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# MINOR PETUNIASTERONES FROM *PETUNIA HYBRIDA*

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**Key Word Index**—*Petunia hybrida*; Solanaceae, phytosterols; ergostanoids; host plant resistance

**Abstract**—Seven new steroids have been isolated from leaves and stems of *Petunia hybrida*. They are 30-hydroxy petuniasterone A (C-30 epimeric mixture), 1α-acetoxy-1,2-dihydropetuniasterone A (designated petuniasterone E), (22*R*,24*S*)-7α,24-dihydroxy-22,25-oxidoergosta-1,4-dien-3-one (petuniasterone F), C-24 epimers of (22*R*)-7α,22,24,25-tetrahydroxyergosta-1,4-dien-3-one (petuniasterones $G_1$ and $G_2$) and C-24 epimers of (22*R*)-1-α-acetoxy-7α,22,24,25-tetrahydroxyergost-4-en-3-one (petuniasterones $H_1$ and $H_2$) The compounds which have 1-α-acetoxy groups may easily be converted into the corresponding A-ring dienes by mild base. The orthoesters and other side-chain variants may be formed from the 24,25-epoxy compounds which co-occur in the plant.

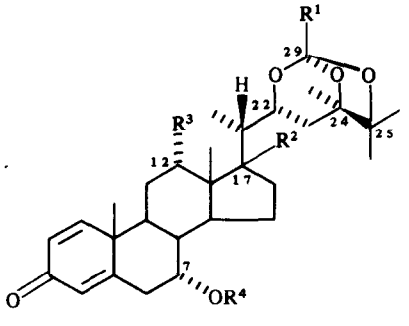
## INTRODUCTION

Petuniasterones [1, 2] are steroidal ketones which occur in varying levels within the commerical varieties of *Petunia hybrida*. The structural aspects of these substances caused us to categorize them into several groups based on common features and designate the trivial names petuniasterones A–D, each of which had a keto group in position-3 and a 7-α-oxygen function The A-rings possessed 1,4-dien-3-one systems or alternatively 1-α-acetoxy-4-en-3-one functionalities. In addition, the side chains at C-17 were highly oxygenated, having bicyclic orthoester systems or a 24,25-epoxy group with a hydroxy or ester substituent at C-22 Compound **1a**, which we have termed petuniasterone A, is generally present in the highest concentration within the plant (up to *ca* 300 ppm dry wt) and it appears to inhibit the growth of *Heliothis zea* (corn earworm) larvae as shown by preliminary experiments using artificial diets (unpublished) Petuniasterone A possesses the unusual thiolester moiety attached to the sidechain orthoester, but this functionality does not appear to be responsible for insect-inhibitory activity since petuniasterone D (**4**) having only the corresponding orthoacetate is also active (unpublished). We have identified several other derivatives of petuniasterones A and D (**2**, **3** and **5**) [2] as well as two sets of compounds (**7**–**9** and **10**–**12**) having 24,25-epoxy substitution [1]. Those having the A-ring dienone are derivatives of petuniasterone C (**9**) and the corresponding 1-acetoxy-4-enones are related to petuniasterone B (**12**). Petuniasterones B and C do not appear to be active against *Heliothis zea* (unpublished)

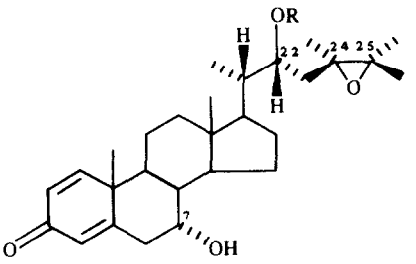
The present communication describes the identification of seven minor petuniasterones. Compound **6a** was shown to be a 30-hydroxy derivative of petuniasterone A The 1-acetoxy-1,2-dihydro- analogue of petuniasterone A (**13**), termed petuniasterone E, was also present. The other substances isolated are less substituted on the side chain. Petuniasterone F (**14a**) has the ring closed 22–25 oxido system whereas the petuniasterones of the G series (**15a**, **b**) have trihydroxy side chains. Petuniasterones $H_1$ (**16a**) and $H_2$ (**16b**) are similar, but have the corresponding 1-acetoxy enone system in the A-ring.

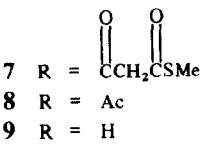
## RESULTS AND DISCUSSION

From material remaining after isolation of several batches of petuniasterones A to D it was possible to obtain small quantities of the seven new substances. Compound **6a**, $C_{32}H_{46}O_7S$ had $^1H$ and $^{13}C$ NMR spectra (Tables 1 and 2) that showed a close relationship of this material to petuniasterone A (**1a**), but one additional hydroxyl was present (mass spectrum). The UV, $\lambda_{max}$ 243 nm, and IR spectra, $\nu_{max}$ 1685 and 1660 $cm^{-1}$ were likewise similar to those of **1a** [1] The characteristic AB quartet ($\delta$ 3.04 and 3.10) of $CH_2$-30 in the $^1H$ NMR of

