X-RAY CRYSTAL STRUCTURE OF PETUNIASTERONE O, A NOVEL ERGOSTANOID FROM *PETUNIA PARODII*

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ABSTRACT.—Petuniasterone O [**3**], which occurs in leaves of *Petunia parodii* at a level of about 100 ppm, was shown to be the 22,24,25-orthoacetate of (22R,24R)-2α-acetoxy-6α,7α-epoxy-5α,22,24,25-tetrahydroxyergostan-1-one by X-ray crystallographic analysis.

We have shown that the resistance of *Petunia* (Solanaceae) species toward feeding by polyphagous larvae of the noctuid *Heliothis zea* (Boddie) is related to the presence of a diverse family of steroidal ketones, which we have termed petuniasterones (1–5). Petuniasterone D [**1**] is a typical example, showing the characteristic side-chain-bridged orthoester as well as the ring-A dienone which is present in most instances. Also significant in conferring resistance to insect attack are certain related lactones (6) such as petuniolide C [**2**]. The petuniolides are probably derived from the petuniasterones by rearrangement of ring A, and their biological activity against *H. zea* is substantially greater than that of the petuniasterones. We now wish to report the occurrence of a new petuniasterone whose structure is similar to that of the petuniolides and which may be an intermediate in the biogenesis of the latter substances. Petuniasterone O [**3**] was isolated from *Petunia parodii* Steere as a crystalline

solid, and its structure was unequivocally determined by X-ray crystallographic analysis. Figure 1 presents a perspective view of its molecular conformation, and the final atomic coordinates and their estimated standard deviations (in parentheses) are listed in Table 1. Figure 2 gives a stereoscopic view of this structure. Carbons 10, 13, 17, 22, and 24 possess *R* configurations and carbons 2, 5, 6, 7, 8, 9, 14, and 20 have *S* con-
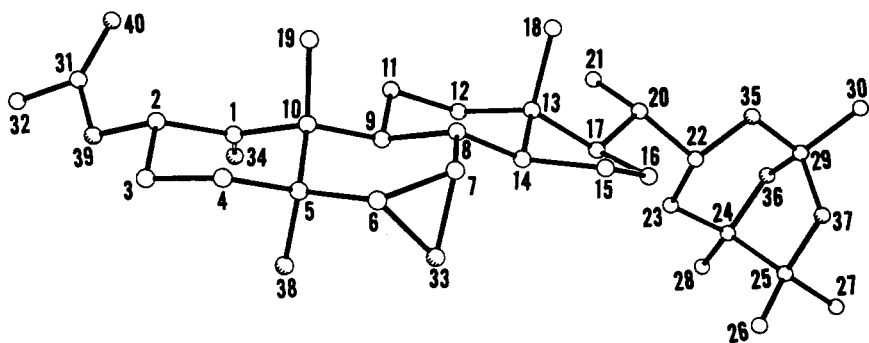

2

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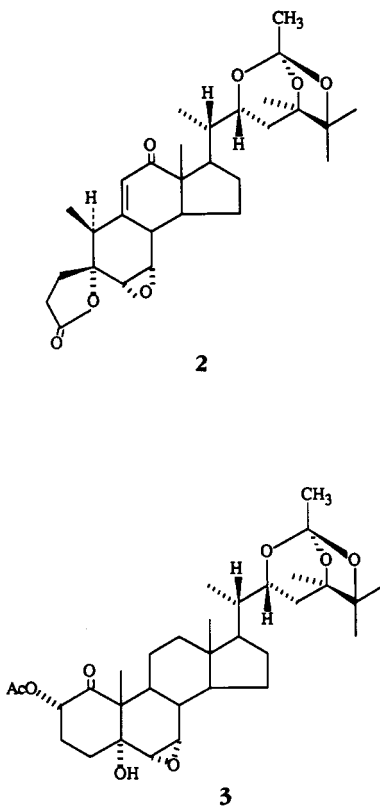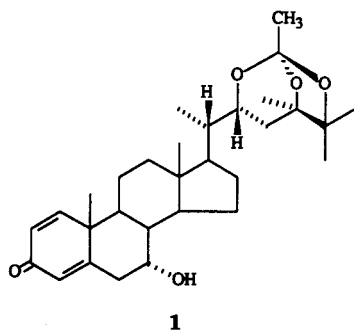
FIGURE 1.   Perspective view of **3** with the crystallographic numbering system. Open bonds represent double bonds, and shaded circles represent oxygen atoms.

