are thus able to compete more successfully with the potassium-ion complexes occurring in a KCl-rich medium.
We have thus demonstrated that pyruvate kinase from *Vibrio costicola* may function as a halophilic enzyme. Its halophilic character requires further testing in vivo.

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Silymarin, an inhibitor of lipoxygenase

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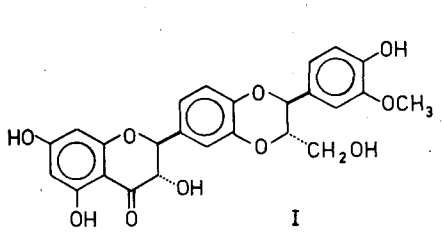
Summary. Silybin (**I**), silydianin (**II**) and silychristin (**III**), the main constituents of silymarin, non-competitively inhibit the lipoxygenase from soybeans (EC 1.13.11.12) in vitro.

Normal lipoid peroxidation is a physiological process which assists the natural decomposition and conversion of membrane lipoids (ML). On the other hand, pathologically increased lipoid peroxidation is regarded as an indication of lasting membrane damage, as under certain circumstances it can have deleterious consequences for the organs involved[3]. The integrity of animal and vegetable membranes is determined by the functional state of the ML's, or of the fatty acids (FA) in them, in particular polyunsaturated fatty acids. The biological properties of membranes, and their functions, such as permeability, active transport, inhibition of contact, conduction in nerves, enzymatic reactions, cellular immune reactions, etc. depend on the presence of ML and on the condition of the FA in the latter[4]. Furthermore, individual FA are precursors of prostaglandins[5].
The natural decomposition of ML is initiated by an attack of oxygen-carrying enzymes on the FA, which are released from the ML by lipolysis. Radical peroxides occur as primary reaction products, and can become the starting point for further oxidative changes in the ML and other cellular components. In an intact and healthy organism, physiological antioxidants and repair mechanisms ensure that these processes are kept within limits[5,6].
Certain toxins (producers of radicals) initiate an autocatalytic peroxidation similar to enzymatic lipoid peroxidation. The effects of this autocatalytic peroxidation exceed by far those of enzymatic lipoid peroxidation, and subsequently cause a rapidly progressive destruction of the pericellular and intracellular membranes[7]. The integrity of the ML is destroyed, and the result is the breaking down of the physiological membrane functions. Externally there are symptoms of poisoning, which, if the natural capacities for compensation and regeneration are overstretched by the excessive or continuous supply of the noxious agent, end in the rapid death of the individual or result in protracted invalidism[3].

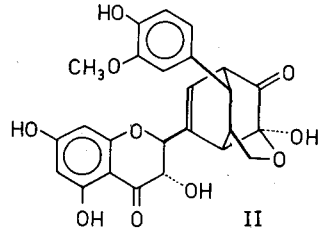
The above-mentioned pathological lipoid peroxidation can be counteracted, in the case of experimental animals, for example, by silymarin, the 'antihepatotoxic principle'[8] of the fruit of the milk thistle[9,10-12]. For this reason silymarin, a complex consisting essentially of the active components silybin (**I**), silydianin (**II**) and silychristin (**III**)[13], is used therapeutically for various liver diseases[14].

For a long time the way in which silymarin works has been linked with a partial or total inhibition of the pathological changes in the ML described above[14]. However, the molecular function of substances **I-III** has not yet been explained completely. In the present investigation we looked into the question of whether, and to what extent, **I-III** influence the activity of lipoxygenase as specific effectors, and whether the effect of silymarin could possibly be due to an inhibition of enzymatic lipoid peroxidation.

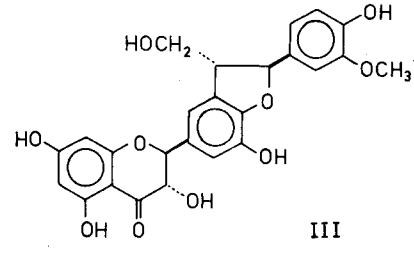
Lipoxygenase (linoleate: oxygen oxidoreductase, EC 1.13.11.12) catalyses the transfer of molecular oxygen to polyunsaturated FA, for example linolic acid, linoleic acid and arachidonic acid, the corresponding FA peroxides being formed in the process[5,6,16]. This enzyme is rich in iron[16] and is ubiquitous in the plant and animal king-



I



II



III

doms[17-17]. It exists as several isoenzymes[18] that are activated by Ca^{++} ions[19]. For practical reasons, the experiments were carried out with vegetable lipoxygenase; one can assume, however, that the function of this enzyme is essentially the same as that of animal lipoxygenase.

