行政院國家科學委員會專題研究計劃成果報告

去甲烏藥鹼之代謝研究

Metabolism Study of Metabolism

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一、中文摘要:

口服消旋性去甲烏藥鹼(HG)，尿液經純化得到八個結晶型代謝物，以 LC/MS 與 2D NMR 判定結構，其代謝物之非糖體光學決定，用酸水解後經對掌管柱與光學活性 HG 標準品比對。八個代謝物結構分別為 *S*-(-)-HG-6,7-*O*-β-D-diglucuronide 、 *S*-(-)-HG-13-*O*-β-D-glucuronide 、 *R*-(+)-HG-7-*O*-β-D-glucuronide 、 *R*-(+)-HG-13-*O*-β-D-glucuronide 、 *S*-(-)-HG-6-*O*-β-D-glucuronide 、 *R*-(+)-HG-6-*O*-β-D-glucuronide 、 *S*-(-)-HG-7-*O*-β-D-glucuronide 及 *S*-(-)-HG-7-*O*-sulfate。由尿液發現(±)-HG 主要代謝以共軛為主，且以單尿甘酸共軛物佔 85 %；右旋 HG 主要共軛於 C-6-OH，而左旋 HG 主要共軛於 C-7-OH。而 *R*-(+)/*S*-(-)之代謝物比率在 C-6-及 C-7-OH，分別為 3:1 及 1:10。去甲烏藥鹼於尿甘酸共軛之位置及光學異構選擇性具有明顯差異

關鍵字：

去甲烏藥鹼，家兔，尿液代謝物分離及確認，尿甘酸共軛反應，代謝光學選擇性。

Abstract:

Eight urinary crystalline metabolites were isolated after oral administration. These metabolites were characterized by the LC/MS, and 2D NMR. After acid hydrolysis, the configuration of each HG metabolite was determined by using a chiral column and comparing with the optically active HG authentic samples. Eight metabolites were characterized as *S*-(-)-HG-6,7-*O*-β-*D*-diglucuronide, *S*-(-)-HG-13-*O*-β-D-glucuronide, *R*-(+)-HG-7-*O*-β-D-glucuronide, *R*-(+)-HG-13-*O*-β-D-glucuronide, *S*-(-)-HG-6-*O*-β-D-glucuronide, *R*-(+)-HG-6-*O*-β-D-glucuronide, *S*-(-)-HG-7-*O*-β-D-glucuronide and *S*-(-)-HG-7-*O*-sulfate. Major glucuronidation occurred at the C-6 and C-7 -OH about 40 % and 45 %, respectively. A great difference in stereoselective glucuronidation between the HG enantiomers was found. The ratios of *R*-(+) / *S*-(-) isomer of HG conjugation at the C-6 and C-7-OH were about 30 and 1, respectively. HG was showed a regioselective and enantioselective metabolism in rabbit.

Keywords :

Higenamine，rabbits，isolation ，identification ，urine metabolites，glucuronic acid conjugation，regioselectivity ， stereoselectivity

二、前言:

去甲烏藥鹼(higenamine；HG)為烏頭、附子之強心成份(1~3)，其他許多生藥亦含有此成分存在(4)。其主要作用機轉係直接與交感神經受体結合所致。本研究室曾完成其於動物之藥物動力學及生体可用率的探討(5,6)。另以老鼠進行急性毒性試驗(7)，發現口服之毒性遠比靜脈注射為低。其可能原因為去甲烏藥鹼於腸胃道或肝臟較易代謝。初步發現口服比靜脈注射極易形成尿甘酸共軛。今次計劃擬以家兔為實驗對象，進行尿液 HG 代謝之分離及確認，因為 HG 可進行尿甘酸共軛之官能基有三個 OH 基及一個 NH-基，是否具有位置選擇性？另本身為消旋化合物，共軛理應有立体選擇性亦可一併探討之。

三、實驗方法與步驟:

