Pergamon

0031-9422(94)00948-1

Phytochemistry, Vol. 39, No. 4, pp. 941–942, 1995

0031-9422/95 $9.50 + 0.00

ECDYSTEROIDS FROM *PENSTEMON VENUSTUS*

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(*Received* 28 *September* 1994)

Key Word Index—*Penstemon venustus*; Scrophulariaceae; ecdysteroids; venustone.

Abstract—For the first time ecdysteroids have been found in the genus *Penstemon*. In addition to the known ecdysteroids taxisterone, 20-hydroxyecdysone, makisterone A and C, the new ecdysteroid venustone has been isolated from the roots of *Penstemon venustus*. This ecdysteroid was shown to be 22-*O*-[(3*R*)-3-hydroxybutanoyl]-20-hydroxyecdysone. The structure has been determined primarily on the basis of NMR spectroscopy. The assignments of the NMR signals were performed by means of ^1H–^1H COSY – 45° and ^1H–^{13}C COSY experiments.

INTRODUCTION

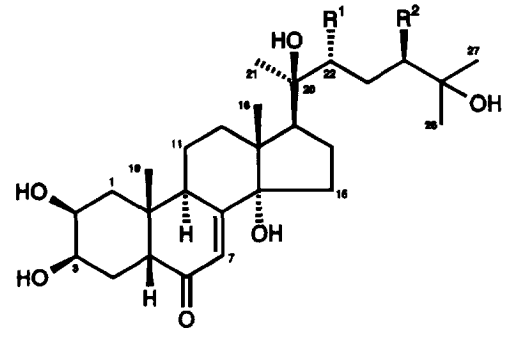
Penstemon venustus Dougl. (Lovely *Penstemon*) is a plant which grows wild on rocky slopes to the northwest of the U.S.A. Its distribution reaches from Idaho and Montana to Washington and Oregon [1]. To our knowledge *P. venustus* has not been phytochemically investigated previously. In the present paper we report on the isolation and the structure elucidation of the new ecdysteroid venustone (**5**) from *P. venustus*.

RESULTS AND DISCUSSION

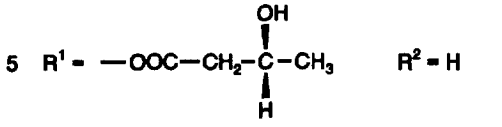
Fresh and minced roots of *P. venustus* were extracted with methanol. CC of the extract on silica gel followed by reversed-phase HPLC (RP-18) yielded the five ecdysteroids taxisterone (**1**) [2], 20-hydroxyecdysone (**2**) [3, 4], makisterone A (**3**) [5], makisterone C (**4**) [4, 5] and venustone (**5**).

The EI-mass spectrum of **5** showed the typical ions for ecdysteroids at *m/z* 363, 345 and 327 corresponding to fragmentation between C-20 and C-22 and loss of one and two molecules of water [6]. The ion at *m/z* 69, caused by cleavage of the ester bond and elimination of water, indicated the presence of an ester of hydroxybutanoic acid. The negative-ion FAB-mass spectrum of **5** exhibited the $[M-1]^-$ ion *m/z* 565, which together with ^1H and ^{13}C NMR data allowed us to propose the molecular formula $C_{31}H_{50}O_9$.

Methanolysis (1.0 M NaOMe) of **5** afforded methyl 3-hydroxybutanoate (**6**) and 20-hydroxyecdysone (**2**). The presence of both compounds was detected by HPLC analysis with authentic samples of **6** and **2**. The configuration at C-3 of **6** was determined by GC comparison with methyl (3*R*)- and methyl (3*S*)-3-hydroxybutanoate as their trimethylsilylated derivatives on a chiral Cyclodex-B phase. The trimethylsilylated **6** showed one peak at R_t 24.28 min in the gas chromatogram. Addition of methyl (3*R*)-3-trimethylsilyloxybutanoate also caused one peak at the same R_t, whereas addition of methyl (3*S*)-3-trimethylsilyloxybutanoate gave two peaks at R_t 24.28 and 24.72 min.



