Petuniasterones, Novel Ergostane-type Steroids of *Petunia hybridia* Vilm. (Solanaceae) having Insect-inhibitory Activity. *X*-Ray Molecular Structure of the 22,24,25-[(Methoxycarbonyl)orthoacetate] of 7α,22,24,25-Tetrahydroxy ergosta-1,4-dien-3-one and of 1α-Acetoxy-24,25-epoxy-7α-hydroxy-22-(methylthiocarbonyl)acetoxyergost-4-en-3-one

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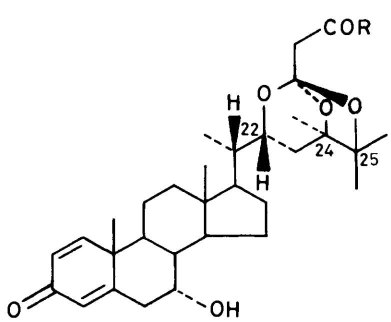
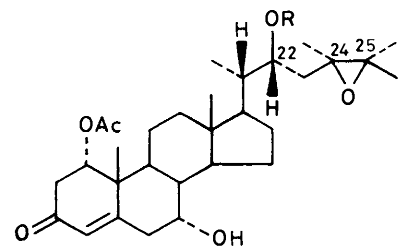
Three new types of ergostanoids with unusual functionalities were isolated from leaves and stems of *Petunia hybrida*. These include the [(methylthio)carbonyl]orthoacetate of (22*R*,24*R*)-7α,22,24,25-tetrahydroxyergosta-1,4-dien-3-one (**1**) and the respective [(methylthio)carbonyl]acetates, acetates, and free 22-alcohols derived from (22*R*,24*S*)-1α-acetoxy-24,25-epoxy-7α,22-dihydroxyergost-4-en-3-one [(**3**), (**4**), and (**5**)] and (22*R*,24*S*)-24,25-epoxy-7α,22-dihydroxyergosta-1,4-dien-3-one [(**6**), (**7**), and (**8**)]. Structures of compounds (**2**) and (**3**) were determined by *X*-ray crystallography. Compound (**1**) reduced the growth of *Heliothis zea* larvae to 50% of control values at *ca.* 40 p.p.m. in artificial diets.

In examining the response of the polyphagous lepidopteran insect *Heliothis zea* (Boddie) towards various non-host plants of the Solanaceae it was observed that on *Petunia hybrida* foliage insect growth was much retarded compared with controls, and mortality was high. Initial extractions of dried plant material followed by bioassay using artificial diets[1] showed that the fraction obtained in chloroform was responsible for all insect-inhibitory activity. When this extract was presented in bioassay diets at a level corresponding to the amount contained within the original plant material, all test insects were dead after three days.

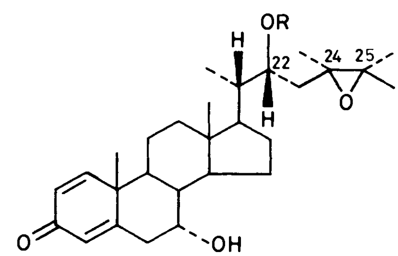
Fractionation of the chloroform extract by selective desorption from silica gel (EtOAc) and chromatography on Sephadex LH-20 yielded a mixture of substances that retained all the original activity and that resisted purification by simple, large-scale chromatography. Final purification of the major component, petuniasterone A (**1**), was carried out by preparative high-performance liquid chromatography (h.p.l.c.) using in succession silica gel, C-18 reverse-phase silica gel, and 'polar amino-cyano' (PAC) columns. Petuniasterone A reduces the growth of *H. zea* larvae to 50% of control values when it is present in test diets at *ca.* 40 mg kg^{-1}. Other related substances were present in smaller quantities, and we have isolated and characterized several of these [compound (**3**)—(**8**)]. Preliminary bioassays of compounds (**3**)—(**8**) indicate less inhibitory activity in general than does (**1**) for the same concentration.



(**1**) R = SMe
(**2**) R = OMe



(**3**) R = $\overset{O}{\|}{C}CH_2\overset{O}{\|}{C}SMe$
(**4**) R = Ac
(**5**) R = H



(**6**) R = $\overset{O}{\|}{C}CH_2\overset{O}{\|}{C}SMe$
(**7**) R = Ac
(**8**) R = H

Petuniasterone A did not form crystals adequate for *X*-ray analysis. However, the corresponding methyl ester (**2**), formed by transesterification of (**1**) with sodium methoxide in methanol, was satisfactory for this purpose. The molecular structure of ester (**2**) was unequivocally established and is shown in Figure 1 with the atom-numbering system used in the *X*-ray investigation. Figure 2 presents a stereoscopic view of its molecular conformation. The final atomic co-ordinates and their estimated standard deviations (in parentheses) are listed in Table 3. The ^{1}H n.m.r. and ^{13}C n.m.r. spectra of compounds (**1**) and (**2**) are consistent with these structures (Tables 1 and 2). N.m.r. skeletal assignments are based upon the magnitudes and

