SUPPLEMENTARY MATERIAL

Title: A new anthraquinone and eight constituents from *Hedyotis caudatifolia* Merr. et Metcalf: isolation, purification and structural identification

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Abstract: *Hedyotis caudatifolia* Merr. et Metcalf. (HC), a folk medicine in Yao nationalities areas in China, was used to investigate the chemical constituents. Through silica gel and Sephadex LH-20 column chromatography, nine compounds were isolated and purified. By physical and chemical properties, IR, MS (EI-MS, high resolution EI-MS), 1D-NMR (\textsuperscript{1}H-NMR, \textsuperscript{13}C-NMR) and 2D-NMR (HSQC, \textsuperscript{1}H-\textsuperscript{1}H COSY, HMBC), their structures were identified as \(\beta\)-sitosterol (1), stigmasterol (2), scopolin (3), 2-hydroxy-1,7,8-trimethoxyanthracene-9,10-dione (4), oleanolic acid (5), ursolic acid (6), methy barbinervate (7), \(\beta\)-daucosterol (8), p-Hydroxybenzoic acid (9). These compounds were isolated from HC for the first time, and 4 is a new anthraquinone whose biological activities is worth to be investigated in future. These compounds may contribution to the HC’s pharmacological effects on treating diseases, and may be used as candidates for control index in establishing the quality control standard of HC.

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List of NMR data and chemico-physical data of the compounds

β-sitosterol (1): White needle crystal, m.p. 137–138°C, soluble in chloroform or benzene and insoluble in water or methanol.

\(^1\)H-NMR (400MHz, CDCl\(_3\)) \(\delta\): 3.52 (1H, m, H-3), 2.23–2.31 (2H, m, H-4), 5.35 (1H, m, H-6), 0.67–0.69 (3H, m, H-18), 0.78–2.00 (42H, m). \(^{13}\)C-NMR and DEPT (100MHz, CDCl\(_3\)) \(\delta\): 37.2 (C-1, CH\(_2\)), 31.6 (C-2, CH\(_2\)), 71.8 (C-3, CH), 42.3 (C-4, CH\(_2\)), 140.7 (C-5), 121.7 (C-6, CH), 31.9 (C-7, CH\(_2\)), 31.9 (C-8, CH), 50.1 (C-9, CH), 36.5 (C-10, C), 21.0 (C-11, CH\(_3\)), 39.7 (C-12, CH\(_2\)), 42.2 (C-13, C), 56.8 (C-14, CH), 24.3 (C-15, CH\(_2\)), 28.2 (C-16, CH\(_2\)), 56.7 (C-17, CH), 11.8 (C-18, CH\(_3\)), 19.4 (C-19, CH\(_3\)), 36.1 (C-20, CH), 18.9 (C-21, CH\(_3\)), 33.9 (C-22, CH\(_2\)), 26.0 (C-23, CH\(_2\)), 45.8 (C-24, CH), 29.1 (C-25, CH), 19.8 (C-26, CH), 19.0 (C-27, CH\(_3\)), 23.0 (C-28, CH\(_2\)), 12.0 (C-29, CH\(_3\)).

Stigmasterol (2): White flake crystal, m.p. 169–171°C, soluble in chloroform. IR \(v_{\text{max}}\) (cm\(^{-1}\), KBr, w-weak, m-middle, s-strong, br-broad): 3735(m), 3450(s, br), 3425(s, br) [v\(_{\text{O-H}}\); 1029(w) [v\(_{\text{C=H}}\); 1665(w), 1658(w), 1641(w); 1464(s), 1382(s), 1332(w, s, H, C, CH), 32.7 (C-3, C), 144.1 (C-9, CH), 36.9 (C-10, C), 21.4 (C-11, CH\(_2\)), 40.0 (C-12, CH\(_2\)), 42.5 (C-13, C), 56.9 (C-14, CH), 24.6 (C-15, CH\(_2\)), 28.6 (C-16, CH\(_2\)), 56.2 (C-17, CH), 12.0 (C-18, CH\(_3\)), 19.2 (C-19, CH\(_3\)), 40.9 (C-20, CH), 19.7 (C-21, CH\(_3\)), 138.9 (C-22, CH), 129.5 (C-23, CH), 51.5 (C-24, CH), 32.3 (C-25, CH), 20.0 (C-26, CH\(_3\)), 19.1(C-27, CH\(_3\)), 25.8 (C-28, CH\(_2\)), 12.2 (C-29, CH\(_3\)).

