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STRUCTURAL ANALYSIS OF SESQUITERPENE LACTONES FROM *HYMENOCLEA SALSOLA*

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Key Word Index—*Hymenoclea salsola*; Asteraceae, Heliantheae, sesquiterpene lactones, pseudoguaianolides, bipinnatin

Abstract—Some characteristic features of the ^{1}H and ^{13}C NMR spectra of pseudoguaianolides in *Hymenoclea salsola* are described in order to facilitate the structural determination of these sesquiterpene lactones.

INTRODUCTION

Hymenoclea salsola T. and G. (Asteraceae, tribe Heliantheae) [1] is a perennial shrub of the Colorado and Mohave Deserts of California [2] and the Sonoran Desert of Baja California, Mexico [3]. Earlier phytochemical analysis of *H. salsola* had indicated the presence of several pseudoguaianolide sesquiterpene lactones [4, 5] as well as flavonoid aglycones isolated from the leaf resin [6].

In continuation of our phytochemical studies of arid land plants from California, we report the isolation and characterization of bipinnatin (**1**) previously not known to occur in leaves and stems of this species. Bipinnatin is of special interest for its only reported occurrence in the Asteraceae prior to this work is in *Parthenium bipinnatifidum* [7].

RESULTS AND DISCUSSION

Ivoxanthin (**2**) isolated from *Cyclachaena xanthifolia* Fresen. [synonym *Iva xanthifolia* Nutt. (Asteraceae)] and bipinnatin showed similar behaviour when treated under mild acetylation conditions with acetic anhydride and pyridine affording the elimination product ambrosin (**3**) rather than the expected acetate. This observation indicated that **1** and **2** are stereoisomers [7, 8].

We have now carried out a detailed examination of the close structural resemblance of these two epimers with respect to their ^{1}H and ^{13}C NMR spectra.

The structure of bipinnatin (**1**) [mp 197–198°, $(\alpha)_D^{23}$ −9.4° in MeOH] was previously assigned by comparison with the ^{1}H NMR spectrum of **2** and based on the deshielding effect produced on the C-5 and C-10 methyl group signals (δ 1.39 and 1.37, respectively) by the *syn*-axial interaction with the β-hydroxyl group. In **2**, the C-2α hydroxy group seems to have released some of this steric strain causing an upfield shift of the C-10 and C-5 methyls to δ 1.11 and 1.06, comparable to those of damsin (**4**) [7, 8].

Analysis of the 400 MHz ^{1}H NMR spectrum of **1** has shown significant differences from that **2**. For instance, the H-3 signals of **1** exhibit a geminal coupling of 18.0 Hz. The C-3 proton with an absorption at δ 2.40 appears to be *cis* to the β-hydroxy group in order to allow for $J_{2,3\beta}$ of ~0 Hz (~90° dihedral angle), whereas the other C-3 proton gives rise to a doublet, $J_{2,3\alpha}$ = 4.5 Hz, at δ 2.62. An NOE difference experiment confirmed these assignments by showing selective enhancement of the H-3α signal when H-2 was irradiated. In **2**, the H-3 signals of the non-equivalent methylene group correspond to two doublets of doublets centred at δ 2.30 (H-3β) and 2.86 (H-3α) with geminal coupling of 18.5 Hz and $J_{2,3\beta}$ = 8.5 Hz, and $J_{2,3\alpha}$ = 7.5 Hz (Table 1).

As expected, a change in the configuration at C-2 would not only affect the H-3 signals but also H-1. Indeed, the absorption of the proton at C-1 (δ 1.96) appears as a triplet (J = 3.1 Hz) caused by the protons at C-2 and C-10 in **1**, whereas in **2** this signal is a doublet of doublets (δ 1.97) showing splitting by H-2 (10 Hz) and H-10 (4.5 Hz). The assignment of the H-1 signal in **1** was also confirmed in the NOE difference spectrum, showing positive enhancement after irradiation of the H-2 signal.

