

## **Picrolide A: An Unusual Sesquiterpene Lactone from *Acroptilon repens***

Kenneth L. Stevens, Sue C. Witt, Saima  
Kint, William F. Haddon, and Mabry Benson

*J. Nat. Prod.*, **1991**, 54 (1), 276-280 • DOI:  
10.1021/np50073a034 • Publication Date (Web): 01 July 2004

Downloaded from <http://pubs.acs.org> on April 3, 2009

### **More About This Article**

---

The permalink <http://dx.doi.org/10.1021/np50073a034> provides access to:

- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article



**ACS Publications**  
High quality. High impact.

Journal of Natural Products is published by the American  
Chemical Society, 1155 Sixteenth Street N.W., Washington,  
DC 20036

PICROLIDE A: AN UNUSUAL SESQUITERPENE LACTONE FROM  
*ACROPTILON REPENS*

KENNETH L. STEVENS,\* SUE C. WITT, SAIMA KINT, WILLIAM F. HADDON, and MABRY BENSON

Western Regional Research Center, Agricultural Research Service,  
U.S. Department of Agriculture, Albany, California 94710

**ABSTRACT.**—A sesquiterpene lactone picrolide A [1], unusual because of the occurrence of a *p*-hydroxybenzoate group, has been isolated from Russian knapweed (*Acroptilon repens*). Its structure was determined mainly by spectroscopic methods.

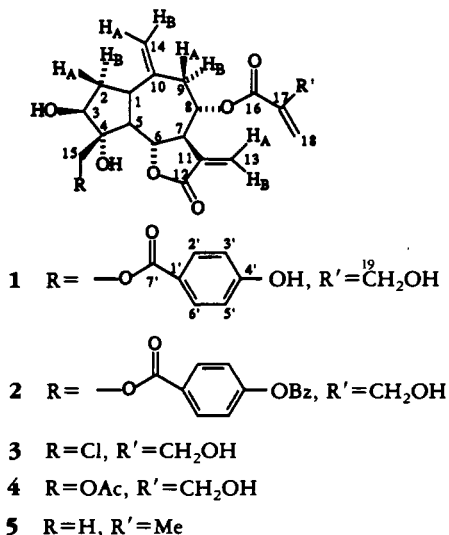
Russian knapweed (*Acroptilon repens* L., Asteraceae) is a rapidly spreading perennial in the northern Great Plains area and is fast becoming a pest of major proportions (1). The noxious weed is reported to be allelopathic (2) as well as toxic to horses (3–5). Certain sesquiterpene lactones isolated from Russian knapweed and other related thistles have demonstrated substantial toxicity in *in vitro* tests on chick embryo neurons (6,7). The present work describes the isolation and structural characterization of a new and unusual sesquiterpene lactone, picrolide A [1], from Russian knapweed.

Picrolide A [1] was isolated as dextrorotatory colorless needles from a  $\text{CH}_2\text{Cl}_2$  extract of *A. repens* by repeated cc on silica using EtOAc/Skellysolve-F, Skellysolve-F/ $\text{Me}_2\text{CO}$ , and  $\text{CH}_2\text{Cl}_2$ /EtOH as solvent systems. Ir spectral analysis showed carbonyl stretching frequencies at 1752, 1718, and  $1695\text{ cm}^{-1}$ , which are characteristic of an  $\alpha$ -methylene- $\gamma$ -butyrolactone, an  $\alpha$ -unsaturated ester, and an aromatic ester, respectively (8). Frequencies occurred at 1610, 1595, 1516, and  $1450\text{ cm}^{-1}$ ; these are suggestive of aromatic ring-breathing. Strong bands observed at 1277, 1060, and  $1155\text{ cm}^{-1}$  were suggestive of a vinyl alcohol. Broad absorption was observed at  $3100\text{--}3600\text{ cm}^{-1}$ , which indicated the presence of hydrogen-bonded hydroxyl groups. The uv spectrum,  $\lambda_{\text{max}}$  EtOH 260 nm ( $\epsilon = 15,500$ ), confirmed the presence of an aromatic moiety, namely a *p*-hydroxybenzoate (9).

The mass spectrum of 1 was unusual in that a cims spectrum in both isobutane and  $\text{NH}_3$  gave essentially no significant peaks. An eims spectrum failed to give a parent ion. However, a peak at  $m/z$  469 was observed, which by accurate mass measurement corresponds to  $\text{C}_{25}\text{H}_{25}\text{O}_9$ . The mass difference between 469 and 332 ( $469.1478 - 332.1239$ ) is 137.0239, which corresponds to  $\text{C}_7\text{H}_5\text{O}_3$ , i.e., the *p*-hydroxybenzoate portion of picrolide A [1].

