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BAKUCHIOL DERIVATIVES FROM THE LEAVES OF *PSORALEA GLANDULOSA*

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Key Word Index—*Psoralea glandulosa*; Papilionaceae; meroterpenes; bakuchiol derivatives; brine shrimp; *Artemia salina*; *Spodoptera littoralis*; disc-choice bioassay.

Abstract—Four new meroterpene derivatives have been isolated in a bioassay-guided study of the methanol extract of the leaves of *Psoralea glandulosa*, in addition to the known compounds bakuchiol and angelicin. Structures for the new compounds are proposed on spectroscopic evidence. The results of brine shrimp (*Artemia salina*) and disc-choice (*Spodoptera littoralis*) bioassays are interpreted in terms of the structural features.

INTRODUCTION

In a continuation of our chemical studies of Chilean plants as sources of bioactive compounds [1], we have now examined the methanol extract of the leaves of *Psoralea glandulosa* L., a native tree with various uses in traditional medicine [2] and observed natural resistance against herbivory. Previous chemical studies on this species [3, 4] reported the antipyretic and anti-inflammatory effect of the methanolic extract and the isolation of psoralene, angelicin, drupanin methyl ester and the meroterpene derivatives bakuchiol and cyclobakuchiols A and B. Bakuchiol has also been isolated from *P. corylifolia* [5] and has been reported to have antimutagenic [6], antimicrobial [7] and insect juvenile hormone [8] activity.

As a general guide for the detection of bioactive compounds [9], we used the brine shrimp bioassay [10] at the different stages of fractionation of the extract. This method led to the isolation of bakuchiol (**1**), angelicin (**2**) and four new meroterpenes whose structures **3–6** are proposed on the evidence discussed below. The antifeedant activity of these compounds was tested in a disc-choice bioassay with fifth instar larvae of *Spodoptera littoralis* [11] in order to assess their potential as natural pesticides.

RESULTS AND DISCUSSION

The methanolic extract was digested in cold dichloromethane to give F_1 and the residue F_2. The results of the brine shrimp bioassay (Table 1) indicated that the activity resided mainly in F_1. Repeated low pressure column chromatography of this fraction led to the isolation of compound **1**, as the main component, and compound **2**, readily identified by their NMR spectral data [3].

Four other minor compounds appeared on TLC as a complex mixture that could only be resolved by circular chromatography and preparative TLC. As the pure compounds easily decomposed on standing, the corresponding acetates were prepared under the usual conditions in order to run ^{13}C NMR and mass spectra.

Comparison of the ^1H and ^{13}C NMR resonances of compounds **3–6** (Tables 2 and 3) indicated that they are all closely related and have the same carbon skeleton as **1**. Resonances in the ^1H NMR spectrum clearly revealed that the aromatic ring, the C-6, C-7 *trans* double bond (δ 6.23 and 6.02, both *d*, H-7 and H-8), the vinyl group (δ 5.86, *dd* and 5.07, *m*) and the C-10 tertiary

Table 1. Lethal concentrations (*A. salina*, LC_{50} in ppm) and feeding ratios (FR_{50}, *S. littoralis*) of compounds **1–6Ac**

	LC_{50}	FR_{50}†
F_1 (DM)	27.4 (36.9–19.2)*	nd
F_2 (MeOH)	46.8 (57.1–33.0)	nd
1	<1	0.64±0.04
1Ac	3.6 (5.6–2.8)	nd
2	25.4 (44.0–17.3)	0.43±0.10
3	9.7 (13.1–8.0)	0.75±0.11
3Ac	46.2 (125.7–28.1)	0.47±0.07
4	18.7 (26.5–14.7)	0.35±0.19
4Ac	13.6 (16.4–10.9)	0.66±0.08
5	65.7 (71.0–60.1)	0.49±0.07
6	20.0 (32.5–13.5)	0.33±0.07
6Ac	36.0 (43.7–30.2)	0.44±0.16

*95% confidence interval [11].
†At a dose of 10 $\mu g\,cm^{-2}$.

