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PETUNIASTERONES FROM *PETUNIA PARODII* AND *P. INTEGRIFOLIA*; UNUSUAL ERGOSTANE-TYPE STEROIDS

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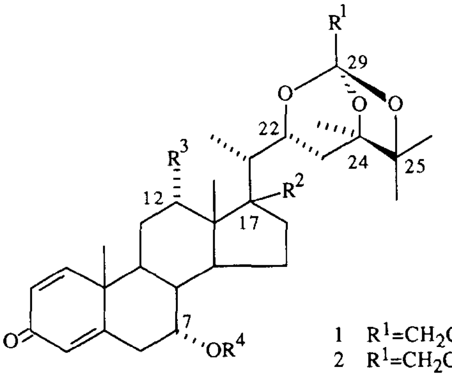
Key Word Index—*Petunia parodii*; *P. integrifolia*; *P. hybrida*; Solanaceae; phytosterols; ergostanoids; host plant resistance; insect-resistance.

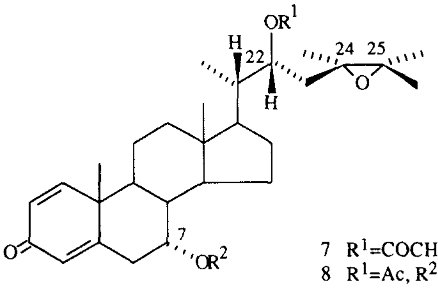
Abstract—From leaves of *Petunia parodii* were isolated four epoxy group-bearing petuniasterones. These were shown to be (22*R*,24*R*)-7α-acetoxy-4β,5β-epoxy-17β,22,24,25-tetrahydroxyergost-1-en-3-one 22,24,25-[(methylthio)carbonyl]orthoacetate (petuniasterone I); (22*R*,24*R*)-7α,12α-diacetoxy-4β,5β-epoxy-22,24,25-trihydroxyergost-1-en-3-one 22,24,25-orthoacetate (petuniasterone J); (22*R*,24*R*)-7α-acetoxy-16β,17β-epoxy-22,24,25-trihydroxyergosta-1,4-dien-3-one 22,24,25-orthoacetate (petuniasterone K); and (22*R*,24*R*)-7α-acetoxy-16β,17β-epoxy-22,24,25-trihydroxyergosta-1,4-dien-3-one 22,24,25-[(methylthio)carbonyl]orthoacetate (petuniasterone L). From leaves of *Petunia integrifolia* were isolated (22*R*,24*R*)-7α,12ξ-diacetoxy-11β,22,24,25-tetrahydroxyergosta-1,4-diene-3-one 22,24,25-orthoacetate (12ξ-acetoxy-11-β-hydroxypetuniasterone D 7-acetate); (22*R*,24*R*)-7α,12ξ-diacetoxy-11β,22,24,25-tetrahydroxyergosta-1,4-dien-3-one 22,24,25-orthopropionate (12ξ-acetoxy-11β-hydroxypetuniasterone M 7-acetate); (22*R*,24*R*)-7α,22,24,25-tetrahydroxyergosta-1,4-dien-3-one 22,24,25-orthopropionate (petuniasterone M); and (22*R*, 24*R*)-12α-acetoxy-7α,22,24,25-tetrahydroxyergosta-1,4-diene-3-one 22,24,25-orthopropionate (12α-acetoxypetuniasterone M). From leaves of *Petunia hybrida* were isolated 30-hydroxypetuniasterone A 7-acetate and petuniasterone C 7-acetate.

INTRODUCTION

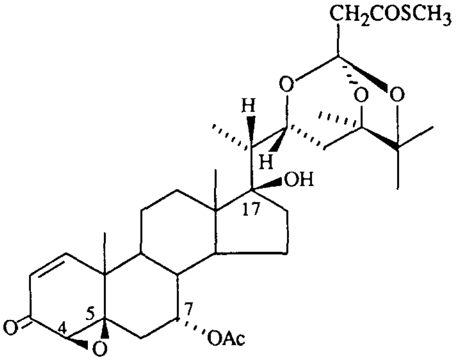
Petuniasterones [1–3] are steroidal ketones that were first reported to occur in *Petunia hybrida*, Vilm (Solanaceae). All of these compounds possessed the common structural features of a keto group at position C-3 and a 7α-oxygen function. Typical examples of the diverse substitution patterns shown by the petuniasterones include the A-ring dienones of the PS-A and -D series (**1**–**5**) which also have a bicyclic orthoester system upon the pendant side chain. Further oxygenation of the steroid nucleus is observed in several cases (**2** and **4**). Dienones with epoxy-bearing side chains also occur in the PS-C series (**7**–**9**). We have found that these epoxides are readily transformed into the orthoesters of the A- and D-series as well as into several related oxygenated derivatives (e.g. **11** and **12**) which also co-occur naturally [3]. As certain of the petuniasterones appear to be involved in the resistance of *Petunia* toward larval attack of the lepidopteran, *Heliothis zea*, Boddie (corn earworm) we have examined the relative potency of these materials [4]. Insect growth inhibition was caused only by those substances bearing the orthoester system (PS-A and PS-D series).



1 $R^1=CH_2COSCH_3, R^2, R^3, R^4=H$
2 $R^1=CH_2COSCH_3, R^2=B\text{-}OH, R^3=H, R^4=$ Ac
3 $R^1=CH_3, R^2, R^3, R^4=H$
4a $R^1=CH_3, R^2=H, R^3=OAc, R^4=Ac$
4b $R^1=CH_3, R^2=H, R^3=OAc, R^4=H$
5 $R^1=CHOHCOSCH_3, R^2, R^3, R^4=H$
6 $R^1=CHOHCOSCH_3, R^2, R^3=H, R^4=Ac$



7 R^1=$COCH_2COSCH_3$, R^2=H
8 R^1=Ac, R^2=H
9 R^1=H, R^2=H
10 R^1=H, R^2=Ac



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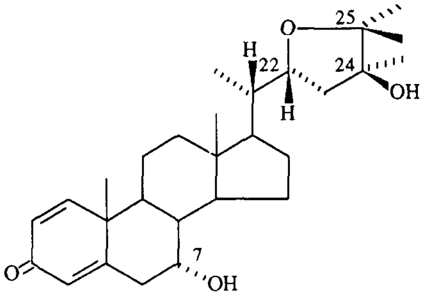

