

Petunianines, Unusual Steroidal Nitrogenous Bases from *Petunia inflata*

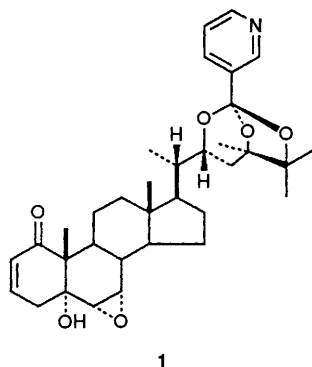
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The structures and stereochemistry of two ergostanoids from *Petunia inflata*, bearing pyridine rings on the side chain, have been established by spectroscopy and X-ray crystallographic analysis.

Numerous novel steroids having insect-inhibitory activity have been isolated from various *Petunia* species.¹ These substances, termed petuniasterones and petuniolides, are formally related to ergostane and typically bear a highly elaborated side chain, that in biologically active examples possesses an ortho ester functionality attached at positions -22, -24 and -25. Previously described ortho esters are derived from acetic, propionic, and (methylthio)carbonylacetic acid.¹ We report here upon ortho esters of *Petunia inflata* Fries which represent the first examples derived from nicotinic acid. These have a pyridine nucleus appended to the ortho ester system, and we have named them petunianines to emphasize their basic character and to distinguish them from non-nitrogenous steroidal compounds previously isolated from *Petunia*.

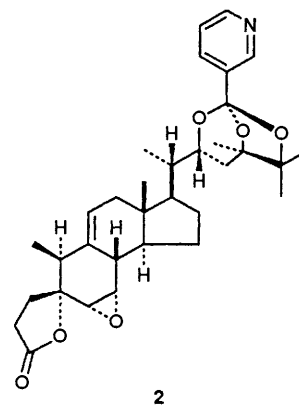
Petunianine A **1** was isolated from CHCl₃ extracts of *P. inflata* leaves (content *ca.* 50 ppm) by successive chromatography on reverse phase and normal phase column media. Its IR spectrum showed absorptions at 3530 (OH) and 1680 cm⁻¹ (conj. CO) as well as below 1600 cm⁻¹ suggestive of further unsaturation. The UV spectrum of **1** was consistent with the



presence of a conjugated ketone (228 nm), and also showed absorption at 258 nm. The ¹H NMR spectrum (200 MHz, CDCl₃) displayed a series of signals [δ 8.91 dd (2,1), 8.60 dd (5,2), 7.95 dt (8,2) and 7.28 ddd (8,5,1) (*J*†)] indicative of a 3-substituted pyridine,² which was in accord with the 258 nm UV absorption. Other ¹H NMR signals were associated with six methyl groups [δ 0.77, 1.18, 1.30(2), 1.43, singlets; 1.06 d (*J* 7)]. Resonances arising from the unsaturated ketone system were observed at δ 6.60 ddd (*J* 10, 5, 2.2, 3-H), 5.86 dd (*J* 10, 2.5, 2-H) and 2.60 multiplet (2 allylic H, 4-H₂). A lowfield proton, δ 4.42 dt (*J* 10, 4, 22-H) was consistent with attachment to a carbon bearing an ether function, and signals at δ 3.32 dd (*J* 4, 2, 6-H) and 3.04 d (*J* 4, 7-H) were ascribed to oxirane protons. As a whole, the ¹H NMR spectrum was very similar to that of petuniasterone O,³ except for signals of ring A, which strongly resembled those of certain withanolides having 2-en-1-one substitution.⁴ The ¹³C NMR spectrum of **1** showed signals at δ_c

203.2 (conj. CO), 129.0 and 139.6 (conj. olefin), 115.9 (ortho ester trioxygenated C), 70.6, 82.5 and 83.4 (ortho ester), 56.3 and 57.3 (oxirane). Pyridine ring resonances occurred at δ_c 150.2, 133.6, 133.7, 122.7 and 147.9 for positions 2–6 respectively.⁵ The structure and stereochemistry of **1** were established unequivocally by X-ray crystallographic analysis; ‡ a perspective view of the structure is presented in Fig. 1.