The ^{1}H-decoupled ^{13}C NMR spectrum of **1** in Me_2CO-d_6 shows the presence of two carbonyl groups at

Table 1. ^{1}H NMR spectral data of compounds **1** and **2** (400 MHz, $CDCl_3$, δ)

H	1	2
1	1.96 *t* (3.1)*	1.97 *dd* (10.0, 4.5)
2	4.61 *ddd*	4.50 *ddd*
	(4.5, 3.0, 2.5)	(10.0, 8.5, 7.5)
OH	1.65 *d* (2.5)	
3	2.40 *d* (18.0)	2.30 *dd* (18.5, 8.5)
3	2.62 *dd*	2.86 *dd* (18.5, 7.5)
	(18.0, 4.5)	
6	4.39 *d* (8.5)	4.61 *d* (8.4)
7	3.28 *m*	3.35 *m*
10	2.52 *m*	2.60 *m*
C-10 methyl	1.37 *d* (7.5)	1.11 *d* (7.5)
C-5 methyl	1.39 *s*	1.06 *s*
13	5.56 *d* (3.0)	5.66 *d* (2.9)
13	6.29 *d* (3.3)	6.26 *d* (3.2)

*Coupling constants (in Hz) are given in parentheses

δ218 3 and 170.4, and two further sp^2 carbon atoms at δ142.1 and 120 3 Signals at δ82 5 and 73.8 correspond to two sp^3 carbon atoms bearing an oxygen functional group, and the other nine signals are due to the remaining sp^3 carbon atoms in the molecule (Table 2).

The signals of the cyclopentanone (δ218.3) and $\Delta^{11,13}$-unsaturated-γ-lactone (δ170.4) carbonyl carbon atoms were assigned by comparison with earlier data on pseudoguaianolides, and from their relative intensities in the spectrum The other two sp^2 carbon atom signals were assigned on the basis of their respective multiplicities, obtained from the ^{1}H-coupled ^{13}C NMR spectrum Thus, the signal at δ142 1 is a singlet (C-11) and that at δ120 3 a triplet (C-13).

Angular-type pseudoguaianolides having a *cis*-fused $\Delta^{11,13}$-unsaturated-6-γ-lactone ring show characteristic chemical shifts for the C-6 and C-7, as well as C-11, C-12, and C-13 carbon atoms, as seen in Table 2 Resonance signals at δ82.5 and 45.2 have, therefore, been assigned to C-6 and C-7, respectively. The other carbon bearing a C–O bond as a secondary hydroxyl group must give rise to the absorption at δ73 8 (C-2).

One of the residual signals corresponds to a quaternary sp^3 carbon atom, known to have a relatively long relaxation time (T_1), and consequently it is of low intensity This sharp singlet at δ53 8 was assigned as C-5.

The signals of the remaining methyl, methylene and methine carbons were assigned by APT and selective proton decoupling experiments. Thus, of the two methine signals, selective proton decoupling at δ2 50 (H-10) collapsed the doublet at δ36 1 to a singlet, identifying the C-10 carbon signal. Therefore, the C-1 carbon signal must be that at δ51 4 The same experiment enabled us to confirm the carbon signal at δ49.4 as C-3, owing to the close proximity of the H-10 resonance to that of the H-3 geminal protons, and also the methyl group at δ17.7 as C-14 Signals at δ36.6 and 27 2 were assigned to C-8 and C-9, respectively, by analogy with known compounds.

It is well established that ^{13}C shieldings are markedly sensitive to molecular geometry and this feature renders ^{13}C NMR a valuable aid for spectral analysis and stereochemical assignments [9]. Comparison of the ^{13}C NMR spectra of **1**, **2**, and **5** in Me_2CO-d_6 has provided data on the long-range deshielding effect of the hydroxyl group in compounds having a *syn*-axial interaction between δ substituents, i e hydroxyl and methyl groups separated by four bonds Compound **1** is a unique example of a sterically crowded structure, where the C-5 and C-10 methyl groups are *syn*-diaxial to the C-2β hydroxyl group The C-5 and C-10 methyl resonances for **1** in comparison with the C-2α epimer (**2**) show that they are deshielded to δ17 9 and 17 7, respectively. At the same time, the carbons bearing these substituents in **1** at δ73.8 (C-2), 36 1 (C-10), and 53 8 (C-5) are appreciably more deshielded than in **2**, with $\Delta\delta_c$ values ranging from 6 0, 5 6, and 2 2 ppm, in that order

