Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No 11, pp. 1323-1326, 1994

0960-894X/94 $7.00+0.00

0960-894X(94)00149-9

CUCURBITACIN F DERIVATIVES, ANTI-HIV PRINCIPLES FROM *COWANIA MEXICANA*[1]

Takao Konoshima,[a] Yoshiki Kashiwada,[b] Midori Takasaki,[a] Mutsuo Kozuka,[a] Ichiro Yasuda,[b] L. Mark Cosentino,[c] and Kuo-Hsiung Lee[b*]

[a]*Kyoto Pharmaceutical University, Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607, Japan*

[b]*Natural Products Laboratory, Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599*

[c]*Biotech Research Laboratories, 3 Taft Court, Rockville, Maryland 20850*

Abstract : 23,24-Dihydro- (**2**) and 15-oxo-cucurbitacin F (**3**), and 15-oxo-23,24-dihydrocucurbitacin F (**4**), isolated from *Cowania mexicana*, demonstrated inhibitory activity against HIV-1 replication in H9 cells with EC_{50} values of 0.8, 0.3, and 2.5 μg/ml, respectively, and therapeutic index values of 11.6, 17.0, and 15.2, respectively.

In our continuing search for novel anti-HIV agents from natural products, we found that the MeOH extract of the leaves of *Cowania mexicana* (Rosaceae) exhibited anti-HIV activity in addition to its antitumor promoter activity.[3] Primary bioactivity-guided solvent fractionation of the MeOH extract with *n*-hexane, CH_2Cl_2, EtOAc, and *n*-BuOH revealed that potent anti-HIV activity was concentrated in the CH_2Cl_2-soluble fraction. Subsequent bioassay-directed fractionation of this fraction led to the isolation and characterization of four cucurbitacins (**1** – **4**). The structure of **1** – **4** were assigned as cucurbitacin F (**1**), 23,24-dihydrocucurbitacin F (**2**), 15-oxocucurbitacin F (**3**), and 15-oxo-23,24-dihydrocucurbitacin F (**4**), respectively.[3]

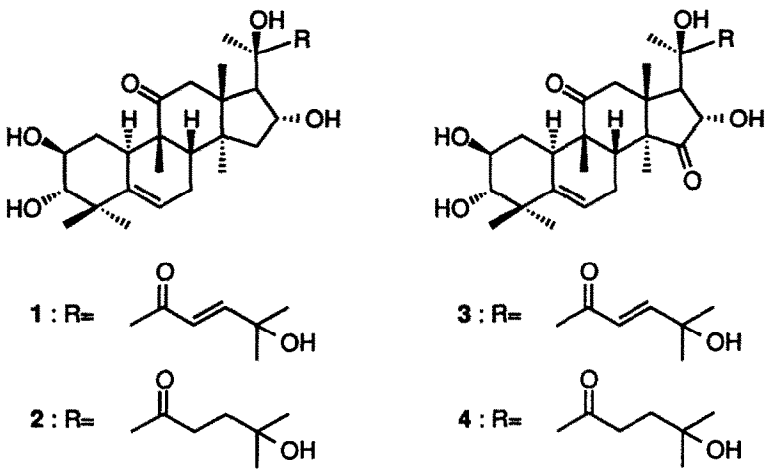

Table 1. Anti-HIV activity of compounds **1** — **4**

	IC_{50} (μg/ml)	EC_{50} (μg/ml)	Therapeutic Index
1	0.16	0.06	2.7
2	9.3	0.8	11.6
3	5.1	0.3	17.0
4	38.0	2.5	15.2

As shown in Table 1, compound **3** demonstrated potent anti-HIV activity in acutely infected H9 cells with an EC_{50} value of 0.3 μg/ml. It also exhibited good therapeutic index value of 17.0. Compounds **2** and **4** also showed anti-HIV activities with EC_{50} values of 0.8 and 2.5 μg/ml, respectively, and therapeutic index values of 11.6 and 15.2, respectively. Compound **1** showed inhibitory activity only in the toxic concentration.

Based on these good preliminary results, biological evaluation of other types of cucurbitacins is in progress.

