Supporting Information

Chemical Constituents and Antitubercular Activity of Formosan *Pisonia umbellifera*

Hsiou-Ting Kuo¹, Chien-Fang Peng², Hung-Yi Huang¹, Chu-Hung Lin³, Ih-Sheng Chen¹³, Ian-Lih Tsai¹³

Affiliation

¹ Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan, R.O.C.

² Department of Medical Laboratory Science and Biotechnology, College of Health Sciences, Kaohsiung Medical University, Kaohsiung, Taiwan, R.O.C.

³ School of Pharmacy, College of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan, R.O.C.

Correspondence

*Prof. Dr. Ian-Lih Tsai*

School of Pharmacy, College of Pharmacy

Kaohsiung Medical University

Kaohsiung, Taiwan 807

Republic of China

Tel.: +886/7/3121101 ext. 2664

Fax: +886/7/3210683

ialits@kmu.edu.tw
Supporting Information Available

Extraction and isolation----------------------------------------------- Page 3

Fig. 1S ^1^H-NMR spectrum of secopisonic acid (1)---------------------Page 6

Fig. 2S ^13^C-NMR spectrum of secopisonic acid (1)-------------------Page 7

Fig. 3S ^1^H-NMR spectrum of 6,8-dimethylisogenistein (2)-------------Page 8

Fig. 4S ^13^C-NMR spectrum of 6,8-dimethylisogenistein (2)-----------Page 9

Fig. 5S ^1^H-NMR spectrum of (+)-ent-ficusol (3)---------------------Page 10

Fig. 6S ^13^C-NMR spectrum of (+)-ent-ficusol (3)-------------------Page 11

Fig. 7S ^1^H-NMR spectrum of pisoninol I (4)------------------------Page 12

Fig. 8S ^13^C-NMR spectrum of pisoninol I (4)-----------------------Page 13

Fig. 9S ^1^H-NMR spectrum of pisoninol II (5)------------------------Page 14

Fig. 10S ^13^C-NMR spectrum of pisoninol II (5)---------------------Page 15

Fig. 11S ^1^H-NMR spectrum of pisoquinoline (6)---------------------Page 16

Fig. 12S ^13^C-NMR spectrum of pisoquinoline (6)-------------------Page 17

Fig. 13S ^1^H-NMR spectrum of pinsodienone (7)----------------------Page 18

Fig. 14S ^13^C-NMR spectrum of pinsodienone (7)-------------------Page 19
Extraction and isolation