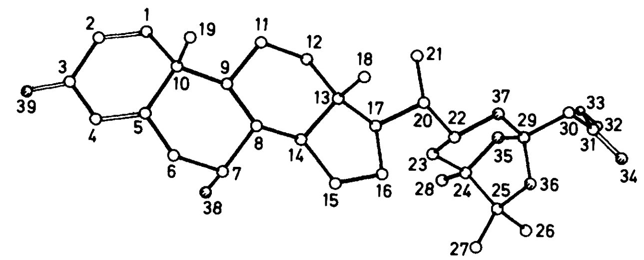


Figure 1. Perspective view of compound (**2**) with crystallographic numbering scheme. Open bonds represent double bonds, and shaded circles represent oxygen atoms

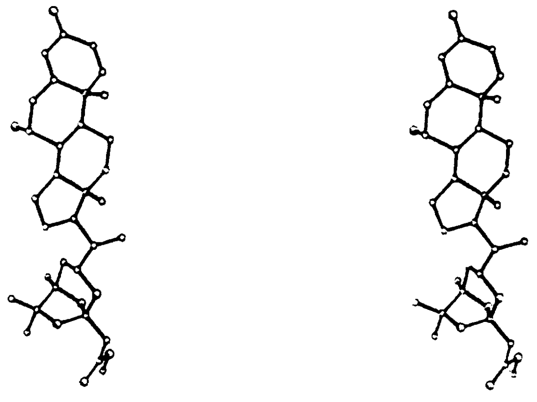


Figure 2. Stereoscopic view of compound (**2**)

multiplicities of the observed signals in conjunction with the unique characteristics revealed by the *X*-ray structure. The thiomethyl ester proton resonance (δ 2.31) is replaced by the methyl ester signal (δ 3.71) upon conversion of (**1**) into (**2**). Similarly, petuniasterone A (**1**) has a chemical shift of δ_C 193.3 for the *S*-alkyl thioester carbonyl which is replaced by that of the ester (δ_C 168.3) in (**2**). For comparison, the analogous carbonyl resonance of *S*-ethyl thioacetate occurs at δ_C 194.9[2] *vs.* δ_C 170.3 for ethyl acetate.[3a] [The i.r. spectrum of compound (**1**) shows a band at 1 685 cm^{-1} for the *S*-alkyl thioester[4] whereas compound (**2**) shows a band at 1 735 cm^{-1} characteristic of the usual ester CO stretch]. Smaller chemical-shift differences are apparent for the methylene group adjacent to the ester carbonyl. The methylene proton signals occur at *ca.* 0.2 p.p.m. to higher field in the methyl ester, and the chemical shift of the carbon is likewise to higher field (*ca.* 8 p.p.m.) than that of the sulphur analogue. The quaternary orthoester carbon resonates at δ_C 115.3 and 115.7 for compounds (**1**) and (**2**) respectively, which values are approximately the same chemical shift as that of the corresponding carbon in the orthoacetate functionality (δ_C 117.6) of pseudrelone B.[5] The cross-conjugated ketone of ring A ($\nu_{max.}$ *ca.* 1 670 cm^{-1}) exhibits a relatively high-field chemical shift of δ_C 185.6 for C-3 which is expected for a dienone of this type.[6] The expected proton couplings are observed in both rings A and B. Of particular interest is the long-range coupling of 4-H to 2-H and to the 6β-proton.[7]

Compound (**3**) possesses spectral characteristics showing structural similarity to petuniasterone A (**1**) and it is possible to establish all significant structural features by n.m.r. spectroscopy. Clearly, the cross-conjugated carbonyl of ring A is no longer present, being replaced by the singly unsaturated 4-en-3-one moiety. Also, the orthoester functionality is absent. Two additional CO resonances appear in the ^{13}C n.m.r. spectrum of (**3**), one of which is associated with the 1α-acetoxy group (^{13}CO, δ_C 170.2 and C^1H_3, δ 2.04). The other new carbonyl (^{13}CO, δ_C 165.6) belongs to the ester attached at C-22 (C^1H, δ 5.25). An *S*-methyl thioester is present as in compound (**1**), showing a similar ^{13}C carbonyl chemical shift (δ_C 191.4) and an SCH_3 proton signal (δ 2.37), but the position observed for the proton resonance of the adjacent methylene (δ 3.61) suggests a hemimalonate structure which must thereby be attached to the C-22 ester. The ^{13}C carbonyl position of ring A, δ_C 194.9, is satisfactory for an α,β-unsaturated ketone.[3b] The mass spectrum (MH^+ = 619) shows that, in addition to the basic steroidal ring system, another ring must be present since no other olefinic carbons are observed. This must be a 24,25-epoxide, inasmuch as the n.m.r. signals of these carbons occur at δ_C 61.3 and 62.6, considerably upfield from the corresponding positions in compounds (**1**) and (**2**) (δ_C 81.8 and 82.9). Treatment of compound (**3**) with dilute sodium methoxide at room temperature not only removed the ester at C-22, but also effected elimination of the acetoxy group. The latter substituent must therefore be at C-1, β to the 3-ketone, for easy elimination to occur under such mild conditions.[8] The resulting dienone (**8**) was also present in the plant extract. The remaining hydroxy group in compounds (**3**) and (**8**) may be assigned the 7α configuration since the 6α and β protons of (**8**) give rise to signals very similar to those of compounds (**1**) and (**2**). The remaining stereochemical features of compound (**3**) were established by *X*-ray crystallography, showing a 1α-acetoxy group and a 17β-side chain with a 22*R*,24*S* configuration.

