Anti-AIDS Agents, 10. Acacetin-7-O-#-D-galactopyranoside, an Anti-HIV Principle from Chrysanthemum morifolium and a Structure-Activity Correlation with Some Related Flavonoids

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J. Nat. Prod., **1994**, 57 (1), 42-51• DOI: 10.1021/np50103a006 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 57, No. 1, pp. 42-51, January 1994

ANTI-AIDS AGENTS, 10.[1] ACACETIN-7-*O*-β-D-GALACTOPYRANOSIDE, AN ANTI-HIV PRINCIPLE FROM *CHRYSANTHEMUM MORIFOLIUM* AND A STRUCTURE-ACTIVITY CORRELATION WITH SOME RELATED FLAVONOIDS

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ABSTRACT.—An active anti-HIV principle, acacetin-7-*O*-β-D-galactopyranoside, has been isolated from *Chrysanthemum morifolium*. Seven additional flavonoids isolated from this plant, 13 known related flavonoids, and 14 synthetic flavonoids were also evaluated as inhibitors of HIV replication in H9 cells. A known flavone, chrysin, was found to be the most promising compound in this series. Flavonoids with hydroxy groups at C-5 and C-7 and with a C-2–C-3 double bond were more potent inhibitors of HIV growth. In general, the presence of substituents (hydroxyl and halogen) in the B-ring increased toxicity and/or decreased activity.

The flowering heads of *Chrysanthemum morifolium* Ramar (Compositae) have been used as an herbal tea in Chinese folklore and are known as "Ju Hua." They have been found to possess antibacterial, antifungal, antiviral, antispirochetal, and anti-inflammatory activities (2). In the course of our continuing search for novel anti-AIDS agents from natural products, the MeOH extract of the flower heads of *C. morifolium* was found to show significant anti-HIV activity. Bioactivity-directed fractionation of the active extract has led to the isolation of eight known flavonoids. One of these compounds has been identified as a new active anti-HIV principle, acacetin-7-*O*-β-D-galactopyranoside. This paper describes the isolation and structural identification of the flavonoids from *C. morifolium* and a structure-activity relationship study with related known and synthetic flavonoids.

RESULTS AND DISCUSSION

The air-dried flowering heads of *C. morifolium* were extracted with 70% Me_2CO. This extract was concentrated *in vacuo* to a syrup and partitioned successively with hexane, $CHCl_3$, EtOH, *n*-BuOH, and H_2O. The EtOAc and *n*-BuOH extracts were active in both HIV-reverse transcriptase and HIV replication inhibition assays. Tlc of both extracts showed several spots giving a deep green or brown color with ferric chloride reagent.

Chromatography of the EtOAc fraction over polyvinylpyrrolidone, sequentially using MeOH-H_2O, MeOH, and Me_2CO as eluents, afforded four components, which were identified as the known flavonoids, acacetin-7-*O*-β-D-galactopyranoside [**1**],

[1]For part 9, see Li *et al.* (1).

apigenin-7-*O*-β-D-galactopyranoside [**2**], luteolin [**3**], and quercetin [**4**]. The *n*-BuOH fraction was chromatographed on Diaion HP-20 with MeOH-H_2O. The 60%-80% MeOH/H_2O fraction was then rechromatographed over polyvinylpyrrolidone with MeOH/H_2O, MeOH, Me_2CO, and NH_4OH resulting in the isolation of the four known flavonoids, acacetin-7-*O*-(6″-rhamnosyl)-β-D-glucopyranoside [**5**], luteolin-7-*O*-β-D-glucopyranoside [**6**], 4′-methoxyluteolin-7-*O*-β-D-glucopyranoside [**7**], and baicalin [**8**]. All compounds were identified on the basis of their spectral properties.

Table 1 shows the inhibitory activity of these compounds against HIV in acutely infected H9 lymphocytes. Compound **1**, a flavone substituted in the A ring with a galactose moiety at C-7 and an hydroxy group at C-5 and in the B ring with a methoxy group at C-4′, showed potent activity (EC_{50}=8 μM) with relatively low toxicity (IC_{50}=37 μM). The therapeutic index (defined as toxicity, IC_{50}, divided by anti-HIV activity, EC_{50}) is 5. Replacement of the methoxy at C-4′ with an hydroxy group [**2**] greatly decreased anti-HIV activity. Addition of a second sugar moiety [**5**] abolished anti-HIV activity. In compound **8**, the phenyl B-ring is unsubstituted, but the sugar moiety and the A-ring (additional OH at C-6) are more polar; **8** also shows low activity. All three luteolin compounds [**3**, **6**, **6a**] show anti-HIV activity similar to **1**, however, they are more toxic. Compound **3**, luteolin, a non-glycosylated flavone with hydroxyl groups at C-5,-7,-3′, and -4′, does show anti-HIV activity (EC_{50}=10 μM) but only at near toxic level (IC_{50}=16 μM). Addition of a glucose moiety C-7 [**6**] has little effect on the biological activities (EC_{50}=7 μM, IC_{50}=25 μM), while acetylation of **6** to give **6a** increases toxicity (EC_{50}=IC_{50}=6 μM). Comparison of luteolin [**3**] (a flavone) and quercetin [**4**] (a flavonol) shows that addition of an hydroxy group at C-3 dramatically decreases activity.

