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SESQUITERPENE LACTONES AND OTHER CONSTITUENTS FROM *HYMENOXYS RICHARDSONII* AND *H. SUBINTEGRA*

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Key Word Index—*Hymenoxys richardsonii*; *Hymenoxys subintegra*; Asteraceae; sesquiterpene lactones; sesquiterpene glucoside eudesmane; butyrolactone.

Abstract—Two *Hymenoxys* species afforded, in addition to known compounds, three new pseudoguaianoloides, a new sesquiterpene glucoside and a new butyrolactone. This is the first report of an eudesmane from the genus *Hymenoxys*. The structures were elucidated by high-field NMR and chemical transformations.

INTRODUCTION

Species of the genus *Hymenoxys* have previously been under intensive chemical investigation, predominantly due to the toxicity of their sesquiterpene lactones which causes severe livestock poisoning in the south western parts of the U.S.A. [1]. Besides, a recent study has demonstrated the taxonomical usefulness of sesquiterpene lactones for the *Hymenoxys* complex [2]. We have now reinvestigated the chemistry of *Hymenxoys richardsonii* var. *floribunda* and *H. subintegra*. Both species belong to subgenus *Picradenia*, a group of biennial or perennial plants with distribution in the dry areas of the southwestern U.S.A. and Mexico [3].

Two varieties of *H. richardsonii* are currently recognized, var. *richardsonii* and var *floribunda*. Previous chemical investigation of *Hymenoxys richardsonii* var. *floribunda* had afforded the sesquiterpene lactone floribundin (= psilotropin) and vermeerin [4] as well as hymenoxon (= hymenovin) [5]. Floribundin was also detected in *H. subintegra* [6]. Chromatographic analysis of glandular trichome exudates from the two taxa confirmed these results and indicated the presence of several additional sesquiterpene lactones, some of which were tentatively assigned to guaianolides, pseudoguaianolides and modified pseudoguaianolides, known from other species of the genus [2].

We now wish to report on new structural data of eight sesquiterpene lactones from *H. subintegra* and six sesquiterpene lactones, an eudesmane and butyrolactone from *H. richardsonii*. This is the first isolation of a compound with the eudesmane skeleton from the genus *Hymenoxys*.

RESULTS AND DISCUSSION

The extract of the aerial parts of *hymenoxys richardsonii* Hooker, in addition to hymenoratin (**1**) [7], hymenograndin (**3**) [8] isohymenoloide (**11**), hymenolide (**12**) [8], 2α-tiglinoyloxydugaldiolide (**14**) [9] and **16** [10], gave two new compounds **15** and **17**.

The structure of **1** followed from its IR, ^{1}H NMR and mass spectra while acetylation of **1** gave the diacetate product **2**; ^{1}H NMR data are reported in Table 1. The spectral data of **3**, and its acetyl product **4**, were identical with those previously reported [4]. Oxidation of **3** gave **5** (^{1}H NMR data in Table 1).

The ^{1}H NMR spectrum of **15** indicated a sugar moiety, and the coupling constants and chemical shifts were in accord with a β-D-glucopyranoside [12]. The signal for the anomeric proton of the sugar appeared as a doublet at δ4.40. The other proton signals could be easily assigned by irradiation initially of the anomeric proton, which collapsed the doublet of doublets at δ3.35 for H-2 to a doublet. Signals for two acetyl groups at δ2.00 and 2.10, as well as downfield signals for H-3 and H-6 (δ4.85 and 4.30, respectively), indicated that the acetyl groups were at C-3 and C-6.

The ^{1}H NMR spectrum of **15** confirmed a guaianolide skeleton. An H-15 singlet appeared at δ1.20 and the two broad singlets were observed at δ5.0 and 4.9 for the $\Delta^{10(14)}$-double bond. Two doublets at δ6.25 and 5.65 are typical for H-13.

Spin decoupling allowed the assignments of all signals with H-7, H-8 and H-9$_\alpha$ at δ3.15, 4.50 and 2.70, respectively. The stereochemistry at C-7 and C-8 was established from the chemical shifts and NOE, irradiation of H-8 at δ4.50 enhanced H-7 at δ3.15 by 10% and H-9$_\alpha$ at δ2.70 by 12% ∴ On the other hand, ^{13}C NMR data (Table 2) showed 25 carbons and DEPT experiments indicated the protonated carbons belonged to three methyls, five methylenes, nine methines, six of which were oxygenated.

