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PETUNIASTERONE R, A NEW ERGOSTANOID FROM *PETUNIA PARODII*

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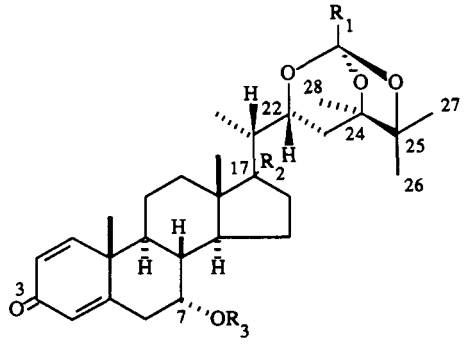
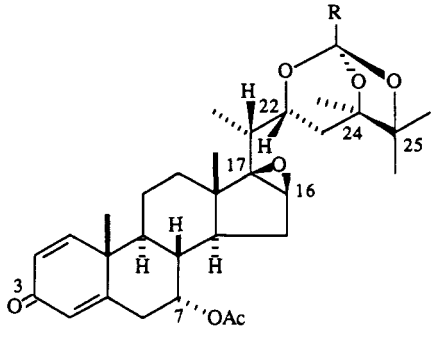
ABSTRACT.—The ergostanoid petuniasterone R [**7**] was present in dry *Petunia parodii* leaf at a concentration of 300 ppm. In addition to typical petuniasterone functionality of a bicyclic orthoester side chain and dienone A ring, **7** has an epoxy group at position 16α,17α of ring D. Petuniasterone R reduces the growth of *Heliothis zea* larvae to 50% of control size at a concentration of ca. 400 ppm in artificial diets.

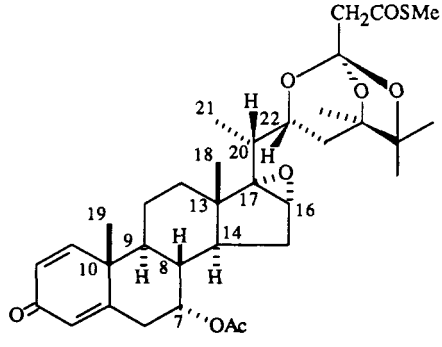
Petunia (Solanaceae) foliage is resistant toward feeding by the larvae of a number of lepidopteran insects, and it has been shown that this resistance is, in part, conferred by a series of closely related steroidal compounds that we have termed petuniasterones (1–6). Petuniasterones, which have an unaltered ergostane carbon skeleton, typically possess a keto group at position 3 and an α-hydroxy or α-acetoxy at position 7. Also present in most instances is a rather unusual bridged orthoester system attached at positions 22, 24, and 25 of the side chain. Petuniasterone A [**1**] shows these features and also exhibits a thiolester moiety linked to the orthoester. Functionalities such as epoxy, hydroxy, and acetoxy groups may be found on the side chain or on the steroid nucleus in numerous other compounds such as **3**, **5**, and **6**. We have observed that insect-growth-inhibiting activity is only found in those compounds that possess an orthoester group. However, the thiolester substituent such as is found in **1** is not necessary for this activity; compound **4**, lacking this group, is nonetheless of similar potency. We now report the occurrence of a new ring-D-oxygenated petuniasterone **7**, isolated from *Petunia parodii* Steere, whose structure incorpo-



1 R_1=CH_2COSMe, R_2=α-H, R_3=H
2 R_1=CH_2COSMe, R_2=α-H, R_3=Ac
3 R_1=CH_2COSMe, R_2=β-OH, R_3=Ac
4 R_1=Me, R_2=α-H, R_3=H



5 R=CH_2COSMe
6 R=Me



7

rates a 16α,17α-oxido bridge, and which is epimeric at this position to petuniasterones K [**6**] and L [**5**]. Compound **7**, which we have termed petuniasterone R, has insect inhibitory activity ca. one third as potent as that of **1** and **4**. The dose of **7** that reduces larval growth of the corn earworm, *Heliothis zea*, a common polyphagous insect pest, to 50% of control weight was approximately 400 ppm in artificial diet.

Petuniasterone R had the elemental composition $C_{34}H_{46}O_8S$, indicative of twelve degrees of unsaturation. Examination of ^{1}H- and ^{13}C-nmr spectra and comparison with previous examples revealed the presence of typical dieneone functionalization of ring A as well as side chain orthoester substitution bearing an associated thiolester moiety. This was consistent with observed uv absorption at 240 nm for the dieneone and with ir bands at 1665 (conjugated CO) and 1685 (COSMe) cm^{-1}. One acetoxy group was also found, and its attachment at position 7α was established by characteristic nmr signals associated with protons on carbons 6 and 7 which were well defined from earlier examples (1,2). No other multiple bonds were indicated by ^{13}C nmr, showing that one additional ring besides the usual steroid skeleton and orthoester system was present. One oxygen atom remained unaccounted for, and the absence of any hydroxyl absorption in the ir suggested that it was incorporated into an ether ring. Thus, **7** is isomeric with compound **5**, and comparison of their ^{1}H- and ^{13}C-nmr spectra (Tables 1 and 2) reveals substantial similarity of their structures. Assignments of carbon and proton signals were facilitated by ^{1}H-^{1}H and ^{1}H-^{13}C correlation spectroscopy (7) and by long range ^{1}H-^{13}C correlation techniques (8). The ^{1}H-nmr and COSY spectra of **7** showed that H-20 was coupled to only H_3-21 and H-22, indicating that C-17 (having no proton) is attached to oxygen. The signal at δ 70.6

