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Effect of dianthrones and their precursors from *Hypericum perforatum* L. on lipoxygenase activity

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The biologically active constituents of *Hypericum* species are flavonoids (quercetin glycosides), biflavonoids and naphthodianthrones (hypericin, pseudohypericin) and hyperforin. When the content of flavonoids, hypericin and pseudohypericin in suspension and cell cultures of different *Hypericum* species was established, they varied significantly depending on the geographical origin of the parental plants [1].

Flavonoids are effective scavengers of free radicals and because of this they exhibit antiinflamatory activity. The sensitivity of lipoxygenase (LOX) towards flavonoids isolated from *Hypericum perforatum* has been reported [2]. Naphthodianthrones, particulary hypericin and pseudohypericin are specific constituents of *Hypericum perforatum* L., (Hypericaceae) plants. Hypericin can act as a photosensibilising agent and recently its antidepressant and antiviral activities are investigated [3, 4].

A variety of redox active compounds have been identified as inhibitors of lipoxygenases. Lipoxygenases (EC.1.13.11.12) (LOXs) catalyze dioxygenation of polyunsaturated fatty acids, with a *cis,cis* 1,4-pentadiene unit, to their corresponding hydroperoxide derivatives [5]. According to the currently used nomenclature [6], LOXs are classified with respect to their positional specificity of arachidonic acid oxygenation (5-LOX, 9-LOX, 12-LOX, 15-LOX). LOX from rat lung cytosol is arachidonate-12-LOX [7].

The products of lipoxygenase catalyzed oxygenations as HPETE, HETE, leukotrienes and lipoxins seem to be involved in inflammatory reactions (rheumatoid arthritis and psoriasis). There is good evidence that leukotrienes are mediators of asthmatic responses and are also involved in glomerular nephritis and myocardial ischemia [8].

In our experiments we have tested the inhibitory effect of anthrones (which are considered to be intermediates in the biosynthesis of naphthodianthrones [4]) and naphthodianthrones on linoleate peroxidation by 12-LOX from the rat lung cytosolic fraction. After purification of LOX on Sephadex G-150 and DEAE-Sephacel columns, two forms of enzyme were eluted: one form with SA= 29.5 (pH optimum 6.5) and a second one with SA= 54.1 (pH optimum 9.0). Km for linoleic acid was 1.9×10^{-4} mol · l^{-1} (pH 6.5) and 1.82×10^{-4} mol · l^{-1} (pH 9.0).

The activity of 12-LOX was monitored as an increase in the absorbance at 234 nm which reflects the formation of hydroperoxylinoleic acid. The effect of anthrone, anthraquinone, emodin, hypericin and pseudohypericin was expressed as $1/IC_{50}$ of inhibition. The final concentrations of anthrones and dianthrones tested was between 1×10^{-5} and 4×10^{-5} mol · l^{-1}.

The results obtained (Fig.) show that all compounds tested caused an inhibition of 12-LOX activity. The second form of 12-LOX active at pH 9.0 appeared to be more sensitive to anthrones tested, than that active at pH 6.5. Pseudohypericin was a highly potent inhibitor for both forms of 12-LOX. Similar inhibitory effect on LOX activity exhibited synthetic anthrones (anthralin derivatives) used in the treatment of psoriasis [9].

Many LOX inhibitors inhibit lipid peroxidation acting by scavengering chain-propagating peroxyl free radicals [10]. Some other studies suggest a relationship betweeen LOX inhibition and the ability of the inhibitors to reduce Fe^{3+} at the active site to the catalytically inactive Fe^{2+} [11, 12]. The results obtained here indicate that naphthodianthrone compounds may be involved in the antiinflammatory effects of *Hypericum perforatum* L.

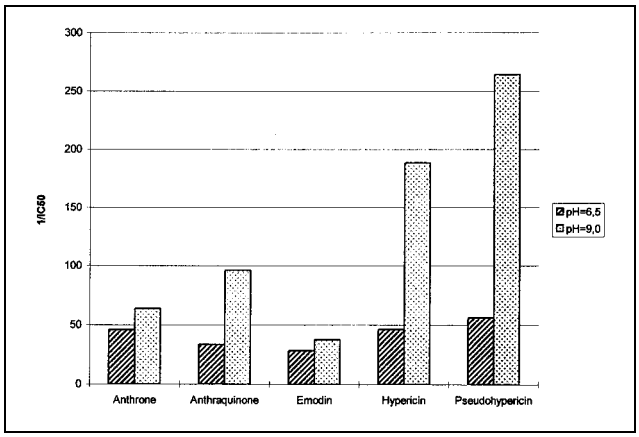

Fig.: Inhibitory effect of anthrones and dianthrones on activity LOX from rat lung cytosol. Values are expressed as $1/IC_5$

Experimental

The cytosolic fraction from rat lung (Wistar rat, male 180 g) as a source LOX was isolated according the procedure of Kulkarni et al. [7]. Linoleic acid (99%, Sigma) was used as a substrate prepared in emulgified state as described [7]. The anthrones and dianthrones are commercially available: anthrone (Sigma/Wien, product number A 1631) anthrachinone (Sigma/Wien, product number A 9706) emodin (Sigma/Wien, product number E 7881) hypericin (Roth, product number 7929.1) pseudohypericin (Hämosan/Austria).

For the LOX activity assay, a Specord M-42 (Carl-Zeiss, Jena) was used. The reaction medium (2.0 ml final volume) contained 50 mM Tris HCl buffer (pH 6.5) or 50 mM borate buffer (pH 9.0), 400 µg of enzyme protein and a solution of linoleic acid (6.0 mM). The reaction was measured at 234 nm for 5 min. For calculation of enzyme activity, an extinction coefficient of 25 mM^{-1} · cm^{-1} was used. The anthrones and dianthrones were tested at final concentrations of 1×10^{-5}–4×10^{-5} mol · l^{-1}.

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