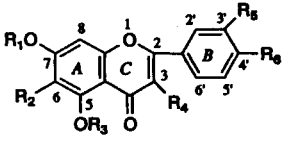
Based on the activity of the lead compound from *C. morifolium*, 13 additional known compounds [**9–21**], including flavones [**9–12**], flavonols [**13–16**], and flavonones [**17–21**] were tested for anti-HIV activity. The results are shown in Table 2.

The most promising compound was a flavone, chrysin [**11**], with slightly greater anti-HIV activity (EC_{50}=5) and slightly lower toxicity (IC_{50}=47) than flavonoid **1** from *C. morifolium*. The therapeutic index of chrysin is 9. Chrysin lacks the galactose moiety at C-7 and the methoxy at C-4′ found in **1**, but retains hydroxy groups at C-5 and C-7.

Other SAR observations with these flavonoids are as follows. Removal of all hydroxy groups gives flavone [**9**] itself; the anti-HIV activity decreases (EC_{50}=50 μM) and the therapeutic index is only 1. Saturation of the C-2–C-3 double bond of **9** gives flavanone **17**, which has cytotoxicity and anti-HIV activity comparable with those of **9**. Addition of an hydroxy group to **9** at C-3 gives the flavonol, 3-hydroxyflavone [**13**]; the anti-HIV activity (EC_{50}=13 μM) and cytotoxicity (IC_{50}=17 μM) both increase. Two additional flavones [**10** and **12**] show anti-HIV activity on the order of chrysin. Compound **12**, apigenin, is hydroxylated at C-5,-7, and -4′; this compound has activity (EC_{50}=9 μM) and toxicity (IC_{50}=35 μM) comparable with compound **1** isolated from *C. morifolium*. However, flavone **10** (OH groups at C-7 and C-8) is more toxic (IC_{50}=14 μM) and has a therapeutic index of only 1. None of the flavonols [**13–16**] or flavonones [**17–21**] tested showed activity in the absence of toxicity. In general, no activity was found with highly polar compounds (e.g., flavonoids **14**, **15**, **20**, or **21**).

Synthetic modification of chrysin at C-2 and in the A-ring led to derivatives **22–29** and **30–34**, respectively. The biological results are shown in Table 3. The anti-HIV activity was decreased by saturation of the C-2 phenyl [**24**] (four-fold) and by its replacement with a propyl [**23**] (>five-fold) or a methyl [**22**] (ten-fold) group. Addition of a halogen to the phenyl B ring (at C-4′-F, **25**; Cl, **28**, Br, **29**; at C-2′-Cl, **26**; at C-3′-Cl, **27**) retained the anti-HIV activity of chrysin, but increased the toxicity, and

TABLE 1. Inhibition by Flavonoids Isolated from *C. morifolium*.

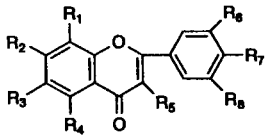


1–8

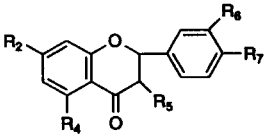
Compounds	R_1	R_2	R_3	R_4	R_5	R_6	IC_{50} (μM)[a]	EC_{50} (μM)[b]	Therapeutic Index
Acacetin-7-*O*-β-D-galactopyranoside [**1**]	gal	H	H	H	H	OMe	37	8	5
Apigenin-7-*O*-β-D-galactopyranoside [**2**]	gal	H	H	H	H	OH	115	61	2
Luteolin [**3**]	H	H	H	H	OH	OH	16	10	2
Quercetin [**4**]	H	H	H	OH	OH	OH	132	132	1
Acacetin-7-*O*-(6″-rhamnopyranosyl)-β-D-glucopyranoside [**5**]	rha$^{1-6}$glu	H	H	H	H	OMe	>231	>231	ND[c]
Luteolin-7-*O*-β-D-glucopyranoside [**6**]	glu	H	H	H	OH	OH	25	7	3
Acetate of **6** [**6a**]	tetraAc-glu	H	Ac	H	OAc	OAc	6	6	1
Baicalin [**8**]	glucosiduronyl	OH	H	H	H	H	72	112	<1

[a]Concentration which inhibits uninfected growth by 50%; AZT has an IC_{50} value of 2000 μM.
[b]Concentration which inhibits virus replication by 50%; AZT has an EC_{50} value of 0.04 μM.
[c]Not determined.

TABLE 2. HIV Inhibition by Known Flavonoids.