TABLE 1.   Atom Coordinates ($\times 10^4$) and Temperature Factors ($\text{Å}^2 \times 10^3$).

Atom	x	y	z	U[a]
C-1	1760 (4)	10049 (2)	10798 (1)	38 (1)
C-2	1168 (5)	10093 (2)	11202 (1)	43 (1)
C-3	32 (5)	11134 (2)	11291 (1)	52 (1)
C-4	− 1672 (5)	11340 (2)	11012 (1)	46 (1)
C-5	− 895 (4)	11270 (2)	10617 (1)	35 (1)
C-6	− 2531 (4)	11595 (2)	10341 (1)	36 (1)
C-7	− 2626 (4)	11098 (2)	9972 (1)	35 (1)
C-8	− 1190 (4)	10253 (2)	9858 (1)	32 (1)
C-9	643 (4)	10105 (2)	10117 (1)	33 (1)
C-10	− 9 (4)	10138 (2)	10527 (1)	32 (1)
C-11	1820 (4)	9098 (2)	9992 (1)	41 (1)
C-12	2492 (4)	9169 (2)	9589 (1)	39 (1)
C-13	725 (4)	9393 (2)	9320 (1)	30 (1)
C-14	− 389 (4)	10385 (2)	9467 (1)	34 (1)
C-15	− 1836 (5)	10679 (3)	9151 (1)	48 (1)
C-16	− 551 (5)	10476 (3)	8810 (1)	49 (1)
C-17	1282 (4)	9785 (2)	8926 (1)	33 (1)
C-18	− 666 (5)	8413 (2)	9302 (1)	45 (1)
C-19	− 1576 (4)	9254 (2)	10604 (1)	37 (1)
C-20	1849 (4)	8964 (2)	8624 (1)	33 (1)
C-21	3498 (5)	8166 (2)	8741 (1)	48 (1)
C-22	2375 (4)	9525 (2)	8265 (1)	33 (1)
C-23	4591 (4)	9874 (2)	8221 (1)	36 (1)
C-24	5178 (4)	10133 (2)	7825 (1)	35 (1)
C-25	3785 (4)	10957 (2)	7633 (1)	35 (1)
C-26	3216 (5)	11911 (2)	7866 (1)	52 (1)
C-27	4525 (5)	11297 (3)	7253 (1)	50 (1)
C-28	7404 (4)	10332 (3)	7784 (1)	49 (1)
C-29	2520 (4)	9208 (2)	7618 (1)	34 (1)
C-30	1645 (4)	8531 (2)	7321 (1)	49 (1)
C-31	3751 (5)	9075 (3)	11486 (1)	49 (1)
C-32	5566 (5)	9136 (3)	11725 (1)	67 (1)
O-33	− 1806 (3)	12166 (1)	10020 (1)	42 (1)
O-34	3500 (3)	10019 (2)	10706 (1)	59 (1)
O-35	1865 (3)	8832 (1)	7963 (1)	34 (1)
O-36	4650 (3)	9200 (2)	7610 (1)	37 (1)
O-37	1982 (3)	10300 (1)	7574 (1)	36 (1)
O-38	713 (3)	12038 (2)	10598 (1)	47 (1)
O-39	2923 (3)	10048 (2)	11435 (1)	50 (1)
O-40	3014 (4)	8287 (2)	11358 (1)	72 (1)