Petunianine B **2** was isolated similarly (*ca.* 625 ppm). Its IR spectrum showed γ -lactone absorption at 1775 cm⁻¹, and its UV spectrum exhibited absorption associated only with a pyridine ring at λ_{\max} 260 nm. The ¹H NMR spectrum of **2** showed signals at precisely the same chemical shifts indicated above for the pyridine ring of **1**. A multiplet at δ 5.55 was assigned to a single proton on an isolated double bond. Six methyl resonances were



‡ Crystal Data for **1**. C₃₄H₄₅NO₆, *M* = 563.8, orthorhombic, space group *P*2₁2₁2₁, *a* = 6.863(1), *b* = 13.416(4), *c* = 32.624(10) Å, *U* = 3.003.7 Å³, *Z* = 4, *D_c* = 1.25 g cm⁻³, *F*(000) = 1216, μ (Cu-K α) = 6.41 cm⁻¹; crystal size: 0.20 × 0.22 × 0.46 mm. Intensity data were collected in the range of $3^\circ \leq 2\theta \leq 114^\circ$ 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⁻¹) at room temperature. The intensity data were corrected for background and Lorentz-polarization effects, but not for absorption. The crystal structures were solved by direct methods and refined by a 'blocked-cascade' full-matrix least-squares procedure with the SHELXTL program package. The function minimized was $[\sum \omega(|F_o| - |F_c|)^2]$, where $\omega = [\sigma^2|F_o| + 0.001|F_o|^2]^{-1}$. Scattering factors of oxygen and nitrogen were corrected for anomalous dispersion.⁷ A secondary extinction correction (0.0026) was included in the final cycles of refinement to minimize the discrepancy between observed and calculated structure factors of the most intense reflections, which led to a small improvement in *R*-index. Least-squares refinement of all atomic parameters and thermal parameters (anisotropic C, O, N and isotropic H with invariant idealized values) converged at *R* = 0.046 and *R_w* = 0.050 for 3019 unique reflections with $|F_o| \geq 3\sigma|F_o|$. The absolute configuration was determined by comparing the *R_w* values for the two enantiomeric structures; according to Hamilton's statistical criteria,⁸ the enantiomer with the lower *R_w* value has a probability of being correct to a significance level better than 0.5%. Atomic co-ordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre, see Notice to Authors (1991), *J. Chem. Soc., Perkin Trans. 1*, 1991, Issue 1.

† *J* Values in Hz throughout.

