Petuniasterones. Part 2.[1] Novel Ergostane-type Steroids from *Petunia hybrida* Vilm. (Solanaceae)

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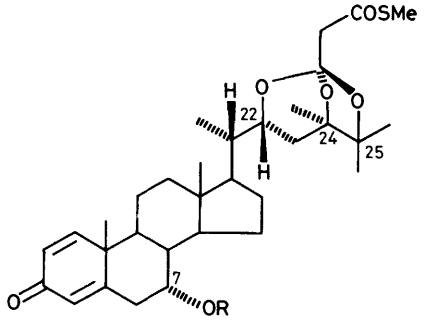
Four new compounds in the petuniasterone series were isolated from leaves and stems of *Petunia hybrida*. These all possess the $\Delta^{1,4}$-diene-3-one structure and have a 7α-oxygenated functionality. Compounds identified include the 17β-hydroxy derivative of petuniasterone A (**9**) and its 7-acetate (**10**) in addition to petuniasterone D (**3**), which is the orthoacetate of (22*R*,24*R*)-7α-22,24,25-tetrahydroxyergosta-1,4-dien-3-one as well as 12α-acetoxypetuniasterone D 7-acetate (**5**).

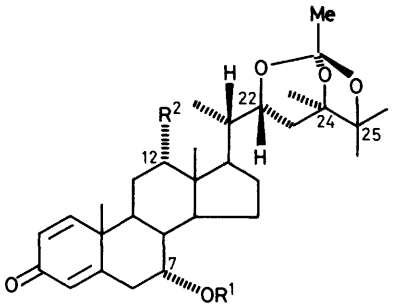
Seven unusually substituted ergostane-type steroids have previously been isolated from leaf and stem tissue of *Petunia hybrida*.[1] These petuniasterones appear to play a role in the defence of the plant against feeding by certain insects. We now report the isolation and structural characterization of four additional, related materials from the same source.



(**1**) R = H
(**2**) R = Ac

Chloroform extracts of plant material were fractionated, as previously described,[1] by initial separation on silica gel followed by preparative h.p.l.c. on several columns. After removal of petuniasterone A (**1**) and several of the other, more abundant, petuniasterones, it was possible to obtain four new compounds. The least polar of these, compound (**3**), which we have named petuniasterone D, is closely related to petuniasterone A (**1**). It has an unsubstituted orthoacetate system on its side chain, analogous to the (methylthio)carbonyl orthoacetate at positions 22, 24, and 25 of compound (**1**). The u.v. spectrum of compound (**3**) shows a band at 246 nm which is consistent with the cross-conjugated dienone of ring A[2] and is essentially the same as that of compound (**1**). The i.r. spectrum shows the expected frequency ($\nu_{max.}$ 1 660 cm^{-1}) as in (**1**). The ^1H n.m.r. (Table 1) and ^{13}C n.m.r. (Table 2) spectra of compounds (**1**) and (**3**) are essentially superposable except for signals associated with the pendant group of the ortho ester moiety. In compound (**3**) the orthoacetate methyl is associated with a 3-proton singlet at δ_H 1.56 in the ^1H n.m.r. spectrum and with ^{13}C signals at δ_C 23.5 and 117.3 (CH_3 and C, respectively). The corresponding values for triethyl orthoacetate are δ_H 1.37 (3 H, methyl)[3] and δ_C 20.4 and 114.3 (CH_3 and C).[4] In pseudrelone B, having similar functionality, the methyl protons of the orthoacetate appeared at δ_H 1.78 and the corresponding carbons were at δ_C 25.8 and 117.6.[5] The excellent correspondence of the characteristic proton couplings in compounds (**1**) and (**3**) is significant evidence that identical stereochemical configuration pertains. The n.m.r. spectral shifts and couplings in the corresponding acetates (**2**) and (**4**) confirms this point, especially with regard to the α-configuration of the 7-hydroxy substituent.

Compound (**5**) has two acetoxy groups. One of these must be at position 7α as is indicated by comparison of the ^1H n.m.r. signals of the 6α and 6β protons in this compound with those of acetate (**4**). Partial methanolysis of diacetate (**5**) gave acetate (**6**)



(**3**) R^1 = R^2 = H
(**4**) R^1 = Ac, R^2 = H
(**5**) R^1 = Ac, R^2 = OAc
(**6**) R^1 = H, R^2 = OAc

which has a free 7α-OH and which shows the complex and highly characteristic coupling pattern for the hydrogens attached to C-6 [*cf.* compounds (**1**) and (**3**), Table 1]. The assignment of the second acetoxy group of compound (**5**) to position 12 was facilitated by the observation that the $^{13}CH_2$ signal at δ *ca.* 39 for (**3**) is no longer present, being replaced by the ^{13}CH signal at δ 74.6. Confirmatory evidence is provided by the magnitude and direction of the substituent effect of the OAc group upon the chemical shifts of carbons in its vicinity. Iida *et al.*[6] have reported Δδ of *ca.* −6, −7, and −9 p.p.m. for carbons 9, 14, and 17 respectively and *ca.* +5 and +2 p.p.m. for carbons 11 and 13 in methyl 12α-acetoxy-5β-cholanoate *vs.* the unsubstituted compound. We observe similar differences between compounds (**4**) and (**5**) or (**6**). The effect (+0.08 p.p.m.) reported for a 12α-acetoxy group upon the ^1H chemical shift of 18-H_3[7] correlates satisfactorily with our data. Although the geometry of the C-19 methyl group is somewhat altered as a

