Vittatalactone, a β-Lactone from the Striped Cucumber Beetle, *Acalymma vittatum*

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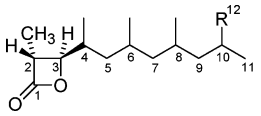
A novel β-lactone, vittatalactone (**1**), was isolated from collections of airborne volatile compounds from feeding male striped cucumber beetles, *Acalymma vittatum*. The structure of **1** was determined to be (3*R*,4*R*)-3-methyl-4-(1,3,5,7-tetramethyloctyl)oxetan-2-one by microderivatization, GC-MS, and NMR studies. The absolute configurations at C-2 and C-3 on the β-lactone ring were assigned by use of the modified Mosher method, applied to the β-hydroxy acid methyl ester resulting from methanolysis of **1**. Biological activity of **1**, possibly as an aggregation pheromone for *A. vittatum*, was indicated by electrophysiological studies using beetle antennae and by the production of **1** by feeding male, and not female, beetles.

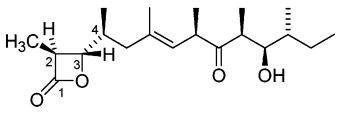
The striped cucumber beetle, *Acalymma vittatum* (F.) (Coleoptera: Chrysomelidae), is a serious pest of curcurbit crops in North America, east of the Rocky Mountains.[1] In spring, adult beetles aggregate and feed on young crops, causing significant damage. A field study of this early season colonization indicated that *A. vittatum* may use a male-produced aggregation pheromone.[2] Consequently, we undertook a chemical study of the volatile compounds released by male beetles feeding on curcurbits, resulting in the isolation and structural elucidation of vittatalactone (**1**), a novel 2,3-disubstituted β-lactone. Electrophysiological responses of male and female *A. vittatum* antennae to **1**, along with its absence in female beetles, is consistent with it being a likely component of the pheromone suggested by the earlier field study.[2]

Several aggregation pheromones have recently been found for chrysomelid beetles: (*S*)-3,7-dimethyl-2-oxo-6-octene-1,3-diol from the Colorado potato beetle, *Leptinotarsa decemlineata* (Say),[3,4] (*E*)-8-hydroxy-6-methyl-6-octen-3-one from the cereal leaf beetle, *Oulema melanopus* L.,[5,6] and himachalene and cadinene sesquiterpenes from *Phyllotreta* and *Aphthona* flea beetles[7] (structures shown in Supporting Information). Although γ-lactones have been identified as female-produced sex pheromones from several scarab beetles,[8] **1** is the first β-lactone to be reported from a beetle, and the third from an insect, after (3*S*,4*S*)-3-hydroxy-3-(1-methylethyl)-4-methyloxetan-2-one and (3*S*,4*S*)-3-ethyl-3-hydroxy-4-methyloxetan-2-one, components of the male-produced courtship pheromone of the giant white butterfly, *Idea leuconoe*.[9,10] The latter two compounds are apparently derived from pyrrolizidine alkaloids sequestered from their host plant.[9,10]

The natural product with the closest structural relationship to **1** is ebelactone A, isolated from a strain of *Streptomyces*,[11,12] which is an inhibitor of esterases,[11] lipases,[11,13] and *N*-formylmethionine aminopeptidases.[11]



1 R = CH_3
2 R = H



Ebelactone A

Biosynthetic studies have indicated that the ebelactones are of polyketide origin, involving propionate-derived subunits,[14] which also appears likely for vittatalactone (**1**).

A possible structure for **1** was indicated initially by microderivatization, along with mass spectrometry, of small collections of volatile compounds released by feeding male beetles. Complete structural elucidation required the collection and purification of a larger amount of **1**, and NMR experiments. Acid methanolysis of **1**, giving β-hydroxy methyl ester **3**, followed by derivatization to give Mosher esters **4a** and **4b** (Scheme 1), allowed the absolute configuration of C-2 and C-3 on the β-lactone ring to be determined.

