Petuniolides. Unusual Ergostanoid Lactones from *Petunia* Species that Inhibit Insect Development

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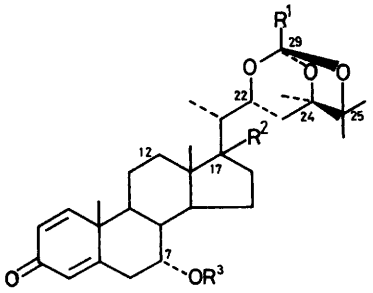
Four new compounds that strongly contribute to the resistance of *Petunia parodii* and *P. integrifolia* against feeding by larvae of the lepidopteran, *Heliothis zea*, were isolated from leaves of these plant species. These consist of the (22*R*,24*R*)-22,24,25-orthoacetates and orthopropionates derived from multiply functionalized ergostanoids in which the A-ring has lost one carbon and has been converted into a spirolactone. An epoxy group is present at position 6α,7α. Petuniolides A (**11**) and B (**12**) have a $\Delta^{9(11)}$-12α-acetoxy group whereas petuniolides C (**13**) and D (**14**) possess the corresponding allylic ketone functionality. The molecular structures of (**12**) and (**14**) were determined by X-ray crystallography.

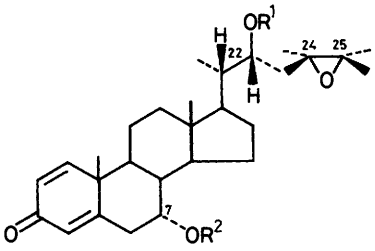
The resistance of *Petunia hybrida* towards attack by the polyphagous larvae of the moth *Heliothis zea* (Boddie) has been shown by us to be correlated with the presence of certain ergostane related allelochemicals found in the leaves of the plant.[1] These substances, which are termed petuniasterones, occur as a diverse family of steroidal compounds,[2–5] of which a few typical examples are shown [compounds (**1**)–(**10**)]. Although various functionalizations of the side chain and of the steroid nucleus occur within the family, the individual members all possess an oxo group at position-3 and an α-hydroxy or -acetoxy at position-7 on an essentially unaltered ergostane system. We have observed, however, that insect-inhibitory activity against *H. zea* is only significant for those compounds having a bicyclic orthoester system on the side chain.[1]

We now report the occurrence of four new compounds from *Petunia* species that, although related to the petuniasterones, represent a new structural type of modified ergostanoid in which ring A has undergone rearrangement with the loss of one carbon atom and formation of a spirolactone. These compounds [(**11**)–(**14**)], which were isolated from the putative ancestors of *P. hydrida* (*i.e.*, *P. integrifolia* and *P. parodii*), are named petuniolides in analogy with the well known withanolides,[6,7] and we propose the numbering system shown in Figure 1 which has the advantage of preserving standard steroid numbering in the unmodified portion of the molecule. In artificial diets, the insect-inhibitory activity of the petuniolides is greater than that of the most active petuniasterones. Petuniolides C (**13**) and D (**14**) reduce larval growth of *H. zea* to 50% of normal at dietary concentrations (ED_{50}) of about 2–4 mg kg^{-1}. Petuniolides A (**11**) and B (**12**) are less inhibitory with ED_{50} values of *ca.* 10–13 mg kg^{-1}.



(**1**) R^1 = CH_2COSMe, R^2 = R^3 = H
(**2**) R^1 = CH_2COSMe, R^2 = OH, R^3 = H
(**3**) R^1 = CH_2COSMe, R^2 = OH, R^3 = Ac
(**4**) R^1 = CHOHCOSMe, R^2 = R^3 = H

Petuniasterone A series

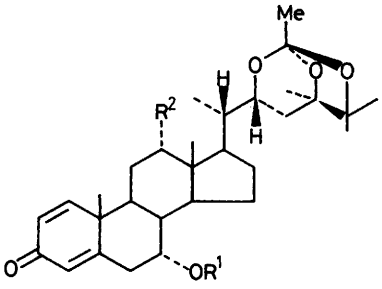


(**5**) R^1 = $COCH_2COSMe$, R^2 = H
(**6**) R^1 = Ac, R^2 = H
(**7**) R^1 = H, R^2 = H

Petuniasterone C series

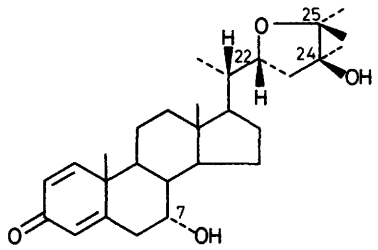
Chloroform extracts of plant materials were fractionated as previously described,[2] and the individual petuniolides were obtained by successive preparative HPLC. Petuniolides A (**11**), B (**12**), and D (**14**) were found in leaves of *P. integrifolia* (Hook.) Schinz and Thelling (= *P. violacea*, Lindley) in amounts of 100, 430, and 200 ppm (dry weight basis), respectively. Petuniolide C (**13**) was obtained at 370 ppm from *P. parodii* (Steere).