[a]Equivalent isotropic U defined as one third of the trace of the orthogonalised U_{ij} tensor.
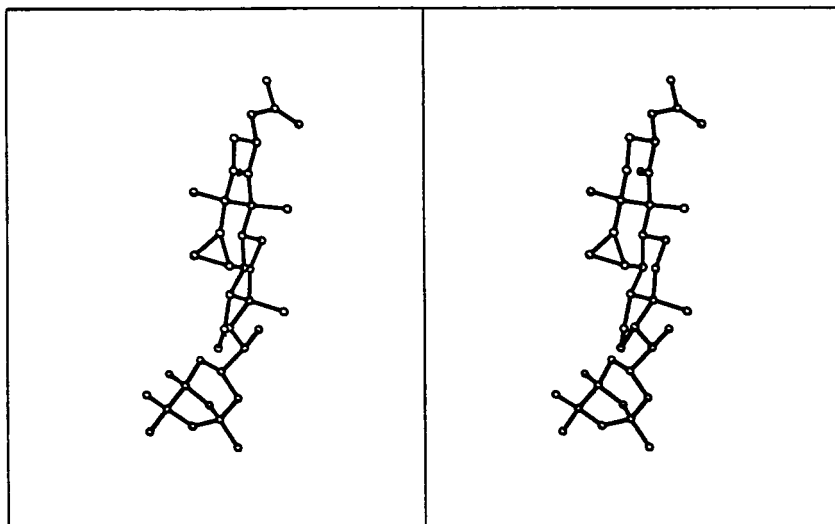
FIGURE 2.   Stereoscopic view of **3**.

figurations. The ¹H-nmr and ¹³C-nmr spectra of **3** (Table 2) are consistent with this structure. Chemical shift assignments, based upon the positions and multiplicities of the observed signals compared with spectra of previously described petuniasterones (1–4) and with the spectra of certain nicandrenones and withanolides (7,8), were facilitated by

¹H-¹H and ¹³C-¹H correlation spectroscopy (9) and by decoupling. The signals assigned to the side chain and its bridged orthoacetate feature are in good agreement with nmr spectra of typical petuniasterones. The chemical shifts of signals associated with the oxygenated positions of the steroid nucleus also are in accord with signal positions in similar

TABLE 2.   ¹H- and ¹³C-nmr Data for **3**.[a]

Position	δ_H	δ_C	Position	δ_H	δ_C
1	—	204.7, C	18	0.72 s	12.2, Me
2	5.66 br t (ca. 9)	72.6, CH	19	1.25 s	15.2, Me
3	2.25 m	26.6, CH₂	20	1.78 m	38.6, CH
4	1.95 and 2.25 m	30.8, CH₂	21	0.96 d (7)	12.5, Me
5	—	74.0, C	22	4.20 dt (11,4.5)	69.8, CH
6	3.06 d (4)	56.4, CH	23	1.55 m	30.2, CH₂
7	3.27 dd (4,2)	57.0, CH	24	—	82.4[c], C
8	1.75 m	34.9, CH	25	—	81.3[c], C
9	1.58 m	35.7, CH	26	1.31 s	19.9, Me
10	—	53.7, C	27	1.17[d] s	20.4[d], Me
11	1.15 and 2.24 m	21.6, CH₂	28	1.20[d] s	25.2[d], Me
12	1.40 and 1.95 m	39.6, CH₂			
13	—	43.7, C		Other signals	
14	51.2[b], CH		orthoacetate: δ_H 1.56 s and δ_C 23.5, Me;		
15	23.4, CH₂		δ_C 117.3, C		
16	1.55 and 1.84 m	27.3, CH₂	acetate: δ_H 2.15 s and δ_C 20.7, Me; δ_C 170.2, CO		
17	51.9[b], CH				

[a]In ppm from internal TMS for CDCl₃ solutions; coupling constants (Hz) in parentheses. Assignments are by decoupling and correlation techniques.
[b-d]Values with like superscripts in each column may be interchanged.

compounds having 5α-hydroxy-6α,7α-epoxy substitution (7,8). The effect of the carbonyl group at position 1 accounts for the chemical shift (δ_H 1.25) of Me-19 (10). The chemical shift of H-2 (δ_H 5.66) is somewhat lower field than is typical (5.05 ppm) for compounds bearing an acetoxy group at a position flanked by a ketone moiety in simple six-membered ring systems (11). By comparison, 2α-acetoxy-5α-cholestan-3-one shows a chemical shift for H-2 of 5.30 ppm, and 2α-acetoxy-5α-hydroxycholestan-3-one exhibited essentially the same value (δ_H 5.33) for this proton (12).