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Table 2. ^{1}H NMR spectral data for compounds **1–6** (300 MHz, $CDCl_3$, δ values); J (Hz)

H	1	1Ac	3	3Ac	4	4Ac	5	5Ac	6	6Ac	2
2	7.22 *d* (7.2)	7.40 *d* (8.6)	6.90 *bs*	7.18 *d* (1.9)	7.24 *d* (8.3)	7.35 *d* (8.6)	7.23 *d* (8.5)	7.36 *d* (8.5)	7.21 *d* (8.5)	7.19 *d* (8.3)	
3	6.74 *d* (7.2)	7.05 *d* (8.6)			6.77 *d* (8.3)	7.02 *d* (8.6)	6.76 *d* (8.5)	7.02 *d* (8.5)	6.75 *d* (8.5)	6.86 *d* (8.3)	7.63 *d* (9.5)
4											6.21 *d* (9.5)
5	6.74 *d* (7.2)	7.05 *d* (8.6)	6.78 *bs*	7.01 *d* (8.8)	6.77 *d* (8.3)	7.02 *d* (8.6)	6.76 *d* (8.5)	7.02 *d* (8.5)	6.75 *d* (8.5)	6.86 *d* (8.3)	7.19 *d* (8.6)
6	7.22 *d* (7.2)	7.40 *d* (8.6)	6.78 *bs*	7.22 *dd* (8.8, 1.8)	7.24 *d* (8.3)	7.35 *d* (8.6)	7.23 *d* (8.5)	7.36 *d* (8.5)	7.21 *d* (8.5)	7.19 *d* (8.3)	7.25 *d* (8.6)
7	6.23 *d* (16.2)	6.34 *d* (16.3)	6.17 *d* (16.2)	6.27 *d* (16.2)	6.26 *d* (16.3)	6.32 *d* (16.3)	6.25 *d* (16.2)	6.30 *d* (16.3)	6.23 *d* (16.2)	6.14 *d* (16.2)	
8	6.03 *d* (16.2)	6.19 *d* (16.3)	6.00 *d* (16.2)	6.14 *d* (16.2)	6.02 *d* (16.3)	6.13 *d* (16.3)	6.03 *d* (16.2)	6.11 *d* (16.2)	6.03 *d* (16.2)	5.99 *d* (16.2)	7.52 *d* (2.1)
10	1.93 *m*	2.00 *m*	1.93 *m*	1.94 *m*					2.20 *d* (5.8)	2.05 *d* (5.9)	6.95 *d* H-9 (1.8)
11	1.47 *m*	1.54 *m*	1.46 *m*	1.46 *m*					5.61 *m*	5.44 *m*	
12	5.09 *bt*	5.15 *t* (7.1)	5.09 *bt*	5.09 *bt*	2.74 *bt*	2.70 *bt*	4.04 *bt*	5.14 *t* (6.6)	5.61 *m*	5.44 *m*	
14	1.65 *s*	1.71 *s*	1.67 *s*	1.67 *s*	1.32 *s*	1.30 *s*	1.70 *s*	1.70 *s*	1.30 *s*	1.13 *s*	
15	1.56 *s*	1.62 *s*	1.57 *s*	1.58 *s*	1.27 *s*	1.25 *s*	4.94 *s* 4.85 *s*	4.94 *s* 4.89 *s*	1.30 *s*	1.13 *s*	
16	1.17 *s*	1.24 *s*	1.17 *s*	1.19 *s*	1.19 *s*	1.21 *s*	1.18 *s*	1.19 *s*	1.16 *s*	1.01 *s*	
17	5.86 *dd* (10.9, 17.3)	5.92 *dd* (10.7, 17.4)	5.86 *dd* (10.9, 17.2)	5.86 *dd* (10.8, 17.4)	5.86 *dd* (17.4, 10.7)	5.86 *dd* (10.7, 17.4)	5.86 *dd* (10.8, 17.4)	5.84 *dd* (10.7, 17.5)	5.87 *dd* (10.8, 17.3)	5.71 *dd* (10.7, 17.4)	
18a	4.98 *m*	5.09 *d* (17.4)	5.00 *m*	5.00 *d* (17.4)	5.04 *d* (17.4)	5.03 *d* (17.4)	5.01 *d* (17.4)	5.02 *d* (17.4)	4.99 *d* (17.4)	4.84 *d* (17.4)	
18b		5.07 *d* (10.8)		5.05 *d* (10.7)	5.06 *d* (10.7)	5.07 *d* (10.7)	5.04 *d* (10.7)	5.06 *d* (10.7)	5.03 *d* (10.8)	4.90 *d* (10.7)	
Ac		2.31 *s*		2.29 *s* 2.28 *s*		2.28 *s*		2.29 *s* 2.06 *s*		2.12 *s*	