A more definitive mass spectrum was obtained from the pertrimethylsilyl derivative of 1, which gave a parent ion (ei) at  $m/z$  788; exact measurement indicated a composition of  $\text{C}_{30}\text{H}_{60}\text{O}_{10}\text{Si}_4$ . Treatment of 1 with benzoyl chloride in  $\text{Me}_2\text{CO}/\text{K}_2\text{CO}_3$  gave the monobenzoate 2, mp 216–218°.

The  $^1\text{H}$ -nmr and  $^{13}\text{C}$ -nmr spectra of 1



and **2** in Me<sub>2</sub>CO-*d*<sub>6</sub> are shown in Tables 1 and 2, respectively. The assignments were made with the help of <sup>1</sup>H-<sup>1</sup>H and <sup>1</sup>H-<sup>13</sup>C correlation spectra. The *p*-substituted benzoate group resonances are readily seen (Table 1) at δ 7.98 and 6.91 (*J*<sub>o,m</sub> = 8.9 and 2.4 Hz, respectively). A carbonyl group and appropriate aromatic carbons occur at 167.1, 122.4, 132.7, 115.7, and 162.6 ppm. These correspond well with known *p*-hydroxybenzoates (10). With the exception of the aromatic group, the <sup>13</sup>C nmr spectrum of **1** exhibits resonances very similar to other guaianolide sesquiterpene lactones, e.g., **3**, previously isolated from Russian knapweed (2,11) (Table 2). For example, the hydroxymethacrylate side chain shows resonances at 165.8,

141.9, 124.7, and 61.0 ppm for C-16 through C-19, respectively, which is in excellent agreement with reported values (1,12). Likewise, the 5-membered carbocyclic ring shows resonances at 39.5, 77.0, 84.7, and 58.3 ppm, again in excellent agreement with those reported for a 3,4-dihydroxyguaianolide sesquiterpene lactone (1,12).

The remaining resonances and coupling constants are consistent with structure **1** for picrolide A. Of particular interest is the high field location of H-2A at δ 1.62. Most guaianolides of similar structure have resonances at lower field (δ 1.7–1.8) for H-2A (2,11,13). Examination of models shows that H-2A (above the plane) lies within the influence of the C-10–C-14 double bond and would be

TABLE 1. <sup>1</sup>H-nmr Spectra of **1** and **2**.<sup>a</sup>

Proton	Compound		Coupling
	<b>1</b>	<b>2</b>	
H-1 <sup>b</sup>	3.65 ddd	3.66	<i>J</i> <sub>1,2A</sub> = 7.1
H-2A <sup>c</sup>	1.62 ddd	1.64	<i>J</i> <sub>1,2B</sub> = 8.6
H-2B	2.56 ddd	2.56	<i>J</i> <sub>1,5</sub> = 11.2
H-3	4.26 dd	4.21	<i>J</i> <sub>2A,2B</sub> = 14.5
H-5	2.50 dd	2.52	<i>J</i> <sub>2A,3</sub> = 0.5
H-6 <sup>d</sup>	5.01 dd	5.04	<i>J</i> <sub>2B,3</sub> = 6.0
H-7	3.24 dddd	3.25	<i>J</i> <sub>5,6</sub> = 11.1
H-8 <sup>e</sup>	5.26 ddd	5.27	<i>J</i> <sub>6,7</sub> = 8.9
H-9A	2.78 dd	2.79	<i>J</i> <sub>7,8</sub> = 9.7
H-9B	2.47 dd	2.47	<i>J</i> <sub>7,13A</sub> = 3.1
H-13A	5.61 dd	5.62	<i>J</i> <sub>7,13B</sub> = 3.5
H-13B	6.03 dd	6.04	<i>J</i> <sub>8,9A</sub> = 1.9
H-14A <sup>f</sup>	5.14 dd	5.16	<i>J</i> <sub>8,9B</sub> = 5.0
H-14B	4.84 dd	4.86	<i>J</i> <sub>9A,9B</sub> = 14.9
H-15A	4.86 d	4.90	<i>J</i> <sub>13A,13B</sub> = 0.4
H-15B	4.51 d	4.62	<i>J</i> <sub>14A,14B</sub> = 2.3
H-18A	6.30 dd	6.29	<i>J</i> <sub>15A,15B</sub> = 11.9
H-18B	5.99 dd	5.99	<i>J</i> <sub>18A,18B</sub> = 1.7
H-19A	4.36 s	4.35	<i>J</i> <sub>18A,19A</sub> = 1.5
H-19B	4.36 s	4.35	<i>J</i> <sub>18B,19A</sub> = 1.9
H-2',6'	7.98 dd	8.22	<i>J</i> <sub>ortho</sub> = 8.9
H-3',5'	6.91 dd	7.44	<i>J</i> <sub>meta</sub> = 2.4
benzoate	—	7.62, 7.77, 8.20	

<sup>a</sup>Spectra obtained at 200 MHz in Me<sub>2</sub>CO-*d*<sub>6</sub> with TMS as an internal standard.