Results and Discussion

Collections of volatile compounds released by *A. vittatum* were made on Porapak R or using solid-phase microextraction (SPME). These were analyzed by gas chromatography with coupled electroantennogram detection (GC-EAD),[15] using *A. vittatum* antennae. Collections from feeding male beetles contained two compounds that consistently gave electrophysiological responses (Figure 1), from both male and female antennae. These compounds were absent, or detected in only trace amounts, from collections from feeding female beetles and nonfeeding male beetles. The major compound (**1**) had an EIMS resembling that of a monounsaturated branched hydrocarbon. There was a weak signal at *m/z* 254 assigned as the molecular ion, which was supported by signals at *m/z* 239 $[M - CH_3]^+$

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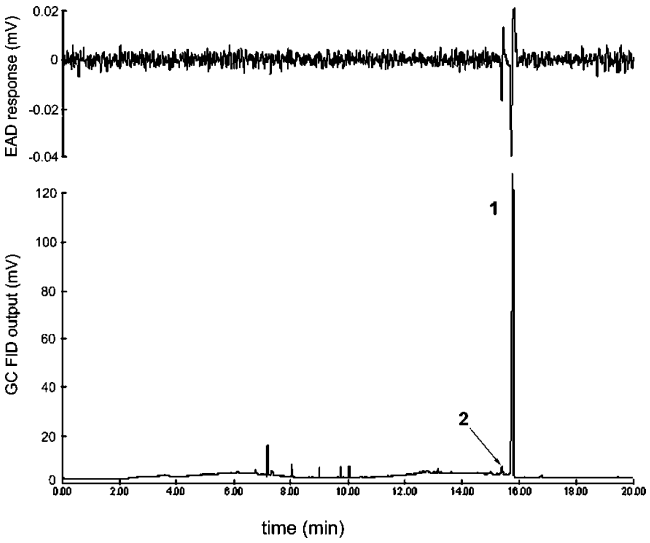
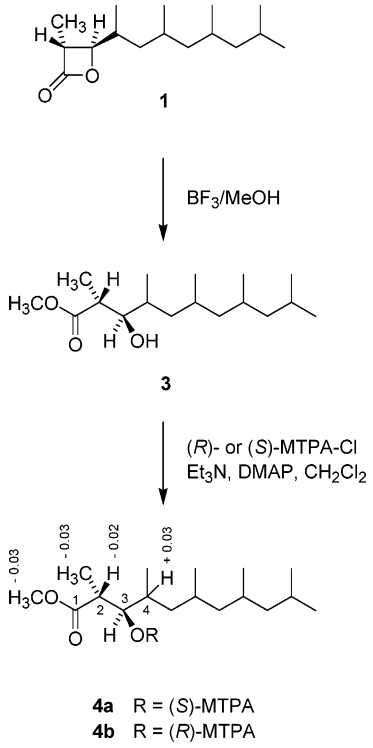


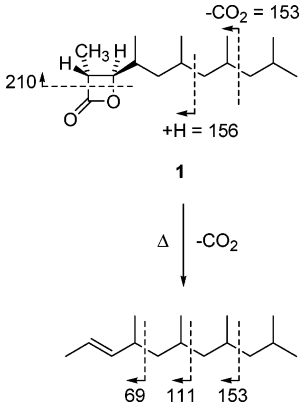
Figure 1. Coupled GC-EAD responses of male *A. vittatum* antennae to a 1.5 h SPME collection from seven male *A. vittatum* feeding on cucumber cotyledons.

Scheme 1. Acid Methanolysis of Vittatalactone (**1**) and Esterification of **3** to Give Mosher Esters



and 236 $[M - H_2O]^+$. Positive CIMS (NH_3) gave a base peak at *m/z* 272 $[M + NH_4]^+$, confirming that the peak at *m/z* 254 in the EIMS was the molecular ion. Purification of Porapak R collections by normal-phase column chromatography gave **1** eluting in hexane−ether (9:1) fractions, indicating that it had polarity similar to an aliphatic ester, aldehyde, or ketone, common insect pheromones.[16] However, treatment with dimethyl disulfide failed to yield an adduct, and **1** could not be hydrogenated, indicating the absence of a carbon−carbon double bond of an unsaturated aliphatic ester. Compound **1** also failed to give an oxime when treated with *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride, suggesting it was not an aldehyde