Petuniolide B (**12**) formed crystals adequate for X-ray

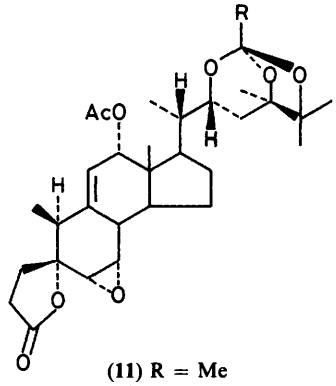


(**8**) R^1 = R^2 = H
(**9**) R^1 = Ac, R^2 = OAc

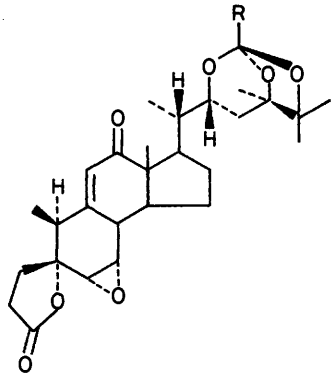
Petuniasterone D series



(10) Petuniasterone F



(11) R = Me
(12) R = Et



(13) R = Me
(14) R = Et

analysis. The molecular structure of (12) was unequivocally established and is shown in Figure 2 with the atom numbering system used in the X-ray investigation. Figure 3 presents a stereoscopic view of its molecular conformation, and the final atomic co-ordinates and their estimated standard deviations (in parentheses) are listed in Table 3. Carbons 5, 13, 17, 22, and 24 possess the (*R*)-configuration, and carbons 6, 7, 8, 10, 12, 14, and 20 have the (*S*)-configuration. The ^1H and ^{13}C NMR spectra of (12) are consistent with this structure (Tables 1 and 2). Their assignments, based upon the positions and multiplicities of the observed signals compared with spectra of the petuniasterones[2–5] were facilitated by ^1H–^1H and ^{13}C–^1H correlation spectroscopy[8] and by decoupling experiments. A complex set of proton signals was observed at δ_H 2.0–2.2 and 2.5–2.8 in all the compounds in the series, and was assigned to the spirolactone methylenes (Table 1); however, their complexity precluded detailed analysis. In the ^{13}C NMR spectrum, the lactone carbonyl signal appeared at δ_C 175.6 with the methylene carbons at positions-3 and -4 at δ_C 28.6 and 29.2 respectively. The proton signals at δ_{H-6} 3.19 (d, *J* 4 Hz) and δ_{H-7} 3.31 (dd, *J* 4, 1.5) were assigned to the 6,7-epoxy functionality. Signals at δ_C 55.1 and 53.8 were correlated with positions 6 and 7 respectively. By comparison, the oxirane carbons of epoxycyclohexane appear at δ_C 51.9.[9] The vinyl signal at δ_H 5.79,

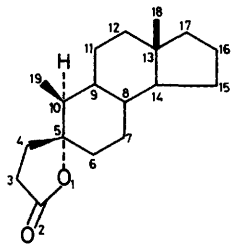


Figure 1. Petuniolide numbering.

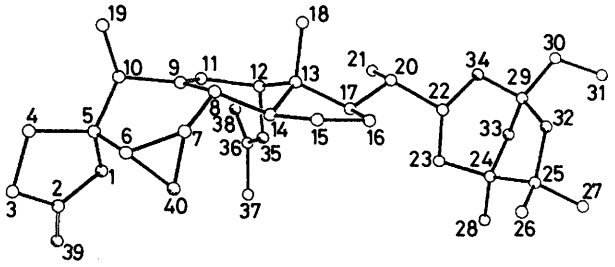


Figure 2. Perspective view of compound (12) with the crystallographic numbering scheme. Open bonds represent double bonds, and shaded circles represent oxygen atoms.

which appears as a broadened doublet as a result of long-range coupling to H-8 was assigned to H-11. H-12 is observed as a sharp doublet at δ_H 4.96 and C-12 is at δ_C 73.2. Even though (12) has a 9–11 double bond and a 12-acetoxy substituent, the position of the 18-methyl group resonance (δ_H 0.71) is essentially unshifted compared to compound (1). Just such a small substituent effect is expected for an α-oriented 12-acetoxy group.[10] NMR signals associated with the side chain and the orthopropionate ester were similar to those previously reported.[5] In the IR spectrum, compound (12) exhibits two carbonyl bands: 1 775 cm^{-1} corresponding to the spirolactone[11] and 1 725 cm^{-1} associated with the acetate at position 12. The UV absorption maximum at 206 nm is consistent with the $\Delta^{9(11)}$ isolated double bond.[12a]

