Furohyperforin, a Prenylated Phloroglucinol from St. John's Wort (*Hypericum perforatum*)

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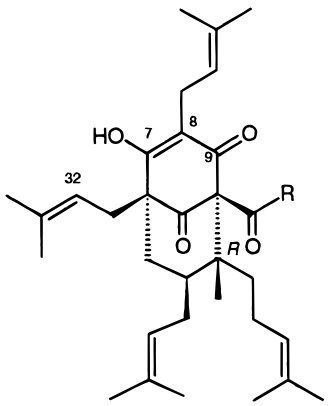
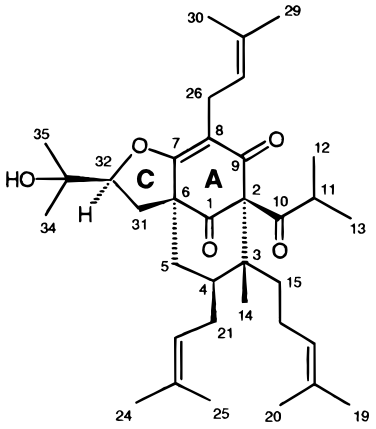
Furohyperforin, an oxygenated analogue of the prenylated phloroglucinol hyperforin, was isolated from the aerial parts of *Hypericum perforatum*. Its structure was elucidated as **2** on the basis of extensive NMR investigations.

The antidepressant activity of St. John's wort (*Hypericum perforatum* L.) (Clusiaceae) extracts has sparked great interest in the chemistry and biochemistry of its constituents, generating coverage also on the mainstream press.[1] More than twenty clinical studies have backed up the use of extracts from this plant to treat mild to moderate depression and improve mood,[2] but several issues have yet to be addressed before *H. perforatum* is accepted in standard pharmacopoeias. The major limitation to a rationale exploitation of the medicinal potential of this plant is our still incomplete knowledge of its active constituent(s). *H. perforatum* L. is a prolific producer of secondary metabolites.[3] Current standardization of plant extracts is based on hypericin, a polar naphthodianthrone under development as an antiviral agent (HIV, hepatitis C) and as a photosensitizer for the photodynamic therapy of cancer.[4] Hypericin shows a moderate anti-monoamine oxidase-A activity,[5] but its relevance for the antidepressant activity of St. John's wort extracts has been strongly questioned,[6] suggesting that activity might be due to other, less polar, constituents.[6] The major lipophilic constituent of St. John's wort extracts is the prenylated phloroglucinol hyperforin (**1a**), an unstable antibacterial compound[7] which has been reported to be at least partially responsible for the antidepressant activity of the plant.[8] Compound **1a** is the major constituent of the flowers and leaves. The presence of its homologue, adhyperforin (**1b**),[9] and more polar analogues of unknown structure has also been described.[9] We report here the isolation and structural characterization of furohyperforin (**2**), a polar analogue of hyperforin occurring in the aerial parts of the plant at a concentration of ca. 5% of **1a**.



1a R= $CH(CH_3)_2$
1b R= $CH(CH_3)CH_2CH_3$



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Compound **2** was obtained as a viscous colorless oil by repeated solvent partitions and chromatographic purifications of the crude extract. The EIMS of **2** disclosed a molecular weight of 552, corresponding to the addition of one oxygen atom to the molecular formula of hyperforin, and the IR spectrum showed hydroxyl and carbonyl absorption bands at 3500 and 1725 cm^{-1}. Since **2** did not react with Ac_2O even under forced conditions (Ac_2O, DMAP), the extra oxygen atom was either a tertiary hydroxyl or part of an ether (epoxide) bridge. The ^1H NMR spectrum of **2** (Table 1) differed from that of hyperforin[10] by the oxidation of one of the four prenyl residues, whose resonances were now replaced by those of an α-hydroxyisopropyl group (δ 1.38 and 1.21, sharp singlets, H-34 and H-35, respectively) and by the ABX system of a $-CH_2-CHOR-$ moiety (δ 2.65 (dd), 1.77 (dd) and 4.55 (dd); $J_{A,B}$ = 13.0 Hz; J_{AX} = 12.5 Hz; J_{BX} = 8.0 Hz, H-31α,β and H-32). In the ^{13}C NMR spectrum, the three broad peaks of the α-substituted enolyzed β-dicarbonyl system of hyperforin (C-7, C-8, and C-9) were replaced by three sharp singlets at δ 172.9, 116.7, and 192.7, suggesting that the keto−enol equilibrium of

