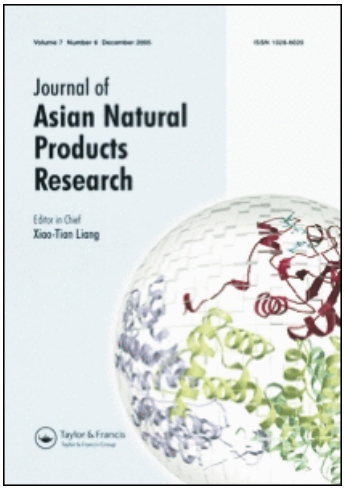


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# Three new diterpenoids from *Coleus forskohlii* Briq

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# Three new diterpenoids from *Coleus forskohlii* Briq

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Three new diterpenoids, forskolin G(2), forskolin H(3), forskolin I(4), were isolated from the whole plant of the *Coleus forskohlii Briq.*, and their structures were elucidated as 1α,6β-diacetoxy-8,13-epoxylabd-14-en-11-one, 1α-hydroxy-6β,7β-diacetoxy-8,13-epoxylabd-14-en-11-one, and 1α,9α-dihydroxy-6β,7α-diacetoxy-8,13-epoxylabd-14-en-11-one on the basis of spectral data.

*Keywords*: *Coleus forskohlii Briq.*; Labiatae; Diterpenes; Forskolin G; Forskolin H; Forskolin I

## 1. Introduction

*Coleus forskohlii Briq.*, a perennial dried herb of Labiatae growing in India as well as Yunnan Province of China, is a traditional folk medicine against cough, asthma, acute and chronic bronchitis, dizziness and fluster [1]. Currently, more than 20 labdane diterpenoids have been isolated from the roots of the different species distributed in India [2–7], and six from those distributed in Yunnan Province of China [8,9]. It has been reported that the diterpenoids are the effective components in *Coleus forskohlii* [10]. In this paper, we describe the isolation and structure elucidation of another three new diterpenoids, forskolin G (**2**), forskolin H (**3**) and forskolin I (**4**) (figure 1) from *Coleus forskohlii* along with the known compound isoforskolin (**1**).

## 2. Results and discussion

The dried whole plant of *Coleus forskohlii* Briq. was extracted with EtOH. The extract was sequentially partitioned with $Et_2O$, EtOAc and $(Me)_2CO$. Repeated chromatography of $Et_2O$ extract on neutral alumina and silica gel column led to the isolation of the known compound isoforskolin (**1**) [3,9], and three new compounds forskolin G (**2**), forskolin H (**3**) and

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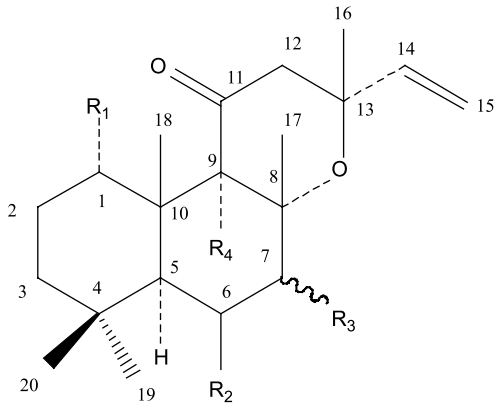


**1**. $R_1 = R_4 = OH$, $R_3 = \beta$ OH, $R_2 = OAC$
**2**. $R_1 = R_2 = OAC$, $R_3 = R_4 = H$
**3**. $R_1 = OH$, $R_2 = R_3 = \beta$ OAC, $R_4 = H$
**4.** $R_1 = R_4 = OH$, $R_2 = OAC$, $R_3 = \alpha$ OAC

Figure 1. Structures of forskolins G (**2**), H (**3**), I (**4**) and isoforskolin (**1**).



forskolin I (**4**). The structures of compounds **2–4** were established by spectroscopic analysis and in comparison with the known compound **1**.