Scheme 2. EIMS Fragmentation of Vittatalactone (**1**) and the Product of Thermal Decarboxylation of **1** in the GC Injector



or ketone. Acid methanolysis of **1** gave a product with a MS of a 2-methyl-3-hydroxyalkanoic acid methyl ester (compound **3**, Scheme 1), with the base peak at *m/z* 117 (α-cleavage next to −OH) and a prominent peak at *m/z* 88 (McLafferty rearrangement of ester). Base hydrolysis (aqueous KOH) of **1** followed by treatment with ethereal CH_2N_2 also gave **3** by GC-MS, which could be acetylated with AcCl to give the corresponding 2-methyl-3-acetoxyalkanoic acid methyl ester (diagnostic ions at *m/z* 297 (0.3) $[M - OCH_3]^+$, 268 (2) $[M - CH_3COOH]^+$, 169 (4), 117 (17), 88 (26), 43 (100)). These results suggested that **1** had a *β*-lactone ring.

HREIMS established the molecular formula of **1** as $C_{16}H_{30}O_2$; therefore, there were two degrees of unsaturation. A second peak in the mass spectrum at *m/z* 210 indicated loss of carbon dioxide (Scheme 2). A GC-MS peak with a shorter retention time than **1**, apparently formed in the injector by thermal decarboxylation of **1**, had a molecular ion of *m/z* 210 and a fragmentation pattern suggesting a 2,4,6,8-tetramethylundecene (Scheme 2; ions at *m/z* 210 (6), 153 (5), 140 (3), 125 (14), 111 (22), 97 (7), 83 (33), 69 (100), 57 (40), 55 (20), 43 (46), 41 (36)). Decarboxylation also occurred in the GC column, with a rising baseline seen at temperatures >180 °C, which dropped after the peak for **1**, as might occur for a *β*-lactone, which typically decompose between 80 and 160 °C.[17] These results indicated a disubstituted *β*-lactone, with a methyl group at C-2 and a branched alkyl chain at C-3. GC-FTIR gave a strong absorption at 1853 cm^{-1}, also suggesting a *β*-lactone,[17] rather than a *γ*- or *δ*-lactone, or an ester. In the mass spectra of *γ*-lactones and also *δ*-lactones, fragments representing the heterocylic ring usually form the base peak of the spectra, which was not the case with **1**.

Two NMR studies were carried out, the first on approximately 0.1 mg of **1** collected in 2002 (^1H and 2D experiments in C_6D_6) and the second on 0.8 mg collected in 2003 (^1H, ^{13}C, and further 2D experiments in $CDCl_3$). The ^1H NMR spectrum of **1** in C_6D_6 contained a one-proton doublet of quartets at 2.71 ppm coupled to a one-proton doublet of doublets at 3.32 ppm (Table 1). These signals were shifted downfield in $CDCl_3$ to 3.25 and 3.87 ppm, closely matching the values published for protons H-2 and H-3 of ebelactone A.[12,18] An authentic sample of ebelactone A in C_6D_6 gave shifts for H-2 and H-3 that were also very similar to those of **1** in this solvent (Table 1), providing further evidence of a 2,3-disubstituted *β*-lactone. The H-2/H-3 coupling constant of 4.0 Hz indicated that the substituents on the *β*-lactone ring were in a *trans* configuration,[19] as for the ebelactones.[12] 2,3-Disubstituted *β*-lactones with *cis* geometry have been found to have $^3J = 6.5$ Hz for H-2/

Table 1. ^{1}H NMR Data (δ in ppm, J in Hz) for Vittatalactone (**1**) and Selected Data for Ebelactone A

proton	**1** ($CDCl_3$)	**1** (C_6D_6)	ebelactone A ($CDCl_3$)	ebelactone A (C_6D_6)
2	3.25 dq (4.0, 7.5)	2.71 dq (4.1, 7.5)	3.28 dq (4.1, 7.4)	2.59 dq (4.1, 7.5)
3	3.87 dd (4.0, 8.1)	3.32 dd (4.1, 8.2)	3.86 dd (4.1, 8.6)	3.32 dd (4.1, 8.4)
4	1.87 m	1.51 m	1.99 m	1.50 m
5a	1.21 m	0.92 m		
5b	1.01 m	0.64 ddd (6.6, 8.8, 13.9)		
6	1.60 m	1.41 m		
7a	1.19 m	1.06 ddd (5.0, 8.2, 13.6)		
7b	0.89 m	0.74 m		
8	1.55 m	1.51 m		
9a	1.10 ddd (4.4, 9.1, 13.5)	1.10 ddd (4.4, 9.1, 13.6)		
9b	0.91 m	0.92 m		
10	1.65 m	1.62 m		
11	0.84 d (6.6)	0.86 d (6.6)		
12	0.88 d (6.6)	0.90 d (6.6)		
Me-2	1.39 d (7.5)	0.88 d (7.5)	1.39 d (7.5)	0.84 (7.5)
Me-4	1.02 d (6.6)	0.78 d (6.6)		
Me-6	0.90 d (6.6)	0.74 d (6.6)		
Me-8	0.84 d (6.6)	0.84 d (6.6)		

