Hyperforin Analogues from St. John's Wort (*Hypericum perforatum*)

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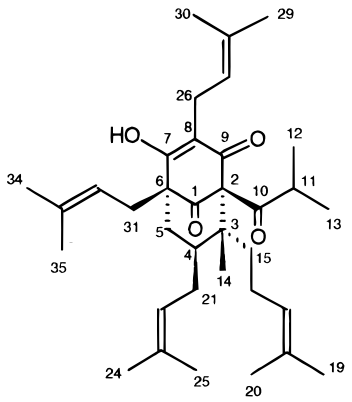
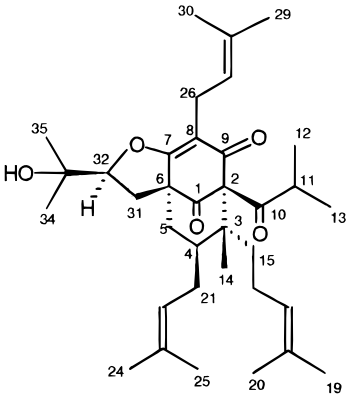
Three oxygenated analogues of the prenylated phloroglucinol hyperforin (**3**−**5**) were isolated from the aerial parts of *Hypericum perforatum* and their structures elucidated by spectroscopic methods.

Over the past few years, St. John's wort (*Hypericum perforatum* L., family Clusiaceae) has been the subject of considerable research aimed at establishing the chemical and pharmacological bases of the antidepressant activity of its extracts.[1] As a response to the great interest generated by this plant and its widespread use as a mood enhancer, the National Institute of Mental Health has sponsored a large placebo-controlled trial of a well-characterized extract from St. John's wort, whose results are expected in two years.[2] In this context, the formulation of an activity-related standardization is still an unresolved issue, because the pharmacologically active constituent(s) of the plant has not yet been identified.[1,2] Recent studies have suggested that the prenylated phloroglucinol hyperforin[3] (**1**) might be a critical component for the antidepressant activity of St. John's wort.[4] This compound is unstable, and its liability to oxidative degradation poses serious problems for standardization and may also dramatically affect the pharmacological activity of the extracts.



Hyperforin **1a**
Adhyperforin **1b**



Furohyperforin **2**

It has been known for some time that *H. perforatum* contains oxidized forms of hyperforin,[5] but it is not clear whether these compounds are genuine plant constituents or artifacts formed en route to the degradation of the natural product. Whatever the origin, their structural characterization and availability has an obvious relevance for the standardization and the pharmacological evaluation of St. John's wort extracts. The identity of the major oxidized form of hyperforin remained unknown until recently, when we[6] and another group[7] independently reported the isolation of the dihydrofuran **2** from a supercritical carbon-dioxide extract of *H. perforatum*. A third group claimed the formation of **2** in an unreported yield during oxidative experiments of degradation of hyperforin in methanol.[8] We have now isolated three further oxidized derivatives of hyperforin from the same type of extracts that afforded **2** and present here their structure elucidation and a preliminary study of their biological activities.

Hyperforin is a mixture of tautomers, as shown by the broad shape of most ^{1}H NMR signals and the poor resolution of many ^{13}C NMR lines. In contrast, the three oxidized analogues **3**−**5** show sharp signals, indicative of a covalent block of the tautomeric equilibrium.

Compound **3** was isolated as a clear oil and analyzed for $C_{35}H_{52}O_5$, corresponding to the addition of two oxygen atoms to the molecular formula of hyperforin (**1**). The ^{1}H NMR spectrum is very similar to that of **2**, except for the presence of a downfield exchangeable signal (δ 7.65 in $CDCl_3$, 11.21 in DMSO-d_6) and a downfield shift for H-32 ($\Delta\delta$ + 0.34, $CDCl_3$). Small differences (±0.10 ppm) were also observed for the protons around the furan ring, while all coupling constants and the rest of the spectrum are virtually identical. Compared to **2**, the ^{13}C NMR spectrum of **3** disclosed the opposite shift for the oxygenated carbons C-33 ($\Delta\delta$ +12.9 ppm) and C-32 ($\Delta\delta$ −3.7) and an upfield shift for the C-33 geminal methyls ($\Delta\delta$ −6.9 for C-34 and −4.2 for C-35). These observations suggest that **2** and **3** have the same basic structure and differ only in the replacement of the tertiary hydroxyl at C-33 with a hydroperoxyl group. The in situ reduction of **3** to **2** with triphenylphosphine in $CDCl_3$ confirmed the results of the spectroscopic analysis and established the configuration of the oxymethine carbon. A hyperforin−cadinane adduct

