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APIGENIN-7-O-β-D-GLUCOPYRANOSIDE, AN ANTI-HIV PRINCIPLE FROM *KUMMEROWIA STRIATA*[1]

Renjiu Tang,[a] Ke Chen,[a] Mark Cosentino,[b]

and Kuo-Hsiung Lee[a]*

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

[b]*Biotech Research Laboratories, Rockville, Maryland 20850*

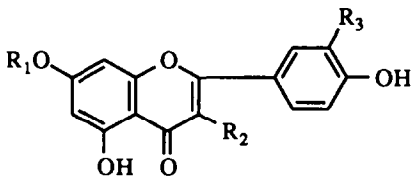
Abstract: An anti-HIV active principle, apigenin-7-O-β-D-glucopyranoside (**3**), has been isolated from *Kummerowia striata*. Four additional flavonoids, apigenin (**1**), quercetin (**2**), kaempferol-3-O-β-D-glucopyranoside (**4**) and apigenin-7-O-neohesperidoside (**5**) have also been isolated from this plant. The structures of these compounds were identified on the basis of their spectral data.

The medicinal herb, *Kummerowia striata (Thunb.)* Schindle (Papilionaceae), a plant widely distributed in Guangxi Province, China, is known as "Renzi-Cao" and has been used for treatment of hepatitis and some other diseases in folklore.[2] *K. striata* is a hitherto phytochemically uninvestigated species. In the course of our continuing search for novel anti-AIDS agents, we found the flavonoid fraction from *K. striata* to possess anti-HIV activity. Bioassay-directed fractionation of this fraction has led to the isolation and characterization of an anti-HIV principle, apigenin-7-O-β-D-glucopyranoside (**3**). In addition, four known flavonoids, apigenin (**1**), quercetin (**2**), kaempferol-3-O-β-D-glucopyranoside (**4**) and apigenin-7-O-neohesperidoside (**5**) were isolated. These compounds were identified by comparison of their spectral data with those of authentic samples and literature values. The bioassay results for anti-HIV activity indicated that compound **3** showed good anti-HIV activity (see Table 1).

Table 1. Anti-HIV activity of the flavonoid fraction and compounds

Fraction and Compounds	IC_{50} (μg/ml)	EC_{50} (μg/ml)	Therapeutic Index
flavonoid fraction	>100	8.5	>11.8
1	35	9	4
2	<4	1.6	<2.5
3	>100	1.8	>55.6
4	<100	80	<1.25
5	>100	80	>1.25

As shown, compound **3** is a flavone, which is glucosylated at C-7 and has hydroxy groups at C-5 and C-4'. We have also investigated the anti-HIV activity of a variety of known flavonoids including compounds **1** and **2**.[3] In this investigation, the flavonol, compound **2**, and the flavonol glucoside, compound **4**, did not show activity in the absence of toxicity. The aglycone, compound **1**, showed less activity and more toxicity than those described here for its glucosylated derivative, compound **3**. Addition of a second sugar moiety, compound **5**, greatly decreased anti-HIV activity. The high potency of **3** represents the first discovery of a bioactive natural product from *K. striata* and prompts further investigation of this plant and of the anti-HIV activity of the class of flavonoids.



1, $R_1 = R_2 = R_3 = H$
2, $R_1 = H$, $R_2 = R_3 = OH$
3, $R_1 = glu$, $R_2 = R_3 = H$
4, $R_1 = R_3 = H$, $R_2 = O$–glu
5, $R_1 =$ rhamnoglucosyl, $R_2 = R_3 = H$

Experimental

GENERAL EXPERIMENTAL PROCEDURES–Melting points were taken on a Fischer-Johns melting point apparatus and are uncorrected. The ir spectra were recorded on a Perkin-Elmer 1320 ir spectrophotometer. The uv spectra were recorded on a UV-2101 PC spectrophotometer. The EI ms spectra were recorded on a TRIO-1000 GC-MS spectrometer. The ^{1}Hnmr and ^{13}Cnmr spectra were recorded on a BRUKER AM 300 spectrometer.

PLANT MATERIAL–*K. striata* was collected in the outskirts of Nanning City of Guangxi, China. It was identified by Professor Fang Ding, medicinal plant toxonomist, Department of Chinese Medicine, Guangxi Institute of Traditional Medical and Pharmaceutical Sciences, where a voucher specimen is deposited.

EXTRACTION AND ISOLATION–The air-dried whole herb (10 kg) was extracted with 95% EtOH at room temperature. The EtOH extract was evaporated under reduced pressure, and the residue was stirred with hot water. This suspension was cooled to room temperature and then filtered. The filtrate was partitioned with hexane and EtOAc respectively. The EtOAc fraction (31 g), which was called the flavonoid fraction and showed good anti-HIV activity, was subjected to silica gel column chromatography and eluted with increasing polarities of a mixture of $CHCl_3$ and MeOH. The MeOH–$CHCl_3$ (5:95) fraction gave apigenin (**1**) (13 mg) and quercetin (**2**) (104 mg). The MeOH–$CHCl_3$ (10:90) fraction gave apigenin-7-O-β-D-glucopyranoside (**3**) (81 mg), kaempferol-3-O-β-D-glucopyranoside (**4**) 24 mg, and apigenin-7-O-neohesperidoside (**5**) (49 mg) using chromatography on polyamide with MeOH as eluent.

The isolated compounds were identified by comparison of their physical and spectral (ms, uv, ^{1}Hnmr or ^{13}Cnmr) data with those reported in the literature (for compounds **1** and **2**, reference 4; for compounds **1-3** and **5**, reference 5; for compound **4**, references 6 and 7). In addition, hydrolysis of compounds **3-5** by treatment with 6% HCl on a steam bath for 1 h gave the corresponding aglycones and sugar moieties, which were identified by comparison of their TLC and ir data with those of authentic samples.

BIOLOGICAL ASSAY-Compounds were dissolved in DMSO for a final working concentration of 100, 20, 4, and 0.8 μg/ml. As the test samples were being prepared, an aliquot of the T cell line, H9, was infected with HIV-1 (IIIB isolate, $TCID_{50} = 10^{-4}$ IU/ml) while another remained uninfected (to be used for toxicity determinations). H9 cells were continuously maintained (mycoplasma-free) in RPMI-1640 with 10% FCS supplemented with L-glutamine. After a 1 hour virus adsorption at 37° C and 5% CO_2, both H9 cell populations were washed 3 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 medium alone (positive infected control/negative drug control). In addition, ddC was also assayed during the 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 the 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 also determined by performing cell counts on the uninfected H9 cell which had either received medium alone (no toxicity) or test drug or ddC.

Non-toxic suppressive agents are presented in the following terms: IC_{50}, the concentration of test sample toxic of 50% of the uninfected H9 cells; EC_{50}, the concentration of test sample which was able to suppress HIV replication by 50%; and Therapeutic Index (TI), the ratio of IC_{50} to EC_{50}.

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References and Notes

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