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

H	Compound (1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
1	7.08 [7.11] d (10)	7.02 d (10)	7.08 d (10)	7.08 d (10)	6.96 d (10)	6.95 d (10)	7.07 d (10)	7.02 d (10)	7.10 [7.06] d (10)	7.10 d (10)	7.08 d (10)
2	6.24 [6.42] dd (10, 2)	6.22 dd (10, 2)	6.25 dd (10, 2)	6.26 dd (10, 2)	6.25 dd (10, 2)	6.23 dd (10, 2)	6.25 dd (10, 2)	6.23 dd (10, 2)	6.22 [6.48] dd (10, 2)	6.26 dd (10, 2)	6.26 dd (10, 2)
4	6.13 [6.46] br t (*ca.* 2)	6.01 br d (*ca.* 2)	6.14 br t (*ca.* 2)	6.02 br d (*ca.* 2)	6.03 br d (*ca.* 2)	6.14 br t (*ca.* 2)	6.00 d (2)	6.00 br s	6.14 [6.44] br t (*ca.* 2)	6.02 br s	6.00 d (2)
6α	2.50 [2.62] dd (14, 3)	2.60 d (14)	2.48 dd (14, 3)	2.60 d (13)	2.60 d (14)	2.50 dd (14, 3)	6-H 6.03 dd (10, 2)	6-H 6.04 dd (10, 2)	2.50[6.62] dd (14, 3)	2.60 d (14)	6-H 6.03 dd (10, 2)
6β	2.75 [2.76] ddd (14, 3, 2)	2.66 d (14)	2.74 ddd (14, 3, 2)	2.65 d (13)	2.66 d (14)	2.76 ddd (14, 3, 2)			2.74 [2.74] br d (14)	2.66 d (14)	
7	4.04 [4.18] br s	5.05 br q (*ca.* 3)	4.04 br s	5.06 br q (*ca.* 3)	5.08 br s	4.06 s	6.23 dd (10, 2.5)	6.22 dd (10, 2)	4.06 [4.24] br s	5.08 br q (*ca.* 3)	6.24 dd (10, 2.5)
8							2.26 br t (10)	2.30 br t (9)			2.32 br t (10)
12β	2.02 dt (12.5, 4)	2.04 m	2.03 dt (12.5, 4)	2.05 m	5.08 br s	5.07 br t (3)	2.10 dt (13, 3.5)	4.09 br s			2.10 ddd (13, 5, 4)
20	1.78[b] (J_{20-22} 4)							*ca.* 1.8[b] (J_{20-22} 4)	[2.36] qd (7, 4)		
22	4.21 [4.34] dt (11.5, 4)	4.20 dt (11, 4)	4.20 dt (11, 5)	4.18 dt (10, 4)	4.16 td (8, 4)	4.18 td (8, 4)	4.20 dt (11, 5)	4.20 ddd (10, 7, 4)	4.29 ddd (11, 7, 4) [5.14] dt (11, 4)	4.30 ddd (11, 7, 4)	4.28 ddd (10, 7, 4)
23	1.47[b] ($J_{22\ 23}$ 4)				1.50 d (8)	1.50 d (8)		*ca.* 1.6[b] (J_{22-23} 7) (J_{22-23} 10)	[1.93] dd (14, 11) [2.15] dd (14, 4)		
	1.52[b] (J_{22-23} 11.5)										
18-H_3	0.76 [0.67] s	0.76 s	0.76 s	0.75 s	0.83 s	0.83 s	0.80 s	0.82 s	0.97 [1.18] s	0.97 s	1.01 s
19-H_3	1.23 [1.24][c] s	1.25 s	1.24 s	1.25 s	1:23 s	1.22 s	1.20[c] s	1.20[c] s	1.22 [1.22][c] s	1.26 s	1.20[c] s
21-H_3	0.96 [1.09] d (7)	0.95 d (7)	0.96 d (7)	0.97 d (7)	0.85 d (7)	0.86 d (7)	0.98 d (7)	1.02 d (7)	0.92 [1.12] d (7)	0.91 d (7)	0.90 d (7)
26-H_3	1.30 [1.35] s	1.31 s	1.30 s	1.30 s	1.30 s	1.30 s	1.31 s	1.32 s	1.33 [1.44] s	1.33 s	1.34 s
27-H_3	1.12 [1.25][c] s	1.12[c] s	1.16[c] s	1.17[c] s	1.16[c] s	1.16[c] s	1.17[c] s	1.18[c] s	1.14[c] [1.18][c] s	1.14[c] s	1.14[c] s
28-H_3	1.21 [1.20][c] s	1.20[c] s	1.20[c] s	1.19[c] s	1.18[c] s	1.18[c] s	1.20[c] s	1.18[c] s	1.22[c] [1.22][c] s	1.20[c] s	1.20[c] s
COSMe	2.31 [2.28] s	2.31 s							2.32 [2.25] s	2.31 s	
CH_2CO	3.04 + 3.10 [3.44 + 3.46] each d (14)	3.04 d (14), 3.10 d (14)							3.01 + 3.07 d (14) [3.45] s	3.02 d (14), 3.07 d (14)	2.78 d (14), 2.90 d (14)
OAc		2.00 s		2.00 s	2.03 s, 2.08 s	2.04 s				1.98 s	
Ortho-acetate			1.56 s	1.56 s	1.54 s	1.56 s	1.56 s	1.57 s			
CO_2Me											3.72 s

[a] δ Values in $CDCl_3$ except [in brackets] [$^2H_5$]pyridine: coupling constants (Hz) in parentheses. [b] Values obtained by stepwise decoupling. [c] Values may be interchanged.

consequence of the A-ring dienone, the small substituent effect (−0.02 p.p.m.) upon its chemical shift is also in agreement with that shown by more typical steroids having 12α-acetoxy groups. It may be noted that those examples for the alternative 11-, 15-, or 16-position of acetoxylation exhibit substantially different effects upon their respective angular methyl groups.