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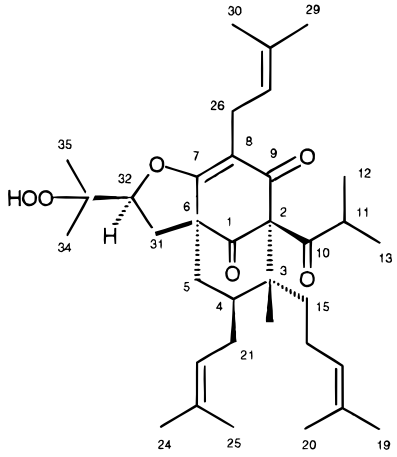

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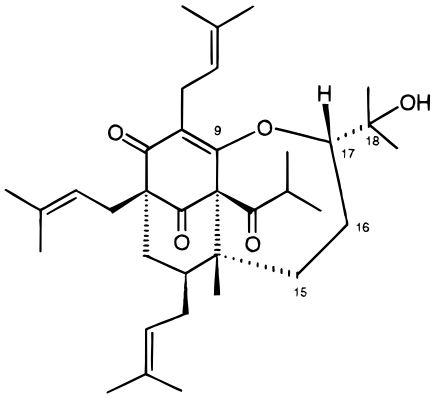

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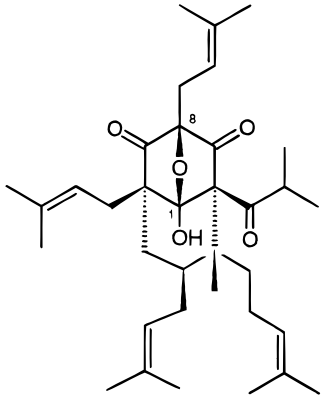

5

bearing a hydroperoxyl group on the sesquiterpene moiety had previously been isolated from the stem and leaves of *H. perforatum*.[9]

Compared to hyperforin, the molecular weight of compound **4** showed the presence of an additional oxygen atom. Because the signals of three prenyl residues could still be observed, oxygenation had taken place in the homoprenyl moiety, whose olefinic carbons were replaced by an oxymethine and a quaternary oxygenated carbon. After analysis of the proton scalar-coupled spin network and association of all the ^{1}H NMR resonances with directly bonded carbons via HMQC experiments, the analysis of the HMBC correlations showed that the enone carbonyl was coupled to the allylic methylenes of two prenyl residues [δ 3.28 (H-26a), 3.14 (H-26b), 2.45 (H-31a,b)], a situation consistent only with the location of the enone carbonyl at C-7. Thus, compound **4** is a derivative of the 7-keto $\Delta^{8,9}$-tautomer of

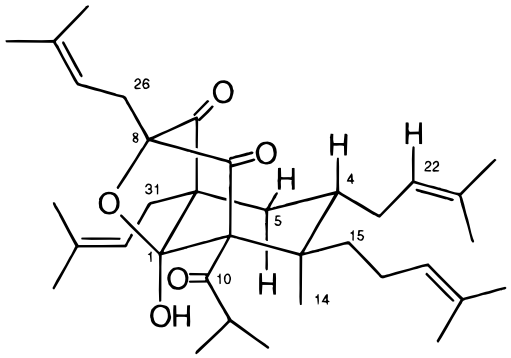

Figure 1. Sterical view of **5**.

hyperforin, where the dioxygenated homoprenyl residue is bound through an ether bond to the enol carbon on ring A. Owing to an unfavorable arrangement for scalar coupling, the oxymethine proton H-17 did not show any correlation with the carbons of the bicyclic core, thus precluding the location of the ether bridge by HMBC data alone. Evidence for the closure of an oxepane ring came from chemical-shift consideration. Ether formation has a marked deshielding effect on the α-carbon. This effect is evident in the downfield resonance of the C-17 oxymethine (δ 87.9), but is inconsistent with the upfield resonance of the oxygenated quaternary carbon C-18 (δ 72.6), which points to the presence of a free hydroxyl on this carbon. NOE difference experiments were used to assess the relative configuration at C-17. These experiments disclosed a spatial relationship between the oxymethine proton H-17 and one of the H-15 methylenes (δ 2.50), assigned as β on account of its strong NOE correlation with the adjacent angular methyl at C-3 (δ 1.00, H-14). A consistent NOE between H-17 and H-13 could also be detected.

