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INHIBITORY EFFECTS OF FLAVONOIDS ON MOLONEY MURINE LEUKEMIA VIRUS REVERSE TRANSCRIPTASE ACTIVITY

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ABSTRACT.—Several flavonoids were tested for their effects on Moloney murine leukemia virus reverse transcriptase activity. Four groups of flavonoids, namely flavones, flavanones, flavonols, and flavanonols, were studied, and it was found that flavonols and flavanonols were very active in this regard while flavones and flavanones displayed very low activity. Among the flavonoids tested, fisetin, quercetin, myricetin, kaempferol, morin, (±)-taxifolin, (+)-catechin, and (−)-epicatechin were shown to be highly effective in inhibiting the reverse transcriptase activity.

Structure-activity relationship analysis of these flavonoids revealed that the simultaneous presence of free hydroxyl groups at positions 3 and 4′ enhanced the reverse transcriptase inhibitory activity. Replacement of the 3-hydroxyl group with a monosaccharide or of the 4′-hydroxyl group with a methyl group reduced inhibitory activity. The double bond at position 2 and 3 of the flavonoid's pyrone ring is not essential for inhibiting reverse transcriptase activity.

The flavonoids studied demonstrated ability to inhibit the reverse transcriptase activity using either $(rA)_n(dT)_{12-18}$ or $(rC)_n(dG)_{12-18}$ as template-primers.

Antiviral drugs have mostly been directed against viral nucleic acid polymerases, and most of the active compounds have been found to be nucleotide analogues. By the use of reverse transcriptase (RT) as a target for antiviral chemotherapy, 3′-azido-2′,3′-dideoxythymidine (AZT) (1) and phosphonoformate (2) were demonstrated to be selective inhibitors of HIV RT. Suramin (3), HPA23 (4), and 2′,3′ dideoxynucleosides (5) were also demonstrated to be potent RT inhibitors. However, clinical trials of these compounds in AIDS patients revealed side effects such as thrombocytopenia for HPA23 (4) and leucopenia for AZT (6). Natural products represent a potential source of RT inhibitors. In the present study, evidence is provided to show that certain phenolic flavonoids, which are widely distributed in Chinese herbs (7), have an inhibitory effect on RT activity of Moloney murine leukemia virus (MMLV).

EXPERIMENTAL

MATERIALS.—Flavonoids were purchased from Sigma Chemical Co., St. Louis, or from Aldrich Chemical Co., Milwaukee. Activated calf thymus DNA, $(Riboadenylic\ acid)_n$ $(deoxythymidylic\ acid)_{12-18}$, and $(ribocytidylic\ acid)_n$ $(deoxyguanylic\ acid)_{12-18}$ were obtained from Pharmacia, Uppsala, Sweden. RT from MMLV is the product of Bethesda Research Laboratories Life Technologies, Inc., Gaithersburg, MD. [^{3}H]dTTP (50 Ci/mmol) and [^{3}H]dGTP (50 Ci/mmol) were obtained from New England Nuclear Corp., Boston. *Escherichia coli* DNA polymerase I (DNAP I) was the product of Boehringer Mannheim GmbH, Penzberg, Germany.

REVERSE TRANSCRIPTASE ASSAY.—The reaction mixture (30 μl) contained 50 mM Tris-HCl buffer, pH 8.3; 75 mM KCl; 10 mM dithiothreitol; 3 mM $MgCl_2$; 3 μg nuclease-free bovine serum albumin; 0.5 μg of $(rA)_n(dT)_{12-18}$ or $(rC)_n(dG)_{12-18}$; 2 mM dTTP or dGTP; 0.5 μCi [^{3}H]-dTTP or [^{3}H]-dGTP; and 5 units of MMLV RT preparation (8). After incubation at 37° for 30 min, 5 μl of 0.2 M EDTA was added to terminate the reaction. The reaction mixture (15 μl, in duplicate) was spotted on Whatman DE-81 cellulose paper and washed 6 times with 5% Na_2HPO_4, 2 times each with H_2O and EtOH, and once with Et_2O (9). The radioactivity of the samples was measured with a Beckman 5801 liquid scintillation counter.

DNA POLYMERASE I ASSAY.—The reaction mixture (30 μl) contained 10 mM Tris-HCl buffer, pH 8.0; 5 mM $MgCl_2$; 10 μg nuclease-free bovine serum albumin; 1.5 mM dithiothreitol; 5 ug of activated calf thymus DNA; 1 μM each of dATP, dGTP, and dCTP; 0.5 μCi [^{3}H]dTTP, and 1 unit DNAP I. The incubation was carried out at 37° for 30 min, and the acid-insoluble precipitates were collected and processed for scintillation counting (10).

