# Spectral Characteries of Forskolins (1)

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**Abstract**: Active diterpenoids, forskolin (**1**, 7β-acetoxy-1α, 6β, 9α-trihydroxy-8, 13-epoxy-labd-14-en-11-one) and isoforskolin (**2**, 6β-acetoxy-1α, 7β, 9α-trihydroxy-8, 13-epoxy-labd-14-en-11-one), were isolated from *Coleus forskohlii* (Willd.) Briq. This paper describes detailedly their spectral characteries (including 1D and 2D NMR data).

**Key words**: *Coleus forskohlii*; forskolin; isoforskolin; spectral characteries

# 佛司可林类成分的光谱特征(一)

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**摘　要**: 活性二萜佛司可林和异佛司可林已从毛喉鞘蕊花分离得到。本文详细描述了它们的光谱特征(包括一维和二维的核磁共振谱)。

**关键词**: 毛喉鞘蕊花; 佛司可林; 异佛司可林; 光谱特征
**中图分类号**: O629.6+1; O433.5

## Introduction

*Coleus forskohlii* (Willd.) Briq was known to contain abundant labdane diterpenoids, which possessed significant bioactivity[1-5]. As a continuation of our study on *C. forskohlii*, has isolated twenty constituents including eight new labdane diterpenoids[6-12]. In this paper, we report detailedly spectral characteries of forkolin (**1**) and isoforskolin (**2**) (including 1D and 2D NMR data).

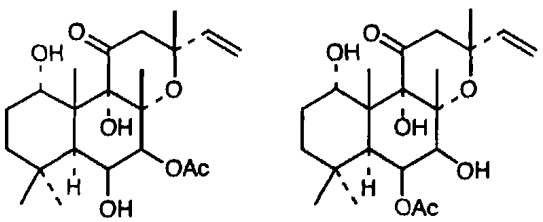


**Fig.1 Forskolin and isoforskolin**

## Results and Discussion

Compound **1** was obtained as colorless needles (MeOH). EIMS *m/z* 410 $[M]^+$, together with $^{13}C$ and DEPT NMR spectra indicated the molecular formula as $C_{22}H_{34}O_7$, DEPT spectra showed five tertiary methyl groups, four methylene groups, five methine groups, five quaternary carbons, two olefinic carbons, one ketonic carbon and one acetoxy signals. Comparison of the data of compound **1** with forskolin B[6,7] suggested that compound **1** had a typical 8,13-epoxy-labd-14-en-11-one skeleton[6,7]. In its $^1H$ NMR spectrum, the five methyl signals at $\delta_H$ 1.06, 1.44, 1.52, 1.85 and 1.90, and the signals of AB coupling system at $\delta_H$ 3.49 (1H, d, $J$ = 16.4 Hz) and 2.61 (1H, d, $J$ = 16.4 Hz), and three olefinic proton signals at $\delta_H$ 6.31, 4.92 and 5.42 also confirmed the above assumption. The HMBC spectrum showed cross-peaks of $\delta_H$ 5.07 (1H, d, 2.7, 1β-H) with $\delta_C$ 36.97 (C-3), 43.79 (C-5), and 43.52 (C-10), $\delta_H$ 5.04 (1H, dd, $J$ = 4.1, 2.5 Hz, 6α-H) with $\delta_C$ 43.79 (C-5), 78.60 (C-7), 81.97 (C-8), and 43.52 (C-10), $\delta_H$ 6.09 (1H, d, $J$ = 4.1Hz, 7α-H) with $\delta_C$ 43.79 (C-

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5),68.93(C-6),81.97(C-8),83.30(C-9), and 170.68 (OAc), which revealed the locations of 1-OH, 6-OH, 9-OH and 7-OAc. The above inferences were also supported by the $^1H$-$^1H$ COSY. Additionally, the relative configurations of 1-OH, 6-OH and 7-OAc were determined respectively as α, β and β orientation due to ROESY correlations of 1-H with 2β-H and 20β-Me; 6-H with 5α-H and 18α-Me; 7-H with 5α-H, 6α-H and 9α-OH respectively. Thus, compound 1 was determined as 7β-acetoxy-1α, 6β, 9α-trihydroxy-8, 13-epoxy-labd-14-en-11-one, named 1-deacetylforskolin B[7], 6-deacetylforskolin J[11], or forskolin.

