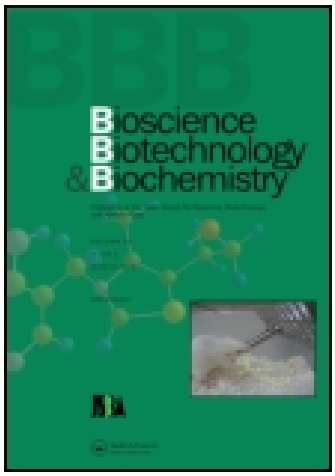

Bioscience, Biotechnology, and Biochemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tbbb20

Antitumoral and Antimicrobial Activities of Bitter Sesquiterpene Lactones of Vernonia amygdalina, a Possible Medicinal Plant Used by Wild Chimpanzees

Mitsuo Jisaka[a], Hajime Ohigashi[a], Kazunori Takegawa[a], Michael A. Huffman[ab] & Koichi Koshimizu[a]

[a] Department of Food Science and Technology, Faculty of Agriculture, Kyoto University, Kyoto 606, Japan

[b] Department of Zoology, Faculty of Science, Kyoto University, Kyoto 606, Japan

Published online: 12 Jun 2014.

To cite this article: Mitsuo Jisaka, Hajime Ohigashi, Kazunori Takegawa, Michael A. Huffman & Koichi Koshimizu (1993) Antitumoral and Antimicrobial Activities of Bitter Sesquiterpene Lactones of Vernonia amygdalina, a Possible Medicinal Plant Used by Wild Chimpanzees, Bioscience, Biotechnology, and Biochemistry, 57:5, 833-834, DOI: 10.1271/bbb.57.833

To link to this article: http://dx.doi.org/10.1271/bbb.57.833

Biosci. Biotech. Biochem., **57** (5), 833–834, 1993

Note

Antitumoral and Antimicrobial Activities of Bitter Sesquiterpene Lactones of *Vernonia amygdalina*, a Possible Medicinal Plant Used by Wild Chimpanzees

Mitsuo JISAKA,† Hajime OHIGASHI, Kazunori TAKEGAWA, Michael A. HUFFMAN,* and Koichi KOSHIMIZU††

Department of Food Science and Technology, Faculty of Agriculture, Kyoto University, Kyoto 606, Japan
**Department of Zoology, Faculty of Science, Kyoto University, Kyoto 606, Japan*
Received August 10, 1992

Vernonia amygdalina (Compositae), which is known for its very bitter taste, is a plant that has been reported for its possible medicinal use by wild chimpanzees.[1] In a previous study, we found two classes of bitter compounds in this plant: novel sigmastane-type steroid glucosides[2,3]; and four known sesquiterpene lactones, vernodalin (**1**), vernolide (**2**), hydroxyvernolide (**3**), and vernodalol (**4**).[4] Based on the presumption that a chimpanzee observed to ingest this plant was likely to be suffering from a parasite-related disease,[1] the *in vitro* antischistosomal activity of these bitter and related compounds has previously been investigated.[5] In addition, antitumoral and antibacterial activities were found in a partially purified fraction containing **1–3**. This report describes the *in vitro* antitumoral activity against mouse leukemia cells, P-388 and L-1210, and the antibacterial activity against Gram-positive bacteria, *Bacillus subtilis* and *Micrococcus lutea*.

Compounds **1**, **2**, and **3** were obtained (2.3 g, 52.5 and 70.1 mg, respectively) from the ethyl acetate-soluble part of a leaf extract (800 g).[4] The occurrence of **3** in *V. amygdalina* was first found in this study. Compound **4** was obtained as an artifact from **1** after extracting with methanol. Compounds **1**, **2**, and **3** tasted more bitter (bitterness: 0.8, 1.2, and 1.0 μg, respectively; see Table) than bitter steroids glucosides.[2,3]

Kupchan *et al.* have reported the antitumoral activity of **1** and **2**, using KB cells, and also pointed out the importance of the α-methylene-γ-lactone for this activity.[6,7] However, the antitumoral activity against such leukemia cells as P-388 and L-1210 have not been investigated for **1–4** and their derivatives, except for the *in vivo* activity of the homologous compounds, vernolepin (**5**) and vernomenin (**6**).[8] Therefore, the *in vitro* antitumoral activity against P-388 and L-1210 cells was tested for **1–4** and some of the derivatives (**5–10**) of **1** shown in Fig.