9–16

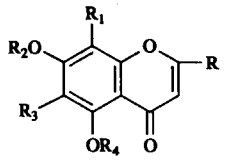


17–21

Compounds	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	IC_{50} (μM)[a]	EC_{50} (μM)[b]	Therapeutic Index
Flavone [9]	H	H	H	H	H	H	H	H	68	50	1
7,8-Dihydroxyflavone [10]	OH	OH	H	H	H	H	H	H	14	10	1
Chrysin [11]	H	OH	H	OH	H	H	H	H	47	5	9
Apigenin [12]	H	OH	H	OH	H	H	OH	H	35	9	4
3-Hydroxyflavone [13]	H	H	H	H	OH	H	H	H	17	13	1
Fisetin [14]	H	H	OH	H	OH	H	OH	H	157	122	1
Morin [15]	OH	H	OH	H	OH	OH	H	OH	>331	>331	ND[c]
Myricetin [16]	H	OH	H	OH	OH	OH	OH	OH	69	35	2
Flavanone [17]		H		H	H	H	H		45	58	1
Galangin [18]		OH		OH	OH	H	H		44	28	2
4′,5,7-Trihydroxyflavone [19]		OH		OH	H	H	OH		294	92	3
(+)-Catechin [20]		OH		OH	OH	OH	OH		>345	>345	ND
Hesperidin [21]		rha[1-6]glu		OH	H	OH	OMe		>164	>164	ND

[a]Concentration which inhibits uninfected growth by 50%; AZT has an IC_{50} value of 2000 μM.
[b]Concentration which inhibits virus replication by 50%; AZT has an EC_{50} value of 0.04 μM.
[c]Not determined.

TABLE 3. HIV Inhibition by Synthetic Flavonoids.



22–34

Compounds	R	R_1	R_2	R_3	R_4	IC_{50} (μM)[a]	EC_{50} (μM)[b]	Theapeutic Index
22	Me	H	H	H	H	214	49	4
23	*n*-propyl	H	H	H	H	73	27	3
24	cyclohexyl	H	H	H	H	42	19	2
25	4′-fluorophenyl	H	H	H	H	13	4	3
26	2′-chlorophenyl	H	H	H	H	17	5	3
27	3′-chlorophenyl	H	H	H	H	14	4	3
28	4′-chlorophenyl	H	H	H	H	16	4	4
29	4′-bromophenyl	H	H	H	H	21	5	4
30	phenyl	H	Ac	H	Ac	>373	56	ND[c]
31	phenyl	H	N-CBZ-glycine	H	H	30	9	3
32	phenyl	NO_2	H	H	H	12	12	1
33	phenyl	NO_2	H	NO_2	H	372	372	1
34	phenyl	NH_2	H	H	H	34	11	3

[a]Concentration which inhibits uninfected growth by 50%; AZT has an IC_{50} value of 2000 μM.
[b]Concentration which inhibits virus replication by 50%; AZT has an EC_{50} value of 0.04 μM.
[c]Not determined.

decreased the therapeutic index. Diacetylation of chrysin [**30**] decreased activity (EC_{50}=56 μM) significantly. Esterification of chrysin's C-7 hydroxyl with a CBZ-protected glycine [**31**] resulted in slightly decreased activity (EC_{50}=9), slightly increased cytotoxicity (IC_{50}=30), and a three-fold decrease in the therapeutic index (TI=3). Addition of an amino group at C-8 [**34**] had the same effects, and addition of a nitro group at this position [**32**] increased cytotoxicity even further (IC_{50}=12, TI=1). Addition of a second nitro group at C-6 [**33**] completely abolished anti-HIV activity.

Previous literature has also reported antiviral activity, both anti-infective and antireplicative, with flavonoids. Examples of virus types are rhino- and coxsackie-viruses (3), herpes simplex virus type 1 (HSV-1), parainfluenza virus type 3 (Pf-3), respiratory syncytial virus (4), polio-virus type 1 (4,5), and influenza viruses (6). Several flavones have been found to be inhibitors of HIV-1 proteinase (7), and a flavonoid alkaloid isolated from *Buchenavia capitata* has shown anti-HIV activity (8). Also, a variety of flavonoids have been reported to have an inhibitory effect on the reverse transcriptase activity of avian (AMV and RAV-2) (9), murine leukemia (Moloney and Rausher strains) (9–12), human immunodeficiency type-1 (11,12) and -2 (13), and human T cell leukemia (14) viruses.

In summary, in our study of the anti-HIV activity of flavonoid compounds, the known flavone, chrysin [**11**], showed the highest therapeutic index. A related flavone isolated from *C. morifolium*, acacetin-7-*O*-β-D-galactopyranoside [**1**], also showed good anti-HIV activity with relatively low toxicity.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were taken on a Fisher-Johns melting point apparatus and are uncorrected. Uv spectra were measured in MeOH on a Varian Cary 2200 spectrophotometer. Ir spectra were recorded on a Perkin-Elmer 1320 spectrophotometer. Nmr spectra were obtained on Varian EM-400 and Bruker AC-300 spectrometers. Mass spectra were determined on VG Micromass 7070 and TRIO-1 GC MS instruments at 70eV with a direct inlet system. Polyvinylpyrrolidione and Diaion HP-20 were used for cc. Pre-coated Si gel plates were used for analytical (Kieselgel 60 F254, 0.25 mm, Merck) and prep. (GF, 1000 μm) tlc. Elemental analyses were performed by Atlantic Microlab, Inc. Norcross, GA.

MATERIALS.—The flower heads of *C. morifolium* were a gift from Brion Research Institute (BRI), Taipei, Taiwan. A voucher specimen is kept at BRI. Flavonoids were purchased from Aldrich Chemical Co., Milwaukee, WI.

