E* isomers, this reversal does not occur with the $M^+ - 60$ and $M^+ - 75$ fragments.

Ethyl (*E*)-3,3-Dimethylcyclohexane-$\Delta^{1,\alpha}$-acetate (23). The GLC–MS of this compound was identical with that of the synthesized sample. The molecular ion (M^+ 196) was more intense than the M/e 181 as expected for the *E* isomer and previously discussed. ^{13}C NMR revealed a shift of 167.1 ppm for the ester carbon with shifts for the vinyl carbons at 162.1 and 115.1 ppm. The C-4 was assigned 29.8 ppm, and C-8 was assigned 51.7 ppm, in harmony with the *E* configuration of IV. The shift for the C-11 carbon adjacent to the ester carbon was 59.9 ppm as expected, and C-12 was appropriate at 14.9 ppm.

3,3-Dimethylcyclohex-6-en-1-yl Propionate (24). The mass spectrum of the compound showed a very intense M/e 75 with a small apparent M^+ of 182, and with a supporting M/e 167. The suggested structure is generally consistent with an unsaturated propionate ester (Stenhagen et al., 1974b).

(*Z*)-3,3-Dimethylcyclohexane-$\Delta^{1,\beta}$-ethyl Formate (II formate). This compound, which is a homologue of 18, was not found in any of the pheromone samples but was inhibitory in the bioassay. The mass spectrum is nearly identical with that of 18 and 21, with M/e 136 the highest mass fragment present. The ^{13}C NMR spectrum showed a 160.9 ppm shift for the formate carbon and 60.4 ppm for the carbon adjacent to the formate carbon. The vinyl carbons are at 118.0 and 122.0 ppm.

The distribution of the compounds present in the several pheromone preparations (STD 1983 = A and B–G) is

Table V. Laboratory Bioassays of Assorted Boll Weevil Pheromone Formulations with and without Synthetic Intermediate and Byproducts and Index of Attraction[a,b]

sample origin	amt of ea used in bioassay,[c] μg																		I/A: $\bar{X} + S_{\bar{X}}$
	I	II	III + IV	TrII	III	IV	3[d]	4	5	0.1	18	19	20	21	22	23	I acetate	II formate	
STD 1980																			
	2.4	3.2	2.4																0.49 ± .03
STD 1983																			
A	2.4	3.2	1.4[d]						0.2	0.1	0.1	0.1	0.1	0.2	0.1	0.1			0.25 ± .04
B	2.4	3.2	0.4					0.2	1.6	0.1			0.1		0.1				0.23 ± .05
C	2.4	3.2	2.0						0.2				0.1		0.1				0.56 ± .06
D	2.4	3.2	1.6						0.1	0.1			0.3		0.3				0.28 ± .04
E	2.4	3.2	2.1						0.1		0.1			0.1					0.52 ± .05
F	2.4	3.2	2.1								0.1			0.1		0.1			0.40 ± .05
III	2.4	3.2			2.4													0.43 ± .06	
IV	2.4	3.2				2.4													0.40 ± .05
E-II	2.4		2.4	3.2															0.05 ± .02
3	2.4	3.2	2.4				1.0												0.42 ± .04
5	2.4	3.2							2.4										−0.03 ± .02
4 + 5	2.4	3.2						1.2	1.2										0.03 ± .02
	2.4	3.2	2.4					1.2	1.2										0.56 ± .07
12	2.4	3.2	2.4							0.3									0.32 ± .03
	2.4	3.2	2.4							1.0									0.26 ± .05
18	2.4	3.2	2.4								0.3								0.32 ± .04
	2.4	3.2	2.4								1.0								0.25 ± .05
19	2.4	3.2	2.4									0.3							0.52 ± .07
	2.4	3.2	2.4									1.0							0.64 ± .05
20 + 22	2.4	3.2	2.4										0.3		0.3				0.11 ± .04
	2.4	3.2	2.4										1.0		1.0				0.12 ± .03
23	2.4	3.2	2.4													0.3			0.53 ± .06
	2.4	3.2	2.4													1.0			0.73 ± .08
I acetate	2.4	3.2	2.4														0.3		0.30 ± .05
	2.4	3.2	2.4														1.0		0.27 ± .05
II formate	2.4	3.2	2.4															0.3	0.23 ± .03
																		1.0	0.23 ± .04

[a] Index of attraction nIA) defined in text. [b] See Figure 1 for structures and Tables I and IV for nomenclature and distribution with percent content. [c] 8 μg of I/II/III/IV (3/4/1.5/1.5) diluted in 0.1 mL of CH_2Cl_2 for standard bioassay. [d] 2.4 μg of aldehyde preparation actually contained 1.4 μg of III and IV plus listed impurities; similar for B–F.

summarized in Table IV. A was chromatographed to provide four fractions graded by polarity as described above. Samples B–G were analyzed by GLC–MS without any prior treatment. Sample B had performed poorly in bioassays; sample C was actually an endo distillation byproduct of the ethyl ester precursor, then converted to the aldehydes, and was not expected to have much activity. Sample D was thought to have relatively few impurities and expected to be active. Sample E was known to contain at least 10% of the acids **20** and **22** so that its potency was thought to be questionable. Samples F and G, like D, were thought to have relatively few impurities so that good potency was anticipated.

In fact, the predictions for degree of potency were generally upheld (see IA, last line of Table IV). The percent of total GLC integration (Table IV) appears to vary in a generally positive manner with the index of attraction. For simplification, a ratio of III + IV/byproducts was calculated for each of the samples. The IA was satisfactory in two tests where the ratio exceeded 6, but it was unsatisfactory where it did not.