As mentioned previously, the deshielding of the C-5 and C-10 methyl protons of **1** relative to **2**, and the analogous effect on the carbons attributable to the δ-effect, is noteworthy. By contrast, the upfield ^{13}C shift associated with the γ-effect on the C-10 carbon of **2** (δ30 5) compared to **1** (δ36.1) is accompanied, as frequently observed [10], by a downfield ^{1}H shift of the corresponding H-10 signal (Table 1)

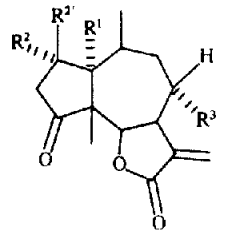
Changes minimizing *syn*-methyl–hydroxyl and methyl–methyl interactions in **1** and **2** may account for the difference in shielding for the C-8 methylene carbon, because of skeletal twist

In order to obtain additional information on the effect of the α-hydroxyl group on a δ-methyl, desacetylconfertiflorin (**5**) isolated from *Ambrosia confertiflora* (Asteraceae), possessing a C-8α hydroxyl substituent which is in a δ array with the C-10 methyl group and five bonds apart from the C-5 methyl group, was chosen for study. In this compound (mp 203–204°, $[\alpha]_D^{24}$ +17.2° in MeOH) the individual ^{13}C NMR signals of carbonyls, sp^2 carbons as well as sp^3 carbons bearing an oxygen functional group were assigned by direct comparison with those observed for **1** and **2**, leading to the ^{13}C shieldings listed in Table 2

The methine and methylene carbons were distinguished from the others by off-resonance decoupling and then assigned on the basis of the expected substituent effects of the hydroxyl group. It had been established that the hydroxyl group deshields both the carbon to which it is bonded and its immediate neighbours named α and β effects, respectively The signal at δ67.0 for **5** was attributed to C-8 and the absorptions at δ52 1 and 44.5 identified the carbons C-7 and C-9. The methine signals at δ32 4 (C-10) and 46.9 (C-1) were assigned from the fact

Table 2 ^{13}C NMR spectral data of compounds **1**, **2**, and **5** (100 MHz, Me_2CO-d_6, δ)

C	1	2	5
4	218 3	215 0	217 3
12	170 4	170 6	170.4
11	142 1	141 6	139 2
13	120 3	120 1	122 0
6	82 5	82 2	81 4
2	73 8	67 8	24 3
5	53 8	51 6	55 3
1	51 4	47 6	46 9
3	49 4	46 3	36 6
7	45 2	45.2	52 1
8	36 6	33 8	67 0
10	36 1	30 5	32 4
9	27 2	26 5	44 5
14 (C-10 methyl)	17 7	16 0	16 0
C-15 (C-5 methyl)	17 9	15 4	13 9



1 R^1 = H, R^2 = H, $R^{2'}$ = OH, R^3 = H
2 R^1 = H, R^2 = OH, $R^{2'}$ = H, R^3 = H
4 R^1 = R^2 = $R^{2'}$ = R^3 = H
5 R^1 = R^2 = $R^{2'}$ = H, R^3 = OH
7 R^1 = OH, R^2 = $R^{2'}$ = R^3 = H

that equatorial hydroxyl groups shield the γ carbon more than the δ carbon [11, 12]. Closely similar γ-effects were observed for C-5, C-10 and the cyclopentanone carbonyl of **2**, relative to **1**.