The molecular weight of **5** showed that this compound was a monooxygenated form of hyperforin. The spectroscopic characterization of **5** was carried out in C_6D_6, since there were many overlapping signals in $CDCl_3$. The presence of a hemiacetal ring was a key feature, readily distinguishable from a cursory analysis of the NMR data (downfield exchangeable proton signal at δ 8.18; deshielded aliphatic carbon at δ 108.8). Three prenyls and one homoprenyl were also observed in the NMR spectra, showing that the additional oxygen atom is located on the cyclic core. Compared to the ^{13}C NMR spectrum of hyperforin, the spectrum of **5** shows no signal for the enone double bond, with ring B now made up by two quaternary aliphatic carbons (δ 71.9, C-2 and δ 55.2, C-6), two carbonyls (δ 209.1, C-7 and δ 207.6, C-9), one acetal carbonyl (δ 108.8, C-1), and one nonprotonated oxygenated carbon (δ 97.7, C-8). These data could be combined in formula **5** using diagnostic HMBC correlations of one of the two carbonyls (δ 209.1, C-7) with the methylenes of the two prenyl groups bound to ring A (H-31a,b; H-26a,b) and with the methylene of ring B (H-5a,b). Compound **5** is the 15-prenyl derivative (hyperforin numbering) of subellinone, a phloroglucinol isolated from *Garcinia subelliptica*.[10] The cage-like structure of hyperforin and the presence of the oxygen bridge dictate the configuration at C-1 and C-8 (Figure 1.)

It is not clear whether compounds **2**−**5** are natural products or artifacts of extraction/isolation procedures, and thus good models or markers for the degradation of hyperforin in plant extracts. Compounds **2**−**5** could be detected by HPLC−MS analysis in the original extract from which they were isolated. On the other hand, they could also be detected as intermediates en route to further

Table 1. ^{1}H NMR Data for Compounds **3**, **4**, and **5**[a−c]

	3		**4**		**5**[c]	
H	*δ*	HMBC	*δ*	HMBC	*δ*	HMBC
4	1.62 m		1.98 m		1.39 m	
5	2.04 dd (13, 4)		1.89α m		2.20 d (13)	1, 3, 4, 6, 7, 21, 31
5′	1.52 dd (13, 13)	6, 7	1.50*β* dd (13, 13)		1.44 dd (13, 12)	
11	1.98 qq (6.5, 6.5)		2.48 m		3.31 qq (7, 7)	10, 12, 13
12	1.09 d (6.5)	10, 11, 13	1.16 d (6.5)	10, 11, 13	1.03 d (7)	10, 11, 13
13	1.00 d (6.5)	10, 11, 12	1.10 d (6.5)	10, 11, 12	0.87 d (7)	10, 11, 12
14	1.04 s	2, 3, 4, 15	1.00 s	2, 3, 4, 15	1.07 s	2, 3, 4, 15
15	2.07 m		2.50*β* m		1.92 ddd (15.5, 13, 4)	2, 3, 4, 14, 16, 17
15′	1.31 m		1.90αm		1.81 ddd (15.5, 13, 5.5)	2, 3, 4, 16, 17
16	2.15 m		1.82 m		2.48 ddd (14, 13, 4, 6.5)	17, 18
16′	1.92 m		1.57 m		1.96 m	
17	5.05 m		3.81 d (9)	15, 18, 19, 20	5.14 br dd (6.5, 6.5)	
19	1.65 br s		1.24 s	17, 18, 20	1.70 br s	17, 18, 20
20	1.59 br s		1.22 s	17, 18, 19	1.61 br s	17, 18, 19
21	2.17 m		2.04 m		2.13 br dd (14, 6.5)	
21′	1.77 m		1.60 m		1.64 m	
22	4.94 br dd (6.5, 6.5)		4.92 br dd (6.5,6.5)		4.98 br dd (6.5, 6.5)	
24	1.70 br s		1.67 br s		1.58 br s	22, 23, 25
25	1.57 br s		1.54 br s		1.51 br s	22, 23, 24
26	3.13 br dd (15, 6.5)	7, 8, 9, 27, 28	3.28 br dd (15, 6.5)	7, 8, 9, 27, 28	3.09 br dd (15, 7.5)	7, 8, 9, 27, 28
26′	3.01 br dd (15, 6.5)	7, 8, 9, 27, 28	3.14 br dd (15, 6.5)	7, 8, 9, 27, 28	2.79 m	7, 8, 9, 27, 28
27	5.06 m		4.98 br dd (6.5, 6.5)		5.34 br dd (7.5, 6.5)	
29	1.63 br s		1.67 br s		1.61 br s	27, 28, 30
30	1.69 br s		1.70 br s		1.61 br s	27, 28, 29
31	2.56 dd (13, 11)	1, 5, 6, 32, 33	2.45 br d (2H) (6.5)	1, 5, 6, 7, 32, 33	2.81 m	1, 5, 6, 7, 32, 33
31′	1.84 dd (13, 6)	5, 6, 7			2.72 br dd (14, 8)	1, 5, 6, 7, 32, 33
32	4.89 m (11, 6)		5.06 br t (6.5)		5.73 br dd (8, 6.5)	
34	1.29 s	32, 33, 35	1.65 br s		1.72 br s	32, 33, 35
35	1.28 s	32, 33, 34	1.65 br s		1.61 br s	32, 33, 34
OH	7.65 s				8.18 s	1, 2, 6