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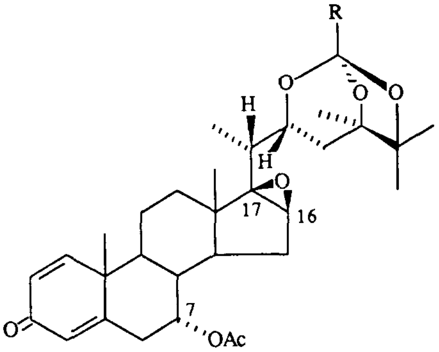
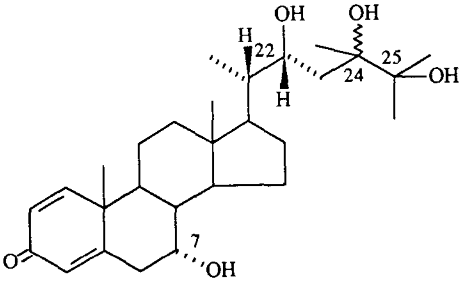


15 R=CH_3
16 R=CH_2COSCH_3



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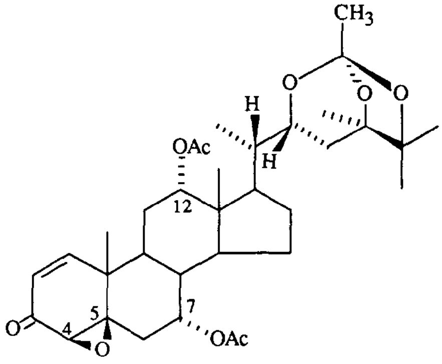
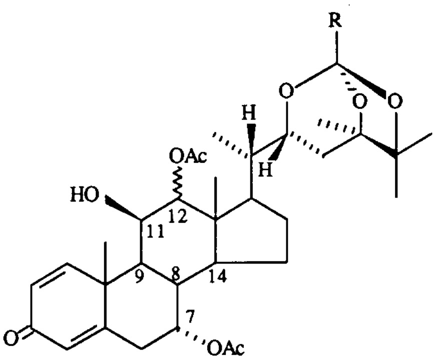
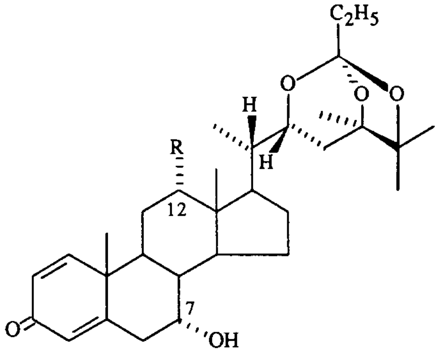


14

The relative content of the various petuniasterones is rather variable among commercial varieties of *P. hybrida*, and we have noted that resistance toward larval feeding of *H. zea* upon fresh leaf material also varied (unpublished results). It was of interest, therefore, to examine the presumed ancestral species that have given rise to present petunia hybrids. In this way, the relative contribution toward insect resistance of the putative progenitors might be ascertained, and a potentially more stable source of genetic material could be obtained for eventual plant breeding experiments. It is likely that *P. hybrida* is derived from one or more combinations of the species, *P. axillaris* Lam., *P. integrifolia* Hook. (=*P. violacea* Lindley), *P. parodii* Steere, or *P. inflata* Fries [5]. We were able to acquire seed of all but the last mentioned species and grow plants for evaluation. Additional plant material was available from natural sources found in Argentina. In addition to the petuniasterones already described, we have identified several new petuniasterone types in extracts from two of these species. From *P. parodii* were obtained the A-ring epoxides **13** (PS-I) and **14** (PS-J) which are otherwise similar to the previously reported **2** and **4a**, respectively. Also obtained were D-ring epoxides **15** (PS-K) and **16** (PS-L). *Petunia integrifolia* afforded an 11-hydroxy-12-acetoxy derivative of PS-D (**17**) as well as three compounds (**18**, **19** and **20**) having ethyl groups at the orthoester bridgehead (orthopropionates vs orthoacetates) which are assigned to the petuniasterone M series. *Petunia axillaris* appears to contain only compounds that are of types previously described in *P. hybrida*. Two new 7-acetates in the PS-A (**6**) and PS-C (**10**) series were found in leaf material from the latter source.



17 R=CH_3
18 R=C_2H_5



19 R=H
20 R=OAc

Table 1. ^1H NMR spectral data* for compounds **2**, **4a**, **13** and **14**.

H	**2**	**4a**	**13**	**14**
H-1	7.10 *d* (10)	6.96 *d* (10)	6.55 *d* (11)	6.44 *d* (11)
H-2	6.26 *dd* (10, 2)	6.25 *dd* (10, 2)	5.90 *dd* (11, 2)	5.86 *dd* (11, 2)
H-4	6.02 *br s*	6.03 *br d* (*ca* 2)	3.12 *d* (2)	3.10 *d* (2)
H_2-6 (α and β)	2.60 *d* (14)	2.60 *d* (14)	2.36 *dd* (14, 2.5)	2.34 *dd* (15, 3)
	2.66 *d* (14)	2.66 *d* (14)	1.54 *dd* (14, 2.5)	*ca* 1.55
H-7	5.08 *br q* (*ca* 3)	5.08 *br s*	5.12 *br q* (*ca* 2.5)	5.12 *br t* (*ca* 3)
H-12β	—	5.08 *br s*	—	5.04, *br s*
H-20	—	—	1.84, *m* $J_{20-21}=7$, $J_{20-22}=8$	—
H-22	4.30 *ddd* (11, 7, 4)	4.16 *td* (3, 4)	4.29 *ddd* (11, 8, 4)	4.16 *td* (8, 3.5)
H_2-23	—	1.50 *d* (8)	1.62, *m* $J_{22-23}=4$ and 11	1.50 *d* (8)
H_3-18	0.97, *s*	0.83, *s*	0.94, *s*	0.81, *s*
H_3-19	1.26, *s*	1.23, *s*	1.33, *s*	1.30, *s*
H_3-21	0.91 *d* (7)	0.85 *d* (7)	0.90 *d* (7)	0.84 *d* (7)
H_3-26	1.33, *s*	1.30, *s*	1.33, *s*	1.30, *s*
H_3-27	1.14†, *s*	1.16†, *s*	1.14,†, *s*	1.16,† *s*
H_3-28	1.20†, *s*	1.18†, *s*	1.20†, *s*	1.19,† *s*
COSMe	2.31, *s*	—	2.31, *s*	—
CH_2CO	3.02, 3.07 *d*'s (14)	—	3.01, 3.06 *d*'s (14)	—
orthoacetate-H_3	—	1.54, *s*	—	1.54, *s*
OAc	1.98, *s*	2.03, 2.08, *s*'s	2.03, *s*	2.07, 2.08, *s*'s

*δ Value in $CDCl_3$; coupling constants (Hz) in parentheses.
Data for **2** and **4a** are from ref. [2].
† Values may be interchanged.