Selective proton decoupling at δ1.16 (C-10 methyl group) collapsed the quartet at δ16.0 to a singlet, identifying the methyl signals of this compound

Although the α-hydroxyl group shifts the α-carbon in **2** and **5** to δ67.8 and 67.0, respectively, it seems that it also exerts a comparable effect at the δC-10 methyl group carbon exhibiting the same shielding of δ16.0 in both compounds On the other hand, the angular methyl group at C-5 (δ13.9), five bonds removed from the α-hydroxyl in **5** is more shielded than the corresponding carbon atom in **2** (δ15.4), and to an even greater extent than that in **1** at δ17.9.

Another examination of structural influences on chemical shifts of the pseudoguaianolides **3**, **6**, and **8** was carried out. Although the ^{13}C shifts for compounds **3** and **8** have been reported, the spectra were determined in $CDCl_3$ solutions to obtain results for a common solvent for more precise comparisons of the hydroxyl substituent effect

The previously unreported ^{1}H-decoupled ^{13}C NMR spectrum of **6** in $CDCl_3$ shows the presence of two carbonyl groups at δ211 2 and 180.6, and two further sp^2 carbon atoms at δ163.3 and 130.9. Signals at δ84.1 and 79.2 correspond to two sp^3 carbon atoms bearing an oxygen group, and the other nine signals are due to the remaining sp^3 carbon in the molecule.

The signals of the cyclopent-2-en-4-one (δ211 2) and γ-lactone (δ180.6) carbonyl carbon atoms, as well as the two sp^2 carbon resonances at δ163.3 (C-2) and 130.9 (C-3), were readily assigned. The other two carbons bearing a C–O bond were assigned on the basis of their respective multiplicities, obtained from the ^{1}H-coupled spectrum. Thus, the signal at δ84.1 is a singlet (C-1) and that at δ79.2 a doublet (C-6) The sharp singlet at δ58.7 identified the C-5 quaternary carbon.

Doublets at δ47.4, 41.3, and 40.3 in the off-resonance spectrum were attributed by selective proton decoupling to the methine carbons at C-7, C-10, and C-11, respectively.

The two methylene functions were distinguished from each other by selective proton decoupling and HETCOR experiments. Hence, the signal at δ29.5 was assigned to C-9 and that at δ25.8 to C-8.

Differentiation of the remaining three methyl groups was obtained from a HETCOR experiment on **6** which unambiguously assigned the upper field signal to the C-11 methyl group (δ16.1), the angular C-5 methyl group to the resonance at δ18.3, and finally the C-10 methyl group to δ17.6.

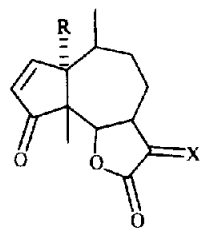
The relatively large coupling constant between H-7 and H-11 (7 0 Hz) in the ^{1}H NMR spectrum of **6** suggests that they are *anti* (or nearly so) and that the C-11 methyl group and C-8 methylene are therefore *trans*. Similarly, a large coupling constant between H-7 and H-11, suggesting a dihedral angle of *ca* 0°, might also have been the case if the two protons were *cis*, as shown by the magnitude of the coupling between H-6 and H-7 (8.0 Hz) However, selective enhancement of the H-6 and C-11 methyl signals in the NOE difference spectrum when H-7 was irradiated assigned this methyl absorption as being in the α-configuration. Therefore H-7 and H-11 must be *trans*. Using this argument, the relative stereochemistry of the substituents on the γ-lactone ring can tentatively be depicted as in **6**.

In view of the results obtained from the ^{13}C NMR spectral data, it appears that introduction of an axial hydroxyl group at C-1 on **3**, not only modifies greatly the chemical shift of the latter but also deshields C-10 and C-9, namely β- and γ-carbons in the seven-membered ring. Similarly, C-5 and the methyl attached to it, are also deshielded, by $\Delta\delta_c = 3.1$ and 1.3 ppm, respectively. Although C-9 and C-15 are γ carbons, upfield shifts are not likely to occur because of the spatial arrangement and the positioning of the hydroxyl group at the bridge of the five- and seven-membered rings. Nevertheless, the C-6 γ-carbon shows an upfield shift of 1.1 ppm in a different conformational array.

