行政院國家科學委員會補助專題研究計畫成果報告

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※ 雲實與黃蝴蝶莢果之鞣質及相關連化合物之研究 ※

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中文摘要

從雲實莢果之含水丙酮萃出物中，分離出 (+)-gallocatechin (**1**), (-)-epicatechin 3-*O*-gallate (**2**), procyanidin B-1 (**3**), procyanidine B-3 (**4**), prodelphinidine B-1 (**5**), gallocatechin-(4α→8)-catechin (**6**), 4-*O*-galloyl–(-)-shikimic acid (**9**), 5-*O*- galloyl-(-)-shikimic acid (**10**) 等化合物。另外，從黃蝴蝶之果實，純化得到化合物 **1**, (-)-epigallocatechin 3-*O*-gallate (**11**), (-)-epicatechin (**12**), 1,6-di-*O*-galloyl-β-D-glucose (**16**), 2,6-di-*O*-galloyl-D-glucose (**17**), 1,2,3,6-tetra-*O*-galloyl-β-D-glucose (**18**) 。這些化合物之結構是藉由光譜解析以及化學之方法而定。其中化合物 **7**, **8**, **13**, **14** 及 **15** 為新穎之成分。.

關鍵詞：雲實、黃蝴蝶、鞣質、豆科

Abstract

From the aqueous acetone extract of the fruit of *C. decapetala*: (+)-gallocatechin (**1**), (-)-epicatechin 3-*O*-gallate (**2**), procyanidin B-1 (**3**), procyanidine B-3 (**4**), prodelphinidine B-1 (**5**), gallocatechin- (4α→8)-catechin (**6**), 4-*O*-galloyl– (-)-shikimic acid (**9**), 5-*O*-galloyl-(-)-shikimic acid (**10**) were isolated. From the fruit of *C. pulcherrima*: **1**, (-)-epigallocatechin 3-*O*-gallate (**11**), (-)-epicatechin (**12**), 1,6-di-*O*-galloyl-β-D-glucose (**16**), 2,6-di-*O*-galloyl-D-glucose (**17**), 1,2,3,6-tetra-*O*-galloyl-β-D-glucose (**18**) were obtained. The structure of those compounds was determinate base on spectral analysis and chemical evidence. Compounds **7**, **8**, **13**, **14** and **15** were novel constituents.

Keywords: *Caesalpinia decapetala*, *Caesalpinia pulcherrima*, Tannin, Leguminosae

INTRODUCTION

Leguminosae, a great plant family, is widely distributed in the whole world and is rich in tannin substance. There are about 60 genera and 170 species in Taiwan, [1] and have been an important source of the economic and medicinal plants. *Caesalpinia decapetala* was traditionally taken as antitussives, expectorants and anthelmintics.[2] On the other hand, *Caesalpinia pulcherrima* have been used for botanical remedies.[3] According to the literatures, the rhizome contains cassane diterpenoids; the root contains caesalpin diterpenoids with anticarcinogenic effect;[4] the seeds are rich in galactomanna.[5] AS a consequence, a wide spectrum of biological activities has been reported for many condensed tannins[6] for example, silymarin for liver problems,[7] ginkgo flavone glycosides for circulatory disorders,[8] pycnogenol from pin tree for antioxidant.[9] This paper deals with the isolation and structure determination of novel condensed tannins of above-mentioned two plants.

RESULTS AND DISCUSSIONS

From the aqueous acetone extract of the fruit of *C. decapetala*: (+)-gallocatechin (**1**),[10] (-)-epicatechin 3-*O*-gallate (**2**),[10] procyanidin B-1 (**3**),[11] procyanidine B-3 (**4**),[11] prodelphinidine B-1 (**5**)[11], gallocatechin-(4α→8)-catechin (**6**),[12] 4-*O*-galloyl–

(-)-shikimic acid (**9**),[13] 5-*O*-galloyl-(-)-shikimic acid (**10**)[13] were isolated. From *C. pulcherrima*: **1**, (-)-epigallocatechin 3-*O*-gallate (**11**),[10] (-)-epicatechin (**12**),[10] 1,6-di-*O*-galloyl-β-D-glucose (**16**),[14] 2,6-di-*O*-galloyl-D-glucose (**17**),[15] 1,2,3,6-tetra-*O*-galloyl-β-D-glucose (**18**)[16] were obtained. The structure of those compound were determinate base on spectral analysis and chemical evidence. Compounds **7**, **8**, **13**, **14** and **15** were novel constituents.