Table 3. ^{13}C NMR spectral data for compounds **1**, **3** and **3–6** acetate derivatives (75.4 MHz, in $CDCl_3$)

C	1	3	3Ac	4Ac	5Ac	6Ac
1	131.7	131.7	—	135.7	—	135.8
2	127.4	112.9	123.3	127.3	127.3	127.3
3	115.4	143.6	—	121.8	121.8	121.8
4	154.5	142.8	—	150.0	—	149.9
5	115.4	115.5	123.3	121.8	121.8	121.8
6	127.4	119.4	120.7	127.3	127.3	127.3
7	135.9	136.3	139.3	137.9	137.7	138.0
8	126.5	126.5	125.8	127.0	126.9	126.8
9	42.5	42.5	42.7	42.6	—	43.0
10	41.3	41.3	41.2	37.7	36.4	44.1
11	23.3	23.3	23.2	25.1	27.5	123.1
12	124.8	124.8	124.7	64.8	77.7	141.5
13	131.4	131.4	—	58.6	—	71.0
14	17.7	17.7	17.7	18.9	18.3	30.1
15	25.7	25.7	25.7	24.5	113.3	30.1
16	23.4	23.3	23.3	23.6	23.4	23.7
17	146.0	145.9	145.5	145.5	145.5	145.5
18	111.9	112.0	112.3	112.8	112.6	112.7
Ac			—	169.7	—	169.7
			20.7	21.3	21.3	21.4
			20.7		21.1	

—, Signal not detected due to low concentration.
Assignments were made from APT experiments.

methyl group (δ 1.17) were present in all compounds. The ^{13}C NMR data for compounds **4–6** suggested that the main differences lie in the region of C-10 to C-15. It is apparent that different oxidative modifications of the C-12, C-13 double bond have occurred. Mass fragmentation and IR data support this conclusion.

While the side chain of **3** and its diacetate **3Ac** [$M^+ = m/z$ 356] is identical to that of **1**, the substitution pattern of the aromatic ring clearly differed from that of **1** and the other derivatives. It was evident that there is another phenolic hydroxyl attached to the ring. The 1,2,4-nature of the aromatic protons [δ_H 7.18, *d*, 1.9 Hz; 7.01, *d*, 8.8 Hz and 7.22, *dd*, 8.8 and 1.8 Hz in **3Ac**) can be accommodated by *ortho*-hydroxyl groups as in **3** or by *meta*-hydroxyl groups. The choice of **3/3Ac** follows from a consideration of the chemical shifts of H-2 and H-6. In a *meta*-dihydroxy system, H-3 would be expected to resonate at higher field as a result of the shielding effect of its neighbouring *ortho*-hydroxyl groups. Thus, **3** is 3-hydroxybakuchiol.

The second compound produced a monoacetate [$M^+ = m/z$ 314]. Resonances at δ_C 64.6 (*d*) and 58.6 (*s*) in the carbon spectrum and a broad triplet (δ_H 2.74) in the proton spectrum of these compounds suggested the presence of a trisubstituted epoxide ring located at C-12, C-13. The observed chemical shifts of the C-13 methyl groups are consistent with this proposal. Thus, **4** is 12,13-dihydro-12,13-epoxybakuchiol. A synthetic propyl ether of this structure has been reported to have high insect juvenile hormone activity [8].