<sup>b</sup>nOe to H-2B, H-5 and H-14A.

<sup>c</sup>nOe to H-2B and H-9A.

<sup>d</sup>nOe to H-8 and H-9A.

<sup>e</sup>nOe to H-9A.

<sup>f</sup>nOe to H-14B.

shifted either downfield or upfield with changes in the conformation of the 7-membered ring (14). Massiot *et al.* (13) reported that opening of the 4,15-epoxide in similar compounds changed the H-8, H-9A coupling constant, which they ascribed to deformation of the 7-membered ring. A shielding effect for H-2A would seem to indicate that it is above the plane of the double bond. This information, coupled with the fact that an nOe (Table 1) is observed between H-2A to 9A, as well as from H-6 to both H-8 and H-9A, would suggest that the conformation of the 7-membered ring is a twist chair (15). This particular conformation, with the C-10-C-14 double bond pointed downward, would place H-2A in the proximity of H-9A as well as H-6, H-8, and H-9A all near each other.

In 1986 Nowak and co-workers, in a series of two papers (16,17), reported the isolation of cebellin G and pterocaulin from *Centaurea bella* and *Chartolepis pterocaula*, respectively, and assigned structures 4 and 5, respectively. Unfortunately, no data were given to substantiate the assignments, hence comparison of the nmr spectra with picrolide A is not possible. Picrolide A is the 15-(*p*-hydroxybenzoate) of deacetylated cebellin G.

Although *p*-hydroxybenzoates and other aromatic esters have been reported for sesquiterpenes, to our knowledge this is the first report of a *p*-hydroxyben-

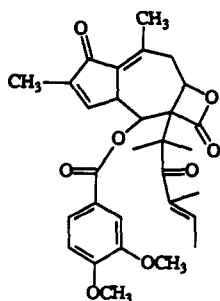
zoate of a sesquiterpene lactone. Kiseleva *et al.* (18) reported the presence of the sesquiterpene lactone 6 containing a 3,4-dimethoxybenzoate group in the

TABLE 2.  $^{13}\text{C}$ -nmr Spectra of 1 and 3.

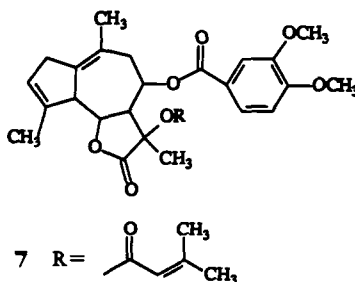
Carbon	Compound		
	1 <sup>a</sup>	1 <sup>b</sup>	3 <sup>b</sup>
C-1 . . . . .	47.6	46.6	46.6
C-2 . . . . .	39.5	40.1	40.3
C-3 . . . . .	77.0	77.1	76.5
C-4 . . . . .	84.7	84.7	85.4
C-5 . . . . .	58.3	58.6	59.6
C-6 . . . . .	77.5	77.7	77.6
C-7 . . . . .	46.9	47.7	48.7
C-8 . . . . .	74.7	74.5	74.5
C-9 . . . . .	35.8	35.7	35.4
C-10 . . . . .	144.8	144.8	144.8
C-11 . . . . .	139.2	139.1	139.0
C-12 . . . . .	169.3	169.3	169.2
C-13 . . . . .	121.2	120.9	121.1
C-14 . . . . .	117.0	116.9	116.9
C-15 . . . . .	67.3	67.6	51.3
C-16 . . . . .	165.8	165.9	165.9
C-17 . . . . .	141.9	142.4	142.4
C-18 . . . . .	124.7	124.7	124.9
C-19 . . . . .	61.0	61.0	61.1
C-1' . . . . .	122.4	122.1	
C-2' . . . . .	132.7	132.6	
C-3' . . . . .	115.7	115.9	
C-4' . . . . .	162.6	163.4	
C-5' . . . . .	115.7	115.9	
C-6' . . . . .	132.7	132.6	
C-7' . . . . .	167.1	167.2	

<sup>a</sup>Me<sub>2</sub>CO-*d*<sub>6</sub> was used as the internal standard set at 29.2 ppm.

<sup>b</sup>Pyridine was used as the internal standard set at 149.8 ppm.



