in contact with the active ingredient. Linearized forms of the dissolution curves are compared in Fig. 1B. Values of rate constants for dissolution [3, 4] are (min^{-1}):

$$K_1 = 5.97 \cdot 10^{-3}; \qquad K_2 = 3.15 \cdot 10^{-3};$$
$$K_3 = 2.81 \cdot 10^{-3}.$$

Experimental

1. Preparation of boluses

Aerosil 200 (colloidal silicon dioxide) (Wacker Chemie GmbH), Albendazole USP 23 (Transchem), Potato starch (AVEBE), Kollidon 30 (polyvinylpyrrolidone) (BASF), Kollidon CL (cross-linked polyvinyl pyrrolidone) (BASF), Lactose monohydrate (Pharmatose 200M) (DMV International), Magnesium stearate (Carasco), Vitacel F120 (powdered cellulose) (J. Rettenmaier & Söhne GmbH).

Boluses of identical composition and the same average mass of 2.5 g (2.375–2.625 g; Size: 28.4 × 13.5 × 7.1 mm; oval, round shape) were prepared using three kinds of technology.

Composition of boluses: Inner phase – albendazole (100%) 24.0 w/w %, Pharmatose 200M 40.0 w/w %, Potato starch 12.5 w/w %, Vitacel F120 10.0 w/w %, Kollidon 30 3.5 w/w % (dissolved in water (3.5 g/3.0 ml) when applying wet granulation); Outer phase – Vitacel F120 6.0 w/w %, Kollidon CL 2.0 w/w %, Magnesium stearate 1.0 w/w %.

Equipment: Lödige FM 130 homogenizing device (Lödige), Manesty oscillating sieve (BWI Manesty), Aeromatic fluid bed dryer (Aeromatic – Fielder AG), Frewitt SMG vibrating sieve (Frewitt Apparatebau GmbH).

IR 520 Chilsonator compaction/granulation system (Fritzpatrick): speed of the vertical feed screw (260 rpm), speed of the horizontal feed screw (55 rpm), velocity of the compaction rolls (20 rpm); (the surface texture of the roll was selected depending on the quality of the material), distance between the rolls (1.9 mm), (the gap between the rolls and the resulting thickness of the compacted product were depending on the setting of the above mentioned parameters), pressure applied (air to hydraulic actuator regulates pressure exerted on rolls) (45 bar), rotor speed (300 rpm), screen type (mesh size: 3.18 mm, rough surface, round holes).

Ed. Frogerais excenter tablet press (ed. Frogerais): compression force 15–16 kN.

2. Physical tests

Pharma Test Friabilator PTF, Pharma Test disintegration tester PTZ 1, Pharma Test hardness tester (Pharmatest Apparatebau GmbH).

3. Dissolution experiments

Acetic acid >99.5% (Fluka), ammonium hydroxide 1 M (Fluka), butyric acid >99.5% (Fluka), citric acid (Reanal), propionic acid >99.5% (Fluka), sodium citrate (Reanal), sodium hydroxide (Merck).

Composition of artifical rumen fluid [5]: acetic acid 65 mmol/l, propionic acid 21 mmol/l, butyric acid 14 mmol/l, ammonium hydroxide 5 mmol/l, sodium hydroxide 98 mmol/l.

Equipment: Sotay CE6 dissolution testing apparatus (Sotax AG) with flow through cell. Sample unit of the preparation: 1 bolus (containing 600 mg albendazole); reservoir for the dissolution medium: 12.5 l; temperature (in accordance with temperature of the biological medium): 40.0 ± 0.5 °C. [6]; sample: 5 ml; 13 mm diameter Millex-HV_{13} filters with 0.45 μm pores (Millipore GmbH).

HPLC: Varian 9010 gradient pump, Varian 9065 detector (Polychrom diode array detector), Varian Star chromatographic software, Nucleosil C-18 (octadecyl silica) column, colum length: 250 mm, column inner diameter: 40.0 mm, particle size: 5 μm. Eluent: 85% methanol – 15% distilled water, 25 mM/l dihydrogene phosphate. Flow rate: 1.0 ml/min, detection wavelength: 234 nm, Injection volume: 10 μm.

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The influence of captopril on unsaturated fatty acids in sunflower oil stabilisation

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In our previous paper captopril (1-[(2*S*)-3-mercapto-2-methyl-propionyl]-L-proline) has been shown to have very strong antioxidant properties *in vitro* [1, 2]. Captopril contains a sulphydryl group which was supposed to act as a free radical scavenger. It was established that captopril significantly inhibited the rate of peroxide formation in sunflower oil. The peroxide value is used as one of the basic analytical parameters to monitor autoxidation changes in oils (BP 93, DAB 10, FPV). However, the peroxide value can only generally characterise the changes in oils.

The aim of this work was to find out the mechanism of the captopril induced delay of unfavourable changes in the unsaturated glycerides of fatty acids during autoxidation. A GC method was used to determine the contents of oleinic and linoleic acids in sunflower oil. The antioxidant properties of captopril were compared with those of octyl gallate (as a reference) which is a well known antioxidant, used in the lipid phase. The sunflower oil samples with or without captopril or octyl gallate in concentrations of 0.05%, 0.1% and 0.2% were incubated at 313 K and 333 K.

All samples were analysed in reference to the fresh sunflower oil incubated at 278 K. The fatty acids composition of lipid samples was determined by GC of the corresponding methyl esters [3]. We determined the percentage decrease of oleinic and linoleic acids in sunflower oil in all samples with and without antioxidants. All samples were examined when they had achieved a peroxide value of approximately 10.0 (a limit determining stability of many lipids according to DAB 10).

The results of percentage changes of oleinic and linoleic acids are given in Table. The investigation confirms our

Table: Content of oleinic and linoleic acid in sunflower oil incubated at different temperatures

Temperature	Sample	Oleinic acid (%)	Linoleic acid (%)
278 K	fresh oil	26.6 ± 0.11	63.6 ± 0.15
313 K	oil	21.9 ± 0.20	62.3 ± 0.23
	oil + captopril 0.05%	22.1 ± 0.15	62.4 ± 0.12
	oil + captopril 0.1%	24.1 ± 0.22	63.6 ± 0.21
	oil + captopril 0.2%	24.5 ± 0.10	63.5 ± 0.15
	oil + octyl gallate 0.05%	22.1 ± 0.18	62.0 ± 0.20
	oil + octyl gallate 0.1%	23.9 ± 0.22	63.0 ± 0.14
	oil + octyl gallate 0.2%	24.1 ± 0.18	63.0 ± 0.17
333 K	oil	21.8 ± 0.10	58.5 ± 0.20
	oil + captopril 0.05%	21.6 ± 0.20	57.9 ± 0.20
	oil + captopril 0.1%	23.3 ± 0.12	63.0 ± 0.21
	oil + captopril 0.2%	23.3 ± 0.14	63.4 ± 0.25
	oil + octyl gallate 0.05%	21.9 ± 0.10	58.7 ± 0.15
	oil + octyl gallate 0.1%	22.0 ± 0.18	60.0 ± 0.10
	oil + octyl gallate 0.2%	22.5 ± 0.18	61.8 ± 0.12

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.