去甲烏藥鹼投藥實驗

取雄性家兔二隻，各口服 1.2 克之 HG.HCl 溶液，於 30 分鐘內分兩次以胃管導入胃

中，並同時以膀胱導尿方式收集尿液24小時並予以酸化以防代謝物分解。並置於零度冰箱存放。

尿液代謝物之純化及分離

尿液(300 mL)於 40 C 以下減壓濃縮，加水溶解過濾後，以 MCI(100g)之層析管柱分離，並以 100 ml 之 50%丙酮溶液與水沖洗至中性為止，最後以 20%甲醇水溶液沖提，前面 200 mL 為 Fraction A 而後 600 mL 為 Fraction B。此二部分各別濃縮至乾為止並加入 40mL 水。Fraction B 即有 M_7(189 mG)之結晶析出。而 Fraction A 之水溶液直接以製備型之高壓液相層析儀分離；層析管柱為 C18 Bondapak (19 x 300 mm, 5 um)；移動相為氰甲烷與水(5%:95%)溶液；流速為 17 mL/min。經分析液相層析確認純度。共得 M_1 (48 mG)，M_2 (30 mG)，M3 (15.4 mG)，M_4 (8.2 mG)，M_5 (116 mG)，M_6 (188 mG)，M_7 (266 mG) 及 M_8 (90.1 mG)。

S-(-)-(13-Hydroxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline-6,7-O-β-D-diglucuronopyranoside (M_1).

m.p.215~218 ℃(dec.) (CH_3CN : H_2O)；$[\alpha]_D^{26}$ -55.14 °(c = 0.4125, H_2O)；UV λ_{max} nm (log ε) (MeOH): 225.2 (4.08), 279.4 (3.43), UV λ_{min} nm (log ε) (MeOH): 249.9 (2.81)；^{1}H NMR (300 MHz, DMSO-d_6): δ 2.72 (2H, br s, H-4), 3.25 (6H, br s, H-2',4',5',2'',4'',5''), 3.40 (1H, d, J = 9.3 Hz, H-3'), 3.47 (1H, d, J = 8.7 Hz, H-5'), 4.23 (1H, t, H-1), 4.65 (1H, d, J = 6.6 Hz, anomeric H-1'), 4.85 (1H, d, J = 6.2 Hz, anomeric H-1''), 6.69 (2H, d, J = 8.3 Hz, H-12, 14), 6.83 (1H, s, H-8), 6.90 (1H, s, H-5), 7.02 (2H, d, J = 8.2 Hz, H-11, 15)；^{13}C NMR (75 MHz, DMSO-d_6) δ 25.85 (t, C-4), 38.75 (t, C-9), 39.32 (t, C-3), 55.42 (d, C-1), 71.74 (d, C-4', C-4''), 73.30 (d, C-2', C- 2''), 74.12, 74.29 (d, C-3' or C-3''), 76.06 (d, C-5', C-5''), 101.80 (d, C-1''), 101.96 (d, C-1'), 115.27 (d, C-12, 14), 116.76 (d, C-8), 119.09 (d, C-5), 127.05 (s, C-4a), 129.20 (s, C-8a), 130.46 (d, C-10, C-11, 15), 145.67 (s, C-7), 146.08 (s, C-6), 156.11 (s, C-13), 172.05 (s, C-6', C-6'')；IR (KBr) cm^{-1}: 3400 (OH), 2200-2700 (>NH_2^+), 1611 (>C=O), 1061 (OH, s, bending)；ESMS (neg.) m/z (%): 644 [(M+Na)-2H]$^-$ (70.6), 622 [M-H]$^-$ (84.5), 446 [M-$C_6H_9O_6$- H]$^-$ (100.0), 270 (33.6), 175 (42.7), 162 (65.1)；HRFABMS (neg.) m/z for $C_{28}H_{33}O_{15}NH$: Calcd. 622.1772；Found: 622.1817。

R-(+)-(13-Hydroxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline-13-O-β-D-glucuronopyranoside (M_2).