The spectroscopic properties of petuniolide A (11) were very similar to those of (12). The elemental composition of (11) compared with that of (12) indicated only the difference of one $-CH_2-$ group. In the ^1H NMR spectrum of (11), the signals associated with the pendant orthopropionate system of the side chain (CH_3, δ 0.99 and CH_2, δ 1.80) in (12) are absent, and a new methyl singlet appears at δ 1.56. Similarly, the ^{13}C NMR spectrum of (11) does not show the orthopropionate CH_3 and CH_2 signals at δ_C 7.7 and 29.3 respectively which are observed in (12) but does exhibit a CH_3 peak at δ_C 23.5. Additionally, the ^{13}C signal at δ_C 118.6 of (12) now appears at δ 117.3. These spectral changes show that an orthoacetate moiety is attached to the side chain of (11), and are in full agreement with the spectra of similar orthoacetates in the petuniasterone D series.[3]

Petuniolides C (13) and D (14) showed lactone IR absorption at 1 775 cm^{-1} similar to that of (11) and (12); however, no acetate band (1 725 cm^{-1}) was observed, and a new carbonyl band at 1 680 cm^{-1} was present. Elemental compositions of (13) and (14) differed from those of (11) and (12) respectively by the loss of C_2H_4O. UV absorption at *ca.* 234 nm was consistent with an α,β-unsaturated ketone, suggesting the presence of a $\Delta^{9(11)}$-12-oxo-system.[12b] In the NMR spectra of (13) and (14), no resonances associated with an acetoxy group were observed, and the signal at δ_H 4.96 for H-12 of the allylic acetates was not present. The signal at δ_C 73.2 (C-12) was missing, and a new carbonyl resonance at δ_C 204.4 appeared in each case. Additionally, olefinic signals in (13) and (14) now were found at δ_C 155.4 (C) and 128.0 (CH), which, together with the above, indicate a conjugated olefinic ketone[13] at position 12.

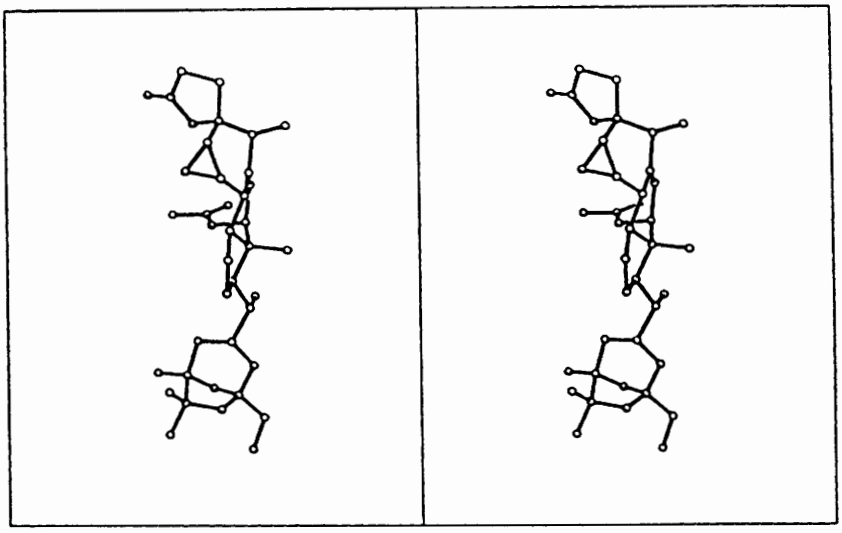


Figure 3. Stereoscopic view of compound (**12**).