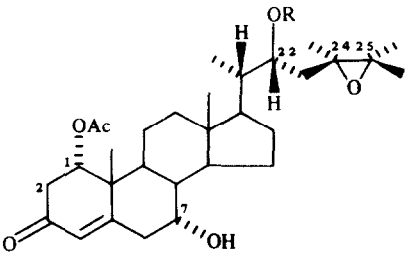


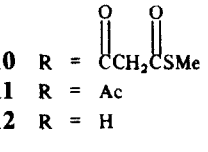

**1a** $R^1$ = $CH_2COSMe$, $R^2$, $R^3$, $R^4$ = H
**1b** $R^1$ = $CH_2COOMe$, $R^2$, $R^3$, $R^4$ = H
**2** $R^1$ = $CH_2COSMe$, $R^2$ = β-OH, $R^3$, $R^4$ = H
**3** $R^1$ = $CH_2COSMe$, $R^2$ = β-OH, $R^3$ = H, $R^4$ = Ac
**4** $R^1$ = Me, $R^2$, $R^3$, $R^4$ = H
**5** $R^1$ = Me, $R^2$ = H, $R^3$ = OAc, $R^4$ = Ac
**6a** $R^1$ = $^{30}CHOHCOSMe$, $R^2$, $R^3$, $R^4$ = H
**6b** $R^1$ = $^{30}CHOAcCOSMe$, $R^2$, $R^3$ = H, $R^4$ = Ac



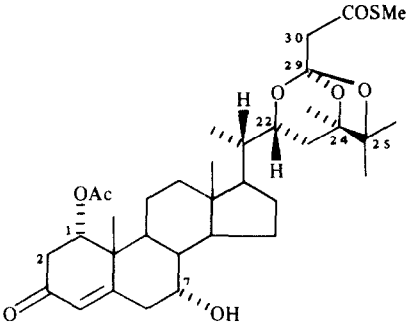


**7** R = $CCH_2CSMe$
**8** R = Ac
**9** R = H





**10** R = $CCH_2CSMe$
**11** R = Ac
**12** R = H



**13**

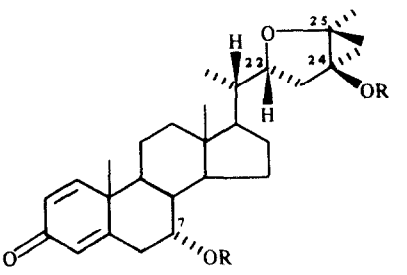
**1a** was no longer observed and new signals at $\delta$ 4 33 and 4.35 appeared This pair of singlets is associated with the proton $\alpha$- to an epimeric OH at C-30. $^1$H NMR signals of $CH_3$-21 and $CH_3$-27 also appeared as double lines. The $^{13}$C NMR spectrum of **6a** confirms the position of hydroxylation since the signals of $CH_2$-30 ($\delta$ 50 3) in **1a** was replaced by a pair of lines at $\delta$ 77 7 and 78 2 corresponding to CHOH-30. In the $^{13}$C NMR spectrum of **6a**, 10 additional peaks associated with carbons in close proximity to the epimeric centre are doubled Treatment of **6a** with acetic anhydride gave the 7,30-diacetate **6b**. The $^1$H NMR spectrum of **6b** (see Experimental) showed typical downfield shifts for the signals of acetylated positions, CH-7 and CH-30 (*ca* 1 06 ppm) [3]. As in the case of **6a**, we noted numerous double peaks for signals associated with carbons and protons near the epimeric center. Although preparative separation of this epimeric mixture by HPLC could not be accomplished, an analytical separation showed partial resolution of **6a** into two components in the ratio of *ca* 2 1 which agrees with the proportions of the NMR signals

Compound **13**, $C_{34}H_{50}O_8S$, resembles petunia sterone A (**1a**), but it has a 1-$\alpha$-acetoxy-4-en-3-one A-ring, and we have given it the name petunia sterone E Comparison of the $^1$H and $^{13}$C NMR spectra of **13** with those of the petunia sterone B series [1] confirms the substitution and stereochemistry of Ring-A Further evidence for the proposed structure is provided by conversion of **13** into the methyl ester corresponding to petunia sterone A (**1b**) [1] by treatment with dilute sodium methoxide in methanol. Facile elimination of the 1-acetoxy group is characteristic of this type of compound [1] and transesterification of the thiolmethyl ester is rapid under these conditions

Petunia sterone F (**14a**), $C_{28}H_{42}O_4$, is isomeric with petunia sterone C (**9**) The $^1$H and $^{13}$C NMR spectra (Tables 1 and 2) show that the A-ring dienone system is present Since no additional unsaturation was observed ($^{13}$C NMR), one more ring is necessary to account for the elemental composition It was possible to convert **9** into **14a** by treatment with weak perchloric acid (0 025 M) in dioxane thereby showing that the additional ring must also be associated with the side chain On treatment with acetic anhydride, **14a** gave the diacetate, **14b** This product had spectral characteristics showing acetylation at position-7 [2] as well as acetylation at a tertiary position Since no acetate shift to lower field was observed in the $^1$H NMR for the proton attached to position-22, it was apparent that the oxygen at this position is involved in ring formation. We have assigned the structure and stereochemistry of **14a** on the basis of concerted ring opening of the 24,25-epoxide of **9** under acid catalysis with formation of the 22,25-oxido system

Petunia sterones $G_1$ and $G_2$ (**15a** and **15b**) are C-24 epimers, $C_{28}H_{44}O_5$, which could be separated by HPLC A mixture of **15a** and **15b** could be prepared from petunia sterone C (**9**) by opening of the epoxide ring in 20% aqueous dioxane About 45% yield of the two epimers was obtained along with 25% of **14a**

