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# Polyprenylated phloroglucinol derivatives from *Hypericum erectum*

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**Abstract**

Three polyprenylated phloroglucinol derivatives, namely erectquione A, B, and C, were isolated from *Hypericum erectum*. Their structures were established using extensive spectral methods. 

*Keywords:* Erectquione; *Hypericum erectum*; Guttiferae

## 1. Introduction

In recent years, the widespread interest in the antidepressant activity of *Hypericum perforatum* (St. John's wort) has attracted much attention in investigating metabolites from the *Hypericum* genus, many of which are biologically active compounds with an acylphloroglucinol moiety (Decossterd et al., 1989; Decosterd et al., 1991; Jayasuriya et al., 1989; Ishigure et al., 1990; Kyoko et al., 1994; Hu and Sim, 1998, 1999, 2000). *Hypericum erectum* (Guttiferae) is an important herb in Chinese medicine as antihemorrhagic agent, astringent and antibiotic agent (Jiangsu New Medical College, 1977), which has been reported to contained some antiviral prenylated phloroglucinol derivatives (Tada et al., 1991) and two antihemorrhagic compounds, otogirin and otogirone (Kosuge et al., 1985). In continuation of our phytochemical work on the Chinese medicinal plants of *Hypericum* genus (Hu and Sim, 1998, 1999, 2000), we wish to report the isolation and structure elucidation of three new polyprenylated phloroglucinol derivatives, erectquione A–C (**1**–**3**), from the whole plant of *H. erectum*.

## 2. Results and conclusion

The molecular formula of erectquione A (**1**) was established as $C_{21}H_{28}O_4$ by its HREIMS, which showed a molecular ion peak at $m/z$ 344.1968. The presence of a 2,5-dihexadiene-1,4-dione skeleton was suggested by the observed UV (273, 214 nm) and IR (1699, 1654, 1625, 1560 $cm^{-1}$) absorptions. Its IR spectrum also showed hydroxyl group absorptions at 3340 and 3324 $cm^{-1}$. The $^1H$ NMR and $^{13}C$ NMR spectral data (Table 1) revealed the presence of one isoprenyl group (C-17–C-21) and one geranyl side chain (C-7–C-15). The $^{13}C$ NMR signals of the 2,5-dihexadiene-1,4-dione skeleton appeared at $\delta$ 180.2 (C-1), $\delta$ 147.9×2 (C-2, C-6), $\delta$ 121.7×2 (C-3, C-5), and $\delta$ 186.7 (C-4), which indicated that the structure of compound **1** should be symmetrical and thus the carbons of C-2 and C-6 were oxygenated. The HMBC relationships between the carbons at C-4, C-2, and C-6 and the methylene protons at $H_2$-17 and $H_2$-7 demonstrated that the isoprenyl and geranyl groups were at C-3 and C-5, respectively. Therefore, erectquione A (**1**) was 2,6-dihydroxyl-3-geranyl-5-isoprenyl-2,5-dihexadiene-1,4-dione.

Erectquione B (**2**), obtained as a yellow oil, showed an $[M]^+$ at $m/z$ 484.2825 in the HREIMS, which corresponded to $C_{29}H_{40}O_6$ (calc. 484.2824). Its UV and IR spectra were very similar to those of **1**, which revealed that **2** has the same skeleton as **1**. The $^1H$ NMR, $^{13}C$ NMR and HMBC spectra of **2** showed signals corresponding to one isoprenyl side chain, one geranyl side chain and two identical isobutyryloxy side chains (C-1′ to C-4′). Its HMBC spectrum (Fig. 1) had long-range relationships between the methylene protons of isoprenyl and geranyl groups ($\delta$ 3.10, $H_2$-7 and $H_2$-17) and the carbons at of C-4, C-6, and C-2. Hence, the isoprenyl

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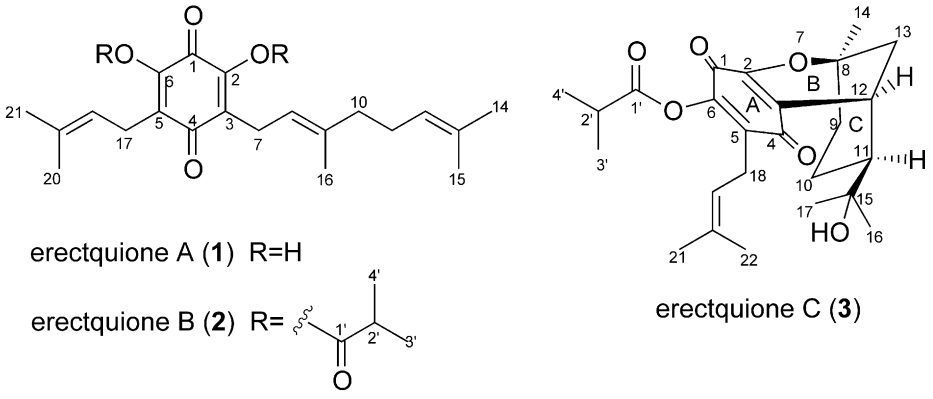