Experimental

PLANT MATERIAL — *C. mexicana* was collected in Utah, USA and was identified by Dr. James R. Estes, Department of Botany, University of Oklahoma at Norman. Voucher specimens are deposited in the herbarium of Kyoto Pharmaceutical University, Kyoto, Japan.

EXTRACTION AND ISOLATION — The air-dried, powdered leaves (1.5 kg) of *C. mexicana* were extracted with hot MeOH. Concentration and extraction with *n*-hexane, CH_2Cl_2, EtOAc, and *n*-BuOH gave subsequent organic fractions. Chromatography of the CH_2Cl_2 fraction on silica gel (eluting with $CHCl_3$ and increasing polarities of $CHCl_3$–MeOH) and with preparative HPLC gave the four cucurbitacins **1** – **4**, which were identified on the basis of spectral determinations.[3] The complete experimental details are given in reference 3.

BIOLOGICAL ASSAY — HIV inhibition was measured as described previously.[4,5] The H9 T cell line was maintained in continuous culture with complete medium (RPMI 1640 and 10% fetal calf serum) at 5% CO_2 and 37 °C and was used in experiments only when in log phase of growth. The cells were incubated with HIV-1 (IIIB isolate, $TCID_{50}$ 10^4 IU/ml, at a multiplicity of infection of 0.1 – 0.01 IU/cell) for 1 hour at 37 °C and 5% CO_2. The cells then were washed throughly to remove unadsorbed virions and resuspended at 4 x 10^5 cells/ml in complete medium. Aliquots (1 ml) were placed in wells of 24-well culture plates containing an equal volume of test compound (diluted in the culture medium). After a four day incubation at 37 °C, cell density of uninfected cultures was determined by counting cells in a Coulter counter to assess toxicity of the test compound. A p24 antigen ELISA assay was used to determine the level of virus released in the medium of the HIV-infected cultures. The p24 antigen assay uses an HIV-1 anti-p24 specific monoclonal antibody as the capture antibody coated-on-96-well plates. Following a sample incubation period, rabbit serum containing antibodies for HIV-1 p24 is used to tag any p24 "captured" onto the microtiter well surface. Peroxidase conjugated goat anti-rabbit serum is then used to tag HIV-1 p24 specific rabbit antibodies that have complexed with captured p24. The presence of p24 in test samples is then revealed by addition of substrate. The cut-off for the p24 ELISA assay is 12.5 pg/ml. P24 in the culture medium was quantitated aganst a standard curve containing

known amounts of p24. The effective (EC_{50}) and inhibitory (IC_{50}) concentrations (for anti-HIV activity and cytotoxicity, respectively) were determined.

Acknowledgment. This investigation was supported by grant AI-33066 from the National Institute of Allergies and Infectious Diseases awarded to K. H. Lee. The authors thank Dr. J. R. Estes, Department of Botany, University of Oklahoma at Norman, for identification of the plant material.

References

1. Anti-AIDS Agents 16. For part 15, see Refernce 2.
2. Huang, L.; Kashiwada, Y.; Cosentino, L.M.; Fan, S.; Chen, C.H.; McPhail, A.T.; Fujioka, T.; Mihashi, K.; Lee, K.H. *J. Med. Chem.*, submitted.
3. Konoshima, T.; Takasaki, M.; Kozuka, M.; Haruna, M.; Ito, K.; Estes, J.R.; Lee, K.H. *Chem. Pharm. Bull.*, **41**, 1612 (1993), and literature cited therein.
4. Huang, L.; Kashiwada, Y.; Cosentino, L. M.; Fan, S.; Lee, K.H. *Bioorg. Med. Chem. Lett.*, **4**, 593 (1994).
5. Fujioka, T.; Kashiwada, Y.; Kikuskie, R. E.; Cosentino, L.M.; Ballas, L.M.; Jiang, J.B.; Janzen, W.P.; Chen, I.S.; Lee, K.H. *J. Nat. Prod.*, **57**, 243 (1994).

(*Received in USA* 1 *March* 1994; *accepted* 20 *April* 1994)