EXPERIMENTAL

Plant material

The fresh pods of *Caesalpinia decapetala* (Roth.) Alston. and *Caesalpinia pulcherrima* Swartz. was collected in Chang-Jhu, Ping-Tung, Taiwan (Jul. 2000), and verified by Dr. Feng-Chi Ho (Taiwan Forestry Research Institute, Heng-Chun Branch, Ping-Tung, Taiwan).

Extraction and isolation

[I] The fresh pods of *C. decapetala* (6.5 kg) were extracted four times with 70 % aqueous acetone at room temp. The extract was concentrated under reduced pressure and the resulting brown precipitates were removed by filtration. The filtrated was concentrated and subjected to Sephadex LH-20 column chromatography with water containing increasing amounts of MeOH and finally with a mixture of H_2O-acetone (1:1) to give fraction 1 (16 g), fraction 2 (7 g). fraction 3 (10.6 g), fraction 4 (14 g). Fraction 1 was chromatographed over Sephadex LH-20, MCI-gel CHP-20P, Cosmosil C_{18}-OPN (H_2O-MeOH) (1:0-0:1) to yield 4-*O*-galloyl-(-)-shikimic acid (**9**) (29 mg). Repeated chromatography of fraction 2 on Sephadex LH-20, MIC-gel CHP-20P, Fuji-gel ODS-G3 (H_2O-MeOH, 1:0-0:1) to obtain 5-*O*-galloyl-(-)-shikimic acid (**10**) (32 mg). Fraction 3 was subjected to Sephdex LH-20, Fuji-ge ODS-G3, MCI-gel CHP-20P (H_2O-MeOH, 1:0-0:1) to yield (+)-gallocatechin (**1**) (851 mg), procyanidin B-1 (**3**) (101 mg), procyanidin B-3 (**4**) (95 mg), and prodelphinidin B-3 (**5**) (28 mg). Fraction 4 was applied to columns of Sephadex LH-20, MIC-gel CHP-20P, Cosomsil C_{18}-OPN, Fuji-gel ODS-G3 (H_2O-MeOH, 1:0-0:1) to give (-)-epicatechin 3-*O*-gallate (**2**) (38 mg), gallocatechin-(4α→8)-catechin (**6**) (63 mg), prodelphinidin B-3 3-*O*-gallate (**7**) (481 mg), and prodelphinidin B-6 3-O-gallate (**8**) (75 mg).

[II] The fresh pods of *C. pulcherrima* (2.1 kg) were extracted four times with 70 % aqueous acetone at room temp. The extract was concentrated under reduced pressure, and the resulting brown precipitates were removed by filtration. The filtrate was concentrated and subjected to Sephadex LH-20 column chromatography with H_2O containing increasing amounts of MeOH and finally with a mixture of H_2O:acetone (1:1) to give fraction 1 (10.2 g), fraction 2 (24.1 g), fraction 3 (16.3 g), and fraction 4 (14.6 g). Fraction 1 was chromatographed over MIC-gel CHP-20P, Sephadex LH-20 (H_2O-MeOH, 1:0-0:1) to yield 1,6-di-*O*- galloyl-β-D-glucose (**16**) (104 mg) and 2,6-di-*O*-galloyl-D-glucose (**17**) (932 mg). Purification of Fraction 2 by MCI-gel CHP-20P, Fujigel ODS-G3, Cosmosil C_{18}-OPN (H_2O-MeOH, 1:0-0:1) to give 1,2,3,4-tetra-*O*-galloyl-β-D-glucose (**18**) (81 mg), 3,4,5-tri-*O*-galloyl-(-)-shikimic acid (**15**) (131 mg), and (+)-gallocatechin (**1**) (581 mg). Fraction 3 repeatedly chromatographed over sephadex LH-20, Fuji-gel ODS-G3, Cosmosil C_{18}-OPN, MIC-gel CHP-20P (H_2O-MeOH, 1:0-0:1) to yield (-)-epigallocatechin 3-*O*-gallate (**11**) (1.3 g), and (-)-epicatechin (**12**) (108 mg). Fraction 4 was applied to columns of MCI-gel CHP-20P, Fuji-gel ODS-G3, Cosmosil C18 OPN, Sephadex LH-20 (H_2O-MeOH, 1:0-0:1) to yiel prodelphinidin B-3 3,3’-di-O-gallate (**13**) (338 mg), and prodelphinidin C-2 3,3’-di-O-gallate (**14**) (420 mg).