The particular substitution pattern of **3** suggests that it or a more fully functionalized derivative of **3** may lie intermediate between the more typical petuniasterones such as **1** and the petuniolides (e.g., **2**). We also note considerable similarity between the structure of **3** and the structures of many of the withanolides.

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 a Hewlett-Packard 8451 spectrophotometer. ^{1}H-nmr and ^{13}C-nmr spectra were obtained on a Nicolet NT-200 spectrophotometer at 200 and 50 MHz, respectively. Chemical ionization mass spectra were obtained using a VG Micromass 70/70 HS instrument at an ion source temperature of 160°. Accurate mass measurement was relative to a perfluoroalkane internal standard (13). Hplc solvents were pumped with an Altex-Beckman Model 110 pump with preparative head. Detection was by uv using a Beckman Model 165 variable wavelength detector.

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 between December 1987 and October 1988, freeze-dried, and stored for later use.

ISOLATION OF PETUNIASTERONE O [3].—After extraction and preliminary chromatography

as previously described (1–5), 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 550 to 900 ml was then rechromatographed on a Rainin Dynamax 21.4 × 250 mm cyano column with 20% iPrOH in hexane to give along with other, strongly uv-absorbing compounds, a zone of minimal uv absorption (220–250 ml) which deposited crystals of **3**. The crystals were recrystallized from MeOH to give material of mp 248–250°. Compound **3** (60 mg) was obtained from 600 g of dry leaf.

Petuniasterone O [3].—$[\alpha]^{26}$ (λ nm) +50° (589), +51° (578), +57° (546), +102° (436), +161° (365); ir ν max (CHCl₃) 3525 br (OH), 1725 (acetate and nonconjugated CO) cm^{-1}; uv λ max (MeOH) 206 nm (log ε 3.32); ^{1}H and ^{13}C nmr see Table 1; ms [MH]$^+$ 561.3363 (67%), $C_{32}H_{49}O_8$ requires 561.3427.

CRYSTAL STRUCTURE OF COMPOUND **3**[1].—Petuniasterone O, $C_{32}H_{48}O_8$, M = 560.8, orthorhombic, space group P2₁2₁2₁, a = 6.626(1), b = 12.554(2), c = 366.197(6) Å, β = 90.0°, U = 3010.9 Å³, D_c = 1.24 g·cm^{-3}, Z = 4, F(000) = 1216, μ(CuKα) = 6.73 cm^{-1}. Final R = 0.043 (362 parameters), R_w = 0.051 for 3030 unique reflections with |F$_o$|≥3σ|F$_o$| in the range 3° ≤2θ≤114°, average parameter shift is ±0.1σ, and difference Fourier synthesis excursions are within ±0.4 e Å^{-3}.

DATA COLLECTION AND STRUCTURE REFINEMENT.—Intensity data were collected on a Nicolet R3 diffractometer with graphite monochromatized CuKα radiation (λ = 1.5418 Å) by the θ–2θ scan technique with variable scan speed (4–30°) at room temperature. The intensity data were corrected for background, Lorentz-polarization effects, and secondary extinction (14), but not for absorption. The crystal structure was solved by direct methods. Atomic coordinates, thermal parameters, and scale factors were refined by a "blocked-cascade" full-matrix least-squares procedure with a SHELXTL (15) program package. The function minimized was $\Sigma w(|F_o| - |F_c|)^2$, with $w = [\sigma^2|F_o| + 0.001|F_o|^2]^{-1}$. Scattering factors were from "International Tables for X-ray Crystallography" (16); 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

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[1]Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.

in subsequent difference Fourier maps and included at invariant idealized values in the respective structure-factor calculation. The absolute configuration was determined by Hamilton's statistical method (17) of comparing the R values for the two enantiomeric structures.

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