# Studies on chemical constituents of *Isodon eriocalyx* var. *laxiflora*

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**Abstract**: **Object** To study the chemical constituents of the leaves of *Isodon eriocalyx* (Dunn) Hara var. *laxiflora* C. Y. Wu et H. W. Li. **Methods** The leaves of *I. eriocalyx* var. *laxiflora* were extracted with 70% $Me_2CO$, and separated and purified by column chromatographies on silica gel or MCI gel. All the compounds were identified on the basis of spectral analysis (including 1D and 2D NMR and MS) or direct co-chromatographies with authentic samples. **Results** Fifteen compounds were isolated from *I. eriocalyx* var. *laxiflora*. Their structures were characterized as blumenol A (Ⅰ), 2-formyl-5-hydroxymethylfuran (Ⅱ), 3, 4-dihydroxyphenacyl alcohol (Ⅲ), caffeic acid (Ⅳ), caffeic acid ethylene ester (Ⅴ), *p*-hydroxybenzoic acid (Ⅵ), *m*-hydroxybenzoic acid (Ⅶ), *p*-methylbenzoic acid (Ⅷ), 3, 4-dimethoxyphenol (Ⅸ), rosmanic acid (Ⅹ), rosmanic acid methyl ester (Ⅺ), cirsimaritin (Ⅻ), 8-hydroxycirsimaritin (ⅩⅢ), ursolic acid (ⅩⅣ), and 2α-hydroxyursolic acid (ⅩⅤ) respectively. **Conclusion** Compounds Ⅰ- Ⅴ, Ⅷ, Ⅸ and Ⅺ were isolated from this plant for the first time.

**Key words**: the leaves of *Isodon eriocalyx* (Dunn) Hara var. *laxiflora* C. Y. Wu et H. W. Li; chemical constituents; blumenol A

# 疏花毛萼香茶菜的化学成分研究

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摘 要: 目的 研究疏花毛萼香茶菜 *Isodon eriocalyx* var. *laxiflora* 叶的化学成分。方法 疏花毛萼香茶菜叶经 70% 丙酮提取, 采用硅胶或 MCI 柱层析进行分离纯化, 通过波谱分析(包括一维和二维核磁共振及质谱)或直接与标准品对照进行结构鉴定。结果 从疏花毛萼香茶菜中分离得到 15 个化合物, 分别鉴定为: 布卢姆醇 A(Ⅰ), 2-醛基-5-羟甲基呋喃(Ⅱ), 3, 4-二羟基苯酰乙醇(Ⅲ), 咖啡酸(Ⅳ), 咖啡酸乙烯酯(Ⅴ), 对-羟基苯甲酸(Ⅵ), 间-羟基苯甲酸(Ⅶ), 对-甲基苯甲酸(Ⅷ); 3, 4-二甲氧基苯酚(Ⅸ), 迷迭香酸(Ⅹ), 迷迭香酸甲酯(Ⅺ), 滨蓟素(Ⅻ), 8-羟基滨蓟素(ⅩⅢ), 乌苏酸(ⅩⅣ)和 2α-羟基乌苏酸(ⅩⅤ)。结论 化合物Ⅰ～Ⅴ, Ⅷ, Ⅸ和Ⅺ为首次从该植物中分离得到。

关键词: 疏花毛萼香茶菜叶; 化学成分; 布卢姆醇 A

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*Isodon eriocalyx* (Dunn) Hara var. *laxiflora* C. Y. Wu et H. W. Li, a perennial shrub, belongs to *Isodon* Kudo, which is known for being rich in *ent*-kaurane diterpenoids. A series of new and known *ent*-kaurane diterpenoids from this plant have been reported[1, 2]. However, it was rarely reported on other chemical constituents except diterpenoids. In this paper, we describe the isolation and structural elucidation of 15 non-diterpenoids compounds (including 12 phenolic compounds, 2 triterpenoids and a carotenoid-like compound) isolated from the leaves of *I. eriocalyx* var. *laxiflora*. Their structures were identified as blumenol A (Ⅰ)[3], 2-formyl-5-hydroxymethylfuran (Ⅱ)[4], 3, 4-dihydroxyphenacyl alcohol (Ⅲ)[5], caffeic acid (Ⅳ)[6], caffeic acid ethylene

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ester (Ⅴ)[7], *p*-hydroxybenzoic acid (Ⅵ), (identical with authentic sample), *m*-hydroxybenzoic acid (Ⅶ), (identical with authentic sample), *p*-methylbenzoic acid (Ⅷ), (identical with authentic sample), 3, 4-dimethoxyphenol (Ⅸ)[8], rosmanic acid (Ⅹ), (identical with authentic sample), rosmanic acid methyl ester (Ⅺ)[6], cirsimaritin (Ⅻ)[9], 8-hydroxycirsimaritin (ⅩⅢ)[10], ursolic acid (ⅩⅣ)[11], 2α-hydroxyursolic acid (ⅩⅤ)[12]. Among these 15 compounds, Ⅰ- Ⅴ, Ⅷ, Ⅸ and Ⅺ were obtained from this plant for the first time.