It was not possible to effect hydrolysis of the 12-acetoxy group without elimination of the group (OH or OAc) at C-7 to yield triene (**8**). This lability is not unusual since these substituents are adjacent to a position from which a proton may be easily abstracted.[8] However, shift comparisons between triene (**8**) and the corresponding non-hydroxylated triene (**7**) were also in agreement with reported values for ^{13}C substituent effects in the androstane series[9] and for bile acids.[6-10]

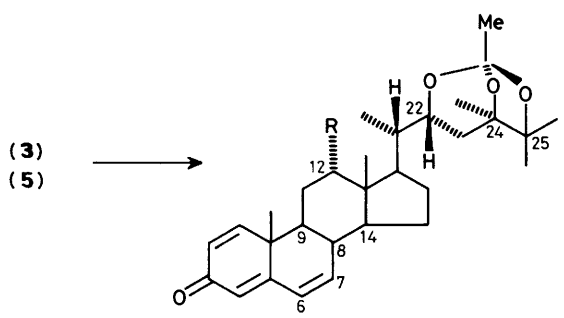
Table 2. ^{13}C N.m.r. data[a]

Carbon[b]	Compound (1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
1[c]	155.6, CH	155.2, CH	155.7, CH	155.2, CH	154.3, CH	154.7, CH	153.0, CH	152.6, CH	155.7, CH	155.3, CH
2[c]	127.6, CH	127.7, CH	127.6, CH	127.7, CH	128.0, CH	128.0, CH	128.1,* CH	128.2,* CH	127.6, CH	127.7, CH
3	185.6, C	185.7, C	185.6, C	185.8, C	185.6, C	185.5, C	186.4, C	186.4, C	185.6, C	185.7, C
4[c]	127.1, CH	126.6, CH	127.1, CH	126.6, CH	126.8, CH	127.4, CH	123.7, CH	123.8, CH	127.1, CH	126.6, CH
5[c]	164.5, C	163.9, C	164.6, C	163.9, C	163.1, C	163.8, C	162.6, C	162.5, C	164.6, C	163.9, C
6[c]	41.0, CH_2	37.3, CH_2	41.0, CH_2	37.3, CH_2	37.2, CH_2	40.9, CH_2	138.4, CH	138.1, CH	41.0, CH_2	37.3, CH_2
7[c]	69.5, CH	72.0, CH	69.5, CH	72.0, CH	71.5, CH	69.2, CH	127.6,* CH	127.7,* CH	69.1, CH	71.7, CH
8	38.5, CH	38.3,* CH	38.5, CH	38.4,* CH	37.8, CH	39.0, CH	38.1, CH	38.2, CH	40.8, CH	39.3, CH
9	44.4, CH	45.1, CH	44.4, CH	45.2, CH	38.2, CH	37.9, CH	52.0,† CH	42.8,† CH	44.3,* CH	45.3,* CH
10	43.4,* C	43.2,† C	43.4,* C	43.2,† C	42.5, C	42.8, C	41.2, C	40.9, C	43.4 C	43.2, C
11	22.5, CH_2	22.4, CH_2	22.5, CH_2	22.4, CH_2	26.8, CH_2	26.8, CH_2	21.8, CH_2	29.3, CH_2	22.5, CH_2	22.5, CH_2
12[c]	39.0, CH_2	38.8, CH_2	38.9, CH_2	38.8, CH_2	74.6, CH	74.8, CH	39.3, CH_2	72.3, CH	36.4, CH_2	36.4, CH_2
13	42.9,* C	43.0,† C	42.9,* C	43.0,† C	45.3, C	45.2, C	43.3, C	47.4, C	48.3, C	48.3, C
14	49.9, CH	49.6, CH	49.9, CH	49.7, CH	42.7, CH	43.0, CH	48.3,† CH	41.5,† CH	44.2,* CH	44.1,* CH
15	23.8, CH_2	23.8, CH_2	23.8, CH_2	23.8, CH_2	23.0, CH_2	23.1, CH_2	23.8, CH_2	23.2, CH_2	23.1, CH_2	23.3, CH_2
16	27.2, CH_2	27.0, CH_2	27.2, CH_2	27.1, CH_2	26.2, CH_2	26.4, CH_2	27.2, CH_2	26.2, CH_2	33.5, CH_2	33.4, CH_2
17	52.0, CH	51.9, CH	52.0, CH	52.1, CH	43.9, CH	43.9, CH	53.2, CH	45.2, CH	85.1, C	84.9, C
18[c]	11.8, CH_3	11.7, CH_3	11.8, CH_3	11.7, CH_3	12.2, CH_3	12.2, CH_3	11.9, CH_3	12.9, CH_3	15.3, CH_3	15.3, CH_3
19[c]	18.3, CH_3	18.4, CH_3	18.2, CH_3	18.5, CH_3	18.1, CH_3	18.0, CH_3	20.0,‡ CH_3	20.0,‡ CH_3	18.3, CH_3	18.5, CH_3
20[c]	39.8, CH	38.4,* CH	39.7, CH	38.5,* CH	39.9, CH	39.7, CH	38.5, CH	39.0, CH	41.5, CH	41.7, CH
21[c]	12.5, CH_3	12.5, CH_3	12.6, CH_3	12.7, CH_3	11.6, CH_3	11.6, CH_3	12.7, CH_3	11.7, CH_3	14.6, CH_3	14.4, CH_3
22[c]	70.2, CH	70.1, CH	69.8, CH	69.8, CH	69.6, CH	69.6, CH	69.8, CH	70.4, CH	71.8, CH	71.7, CH
23[c]	30.3, CH_2	30.3, CH_2	30.2, CH_2	30.3, CH_2	30.1, CH_2	30.1, CH_2	30.2, CH_2	31.8, CH_2	31.5, CH_2	31.3, CH_2
24	82.9,† C	82.8,‡ C	82.5,† C	82.5,‡ C	82.4,* C	82.5,* C	82.5,§ C	82.6,§ C	83.4,† C	83.5,† C
25	81.8,† C	81.7,‡ C	81.3,† C	81.3,‡ C	81.1,* C	81.2,* C	81.3,§ C	81.4,§ C	82.1,† C	82.1,† C
26	19.9, CH_3	19.9, CH_3	19.9, CH_3	20.0, CH_3	19.9, CH_3	20.0, CH_3	20.5,‡ CH_3	20.4,‡ CH_3	19.7, CH_3	19.7, CH_3
27	20.4,‡ CH_3	20.4,§ CH_3	20.5,‡ CH_3	20.5,§ CH_3	20.5,† CH_3	20.5,† CH_3	20.7,‡ CH_3	20.5,‡ CH_3	20.2,‡ CH_3	20.5,‡ CH_3
28	24.9,‡ CH_3	24.9,§ CH_3	25.2,‡ CH_3	25.2,§ CH_3	25.1,† CH_3	25.2,† CH_3	25.2,‡ CH_3	25.1,‡ CH_3	24.8,‡ CH_3	24.8,‡ CH_3
Ortho ester	115.3, C	115.3, C	117.3, C	117.3, C	117.3, C	117.3, C	117.3, C	117.3, C	114.9, C	115.0, C
$^{13}CH_2CO$	50.3m CH_2	50.2, CH_2							50.2, CH_2	50.2, CH_2
^{13}COS	193.3, C	193.2, C							193.2, C	193.2, C
SMe	12.0, CH_3	12.0, CH_3							12.0, CH_3	12.0, CH_3
Acetate		170.2, C		170.3, C	170.2, C, 170.2, C	170.3, C				170.3, C
Acetate		21.0, CH_3		21.1, CH_3	21.1, CH_3, 21.2, CH_3	21.2, CH_3				21.0, CH_3
Orthoacetate			23.5, CH_3	23.5, CH_3	23.4, CH_3	23.5, CH_3	23.5, CH_3	23.5, CH_3		