TABLE 1. Comparison of ^{1}H-nmr Data for Compounds **2**, **3**, **5**, and **7**.[a]

Proton	Compound			
	2[b]	**3**[c]	**5**[c]	**7**
H-1	7.0 d (10)	7.10 d (10)	7.05 d (10)	7.06 d (10)
H-2	6.22 dd (10, 2)	6.26 dd (10, 2)	6.28 dd (10, 2)	6.26 dd (10, 2)
H-4	6.01 br d (ca. 2)	6.02 br s	6.00 br d (ca. 2)	6.02 br s
H_2-6	2.60, 2.66 d's (14)	2.60, 2.66 d's (14)	2.60 d (3)	2.60, 2.66 d's (14)
H-7	5.05 br q (ca. 3)	5.08 br q (ca. 3)	4.94 br s	5.04 br q (ca. 3)
H-8	—	—	—	1.86 br m
H-16	—	—	3.33 d (4)	3.43 s
H-20	—	—	2.20 br m	2..14 dq (10, 7)
H-22	4.20 dt (11, 4)	4.30 ddd (11, 7, 4)	4.31 ddd (11, 6, 5)	3.59 td (10, 4)
Me-18	0.76 s	0.97 s	0.93 s	0.88 s
Me-19	1.25 s	1.26 s	1.24 s	1.26 s
Me-21	0.95 d (7)	0.91 d (7)	0.82 d (7)	0.90 d (7)
Me-26	1.31 s	1.33 s	1.36 s	1.30 s
Me-27	1.12[d] s	1.14[d] s	1.13[d] s	1.15[d] s
Me-28	1.20[d] s	1.20[d] s	1.21[d] s	1.21[d] s
COSMe	2.31 s	2.31 s	2.30 s	2.30 s
CH_2CO	3.04, 3.10 d's (14)	3.02, 3.07 d's (14)	3.02, 3.09 d's (14)	3.00, 3.05 d's (14)
OAc	2.00 s	1.98 s	2.01 s	1.99 s

[a]δ values in $CDCl_3$; coupling constants (Hz) in parentheses.
[b]Data for this compound are from Elliger *et al.* (2).
[c]Data for this compound are from Elliger *et al.* (4).
[d]Values may be interchanged.

TABLE 2. Comparison of ^{13}C-nmr Data for Compounds **2**, **3**, **5**, and **7**.[a]

Carbon	Compound			
	2[b]	**3**[c]	**5**[c]	**7**
C-1	155.2	155.3	154.8	154.9
C-2	127.7	127.7	127.8	127.8
C-3	185.7	185.7	185.6	185.7
C-4	126.6	126.6	126.7	126.7
C-5	163.9	163.9	163.5	163.6
C-6	37.3	37.3	37.1	37.3
C-7	72.0	71.7	71.7	71.5
C-8	38.3[d]	39.3	36.7	37.0
C-9	45.1	45.3[d]	44.6	45.3
C-10	43.2	43.2	43.2	43.2
C-11	22.4	22.5	22.6	22.2
C-12	38.8	36.4	33.8	32.2
C-13	43.0	48.3	42.4	42.6
C-14	49.6	44.1[d]	56.2	39.2
C-15	23.8	23.3	28.0	27.0
C-16	27.0	33.4	63.6	61.9
C-17	51.9	84.9	75.0	70.6
C-18	11.7	15.3	14.2	15.5
C-19	18.4	18.5	18.2	18.4
C-20	38.4[d]	41.7	37.1	35.4
C-21	12.5	14.4	10.4	11.9
C-22	70.1	71.7	69.9	68.5
C-23	30.3	31.3	34.5	36.3
C-24	82.8[e]	83.5[e]	82.9[d]	83.3[d]
C-25	81.7[e]	82.1[e]	82.0[d]	81.8[d]
C-26	19.9	19.7	19.9	19.7
C-27	20.4[f]	20.5[f]	20.2[e]	20.2[e]
C-28	24.9[f]	24.8[f]	24.8[e]	24.9[e]
orthoester	115.3	115.0	115.1	115.0
*C*H$_2$COS	50.2	50.2	50.2	50.7
*C*OSMe	193.2	193.2	192.9	192.9
SMe	12.0	12.0	12.0	12.0
*C*H$_3$CO	21.0	21.0	21.1	21.0
MeCO	170.2	170.3	170.3	170.6

[a]In ppm from TMS for $CDCl_3$ solution.
[b]Data for this compound are from Elliger *et al.* (2).
[c]Data for this compound are from Elliger *et al.* (4).
[d–f]Values with like superscripts in each column may be interchanged.

of a quaternary, oxygenated carbon exhibited long range connectivity to H_3-18/21, supporting this conclusion, and it was assigned to C-17. It was possible by these means to assign carbon resonances for all carbons with the exception of those at positions 15 and 16, one of which (δ 61.9, CH) must bear an oxygen.

