Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
ELSEVIER
Bioorganic & Medicinal Chemistry Letters 14 (2004) 1329–1332

Anti-AIDS Agents. Part 57:† Actein, an anti-HIV principle from the rhizome of *Cimicifuga racemosa* (black cohosh), and the anti-HIV activity of related saponins☆

Nobuko Sakurai,[a] Jiu-Hong Wu,[a] Yutaka Sashida,[b] Yoshihiro Mimaki,[b] Tamotsu Nikaido,[c] Kazuo Koike,[c] Hideji Itokawa[a] and Kuo-Hsiung Lee[a,*]

[a]*Natural Products Laboratory, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599-7360, USA*
[b]*Tokyo University of Pharmacy and Life Science, Horinouchi, Hachioji, Tokyo, Japan*
[c]*Toho University School of Pharmaceutical Sciences, Funabashi, Chiba, Japan*

Received 2 July 2003; accepted 2 December 2003

Abstract—Actein (**1**), a tetracyclic triterpenoid from the rhizome of *Cimicifuga racemosa* (black cohosh), and 82 related triterpenoid and steroidal saponins isolated from higher plants were evaluated for anti-HIV activity as a continuing study to discover potential anti-AIDS agents from natural products. Actein showed potent activity and another twelve saponins showed moderate activity. The active compounds included two steroidal, seven tetracyclic triterpenoid, and four pentacyclic triterpenoid compounds.

In our continuing search for novel anti-HIV agents from natural products, we found that a methanol extract from the rhizome of *Cimicifuga racemosa*, commonly known as black cohosh, exhibited anti-HIV activity. In Europe and the United States, this herb is gaining popularity as a phytoestrogen for menopausal symptoms;[2] and in Japan and China, some *Cimicifuga* spp. are used as antipyretic and analgesic agents in traditional Chinese medicine.[3] The terpenoid saponin actein (**1**) was identified as the anti-HIV principle of *C. racemosa* by bioactivity-directed fractionation and isolation. As our group has been interested in the anti-HIV activity of various natural products,[4,5] including a recent investigation of triterpene derivatives of betulinic acid[6] and ursolic acid,[7] we also evaluated 83 related saponins (**1**–**83**), which are isolated from various plant species, including Ranunculaceae,[3,8] Liliaceae,[9] Leguminoseae,[10] Caryophyllaceae,[11] Myrsinaceae,[12] and Hippocastanaceae,[13] (Tables 1–3, Supporting Information),[14] for inhibition of HIV replication in H9 lymphocytes.

Saponins are classified into several structural types: S series are steroidal saponins, T series are tetracyclic triterpenes, and G series are oleanane-type triterpenes. Our study included 32 T-type (**1**–**32**), 5 S-type (**33**–**37**), and 46 G-type (**38**–**83**) saponins. T-type and S-type saponins have fewer sugar moieties than G series saponins, which are glycosylated at C-3 and C-28 and contain one to three geranyl moieties. Some T- and S-type saponins contain spiroketal moieties.

Thirteen out of the 83 tested saponins showed activity against HIV replication in H9 lymphocytes.[15] The data are shown in Table 4 and the active structures are given in Figure 1. The most promising compounds were T-type saponins, with actein (**1**) being the most potent compound with an EC_{50} of 0.375 μg/mL and TI of 144. Six additional T type saponins (**2**, **3**, **4**, **6**, **14**, **22**) had TI values ranging from 10.5 to 30.2. Compounds **1**–**4** and **6** were isolated from *Cimicifuga racemosa* and are 9,19-cyclolanostanol xylosides.[8] Compound **14** was obtained by acetylation of acetylshengmanol xyloside, which was isolated from the rhizome of *Cimicifuga dahurica*.[3] Compound **22**, scillacilloside D-2, was isolated from *Scilla perviana*.[9] Two steroidal saponins (**33**[16] and **34**[17]) had EC_{50} values <0.25 μg/mL, but were also quite cytotoxic (IC_{50} ~ 0.5 μg/mL). Four G type saponins (**38**–**41**)[11] showed only moderate activity and had low TI values. The remaining saponins showed no HIV suppression. Actein (**1**) appears to be a promising lead compound for future structure modification studies.