Prodelphinidin B-3 3-*O*-gallate: A tan amorphous powder, $[\alpha]_D$ –140.0° (c=1.0, acetone). Anal. Calcd for $C_{37}H_{30}O_{18} \cdot 7/2\ H_2O$: C, 53.82; H, 4.52. Found: C, 53.93; H, 4.38. negative FAB-MS: 76.1 $[M-H]^-$. ^{1}H-NMR (acetone-d_6+D_2O): δ 2.38-3.11 (2H, m, 4’-H), 4.21-4.82 (4H in total, m, 2, 4, 2’, 3’-H), 5.84-6.04 (4H, in total, m, 3, 6, 8, 6’-H), 6.26, 6.43, 6.53, 6.59 (4H in total, each s, 12, 16, 12’, 16’-H), 6.91, 7.00, 7.04 (2H in total, each s, galloyl H). ^{13}C-NMR (acetone-d_6+D_2O): δ 28.7 (C-4’), 67.8 68.5 (C-3’), 73.3 (C-3), 81.5, 81.9 (C-2’), 83.4 (C-2), 95.2, 95.7, 96.1, 96.7, 97.3 (C-6, 8, 6’) 100.5, 101.3, 101.6 (C-10, 10’), 105.7 (C-8’), 108.1 (galloyl C-2, 6), 105.7, 109.9,110.2 (C-12, 16, 12’, 16’), 121.5 (galloyl C-1), 129.7, 130.9 (C-11, 11’), 133.2, 134.4 (galloyl C-4), 158.6 (C-14, 14’), 145.6, 145.8 (C-13, 15, 13’, 14’, galloyl C-3, 5), 154.7, 155.0, 155.5, 156.6, 156.7, 157.7, 158.0 (C-5, 7, 9, 5’, 7’, 9’, 5”, 7”, 9”), 165.4, 165.9 (-COO-).

Enzymatic hydrolysis of **7** (prodelphinidin B-3 3-*O*-gallate): A solution of **7** (80 mg) in H_2O (4 ml) was incubated with tannase at 37° C for 4 hr. The reaction mixture as concentrated to dryness *in vacuo* and the residue was treated with MeOH. The MeOH soluble portion was purified by Sephadex LH-20 with 80% aqueous MeOH to furnish gallic acid (19.2 mg) and prodelphinidin B-3 (**5**) (41 mg), off-whit amorphous, $[\alpha]_D$ –143.5° (c=1.0, acetone). ^{1}H-NMR (acetone-d_6+D_2O): δ 2.58 (1H, dd, J=7, 15 Hz, 4’-H), 2.94 (1H, dd, J=5, 15 Hz, 4’-H), 4.25-4.67 (3H, m, 2, 4, 2’-H), 5.78-6.14 (3H in total, m, 6, 8,6’-H), 6.19, 6.41, 6.58, 6.62 (4H in total, each s, 12, 16, 12’, 16’-H).

