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ENT-CLERODANE DITERPENOIDS FROM *RHYNCHOSPERMUM VERTICILLATUM*

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Key Word Index—*Rhynchospermum verticillatum*; Compositae; *ent*-clerodane diterpenoids; rhynchosperin A; rhynchosperin B; rhynchosperin C; rhynchospermoside A; rhynchospermoside B.

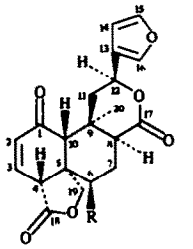
Abstract—From the whole plants of *Rhynchospermum verticillatum*, five new *ent*-clerodane diterpenoids, rhynchosperin A, rhynchosperin B, rhynchosperin C, rhynchospermoside A and rhynchospermoside B, have been isolated. The structures were elucidated by spectroscopic methods.

INTRODUCTION

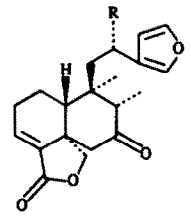
The genus *Rhynchospermum* (Compositae) has so far not been studied chemically. We have therefore examined the whole plants of *R. verticillatum* and isolated three new diterpenes and two new diterpene glycosides.

RESULTS AND DISCUSSION

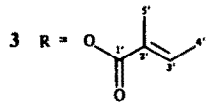
The IR spectrum of rhynchosperin A (**1**) was consistent with the presence of a δ-lactone group (1785 cm^{-1}), a γ-lactone group (1720 cm^{-1}) and an α,β-unsaturated carbonyl group (1680 cm^{-1}) that was supported by ^{13}C NMR data. However, it was the ^1H NMR spectrum of **1** that provided most of the relevant information. It showed signals of a tertiary methyl group at δ1.34, a β-substituted furan ring at δ6.64, 7.65 and 7.79 and olefinic protons at δ6.23 and 6.64 which can be assigned to olefinic α- and β-protons of an α,β-unsaturated carbonyl group possessing one proton in the γ-position. *W*-Coupling between H-10 and H-20 was observed. Furthermore the AB system at δ4.14 and 4.63 was assigned to the C-19 methylene group, one of the protons of which was in turn *W* coupled with the C-6β proton, a characteristic of C-19 lactonized *ent*-clerodanes [1–3]. A slightly broadened double-doublet at δ5.61 was coupled with one of the furan protons. Accordingly this signal could be assigned to H-12, which must be located at an oxygen-bearing carbon. These results led us to conclude the structure of rhynchosperin A to be **1**.



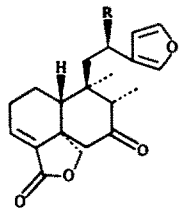
1 R = H
2 R = OH



4 R = O-glc
4a R = OH



3 R =



5 R = O-glc
5a R = OH

The molecular formula of **2** was $C_{20}H_{20}O_7$ indicating the presence of an additional oxygen, which, according to the IR band at 3500 cm^{-1}, obviously was a hydroxy group; the ^1H NMR spectrum was similar to that of **1**. Careful spin decoupling showed that this group was located at C-6 of rhynchosperin B (**2**). *W*-Coupling between H-19 and H-6 observed in **1** was not present but the signals of H-4 and H-10 shifted upfield so that the hydroxy group in **2** might be located β in a 1,3-diaxial relationship. Accordingly H-6 is α-oriented. Inspection of the Dreiding stereo model indicated that H-8 is α-oriented and irradiation of this proton showed a clear NOE with H-12, so that H-12 is also α-oriented. These results led us to conclude rhynchosperin B is **2**.