Structures (1-3)

and geranyl side chains were at C-3 and C-5, respectively, and thus the two isobutyryloxy groups should be at C-2 and C-6. Accordingly, the structure of erectquione B (**2**) was 2,6-diisobutyryloxy-3-geranyl-5-isoprenyl-2,5-dihexadiene-1,4-dione.

Erectquione C (**3**) was obtained as a yellow oil. Its HREIMS (found 430.2349; calc. 430.2355) established the molecular formula as $C_{25}H_{34}O_6$, revealed the presence eight degrees of unsaturation in compound **3**. The UV (273, 214 nm) and IR (1766, 1683, 1652, 1624 $cm^{-1}$) absorptions indicated the presence of a 2,5-dihexadiene-1,4-dione moiety and the presence of a hydroxyl group was also shown in IR spectrum (3434 $cm^{-1}$). Its NMR spectroscopic data (Table 2) showed the presence of one isoprenyl side chain (C-18–C-22), one isobutyryloxy side chain, and the moiety of 2,5-dihexadiene-1,4-dione (C-1–C-6), two of which (C-2 and C-6) should be oxygenated. Furthermore, the signals of three methyls, three methylenes, two methines and two oxygenated quaternary carbons were also noted from the $^{13}C$ NMR spectrum. Combination of the HREIMS with the NMR spectrum signals allowed the establishment of compound **3** as a tricyclic derivative.

Table 1
$^{13}C$ NMR ($CDCl_3$, 100 Hz) and $^{1}H$ NMR ($CDCl_3$, 400 Hz) spectral data for compounds **1** and **2**

| Position | **1** | | **2** | |
|---|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 1 | | 180.2 | | 174 |
| 2 | | 147.9 | | 147.7 |
| 3 | | 121.7 | | 136.1 |
| 4 | | 186.7 | | 185.5 |
| 5 | | 121.7 | | 136.1 |
| 6 | | 147.9 | | 147.7 |
| 7 | 3.13, *m* | 22.3 | 3.10, *m* | 23.4 |
| 8 | 5.02, *m* | 119.6 | 4.98, *m* | 118.3 |
| 9 | | 137.3 | | 138.2 |
| 10 | 1.94, *m* | 39.6 | 1.95, *m* | 39.6 |
| 11 | 2.01, *m* | 26.5 | 2.03, *m* | 26.4 |
| 12 | 5.08, *m* | 124.1 | 4.98, *m* | 123.9 |
| 13 | | 131.3 | | 131.6 |
| 14 | 1.67, *s* | 25.6 | 1.69, *s* | 25.7 |
| 15 | 1.59, *s* | 17.6 | 1.65, *s* | 17.7 |
| 16 | 1.51, *s* | 16.1 | 1.69, *s* | 16.3 |
| 17 | 3.13, *m* | 22.3 | 3.10, *m* | 23.3 |
| 18 | 5.08, *m* | 119.4 | 5.02, *m* | 118.1 |
| 19 | | 133.8 | | 134.7 |
| 20 | 1.66, *s* | 25.6 | 1.58, *s* | 17.8 |
| 21 | 1.79, *s* | 17.7 | 1.65, *s* | 25.7 |
| 1′ | | | | 173.8 |
| 2′ | | | 2.83, *m* | 33.9 |
| 3′ | | | 1.35, *d*, 7.2 | 18.8 |
| 4′ | | | 1.35, *d*, 7.2 | 18.8 |
| 2-OH | 6.47, *s* | | | |
| 6-OH | 6.47, *s* | | | |

The gross structure of **3** and all the $^{1}H$ and $^{13}C$ chemical shifts associated with the molecule were determined using 2D NMR spectroscopic analyses (HMBC and HMQC). Analysis of its HMBC spectrum established the connectivity of the B-ring and the methyl group at C-8 i.e. from the crosspeaks (Fig. 2) between the methylene protons at $\delta$ 1.78 and $\delta$ 1.60 ($H_2$-13) and the carbons at $\delta$ 119.6 (C-3), $\delta$ 26.4 (C-12), and $\delta$ 27.5 (C-14), the methine proton at $\delta$ 3.43 (H-12) and the carbons at $\delta$ 80.0 (C-8) and $\delta$ 37.4 (C-13), and the methyl proton at $\delta$ 1.37 ($H_3$-14) and the carbon of C-8 and C-13. The long-range HMBC interactions between

