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Bruchins—Mitogenic 3-(Hydroxypropanoyl) Esters of Long Chain Diols from Weevils of the Bruchidae

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Abstract—Mono- and bis 3-(hydroxypropanoyl) esters of long chain, unsaturated diols have been isolated and identified from two genera of the family Bruchidae, and have been shown to be responsible for the mitogenic activity observed on pea pods resulting from oviposition by the pea weevil, *Bruchus pisorum*. The mitogenic compounds have been characterized and synthesized. Published by Elsevier Science Ltd.

Pods of the *Np* mutant of pea (*Pisum sativum* L.) respond to oviposition by the pea weevil (*Bruchus pisorum* L.) with callus formation at the point of egg attachment.[1–3] The response can also be stimulated by a whole body extract of adult weevils, and although activity can be detected from extracts of either sex, egg-bearing adult females provide extracts of highest activity. In a recent communication[2] we reported that members of an unprecedented class of natural products are responsible for mitogenesis. We here provide a more complete account of the identification and syntheses of these novel and remarkably active (measurable callus formation is produced by applications as low as 0.5 fmol) compounds, for which we have proposed the name bruchins.

Compound Identification

After finding that the response elicited by extracts of pea weevils was also elicited by extracts of the more easily reared cowpea weevil (*Callosobruchus maculatus* L.), we began a bioassay-guided fractionation of whole body extracts of the latter. Two complications quickly became apparent: the active material(s) tended to co-chromatograph with common fatty acids, and the extreme sensitivity of the bioassay could be misleading in that very high activity in the bioassay did not necessarily ensure high purity of a fraction. In our first successful separation, chromatography on Florisil followed by low-pressure liquid chromatography on an ODS column gave an active fraction that was reacted with phenacyl bromide and re-chromatographed on the C18 column. The esterified fatty acids were moved to a different chromatographic window, whereas the chromatographic characteristics of the active material(s) were unchanged. One of two active fractions thus obtained was then subjected to preparative thin layer chromatography on silver nitrate-impregnated silica gel. A band with R_f 0.4 (95:5 $CHCl_3$/MeOH) provided about 0.4 mg of a white solid **1** that was highly active in the pea pod bioassay. The major component of this fraction had limited volatility, but could be successfully analyzed by gas chromatography-mass spectrometry (GC–MS) using a relatively short (15 m) capillary column. Its electron ionization mass spectrum (EI-MS) did not provide a reliable molecular ion, but in many respects resembled the spectrum of oleyl alcohol. Two noteworthy differences were prominent ions with *m*/*z* 73 and 91 in the spectrum of the unknown. Chemical ionization mass spectrometry (CI-MS) with ammonia as reagent gas provided an ammonium ion adduct with *m*/*z* 430, indicating a molecular weight of 412. CI-MS with deuteroammonia established the presence of two exchangeable hydrogens. Consistent with the latter was the formation of a bis-TMS derivative (M_w 556) upon silylation with BSTFA. Although the mass spectrum of the TMS derivative had some features in common with those of certain glycerol ethers, the spectrum of the unknown could not be reconciled with either a glycerol ether or ester.

Treatment of the unknown (**1**) with NaOH in MeOH gave a new compound **2** (Scheme 1), M_w 340, that also contained two exchangeable hydrogens, but whose mass spectrum lacked the ions with *m*/*z* 73 and 91, and also lacked ions likely to be indicative of rings or chain branches. The

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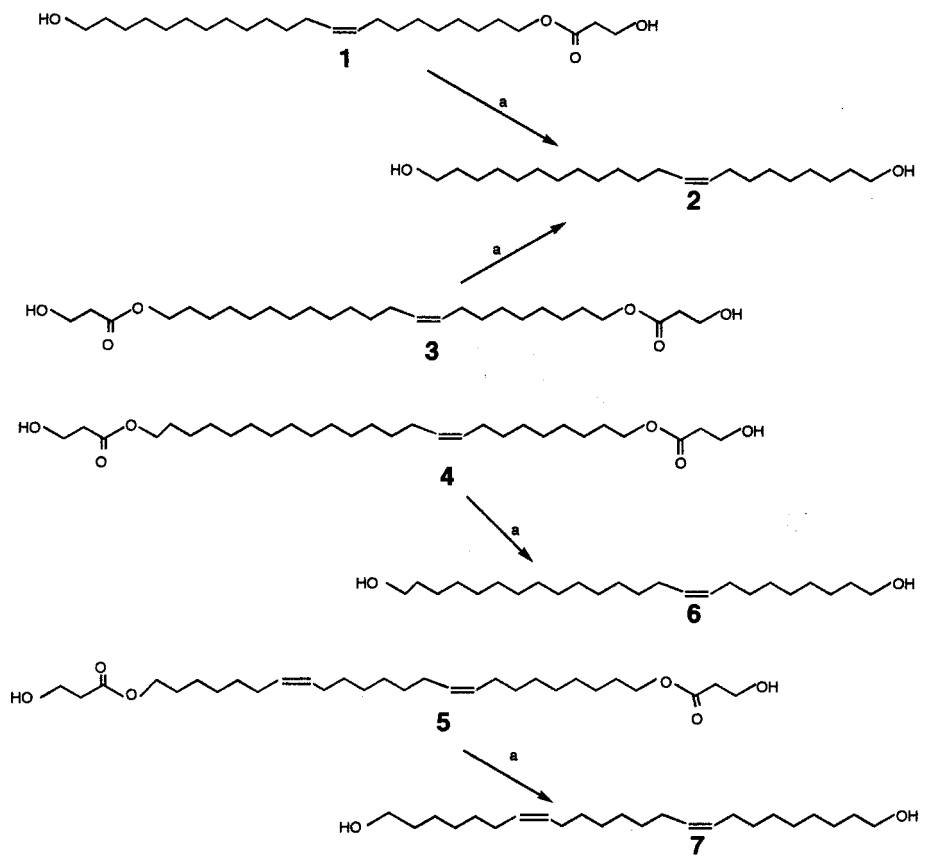


Scheme 1.