m.p. 218~220℃(dec.) (CH_3CN : H_2O)；$[\alpha]_D^{26}$ -72.69 °(c = 0.214, H_2O)；UV λ_{max} nm (log ε) (MeOH) : 222.0 (4.23), 286.7 (3.61), UV λ_{min} nm (log ε) (MeOH): 252.7 (2.96)；^{1}H NMR (300 MHz, DMSO-d_6): δ 2.67 (2H, br s, , H-4), 2.85-2.99 (2H, br m, H-3), 3.05-3.18 (2H, br m, H-9), 3.58 (1H, d, J = 9.0 Hz, H-3'), 4.27 (1H, br t, H-1), 4.87 (1H, d, J = 6.8 Hz, anomeric H-1'), 6.49 (1H, s, H-5), 6.57 (1H, s, H-8), 6.96 (2H, d, J = 8.2 Hz, H-12, 15), 7.19 (2H, d, J = 8.2 Hz, H-12, 14)；^{13}C NMR (75 MHz, DMSO-d_6): δ 25.26 (t, C-4), 39.27 (t, C-3, C-9), 55.23 (d, C-1), 71.74 (d, C-4'), 72.97 (d, C-2'), 74.45 (d, C-3'), 76.32 (d, C-5'), 100.09 (d, C-1'), 113.41 (d, C-8), 115.25 (d, C-5), 116.12 (d, C-12, 14), 123.18 (s, C-4a), 124.75 (s, C-8a), 130.28 (s, C-10), 130.28 (d, C-11, 15), 143.76 (s, C-7), 144.59 (s, C-6), 156.07 (s, C-13), 171.83 (s, C-6')；IR (KBr) cm^{-1}: 3289 (OH)；ESMS (neg.) m/z (%):446 [M-H]$^-$ (100.0), 270 (5.7), 162 (11.6)；HRFABMS (neg.) m/z: for $C_{22}H_{25}O_9NH$: Calcd. 446.1451；Found: 446.1483。

R-(+)-(13-Hydroxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline-7-O-β-D-glucuronopyranoside (M_3).

m.p.225~227 ℃(dec.) (CH_3CN : H_2O)；$[\alpha]_D^{26}$ -29.55 °(c = 0.288, MeOH)；UV λ_{max} nm (log ε) (MeOH): 226.8 (4.22), 280.0 (3.67), UV λ_{min} nm (log ε) (MeOH): 250.9 (3.00)；^{1}H NMR (300 MHz, DMSO-d_6): δ 2.70 (2H, br s, H-4), 2.79-2.99 (2H, br m, H-3), 3.05-3.17 (2H, br m,

H-9), 3.55 (1H, d, J = 9.1 Hz, H-3'), 4.22 (1H, br t, H-1), 4.63 (1H, d, J = 7.0 Hz, anomeric H-1'), 6.57 (1H, s, H-5), 6.68 (2H, d, J = 8.2 Hz, H-11, 15), 6.99 (1H, s, H-8), 7.03 (2H, d, J = 8.2 Hz, H-12, 14) ; ^{13}C NMR (75 MHz, DMSO-d_6) δ 25.92 (t, C-4), 39.09 (t, C-9), 39.28 (t, C-3), 55.46 (d, C-1), 71.77 (d, C-4'), 73.09 (d, C-2'), 74.55 (d, C-3'), 75.79 (d, C-5'), 102.58 (d, C-1'), 115.74 (d, C-5), 116.30 (d, C-8), 115.17 (d, C-12, 14), 125.35 (s, C-8a), 127.05 (s, C-10), 128.26 (s, C-4a), 130.30 (d, C-11, 15), 143.33 (s, C-7), 146.32 (s, C-6), 156.00 (s, C-13), 171.40 (s, C-6') ; IR (KBr): 3376 (OH, s, br), 2200-2700 (>NH_2^+), 1613 (>C=O, s, br), 1063 (OH, s, bending) cm^{-1} ; ESMS (neg.) m/z (%): 468 [M+Na-2H]$^-$ (13.8), 446 [M-H]$^-$ (100.0), 270 (32.2), 175 (21.3), 162 (23.7), 134 (29.4), 107 (33.9) ; HRFABMS (neg.) m/z : for $C_{22}H_{25}O_9NH$: Calcd. 446.1451 ; Found: 446.1435 ◦

S-(-)-(13-Hydroxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline-13-O-β-D-glucuronopyranoside (M_4).

