the local market. The plant samples were air dried at room temperature and then finely powdered. The powder (10 g) was extracted with 70% acetone overnight and filtered. The filtrate was evaporated under reduced pressure below 40 °C. The aqueous residue was lyophilised and stored in a dessicator at −20 °C. Total polyphenols present in the lyophilisates was determined by the modified Prussian bleu assay [9, 10] using tannic acid as a standard. The extraction yield was 229 and 118 mg of equivalent tannic acid per gram of *P. granatum* and *Q. ilex* dry matter, respectively.
Fasted male Wistar rats (200–300 g) were randomly divided into 5 groups of 10 rats. The first four treated groups received 5 or 50 mg of polyphenols per kg body weight by oro-gastric intubation; whereas control group received proportionate amount of water by the same route. One hour later, all animals were gavaged with absolute ethanol (5 ml/kg). Fifteen min after administration of the necrotizing agent, animals were killed by cervical dislocation and their stomach rapidly removed. Each stomach was opened along the greater curvature, pinned flat on a corkboard and fixed with 10% formalin. Stomachs were photographed at about 2.3 magnification for lesions evaluation. The total length hemorrhagic lesions was evaluated in blind conditions.

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Orthoforin: The main degradation product of hyperforin from *Hypericum perforatum* L.

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Hyperforin is well documented in literature [1–5]. Recent publications show, that it is a highly important principle of the antidepressant activity of alcoholic Hypericum extracts [6–8]. Pure hyperforin is highly unstable in solution as well as in the solid state due to oxidation. The isolation, purification and structure elucidation of the main degradation product is described here.
The mass spectrum of pure hyperforin, which was treated in methanolic solution by a stream of compressed air for 6.5 h is shown in Fig. 1. The main oxidation product at 551.6 Da in the APCIneg. mode showed a total mass increase of 16 Da compared to hyperforin (535.8 Da). Other signals at 567.6, 583.7 and 599.3 Da indicated further oxidation processes. The main degradation product is an intermediate product undergoing further degradation which could be shown by prolonged oxidation experiments. The oxidized sample was separated by preparative chromatography. The main degradation product was collected between 11.8 and 12.5 min, resulting in a HPLC-purity of 99.9%. Other minor oxidation products were obvious in the chromatogram. Storage in methanol for 4 weeks at −20 °C showed a degradation of only 1%. Structure elucidation was performed using one- and two-dimensional ^{1}H and ^{13}C NMR techniques (COSY, HMBC, HMQC, NOESY). In contrast to the spectrum of hyperforin the ^{13}C signals of one olefinic side chain are missing and new signals at 83.0 and 88.8 ppm are detected. Furthermore, narrow signals for C-2, C-3 and C-4 indicate that there is no tautomerism as in the enol system of hyperforin. Since the major parts of the spectra of orthoforin do not strongly differ from those of hyperforin, a cyclic ether without changes in the central bicyclic ring system is suggested to be present in orthoforin. HMBC correlations between