Material and method. Test substances[20]. Stable, water-soluble, uniform TLC derivates of **I-III** (Na salts of dihemisuccinates); solutions of the effectors in the buffer (0.1 M Na-K-phosphate pH 6.8) were prepared ad hoc.

Substrate. Linoleic acid, 70-80% (GLC) with the addition of 20-25% linolic acid (Fluka AG, Buchs); Tween 20 (Fluka AG). 0.14 ml of linoleic acid were dispersed in a solution of 0.1 ml of Tween in 10 ml of buffer and mixed drop by drop with 0.1 M NaOH until a clear solution was obtained. Concentrated H_3PO_4 was added to adjust the pH value to 6.8 and the solution diluted with buffer to the specified concentrations (figures 1-3)[1,21].

Enzyme. Commercial lipoxygenase (lipoxidase from soybeans, 50,000 U/mg; Fluka AG); 2 mg of enzyme were dissolved in 1 ml of buffer.

Equipment. Biological oxygen monitor, model 53 (Yellowsprings Instruments, USA); Omniscribe recorder (Houston Instruments, USA); digital pH/mV/temperature meter, model 671 (Extech, Boston/Mass., USA); HP67 programmable electronic computer (Hewlett-Packard), STD-03 programme.

Experimental method[1]. 1 ml substrate, 1 ml effector and 1 ml buffer were saturated with O_2 for 3 min at 20 °C, the mixture being stirred all the while. After the electrode had been inserted and the measuring vessel sealed, 50 µl of enzyme solution were quickly injected. The decrease in O_2 was recorded for 3 min and the O_2 consumption in µM/h calculated from the readings.

Evaluation. The data obtained were transformed according to Lineweaver and Burk[22] and Dixon[23] and the straight lines fitting the measured values optimally were determined by regression calculation.

Results. The oxidation of linoleic acid in the presence of lipoxygenase and molecular oxygen follows Michaelis-Menten kinetics[24]. The value of $K_m = 0.130 \times 10^{-3}$ mol/l measured in the standard effectorless system is of the same order of magnitude as the value given in the literature[25].

The fact that the K_m value is around 0.127×10^{-3} moles/l ($s = 0.003$) with increasing concentrations of **I** points to a non-competitive inhibitory mechanism (figure 1). In this case K_i was 8.32×10^{-3} moles/l ($s = 0.36$). In both methods of representation the correlation coefficients of the regression lines are > 0.992.

Increasing concentrations of **II** also bring about a non-competitive type of inhibition of the lipoxygenase (figure 2), though the effect of **II** is about 5 times more pronounced than that of **I**. The values calculated were 0.136×10^{-3} moles/l for K_m ($s = 0.013$), and 1.62×10^{-3}

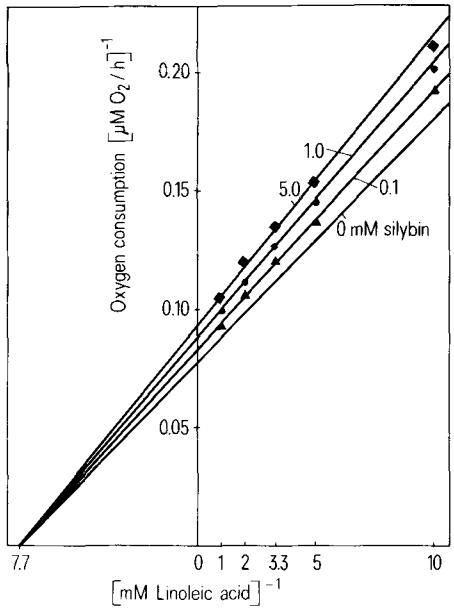


Fig. 1. Lineweaver-Burk plot showing the effect of silybin (**I**) on the standard system.