Thiolytic degradation[17] of **7**: A mixture of **7** (312 mg), benzylmercaptan (3 ml) and acetic acid (1 ml) in EtOH (5 ml) was refluxed for 4 h. The solvent was evaporated off under reduced pressure, and the residue was subjected to Sephadex LH-20 chromatography with chlorofom-MeOH (4:1-1:1) to yield (+)-gallocatechin (81 mg), **7b** (62 mg) and **7c** (75 mg). 7b is an amorphous powder, $[\alpha]_D$ +74.7° (c=0.9, acetone), ^{1}H-NMR (acetone-d_6+D_2O):δ 3.87 (2H, s, -S-CH_2-), 4.62 (1H, d, J=4Hz, 4-H), 5.32 (1H, d, J=10 Hz, 2-H), 5.46 (1H, dd, J=4,10 Hz, 3-H), 5.89, 6.59 (each 1H, d, J=2 Hz, 6, 8-H), 6.59 (2H, s, 12, 16-H), 7.14 (2H, s, galloyl-H), 7.16-7.44 (5H, m, aromatic H). Anal. Calcd for $C_{29}H_{24}O_{11}S \cdot 5/2\ H_2O$: C, 57.91; H, 4.49, Found: C, 57.94; H, 4.33. Negtive FAB-MS m/z: 579 $[M-H]^-$. ^{1}H-NMR (acetone-d_6+D_2O): δ 3.79, 3.89 (each 1H, d, J=12 Hz, -S-CH_2-), 4.20 (1H, d, J=4 Hz, 4-H), 5.10 (1H, d, J=6 Hz, 2-H), 5.76 (1H, dd, J=4, 6 Hz, 3-H), 6.04, 6.12 (each 1H, d, J=2 Hz, 6, 8-H), 6.53 (2H, s, 12, 16-H), 7.25 (2H, S, galloyl H), 7.15-7.30 (5H, m, aromatic H). Desulfurization: **7c** (45 mg) in EtOH (8 ml) was treated at room temperature with an EtOH slurry of Raney-nikel (W-4), for 30 min. After filtration of the catalyst, the filtrate was concentrated under reduced pressure to dryness, and the residue was purified by Sephadex LH-20 with H_2O-MeOH to give (+)-galloylcatechin 3-*O*-gallate (**20**) (7.5 mg).

Prodelphinidin B-6 3-*O*-gallate (**8**):
A tan amorphous powder, $[\alpha]_D$ –118.4° (c=0.1, acetone), Anal. Calcd for $C_{37}H_{30}O_{18} \cdot 2H_2O$: C, 55.46; H, 4.29. Found: C, 55.17, H, 4.40. Negative FAB-MS m/z: 764 $[M-H]^-$. ^{1}H-NMR (acetone-d_6+D_2O): δ 2.40 (1H, dd, J=9, 16 Hz, 4'-H), 2.98 (1H, dd, J=6, 16 Hz, 4'-H), 4.36 (1H, d, J=8 Hz, 2'-H), 4.68 (1H, d, J=10 Hz, 4-H), 4.82 (1H, d, J=7 Hz, 2-H), 5.86-6.32 (4H, m, 3, 6, 8, 8'-H), 6.46. 6.62 (each 2H, s, 12, 16, 12', 16'-H), 6.97 (2H, s, galloyl H). ^{13}C-NMR (acetone-d_6+D_2O): δ 30.4 (C-4'), 35.7 (C-4), 68.1 (C-3'), 74.5 (C-3), 81.8 (C-2'), 82.4 (C-2), 96.1, 96.3, 97.3, 97.7 (C-6, 8, 8'), 107.8, 106.6 (C-10, 10'), 107.1, 107.6, 108.0 (C-12, 16, 12', 16'), 107.9 (C-6'), 110.0, 110.3, 110.5 (galloyl C-2, 6), 121.6 (galloyl C-1), 129.6, 130.8 (C-11, 11'), 133.4, 135.5 (C-14, 14'), 138.6 (galloyl C-4), 145.6 145.9, 146.0 (C-13, 15, 13', 15', galloyl C-3, 5), 154.5, 154.6, 155.2, 157.3, 158.1, 158.5 (C-5, 7, 9, 5', 7', 9'), 165.8 (-COO-).

Enzymatic hydrolysis of **8**: Hydrolysis of **8** (25 mg) by tannase as describes for previous process to give gallic acid (4 mg) and 8a (12 mg). **8a**: an amorphous powder, $[\alpha]_D$ –94.6° (c=1.0, acetone). ^{1}H-NMR (acetone-d_6 +D_2O): δ 2.58-3.13 (2H, m, 4'-H), 3.95 (1H, m, 3-H), 4.30-4.50 (3H, m, 2, 4, 2'-H), 5.83-6.25 (3H in total, m, 6, 8, 6'-H), 6.48, 6.57 (each 2H, 12, 16, 12', 16'-H).