RESULTS AND DISCUSSION

Individual petuniasterones were isolated by HPLC after preliminary chromatography of chloroform extracts of leaf material [1]. Compound **13** (PS-I), $C_{34}H_{48}O_9S$, had ^1H and ^{13}C NMR spectra (Tables 1 and 2) that showed a close relationship to 17β-hydroxypetuniasterone A, 7-acetate (**2**, $R^4=Ac$). The dieneone system of **2** was no longer present, however, being replaced by the 4,5-epoxy-1,2-en-3-one as indicated by the UV (232 nm) and IR spectra (1660 cm^{-1}) as well as by ^1H NMR signals at δ6.61 and 6.10 for 1-H and 2-H, respectively. The signal of H-4, δ3.12, was consistent with the presence of a 4,5-epoxy group [6] as were the ^{13}C signals at δ62.5 and

Table 2. ^{13}C NMR spectral data* for compounds **2**, **4a**, **13** and **14**

C	**2**	**4a**	**13**	**14**
1	155.3	154.3	154.9	154.0
2	127.7	128.0	123.3	123.7
3	185.7	185.6	195.9	195.4
4	126.6	126.8	62.5	62.4
5	163.9	163.1	63.9	63.7
6	37.3	37.2	34.6	34.6
7	71.7	71.5	70.1	70.1
8	39.3	37.8[a]	39.1	37.8[a]
9	45.3[a]	38.2[a]	46.5[a]	38.1[a]
10	43.2	42.5	42.0	41.5
11	22.5	26.8	22.1	26.5
12	36.4	74.6	36.5	74.4
13	48.3	45.3	48.2	45.2
14	44.1[a]	42.7	43.8[a]	42.4
15	23.3	23.0	23.3	23.0
16	33.4	26.2	33.2	26.1
17	84.9	43.9	84.9	43.9
18	15.3	12.2	15.2	12.2
19	18.5	18.1	15.7	15.5
20	41.7	39.9	41.7	41.1
21	14.4	11.6	14.5	11.6
22	71.7	69.6	71.7	69.6
23	31.3	30.1	31.1	30.1
24	83.5[b]	82.4[b]	83.5[b]	82.5[b]
25	82.1[b]	81.1[b]	82.1[b]	81.2[b]
26	19.7	19.9	19.7	20.0
27	20.5[c]	20.5[c]	20.1[c]	20.6[c]
28	24.8[c]	25.1[c]	24.8[c]	25.2[c]
orthoester	115.0	117.3	114.8	117.3
CH_2CO	50.2	—	50.2	—
CO S	193.2	—	193.2	—
S Me	12.0	—	12.0	—
acetate	21.0	21.1, 21.2	20.9	21.1, 21.3
acetate	170.3	170.2, 170.2	170.1	170.1, 170.1
orthoacetate	—	23.4	—	23.5

*In ppm from internal TMS for $CDCl_3$ solutions. Data for **2** and **4a** are from ref. [2].

[a–c] Values with like superscripts may be interchanged in each column.

63.9 for C-4 and C-5, respectively [7]. The respective ^{1}H–^{13}C correlations were established by two dimensional spectroscopy [8]. However, it was not possible to assign the stereochemistry of the epoxy group by NMR since significant perturbation of the system by the associated 1,2-double bond rendered close comparison with fully saturated model systems [6] invalid. Examination of the specific rotation of **13** (Experimental) at several wavelengths revealed a trend toward the value of $[\alpha]_{365} = +1420°$. By comparison, 4β,5β-epoxy-3-cholestanone shows a strong positive Cotton effect with a value for rotation at 365 nm of *ca* +3 300° [9]. Although the influence of the double bond in **13** surely alters the magnitude of rotation, the observed value suggests that the β-epoxy configuration is present in **13** as well. Comparison with the specific rotation of **14** (*vide infra*) which has a very similar system confirms this assignment. The remaining features of **13** are easily established by comparison of its NMR spectra with those of **2**. Thus, the ^{1}H NMR signals associated with H_2-6 and H-7 are essentially superimposable for each compound and indicate a 7α-acetoxy group. The hydroxy group is attached at position C-17 and possesses the β-configuration as is shown by the close match of all relevant signals in the ^{1}H and ^{13}C NMR spectra of **2** and **13**. It may also be noted that in deuteropyridine, compound **13** exhibits a distinct quartet of doublets for H-20 at δ2.38 ($J = 7$ and 4 Hz) indicative of lack of proton substitution at position C-17.

The molecular structure of **14** (PS-J), $C_{34}H_{48}O_9$, was unequivocally established by X-ray analysis. The established molecular conformation with the atom-numbering system used in the X-ray investigation is shown in Fig. 1 and its stereoscopic view showing absolute configuration is presented in Fig. 2. Table 3 gives values for the observed bond lengths. The ^{1}H and ^{13}C NMR spectra of **14** (Tables 1 and 2) are in agreement with this structure and, in addition, resemble closely the major portions of the NMR spectra of **4a** except for the signals associated with ring-A. The close correspondence of the ring-A signals of **14** with those of **13** along with the observed specific rotation at 365 nm (+1 750° in **14**) indicates that the epoxy group in each case possesses the same β-orientation.

Compounds **15** (PS-K), $C_{32}H_{44}O_7$, and **16** (PS-L),

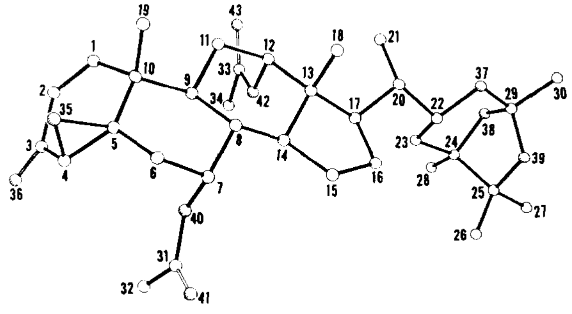


Fig. 1. Perspective view of compound **14** with crystallographic numbering scheme. Open bonds represent double bonds, and shaded circles represent oxygen atoms.

