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# PETUNIASTERONE N, AN UNUSUAL ERGOSTANOID FROM *PETUNIA* SPECIES

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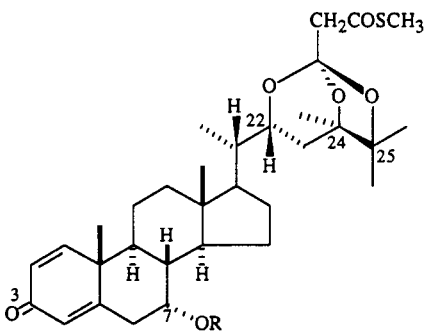
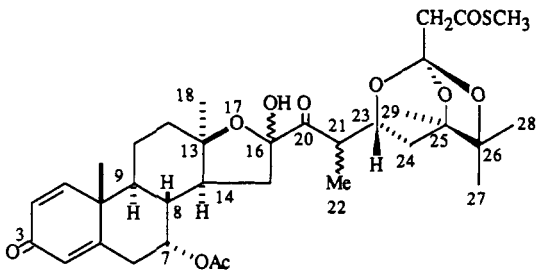
ABSTRACT.—The novel steroid, petuniasterone N [**3**], was isolated from leaves of *Petunia hybrida*, *Petunia parodii*, and *Petunia integrifolia*. Petuniasterone N, which is present as a mixture of epimeric forms, has 17-oxa substitution in the D ring with side chain attachment at C-16. Compound **3** reduces the larval growth of *Heliothis zea* to 50% of control size at a dietary concentration of about 120 ppm.

The resistance of *Petunia* toward leaf-feeding by larvae of the lepidopteran insect *Heliothis zea* has been shown by us to be determined by a series of closely related steroids which we have termed petuniasterones (1–3). The petuniasterones, which have the basic ergostane carbon skeleton, all possess a keto group at position 3 and an α-hydroxy or α-acetoxy at position 7. Typical of these is petuniasterone A [**1**], which has a bicyclic orthoester system as part of the side chain. Functionalities such as epoxy, hydroxy, and acetoxy groups may be found on the side chain or on the unmodified steroid nucleus in numerous other examples. However, we have observed that insect-growth-inhibiting activity is only found in those compounds that have an orthoester side chain. It does not appear that the unusual thiolester substituent of **1** is necessary for this activity to occur inasmuch as simple orthoacetate derivatives are equally inhibitory toward *H. zea* in our bioassays (4). We now wish to report the occurrence of a D-ring-rearranged analogue of **1** which we have named petuniasterone N [**3**]. Compound **3** possesses the same (methylthio)carbonyl orthoacetate as does **1**, but the relative configuration at C-13 is inverted, and attachment of the side chain to the now oxygenated ring D is at position 16. Insect growth inhibition studies using artificial diets (5) showed that **3** has about the same activity as **1**. The dose of **3** required to reduce larval growth to 50% of control weights was in the range of 120 ppm in prepared diet.

Compound **3** was obtained as a mixture of two epimers which could be enriched in the major component by hplc. At room temperature, solutions of **3** reverted to the original epimeric proportions (ca. 80:20) upon overnight standing. Spectral data are presented for the major component of the mixture. The molecular formula of **3** is $C_{34}H_{46}O_{10}S$, indicating twelve degrees of unsaturation. The $^1H$- and $^{13}C$-nmr spectra




**1** R=H
**2** R=Ac



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(Tables 1 and 2) are consistent with the proposed structure and clearly show the presence of a 1,4-dien-3-one system (cf. nmr data of **2**) as well as acetoxy, methyl(thiocarbonyl), and nonconjugated carbonyl groups. The uv spectrum of **3** is in agreement with the ring-A dienone (241 nm) as is the ir (1660 $cm^{-1}$), which also showed bands at 1680 (COS), and 1725 $cm^{-1}$ (ester and nonconjugated CO). The position of the 7α-acetoxy group in **3** was established by examination of the coupling constants of H-8 ($\delta_H$ 2.46) in the $^1H$ nmr. Small coupling (ca. 2 Hz) was observed for $J_{7,8}$, and two large couplings were found for $J_{8,9}$ and $J_{8,14}$ (ca. 11 and 12 Hz). This result is indicative of axial proton