Air-dried stem (15 kg) were extracted with cold MeOH to yield a MeOH extract (780 g), which was partitioned into \( n \)-hexane- (140 g), EtOAc- (18.9 g), \( n \)-BuOH- (70 g), and H\(_2\)O- (540 g) soluble layers. Both the \( n \)-hexane-soluble and EtOAc-soluble fractions tested active against *Mycobacterium tuberculosis* H37Rv. The active \( n \)-hexane-soluble layer (100 g) was subjected to silica gel (70-230 mesh, 22 × 72 cm, 2.4 kg) column chromatography (CC) eluted with \( n \)-hexane, gradually increasing the polarity with EtOAc to give 23 fractions (Frs. A1–A23). Fr-A8 (810 mg) was further purified by silica gel (70-230 mesh, 2 × 52 cm, 73 g) CC eluted with CH\(_2\)Cl\(_2\), gradually increasing the polarity with acetone to give 19 (6.3 mg). Fr-A13 (2 g) was subjected to silica gel (70-230 mesh, 1.5 × 50 cm, 35 g) CC eluted with \( n \)-hexane-EtOAc (20:1), gradually increasing the polarity with EtOAc and MeOH to give 10 fractions (Frs. A13-1–A13-10). Fr-A13-6 (216 mg) was purified by silica gel (70-230 mesh, 2 × 53 cm, 73 g) CC to furnish 15 (14.6 mg). The active Fr-A15 (2 g) was recrystallized with MeOH to obtain 2 (7.1 mg), and its mother liquid (535 mg) was repeatedly subjected to silica gel CC eluted with \( n \)-hexane-EtOAc, and then purified by preparative TLC (CHCl\(_3\)-acetone, 15:1) to obtain 16 (6.7 mg). The active Fr-A19 (160 mg) was subjected to silica gel (230-400 mesh, 1 × 30 cm, 5 g) CC eluted with \( n \)-hexane-EtOAc (50:1), gradually increasing the polarity with EtOAc to give 12 fractions (Frs. A19-1–A19-12). Fr-A19-11 (15 mg) was further purified by preparative TLC (\( n \)-hexane-EtOAc, 2:1) to afford 1 (6.7 mg). The active Fr-A22 (280 mg) was subjected to silica gel (70-230 mesh, 1.5 × 31 cm, 9 g) CC eluted with \( n \)-hexane-EtOAc (10:1), gradually increasing the polarity with EtOAc to give 7 fractions (Frs. A22-1–A22-7). Fr-A22-6 (13 mg) was further purified by preparative TLC (\( n \)-hexane-EtOAc, 1:1) to obtain 3 (1.4 mg).
The active EtOAc-soluble layer (18.9 g) was applied to silica gel (230-400 mesh, 5 × 71 cm, 567 g) CC eluted with CH₂Cl₂, gradually increasing the polarity with MeOH to give 13 fractions (Frs. B1–B13). Fr-B3 (1 g) was separated by MPLC on silica gel (RP-18, 2.0 x 30 cm, 55 g) column eluted with acetone-MeOH (1:50) to give 3 fractions (Frs. B3-1–B3-3). Fr-B3-1 (291 mg) was further purified by MPLC on a silica gel (RP-18, 1.5 x 30 cm, 30 g) column eluted with acetone-H₂O (5:1) to give 4 fractions (Frs. B3-1-1–B3-1-4). The crystalline Fr-B3-1-1 (52 mg) was recrystallized with MeOH to obtain 8 (2.1 mg), and the mother liquid (52 mg) was repeatedly purified by preparative TLC (n-hexane-EtOAc, 1:1) to yield 7 (1.0 mg), 10 (1.3 mg), and 22 (1.3 mg). Fr-B3-1-2 (63 mg) was subjected by MPLC on a silica gel (1.0 x 30 cm, 20 g) column eluted with n-hexane-EtOAc (3:1) to give 4 (2.0 mg), 12 (2.5 mg), 13 (1.0 mg), 17 (2.9 mg), and 18 (1.1 mg). Fr-B5 (538 mg) was separated by MPLC on a silica gel (RP-18, 2.0 x 30 cm, 55 g) column eluted with MeOH-H₂O (1:5) to give 5 fractions (Frs. B5-1–B5-5). Fr-B5-1 (21 mg) was further purified by preparative TLC (n-hexane-EtOAc, 1:1) to afford 9 (5.0 mg). Fr-B5-2 (24 mg) was purified by preparative TLC (n-hexane-EtOAc, 1:1) to obtain 11 (1.3 mg). Fr-B7 (597 mg) was applied to MPLC on a silica gel (RP-18, 2.0 x 30 cm, 55 g) column eluted with MeOH-H₂O (1:1) to give 20 (5 mg). Fr-B8 (500 mg) was applied to MPLC on a silica gel (RP-18, 2.0 x 30 cm, 55 g) column eluted with acetone-H₂O (1:3) to give 5 fractions (Frs. B8-1–B8-5). Fr-B8-1 (19 mg) was repeatedly purified by preparative TLC (n-hexane-EtOAc, 1:2) to give 14 (4.4 mg) and 5 (1.4 mg). Fr-B8-2 (16 mg) was purified by preparative RP-18 TLC (acetone-H₂O, 1:5) to afford 6 (2.0 mg). Fr-B8-4 (16 mg) was purified by preparative TLC (n-hexane-EtOAc, 1:1) to obtain 21 (12.5 mg).
**Pisodienone (7):** Colorless needles; mp 120-122°C. UV(MeOH) \( \nu_{\text{max}} \) (log\( \varepsilon \)): 239 (4.36), 294 (3.33) nm; IR (KBr) \( \nu_{\text{max}} \) 1659 (C=O), 1598 (C=C) cm\(^{-1}\); \(^1\)H-NMR (CDCl\(_3\), 400 MHz) \( \delta \) 3.28 (6H, s, OCH\(_3\)-4×2), 3.81 (6H, s, OCH\(_3\)-3, 5), 5.65 (2H, s, H-2, 6); \(^13\)C-NMR (CDCl\(_3\), 100 MHz) \( \delta \) 52.3 (OCH\(_3\)-4×2), 56.3 (OCH\(_3\)-3, 5), 104.5 (C-2, 6), 111.2 (C-4), 166.1 (C-3, 5), 186.2 (C=O); El MS (pos. ion mode) \( m/z \) (%) 214 [M]+ (10), 199 (100), 183 (78), 168 (40), 155 (38), 153 (25), 140 (35), 125 (50); HR ESIMS 237.0739 [M+Na]+ (calcd. for C\(_{10}\)H\(_{14}\)O\(_5\)Na: 237.0737).
Fig. 1S ¹H-NMR spectrum of secopisonic acid (1).
Fig. 2S $^{13}$C-NMR spectrum of secopisonic acid (1).

Fig. 3S $^1$H-NMR spectrum of 6,8-dimethylisogenistein (2).
Fig. 4S $^{13}$C-NMR spectrum of 6,8-dimethylisogenistein (2).
Fig. 5S ¹H-NMR spectrum of (+)-ent-ficusol (3).
Fig. 6S $^{13}$C-NMR spectrum of (+)-ent-ficusol (3).
Fig. 7S $^1$H-NMR spectrum of pisoninol I (4).
Fig. 8S $^{13}$C-NMR spectrum of pisoninol I (4).
Fig. 9S $^1$H-NMR spectrum of pisoninol II (5).
Fig. 10S $^{13}$C-NMR spectrum of pisoninol II (5).
Fig. 11S $^1$H-NMR spectrum of pisoquinoline (6).
Fig. 12S $^{13}$C-NMR spectrum of pisoquinoline (6).
Fig. 13S $^1$H-NMR spectrum of pinsodienone (7).
Fig. 14S $^{13}$C-NMR spectrum of pinsodienone (7).