**Table 1 The $^{13}C$ NMR data of 1 and 2(in $C_5D_5N$)**

| Carbon | 1 | 2 | Carbon | 1 | 2 |
|---|---|---|---|---|---|
| 1 | 74.16 | 73.78 | 12 | 49.80 | 49.86 |
| 2 | 27.46 | 27.22 | 13 | 75.52 | 75.46 |
| 3 | 36.97 | 37.22 | 14 | 147.94 | 148.27 |
| 4 | 34.75 | 34.27 | 15 | 109.99 | 109.78 |
| 5 | 43.79 | 43.12 | 16 | 31.14 | 30.49 |
| 6 | 68.93 | 73.29 | 17 | 24.57 | 23.54 |
| 7 | 78.60 | 73.36 | 18 | 33.27 | 33.04 |
| 8 | 81.97 | 83.33 | 19 | 24.49 | 23.46 |
| 9 | 83.30 | 83.01 | 20 | 20.25 | 20.17 |
| 10 | 43.52 | 43.49 | OAc | 170.68 | 170.57 |
| 11 | 207.25 | 207.40 | | 21.26 | 21.85 |

**Table 2 The $^1H$ NMR and COSY data of 1 and 2 (in $C_5D_5N$, $J$ in Hz)**

| 1 | | | 2 | | |
|---|---|---|---|---|---|
| H | $^1H$ NMR | COSY | H | $^1H$ NMR | COSY |
| 1β-H | 5.07(d,2.7) | 2-H, | 1β-H | 5.07(brs) | 2β-H,1-OH |
| 2β-H | 2.31(m) | 1β-H,2α-H,3-$H_2$ | 2β-H | 2.21(m) | 1β-H,2α-H,3-$H_2$ |
| 2α-H | 1.60(m) | 1β-H,2β-H,3-$H_2$ | 2α-H | 1.60(m) | 2β-H,3-$H_2$ |
| 3α-H | 2.10(m) | 2-$H_2$,3β-H | 3α-H | 2.12(m) | 2-$H_2$,3β-H |
| 3β-H | 1.12(m) | 2-$H_2$,3α-H | 3β-H | 1.10(m) | 2-$H_2$,3α-H |
| 5α-H | 2.62(d,2.5) | 6α-H | 5α-H | 2.73(d,2.6) | 6α-H |
| 6α-H | 5.04(dd,2.5,4.1) | 5α-H,7α-H | 6α-H | 6.29(dd,4.6,2.6) | 5α-H,7α-H |
| 7α-H | 6.09(d,4.1) | 6α-H | 7α-H | 4.88(d,4.6) | 6α-H |
| 12α-H | 3.49(d,16.4) | 12β-H | 12α-H | 3.46(d,16.5) | 12β-H |
| 12β-H | 2.61(d,16.4) | 12α-H | 12β-H | 2.62(d,16.5) | 12α-H |
| 14-H | 6.31(dd,17.3,10.7) | 15-$H_2$ | 14-H | 6.42(dd,17.5,10.7) | 15-$H_2$ |
| 15-Hcis | 4.92(d,10.7) | 14-H | 15-Hcis | 4.93(d,10.7) | 14-H |
| 15-Htrans | 5.42(d,17.3) | 14-H | 15-Htrans | 5.25(d,17.5) | 14-H |
| 16-Me | 1.44(s) | | 16-Me | 1.41(s) | |
| 17-Me | 1.85(s) | | 17-Me | 1.92(s) | |
| 18-Me | 1.06(s) | 19-Me | 18-Me | 1.17(s) | 19-Me |
| 19-Me | 1.52(s) | 18-Me | 19-Me | 1.09(s) | 18-Me |
| 20-Me | 1.90(s) | | 20-Me | 1.69(s) | |
| OAc | 2.13(s) | | OAc | 2.13(s) | |