Forskolin G (**2**) was obtained as colourless needles, The molecular formula was determined by HREI-MS to be $C_{24}H_{36}O_6$ (*m/z* 420.2513, $M^+$). EI-MS showed $[M]^+$ at *m/z* 420 and major fragments at *m/z* 377 $[M - CH_3CO]^+$, 360 $[M - CH_3COOH]^+$, 300 $[M - 2CH_3COOH]^+$, 285 $[M - 2CH_3COOH\text{-}CH_3]^+$. The $^1$H NMR spectrum showed signals for five tertiary methyl groups at $\delta$ 1.45, 1.41, 1.25, 0.96, 0.98 (s,5 × $CH_3$), a vinyl group linked to a fully substituted carbon (ABX system, $\delta_A$ 5.19, $\delta_B$ 5.04, $\delta_X$ 5.91, $J_{AB}$ 1.5, $J_{AX}$ 17.4, $J_{BX}$ 10.4 Hz) and two germinal protons adjacent to carbonyl (AB system, $\delta_A$ 2.60, $\delta_B$ 2.66, $J_{AB}$ 18.6 Hz).

The splitting patterns of the above-mentioned protons were similar to those of the corresponding protons of isoforskolin (**1**) which has been reported. It is a labdane diterpene and has a basic skeleton identical to that of **1** (11-keto-manoyl oxide). According to the previous studies on the labdane diterpenes from the whole plant of *Coleus forskohlii*, the differences of the series compounds embodied in the substituting groups at C-1, C-6, C-7 and C-9, meanwhile the substituting groups included only -OH and -OAc. In the $^1$H NMR spectrum of **2**, two sharp peaks for acetyl protons appeared at $\delta$ 1.95 (3H, s) and 2.05 (3H, s).

The $^{13}$C NMR spectrum (table 1) of **2** showed 24 carbon signals, consistent with the molecular formula. From the DEPT experiment, they could be assigned to seven quaternary carbons including two ester carbonyl carbons at $\delta$ 170.1, 169.8, a carbonyl carbon at $\delta$ 206.5 and two carbons bearing an oxygen atom at $\delta$ 75.9 (C-8), 74.8 (C-13), five tertiary carbons ($\delta$ 49.3, $\delta$ 58.4, $\delta$ 69.7, $\delta$ 75.3, $\delta$ 146.9) containing two carbons carrying the acetoxyl group at $\delta$ 75.3 (C-1), 69.7 (C-6) and a vinyl carbon at $\delta$ 146.9, five secondary carbons ($\delta$ 21.9, 37.1, 46.3, 49.3, 112.6), including a carbon holding a carbonyl presented at $\delta$ 49.3 (C-12) and seven primary carbons ($\delta$ 17.6, 21.6, 22.0, 23.1, 29.7, 31.9, 33.1), among which two acetyl carbons appeared at $\delta$ 22.0, 21.6.

Table 1. $^{1}H$ NMR (600 MHz) and $^{13}C$ NMR (150 MHz) data ($\delta$, multiplicity, $J$) for **2**, **3** and **4** in $CDCl_3$.

