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PROCHAMAZULENE SESQUITERPENE LACTONES FROM *STEVIA SERRATA*

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Key Word Index—*Stevia serrata*; Compositae; Eupatorieae; sesquiterpene lactones; guaianolides; prochamazulenes.

Abstract—The leaves of *Stevia serrata* afforded two new sesquiterpene lactones. Their structures and stereochemistry were established by spectral methods, mainly ^{1}H NMR.

INTRODUCTION

In the course of the column chromatographic separation of the components from *Stevia serrata* Cav. on silica gel, we noticed the presence of chamazulene in the non-polar fractions. This observation led to the isolation of two new prochamazulene sesquiterpene lactones, **1a** and **1b**, and the known lactones christinine I and III [1, 2]. To our knowledge, the only three known prochamazulenes are artabsin, matricin and 4-epimatricin from *Artemisia arborescens* [3] and *A. absinthium* [4].

RESULTS AND DISCUSSION

Steviserrolide A (**1a**), $C_{17}H_{22}O_5$, was an unstable colourless oil, which exhibited the typical IR absorption band of a γ-lactone at 1773 cm^{-1}. Further absorptions at 3696, 3577 and 1736 cm^{-1} indicated the presence of hydroxyl and ester functions, respectively.

The ^{1}H NMR spectrum (Table 1) of **1a** lacked the typical doublets of the exocyclic methylene conjugated with the γ-lactone, which must be saturated as indicated by a doublet at δ1.20 (J = 7.0 Hz). The two doublets at δ6.45 and 5.90 (J = 5.5 Hz) were assigned to the double bond at C-2 in the five-membered ring of the guaiane skeleton.

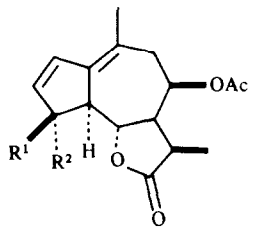
A sharp singlet at δ2.03 in the ^{1}H NMR spectrum, together with strong mass spectral peaks at m/z 43 and 246 [M − HOAc]$^+$ indicated the presence of an acetate group. Their position and stereochemistry could be deduced from the small coupling constants and the downfield chemical shift observed for H-8 (δ5.28), which were almost identical to those of 11,13-dihydroguaianolides with a β-oriented acetate group [1, 5] and markedly different to those of 11,13-dihydroguaianolides with an α-oriented ester group [3, 6]. Assuming the α-orientation of H-7, as in all the sesquiterpene lactones of higher plants, [7], the stereochemistry at C-5 and C-6 was assigned based on the coupling constants of the corresponding protons ($J_{5,6}$ = 10 Hz, $J_{6,7}$ = 10 Hz) confirming the *trans* ring attachment of the γ-lactone. The assignment of the configuration of the C-11 methyl group in **1a** as β pseudoaxial was suggested on the basis of the observed

Table 1. ^{1}H NMR spectral data for compounds **1a**, **1b** and **2** (80 MHz, $CDCl_3$ TMS as int. standard)

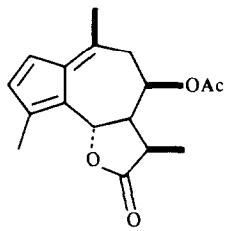
H	1a		1b	2
2	6.45 *d*	(6.08)	6.32 *d*	6.50 *d*
3	5.90 *d*	(5.78)	5.90 *d*	6.20 *d*
6	4.82 *t*	(4.50)	4.70 *t*	5.72 *br d*
8	5.28 *br d*	(4.90)	5.27 *m*	5.40 *t*
13	1.20 *d*	(0.90)	1.20 *d*	1.20 *d*
14	1.78 *br s*	(1.50)	1.78 *br s*	2.15 *br s*
15	1.57 *s*	(1.40)	1.48 *s*	2.15 *br s*
OAc	2.03 *s*	(1.55)	2.05 *s*	2.05 *s*

J (Hz) **1a**: 2,3 = 5.5; 5.6 = 6,7 = 10; 7.8 = 8,9 ~ 1.5; 8,9′ = 5.0; 11,13 = 7.0; **1b**: 8,9′ = 3.0; **2**: 2,3 = 6.0; 6,7 = 10; 7,8 = 0; 8,9 = 8,9′ = 4.0; 11,13 = 7.0.

Numbers in parentheses are chemical shifts in C_6D_6.