The molecular formula of **3** was $C_{25}H_{26}O_8$. The ^1H NMR spectrum again was similar to that of **2**. The loss of 99 mass units in the mass spectrum and a strong fragment *m/z* 83 indicated the presence of an unsaturated C_5-ester. The ^1H NMR spectrum showed that this ester group was a tiglate as followed from typical ^1H NMR

Table 1. ^{1}H NMR spectral data of 1, 4, 5 (90 MHz) and 2, 3, 4a, 5a (400 MHz)

Hydrogen	1	2	3
2	6.23 (1H, *dd*, 10, 3 Hz)	6.20 (1H, *dd*, 10, 3 Hz)	6.31 (1H, *dd*, 10, 3 Hz)
3	6.64 (1H, *dd*, 10, 3 Hz)	6.68 (1H, *dd*, 10, 3 Hz)	6.76 (1H, *dd*, 10, 3 Hz)
4	3.43 (1H, *t*, 3 Hz)	4.37 (1H, *t*, 3 Hz)	4.07 (1H, *t*, 3 Hz)
6		3.98 (1H, *dd*, 3, 3.3 Hz)	5.36 (1H, *t*, 3 Hz)
6		5.92 (1H, *d*, 3.3 Hz)	
7α		2.32 (1H, *ddd*, 16, 6, 3 Hz)	2.40 (1H, *ddd*, 16, 7, 3 Hz)
7β		2.70 (1H, *br d*, 16 Hz)	3.28 (1H, *br d*, 16 Hz)
8		2.85 (1H, *d*, 6 Hz)	2.83 (1H, *d*, 7 Hz)
10	2.96 (1H, *br s*)	3.39 (1H, *br s*)	3.51 (1H, *br s*)
11α		2.91 (1H, *dd*, 15, 1.5 Hz)	3.02 (1H, *dd*, 15, 2 Hz)
11β		2.17 (1H, *dd*, 15, 12 Hz)	2.31 (1H, *dd*, 15, 12 Hz)
12	5.61 (1H, *dd*, 12, 1.5 Hz)	5.63 (1H, *br d*, 12 Hz)	5.07 (1H, *br d*, 12 Hz)
14	6.64 (1H, *m*)	6.66 (1H, *m*)	6.61 (1H, *m*)
15	7.65 (1H, *m*)	7.62 (1H, *m*)	7.57 (1H, *m*)
16	7.79 (1H, *m*)	7.58 (1H, *m*)	7.62 (1H, *m*)
19α	4.14 (1H, *br d*, 10 Hz)	4.29 (1H, *d*, 10 Hz)	4.44 (1H, *d*, 10 Hz)
19β	4.63 (1H, *d*, 10 Hz)	4.63 (1H, *d*, 10 Hz)	4.92 (1H, *d*, 10 Hz)
20	1.34 (3H, *br s*)	1.35 (3H, *br s*)	1.43 (3H, *br s*)
3′			6.01 (1H, *dq*, 8, 1.5 Hz)
4′			2.09 (3H, *dd*, 8, 1.5 Hz)
5′			1.91 (3H, *s*)

signals (δ6.01 *dq*, 2.33 *dd*, 1.91 *s*), that was supported by ^{13}C NMR data and acid hydrolysis. These results led us to conclude the structure of rhynchosperin C to be **3**.

The molecular formula of rhynchospermoside A (**4**) was $C_{26}H_{34}O_{10}$ and its IR spectrum indicated the presence of a γ-lactone (1770 cm^{-1}). The ^{1}H NMR spectrum showed signals of a secondary and a tertiary methyl group at δ1.06 and 0.53, respectively, of an olefinic proton at δ6.76 and an anomeric proton at δ4.93. However, *W*-coupling between H-19 and H-6 indicated the connection between the two sequences and showed that C-5 was quarternary. Only the signal at δ3.52 was coupled with H-17. The remaining group had to be placed in a clerodane skeleton. Acid hydrolysis of **4** gave glucose and enzymatic hydrolysis of **4** gave an aglycone **4a**. In the NOE difference spectroscopy of **4a** an effect was observed between H-12 and H-8. Accordingly **4a** required the given stereochemistry [4].

The molecular formula of rhynchospermoside B (**5**) was $C_{26}H_{34}O_{10}$ from FAB-mass spectrometry. The ^{1}H NMR spectrum was similar to that of **4** except for the anomeric proton and the H-12 signals. The ^{13}C NMR was also similar to **4** except for the anomeric carbon, the C-12 carbon and the C-20 carbon signals. These results led us to suggest that **5** was an epimer of **4** at C-12. Acid hydrolysis of **5** gave glucose and enzymatic hydrolysis of **5** gave an aglycone **5a**. The spectral data of **5a** supported the proposed suggestion.