Table 3. Bond lengths (Å) in petuniasterone J (**14**)

C(1)–C(2)	1.322(7)	C(1)–C(10)	1.509(6)
C(2)–C(3)	1.440(6)	C(3)–C(4)	1.500(6)
C(3)–O(36)	1.218(6)	C(4)–C(5)	1.455(6)
C(4)–O(35)	1.432(5)	C(5)–C(6)	1.489(6)
C(5)–C(10)	1.525(5)	C(5)–O(35)	1.440(5)
C(6)–C(7)	1.520(5)	C(7)–C(8)	1.531(5)
C(7)–O(40)	1.451(5)	C(8)–C(9)	1.535(5)
C(8)–C(14)	1.521(5)	C(9)–C(10)	1.569(5)
C(9)–C(11)	1.540(5)	C(10)–C(19)	1.533(6)
C(11)–C(12)	1.522(5)	C(12)–C(13)	1.531(5)
C(12)–O(42)	1.459(4)	C(13)–C(14)	1.533(5)
C(13)–C(17)	1.557(5)	C(13)–C(18)	1.540(5)
C(14)–C(15)	1.533(5)	C(15)–C(16)	1.524(6)
C(16)–C(17)	1.541(5)	C(17)–C(20)	1.540(5)
C(20)–C(21)	1.521(5)	C(20)–C(22)	1.535(5)
C(22)–C(23)	1.522(5)	C(22)–O(37)	1.457(5)
C(23)–C(24)	1.527(6)	C(24)–C(25)	1.542(6)
C(24)–C(28)	1.518(6)	C(24)–O(38)	1.440(5)
C(25)–C(26)	1.503(6)	C(25)–C(27)	1.525(6)
C(25)–O(39)	1.457(5)	C(29)–C(30)	1.495(6)
C(29)–O(37)	1.394(5)	C(29)–O(38)	1.407(5)
C(29)–O(39)	1.420(5)	C(31)–C(32)	1.483(7)
C(31)–O(40)	1.336(5)	C(31)–O(41)	1.180(6)
C(33)–C(34)	1.476(6)	C(33)–O(42)	1.336(4)
C(33)–O(43)	1.198(5)		

$C_{34}H_{46}O_8S$, may be considered together since their NMR spectra showed that they are identical except for the substituent located upon the orthoester functionality of the side chain (Tables 4 and 5). Together with the UV and IR spectra the NMR spectra clearly showed the presence of a ring-A dienone and a 7-acetoxy group. The elemental compositions of **15** and **16** indicated that one additional ring was present as well. The structure of **15** was determined by X-ray methods (Figs 3 and 4, Table 6) which showed the presence of a 16β,17β-epoxy group in addition to the features already mentioned.

From *P. integrifolia* extracts were isolated a pair of compounds (**17** and **18**), which differed only in bearing a methyl (**17**), $C_{34}H_{48}O_9$, or an ethyl group (**18**), $C_{35}H_{50}O_9$, upon the orthoester system. The ring-A dienone system was present as indicated by NMR (Tables 7 and 8) and the UV and IR spectra as was the typical 7-acetoxy group. An additional acetoxy and a hydroxy group were present in each, and these were shown to be on adjacent carbons of the steroid nucleus by decoupling of the respective ^1H NMR signals at δ4.86 and 4.22. The signal of H-8 (δ2.20) appears as a triplet of doublets in which the two larger couplings (12 Hz) arise from the respective diaxial orientations of H-8, H-9 and H-14. The small (3 Hz) axial–equatorial coupling between H-8 and H-7β serves to confirm the axial, α-orientation of the 7-acetoxy group. In compound **17**, the signal of H-9 could be observed directly (δ1.65), and it appeared as a double doublet ($J_{8,9} = 12$ Hz, $J_{9,11} = 3$ Hz) which was consistent with an equatorial orientation for H-11. Decoupling

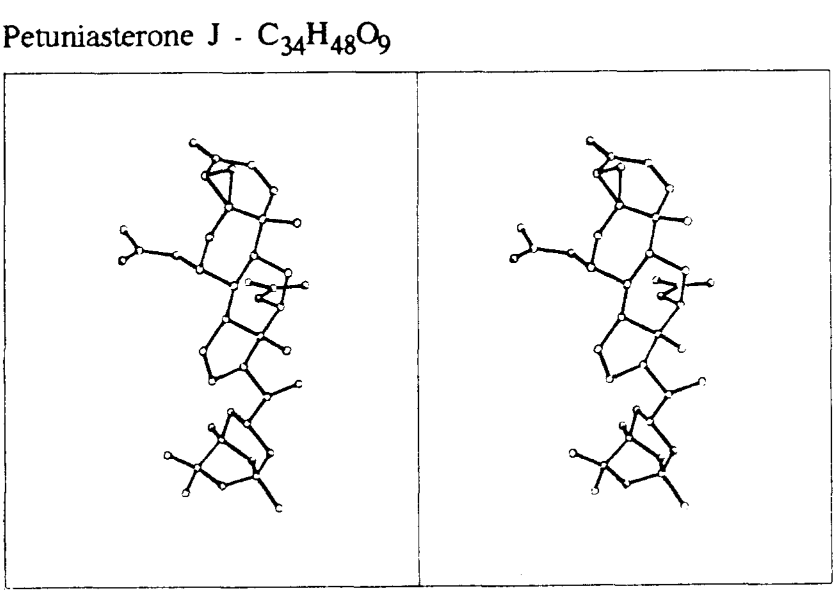


Fig. 2. Stereoscopic view of compound **14**.

Table 4. ^{1}H NMR spectral data* for compounds **15** and **16**

H	**15**	**16**
H-1	7.05 *d* (10)	7.05 *d* (10)
H-2	6.28 *dd* (10, 2)	6.28 *dd* (10, 2)
H-4	6.00 *br d* (*ca* 2)	6.00 *br d* (*ca* 2)
H_2-6	2.60 *d* (3)	2.60 *d* (3)
H-7	4.95, *br s*	4.94, *br s*
H-16	3.34 *d* (4)	3.33 *d* (4)
H-20	2.20, *br m*	2.20, *br m*
H-22	4.26 *ddd* (11.5, 6, 4.5)	4.31 *ddd* (11, 6, 5)
H_3-18	0.93, *s*	0.93, *s*
H_3-19	1.24, *s*	1.24, *s*
H_3-21	0.81 *d* (7)	0.82 *d* (7)
H_3-26	1.34, *s*	1.36, *s*
H_3-27	1.18†, *s*	1.13†, *s*
H_3-28	1.19†, *s*	1.21†, *s*
$COSCH_3$	—	2.30, *s*
CH_2CO	—	3.02, 3.09 *d*'s (14)
orthoacetate-H_3	1.55, *s*	—
OAc	2.01, *s*	2.01, *s*

*δValues in $CDCl_3$; coupling constants (Hz) in parentheses.
†Values may be interchanged.