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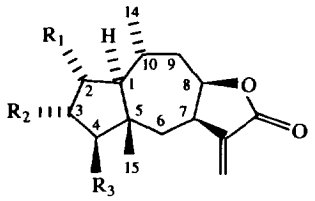
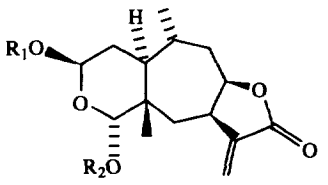
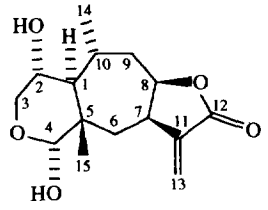


	R_1	R_2	R_3
1	H	OH	OH
2	H	OAc	OAc
3	OAc	OAc	OH
4	OAc	OAc	OAc
5	OAc	OAc	O
6	OH	OH	OAc
7	OAc	OH	OAc



	R_1	R_2
8	Me	H
9	H	Me
10	Me	Me
11	Et	H
12	H	Et



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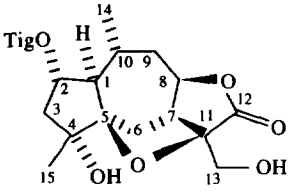

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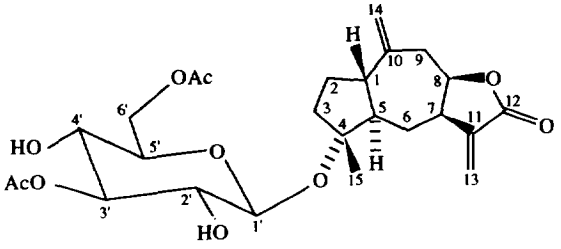

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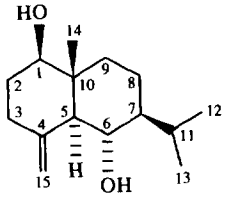

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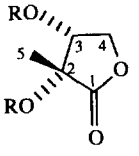

	R
17	H
18	Ac

The oxygenated carbons could be easily assigned by ^1H–^{13}C correlation. The CI mass spectrum of **15** gave a molecular ion peak at *m/z* 495 for a formula of $C_{25}H_{34}O_{10}$.

^1H and ^{13}C NMR spectra of **16** (Tables **2** and **3**, respectively), indicated a eudesmane skeleton, namely the typical chemical shift for H-14 at δ0.75. The ^1H NMR spectrum exhibited two doublets at δ0.86 and 0.95, both of which were coupled to a multiplet at δ2.21 in accordance with an isopropyl group. Carbons containing two hydroxyl groups were detected at δ77.2 and 79.0 in the ^{13}C NMR spectrum and spin decoupling experiments

Table 1. ^{1}H NMR data of **2**, **5**, **6** and **7** (200 MHz, $CDCl_3$, TMS as int. standard, δ values)

	2	**5**	**6**	**7**
H-2		5.10 *m*	3.85 *dd* (J = 10 and 12 Hz)	4.75
H-3	5.00 *m*	5.10 *m*	3.95 *dd* (J = 10 and 7 Hz)	4.20
H-4	5.10 *d*		4.61 *d* (J = 7 Hz)	4.65
	(J = 7 Hz)		1.75 *dd* (J = 13 and 7 Hz)	1.70
H-6			1.25 *dd* (J = 15 and 13 Hz)	1.20
H-7	3.20 *m*	3.19 *m*	3.17	3.20
H-8	4.68 *ddd*	4.75	4.75	4.80
	(J = 12.8, 9 and 3 Hz)			
H-9			2.00 *m*	
	6.20 *d* (J = 2.2 Hz)	6.25	6.20	6.20
H-13	5.55 *d* (J = 2.2 Hz)	5.68	5.52	5.60
H-14	1.00 *d*	1.00	1.20	1.05
	(J = 7 Hz)			
H-15	0.70 *s*	1.05	0.90	0.90
AcO	2.05 *s*	2.04	2.05	2.05
	1.95 *s*	2.01		2.10

Table 2. ^{1}H NMR data of **10**, **15** and **16** (200 MHz, $CDCl_3$, TMS as int. standard, δ values)