EXTRACTION AND ISOLATION.—The air-dried flower heads of *C. morifolium* (2 kg) were extracted with 70% Me_2CO at room temperature. The extract was concentrated *in vacuo* to a syrup and partitioned successively with hexane, $CHCl_3$, EtOAc, *n*-BuOH, and H_2O. The EtOAc and *n*-BuOH extracts were active in both HIV reverse transcriptase and HIV replication inhibition assays.

The EtOAc extract was chromatographed over polyvinylpyrrolidone employing MeOH-H_2O, MeOH and Me_2CO as eluents. The 80%–90% MeOH and MeOH-Me_2CO (2:1) fractions were combined and further purified, respectively. After evaporation of solvent, the residue (1.5 g) from the 80%–90% MeOH fraction was rechromatographed over polyvinylpyrrolidone with 80% MeOH and MeOH as eluents. The 80% MeOH fraction was evaporated, and the residue was recrystallized from Me_2CO to yield 110 mg of yellow needles [**1**]. The 90% MeOH fraction was further chromatographed with the same conditions to afford 143 mg of **2**. The MeOH fraction yielded 420 mg of **3**, which was purified by recrystallization from MeOH. After removal of solvent, the residue from the MeOH-Me_2CO (2:1) fraction was passed over a short Si gel column to remove an impurity, giving 143 mg of **4**.

The *n*-BuOH extract was chromatographed over Diaion HP-20 with MeOH-H_2O. The 60%–80% MeOH fractions were combined, and rechromatographed over polyvinylpyrrolidone with MeOH-H_2O, MeOH, Me_2CO, and NH_4OH as eluents. The 80% MeOH fraction was rechromatographed over Si gel with MeOH as eluent to obtain 31 mg of **5**. The 90% MeOH fraction was concentrated *in vacuo*, and the residue recrystallized from Me_2CO to yield 280 mg of **6**. The mother liquor of **6** was rechromatographed over polyvinylpyrrolidione with MeOH as eluent to afford 21 mg of **7**. The MeOH fraction was further chromatographed to yield 11 mg of **8**.

ACACETIN-7-*O*-β-D-GALACTOPYRANOSIDE [**1**].—Yellow needles (Me_2CO); mp 254–256°; ^{1}H nmr (DMSO-d_6) δ 8.04 (2H, d, J=8.0 Hz, H-2′,6′), 7.14 (2H, d, J=8.0 Hz, H-3′,5′), 6.97 (1H, d, J=2.0 Hz, H-8), 6.86 (1H, d, J=2.0 Hz, H-6), 6.44 (1H, s, H-3), 5.1–3.2 (m, galactosyl), 3.86 (3H, s, OCH_3-4′); fabms *m/z* [M]$^+$ 446 ($C_{22}H_{22}O_{10}$), 389, 343, 329, 287 (100), 271, 237, 183, 145, 115, 103, 87. Hydrolysis with 10% H_2SO_4 at 100° for 3 h gave acacetin and galactose, which were identified by comparison of their tlc and ir properties with those of authentic samples.

APIGENIN-7-*O*-β-D-GALACTOPYRANOSIDE [**2**].—Yellow needles (MeOH); mp 208–210°. Compound **2** was identified as apigenin-7-*O*-β-D-galactopyranoside by comparison with literature sources (15).

LUTEOLIN [**3**].—Yellow needles (MeOH); mp>300°. Compound **3** was identified as luteolin by comparison with literature sources (16). Methylation of **3** with CH_2N_2 gave yellowish needles (MeOH); mp 162–163°, ^{1}H nmr showed three methoxy groups at 3.99, 3.97, and 3.89 ppm (7,3′,4′-OCH_3); the acetate of **3** showed four acetate groups at 2.45, 2.44, 2.36, 2.34 ppm.

QUERCETIN [**4**].—Yellow needles (MeOH): mp>300°. Compound **4** was identified as quercetin by tlc and ir comparison with an authentic sample.