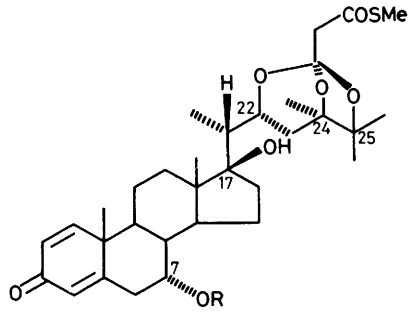
[a] In p.p.m. from internal $SiMe_4$ for $CDCl_3$ solutions. [b] Values with like superscripts in each column may be interchanged. [c] Assigned by C–H correlation spectroscopy in compounds (1), (2), (5), (7), (8), and (9), except for C-12 in (9).

Comparative ^1H n.m.r. shifts of 18- and 19-H_3 between trienes (7) and (8) were not definitive. We have assigned the α-configuration to the 12-acetoxy group of compounds (5) and (6) on the basis of the multiplicity and magnitude of the 12-H signal (δ 5.07, t, *J* 3 Hz) indicative of two *gauche* proton couplings necessitating an axial orientation (α) for the acetoxy group.

Compound (9) is similar to petuniasterone A (1) but bears an additional OH on a quaternary centre as is shown by the ^{13}C n.m.r. spectrum (δ_C 85.1) and by the absence of a proton signal geminal to this group. Acetylation of (9) yields compound (10), which is also found in the plant. The ^1H n.m.r. spectra of this pair show the same comparative features for the C-6 protons as

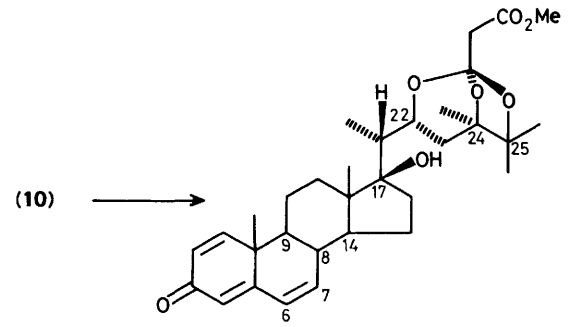


(7) R = H
(8) R = OH



(9) R = H
(10) R = Ac

do (**1**) *vs.* (**2**), and (**3**) *vs.* (**4**). Treatment of compound (**10**) with methoxide gives triene (**11**) in which the thiol ester moiety has been converted into methyl ester. The 8β hydrogen of trienone (**11**) appears as a triplet (δ 2.32, *J* 10 Hz) in the ^{1}H n.m.r.