Thiolytic degradation and desulfurization of **8**: **8** (21 mg) dissolved in EtOH (5 ml) and thyolysis with AcOH-benzylmercaptan to give **7b** (3-*O*-galloyl gallocatechin 4α-bezylthioether (3.5 mg), **7c** (3-O-galloyl gallocatechin 4β-bezylthioether (2.3 mg) and (+)-gallocatechin (6 mg). As usual process after desulfurization of **7c** to yield **20**.

Prodelphinidin B-3 3,3-di-*O*-gallate (**13**):
An amorphous powder, $[\alpha]_D$ –135.2° (c=0.9, acetone), Anal. Calcd for $C_{44}H_{32}O_{22} \cdot 2H_2O$: C, 56.66; H, 3.89. Found: C, 56.52, H, 4.04. Negative FAB-MS m/z: 913 $[M-H]^-$. ^{1}H-NMR (acetone-d_6+D_2O): δ 2.60-2.72 (2H, m, 4'-H), 4.54 (1H, d, J=10 Hz, 4-H), 4.84 (2H, d, J=8 Hz, 2, 2'-H), 5.17-5.37 (1H, m, 3'-H), 5.81-6.28 (4H in total, m, 3, 6, 8, 6'-H), 6.50, 6.53, 6.59, 6.66 (4H in total, each s, 12, 16, 12', 16'-H), 7.01, 7.07, 7.17 (4H in total, each s, 2 x galloyl H). ^{13}C-NMR (acetone-d_6+D_2O): δ 23.1 (C-4'), 36.2 (C-4), 67.2 (C-3'), 76.2 (C-3), 78.7 (C-2'), 81.8 (C-2), 95.9, 96.7, 97.8 (C-6, 8, 6'), 106.8 (C-8'), 107.0, 107.8, 110.0, 130.3 (C-11, 11'), 133.1, 133.4 (C-14, 14'), 138.7, 138.9 (galloyl C-4), 145.4, 146.3, 145.9 (C-13, 15, 13', 15', galloyl C-3, 5), 153.9, 154.6, 155.1, 155.4, 155.9, 156.8, 157.3, 158.1, 158.6 (C-5, 7, 9, 5', 7', 9'), 165.0, 166.0, 167.1 (-COO-).

Enzymatic hydrolysis of **13**: Hydrolysis of **13** (35 mg) with tannase as describes for previous process to obtain gallic acid (**8** mg) and prodelphinidin B-3 (14 mg).

Prodelphinidin C-2 3,3-di-*O*-gallate (**14**):
An amorphous powder, $[\alpha]_D$ –188.5° (c=1.0,

acetone), Anal. Calcd for $C_{59}H_{46}O_{29}$ · 8/3 H_2O: C, 55.93; H, 4.08. Found: C, 55.45, H, 4.22. Negative FAB-MS m/z: 1217 $[M-H]^-$. ^{1}H-NMR (acetone-d_6+D_2O): δ 2.31-3.16 (2H, m, 3”-H), 3.49-3.84 (1H, m, 3’-H), 4.31 (1H, d, J=9 Hz, 2”-H), 4.54-4.94 (4H in total, m, 2, 4, 2’, 4’-H), 5.58-6.12 (6H in total, m, 3, 6, 8, 3’, 6’, 6”-H), 6.29, 6.53, 6.67 (6H in total, each s, 12, 16, 12’,16, 12”, 16”-H), 6.85, 6.93, 7.00, 7.08 (4H in total, each s, galloyl H). ^{13}C-NMR (acetone-d_6+D_2O): δ 28.3 (C-4”), 36.1 (C-4. 4’), 68.7 (C-3”), 72.7 (C-3, 3’), 81.4, 81.7 (C-2’, 2”), 83.7 (C-2), 96.5, 97.6 (C-6, 8, 6’, 8’), 100.7 (C-10, 10’, 10”), 105.6, 106.3 (C-8’, 8”), 106.6, 107.1, 110.0, 110.3 (C-12, 16, 12’, 16’, 12”, 16”), 121.9 (galloyl C-1), 129.6, 130.0, 130.8 (C-11, 11’, 11”), 133.6, 133.9 (C-14, 14’, 14”), 138.2, 138.6 (galloyl C-4), 145.4, 145.8 (C-13, 15, 13’, 15’, 13”, 15”, galloyl C-3, 5), 155.4,155.9, 158.0 (C-5, 7, 9, 5’, 7’, 9’, 5”, 7”, 9”), 164.9 (-COO-).

