# Three New Hyperforin Analogues from *Hypericum perforatum*

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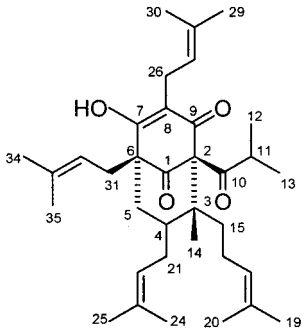
Three new analogues of hyperforin (**1**), pyrano[7,28-*b*]hyperforin (**2**), (2*R*,3*R*,4*S*,6*R*)-6-methoxycarbonyl-3-methyl-4,6-di(3-methyl-2-butenyl)-2-(2-methyl-1-oxopropyl)-3-(4-methyl-3-pentenyl)cyclohexanone (**3**), and (2*R*,3*R*,4*S*,6*S*)-3-methyl-4,6-di(3-methyl-2-butenyl)-2-(2-methyl-1-oxopropyl)-3-(4-methyl-3-pentenyl)-cyclohexanone (**4**), were isolated from the aerial parts of *Hypericum perforatum* (St. John's wort). The structures of **2**−**4** were elucidated by spectroscopic methods.

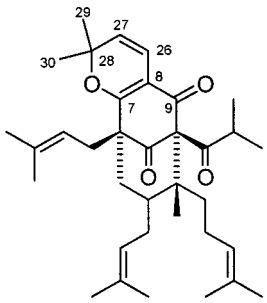
*Hypericum perforatum* L. (St. John's wort) is a herbaceous perennial plant, belonging to the family Clusiaceae, and is distributed in Europe, North Africa, Asia, and North America. Extracts of its aerial parts are used in many countries to treat mild to moderately severe depressive disorders. Several reviews of controlled clinical studies with extracts of *H. perforatum* are supportive of its antidepressant activity.[1−3] Pharmacological studies are also suggestive of an antidepressant activity.[4−6] Initially, the antidepressant activity of *H. perforatum* was associated with hypericin, a naphthodianthrone,[7] but several subsequent studies have questioned this assumption.[8,9] Recent studies have suggested that the prenylated phloroglucinol hyperforin (**1**) might be a critical *H. perforatum* constituent for its antidepressant activity.[10−12]

Previous investigations on hyperforin analogues of *Hypericum perforatum* have yielded furohyperforin, an oxidized form of hyperforin.[13,14] More recently, three new oxidation analogues of hyperforin were reported, comprising oxepahyperforin, 33-deoxy-33-hydroperoxyfurohyperforin, and 8-hydroxyhyperforin-8,1-hemiacetal.[15] In this paper, we report the isolation and structure elucidation of three new hyperforin analogues (**2**−**4**).

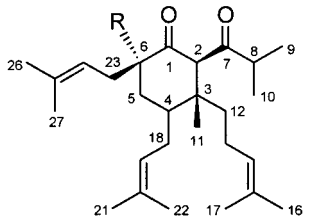
Compound **2** was obtained as a colorless viscous oil. The molecular formula $C_{35}H_{50}O_4$ was established by HREIMS (*m*/*z* 458.3392 $[M]^+$). Evidence from the UV, IR, and NMR data indicated that **2** was an analogue of hyperforin (**1**). A comparison of the elemental formula of **2** with that of **1** revealed that the new compound had two hydrogen atoms less. In the $^{13}C$ NMR spectrum, the three broad peaks of the α-substituted enolyzed *β*-dicarbonyl system of hyperforin (C-7, C-8, C-9) were replaced by three sharp singlets at *δ* 171.0 (C-7), 114.7 (C-8), and 188.6 (C-9), suggesting that the keto−enol equilibrium of the *β*-dicarbonyl system was covalently blocked by the formation of an enol ether. From the IR spectrum, it was apparent that there was no free hydroxyl group in the molecule of **2**. It was probable that the differences between **1** and **2** resulted from the formation of a pyran ring in **2**, which was composed of one of the four prenyl residues indicated by the NMR data (Table 1). Compound **2** was assigned with *Z* geometry of the −CH=CH− unit, from data observed at *δ* 6.74 d (H-26), 5.33 d (H-27) (*J* = 10.0 Hz), and *δ* 115.5 (C-26) and 123.8 (C-27). In the HMBC spectrum, the methyl protons of C-29 (*δ* 1.45) correlated to C-27 (*δ* 123.8), C-28 (*δ* 82.0), and C-30 (*δ* 28.7), while the methyl protons of C-30 (*δ* 1.40) correlated to the signals of C-27 (*δ* 123.8), C-28 (*δ* 82.0), and C-29 (*δ* 28.5). The H-26 (*δ* 6.74 d) and H-27 (*δ* 5.33 d) protons also showed correlations with the C-28 (*δ* 82.0) signals. Therefore, the linkages between C-29−C-28−C-27−C-26 and C-30−C-28−C-27−C-26 were deduced. The chemical shift of C-28 (*δ* 82.0) indicated it was an oxygen-bearing quaternary carbon. Since **2** has three carbonyls at