6



7 R =

8 R = Ac

roots of *Ferula diversivittata*. Two compounds, malphyll [7] and malphyllin [8], isolated from *Ferula malacophylla* by Bagirov *et al.* (19), also contain the 3,4-dimethoxybenzoate moiety.

## EXPERIMENTAL

**SPECTROSCOPIC DATA.**—Nmr spectra were obtained at 200 MHz for  $^1\text{H}$  and 50 MHz for  $^{13}\text{C}$  on a Nicolet NT-200 operating at 20°. Assignments were facilitated by the use of  $^1\text{H}$ - $^1\text{H}$  correlation (COSY) (20),  $^{13}\text{C}$ - $^1\text{H}$  correlation (21), attached proton test (APT) for carbon (23), and nOe techniques (24,25). Mass spectra were measured on an MM 70/70 HS magnetic mass spectrometer (VG Analytical, Manchester, England) using direct probe sample introduction and ion source temperature 150°. Uv spectra were obtained on a Hewlett-Packard model 8451A diode array spectrophotometer. Rotations were obtained on a Perkin-Elmer model 241 polarimeter. Ir spectra were obtained on an IBM (Bruker Instruments) model IR/98 FTIR spectrophotometer.

**PLANT MATERIAL.**—*A. repens* was collected during the summer of 1989 near Discovery Bay, Contra Costa County, California. A voucher sample is deposited with K.L. Stevens, USDA, Albany, California.

**EXTRACTION AND ISOLATION.**—Dried aerial parts of *A. repens* (82.1 kg) were extracted with Skellysolve-F for 5 days at room temperature. The solvent was removed, and  $\text{CH}_2\text{Cl}_2$  was added to the plant material and allowed to stand at room temperature for 5 days. The  $\text{CH}_2\text{Cl}_2$  extract was evaporated and the residue dissolved in 5 liters of EtOH. This was added to 5 liters of 4% aqueous lead acetate to precipitate the chlorophyll. This mixture was stirred continuously and allowed to cool for 1 h; then it was filtered and the EtOH removed under vacuum. The resulting aqueous mixture was extracted with  $\text{CHCl}_3$  and dried over anhydrous  $\text{MgSO}_4$ . Repeated cc on Si gel using EtOAc/Skellysolve-F, Skellysolve-F/ $\text{Me}_2\text{CO}$ ,  $\text{CH}_2\text{Cl}_2$ /EtOH, and Skellysolve-F/EtOAc gave 650 mg of picrolide A [1]: mp 254–256°;  $[\alpha]_D^{21} =$

81.4	85.3	95.7	153.9	214.7
589	578	546	436	365

uv (EtOH)  $\lambda$  max 260 nm ( $\epsilon = 15500$ ); ir (KBr)  $\text{cm}^{-1}$  3100–3600 (broad), 1752, 1718, 1610, 1595, 1516, 1450, 1405, 1380, 1277, 1170, 1155, 1030, 1060, 1020, 976, 922, 855, 821, 773, 700;  $^1\text{H}$  nmr see Table 1;  $^{13}\text{C}$  nmr see Table 2.

**Monobenzoate of 1.**—Picrolide A [1] (40 mg) was dissolved in 5 ml of  $\text{Me}_2\text{CO}$  along with 120 mg of  $\text{K}_2\text{CO}_3$  and 37  $\mu\text{l}$  (4 molar equiv.) of ben-

zoyl chloride. The mixture was stirred at room temperature for 2.5 h, then poured into 100 ml  $\text{H}_2\text{O}$ . The product was extracted with  $\text{Et}_2\text{O}$  (3 $\times$ ), washed with 10% aqueous  $\text{NaHCO}_3$  (3 $\times$ ) and  $\text{H}_2\text{O}$  (3 $\times$ ), and dried over anhydrous  $\text{MgSO}_4$ . After filtration and evaporation the product was crystallized from  $\text{Me}_2\text{CO}$ /hexane: mp 216–218°; uv (EtOH)  $\lambda$  max 240 nm ( $\epsilon = 61430$ );  $[\alpha]_D^{26} =$