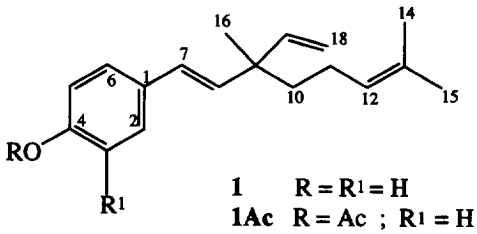
The third compound **5** has a secondary alcohol group. Thus, the ^1H NMR spectrum shows a broad triplet at δ_H 4.04 which shifts to δ_H 5.14 (J = 6.6 Hz) in the spectrum of the diacetate **5Ac** [$M^+ = m/z$ 356]. In order to account for the observed couplings the hydroxyl group must be placed at C-12. Other resonances in the NMR spectra [δ_C 113.1 (*t*), δ_H 4.94 (*s*) and 4.85 (*s*), 1.70 (3H, *s*)] are characteristic of an isopropenyl group and lead to structures **5** and **5Ac**. The reported data for similar structures [12] support this conclusion. Compound **5** is, therefore, 12-hydroxyisobakuchiol.

The side chains of **6** and its monoacetate **6Ac** contain a tertiary alcohol (ν_{max} 3404 cm^{-1}), a new disubstituted double bond (δ_H 5.61, 2H, *m*) and geminal methyl resonances at δ_H 1.30. These data suggest an allylic alcohol side chain as in **6** and **6Ac**. The carbon shifts associated with this side chain [δ_C 141.5 (*d*), 123.1 (*d*), 71.0 (*s*), 30.1 (2 × *q*) and 44.1 (*t*)] are virtually identical with those reported for a corresponding side-chain in 2,9-bisaboladiene-7,11-diol, isolated from *Achillea odorata* [12].

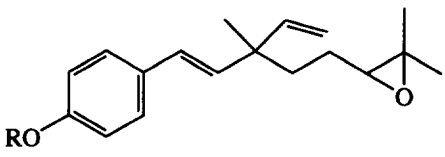
A series of diterpenoids with the same types of side chain modification as the bakuchiol congeners **4–6** has been isolated from *Halimium viscosum* and a comparison of spectral data lends further support for the proposed structures [13].

The results of the brine shrimp bioassay (Table 1) of the pure compounds indicated that **1** is the most active in this meroterpene series. The side chain and the *p*-phenolic hydroxyl group both seem to be important features for activity in this bioassay. Acetylation and/or functionalization of the terminal double bond resulted in a reduction of the toxicity.

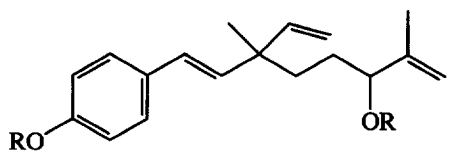
The antifeedant activity of the compounds isolated in this study is moderate at the dose tested (Table 1) and, in this case, the presence of oxygen at C-12 and C-13



1 R = R¹ = H
1Ac R = Ac ; R¹ = H
3 R = H ; R¹ = OH
3Ac R = Ac ; R¹ = OAc



4 R = H
4Ac R = Ac



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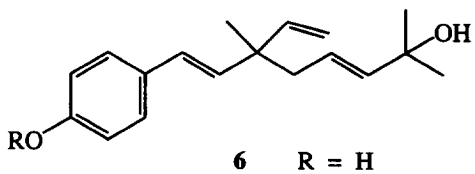
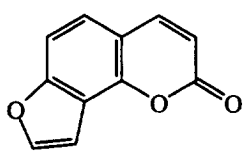
5Ac R = Ac



6 R = H
6Ac R = Ac



2

apparently enhances the feeding deterrence of **1** derivatives, while the acetylation of the phenolic hydroxyl does not have much effect on the activity.