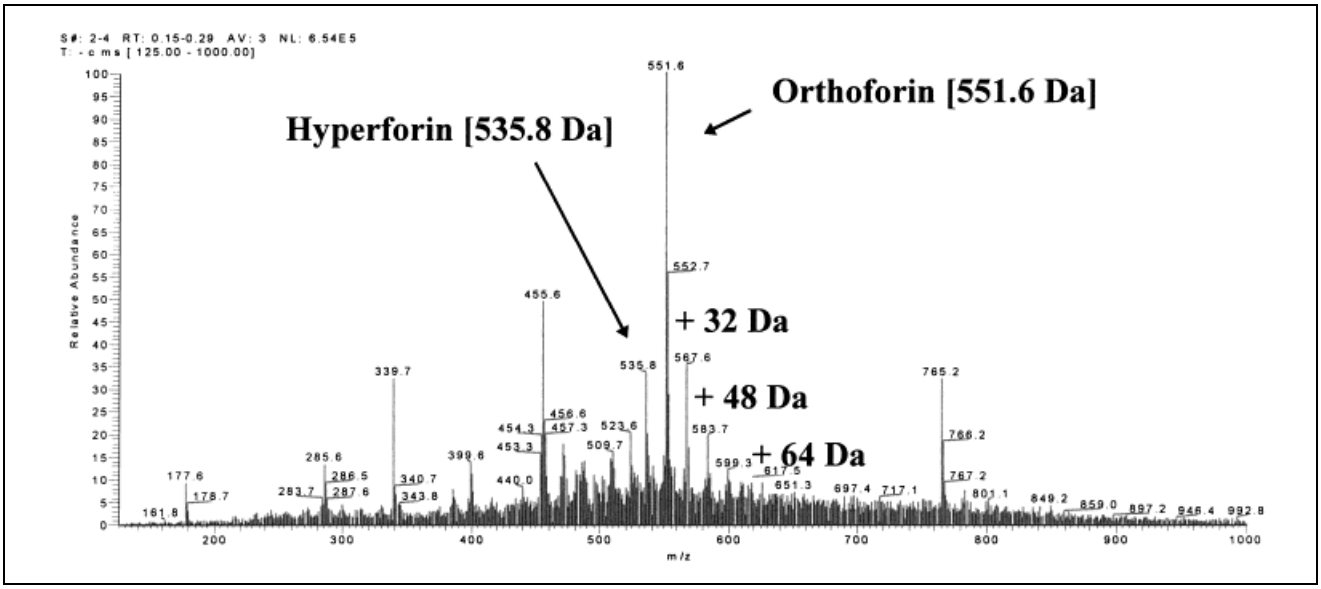

Fig. 1: The mass spectrometric APCIneg.-scan of hyperforin, after 6.5 h of oxidation with compressed air. The main degradation product orthoforin (551.6 Da) shows a mass gain of +16 Da compared to hyperforin. Additionally, signals of 567.6, 583.7 and 599.3 Da can be detected. This could be an indicator for further oxidation processes on the side chains.

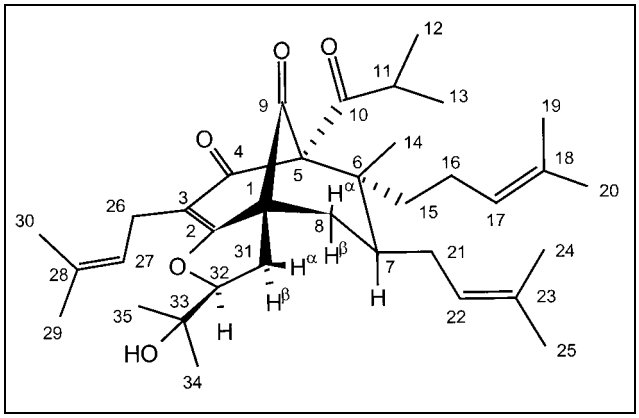

Fig. 2: Structure of orthoforin – numbering according to ref. [4]

H-26 and C-2, C-3, C-4, C-27 and C-28 show, that the side chain at C-3 is unchanged. On the other hand, H-31$^{\alpha}$ correlates to C-1 and C-9 and to C-32 (88.8 ppm) and C-33 (83.0 ppm). Thus, only an additional ring from C-1 to C-2 is possible. The relative configuration of orthoforin is derived from NOESY correlations between H-31$^{\alpha}$ and H-34 and H-35 and between H-8$^{\beta}$ and H-32. The absolute stereochemistry is assumed to be the same as in hyperforin (Fig. 2).

The new compound was named orthoforin. Similar chromatographic behaviour was found for the oxidation product of adhyperforin, so its likely, that this closely related molecule with one additional methylene group at C-12 has the same degradation pattern as hyperforin. Preliminary data show that orthoforin in contrast to hyperforin does not display pharmacological activity. Therefore it is unlikely, that it may contribute to antidepressent effects of St. John's wort extracts.

Experimental

1. Materials and methods

Preparative HPLC was performed on a LCP 4000 pump and a LCD 2082 UV-detector (Biotek, Sulzbach, Germany). UV-spectra were obtained on a diode array detector (Hewlett-Packard 1040A, Böblingen, Germany) and compared to hyperforin. The oxidation products of hyperforin were identified by negative-ion atmospheric pressure chemical ionization mass spectrometry (LCQ, Finnigan, Hemel Hempstead, USA). NMR spectra were acquired on a Bruker Avance 200 spectrometer. The chemical shifts refer to TMS (^{1}H NMR, $\delta = 0$ ppm) or to CD_3OD (^{13}C NMR, $\delta = 49.0$ ppm), respectively. CD_3OD was obtained from Promochem GmbH (Wesel, Germany), all other solvents from Merck KGaA (Darmstadt, Germany) in p.a. or gradient grade quality.