Petunia sterones $H_1$ and $H_2$ (**16a** and **16b**), $C_{30}H_{48}O_7$, are related to $G_1$ and $G_2$, respectively, but have the 1-acetoxy-4-en-3-one system Both **16a** and **16b** could be converted into **15a** and **15b** by dilute sodium methoxide in methanol Comparison of the respective $^1$H NMR and $^{13}$C NMR spectra of the G and H series revealed minor chemical shift differences associated with the side chains that enabled the members of each set to be correlated with the other (Tables 3 and 4)



**14a** R = H
**14b** R = Ac

Table 1 $^1$H NMR data* for compounds **1a**, **6**, **13**, **14a** and **14b**

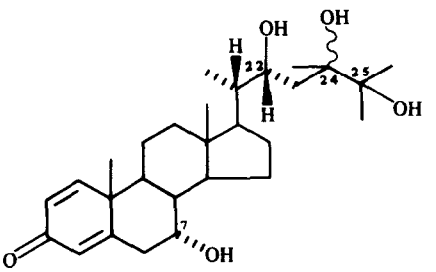
| | Compound | | | | |
|---|---|---|---|---|---|
| | **1a** | **6a** epimers | **13** | **14a** | **14b** |
| 1-H | 7 08 *d* (10) | 7.08 *d* (10) | 5 26 *t* (3) | 7.08 *d* (10) | 7.07 *d* (10) |
| 2-H | 6.24 *dd* (10, 2) | 6.24 *dd* (10, 2) | 2.63 *mult.* | 6.25 *dd* (10, 2) | 6.27 *dd* (10, 2) |
| 4-H | 6.13 *br t* (*ca* 2) | 6.14 *br s* | 5 88 *br s* | 6 15 *t* (2) | 6.02 *d* (2) |
| 6-H$\alpha$ | 2 50 *dd* (14, 3) | 2.49 *dd* (14, 3) | 2.50 *dd* (16, 3) | 2.49 *dd* (14, 3) | 6-H's 2 62 *d* (3) |
| 6-H $\beta$ | 2.75 *ddd* (14, 3, 2) | 2.70 *br d* (14) | 2.70 *dd* (16, 3) | 2.74 *ddd* (14, 3, 2) | |
| 7-H | 4.04 *br s* | 4 04 *br s* | 3 95 *br q* (2) | 4 04 *br s* | 5.04 *br q* (*ca* 3) |
| 12-H $\beta$ | 2.02 *dt* (12.5, 4) | 2.02 *br d* (12.5) | | 2.04 *dt* (13, 4) | |
| 20-H | 1 78† $H_{20-22}=4$) | | | | *ca*.1.9, m $J_{20-21}=7$) $J_{21-22}=4$) |
| 22-H | 4.21 *dt* (11.5, 4) | 4.23 *dt* (11.5, 4) | 4.22 *dt* (11, 4 5) | 4.24 *dt* (11, 5) | 4.06 *ddd* (10, 6, 4) |
| 23-H's | 1.47† $J_{22-23}=4$ | | | | 2.49, *dd* (14, 6) 1.88, *dd* (14, 10) |
| 18-Me | 0 76, *s* | 0 76, *s* | 0 72, *s* | 0.75, *s* | 0.73, *s* |
| 19-Me | 1 23, *s* | 1 23, *s* | 1.27‡, *s* | 1 23, *s* | 1.24, *s* |
| 21-Me | 0.96 *d* (7) | 0.92, 0.95 *d*'s (7) | 0.95 *d* (7) | 0.92 *d* (7) | 0 90 *d* (7) |
| 26-Me | 1.30, *s* | 1.33, *s* | 1.31, *s* | 1.23‡, *s* | 1 48, *s* |
| 27-Me | 1 12‡, *s* | 1.14‡, 1.17‡ *s*, *s* | 1.12†, *s* | 1.13‡, *s* | 1.16‡, *s* |
| 28-Me | 1.21‡, *s* | 1.22‡, *s* | 1.20‡, *s* | 1 26‡, *s* | 1.27‡, *s* |
| COSMe | 2.31, *s* | 2 34, *s* | 2.31, *s* | | |
| $C\underline{H}_2CO$ | 3 04, 3.10 *d*'s (14) | | 3.04, 3.10 *d*'s (14.5) | | |
| $C\underline{H}OHCO$ | | 4.33, 4.35§, *s*, *s* | | | |
| OAc | | | 2.03, *s* | | 2 02, 2 03, *s*'s |

*$\delta$ value in $CDCl_3$; coupling constants (Hz) in parentheses

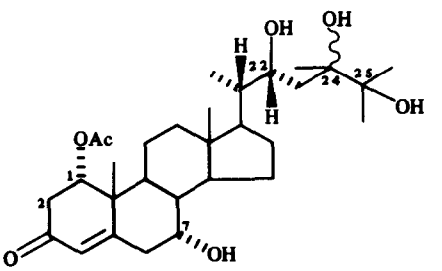
†Value obtained by stepwise decoupling.

‡Values may be interchanged.

§After $D_2O$ exchange, two lines are distinguishable.