TABLE 1. $^1H$-nmr Data for Compounds **2** and **3**.[a]

| Proton | Compound | |
|---|---|---|
| | **2**[b] | **3** |
| H-1 . . . | 7.02, d(10) | 7.16, d(10) |
| H-2 . . . | 6.22, dd(10,2) | 6.28, dd(10,2) |
| H-4 . . . | 6.01, br d(2) | 6.03, br d(2) |
| $H_2$-6 . . . | 2.60, d; 2.66, d(14) | 2.68, d(3) |
| H-7 . . . | 5.05, br q(3) | 5.19, br q(3) |
| H-8 . . . | — | 2.46, ddd(12,11,2) |
| H-9 . . . | — | ca. 1.52 |
| $H_2$-11 . . . | — | ca. 1.80 |
| $H_2$-12 . . . | (12β) 2.04, m | (12α) 2.12, br dd(14,10) |
| H-14 . . . | — | 1.72, dd(11,7) |
| $H_2$-15 . . . | — | 2.90, dd(14,7); 1.92, d(14) |
| $H_3$-18 . . . | 0.76, s | 1.04, s |
| $H_3$-19 . . . | 1.25, s | 1.26, s |
| H-21 . . . | — | 3.36, dq(11,7) |
| $H_3$-21 . . . | 0.95, d(7) | — |
| H-22 . . . | 4.20, dt(11,4) | — |
| $H_3$-22 . . . | — | 1.03, d(7) |
| H-23 . . . | — | 4.18, td(11,4) |
| $H_2$-24 . . . | — | 1.55, dd(14,11); 1.88, dd(14,4) |
| $H_3$-26 . . . | 1.31, s | — |
| $H_3$-27 . . . | 1.12[c], s | 1.34, s |
| $H_3$-28 . . . | 1.20[c], s | 1.14[c], s |
| $H_3$-29 . . . | — | 1.23[c], s |
| COSMe . . . | 2.31, s | 2.31, s |
| $CH_2CO$ . . | 3.04, d; 3.10, d(14) | 2.98, d; 3.01, d(14) |
| OAc . . . . | 2.00, s | 2.01, s |

[a]Values in $CDCl_3$; coupling constants (Hz) in parentheses.
[b]Data for compound **2** are taken from Elliger *et al.* (2).
[c]Values may be interchanged within each column.

TABLE 2. $^{13}C$ nmr Data for **2** and **3**.[a]

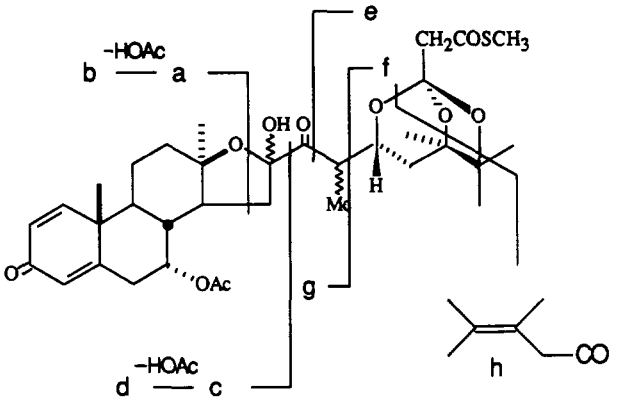
| Carbons | Steroid Nucleus | | Carbons | Side Chain | |
|---|---|---|---|---|---|
| | Compound | | | Compound | |
| | **2**[b] | **3**[c] | | **2**[b] | **3**[c] |
| C-1 | 155.2, CH | 154.8, CH | C-20 | 38.4[d], CH | 207.8, C |
| C-2 | 127.7, CH | 127.7, CH | C-21 | 12.5, Me | 45.1, CH |
| C-3 | 185.7, C | 185.7, C | C-22 | 70.1, CH | 13.2, Me |
| C-4 | 126.6, CH | 126.7, CH | C-23 | 30.3, $CH_2$ | 72.0, CH |
| C-5 | 163.9, C | 163.8, C | C-24 | 82.8[f], C | 35.8, $CH_2$ |
| C-6 | 37.3, $CH_2$ | 37.3, $CH_2$ | C-25 | 81.7[f], C | 83.4[e], C |
| C-7 | 72.0, CH | 71.3, CH | C-26 | 19.9, Me | 82.4[e], C |
| C-8 | 38.3[d], CH | 39.9, CH | C-27 | 20.4[g], Me | 19.7, Me |
| C-9 | 45.1, CH | 43.4[d], CH | C-28 | 24.9[g], Me | 20.1[f], Me |
| C-10 | 43.2[e], C | 42.9, C | C-29 | — | 24.7[f], Me |
| C-11 | 22.4, $CH_2$ | 21.3, $CH_2$ | Orthoester | 115.3, C | 115.0, C |
| C-12 | 38.8, $CH_2$ | 35.3, $CH_2$ | $CH_2COS$ | 50.2, $CH_2$ | 49.9, $CH_2$ |
| C-13 | 43.0[e], C | 84.4, C | COSMe | 193.2, C | 193.5, C |
| C-14 | 49.6, CH | 42.4[d], CH | SMe | 12.0, Me | 12.1, Me |
| C-15 | 23.8, $CH_2$ | 37.2, $CH_2$ | | | |
| C-16 | 27.0, $CH_2$ | 104.6, C | | | |
| C-17 | 51.9, CH | — | | | |
| C-18 | 11.7, Me | 28.5, Me | | | |
| C-19 | 18.4, Me | 18.7, Me | | | |
| Acetate | 21.0, Me | 21.1, Me | | | |
| Acetate | 170.2, C | 170.5, C | | | |