Table 1. ^{1}H NMR data.*

	Compound			
	(**11**)	(**12**)	(**13**)	(**14**)
3-H_2	*ca.* 2.5–2.8m	*ca.* 2.5–2.8m	*ca.* 2.5–2.8m	*ca.* 2.5–2.8m
4-H_2	*ca.* 2.0–2.2m	*ca.* 2.0–2.2m	*ca.* 2.2m	*ca.* 2.2m
6-H	3.20d (4)	3.19d (4)	3.29d (4)	3.29d (4)
7-H	3.29dd (4, 1.5)	3.31dd (4, 1.5)	3.35br d (4)	3.35br d (4)
8-H	2.26br d (10)	2.26br d (10)	2.72m	2.72m
10-H	2.58q (7)	2.59q (7)	2.78m	2.78m
11-H	5.79br d (5)	5.78br d (5)	5.84br s	5.84br s
12-H	4.96d (5)	4.96d (5)	—	—
14-H	*ca.* 2.0m	*ca.* 2.0m	*ca.* 2.2m	*ca.* 2.2m
15-H_2	*ca.* 1.4 and 2.1 m[a]	*ca.* 1.4 and 2.1m[a]	1.6 and 1.9m[a]	1.6 and 1.9m[a]
16-H_2	*ca.* 1.6 and 1.9m[a]	*ca.* 1.6 and 1.9m[a]	1.6 and 2.0m[a]	1.6 and 2.0m[a]
17-H	*ca.* 1.95m	*ca.* 1.95m	*ca.* 1.9m	*ca.* 1.9m
18-H_3	0.71s	0.71s	1.00s	1.01s
19-H_3	1.06d (7)	1.06d (7)	1.15d (7)	1.16d (7)
20-H	*ca.* 1.8m	*ca.* 1.8m	*ca.* 1.7m	*ca.* 1.7m
21-H_3	0.85d (6)	0.85d (6)	1.08d (7)	1.08d (7)
22-H	4.21td (8, 4)	4.21td (8, 4)	4.25dt (11.5, 4)	4.25dt (11.5, 4)
23-H_2	1.53d (8)	1.54d (8)	1.48dd (14,5) and 1.80br d (14)	1.50dd (14, 5) and 1.78m
26-H_3	1.33s	1.34s	1.31s	1.33s
27-H_3	1.18s[b]	1.17s[b]	1.18s[b]	1.17s[b]
28-H_3	1.22s[b]	1.21s[b]	1.22s[b]	1.22s[b]
Orthoacetate	1.56s	—	1.56s	—
Orthopropionate				
CH_2	—	1.80m	—	1.82m
CH_3	—	0.99t (7.5)	—	0.99t (7.5)
OAc	2.07s	2.06s	—	—

* δ values in $CDCl_3$; coupling constants (Hz) in parentheses. Assignments are by decoupling and correlation techniques. Values with identical superscripts in each column may be interchanged.

Confirmatory evidence for this position of functionalization was provided by the proton at position-11 which appeared at δ_H 5.84 as a broadened singlet showing coupling to H-8. CH_3-18 in (**13**) and (**14**) exhibits a substituent-induced chemical-shift difference in the ^{1}H NMR of +0.30 ppm which agrees closely with the value reported for an unsaturated ketone at position-12 of steroids.[10] Methyl groups at positions 19 and 21 also show shifts to lower field for their proton resonances, but in other respects the NMR spectra of (**13**) and (**14**) are quite similar to those of (**11**) and (**12**) and show signals ascribable to the oxirane and lactone moieties. Evidence analogous to that above indicates that compound (**13**) possesses orthoacetate functionality and (**14**) has an orthopropionate group. The remaining features in the NMR spectra of (**13**) and (**14**) compared to those of (**11**) and (**12**) and to those of the petuniasterones did not provide sufficient information for the assignment of further stereochemical details.

The molecular structure and absolute stereochemistry of (**14**) were determined by X-ray crystallography (Figures 4 and 5). Atomic co-ordinate data are given in Table 4. Compound (**14**) [and by comparison (**13**)] have the same absolute configuration as compounds (**11**) and (**12**). In the molecular structures of both