Figures 3 and 4 show perspective and stereoscopic views respectively for the *X*-ray structure of compound (**3**) and the corresponding crystallographic data are listed in Table 4.

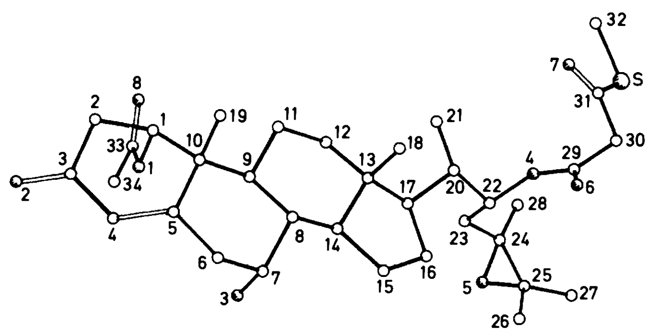


Figure 3. Perspective view of compound (**3**) with crystallographic numbering scheme. Open bonds represent double bonds, and smaller shaded circles represent oxygen atoms

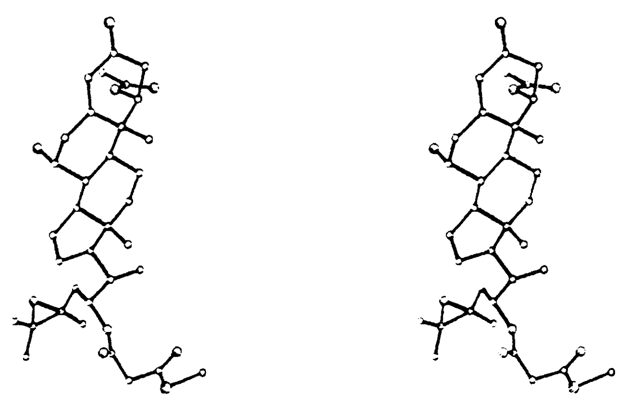


Figure 4. Stereoscopic view of compound (**3**)

Compounds (**4**) and (**5**) differ from (**3**) only in their substituents at C-22 by having acetoxy and hydroxy groups respectively. Mild methoxide treatment produces compound (**8**) from each which, along with spectral comparisons, shows that

Table 1. ^{1}H N.m.r. data[a]

Compound	1-H	2-H	4-H	6-H_{α}	6-H_{β}	7-H	12-H_{β}	22-H	18-H_3	19-H_3	21-H_3	26-H_3	27-H_3	28-H_3	Other	
(1)	7.08d (10)	6.24dd (10, 2)	6.13br t (*ca.* 2)	2.50dd (14, 3)	2.75ddd (14, 3, 2)	4.04br s	2.02dt (12.5, 4)	4.21dt (11.5, 4)	0.76s	1.23s	0.96d (7)	1.12s[b]	1.21s[b]	1.30s[b]	COSMe 2.31s	CH_2CO 3.04d, 3.10d (14)
(2)	7.07d (10)	6.25dd (10, 2)	6.15br t (*ca.* 2)	2.47dd (14, 3)	2.75ddd (14, 3, 2)	4.03br s	2.03dt (12.5, 4)	4.21dt (11,4)	0.75s	1.23s	0.95d (7)	1.13s[b]	1.21s[b]	1.31s[b]	CO_2Me 3.71s	CH_2CO 2.84d, 2.93d (14)
(3)	5.25t (3)	2.63m	5.88br s	2.53dd (16, 3)	2.70dd (16, 3)	3.96br s		5.25m	0.73s	1.27s[b]	0.94d (7)	1.28s[b]	1.30s[b]	1.33s[b]	COSMe 2.37s	$COCH_2CO$ 3.61s OAc 2.04s
(4)	5.27t (3)	2.63m	5.88br s	2.53dd (16, 3)	2.70dd (16, 3)	3.97br s		5.20br t (3)	0.72s	1.27s[b]	0.93d (7)	1.28s[b]	1.29s[b]	1.34s[b]	2 × OAc 2.03s, 2.07s	
(5)	5.27t (3)	2.63m	5.88br s	2.53dd (16, 3)	2.70dd (16, 3)	3.98br s		4.16br d (9)	0.75s	1.27s[b]	0.92d (7)	1.36s[b]	1.36s[b]	1.38s[b]	OAc 2.03s	
(6)	7.07d (10)	6.25dd (10, 2)	6.14br t (*ca.* 2)	2.50dd (14, 3)	2.75ddd (14, 3, 2)	4.05br s		5.26dt (12, 2)	0.75s	1.23s	0.93d (7)	1.28s[b]	1.30s[b]	1.33s[b]	COSMe 2.36s	$COCH_2CO$ 3.61s
(7)	7.07d (10)	6.26dd (10, 2)	6.15br t (*ca.* 2)	2.48dd (14, 3)	2.75ddd (14, 3, 2)	4.04br s		5.24dt (11, 2)	0.75s	1.24s	0.94d (7)	1.28s[b]	1.30s[b]	1.35s[b]	OAc 2.07s	
(8)	7.08d (10)	6.26dd (10, 2)	6.15t (2)	2.50dd (14, 3)	2.76ddd (14, 3, 2)	4.06br s	2.04dt (13, 4)	4.17dt (10, 3)	0.78s	1.24s	0.93d (7)	1.35s[b]	1.36s[b]	1.37s[b]		

[a] Values in $CDCl_3$; coupling constants (Hz) in parentheses. [b] Values may be interchanged within a row.