Saturation of the exocyclic double bond of **8**, as shown in **6**, causes the C-9 to move further downfield, whereas C-8 becomes more shielded. As expected, pseudoequatorial orientation of the methyl group at C-11 (α-isomer) should effect an increase in shielding at both C-13 and C-8, presumably because of the *gauche* interaction They should exert reciprocal γ-effects due to the increased nonbonded interactions between the atoms attached to this carbon when the methyl is in the pseudoequatorial position. The observed differences for **8** and **6**, $\Delta\delta_c = 3\,6$ ppm for C-8 and the shift at δ16.1 (C-13), the most shielded methyl resonance of **6** and other compounds of this series, are in agreement with this expectation. These data, together with the ^{1}H NMR measurements as stated above, support the relative stereochemistry at C-11 in the γ-lactone ring of **6**

A β-effect associated with the methyl group at C-11 of **6** is noticeable at C-7 and C-12, relative to **8**, with the deshielding effect exhibited at C-12 ($\Delta\delta_c = 3\,7$ ppm). A carbonyl rather than carbon–carbon sp^2 character arising from resonance between C-11 and C-12 of the α,β'-unsaturated-γ-lactone function, where the ^{13}C signal involved for the carbonyl is frequently more shielded, may explain in part the difference in shielding for C-12 of **6**, relative to **8**.

Correlations of this type in related compounds are highly useful for assignments of ^{13}C resonances, allowing for ready assessment of structural influences on chemical shifts as well as on the stereochemistry.



	3	**6**	**8**
R	H	OH	OH
X	CH_2	αMe,H	CH_2

EXPERIMENTAL

^{13}C NMR: 75 and 100 6 MHz (Me_2CO-d_6 and $CDCl_3$; δ, relative to TMS). Chemical shifts are referenced to the partly deuterated signals of Me_2CO-d_6 ($\delta_c = 77.0$ ppm) and $CDCl_3$ ($\delta_c = 29.8$ ppm) Mp: uncorr.

Hymenoclea salsola T and G. was collected and identified in May 1983 at the 8836-Intersection, Pipe Canyon Road, Baja California, Mexico on an expedition led by Dr E. Rodriguez of the University of California, Irvine. Ground leaves and stems (1.0 kg) were extracted with $CHCl_3$ (4.0 l) for 48 hr. The filtered extract was concd *in vacuo* and mixed with 4% aq $Pb(OAc)_2$ (40 ml). The solid was filtered off and the filtrate diluted with dist H_2O, concd and reextracted with $CHCl_3$. The $CHCl_3$ soln was finally treated with activated charcoal and filtered, dried over dry $MgSO_4$ and evapd to dryness, yielding 62.7 g of crude extract.

The crude extract (40.0 g) was chromatographed on a silica gel (Merck 70–230 mesh) column (95 × 3.5 cm) packed and eluted with $CHCl_3$–Me_2CO (6:1) gradually changing to (7:3), (3:7) and finally Me_2CO. Eluted fractions (25 × 200 ml) were screened for sesquiterpene lactones on precoated silica gel 60 F_{254} TLC plates (0.2 mm) with $CHCl_3$–Me_2CO (6:1) as solvent system and vanillin as spray reagent.