2. Isolation of orthoforin

Pure hyperforin was directly oxidized in methanolic solution by a stream of compressed air for 6.5 h. A VarioPrep column (21 mm × 25 cm) containing 100–7 µm C_{18} material combined with a guard column (10 mm × 4 cm) filled with Nucleoprep 100–20 µm C_{18} material was applied. The mobile phase containing acetonitrile/bidistilled water 89.5/10.5 (v/v) was sparged with helium. The flow rate was 26 ml · min^{-1}. The detector wavelength was set at 272 nm. A 200 µl loop was attached containing 40 mg oxidized hyperforin. The analysis was supported by software Class-CR 10, version 1.3 (Shimadzu, Düsseldorf, Germany). The orthoforin peak was cut out of the flow between 11.8 and 12.5 min, resulting in a k-value of orthoforin = 2.5. The azeotrop phase was removed using a rotary evaporator, the remaining orthoforin/water emulsion was freeze dried using a Lyovac GT2 freeze dryer (Finn-Aqua, Hürth, Germany). The yield was 1.5 mg per run. The samples were stored at −70 °C under nitrogen.

3. Characterization of orthoforin

UV-Spectrum (AcCN/H_2O 89.5/10.5 [v/v]), λ_{max} 272 nm; APCI-MS (neg.) m/z 551.6 [M–H]; ^{1}H NMR (CD_3OD, 200 MHz): $\delta = 0.97$ (d, 6.5 Hz) and 1.05 (d, 6.5 Hz, CH_3-12 and CH_3-13), 1.03 (s, CH_3-14), 1.24 and 1.25 (2 s, CH_3-34 and CH_3-35), ∼ 1.39–1.50 (m, 1 H-15), between 1.55 and 2.20 (not exactly detectable, H-16, H-21), ∼ 1.58–1.73 (m, H-7, H-8$^{\alpha}$), 1.60 (s, CH_3-19), 1.62 (s, CH_3-24), 1.63 (s, CH_3-29), 1.67 (s, CH_3-25), 1.70 (s, CH_3-20, CH_3-30), ∼ 1.89–2.00 (m, 1 H-15), 1.95 (dd, 13.3 Hz, 5.9 Hz, H-31$^{\beta}$), 1.98 (sept. 6.5 Hz, H-11), ∼ 2.05–2.20 (m, H-8$^{\beta}$), 2.56 (dd, 13.3 Hz, 11.0 Hz, H-31$^{\alpha}$), 2.94–3.14 (m, H-26), 4.97 (dd, 11.0 Hz, 5.9 Hz, H-32), ∼ 4.98–5.06 (m, H-17, H-22), ∼ 5.06–5.14 (m, H-27). The chemical shifts of ^{1}H multiplets were mainly derived from 2 D spectra. ^{13}C NMR (CD_3OD, 50 MHz): $\delta = 14.5$ (C-14), 17.8 (C-24), 18.1 (C-19), 18.1 (C-30), 20.6, 20.7 (C-34, C-35), 20.8, 21.9 (C-12, C-13), 22.9 (C-26), 25.9, 26.0 (C-25, C-29), 26.0 (C-16), 26.1 (C-20), 28.2 (C-21), 31.5 (C-31), 37.7 (C-15), 39.0 (C-8), 43.1 (C-11), 44.3 (C-7), 49.3 (C-6), 60.8 (C-1), 83.0 (C-33), 84.6 (C-5), 88.8 (C-32), 117.7 (C-3), 122.4 (C-27), 123.4 (C-17), 125.9 (C-22), 132.0 (C-23), 133.3 (C-28), 134.6 (C-18), 175.7 (C-2), 194.5 (C-4), 205.8 (C-9), 211.4 (C-10). – The ^{1}H and ^{13}C NMR assignments of the fragments C-17 to C-20 and C-22 to C-25 may be interchanged as a whole.

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