Table 2. ^{13}C NMR Data (δ in ppm) for Vittatalactone (**1**) and Selected Data for Ebelactone A, in $CDCl_3$

carbon	**1**	ebelactone A (lit.[18])
1	172.1	171.7
2	48.9	49.2
3	83.8	82.9
4	34.8	
5	39.8	
6	27.3	
7	45.2	
8	27.7	
9	46.0	
10	25.2	
11	21.8[a]	
12	23.9	
Me-2	12.9	12.8
Me-4	15.8	
Me-6	21.0	
Me-8	20.8[a]	

[a] Assignments may be interchanged.

H-3.[19] Proton signals for the alkyl chain consisted of 5 three-proton doublets, due to methyl branches, and 10 one-proton multiplets for diastereotopic methylene or methine protons. The ^{13}C NMR spectrum of **1** contained 16 resonances (Table 2), with their multiplicities determined from edited DEPT spectra. Apart from 14 signals for aliphatic carbons, there was a signal for a carbonyl carbon at 172.1 ppm and one for a C−O carbon at 83.8 ppm, very similar to the shifts for C-1 and C-3 of ebelactone A (Table 2).[18]

A gHMBC NMR experiment showed that **1** had a 1,3,5,7-tetramethyloctyl chain at C-3 on the β-lactone ring, with correlations from Me-4 protons to C-3 and C-5, from Me-6 protons to C-5 and C-7, and from Me-8 protons to C-7 and C-9; C-9 also had correlations from H-11 and H-12. This confirmed the structure indicated by the MS fragmentation pattern that the chain had a "skipped" methylene motif, also found in several other insect pheromones,[16] in lardolure, the aggregation pheromone of the mite *Lardoglyphus konoi*,[20] and in polypropionates found in marine organisms, especially mollusks.[21−23] Proton and carbon shifts were assigned with the aid of gCOSY, phase-sensitive gDQF-COSY, gHSQC, and gHMBC NMR experiments. gHMBC correlations across the β-lactone ring were present between Me-2 protons and C-1, C-2, and C-3, while the gCOSY experiments showed coupling in this part of the molecule of Me-2 protons to H-2, of H-2 to H-3, and between H-3 and H-4.

The absolute configuration at C-3 was determined by the modified Mosher method.[24] Acid methanolysis of **1** with BF_3−MeOH gave β-hydroxy methyl ester **3** as the major product. After purification by column chromatography, **3** was reacted with either (*R*)- or (*S*)-MTPA-Cl (Scheme 1), to give Mosher esters **4a** and **4b**, respectively. The $\Delta\delta = \delta_S - \delta_R$ values for 1-OMe (−0.03 ppm), H-2 (−0.02 ppm), and 2-Me (−0.03) on one side of the MTPA plane, and H-4 (+0.03) on the other, indicated that the configuration at C-3 was *R*. This is a result similar to that previously obtained for the 3-hydroxy-2,4,6-trimethylheptanoic acid fragment from callipeltin A, which was also 3*R*.[25] Since the H-2/H-3 coupling constant for **1** showed the substituents on the β-lactone ring to be *trans*, the configuration at C-2 must also be *R*, and **1** is 2*R*,3*R*. This is the opposite configuration of the ebelactones which are 2*S*,3*S*, determined by single-crystal X-ray crystallography.[12] Therefore **1** is (3*R*,4*R*)-3-methyl-4-(1,3,5,7-tetramethyloctyl)oxetan-2-one, with unknown stereochemistry at positions 1, 3, and 5 on the tetramethyloctyl chain.