The following major compounds were eluted, purified, and characterized. Ambrosin (**3**) R_f 0.62 (130 mg), mp 146–147°, coronopilin (**7**) R_f 0.40 (26 mg), mp 177–179°, hymenolin (**6**) R_f 0.33 (85 mg), mp 186–188°, bipinnatin (**1**) R_f 0.25 (318 mg) and a mixture of **6** and **7** (120 mg). ^{13}C NMR for ambrosin (**3**) ($CDCl_3$, ppm): C-4 (210.5), C-12 (170.2), C-2 (163.8), C-11 (137.7), C-3 (130.2), C-13 (119.3), C-6 (79.8), C-5 (55.5), C-1 (47.1), C-7 (43.9), C-10 (33.0), C-8 (28.9), C-9 (24.0), C-14 (16.9), C-15 (16.6), for hymenolin (**6**), C-4 (211.2), C-12 (180.6), C-2 (163.3), C-3 (130.9), C-1 (84.1), C-6 (79.2), C-5 (58.7), C-7 (47.4), C-10 (41.5), C-11 (40.3), C-8 (29.5), C-9 (25.8), C-15 (18.3), C-14 (17.6), C-13 (16.1), for coronopilin (**7**), C-4 (218.6), C-12 (170.8), C-11 (141.1), C-13 (121.4), C-1 (84.5), C-6 (79.9), C-5 (58.8), C-7 (44.5), C-10 (42.1), C-2 (32.3), C-3 (32.1), C-8 (30.0), C-9 (27.5), C-14 (17.1), C-15 (14.4). Parthenin (**8**) isolated from *Parthenium hysterophorus* L., mp 164–166°. ^{13}C NMR for **8** ($CDCl_3$, ppm): C-4 (210.7), C-12 (170.8), C-2 (163.4), C-11 (140.1), C-3 (130.9), C-13 (121.3), C-1 (83.8), C-6 (78.7), C-5 (58.6), C-7 (43.7), C-10 (40.0), C-8 (29.4), C-9 (27.9), C-14 (16.8), C-15 (17.9).

^1H NMR for hymenolin (**6**) (400 MHz, $CDCl_3$): δ 7.63 (1H, *d*, J = 6.0 Hz, H-2), 6.12 (1H, *d*, J = 6.0 Hz, H-3), 5.03 (1H, *d*, J = 8.0 Hz, H-6), 3.35 (1H, *br s*, OH), 2.67 (1H, *dddd*, J_1 = 9.0, J_2 = 8.0, J_3 = 7.0, J_4 = 4.5 Hz, H-7), 2.38 (1H, *br q*, J = 7.0 Hz, H-11), 2.37 (1H, *br q*, J = 7.5 Hz, H10), 2.12 (1H, *m*, H-9), 1.96 (2H, *m*, H-8), 1.65 (1H, *m*, H-9), 1.30 (3H, *s*, C-5 methyl), 1.29 (3H, *d*, J = 7.0 Hz, C-11 methyl), 1.12 (3H, *d*, J = 7.5 Hz, C-10 methyl).

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REFERENCES

1. Stuessy, T. F. (1977) in *The Biology and Chemistry of the Compositae* (Heywood, V. H., Harborne, J. B., and Turner, B. L., eds), p. 261. Academic Press, London.
2. Jepson W. L. (1966) in *A Manual of the Flowering Plants of California*, p. 1108. University of California Press, Berkeley.
3. Wiggins, I. L. (1977) in *Flora of Baja California*, p. 244. Stanford University Press, Stanford.
4. Geissman, T. A. and Toribio, F. P. (1967) *Phytochemistry* **6**, 1563.
5. Toribio, F. P. and Geissman, T. A. (1968) *Phytochemistry* **7**, 1623.
6. Proksch, P., Wollenweber, E. and Rodriguez, E. (1983) *Z. Naturforsch. C* **38**, 668.
7. Rodriguez, E., Yoshioka, H. and Mabry, T. J. (1971) *Phytochemistry* **10**, 1145.
8. Samek, Z., Holub, M., Novikov, V. J., Forostjan, J. N. and Popa, D. N. (1970) *Collect. Czech. Chem. Commun.* **35**, 3818.
9. Wilson, N. K. and Stothers, J. B. (1974) in *Topics in Stereochemistry* (Eliel, E. L. and Allinger, N. L., eds). Wiley-Interscience, New York.
10. Perlin, A. S. (1977) in *Isotopes in Organic Chemistry* (Buncel, E. and Lee, C. C., eds). Vol. 3, Chap. 4. Elsevier, Amsterdam.
11. Pehk, T. and Lippmaa, E. (1971) *Org. Magn. Res.* **3**, 679.
12. Roberts, J. D., Weigert, F. J., Kroschwitz, J. J. and Reich, H. J. (1970) *J. Am. Chem. Soc.* **92**, 1338.