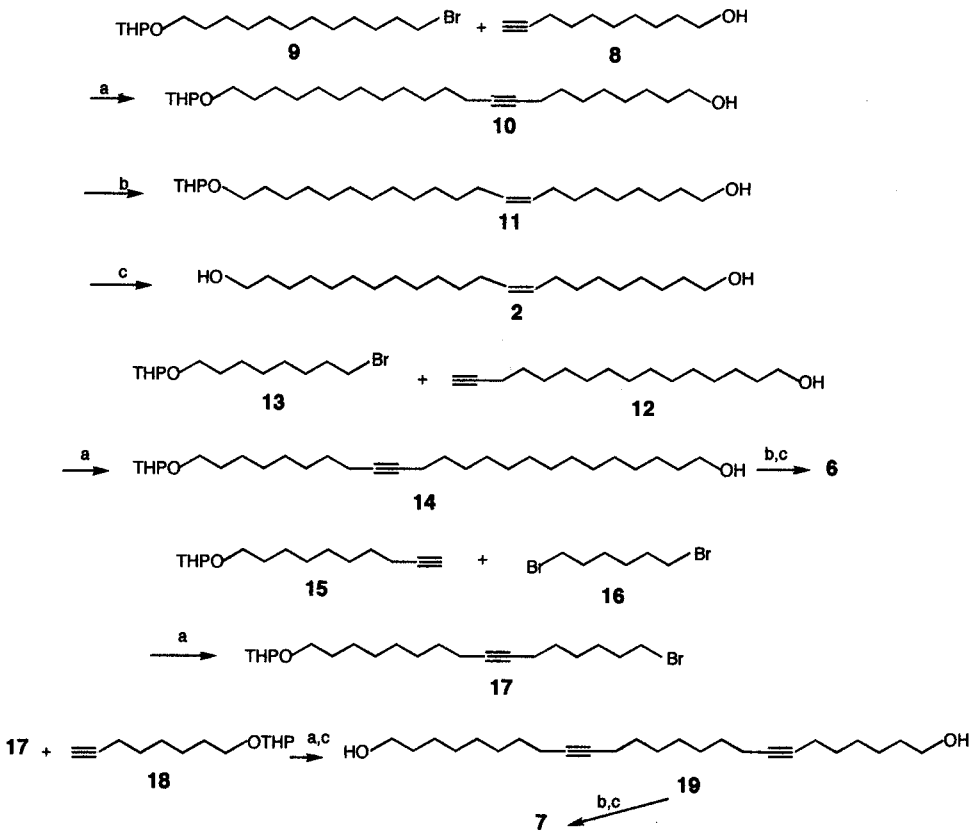
hydrolysis product **2** also formed a bis-TMS derivative whose mass spectrum contained no ions characteristic of TMS ethers of secondary or tertiary alcohols, suggesting an α,ω,-diol. Exhaustive hydrogenation/hydrogenolysis of underivatized **2** with $LiAlH_4/Pt/Al_2O_3$ at 300°C[4] provided *n*-docosane, *n*-heneicosane and *n*-eicosane, indicating that **2** was a docosene-1,22-diol. This was confirmed by catalytic hydrogenation (Pd on C, 1 atm) to produce the known[5] docosane-1,22-diol. Ozonolysis provided fragments corresponding to C_9 and C_{13} hydroxyaldehydes, implying 9-docosene-1,22-diol with double bond geometry yet to be determined.

These facts indicated that the smaller fragment (not observed) produced by base hydrolysis had itself contained an active hydrogen; possible candidates for **1** would thus be monoesters of lactic or 3-(hydroxypropanoic) acids. Available mass spectra of lactate esters did not display prominent *m/z* 73 or 91 ions (and neither did the spectra of either the mono- or diesters from an acid-catalyzed reaction of nonane-1,9-diol and lactic acid). Our mass spectra library contained no spectra of esters of 3-(hydroxypropanoic) acid.

Esters of 3-(hydroxypropanoic) acid are relatively uncommon, and as natural products appear to be virtually unknown. The free acid is a hygroscopic liquid that is usually obtained only in a hydrated form.[6] We initially prepared a sample of *n*-decyl 3-(hydroxypropanoate) as a model compound, and after establishing the presence of the *m/z* 73 and 91 ions in its EI-MS, synthesized a small sample of **1** using the Fleming[7] (vide infra) oxidative desilylation technique for introducing an OH into the hydroxypropanoyl ester.[2] Synthetic **1** matched the isolated compound in all respects including activity in the pea pod bioassay.[2]

At this time we began another bioassay-directed fractionation on a somewhat larger scale. After derivatization of acids to facilitate chromatography[2] and multiple column chromatographies, three major compounds, **3**, **4** and **5**, were obtained, all highly active in the pea pod assay. These were all slightly slower moving on silica gel than **1**, which was, at best, a minor component; they were also later-eluting from the GC, again requiring relatively short columns. The diagnostic *m/z* 73 and 91 ions were evident in the mass spectra of each. All formed bis-TMS ethers, which, although also of limited volatility, performed satisfactorily for GC–MS analysis. Chemical ionization mass spectra of these bis-TMS ethers indicated molecular weights of 628, 656, and 654, respectively. EI-Mass spectra of the TMS ethers all contained ions with *m/z* 103, 145, 147, and 163; although the first three of these ions are not uncommon in spectra of TMS-ethers, especially of polyhydroxylated compounds, collectively the four ions have proved useful for selected ion monitoring-based identifications of bruchin–TMS-derivatives.

Saponification of **3** gave the same C_{22} monounsaturated diol **2** encountered earlier, indicating that **3** was the



Scheme 2.

bis-3-(hydroxypropanoyl) ester of **2**. Compounds **4** and **5** also proved to be bis-3-(hydroxypropanoyl) esters; saponification provided mono- and diunsaturated C_{24}-diols **6** and **7**, respectively, whose double bond locations were again determined by ozonolysis. We suspected these compounds to be lipid-derived, and that double bond geometries would be *Z*. Note that all the three diols have the conventional (*Z*)-9 double bond, i.e. analogous to oleic acid, and that **6** is simply a two carbon homolog of **2**. The diunsaturated diol **7** also contains a (*Z*)-9 double bond (if numbered from the longer end of the chain), but contrary to our expectation, was not a 'skipped' diene analogous to many polyunsaturated fatty acids, but instead had the two double bonds separated by six methylenes.

Adequate quantities of **3**, **4** and **5** were isolated to permit NMR analyses. NMR spectra supported the assignments discussed, and confirmed the (*Z*)-configuration of all double bonds. This was achieved by a recently described procedure:[8] an experiment was designed, which isolates the individual olefinic protons that are coupled to the attached ^{13}C atoms and are also coupled to the vicinal olefinic proton as well as to the two vicinal methylene protons. In order to simplify this pattern and to facilitate spectral interpretation, the vicinal methylene protons were simultaneously decoupled during acquisition. Olefinic homonuclear coupling constants of ~11 Hz for the natural bruchins indicated that the configurations of these double bonds were indeed (*Z*).[8]