EXPERIMENTAL

All mps are uncorr. ^{1}H and ^{13}C NMR spectra were recorded at 89.55 and 22.5 MHz, respectively, and 399.65 MHz. TMS was used as an int. std.

Plant material. Whole plants of *R. verticillatum* REINW. were collected in Shizuoka, Japan, in November 1985. Plants were identified by Dr A. Ueno, and a voucher specimen has been deposited in the Herbarium, Shizuoka College of Pharmacy.

Extraction and isolation. Dried whole plants (2.15 kg) were extd twice with MeOH under reflux. The ext was concd under red. pressure and the residue was suspended in H_2O. This suspension was extracted with Et_2O. The H_2O layer was passed through an Amberlite XAD-2 column and the MeOH eluate conc. under red. pressure. The residue (18.8 g) was rechromatographed on a polyamide column with H_2O as an eluent to give fraction A (9.5 g). The Et_2O layer was rechromatographed on a silica gel column with C_6H_6–Me_2CO (9:1) to give 400 mg **1**, 22 mg **2**, 18 mg **3**. The polar fraction A was also rechromatographed on a silica gel column with $CHCl_3$–MeOH (19:1) to give 100 mg **4** and 16 mg **5**.

Rhynchosperin A (**1**). Needles from MeOH, mp. 236–237° (Found: C, 67.49; H, 5.63. $C_{20}H_{20}O_6$ requires: C, 67.41; H, 5.66%). $[\alpha]_D^{25}$ −195 ($CHCl_3$; *c* 0.37). IR ν_{max}^{KBr} cm^{-1}: 1785, 1720, 1680, 875. ^{1}H NMR and ^{13}C NMR: Tables 1 and 2. UV λ_{max}^{MeOH} nm (log ε): 212 (3.87), 234 sh (3.49). EIMS 75 eV *m/z* (rel. int.): 356 $[M]^+$ (31), 262 (100), 217 (61), 107 (87), 95 (59), 91 (63).

Rhynchosperin B (**2**). Needles from MeOH, mp. 277–279°. $[\alpha]_D^{25}$ −202 ($CHCl_3$; *c* 0.35). IR ν_{max}^{KBr} cm^{-1}: 3500, 1785, 1695, 1685, 875. ^{1}H and ^{13}C NMR: Tables 1 and 2. UV λ_{max}^{MeOH} nm (log ε): 212 (4.29), 233 sh (3.98). EIMS 75 eV *m/z* (rel. int.): 372 $[M]^+$ (18), 354 $[M-H_2O]^+$ (15), 271 (63).

Rhynchosperin C (**3**). Amorphous powder. $[\alpha]_D^{25}$ −153 ($CHCl_3$ *c* 1.76). IR ν_{max}^{KBr} cm^{-1}: 1790, 1750, 1690, 1265, 1235, 1205. ^{1}H NMR and ^{13}C NMR: Tables 1 and 2. UV λ_{max}^{MeOH} nm (log ε): 216 (4.31). EIMS 75 eV *m/z* (rel. int.): 454 $[M]^+$ (30), 372 (100), 355 (65).

Rhynchospermoside A (**4**). Amorphous powder. $[\alpha]_D^{25}$ −162 (MeOH; *c* 1.93). IR ν_{max}^{KBr} cm^{-1}: 3450, 1770, 1710, 1675, 875. ^{1}H and ^{13}C NMR: Tables 1 and 2. FAB (MeOH + glycerol) *m/z* (rel. int.): 507 $[M+H]^+$ (5), 489 $[M-H_2O]^+$ (1.5), 327 [M + H − glucose]$^+$ (44).

Rhynchospermoside B (**5**). Amorphous powder. $[\alpha]_D^{25}$ −165 (MeOH, *c* 0.98). IR ν_{max}^{KBr} cm^{-1}: 3450, 1775, 1705, 1670, 875. ^{1}H and ^{13}C NMR: Tables 1 and 2. FAB MS (MeOH + glycerol) *m/z* (rel. int.): 507 $[M+H]^+$ (6), 489 $[M+H-H_2O]$ (1), 327 [M + H − glucose]$^+$ (54).