m.p. 218~219℃(dec.) (CH_3CN : H_2O) ; $[\alpha]_D^{26}$ +72.27 °(c = 0.210, H_2O) ; UV λ_{max} nm (log ε) (MeOH): 222.2 (4.21), 285.2 (3.65), UV λ_{min} nm (log ε) (MeOH): 254.5 (3.26) ; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.72 (2H, br m, H-4), 2.99 (2H, br m, H-3), 3.05-3.20 (2H, br m, H-9), 3.57 (1H, d, J = 8.9 Hz, H-3'), 4.29 (1H, br t, H-1), 4.88 (1H, d, J = 6.8 Hz, anomeric H-1'), 6.50 (1H, s, H-5), 6.52 (1H, s, H-8), 6.97 (2H, d, J = 7.8 Hz, H-12, 14), 7.17 (2H, d, J = 7.9 Hz, H-11, 15) ; ^{13}C NMR (75 MHz, DMSO-d_6) δ 25.46 (t, C-4), 39.15 (t, C-9), 39.30 (t, C-3), 55.32 (d, C-1), 71.80 (d, C-4'), 73.00 (d, C-2'), 74.28 (d, C-3'), 76.37 (d, C-5'), 100.13 (d, C-1'), 113.46 (d, C-8), 115.26 (d, C-5), 116.13 (d, C-12, 14), 123.24 (s, C-4a), 124.74 (s, C-8a), 130.26 (s, C-10), 130.26 (d, C-11, 15), 143.67 (s, C-7), 144.51 (s, C-6), 156.06 (s, C-13), 171.88 (s, C-6'); IR (KBr) cm^{-1}: 3405 (OH, s, br), 2200-2700 (>NH_2^+), 1611 (>C=O, s, br), 1067 (OH, s, bending) ; ESMS (neg.) m/z (%): 468 [M+Na-2H]$^-$ (5.3), 446 [M-H]$^-$ (100.0), 270 (12.2), 175 (9.5), 162 (35.5) ; HRFABMS (neg.) m/z for $C_{22}H_{25}O_9NH$: Calcd. 446.1451 ; Found: 446.1430 ◦

S-(-)-(13-Hydroxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline-6-O-β-D-glucuronopyranoside (M_5).

m.p. 223~225℃(dec.) (CH_3CN : H_2O) ; $[\alpha]_D^{25}$ -76.53 °(c = 0.3075, MeOH) ; UV λ_{max} nm (log ε) (MeOH): 226.3 (4.16), 281.8 (3.64), UV λ_{min} nm (log ε) (MeOH): 249.4 (2.61) ; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.71 (2H, br s, H-4), 2.84-3.05 (2H, br m, H-3), 3.05-3.20 (2H, br m, H-9), 3.57 (1H, d, J = 8.6 Hz, H-3'), 4.32 (1H, br t, H-1), 4.70 (1H, d, J = 5.8 Hz, anomeric H-1'), 6.57 (1H, s, H-8), 6.71 (2H, d, J = 8.4Hz, H-12, 14), 6.87 (1H, s, H-5), 7.08 (2H, d, J = 8.3 Hz, H-11, 15) ; ^{13}C NMR (75 MHz, DMSO-d_6) δ 25.53 (t, C-4), 38.96 (t, C-9), 39.29 (t, C-3), 55.39 (d, C-1), 71.82 (d, C-4'), 73.02 (d, C-2'), 74.43 (d, C-3'), 75.66 (d, C-5'), 102.12 (d, C-1'), 113.95 (d, C-5), 115.24 (d, C-12, 14), 117.17 (d, C-8), 123.37 (s, C-4a), 126.88 (s, C-1'), 128.72 (s, C-8a), 130.33 (d, C-12, 15), 144.21 (s, C-6), 145.37 (s, C-7), 156.10 (s, C-13), 171.83 (s, C-6'); IR (KBr) cm^{-1}: 3384 (OH, s, br), 2200-2700 (>NH_2^+), 1613 (>C=O, s, br), 1064 (OH, s, bending); ESMS (neg.) m/z (%): 468 [M+Na -2H]$^-$ (6.5), 446 [M-H]$^-$ (100.0), 270 (18.9), (175, 13.5), 162 (12.5), 107 (77.5) ; HRFABMS (neg.) m/z for $C_{22}H_{25}O_9N$-H: Calcd. 446.1451 ; Found: 446.1452 ◦

