concentration of inorganic phosphate in the growth medium.[1] It is capable of exerting an inhibitory effect on cells containing an active glycerol-3-phosphate dehydrogenase.[1] Recent studies indicate that lipid synthesis *in vivo* is more sensitive to inhibition by **6** than DNA, RNA, and protein synthesis.† It appears to be a competitive inhibitor of glycerol 3-phosphate in the glycerol 3-phosphate:CMP phosphatidyl transferase reaction.† It is also a substrate for rabbit muscle glycerol-3-phosphate dehydrogenase.‡

Experimental Section§

3-Butenyl Bromide (2). A prior synthesis of this compound is reported by Juvala.[2] To 651 g (1.386 moles) of freshly prepared triphenyl phosphite dibromide were added 54.8 g (0.693 mole) of 3-buten-1-ol, 1 (prepared according to the method of Linstead and Rydon[3]), and 55.7 g (0.705 mole) of pyridine, and the mixt was stirred for 2 hr. The precipitate of pyridine hydrobromide was removed with suction filtration and the volatile components of the filtrate were collected in a cold trap under vacuum. Upon fractional distillation there was isolated 44.9 g (48%) of pure **2**, bp 32° (47 mm).

Diethyl 3-Butene-1-phosphonate (3). To the refluxing bromide, **2** (49.9 g, 0.333 mole), was added dropwise 66.4 g (0.400 mole) of freshly distilled triethyl phosphite, and the mixt was refluxed for 2 hr. The reaction mixture was then distilled using an 18-in. spinning band column, EtBr being removed first at atmospheric pressure. Under reduced pressure was then isolated 43.6 g (68%) of pure **3**, bp 78° (1.75 mm). The ir and nmr spectra were in accord with the proposed structure. *Anal.* ($C_8H_{17}O_3P$) C, H.

Dilithium 3,4-Dihydroxybutyl-1-phosphonate (6). To 28.5 g (0.150 mole) of phosphonate **3** were added 76 ml of 88% formic acid and 22.1 ml of 30% H_2O_2. The reaction mixture was maintained overnight in the temperature range 40–50°. Volatile materials were removed under reduced pressure on a rotary evaporator leaving as a syrupy, clear liquid, impure diethyl 3,4-dihydroxybutyl-1-phosphonate, **4**. This material was not purified but hydrolyzed directly. To it was added 220 ml of hot, saturated LiOH solution. The reaction mixture was heated in an autoclave at 120° for 5 hr. Volatile components were removed under reduced pressure to give a syrupy liquid which upon washing with three 300-ml portions of acetone yielded a white precipitate, isolated by suction filtration, and dried under vacuum. Spectra indicated this to be the partial ester, lithium ethyl 3,4-dihydroxybutyl-1-phosphonate, **5**, although further purification was not attempted. This material was dissolved in 350 ml of water with 20 ml of saturated LiOH solution, and the reaction mixture was heated in an autoclave for 3 hr at 120°. A white precipitate formed which was isolated by suction filtration. The filtrate was returned to the autoclave for 2 hr at 120°; the second crop of crystals was isolated and combined with the first. The combined materials were washed with several portions of 95% ethanol and ether and dried under vacuum to yield 11.0 g (49%) of **6**. The ir and nmr spectra were in accord with the proposed structure. *Anal.* ($C_4H_9O_5PLi_2$) C, H.

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§All nmr spectra were measured using a Varian A60-A instrument. The ir spectra were measured on a Perkin-Elmer 237B spectrophotometer. The microanalyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y. Structures were confirmed by ir and nmr spectra, all of which agreed with those predicted. Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for those elements or functions were within ±0.4% of the theoretical values.

Synthesis and Antiviral Activity of 4′-Hydroxy-5,6,7,8-tetramethoxyflavone

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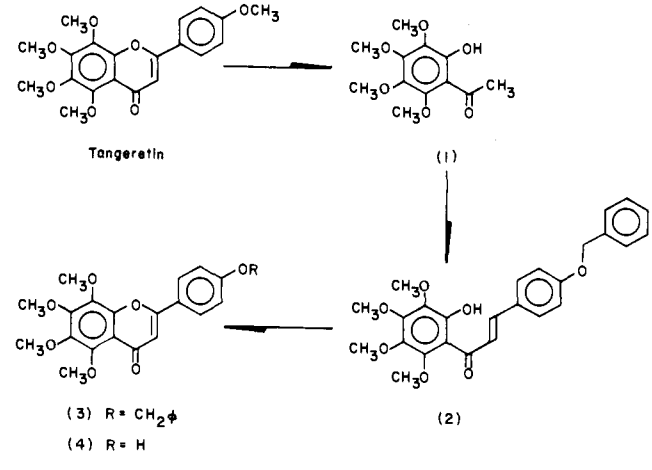
Many pharmacological properties have been ascribed to various flavonoids. Our current interest has centered in those with fully hydroxylated A rings. Tangeretin (4′,5,6,7,-8-pentamethoxyflavone) has shown marked cytotoxicity against the rapidly developing zebra fish embryo.[1] Nobiletin (3′,4′,5,6,7,8-hexamethoxyflavone) and tangeretin have been described as having fungistatic properties toward *Deuterophoma tracheiphila*, which causes a widespread disease of citrus trees in the Mediterranean.[2]