(**11**)

spectrum thereby confirming the presence of the axial hydrogens at C-9 and C-14. The remaining OH must therefore be attached to C-17. In pentadeuteriopyridine solvent, the ^{1}H n.m.r. spectrum of compound (**9**) exhibits a quartet of doublets at δ 2.36 (*J* 7 and 4 Hz) for 20-H. Irradiation at this position caused the 21-H_3 doublet to collapse to a singlet and the 22-H signal (δ 5.14) to change from a double triplet to a double doublet (*J* 11 and 4 Hz) in agreement with the assigned position. Decoupling of the 22-H proton by irradiation at δ 5.14 resulted in simplification of the 20-H signal to the quartet (*J* 7 Hz). This result is in accord with the absence of a proton at C-17 which therefore must be the position of hydroxylation.

In an effort to assign the stereochemistry at C-17 we considered the magnitude of the substituent-generated proton chemical-shift difference between the C-18 methyl groups of compounds (**1**) and (**9**). The observed shift difference attributable to the 17-hydroxy group (+0.21 p.p.m.) of (**9**) *vs.* (**1**) in $CDCl_3$ is much greater than the values reported by Page[7] for either α or β 17-hydroxy substituents (0.06 and 0.03 p.p.m. respectively) in this solvent. In pentadeuteriopyridine, the observed C-18 methyl signal of compound (**9**) is shifted 0.51 p.p.m. to lower field compared with that in compound (**1**), but it must be noted that the C-18 methyl group of compound (**1**) appears at δ_H 0.67 in this solvent, which is surprisingly higher. The reported chemical-shift differences of 18-H_3 for C-17 α- and β-hydroxy substituents in pentadeuteriopyridine are 0.00 and 0.20 p.p.m. respectively.[7] It appears that the additional oxygenation of the side chain may be influencing the association of pyridine with the C-17 hydroxy group. Clearly the large downfield shift in pentadeuteriopyridine of 22-H for compound (**9**) (Δδ +0.85 p.p.m.) indicates that the side chain can assume a conformation in close proximity to the 17-OH which is possibly stabilized by hydrogen bonding. Where there is no C-17 hydroxy group, *i.e.* structure (**1**), 22-H is only shifted 0.13 p.p.m. downfield in pentadeuteriopyridine. Although the large magnitude of the substituent shift for the C-18 methyl implies close proximity to the 17-OH and thereby a β orientation for that group, the perturbing influence of the side chain lends significant uncertainty to the assignment. We therefore chose to subject compound (**9**) to *X*-ray analysis.

The molecular stereochemistry of compound (**9**) with its 17β-OH group was unequivocally determined in this manner. The established molecular conformation with the atom-numbering system used in the *X*-ray investigation is shown in Figure 1 and its stereoscopic view is presented in Figure 2. The final atomic co-ordinates are listed in Table 3. The asymmetric centres C(20), C(22), and C(24) have the *R* configuration. Molecules of

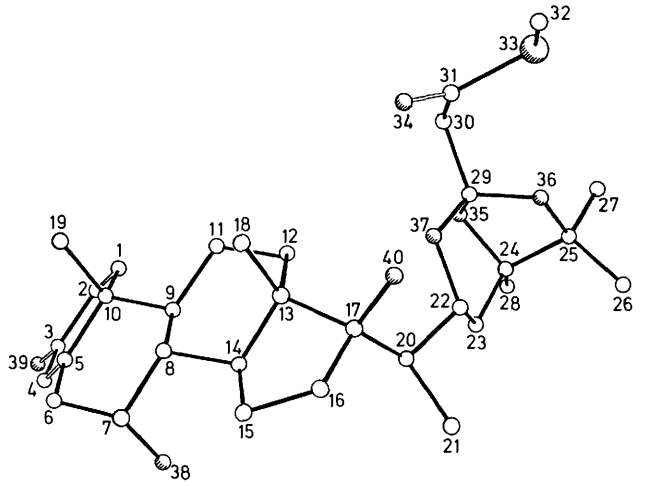


Figure 1. Perspective view of compound (**9**) with the crystallographic numbering scheme. Open bonds represent double bonds; small and large shaded circles represent oxygen and sulphur atoms, respectively

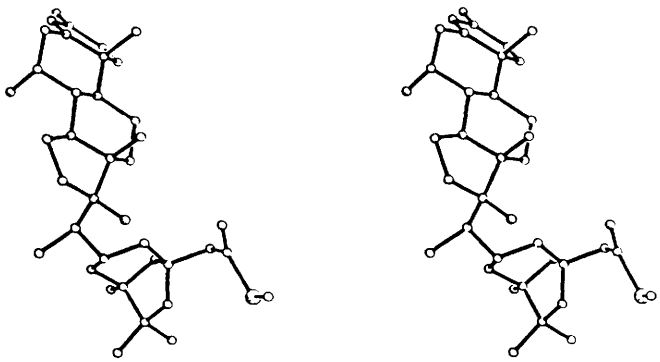


Figure 2. Stereoscopic view of compound (**9**)

compound (**9**) are weakly held together in the crystal structure by van der Waals interaction and two intermolecular hydrogen bonds [O(38)···O(39) 2.94 and O(34)···O(40) 3.32 Å].

Experimental

M.p.s were taken with a Fisher-Johns apparatus and are corrected. Optical rotations were obtained on a Perkin-Elmer model 241 automatic polarimeter at *ca.* 26 °C using 1% concentration in chloroform solutions. 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 spectrophotometer using 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.[11] Mass spectra were run using a VG Micromass 70/70HS instrument either by electron impact or by using ammonia chemical ionization. Listed relative abundances are those of the chemical ionization spectra. *X*-Ray intensities were collected with a Nicolet R3 automatic diffractometer at room temperature. Microanalyses were determined by Galbraith Enterprises, Knoxville, Tennessee.