Table 5. ^{13}C NMR spectral data* for compounds **15** and **16**

C	**15**	**16**
1	154.8	154.8
2	127.8	127.8
3	185.6	185.6
4	126.7	126.7
5	163.4	163.5
6	37.1	37.1
7	71.7	71.7
8	36.8	36.7
9	44.7	44.6
10	43.2^a	43.2^a
11	22.7	22.6
12	33.9	33.8
13	42.4^a	42.4^a
14	56.2	56.2
15	28.0	28.0
16	63.5	63.6
17	75.2	75.0
18	14.1	14.2
19	18.2	18.2
20	37.1	37.1
21	10.5	10.4
22	69.6	69.9
23	34.5	34.5
24	82.5^b	82.9^b
25	81.4^b	82.0^b
26	20.0^c	19.9^c
27	20.3^c	20.2^c
28	25.1^c	24.8^c
orthoester	117.0	115.1
CH_2CO	—	50.2
COS	—	192.9
S Me	—	12.0
acetate	21.0	21.1
acetate	170.2	170.3
orthoacetate	23.4	—

*In ppm from internal TMS for $CDCl_3$ solutions.
$^{a-c}$Values with like superscripts may be interchanged in each column.

experiments confirmed these assignments and served to locate the hydroxyl at position-11 (δ4.22) in the axial β-configuration. In agreement with this was the appearance of the H-12 signal (δ4.86) as a 3 Hz doublet ($J_{a,e}$ or $J_{e,e}$) which, however, does not uniquely specify the orientation of the 12-acetoxy group. In compound **18**, overlapping peaks associated with the ethyl group of the orthopropionate coincidentally obscure H-9. It may be noted that both H_3-18 and H_3-19 in compounds **17** and **18** give signals that appear at much lower field than those of the corresponding methyls of **4a** which possesses only a 12β-acetoxy substituent. The observed shifts in **17** and **18** of +0.21 ppm and +0.24 ppm for H_3-18 and H_3-19, respectively, are attributable to an 11β-hydroxyl. Typical steroids possessing this functionality exhibits shifts of *ca* +0.21 ppm for H_3-18 and +0.23 ppm for H_3-19 in A-ring dienones [10]. The effect of α- versus β-orientation of an acetoxy at position C-12 upon the resultant positions of methyls C-18 and C-19 is unfortunately nearly equal, i.e. +.08 and +.09 ppm for H_3-18 and −.01 ppm and +.02 ppm for H_3-19 [10]. We therefore allow the configuration at position C-12 to remain unassigned.

The compounds **19** (PS-M), $C_{31}H_{46}O_5$, and **20** (12-α-acetoxy PS-M), $C_{33}H_{48}O_7$ also have bicyclic orthopropionate esters upon the steroid side chain. They are analogous to similar compounds in the petuniasterone-D series (**3** and **4b**) [2]. Compounds **19** and **20** exhibit NMR spectra (Tables 9 and 10) that are essentially superimposable with those of **3** and **4b** respectively, except for the ^{1}H NMR signals associated with the ethyl or methyl of the side chain, and for the position of the quaternary orthopropionate carbon, δ118.8 or 117.3 for orthoacetates.

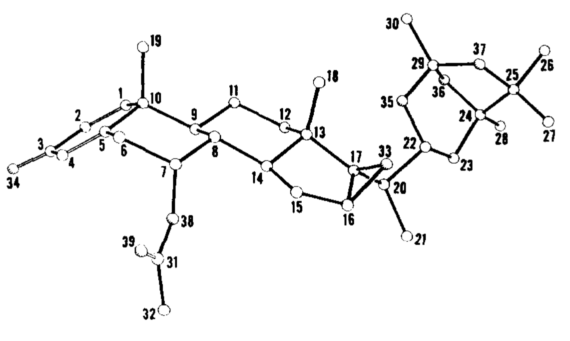


Fig. 3. Perspective view of compound **15** with crystallographic numbering scheme. Open bonds represent double bonds, and shaded circles represent oxygen atoms.

30-Hydroxy petuniasterone A, 7-acetate (**6**) and petuniasterone C, 7-acetate (**10**) were present in extracts from *P. hybrida*. Compound **6** was identified by conversion to the previously prepared PS-A 7,30-diacetate [3] and

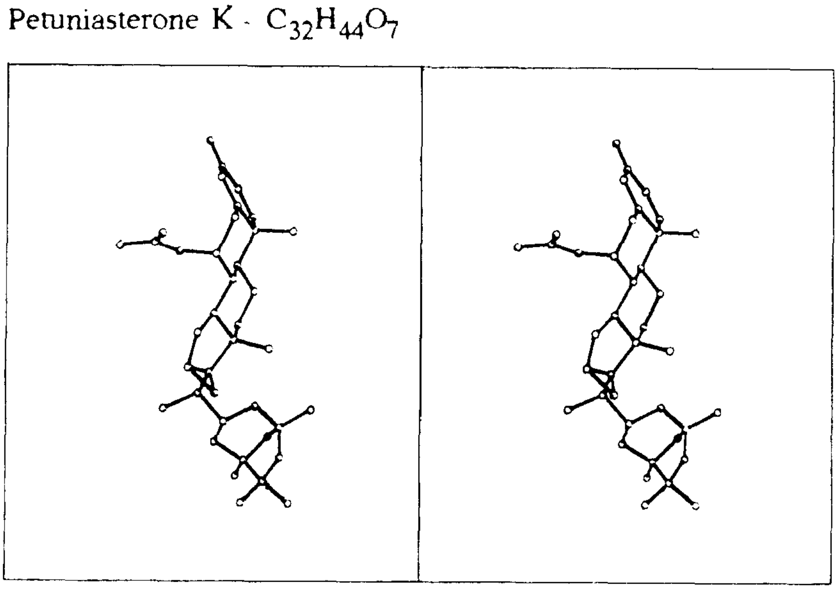


Fig. 4. Stereoscopic view of compound **15**.