Enzymatic hydrolysis of **14**: Hydrolysis of **14** (50 mg) with tannase as describes for previous process to give gallic acid (8 mg) and **14a** (27 mg). **14a**: $[\alpha]_D$ –189.5° (c=0.9, acetone), Anal. Calcd for $C_{45}H_{38}O_{21}$ · 6 H_2O: C, 52.84; H, 4.92. Found: C, 52.97, H, 4.71. Negative FAB-MS m/z: 913 $[M-H]^-$. ^{1}H-NMR (acetone-d_6+D_2O): δ 2.46-3.16 (2H, m, 4”-H), 4.16-4.91 (5H, m, 2, 4, 2’, 4’, 2”-H), 5.84-6.27 (4H in total, m, 6, 8, 6’, 6”-H), 6.56, 6.60, 6.63, 6.69 (6H in total, each s, 12, 16, 12’, 16’, 12”, 16”-H).

Partial thiolysis of **14**: A mixture of **14** (130 mg), benzylmercaptan (4 ml), acetic acid (2 ml) and EtOH (8 ml) was heated under reflux for 1 h. Work-up as described above gave partial degradation products, (+)-gallocatechin (9 mg), (+)-gallocatechin 4α-benzylthioether (**7b**) and **14b** (38 mg). **14b**: A tan amorphous powder, $[\alpha]_D$ –84.55° (c=0.9, acetone), Anal. Calcd for $C_{51}H_{40}O_{22}$ · 4 H_2O: C, 55.24; H, 4.36. Found: C, 55.56, H, 4.46. Negative FAB-MS m/z: 1035 $[M-H]^-$. ^{1}H-NMR (acetone-d_6+D_2O): δ 3.79 (2H, S, -S-CH_2-), 4.49-5.55 (5H, m, 2, 4, 2’, 3’, 4’-H), 5.81-6.11 (4H, m, 3-H, A-ring H), 6.35, 6.42, 6.59, 6.72 (4H, m, 12, 16, 12’, 16’-H), 6.92-7.14 (5H, m, aromatic H), 7.21 (4H, s, 2 x galloyl H).

Desulfurization of **14b**: **14b** (20 mg) dissolved in 20 % AcOH-EtOH (5 ml) then sterring with Raney nikel under room temperature for 2 h. Filtrated the Raney nikel, concentrated the filtrate, at TLC the spot of **13** was observed [Rf: 0.25, silica gel, bezene-HCOOEt-HCOOH (1:7:1)]

3,4,5-Tri-*O*-galloyl-(-)-shikimic acid (**15**):
An off-whit amorphous powder, $[\alpha]_D$ –223.1° (c=1.0, acetone), Anal. Calcd for $C_{28}H_{22}O_{17}$ · 2 H_2O: C, 50.45; H, 3.93. Found: C, 50.26, H, 4.17. Negative FAB-MS m/z: 621 $[M-H]^-$. ^{1}H-NMR (acetone-d_6+D_2O): δ 2.72 (1H, dd, J=5.6, 19.0 Hz, 2-H), 3.12 (1H, dd, J=5.1, 19.0 Hz, 2-H), 5.66 (1H, dd, J=3.8, 8.1 Hz, 4-H), 5.73 (1H, ddd, J=5.1, 5.6, 8.1 Hz, 3-H), 6.04 (1H, t, J=3.8 Hz, 5-H), 6.93 (1H, d, J=3.8 Hz, 6-H), 7.06, 7.12, 7.14 (each 2H, s, galloyl H).

Enzymatic hydrolysis of **15**: **15** (45 mg) hydrolysis with tannase uder room temperature, and purified by Sephadex LH-20 to give gallic acid (22 mg) and **15a** (4 mg). **15a**: whit amorphous powder, $[\alpha]_D$ –19.1 (c=0.9, MeOH), identified by co-TLC with authentic sample on silica gel [Rf 0.45, benzene-HCOOEt-HCOOH

(1:5:1.5)] and cellulose [Rf 0.60, n-BuOH-AcOH-H_2O (4:1:5)].

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