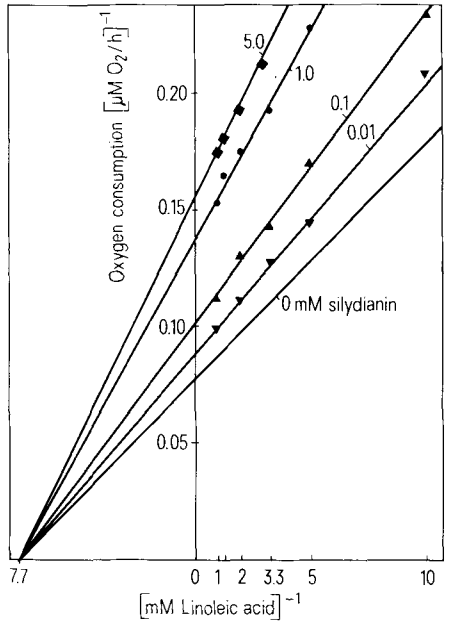


Fig. 2. Lineweaver-Burk plot showing the effect of silydianin (**II**) on the standard system.

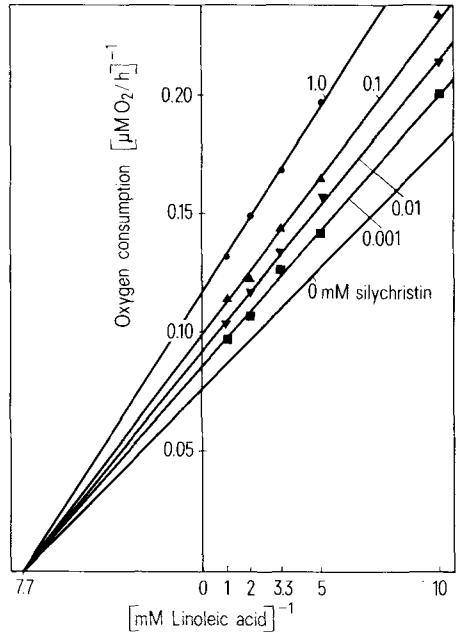


Fig. 3. Lineweaver-Burk plot showing the effect of silychristin (**III**) on the standard system.

moles/l for K_i (s = 0.12), in both cases the correlation coefficients of the regression lines being > 0.989.
The enzyme inhibition caused by **III** was also of the non-competitive type (figure 3). The values calculated in this case were 0.130×10^{-3} moles/l for K_m (s = 0.007) and 2.02×10^{-3} moles/l for K_i (s = 0.11), and r > 0.992. This means that **III** inhibits about 4 times as strongly as **I**.
From these results obtained in vitro it can be seen that silymarin components **I–III** clearly inhibit, depending on concentration, the peroxidation of linoleic acid catalysed by lipoxygenase. An analogous interaction with animal lipoxygenase and thus an inhibition of the peroxidation of the FA in vivo is self-evident. It therefore appears that the assumption that silymarin has a protective function for the ML also holds true experimentally.

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Silymarin, an inhibitor of prostaglandin synthetase

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Summary. Silybin (**I**), silydianin (**II**) and silychristin (**III**), the main constituents of silymarin, inhibit the formation of prostaglandins in vitro. The inhibition is log-linearly dependent on the concentration of the effectors.

The antihepatotoxic effect[3] of silymarin is due, amongst other things, to a particular 'protective effect' which the active components of this natural substance[4] have on certain membrane lipoids[5–7]. In the preceding report[7a] we show that these substances inhibit the activity of lipoxygenase in a specific manner. We have already found a similar inhibitory effect on vegetable peroxidases[8].
Lipoid peroxidation[5–7,9], which is increased by certain hepatic toxins until clinical-pathological symptoms occur, is closely linked to the formation of prostaglandins (PG). There is a high degree of correlation between the 2 processes[10]. The primary substrate for the formation of PG is formed by the polyunsaturated C20 fatty acids, especially arachidonic acid, which are released from the membranes by lipolysis[11]. We were able to show that the effect of arachidonic acid on lipoid peroxidation and PG formation in individual organs depends directly exactly on dosage[10]. On the other hand, the inflammatory processes that can occur in tissues and organs as a result of this and similar pathogenic phenomena are linked to the formation of PG[11,12]. The inhibition of prostaglandin synthetase (PG synthetase) by various corticosteroids[13] and non-steroid antiinflammatory medicaments[14,15], and the fact that inflammatory symptoms can be induced by the application of exogenic PG[12,16], are conclusive indications of the involvement of PG and its precursors in the inflammation process. Investigation into the effect of substances **I–III** on the formation of PG acquires particular significance because various liver diseases are also accompanied by inflammatory processes (hepatitis).
PG synthetase is a membrane bound enzyme complex. This complex combines the properties of a phospholipase, a