[a]In ppm from internal TMS for $CDCl_3$ solutions.
[b]Data for compound **2** are taken from Elliger *et al.* (2).
[c]Numbering of compound **3** is according to structure shown.
[d-g]Values with like superscripts may be interchanged for each compound.

substitution at positions 8, 9, and 14 and for an equatorial proton at position 7 (7α-OAc).

The cims ($NH_3$) showed intense peaks for fragment ions at *m/z* 345 and 285 (Table 3 and Scheme 1). Loss of the entire side chain and retention of the two oxygens associated with ring D give rise to these fragments and provide evidence that a 17-oxa D ring is present in **3**. Because C-15 already bears two protons, further substitution is only possible at C-16. The chemical shift of C-16, $\delta_C$ 104.6, is in agreement with a hemiketal carbon (7) at this position. The essential features of the rearranged steroid nucleus are thus accounted for.

The presence of a bicyclic orthoester system on the side chain is clearly indicated by comparison of the nmr spectra of **2** and **3**. In each case, a characteristic $^{13}C$-nmr signal at $\delta_c$ ca. 115 ppm is present (6), and signals for the appropriate mono-oxygenated carbons also appear. Methyl resonances in the $^1H$-nmr spectrum associated with the orthoesters of **2** and **3** correspond closely as well. Vicinal proton couplings between H-23 and H-21 (11 Hz) as well as between H-21 and $H_3$-22 (7 Hz) in compound **3** show that this portion of the side chain is unaltered. The absence of further coupling at C-21 indicates that this chain is attached to a nonprotonated carbon; therefore, the remaining carbonyl group (C-20) is placed between C-21 and C-16. This point of attachment also is indicated by the absence of coupling between $H_2$-15 and any proton other than H-14. Single frequency irradiation of the H-21 signal at $\delta_H$ 3.36 ppm with just sufficient power to decouple the carbon-proton long range couplings sharpened the C-20 signal at




SCHEME 1. Major fragment ions of compound **3**.

$\delta_C$ 207.8 ppm by 1.2 Hz compared to the fully coupled signal. This provides further evidence that H-21 is on a carbon adjacent to C-20.

The stereochemistry of the C/D ring junction of **3** is inferred from the chemical shift of H-8 ($\delta_H$ 2.46). The H-8 signal in **1** is not directly observable; however, stepwise decoupling with examination of the H-7 signal (at $\delta_H$ 4.04 in **1**) showed that H-8 appears at ca. 1.62 in this compound. In compound **3**, cis geometry of the C/D ring junction with the position-17 oxygen in close proximity to H-8 would be expected to shift the signal of that proton to lower field (8).

The stereochemistry at C-16 and C-21 is allowed to remain unassigned. It may be noted that either enolization of the C-20 keto group or opening and reclosure of the C-16 hemiketal group would account for the ready formation of the equilibrium population of epimers.

TABLE 3. Cims ($NH_3$) Data for Compound **3**.

| Ion | *m/z* | Formula | Abundance (%) |
|---|---|---|---|
| a | 287.1641 | $C_{18}H_{23}O_3$ | 47 |
| b | 227 | $C_{16}H_{19}O$ | 24 |
| c | 345.1695 | $C_{20}H_{25}O_5$ | 89 |
| c + $NH_3$ | 362.1949 | $C_{20}H_{28}NO_5$ | 15 |
| d | 285.1499 | $C_{18}H_{21}O_3$ | 100 |
| d + $NH_3$ | 302.1765 | $C_{18}H_{24}NO_3$ | 21 |
| e | 275.1294 | $C_{13}H_{23}O_4S$ | 18 |
| f | 245.0869 | $C_{11}H_{17}O_4S$ | 14 |
| g | 403.2158 | $C_{23}H_{31}O_6$ | 11 |
| h | 111.0811 | $C_7H_{11}O$ | 48 |
| M + H | 647.2870 | $C_{34}H_{47}O_{10}S$ | 4 |
| M + $NH_4$ | 664.3075 | $C_{34}H_{50}NO_{10}S$ | 1 |
| M + H − $H_2O$ | 629.2733 | $C_{34}H_{45}O_9S$ | 22 |
| M + $NH_4$ − $H_2O$ | 646.3023 | $C_{34}H_{48}NO_9S$ | 1 |
| M + H − HOAc | 587 | $C_{32}H_{43}O_8S$ | 2 |
| M + H − HOAC − $H_2O$ | 569.2598 | $C_{32}H_{41}O_7S$ | 9 |