| *Position* | ***2*** | | ***3*** | | ***4*** | |
|---|---|---|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 1 | 5.52 (brs) | 75.3 | 4.38 (brs) | 71.0 | 4.61 (brs) | 74.2 |
| | | | 2.83 (*OH*) | | 2.90 (*OH*) | |
| 2 | 1.70 (dd, 15.6, 2.4) | 21.9 | 1.45 (dd, 15.6, 3.0) | 25.5 | | 26.7 |
| | 1.98 (d, 2.4) | | 1.48 (d, 3.0) | | 1.45 (dd, 11.0, 3.6) | |
| 3 | 1.10 (d, 13.8) | 37.1 | 1.10 (d, 13.2) | 36.2 | 1.10 (d, 12.9) | 36.4 |
| | 1.46 (m) | | 1.65 (m) | | 1.73 (m) | |
| 4 | | 34.0 | | 33.9 | | 34.3 |
| 5 | 1.45 (s) | 49.3 | 1.62 (s) | 46.1 | 2.39 (s) | 42.0 |
| 6 | 5.56 (brs) | 69.7 | 5.74 (brs) | 69.8 | 5.80 (brs) | 69.9 |
| 7 | 2.25 (dd) | 46.4 | 5.09 (d, 3.6) | 78.6 | 5.51 (d, 4.2) | 74.4 |
| | 1.89 (d, 15.0) | | | | | |
| 8 | | 76.0 | | 77.8 | | 81.2 |
| 9 | 3.22 (s) | 58.4 | 3.59 (s) | 57.7 | 6.03 (*OH*) | 82.6 |
| 10 | | 40.7 | | 41.8 | | 43.3 |
| 11 | | 206.5 | | 207.3 | | 205.6 |
| 12 | 2.65 (d, 18.6) | 49.3 | 2.71 (d, 18.0) | 49.6 | 3.22 (dd, 16.8) | 49.0 |
| | 2.59 (d, 18.6) | | 2.59 (d, 18.0) | | 2.49 (dd, 16.8) | |
| 13 | | 74.8 | | 74.8 | | 75.4 |
| 14 | 5.91 (dd) | 146.9 | 5.97 (dd) | 145.7 | 5.95 (dd) | 146.3 |
| 15 | 5.18 (d, 17.4) | 112.6 | 5.22 (d, 16.8) | 112.8 | 5.27 (d, 17.4) | 111.1 |
| | 5.03 (d, 10.4) | | 5.07 (d, 11.4) | | 4.99 (d, 10.8) | |
| 16 | 1.25 (s) | 31.9 | 1.24 (s) | 31.5 | 1.35 (s) | 31.6 |
| 17 | 1.44 (s) | 29.7 | 1.50 (s) | 24.0 | 1.65 (s) | 23.2 |
| 18 | 1.41 (s) | 17.6 | 1.40 (s) | 17.8 | 1.43 (s) | 19.8 |
| 19 | 0.96 (s) | 33.1 | 0.97 (s) | 32.6 | 1.03 (s) | 33.0 |
| 20 | 0.97 (s) | 23.1 | 0.93 (s) | 22.8 | 0.98 (s) | 23.7 |
| $COCH_3$ | | 170.1 | | 170.3 | | 170.1 |
| $COCH_3$ | | 169.8 | | 170.0 | | 170.1 |
| $COCH_3$ | 2.04 (s) | 22.0 | 2.08 (s) | 21.4 | 2.09 (s) | 21.7 |
| $COCH_3$ | 1.94 (s) | 21.6 | 2.07 (s) | 21.0 | 2.03 (s) | 21.1 |



In the HMBC spectrum (figure 2), the H-9 at $\delta$ 3.22 showed correlations to C-11 ($\delta$ 206.5), C-8 ($\delta$ 75.9), C-10 ($\delta$ 40.7), C-17 ($\delta$ 29.7) and C-18 ($\delta$ 17.6). The long-range correlations between the methylene protons at $\delta$ 2.25 and 1.19 (2H-7, dd, $J = 15.0$ Hz) and four carbon signals at $\delta$ 58.4 (C-9), 49.3 (C-5), 75.9 (C-8), 69.7 (C-6) indicated that two acetoxy groups must be linked to C-1 and C-6.

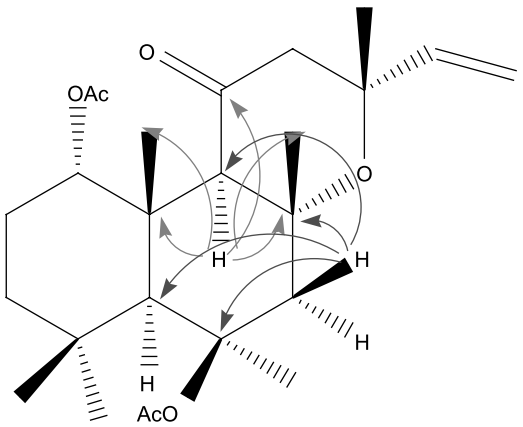


Figure 2. Key HMBC correlations for forskolin G.