	10	**15***	**15†‡**	**16§**
H-2				1.84 *dddd*
				(J = 12, 5, 2.5 and 2.5 Hz)
				1.52 *m*
H-3	5.00 *dd*			2.3 *ddd*
	(J = 10 and 3 Hz)			(J = 14, 13 and 2.5 Hz)
				2.05 m
H-4	4.05 *s*			
H-6	1.49 *dd*			3.70 *dd*
	(J = 15 and 3.5 Hz)			(J = 10 and 10 Hz)
H-7	3.38	3.15	2.70	2.00 *m*
H-8	4.75 *ddd*	4.50 *ddd*	4.10	
	(J = 12.8, 9 and 3 Hz)	(J = 12,9 and 4 Hz)		
H-9		2.70 *dd*	2.60	
		(J = 12 and 4 Hz)		
H-13	6.15	6.25	6.15	0.95 *d*
	5.49	5.65	4.85	(J = 7 Hz)
H-14	1.05	5.00 *br s*	4.74	0.75 *s*
		4.90 *br s*	4.70	
H-15	1.00	1.20 *s*	1.20	5.01, 4.74
AcO		2.10, 2.00	2.10, 2.00	
OMe	3.35, 3.45			

*O-Glucosyl: H-1, 4.40 *d* (J = 8 Hz), H-2, 3.55 *dd* (J = 9 and 8 Hz), H-3, 4.85 *dd* (J = 9 and 9 Hz), H-4, 3.46 *dd* (J = 9 and 9 Hz), H-5, 3.46 *ddd* (J = 9,5 and 3 Hz), H-6, 4.40 *m*.

†O-Glucosyl: H-1, 4.25, H-2, 3.55, H-3, 5.10, H-4, 3.40, H-5, 3.10, H-6, 4.40.

‡In benezene-d_6.

§H-1$_\alpha$, 3.35 *dd* (J = 11 and 5 Hz), H-5$_\alpha$, 1.75 *br d* (J = 10 Hz), H-11, 2.21 *m*, H-12, 0.86 *d* (J = 7 Hz).

placed the two hydroxyl groups at C-1 and C-6. The stereochemistry at C-1, C-5, C-6 and C-10 was proved by NOEs. Irradiation of H-14, at δ0.75, enhanced H-6$_\beta$, at δ3.70, by 12%, while irradiation of H-1$_\alpha$, at δ3.35, enhanced H-5$_\alpha$, at δ1.75, by 8%. The CI mass spectrum exhibited a molecular ion peak at m/z 239 for $C_{15}H_{26}O_2$, followed by the consecutive loss of a molecule of water as indicated by peaks at m/z 221 and 203. A similar compound has been reported from *Senecio* species [10].

The ^{1}H NMR spectral data of **17** (see Experimental) showed a sharp singlet signal integrated for three protons at δ1.3, two doublets at δ4.01 and 4.15 and a doublet of doublets at δ4.30. While, the stereochemistry of the chiral centres were established by NOE, irradiation of H-5 at

Table 3. ^{13}C NMR spectral data of **6**, **7**, **15–18** (67.5 MHZ, $CDCl_3$)

C	6*	7	15*†	16	17*	18*
1	55	50	50.5	77.2	179	173
2	72	77	36	31.9	73	75
3	73	75	26.5	35.1	72.5	72
4	86	86	89	146.2	72	70
5	41	42	54.5	55.9	21	22
6	40	41	30.0	67		
7	38	38	42.5	49.3		
8	78	71	81.5	16.2		
9	35	35	40.5	36.3		
10	30	30	145	41.7		
11	140	140	139	26		
12	170	169	168	16.2		
13	123	123	123	21.1		
14	18	18	112	11.6		
15	20	20	23.2	107.8		
AcO	174, 20.0	177, 20.1	170,2			169.0, 20.0
		174, 20.1	169,2			169.2

*Assigned by 2D-hetero COSY.
†Glucosyl:9 8.3, C-1; 73.9, C-2; 78.2, C-3; 69.1, C-4, 73.9, C-5; 69.5, C-6.

δ1.3 enhanced H-3 at δ4.01 by 13% and H-4$_B$ at δ4.30, by 9%.