Table 2. ^{13}C NMR data.*

Carbon	Compound (11)	(12)	(13)	(14)
2	175.7, C	175.6, C	175.2, C	175.2, C
3	28.6, CH_2	28.6, CH_2	28.5, CH_2	28.5, CH_2
4	29.2, CH_2	29.2, CH_2	28.8, CH_2	28.8, CH_2
5	85.8, C	85.8, C	85.3, C	85.3, C
6	55.1, CH	55.2, CH	55.4, CH	55.5, CH
7	53.8, CH	53.8, CH	53.3, CH	53.3, CH
8	36.5, CH	36.6, CH	37.7, CH	37.7, CH
9	139.9, C	139.9, C	155.4, C	155.4, C
10	41.4, CH	41.4, CH	41.4, CH	41.4, CH
11	123.0, CH	123.0, CH	128.0, CH	128.0, CH
12	73.2, CH	73.2, CH	202.4, C	202.4, C
13	44.3, C	44.3, C	53.8, C	53.8, C
14	42.2,[a] CH	42.2,[a] CH	49.7, CH	49.8, CH
15	24.0,[b] CH_2	24.1,[b] CH_2	23.8,[a] CH_2	23.8,[a] CH_2
16	27.0,[b] CH_2	27.1,[b] CH_2	27.5,[a] CH_2	27.4,[a] CH_2
17	43.3,[a] CH	43.3,[a] CH	43.4, CH	43.5, CH
18	10.9, CH_3	10.9, CH_3	10.1, CH_3	10.2, CH_3
19	14.4, CH_3	14.4, CH_3	13.7, CH_3	13.7, CH_3
20	38.1, CH	38.2, CH	39.4, CH	39.5, CH
21	11.1, CH_3	11.1, CH_3	13.3, CH_3	13.4, CH_3
22	69.7, CH	69.5, CH	70.1, CH	69.9, CH
23	30.1, CH_2	30.4, CH_2	30.4, CH_2	30.8, CH_2
24	82.5,[c] C	82.1,[c] C	82.5,[b] C	82.1,[b] C
25	81.2,[c] C	81.0,[c] C	81.4,[b] C	81.2,[b] C
26	20.0, CH_3	20.2, CH_3	20.0, CH_3	20.1, CH_3
27	20.6,[d] CH_3	20.6,[d] CH_3	20.4,[c] CH_3	20.5,[c] CH_3
28	25.2,[d] CH_3	25.4,[d] CH_3	25.1,[c] CH_3	25.3,[c] CH_3
Orthoester	117.3, C	118.6, C	117.2, C	118.7, C
Acetate	21.3, CH_3	21.2, CH_3	—	—
Acetate	170.3, C	170.2, C	—	—
Orthoacetate	23.5, CH_3	—	23.5, CH_3	—
Orthopropionate	—	7.7, CH_3	—	7.7, CH_3
Orthopropionate	—	29.3, CH_2	—	29.3, CH_2

* In ppm from internal $SiMe_4$ for $CDCl_3$ solutions. Values with identical superscripts in each column may be interchanged. Assignments were facilitated by C–H correlation spectroscopy.

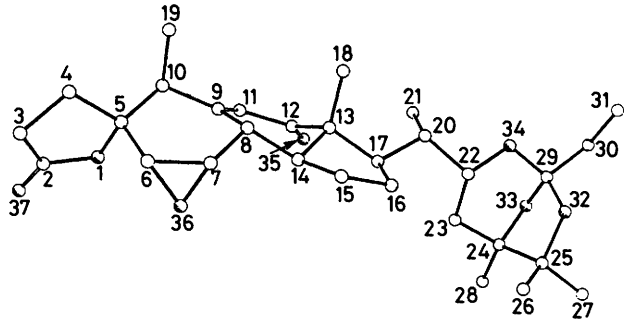


Figure 4. Perspective view of compound (**14**) with the crystallographic numbering scheme. Open bonds represent double bonds, and shaded circles represent oxygen atoms.

(**12**) and (**14**) the dihedral angle between the normals of the two best least-squares ring planes defined by (O-1, C-2, C-3, C-4, and C-5) and (C-5, C-6, C-7, C-8, C-9, and C-10) is nearly orthogonal: petuniolide B, 93.0° and petuniolide D, 83.4°.

Experimental

M.p.s were taken with a Thomas–Hoover apparatus and are corrected. Optical rotations were obtained for chloroform solutions on a Perkin-Elmer Model 241 automatic polarimeter at *ca.* 26 °C. IR spectra were recorded on a Perkin-Elmer Model 237 spectrophotometer and refer to chloroform solutions; UV spectra were taken on Cary 219 and Hewlett-Packard 8451A spectrophotometers using methanol solutions; ^1H NMR spectra were obtained in $CDCl_3$ at 90 MHz on a Varian EM-390 instrument or at 200 MHz on a Nicolet NT-200, and ^{13}C NMR spectra were taken at 50 MHz on the latter instrument. NMR assignments were facilitated by decoupling methods and by the use of two-dimensional proton–proton and carbon–proton correlation techniques.[8] Mass spectra were obtained using a VG Micromass 70/70 HS instrument either by electron impact or using ammonia chemical ionization. X-Ray intensities were collected with a Nicolet R3 automatic diffractometer at room temperature.

HPLC columns were from Rainin Instruments, Alltech Associates and IBM, Inc. Solvents were HPLC grade and were pumped using an Altex-Beckman Model 110A pump. Detection was by UV spectroscopy either at 254 nm with an Altex Model 150 monitor or at lower wavelengths with a Beckman Model 165 variable wavelength detector. Bulk RP-18 preparative column packing was obtained from Waters Chromatography Division.