EXPERIMENTAL

^{1}H and ^{13}C NMR: 300 and 75.4 MHz, respectively; EIMS (probe): 75 eV.

Plant material. Psoralea glandulosa was collected at Cuesta La Dormida (V Region, Chile) in March 1994 and was identified by Dr Sebastian Teillier. A voucher specimen (M-3-94) is deposited in the Chemistry Department, Universidad de Chile, Santiago.

Extraction and isolation. Ground air-dried leaves (747 g) were macerated in MeOH (5 l, room temp., 8 hr). Evapn of solvent gave 120 g crude extract which was treated with CH_2Cl_2 (1.2 l) to afford F_1 (78 g) and a residue F_2 (44.2 g). Repeated low pressure CC of F_1 using silica gel (Merck 60G) and different solvent systems (CH_2Cl_2–EtOAc; petrol–EtOAc or CCl_4–EtOAc) in frs of increasing polarity, afforded **1** (10 g) and **2** (0.1 g). Further purification of frs was achieved using a Chromatotron apparatus and prep. TLC to give **3** (112 mg), **4** (86 mg), **5** (80 mg) and **6** (96 mg).

Bakuchiol acetate (**1Ac**). Oil. UV λ_{max}^{MeOH} nm: 255; IR ν_{max}^{film} cm^{-1}: 1755 (C=O), 1617, 1600, 967 (C=C), 1497, 1433, 1359, 1200, 1091, 1002, 911, 856, 811; EIMS *m/z* (rel. int.): 298.20 [M]$^+$ (3), 215 (5.4), 173 (100), 145 (27.6), 107 (32.4), 83 (34.8), 55 (54.3), 41 (68.2), 43 (46.4); ^{1}H NMR: Table 2: ^{13}C NMR: Table 3.

3-*Hydroxybakuchiol* (**3**). Oil. UV λ_{max}^{MeOH} nm: 262; IR ν_{max}^{film} cm^{-1}: 3371 (–OH), 1600, 967 (C=C), 3044, 3000, 1511, 1439, 1364, 1278, 1194, 1100, 900, 794; ^{1}H NMR: Table 2; ^{13}C NMR: Table 3. Treatment of **3** (70 mg) with Ac_2O–pyridine yielded the acetate **3Ac** (45 mg) as an oil. EIMS *m/z* (rel. int.): 356 [M]$^+$ (2.3), 231 (65.1), 189 (100), 171 (21.3), 143 (18.4), 123 (25.1), 83 (35.4), 55 (43.3), 43 (97), 41 (50); UV λ_{max}^{MeOH} nm: 254; IR ν_{max}^{film} cm^{-1}: 1772, 1656, 1574, 1497, 1211, 1100, 1009, 967, 900; ^{1}H NMR: Table 2; ^{13}C NMR: Table 3.

12,13-*Dihydro*-12,13-*epoxybakuchiol* (**4**). Oil. UV λ_{max}^{MeOH} nm: 261; IR ν_{max}^{film} cm^{-1}: 3356 (OH), 1605, 967 (C=C), 1511, 1267, 1233, 1167, 1111, 911, 850, 819, 744, 662; ^{1}H NMR: Table 2. Treatment of **4** (40 mg) with Ac_2O–pyridine yielded the acetate **4Ac** (25 mg) as an oil. EIMS *m/z* (rel. int.): 314 [M]$^+$ (2.5), 215 (11.6), 173 (100), 145 (19.2), 107 (27.1), 79 (10.9), 43 (41.4), 41 (16.5); UV (MeOH) λ_{max} nm: 253; IR (film) ν_{max} cm^{-1}: 1756, 1622, 1594, 1511, 1367, 1200, 1000, 967, 911, 850, 819; ^{1}H NMR: Table 2; ^{13}C NMR: Table 3.