Acetylation of **17** gave the diacetyl derivative **18**. Comparison of ^{1}H NMR data (see Experimental) with those for **17** showed a downfield shift of the H-3 signal from δ4.01 to 5.3, confirming a hydroxyl group at C-3 in **17**. The two signals, doublet of doublets at δ4.25 and 4.55, were assigned to H-4. The ^{13}C NMR spectrum showed C-2 and C-4 at δ75, 72 and 70, respectively, which could be assigned by hetero-COSY. The CI mass spectrum had a molecular ion peak at *m/z* 217 for $C_9H_{12}O_6$, followed by peak at *m/z* 174 for the loss of an acetyl group.

The aerial parts of *H. subintegra* Cockerell gave, in addition to the known compounds 3-methyl-hymenoxon (**8**) and 4-methyl-hymenoxon (**9**) [13], isohymenoloide (**11**) [8], bennin A (**13**) [14] and 2α-tiglinoyloxydugaldiolide (**14**) [9], three new sesquiterpene lactones **6**, **7** and **10**. The structure of **6** could be easily deduced from the ^{1}H NMR data. The spectrum of **6** indicated one acetyl group (δ2.05), four protons geminal to hydroxyl groups, one of which is typical for H-8 (δ4.75). Comparison of the coupling constants and chemical shifts for H-2, H-3 and H-4 with those of **3** located the acetyl group at C-4: downfield shift of H-4 at δ4.61; H-2 and H-3 were found at δ4.85 and 3.95, respectively. The two narrow doublets at δ6.2 and 5.52 were assigned to H-13, the sharp singlet at δ0.9 for H-15 and doublet at δ1.20 for H-14. The assignments of the ^{13}C NMR spectrum were made by the use of ^{1}H–^{13}C correlation. Additionally, the CI mass spectrum showed the molecular peak $[M + H]^+$ at *m/z* 325, followed by elimination of either a molecule of water at *m/z* 307 or a methyl group at *m/z* 310.

Although, the CI mass spectral data and fragmentation pattern (see Experimental) of **7** were identical with those of **3**, ^{1}H and ^{13}C NMR data showed differences. In the ^{1}H NMR spectrum, the two acetyl groups appeared at δ2.1 and 2.05 and their geminal proton was at δ4.65 and 4.75. Again, comparison of both the coupling constants and the chemical shifts of H-2, H-3 and H-4 with those of **3** and **6** placed the two acetyl groups at δC-2 and C-4. H-8 appeared at δ4.80, H-13 at δ6.20 and 5.60, H-14 at δ1.05, H-15 at δ0.9 and H-7 at δ3.20. Thus, **7** must be a new isomer of **3**.

The structures of **8** and **9** were determined from their IR, MS, ^{1}H and ^{13}C NMR and NOEs, which were identical with the previous report [13]. In the ^{1}H NMR of **10**, two methoxyl signals were found at δ3.35 and 3.45. While H-3 and H-4 could be detected at δ5.0 and 4.05, respectively. The other signals were identical with those of **8** and **9**. However, the stereochemistry of H-3 was established from the coupling constant, NOEs proved that the methoxyl group at C-4 could be in the α-configuration, irradiation of H-4 at δ4.10 enhanced H-6 at δ1.49 by 12% and H-15 by 7%. The CI mass spectrum (see Experimental) confirmed the proposed structure.

The isolation of hymenoratin, hymenograndin, 2α-tiglinoyloxydugaldiolide and various isomers of hymenolide from *H. richardsonii* var.*floribunda*, of biennin A, 2α-tiglinoyloxydugaldiolide and isomers of hymenoxon from *H. subintegra* confirmed a major part of the results of a recent chemotaxonomical investigation of *Hymenoxys* and related genera, which was based on chromatography of trichome exudates [2]. Floribundin, a secohelenandolide previously isolated from the two species [3, 4], could not be detected in our present bulk samples.

The two species, now investigated in this study afforded sesquiterpene lactone skeletons which are typical for the genus *Hymenoxys*. These include guaianolides (**14**, **15**) and pseudoguaianolides (**1**, **3**, **6**, **7**) as well as modified pseudoguaianolides (**8–13**, known also as secohelenanolides). The latter type of compound is particularly characteristic for the genus *Hymenoxys* (including subgenera

Hymenoxys, Phileozera, Picradenia, Rydbergia) and the closely related genera *Dugaldia, Plummera* and *Macdougalia. Tetraneuris*, a genus occasionally submerged in *Hymenoxys* senu lato, lacks the ability to produce modified pseudoguaianolides.