R-(+)-(13-Hydroxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline-6-O-β-D-glucuronopyranoside (M_6).

m.p. 211~213℃(dec.) (CH_3CN : H_2O) ; $[\alpha]_D^{25}$ -51.87 °(c = 0.3205, MeOH) ; UV λ_{max} nm (log ε) (MeOH): 227.0 (4.22), 281.9 (3.72), UV λ_{min} nm (log ε) (MeOH): 250.3 (2.90) ; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.67 (2H, br m, H-4), 2.82-

3.02 (2H, br m, H-3), 3.02-3.25 (2H, br m, H-9), 3.56 (1H, d, J = 8.6 Hz, H-3'), 4.30 (1H, t, br, H-1), 4.70 (1H, d, J = 6.6 Hz, anomeric H-1'), 6.50 (1H, s, H-8), 6.70 (2H, d, J = 8.2 Hz, H-12,14), 6.84 (1H, s, H-5), 7.07 (2H, d, J = 8.2 Hz, H-11, 15) ; ^{13}C NMR (75 MHz, DMSO-d_6) δ 25.31 (t, C-4), 38.18 (t, C-3), 38.96 (t, C-9), 55.01 (d, C-1), 71.90 (d, C-4'), 73.04 (d, C-2'), 74.25 (d, C-3'), 75.72 (d, C-5'), 101.91 (d, C-1'), 114.04 (d, C-8), 115.29 (d, C-12, 14,), 116.51 (d, C-5), 123.15 (s, C-4a), 126.60 (s, C-10), 127.96 (s, C-8a), 130.48 (d, C-11, 15), 144.36 (s, C-6), 145.12 (s, C-7), 156.20 (s, C-13), 172.40 (s, C-6');IR (KBr) cm^{-1}: 3382 (OH, s, br), 2200-2700 (>NH_2^+), 1614 (>C=O, s, br), 1063 (OH, s, bending) ; ESMS (neg.) m/z (%): 468 [M+Na -2H]$^-$ (5.6), 446 [M-H]$^-$ (100.0), 270 (25.2), 175 (14.5), 162 (15.0) ; HRFABMS (neg.) m/z for $C_{22}H_{25}O_9NH$: Calcd. 446.1451 ; Found: 446.1447, ◦

S-(-)-(13-Hydroxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline-7-O-β-D-glucuronopyranoside (M_7).

m.p. 225~227℃(dec.) (CH_3CN : H_2O) ; $[\alpha]_D^{26}$ -25.14 °(c = 0.2705, MeOH) ; UV λ_{max} nm (log ε) (MeOH): 227.3 (4.21), 281.0 (3.66), UV λ_{min} nm (log ε) (MeOH): 250.7 (2.89) ; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.74 (2H, m, H-4), 2.99 (2H, br s, H-9), 3.04-3.17 (2H, br m, H-3), 3.41 (1H, d, J = 9.48 Hz, H-3'), 4.33 (1H, br t, H-1), 4.33 (1H, d, J = 6.5 Hz, anomeric H-1'), 6.56 (1H, s, H-5), 6.61 (1H, s, H-8), 6.65 (2H, d, J = 8.1 Hz, H-12, 14), 6.89 (2H, d, J = 8.0 Hz, H-11, 15);^{13}C NMR (75 MHz, DMSO-d_6) : δ 24.93 (t, C-4), 38.12 (t, C-9), 38.94 (t, C-3), 55.02 (d, C-1), 71.80 (d, C-4'), 72.90 (d, C-2'), 73.92 (d, C-3'), 75.50 (d, C-5'), 102.02 (d, C-1'), 115.19 (d, C-12, 14、 d, C-5、 d, C-8), 123.60 (s, C-8a), 126.34 (s, C-10), 126.49 (s, C-4a), 130.60 (d, C-11, 15), 143.30 (s, C-7), 145.84 (s, C-6), 156.19 (s, C-13), 172.55 (s, C-6') ; IR (KBr) cm^{-1}: 3432, 3280 (OH, s, br), 2200-2700 (>NH_2^+), 1598 (>C=O, s, br), 1065 (OH, s, bending) ; ESMS (neg.) m/z (%): 468 [M+Na -2H]$^-$ (6.4), 446 [M-H]$^-$ (100.0), 270 (13.5), 175 (8.1), 162 (16.3) ; HRFABMS (neg.) m/z for $C_{22}H_{25}O_9NH$: Calcd. 446.1451 ; Found: 446.1445 ◦