RESULTS AND DISCUSSION

INHIBITORY EFFECTS OF FLAVONOIDS ON MMLV RT.—The effects of flavonoids on RT activity are shown in Table 1 for template-primers $(rA)_n(dT)_{12-18}$ or

TABLE 1. Inhibition of Moloney Murine Leukemia Virus Reverse Transcriptase by Flavonols, Flavanonols, Flavananes, and Flavones Using $(rA)_n(dT)_{12-18}$ and $(rC)_n(dG)_{12-18}$ as Template-primers (Table values are ID_{50}).

Drug	Template-primer			
	$(rA)_n(dT)_{12-18}$		$(rC)_n(dG)_{12-18}$	
	μg/ml	(μM)	μg/ml	(μM)
Flavonols				
Fisetin	0.08	(0.28)	0.07	(0.24)
Kaempferol	0.35	(1.22)	0.37	(1.29)
Morin	0.35	(1.03)	0.34	(1.01)
Quercetin	0.06	(0.20)	0.05	(0.17)
Myricetin	0.08	(0.25)	0.09	(0.28)
Myricitrin	4.67	(10.05)	4.50	(9.69)
Rutin	4.83	(7.27)	4.87	(7.33)
Flavanonols				
(±)-Taxifolin	0.74	(2.43)	0.75	(2.46)
(+)-Catechin	0.80	(2.75)	0.83	(2.86)
(−)-Epicatechin	0.75	(2.58)	0.77	(2.65)
Flavanones				
Flavanone	>100	(>445.9)	>100	(>445.9)
Hesperetin	6.33	(20.9)	6.67	(22.1)
Flavones				
3-Hydroxyflavone	>100	(>419.7)	>100	(>419.7)
7-Hydroxyflavone	>100	(>419.7)	>100	(>419.7)

$(rC)_n(dG)_{12-18}$. The flavonoids used in these experiments belong to four different structural groups, flavones, flavanones, flavonols, and flavanonols. As indicated in Table 1, with $(rA)_n(dT)_{12-18}$ as a template-primer, the flavonols such as fisetin, quercetin, and myricetin were the most effective inhibitors, having ID_{50}'s between 0.06 and 0.08 μg/ml. Two other flavonols, kaempferol and morin, have weaker inhibitory effects, with ID_{50} of 0.35 μg/ml. Flavanonols, such as (+)-catechin, (−)-epicatechin, and (±)-taxifolin, exhibited similar inhibitory potencies (ID_{50}'s = 0.74–0.80 μg/ml), which were less than those of the flavonols. Hesperetin (a flavanone) has moderate inhibitory effects on the RT activity, with an ID_{50} value of 6.33 μg/ml. Flavones (3-hydroxyflavone and 7-hydroxyflavone) and flavanone have very low inhibitory effects on the RT activity. The extent of inhibition exhibited by these flavonoids was very similar for the two template-primers used.

EFFECTS OF FLAVONOIDS ON DNA POLYMERASE I.—The inhibitory effects of flavonoids such as myricetin, morin, quercetin, kaempferol, and taxifolin on DNAP I were studied. Concentrations that inhibited DNAP I activity were observed to be tenfold to thirtyfold higher than those required to inhibit MMLV RT using activated calf thymus DNA template (Figure 1).

Many flavonoids have been shown to possess antioxidant (12), antimutagenic (13–15), and anticarcinogenic (16,17) properties. It is of particular interest to note that some flavonoids have also been shown to possess antiviral activities (18). The present in-