Table 6. Bond lengths (Å) in petuniasterone K (**15**)

C(1)–C(2)	1.343(7)	C(1)–C(10)	1.500(8)
C(2)–C(3)	1.448(10)	C(3)–C(4)	1.430(9)
C(3)–O(34)	1.230(8)	C(4)–C(5)	1.373(8)
C(5)–C(6)	1.470(8)	C(5)–C(10)	1.525(8)
C(6)–C(7)	1.543(8)	C(7)–C(8)	1.518(8)
C(7)–O(38)	1.472(7)	C(8)–C(9)	1.503(7)
C(8)–C(14)	1.533(7)	C(9)–C(10)	1.575(7)
C(9)–C(11)	1.547(8)	C(10)–C(19)	1.540(9)
C(11)–C(12)	1.541(8)	C(12)–C(13)	1.518(7)
C(13)–C(14)	1.548(8)	C(13)–C(17)	1.569(7)
C(13)–C(18)	1.543(8)	C(14)–C(15)	1.530(7)
C(15)–C(16)	1.508(9)	C(16)–C(17)	1.469(7)
C(16)–O(33)	1.450(7)	C(17)–C(20)	1.528(8)
C(17)–O(33)	1.457(6)	C(20)–C(21)	1.524(7)
C(20)–C(22)	1.551(7)	C(22)–C(23)	1.515(9)
C(22)–O(35)	1.430(6)	C(23)–C(24)	1.554(8)
C(24)–C(25)	1.513(8)	C(24)–C(28)	1.513(9)
C(24)–O(36)	1.441(6)	C(25)–C(26)	1.546(9)
C(25)–C(27)	1.540(7)	C(25)–O(37)	1.429(8)
C(29)–C(30)	1.477(7)	C(29)–O(35)	1.438(7)
C(29)–O(36)	1.384(7)	C(29)–O(37)	1.429(6)
C(31)–C(32)	1.470(9)	C(31)–O(38)	1.343(7)
C(31)–O(39)	1.180(8)		

compound **10** was hydrolysed to PS-C for comparison to an authentic sample [1].

EXPERIMENTAL

Mps: corr. Optical rotations were measured at *ca* 26° in $CHCl_3$ solns, IR spectra were recorded in $CHCl_3$ solns, UV spectra in MeOH. ^1H NMR spectra were obtained at 90 or 200 MHz and ^{13}C NMR spectra at 50 MHz. NMR assignments were facilitated by decoupling methods and by the use of 2D proton–proton and carbon–proton correlation techniques. Mass spectra were obtained with NH_3 chemical ionization. HPLC columns were commercially obtained and solvents were HPLC grade. Detection was by UV at 254 nm using a monitor equipped with 0.5 mm pathlength prep. cell.

X-Ray 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 temp. The intensity data were corrected for background and Lorentz-polarization effects [11], but not for absorption or secondary extinction. 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 [12] program package. The function minimized was $\Sigma\omega\ (|F_0|\text{-}|F_c|)^2$, where $\omega=[\sigma^2|F_0|\ +0.001\ |F_0|^2]^{-1}$. Scattering factors were from International Tables for X-ray Crystallography [13]; 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 **14** and **15** were determined by Hamilton's statistical method [14] of comparing the R-values for the two enantiomeric structures.

Plant material. Petunia parodii (Steere) seeds were obtained from the National Seed Storage Lab., Colorado State Univ., Fort Collins, Co., U.S.A. *P. 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 Lab., Hurlingham, Buenos Aires Prov., Argentina. Plants were grown in the greenhouse and in outdoor beds in Albany, CA. Leaf material was harvested at intervals between December 1987 and June 1988.

Isolation procedure. Freeze-dried leaf material was ground in $CHCl_3$ with a high speed homogenizer followed by filtration and evapn. The $CHCl_3$ soluble material was stirred with 10 vols of boiling MeCN, cooled to 5°, and filtered to remove waxy material. The soln was concd and passed through a column of

Table 7. ^{1}H NMR spectral data* for compounds **17** and **18**

H	**17**	**18**
H-1	7.20 *d* (10)	7.20 *d* (10)
H-2	6.30 *dd* (10, 2)	6.30 *dd* (10, 2)
H-4	5.98 *br s*	5.98 *br s*
H_2-6 (α and β)	2.56 *dd* (14, 3) 2.74 *ddd* (14, 3, 2)	2.56 *dd* (14, 3) 2.74 *ddd* (14, 3, 2)
H-7	5.18 *br q* (*ca* 3)	5.18 *br q* (*ca* 3)
H-8	2.20 *td* (12, 3)	2.20 *td* (12, 3)
H-9	1.65 *dd* (12, 3)	—
H-11	4.22 *m*	4.22 *m*
H-12	4.86 *d* (3)	4.87 *d* (3)
H-20	1.72†	—
H-22	4.16 *td* (8, 4)	4.16 *td* (8, 4)
H_3-18	1.04 *s*	1.04, *s*
H_3-19	1.48 *s*	1.48 *s*
H_3-21	0.84 *d* (6)	0.83 *d* (6.5)
H_3-26	1.30 s	1.32 s
H_3-27	1.17‡ *s*	1.16‡ *s*
H_3-28	1.19‡ *s*	1.18‡ *s*
orthoacetate-H_3	1.55, *s*	—
orthopropionate-H_2	—	*ca* 1.8 *m*
orthopropionate-H_3	—	0.98 t (8)
OAc	2.03 *s*	2.04 *s*
OAc	2.09 *s*	2.09 *s*

*δ Values in $CDCl_3$; coupling constants (Hz) in parentheses.

†By stepwise decoupling.

‡Values may be interchanged.

Table 8. ^{13}C NMR spectral data* for compounds **17** and **18**

C	**17**	**18**
1	155.1	155.1
2	128.3	128.3
3	186.1	186.1
4	125.2	125.2
5	164.6	164.7
6	36.9	36.9
7	71.8	71.9
8	34.8	34.8
9	43.2	43.2
10	44.1[a]	44.1[a]
11	71.3	71.3
12	78.7	78.7
13	43.5[a]	43.5[a]
14	37.8[b]	37.8[b]
15	23.2	23.2
16	26.0	26.0
17	44.7[b]	44.7[b]
18	12.0	12.1
19	21.3	21.3
20	44.6[b]	44.6[b]
21	11.6	11.5
22	69.6	69.3
23	30.2	30.5
24	82.5[c]	82.1[c]
25	81.2[c]	81.0[c]
26	20.0	20.1
27	20.6[d]	20.6[d]
28	25.2[d]	25.3
orthoester	117.3	118.9
acetate	21.2, 21.2	21.2, 21.2
acetate	170.2, 170.3	170.2, 170.3
orthoacetate	23.5	—
orthopropionate	—	29.3
orthopropionate	—	7.7

*In ppm from internal TMS for $CDCl_3$ solutions.

[a–d]Values with like superscripts may be interchanged in each column.

25 μ RP-18 preparative packing (50 mm dia × 250 mm) with MeCN. All petuniasterones appeared in an eluate volume of *ca* 400–900 ml whereas chlorophyll and other less polar lipids remained on the column. Further fractionation was by prep HPLC as previously described [1–3].

Columns and conditions: Rainin Dynamax Silica, 21.4 × 250 mm with guard, 20% 2-propanol in hexane; Alltech R-Sil C-18, 10 × 250 mm, 30% H_2O in CH_3CN; Dynamax C-18, 21.4 × 250 mm, 30% H_2O in MeCN; Whatman PAC, 9 × 500 mm, 10% 2-propanol in hexane. Elution volumes of products are shown in Table 11.