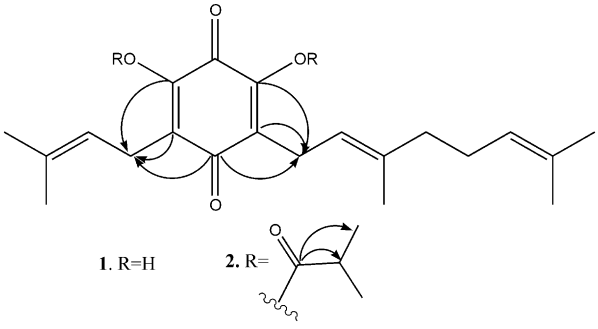


Fig. 1. Key HMBC relationships of compounds **1** and **2**.

Table 2
$^{13}C$ NMR ($CDCl_3$, 100 Hz) and $^{1}H$ NMR ($CDCl_3$, 400 Hz) spectral data and HMBC for compound **3**

| Position | **3** | | |
|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | HMBC[a] |
| 1 | | 174.7 | |
| 2 | | 150 | |
| 3 | | 119.6 | |
| 4 | | 187.3 | |
| 5 | | 135.8 | |
| 6 | | 146.8 | |
| 8 | | 80 | |
| 9 | 1.54, *m* | 39.6 | 8, 10, 13 |
| | 2.04, *m* | | |
| 10 | 1.50, *m* | 21.8 | 8, 9, 11, 12 |
| | 1.17, *m* | 8, 9 | |
| 11 | 1.72, *dt*, (12.8, 2.7) | 53 | 3, 12, 16,17 |
| 12 | 3.43, *m* | 26.4 | 8, 13 |
| 13 | 1.78, *dd*, (13.3, 2.5) | 37.4 | 3, 11, 12, 14 |
| | 1.60, *dd*, (13.3, 3.2) | 9, 12 | |
| 14 | 1.37, *s* | 27.5 | 8, 9, 13 |
| 15 | | 71.7 | |
| 16 | 1.15, *s* | 30.1 | 11, 15, 17 |
| 17 | 0.76, *s* | 25.4 | 11, 15, 16 |
| 18 | 3.04, *m* | 23.4 | 4, 5, 6, 19, 20 |
| 19 | 4.90, *m* | 118.6 | 21, 22 |
| 20 | | 134.9 | |
| 21 | 1.62, *s* | 18 | 22, 20 |
| 22 | 1.56, *s* | 25.8 | 20, 21 |
| 1′ | | 174.3 | |
| 2′ | 2.78, *m* | 34 | 3′, 4′ |
| 3′ | 1.28, *d* (4.2) | 18.9 | 4′, 2′ |
| 4′ | 1.28, *d* (4.2) | 18.9 | 3′, 2′ |

[a] Carbons that correlate with the proton resonance.

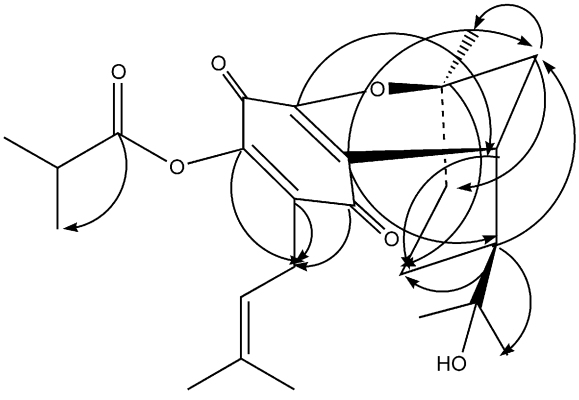


Fig. 2. Key HMBC relationships of compound **3**.

the methylene protons at $\delta$ 2.04 and 1.54 ($H_2$-9) and C-8, C-10, and C-13, the methylene protons at $\delta$ 1.50 and $\delta$ 1.17 ($H_2$-10) and the carbons at $\delta$ 80.0 (C-8), $\delta$ 39.6 (C-9), and $\delta$ 26.4 (C-12), and the methine proton at $\delta$ 1.72 (H-11) and the carbons of C-3 and C-12, determined the connectivity of C-8/C-9/C-10/C-11/C-12 in the C-ring. A 2-(2-hydroxy) propyl group at C-11 was also assigned by the HMBC correlations observed between the methyl protons at $\delta$ 1.15 ($H_3$-16) and $\delta$ 0.76 ($H_3$-17) and C-11 and C-15. The HMBC interactions between the $H_2$-18 and C-4 and C-6 established the isoprenyl at C-5. Thus the remaining isobutyryloxy side chain should be located at C-6.