From the evidence described above, the structure of **2** was established as 1α,6β-diacetoxy-8,13-epoxylabd -14-en-11-one.

Forskolin H (**3**) appeared as colourless cubic crystals. The molecular formula was determined by HREI-MS to be $C_{24}H_{36}O_7$ (*m/z* 436.2457, $M^+$). The main structural features shown by $^1H$ NMR and $^{13}C$ NMR spectra were consistent with a diterpenic tricyclic structure (labdane), all having a vinyl group as common characteristic. From its spectral data and TLC plots it clearly has the basic skeleton identical to **1**. Its $^1H$ NMR spectrum exhibited two acetyl protons at $\delta$ 2.08 (3H, s), 2.07 (3H, s) and one hydroxyl proton at $\delta$ 2.84 which disappeared by $D_2O$ exchange.

Its $^{13}C$ NMR (table 1) and DEPT spectra displayed the presence of seven methyl groups including two acetyl carbons at $\delta$ 21.4, 21.0, four methylene groups, six methine groups and four quaternary carbons. It indicated no substituting group at C-9. In the HMBC spectrum, long-range correlations between H-18 ($\delta$ 1.40) and C-9 ($\delta$ 57.7), C-10 ($\delta$ 41.8), C-5 ($\delta$ 46.1) and C-1 ($\delta$ 71.0) were observed. In the HSQC experiment, H-1 ($\delta$ 4.39) showed a correlation with C-1 ($\delta$ 71.0). Meanwhile, in the HMBC spectrum, two ester carbonyls at $\delta$ 170.3, 170.0 showed a correlation not with the proton at $\delta$ 4.39 (H-1) but with the protons at $\delta$ 5.74 (1H, s, H-6) and 5.09 (1H, s, H-7). It was thus revealed that two acetoxy groups must be located in the C-6 and C-7 positions and one hydroxyl group is linked to C-1.

The configuration of **3** was suggested by correlations observed in the NOE difference spectrum. The NOE effects were observed from H-7 to H-6, H-5 and H-9; H-6 to H-7, H-9, H-5 and H-19. The signal intensity of these protons at $\delta$ 5.74 (H-6), 1.62 (H-5) and 3.40 (H-9) were enhanced from the NOE difference spectra with decoupling of the proton at $\delta$ 5.09 (H-7). From the coaxial relationships it was shown that the configuration of the proton at C-7 was *axial* and this established the *equatorial* position of the acetoxy group attached to C-7.

From these data, forskolin H was therefore assigned the structure of 1α-hydroxy-6β, 7β-diacetoxy-8,13-epoxylabd-14-en-11-one (**3**).

Forskolin I (**4**) was obtained as colourless needles. The molecular formula of $C_{24}H_{36}O_8$ was determined by HREI-MS (*m/z* 452.2413, $M^+$). From its spectral data and TLC plots it has the same skeleton as **1**. In the $^1H$ NMR spectrum, two sharp singlets of the methyl at $\delta$ 2.10 and 2.03 exhibited two acetoxy groups, whereas two signals at $\delta$ 2.90 and 6.04 which disappeared by $D_2O$ exchange exhibited two hydroxyl groups. In comparison with **1**, its $^1H$ NMR spectrum increased one acetoxy signal and the signal at $\delta$ 4.27 (1H, d, H-7) of **1** was shifted downfield to $\delta$ 5.52 (1H, d) of **4**. This suggested that the hydroxyl group linked to C-7 was acetylated.

The $^{13}C$ NMR (table 1) and DEPT experiments showed that **4** contained seven methyl groups, four methylene units (including C-2, C-3, C-12 and C-15), five methine units (including C-1, C-5, C-6, C-7 and C-14) and five quaternary carbons (including C-4, C-8, C-9, C-10 and C-13). Compared with **1** it was shown that C-1, C-6, C-7 and C-9 of **4** were linked to respective oxygen substituting groups. In the HMBC spectrum, H-7 ($\delta$5.52) and H-6 ($\delta$5.81) showed long-range correlations between the two ester carbonyl carbons at $\delta$ 170.1. This indicated that two acetoxy groups were linked to C-7 and C-6, while two hydroxyl groups were attached to C-1 and C-9.