1 R^1 = H, R^2 = H
2 R^1 = OH, R^2 = H
3 R^1 = OH, R^2 = CH_3
4 R^1 = OH, R^3 = C_2H_5



5 R^1 = —OOC—CH_2—C(OH)(H)—CH_3, R^2 = H

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The esterification of the 22-hydroxy group of 20-hydroxyecdysone (**2**) with (3*R*)-3-hydroxybutanoic acid in venustone (**5**) caused the ^{13}C downfield shift of the C-22 signal ($\Delta\delta$ + 2.40). In the ^1H NMR spectrum of **2** the signal of H-22 was observed at δ 3.33, whereas the H-22 signal in **5** was shifted downfield to δ4.92. The stereochemical arrangements of the ecdysteroid protons were assigned according to those of 20-hydroxyecdysone (**2**) [4]. Besides the 27 ecdysteroid signals the ^{13}C NMR spectrum of **5** showed additional signals of the 3-hydroxybutanoic acid moiety at δ 173.5 (C-1'), 45.2 (C-2'), 65.6 (C-3') and 23.2 (C-4'). The ^1H NMR signals of the 3-hydroxybutanoic acid moiety in **5** were observed at δ 1.23 (3H, *d*, $J_{3',4'}$ = 6.2 Hz, 3H-4'), 4.23 (1H, *m*, H-3'), 2.43 (1H, $J_{2'a,2'b}$ = 15.1 Hz, $J_{2'a,3'}$ = 8.3 Hz, H-2'$_a$) and 2.57 (1H, $J_{2'a,2'b}$ = 15.1 Hz, $J_{2'b,3'}$ = 4.8 Hz, H-2'$_b$). Based on the above data the structure of venustone was as shown in formula **5**. It is notable, that we could not find any iridoid glucosides in the roots of *P. venustus*.

EXPERIMENTAL

General. NMR: 300 MHz (^1H) and 75.5 MHz (^{13}C), δ in ppm, solvent CD_3OD. The NMR spectra of **5** were recorded at 500 MHz (^1H) and 125 MHz (^{13}C). Negative-ion FAB- and EI-(70 eV) mass spectra: Finnigan MAT 8500. The matrix for the FAB-MS was glycerol. $[\alpha]_D$: MeOH; CC: silica gel 60 (0.063–0.2 mm); TLC: silica gel (0.25 mm, $CHCl_3$–MeOH (4:1)), the spots were visualized by UV light and spraying (1% soln of vanillin in 50% H_3PO_4); analyt. and prep. HPLC: LiChrosorb RP-18 pre-packed columns (Knauer, 250 × 4 mm, 5 μm; 250 × 8 mm, 5 μm; 250 × 16 mm, 5–20 μm). The system was equipped with a Knauer variable wavelength monitor; GC: fused silica capillary column (30 m × 0.25 mm) coated with chiral Cyclodex-B phase, the oven temp. was held at 40° for 10 min, then raised to 120° at 3° min^{-1}, trimethylsilylation with MSTFA (N-methyl-N-trimethylsilyltrifluoracetamide).

Isolation. The plants were cultivated from seeds (Botanical Garden of the University of Warsaw, Poland) in the garden of the Institute for Plant Biochemistry Halle/Saale Germany (a voucher specimen is deposited in the herbarium of the institute). The fresh and minced roots (3.5 kg) were extracted with MeOH (4 × 7 l). The extract was concd under red. pres. to give a 89.4 g crude extract. The residue was chromatographed on silica gel (1.8 kg) eluting with $CHCl_3$ and $CHCl_3$–MeOH (19:1 to 1:1) to give the ecdysteroid frs 19/20 (111 mg) and 22 (202 mg). The HPLC sepn of frs 19/20 on LiChrosorb RP-18 (H_2O–MeOH, gradient: 0–32 min: 10–93% MeOH, flow rate: 2.0 ml min^{-1}, detection: 250 nm) yielded **4** (4.9 mg, R_f 0.57). Fr. 22 afforded under the same conditions **1** (6.9 mg, R_f 0.56), **2** (88.8 mg, R_f 0.50), **3** (3.7 mg, R_f 0.53) and **5** (8.9 mg, R_f 0.58).