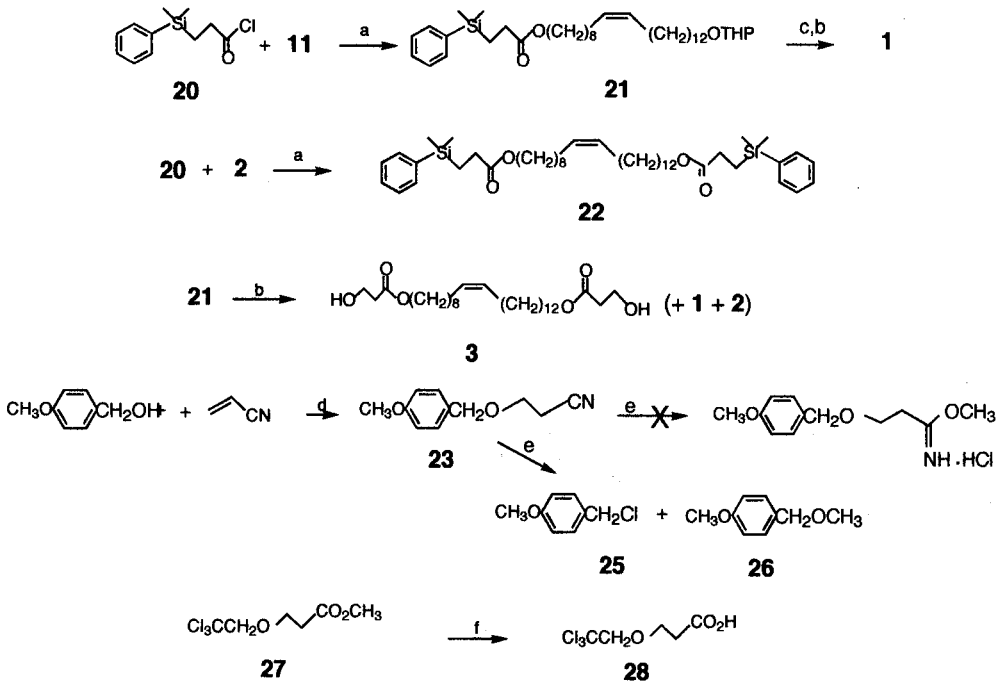
α,ω-Diol Syntheses

Construction of C_{22} and C_{24} mono- and diunsaturated diols were achieved using conventional acetylene alkylation reactions and semihydrogenations with Lindlar[9] catalyst (Scheme 2). 9-Decyn-1-ol **8** was alkylated with the tetrahydropyranyl (THP) ether of 12-bromododecan-1-ol **9**,[10] and the product was subjected to semihydrogenation to give the monoprotected (*Z*)-9-docosen-1,22-diol **11**. Removal of the THP ether gave diol **2**, indistinguishable (GC and GC–MS of the diol and of its TMS–ether) from the saponification product of **1**.

Diol **6** was prepared by alkylating 15-hexadecyn-1-ol[11] **12** with the THP ether of 8-bromooctanol **13** to give the mono THP **14**, followed by semihydrogenation and deprotection (Scheme 2). Two acetylenic units were used in the synthesis of **7**: the THP ether of 9-decyn-1-ol **15** was deprotonated with butyllithium and alkylated with excess 1,6-dibromohexane **16**. Monoadduct **17** was obtained in 94% yield after flash chromatography; the diadduct accounted for no more than 3% of the product. The THP ether of 7-octyn-1-ol **18** was similarly deprotonated and allowed to react with **17** to afford, after deprotection, diyne **19**. Semihydrogenation and deprotection provided (*Z*,*Z*)-7,15-tetracosadiene-1,24-diol **7**.

3-Hydroxypropanoyl Esters

Because monoester **1** was the first compound identified,

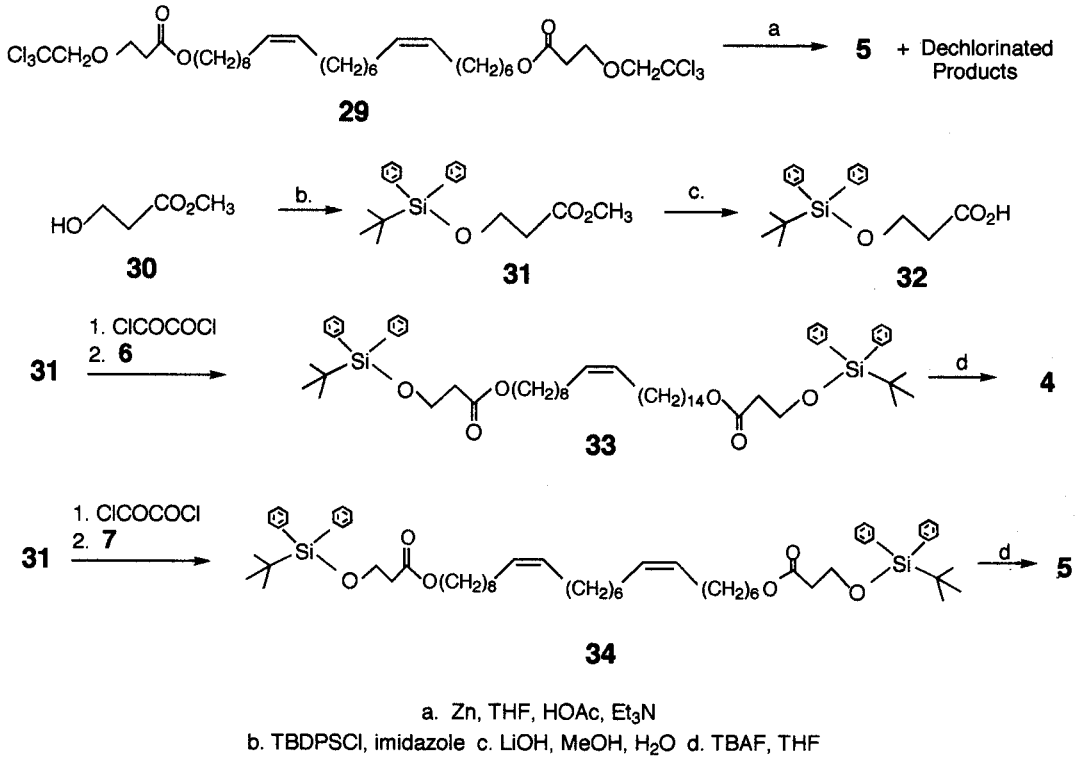


Scheme 3.

mono THP **11** was esterified with acid chloride **20** (from the known[12] methyl 3-(phenyldimethylsilyl) propanoate (Scheme 3) to give **21**. Removal of the THP protecting group followed by oxidative desilylation (KF, $NaHCO_3$, H_2O_2)[7,13] gave compound **1** accompanied by diol **2**. Esterification of diol **2** with **20** (2 equiv.) gave diester **22**; the same oxidative desilylation procedure afforded bis 3-(hydroxypropanoyl) ester **3**. Yields, however, were disappointing, and **3** was accompanied by the alcohols **1** and **2** resulting from ester hydrolysis. A recent review[14] indicates that although a number of methods have been developed for oxidative desilylation, very few are likely to be compatible with both double bonds and esters. We accordingly turned our attention to alternative methods for constructing 3-(hydroxypropanoyl) esters.

Acylation of long chain alcohols with propiolactone did not proceed well, and in any event, the products, themselves being primary alcohols, might be expected to continue to react with additional reagent. Limited attempts toward deprotonation of acetates with subsequent reaction with formaldehyde were unrewarding (small amounts of 3-(hydroxypropanoyl)esters were detectable, but were accompanied by larger amounts of unchanged acetate along with the primary alcohol resulting from deacetylation), as were reactions of bromoacetates with zinc and formaldehyde (Reformatsky conditions). Hydrogenolysis of 3-(benzyloxy)propanoate esters has been described,[15,16] the acids having been obtained by addition of benzyl alcohol to acrylonitrile followed by methanol/HCl conversion to a methyl imidate and finally hydrolysis to the methyl ester or free acid (analogous to line 4 of Scheme 3). We have used this route for the construction of several saturated analogs of **3**–**5**, but the hydrogenolysis used to remove the benzyl protecting group is incompatible with olefinic compounds. We intended to explore the use of 3-(*p*-methoxybenzyloxy)propanoate esters because the *p*-methoxybenzyl protecting group should be removable under oxidative conditions;[17] *p*-methoxybenzyl alcohol reacted exothermically with acrylonitrile to form the desired 1,4 adduct **23** (Scheme 3); **23** in turn reacted readily with methanol and anhydrous HCl (using conditions employed for methanolysis of 3-(benzyloxy)propanenitrile), but did not give the expected imidate **24**. Instead, two volatile compounds were formed, and although they have not been exhaustively characterized, their mass spectra indicate them to be *p*-methoxybenzyl chloride **25** and *p*-methoxybenzyl methyl ether **26**. This approach was not pursued further.