☆Supplementary data associated with this article can be found at, doi:10.1016/j.bmcl.2003.12.035
† For Part 56, see ref 1.
* Corresponding author. Tel.: +1-919-962-0066; fax: +1-919-966-3893; e-mail: khlee@unc.edu

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2003.12.035

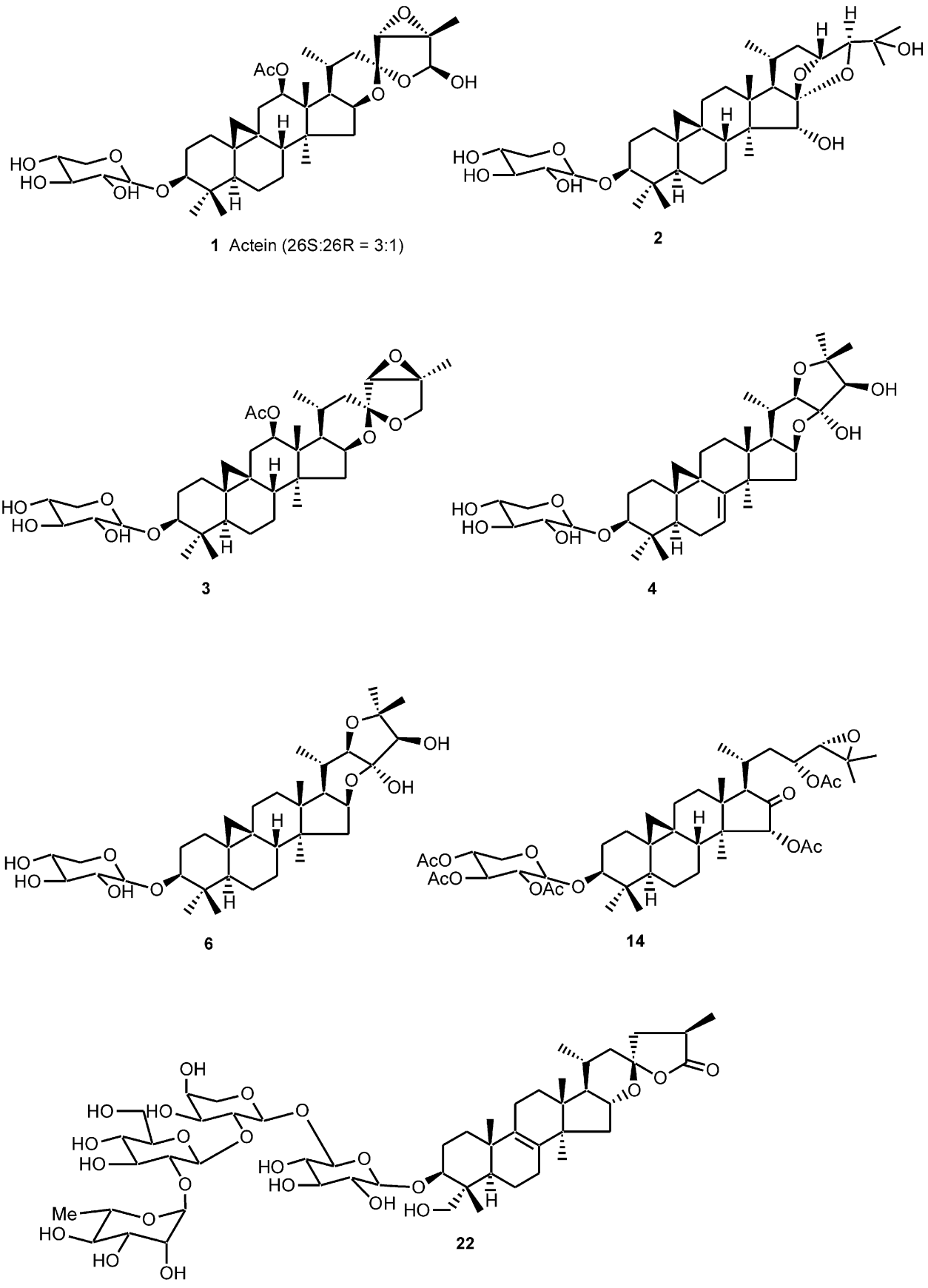

Figure 1.