Reductive cleavage of **1** with $LiAlH_4$ gave two hydrocarbons by GC-MS, a branched hexadecane and a branched hexadecene (molecular ions at *m/z* 226 and 224, respectively), which were analyzed on a chiral GC column (Hydrodex β-6TBDMS). As stereoisomers were not detected, beetle-produced **1** may be enantiomerically pure. The absence of peaks for the 2*S*,3*S* enantiomer in the ^{1}H NMR spectrum of either MTPA ester of **1** also indicates that the beetle-produced compound is comprised of the 2*R*,3*R* enantiomer only. Since, in the case of γ-lactone sex pheromones of several scarab beetles, the correct enantiomer is required for attractiveness,[8] the absolute configuration of **1** may be important for pheromonal activity.

The second, minor, compound (**2**) giving a GC-EAD response, with an earlier GC retention time than **1** (Figure 1), had a positive CIMS (NH_3) base peak at *m/z* 258 $[M + NH_4]^+$ and a molecular formula of $C_{15}H_{28}O_2$ by HREIMS. There was a MS fragment ion at *m/z* 196 due to loss of CO_2 and ions at *m/z* 156 $[M - C_6H_{12}]^+$ and 153 $[M - CO_2 - C_3H_7]^+$ similar to those seen in the MS of **1** (Scheme 2). The MS of the thermal decarboxylation products of **1** and **2** were also very similar, apart from the molecular ion being at *m/z* 196 for the latter. From these results **2** appeared to be the nor-analogue of **1**, with a terminal methyl group missing.

While compound **2** may be biosynthesized from five propionate units, in vittatalactone (**1**) valine may serve as the starter (accounting for the *iso*-branching), which is chain-elongated with four propionate units (structures with possible biosynthetic units shown in Supporting Informa-

tion). With five stereocenters, vittatalactone (**1**) and 12-norvittatalactone (**2**) represent the most complex structures among physiologically active insect volatiles identified so far. We are currently attempting synthesis of **1**, to confirm the absolute configuration at C-4, C-6, and C-8 and for use in behavioral and field studies with *A. vittatum* to determine biological activity.

Experimental Section

General Experimental Procedures. NMR spectra were obtained using 11.7 T instruments at 25 °C, either a Bruker DRX 500 (operated at 500.13 MHz for ^{1}H and 125.77 MHz for ^{13}C), for the sample dissolved in C_6D_6, or a Varian Inova (operated at 499.67 MHz for ^{1}H and 125.65 MHz for ^{13}C) for samples dissolved in $CDCl_3$. gCOSY, phase-sensitive gDQF-COSY, gHSQC, and gHMBC experiments (g = gradient-selected) were run using standard pulse sequences. The edited DEPT spectra were acquired on a 9.4 T Varian Inova spectrometer operated at 100.57 MHz. NMR chemical shifts for spectra using C_6D_6 were referenced to TMS; spectra using $CDCl_3$ were referenced to the residual $CHCl_3$ signal at 7.26 ppm for ^{1}H and the solvent signal at 77.0 ppm for ^{13}C. Gas chromatography with electroantennogram detection (GC-EAD) used a Varian 3800 instrument with a flame ionization detector (FID) and an HP-Innowax capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness, Agilent). A temperature program of 40 °C (2 min hold) to 180 °C at 15 °C/min, 180−190 °C at 1 °C/min, then 190−230 °C at 15 °C/min was used, with splitless injection and He as the carrier gas at 30 cm/s linear velocity. The same GC and column were used to compare collections from feeding male and female beetles, with a temperature program of 40 °C (2 min hold) to 230 °C at 15 °C/min. GC-MS was performed on an HP 5890 Series II Plus gas chromatograph fitted with a ZB-wax column (30 m, 0.25 mm i.d., 0.25 μm film thickness, Phenomonex), interfaced with an HP 5972 quadrupole mass spectrometer operated in EI mode with a 70 eV ionizing energy, or in CI mode, with NH_3. Further GC-MS and HREIMS was carried out on an HP 5890 GC, with a BPX5 column (30 m, 0.25 mm i.d., 0.25 μm film thickness, SGE) or a Hydrodex β-6TBDMS column (0.25 mm i.d. × 25 m, Macherey-Nagel), coupled to a VG 70-250 SE magnetic sector machine, at 70 eV ionizing energy. EIMS reported for **1** and the 2,4,6,8-tetramethylundecene thermal decarboxylation product of **1** were recorded on this instrument. The temperature program used for the Hydrodex β-6TBDMS column was 60 °C (3 min hold) to 220 °C at 3 °C/min. GC-FTIR used the HP 5890 GC and a BPX5 column (50 m, 0.32 mm i.d., 0.5 μm film thickness, SGE), coupled to an HP 5965A IRD. Column chromatography used silica gel (Aldrich 60 Å, 130−270 mesh). Solvents were HPLC grade or redistilled prior to use. A sample of ebelactone A (1 mg) was obtained from MP Biomedicals (Irvine, CA).