Scopolin (3): Light yellow needle crystal, m.p. 203–204°C. IR \(v_{\text{max}}\) (cm\(^{-1}\), KBr): 3830(w), 3650(w), 3339(s, br) [v\(_{\text{O-H}}\); 3008(w) [v\(_{\text{C=O}}\); 2948(w) [v\(_{\text{C-H}}\); 2485(s, br), 2346(w); 1704(s, br)] [v\(_{\text{C=O}}\); 1627(m), 1607(s), 1565(s), 1510(s), 1454(m) [v\(_{\text{C-C}}\]; 1435(m), 1375(m), 1290(s), 1263(s), 1219(m), 1190(m), 1163(w), 1141(s), 1099(m), 1019(s), 923(s), 857(s), 821(m), 746(m), 592(s). Ei-MS m/z (%):192(M\(^{+}\),100),177,164,149,121,69. \(^1\)H-NMR (400MHz, C\(_6\)D\(_6\)N) \(\delta\): 6.29 (1H, d, J=9.4Hz, H-3), 7.67 (1H, d, J=9.4Hz, H-4), 7.10 (1H, s, H-5), 7.02 (1H, s, H-8), 3.75 (3H, s, OCH\(_3\)). \(^{13}\)C-NMR and DEPT (100MHz, C\(_6\)D\(_6\)N) \(\delta\): 161.5 (C-2), 112.3 (C-3), 144.1 (C-4), 109.4 (C-5), 151.0 (C-6), 153.0 (C-7), 104.1 (C-8), 146.2 (C-9), 111.1 (C-10), 56.1 (OCH\(_3\)).

2-hydroxy-1,7,8-trimethoxyanthracene-9,10-dione (4): Light green featherlike crystal, m.p. 237–239°C, soluble in chloroform. IR \(v_{\text{max}}\) (cm\(^{-1}\), KBr, Figure S2): 3740(w), 3425(s, br) [v\(_{\text{O-H}}\); 2925(s, br), 2852(w) [v\(_{\text{C-H}}\); 1844(w); 1772(w), 1734(w) [v\(_{\text{C=O}}\); 1666(s), 1573(s), 1521(w), 1507(w), 1480(m), 1456(m) [v\(_{\text{C=O}}\); 1412(m), 1381(s), 1274(s), 1075(m), 1036(m), 978(m), 938(m), 840(w), 801(m), 741(m). Ei-MS m/z (%) (Figure S1):314(M\(^{+}\),100),299,281,253, 239, 225.
HR-EI-MS m/z (Figure S1): 314.0791. $^1$H-NMR (600MHz, CDCl$_3$, Figure S3) $\delta$: 7.25~7.27 (2H, d, J=12.0 Hz, H-3), 7.99~8.01 (2H, d, J=12.0 Hz, H-4), 8.03~8.05 (2H, d, J=12.0 Hz, H-5), 7.18~7.20 (2H, d, J=12.0 Hz, H-6), $\delta$H 3.95 (3H, s, H-11), 3.92 (3H, s, H-12), 3.93 (3H, s, H-13), 6.54 (br, OH). $^{13}$C-NMR and DEPT (150MHz, CDCl$_3$, Figure S4) $\delta$: 144.8 (C-1, C), 153.8 (C-2, C), 119.1 (C-3, CH), 124.5 (C-4, CH), 128.1 (C-4a , C), 124.1 (C-5, CH), 114.8 (C-6, CH), 157.7 (C-7, C), 148.0 (C-8, C), 125.4 (C-8a, C), 180.8 (C-9, C), 124.2 (C-9a, C), 180.9 (C-10, C), 127.2 (C-10a, C), 61.3 (C-11, OCH$_3$), 55.3 (C-12, OCH$_3$), 60.3 (C-13, OCH$_3$).