Table 2. ^{13}C N.m.r. data*

Carbon†	Compound (1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
1‡	155.6	155.6	73.7	73.7	73.6	155.5	155.4	155.7
2‡	127.6	127.6	39.3	39.4	39.3	127.7	127.7	127.6
3	185.6	186.5	194.9	194.9	194.9	185.6	185.5	185.6
4‡	127.1	127.2	126.4	126.4	126.3	127.2	127.2	127.1
5	164.5	164.5	163.6	163.6	163.8	164.4	164.3	164.7
6‡	41.0	40.9	40.9	40.9	40.9	41.0	40.9	41.0
7‡	69.5	69.6	67.7	67.7	67.7	69.5	69.5	69.6
10	43.4[a]	43.4[a]	41.7[a]	41.7[a]	41.7[a]	43.4[a]	43.4[a]	43.5[a]
11	22.5	22.5	20.3	20.3	20.3	22.4	22.4	22.5
12‡	39.0	38.9	39.0	39.0	39.1	38.9	38.9	39.0
13	42.9[a]	42.9[a]	42.8[a]	42.8[a]	42.7[a]	43.0[a]	42.9[a]	42.9[a]
15	23.8[b]	23.8[b]	23.6[b]	23.6[b]	23.6[b]	23.8[b]	23.9[b]	23.9[b]
16	27.2[b]	27.2[b]	27.2[b]	27.2[b]	27.4[b]	27.2[b]	27.2[b]	27.3[b]
18‡	11.8	11.8	11.8	11.7	11.7	11.8	11.8	11.9
19‡	18.3	18.2	18.1	18.1	18.1	18.3	18.3	18.3
20	39.8	39.7	39.8	40.0	40.7	39.8	40.0	40.7
21‡	12.5	12.5	12.8	12.8	12.3	12.8	12.9	12.4
22‡	70.2	70.1	75.0	73.2	71.0	75.1	73.2	71.0
23	30.3	30.2	32.3	32.4	31.2	32.3	32.4	31.2
24	82.9[c]	82.9[c]	62.6[c]	62.8[c]	65.4[c]	62.6[c]	62.7[c]	65.4[c]
25	81.8[c]	81.8[c]	61.3[c]	61.3[c]	62.5[c]	61.3[c]	61.3[c]	62.6[c]
26	19.9[d]	19.9[d]	19.6[d]	19.4[d]	19.5[d]	19.5[d]	19.3[d]	19.5[d]
27	20.4[d]	20.4[d]	21.1[d]	21.3[d]	20.7[d]	21.1[d]	21.3[d]	20.7[d]
28	24.9[d]	24.9[d]	21.3[d]	21.5[d]	21.4[d]	21.3[d]	21.5[d]	21.5[d]
	115.3	115.7	165.6			165.7		
	(orthoester)	(orthoester)	(malonate CO_2)			(malonate CO_2)		
	50.3	42.4	49.6			49.6		
	($^{13}CH_2COSMe$)	($^{13}CH_2CO_2Me$)	(malonate CH_2)			(malonate CH_2)		
	193.3	168.3	191.4			(malonate COS)		
	($^{13}COSMe$)	($^{13}CO_2Me$)	(malonate COS)			(not obs.)		
	12.0 SMe	51.8 OMe	12.2 SMe			12.1 SMe		
			170.2	170.2, 170.8	170.2		170.6	
			acetate CO	acetate COs	acetate CO		acetate CO	
			21.0[d]	21.0,[d] 21.2[d]	20.9[d]		21.2[d]	
			acetate Me	acetate Mes	acetate Me		acetate Me	
	Non-assigned Signals							
	38.5	38.4	36.7	36.7	36.7	39.7	39.7	39.7
	44.4	44.4	39.4	39.3	39.2	44.3	44.3	44.4
	49.9	49.9	50.2	50.2	50.3	50.0	50.0	50.0
	52.0	52.0	52.4	52.5	52.7	52.5	52.6	52.8

* δ Values in p.p.m. from internal $SiMe_4$ for $CDCl_3$ solutions. † Values with identical superscripts in each column may be interchanged. ‡ Assigned by ^{13}C–^{1}H correlation spectroscopy in compounds (**1**) and (**2**).

compounds (**3**), (**4**), and (**5**) all have the same overall stereochemical configurations. We have designated compound (**5**) as petuniasterone B.

A-Ring dienones (**6**), (**7**), and (**8**) have spectral characteristics similar to those of derivatives of both petuniasterones A and B. Hemithiomalonate and acetate functionalization at C-22 are indicated for compounds (**6**) and (**7**) respectively, and de-esterification of each with methoxide under mild conditions produces the naturally occurring compound (**8**). Again, this serves to indicate identical stereochemistry for this set of compounds and for the petuniasterone B series. Compound (**8**) has been named petuniasterone C.