Insects. Adult *A. vittatum* were collected near Ithaca, NY, from 2000 to 2003. A colony was maintained at the Cornell University Insectary, to supply beetles during winter, and beetles from this colony, or wild adults, were used for experiments. Beetles were sexed and held in separate containers prior to use. Voucher specimens are lodged in the Cornell University Insect Collection, lot no. 1238.

Airborne Collection and Isolation. Air (80 mL/min flow rate) was passed through activated carbon, then through a glass chamber containing male beetles (10−50), feeding on summer squash (*Curcurbita pepo* L.) cotyledons, which were replenished daily. The collection apparatus was placed under a fluorescent tube set to a 16:8 h light:dark cycle. A glass pasteur pipet filled with Porapak R (0.2 g) was attached to the outlet. This was rinsed with CH_2Cl_2 (2 mL) every 2−7 days to recover collected compounds. GC-MS of each collection was used to indicate the amount of **1** relative to other components, and collections with significant amounts of **1** were combined and fractionated by column chromatography on silica gel (0.6 g), in a pasteur pipet, using a steep step-gradient from hexane to Et_2O. Approximately 3000 "beetle-days" of collections yielded 0.8 mg of **1** (mean of 11 ng/h/beetle), eluting in the hexane−Et_2O (9:1) fraction, which was pure **1** by NMR spectroscopy.

Solid-phase microextraction (SPME) (100 μm PDMS fiber, Supelco) was used for GC-EAD experiments, to show that *A. vittatum* antennae gave an electrophysiological response to **1** and to show that **1** was released by male beetles but not by females. Separate collections (1−16 h) were made for male and female beetles (5−10) placed with cucumber (*Cucumis sativus* L.) cotyledons in 5 cm × 2 cm diameter plastic containers, with a small hole in the lid to accommodate the SPME fiber. Collections from males contained **1** as the major compound and were cleaner than equivalent Porapak R collections, which contained greater relative amounts of mono- and sesquiterpenes, along with other contaminants. Compound **1** was absent, or detected in only trace amounts by the GC FID, in parallel collections from females (shown in Supporting Information).

Electrophysiology. GC-EAD used a glass X-connector (Restek) to split the capillary column effluent between the FID and EAD, with the fourth arm admitting N_2 makeup gas. The EAD used an acrylic stage[26,27] to hold two male or two female *A. vittatum* antennae, placed in parallel between wells containing 0.9% aqueous NaCl, which were connected via gold electrodes to a custom-made preamplifier with a circuit similar to one published previously.[28] The acrylic stage was placed 3 cm from the column outlet inside a modified condenser (1.4 cm i.d.), cooled with 0 °C water.[29] Humidified air at 30 mL/min flowed through the condenser and carried GC effluent over the antennae. FID and EAD signals were passed through a Syntech (Hilversum, Netherlands) IDAC-232 unit and recorded using Syntech software.