In another report[3] nobiletin showed antiinflammatory activity which was described as being 46% as effective as hydrocortisone phosphate on a weight basis in the test system used. The capacity of nobiletin and tangeretin to stimulate the enzyme, benzpyrene hydroxylase, which detoxifies carcinogenic hydrocarbons and hydroxylates steroids has been reported by Wattenberg, *et al.*[4]

The present report details the pronounced antiviral effect of 4′-hydroxy-5,6,7,8-tetramethoxyflavone (**4**, 4′-desmethyltangeretin) against type 13 rhinovirus in cell culture. 4′-Hydroxy-5,6,7,8-tetramethoxyflavone (**4**) was prepared in 3 steps starting with 2′-hydroxy-3′,4′,5′,6′-tetramethoxyacetophenone (**1**) prepared by the basic degradation of tangeretin. The condensation of **1** with *p*-benzyloxybenzaldehyde in EtOH and 50% KOH gave a 71% yield of 4-*p*-benzyloxy-2′-hydroxy-3′,4′,5′,6′-tetramethoxychalcone (**2**). Oxidative closure of **2** in *n*-amyl alcohol with SeO_2 gave an 86% yield of 4′-benzyloxy-5,6,7,8-tetramethoxyflavone (**3**).[5] Hydrogenation of **3** in AcOH with 5% Pd/C as catalyst provided 4′-hydroxy-5,6,7,8-tetramethoxyflavone (**4**).



Antiviral Evaluation. 4′-Hydroxy-5,6,7,8-tetramethoxyflavone (**4**) was tested for *in vitro* activity against types 1A, 13, and 56 rhinoviruses grown in continuous-passaged cells of human carcinoma of the nasopharynx (KB). The tests were carried out in disposable plastic microplates using inhibition of virus-induced cytopathogenic effect (CPE), previously described,[6] as the primary criterion for evaluation of antiviral activity. The virus rating (VR) system[6] was used for determining the degree of significance of CPE inhibition. In this VR system, activity of 0.5 or greater was considered indicative of significant antiviral effect. 4′-

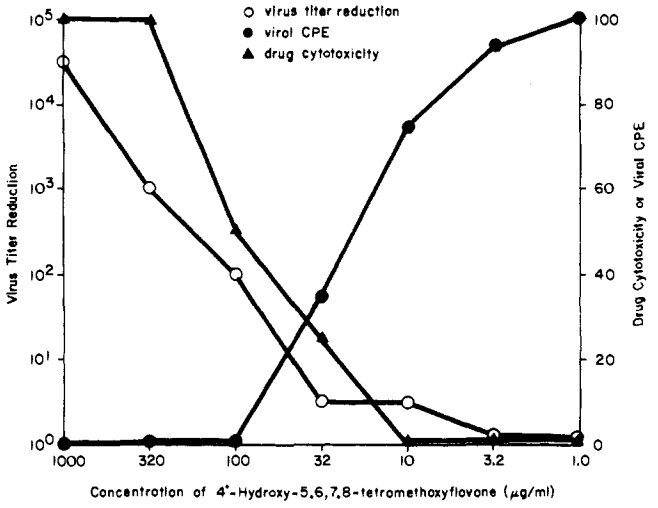


Figure 1.

Hydroxy-5,6,7,8-tetramethoxyflavone (4) had activity in repeated experiments against two of the three rhinoviruses tested. Against the type 13 virus, it had VR's ranging from 0.5 to 0.7. Against the type 56 virus a VR of 0.5 was obtained. Little activity was seen against the type 1A virus (VR 0.1-0.2). For comparative purposes, 2-methyl-4-[(5-methyl-5*H*-*as*-triazino[5,6-*b*]indol-3-yl)amino]-2-butanol (SK & F 30097), a compound known to possess activity against type 13 rhinovirus,[7] was tested in parallel experiments. This compound had VR's also ranging from 0.5 to 0.7 against all three rhinoviruses tested.