During this work we have noticed the presence of other, related compounds which are, at present, incompletely characterized. We have observed varietal (and/or seasonal) differences in the relative proportions of components in the petuniasterone fractions from various petunia sources. One batch of material from a red variety contained almost exclusively petuniasterone C (**8**), although in relatively low concentration. Orthoesters of natural occurrence are found in a number of classes of compounds including limonoids,[9] steroidal alkaloids,[10] and the phorbol-related daphnetoxin.[11] However, the above combination with the other structural features of the petuniasterones, especially the uncommon thioester substituents, is unusual in the extreme and is surely responsible for the observed insect-inhibitory activity of these substances. This makes them of particular interest as a class of compounds that is of defensive utility in the plant and which may provide biochemical insight into the mechanism of insect antibiosis.

Experimental

M.p.s were taken with a Fisher-Johns apparatus and are corrected. Optical rotations were obtained for chloroform solutions on a Perkin-Elmer model 241 automatic polarimeter at *ca.* 21 °C. I.r. spectra were recorded on a Perkin-Elmer model 237 spectrophotometer and refer to chloroform solutions; u.v. spectra were taken on a Cary 219 spectro-

Table 3. Atom co-ordinates (× 10⁴) for compound (2), with e.s.d.s in parentheses

Atom	x	y	z
C(1)	2 847(6)	2 566(2)	1 011(2)
C(2)	3 104(6)	2 045(3)	591(2)
C(3)	4 874(6)	1 853(3)	402(2)
C(4)	6 267(6)	2 258(2)	692(2)
C(5)	6 005(6)	2 773(2)	1 123(2)
C(6)	7 484(5)	3 212(2)	1 382(2)
C(7)	7 240(5)	4 041(2)	1 247(2)
C(8)	5 495(5)	4 296(2)	1 508(2)
C(9)	3 970(5)	3 849(2)	1 231(2)
C(10)	4 220(5)	2 992(2)	1 350(2)
C(11)	2 216(6)	4 140(3)	1 452(3)
C(12)	2 027(6)	4 962(2)	1 377(3)
C(13)	3 518(6)	5 422(2)	1 656(2)
C(14)	5 196(5)	5 098(2)	1 382(2)
C(15)	6 606(5)	5 645(2)	1 566(2)
C(16)	5 638(5)	6 382(2)	1 585(2)
C(17)	3 704(5)	6 228(2)	1 466(2)
C(18)	3 499(7)	5 354(3)	2 368(2)
C(19)	4 073(8)	2 796(3)	2 038(2)
C(20)	2 507(6)	6 810(2)	1 757(2)
C(21)	544(6)	6 661(3)	1 642(3)
C(22)	3 027(6)	7 583(2)	1 546(2)
C(23)	2 954(7)	7 722(3)	851(2)
C(24)	2 894(7)	8 524(3)	667(2)
C(25)	4 332(8)	8 984(3)	954(3)
C(26)	4 370(11)	9 783(3)	698(4)
C(27)	6 184(8)	8 659(4)	944(4)
C(28)	2 540(10)	8 594(4)	−16(3)
C(29)	2 025(6)	8 813(2)	1 619(2)
C(30)	892(8)	9 310(3)	2 016(3)
C(31)	689(8)	10 062(3)	1 751(3)
C(32)	−841(10)	10 799(4)	1 057(4)
O(33)	−595(5)	10 106(2)	1 374(2)
O(34)	1 634(7)	10 571(2)	1 865(2)
O(35)	1 439(4)	8 816(2)	1 006(1)
O(36)	3 769(4)	9 042(2)	1 597(2)
O(37)	1 910(4)	8 093(2)	1 865(1)
O(38)	7 269(4)	4 178(2)	604(1)
O(39)	5 164(4)	1 395(2)	−10(2)

photometer for solutions in methanol; ^{1}H n.m.r. spectra were obtained at 90 MHz on a Varian EM-390 instrument or at 200 MHz on a Nicolet NT-200, and ^{13}C n.m.r. spectra were taken at 50 MHz on the latter instrument. N.m.r. assignments were facilitated by decoupling methods and by use of two-dimensional proton–proton and carbon–proton correlation techniques.[12] Mass spectra were run using a VG Micromass 70/90HS instrument either by electron impact or using ammonia chemical ionization. *X*-Ray intensities were collected with a Nicolet R3 automatic diffractometer at room temperature. Microanalyses were determined by Galbraith Enterprises, Knoxville, Tennessee.

Silica gel was from E. Merck, Si60, 70—230 mesh; Sephadex, LH-20 from Pharmacia Co.; h.p.l.c. columns were from Rainin Instruments, Alltech Associates and Whatman, Inc. Solvents were h.p.l.c. grade and were pumped using an Altex/Beckman Model 110A pump. Detection was by u.v. at 254 nm with an Altex Model 150 monitor equipped with 0.5 mm pathlength preparative cell.