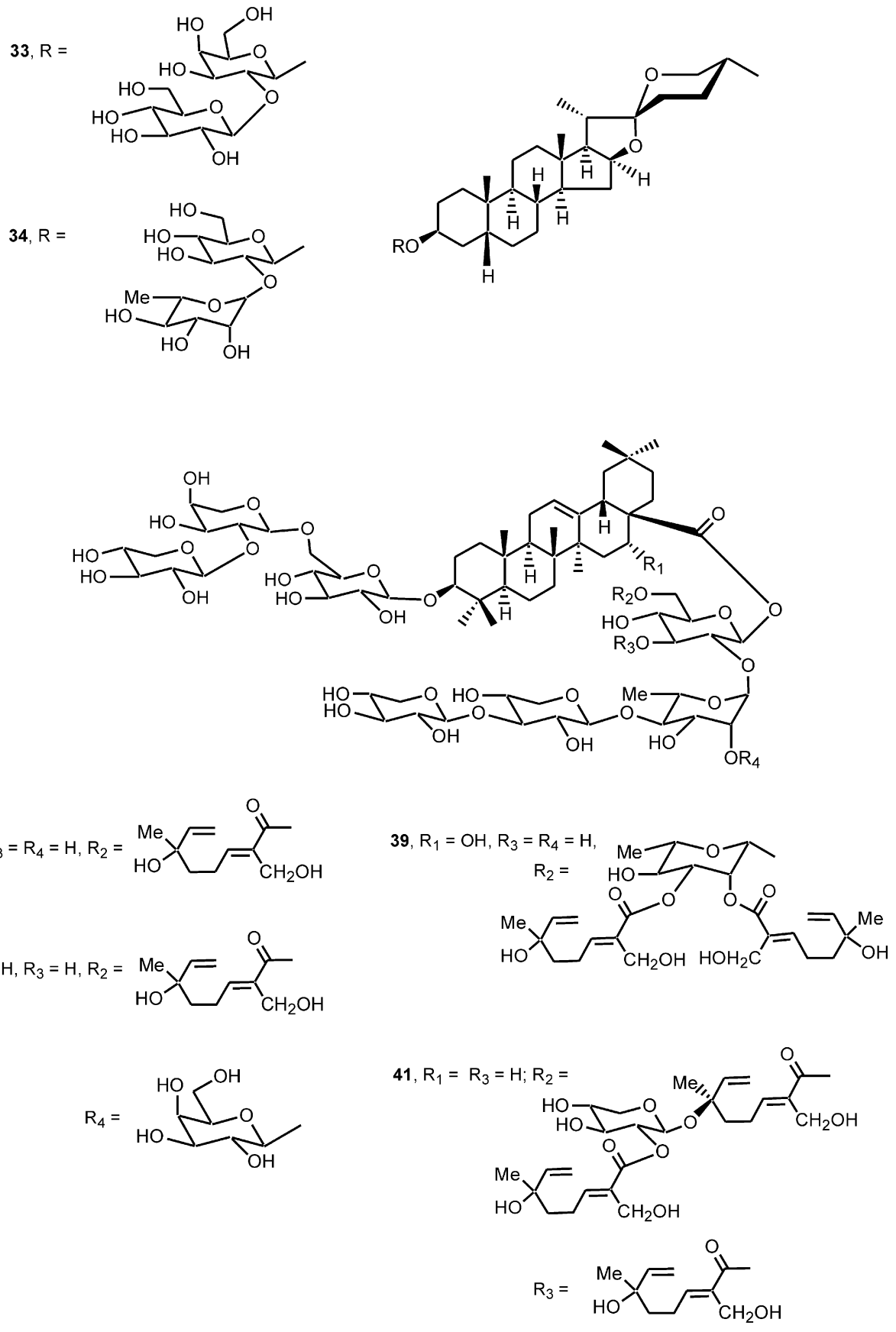

Figure 1 (continued).

Acknowledgements

This investigation was supported by a grant from the National Institute of Allergies and Infectious Diseases, NIH (AI-33066) awarded to K.-H. Lee.