As shown in Table, vernodalin (**1**) and vernolide (**2**) exhibited potent activity (IC_{50} for P-388 and L-1210 cells: 0.11 and 0.17 μg/ml for **1** and 0.13 and 0.11 μg/ml for **2**, respectively), while the activity of hydroxyvernolide (**3**) and vernodalol (**4**) was weak. The lower activity of **3** compared with **2** could be explained by the loss of hydrophobicity in the acyl moiety. On the basis of the results for **1**, **5**, and **6**, the presence of the hydroxymethacryloyl moiety was not significant for the activity of the vernodalin-related compounds. However, it may still be possible that the activity of **1** can be increased if the acyl moiety is changed to a more hydrophobic group (methacryloyl group) as in the case of **2**. 4,15-Dihydrovernodalin (**7**) and 1,2,2′,3′-tetrahydrovernodalin (**8**) showed no significant change in activity compared to that of **1**. However, when the $\Delta^{11,13}$ double bond of **8** was saturated (compound **9**), the activity was much less. Moreover, **4** was less active than **1**, and **10** was inactive even at 50 μg/ml. Thus, the importance of the α-methylene-γ-lactone was also indicated for the antitumoral activity of **1** against P-388 and L-1210 cells.

The antibacterial activity of **1–10** against two Gram-positive (*Bacillus subtilis* and *Micrococcus lutea*) and two Gram-negative bacteria (*Escherichia coli* and *Agrobacterium tumefaciens*) (see Table) was measured by the pulp disc method. None of the compounds tested here exhibited activity against the two Gram-negative bacteria. However, compounds **1**, **2**, **3**, **5**, **6**, and **7** strongly inhibited the growth of *B. subtilis* and *M. lutea* at 5 μg/disk. The activity of the vernodalin derivatives decreased in the order of **8**>**9**=**4**, and **10** was inactive at 50 μg/disk. This structure–activity relationship correlates with that found for the antitumoral activity.

Interestingly, while the antitumoral and antibacterial activities of **10** were insignificant, its degree of bitterness was quite high (see Table). Only vernodalol (**4**) had low bitterness, suggesting that either α-methyl- or α-methylene-γ-lactone is important for the bitter taste of the vernodalin-related compounds.

Experimental

Preparation of the derivatives of 1. The structures of the derivatives of **1** prepared as next described were confirmed by IR, MS, and ^{1}H-NMR spectra (data not shown), although the configuration of the newly formed methyl groups is unknown.

Vernolepin (**5**) and vernomenin (**6**): Vernodalin (**1**, 50.7 mg) in dioxane (3.5 ml) was hydrolyzed with 5% KOH (0.7 ml) at room temperature for 1 h. The product was then refluxed in dry benzene (9 ml) containing

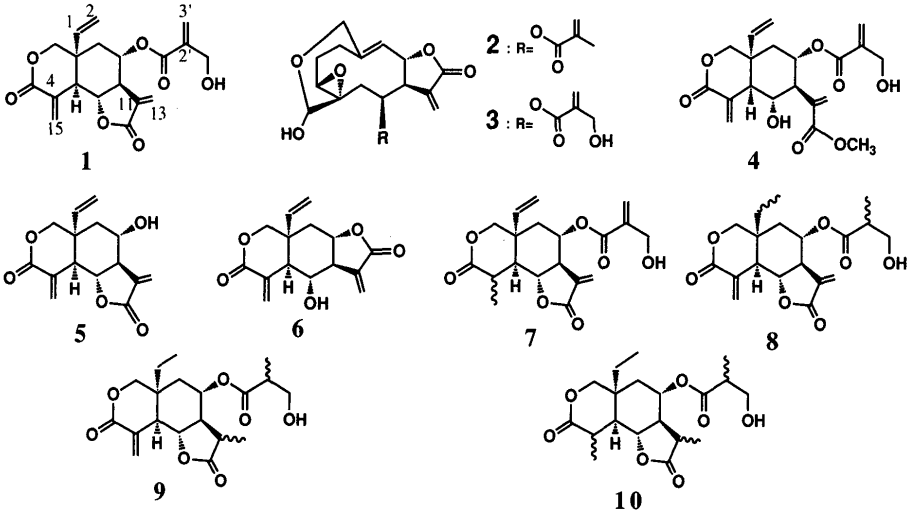

Fig. Structures of the Sesquiterpene Lactones.

† Present address: *Department of Bioresources Sciences, Faculty of Agriculture, Shimane University, Matsue 1060, Japan.*
†† To whom correspondence should be addressed.