ACACETIN-7-*O*-(6″-RHAMNOPYRANOSYL)-β-D-GLUCOPYRANOSIDE [**5**].—Light yellow needles (MeOH): mp 248–251°; uv λ max 267, 325 nm; ir (KBr) 3480, 3420, 3080, 2940, 1655, 1605, 1580, 1500, 1300, 1245, 1180, 1070, 980, 910, 840 cm^{-1}; ^{13}C nmr (DMSO-d_6) δ 164.1 (C-2), 103.9 (C-3), 182.1 (C-4), 162.5 (C-5), 99.6 (C-6), 163.1 (C-7), 94.9 (C-8), 161.2 (C-9), 105.9 (C-10), 122.8 (C-1′), 114.7 (C-2′), 128.6 (C-3′), 157.1 (C-4′), 114.8 (C-5′), 100.6 (C-6′), 99.8 (C-1″), 73.2 (C-2″), 75.8 (C-3″), 70.5 (C-4″), 76.4 (C-5″), 66.2 (C-6″), 100.0 (C-1‴), 70.8 (C-2‴), 69.7 (C-3‴), 72.2 (C-4‴), 68.5 (C-5‴), 17.9 (C-6‴), 55.7 (OCH_3). Hydrolysis of **5** with 3N HCl at 60° for 3 h, followed by neutralization and extraction with EtOAc, gave acacetin and acacetin-7-*O*-β-D-glucopyranoside and rhamnose, which were identified on tlc developed with *n*-BuOH-HOAc-H_2O (4:1:5) by comparison with authentic samples. Acetylation of **5** with Ac_2O/pyridine afforded the hepta-acetate: ^{1}H nmr ($CHCl_3$) δ 7.85 (2H, d, J=9.0 Hz, H-2′,6′), 7.03 (2H, d, J=9.0 Hz, H-3′,5′), 6.60 (1H, d, J=2.0 Hz, H-8), 6.57 (1H, d, J=2.0 Hz, H-6), 6.41 (1H, s, H-3), 5.41 (1H, d, J=6.5 Hz, H-1″), 5.23 (1H, br, H-1‴), 5.2–3.6 (m, glycosyl), 3.90 (3H, s, OCH_3), 2.18, 2.09, 2.07, 2.06, 2.05, 2.03, 1.93 (3H each, $COCH_3$×7), 1.15 (3H, d, J=6.5 Hz, rhamnose CH_3); fabms *m/z* [M+1]$^+$ 887, [M+1−CH_2CO]$^+$ 845, 803, 727, 601, 313, 285, 217, 153.

LUTEOLIN-7-*O*-β-D-GLUCOPYRANOSIDE [**6**].—Yellowish needles (Me_2CO); mp 238–239°. Hydrolysis of **6** with 5% H_2SO_4 at 90° for 5 h gave luteolin [**3**] and glucose, which were identified on cellulose tlc using *n*-BuOH-HOAc-H_2O (4:1:5). Acetylation of **6** with Ac_2O/pyridine gave colorless needles [**6a**] (95% EtOH): mp 242°; ^{1}H nmr ($CDCl_3$) δ 5.32 (1H, d, J=8.0 Hz, H-1″) indicated that the glucoside has a β configuration; fabms *m/z* [M−(CH_2CO)×3]$^+$ 616, [M−(CH_2CO)×6] 490, [luteolin M]$^+$ 286.

4′-METHOXYLUTEOLIN-7-*O*-β-D-GLUCOPYRANOSIDE [**7**].—Amorphous powder (MeOH); mp 251–254°; ^{1}H nmr (DMSO-d_6) δ 7.59 (1H, dd, J=8.0, 2.0 Hz, H-6′), 7.43 (1H, d, J=2.0 Hz, H-2′), 7.12 (1H, d, J=8.0 Hz, H-5′), 6.85 (1H, s, H-3), 6.81 (1H, d, J=2.0 Hz, H-8), 6.46 (1H, s, J=2.0 Hz, H-6), 5.1–3.6 (m, glucosyl), 3.87 (3H, s, 4′-OCH_3). When compounds **6** and **7** were methylated with CH_2N_2, the identical product was obtained. From comparison of the ^{1}H-nmr data of the B-ring of **6** and **7**, the methoxy group in the latter was identified at the 4′ position of the B-ring.

BAICALIN [**8**].—Yellow needles (MeOH): mp 228–231°; ir showed a carboxylic group and a glucoside absorption. Compound **8** was identified as baicalin by tlc comparison with an authentic sample.

2-METHYL-5,7-DIHYDROXYCHROMONE [**26**].—A mixture of 2′,4′,6′-trihydroxyacetophenone (0.5 g), Ac_2O (1.3 g), and pyridine (2 ml) was refluxed for 1 h. After cooling, the mixture was poured into ice water (30 ml) and stirred to give an oil, which solidified upon cooling to 0° overnight. 2′,4′6′-Triacetoxyactophenone (1 g) was obtained by filtration. To a solution of this triacetate (0.5 g) in pyridine (2 ml), powdered KOH (0.2 g) was added with stirring, and the resulting mixture was heated at 50° for 1 h. The solution was then poured into 10% H_2SO_4 (20 ml), and the solid was filtered and dissolved in EtOH (3 ml) containing 0.2 ml of H_2SO_4. The solution was refluxed for 1 h followed by alkalinization to pH 10 with 20% NaOH and refluxed for another 30 min. After cooling, the solution was neutralized with 10% H_2SO_4 to give a solid, which was recrystallized from MeOH. Compound **26** was obtained as colorless needles (0.5 g): mp 285–286°; ir (KBr) 3420, 3100, 3030, 2900, 1650, 1620, 1565, 1505, 1415, 1345, 1160, 1015, 940, 845, 810 cm^{-1}; ^{1}H nmr (Me_2CO-d_6) δ 2.32 (3H, s, CH_3), 6.06 (1H, s, H-3), 6.18 (1H, s, H-6), 6.33 (1H, s, H-8); eims *m/z* [M]$^+$ 192 (100%), 163, 152, 135, 124, 111, 96, 69; *anal.* calcd for $C_{10}H_8O_4$, C 62.50, H 4.17, found 62.31, H 4.21.