Oleanolic acid (5): White solid, m.p. 308~310°C, soluble in mixture solution of chloroform-methanol. IR $\nu_{\text{max}}$ (cm$^{-1}$, KBr): 3855(w), 3822(w), 3676(w), 3651(w), 3428(s, br) [v$_{\text{OH}}$]; 2942(s, br), 2876(s, br) [v$_{\text{CH}}$]; 2653(w), 2346(w), 1697(s, br), 1560(w), 1541(w), 1508(w), 1463(s), 1387(s), 1365(m), 1321(w), 1304(m), 1268(m), 1206(w), 1183(m), 1163(w), 1138(w), 1107(w), 1094(w), 1030(s), 1009(w), 996(m), 974(w), 950(w), 916(w), 884, 826(m), 816(w), 758(m), 680(w), 655, 642(w), 601(w), 565(m), 532(m). EI-MS m/z(%):456(M$^+$),438, 423, 410, 395, 300, 287, 257, 248(100), 233, 219, 203,189,175, 161, 147, 133,119, 95,81,69, 55. $^1$H-NMR (400MHz, Cd$_2$D$_2$N) $\delta$: 3.43 (1H, m, H-3), 5.49 (1H, brs, H-12), 3.31 (1H, m, H-18), 1.24(3H, s, H-23), 1.02(3H, s, H-24), 0.88(3H, s, H-25),1.02(3H, s, H-26), 1.28(3H, s, H-27), 0.94(3H, s, H-29), 1.00(3H, s, H-30). $^{13}$C-NMR and DEPT (100MHz, Cd$_2$D$_2$N) $\delta$: 39.0 (C-1, CH$_2$), 28.2 (C-2, CH$_3$), 78.1 (C-3, CH), 39.5 (C-4, C), 55.9 (C-5, CH), 18.9 (C-6, CH$_2$), 33.3 (C-7, CH$_2$), 39.8 (C-8, C), 48.2 (C-9, CH), 37.4 (C-10, C), 23.8(C-11, CH$_2$), 122.6 (C-12, CH), 144.9 (C-13, C), 42.1(C-14, C), 28.4 (C-15, CH$_2$), 23.9 (C-16, CH$_2$), 46.5 (C-17, C), 42.2 (C-18, CH), 46.7 (C-19, CH$_2$), 31.0 (C-20, C), 34.3 (C-21, CH$_2$), 33.3 (C-22, CH$_2$), 28.9 (C-23, CH$_3$), 15.6 (C-24, CH$_3$), 16.6 (C-25, CH$_3$), 17.5 (C-26, CH$_3$), 26.3 (C-27, CH$_3$), 180.3(C-28, COOH), 33.4 (C-29, CH$_3$), 23.8 (C-30, CH$_3$).