[a] 500 MHz, $CDCl_3$ for **3** and **4**, C_6D_6 for **5**. [b] *J* values in Hz. [c] Selected NOEs. For **3**: H-5, H-14; H-5, H-32; H-14, H-16′; H-14, H-21; H-14, H-21′; H-31, H-32; H-31, H-35; H-32, H-34; H-32, H-35. For **4**: H-5*β*, H-14; H-13, H-17; H-13, H-19; H-14, H-15*β*; H-14, H-21; H-14, H-32; H-16, H-19; H-17, H-19; H-19, H-26; H-19, H-27; H-19, H-30.

degradation in solutions of hyperforin left standing in an air atmosphere for variable spans of time.[11]

When tested in a 5HT reuptake inhibition assay in rat brain cortical synaptosomes, compounds **3**−**5** showed a more than 10-fold decrease of activity compared to hyperforin,[12] suggesting that oxidation resulting in a covalent block of the tautomeric equilibrium is detrimental for activity. The lability of hyperforin (**1**) toward oxidation and the decreased activity of its oxidized derivatives **2**−**5** highlight the challenges facing the development of preparations of *H. perforatum* that are stable and standardized in their active principle(s).

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}). EIMS (EI, 70 eV) were obtained on a VG 7070 EQ spectrometer. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker DRX-500 spectrometer (500 and 125 MHz respectively). Si gel 60 (70−230 mesh, Merck) was used for open-column chromatography. Most frequently used eluents for TLC were petroleum ether−*t*-BuOMe 4:1 and 20:1. Spots were revealed by UV absorption (254 nm) and by spraying with $(NH_4)_2MoO_4$, followed by heating. The high-speed countercurrent chromatography (HSCCC) was carried out with a centrifugal countercurrent chromatograph from Pharma-Tech Research Corp., Baltimore, MD (model CCC 1000). The equipment consisted of three multilayer coiled columns (2.6 mm i.d. PTFE, total volume capacity 800 mL) in symmetrical positions 60° apart, 3 inches away from the central axis of the centrifuge. The HSCCC system is equipped with a SSI liquid chromatography pump (model 300), a PTRC speed controller, and an injection valve (Rheodyne). A biphasic solvent system petroleum ether−CH_3CN−MeOH 8:5:2 was prepared by thoroughly mixing the solvents in a separatory funnel at room temperature and leaving the mixture to settle for 12 h. The multilayer coiled column was first entirely filled with the stationary phase (upper phase), then the mobile phase (lower phase) was pumped into the inlet of the column at a rotation speed of 1040 rpm and a flow rate of 2 mL/min. The retention volume ($V_c - V_s = 680$ mL) and the stationary phase retention volume ($R_v = V_c - V_s/V_c = 0.85$) were calculated from the volume of the stationary phase ($V_s = 120$ mL) replaced by the mobile phase. The samples were always filtered on a Millex HV filter (0.45 μm, Millipore).

Plant Material. *H. 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, 1.5%). A portion 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 pooled acetonitrile phases were counter-extracted with hexane (3 × 50 mL), then evaporated to dryness under reduced pressure (2.4 g). The acetonitrile residue (10.5 g) was partitioned in petroleum ether−CH_3CN−MeOH 8:5:2 (500 mL), and the upper layer (1.450 g) was purified by countercurrent chromatography using as eluent the same mixture, in the "head-to-tail" mode (lower phase as mobile phase). Fractions (2 mL each) were collected and pooled according to their composition.