The molecular model showed that the formation of the B-ring and C-ring required the $CH_3$-14 and H-12 to be in an equatorial position. The 2D NOESY spectrum did not, however, give much information about the stereochemistry of C-11 due to overlapping of key proton signals ($H_2$-9). The axial orientation of H-11 was finally deduced from its coupling constant with the axial proton of C-10 ($J = 12.8$ Hz) (Lambert et al., 1987).

Antibiotic testing of erectquione A, B and C (**1**–**3**) were performed against *Staphylococcus aureus, Micrococcus lutens, Pseudomonas aeruginosa* and *Escherichia coli*. All of them showed no antibiotic activity.

## 3. Experimental

### 3.1. General experimental procedures

Optical rotations were recorded on a Perkin-Elmer 241 polarimeter using $CHCl_3$ as solvent. IR spectra were recorded on a Perkin-Elmer 599B spectrophotometer. UV spectra were measured on a Shimadzu UV-250 spectrophotometer. NMR spectra were acquired on Brüker AM-400 and DRX-400 spectrophotometer and mass spectra (EIMS and HREIMS) were recorded on a MAT-711 instrument.

### 3.2. Plant material

The whole plant of *H. erectum* was collected from Xucheng County, Anhui Province of China, in August 2000. The plant was identified by Mr. Liu, S. J. (Department of Pharmacy, Anhui Chinese Medicine University). A voucher specimen (20000801) was deposited at the herbarium of Shanghai Institute of Materia Medica, Shanghai, China.

### 3.3. Extract and isolation

The dried and powdered whole plant of *H. erectum*. (4.0 kg) was extracted with petroleum ether (8 l×3) at room temperature. The concentrated extract obtained under reduced pressure was then stirred with 200 ml of acetone. After the insoluble solid was filtered, the filtration was concentrated to a residue (32 g). Then the residue was subjected to Si gel chromatography, eluted with petroleum ether containing increasing amounts of $Me_2CO$ to yield four fractions (fractions 1–4). Fraction 1 (yellow oil, eluted with petroleum ether–$Me_2CO$, 20:1) was resubjected to Si gel chromatography, eluted with petroleum ether–$CHCl_3$ (15:1–10:1), and then applied to MCI column, eluted with $Me_2CO$–$H_2O$ (3:1), to afford

erectquione B (**2**) (27 mg). Fraction 2 (red oil, eluted with petroleum ether–$CHCl_3$, 10:1) was applied to Si gel chromatography, (petroleum ether–$CHCl_3$, 15:1 to 10:1) and then to MCI column ($Me_2CO$–$H_2O$, 3:1) and Sephadex LH-20 (MeOH) to afford erectquione A (**1**) (20 mg) and erectquione C (**3**) (15 mg).

### 3.4. Erectquione A (*1*)

Red oil, UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 214 (3.91), 273 (4.04) nm; IR (film) $\nu_{max}$: 3340, 3324, 1699, 1654, 1625, 1560 $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR spectroscopy data, see Table 1; EIMS m/z (rel. Int): 344 $[M]^+$ (27), 301 (24), 261 (67), 204 (100), HREIMS *m/z* 344.1968 (calc. for $C_{21}H_{28}O_4$, 344.1988).

### 3.5. Eerectquione B (*2*)

Yellow oil, UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 214 (4.02), 273 (4.01) nm; IR (film) $\nu_{max}$: 3408, 1707, 1699, 1666, 1620, 1552 $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR spectroscopy data, see Table 1; EIMS *m/z* (rel. int): 484 $[M]^+$ (28), 374 (45), 291 (63), 221 (100), HREIMS m/z 412.2598 (calc. for $C_{26}H_{36}O_4$, 412.2614).

### 3.6. Erectquione C (*3*)

Yellow oil, $[\alpha]_D^{25}$ $-10^\circ$ (c 0.10, $CHCl_3$);UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 214 (3.98), 273(3.99) nm; IR (film) $\nu_{max}$: 3434, 1766, 1683, 1652, 1624 $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR spectroscopy data, see Table 2; EIMS *m/z* (rel. int): 430 $[M]^+$ (6), 360 (31), 327 (100), 259 (27), HREIMS m/z 430.2349 (calc. for $C_{25}H_{34}O_6$, 430.2355).

### 3.7. Biological activity

Antibiotic testings were performed against *S. aureus, M. lutens, P. aeruginosa* and *E. coli* using microtitre plate broth dilution method (Hammer et al., 1996).

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