Ursolic acid (6): White solid, m.p. 274~276°C, soluble in mixture solution of chloroform-methanol. IR $\nu_{\text{max}}$ (cm$^{-1}$, KBr): 3739(w), 3425(s, br) [v$_{\text{OH}}$]; 2966(s, br), 2926(s, br), 2871(s, br) [v$_{\text{CH}}$]; 1703(s, br), 1692(s, br), 1635(w), 1554(m), 1454(s), 1388(m), 1378(m), 1311(m), 1274(m), 1237(m), 1187(w), 1140(w), 1092(w), 1029(s), 997(m), 974(w), 828(w), 807(w), 761(m), 662(m), 532(m). EI-MS m/z: 457(M+1),458,439,411,396,248(100),249,207,203,133,119. $^1$H-NMR (400MHz, Cd$_2$D$_2$N) $\delta$: 3.45 (1H, dd, J=9.5,6.3Hz, H-3),5.49 (1H, d, J=3.2Hz , H-12),2.63 (1H, d, J=11.2Hz , H-18),2.32 (2H, m, H-15),2.11 (2H, m, H-16),1.22 (3H, s, H-23),1.02 (3H, s, H-24),1.04(3H, s, H-25),0.87(3H, s, H-26),1.24 (3H, s, H-27),0.94 (3H, s, J=6.1Hz, H-29),0.99 (3H, s, J=6.4Hz, H-30). $^{13}$C-NMR and DEPT (100MHz, Cd$_2$D$_2$N) $\delta$: 39.1 (C-1, CH$_2$), 28.1 (C-2, CH$_2$), 78.1 (C-3, CH), 39.4 (C-4, C), 55.8 (C-5, CH), 18.8 (C-6, CH$_2$), 33.6 (C-7, CH$_2$), 40.0 (C-8, C), 48.1(C-9, CH), 37.5 (C-10, C), 23.6(C-11, CH$_2$), 125.7 (C-12, CH), 139.3 (C-13, C), 42.5(C-14, C), 28.7 (C-15, CH$_2$), 24.9 (C-16, CH$_2$), 48.1 (C-17, C), 53.6 (C-18, CH), 39.5 (C-19, CH), 39.4(C-20, CH), 31.1 (C-21, CH$_2$), 37.3 (C-22, CH$_2$), 28.8 (C-23, CH$_3$), 16.6 (C-24, CH$_3$), 15.7 (C-25, CH$_3$), 17.5 (C-26, CH$_3$), 23.9 (C-27, CH$_3$), 179.9 (C-28, COOH), 17.6 (C-29, CH$_3$), 21.4 (C-30, CH$_3$).
Methy barbinervate (7): Colorless needle crystal, m.p. 321~322°C. IR νmax (cm⁻¹, KBr): 3855(w), 3822(w), 3752(w), 3568(s, br), 3448(s, br) [vO-H]; 2970(s, br), 2935(s, br), 2878(s, br) [νC-H]; 2346(w), 1689(s, br), 1560(w), 1542(w), 1508(w), 1459(s), 1379(s), 1271(m), 1235(m), 1157(m), 1091(w), 1068(m), 1027(m), 1004(m), 957(w), 931(m), 910(w), 865(m), 804(w), 766(m), 690(w), 653(m), 601(m), 534(m). El-MS m/z(%): 471,443,370,264,246,231,218,206(100),187,175,146,119. ¹H-NMR (400MHz, CD₃D₂N) δ: 4.44 (1H, brs, H-3), 5.60 (1H, brs, H-12), 3.03 (1H, s, H-18), 1.61(3H, s, H-23), 1.01(3H, s, H-25), 1.09(3H, s, H-26), 1.69(3H, s, H-27), 1.64(3H, s, H-29), 1.11(3H, s, H-30), 3.59(3H, s, OCH3). ¹³C-NMR and DEPT (100MHz, CD₃D₂N) δ: 34.0 (C-1, CH₂), 26.5 (C-2, CH₂), 70.0 (C-3, CH), 43.9 (C-4, C), 50.2 (C-5, CH), 19.2 (C-6, CH₂), 34.0 (C-7, CH₂), 40.6 (C-8, C), 47.8 (C-9, CH), 37.5 (C-10, C), 24.2 (C-11, CH₂), 128.1 (C-12, CH), 139.9 (C-13, C), 42.1(C-14, C), 29.3 (C-15, CH₂), 26.4 (C-16, CH₂), 48.3 (C-17, C), 54.6 (C-18, CH), 72.7 (C-19, C), 42.4(C-20, CH), 27.0 (C-21, CH₂), 38.5 (C-22, CH₂), 23.6 (C-23, CH₃), 65.7 (C-24, CH₂), 16.0 (C-25, CH₃), 17.1 (C-26, CH₃), 24.6 (C-27, CH₃), 180.7 (C-28, COOR), 27.1(C-29, CH₃), 16.8 (C-30, CH₃), 49.7 (OCH₃).

β-daucosterol (8): White amorphous powder, m.p. 286~287°C. positive reaction in Liebermann-Burchard detection or Molish detection. IR νmax (cm⁻¹, KBr): 3739(w), 3571(s), 3451(s, br), 3405(s, br) [vO-H]; 2959(s, br), 2934(s, br), 2869(s, br) [vC-H]; 1659(w), 1641(w), 1631(w), 1463(s), 1436(w), 1419(w), 1379(s), 1367(s), 1254(w), 1197(w), 1164(m), 1105(s), 1074(s), 1025(s), 960(w), 800(m), 620(w), 601(w). El-MS m/z(%):396(100),383,255,161,135. ¹H-NMR (400MHz, CD₃D₂N) δ: 3.96 (1H, m, H-3), 2.70(1H, m, H-4a), 2.46(1H, m, H-4b), 5.33 (1H, m, H-6), 0.65 (3H, m, H-18), 0.84~2.20 (42H, m), 5.04~5.06 (1H, d, J=7.6Hz, H-1'), 4.06~4.07 (1H, m, H-2'), 4.28~4.30 (2H, m, H-3', 4'), 3.97 (1H, m, H-5'), 4.55~4.58 (1H, m, H-6a'), 4.39~4.43 (1H, m, H-6b'). ¹³C-NMR and DEPT (100MHz, CD₃D₂N) δ: 37.5 (C-1, CH₂), 30.3 (C-2, CH₂), 78.5 (C-3, CH), 40.0 (C-4, CH₂), 140.9 (C-5), 122.0 (C-6, CH), 32.2 (C-7, CH₂), 32.1 (C-8, CH), 50.4 (C-9, CH), 36.9 (C-10, C), 21.4 (C-11, CH₂), 39.4 (C-12, CH₂), 42.5 (C-13, C), 56.8 (C-14, CH), 24.5 (C-15, CH₂), 28.6 (C-16, CH₂), 56.3 (C-17, CH), 12.0 (C-18, CH₃), 19.5 (C-19, CH₃), 36.4 (C-20, CH), 19.0 (C-21, CH₃), 34.2 (C-22, CH₂), 26.4 (C-23, CH₂), 46.1 (C-24, CH), 29.5 (C-25, CH), 19.2 (C-26, CH₃), 20.0 (C-27, CH₃), 23.4 (C-28, CH₂), 12.2 (C-29, CH₃), 102.6(C-1', CH), 75.4 (C-2', CH), 78.6 (C-3', CH), 71.7 (C-4', CH), 78.1 (C-5', CH), 62.8 (C-6', CH₂).

