previous suggestion [1] that captopril shows antioxidant properties. The optimum concentration of captopril was established at 0.2%. In most cases the concentration of linoleic acid in the stabilised oil at 313 K is the same as in the fresh oil. It should be mentioned that the concentration of linoleic acid in the samples stabilised with 0.2% captopril decreases only slightly more at 333 K then at 313 K. The sample without antioxidant incubated at a temperature of 333 K indicated a 5% decrease of linoleic acid. For oleinic and linoleic acid the captopril antioxidant properties were significant. The decrease in the linoleic and oleinic acid contents in the samples with octyl gallate was higher then in samples with captopril. In conclusion, we found that captopril can be defined as a substance whose presence in a relatively low concentration (0.2%), significantly inhibits the rate of oxidation of sunflower oil *in vitro*. The results confirmed that captopril appears to be a more effective antioxidant then octyl gallate [4, 5].

Experimental

1. Sample preparation

Working solutions were prepared in open glass containers. Each container included 100.0 g sunflower oil without or with antioxidant at concentrations of 0.05%, 0.1% or 0.2%. All samples were kept in an incubator at 313 K and 333 K protected from light. The reference was sunflower oil incubated at 278 K. The contents of oleinic and linoleic fatty acids were determined after different time intervals.

2. Preparation of fatty acid methyl esters

The fatty acids composition of lipids is usually determined by GC of the corresponding methyl esters. Preparation of fatty acid methyl esters (FAMES) by transmethylation of lipids was made according to the method described by Garces and Mancha [3]. Samples of 50 mg of sunflower oil with methylating mixtures containing methanol/toluene/dimethoxypropan/H_2SO_4 (30 : 20 : 5 : 2 v/v) were placed in tubes with teflon caps. To the methylating mixtures in the volume of 3.3 ml, heptane was added to a total volume of 5 ml. The tubes were placed in a water bath at 80 °C for 90 min and then subjected to vigorous shaking for 3 min. Heating was necessary to mix all components into a single phase. After heating, the tubes were cooled to room temperature and shaken. Two phases were formed, the upper one contained the fatty acid methyl esters.

3. Chromatographic conditions and instrumentation

The fatty acid composition was determined by GC-MS in a silica capillary column with high polarity. The capillary column 25 m × 0.25 mm (Macherey-Nagel) was operated from 96 °C to 196 °C at the rate of 25 °C/2 min. The temperatures of the injector and the detector (FID) were 200 °C and 220 °C, respectively. The carrier gas was helium. Gas chromatograph: Hewlett Packard model 5890 Series II.

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Cyclic AMP phosphodiesterase inhibition by coumarins and furanocoumarins

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Identifying the mechanisms of action of known drugs or new agents is an ongoing process in drug development. In many cases, novel actions that are not identified before may be found and this could lead to refining of their therapeutic profile. Coumarins are shown to have many different bioactivities. Among the bioactivities of coumarins, photoreactivation has been the subject of many studies [1]. However, only a few reports have studied other cellular processes.

The circulatory effects of coumarins have been studied on the basis of the phosphodiesterase inhibitory activity. The reports indicate the involvement of cAMP-phosphodiesterase inhibition in coronary vasodilatory effects of acyloxydihydropyrano- and acyloxydihydrofuranocoumarins [2]. Inhibition of platelet aggregation also occurred by increasing intraplatelet cAMP concentration due to the application of coumarins [3, 4].

Certain esters of dihydropyranocoumarin, dihydrofuranocoumarin alcohols and isocoumarins have previously been shown to inhibit the cAMP-phosphodiesterase from bovine heart. These naturally occurring coumarins also inhibit the high affinity cAMP-phosphodiesterase from human platelets with activities that closely correlate with those obtained using phosphodiesterase from bovine heart tissue [5, 6]. 3-Arylcoumarin derivatives, from *Glycyrrhiza radix*, which is a crude drug of kampo herbal medicines, have been shown to inhibit platelet aggregation, phosphorylation of 40 K and 20 K dalton proteins, inositol 1,4,5-trisphosphate production, intraplatelet calcium increase and phosphodiesterase activity *in vitro* [3].

Coumarins have other related activities on the circulatory system as well. For example, AD6 is a coumarin derivative which is able to inhibit platelet aggregation and release due to various agonists as adrenaline, PAF (platelet activating factor), Ca^{2+} ionophore and others. It has been demonstrated that this compound reduces the production of free arachidonate and diglyceride from human platelets pulse-labeled with radioactive arachidonic acid, thus suggesting a possible interference with the activity of phospholipase A2 and/or phospholipase C [7].

In a separate study, we reported that the cAMP-phosphodiesterase inhibitory activity of naturally occurring compounds from *Fraxinus americana* such as syringin (**3**), verbascoside (**4**), ligstroside (**5a**) and 10-hydroxyligstroside (**5b**) are not comparable to coumarins, such as esculetin (**1c**) and fraxin (**1f**), which are usually found in several species of *Fraxinus* (Table) [8]. In this paper, we would like to report the phosphodiesterase inhibitory property of several synthetic as well as natural coumarins. The activities of these novel derivatives have been reported for the first time.

The results of cAMP phosphodiesterase inhibition by these agents are shown in the Table. In comparison to caffeine, it can be seen that many of the coumarins tested, show strong inhibitory activity on phosphodiesterase.