Greene and Wuts[17] list a single example of a 2,2,2-trichloroethyl ether as a protecting group for alcohols, although the use of trichloroethyl esters, carbonates, and carbamates along with trichloroethoxymethyl ethers has been better documented.[17] Zinc, in acetic acid and other media, has frequently been used to remove these groups, conditions we felt likely to be compatible with both esters and double bonds. 3-(2,2,2-Trichloroethoxy)propanoic acid **28** was prepared by HBr hydrolysis of the known[18] methyl ester **27** (hydrolysis of **27** under alkaline conditions was accompanied by considerable retroadditon of trichloroethanol). Esters of **28** such as **29** (Scheme 4) could be converted to 3-(hydroxypropanoyl) esters with zinc in methanol containing triethylamine and acetic acid;[19] however, yields were unsatisfactory and the products were accompanied by byproduct(s) that, although not rigorously characterized, appeared to provide the analogous esters containing one less chlorine (and which were unreactive toward the desired reductive elimination). Precedent for this type of



Scheme 4.

dechlorination has been reported during the removal of the trichloroethoxymethyl group with zinc–copper or zinc–silver couples in methanol.[19] Other reductive conditions including zinc in acetic acid, zinc in buffered THF[20] or chromium perchlorate in DMF[21] failed to produce more satisfactory results.

Although 3-hydroxypropanoic acid is inconvenient to work with, its methyl ester **30** can be conveniently prepared from 3-hydroxypropionitrile[22] (Scheme 4). We converted **30** to the known *t*-butyldiphenylsilyl ether **31**.[23] Saponification with lithium hydroxide in methanol–water was accompanied by some desilylation, but provided satisfactory yields of acid **32** (the previous preparation featured monosilylation of propane-1,3-diol and Jones oxidation[24]). This acid, via its acid chloride, was readily esterified with diols **6** and **7** to provide esters **33** and **34**, respectively. Tetrabutylammonium fluoride in THF smoothly promoted deprotection at room temperature to give the bis-3-(hydroxypropanoyl) esters **4** and **5**, respectively. Both proved indistinguishable from the isolated materials, and both stimulated callus formation at applications of 1 pg or less in the pea pod assay.

In summary, 3-(benzyloxy)propanoic acid is probably the most practical precursor of 3-(hydroxypropanoyl) esters of alcohols compatible with catalytic hydrogenation. However, esters of 3-((*t*-butyldiphenylsilyl)oxy)propanoic acid **32** provide viable alternatives in cases where hydrogenation is not an option.

Bruchins

As stated in the introduction, the isolation and characterization of **1** and **3**–**5** was from extracts of the cowpea weevil (*Callosobruchus maculatus* L.). We next turned our attention back to the species with which the mitogenic activity had originally been associated, the pea weevil *Bruchus pisorum* L. Active chromatographic fractions of a whole body extract were assayed by GC–MS using selected ion monitoring (the *m/z* 73 and 91 ions for underivatized samples, and *m/z* 103, 145, 147 and 163 for trimethylsilylated samples). Although we detected several additional, albeit very minor, components providing spectra characteristic of 3-(hydroxypropanoyl) esters, the major components were the same bis-3-(hydroxypropanoyl) esters **3**–**5** previously characterized. This was somewhat surprising in view of the fact that although belonging to the same family (Bruchidae), the two insects occupy different genera. Thus our results indicate that in two different bruchids, bis 3-(hydroxypropanoyl) esters **3**–**5** are the principal mitogens, and that monoesters such as **1**, although active mitogens, are in general less abundant. Neither these esters (**1**,**3**,**4**,**5**) nor their diol precursors (**2**, **6**, **7**) appear to have been described previously, and it is noteworthy that the diols themselves do not promote callus formation. With the exception of **5** and **7**, which are liquids at room temperature but solidify in a refrigerator, the esters and diols are crystalline solids that are stable for at least several months at room temperature. We are continuing to explore the scope and generality of this insect–plant interaction, and to examine the structure–activity relationships of these compounds and synthetic analogs.

Experimental

Melting points are uncorrected. NMR spectra were obtained

with a Bruker DRX 600 spectrometer (Billerica, MA) on deuterochloroform solutions at 600 MHz for ^{1}H, and 150 MHz for ^{13}C. Mass spectra were obtained from a Finnigan-MAT Incos-50 GC–MS equipped with a 15 m DB-5 fused silica column. Electron ionization spectra were collected at 70 eV and a source block temperature of 150°. Chemical ionization spectra were collected using ammonia as reagent gas (reagent gas pressure 0.5 Torr.) at a source temperature of 60°. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. Mention of a proprietary product does not imply endorsement by the US Department of Agriculture.

Microreactions of unknowns

Saponification. Typically, a solution containing 10–30 μg of the unknown ester was evaporated to dryness in a conical vial, and the residue was treated with 10 μL MeOH plus 5 μL of 25% NaOH in 1:1 MeOH/H_2O. The vial was tightly capped and heated 30 min at 70°, then was cooled and the contents neutralized with 10 μL 2N HCl and concentrated with a stream of N_2. The white residue was extracted with several 10 μL portions of EtOAc which were passed through a micro column of silica gel (contained in a capillary drawn from a Pasteur pipette) into a clean conical vial. The volume was adjusted for GC or subsequent transformations.

Trimethylsilylation. A solution containing ca. 10 μg of the compound(s) to be derivatized was concentrated with a stream of nitrogen, and the residue was treated with 10 μL of bis(trimethylsilyl)trifluoroacetamide (BSTFA) that had been amended with 1% chlorotrimethylsilane. The vial was tightly capped and heated at 60° for 15–20 min, then a stream of N_2 was used to evaporate excess reagent and an appropriate volume of hexane was added for GC or GC–MS.*Ozonolysis.* A solution of ca. 10 μg of an olefin in 5 μL CH_2Cl_2 was cooled with dry ice–acetone and treated with 20 μL of a blue solution of freshly generated ozone trapped in CH_2Cl_2 at −78°. The vial was removed from the cooling bath for several seconds, then again cooled and dimethylsulfide (2–3 μL) was added. After warming to room temperature and concentrating with a stream of N_2, an appropriate volume of solvent was added for GC or GC–MS. In addition to the expected hydroxyaldehydes and dialdehyde from unsaturated diols **2**, **6** and **7**, aldehyde–acids were in some cases also observed, apparently from oxidation of the primary alcohols under the conditions.

Semihydrogenations. Reductions of alkynes to (*Z*)-alkenes were conducted with lead-poisoned, 5% palladium on calcium carbonate (Lindlar[9] catalyst, Strem Chemicals, Inc., 50–75 mg per g of alkyne) at one atmosphere and room temperature, using freshly distilled cyclohexene as solvent or as a cosolvent with absolute ethanol or ethyl acetate. The use of an olefinic solvent eliminates or greatly reduces possible overreduction, and the reactions, sometimes difficult to monitor with precision, could be allowed to proceed several hours, or even overnight in at least two cases, to ensure completion.