Insect Bioassays.—Solutions containing materials for bioassay were evaporated on to cellulose powder. The powder was mixed thoroughly and incorporated into modified Berger-diet premix.[14] The test diets were divided into ten portions, placed in individual plastic containers, and newly hatched larvae of *Heliothis zea* were added. The insects were maintained at 26 °C

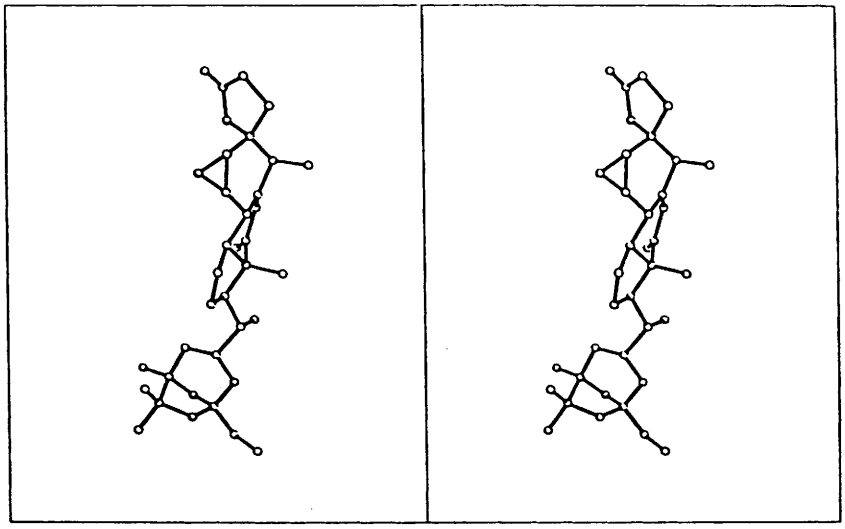


Figure 5. Stereoscopic view of compound (**14**).

Table 3. Petuniolide B: atomic co-ordinates ($\times 10^4$) with e.s.d.s in parentheses.

Atom	*x*	*y*	*z*
O(1)	691(3)	1 171	8 695(2)
C(2)	1 342(5)	−142(7)	8 705(3)
C(3)	2 698(4)	200(6)	8 853(3)
C(4)	2 683(4)	1 823(5)	9 194(3)
C(5)	1 517(4)	2 480(5)	8 779(3)
C(6)	1 781(4)	3 087(5)	7 919(3)
C(7)	1 088(4)	4 380(5)	7 574(3)
C(8)	8(4)	5 080(5)	8 039(2)
C(9)	−298(4)	4 235(5)	8 833(3)
C(10)	803(4)	3 631(5)	9 332(3)
C(11)	−1 451(4)	4 036(5)	9 112(3)
C(12)	−2 614(4)	4 581(5)	8 611(3)
C(13)	−2 310(4)	5 725(5)	7 941(2)
C(14)	−1 134(4)	5 186(5)	7 479(2)
C(15)	−1 054(4)	6 206(6)	6 698(3)
C(16)	−2 423(4)	6 572(6)	6 475(3)
C(17)	−3 244(4)	5 904(5)	7 199(3)
C(18)	−2 083(4)	7 254(5)	8 360(3)
C(19)	1 676(4)	4 931(5)	9 631(3)
C(20)	−4 438(4)	6 828(5)	7 348(3)
C(21)	−5 250(4)	6 196(6)	8 062(3)
C(22)	−5 182(4)	7 002(5)	6 515(3)
C(23)	−5 767(4)	5 532(5)	6 187(3)
C(24)	−6 895(4)	5 814(5)	5 615(3)
C(25)	−6 612(4)	6 845(5)	4 848(3)
C(26)	−5 388(4)	6 529(6)	4 396(3)
C(27)	−7 670(4)	6 921(6)	4 211(3)
C(28)	−7 575(5)	4 374(5)	5 406(3)
C(29)	−7 057(4)	8 199(5)	6 085(3)
C(30)	−7 889(5)	9 497(6)	6 298(3)
C(31)	−8 985(5)	9 734(7)	5 737(3)
O(32)	−6 533(3)	8 325(3)	5 274(2)
O(33)	−7 728(3)	6 809(3)	6 071(2)
O(34)	−6 122(3)	8 132(3)	6 690(2)
O(35)	−3 139(3)	3 225(3)	8 215(2)
C(36)	−3 787(4)	2 270(6)	8 694(4)
C(37)	−4 094(5)	889(6)	8 225(4)
O(38)	−4 040(4)	2 521(5)	9 424(2)
O(39)	867(4)	−1 344(4)	8 617(3)
O(40)	883(3)	2 844(4)	7 276(2)

for ten days, and their weights were determined and compared with those of control subjects maintained on diets containing as additive only the standard quantity of cellulose powder.

Plant Material.—*Petunia parodii* (Steere) seeds were obtained from the National Seed Storage Laboratory, Colorado State University, Fort Collins, CO, USA. *Petunia integrifolia* (Hook.) seeds were from the Northeastern Plant Introduction Station, US Dept. of Agriculture, Geneva, New York. Leaf material and seed of *P. integrifolia* were also supplied by Ing. Agr. Hugo A. Cordo, Biological Control of Weeds Laboratory, Hurlingham, Buenos Aires Prov., Argentina. Plants were grown in a greenhouse and in outdoor beds in Albany, California. Leaf material was harvested at intervals between December 1987 and June 1988.