Fractions 29−32 (40 mg) and 47−50 (108 mg) were purified by open column chromatography on Si gel (4 and 13 g, respectively, elution with petroleum ether−*t*-BuOMe 9:1), affording **3** (20 mg) and **4** (19 mg), respectively. The stationary phase of the countercurrent chromatographic separation was evaporated to dryness (500 mg) and purified by open column

Table 2. ^{13}C NMR Chemical Shifts of Compounds **3**−**5**[a]

C	3	4	5
1	204.6 s	206.6 s	108.8 s
2	83.2 s	74.5 s	71.9 s
3	48.2 s	45.2 s	47.1 s
4	43.3 d	36.9 d	41.7 d
5	38.0 t	40.3 t	33.5 t
6	59.0 s	64.1 s	55.2 s
7	172.8 s	194.4 s	209.1 s
8	116.8 s	129.5 s	97.7 s
9	192.7 s	166.2 s	207.6 s
10	209.5 s	211.4 s	218.3 s
11	41.9 d	41.1 d	39.5 d
12	20.4 q	20.7 q	17.5 q
13	21.3 q	21.6 q	19.1 q
14	13.4 q	16.9 q	15.8 q
15	36.3 t	33.4 t	37.4 t
16	25.2 t	24.2 t	24.3 t
17	124.7 d	87.9 d	124.8 d
18	131.1 s	72.6 s	131.7 s
19	25.6 q	25.6 q	25.8 q
20	17.7 q	25.9 q	17.8 q
21	27.1 t	26.9 t	28.9 t
22	122.3 d	122.0 d	122.6 d
23	133.5 s	133.6 s	133.4 s
24	25.9 q	25.5 q	25.7 q
25	18.0 q	17.9 q	17.9 q
26	22.2 t	22.9 t	24.4 t
27	121.0 d	122.0 d	116.9 d
28	132.5 s	133.6 s	135.7 s
29	25.7 q	26.0 q	25.9 q
30	17.8 q	18.1 q	17.9 q
31	31.1 t	29.5 t	31.7 t
32	86.4 d	119.7 d	119.9 d
33	83.0 s	134.3 s	133.5 s
34	20.2 q	25.9 q	26.1 q
35	19.8 q	18.1 q	17.7 q

[a] 125 MHz, $CDCl_3$ for **3** and **4**, C_6D_6 for **5**.

chromatography on Si gel (50 g, elution with petroleum ether−CH_2Cl_2), yielding **5** (200 mg).

33-Deoxy-33-hydroperoxyfurohyperforin (3): colorless viscous oil; $[\alpha]^{20}_D$ +75.0° (*c* 1.2, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 272 (3.83) nm; CD ($CHCl_3$) λ_{max} ($\Delta\epsilon$) 247 (−2.55), 271 (+6.91), 302 (−1.1), 313 (−0.85), 333 (+0.64) L mol^{-1} cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS *m*/*z* 568 $[M]^{+3}$, 552 (11), 494 (4), 361 (8), 347 (32), 305 (34), 293 (26), 204 (72), 147 (55), 135 (100).

Oxepahyperforin (4): colorless viscous oil: $[\alpha]^{20}_D$ −73.7° (*c* 0.8, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 272 (3.83) nm; CD ($CHCl_3$) λ_{max} ($\Delta\epsilon$) 266 (+13.28), 305 (−12.84) L mol^{-1} cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS *m*/*z* 552 $[M]^{+,63}$ 482 (22), 426 (12), 331 (38), 315 (22), 275 (100), 257 (23), 135 (37).

8-Hydroxyhyperforin 8,1-hemiacetal (5): colorless viscous oil; $[\alpha]^{20}_D$ + 34.0° (*c* 1.0, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 270 (3.21), 380 (1.66) nm; CD ($CHCl_3$) λ_{max} ($\Delta\epsilon$) 260 (−2.25), 300 (+8.84) 331 (+0.12), 365 (−1.79) L mol^{-1} cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HREIMS *m*/*z* 552.3757 $[M]^+$ (12) (calculated for $C_{35}H_{52}O_5$ 552.3814), 483 (29), 455 (85), 413 (55), 387 (48), 369 (34), 319 (32), 301 (27), 251 (47), 225 (38), 203 (100), 195 (66), 135 (58), 123 (59).

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