9-Docosyne-1,22-diol, 22-tetrahydropyranyl ether 10 and (*Z*)-9-docosene-1,22-diol, 22-tetrahydropyranyl ether 11. A solution of 9-decyn-1-ol **8** (3.85 g, 25 mmol) in dry THF (50 mL) was cooled to −70° and treated dropwise with 22 mL of 2.5 M butyllithium in hexanes. A thick precipitate separated. Hexamethylphosphoric triamide (HMPA, 10 mL) was added, and the mixture was mechanically stirred and warmed to room temperature, then again cooled to −70°. Sodium iodide (ca. 200 mg) was added followed by the THP ether of 12-bromo-1-dodecanol[25] **9** (8.4 g, 24 mmol). The mixture was allowed to warm to room temperature and stir overnight, then was partitioned between water and ether–hexane (1:1). The crude product (10.8 g) was flash chromatographed (20–25% EtOAc in hexanes) to give 5.30 g of the mono THP ether of 9-docosyne-1,22-diol **10**. This material (CI-MS *m/z* 440, M+18) was used without further characterization. A portion was semihydrogenated as described above, and the resulting **11** (CI-MS *m/z* 442, M+18) was used without further purification.

(*Z*)-9-Docosene-1,22-diol 2. A solution of **11** (3.0 g) in methanol (50 mL) containing toluenesulfonic acid monohydrate (0.15 g) was stirred at room temperature for 5 h. The solvent was concentrated and the residue was partitioned between ether and aq. Na_2CO_3. The ether solution was dried and concentrated, and the residue was crystallized from isooctane and 1–2% EtOAc to give 1.38 g of diol **2**, mp 56–57°. ^{1}H NMR: 5.25 (2H, m), 3.64 (4H, t, *J*=7.4 Hz), 2.01 (2H, m), 1.56 (2H, q, *J*=6.6 Hz), 1.39–1.22 (12H, ovlp). ^{13}C NMR: 129.9, 129.8, 63.1, 63.1, 32.8, 32.8, 29.7, 29.6, 29.6, 29.5, 29.5, 29.4, 29.4, 29.2, 29.2, 27.2, 25.7, 25.7. CI-MS *m/z* 358, M+18; EI-MS, *m/z* (%) 123 (10), 110 (16), 109 (21), 97 (19), 96 (46), 95 (41), 94 (12), 83 (30), 82 (62), 81 (55), 80 (22), 79 (14), 69 (45), 68 (36), 67 (58), 56 (12), 55 (100), 54 (28), 43 (32), 42 (16), 41 (66). Further chilling of the mother liquor afforded a second crop (0.19 g). Anal. Calcd for $C_{22}H_{44}O_2$: C, 77.58; H, 13.02. Found: C, 77.34; H, 13.33.

(*Z*)-9-Tetracosene-1,24-diol 6. A solution of 15-hexadecyn-1-ol[11] (**12**, 4.76 g, 20 mmol) in THF (40 mL) was stirred mechanically and cooled to −75° under nitrogen and treated dropwise with 16 mL 2.5 M butyllithium. After warming to about 0° then again cooling with dry ice–acetone, a solution of the THP ether of 8-bromooctanol (**13**, 5.37 g, 18 mmol) in THF (10 mL) and HMPA (5 mL) was added. The mixture was allowed to warm to room temperature and stir overnight, then was partitioned between hexane and water to give 9.21 g of an amber oil. This material was deprotected by stirring in methanol (200 mL containing 0.5 g toluenesulfonic acid hydrate) for 1 h; after removal of most of the methanol, the residue was partitioned between EtOAc and dilute HCl. After rinsing with water and aq. $NaHCO_3$, the solution was dried and concentrated, and the residue (7.6 g) was flash chromatographed (20, 40 and 60% EtOAc in hexanes). A major component was unreacted 15-hexadecyn-1-ol **12** (3.03 g); also obtained was the desired 9-tetracosyne-1,24-diol (2.83 g, 43%), mp 73° (isooctane), ^{1}H NMR ($CDCl_3$, 600 MHz) 3.36 (4H, q, 6.6 Hz), 2.10 (4H, t, 6.6 H), 1.42–1.20 (32 H, ovlp). ^{13}C NMR 85.0, 65.8, 37.6, 34.2, 34.2, 34.1, 34.1, 34.1, 34.0, 33.7, 34.1, 33.6, 33.6, 33.2, 33.2, 23.1. CI-MS *m/z* 384, M+18). Two grams of this material was semihydrogenated and the olefin was recrystallized

from isooctane to give **6** (1.65 g), mp 62–63°. ^{1}H NMR: 5.32 (2H, m), 3.36 (4H, t, J=6.6 Hz), 1.98 (4H, q, J=6.4 Hz), 1.39 (m), 1.32–1.2 (ovlp). ^{13}C NMR: 129.6, 129.6, 60.7, 32.5, 29.1, 29.1, 29.0, 29.0, 29.0, 28.9, 28.9, 28.9, 28.9, 28.8, 28.6, 28.5, 26.6, 26.5, 25.5. CI-MS *m/z* 386 (M+18); EI-MS *m/z* (%): 125 (13), 123 (11), 111 (21), 110 (16), 109 (29), 97 (26), 95 (37), 94 (11), 83 (37), 82 (45), 81 (46), 80 (18), 71 (13), 70 (12), 69 (100), 68 (32), 67 (50), 57 (29), 56 (14), 55 (99), 54 (23), 43 (32), 42 (12), 41 (47). Anal. Calcd for $C_{24}H_{48}O_2$: C, 78.19; H, 13.12. Found: C, 77.80; H, 13.42.