Isolation Procedure.—Freeze-dried leaf material (typically 100–300 g) was successively ground with chloroform (3 × 1 400 ml) using a Tekmar SD-45 homogenizer at maximum speed followed by filtration. After evaporation of the combined filtrate under reduced pressure, the resulting green oil (*ca.* 10% of original dry wt.) was suspended in boiling acetonitrile (*ca.* 10–15 volumes) with stirring for 1 h. Upon cooling to 5 °C, the solution was easily decanted from waxy, solid material. Approximately 50% of the original extract remained in solution. This solution was evaporated and redissolved in four volumes of acetonitrile for application to a 50 mm × 250 mm column of 25 μ RP-18 packing. Elution with acetonitrile gave a zone of active material (elution volume 375–900 ml) containing numerous petuniasterones as well as the petuniolides reported herein. All chlorophyll and considerable amounts of less polar lipid material were removed in this way to yield a mixture representing about 4% of the original plant weight. Further fractionation was accomplished by preparative HPLC. Columns and conditions are as follows: Dynamax C-18, 21.4 × 250 mm + guard column (30% water in acetonitrile); R Sil C-18, 10 mm × 250 mm (30% water in acetonitrile); and IBM Cyano, 10 mm × 250 mm (20% propan-2-ol in hexane). Results are given in Table 5.

Compound (**11**), *petuniolide A*, m.p. 232–235 °C (from MeOH); [α] (λ/nm) (589) +99°, (578) +104°, (546) +118°, (436) +209°, (365) +321°; ν_{max} 1 775 (lactone) and 1 725 cm^{-1} (acetate); λ_{max} 206 nm (log ε 3.75); *m/z* 562.3370 (MNH_4^+, 37%), 545 (MH^+, 4), and 485 ($MH^+ - C_2H_4O_2$, 63). $C_{31}H_{48}NO_8$ requires 562.3379.

Compound (**12**), *petuniolide B*, m.p. 249–252 °C (from MeOH–H_2O); [α] (λ/nm) (589) +113°, (578) +117°, (546) +134°, (436) +237°, (365) +390°; ν_{max} 1 775 (lactone) and 1 725 cm^{-1} (acetate); λ_{max} 206 nm (log ε 3.63); *m/z* (EI) 558.3220

Table 4. Petuniolide D: atomic co-ordinates ($\times 10^4$) with e.s.d.s in parentheses.

Atom	*x*	*y*	*z*
O(1)	12 218(3)	7 357	10 482(1)
C(2)	13 368(5)	7 326(6)	10 984(2)
C(3)	14 504(5)	8 072(7)	10 611(3)
C(4)	13 752(4)	9 055(5)	9 929(3)
C(5)	12 426(3)	8 125(5)	9 736(2)
C(6)	12 612(4)	6 747(5)	9 151(2)
C(7)	11 593(3)	6 444(5)	8 454(2)
C(8)	10 342(3)	7 445(5)	8 285(2)
C(9)	10 028(4)	8 331(4)	9 007(2)
C(10)	11 196(6)	9 213(5)	9 483(2)
C(11)	8 783(3)	8 425(4)	9 195(2)
C(12)	7 609(3)	7 748(5)	8 707(2)
C(13)	7 802(3)	7 363(5)	7 854(2)
C(14)	9 119(3)	6 386(5)	7 954(2)
C(15)	9 152(4)	5 596(7)	7 148(2)
C(16)	7 657(3)	5 209(7)	6 860(2)
C(17)	6 808(3)	6 142(5)	7 412(2)
C(18)	7 914(4)	8 945(5)	7 384(2)
C(19)	11 554(5)	10 681(6)	8 991(3)
C(20)	5 494(3)	6 813(5)	6 947(2)
C(21)	4 631(4)	7 804(6)	7 450(2)
C(22)	4 691(3)	5 434(5)	6 511(2)
C(23)	4 259(4)	4 102(5)	7 036(2)
C(24)	3 084(3)	3 124(5)	6 660(2)
C(25)	3 269(4)	2 324(6)	5 858(2)
C(26)	4 625(4)	1 601(7)	5 819(2)
C(27)	2 163(5)	1 153(9)	5 541(3)
C(28)	2 553(4)	2 017(6)	7 264(3)
C(29)	2 560(4)	5 058(6)	5 763(2)
C(30)	1 416(4)	5 940(8)	5 253(3)
C(31)	1 818(4)	6 774(9)	4 534(3)
O(32)	3 128(2)	3 805(5)	5 357(1)
O(33)	2 034(2)	4 297(4)	6 391(2)
O(34)	3 538(2)	6 185(4)	6 049(2)
O(35)	6 580(2)	7 469(4)	8 981(1)
O(36)	11 673(3)	5 420(3)	9 129(1)
O(37)	13 422(5)	6 721(6)	11 607(2)

(M^+, 2%). $C_{32}H_{46}O_8$ requires 558.3192; *m/z* (CI) 576 (MNH$_4^+$, 12%), 559 (MH$^+$, 6%) and 499 (MH$^+$ − $C_2H_4O_2$, 32).