Irradiation of H-7 ($\delta$ 5.52) led to no NOE enhancement of H-5 and H-9. It confirmed H-7 was far away from 5a-H. This established the *equatorial* configuration of H-7 and *axial* position of the acetoxy group attached to C-7. HMBC (table 2) and NOE difference spectra of **4** led to the assignment of all protons and carbons.

Table 2. HMBC correlations for **2**, **3** and **4**.

| *Position* | ***2*** | ***3*** | ***4*** |
|---|---|---|---|
| 1 | - | - | - |
| 2 | - | - | - |
| 3 | - | - | - |
| 4 | - | - | - |
| 5 | 4, 10, 18, 20 | 1, 3, 4, 7, 9, 10, 18, 20 | 4, 10, 18, 20 |
| 6 | - | OAc, 7, 10 | OAc, 7, 8, 10 |
| 7 | 5, 6, 8, 9 | OAc, 6, 8, 17 | OAc, 7, 8, 17 |
| 8 | - | - | - |
| 9 | 8, 10, 11, 17, 18 | 1, 5, 8, 10, 11, 17, 18 | - |
| 10 | - | - | - |
| 11 | - | - | - |
| 12 | 11, 13, 14, 16 | 11, 13, 14, 16 | 11, 13, 14, 16 |
| 13 | - | - | - |
| 14 | 13 | 13 | 13 |
| 15 | 13, 14 | 13, 14 | 13, 14 |
| 16 | 12, 13, 14 | 12, 13, 14 | 12, 13, 14 |
| 17 | 7, 8, 9 | 8, 9 | 7, 8, 9 |
| 18 | 1, 5, 9, 10 | 1, 5, 9, 10 | 1, 5, 9, 10 |
| 19 | 3, 4, 5, 20 | 3, 4, 5, 20 | 3, 4, 5, 20 |
| 20 | 3, 4, 5, 19 | 3, 4, 5, 19 | 3, 4, 5, 19 |

Therefore, the new compound **4** was identified as 1α,9α-dihydroxy-6β,7α-diacetoxy-8,13-epoxylabd-14-en-11-one.



## 3. Experimental

### *3.1 General experimental procedures*

Melting points were measured using a XRC-1 microscope melting point apparatus and are uncorrected. Optical rotations were taken on a WZZ-1 polarimeter. NMR spectra were acquired in $CDCl_3$ solution and recorded at 600 MHz for $^1H$ and 150 MHz for $^{13}C$ on a Varian Mercury VX-300/600 and INOVA-150 spectrophotometer. Chemical shifts ($\delta$) are expressed in ppm relative to tetramethylsilane (TMS) as internal standard and coupling constants are given in Hz. HMBC and HMQC experiments were recorded with gradient enhancements using sine-shaped gradient pulses. $^1H$ NMR spectra were referenced against the $CHCl_3$ signal at $\delta_H$ 7.27 and $^{13}C$ NMR spectra to the corresponding signal at $\delta_C$ 77.0. IR spectra were recorded on KBr discs with a Nicolet 170SX Fourier-transform infrared (FT-IR) spectrometer. MS spectra were obtained with a VG ZAB-3F mass spectrometer (EI mode). Semi-preparative HPLC was performed using Agilent Zorbax Eclipse XDB-$C_{18}$ (5 μm, 250 × 9.4 mm i.d., Agilent 1100 series) columns. Precoated (silica gel 60G) TLC plates were used to monitor the fractions and examine the purity of the compounds. Visualization was done by spraying with anisaldehyde/$H_2SO_4$ or vanillin/$H_2SO_4$ and heating the plate at 110°C. Column chromatography was performed on silica gel 60G or on neutral alumina. All solvents were analytical grade.