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Optical rotations were obtained on a Perkin-Elmer 241 automatic polarimeter at ca. 26° in $CHCl_3$ solutions. Ir spectra were recorded on a Perkin-Elmer 237 spectrometer in $CHCl_3$, and uv spectra were taken on a Cary 219 spectrophotometer using MeOH solutions. $^1$H-nmr spectra were obtained at 200 MHz on a Nicolet NT-200 spectrophotometer, and $^{13}$C-nmr spectra

were taken at 50 MHz on the same instrument. Nmr assignments were facilitated by proton-proton decoupling and by use of 2D proton-proton and carbon-proton correlation techniques. Mass spectra were run by chemical ionization using a VG Micromass 70/70 HS instrument at an ion source temperature of 160°. Accurate masses were measured relative to a perfluoroalkane internal standard (9). Hplc columns were commercially obtained; all solvents were hplc grade. Solvents were pumped with an Altex-Beckman Model 110 pump with preparative head, and detection was by uv at 254 with a Model 150 monitor using a 0.5 mm preparative cell.

PLANT MATERIAL.—*Petunia parodii* Steere (Solanaceae) seeds were obtained from the National Seed Storage Laboratory, Colorado State University, Fort Collins, Colorado. *Petunia integrifolia* Hook. seeds were from the Northeastern Plant Introduction Station, U.S. Department of Agriculture, Geneva, New York. Leaf material and seed of the latter plant were also supplied by Ing. Agr. Hugo A. Cordo, Biological Control of Weeds Laboratory, Hurlingham, Buenos Aires Prov., Argentina. *Petunia hybrida* Vilm. was commercial variety, "Royal Cascade," whose seeds were obtained from A.H. Hummert Co., St. Louis, Missouri. Plants were grown in the greenhouse and outdoor beds in Albany, California. Leaf material was harvested at intervals between December 1987 and June 1988.

ISOLATION OF PETUNIASTERONE N [**3**].—Freeze-dried leaf material was ground in $CHCl_3$ with a high speed homogenizer (Tekmar SD-45) three times in succession using 10 ml of solvent per gram of plant weight followed by vacuum filtration. The solvent was evaporated on the rotary evaporator, and the resulting oil was suspended in boiling MeCN (10–15 ml per gram of extract) and stirred 1 h. The mixture was cooled to 5°, and the solution was decanted from waxy, solid material. About 50% of the original extract remained in solution. This supernatant was evaporated and redissolved in four volumes of MeCN for application onto a 50 × 250 mm column of Waters RP-18 preparative packing. Elution with MeCN gave a zone (375–900 ml) which contained all petuniasterones and was free of most nonpolar lipids and chlorophyll (ca. 4% of original plant wt). Further fractionation was by preparative hplc (Rainin Dynamax C-18, 21.4 × 250 mm, 30% $H_2O$ in MeCN, elution volume 125–150 ml; Alltech R Sil C-18, 10 × 250 mm, 30% $H_2O$ in MeCN, elution volume 25–40 ml; and Whatman PAC, 9 × 500 mm, 10% iPrOH in hexane, elution volume 105–125 ml) to give compound **3** as a mixture of epimers that could not be purified by crystallization, but that could be enriched in the major component by further hplc on a Whatman 9 × 500 mm 10 μ silica column (20% iPrOH in hexane, elution volume 60–65 ml). Upon standing 24 h in $CHCl_3$ solution, compound **3** reverted to approximately the original epimeric ratio. The approximate content of **3** in plant material (dry basis) was: *P. integrifolia*, 1500 ppm; *P. parodii*, 700 pm; and *P. hybrida*, 200 ppm.

PETUNIASTERONE N (EPIMERS).—[α] (λ nm) (589) −70°, (578) −75°, (546) −86°, (436) −180°, (365) −447°; ir ν max ($CHCl_3$) 3450 br (OH), 1725 (acetate and nonconjugated CO), 1680 (COS), 1660 (conjugated CO), 1625(conjugated olefin) $cm^{-1}$; uv λ max (MeOH) 241 nm (log ε 4.23); $^1H$ nmr see Table 1; $^{13}C$ nmr see Table 2; ms (Table 3) *m/z* $[MH]^+$ 647.2870 (4%); $C_{34}H_{47}O_{10}S$ requires $[MH]^+$ 647.2889.

## ACKNOWLEDGMENTS

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