Silica gel Si60, 70—230 mesh, was from E. Merck; Sephadex, LH-20, from Pharmacia Co.; h.p.l.c. columns were from Rainin

Table 3. Atom co-ordinates ($\times 10^4$) for compound (**9**), with e.s.d.s in parentheses

Atom	*x*	*y*	*z*
C(1)	5 399(3)	7 908	−2 618(2)
C(2)	6 122(2)	8 509(5)	−2 638(2)
C(3)	6 760(2)	7 528(5)	−2 790(2)
C(4)	6 581(2)	5 859(5)	−2 885(2)
C(5)	5 855(2)	5 262(5)	−2 865(2)
C(6)	5 709(2)	3 506(5)	−2 927(2)
C(7)	5 386(2)	2 853(5)	−2 399(2)
C(8)	4 634(2)	3 731(5)	−2 291(2)
C(9)	4 788(2)	5 505(5)	−2 222(2)
C(10)	5 156(2)	6 250(5)	−2 749(2)
C(11)	4 042(2)	6 386(5)	−2 091(2)
C(12)	3 658(2)	5 670(5)	−1 583(2)
C(13)	3 492(2)	3 903(5)	−1 672(2)
C(14)	4 281(2)	3 105(5)	−1 759(2)
C(15)	4 110(3)	1 339(5)	−1 732(2)
C(16)	3 451(3)	1 202(5)	−1 334(2)
C(17)	3 255(2)	2 877(5)	−1 138(2)
C(18)	2 832(2)	3 676(6)	−2 225(2)
C(19)	4 525(3)	6 276(7)	−3 345(2)
C(20)	3 708(2)	3 362(5)	−506(2)
C(21)	3 869(3)	1 971(6)	−78(2)
C(22)	3 292(2)	4 596(5)	−175(2)
C(23)	3 879(2)	5 607(5)	248(2)
C(24)	3 566(2)	7 176(5)	441(2)
C(25)	2 792(2)	7 037(6)	723(2)
C(26)	2 791(3)	5 721(7)	1 182(2)
C(27)	2 540(3)	8 591(7)	957(2)
C(28)	4 213(3)	8 183(6)	765(2)
C(29)	2 562(2)	6 989(5)	−317(2)
C(30)	1 976(2)	7 861(6)	−785(2)
C(31)	1 266(3)	6 820(6)	−1 001(2)
C(32)	−198(3)	5 559(8)	−952(3)
S(33)	480(1)	7 006(2)	−609(1)
O(34)	1 250(2)	5 963(6)	−1 434(2)
O(35)	3 223(2)	7 944(4)	−123(1)
O(36)	2 216(1)	6 636(4)	195(1)
O(37)	2 793(1)	5 635(3)	−582(1)
O(38)	5 957(2)	2 923(4)	−1 865(1)
O(39)	7 414(2)	8 065(4)	−2 832(2)
O(40)	2 409(2)	2 845(4)	−1 113(1)
C(41)	0	2 228(8)	0
C(42)	701(4)	1 487(8)	122(3)
C(43)	1 411(5)	2 473(11)	172(4)
C(44)	717(8)	390(26)	401(7)

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 using an Altex Model 150 monitor equipped with a 0.5 mm pathlength preparative cell.

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 during the growing seasons of 1986 and 1987.

Isolation Procedure.—This was carried out on freeze-dried plant material as previously described[1] by preliminary enrichment of the petuniasterone fraction on silica gel followed by preparative h.p.l.c. Columns and conditions were as follows: Rainin Dynamax Silica, 21.4 mm dia. × 250 mm with guard, 20% propan-2-ol in hexane; Alltech R-Sil C-18, 10 mm dia. × 250 mm, 30% water in acetonitrile; and Whatman Partisil-10 PAC, 9 mm dia. × 500 mm, 10% propan-2-ol in hexane. Products were eluted as indicated in Table 4.

Table 4. Elution zone (ml)

Compd.	Dynamax silica	R Sil C-18	PAC
(**3**)	140—160	45—60	60—70
(**5**)	230—265	30—43	100—120
(**9**)	260—310	28—34	210—240
(**10**)	210—250	25—43	145—160

Compound (**3**), *Petuniasterone D.*—M.p. 209—212 °C (from heptane–EtOAc); [α](λ/nm) +47.7° (589), +49.6° (578), +55.3° (546), +79.4° (436), and +38.9° (365); $\nu_{max.}$ 3 450br (OH) and 1 660 cm^{-1} (conj. CO); $\lambda_{max.}$ 246 nm (log ε 4.18); *m/z* 485 (MH^+, 86%) and 467 ($MH^+ - H_2O$, 12) (Found: C, 74.75; H, 9.0. $C_{30}H_{44}O_5$ requires MH^+, 485; C, 74.34; H, 9.15%).

Compound (**5**), *12α-Acetoxypetuniasterone D 7-Acetate.*—M.p. 241—243 °C (from heptane–EtOAc); [α](λ/nm) +60.3° (589), +62.6° (578), +69.8° (546), +105.8° (436), and +97.6° (365); $\nu_{max.}$ 1 730 (OAc) and 1 665 cm^{-1} (conj. CO); $\lambda_{max.}$ 244 nm (log ε 4.15); *m/z* 585 (MH^+, 18%), 525 (MH^+ − HOAc, 19), and 465 ($MH^+ - 2$ HOAc, 6) (Found: C, 69.9; H, 8.8. $C_{34}H_{48}O_8$ requires MH^+, 585; C, 69.84; H, 8.27%).