Compound **7** must have an oxirane (three-membered) or oxetane (four-membered) ring system involving position 17 and either position 15 or 16. The chemical shift of the proton α to oxygen on this ring (δ 3.43 compared to 3.33 in **5**) points conclusively to the oxirane system since the chemical shift of corresponding protons in a typical four-membered system is somewhat greater than 1 ppm farther downfield (9). Also, the position observed for C-17 is at much higher field than would be expected for an oxetane, which would have a value for this carbon close to that observed for

compound **3** (δ 84.9). Similarly, the chemical shift of C-16 (δ 61.9) is consistent with a three-membered ring (10) and is close in value to that observed for C-16 in compound **5**. It is interesting that H-16 appears as a rather sharp singlet ($w_{1/2}$ ca. 2.5 Hz); however, examination of the COSY spectrum showed that this proton is weakly coupled to two adjacent protons. Because the epoxy group of **5** has the β configuration (established conclusively by X-ray determination), this group must have the opposite configuration in petuniasterone R. The considerably higher field chemical shift of C-14 in **7** compared to that observed for this carbon in **5** is in agreement with the presence of an α-epoxide in the former compound, since it is known that close proximity of an epoxy group gives rise to a very pronounced upfield shift for the resonance of a carbon located above its ring plane. It has been shown that the chemical shifts of C-7 in *exo-* and *endo-*epoxynorboranes can differ by as much as 23 ppm (11). Dreiding models show that the epoxy group of **7** is about 0.6 Å closer to C-14 than in **5**, and that the orientation of the three-membered ring with respect to this carbon is very similar to the stereochemistry of the bicyclo-2:2:1 system examined above. We note also that the position of H-22, δ 3.59, is very much higher than the values observed in other petuniasterones. Protons lying above epoxy rings also are strongly shielded (12), and it is possible that the preferred conformation of the side chain in **7** places H-22 within this shielding zone.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Thomas-Hoover apparatus and are corrected. Ir spectra and specific rotations were determined on Perkin-Elmer 237 and 241 instruments, respectively, and uv spectra were taken using Cary 219 and Hewlett-Packard 8451 spectrophotometers. ^{1}H- and ^{13}C-nmr spectra were obtained on a Nicolet NT-200 spectrophotometer at 200 and 50 MHz, respectively. NH_3 cims were obtained using a VG Micromass 70/70 HS instrument at an ion source temperature of 130°. Accurate mass measurement was relative to a perfluoroalkane internal standard. Hplc solvents were pumped with an Altex-Beckman Model 110A pump with preparative head. Detection was by uv using a Beckman Model 150 detector at 254 nm with preparative flow cell.

PLANT MATERIAL.—*P. parodii* seeds were obtained from the National Seed Storage Laboratory, Colorado State University, Fort Collins, Colorado. Plants were grown in the greenhouse and in outside beds in Albany, California. Leaves were harvested at intervals, freeze-dried, and stored for later use.

ISOLATION OF PETUNIASTERONE R [7].—After extraction of 600 g of dry leaf, and preliminary chromatography as previously described (1–4), the petuniasterone/petuniolide-containing material was further fractionated by hplc on a Rainin Dynamax 41.4 × 250 mm C-18 column using 30% H_2O in MeCN. The eluate from 570 to 780 ml (9.6 g) was then rechromatographed on a Rainin Dynamax 21.4 × 250 mm cyano column with 20% iPrOH in hexane to give a zone eluting at 380–445 ml which contained impure **7**, 0.22 g. Final chromatography on a Whatman M-9, 9 mm × 500 mm Partisil 10 PAC column with 10% iPrOH in hexane gave pure **7**, 180 mg, in an elution volume of 75–100 ml. Compound **7** deposited poorly formed crystals from heptane/EtOAc, mp 130–132°.

Petuniasterone R [7].—$[\alpha]^{26}$ (λ nm) −29° (589), −31° (578), − 36° (546), −75° (436), −180° (365); ir ν max ($CHCl_3$) 1725 (acetate), 1680 (COS), 1660 (conjugated CO), 1630 (conjugated olefin) cm^{-1}; uv λ max (MeOH) 240 nm (log ε 4.21); ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; ms $[MH]^+$ 615.2960 (65%) ($C_{34}H_{47}O_8S$ requires 615.2991).

BIOASSAYS.—Solutions containing material for bioassay were evaporated onto cellulose powder (5% of final diet wt) at varying concentrations. The powder was mixed thoroughly and incorporated into modified Berger-diet premix (13). The test diets were divided into 10 portions and placed in individual plastic containers, and newly hatched larvae of *H. zea* were added. The insects were maintained at 26° for 10 days, and their wts were determined and compared with those of control larvae grown on diets containing as additive only cellulose powder.

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