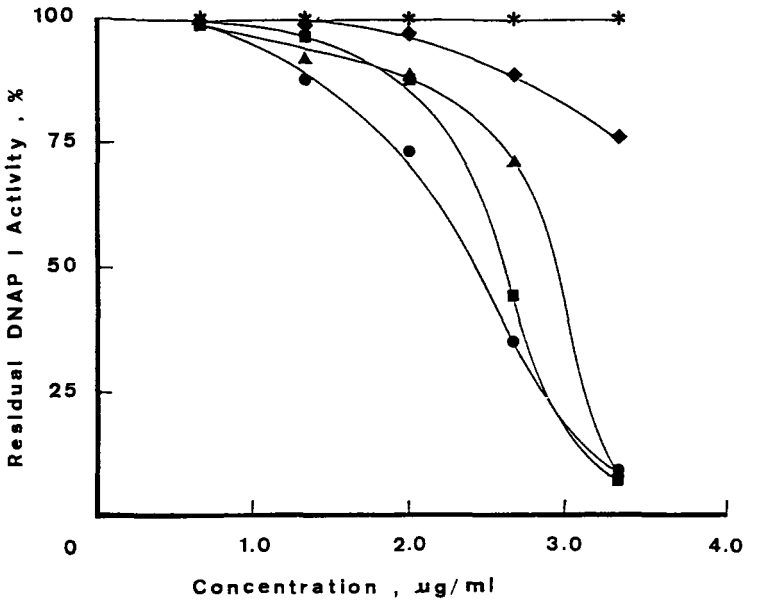


FIGURE 1. Effects of various flavonoids on the activity of DNA polymerase I. Quercetin (● -- ●), myricetin (■ -- ■), morin (◆ -- ◆), kaempferol (▲ -- ▲), and (±)-taxifolin (* -- *).

vestigation demonstrates that flavonoids have an inhibitory effect on MMLV RT. These flavonoids are ubiquitous in vascular plants, including food plants, and are therefore consumed almost daily (11).

Among the four groups of flavonoids tested, flavonols and flavanonols showed the highest activity in inhibiting the MMLV RT. It was observed that the flavones and flavanones show a lower inhibitory activity against RT. This strongly suggests that flavonoids that simultaneously have free hydroxyl groups at position 3 and 4′ exhibit a higher inhibitory activity against RT. When the hydroxyl group at position 3 is glycosylated with rutinose or rhamnose, for example, to form rutin or myricitrin, respectively, the inhibitory activity of these flavonoids is decreased moderately. A similar phenomenon was also observed for the flavonoids when the 4′-hydroxyl group was blocked with a methyl group, such as occurs in hesperetin. The double bond at position 2 and 3 seems not to be essential for the inhibitory activity, as similar high inhibitory activity is found for flavonols and flavanonols (Table 2).

It was of great interest to observe that flavonoids were structurally different from AZT or dideoxycytidine. In contrast to those compounds, the flavonoids studied can inhibit RT activity by using either $(rA)_n(dT)_{12-18}$ or $(rC)_n(dG)_{12-18}$ as template-primers.

TABLE 2. Structural Formulae of the Flavonoids.

Flavonols	
Fisetin	3-, 7-, 3′-, 4′-OH
Kaempferol	3-, 5-, 7-, 4′-OH
Morin	3-, 5-, 7-, 2′-, 4′-OH
Quercetin	3-, 5-, 7-, 3′-, 4′-OH
Myricetin	3-, 5-, 7-, 3′-, 4′-, 5′-OH
Myricitrin	5-, 7-, 3′-, 4′-, 5′-OH; 3-*O*-L-rhamnose
Rutin	5-, 7-, 3′-, 4′-OH; 3-*O*-rutinose

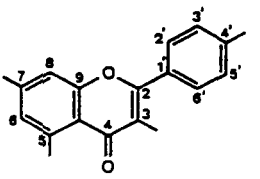
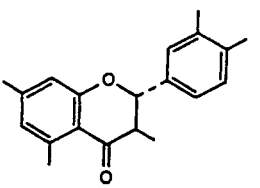
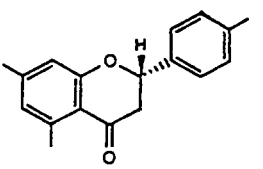
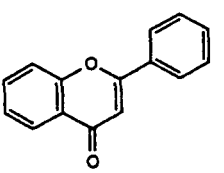


TABLE 2. Continued

Flavanonols		
(±)-Taxifolin	3-, 5-, 7-, 3′-, 4′-OH	
(+)-Catechin	3-, 5-, 7-, 3′-, 4′-OH	
(−)-Epicatechin	3-, 5-, 7-, 3′-, 4′-OH	
Flavanones		
Flavanone		
Hesperetin	5-, 7-, 3′-OH; 4′-Me	
Flavones		
3-Hydroxyflavone	3-OH	
7-Hydroxyflavone	7-OH	

This suggests that flavonoids can inhibit RT by competing with either $(rA)_n(dT)_{12-18}$ or $(rC)_n(dG)_{12-18}$ as template-primers while AZT can compete only with $(rA)_n(dT)_{12-18}$ (8,19). Many natural flavonoids, including some studied in the present investigation, form part of the daily diet, particularly for Chinese people, who traditionally eat more vegetables than meat.

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