Compound (**9**), *17β-Hydroxypetuniasterone A.*—M.p. 145—147 °C with gas evolution (from heptane–EtOAc); [α](λ/nm) +44.2° (589), +46.1° (578), +52.0° (546), +83.6° (436), and +82.6° (365); $\nu_{max.}$ 3 475br (OH), 1 685 (COSMe), and 1 665 cm^{-1} (conj. CO); $\lambda_{max.}$ 242 nm (log ε 4.20); *m/z* 575 (MH^+, 13%) and 557 ($MH^+ - H_2O$, 21) (Found: C, 66.4; H, 7.3; S, 5.5. $C_{32}H_{46}O_7S$ requires MH^+, 575; C, 66.87; H, 8.07; S, 5.58%).

Compound (**10**), *17β-Hydroxypetuniasterone A 7-Acetate.*—[α](λ/nm) +12.4° (589), +12.7° (578), +13.5° (546), +14.9° (436), and −35.9° (365); $\nu_{max.}$ 3 500br (OH), 1 730 (ester), 1 685 (COSMe), and 1 665 cm^{-1} (conj. CO); $\lambda_{max.}$ 243 nm (log ε 4.15); *m/z* 617 (MH^+, 84%), 599 ($MH^+ - H_2O$, 63), and 557 (MH^+ − HOAc, 5.3) ($C_{34}H_{48}O_8S$ requires MH^+, 617).

Petuniasterone A Acetate (**2**).—Petuniasterone A (**1**) (56 mg) was dissolved in acetic anhydride (5 ml) and the mixture was warmed at 100 °C for 15 h. Most of the Ac_2O was removed at 80 °C on a rotary evaporator and MeOH (5 ml) was added. After being kept for 1 h at room temperature, the mixture was taken to dryness and the residue redissolved in CH_2Cl_2 (1 ml). Chromatography of this solution on the PAC column gave pure acetate (**2**) (50 mg), retn. vol. 55—65 ml (10% propan-2-ol–hexane), *m/z* 601 (MH^+, 83%) and 541 (MH^+ − HOAc, 14) ($C_{34}H_{48}O_7S$ requires MH^+, 601).

Petuniasterone D Acetate (**4**).—Petuniasterone D (**3**) was converted into the acetate as described above. H.p.l.c. on the PAC column gave pure acetate (**4**), retn. vol. 50—58 ml (10% propan-2-ol–hexane).

Acetylation of Compound (**9**).—Conversion of compound (**9**) to the 7-acetate was carried out as described above. H.p.l.c. on the RSil C-18 column, retn. vol. 30—38 ml (30% water–MeCN) gave pure monoacetate that was chromatographically and spectroscopically identical with compound (**10**).

Conversion of Dienone (**3**) *into Trienone* (**7**).—Petuniasterone D (**3**) (27 mg) was dissolved in pyridine (1.0 ml) and methanesulphonyl chloride (0.1 ml) was added. The mixture was warmed to ~50 °C for 15 min, and the development of a slight brown colour was observed. Most of the pyridine was

removed under reduced pressure and the remaining material was dissolved in MeOH (25 ml). After evaporation of MeOH, the crude product was dissolved in CH_2Cl_2 (1.0 ml) and the *solution* was filtered through a 0.5 μm PTFE filter. H.p.l.c. on the PAC column gave mainly one substance, retn. vol. 110—130 ml (10% propan-2-ol–hexane), showing a ^1H n.m.r. signal at δ 3.00 ($CDCl_3$; mesyl ester). To the mesyl ester was added 0.5M NaOMe–MeOH (1 ml) and this mixture was warmed briefly to reflux then kept for 1 h at ambient temperature. Acetic acid (0.025 ml) was added and the solution was taken to dryness. The mixture was redissolved in CH_2Cl_2 (1.0 ml), and the solution was filtered through a 0.5 μm PTFE filter and chromatographed on the PAC column to give pure trienone (**7**), retn. vol. 38—46 ml (10% propan-2-ol–hexane) (13 mg); $\lambda_{max.}$ 223, 257, and 301 nm; *m/z* 467 (MH^+, 97%) ($C_{30}H_{42}O_4$ requires MH^+, 467).

Treatment of Diacetate (**5**) *with* NaOMe.—Compound (**5**) (20 mg) was warmed at reflux for 30 min with 0.5M NaOMe–MeOH (1 ml). Acetic acid (0.05 ml) was added and the mixture was evaporated. The residue was dissolved in CH_2Cl_2 (1 ml), and the solution was filtered through a 0.5 μm PTFE filter and chromatographed on a Whatman M-9 silica column, 9 mm dia. × 50 cm, with 20% propan-2-ol–hexane to give two products (**6**), retn. vol. 52—62 ml and (**8**), retn. vol. 82—100 ml. Compound (**6**) resulted from methanolysis of the C-7 acetate, and had $\lambda_{max.}$ 245 nm (log ε 4.15); *m/z* 543 (MH^+, 15%) and 483 (MH^+ − HOAc, 8.0) ($C_{32}H_{46}O_7$ requires MH^+, 543). Compound (**8**), m.p. 270 °C (decomp.) (from hexane–Pr^{i}OH), is the trienone resulting from elimination of the 7-acetate and methanolysis of the 12-acetoxy group; it had $\lambda_{max.}$ 224, 258, and 302 nm; *m/z* 483 (MH^+, 49%) ($C_{30}H_{42}O_5$ requires MH^+, 483).