## 1 Experiment

1.1 General. MS spectra were recorded on a VG Auto Spec-3000 spectrometer. NMR spectra were run on a Bruker AM-400 and DRX-500 instrument.

1.2 Plant materials. The leaves of *I. eriocalyx* (Dunn) Hara var. *laxiflora* C. Y. Wu et H. W. Li were collected in Xishuangbanna Prefecture, Yunnan Province, People's Republic of China, in November 1999. It was identified by Prof. Tao G D at Xishuangbanna Botanic Garden. A voucher specimen has been deposited in the Herbarium of Kunming Institute of Botany, the Chinese Academy of Sciences.

1.3 Extraction and isolation. The dried and powdered leaves (25 kg) were extracted with 70% $Me_2CO$ at room temperature for three times and filtered. The filtrate was concentrated and extracted with EtOAc. The EtOAc layer was evaporated in vacuo to give the residue (1 000 g), which was subjected to silica gel column chromatography, using $CHCl_3$, $CHCl_3$-$Me_2CO$ (9∶1, 8∶2, 7∶3, 6∶4, and 1∶1) and $Me_2CO$ as eluents to afford fractions 1, 2, 3, 4, 5, 6 and 7, respectively. The fractions were collected and combined by monitoring with TLC. Compounds ⅩⅣ (30 mg) and ⅩⅤ (40 mg) were obtained from fraction 1. In the same way, compounds Ⅻ (200 mg) and ⅩⅢ (300 mg) were afforded from fractions 2 and 3, respectively. Fraction 2 was further purified on silica gel CC eluting with petroleum-$Me_2CO$ (4∶1, 3∶1, 2∶1, 1∶1) to give Ⅱ (6 mg), Ⅵ (11 mg), Ⅶ (9 mg) and Ⅷ (7 mg). Fraction 4 was repeatedly treated by middle-pressure column chromatography over silica gel developing with $CHCl_3$-MeOH (15∶1, 9∶1) and followed by recrystallization to yield Ⅰ (16 mg). Fraction 5 was subjected to middle-pressure column chromatography on silica gel column using $CHCl_3$-MeOH (9∶1 and 8∶2) as eluents and the portion eluting by $CHCl_3$-MeOH (8∶2) was purified by column chromatography over MCI-gel CHP-20P developing with aqueous MeOH (40%, 50%, 60%) to afford compounds Ⅲ (40 mg), Ⅳ (70 mg), Ⅴ (13 mg), Ⅹ (100 g) and Ⅺ (20 mg).

## 2 Identification

Compound Ⅰ (blumenol A): $C_{13}H_{20}O_3$, colorless needles. EI-MS (70 eV) *m*/*z* (%): 224 [$M^+$] (7), 206 (29), 191 (4), 181 (9), 168 (48), 162 (8), 150 (40), 135 (43), 124 (100), 111 (44), 107 (37), 95 (37), 79 (54), 69 (42), 55 (83). $^1$HNMR (400 MHz, $C_5D_5N$) δ: 2.68 (1H, d, *J* = 16.6 Hz, H-2a), 2.40 (1H, d, *J* = 16.6 Hz, H-2b), 6.25 (1H, d, *J* = 15.6 Hz, H-7), 6.33 (1H, dd, *J* = 5.0, 15.6 Hz, H-8), 4.70 (1H, m, H-9), 1.45 (3H, d, *J* = 6.6 Hz, Me-10), 1.12 (3H, s, Me-11), 1.28 (3H, s, Me-12), 2.01 (3H, s, Me-13). $^{13}$CNMR (100 MHz, $C_5D_5N$) δ: 41.5 s (C-1), 50.3 t (C-2), 197.6 s (C-3), 126.6 d (C-4), 164.3 s (C-5), 78.9 s (C-6), 137.2 d (C-7), 129.1 d (C-8), 67.3 d (C-9), 24.4 q (C-10), 23.4 q (C-11), 24.4 q (C-12), 19.3 q (C-13).