Compound **2**, colorless prisms (MeOH), was assigned the molecular formula $C_{22}H_{34}O_7$ by EIMS $m/z$ 410 $[M]^+$, $^1H$ and $^{13}C$ NMR spectra. The NMR data of compound **2** were very similar to those of compound **1**. Further comparison of $^{13}C$ NMR of compound **2** with that of compound **1** showed that compound **2** also possessed the same typical 8,13-epoxy-labd-14-en-11-one skeleton [6,7]. Moreover, the correlations of HMBC between $\delta_H$ 5.07 (1H, brs, 1β-H) with $\delta_C$ 37.22(C-3) 43.02(C-5) and 20.17(C-20); $\delta_H$ 6.29 (1H, dd, $J$ = 4.6, 2.6 Hz, 6α-H) with $\delta_C$, 43.02(C-5), 73.36(C-7), 83.33(C-8), 43.49(C-10); $\delta_H$ 2.13(3H, s, 6-OAc) with $\delta_C$ 73.29(C-6); $\delta_H$ 4.88(1H, $J$ = 4.6 Hz, 7α-H) with $\delta_C$ 73.29(C-6), 83.33(C-8); indicated the presence of 1-OH, 6-OAc and 7-OH substitution and located at 1α, 6β and 7β position respectively in compound **2**, which were confirmed by the ROESY correlations of 1-H with 20β-Me; 6-H with 5α-H, 18α-Me and 9α-OH; 7-H with 5α-H and 6α-H. Therefore, compound **2** was deduced as 6β-acetoxy-1α, 7β, 9α-trihydroxy-8, 13-epoxy-labd-14-en-11-one, named coleonol B [6], 1-deacetylforskolin I [11], or isoforskolin.

## Experimental Section

### General experimental procedures

Melting points were measured on an XRC-1 micromelting apparatus and are uncorrected. IR were obtained on a Bio-Rad FTS-135 infrared spectrometer with KBr pellets. The MS spectra were performed on a VG Autospec-3000 spectrometer with 70 eV. $^1H$ NMR, $^{13}C$ NMR and 2D NMR were recorded on a Bruker AM-400 and DRX-500 spectrometer with TMS as internal standard. The silica gel for TLC and column chromatography was obtained from Qingdao Marine Chemical Inc., China.

### Plant material

The roots of *Coleus forskohlii* (Willd.) Briq. which seed came from India, were collected in Yunnan Province, China, in September 2001, and identified by Professor Li HW, Kunming Institute of Botany. The voucher specimen has been deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

## Extraction and isolation

5 kg dried roots of *Coleus forskohlii* were extracted with 25 L × 3 of 95% ethanol at room temperature and filtered. The filtrate was concentrated *in vacuo* and partitioned with petroleum ether, chloroform and n-butanol. The chloroform extract was evaporated to afford 50 g of residues. The residues were subjected to column chromatography on silica gel, eluted with petroleum ether-acetone (from petroleum ether to petroleum ether-acetone 1 : 1). The fractions were combined by monitoring with TLC to obtain fractions 1 ~ 6. Then the fraction 1 was recrystallized with MeOH to afford **2**. The fraction 4 was chromatographied repeatedly on silica gel eluted with MeOH to give **1**.