Table Biological Activity of the Sesquiterpene Lactones

Compound	Bitterness (μg)	Antitumoral activity IC_{50} (μg/ml)		Antimicrobial activity (μg/disk)	
		P-388	L-1210	*B. subtilis*	*M. lutea*
Vernodalin (**1**)	0.8	0.11	0.17	5	5
Vernolide (**2**)	1.2	0.13	0.11	5	5
Hydroxyvernolide (**3**)	1.0	0.92	1.25	5	5
Vernodalol (**4**)	70.0	0.32	0.61	50	50
Vernolepin (**5**)	1.2	0.12	0.29	5	5
Vernomenin (**6**)	1.0	0.17	0.32	5	5
4,15-Dihydro-vernodalin (**7**)	0.8	0.07	0.15	5	5
1,2,2′,3′-Tetrahydro-vernodalin (**8**)	2.5	0.14	0.26	10	10
1,2,11,13,2′,3′-Hexa-hydrovernodalin (**9**)	2.4	0.52	1.00	50	50
1,2,4,15,11,13,2′,3′-Octahydroverno-dalin (**10**)	0.4	>50	>50	>50	>50

p-toluenesulfonic acid (8 mg) for 90 min to yield **5** and **6** (10.1 and 7.2 mg, respectively).[8] Dihydrovernodalin (**7**): Vernodalin (**1**, 57.7 mg) in MeOH (2 ml) was reduced with $NaBH_4$ (2 mg) at 0°C for 8 min to yield **7** (8.2 mg). 1,2,2′,3′-Tetrahydrovernodalin (**8**) and 1,2,11,13,2′,3′-hexahydrovernodalin (**9**): Vernodalin (**1**, 138 mg) in EaOAc (20 ml) was hydrogenated over 5% Pd–C (50 mg) to yield **8** and **9** (12.0 and 18.3 mg, respectively). 1,2,4,15,11,13,2′,3′-Octahydrovernodalin (**10**): Hexahydrovernodalin (**9**, 9.48 mg) in EtOH (1 ml) was reduced with $NaBH_4$ (1.13 mg) at 0°C for 10 min to yield **10** (4.89 mg).

Determination of bitterness. The minimum threshold level for bitterness was determined by tasting a filter paper (1 cm^2 × 0.2 mm thick) soaked with a known amount of the test compound.[4]

Determination of the in vitro antitumoral activity. P-388 or L-1210 mouse leukemia cells (1×10^4 cells) were incubated in RPMI 1640 medium supplemented with 10% fetal calf serum and a known amount of the test compound under 5% CO_2 at 37°C. After a 3-day incubation period, the number of cells was compared with that in the control medium without the test compound.[9] The concentration for a 50% inhibition (IC_{50}) of cell proliferation was determined.

Determination of the antibacterial activity. The bacteria were preincubated for 24 h in a shaken liquid medium (meat extract, 0.5%; peptone, 1%; NaCl, 0.5%). A portion of the precultivated medium (0.1 ml) was poured uniformly onto an agar medium (agar, 1.5%; extract, 0.5%; poptone, 1%; NaCl, 0.5%) in a Petri dish (8.5 cm diameter, 5 ml of medium). A paper disk (8 mm diameter × 0.5 mm thick) containing a known amount of the test compound was placed on the agar medium, which was then incubated for 24 h at 37°C. The minimum inhibiting dose per disk to form an antimicrobial zone was determined.

Acknowledgments. This study was supported by a Grant-in-Aid for General Scientific Research from the Ministry of Education, Science, and Culture of Japan. We thank members of the Institute of Life Science of Snow Brand Milk Products Co., Ltd. for the *in vitro* antitumoral activity tests.

References

1) M. A. Huffman and M. Seifu, *Primates*, **30**, 51–63 (1989).
2) H. Ohigashi, M. Jisaka, T. Takagaki, H. Nozaki, T. Tada, M. A. Huffman, T. Nishida, M. Kaji, and K. Koshimizu, *Agric. Biol. Chem.*, **55**, 1201–1203 (1991).
3) M. Jisaka, H. Ohigashi, T. Takagaki, H. Nozaki, T. Tada, M. Hirota, R. Irie, M. A. Huffman, T. Nishida, M. Kaji, and K. Koshimizu, *Tetrahedron*, **48**, 625–632 (1992).
4) H. Ohigashi, M. Jisaka, T. Takagaki, K. Koshimizu, M. A. Huffman, T. Nishida, H. Takasaki, and M. Kaji, *AFRICA-KENKYU* (*Journal of African Studies*), **39**, 15–27 (1992) (in Japanese with English summary).
5) M. Jisaka, M. Kawanaka, H. Sugiyama, K. Takegawa, M. A. Huffman, H. Ohigashi, and K. Koshimizu, *Biosci. Biotech. Biochem.*, **56**, 845–846 (1992).
6) S. M. Kupchan, R. J. Hemingway, A. Karim, and D. Werner, *J. Org. Chem.*, **34**, 3908–3911 (1969).
7) S. M. Kupchan, M. A. Eakin, and A. M. Thomas, *J. Med. Chem.*, **14**, 1147–1154 (1971).
8) S. M. Kupchan, R. J. Hemingway, D. Werner, and A. Karim, *J. Org. Chem.*, **34**, 3903–3908 (1969).
9) T. W. Doyle and W. T. Brander, in "Anticancer Agents Based on Natural Product Models," ed. by J. M. Cassady and J. D. Douros, Acedemic Press, New York, 1980, pp. 43–72.