	4	4a	5	5a
1α		1.18 (1H, *m*)		1.21 (1H, *m*)
1β		2.26 (1H, *m*)		1.99 (1H, *m*)
2α		2.47 (1H, *m*)		2.43 (1H, *m*)
2β		2.38 (1H, *m*)		2.23 (1H, *m*)
3	6.76 (1H, *br d*, 6 Hz)	6.89 (1H, *dd*, 8, 3 Hz)	6.81 (1H, *br d*, 7 Hz)	6.91 (1H, *dd*, 8, 3 Hz)
6α	3.14 (1H, *d*, 12 Hz)	2.71 (1H, *d*, 13 Hz)	2.21 (1H, *d*, 16 Hz)	2.77 (1H, *br d*, 18 Hz)
6β	2.79 (1H, *br d*, 12 Hz)	2.42 (1H, *br d*, 13 Hz)	2.09 (1H, *br d*, 16 Hz)	2.30 (1H, *dd*, 18, 2 Hz)
8	3.52 (1H, *q*, 7 Hz)	2.82 (1H, *q*, 7 Hz)	2.89 (1H, *q*, 7 Hz)	2.55 (1H, *q*, 7 Hz)
10	3.65 (1H, *br d*, 12 Hz)	2.97 (1*H*, *d*, 12 Hz)	3.23 (1H, *br* d, 12 Hz)	2.75 (1H, *br d*, 12 Hz)
11β	2.33 (1H, *dd*, 16, 9 Hz)	2.22 (1H, *dd*, 16, 9 Hz)	2.11 (1H, *dd*, 16, 9 Hz)	1.95 (1H, *dd*, 16, 10 Hz)
11α	1.63 (1H, *br d*, 16 Hz)	1.66 (1H, *dd*, 16, 3 Hz)	1.64 (1H, 16 Hz)	1.66 (1H, *d*, 16 Hz)
12	5.43 (1H, *br d*, 9 Hz)	4.94 (1H, *br d*, 9 Hz)	5.07 (1H, *br d*, 9 Hz)	4.52 (1H, *br d*, 9 Hz)
14	6.67 (1H, *m*)	6.43 (1H, *m*)	6.70 (1H, *m*)	6.38 (1H, *m*)
15	7.69 (1H, *m*)	7.42 (1H, *m*)	7.62 (1H, *m*)	7.38 (1H, *m*)
16	7.69 (1H, *m*)	7.42 (1H, *m*)	7.62 (1H, *m*)	7.38 (1H, *m*)
17	1.06 (3H, *d*, 7 Hz)	0.98 (3H, *d*, 7 Hz)	1.16 (3H, *d*, 7 Hz)	1.07 (3H, *d*, 7 Hz)
19α	4.13 (1H, *br d*, 10 Hz)	3.93 (1H, *dd*, 8, 2 Hz)	4.38 (1H, *br d*, 8 Hz)	4.33 (1H, *dd*, 8, 2 Hz)
19β	4.51 (1H, *d*, 10 Hz)	4.00 (1H, *d*, 8 Hz)	4.83 (1H, *d*, 8 Hz)	4.45 (1H, *d*, 8 Hz)
20	0.53 (3H, *s*)	0.62 (3H, *s*)	0.86 (3H, *s*)	0.99 (3H, *s*)
anomeric	4.93 (1H, *d*, 8 Hz)		4.75 (1H, *d*, 8 Hz)	

Compounds **1**, **4** and **5** run at 89.55 MHz in pyridine-d_5.
Compounds **2**, **3**, **4a** and **5a** run at 399.65 MHz in pyridine-d_5.