Petuniasterone I (**13**). $[\alpha]_{(\lambda,\ \mathrm{nm})}$: (589) + 134°, (578) + 142°, (546) + 171°, (436) + 427°, (365) + 1420° ($CHCl_3$; *c* 1.00); IR $\nu_{\max}^{CHCl_3}$ cm^{-1}: 3450 *br* (OH), 1725 (acetate), 1675 (COSMe), and 1665 (conj. CO); UV $\lambda_{\max}^{MeOH}$ nm: 232 (log ε 4.09); MS *m/z* (rel. int.): 633.3098 $[MH]^+$ (8), 615 $[MH-H_2O]^+$ (6). $C_{34}H_{49}O_9S$ requires $[MH]^+$ 633.3097.

Petuniasterone J (**14**). From MeOH, mp 305–308°; $[\alpha]_{(\lambda,\ \mathrm{nm})}$: (589) + 186°, (578) + 197°, (546) + 233°, (436) + 536°, (365) + 1750° ($CHCl_3$; *c* 0.80); IR $\nu_{\max}^{CHCl_3}$ cm^{-1}: 1735 (acetate), 1675 (conj. CO); UV $\lambda_{\max}^{MeOH}$ nm: 232 (log ε 3.87); MS *m/z* (rel. int.): 618.3649 $[MNH_4]^+$ (23), 601 $[MH]^+$ (10), 558 $[MNH_4-HOAc]^+$ (10), 541 $[MH-HOAc]^+$ (13). $C_{34}H_{52}NO_9$ requires $[MNH_4]^+$ 618.3641.

Petuniasterone K (**15**). From (heptane–EtOAc), mp 208–209°; $[\alpha]_{(\lambda,\ \mathrm{nm})}$: (589) + 30°, (578) + 31°, (546) + 34°, (436) + 50°, (365) + 26° ($CHCl_3$; *c* 1.00); IR $\nu_{\max}^{CHCl_3}$ cm^{-1}: 1730 (acetate), 1665 (conj. CO); UV $\lambda_{\max}^{MeOH}$ nm: 244 (log ε 4.16); MS *m/z* (rel. int.): 541.3126 $[MH]^+$ (87), 481 $[MH-HOAc]^+$ (76). $C_{32}H_{45}O_7$ requires $[MH]^+$ 541.3165.

Petuniasterone L (**16**). $[\alpha]_{(\lambda,\ \mathrm{nm})}$: (589) + 25°, (578) + 25°, (546) + 28°, (436) + 40°, (365) + 12° ($CHCl_3$; *c* 1.00); IR $\nu_{\max}^{CHCl_3}$ cm^{-1}: 1725 (acetate), 1680 (COSMe), 1665 (conj. CO); UV $\lambda_{\max}^{MeOH}$ nm: 241 (log ε 4.24); MS *m/z* (rel. int.): 615.3018 $[MH]^+$ (11), 555 $[MH-HOAc]^+$ (7), $C_{34}H_{47}O_8S$ requires $[MH]^+$ 615.2991.

12ξ-*Acetoxy*-11β-*hydroxypetuniasterone D 7-acetate* (**17**). $[\alpha]_{(\lambda,\ \mathrm{nm})}$: (589) + 40°, (578) + 41°, (546) + 46°, (436) + 65° (365) + 65° ($CHCl_3$; *c* 1.00); IR $\nu_{\max}^{CHCl_3}$ cm^{-1}: 3400 *br* (OH), 1725 (acetate), 1660 (conj. CO); UV $\lambda_{\max}^{MeOH}$ nm: 242 (log ε 4.01); MS *m/z* (rel. int.): 601.3379 $[MH]^+$ (35), 541 $[MH-HOAc]^+$ (15). $C_{34}H_{49}O_9$ requires $[MH]^+$ 601.3376.

12ξ-*Acetoxy*-11β-*hydroxypetuniasterone M 7-acetate* (**18**).

Table 9. ^{1}H NMR spectral data* for compounds **3**, **4b**, **19** and **20**

H	**3**	**4b**	**19**	**20**
H-1	7.08 *d* (10)	6.95 *d* (10)	7.08 *d* (10)	6.95 *d* (10)
H-2	6.25 *dd* (10, 2)	6.23 *dd* (10, 2)	6.26 *dd* (10, 2)	6.22 *dd* (10, 2)
H-4	6.14 *br t* (*ca* 2)	6.14 *br t* (*ca* 2)	6.14 *br t* (*ca* 2)	6.14 *br t* (*ca* 2)
H_2-6	2.48 *dd* (14, 3)	2.50 *dd* (14, 3)	2.48 *dd* (14, 3)	2.50 *dd* (14, 3)
(α and β)	2.74 *ddd* (14, 3, 2)	2.76 *ddd* (14, 3, 2)	2.75 *ddd* (14, 3, 2)	2.75 *ddd* (14, 3, 2)
H-7	4.04 *br s*	4.06 *br s*	4.05 *br s*	4.06 *br s*
H-12 β	2.03 *dt* (12.5, 4)	5.07 *br t* (3)	2.04 *dt* (12.5, 4)	5.06 *br t* (3)
H-22	4.20 *dt* (11, 5)	4.18 *td* (8, 4)	4.20 *dt* (11, 5)	4.18 *td* (8, 4)
H_2-23	—	1.50 *d* (8)	—	1.47 *d* (8)
H_3-18	0.76 *s*	0.83 *s*	0.76 *s*	0.83 *s*
H_3-19	1.24 *s*	1.22 *s*	1.23 *s*	1.21 *s*
H_3-21	0.96 *d* (7)	0.86 *d* (7)	0.97 *d* (7)	0.84 *d* (7)
H_3-26	1.30 *s*	1.30 *s*	1.31 *s*	1.31 *s*
H_3-27	1.16† *s*	1.16† *s*	1.16† *s*	1.14† *s*
H_3-28	1.20† *s*	1.18† *s*	1.19† *s*	1.18† *s*
orthoacetate-H_3	1.56 *s*	1.56 *s*	—	—
orthopropionate-H_2	—	—	*ca* 1.8 *m*	*ca* 1.8 m
orthopropionate-H_3	—	—	0.99 t (7.5)	0.97 t (7.5)
OAc	—	2.04 *s*	—	2.03 *s*

*δValue in $CDCl_3$; coupling constants (Hz) in parentheses. Data for **3** and **4b** are from ref. [2].

†Values may be interchanged.