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Table 1. NMR Data of Furohyperforin (**2**)[a]

H[b]	δ_H	δ_C	HMBC (H→C)
1		204.5 (s)	
2		83.2 (s)	
3		48.3 (s)	
4	1.62 (m)	43.3 (d)	
5	2.01 (brdd, 13, 4) (α)	38.0 (t)	C-1, C-6, C-7, C-31, C-4, C-21
	1.51 (dd, 13,13) (β)		
6		59.4 (s)	
7		172.9 (s)	
8		116.7 (s)	
9		192.7 (s)	
10		209.5 (s)	
11	1.99 (qq, 6.5, 6.5)	42.0 (d)	C-10, C-12, C-13
12	1.09 (d, 6.5)	20.4 (q)	C-10, C-11, C-13
13	1.00 (d, 6.5)	21.4 (q)	C-10, C-11, C-12
14	1.04 (s)	13.5 (q)	C-2, C-3, C-4, C-15
15	2.06 m	36.3 (t)	
	1.32 m		
16	2.15 m	25.2 (t)	
	1.93 m		
17	5.06 m	124.7 (d)	
18		131.1 (s)	
19	1.65 (br s)	25.6 (q)	C-17, C-18, C-20
20	1.59 (br s)	17.7 (q)	C-17, C-18, C-19
21	2.17 (brd, 15)	26.9 (t)	
	1.77 (m)		
22	4.94 (m)	122.3 (d)	
23		133.5 (s)	
24	1.70 (br s)	25.9 (q)	C-22, C-23, C-25
25	1.57 (br s)	18.0 (q)	C-22, C-23, C-24
26	3.14 (brdd 15, 6.5)	22.1 (t)	C-7, C-8, C-9, C-27, C-28
	3.01 (brdd, 15, 6.5)		C-7, C-8, C-9, C-27, C-28
27	5.08 (brdd, 6.5, 6.5)	121.2 (d)	
28		132.5 (s)	
29	1.65 (brs)	25.7 (q)	C-27, C-28, C-30
30	1.70 (brs)	17.8 (q)	C-27, C-28, C-29
31	2.65 (dd 13,12.5) (α)	30.2 (t)	C-1, C-6, C-7, C-32, C-33
	1.77 (dd 13, 8) (β)		C-1, C-5, C-6, C-7, C-32, C-33
32	4.55 (dd, 12.5, 8)	70.9 (d)	
33		90.1 (s)	
34	1.38 (s)	27.1 (q)	C-32, C-33, C-35
35	1.21 (s)	24.0 (q)	C-32, C-33, C-34

[a] Chemical shifts were determined at 500 (^{1}H) and 125 (^{13}C) MHz in $CDCl_3$. ^{1}H and ^{13}C NMR chemical shifts refer to C*H*Cl_3 at 7.26 ppm and *C*DCl_3 at 77.0 ppm, respectively. *J* values in Hz are in parentheses. [b] Numbering according to ref 9.