Microchemical Reactions. Dimethyl disulfide (DMDS) derivatization was carried out as described previously,[30] using 50 μL of DMDS (Aldrich) and 5 μL of 5% I_2 in Et_2O added to a subsample of **1** in 50 μL of heptane, reacted for 20 h at 40 °C. GC-MS revealed no reaction of **1**. Hydrogenation was carried out by addition of a subsample of **1** to $PdCl_2$ on Celite in a pasteur pipet, followed by flushing with H_2 until color change was complete.[31] The sample was eluted with heptane for GC-MS analysis, which showed that **1** was recovered unchanged. Treatment of a subsample of **1** with 5 μg of *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride in 5 μL of MeOH for 0.5 h at room temperature[32] resulted in no reaction of **1** by GC-MS. Acid methanolysis used either 15% BF_3−MeOH (Pierce, Rockford, IL) or saturated HCl−MeOH. Both reactions gave **3** by GC-MS, while the latter reaction also gave what appeared to be two 2,4-dimethyl-4-decyl-γ-lactone diastereomers (EIMS (VG 70-250 SE instrument) *m/z* 155 (5), 125 (7), 113 (100), 97 (10), 83 (18), 69 (25), 57 (13), 55 (12), 43 (56) and 254 (5) $[M]^+$, 221 (14), 155 (5), 125 (8), 113 (100), 97 (4), 83 (24), 69 (30), 57 (13), 55 (12), 43 (55)) due to rearrangement of **1**. Base hydrolysis of **1** was carried out in 0.5 M aqueous KOH (reacted 30 h at room temperature), and resulting products were reacted with ethereal CH_2N_2, prepared on a small scale as described previously,[33] giving **3** by GC-MS. Further reaction of **3** with neat AcCl (5 μL) gave **3**-acetate by GC-MS. Reductive cleavage was carried out by heating a subsample of **1** with <1 mg of $LiAlH_4$ in a sealed tube for 18 h at 220 °C.

Vittatalactone (1): colorless oil; GC-FTIR ν_{max} 1853, 1387, 1113 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; EIMS *m/z* 254 (0.4) $[M]^+$, 239 (0.1) $[M - CH_3]^+$, 236 (0.1) $[M - H_2O]^+$, 221 (0.4), $[M - CH_3 - H_2O]^+$, 210 (3) $[M - CO_2]^+$, 197 (1), 183 (1), 180 (2), 167 (2), 165 (2), 156 (8) $[M - C_7H_{14}]^+$, 153 (4) $[M - CO_2 - C_4H_9]^+$, 140 (8), 138 (5), 125 (17), 123 (10), 113 (25), 111 (25), 97 (12), 85 (47), 83 (50), 71 (40), 69 (100), 57 (58), 55 (30), 43 (77), 41 (42); HREIMS *m/z* 254.2241 $[M]^+$ (calcd for $C_{16}H_{30}O_2$ 254.2246).

12-Norvittatalactone (2): EIMS *m/z* 240 (0.1) $[M]^+$, 196 (0.5) $[M - CO_2]^+$, 182 (1), 169 (2), 156 (6) $[M - C_6H_{12}]^+$, 153 (8) $[M - CO_2 - C_3H_7]^+$, 140 (7), 138 (6), 127 (8), 123 (14), 111 (37), 97 (13), 85 (45), 83 (28), 71 (52), 69 (99), 57 (71), 55 (54),

43 (100), 41 (90); HREIMS *m/z* 240.2077 [M$^+$] (calcd for $C_{15}H_{28}O_2$ 240.2089).

Methyl 3-Hydroxy-2,4,6,8,10-pentamethylundecanoate (3). A subsample of **1** (0.1 mg) was treated with 50 μL of 15% BF_3−MeOH for 17 h at room temperature. The reaction mixture was diluted with 100 μL of aqueous saturated $NaHCO_3$ and extracted with hexane to give **3**, plus a minor amount of β-methoxy acid (from nucleophilic attack on C-3 of **1**), as indicated by a methoxyl singlet in the ^{1}H NMR spectrum at 3.49 ppm. The product mixture was fractionated by column chromatography on silica gel (0.6 g), using a steep step-gradient from hexane to Et_2O; the hexane−Et_2O (7:3) fraction contained **3**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.72 (3H, s, COOMe), 3.62 (1H, m, H-3), 2.64 (1H, dq, J = 7.4, 7.4 Hz, H-2), 2.33 (1H, d, J = 5.8 Hz, OH-3), 1.71 (1H, m, H-4), 1.55−1.67 (3H, m), 1.44 (1H, ddd, J = 6.9, 6.9, 13.8 Hz), 1.19 (1H, m), 1.16 (3H, d, J = 7.3 Hz, Me-2), 1.10 (1H, ddd, J = 4.7, 9.1, 13.4 Hz), 0.97 (1H, ddd, J = 7.1, 7.1, 13.7 Hz), 0.89−0.94 (2H, m), 0.87 (3H, d, J = 7.3 Hz), 0.86 (3H, d, J = 6.7 Hz), 0.85 (3H, d, J = 6.3 Hz), 0.84 (3H, d, J = 6.2 Hz), 0.83 (3H, d, J = 6.5 Hz); EIMS *m/z* 286 (0.01) [M]$^+$, 271 (0.2) [M − CH_3]$^+$, 255 (0.1) [M − OCH_3]$^+$, 237 (0.3) [M − H_2O − OCH_3]$^+$, 211 (0.4), 199 (0.4), 169 (0.7), 125 (2), 117 (100) [M − $C_{12}H_{25}$]$^+$, 88 (63) [M − $C_{13}H_{26}O$]$^+$, 85 (30), 83 (8), 71 (8), 69 (13), 57 (37), 55 (17), 43 (36), 41 (23).