( 24 - epimers ) **15a** **15b**




( 24 - epimers ) **16a** **16b**

Table 2 $^{13}$C NMR data* for compounds **1a**, **6a**, **13**, **14a** and **14b**

| C | **1a** | **6a** epimers | **13** | **14a** | **14b** |
|---|---|---|---|---|---|
| 1 | 155 6, CH† | 155 7, CH | 73 8, CH | 155 6, CH | 155 2 |
| 2 | 127 6, CH† | 127 5, CH | 39 4, $CH_2$ | 127 6, CH | 127 8 |
| 3 | 185 6, C | 185 7, C | 194 9, C | 185 6, C | 185 8 |
| 4 | 127 1, CH† | 127 0, CH | 126 4, CH | 127 1, CH | 126 6 |
| 5 | 164 5, C | 164 9, C | 163 6, C | 164 7, C | 164 0 |
| 6 | 41 0, $CH_2$† | 41 0, $CH_2$ | 40 9, $CH_2$ | 40 9, $CH_2$ | 37 3 |
| 7 | 69 5, CH† | 69 5, CH | 67 8, CH | 69 6, CH | 72 0 |
| 8 | 38 5, CH | 38 3, CH | 39 3[a], CH | 37 8, CH | 38 3 |
| 9 | 44 4, CH | 44 2, CH | 36 8[a], CH | 44 4, CH | 45 2 |
| 10 | 43 4[a], C | 43 4[a], C | 41 7[b], C | 43 5[a], C | 43 2[a] |
| 11 | 22 5, $CH_2$ | 22 4, $CH_2$ | 20 3, $CH_2$ | 22 5, $CH_2$ | 22 5 |
| 12 | 39 0, $CH_2$† | 38 9, $CH_2$ | 39 1, $CH_2$ | 38 9, $CH_2$ | 38 8 |
| 13 | 42 9[a], C | 42 8[a], C | 42 7[b], C | 43 1[a], C | 43 0[a] |
| 14 | 49 9, CH | 49 8, CH | 50 2, CH | 49 8, CH | 49 6 |
| 15 | 23 8, $CH_2$ | 23 7, $CH_2$ | 23 6, $CH_2$ | 23 9, $CH_2$ | 23 7 |
| 16 | 27 2, $CH_2$ | 27 1, 27 2, $CH_2$ | 27 3, $CH_2$ | 27 4, $CH_2$ | 27 3 |
| 17 | 52 0, CH | 51 8, CH | 52 0, CH | 53 5, CH | 53 3 |
| 18 | 11 8, $CH_3$† | 11 4, $CH_3$ | 11 7, $CH_3$ | 11 7, $CH_3$ | 11 6 |
| 19 | 18 3, $CH_3$† | 18 2, $CH_3$ | 18 1, $CH_3$ | 18 3, $CH_3$ | 18 4 |
| 20 | 39 8, CH† | 39 6, CH | 38 5[a], CH | 39 8, CH | 37 7 |
| 21 | 12 5, $CH_3$† | 12 4, 12 5, $CH_3$ | 12 5, $CH_3$ | 12 5, $CH_3$ | 12 3 |
| 22 | 70 2, CH† | 70 4, 70 7, CH | 70 2, CH | 77 8, CH | *ca* 77 |
| 23 | 30 3, $CH_2$† | 30 6, $CH_2$ | 30 3, $CH_2$ | 38 9, $CH_2$ | 37 7 |
| 24 | 82 9[b], C | 83 16[b], 83 22[b], C | 82 9[c], C | 84 2[b], C | 89 2[b] |
| 25 | 81 8[b], C | 82 19[b], 82 25[b], C | 81 8[c], C | 81 0[b], C | 84 2[b] |
| 26 | 19 9, $CH_3$ | 19 8, $CH_3$ | 19 9, $CH_3$ | 21 8[c], $CH_3$ | 19 3[c] |
| 27 | 20 4[c], $CH_3$ | 20 2[c], 20.3[c], $CH_3$ | 20.4[d], $CH_3$ | 22 9[c], $CH_3$ | 22 2[c] |
| 28 | 24 9[c], $CH_3$ | 24 7[c], 24 9[c], $CH_3$ | 24.9[d], $CH_3$ | 27 8[c], $CH_3$ | 25 9[c] |
| 29-orthoester | 115 3, C | 115 29, 115 32, C | 115 4, C | | |
| 30-$^{13}CH_2CO$ | 50 3, $CH_2$ | | 50.2, $CH_2$ | | |
| 30-$^{13}CHOHCO$ | | 77 7, 78 2, CH | | | |
| 31-$^{13}COS$ | 193 3, C | 198 5, 198 6, C | 193 3, C | | |
| S-Me | 12 0, $CH_3$ | 11 8, 12 4, $CH_3$ | 12 0, $CH_3$ | | |
| Acetate | | | 170 2, C | | |
| | | | 21 0, $CH_3$ | | |

*In ppm from internal TMS for $CDCl_3$ solutions

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

†Assigned by C-H correlation spectroscopy

The extremely facile opening of the epoxide ring of **9** prompted us to examine the reactivity of other compounds having the same functionality Thus, **7** was treated with 0.01 M perchloric acid in dioxane to give **1a** in 50% yield after chromatography This reaction was very rapid, being complete in less than one min and formed the orthoester without significant byproducts The reaction of **8** to produce **4** was equally rapid and a 60% yield of the latter compound was obtained upon work-up after one min Acid treatment of the more sensitive **10** gave a mixture of several components, from which could be obtained 20% of **13** and 5% of **1a** In this case, it appears that acid catalysed elimination of the 1-acetoxy group is competitive with formation of the orthoester system of the side chain. Several other, more polar compounds that were produced in this run were not identified