$[\alpha]_{(\lambda,\ \mathrm{nm})}$: (589) + 52 °, (578) + 59 °, (546) + 60 °, (436) + 93 °, (365) + 114 ° ($CHCl_3$, *c* 1.00); IR $\nu_{\max}^{CHCl_3}$ cm^{-1}: 3400 *br* (OH), 1725 (acetate), 1660 (conj. CO), UV $\lambda_{\max}^{MeOH}$ nm: 242 (log ε 4.15); MS *m/z* (rel. int.): 615.3498 $[MH]^+$ (56), 555 $[MH-HOAc]^+$ (3). $C_{35}H_{51}O_9$ requires $[MH]^+$ 615.3533.

Petuniasterone M (**19**). From heptane–EtOAc, mp 217–220 °; $[\alpha]_{(\lambda,\ \mathrm{nm})}$: (589) + 56 °, (578) + 53 °, (546) + 52 °, (436) + 52 °, (365) + 52° ($CHCl_3$; *c* 0.45), IR $\nu_{\max}^{CHCl_3}$ cm^{-1}: 3400 *br* (OH), 1650 (conj. CO); UV $\lambda_{\max}^{MeOH}$ nm: 246 (log ε 4.20); MS *m/z* (rel. int.): 499.3408 $[MH]^+$ (53), 481 $[MH-H_2O]^+$ (10). $C_{31}H_{47}O_5$ requires $[MH]^+$ 499.3423.

12α-*Acetoxypetuniasterone M* (**20**). From MeCN–H_2O, mp 217–218 °; $[\alpha]_{(\lambda,\ \mathrm{nm})}$: (589) + 96 °, (578) + 100 °, (546) + 113 °, (436) + 186 °, (365) + 241 ° ($CHCl_3$; *c* 1.00); IR $\nu_{\max}^{CHCl_3}$ cm^{-1}: 3400 *br* (OH), 1725 (acetate), 1655 (conj. CO); UV $\lambda_{\max}^{MeOH}$ nm: 244 (log ε 4.18); MS *m/z* (rel. int.): 557.3569 $[MH]^+$ (21), 539 $[MH-H_2O]^+$ (8), 497 $[MH-HOAc]^+$ (3), 479 $[MH-H_2O-HOAc]^+$ (5). $C_{33}H_{49}O_7$ requires $[MH]^+$ 557.3478.

Crystal structure of petuniasterone J (**14**). $C_{34}H_{48}O_9$, M = 600.8, orthorhombic, space group $P2_12_12_1$, $a = 8.447(3)$, $b = 10.465(5)$, $c = 37.216(12)$ Å, $\beta = 90.0$ °, $U = 3289.6$ Å^3, $D_c = 1.21$ g/cm^3, $Z = 4$, $F(000) = 1296$, $\mu(\mathrm{Cu}K\alpha) = 6.72$ cm^{-1}. Final R = 0.048 (389 parameters), $R_w = 0.056$ for 3283 unique reflections with $|F_0| \geq 3\sigma|F_0|$ in the range $3° \leq 2\theta \leq 114°$, average parameter shift is $\pm 0.2\sigma$, and difference Fourier synthesis excursions are within ± 0.4 eÅ^{-3}. Crystals were obtained from methanol by slow evapn.

Crystal structure of petuniasterone K (**15**): $C_{32}H_{44}O_7$, M = 540.8, monoclinic, space group $P2_1$, $a = 11.597(3)$, $b = 9.595(2)$, $c = 13.874(3)$ Å, $\beta = 106.67(2)$ °, $U = 1478.9$ Å, $D_c = 1.21$ g cm^{-3}, $Z = 2$, $F(000) = 584$, $\mu(\mathrm{Cu}K\alpha) = 6.45$ cm^{-1}. Final $R = 0.072$ (352 parameters), $R_w = 0.070$ for 2766 unique reflections with $|F_0| \geq 3\sigma|F_0|$ in the range $3° \leq 2\theta \leq 114°$, average parameter shift is $\pm 0.1\sigma$, and difference Fourier synthesis excursions are within ± 0.5 eÅ^{-3}. Crystals were obtained from a mixture of heptane and ethyl acetate by slow evaporation.

[*Supplementary publication.* For both **14** and **15**, a complete list of final atomic co-ordinates, table of bond 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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Table 10. ^{13}C NMR spectral data* for compounds **3**, **4b**, **19** and **20**

C	**3**	**4b**	**19**	**20**
1	155.7	154.7	155.6	154.8
2	127.6	128.0	127.6	127.9
3	185.6	185.5	185.6	185.5
4	127.1	127.4	127.2	127.3
5	164.6	163.8	164.5	164.0
6	41.0	40.9	40.9	40.9
7	69.5	69.2	69.5	69.2
8	38.5	39.0	38.6	39.0
9	44.4	37.9	44.4	37.9
10	43.4^{a}	42.8	43.4^{a}	42.8
11	22.5	26.8	22.5	26.8
12	38.9	74.8	39.0	74.8
13	42.9^{a}	45.2	42.9^{a}	45.2
14	49.9	43.0	49.9	43.0
15	23.8	23.1	23.8	23.1
16	27.2	26.4	27.2	26.4
17	52.2	43.9	52.1	43.8
18	11.8	12.2	11.8	12.2
19	18.2	18.0	18.2	18.0
20	39.7	39.7	39.7	39.7
21	12.6	11.6	12.6	11.5
22	69.8	69.6	69.5	69.3
23	30.2	30.1	30.5	30.4
24	82.5^{b}	82.5^{a}	82.1^{b}	82.1^{a}
25	81.3^{b}	81.2^{a}	81.1^{b}	81.0^{a}
26	19.9	20.0	20.1	20.1
27	20.5^{c}	20.5^{b}	20.5^{c}	20.6^{c}
28	25.2^{c}	25.2^{b}	25.3^{c}	25.3^{c}
orthoester	117.3	117.3	118.8	118.8
acetate	—	21.2	—	21.2
acetate	—	170.3	—	170.3
orthoacetate	23.5	23.5	—	—
orthopropionate	—	—	29.3	29.3
orthopropionate	—	—	7.7	7.7

*In ppm from internal TMS for $CDCl_3$ solutions. Data for **3** and **4b** are from ref. [2].

$^{a-c}$Values with like superscripts may be interchanged in each column.

Table 11. Elution zone (ml) for chromatographic separation of petuniasterones (see Experimental for details)

Compound	Dynamax Silica	Dynamax C-18	R Sil C-18	PAC
6	120–240	—	40–45	110–130
10	120–240	—	45–50	80–100
13	—	165–205	33–38	65–75
14	115–130	205–230	31–35	—
15	145–155	210–230	43–50	60–70
16	155–170	210–230	43–50	75–90
17	—	150–170	26–32	75–90
18	—	180–200	35–42	70–80
19	—	380–430	—	55–70
20	—	180–200	35–42	85–105

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