Hyperforin **1**



**2**



**3** R=$\overset{28}{C}OO\overset{29}{C}H_3$

**4** R=H

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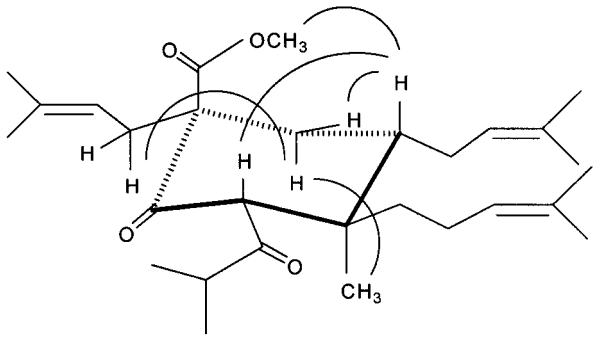
**Table 1.** NMR Data of Compound **2**

| position | $^1$H[a] | $^{13}$C[b] | HMBC (H→C) |
|---|---|---|---|
| 1 | | 206.3 | |
| 2 | | 84.4 | |
| 3 | | 49.1 | |
| 4 | 1.63 m | 43.5 | 3, 5, 6, 15 |
| 5 | 1.42 m | 38.9 | 3, 4, 6, 7, 31 |
| | 1.88 m | | 1, 3, 4, 6, 7, 21, 31 |
| 6 | | 56.6 | |
| 7 | | 171.0 | |
| 8 | | 114.7 | |
| 9 | | 188.6 | |
| 10 | | 209.5 | |
| 11 | 2.10 m | 42.6 | 10, 12, 13 |
| 12 | 1.12 d (6.5) | 20.6 | 10, 11, 13 |
| 13 | 1.07 d (6.5) | 21.5 | 10, 11, 12 |
| 14 | 1.01 s | 13.6 | 2, 3, 4, 15 |
| 15 | 1.43 m | 36.6 | 3, 4, 14, 16 |
| | 1.89 m | | 2, 3, 4, 14 |
| 16 | 1.70 m | 27.2 | 15, 18 |
| | 2.10 m | | 17, 18 |
| 17 | 4.95 m | 122.8 | 19, 20 |
| 18 | | 133.4 | |
| 19 | 1.66 s | 25.8 | 17, 18, 20 |
| 20 | 1.56 s | 17.7 | 17, 18, 19 |
| 21 | 1.86 m | 25.0 | 3, 4, 22, 23 |
| | 2.08 m | | 4, 5, 22, 23 |
| 22 | 5.05 m | 124.8 | 24, 25 |
| 23 | | 131.2 | |
| 24 | 1.63 s | 25.7 | 22, 23, 25 |
| 25 | 1.58 s | 18.0 | 22, 23, 24 |
| 26 | 6.47 d (10.0) | 115.5 | 7, 8, 9, 28 |
| 27 | 5.33 d (10.0) | 123.8 | 7, 8, 28, 29, 30 |
| 28 | | 82.0 | |
| 29 | 1.45 s | 28.5 | 27, 28, 30 |
| 30 | 1.40 s | 28.7 | 27, 28, 29 |
| 31 | 2.40 dd (15, 8.4) | 29.0 | 1, 5, 6, 32, 33 |
| | 2.48 dd (15, 6.7) | | 1, 6, 7, 32, 33 |
| 32 | 4.99 m | 119.4 | 31, 34, 35 |
| 33 | | 133.9 | |
| 34 | 1.66 s | 26.0 | 32, 33, 35 |
| 35 | 1.67 s | 18.2 | 32, 33, 34 |