### *3.2 Plant material*

The whole plant of *Coleus forskohlii* was collected in the Tongcheng of HuBei Province in China and identified by the staff at the Department of Identification in HuBei College of TCM. A voucher specimen is deposited in the Herbarium of HuBei College of TCM.

### *3.3 Extraction and isolation*

The whole plant (10 kg) was refluxed with 95% alcohol (80 L/60 L/40 L) three times (2/1/1 h for each). The extract was concentrated. The residue was taken up with tripolite and extracted with ether in a Soxhlet apparatus. After removal of the solvent, the crude extract (120 g) was passed through a neutral alumina column, eluted with petroleum/acetone [from petroleum ether/acetone (9:1) to petroleum ether/acetone (1:1)], and separated into three major fractions. Fraction 1 was subjected to rechromatography on neutral alumina with petroleum ether/ethyl acetate to furnish compound **2** (30 mg); fraction 2 on further chromotography by passing over a silica gel column in hexane/ethyl acetate yielded compound **3** (32 mg); fraction 3 was purified by silica gel column with petroleum ether/ethyl acetate and by semi-prepared HPLC on RP-18 columns to give compounds **1** (100 mg) and **4** (15 mg).

**3.3.1 Forskolin G (2)**. Colourless needles (petroleum ether-ethyl acetate), mp 235–237°C. $[\alpha]_D^{20}$ − 79.7 (c 0.05, MeOH). $^1$H NMR ($CDCl_3$, 600 MHz): see table 1; $^{13}$C NMR ($CDCl_3$, 150 MHz) see table 1; EI-MS *m/z* (rel. int.): [$M^+$] 420, 377, 360, 300, 285, 247, 233, 215, 109, 95, 81, 69, 55,43. HREI-MS *m/z* 420.2513 (calcd for $C_{24}H_{36}O_6$: 420.2512).

**3.3.2 Forskolin H (3)**. Colourless cubic crystals (EtOAc), mp 238–240°C. $[\alpha]_D^{20}$ − 12.7 (c 0.16 MeOH). $^1$H NMR ($CDCl_3$, 600 MHz): see table 1; $^{13}$C NMR ($CDCl_3$, 150 MHz) see table 1; EI-MS *m/z* (rel. int.): [$M^+$] 436, 421, 325, 231, 203, 109, 99, 81, 69, 55, 43. HREI-MS *m/z* 436.2457 (calcd for $C_{24}H_{36}O_7$: 436.2461).

**3.3.3 Forskolin I (4)**. Colourless needles (petroleum ether/acetone), mp 265.5–267.5°C. $[\alpha]_D^{20}$ + 137.9 [c 0.06, $CHCl_3$/MeOH (1:4)]. $^1$H NMR ($CDCl_3$, 600 MHz): see table 1; $^{13}$C NMR ($CDCl_3$, 150 MHz): see table 1; EI-MS *m/z* (rel. int.): [$M^+$] 452, 419, 392, 375, 342, 282, 233, 207, 109, 95, 81, 69, 55, 43. HREI-MS *m/z* 452.2413 (calcd for $C_{24}H_{36}O_8$: 452.2410).

**3.3.4 Isoforskolin (1)**. IR $\nu_{max}$ (KBr): 3476, 1724, 1697, 1249, 1050. $^1$H NMR ($CDCl_3$, 600 MHz): 6.07, 5.18, 4.98 (3H, ABX system, H-14, 15), 6.39 (1H, s, OH-9), 5.84 (1H, s,H-6), 4.27 (1H, d, H-7), 4.65 (1H, s, H-1), 3.18, 2.53 (2H, dd, 2H-12), 2.68 (1H, s, OH-1), 2.31 (1H, d, H-5), 2.10 (s, OAc), 1.61, 1.41, 1.40, 1.07, 0.99 (s, 5 × $CH_3$); EI-MS *m/z* (rel. int.): [$M^+$] 410, 392, 281, 264, 221, 193, 165, 149, 123, 99, 81, 69, 55, 43.

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