Bioassays.—Solutions containing compounds for bioassay were allowed to evaporate onto cellulose powder. The powder was mixed thoroughly and incorporated into modified Berger-diet premix.[1] The prepared test diets were divided into ten portions, placed in individual plastic containers, and two newly hatched larvae of *Heliothis zea* were added. The insects were

Table 4. Atom co-ordinates (× 10⁴) for compound (3), with e.s.d.s in parentheses

Atom	x	y	z
S	4 230(1)	7 643	−4 186(1)
O(1)	10 475(2)	4 676(4)	3 841(2)
O(2)	12 969(3)	3 044(6)	5 217(2)
O(3)	9 390(2)	215(4)	2 655(2)
O(4)	5 463(2)	4 240(4)	−2 276(2)
O(5)	3 449(2)	2 335(7)	−1 083(2)
O(6)	6 421(3)	2 603(5)	−2 852(2)
O(7)	6 027(3)	7 283(6)	−3 232(3)
O(8)	10 114(4)	7 452(5)	3 814(3)
C(1)	11 202(3)	4 983(5)	3 385(2)
C(2)	12 214(3)	4 937(7)	4 074(3)
C(3)	12 423(3)	3 229(7)	4 485(3)
C(4)	12 008(3)	1 741(6)	3 977(3)
C(5)	11 404(3)	1 847(5)	3 147(2)
C(6)	11 086(3)	231(5)	2 614(3)
C(7)	10 013(3)	239(5)	2 113(3)
C(8)	9 757(2)	1 864(5)	1 561(2)
C(9)	9 983(3)	3 483(5)	2 121(2)
C(10)	11 088(2)	3 564(5)	2 712(2)
C(11)	9 681(3)	5 141(5)	1 585(3)
C(12)	8 604(3)	5 078(5)	956(2)
C(13)	8 443(2)	3 493(5)	388(2)
C(14)	8 679(2)	1 907(5)	992(2)
C(15)	8 262(3)	377(5)	405(3)
C(16)	7 350(3)	1 093(6)	−263(3)
C(17)	7 335(2)	3 096(5)	−128(2)
C(18)	9 076(3)	3 545(6)	−219(2)
C(19)	11 797(3)	4 007(6)	2 183(3)
C(20)	6 883(3)	4 106(5)	−969(2)
C(21)	6 857(3)	6 076(6)	−850(3)
C(22)	5 853(2)	3 374(6)	−1 446(2)
C(23)	5 085(3)	3 606(8)	−983(3)
C(24)	4 015(3)	3 312(8)	−1 527(3)
C(25)	3 685(4)	1 577(10)	−1 787(3)
C(26)	4 331(5)	34(9)	−1 564(4)
C(27)	2 770(4)	1 317(15)	−2 586(4)
C(28)	3 483(4)	4 831(13)	−1 991(5)
C(29)	5 782(3)	3 675(6)	−2 918(2)
C(30)	5 234(4)	4 642(7)	−3 738(3)
C(31)	5 281(3)	6 548(7)	−3 647(3)
C(32)	4 656(4)	9 841(8)	−3 980(4)
C(33)	9 996(3)	5 999(6)	4 028(3)
C(34)	9 391(4)	5 553(9)	4 554(4)

maintained at 26 °C; after five days the excess of larvae were removed so that one individual per container remained (as a precaution against competition and cannibalism). Larval weights were measured on the tenth day and were compared with control subjects reared on diets containing only cellulose powder as additive.

Plant Material.—*Petunia hybrida* Vilm., commercial variety 'Royal Cascade,' was grown in outdoor beds in Albany, California. Leaf and stem material was harvested at intervals between July and November 1986.

Isolation Procedure.—Freeze-dried leaf and stem material (250 g) was ground with chloroform (3 × 2 l) in a 1 gallon Waring Blendor at maximum speed for 5 min. The resulting, boiling hot solutions were filtered by Büchner funnel, combined, and evaporated under reduced pressure to give a green oil (33 g). The oil was stirred with methanol (500 ml) followed by filtration and evaporation to yield wax-free material (23 g) which was then applied in chloroform (50 ml) to a column of silica gel (500 g; 50 mm dia. × 450 mm). The column was eluted with methylene dichloride (1.8 l) followed by ethyl acetate (2 l) and

Table 5. Elution zone (ml)

Compound	Silica	R Sil C-18	Partisil-10 PAC
(3)	230—265	30—43	160—200
(4)	230—265	30—43	125—145
(5)	230—265	25—30	115—145
(6)	210—230	43—60	150—175
(7)	210—230	43—60	115—135
(8)	210—230	25—43	120—140

methanol (1 l). Insect-inhibitory activity was present only in the ethyl acetate eluate (8.1 g on evaporation). Chromatography of this material on Sephadex LH-20/methanol, 50 mm dia. × 950 mm, gave a broad zone having inhibitory activity, elution volume 1 250—1 750 ml (4.0 g). Further fractionation on a 9 mm dia. × 500 mm Partisil-10 silica h.p.l.c. column, 20% propan-2-ol in hexane, gave one major zone of activity (elution volume 60—65 ml; 0.3 g) followed by a complex series of peaks which, on recombination, also were active against *H. zea* larvae. Passage of the former material through an R-Sil C-18 h.p.l.c. column (10 mm dia. × 250 mm) with 30% water in acetonitrile, elution volume 62—70 ml, provided petuniasterone (**1**) (70 mg) as a poorly crystalline solid on evaporation. Rechromatography of the combined material eluted after compound (**1**) was carried out in succession by h.p.l.c. on Dynamax silica, 21.4 mm dia. × 250 mm (20% propan-2-ol in hexane); R Sil C-18, 10 mm dia. × 250 mm (30% water in acetonitrile), and Partisil-10 PAC, 9 mm dia. × 500 mm (10% propan-2-ol in hexane) to yield compounds (**3**)—(**8**). Results are given in Table 5.