2-*n*-PROPYL-5,7-DIHYDROXYCHROMONE [**27**].—To a solution of 2′,4′,6′-trihydroxyacetophenone (0.5 g) in pyridine (3 ml), butyryl chloride (1 g) was added dropwise with stirring. The mixture was heated for 20 min in an oil bath at 110°. After workup as for **26**, the resulting product was recrystallized from Me_2CO to yield **27** (150 mg) as colorless needles: mp 178–180°; ir (KBr) 3420, 3100, 2985, 1640, 1615, 1558, 1500, 1415, 1355, 1160, 1010, 945, 840, 810 cm^{-1}; ^1H nmr ($CDCl_3$) δ 6.32 (1H, d, J=1.5 Hz, H-8), 6.25 (1H, d, J=1.5 Hz, H-6), 6.01 (1H, s, H-3), 2.55 (2H, t, J=7.5 Hz, $CH_2CH_2CH_3$), 1.74 (2H, sextet, J=7.5 Hz, $CH_2CH_2CH_3$), 1.01 (3H, t, J=7.5 Hz, $CH_2CH_2CH_3$); ^{13}C nmr ($CDCl_3$) δ 183.6 (s), 171.7 (s), 165.4 (s), 162.7 (s), 159.4 (s), 129.0 (s), 108.1 (d), 99.8 (d), 94.7 (d), 36.6 (t), 20.9 (t), 13.7 (q); eims *m/z* $[M]^+$ 220 (100%), 205, 192, 163, 153, 152, 124, 111, 96, 69; *anal.* calcd for $C_{12}H_{12}O_4$, C 65.45, H 5.45, found C 65.51, H 5.51.

2-CYCLOHEXYL-5,7-DIHYDROXYCHROMONE [**28**].—A solution of 2′,4′6′-trihydroxyacetophenone (0.5 g) in pyridine (3 ml) was acylated with cyclohexylcarbonyl chloride (1.5 g). After workup as for **26**, the precipitate was filtered and washed with sat. $NaHCO_3$ solution then with H_2O. The product was recrystallized from Me_2CO to afford **28** (0.5 g) as needles: mp 220–222°; ir (KBr) 3380, 3100, 2930, 2860, 1657, 1615, 1565, 1505, 1430, 1355, 1295, 1165, 1080, 945, 850 cm^{-1}; ^1H nmr ($CDCl_3$) δ 6.23 (1H, d, J=2.0 Hz, H-8), 6.15 (1H, d, J=2.0Hz, H-6), 5.89 (1H, s, H-3), 2.39 (1H, m, H-1′), 1.90 (2H, d, J=10.5 Hz), 1.78 (2H, dd, J=2.0, 9.5 Hz), 1.67 (1H, d, J=11.0 Hz), 1.32 (5H, m); eims *m/z* $[M]^+$ 260 (100%), 231, 219, 205, 192, 179, 163, 153, 139, 124, 91, 69; *anal.* calcd for $C_{15}H_{16}O_4$, C 69.23, H 6.15; found C 69.12, H 6.21.

4′-FLUORO-5,6-DIHYDROXYFLAVONE [**29**].—A solution of 2′,4′,6′-trihydroxyacetophenone (0.5 g) in pyridine (5 ml) was acylated with 4-fluorobenzoyl chloride (1.5 g) as for **28**. After the usual workup, the residue was chromatographed over Si gel (10 g) with C_6H_6-Me_2CO (5:1) as eluent to afford **29** as long flat yellow needles (0.29 g); mp 270–272°; ir (KBr) 3080, 1640, 1620, 1605, 1505, 1420, 1360, 1290, 1235, 1165, 1100, 1025, 830 cm^{-1}; ^1H nmr (DMSO-d_6) δ 12.87 (1H, s, OH-5), 9.74 (1H, s, OH-7), 8.14 (2H, m, H-3′,5′), 7.37 (2H, t, J=8.5 Hz, H-2′,6′), 6.77 (1H, s, H-3), 6.58 (1H, s, H-8), 6.28 (1H, s, H-6); eims *m/z* $[M]^+$ 272 (100%), 244, 152, 133, 124, 122, 120, 104, 96, 91, 69; *anal.* calcd for $C_{15}H_9FO_4$, C 66.18, H 3.31, found C 65.98, H 3.41.

2′-CHLORO-5,6-DIHYDROXYFLAVONE [**30**].—A solution of 2′,4′6′-trihydroxyacetophenone (0.5 g) in pyridine (5 ml) was acylated with 2-chlorobenzoyl chloride (1.5 g) as for **29**. After the usual workup, the resulting product was recrystallized from Me_2CO to afford **30** (120 mg) as light yellow needles: mp 273–275°; ir (KBr) 3360, 3080, 1650, 1610, 1500, 1465, 1430, 1410, 1350, 1270, 1155, 1060, 920, 835 cm^{-1}; ^1H nmr (DMSO-d_6) δ 7.77 (1H, dd, J=1.5, 7.0 Hz, H-3′), 7.67 (1H, br d, J=7.0 Hz, H-6′), 7.62 (1H, dt, J=1.5, 7.0 Hz, H-4′), 7.54 (1H, br t, J=7.0 Hz, H-5′); eims *m/z* $[M+2]^+$ 290 (28.2%), $[M]^+$ 288 (100), 152, 139, 124, 111, 104, 96, 75, 69; *anal.* calcd for $C_{15}H_9ClO_4$, C 62.39, H 3.12, Cl 12.31, found C 62.46, H 3.16, Cl 12.33.