The ease of formation of the pendant orthoesters from the epoxy side chain esters and production of the 22,25-oxido-ring as well as trihydroxy side chain derivatives from the hydroxy epoxide points to the epoxides as biogenetic precursors It is likely that a 1-acetoxy-4-en-3-one having the 24,25-epoxide such as **10** is initially formed. Successive esterification of the 22-OH, followed by the steps illustrated above yields all of the main petuniasterone classes found in the plant Further hydroxylation and acetoxylation can give the more highly oxygenated derivatives We have observed that a number of steroidal materials from these *Petunia* isolates remain unidentified and suggest that they should mainly fit into the missing spaces within the scheme suggested

## EXPERIMENTAL

Mps are corr Optical rotations were measured at *ca* 26° IR spectra were recorded in $CHCl_3$ solns. UV spectra in MeOH $^1$H 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 MS spectra were obtained with $NH_3$ chemical ionization Silica gel was Merck,

Si60, 70–230 mesh; Sephadex, LH-20 from Pharmacia, HPLC columns were commercially obtained. Solvents were HPLC grade. Detection was by UV at 254 nm using a monitor equipped with 0 5 mm pathlength prep cell

*Plant material* *P. 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 as previously described on freeze-dried plant material [1] After removal of the more abundant sterones, rechromatography of the residue yielded compounds **6**, **13**, **14**, **15a** and **b** and **16a** and **b**. Columns and conditions are as follows: Rainin Dynamax Silica, 21 4 mm diam × 250 mm with guard, 20% 2-propanol in hexane, Alltech R-Sil C-18, 10 mm diam × 250 mm, 30%, 40% or 50% $H_2O$ in MeCN; and Whatman Partisil-10 PAC, 9 mm diam × 500 mm, 10% 2-propanol in hexane

| | Elution Zone (ml) | | |
|---|---|---|---|
| Compound | Dynamax silica | R Sil C-18 (% Water) | PAC |
| **6a** | 164–216 | 28–34(30) | 148–170 |
| **13** | 164–216 | 42–45(30) | 87–100 |
| **14** | 300–400 | 34–45(30) | 190–250 |
| **15a** | | 24–27(40) | |
| **15b** | | 27–32(40) | |
| **16a** | | 23–28(50) | |
| **16b** | | 28–33(50) | |

*30-Hydroxy petuniasterone A* (**6a**). Compound **6a** was obtained as a *ca* 2·1 mixt of C-30 epimers (Tables 1,2) that could not be sepd preparatively under the chromatographic conditions employed. $[\alpha]_{(\lambda,nm)}$: (589) +50°, (578) +52°, (546) +58°, (436) +88°, (365) +64° ($CHCl_3$, *c*1); IR $\nu_{max}^{CHCl_3}$ 3550 *br* (OH), 1685 (COSMe), and 1660 $cm^{-1}$ (conj. CO); UV $\lambda_{max}^{MeOH}$ 243 nm (log $\varepsilon$ 4.35); MS *m/z* (rel. int %): 575.3020 ($[MH]^+$, 19), 557 ($[MH-H_2O]^+$, 4). $C_{32}H_{46}O_7S+H$ requires: $[MH]^+$ 575 3042.

*30-Acetoxy petuniasterone A 7-acetate* (**6b**). Acetylation of **6a** was carried out in refluxing $Ac_2O$ for 3.5 hr Excess reagent was evapd and the product isolated by HPLC on the PAC column (elution vol. 84–105 ml, 10% 2-propanol–hexane). IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$ 1750, 1730, 1680, 1665; $^1H$ NMR (200 MHz, $CDCl_3$): $\delta$ 0 76 (3H, *s*, 18-Me) 0 93 and 0.95 (3H, two *d*, *J* = 7, 21-Me), 1.14 and 1 16 (3H, two *s*, 27-Me), 1.22 (3H, *s*, Me-28), 1 25 (3H, *s*, Me-19), 1.32 (3H, *s*, Me-26), 1.99 (3H, *s*, 7-OAc), 2.21 and 2 22 (3H, two *s*, 30-OAc), 2 30 and 2 31 (3H, two *s*, SMe), 2 63 (2H, *br s*, $CH_2$-6), 4 21 (1H, *dt*, *J* = 11 5 and 4, H-22), 5 10 (1H, *br q*, *J* = 3, H-7), 5.40 and 5.41 (1H, two *s*, H-30), 6.00 (1H, *br d*, *J* = 2, H-4), 6.24 (1H, *dd*, *J* = 10 and 2, H-2), 7 04 (1H, *d*, *J* = 10, H-1); $^{13}C$ NMR (50 MHz, $CDCl_3$): $\delta$, *inter alia*, 114.6 and 114 7 (C-29), 76.5 and 77.6 (CH-30), 194 04 and 193 99 (COS), 11.78 and 11 83 (SMe) 170.3 and 21.1 (OAc-7), 169.25, 169.39 and 20.72, 20 76 (OAc-30); MS *m/z* (rel. int. %): 659.3201 ($[MH]^+$, 43), 599 ($[MH-HOAc]^+$, 14) $C_{36}H_{50}O_9S+H$ requires: $[MH]^+$ 659.3250.