EXPERIMENTAL

Air-dried plant material of *Hymenoxys richardsonii* var *floribunda* (600 g) was collected in U.S.A. State County, Colorado Park C. (voucher specimen, Bierner 88-66, is deposited in the Plant Resources Center at the University of Texas at Austin, (TEX). Extraction and sepn was achieved as reported previously [15] to give 100 mg **1**, 150 mg **3**, 10 mg **11**, 15 mg **12**, 18 mg **14**, 120 mg **15** and 70 mg **17**.

The extract of aerial parts (500 g) of *H. subintegra* Cockerell collected from U.S.A. Arizona State, Coconina County, voucher specimen Bierner 88/67 is deposited in the Plant Resources Center at the University of Texas at Austin, (TEX) gave 12 mg **6**, 7 mg **7**, 9 mg **8**, 11 mg **9**, 7 mg **10**, 5 mg **11**, 15 mg **13**, 20 mg **14**.

Acetylation of hymenoratin (**1**). (Ac_2O, pyridine, DMAP, 2 hr, 70°) gave 3,4-diacetylhymenoratin (**2**) IR $\nu_{max}^{CHCl_3}$ cm^{-1}. : 1780, 1740, 1050; CIMS *m/z* (rel. int.) : 351 $[M + H]^+$(50), 308 $[M - MeCO]^+$(30).

Oxidation of hymenograndin (**3**). Oxidation of **3** with Jones reagent gave 4-oxohymenograndin (**5**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1790, 1760, 1745, 1715; CIMS *m/z* (rel. int.): 365 $[M + H]^+$ (65), 337 $[M - CO]$ (20), 305 $[M - MeCOOH]^+$(55).

2-Hydroxy-4-acetyl-hymenoratin (**6**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 3500, 1755, 1740, 1250; CIMS *m/z* (rel. int.): 325 $[M]^+$ (100), 307 $[M - H_2O]^+$ (3), 282 $[M - MeCO]^+$ (40).

Isohymenograndin B (**7**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3540, 1770, 1750, 1270; CIMS *m/z* (rel. int.) 306 $[M - MeCOOH]^+$ (40), 263 $[M - MeCOOH - MeCO]^+$, 246 $[M_2MeCOOH]^+$ (10).

3,4-Dimethoxylhymenoxon (**10**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 1790, 1300; CIMS *m/z* (rel. int.): 311 $[M]^+$ (35), 279 $[M - MeOH]^+$ (15), 247 $[M - 2MeOH]^+$ (50).

3-Acetyl-lemmonin A (15). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3590, 3580, 3575,1730, 1290; CIMS *m/z* (rel. int.): 495 $[M + H]^+$ (42), 477 $[M + H - H_2O]^+$ (40), 248 [M + H-diacetylglucose]$^+$ (100).

1β, 6α-Dihydroxyeudesm-4(15)-ene (**16**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3610, EIMS *m/z* (rel. int.): 238 $[M]^+$(6), 220 $[M - H_2O]^+$(20), 202 $[M - 2H_2O]^+$(15), 177 $[M - H_2O - 3CH_7]^+$ (8).

2,3-Dihydroxy-2-methylbutyrolactone (**17**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3615, 1780; CIMS *m/z* (rel. int.): 133 $[M]^+$ (100), 115 $[M - H_2O]^+$ (35), 97 $[M - 2H_2O]^+$ (5). ^1H NMR (200 MHz, $CDCl_3$, δ values): 4.0 (*d*, J = 3.5 Hz, H-3_β), 4.15 (*d*, J = 10.5 Hz, H-4_α), 4.30 (*dd*, J = 3.5 and 10.5 Hz, H-4_β), 1.3 (*s*, H-5_β).

Diacetyl-2-methylbutyrolactone (**18**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1790, 1760; CIMS *m/z* (rel. int.): 217 $[M]^+$ (8); 174 $[MeO]^+$(35). ^1H NMR (200 MHz, $CDCl_3$, δ values): 5.3 (*dd*, J = 3.5 and 6.5, H-3_β), 4.55 (*dd*, J = 6.5 and 10.5 Hz, H-4_α), 4.25 (*dd*, J = 3.5 and 10.5 Hz, H-4_β), 1.6 (*s*, H-5_β).

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