S-(-)-(13-Hydroxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline-7-O-sulfate (M_8).

m.p. 254~256 ℃(CH_3CN : H_2O) ; $[\alpha]_D^{26}$ -117.75 ° (c = 0.220, MeOH-H_2O,1:1) ; UV λ_{max} nm (log ε) (MeOH) : 226.0 (4.20), 279.4 (3.61), UV λ_{min} nm (log ε) (MeOH) : 249.2 (2.73) ; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.79 (2H, m, H-4), 2.87-3.13 (2H, br m, H-3), 3.13-3.30 (2H, m, H-9), 4.39 (1H, br t, H-1), 6.64 (1H, s, H-5), 6.72 (2H, d, J = 8.2 Hz, H-12, 14), 6.97 (1H, s, H-8), 7.10 (2H, d, J = 8.5 Hz, H-11, 15);^{13}C NMR (75 MHz, DMSO-d_6) δ 25.37 (t, C-4), 38.95 (t, C-3, C-9), 55.37 (d, C-1), 115.28 (d, C-12, 14), 116.77 (d, C-5), 121.20 (d, C-8), 124.69 (s, C-8a), 126.38 (s, C-10), 129.25 (s, C-4a), 130.43 (d, C-11, 15), 139.36 (d, C-7), 148.34 (d ,C-6), 156.14 (s, C-13) ; IR (KBr) cm-1: 3354 (OH, s, br), 2200-2700 (-$SO_3^-NH_2^+$), 1590, 1519 (aromatic -C=C-, s), 1063 (OH, s, bending) ; ESMS (neg.) m/z (%): 350 [M-H]$^-$ (18.8), 270 (18.8), 162 (100), 135 (17.4);HRFABMS m/z for $C_{16}H_{17}O_6NS$+H: Calcd. 352.0855 ; Found: 352.0858 ◦

Methylation of HG metabolite (M_7)

取 2.0 g 氫氧化鉀(KOH)及 10 g Diazald 新鮮備製成 diazomethane 乙醚溶液(呈黃色液體)◦取 25 mg (0.056 mmole)代謝物 M_7 溶於 15 mL 甲醇，置於 50 mL 之梨形瓶中，滴加新鮮備製之 diazomethane 至呈黃色，於室溫下反應 24 小時，減壓濃縮至乾，得約 15 mg 白色結晶之 M_{7a} ; ESMS (neg.) m/z (%): 548 [M+2Na -H]$^-$ (8.4), 502 [M-H]$^-$ (3.3), 488 (6.4), 470 (3.3), 312 (16.8), 267 (9.2), 265 (100), 135 (4.9) ; IR (KBr) cm $^{-1}$:

3384 (OH, s, br), 2853 (OCH_3, w), 1748 (ester, >C=O, s), 1513 (aromatic -C=C-, s), 1274, 1228 (SO_3, s), 1049 (OH, s, bending); ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.39 (3H, s, N-CH_3), 2.56 - 2.76 (2H, br s, H-4), 2.94 - 3.10 (2H, br s, H-9), 3.51 - 3.58 (2H, m, H-3), 3.66 (1H, d, J = 9.5 Hz, H-3'), 3.70 (6H, s, C-13, C-6' 2 X OCH3), 3.72 (3H, s, C-6 OCH_3), 4.59 (1H, d, J = 7.2 Hz, anomeric H-1'), 6.35 (1H, s, H-8), 6.64 (1H, s, H-5), 6.78 (2H, d, J = 8.4 Hz, H-12, 14), 7.00 (2H, d, J = 8.3 Hz, H-11, 15); ^{13}C NMR (75 MHz, DMSO-d6) : δ 25.17 (C-4), 38.94 (C-9), 42.23 (N-CH_3), 46.64 (C-3), 54.72 (C-6, C-6' 2 X OCH_3), 55.56 (C-13-OCH_3), 64.09 (C-1), 71.08 (C-4'), 72.70 (C-2'), 74.93 (C-3'), 75.77 (C-5'), 100.11 (C-1'), 112.40 (C-8), 113.13 (C-12, 14), 114.61 (C-8), 127.82 (C-4a), 129.24 (C-8a), 131.55 (C-10), 130.40 (C-11, 15), 143.50 (C-7), 147.01 (C-6), 157.26 (C-13) 168.87 (C-6').