Considering the apparent relative potency contributions of the various byproducts, it appears that a variety may have been involved in the poor performance of B. The endo aldehydes were present in large quantities in C but may only have been diluents; the acids in E appeared to have exerted a negative effect.

A statistical determination of r^2 was made for the ratios of III + IV/byproducts (X) and the index of attraction (Y), employing the data from Table IV and also that obtained from eight additional aldehyde preparations. The correlation ($r^2 = 0.60$) was significant at the 5% level ($r^2 = 0.53$), and $Y = 7.28 + 6.81X$. Thus, the magnitude of byproducts can be a statistical predictor of decreased potency.

Table V summarizes the results of bioassays (IA) obtained from testing of the aldehyde preparations, III, IV, *E*-II, **4 + 5**, and **5** as substitutes for III + IV and seven compounds added to the pheromone in an effort to elicit an inhibitory response. The results of the bioassays of aldehyde preparations B–G were discussed in Table IV but are included here also to show the relative quantities used.

Aldehydes III and IV were each found to be active (when added to I and II) in confirmation of the earlier work of Tumlinson et al. (1969). As with the earlier work, IV was slightly less active than III. *E*-II was not active as a replacement for *Z*-II, again confirming the earlier work.

4 + 5 and **5**, the endo aldehydes, had no potency as replacements for the pheromone aldehydes (IA = 0.03, −0.03) but were evidently not inhibitory, because an IA of 0.56 was obtained when **4 + 5** was added to the complete pheromone. Likewise, **3** was not inhibitory when added to the complete pheromone.

12, when added to the pheromone at 0.3 and 1.0 μg depressed the potency by about 50%. The potency was not further depressed at 3.0 and 10 μg, evidently a common occurrence because this phenomenon was also observed with the other inhibitors.

Quantitatively very similar inhibitions of potency were observed for **18** (II acetate), I acetate, and II formate. The ethyl esters **19** and **23** were not inhibitory. Somewhat greater inhibition of potency was achieved with the **20** and **22** acids (IA = 0.11, 0.12).

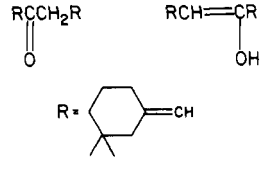
Thus, some but not all of the intermediates and byproducts were demonstrated to be inhibitory at levels at which they had been shown to be present in lower potency aldehyde preparations. Consequently, their presence in aldehyde preparations at a ratio of greater than 1/6 (see Table IV) may be a predictor of poor potency.

As indicated in the experimental section, a 4-year-old sample of IV, though stored in a closed bottle in the freezer, was found to be converted almost totally from IV to about 30% of **12**, plus a number of other products. To assess the magnitude and expected products from some accelerated degradations, two types of studies were carried out.

Using thin films of the aldehydes placed in a desiccator at room temperature, after 24 h small quantities of **9**, **20**, and **22** (an acetate and the acids) arose, but the aldehydes were not noticeably decreased. However, after 120 h, the aldehydes had virtually disappeared, being converted almost totally to the acids **20** and **22**, with some **9**.

In a second study, the aldehydes were dissolved in CH_2Cl_2, the solution was saturated with either O_2 or N_2, and the vial was sealed and maintained at either 25 or 60 °C for stated times. In the treatments using N_2 at either 25 or 60 °C, there was little apparent change after 24 h. However after 72 h at 60 °C, the aldehydes were 90% degraded, being converted mostly to the acids **20** and **22**, and also to some **12**. Using O_2, capping the vial, and holding at 25 °C, after 24 h, little degradation had occurred. Even with O_2 at 60 °C for 24 h, little degradation occurred. However, exposing the sample to O_2 for 72 h at 60 °C caused nearly total degradation of the aldehydes to **9**, **20**, and **22**. It is supposed that the experiments attempting to deny O_2 were flawed, considering the large amounts of acid formed. In any event, the vulnerability of the aldehydes was demonstrated.

Synthetic pheromone sample "H", received after preparation of the manuscript, was observed to contain small amounts of oillike droplets that rose to the top on standing. They were collected with the aid of a separatory funnel. By GLC–MS, the droplets were found to contain approximately a 50/50 mixture of the pheromone components and a second four-component cluster, all with M^+ 288, but of varying fragmentation patterns. The lower mass fragments of two were virtually the same as III, and the other two were virtually the same as IV. One each of the components giving the low mass fragments of III and IV gave M/e 270 ($M^+ - 18$), while the other comparable pair each gave M/e 273 ($M^+ - 15$). Consideration of these data led to the presumptive conclusion that acetals had been formed and that the appearance of the four components could be explained by the *Z* and *E* isomers of the keto and enol forms:



When the droplets were added to the four pheromone components in the ratio 1/10 (w/w), the potency of these formulations deployed in the field was decreased by 10 and 40% in two tests (McKibben, 1985). No droplets were found on reexamination of preparations A–G, nor were these components found in A–G by GLC–MS analysis. Additional work including laboratory evaluations is planned when feasible.

It might be considered that one or several of the inhibitory compounds could be formulated for field use as a confusion agent. This approach has not been very successful, using, for example, the *E* isomer of Lepidopteran pheromones where the *Z* isomer is active although a small amount of the *E* isomer had been shown to inhibit male responses in the laboratory. However, the acids of this study may be adequately stable and may be otherwise more suitable for use as confusion agents.

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