p-Hydroxybenzoic acid (9): White solid, m.p. 217~219°C, soluble in methanol. El-MS m/z(%):138(M⁺). IR νmax (cm⁻¹, KBr): 3854(w), 3394(s, br) [vO-H]; 3199(w) [v s-c-H]; 2964(s, br), 2835(s, br) [νC-H]; 2669(s, br), 2519(s, br), 2226(w), 2055(m), 1925(m), 1890(w), 1794(w), 1750(w); 1685(s, br), 1608(s, br), 1509(s), 1448(s) [νC=C]; 1425(s), 1364(s), 1318(s), 1292(s), 1242(s), 1170(s), 1128(m), 1103(m), 1063(w), 1046(w), 1014(s), 931(s), 855(s), 770(s), 641(m), 639(s), 619(s), 548(s), 504(s). ¹H-NMR (400MHz, CD₃OD) δ: 5.34 (2H, d, J=12.0Hz, H-3,5), 6.40 (2H, d, J=8.0Hz, H-2,6). ¹³C-NMR and DEPT (100MHz, CD₃OD) δ: 122.7 (C-1), 116.0 (C-2,6), 133.0 (C-3, 5), 163.3 (C-4), 170.1 (COOH).
Table S1. 1D-NMR data and 2D-NMR correlations of compound 4 (in CDCl₃ at 600/150 MHz to TMS)

| Position(carbon)* | δ_c  | DEPT | δ_H** | ¹H-¹H COSY | HMBC*** (proton) |
|-------------------|------|------|--------|------------|------------------|
| 1                 | 144.8| C    |        |            |                  |
| 2                 | 153.8| C    |        |            |                  |
| 3                 | 119.1| CH   | 7.25–7.27 (d, J=12.0 Hz) | 4 |
| 4                 | 124.5| CH   | 7.99–8.01 (d, J=12.0 Hz) | 3 |
| 4a                | 128.1| C    |        |            |                  |
| 5                 | 124.1| CH   | 8.03–8.05 (d, J=12.0 Hz) | 6 |
| 6                 | 114.8| CH   | 7.18–7.20 (d, J=12.0 Hz) | 5 |
| 7                 | 157.7| C    |        |            | 5, (6), 13       |
| 8                 | 148.0| C    |        |            | 6, 12            |
| 8a                | 125.4| C    |        |            | 5                |
| 9                 | 180.8| C    |        |            |                  |
| 9a                | 124.2| C    |        |            | 4                |
| 10                | 180.9| C    |        |            | 5, 4             |
| 10a               | 127.2| C    |        |            | 6                |
| 11                | 61.3 | CH₃  | 3.95(s) |            |                  |
| 12                | 55.3 | CH₃  | 3.92(s) |            |                  |
| 13                | 60.3 | CH₃  | 3.93(s) |            |                  |

*Serial numbers see Fig 1; ** Assign ¹H to ¹³C by HSQC; *** Two-bond correlations were shown in brackets.
Elemental Composition Report

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -10.0, max = 120.0
Selected filters: None

Monoisotopic Mass, Odd and Even Electron Ions
14 formula(e) evaluated with 1 results within limits (up to 51 closest results for each mass)
Elements Used:
C: 0.200  H: 0.400  O: 5.7

Mass  Calc. Mass  mDa  PPM  DBE  i-FIT  Formula
314.0791  314.0790  0.1  0.3  11.0  5546337.5  C17 H14 O6

Figure S1  EI-MS and HREI-MS spectra of compound 4
Figure S2  IR spectra of compound 4
Figure S3 $^1$H-NMR spectra (400MHz or 600MHz) of compound 4
Figure S4 $^{13}$C-NMR and DEPT spectra (100MHz or 150MHz) of compound 4
Figure S5 $^1\text{H}^1\text{H}$ COSY spectra of compound 4
Figure S6 HSQC spectra of compound 4
Figure S7  HMBC spectra of compound 4