(*Z*,*Z*)-7,15-Tetracosadiene-1,24-diol 7. A solution of the THP ether of 9-decyn-1-ol (**15**, 2.35 g, 10 mmol) in dry THF was cooled to −75° under nitrogen, and butyllithium (4.4 mL, 2.5 M) was added by syringe. The solution was allowed to warm to about 10°, then was again cooled to −75° and HMPA (2 mL) and NaI (about 50 mg) were added, followed by 1,6-dibromohexane (**16**, 5 mL, 32 mmol). The solution was allowed to warm to room temperature and stir overnight, then was partitioned between water and hexanes. Evaporation of solvent left 9.17 g of a liquid that was added to a silica gel column (35 g). Elution with hexane gave 4.44 g of unreacted **16**; subsequent elution with 10% ethyl acetate in hexanes provided 4.93 g of an oil that was flash chromatographed on 100 g silica gel, eluting with 2.5–10% ethyl acetate in hexanes to provide 3.79 g (94%) of the tetrahydropyranyl ether of 16-bromohexadec-9-yn-1-ol **17** (CI-MS *m/z* 418 and 420, NH_4^+ adducts). A solution of the THP ether of 7-octyn-1-ol (**18**, 1.05 g, 5 mmol) in dry THF (20 mL) was cooled to −75° under nitrogen, and 2.4 mL of 2.5 M butyllithium was added. The cooling bath was removed for a few minutes, then reapplied. HMPA (1 mL), NaI (50 mg), and **17** (2.0 g, 5 mmol) were added. The solution was allowed to warm to room temperature and stirred overnight, then was partitioned between water and hexanes. The crude product, which also contained both reactants **17** and **18**, was flash chromatographed (5 then 10% ethyl acetate in hexanes) to afford 1.27 g (48%) of the bis-tetrahydropyranyl ether of 7,15-tetracosadiyne. Nearly all of this material was semihydrogenated to give 1.23 g of crude diene that was added to methanol (25 mL) and treated with toluenesulfonic acid (25 mg). The initially insoluble diene gradually dissolved as deprotection occurred; after stirring overnight most of the methanol was removed and the residue was partitioned between methyl *t*-butyl ether and aqueous sodium carbonate. A colorless oil (0.84 g) was thus obtained that was flash chromatographed with 40, then 50% ethyl acetate in hexanes to provide 0.53 g of **7** as a clear viscous oil. ^{1}H NMR: 5.39 (4H, m), 3.64 (4H, t, J=5.8 Hz), 2.01 (8H, m), 1.56 (4H, q, J=5.8 Hz), 1.45–1.25 (26 H, ovlp). ^{13}C NMR: 130.4, 130.3, 130.1, 63.5, 63.4, 33.1, 30.1, 30.1, 30.1, 30.1, 29.9, 29.8, 29.6, 29.5, 29.5, 27.6. 27.5, 26.1, 26.0. CI-MS *m/z* 384 (*M*=18); EI-MS, *m/z* (%), 135 (17), 123 (11), 121 (23), 110 (14), 109 (26), 108 (12), 107 (14), 97 (22), 96 (48), 95 (62), 94 (28), 93 (24), 83 (27), 82 (54), 81 (73), 80 (41), 79 (31), 69 (43), 68 (39), 67 (99), 57 (15), 56 (11), 55 (100), 54 (35), 53 (11), 43 (25), 41 (75). Anal. Calcd for $C_{24}H_{46}O_2$: C, 78.62; H, 12.65. Found: C, 78.67; H, 12.86.

(*Z*)-9-Docosene-1,22-diol,3-(phenyldimethylsilyl)propanoate ester, 22-tetrahydropyranyl ether 21. A solution of **11** (424 mg, 1 mmol) in benzene (5 mL) and pyridine (0.25 mL) was treated with 3-(phenyldimethylsilyl)propanoyl chloride **20** (prepared from 0.33 g of the corresponding acid[13] and excess oxalyl chloride). The mixture was stirred overnight, then partitioned between hexanes and water to give 0.68 g of a clear oil. Flash chromatography (5 then 10% EtOAc in hexanes) gave 0.30 g of **22** as an oil, CI-MS *m/z* 632 (M+NH_4).

(*Z*)-9-Docosene-1,22-diol mono 3-(hydroxypropanoyl) ester 1. *A. By oxidative desilylation:* A solution of **22** (73 mg) in MeOH (2.5 mL) containing about 10 mg TsOH was stirred for 1.5 h at room temperature, then was concentrated and the residue partitioned between aq. $NaHCO_3$ and ether–hexane (1:1) to give 60 mg crude product. To 53 mg of this material was added dichloromethane (400 μL) and fluoroboric acid etherate (125 μL). After 5 h at room temperature the product was partitioned between ether and aq. $NaHCO_3$; the organic portion was treated with THF (400 μL), MeOH (400 μL), $NaHCO_3$ (44 mg), KF (44 mg), and 30% H_2O_2 (200 μL). After 45 min the mixture was partitioned between dichloromethane and water, and the organic products (9 mg) were flash chromatographed with 25–50% EtOAc in hexanes. About 2.8 mg of **1** was obtained that was indistinguishable from the natural product and that was very active in the pea pod assay.

B. By F-catalyzed desilylation: A solution of **32** (0.90 g) in benzene (5 mL) was treated with 0. 5 mL oxalyl chloride and 1 μL DMF. After 1.5 h at room temperature the solvent and excess oxalyl choride were stripped followed by another 5 mL portion of benzene. The residue was again dissolved in 5 mL benzene, and a solution of **10** (1.06 g, 2.5 mmol) in benzene (5 mL) and pyridine (0.6 mL) was added dropwise. After stirring at room temperature for 1.25 h, water was added and the mixture was partitioned between water and ether–hexane. The organic extracts were rinsed with dilute HCl and aq. $NaHCO_3$, dried, and concentrated to yield 2.12 g of a clear liquid. Flash chromatography (5 and 10% EtOAc in hexanes) provided 1.01 g of the desired ester (56%) as a clear glass. Most of this sample was semihydrogenated as described above. To the product was added THF (6 mL) and 1 M tetrabutylammonium fluoride in THF (2.7 mL), and the solution was allowed to remain at room temperature 1.5 h. It was then partitioned between 1N HCl and ether to give 1.01 g of a product that was treated directly with methanol (40 mL) and toluenesulfonic acid hydrate (95 mg). After 2.5 h at room temperature, most of the methanol was evaporated and the residue was partitioned between ethyl acetate and water. The organic phase was rinsed with aq. $NaHCO_3$, then dried and concentrated to give 0.86 g of an oil that solidified upon standing. Recrystallization from hexane gave 0.31 g of **1**, mp 47–48° that was identical to the sample described above. ^{1}H NMR: δ 5.34 (2H, m, olefinic), 4.11 (2H, t, J=4.4 Hz, CH_2CO_2R), 3.86 (2H, t, J=3.9 Hz, $-O_2CCH_2CH_2OH$), 3.63 (2H, t, J=4.6 Hz, alkyl-CH_2OH), 2.70 (2H, t J= 3.8 Hz, $-O_2CCH_2CH_2OH$), 2.09 (4H, m, allylic), 1.78 (br. s., O*H*). EIMS, *m/z* (%): 412 $[M]^+$ (ca. 0.4), 124 (14), 123 (16), 121 (14), 111 (11), 109 (28), 97 (25), 96 (59), 95 (56), 94 (21), 93 (12), 91 (58), 83 (34), 82 (74), 81 (69), 80 (36), 79 (18), 73 (83), 71 (11), 69 (51), 68 (36), 67 (67), 57 (19), 56 (14), 55 (100), 54 (31), 45 (13), 43 (42), 42 (14), 41 (58).

Anal. Calcd for $C_{25}H_{48}O_4$: C, 72.76; H, 11.72. Found: C, 72.49; H, 12.04. **1-bis(Trimethylsilyl) ether**, EIMS *m/z* (%): 556 $[M]^+$ (ca. 0.2), 541 (ca. 0.2), 235 (10), 219 (22), 163 (31), 149 (10), 147 (100), 109 (13), 105 (28), 103 (79), 97 (12), 96 (14), 95 (20), 91 (12), 83 (17), 82 (14), 81 (23), 75 (58), 73 (57), 69 (24), 67 (23), 55 (41), 43 (15), 41 (16).