Table: cAMP Phosphodiesterase (bovine heart muscle) inhibitory activity of several coumarin derivatives

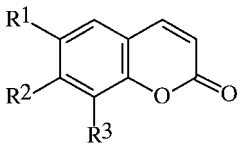
Compd.	PDI activity ($IC_{50} \times 10^{-5}$ M)
1a	178.58
1b	40.34
1c	19.7
1d	>500
1e	>500
1f	9.0
1g	5.77
1h	11.23
1i	>500
1j	36.66
1k	54.92
1l	121.56
1m	13.50
1n	2.90
2a	31.60
2b	4.71
2c	7.47
2d	25.33
2e	22.71
2f	22.38
2g	15.34
2h	>500
3	>500
4	>500
5a	>500
5b	>500
Caffeine	58.6

Among the simple coumarins, **1a**, **1b** and **1g**, the iodo derivative is the strongest enzyme inhibitor. Among the higher molecular weight derivatives, **1d**–**l**, compounds **1f** and **1h** are clearly superior. Trisubstituted coumarins **1m** and **1n**, both show strong phosphodiesterase inhibitory activity, however, **1n** is the stronger derivative. Although many of the furanocoumarins **2a**–**h** have moderate to strong inhibitory activity, **2b** and **2c** are the strongest and **2h** is not active.

Two factors could be important for a coumarin to show phosphodiesterase inhibition. First is the lipophilicity. A comparison among the following similar pairs revealed that the more lipophilic one is usually the stronger inhibitor (the latter in each pair): **1b** and **1g**, **1i** and **1j**, **1i** and **1h**, **1l** and **1k**, **1m** and **1n**, **2d** and **2e**.

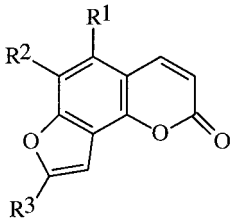
The second factor is the presence of a phenolic hydroxyl group at positions 6 or 7. Representations of this group are the following structurally comparable pairs of coumarins, in which the stronger inhibitor of each pair has one free phenolic hydroxyl (the latter in each pair): **1a** and **1b**, **1i** and **1h**, **2a** and **2b**. It should be noted that the strongest inhibitors of all coumarins tested, ie., compounds **1n** and **2b**, both have one phenolic hydroxyl and can be considered lipophilic as well. The position of the hydroxyl group can be variable at either location 6 or 7. However, the position 8 may not contain a phenolic hydroxyl to show a strong activity, which is suggested by comparing structures **1e** and **1f**. Compound **1d** with phenolic hydroxyl shows very weak or no activity. This could be explained by its lower lipophilicity due to the glucosyl moiety.

In a study, common furanocoumarins, like 8-MOP (8-methoxypsoralene), psoralen, and 5-MOP have been tested together with UV light at 365 nm on macrophage cells. They inhibited the enzyme phosphodiesterase in a dose dependent manner. 8-MOP was the strongest in this respect [9]. In another study, psoralen, 8-MOP, 4,5′,8-trimethylpsoralene (TMP), 4,6,4′-trimethylangelicin (TMA) and khellin were tested for phosphodiesterase inhibitory activity. TMA and TMP, which are more lipophilic than angelicin and psoralen, showed significant activity [10]. Licoarylcoumarin was identified as a strong inhibitor of adenosine 3′,5′-cyclic monophosphate (cAMP) phosphodiesterase. The structure-activity relationships of many 4-arylcoumarins have been studied. In this group, 5,7-dihydroxy derivatives were generally highly inhibitory towards cAMP phosphodiesterase [11]. These reports support our findings that lipophilicity and a phenolic hydroxyl are two important factors in the phosphodiesterase inhibitory activity of coumarins. In addition to these structural features, the dichromone group has been shown to be important as well [12].



1

a: R^1, R^2, R^3 = H
b: R^1, R^3 = H, R^2 = OH
c: R^1, R^2 = OH, R^3 = H
d: R^1= OGlu, R^2 = OH, R^3 = H
e: R^1= OMe, R^2, R^3 = OH
f: R^1= OMe, R^2 = OH, R^3 = OGlu
g: R^1 = H, R^2 = OH, R^3 = I
h: R^1 = OH, R^2 = OCH_2CHCH_2, R^3 = H
i: R^1 = OGlu, R^2 = OCH_2CHCH_2, R^3 = H
j: R^1 = $OGlu(Ac)_4$, R^2 = OCH_2CHCH_2, R^3 = H
k: R^1 = OCH_2Ph, R^2 = OCH_2CHCH_2, R^3 = H
l: R^1 = OCH_2Ph, R^2 = $OCH_2CHOHCH_2OH$, R^3 = H
m: R^1 = OCH_2Ph, R^2 = OH, R^3 = $CH_2CHOHCH_2OH$
n: R^1 = OCH_2Ph, R^2 = OH, R^3 = CH_2CHCH_2



2

a: R^1, R^2, R^3 = H
b: R^1= H, R^2 = OH, R^3 = H
c: R^1= OCH_2Ph, R^2 = OH, R^3 = H
d: R^1, R^2 = H, R^3 = COOH
e: R^1, R^2 = H, R^3 = COOEt
f: R^1, R^2 = H, R^3 = CONLeuOEt
g: R^1, R^2 = H, R^3 = CONPheOEt
h: R^1, R^2 = H, R^3 = CONZ-LysOMe

Experimental

1. Chemistry

The preparation of compounds **1**–**3** has been reported elsewhere [13].

2. Assay method for cyclic AMP phosphodiesterase inhibition

The inhibitory effect of compounds on the enzyme activity was assayed by the method described easrlier [14]. Samples were dissolved in DMSO and tested for their activity against phosphodiesterase of beef heart (Sigma).

The standard reaction mixture that includes [^{3}H]-cyclic AMP, beef heart phosphodiesterase, and the sample was incubated for 30 min at 37 °C, and the unchanged [^{3}H]-cyclic AMP was measured with liquid scintillation counter. IC_{50} value is the concentration of compound required to give 50% inhibition of phosphodiesterase activity.

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