Compound (**1**), *Petuniasterone A*.—M.p. 130—135 °C did not form satisfactory crystals from any solvent; [α] (λ/nm) (589) +52.1°, (578), +54.3°, (546) +60.9° (436) +92.9°, and (365) +80.0°; $\nu_{max.}$ 3 450br (OH), 1 685 (COSMe), and 1 660 cm^{-1} (conjugated CO); $\lambda_{max.}$ 244 nm (log ε 4.29); *m/z* 558.3023 (M^+, 1.2%) ($C_{32}H_{46}O_6S$ requires *M*, 558.3015) and 510.2971 (M^+ − MeSH, 5.3) ($C_{31}H_{42}O_6$ requires *m/z*, 510.2981) (Found: C, 69.2; H, 8.6; S, 5.6. $C_{32}H_{46}O_6S$ requires C, 68.79; H, 8.30; S, 5.74%).

Conversion of (**1**) *into Methyl Ester* (**2**).—Transesterification of petuniasterone A (**1**) (10 mg) was carried out in 0.1M-NaOMe in MeOH (5 ml) for 3.25 h at room temperature. After addition of HOAc (0.05 ml) the solution was evaporated under reduced pressure, the residue redissolved in CH_2Cl_2, and the solution filtered and taken to dryness to give crude ester (**2**) (9 mg), m.p. 218—219 °C (from MeOH); $\nu_{max.}$ 3 500br (OH), 1 735 (CO_2Me), and 1 660 cm^{-1} (conjugated CO); $\lambda_{max.}$ 246 nm (log ε 4.20); *m/z* 542 (M^+, 0.3%) and 510 (M^+ − MeOH, 0.6) (Found: C, 70.5; H, 8.55. $C_{32}H_{46}O_7$ requires *M*, 542; C, 70.82; H, 8.54%).

Compound (**3**), *Petuniasterone B* 22-O-[(*Methylthio*)-*carbonyl*]*acetate*.—M.p. 182—183 °C (from MeOH); [α] (λ/nm) (589) +65.7°, (578) +68.4°, (546) +77.2°, (436) +116.0°, and (365) −78.5°; $\nu_{max.}$ 3 450br (OH), 1 735 (ester), and 1 680 cm^{-1} (COSMe and conjugated CO); $\lambda_{max.}$ 242infl nm (log ε 4.21); *m/z* 636 (MNH_4^+, 19.4%), 619 (MH^+, 7.6), and 485 (MH^+ − $C_4H_6O_3S$, 38.9) (Found: C, 65.55; H, 8.2; S, 5.4. $C_{34}H_{50}O_8S$ requires *M*, 618; C, 65.99; H, 8.14; S, 5.18%).

Compound (**4**), *Petuniasterone B* 22-O-*Acetate*.—M.p. 195—196 °C (from heptane–30% EtOAc); [α] (λ/nm) (589) +39.5°, (578) +41.1°, (546) +46.2°, (436) +68.5°, and (365) −47.9°; $\nu_{max.}$ 3 450br (OH), 1 730 (ester), and 1 670 cm^{-1} (conjugated CO); $\lambda_{max.}$ 243 nm (log ε 4.26); *m/z* 562 (MNH_4^+, 15.9%), 545.3457 (MH^+, 79.2), and 485 (MH^+ − $C_2H_4O_2$) ($C_{32}H_{49}O_7$ requires *m/z*, 545.3474).

Compound (**5**), *Petuniasterone B*.—M.p. 191—192 °C (from heptane–30% EtOAc); [α] (λ/nm) (589) +88.8°, (578) +92.7°, (546) +104.2°, (436) +156.0°, and (365) −96.4°; $\nu_{max.}$ 3 500br (OH), 1 730 (ester), and 1 670 cm^{-1} (conjugated CO); $\lambda_{max.}$ 240 nm (log ε 4.22); *m/z* 520 (MNH_4^+, 100%), 503 (MH^+, 9.9), and 485 (MH^+ − H_2O, 82.2) (Found: C, 71.8; H, 9.4. $C_{30}H_{46}O_6$ requires *M*, 502; C, 71.68; H, 9.22%).

Compound (**6**), *Petuniasterone C* 22-O-[(*Methylthio*)-*carbonyl*]*acetate*.—M.p. 141—142 °C (from heptane–30% EtOAc); [α] (λ/nm) (589) +17.4°, (578) +17.4°, (546) +18.8°, (436) +22.0°, and (365) −41.0°; $\nu_{max.}$ 3 450br (OH), 1 730 (ester), 1 680 (COSMe), and 1 660 cm^{-1} (conjugated CO); $\lambda_{max.}$ 243 nm (log ε 4.18); *m/z* 576 (MNH_4^+, 4.9%), 559.3162 (MH^+, 21.2), 425 (MH^+ − $C_4H_6O_3S$, 19.0), and 407 (MH^+ − $C_4H_6O_3S$ − H_2O, 48.0) (Found: C, 68.7; H, 8.4. $C_{32}H_{46}O_6S$ requires MH^+, 559.3091; C, 68.79; H, 8.30%).