Preparation of (*S*)- and (*R*)-MTPA Esters 4a and 4b. To 0.1 mg of compound **3** in a 2 mL vial was added 4 or 5 small crystals of 4-(dimethylamino)pyridine (DMAP). Distilled dry CH_2Cl_2 (40−50 μL) was added, followed by 0.3 μL of distilled Et_3N and 0.4 μL of either (*R*)-(+)- or (*S*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) chloride (Sigma-Aldrich, 98%). After reaction at room temperature for 19 h, 3-dimethylaminopropylamine (0.4 μL) was added, and the reaction mixture was allowed to stand for 10 min. Addition of 100 μL of 1 M HCl, followed by extraction with 3 × 100 μL of hexane, gave either **4a** (from (*R*)-MTPA-Cl) or **4b** (from (*S*)-MTPA-Cl) after purification by column chromatography on silica gel (0.6 g), using a steep step-gradient from hexane to Et_2O. **4a** or **4b** eluted in the hexane−Et_2O (4:1) fraction.

(*S*)-MTPA ester of methyl 3-hydroxy-2,4,6,8,10-pentamethylundecanoate (4a): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.57 (2H, m, MTPA-ArH), 7.39 (3H, m, MTPA-ArH), 5.39 (1H, dd, J = 2.2, 9.5 Hz, H-3), 3.53 (3H, s, COOMe), 3.49 (3H, s, MTPA-OMe), 2.85 (1H, dq, J = 9.4, 7.2 Hz, H-2), 1.94 (1H, m, H-4), 1.69 (1H, m, H-10), 1.58−1.65 (2H, m, overlapping with H_2O), 1.27 (1H, m), 1.16 (3H, d, J = 7.1 Hz, Me-2), 1.05−1.13 (2H, m), 0.80−0.90 (3H, m, overlapping with Me signals), 0.87 (3H, d, J = 6.7 Hz), 0.86 (3H, d, J = 6.5 Hz), 0.82 (6H, d, J = 6.5 Hz), 0.80 (3H, d, J = 6.5 Hz).

(*R*)-MTPA ester of methyl 3-hydroxy-2,4,6,8,10-pentamethylundecanoate (4b): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.58 (2H, m, MTPA-ArH), 7.39 (3H, m, MTPA-ArH), 5.35 (1H, dd, J = 2.5, 9.2 Hz, H-3), 3.56 (3H, s, COOMe), 3.55 (3H, s, MTPA-OMe), 2.87 (1H, dq, J = 9.2, 7.2 Hz, H-2), 1.91 (1H, m, H-4), 1.62 (1H, m, H-10), 1.59−1.67 (2H, m, overlapping with H_2O), 1.19 (1H, m), 1.19 (3H, d, J = 7.1 Hz, Me-2), 1.07−1.13 (2H, m), 0.80−0.90 (3H, m, overlapping with Me signals), 0.87 (3H, d, J = 6.5 Hz), 0.83 (3H, d, J = 6.5 Hz), 0.83 (3H, d, J = 6.5 Hz), 0.80 (6H, d, J = 6.9 Hz).

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Supporting Information Available: Structures of insect pheromones referred to in the Introduction (chrysomelid aggregation pheromones, β-lactones from the giant white butterfly, and lardolure). GC comparison of SPME collections from male and female striped cucumber beetles. Structures of **1** and **2** showing possible biosynthetic units, compared with ebelactone A. EIMS of compounds **1**−**3** and **3**-acetate and thermal decarboxylation product of **1**. GC-FTIR of **1**. ^{1}H and ^{13}C NMR spectra of **1** in $CDCl_3$. This material is available free of charge via the Internet at http://pubs.acs.org.

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