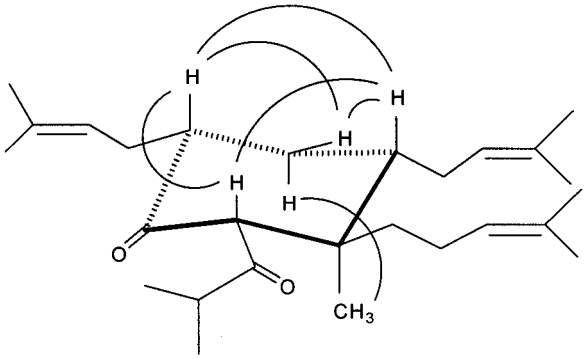
[a] Recorded in $CDCl_3$ at 400 MHz, *J* values in Hz. [b] Recorded in $CDCl_3$ at 100 MHz.

$\delta$ 206.3 (C-1), 188.6 (C-9), 209.5 (C-10), and the number of oxygen atoms in the formula was four, a linkage between C-7−O−C-28 was apparent. The HMBC spectrum also showed correlations between H-26 ($\delta$ 6.74 d) and C-9 ($\delta$ 188.6), and between H-27 ($\delta$ 5.33 d) and C-8 ($\delta$ 114.7). Accordingly, the pyran ring was confirmed. All other proton and carbon signals of **2** could be assigned using a combination of 1D and 2D NMR techniques and by comparison with hyperforin and furohyperforin. On the basis of the analysis of its spectra, the structure **2** was established as pyrano-[7,28-*b*]hyperforin.

Compound **3** was isolated as a viscous colorless oil. The molecular formula, $C_{29}H_{46}O_4$, was determined by HREIMS (*m*/*z* 458.3392 $[M]^+$). The $^1$H NMR spectrum of **3** indicated the presence of seven tertiary methyl groups [$\delta$ 0.96 (s), 1.55 (s), 1.57 (s), 1.60 (s), 1.64 (s), 1.68 (s), 1.73 (s)], two secondary methyl groups [$\delta$ 1.09 (d, $J$ = 6.8), 1.40 (d, $J$ = 7.1 Hz)], one oxygenated methyl group [$\delta$ 3.78 (s)], one methine proton [$\delta$ 3.92 (s)], and three vinylic protons [$\delta$ 4.94 (dd, $J$ = 7.0, 7.0), 5.05 (dd, $J$ = 7.6, 7.6), 5.13 (dd, $J$ = 6.5, 6.2 Hz)]. This information indicated the presence of one isopropyl group and three prenyl side chains. The $^{13}$C NMR spectrum of **3** revealed 29 carbon signals, which were sorted by a DEPT experiment into nine methyls, one methoxyl, five methylenes, three methines, three olefinic carbons, three carbonyls, and five quaternary carbons. By comparison of the NMR data of **3** and those of hyperforin (**1**), the skeleton of **3** was deduced to be part of that of **1**.




**3**




**4**

**Figure 1.** Significant NOE correlations for **3** and **4**.

This was supported by interpretation of the HMQC and HMBC spectra (Table 2). The locations of the methoxyl group and methine proton (H-2) were determined by the observation of correlations in the HMBC spectrum between C-28 and H-5a and H-5b, C-6, C-28 and H-29, and C-1, C-3, C-4, C-7, C-11 and H-2. The configuration at C-2, C-3, C-4, and C-6 was assigned on the basis of NOE correlations of H-4/H-2, H-4/H-5b, H-4/H-29, H-5a/H-11, and H-5a/H-23a (Figure 1). Accordingly, the structure of compound **3** was elucidated as (2*R*,3*R*,4*S*,6*R*)-6-methoxycarbonyl-3-methyl-4,6-di(3-methyl-2-butenyl)-2-(2-methyl-1-oxopropyl)-3-(4-methyl-3-pentenyl)cyclohexanone.