To further substantiate the antiviral activity seen, the effect of 4 on the quantity of infectious virus recoverable from treated KB cells was determined. In this experiment, 1000, 320, 100, 32, 10, 3.2, and 1.0 μg/ml of the compound were added to separate sets of cells 5 min before they were exposed to 100 cell culture infectious doses of type 13 rhinovirus. After a 3-day incubation at 33°, each group of cells was examined for inhibition of viral CPE as compared with untreated virus control cells. The cells were then disrupted by alternately freezing at −12° and thawing a total of three times. The supernatant fluid was then removed and varying dilutions of it added to a monolayer of KB cells which were in turn incubated for 3 days and examined for the presence of virus as evidenced by CPE. Reductions in virus titer ranging from 1 to 5 logs were seen in this experiment, with this titer reduction varying directly with the amount of compound originally added to the cells. The viral CPE inhibition and the titer reduction brought about by 4 is indicated in Figure 1.

Experimental Section

Infrared spectra were determined on a Perkin-Elmer 257 spectrophotometer (KBr), uv on a Cary 15 spectrophotometer, nmr on a Perkin-Elmer MR-20 using TMS as an internal standard, and mp on a Fisher-Johns block (corrected). C, H, and N were determined by M. H. W. Laboratories, Garden City, Mich., and Huffman Microanalytical Labs., Wheatridge, Colo.

2'-Hydroxy-3',4',5',6'-tetramethoxyacetophenone (1). Tangeretin† (4',5,6,7,8-pentamethoxyflavone) was degraded by treatment of 50 g in a refluxing mixture of 500 ml of EtOH and 500 ml of 40% KOH for 6 hr. The mixture was cooled and filtered and the filtrate was brought to pH 7-8 by addition of solid CO_2 (the pH adjustment can be facilitated by heating the basic solution during the addition of CO_2; this greatly reduces the foaming). The neutralized solution was extracted with Et_2O (3X). The combined Et_2O solutions were extracted 6 times with 5% NaOH. The combined basic washes were acidified with HCl and then extracted with Et_2O (3X). The Et_2O washes were combined, washed 2 times with saturated NaCl solution, and then dried over Na_2SO_4. The Et_2O was removed to yield 27 g (79%) of 1. The acetophenone (1) can be distilled under vacuum at 122–124° (0.75 mm).

4-Benzyloxy-2'-hydroxy-3',4',5',6'-tetramethoxychalcone (2). To a solution of 5.0 g (0.026 mmole) of *p*-benzyloxybenzaldehyde and 5.5 g (0.0212 mmole) of 1 in 100 ml of EtOH was added 10 ml of 50% KOH. The red solution stood at room temp for 24 hr and was then acidified to pH 3–4 with concd HCl. Water (125 ml) was added, and the solution extracted 3 times with Et_2O. The Et_2O was washed with H_2O, dried over Na_2SO_4, and evaporated to dryness. The residue was crystallized from MeOH to give 6.6 g (71%) of **2**, which melted at 95–98°. An analytical sample was crystallized from MeOH. *Anal.* ($C_{26}H_{26}O_7$) C, H.

4'-Benzyloxy-5,6,7,8-tetramethoxyflavone (3). The chalcone 2 was cyclized to the flavone by heating and stirring 5.0 g of 2 and 4.0 g of SeO_2 in 50 ml of *n*-amyl alcohol at 125° for 6 hr. The Se was removed by filtration of the mixture while still hot, and the filtrate was then cooled in the refrigerator giving crystalline 3. Recrystallization of the solid from MeOH gave 4.3 g (86%) of tan needles which melted at 145–146°. The reported mp is 145–146°.[5]

4'-Hydroxy-5,6,7,8-tetramethoxyflavone (4). Ease of removal of the benzyl group by hydrogenation was dependent on the purity of 3. A mixture of 2.2 g of 3 and 0.5 g of 5% Pd/C catalyst in 50 ml of AcOH was shaken at 1.6 atm of H_2 pressure (gauge) for 2.5 hr. The catalyst was removed by filtration and the filtrate evaporated. The residue was crystallized from MeOH to give 1.3 g (74%) of 4. An analytical sample was crystallized from MeOH and melted at 200–202°. *Anal.* ($C_{19}H_{18}O_7$) C, H.

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Mechanism of the Reaction of a 1,4-Benzodiazepine *N*-Oxide with Acetic Anhydride†

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As part of our studies on the mechanism of microsomal mixed function oxygenations of nitrogen-containing compounds, we recently demonstrated that molecular oxygen and not water is the source of the C-3 oxygen atom found in the diazepam (1) biotransformation product, 3-hydroxydiazepam (2).[1] This carbinolimine metabolite is analogous to carbinolamines which have been proposed as unstable intermediates in microsomal oxidative N-dealkylations.[2] Evidence suggesting that N-oxidation species may be involved in the metabolic formation of carbon-oxygen bonds has been reviewed,[3] and several reports given at a recent symposium on the biological oxidation of nitrogen in organic molecules are concerned with the question of the possible intermediary role of nitrogen-oxygen bonds in microsomal C-oxidation.[4] The oxygen atom in metabolically formed N-O systems is reported to be O_2 derived.[5] There-

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