12-*Hydroxyisobakuchiol* (**5**). Oil. UV λ_{max}^{MeOH} nm: 262; IR ν_{max}^{film} cm^{-1}: 3322 (OH), 1667, 1639, 1600 (C=C), 3078, 3022, 1510, 1443, 1361, 1233, 1167, 1002, 972, 911, 850, 811, 755; ^{1}H NMR: Table 2. Treatment of **5** (30 mg) with Ac_2O–pyridine yielded the acetate **5Ac** (19 mg) as an oil. EIMS *m/z* (rel. int.): 356 [M]$^+$ (1.1), 215 (15.9), 173 (100), 145 (16.1), 79 (8.7), 43 (52.7), 41 (9.2); UV λ_{max}^{MeOH} nm: 253; IR ν_{max}^{film} cm^{-1}: 1755, 1728 (C=O), 3078, 1639, 1510, 1443, 1361, 1239, 1200, 1002, 972, 911, 854, 811; ^{1}H NMR: Table 2; ^{13}C NMR: Table 3.

Compound **6**. Oil. UV λ_{max}^{MeOH} nm: 262: IR ν_{max}^{film} cm^{-1}: 3356 (OH), 1600, 967 (C=C), 3078, 3033, 1511, 1442, 1361, 1233, 1167, 909, 852, 811, 746; ^{1}H NMR: Table 2. Treatment of **6** (70 mg) with Ac_2O–pyridine yielded the acetate **6Ac** (54 mg) as an oil. EIMS *m/z* (rel. int.): 314 [M]$^+$ (0.4), 215 (23.7), 173 (100), 145 (12.9), 107 (8.1), 79 (7.4), 43 (28.0), 41 (5.2); UV λ_{max}^{MeOH} nm: 253; IR ν_{max}^{film} cm^{-1}: 3404 (OH), 1755 (C=O), 3033, 1622, 1600, 1511, 1361, 1200, 1006, 967, 909, 852, 818, 778; ^{1}H NMR: Table 2; ^{13}C NMR: Table 3.

Bioassays with *Antemia salina* and *Spodoptera littoralis* were performed as described in refs [10] and [11], respectively.

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REFERENCES

1. Labbé, C., Castillo, M., Faini, F., Coll, J. and Connolly, J. D. (1994) *Phytochemistry* **36**, 735.
2. Muñoz, M., Barrera, E. and Meza, Y. (1981) *El Uso Medicinal de Plantas Nativas y Naturalizadas en Chile*, Publicación Ocasional No. 33. Museo Nacional de Historia Natural, Santiago, Chile.
3. Erazo, S., García, R. and Delle Monache, F. (1990) *Rev. Latinoam. Quím.* **21**, 62.
4. Backhouse, N., Delporte, C. L., Negrete, R. E., Cassels, B. K., Schneider, C., Breitmaier, E. and San Feliciano, A. (1995) *Phytochemistry* **40**, 325.
5. Mehta, G., Nayak, U. R. and Sukh, D. (1966) *Tetrahedron* **29**, 1119.
6. Wall, M., Want, M., Manikumar, G., Abraham, P., Taylor, H., Hughes, T., Warner, J. and McGirney, R. (1988) *J. Nat. Prod.* **51**, 1084.
7. Mehta, G. and Nayack, U. R. (1972) *Tetrahedron* **29**, 1115.
8. Chadha, M. S. (1986) *Proc. Indian Acad. Sci.* **B52**, 25.
9. Hamburger, M. and Hostettmann, K. (1991) *Phytochemistry* **30**, 3864.
10. Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E. and McLaughlin, J. L. (1982) *Planta Med.* **45**, 31.
11. Belles, X., Camps, F., Coll, J. and Piulachs, M. D. (1985) *J. Chem. Ecol.* **11**, 1439.
12. Barrero, A. F., Alvarez-Manzaneda, E. J. and Alvarez-Manzaneda, R. (1980) *Phytochemistry* **29**, 3213.
13. Urones, J. G., Marcos, I. S. and Garrido, N. M. (1990) *Phytochemistry* **29**, 3243.