(*Z*)-9-Docosen-1,22-diol bis (3-hydroxypropanoate) ester 3. Diol **2** (0.5 mmol) was esterified with **32** (1 mmol) as described above. The crude diester (^{1}H NMR: 5.34 (2H, m), 4.11 (4H, *J*=5.8 Hz), 3.87 (4H, q, *J*=5.8 Hz), 2.57 (4H, t, *J*=5.8 Hz), 2.43 (2H, t, *J*=5.8 Hz), 2.01 (4H, *J*=5.8 Hz), 1.63 (4H, quin, *J*=7 Hz), 1.39–1.22 (28 H, ovlp); ^{13}C NMR 173.0, 173.0, 130.0, 129.8, 70.6, 70.6, 64.9, 64.9, 58.3, 36.7, 36.7, 29.7, 29.7, 29.6, 29.5, 29.5, 29.5, 29.4, 29.3, 29.2, 29.2, 28.6, 28.5, 27.2, 27.1 ovlp, 25.9. CI-MS M/Z 978, M+18) was passed through a short column of silica gel with 5% EtOAc in hexanes, then was treated with 1.1 mmol tetrabutylammonium fluoride in THF (4 mL). After 2 h at room temperature, the desilylated product was partitioned between EtOAc and dilute HCl, and the organic phase was rinsed with aq. $NaHCO_3$, dried, and concentrated. Flash chromatography of the residue (50 then 60% EtOAc in hexanes) gave 114 mg of **3** (47%) that crystallized on standing. Recrystallization from hexanes gave 107 mg, mp 47°. ^{1}H NMR: 5.35 (2H, m), 4.11 (4H, t, *J*=6.62), 3.86 (4H, q, *J*=6.6 Hz), 2.57 (4H, t, *J*=5.52), 2.43 (2H, t, *J*=5.8 Hz), 2.01 (4H, q, *J*=5.8 Hz), 1.63 (4H, quin, *J*=7.0 Hz), 1.39–1.22 (28 H, ovlp). ^{13}C NMR 173.0, 173.0, 130.0, 129.8, 64.9, 64.9, 58.3, 58.3, 36.7, 36.7, 29.7, 29.7, 29.6, 29.6, 29.5, 29.5, 29.4, 29.3, 29.2, 29.2, 29.2 ovlp. CI-MS *m/z* 502 (M+18); EI-MS, *m/z* (%), 121 (11), 110 (13), 109 (17), 97 (16), 96 (28), 95 (32), 94 (14), 91 (57), 83 (19), 82 (34), 81 (40), 80 (23), 79 (12), 73 (100), 69 (28), 68 (22), 67 (37), 57 (11), 55 (74), 54 (21), 45 (13), 43 (35), 41 (31). Anal. Calcd for $C_{28}H_{52}O_6$: C, 69.38; H, 10.81. Found: C, 69.18; H, 11.21. **3-Bis(trimethylsilyl ether)** EI-MS, *m/z* (%), 628 (M^+, ca. 1%), 467 (ca. 3%), 235 (14), 163 (34), 147 (71), 146 (16), 145 (100), 105 (41), 104 (12), 103 (92), 95 (11), 81 (14), 75 (19), 73 (33), 69 (14), 67 (15), 55 (29), 43 (11), 41 (13).

(*Z*)-9-Tetracosene-1,24-diol bis (3-hydroxypropanoate) ester 4. Diol **6** (0.6 mmol) was esterified with **32** as described above, and the crude product was worked up and flash chromatographed (5 then 10% EtOAc in hexanes) to give 421 mg (79%) of the bis *t*-butyldiphenylsiloxypropanoate ester as a clear glass. Desilylation as described above gave 0.45 g crude product that was crystallized from hexane to give 0.16 g (66%) of **4**, mp 53–54°. CI-MS *m/z* 530 (M+18); EI-MS, *m/z* (%), 123 (10), 110 (13), 109 (18), 97 (17), 96 (27), 95 (32), 94 (15), 93 (11), 93 (11), 91 (60), 83 (22), 82 (34), 81 (43), 80 (25), 79 (11), 73 (100), 69 (32), 68 (20), 67 (42), 57 (12), 55 (69), 54 (19), 45 (12), 43 (34), 42 (10), 41 (29). Anal. Calcd for $C_{30}H_{56}O_6$: C, 70.27; H, 11.01. Found: C, 69.52; H, 11.21. **4-Bis(trimethylsilyl ether)** EI-MS, *m/z* (%), 163 (38), 147 (75), 146 (16), 145 (100), 105 (35), 104 (10), 103 (91), 96 (12), 95 (17), 83 (13), 82 (13), 81 (22), 75 (59), 73 (38), 69 (22), 55 (45), 45 (11), 43 (14), 41 (19).

(*Z,Z*)-7,15-Tetracosadiene-1,24-diol bis 3-hydroxypropanoate ester 5. *A. By F-catalyzed desilylation:* The above procedure applied to diol **7** (173 mg, 0.47 mmol) gave 159 mg of **5** (66% overall) as a clear glass, ^{1}H NMR: 5.39 (m), 4.10 (t, *J*=5.8 Hz), 3.86 (t, *J*=5.8 Hz), 2.01 (m), 1.64 (q, *J*=5.8 Hz), 1.45–1.25 (ovlp). ^{13}C NMR 173.0, 130.0, 129.8, 129.7, 129.5, 64.8, 58.2, 36.7, 29.6, 29.6, 29.5, 29.3, 29.1, 29.1, 28.7, 28.5, 28.5, 28.4, 27.1 ovlp, 27.0, 25.8, 25.7 CI-MS *m/z* 528, (M+18), EI-MS, *m/z* (%), 149 (11), 136 (10), 135 (18), 123 (11), 122 (13), 121 (25), 110 (11), 109 (26), 108 (16), 107 (16), 97 (16), 96 (26), 95 (50), 94 (27), 93 (22), 91 (36), 83 (19), 82 (28), 81 (63), 80 (38), 79 (28), 73 (100), 69 (33), 68 (23), 67 (73), 55 (82), 54 (28), 45 (15), 443 (32), 41 (39). Anal. Calcd for $C_{24}H_{46}O_2$: C, 70.55; H, 10.66. Found: C, 70.20; H, 10.87. **5-Bis(trimethylsilyl ether)** EI-MS, *m/z* (%), 163 (30), 147 (59), 146 (14), 145 (91), 135 (11), 121 (13), 109 (11), 105 (38), 104 (11), 103 (100), 95 (23), 94 (13), 93 (13), 91 (14), 83 (11), 82 (10), 81 (29), 80 (24), 79 (18), 75 (37), 73 (42), 69 (16), 67 (34), 55 (37), 43 (11), 41 (16).