Methylation of HG metabolite (M_8)

取約 25 mg 之 M_8 (0.071 mmole)，操作條件如前得 15 mg 白色結晶 M_{8a}; ESMS (neg.) m/z (%): 452 $[M+2Na-H]^-$ (3.3), 406 $[M-H]^-$ (30.0), 392 (7.9), 378 (9.1), 265 (100); IR (KBr) cm^{-1}: 2852 (OCH_3, m), 1612, 1514 (aromatic -C=C-, s), 1270, 1248 (SO_3, s); ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.92 (2H, dd, J=7.5, 7.5, H-4), 3.08 (6H, s, N-CH_3, C-7-OSO_2OCH_3), 3.53 (2H, dd, J=3.7, 3.5, H-3), 3.60-3.70 (2H, m, H-9), 3.73 (3H, s, C-13-OCH_3), 3.74 (3H, s, C-6-OCH_3), 4.76 (1H, br t, H-1), 6.84 (1H, s, H-5), 6.84 (2H, d, J = 8.4 Hz, H-12, 14), 6.93 (1H, s, H-8), 7.07 (2H, d, J = 8.3 Hz, H-11, 15); ^{13}C NMR (75 MHz, DMSO-d_6) δ 22.92 (C-4), 36.71 (C-9), 50.26 (N-CH_3), 50.41 (C-3), 54.48 (C-7-OSO_2-OCH_3), 54.84 (C-13-OCH_3), 55.42 (C-6-OCH_3), 71.15 (C-1), 113.85 (C-12, 14), 111.97 (C-5), 120.60 (C-8), 122.43 (C-8a), 127.74 (C-10), 123.86 (C-4a), 130.26 (C-11, 15), 140.82 (C-7), 150.69 (C-6), 157.96 (C-13)

Higenamine 代謝物之水解反應

(1) β-Glucuronidase 酵素水解 glucuronide conjugates

配製β-Glucuronidase (Helix pomatia,含 6000 Units/mL β-glucuronidase 及 252 Units/mL sulfatase; Bovine liver,含 6000 Units/mL β-glucuronidase 之酵素)，於 pH 值為 5.0 之 0.5 M 檸檬酸鈉緩衝液中，HG 代謝物及稀釋後之尿液檢體置於內徑 10 mm、長 160 mm 之拋棄式硼矽玻璃管內，加入 0.5 mL 此濃度酵素，浸在 37℃水浴中並振搖之，24 小時取出，加入 0.4 mL 4 M 過氯酸除蛋白，於 6000 g 離心，上清液打入 HPLC-PDA。

(2) 以酸水解 glucuronide conjugates

取收集 24 小時之尿液 0.1 mL，置於內徑 10 mm、長 160 mm 之拋棄式硼矽玻璃管內，加入 0.1 M 過氯酸 (pH = 1.12) 0.5 mL，浸於 100℃沸騰水浴中 6 小時，並隨時振搖及補充水。

(3) 代謝物非糖體(aglycone)立體光學之決定

取純化代謝物約 0.3 mg，水解方式同酸水解方法，以移動相稀釋後，打入 50 uL 至光學活性層析管柱(chiral column)分析，與左旋、右旋及消旋體之去甲烏藥鹼標準品的 retention time 作比對判定。Chiral column 分析條件：HPLC 幫浦：Waters delta prep 4000 preparative HPLC 層析管柱：E. Merck ChiraDex ® (β-cyclodextran)，(4.0 mm X 250 mm，5 um)。移動相：氯甲烷與 0.125 M 磷酸二氫鈉以 1：99 之比例混合，以濃磷酸調 pH 值至 3.5。流速：每分鐘 0.5 mL；管柱溫度：室溫。