*Petuniasterone E* (**13**). $[\alpha]_{(\lambda,nm)}$: (589) + 78°, (578) + 81°, (546) + 91°, (436) + 146°, (365) + 13° ($CHCl_3$, *c*1.00); IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$ 3500 *br* (OH), 1735 (acetate), and 1675 (COSMe and conj CO), UV $\lambda_{max}^{MeOH}$ nm 241 (log$\varepsilon$ 4.19); MS: *m/z* (rel. int %): 619.3298 ($[MH]^+$, 40), 559 ($[MH-HOAc]^+$, 4). $C_{34}H_{50}O_8S$ requires: $[MH]^+$ 619.3304.

Table 3. $^1H$ NMR data* for compounds **15** and **16**

| | Compound | | | |
|---|---|---|---|---|
| | **15a** | **15b** | **16a** | **16b** |
| 1-H | 7 30 | 7.30 | 5 24 | 5.25 |
| | *d* (10) | *d* (10) | *t* (3) | *t* (3) |
| 2-H | 6.22 | 6.22 | *ca* 2 7 | *ca* 2.7 |
| | *dd* (10, 2) | *dd* (10, 2) | *mult* | *mult* |
| 4-H | 6.09 | 6 09 | 5.80 | 5 80 |
| | *br t* (*ca* 2) | *br t* (*ca* 2) | *br s* | *br s* |
| 6-H$\alpha$ | 2 48 | 2.48 | 2 48 | 2.48 |
| | *dd* (14, 3) | *dd* (14, 3) | *dd* (17, 3) | *dd* (17, 3) |
| 6-H$\beta$ | 2 79 | 2.79 | 2 75 | 2.76 |
| | *ddd* (14, 3, 2) | *ddd* (14, 3, 2) | *dd* (17, 3) | *dd* (17, 3) |
| 7-H | 4.02 | *ca.* 4.05 | *ca* 3.95 | 3.92 |
| | *br q* (2) | *mult* | *mult* | *br q* (*ca* 2) |
| 12-H$\beta$ | 2.06 | 2.06 | | |
| | *dt* (13, 4) | *dt* (13, 4) | | |
| 22-H | 3.96 | ca 4.05 | ca. 3.95 | 4.08 |
| | *mult* | *mult* | *mult* | *dd* (11, 3) |
| 18-Me | 0.82, *s* | 0.82, *s* | 0.78, *s* | 0.78, *s* |
| 19-Me | 1.24†, *s* | 1 22†, *s* | 1.24†, *s* | 1.23, *s* |
| 21-Me | 0.96 | 0.96 | 0.94 | 0.96 |
| | *d* (7) | *d* (7) | *d* (7) | *d* (7) |
| 26-Me | 1 19†, *s* | 1.17†, *s* | 1.18†, *s* | 1.17†, *s* |
| 27-Me | 1.22†, *s* | 1.19†, *s* | 1.22†, *s* | 1.19†, *s* |
| 28-Me | 1 28†, *s* | 1.27†, *s* | 1.31†, *s* | 1.31†, *s* |
| OAc | | | 2.01, *s* | 2.01, *s* |

*$\delta$ value in $CD_3OD$, coupling constants (Hz) in parentheses.

†Value may be interchanged.

*Conversion of petunıasterone E* (**13**) *ınto petunıasterone A methyl ester* (**1b**). A soln of **13** (*ca* 13 mg) ın 0 5 M NaOMe–MeOH (1 ml) was allowed to stand 2 hr at room temp, HOAc 50 $\mu$l was added, the mıxt taken to dryness *ın vacuo* and the resıdue trıturated wıth $CH_2Cl_2$ After filtratıon of the $CH_2Cl_2$ soln through a 0 45 $\mu$m Teflon membrane filter followed by concn, the product was chromatographed on the PAC column (10% 2-propanol/hexane) to yıeld **1b** Thıs materıal was chromatographically and spectroscopically ıdentıcal to preVıously ıdentıfied **1b** [1]

*Petunıasterone F* (**14a**) From $CHCl_3$, mp 266–268° (darkens), $[\alpha]_{(\lambda,nm)}$: (589) + 22°, (578) + 23°, (546) + 24°, (436) + 23 °, (365) −87° ($CHCl_3$, *c* 0.5), IR $\nu_{max}^{CHCl_3}$ 3450 *br* (OH), and 1670 $cm^{-1}$ (conj CO); UV $\lambda_{max}^{MeOH}$ nm 246 (log$\varepsilon$ 4 14); MS *m/z* (rel ınt %) 443 3209 ([MH]$^+$, 100), 425 ([MH]$^+$ $-H_2O$, 95), 407 ([MH]$^+$ $-2H_2O$, 21) $C_{28}H_{42}O_4$ + H requıres [MH]$^+$ 443 3161

*Acetylatıon of petunıasterone F* (**14a**) Petunıasterone F, 4 mg, was acetylated as descrıbed for the prepn of **6a** HPLC on the PAC column gave **14b** (elutıon vol 54–60 ml. 10% 2-propanol–hexane)

*Epımerıc petunıasterone* $G_1$ (**15a**) *and* $G_2$ (**15b**) **15a** from MeCN–$H_2O$, mp 218–220°, $[\alpha]_{(\lambda,nm)}$ (589) + 16°, (578) + 17°, (546) + 18°, (436) + 10°, (365) − 108° (MeOH, *c*1), IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$ 3450 *br* (OH), and 1670 (conj CO), UV $\lambda_{max}^{MeOH}$ nm 246 (log$\varepsilon$ 4 15), MS *m/z* (rel ınt %) 461 3269 ([MH]$^+$, 9), 443 ([MH]$^+$ − $H_2O$, 9), 425 ([MH]$^+$ $-2H_2O$, 5), 407 ([MH]$^+$ −3 $H_2O$, 5) $C_{28}H_{44}O_5$ + H requıres 461 3267