Compound Ⅱ (2-formyl-5-hydroxymethylfuran): $C_6H_6O_3$, brown powder. EI-MS (70 eV) *m*/*z* (%): 126 [$M^+$] (89), 109 (50), 97 (100), 81 (45), 69 (80), 59 (21), 53 (54). $^1$HNMR (400 MHz, $CD_3OD$) δ: 9.52 (1H, s, CHO), 7.38 (1H, d, *J* = 3.5 Hz, H-3), 6.58 (1H, d, *J* = 3.5 Hz, H-4), 4.60 (2H, s, $CH_2$); $^{13}$CNMR (100 MHz, $CD_3OD$) δ: 179.5 d (CHO), 163.3 s (C-2), 124.7 d (C-3), 110.9 d (C-4), 153.9 s (C-5), 57.7 t ($CH_2$).

Compound Ⅲ (3, 4-dihydroxyphenacyl alcohol): $C_8H_8O_4$, brown needles. EI-MS (70 eV) *m*/*z* (%): 168 [$M^+$] (57), 152 (5), 149 (10), 137 (100), 123 (4), 109 (65), 91 (10), 81 (52),

63 (30), 53 (51). $^1$HNMR (500 MHz, $CD_3OD$) $\delta$: 7.38 (1H, d, $J$= 2.0 Hz, H-2), 6.83 (1H, d, $J$= 8.0 Hz, H-5), 7.36 (1H, dd, $J$= 2.0, 8.0 Hz, H-6), 4.79 (2H, s, H$_2$-8). $^{13}$CNMR (125 MHz, $CD_3OD$) $\delta$: 127.7 s (C-1), 115.4 d (C-2), 146.7 s (C-3), 152.6 s (C-4), 116.0 d (C-5), 122.3 d (C-6), 198.6 s (C= O), 65.8 t (C-8).

Compound Ⅳ (caffeic acid): $C_9H_8O_4$, brown needles. Negative FAB-MS (70 eV) $m/z$ (%): 179 [M - H]$^-$ (100). $^1$HNMR (500 MHz, $CD_3OD$) $\delta$: 7.04 (1H, d, $J$= 2.0 Hz, H-2), 6.77 (1H, d, $J$= 8.2 Hz, H-5), 6.93 (1H, dd, $J$= 2.0, 8.2 Hz, H-6), 7.54 (1H, d, $J$= 15.8 Hz, H-7), 6.22 (1H, d, $J$= 15.8 Hz, H-8). $^{13}$CNMR (125 MHz, $CD_3OD$) $\delta$: 127.8 s (C-1), 115.5 d (C-2), 146.7 s (C-3), 149.4 s (C-4), 116.5 d (C-5), 122.9 d (C-6), 147.1 d (C-7), 115.1 s (C-8), 171.1 s (C-9).

Compound Ⅴ (caffeic acid ethylene ester): $C_{11}H_{10}O_4$, yellow needles. EI-MS (70 eV) $m/z$ (%): 206 [M$^+$] (45), 163 (100), 145 (40), 135 (63), 117 (48), 107 (18), 105 (20), 97 (20), 89 (67), 77 (47), 71 (37), 63 (41), 55 (58). $^1$HNMR (500 MHz, $CD_3OD$) $\delta$ 7.06 (1H, d, $J$= 2.0 Hz, H-2), 6.78 (1H, d, $J$= 8.2 Hz, H-5), 6.97 (1H, dd, $J$= 2.0, 8.2 Hz, H-6), 7.64 (1H, d, $J$= 15.9 Hz, H-7), 6.27 (1H, d, $J$= 15.9 Hz, H-8), 7.38 (1H, dd, $J$= 6.3, 14.0 Hz, H-10), 4.93 (1H, d, $J$= 14.0 Hz, H-11a), 4.60 (1H, d, $J$ = 6.3 Hz, H-11b). $^{13}$CNMR (125 MHz, $CD_3OD$) $\delta$: 127.5 s (C-1), 115.3 d (C-2), 146.9 s (C-3), 150.1 s (C-4), 116.6 d (C-5), 123.4 d (C-6), 148.7 d (C-7), 113.6 s (C-8), 165.9 s (C-9), 142.5 d (C-10), 97.7 t (C-11).