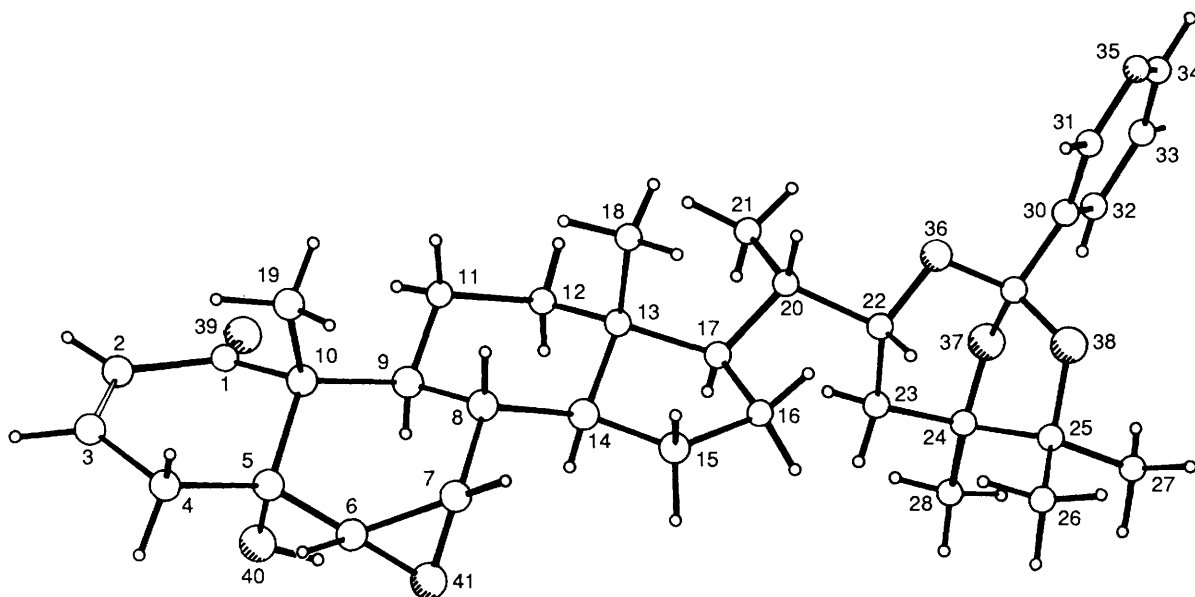


Fig. 1 Perspective view of compound 1 with crystallographic numbering scheme. Open bonds represent double bonds. Smaller shaded circle represents nitrogen, larger shaded circles represent oxygen, and smaller open circles hydrogen atoms.

observed: δ 0.71, 1.30 (2), 1.44 (singlets), 1.04 d (J 7), and 1.06 d (J 6.5). Signals of protons on oxygenated carbons were observed at δ 4.42 dt (J 11, 4.5, 22-H) and at 3.29 dd (J 4, 2), and 3.14 d (J 4) for an oxirane system. The ^{13}C NMR showed signals at δ_{C} 175.7 (lactone CO), 85.6 (lactone), 132.3 and 125.3 (isolated double bond), 115.8 (ortho ester), 70.6, 82.4 and 83.5 (ortho ester) and 54.6 and 54.7 (oxirane). Pyridine ring resonances occurred at δ_{C} 150.2, 133.5, 133.7, 122.9 and 147.8. With the exception of signals arising from positions adjacent to the pyridine substituent, both ^1H NMR and ^{13}C NMR spectra of **2** are superposable with those of petuniolide E,⁶ and we have assigned the structure and stereochemistry on this basis. The biological activity of **2** is similar to that of other petuniolides¹ with an ED_{50} of ca. 15 ppm for the lepidopteran insect, *Heliothis zea* Boddie.

We have previously shown that the ortho ester system in compounds of this type may arise from steroids esterified at position-22 that contain a 24, 25-oxirane system.¹ We observe that nicotinic acid esters of this type are present in extracts of *P. inflata* and are undoubtedly precursors of these pyridine derivatives.

Experimental

Petunianine A 1 and B 2.—Extraction of freeze-dried *P. inflata* leaf (125 g) with CHCl_3 gave an oil which was dissolved in MeCN, filtered from insoluble lipid, and purified by preliminary passage through preparative C-18 bonded media. HPLC was then carried out using C-18 reverse phase (70% aq. MeCN) and silica normal phase (25% propan-2-ol in hexane) columns. Petunianine A **1** (7 mg), m.p. 198–200 °C (heptane–EtOAc); $[\alpha]_{\text{D}} + 76^*$ (c 0.25, CHCl_3); $\nu_{\text{max}}(\text{CHCl}_3)/\text{cm}^{-1}$ 3530 and 1680; $\lambda_{\text{max}}(\text{MeOH})/\text{nm}$ 228 (log ϵ 3.85) and 258 (log ϵ 3.42); m/z

563.3287 (M^+ , 10%). $\text{C}_{34}\text{H}_{45}\text{NO}_6$ requires 563.3249. Petunianine B **2** (78 mg), m.p. 260–262 °C (isooctane–EtOAc); $[\alpha]_{\text{D}} + 30$ (c 1.00, CHCl_3); $\nu_{\text{max}}(\text{CHCl}_3)/\text{cm}^{-1}$ 1775; $\lambda_{\text{max}}(\text{MeOH})/\text{nm}$ 260 (log ϵ 3.41); m/z 549.3159 (M^+ , 6%). $\text{C}_{33}\text{H}_{43}\text{NO}_6$ requires 549.3093.

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* $[\alpha]_{\text{D}}$ Values recorded in 10^{-1} deg cm^2 g^{-1} .