**15b** from MeOH, mp 271–275° (darkens gas evol), $[\alpha]_{(\lambda,nm)}$ (589) + 19°, (578) + 20°, (546) + 21°, (436) + 17°, (365) − 86° (MeOH, *c*1), IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$ 3450 *br* (OH), and 1670 $cm^{-1}$ (conj CO), UV $\lambda_{max}^{MeOH}$ nm 247 (log$\varepsilon$ 4 16), MS *m/z* (rel ınt %) 461 3285 ([MH]$^+$, 1 5), 443 ([MH − $H_2O$]$^+$, 6), 425 ([MH]$^+$ −2 $H_2O$, 4), 407 ([MH]$^+$ −3 $H_2O$, 3) $C_{28}H_{44}O_5$ + H requıres [MH]$^+$ 461 3267

*Conversion of petunıasterone C* (**9**) ınto petunıasterones F (**14a**), $G_1$ (**15a**) *and* $G_2$ (**15b**) In 4 ml of dıoxane (purıfied through alumına) was dıssolved 40 mg of **9** To thıs soln was added 1 ml of $H_2O$ followed by 50 $\mu$l of N $HClO_4$ Progress of the reactıon was followed by HPLC on the R Sıl C-18 column (MeCN–$H_2O$, 7 3) All startıng materıal was consumed after *ca* 2 hr and the three majOr zones correspondıng to **14a**, **15a** and **15b** were sepd preparatıvely on the same column (respectıve yıelds 8, 10, and 10 mg) These materıals were chromatographically and spectroscopically ıdentıcal to the compounds descrıbed above One addıtıonal substance (elutıon vol 53–57 ml, MeCN–$H_2O$, 7 3), 3 mg, had a structure sımılar to **14a** (by NMR) but was not ıdentıfied

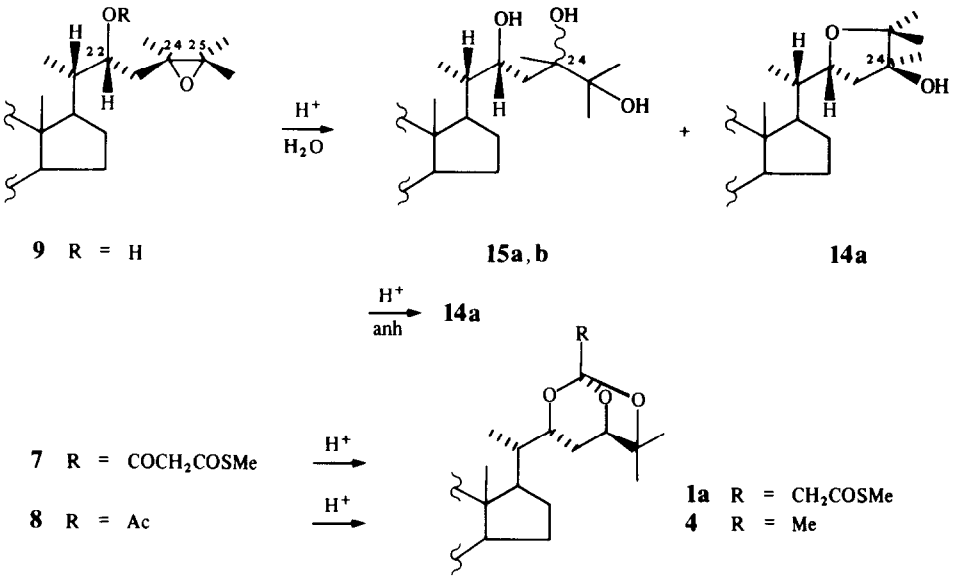
In a separate expt, 40 mg of **9** was dıssolved ın 1 ml of dıoxane and 25 $\mu$l of 0 5 M $HClO_4$ ın dıoxane (prepared by dıl conc $HClO_4$ wıth 10 parts dıoxane) was added HPLC under the above condıtıons showed no startıng materıal after 1 mın and no further reactıon on longer standıng The maın product, 27 mg, was pure crystallıne **14a** accompanıed by *ca* 6 mg of unıdentıfied related materıal (elutıon vol *ca* 35–40 ml)

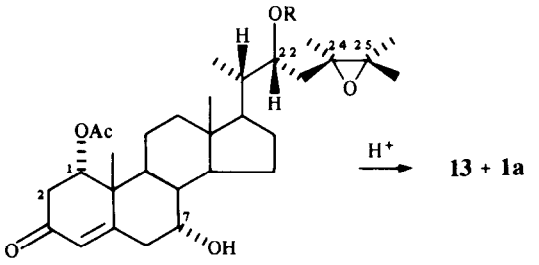
Table 4 $^{13}$C NMR data* for compounds **15** and **16**