Compound Ⅵ ($p$-hydroxybenzoic acid): $C_7H_6O_3$, white plates. EI-MS (70 eV) $m/z$ (%): 138 [M$^+$] (95), 121 (100), 110 (22), 93 (75), 81 (40), 74 (28), 65 (86), 53 (84). $^1$HNMR (400 MHz, acetone-$d_6$) $\delta$: 7.92 (2H, d, $J$= 8.6 Hz, H-2 and H-6), 6.92 (2H, d, $J$= 8.6 Hz, H-3 and H-5). $^{13}$CNMR (100 MHz, acetone-$d_6$) $\delta$ 169.0 s (COOH), 122.3 s (C-1), 132.8 d (2C, C-2 and C-6), 116.0 d (2C, C-3 and C-5), 162.7 s (C-4). Rf values on TLC are consistent with those of authentic sample.

Compound Ⅶ ($m$-hydroxybenzoic acid): $C_7H_6O_3$, colorless needles. EI-MS (70 eV) $m/z$ (%): 138 [M$^+$] (68), 120 (100), 92 (96), 84 (32), 64 (38), 53 (13). $^1$HNMR (400 MHz, $C_5D_5N$) $\delta$ 10.88 (1H, brs, COOH), 6.95 (1H, brs, H-2), 7.18 (1H, d, $J$= 8.4 Hz, H-4), 7.49 (1H, m, H-5), 8.26 (1H, d, $J$= 8.0 Hz, H-6). $^{13}$CNMR (100 MHz, $C_5D_5N$) $\delta$: 174.5 s (COOH), 115.4 s (C-1), 117.8 d (C-2), 163.0 s (C-3), 119.2 d (C-4), 131.4 d (C-5), 135.4 d (C-6). Rf values on TLC are consistent with those of authentic sample.

Compound Ⅷ ($p$-methylbenzoic acid): $C_8H_8O_2$, colorless needles. EI-MS (70 eV) $m/z$ (%): 136 [M$^+$] (80), 121 (100), 107 (8), 93 (63), 77 (13), 65 (65), 53 (16). $^1$HNMR (400 MHz, $C_5D_5N$) $\delta$: 12.67 (1H, brs, COOH), 8.08 (2H, m, H-2 and H-6), 7.18(2H, m, H-3 and H-5), 2.51 (3H, s, $CH_3$). $^{13}$CNMR (100 MHz, $C_5D_5N$) $\delta$ 193.1 s (COOH), 129.8 s (C-1), 131.5 d (2C, C-2 and C-6), 116.2 D (2C, C-3 and C-5), 162.7 s (C-4), 26.3 q ($CH_3$). Rf values on TLC are consistent with those of authentic sample.

Compound Ⅸ (3, 4-dimethoxyphenol): $C_8H_{10}O_3$, brown powder. EI-MS (70 eV) $m/z$ (%): 154 [M$^+$] (60), 136 (100), 108 (40), 97 (5), 80 (37), 69 (7), 63 (12), 55 (64). $^1$HNMR (400 MHz, $CD_3OD$) $\delta$: 7.24 (1H, d, $J$= 3.0 Hz, H-2), 6.77 (1H, d, $J$= 8.8 Hz, H-5), 6.93 (1H, dd, $J$= 3.0, 8.8 Hz, H-6), 3.31 and 3.28 (each 3H, s, $OCH_3$).

Compound Ⅹ (rosmanic acid): $C_{18}H_{16}O_8$, brown amorphous solid. EI-MS (70 eV) data and Rf values on TLC are consistent with those of authentic sample.

Compound Ⅺ (rosmanic acid methyl ester): $C_{19}H_{18}O_8$, brown powder. EI-MS (70 eV) $m/z$ (%): 374 [M$^+$] (3), 314 (23), 208 (3), 194 (100), 180 (41), 163 (79), 153 (7), 136 (28), 123 (57), 89 (28), 77 (18), 63 (11). $^1$HNMR (400 MHz, $CD_3OH$) $\delta$: 7.04 (1H, d, $J$= 2.0 Hz, H-2), 6.77 (1H, d, $J$= 8.0 Hz, H-5), 6.94 (1H, dd, $J$= 2.0, 8.0 Hz, H-6), 7.55 (1H, d, $J$=