Venustone (**5**). $C_{31}H_{50}O_9$, M_r 566.75. $[\alpha]_D^{23}$ + 57° (*c* 0.2); FAB-MS (negative-ion mode) *m/z* (rel. int.): 565 $[M - H]^-$(7), 479 (12), 183 (100), 103 (20), 91 (43); EI-MS *m/z* (rel. int.): 530 $[M - 2H_2O]^+$ (0.5), 512 (0.9), 494 (1), 468 (2), 444 (2), 426 (11), 387 (6), 363 (32), 345 (100), 327 (40), 285 (18), 269 (16), 215 (16), 173 (19), 99 (51), 81 (28), 69 (40), 45 (56); ^1H NMR: δ 0.87 (*s*, 3H-18), 0.95 (*s*, 3H-19), 1.14 (H-23$_a$), 1.15 (*s*, 3H-27), 1.17 (*s*, 3H-26), 1.23 (*d*, $J_{3',4'}$ = 6.2 Hz, 3H-4'), 1.28 (*s*, 3H-21), 1.42 (H-1$_{ax}$, H-24$_b$), 1.47 (H-23$_b$), 1.61 (H-15$_b$), 1.69 (H-11$_{ax}$), 1.70 (2H-4), 1.75 (H-16$_b$), 1.77 (H-24$_a$), 1.79 (H-1$_{eq}$), 1.81 (H-11$_{eq}$), 1.86 (H-12$_{eq}$) 1.95 (H-15$_a$), 2.03 (H-16$_a$), 2.15 (*ddd*, $J_{12ax,12eq}$ = $J_{11ax,12ax}$ = 13.1 Hz, $J_{11eq,12ax}$ = 4.9 Hz, H-12$_{ax}$), 2.38 (H-5, H-17), 2.43 ($J_{2'a,2'b}$ = 15.1 Hz, $J_{2'a,3'}$ = 8.3 Hz, H-2'$_a$), 2.57 ($J_{2'a,2'b}$ = 15.1 Hz, $J_{2'b,3'}$ = 4.8 Hz, H-2'$_b$), 3.15 (H-9$_{ax}$), 3.83 (H-2$_{ax}$), 3.94 (H-3$_{eq}$), 4.23 (H-3'), 4.92 (H-22), 5.80 (*d*, $J_{7,9}$ = 2.0 Hz, H-7); ^{13}C NMR: δ 18.1 (C-18), 21.5 (C-11, C-16), 21.9 (C-21), 23.2 (C-4'), 24.4 (C-19), 26.1 (C-23), 28.9 (C-27), 29.5 (C-26), 31.8 (C-15), 32.6 (C-12), 32.8 (C-4), 35.1 (C-9), 37.4 (C-1), 39.3 (C-10), 41.5 (C-24), 45.2 (C-2'), 48.7 (C-13), 50.8 (C-17), 51.8 (C-5), 65.6 (C-3'), 68.5 (C-3), 68.7 (C-2), 71.0 (C-25), 77.6 (C-20), 80.8 (C-22), 85.2 (C-14), 122.2 (C-7), 167.8 (C-8), 173.5 (C-1'), 206.4 (C-6).

Methanolysis of **5**. Venustone (**5**, 1.0 mg) was dissolved in 0.5 ml of 1.0 M methanolic NaOMe soln and kept for 90 min at 22°. After addition of 1 ml of H_2O and neutralization with Dowex 50W × 4 (H^+-form) 1 ml of $CHCl_3$ was added. The organic phase was sepd, washed with 2 ml of H_2O and dried with Na_2SO_4. In the $CHCl_3$ phase **6** could be detected by HPLC and, after trimethylsilylation, by GC (R_t 24.28). In the aq. phase **2** was identified by HPLC.

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