| Carbon | Compound 15a | 15b | 16a | 16b |
|---|---|---|---|---|
| 1 | 159 5, CH | 159 4 | 75 4, CH | 75 4, CH |
| 2 | 127 6, CH | 127 6 | 40 3[a], $CH_2$ | 40 3[a], $CH_2$ |
| 3 | 188 3, C | 188 3 | 198 0, C | 198 0, C |
| 4 | 126.9, CH | 126 9 | 126 1, CH | 126 2, CH |
| 5 | 170 1, C | 170 0 | 168 7, C | 168 7, C |
| 6 | 42 2[a], $CH_2$ | 42 2[a] | 42 0, $CH_2$ | 42 0, $CH_2$ |
| 7 | 70 5[b], CH | 70 5[b] | 68 5, CH | 68 5, CH |
| 8 | 40 8, CH | 40 9 | 40 5, CH | 40 5, CH |
| 9 | 45 8, CH | 45 8 | 38 1, CH | 38 1, CH |
| 10 | 45 3[c], C | 45 3[c] | 43 8[b], C | 44 2[b], C |
| 11 | 23 7, $CH_2$ | 23 7 | 21 5, $CH_2$ | 21 5, $CH_2$ |
| 12 | 40 5[a], $CH_2$ | 40 5[a] | 40 5[a], $CH_2$ | 40 5[a], $CH_2$ |
| 13 | 43.9[c], C | 44 0[c] | 43 3[b], C | 43 3[b], C |
| 14 | 51 3, CH | 51 2 | 51 4, CH | 51 4, CH |
| 15 | 24.8, $CH_2$ | 24 7 | 24 6, $CH_2$ | 24 6, $CH_2$ |
| 16 | 28.7, $CH_2$ | 28 5 | 28 7, $CH_2$ | 28 5, $CH_2$ |
| 17 | 54 2, CH | 54 3 | 54 2, CH | 54 3, CH |
| 18 | 12 3, $CH_3$ | 12 3 | 12 2, $CH_3$ | 12 2, $CH_3$ |
| 19 | 18 9, $CH_3$ | 18.8 | 18 3, $CH_3$ | 18 3, $CH_3$ |
| 20 | 44 4, CH | 43 2 | 44 4, CH | 43 2, CH |
| 21 | 12 7, $CH_3$ | 12 9 | 12 7, $CH_3$ | 13 1, $CH_3$ |
| 22 | 70 9[b], CH | 71 4[b] | 70 9, CH | 71 4, CH |
| 23 | 36 8, $CH_2$ | 34 1 | 38 5, $CH_2$ | 34 1, $CH_2$ |
| 24 | 76 1[d], C | 76 2[d] | 76 1[c], C | 76 2[c], C |
| 25 | 77 3[d], C | 78 3[d] | 77 3[c], C | 78 3[c], C |
| 26 | 25 3[e], $CH_3$ | 24 8[e] | 25 3[d], $CH_3$ | 24 8[d], $CH_3$ |
| 27 | 25 2[e], $CH_3$ | 24 8[e] | 25 2[d], $CH_3$ | 24 8[d], $CH_3$ |
| 28 | 22 6[e], $CH_3$ | 22 1[e] | 22 6[d], $CH_3$ | 22 2[d], $CH_3$ |
| Acetate | | | 172 0, C | 172 0, C |
| | | | 20 8, $CH_3$ | 20 8, $CH_3$ |

*In ppm from ınternal TMS for $CD_3OD$ solutıons

[a–e]Values wıth lıke superscrıpts may be ınterchanged




**10** R = $COCH_2COSMe$

*Petuniasterones* $H_1$ (**16a**) *and* $H_2$ (**16b**) These compounds are C-24 epimers and could not be completely sepd from each other under the chromatographic conditions used **16a**, IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$ 3450 *br* (OH), 1730 (acetate) and 1670 (conj CO), UV $\lambda_{max}^{MeOH}$ nm 241, MS *m/z* (rel int %) 538 ($[MNH_4]^+$, 100) $C_{30}H_{48}O_7 + NH_4$ requires $[MNH_4]^+$ 538

**16b**, IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$ 3450 *br* (OH), 1730 (acetate) and 1670 (conj. CO), UV $\lambda_{max}^{MeOH}$ nm 240, MS *m/z* (rel int %) 538.3621 ($[MNH_4]^+$, 100) $C_{30}H_{48}O_7 + NH_4$ requires $[MNH_4]^+$ 538 3743

*Conversion of petuniasterones* $H_1$ *and* $H_2$ *into petuniasterones* $G_1$ *and* $G_2$ Compounds **16a** and **16b** were treated with 0 5 M NaOMe–MeOH as described for the conversion of **13** into **1b** HPLC on the RSil C-18 column (MeCN–$H_2O$, 7 3) of the products gave **15a** from **16a** and **15b** from **16b** which were identified by chromatographic and spectral comparison.

*Acid treatment of* **7**. In 1 ml of dioxane was dissolved 16 mg of **7** To this soln was added 10 $\mu$l. of N $HClO_4$ in dioxane. Chromatography on the PAC column (10% 2-propanol in hexane) showed complete reaction in less than 1 min Prep isolation of product on the same column gave **1a**, 8 mg, identified by comparison with authentic material

*Acid treatment of* **8** This was carried out on 10 mg of **8** as described above After less than 1 min, prep chromatography gave **4**, 6 mg, identical with authentic material.

*Acid treatment of* **10** This was carried out on 40 mg of **10** as described for **7** Chromatographic examination on the R Sil C-18 column (MeCN–$H_2O$, 7.3) indicated formation of **13** within *ca* 1 min along with several other, more polar products. Rechromatography on the PAC column yielded 8 mg of **13** and 2 mg of **1a**.

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