*B. By reductive elimination of the bis-3-(2,2,2-trichloroethoxy)propanoate ester of (Z,Z)-7,15-tetracosadiene-1,24-diol **29**:* Diol **7** (366 mg, 1 mmol) was esterified with 2.5 mmol of 3-(2,2,2-trichloroethoxy)propanoyl chloride generated from **28** and oxalyl chloride. The crude product (0.75 g, 97%, single spot TLC, R_f 0.55 15% EtOAc in hexanes) was stirred in MeOH (18 mL) containing HOAc (2 mL) and Et_3N (1.4 mL) at 35° and zinc dust (2.7 g) was added in portions over about 20 min. Within 2 h the starting diester had been consumed, and the composition underwent no further change with time. The mixture was cooled, diluted with ether (50 mL) and filtered through Celite, then was washed twice with water, with aq. $NaHCO_3$, and finally with sat. NaCl. After drying and removal of solvent, a clear syrup (0.41 g) remained that was flash chromatographed (25–50% EtOAc in hexanes). One product moved just behind the solvent front (132 mg) and may have consisted of dechlorinated diester (CI-MS *m/z* 624, 626, 628 ca. 100, 80, 12%, respectively, M+18 ions consistent with two chlorines), but was not investigated further. A later eluting fraction (133 mg) was followed by 111 mg **5** that was rechromatographed with 30–50% methyl *t*-butyl ether in benzene to give 96 mg (19% from **7**) of bis 3-(hydroxypropyl)ester **5**, identical to the sample described above.

3-[(2,2,2-trichloroethoxy)propanoic acid 28. A mixture of methyl 3-[(2,2,2-trichloroethoxy)propanoate[18] (11.78 g, 50 mmol) and 48% HBr (50 mL) plus tetrabutylammonium bromide (1 g) was stirred and heated at 90° 2 h, then was cooled and partitioned between ice and EtOAc. The EtOAc solution was rinsed with water, diluted with one-half its volume of hexane, and again rinsed with water, then with sat. NaCl, and finally dried, concentrated, and distilled to give 9.7 g (88%) of **28**, bp 104–106, 0.05 Torr. ^{1}H NMR: 4.13 (2H, s), 4.08 (2H, t, *J*=6.2 Hz), 2.74 (2H, t, *J*=6.2 Hz), ^{13}C NMR 177.5, 97.4, 84.0, 68.5, 35.3. Anal. Calcd for $C_5H_7Cl_3O_3$: C, 27.11; H, 3.19. Found: C, 27.21; H, 3.29.

3-[[*t*-Butyldiphenylsilyl]oxy]propanoic acid 32. A solution of methyl 3-[[*t*-butyldiphenylsilyl]oxy]propanoate[23] **31** (15.8 g, 46 mmol) in methanol (100 mL) was treated dropwise at room temperature with 1N LiOH (60 mL). After stirring 4 hr at room temperature, the solution was diluted with ice water and extracted three times with

small volumes of ether–hexanes (1:1). It was then neutralized with 35 mL 2N $KHSO_4$ and extracted thoroughly with ether–hexanes (1:1). The organic extracts were rinsed with water, then dried and concentrated to provide 8.47 g of **32** as a white solid. The neutral portion, which consisted of a mixture of unsaponified **31** and *t*-butyldiphenylsilanol, was concentrated (5.16 g) and dissolved in MeOH. The resulting solution was treated with 25 mL 1N LiOH as described above, and after stirring at room temperature and partitioning, afforded an additional 2.81 g of **32**. The two portions were combined and recrystallized from hexanes to give 10.55 g (70%) of pure **32**, mp 109°. ^{1}H NMR: 7.52–7.49 (2H, m), 7.67 (2H, d, J=7.3 Hz), 7.45–7.37 (3H, m, ovlp), 3.95 (2H, t, J=5.8 Hz), 2.61 (2H, t, J=5.8 Hz), 1.05 (9H,s). ^{13}C NMR 135.5, 135.5, 133.2, 129.7, 129.7, 59.5, 37.3, 26.6, 19.0. CI-MS *m/z* 346 (M+18). EI-MS *m/z* (%), 271 (36), 200 (17), 199 (100), 197 (16), 193 (19), 181 (13), 139 (23), 105 (11), 91 (10), 78 (11), 77 (30), 57 (15), 45 (19), 41 (19).

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References

1. Berdnikov, V. A.; Trusov, Y. A.; Bogdanova, V. S.; Kosterin, O. E.; Rozov, S. M.; Nedel'kina, S. U.; Nikulina, Y. N. *Pisum Genet.* **1992**, *24*, 37.
2. Doss, R. P.; Oliver, J. E.; Proebsting, W. M.; Potter, S. W.; Kuy, S-R.; Clement, S. L.; Williamson, R. T.; Carney, J. R.; DeVilbiss, E. D. *Proc. Nat. Acad. Sci. USA* **2000**, *97*, 6218.
3. Doss, R. P.; Proebsting, W. M.; Potter, S. W.; Clement, S. L. *J. Chem. Ecol.* **1995**, *21*, 97.
4. Bierl-Leonhardt, B. A.; DeVilbiss, E. D. *Anal. Chem.* **1981**, *53*, 936.
5. Slezak, F. B.; Stallings, J. P.; Wagner, D. H.; Wotiz, J. H. *J. Org. Chem.* **1961**, *26*, 3137.
6. Read, R. R. *Organic Synthesis Collective Volume 1*; 1964; p 321.
7. Fleming, I. *Pure Appl. Chem.* **1988**, *60*, 71.
8. Williamson, R. T.; Carney, J. R.; Gerwick, W. H. *J. Nat. Prod.* **2000** (in press).
9. Lindlar, H. *Helv. Chim. Acta* **1952**, *35*, 446.
10. Kulkarni, S. M.; Mamdapur, V. R.; Chandha, M. S.; Indian *J. Chem., Sect. B.* **1983**, *22*, 683.
11. Brown, C. A.; Yamashita, A. *J. Chem. Soc., Chem. Commun.* **1976**, 959.
12. Takeshita, K.; Seki, Y.; Kawamoto, K.; Murai, S.; Sonoda, N. *J. Org. Chem.* **1987**, *52*, 4864.
13. Polniaszek, R. P.; Dillard, L. W. *J. Org. Chem.* **1992**, *57*, 4103.
14. Jones, G. R.; Landais, Y. *Tetrahedron* **1996**, *52*, 7599.
15. Gutman, A. L.; Meyer, E.; Boltanski, A. *Synth. Commun.* **1988**, *18*, 1311.
16. Malmberg, M.; Rehnberg, N. *J. Carbohydr. Chem.* **1996**, *15*, 459.
17. Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*, 2nd ed.; Wiley: New York, 1991; p 53.
18. Mamedov, S.; Ismiev, I.; Khydyrov, D. N. *J. Gen. Chem. USSR (Eng. Transl.)* **1964**, *34*, 1072.
19. Jacobson, R. M.; Clader, J. W. *Synth. Commun.* **1979**, *9*, 57.
20. Just, G.; Grozinger, K. *Synthesis* **1976**, 457.
21. Kochi, J. M.; Singleton, D. M. *J. Am. Chem. Soc.* **1968**, *90*, 1582.
22. Ogawa, T.; Nakazato, A.; Sato, M.; Hatayama, K. *Synthesis* **1990**, 459.
23. Lakanen, J. R.; Pegg, A. E.; Coward, J. K. *J. Med. Chem.* **1995**, *38*, 2714.
24. Nicolaou, K. C.; Ladduwahetty, T.; Taffer, I. M.; Zipkin, R. E. *Synthesis* **1986**, 344.
25. Girlanda-Jurges, C.; Keyling-Bilger, F.; Schmidt, G.; Lun, B. *Tetrahedron* **1998**, *54*, 7735.