四、結果及討論:

八個結晶性之 HG 代謝物 M_1 ~ M_8 純品於 HPLC 圖譜滯留時間，分別依序為 4.84、6.70、7.05、7.39、8.54、11.17、14.65 及 22.69 min。八個代謝物 M_1 ~ M_8 經 β-glusulase (β-glucuronidase 及 sulfatase 混合物)水解後，生成單一波峰於 19 min 與標準品 HG 滯留時間一致，故 M_1 ~ M_8 為尿甘酸化合物或硫酸酯代謝物。再經專一性β-gucuronidase 水解試驗，於 19 min 有 HG 之波峰及 22 min

min 有 HG 之波峰及 22 min 波峰的 M_8 代謝物並未水解，故 M_8 應為硫酸共軛型代謝物。以上結果可得代謝物 M_1 ~ M_7 為 β-尿甘酸共軛物，而代謝物 M_8 為硫酸酯共軛物。

至於代謝物連結之尿甘酸或硫酸分子的個數及其 HG 之位置，必需運用 LC/MS、2D-NMR 及衍生化反應判定。FAB 或電灑法質譜儀測定分子量，八個代謝物之[M-H]$^-$分別為 622 (M_1)、446 (M_2 ~ M_7)、350 (M_8)，LC/MS 結果顯示 M_1 為雙取代之尿甘酸共軛物，M_2 - M_7 為單取代之尿甘酸共軛物，M_8 為單取代之硫酸共軛物。利用 2D NMR 之 HMBC 方式判定，因 ^{1}H 與 ^{13}C 相隔二至三個鍵有耦合(coupling)關係(^2J, ^3J)，而 HMBC 之等高線圖譜中以 $^3J_{HC}$ 較大、明顯。依 HMBC 之結果及 ^{1}H-^{13}C $^3J_{HC}$ 關係原則，可以斷定七個 HG 尿甘酸共軛物 (M_1-M_7)之尿甘酸鍵結之位置；但硫酸酯代謝(M_8)因- SO_3H 於 2D NMR 無訊號，無法判定其位置，故進行甲基衍生化反應，得到甲氧基化衍生物，甲基反應得到 *N*-methyl-6,13-dimethoxyhigenamine-7-*O*-methyl sulfate (M_{8a})，其化學構造經 LC/MS 、IR 圖譜、^{1}H NMR、HMBC 圖譜及 nOe 解析，確定甲基化於 catechol 為 C-6 之 OH，非 C-7 之 OH。^{1}H-^{13}C COSY 解析亦符合結構之判定，故硫酸酯基於 C-6 發生代謝共軛反應。各代謝物中所含非糖體 HG 分子，其光學活性則必藉 chiral column 來判定。消旋之 HG 與尿甘酸或硫酸酯於體內 conjugation 時，係經 UDPG 或 Sulfotransferase 酵素參與，可能會有 HG 對掌異構物選擇性(enantioselectivity)及配位選擇性(regioselectivity)。採用酵素水解或以酸水解代謝物，其水解物係以旋光或利用 HPLC chiral column 解析與標準品 *R*-(+)-HG、*S*-(-)-HG 作對比而定。*S*-(-)-HG 與 *R*-(+)-HG 標準品之滯留時間約於 14.8 與 15.8 分鐘，八個 M1~M8 代謝物水解之非糖體經與標準品比對，發現屬 *R*-(+)-HG 者為 M_2、M_3 及 M_6，而屬 *S*-(-)-HG 為 M_1、M_4、M_5、M_7 及 M_8。

由家兔尿液發現(±)-HG 之代謝係以共軛為主，其中單尿甘酸共軛物佔 85 %；右旋 HG 主要共軛於 C-6-OH，但左旋主要共軛於 C-7-OH。而且 *R*-(+)/*S*-(-)之代謝物比率在 C-6-及 C-7-OH 位置上，分別為 3:1 及 1:10。依實驗結果，去甲烏藥鹼於家兔体內之尿甘酸共軛位置及光學異構選擇性具有明顯差異性存在的。

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