Compound **4** was obtained also as a colorless viscous oil. HREIMS (*m*/*z* 400.3340 $[M]^+$) gave its molecular formula as $C_{27}H_{44}O_2$. The $^1$H NMR spectrum of **4** was similar to that of **3** except for the absence of a methoxyl group. The $^{13}$C NMR spectrum gave 27 carbon signals, which consisted of nine methyls, five methylenes, four methines, three olefinic carbons, two cabonyls, and four quaternary carbons. The structure of **4** was then deduced by direct comparison with **3** and confirmed by analysis of its HMQC and HMBC spectra (Table 2). The stereochemistry of **4** was established from the NOESY spectrum. Significant NOE enhancements (Figure 1) were observed between H-4 and H-2, H-5b, and H-6, and between H-6 and H-2 and H-5b, and between H-5a and H-11. Hence, the structure of compound **4** was assigned as (2*R*,3*R*,4*S*,6*S*)-3-methyl-4,6-di(3-methyl-2-butenyl)-2-(2-methyl-1-oxopropyl)-3-(4-methyl-3-pentenyl)-cyclohexanone.

## Experimental Section

**General Experimental Procedures.** Optical rotation data were measured on a Perkin-Elmer 341 polarimeter. UV spectra were obtained on a Shimadzu UV 250 spectrophotometer. IR spectra were recorded on a Nicolet Magna-FTTR-750 spectro-