Compound (**13**), *petuniolide C*, m.p. 235–238 °C (from EtOAc–heptane); [α] (λ/nm) (589) +7°, (578) +7°, (546) +3°, (436) −60°, (365) −699°; ν_{max} 1 775 (lactone) and 1 680 cm^{-1} (conjugated CO); λ_{max} 233 nm (log ε 4.09); *m/z* 501.2831 (MH$^+$, 100%) and 457 (MH$^+$ − CO_2). $C_{29}H_{41}O_7$ requires 501.2852.

Compound (**14**), *petuniolide D*, m.p. 200–206 °C (from MeOH); [α] (λ/nm) (589) +9°, (578) +8°, (546) +6°, (436) −50°, (365) −651°; ν_{max} 1 775 (lactone) and 1 680 cm^{-1} (conjugated CO); λ_{max} 234 nm (log ε 4.14); *m/z* 515.3001 (MH$^+$, 100%). $C_{30}H_{43}O_7$ requires 515.3008.

Crystal Structure of Compound (**12**).—Petuniolide B, $C_{32}H_{46}O_8$, M = 558.8, monoclinic, space group $P2_1$, a = 10.739(1), b = 8.824(1), c = 15.903(2) Å, β = 90.26(1)°, U = 1 507.1 Å^3, D_c = 1.23 g cm^{-3}, Z = 2, F(000) = 603.9, μ(Cu-K_α) = 6.72 cm^{-1}. Final R = 0.053 (361 parameters), R_w = 0.056 for 2 779 unique reflections with $|F_o| \geqslant 3\sigma|F_o|$ in the range 3° ⩽ 2θ ⩽ 114°, average parameter shift is 0.05σ, and difference Fourier synthesis excursions are within ±0.6 eÅ^{-3}. Crystals were obtained from methanol.

Table 5. Elution zone (ml).

Compound	Dynamax C18	R Sil C18	Cyano
(**11**)	160–220	33–40	90–100
(**12**)	260–300	43–53	85–95
(**13**)	100–140	25–35	135–150
(**14**)	160–220	30–38	130–140

Crystal Structure of Compound (**14**).—Petuniolide D, $C_{30}H_{42}O_7$, M = 514.7, monoclinic, space group $P2_1$, a = 10.066(3), b = 8.192(2), c = 17.076(3) Å, β = 97.94(2)°, U = 1 394.6 Å^3, D_c = 1.23 g cm^{-3}, Z = 2, F(000) = 555.9, μ(Cu-K_α) = 6.60 cm^{-1}. Final R = 0.059 (334 parameters), R_w = 0.074 for 2 754 unique reflections with $|F_o| \geqslant 3\sigma|F_o|$ in the range 3° ⩽ 2θ ⩽ 114°, average parameter shift is 0.08σ, and difference Fourier synthesis excursions are within ±0.4 eÅ^{-3}. Crystals were obtained from methanol by slow evaporation.

Data collection and Structure Refinement.—Intensity data were collected on a Nicolet R3 diffractometer with graphite monochromatized Cu-K_α radiation (λ = 1.5418 Å) by the θ–2θ scan technique with variable scan speed (4–30°/min) at room temperature. The intensity data were corrected for background and Lorentz–polarization effects,[15] but not for absorption. In the final cycles of refinement of (**14**), a secondary extinction correction (0.0593) was included to minimize the discrepancy between $|F_o|$ and $|F_c|$ of the most intense reflections and led to a significant improvement in R. 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[16] 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';[17] those of oxygen 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 (**12**) and (**14**) 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 1.014 and 1.026 for (**12**) and (**14**) respectively. According to Hamilton's statistical test,[18] the enantiomer with the lower R_w value has a probability of being correct to a significance level better than 0.5%.*

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* *Supplementary data* (see section 5.6.3 of Instructions for Authors in the January issue). For both (**12**) and (**14**), a complete list of final atomic bond lengths and angles, anisotropic thermal parameters for the non-hydrogen atoms, and positional parameters for the hydrogen atoms have been deposited at the Cambridge Crystallographic Data Centre.

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