3′-CHLORO-5,6-DIHYDROXYFLAVONE [**31**].—A solution of 2′,4′,6′-trihydroxyacetophenone (0.5 g) in pyridine (5 ml) was acylated with 3-chlorobenzoyl chloride (1.5 g) as for **29**. After the usual workup, the resulting product was recrystallized from Me_2CO to afford **31** (110 mg) as light yellow needles: mp 290–292°; ir (KBr) 3430, 3160, 3080, 1650, 1615, 1580, 1510, 1430, 1365, 1240, 1165, 1030, 835 cm^{-1}; ^1H nmr (DMSO-d_6) δ 8.15 (1H, d, H-2′), 8.04 (1H, d, J=7.5 Hz, H-4′), 7.67 (1H, d, J=7.5 Hz, H-6′), 7.59 (1H, t, J=7.5 Hz, H-5′), 7.07 (1H, s, H-3), 6.56 (1H, d, J=1.5 Hz, H-8), 6.22 (1H, d, J=1.5 Hz, H-6); eims *m/z* $[M+2]^+$ 290 (30.3%), $[M]^+$ 288 (100), 260, 152, 139, 130, 124, 111, 96, 75, 69; *anal.* calcd for $C_{15}H_9ClO_4$, C 62.39, H 3.12, Cl 12.31, found C 62.42, H 3.15 Cl 12.23.

4′-CHLORO-5,6-DIHYDROXYFLAVONE [**32**].—A solution of 2′,4′,6′-trihydroxyacetophenone (0.5 g) in pyridine (5 ml) was acylated with 4-chlorobenzoyl chloride (1.5 g) as for **29**. After the usual workup, the resulting product was recrystallized from Me_2CO to yield **32** (100 mg) as light yellow needles: mp 298–300°; ir (KBr) 3360, 3090, 1650, 1615, 1585, 1515, 1490, 1425, 1365, 1270, 1245, 1165, 1095, 1025, 910, 820 cm^{-1}; ^1H nmr (DMSO-d_6) δ 8.06 (2H, d, J=8.5 Hz, H-3′,5′), 7.60 (2H, d, J=8.5 Hz, H-2′,6′), 6.97 (1H, s, H-3), 6.49 (1H, s, H-8), 6.21 (1H, s, H-6); eims *m/z* $[M+2]^+$ 290 (30.7%), $[M]^+$ 288 (100), 260, 152, 139, 130, 124, 111, 96, 75, 69; *anal.* calcd for $C_{15}H_9ClO_4$, C 62.39, H 3.12, Cl 12.31, found C 62.12, H 3.20 Cl 12.22.

4′-BROMO-5,6-DIHYDROXYFLAVONE [**33**].—A solution of 2′,4′,6′-trihydroxyacetophenone (0.5 mg) in pyridine (5 ml) was acylated with 4-bromobenzoyl chloride (1.5 g) as for **29**. After the usual workup, the resulting product was recrystallized from Me_2CO to give **33** (120 mg) as light yellow needles: mp 295–297°; ir (KBr) 3360, 3090, 1650, 1615, 1585, 1510, 1490, 1425, 1405, 1365, 1270, 1165, 1075, 1030, 1010, 910, 820 cm^{-1}; ^1H nmr (DMSO-d_6) δ 8.00 (2H, d, J=8.0 Hz, H-3′,5′), 7.76 (2H, d, J=8.0 Hz, H-2′,6′), 6.99 (1H, s, H-3), 6.50 (1H, s, H-8), 6.21 (1H, s, H-6); eims *m/z* $[M+2]^+$ 334 (100%), $[M+1]^+$ 333 (19.5),

[M]$^+$ 332 (100), 306 (12.9) 304 (14.2), 153, 152, 139, 124, 111, 101, 96, 75, 69; *anal.* calcd for $C_{15}H_9BrO_4$, C 54.05, H 2.70, Br 24.02, found C 53.90, H 2.79, Br 23.86.

CHRYSIN DIACETATE [**34**].—A solution of chrysin (40 mg) in pyridine (2 ml) was acetylated with Ac_2O at room temperature overnight. The product was recrystallized from 95% EtOH to give chrysin diacetate [**34**] (31 mg): mp 218–220°; ^{1}H nmr ($CDCl_3$) δ 7.86 (2H, dd, *J*=7.0, 2.0 Hz, H-2′,6′), 7.54 (1H, m, H-4′), 7.52 (2H, m, H-3′,5′), 7.36 (1H, d, *J*=2.0 Hz, H-8), 6.85 (1H, d, *J*=2.0 Hz, H-6), 6.67 (1H, s, H-3), 2.45 (3H, s, $COCH_3$), 2.36 (3H, s, $COCH_3$); *anal.* calcd for $C_{19}H_{14}O_6$, C 67.46, H 4.14, found C 67.42, H 4.21.