Compound (**7**), *Petuniasterone C* 22-O-*Acetate*.—[α] (λ/nm) (589) +40.3°, (578) +42.2°, (546) +47.4°,(436) +73.5°, and (365) +76.0°; $\nu_{max.}$ 3 500br (OH), 1 730 (ester), and 1 670 (conjugated CO); $\lambda_{max.}$ 246 nm (log ε 4.34); *m/z* 485.3239 (MH^+, 91.2%), 467 (MH^+ − H_2O, 43.1), 425 (MH^+ − $C_2H_4O_2$), and 407 (MH^+ − H_2O-$C_2H_4O_2$) ($C_{30}H_{45}O_5$ requires *m/z*, 485.3265).

Compound (**8**), *Petuniasterone C*.—M.p. 183—185 °C (from heptane–30% EtOAc); [α] (λ/nm) (589) +31.7°, (578) +32.9°, (546) +36.5°, (436) +49.4°, and (365) +8.8°; $\nu_{max.}$ 3 500br (OH) and 1 670 cm^{-1} (conjugated CO); $\lambda_{max.}$ 245 nm, (log ε 4.23); *m/z* 443 (MH^+, 24.9%), 425 (MH^+ − H_2O, 9.6), and 407 (MH^+ − $2H_2O$, 11.0) (Found: C, 76.0; H, 9.6. $C_{28}H_{42}O_4$ requires *M*, 442; C, 75.98; H, 9.56%).

Conversion of Compounds (**3**), (**4**), (**5**), (**6**), *and* (**7**) *into Compound* (**8**).—Each of the above substrates (5—10 mg) was dissolved in 0.5M-NaOMe in MeOH (1 ml). The solutions were kept 1—2 h at room temperature and then were acidified with HOAc (0.025 ml). After evaporation under reduced pressure, the resulting products were dissolved in CH_2Cl_2, the solutions were filtered, and the products were purified by h.p.l.c. on the Partisil-10 PAC column (10% propan-2-ol–hexane). All products were chromatographically and spectroscopically identical with compound (**8**).

Crystal Data.—*Compound* (**2**). $C_{32}H_{46}O_7$, $M = 542.8$, orthorhombic, space group $P2_12_12_1$, $a = 7.701(1)$, $b = 18.173(4)$, $c = 21.642(5)$ Å, $\beta = 90.0(0)°$, $V = 3\,029$ Å^3, $D_c = 1.19$ g cm^{-3}, $Z = 4$, $F(000) = 1\,176$, $\mu(Cu\text{-}K_\alpha) = 6.30$ cm^{-1}; $R = 0.050$; 353 parameters, $R' = 0.060$ for 2 187 unique reflections with $|F_o| \geq 3\sigma|F_o|$ in the range $3° \leq 2\theta \leq 114°$; crystals were obtained from methanol by slow evaporation.

Compound (**3**). $C_{34}H_{50}O_8S$, $M = 618.9$, monoclinic, space group $P2_1$, $a = 14.192(5)$, $b = 7.727(2)$, $c = 16.373(5)$ Å, $\beta = 107.78(3)°$, $V = 1\,710$ Å^3, $D_c = 1.20$ g cm^{-3}, $Z = 2$, $F(000) = 668$, $\mu(Cu\text{-}K_\alpha) = 11.89$ cm^{-1}, $R = 0.056$; 387 parameters, $R' = 0.066$ for 3 354 unique reflections with $|F_o| \geq 3\sigma|F_o|$ in the range $3° \leq 2\theta \leq 114°$; crystals were obtained from ethanol by slow evaporation.

Data Collection and Structure Refinement.—Intensity data were collected on a Nicolet R3 diffractometer with graphitemonochromatized Cu-K_α radiation (λ = 1.5418 Å) by the θ–2θ scan technique with variable scan speed (4—30°) at room temperature. The intensity data were corrected for background, Lorentz-polarization effects,[13] and secondary extinction, but not for absorption. The crystal structures were solved by direct methods. Atomic co-ordinates, thermal parameters, and scale factors were refined by a 'blocked-cascade' full-matrix least-squares procedure with the SHELXTL[14] program package. The function minimized was $\Sigma\omega(|F_o| - |F_c|)^2$, where $\omega = [\sigma^2|F_o| + 0.001|F_o|^2]^{-1}$. Scattering factors were from 'International Tables for *X*-ray Crystallography';[15] those of oxygen and sulphur were corrected for anomalous dispersion. Positions of all non-hydrogen atoms were refined anisotropically, and all hydrogen positions were estimated but verified in subsequent difference Fourier maps and included at invariant idealized values in the respective structure-factor calculation. The absolute configurations of both compounds (**2**) and (**3**) were determined by least-squares refinement of the parameters of both enantiomers in each structure, giving a ratio of the two final R_w values of 1.026 and 1.041 for (**2**) and (**3**), respectively. According to Hamilton's statistical test,[16] the enantiomer with the lower R_w value has a probability of being correct to a significance level better than 5%.

Tables of bond lengths and angles, anisotropic thermal parameters with their estimated standard deviations for the non-hydrogen atoms, and positional and thermal parameters for the hydrogen atoms have been deposited at the Cambridge Crystallographic Data Centre.*

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* *Supplementary data* (see section 5.6.3 of Instructions for Authors, January issue).

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