46.4	48.6	55.2	90.6	131.5
589	578	546	436	365

**TMSi derivative of 1.**—Picrolide A [1] (1 mg) was dissolved in 100  $\mu\text{l}$  of dry pyridine and treated with 100  $\mu\text{l}$  of BSTFA [*N,O*-bis(trimethylsilyl) trifluoroacetamide] at 60° for 0.5 h. Mass spectral analyses were run without further purification and gave:  $m/z$  [M] $^+$  788 (2.6%), measured 788.3242 (calcd for  $\text{C}_{30}\text{H}_{40}\text{O}_{10}\text{Si}_4$ , 788.3263),  $m/z$  [M-15] $^+$  773 (5.1%), [M-72] $^+$  716 (4.2%) (loss of Si  $\text{Me}_3$ ), [M-173] $^+$  615 (5.1%) (loss of  $\text{C}_7\text{H}_{13}\text{O}_3\text{Si Me}_3$ ), [M -  $\text{C}_{10}\text{H}_{14}\text{O}_3\text{Si}$ ] $^+$  578 (20.8%), [773 - 209 (H +  $\text{C}_{10}\text{H}_{13}\text{O}_3\text{Si}$ )] $^+$  565 (9.9%).

## LITERATURE CITED

1. K.L. Stevens and G.B. Merrill, in: "Chemistry of Allelopathy. Biochemical Interactions Among Plants." Ed. by A.C. Thompson, ACS Symposium Series 26B, American Chemical Society, Washington, D.C., 1985, p. 83.
2. K.L. Stevens, *Phytochemistry*, **21**, 1093 (1982).
3. D.R. Cordy, in: "Effects of Poisonous Plants on Livestock." Ed. by R.F. Keeler, K.R. Van Kampem, and L.F. James, Academic Press, New York, 1978, p. 327.
4. S. Young, W.W. Brown, and B. Klinger, *Am. J. Vet. Res.*, **31**, 1393 (1970).
5. G.W. Ivie and D.A. Witzel, in: "Handbook of Natural Toxins." Ed. by R.F. Keeler and A.T. Tu, Marcel Dekker, New York, 1983, p. 543.
6. K.L. Stevens, R.J. Riopelle, and R.Y. Wong, *J. Nat. Prod.*, **53**, 218 (1990).
7. R.J. Riopelle, K.E. Dow, and E. Eisenhauer, *Can. J. Phys. and Pharm.*, **62**, 1032 (1984).
8. L. Ananthasubramanian, H. Gopinath, and S.C. Bhattacharyya, *Indian J. Chem.*, **25B**, 380 (1986).
9. M. Kotake, Ed., "Constants of Organic Compounds," Asakura Publishing Co., Tokyo, 1963, p. 86.
10. "The Sadtler Standard Spectra," Sadtler Research Laboratory, Philadelphia, 1977, Vol. 13, No. 2403C.
11. A. Rusthossein, L. Nazarians, and F. Bohlmann, *Phytochemistry*, **20**, 1152 (1981).

12. M. Budesinsky and D. Saman, *Collect. Czech. Chem. Commun.*, **52**, 453 (1987).
13. G. Massiot, A.M. Morfaux, L.L. Men-Olivier, J. Bouquant, A. Madaci, A. Mahamoud, M. Chopova, and P. Aclinou, *Phytochemistry*, **25**, 258 (1986).
14. L.M. Jackman and S. Sternhell, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," 2nd ed., Pergamon Press, New York, 1969, p. 83.
15. R. Bucourt, in: "Topics in Stereochemistry." Ed. by E.L. Eliel and N.L. Allinger, John Wiley & Sons, New York, 1974, Vol. 8, p. 159.
16. G. Nowak, B. Drozd, M. Holub, M. Budesinsky, and D. S'Man, *Acta Soc. Bot. Pol.*, **55**, 227 (1986).
17. G. Nowak, B. Drozd and M. Holub, *Acta Soc. Bot. Pol.*, **55**, 233 (1986).
18. V.V. Kiseleva, A.I. Saidkhodzhaev, and G.K. Nikonov, *Izv. Akad. Nauk Turkm. SSR, Ser. Fiz. Tekh., Khim. Geol. Nauk*, 126 (1975).
19. V.Y. Bagirov, V.I. Sheichenko, R.Y. Gasanova and M.G. Pimenov, *Khim. Prir. Soedin.*, 445 (1978).
20. R. Freeman and G. Morris, *J. Magn. Reson.*, **42**, 164 (1981).
21. A. Bax and G.A. Morris, *J. Magn. Reson.*, **42**, 501 (1981).
22. S.L. Patt and J.N. Shoolery, *J. Magn. Reson.*, **46**, 535 (1982).
23. A.E. Derome, "Modern NMR Techniques for Chemistry Research," Pergamon Press, Oxford, England, 1987, pp. 112-121.
24. D. Neuhaus and M.P. Williamson, "The Nuclear Overhauser in Structural and Conformational Analysis," VCH, New York, 1989.
25. A.J. Shaka, C.J. Bauer, and R. Freeman, *J. Magn. Reson.*, **60**, 479 (1984).

Received 18 June 1990