**Forskolin (1)** Colorless needles (MeOH), mp. 228 ~ 230 ℃; IR (KBr): 3444, 2949, 2926, 2870, 1703, 1633, 1460, 1411, 1375, 1267, 1219, 1177, 1163, 1115, 1092, 1056, 1036, 993, 976 $cm^{-1}$; $^1H$ NMR data see Table 2; $^{13}C$ NMR data see Table 1; EIMS: (rel %) *m/z*: 410 (0.5, $M^+$), 393 (8, $M^+$-OH), 392 (35, $M^+$-H2O), 377 (3, $M^+$- $H_2O$-$CH_3$), 364 (8), 350 (1, $M^+$-HOAc), 332 (2, $M^+$-HOAc-$H_2O$), 331 (3), 324 (7), 319 (8), 289 (4), 240 (12), 221 (15), 209 (31), 208 (33), 193 (38), 191 (51), 177 (25), 165 (68), 147 (34), 139 (42), 137 (39), 123 (80), 109 (79), 99 (62), 95 (84), 85 (47), 81 (100), 71 (36), 69 (60), 68 (47), 67 (52), 57 (24), 55 (64);

**Isoforskolin (2)** Colorless prisms (MeOH), mp. 209 ~ 210 ℃; IR (KBr): 3444, 3008, 2981, 2955, 2926, 2875, 1723, 1637, 1456, 1397, 1376, 1365, 1290, 1245, 1198, 1176, 1164, 1111, 1102, 1078, 1017, 995, 978, 956, 938 $cm^{-1}$; $^1H$ NMR data see Table 2; $^{13}C$ NMR data see Table 1; EIMS (rel %) *m/z*: 410 (2, $M^+$), 393 (4, $M^+$-OH), 392 (17, $M^+$-$H_2O$), 377 (2, $M^+$- $H_2O$-$CH_3$), 350 (2, $M^+$-HOAc), 324 (1), 306 (3), 289 (5), 281 (13), 264 (6), 233 (12), 221 (40), 209 (17), 193 (34), 191 (36), 166 (24), 165 (76), 152 (34), 123 (58), 109 (47), 99 (53), 95 (65), 85 (40), 81 (75), 69 (44), 68 (47), 67 (40), 57 (17).

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## References

1 Tandon JS, Dhar MM, Ramakumar S, *et al*. Structure of coleonol, a biologically active diterpene from *Coleus forskohlii*. *Indian J Chem*, 1977, 15B(10): 880-883

2 Katti SB, Jauhari PK, Tandon JS. New diterpenes from *Coleus forskohlii*: Structures of diterpenes, coleonol-D, coleol & coleonone. *Indian J Chem*, 1979, 17B(4): 321-323

3 Painuly P, Katti SB, Tandon JS. Diterpenes from *Coleus forskohlii*: structures of coleonol-E & coleonol-F. *Indian J Chem*, 1979, 18B(3): 214-216

4 Seamon KB, Daly JW, Metzger H, *et al*. Structure-activity relationships for activation of adenylate cyclase by the diterpene forskolin and its derivatives. *J Med Chem*, 1983, 26(3): 436-439

5 Bhat SV, Dohadwalla AN, Bajwa BS, *et al*. The antihypertentive and positive inotropic diterpene forskolin: effects of structural modifications on its activity. *J Med Chem*, 1983, 26(4): 486-492

6 Jin QD, Xie XH, Mu Qz. Study on the chemical constituents from *Coleus forskohlii* Briq. *Natural Product R & D*, 1990, 2(3): 6-9

7 Jin QD, He BH. Minor constituents from *Coleus forskohlii*. *Acta Botanica Yunnanica*, 1998, 20(4): 469-473

8 Yao CS, Xu YL. The diterpenoid quinones from *Coleus forskohlii*. *Chinese Chemical Letters*, 2001, 12(4): 339-342

9 Yao CS, Shen YH, Xu YL. The chemical constituents of *Coleus forskohlii*. *Natural Product R & D*, 2002, 14(2): 1-6

10 Shen YH, Yao CS, Xu YL. New Diterpenoids from *Coleus forskohlii*. *Chinese Chemical Letters*, 2002, 13(8): 740-743

11 Shen YH, Wu M, Xu YL. Two new diterpenoids from *Coleus forskohlii*. *Journal of Asia Natural Products Research*, 2004, (Accepted)

12 Shen YH, Yao CS, Dong XJ, *et al*. Research advance on chemistry and physiological activities for *Coleus forskohlii*. *Natural Product R & D*, (Accepted)