Table 2. ^{13}C NMR spectral data

Carbon No.	1	2	3	4	4a	5
Aglycone moiety						
1	198.8	197.1	195.9	21.0	21.4	20.9
2	132.1	131.8	132.2	27.1	27.6	26.5
3	138.8	139.5	138.5	144.0	143.8	144.0
4	57.0	51.3	52.9	128.4	132.6	127.4
5	43.6	49.3	44.7	44.7[c]	44.6[d]	44.9[e]
6	30.9	66.6	69.1	50.8	50.8	49.9
7	18.1	23.9	21.0	210.5	209.8	214.3
8	52.6	47.0[a]	47.4[b]	51.1	51.7	52.4
9	34.7	34.8	34.5	48.7[c]	48.7[d]	41.6[e]
10	45.0	44.7[a]	44.0[b]	46.2	48.5	44.2
11	47.9	47.5	47.4	44.8	45.8	44.2
12	70.2	71.0	69.7	70.0	63.0	68.8
13	124.9	122.5	125.1	136.7	139.0	136.4
14	109.3	109.5	109.5	109.6	109.5	109.4
15	144.1	144.3	144.2	140.6	137.9	140.6
16	140.5	140.9	140.7	136.9	136.8	136.9
17	173.6	173.8	173.0	8.1	8.2	9.3
18	173.6	177.3	173.0	168.5	168.0	169.0
19	71.6	71.5	71.2	71.7	71.9	73.0
20	23.0	22.6	23.2	19.3	19.1	25.0
Sugar moiety or ester moiety						
1			166.7	102.2		100.7
2			128.4	75.3		75.5
3			139.8	78.5		78.7
4			16.2	72.3		72.1
5			20.5	78.5		78.3
6				63.1		63.4

Run at 22.5 MHz in pyridine-d_5.
[a–e]Assignment may be interchanged in each column.

Alkaline hydrolysis of **3**. A soln of rhynchosperin C (**3**) (*ca* 1 mg) in dioxan (1 drop) and 4% NaOH (1 drop) was stirred under N_2 for 1 hr at room temp. The reaction mixt. was acidified with 2% HCl extd with CH_2Cl_2 and the ext. treated with *O*-*p*-nitrobenzyl-*N*,*N*'-diisopropyl isourea in a sealed ampoule for 1 hr at 90°. The reaction product was identified as *p*-nitrobenzyl tiglate by HPLC. Conditions: Develosil ODS-7 4.6 mm × 25 cm; eluent, H_2O – MeCN (2:3); R_t = 10.6 min.

Enzymatic hydrolysis of **4** *and* **5**. **4** (20 mg) was dissolved in H_2O (3 ml) and the soln treated with cellulase (50 mg) for 8 hr at 35° with stirring. The soln was passed through an Amberlite XAD-2 column and the MeOH eluate purified on a silica gel column to give **4a** (5 mg) as an amorphous powder. IR ν_{max}^{KBr} cm^{-1}: 3500, 1775, 1710, 1665, 880. ^1H and ^{13}C NMR: Tables 1 and 2. EIMS 75 eV *m/z* (rel. int.): 344 $[M]^+$ (12), 326 $[M - H_2O]^+$ (3), 254 (16), 233 (45). **5** (10 mg) was hydrolysed in the same way to give **5a** (1 mg) as an amorphous powder. ^1H NMR: Table 1.

Acid hydrolysis of **4** *and* **5**. A solution of glycoside (*ca* 0.1 mg) in 10% H_2SO_4) (2 drops) was heated at 100° for 30 min. The soln was passed through an Amberlite IR-45 column, and concd to give a residue which was reduced with $NaBH_4$ (*ca* 1 mg) for 1 hr at room temp. The reaction mixt. was passed through an Amberlite IR-120 column and concd to dryness. Boric acid was removed by dist with MeOH and the residue acetylated with Ac_2O (1 drop) and pyridine (1 drop) at 100° for 1 hr. The reagents were evapd *in vacuo*. Glucitol acetate was detected by GC. GC conditions: 1.5% OV-17, 3 mm × 1 m; column temp. 200°; carrier gas, N_2; R_t = 8.2 min.

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REFERENCES

1. Herz, W. (1978) *J. Org. Chem.* **43**, 3339.
2. Bohlmann, F. and Wegner, P. (1982) *Phytochemistry* **21**, 1693.
3. Bohlmann, F., Jakupovic, J., Hashemi-Nejad, M. and Huneck, S. (1985) *Phytochemistry* **24**, 608.
4. Bohlmann, F., Zdero, C. and Huneck, S. (1985) *Phytochemistry* **24**, 1027.