the β-dicarbonyl system was covalently blocked by formation of an enol ether.[11] Taken together, these data suggested that **2** differed from **1a** in that either the prenyl on the enolized β-dicarbonyl system or the one on the adjacent quaternary carbon was oxidized and covalently bound with a furan-type oxygen bridge to the enolyzed carbonyl. These suggestions were confirmed by a detailed (500 MHz) analysis of the ^{1}H and ^{13}C NMR spectra, which established the regiochemistry of furan ring formation and the configuration of the new stereogenic center formed in the closure of the oxygen ring. All proton and carbon signals of **2** could be assigned using a combination of 1D and 2D techniques (COSY, HMBC, HMQC, NOE-difference experiments). Since only partially assigned NMR data are available for hyperforin,[10] the data reported in Table 1 represent a significant spectroscopic acquisition for this type of compound. The location of the oxidized prenyl was revealed by the observation that the allylic methylene of one of the three prenyl residues of **2** showed HMBC correlations with two sp^2-hybridized carbons, one carbonyl (δ 192.7, s, C-9) and one oxygen substituted olefin carbon (δ 172.9, s, C-7), an observation consistent with the presence of a prenyl residue on central carbon of the enolized β-dicarbonyl system. The oxidized prenyl was thus the one adjacent to the enolized β-dicarbonyl system. Further confirmation of this somewhat unexpected topology came from the detection of mutual HMBC correlations between the furan C-ring and the cyclohexane B ring methylenes (C-31 and C-5, respectively; correlations C-31/H-5α and C-5/H-31β) (Table 1). The lack of HMBC correlation between the furan oxymethine proton (H-32, δ 4.55) and the quaternary aliphatic γ-carbon (C-6, δ 59.4) is presumably due to a conformation in which the dihedral angle is inappropriate for scalar coupling, an observation supported by the inspection of models (Figure 1), which shows an orthogonal relationship between H-32 and C-6. The oxymethine furan proton (H-32, δ 4.55) was placed α on the basis of a diagnostical NOE-correlation with the "endo" 5α proton on ring B (δ 2.01) (Figure 1).

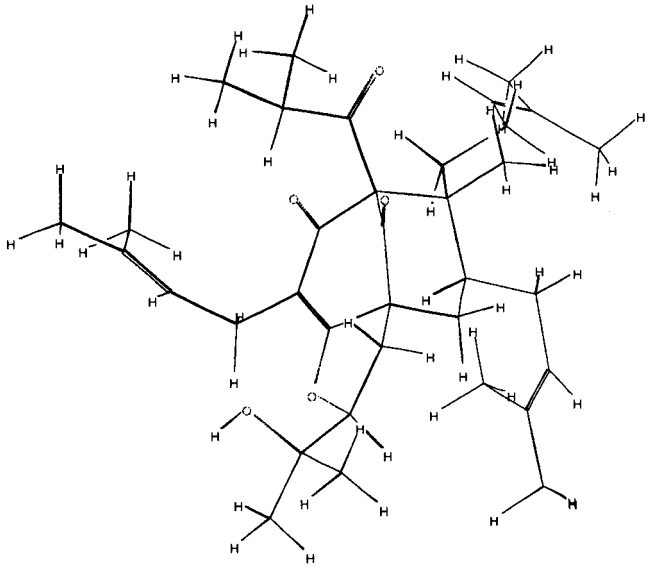

Figure 1. Tridimensional representation of furohyperforin (**2**) (PC-Model).

Furohyperforin is presumably formed by a 5-*exo*-trig opening of the 32-epoxide of hyperforin, a type of reaction well precedented in ortho-prenylated phenols and enols but less documented for α-prenylated ketones.[12] Attempts to prepared **2** by degradation of hyperforin in an oxygen atmosphere or by treatment with various oxidants (*m*CPBA, TBHP-VO(acac)$_2$) gave untreatable mixtures, whose ^{1}H NMR spectra did not show the presence of **2**. These data suggest that furohyperforin is presumably not an artifact of isolation and/or purification procedures but a genuine constituent of the plant.