**Table 2.** NMR Data of Compounds **3** and **4**

| | **3** | | | **4** | | |
|---|---|---|---|---|---|---|
| position | $^1$H[a] | $^{13}$C[b] | HMBC (H→C) | $^1$H[a] | $^{13}$C[b] | HMBC (H→C) |
| 1 | | 205.1 | | | 210.0 | |
| 2 | 3.92 s | 65.6 | 1, 3, 4, 7, 11, 12 | 3.81 s | 66.9 | 1, 3, 4, 7, 11, 12 |
| 3 | | 45.5 | | | 45.7 | |
| 4 | 1.82 m | 39.9 | | 1.80 m | 42.5 | 3, 5, 12, 18 |
| 5 | 1.30 m (a) | 36.3 | 3, 4, 6, 18, 23, 28 | 1.18 m (a) | 34.6 | 1, 3, 4, 6, 23 |
| | 2.50 m (b) | | 1, 3, 4, 6, 18, 28 | 2.13 m (b) | | 1, 3, 4, 6 |
| 6 | | 61.4 | | 2.35 m | 51.3 | 1, 5, 23, 24 |
| 7 | | 210.2 | | | 211.0 | |
| 8 | 2.55 m | 42.2 | 7, 9, 10 | 2.45 m | 42.8 | 7, 9, 10 |
| 9 | 1.09 d (6.8) | 17.4 | 7, 8, 10 | 1.03 d (6.8) | 17.7 | 7, 8, 10 |
| 10 | 1.40 d (7.1) | 18.6 | 7, 8, 9 | 1.05 d (7.0) | 18.6 | 7, 8, 9 |
| 11 | 0.96 s | 17.0 | 2, 3, 4, 12, 13 | 1.01 s | 17.3 | 2, 3, 4, 12 |
| 12 | 1.47 m | 36.4 | 2, 3, 4, 11, 13, 14 | 1.48 m | 36.7 | 2, 3, 4, 11, 13, 14 |
| 13 | 1.68 m (a) | 21.9 | 12, 14, 15 | 1.78 m (a) | 22.0 | 12, 14, 15 |
| | 1.98 m (b) | | 12, 14, 15 | 2.03 m (b) | | 12, 14, 15 |
| 14 | 4.94 dd (7.0, 7.0) | 123.5 | 12, 13, 16, 17 | 4.98 dd (7.0, 6.9) | 123.8 | 12, 13, 16, 17 |
| 15 | | 131.5 | | | 131.8 | |
| 16 | 1.64 s | 25.7 | 14, 15, 17 | 1.64 s | 25.7 | 14, 15, 17 |
| 17 | 1.55 s | 17.6 | 14, 15, 16 | 1.57 s | 17.8 | 14, 15, 16 |
| 18 | 1.65 m (a) | 26.9 | 4, 5, 19, 20 | 1.66 m (a) | 27.0 | 4, 5, 19, 20 |
| | 2.11 dd (14.1, 6.5) (b) | | 4, 5, 19, 20 | 2.12 m (b) | | 3, 4, 5, 19, 20 |
| 19 | 5.13 dd (6.5, 6.2) | 122.5 | 21, 22 | 5.12 dd (7.6, 7.8) | 123.1 | 21, 22 |
| 20 | | 132.9 | | | 133.0 | |
| 21 | 1.73 s | 25.9 | 4, 19, 20, 22 | 1.73 s | 26.0 | 19, 20, 22 |
| 22 | 1.60 s | 18.0 | 4, 19, 20, 21 | 1.60 s | 18.0 | 19, 20, 21, |
| 23 | 2.30 dd (14.5, 7.6) (a) | 33.2 | 1, 5, 6, 24, 25, 28 | 1.68 m (a) | 27.7 | 4, 5, 6, 24, 25 |
| | 2.52 m (b) | | 1, 5, 6, 24, 25, 28 | 2.11 m (b) | | 1, 5, 6, 24, 25 |
| 24 | 5.05 dd (7.6, 7.6) | 118.0 | 23, 26, 27 | 5.07 dd (7.8, 7.9) | 121.6 | 23, 26, 27 |
| 25 | | 135.2 | | | 133.3 | |
| 26 | 1.68 s | 26.0 | 24, 25, 27 | 1.67 s | 25.8 | 24, 25, 27 |
| 27 | 1.57 s | 17.8 | 24, 25, 26 | 1.60 s | 17.9 | 24, 25, 26 |
| 28 | | 172.1 | | | | |
| 29 | 3.78 s | 52.4 | 6, 28 | | | |

[a] Recorded in $CDCl_3$ at 400 MHz, *J* values in Hz. [b] Recorded in $CDCl_3$ at 100 MHz.

photometer. $^1$H (400 MHz) NMR, $^{13}$C (100 MHz) NMR, and all 2-D NMR spectra were recorded on Bruker AM-400 NMR instrument. $^1$H and $^{13}$C NMR chemical shifts refer to $CDCl_3$ at 7.26 ppm and $CDCl_3$ at 77.0 ppm, respectively. Low-resolution EIMS were obtained on a MAT-95 spectrometer and the HREIMS on a MAT-77 spectrometer.

**Plant Material.** The aerial parts of *Hypericum perforatum* were collected in Longxi County, Gansu Province, People's Republic of China, in June 1998. A voucher specimen (98001) was deposited at the herbarium of Shanghai Institute of Materia Medica, Shanghai, People's Republic of China.