References and notes

1. For Anti-AIDS Agents, Part 56, see: Chen, Y.; Zhang, Q.; Zhang, B.; Xia, P.; Xia, Y.; Yang, Z. Y.; Kilgore, N.; Wild, C.; Morris-Natschke, S. L.; Lee, K. H. *Bioorg. Med. Chem.,* to be submitted.
2. McKenna, D. J.; Jones, K.; Humphrey, S.; Hughes, K. *Alternative Therapies* **2001**, *7*, 93.
3. Sakurai, N.; Nagai, M. *Yakugaku Zasshi* **1996**, *116*, 850.
4. Yu, D.; Suzuki, M.; Xie, L.; Morris-Natschke, S. L.; Lee, K. H. *Med. Res. Rev.* **2003**, *23*, 322.
5. Sun, I. C.; Kashiwada, Y.; Morris-Natschke, S. L.; Lee, K. H. *Curr. Topics Med. Chem.* **2003**, *3*, 155.
6. Sun, I. C.; Chen, C. H.; Kashiwada, Y.; Wu, J. H.; Wang, H. K.; Lee, K. H. *J. Med. Chem.* **2002**, *45*, 4271 and literature cited therein.
7. Kashiwada, Y.; Nagao, T.; Hashimoto, H.; Ikeshiro, Y.; Okabe, H.; Cosentino, L. M.; Lee, K. H. *J. Nat. Prod.* **2000**, *63*, 1612.
8. (a) Watanabe, K.; Mimaki, Y.; Sakagami, H.; Sashida, Y. *Chem. Pharm. Bull.* **2002**, *50*, 121. (b) Chen, S. N.; Wenkui, L.; Fabricant, D. S.; Santarsiero, B. D.; Mesecar, A.; Fitzloff, J. F.; Fong, H. H. S.; Farnsworth, N. R. *J. Nat. Prod.* **2002**, *65*, 601.
9. (a) Ori, K.; Kuroda, M.; Mimaki, Y.; Hasegawa, H.; Sashida, Y. *Chem. Pharm. Bull.* **2003**, *51*, 92 and literature cited therein. (b) Mimaki, Y.; Nishino, H.; Ori, K.; Kuroda, M.; Matsui, T.; Sashida, Y. *Chem. Pharm. Bull.* **1994**, *42*, 327.
10. (a) Zhang, Z.; Koike, K.; Jia, Z.; Nikaido, T.; Guo, D.; Zheng, J. *J. Nat. Prod.* **1999**, *62*, 740. (b) Zhang, Z.; Koike, K.; Jia, Z.; Nikaido, T.; Guo, D.; Zheng, J. *J. Nat. Prod.* **1999**, *47*, 388. (c) Zhang, Z.; Koike, K.; Jia, Z.; Nikaido, T.; Guo, D.; Zheng, J. *J. Nat. Prod.* **1999**, *62*, 877.
11. Jia, Z.; Koike, K.; Sahu, N. P.; Nikaido, T. *Studies in Natural Products Chemistry—Bioactive Natural Products, Part G* **2002**, *26*, 3.
12. Koike, K.; Jia, Z.; Nikaido, T. *Chem. Pharm. Bull.* **2001**, *49*, 758.
13. Zhang, Z.; Koike, K.; Jia, Z.; Nikaido, T.; Guo, D.; Zheng, J. *J. Nat. Prod.* **1999**, *62*, 740.
14. Names and plant sources of all compounds studied are available in Tables 1–3 as Supporting Information.
15. Anti-HIV activity was evaluated at Panacos Pharmaceuticals. The biological evaluation of HIV-1 inhibition was carried out according to established protocols. The H9 T cell line was maintained in continuous culture with complete medium (RPMI 1640 with 10% fetal calf serum supplemented with L-glutamine at 5% CO_2 and 37 °C). Aliquots of this cell line were only used in experiments when in log-phase growth. Test samples were first dissolved in dimethyl sulfoxide. The following final drug concentrations were routinely used for screening: 100, 20, 4, and 0.8 μg/mL. For active agents, additional dilutions were prepared for subsequent testing so that an accurate EC_{50} value (defined below) could be achieved. As the test samples were being prepared, an H9 cell aliquot was infected with HIV-1 (IIIB isolate) while another aliquot was mock-infected with complete medium. The stock virus used for these studies typically had a $TCID_{50}$ value of 10^4 Infectious Units/mL. The appropriate amount of virus for a multiplicity of infection (moi) between 0.1 and 0.01 Infectious Units/cell was added to the first aliquot of H9 cells. The other aliquot received only culture medium, and these mock-infected cells were used for toxicity determinations (IC_{50}, defined below). After a 4 h incubation at 37 °C and 5% CO_2, both cell populations were washed three times with fresh medium and then added to the appropriate wells of a 24 well-plate containing the various concentrations of the test drug or culture medium (positive infected control/negative drug control). In addition, AZT was also assayed during each experiment as a positive drug control. The plates were incubated at 37 °C and 5% CO_2 for 4 days. Cell-free supernatants were collected on Day 4 for use in our in-house p24 antigen ELISA assay. P24 antigen is a core protein of HIV and therefore is an indirect measure of virus present in the supernatants. Toxicity was determined by performing cell counts by a Coulter Counter on the mock-infected H9 cells, which had either received culture medium (no toxicity), test sample, or AZT. If a test sample had suppressive capability and was not toxic, its effects were reported in the following terms: IC_{50}, the concentration of test sample that was toxic to 50% of the mock-infected H9 cells; EC_{50} the concentration of the test sample that was able to suppress HIV replication by 50%; and Therapeutic Index (TI), the ratio of IC_{50} to EC_{50}.
16. Inoue, T.; Mimaki, Y.; Sashida, Y.; Kobayashi, M. *Chem. Pharm. Bull.* **1995**, *43*, 1162.
17. Mimaki, Y.; Kuroda, M.; Obata, Y.; Sashida, Y. *Nat. Prod. Lett.* **2000**, *14*, 357.