The molecular basis for the antidepressant activity of St. John's wort extracts is presently unknown. Interaction with the synaptosomal uptake of 5-HT and other biogenic amines has recently been suggested.[10] When tested in a 5-HT accumulation test in rat brain cortical synaptosomes, furohyperforin (**2**) showed only 1/10 of the activity of hyperforin,[13] suggesting that this compound, also on account of its low concentration, is not seemingly one of the major neuroactive constituents of *H. perforatum*.[14]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. IR spectra were recorded on a Perkin-Elmer model 681 spectrophotometer. UV and CD spectra were measured on a JASCO J-500 spectrophotometer (*c* 1.8 × 10^{-4}). HRMS (EI, 70 eV) were taken on a VG 7070 EQ spectrometer. ^{1}H and ^{13}C NMR spectra were taken on a Bruker DRX-500 spectrometer (500 and 125 MHz, respectively). Silica gel 60 (70−230 mesh, Merck) was used for open-column chromatography. A Waters Microporasil column (0.8 × 30 cm) was used for HPLC, with detection by a

Waters differential refractometer 340. The most frequently used eluants for TLC were petroleum ether−*t*-BuOMe (4:1) and hexanes−EtOAc (95:5). Spots were revealed by UV absorption (254 nm) and by spraying with $(NH_4)_2MoO_4$, followed by heating. Figure 1 was generated with PCMODEL, Serena Software, Version 4.0.

Plant Material. *Hypericum perforatum* L. flowering tops collected during the flowering period (December 1997) were purchased in Chile. A voucher specimen (# BA 52673) is deposited at the Indena Research Centre in Settala.

Extraction and Isolation. The plant material (1 kg) was extracted with supercritical CO_2, affording a waxy brown residue (15 g, 15%), whose content of hyperforin was quantified as 30% by HPLC.[15] A sample of this extract (10 g) was dissolved in a mixture of acetonitrile (300 mL) and hexane (300 mL). After standing overnight, the two phases were separated, and the upper hydrocarbon phase was further extracted with acetonitrile (2 × 50 mL). The hexane phase, containing mainly fats and hydrocarbons, was discarded, while the pooled acetonitrile phases were counter-extracted with hexane (3 × 50 mL) to remove part of hyperforin. Evaporation of the acetonitrile phase left a residue (2.4 g) which was purified by column chromatography on silica gel (25 g silica gel). Elution was started with hexane−EtOAc (95:5), and then continued with hexane−EtOAc (9:1 and 8:2). Fractions eluted with hexane−EtOAc (8:2) afforded crude **2a** (315 mg). Final purification was achieved by HPLC, using hexane−EtOAc (8:2) as eluant. Altogether, 110 mg **2** (1% based on the CO_2 extract) could be eventually obtained.

Furohyperforin (2). Colorless viscous oil; $[\alpha]^{20}_D$ + 62.4° (*c* 0.9, $CHCl_3$); $[\alpha]^{20}_D$ +81.9° (*c* 0.9, MeOH); UV (EtOH) λ_{max} (log ϵ) 272 (4.06) nm; IR (Nujol) ν_{max} 3500, 1725, 1617, 1450, 1370, 1240, 1210 cm^{-1}; CD (EtOH) λ_{max} ($\Delta\epsilon$) 250 (+4.34), 272 (+16.96), 301 (−5.87), 330 (+0.98) L mol^{-1} cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HREIMS *m/z* 552.38310 $[M]^+$ (7) (calcd for $C_{35}H_{52}O_5$, 552.38147), 483 (5), 347 (42), 305 (53), 293 (59), 204 (59), 135 (100).

Oxidative Degradation of Hyperforin (1a). (a) A sample of **1a** (15 mg) was dissolved in CH_2Cl_2 presaturated with oxygen and stirred under an oxygen atmosphere (balloon). After standing for ca. 240 min at room temperature, TLC (hexane−EtOAc (9:1)) showed the complete disappearance of the starting material and the formation of a complex mixture, where no major spot could be observed. After removal of the solvent, the residue was analyzed by ^1H NMR, showing no evidence for the presence of **2**, as indicated by the lack of the ABX system of H-31α,β and H-32.

(b) A sample of **1a** was dissolved in CH_2Cl_2 and then treated with 1 molar equiv of *m*CPBA or with $VO(Acac)_2$ (trace) and TBHP (2 molar equiv). After 10 min at room temperature no trace of the starting material could be evidenced by TLC, and the reactions were worked up by washing with brine and evaporation. The residue was analyzed by ^1H NMR, showing no evidence for the presence of **2**.

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