N-CBZ-GLYCINE ESTER OF CHRYSIN [**35**].—A mixture of chrysin (0.27 g), N-CBZ-glycine (0.18 g), and DCC (0.21 g) in CH_2Cl_2 (8 ml) was stirred at room temperature for 5 h. The usual workup procedure gave the N-CBZ-glycine ester of chrysin [**35**] after elution over Si gel with $CHCl_3$-Me_2CO (1:1): mp 180–183°; ^{1}H nmr ($CDCl_3$) δ 12.70 (1H, s, OH), 7.89 (2H, dd, *J*=6.0, 2.0 Hz, H-2′,6′), 7.57 (1H, m, H-4′), 7.54 (2H, dt, *J*=2.0, 6.0 Hz, H-3′,5′), 7.4–7.3 (5H, m, CBZ protons), 6.89 (1H, d, *J*=2.0 Hz, H-8), 6.74 (1H, s, H-3), 6.60 (1H, d, *J*=2.0 Hz, H-6), 5.38 (1H, br, NH), 5.19 (2H, s, ArCH_2), 4.24 (2H, d, *J*=8.0 Hz, NHCH_2CO); *anal.* calcd for $C_{25}H_{19}O_7N$, C 67.42, H 4.27, N 3.15, found C 67.33, H 4.37, N 3.07.

8′-NITRO-CHRYSIN [**36**] AND 6,8-DINITRO-CHRYSIN [**37**].—A mixture of chrysin (1.2 g), 70% HNO_3 (40 ml), and glacial HOAc (70 ml) was heated at 50° for 1.5 h. The mixture was poured into ice water (100 ml) and neutralized to pH 6–7 with 6N NaOH. The precipitate was filtered, washed with H_2O, and chromatographed over Si gel with Me_2CO-CH_2Cl_2 as eluent. The 1:1.5 fractions were combined, concentrated, and recrystallized from Me_2CO to give **36** (210 mg): mp 225–227°; ^{1}H nmr (DMSO-d_6) δ 13.2 (1H, s, OH), 7.96 (2H, dd, *J*=6.0, 2.0 Hz, H-2′,6′), 7.61 (1H, m, H-4′), 7.60 (2H, m, H-3′,5′), 7.20 (1H, s, H-3), 6.40 (1H, s, H-6); *anal.* calcd for $C_{15}H_9O_6N$, C 60.20, H 3.01, N 4.68, found C 60.07, H 3.14, N 4.51.

Elution with CH_2Cl_2-Me_2CO (5:1) gave **37**: mp 228–230°; ^{1}H nmr (DMSO-d_6) δ 14.3 (1H, s, 5-OH), 13.2 (1H, s, 7-OH), 7.96, (2H, dd, *J*=8.0, 2.0 Hz, H-2′,6′) 7.61 (2H, m, H-3′,5′), 7.60 (1H, m, H-4′), 6.98 (1H, s, H-3); *anal.* calcd for $C_{15}H_8O_8N_2$, C 52.94, H 2.35, N 8.24, found C 52.77, H 2.46, N 8.04.

8′-AMINO-CHRYSIN [**38**].—A mixture of 8-nitro-chrysin [**36**] (30 mg), $SnCl_2 \cdot H_2O$ (250 mg) in 15 ml of absolute EtOH was heated at 70° under N_2. After 30 min, the mixture was poured into ice and extracted with EtOAc. The organic phase was washed with H_2O and dried over Na_2SO_4. After evaporation of the solvent, the residue was purified by prep. tlc to give 8-amino-chrysin [**38**] (6 mg): mp 241–244°; ^{1}H nmr ($CDCl_3$) δ 7.90 (2H, m, H-2′,6′), 7.60 (2H, m, H-3′,5′), 7.59 (1H, m, H-4′), 6.77 (1H, s, H-3), 6.66 (1H, s, H-6); *anal.* calcd for $C_{15}H_{11}O_4N$, C 66.91, H 4.09, N 5.20, found C 66.80, H 4.21, N 5.12.

HIV GROWTH INHIBITION ASSAY.—HIV inhibition was measured as described previously (17). Briefly, this assay was performed by incubation of H9 lymphocytes (1×10^7 cells/ml) in the presence or absence of HIV-1 (HTLV-IIIB) for 1 h at 37°. Cells were washed thoroughly to remove unadsorbed virions and resuspended at 4×10^5 cells/ml in culture medium. Aliquots (1 ml) were placed in wells of 24-well culture plates containing an equal volume of test compound (diluted in test medium). After incubation for 3 days at 37°, the cell densities of uninfected cultures were determined to assess toxicity of the test compound. A p24 antigen capture assay was used to determine the level of HIV infection in HIV-treated cultures. The ability of test compounds to inhibit HIV replication was measured at four different concentrations of test compound relative to infected, untreated cultures. Test compounds were considered to be active if p24 levels were less than 70% of infected, untreated cultures.

ACKNOWLEDGMENTS

This investigation was supported by grant AI-33066 from the National Institute of Allergy and Infectious Diseases awarded to K.-H. Lee.

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Received 22 April 1993