1a R¹ = OH, R² = Me
1b R¹ = Me, R² = OH



2

solvent shift [8], ($\Delta\delta$ $CDCl_3 - C_6D_6$) = 0.30. This value was identical to those of christinine I [1] and deacetoxymatricin [6], and larger than those published for matricin ($\Delta\delta$ = 0.20) and 4-epimatricin ($\Delta\delta$ = 0.18) [3], both with an α pseudo equatorial methyl group at C-11. The configuration at C-4 was established from the observed chemical shifts for H-6 and H-15 in similar C-4 hydroxy epimeric guaianolides [3, 9]. In agreement with the β-orientation

of the C-4 hydroxyl group in **1a**, the chemical shifts for H-6 and H-15 were shifted to lower field than those of **1b**.

Steviserrolide B (**1b**), $C_{17}H_{22}O_5$ is also a colourless oil. Comparison of the ^{1}H NMR spectra of **1b** and **1a** (Table 1), clearly showed differences for the proton signals at C-2, C-15 and C-6, indicating that both are epimers at C-4. Consequently, the structure for steviserrolide B is depicted as in **1b**.

Addition of trichloroacetyl isocyanate to the lactone **1b** in the NMR sample tube, gave the fulvene **2**. This elimination instead of acylation of the hydroxyl group at C-4, has been also observed for matricin [3, 11] under the same conditions. Finally, an inspection of a Dreiding model of **2**, clearly showed that the dihedral angle value between H-7 and H-8 was nearly 90°, in agreement with the $J_{7,8}$ value of zero.

EXPERIMENTAL

Stevia serrata Cav., was collected near Chapala, State of Jalisco, in September 1983, and near Cuernavaca, State of Morelos, on the Mexico–Cuernavaca road (Km 64), in August 1983. Voucher specimens were deposited in the Herbarium of the Instituto de Biología, UNAM.

Isolation of christinines and chamazulene. Dried leaves (187 g) of *S. serrata* from Chapala, were extracted with petrol at room temp. The petrol extract (8.7 g) was separated by CC over silica gel (270 g) using petrol and petrol–EtOAc as eluants, to yield 39 fractions. Fraction 7 (85 mg) was purified by prep. TLC (petrol) affording 47 mg of chamazulene [10]. Fractions 29–31 eluted with petrol–EtOAc (1:1) were combined to give 1.18 g. A 450 mg sample was purified by prep. TLC (CH_2Cl_2–Me_2CO 19:1) yielding 126 mg of christinine III [2] and 45 mg of christinine I [1].

Chamazulene. ^{1}H NMR (80 MHz, $CDCl_3$): δ1.31 (3H, J = 7.0 Hz, $-CH_2-\underline{Me}$), 2.63 (3H, *s*, C-1-Me), 2.80 (3H, *s*, C-4-Me), 2.83 (2H, *q*, J = 7.0 Hz, $-\underline{CH}_2-Me$), 6.9 (1H, *d*, J = 11 Hz, H-5), 7.17 (1H, *d*, J = 4 Hz, H-3), 7.35 (1H, *dd*, J = 11, 1.5 Hz, H-6), 7.68 (1H, *d*, J = 4 Hz, H-2), 8.12 (1H, *d*, J = 1.5 Hz, H-8); EIMS (probe) 70 eV *m/z* (rel. int.): 184 $[M]^+$ (100), 169 $[M-Me]^+$ (86), 155 (30), 153 (35).

Isolation of steviserrolides A and B. Due to the extreme sensitivity of the prochamazulene lactones to weak acid conditions [4], these were separated using prep. TLC plates, with loss of large amounts of material. Dried leaves (155 g) of *S. serrata* from Cuernavaca were extracted with CH_2Cl_2 at room temp. and the extract was evapd *in vacuo* to give a greenish syrup (9.75 g). A 0.75 g sample was separated by prep. TLC (CH_2Cl_2–Me_2CO, 19:1, ×2) to give 75 mg of crude **1a** and 67 mg of crude **1b**. Further purification using analytical TLC plates (CH_2Cl_2–Me_2CO, 9:1, ×2) afforded 19 mg of **1a** and 15 mg of **1b**.

Steviserrolide A (**1a**). Unstable colourless oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3696, 3577, 1773, 1736, 1601; EIMS 70 eV, *m/z* (rel. int.): 306 $[M]^+$ (2.3), 246 $[M-HOAc]^+$ (33), 231 $[M-HOAc-Me]^+$ (100), 185 (24), 173 (36), 43 $[Me-CO]^+$ (78).

Steviserrolide B (**1b**). Unstable colourless oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3686, 3592, 1774, 1737, 1602; EIMS 70 eV *m/z* (rel. int.): 306 $[M]^+$ (1.6), 246 $[M-HOAc]^+$ (18), 231 $[M-HOAc-Me]^+$ (26), 145 (23), 43 $[MeCO]^+$ (100).

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