**Extraction and Isolation.** The dried and powdered aerial parts of *H. perforatum* (25 kg) were extracted with petroleum ether (3 × 50 L) at room temperature. Removal of the solvent under reduced pressure left an oily extract (200 mL). Into this extract, 500 mL acetone was added, and after the insoluble solid was filtered, the mother liquid was concentrated to a residue. Next, the residue was passed through a Sephadex LH-20 column, eluted with dichloromethane−ethanol (1:1), to give a fraction (60 g) that contained mainly hyperforin (**1**) analogues. The fraction was subjected to Si gel column chromatography, eluted with petroleum ether−ethyl acetate (40:1, 20:1, 10:1), to yield three residues (A−C). Residue A (15 g) was further purified on Si gel and eluted with *n*-hexanes−ether (50:1) to give mixture I. Mixture I (3 g) was then subjected to RP-18 column chromatography eluted with acetone−water (4:1) to afford 8-hydroxyhyperforin-8,1-hemiacetal (300 mg) (characterized by detailed NMR and MS analysis and comparison with authentic data[15]). Residue B (20 g) was further purified on Si gel by elution with *n*-hexanes−ether (25:1) to give subfractions 1−3. Subfraction 1 (2 g) was then subjected to Si gel column chromatography, eluted with *n*-hexane−chloroform−acetone (8:1:0.1), to give compound **3** (60 mg) and mixture II. Mixture II was finally purified on preparative TLC plates, developed with *n*-hexane−chloroform−acetone (5:1:0.1), to give compound **2** (30 mg). Subfraction 2 (3 g) was separated on a Si gel column, eluted with *n*-hexanes−ether (15:1), to give compound **4** (50 mg). Residue C (10 g) was chromatographed on a RP-18 column, eluted with acetone−water (3:2), to afford mixture III (200 mg), and then mixture III was finally purified on preparative TLC plates, developed with *n*-hexane−chloroform-acetone (5:1:0.3), to give furohyperforin (40 mg) (characterized by detailed NMR and MS analysis and comparison with authentic data[13,14]).

**Pyrano[7,28-*b*]hyperforin (2):** colorless viscous oil; $[\alpha]_D^7$ +83.5° (*c* 0.28, $CHCl_3$); UV ($CHCl_3$) $\lambda_{max}$ (log $\epsilon$) 254 (3.77), 315 (3.53) nm; IR (film) $\nu_{max}$ 1728, 1637, 1585, 1448, 1379, 1221, 1117, 1045 cm$^{-1}$; $^1$H and $^{13}$C NMR data, see Table 1; EIMS *m*/*z* 534 $[M]^+$ (8), 465 (67), 397 (13), 330 (10), 275 (100), 231 (19), 109 (33), 69 (71); HREIMS *m*/*z* 534.3717 $[M]^+$ (calcd for $C_{35}H_{50}O_4$, 534.3709).

**(2*R*,3*R*,4*S*,6*R*)-6-Methoxycarbonyl-3-methyl-4,6-di(3-methyl-2-butenyl)-2-(2-methyl-1-oxopropyl)-3-(4-methyl-3-pentenyl)cyclohexanone (3):** colorless viscous oil; $[\alpha]_D^{26}$ +95.5° (*c* 1.1, $CHCl_3$); UV ($CHCl_3$) $\lambda_{max}$ (log $\epsilon$) 245 (2.83), 274 (2.70) nm; IR (film) $\nu_{max}$ 2968, 2928, 1728, 1448, 1383, 1221, 1113, 1045 cm$^{-1}$; $^1$H and $^{13}$C NMR data, see Table 2; EIMS *m*/*z* 458 $[M]^+$ (8), 415 (13), 389 (6), 358 (30), 343 (85), 270 (20), 207 (35), 124 (100), 121 (50), 109 (10), 85 (15); HREIMS *m*/*z* 458.3392 $[M]^+$ (calcd for $C_{29}H_{46}O_4$ 458.3396).

**(2*R*,3*R*,4*S*,6*S*)-3-Methyl-4,6-di(3-methyl-2-butenyl)-2-(2-methyl-1-oxopropyl)-3-(4-methyl-3-pentenyl)cyclohexanone (4):** colorless viscous oil; $[\alpha]_D^{26}$ +18.3° (*c* 1.8, $CHCl_3$); UV ($CHCl_3$) $\lambda_{max}$ (log $\epsilon$) 245 (3.02), 265 (2.96) nm; IR (film) $\nu_{max}$ 2976, 2930,1722, 1452, 1381, 756 cm$^{-1}$; $^1$H and $^{13}$C NMR data, see Table 2; EIMS *m*/*z* 400 $[M]^+$ (7), 358 (13), 343 (55), 325 (9), 275 (10), 205 (8), 149 (100), 109 (20), 69 (66), 57 (41); HREIMS *m*/*z* 400.3340 $[M]^+$ (calcd for $C_{27}H_{44}O_2$ 400.3341).

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