16.0 Hz, H-7), 6.25 (1H, d, $J$= 16.0 Hz, H-8), 6.71 (1H, d, $J$= 2.0 Hz, H-2′), 6.56 (1H, d, $J$= 8.4 Hz, H-5′), 6.66 (1H, dd, $J$= 2.0, 8.4 Hz, H-6′), 2.97 (2H, m, H-7′), 5.19 (1H, dd, $J$= 7.3, 5.4 Hz, H-8′), 3.68 (3H, s, $OCH_3$). $^{13}$CNMR (100 MHz, $CD_3OD$) $\delta$: 127.7 s (C-1), 114.3 d (C-2), 146.8 s (C-3), 149.8 s (C-4), 116.6 d (C-5), 123.2 d (C-6), 147.9 d (C-7), 116.4 d (C-8), 169.3 s (C-9), 128.9 s (C-1′), 115.8 d (C-2′), 145.4 s (C-3′), 146.2 s (C-4′), 117.6 d (C-5′), 121.9 d (C-6′), 37.9 t (C-7′), 74.7 d (C-8′), 172.2 s (C-9′), 52.9 q ($OCH_3$).

Compound XII (cirsimaritin): $C_{17}H_{14}O_6$, yellow crystal. EI-MS (70 eV) $m/z$ (%): 314 [$M^+$] (100), 299 (94), 285 (60), 271 (65), 254 (21), 239 (8), 208 (3), 200 (14), 181 (52), 167 (13), 153 (74), 119 (47), 69 (75). $^1$HNMR (400 MHz, $C_5D_5N$) $\delta$: 6.79 (1H, s, H-3), 13.63 (1H, brs, 5-OH), 3.88 (3H, s, 6-$OCH_3$), 6.93 (1H, s, H-8), 7.94 (2H, d, $J$= 8.4 Hz, H-2′ and H-6′), 7.27 (2H, d, $J$= 8.4 Hz, H-3′ and H-5′). $^{13}$CNMR (100 MHz, $C_5D_5N$) $\delta$: 164.6 s (C-2), 103.4 d (C-3), 182.8 s (C-4), 153.4 s (C-5), 132.9 s (C-6), 159.0 s (C-7), 91.3 d (C-8), 153.2 s (C-9), 106.1 s (C-10), 122.0 s (C-1′), 128.6 d (2C, C-2′ and C-6′), 162.5 s (C-4′), 116.8 d (2C, C-3′ and C-5′), 56.1 q (6-$OCH_3$), 60.3 q (7-$OCH_3$).

Compound XIII (8-hydroxycirsimaritin): $C_{17}H_{14}O_7$, orange crystal. EI-MS (70 eV) $m/z$ (%): 330 [$M^+$] (95), 329 (16), 315 (100), 297 (36), 287 (8), 212 (2), 197 (58), 169 (45), 119 (38), 118 [$B_1^+$] (54), 77 (4). $^1$HNMR (500 MHz, DMSO-$d_6$) $\delta$: 6.80 (1H, s, H-3), 12.43 (1H, s, 5-OH), 3.81 (3H, s, 6-$OCH_3$), 3.92 (3H, s, 7-$OCH_3$), 9.26 (1H, brs, 8-OH), 10.39 (1H, brs, 4′-OH), 8.00 (2H, dd, $J$= 2.0, 8.3 Hz, H-2′ and H-6′), 6.93 (2H, dd, $J$= 2.0, 8.3 Hz, H-3′ and H-5′). $^{13}$CNMR (125 MHz, DMSO-$d_6$) $\delta$: 164.2 s (C-2), 102.5 d (C-3), 182.8 s (C-4), 144.7 s (C-5), 136.2 s (C-6), 148.0 s (C-7), 130.6 s (C-8), 141.4 s (C-9), 106.4 s (C-10), 121.2 s (C-1′), 128.8 d (2C, C-2′ and C-6′), 161.4 s (C-4′), 116.0 d (2C, C-3′ and C-5′), 60.5 q (6-$OCH_3$), 61.2 q (7-$OCH_3$).

Compound XIV (ursolic acid): $C_{30}H_{48}O_3$, white powder. EI-MS (70 eV) $m/z$ (%): 456 [$M^+$] (10), 438 (3), 410 (5), 395 (2), 300 (5), 248 (100), 233 (10), 219 (15), 203 (75), 189 (31), 175 (23), 147 (24), 133 (62), 119 (46), 105 (40), 95 (41), 81 (44), 69 (55), 55 (61).

Compound XV (2α-hydroxyursolic acid): $C_{30}H_{48}O_3$, white powder. EI-MS (70 eV) $m/z$ (%): 472 [$M^+$] (4), 454 (2), 426 (11), 408 (3), 248 (100), 223 (7), 203 (43), 189 (17), 145 (11), 133 (26), 119 (22), 105 (21), 81 (18), 69 (27), 55 (35).

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