Treatment of Acetate (**10**) *with* NaOMe.—Compound (**10**) (20 mg) was treated with 0.5M NaOMe–MeOH (1 ml) as in the above case. After work-up, the mixture was chromatographed on the M-9 silica column to give two components. retn. vols. 58—66 ml (**11**) and 108—128 ml (20% propan-2-ol–hexane). The material of greater retention volume results from methanolysis of the C-7 acetate to the alcohol with simultaneous formation of methyl ester on the side chain, and had *m/z* 559 (MH^+, 11%) and 541 ($MH^+ - H_2O$, 9.2) ($C_{32}H_{46}O_8$ requires MH^+, 559).

Compound (**11**) is the trienone resulting from elimination of the 7-acetoxy group with concomitant conversion of methyl thiolester into methyl ester; it had $\lambda_{max.}$ 225, 251, and 298 nm; *m/z* 541 (MH^+, 53%) and 523 ($MH^+ - H_2O$, 8.5) ($C_{32}H_{44}O_7$ requires MH^+, 541).

Crystal Data.—Compound (**9**) with solvent (ethyl acetate) of crystallization, $C_{32}H_{46}O_7S{\cdot}0.5[C_4H_8O_2]$: M = 618.9, monoclinic, space group *I*2; a = 16.932(9), b = 8.514(3), c = 22.600(12) Å, β = 98.79(3)°, V = 3 219.7 Å^3, Z = 4, D_c = 1.28 g cm^{-3}, D_o = 1.30 g cm^{-3}, $F(000)$ = 1 335.9, μ(Cu-K_α) = 12.6 cm^{-1}; colourless prismatic crystals were obtained from ethyl acetate by slow evaporation.

Data Collection and Structure Refinement.—Intensity data were measured 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^{-1}) at room temperature. The lattice constants were refined by least-squares fit to setting angles of 20 independent reflections measured on the diffractometer. Intensity data were recorded as space group *C*2 with lattice constants a = 26.087, b = 8.514, c = 22.600 Å, and β = 140.10°. Two standard reflections were monitored periodically for crystal and instrument stability; no significant change in their intensities was noted during the course of the experiment. Intensity data were corrected for background, Lorentz, and polarization factors,[12] but not for absorption or secondary extinction. The unit-cell dimensions and all hkl indices were transformed to space group *I*2; the crystal structure was 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[13] program package on a Nova-3 computer. The function minimized was $\Sigma w(|F_o| - |F_c|)^2$, where $w = [\sigma^2|F_o| + 0.001|F_o|^2]^{-1}$. Scattering factors were from 'International Tables for X-ray Crystallography,'[14] those of oxygen and sulphur being corrected for anomalous dispersion. Least-squares refinement of atomic parameters of the 40 non-hydrogen atoms with anisotropic temperature factors converged at R = 0.151. Inclusion of 46 hydrogen atoms, whose positions were calculated, and of hydroxy groups, which were located on subsequent difference Fourier maps, in the structure-factor calculation with isotropic temperature factors and constraint on their positional parameters, reduced the R index to 0.141.

Both the n.m.r. analysis and the observed density indicate that a solvate is present in the crystal structure. The n.m.r. spectrum of crystalline (**9**) dissolved in $CHCl_3$ indicated that there is half a molecule of ethyl acetate per formula unit of (**9**). The subsequent difference Fourier map revealed a cluster of four residual peaks, C(41), C(42), C(43), and C(44) with significantly high electron densities of 6.0, 4.2, 3.3, and 1.6 e Å^{-3}, respectively. These four peaks are incompatible with the geometry of possible solvate molecules; however, the distances C(41)–C(42) and C(42)–C(44) of 1.34 and 1.13 Å, respectively, are close to those for C–O and C=O. Inclusion of these peaks, with isotropic temperature factors and assuming that they were all carbon atoms, in the least-squares refinement reduced the R value to 0.063 and R_w = 0.071 (375 parameters for 3 049 unique reflections with $|F_o| \geq 3\sigma|F_o|$ in the range 3° ≤ 2θ ≤ 110°). At convergence, the four solvent atoms exhibit extremely high thermal vibrational motions, the average parameter shifts were less than 0.09σ, and the final difference Fourier synthesis excursion are within ±0.7 e Å^{-3}. The first five residual peaks are within the range (0.7—0.5 e Å^{-3}) and (1.1—0.4 Å) from the four solvent atoms. The structure refinement indicated that solvent of crystallization is present in the crystal structure but failed to establish the definite crystallographic position of the complete solvate molecule from the *X*-ray data presumably owing to poor intensity data. Crystals of compound (**9**), crystallized from ethyl acetate, appeared opaque and slightly fractured; nevertheless, they seemed to remain stable during data collection. Attempts to improve crystal quality by recrystallization from a variety of solvents were unsuccessful. There are no obvious intermolecular hydrogen bonds between the parent and solvent molecules; this perhaps accounts for the fact that the ethyl acetate molecules are partially disordered. An attempt to determine the absolute configuration by change of chirality in the structure refinement did not change R and R_w significantly.*

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* *Supplementary data* (see section 5.6.3 of Instructions for Authors, in the January issue). Tables of bond lengths and angles, anisotropic thermal parameters with their estimated standard deviations for the non-hydrogen atoms, and positional and thermal parameters of hydrogen atoms have been deposited at the Cambridge Crystallographic Data Centre.

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