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Piscicidal Constituents of *Stellera chamaejasme* L.

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Four piscicidal diterpenes have been isolated from the roots of *Stellera chamaejasme* L. (Thymelaeaceae). These compounds were identified as huratoxin, subtoxin A, simplexin and pimelea factor P_2.

Keywords——*Stellera chamaejasme*; Thymelaeaceae; piscicidal activity; daphnane type diterpene; huratoxin; subtoxin A; simplexin; pimelea factor P_2

Introduction

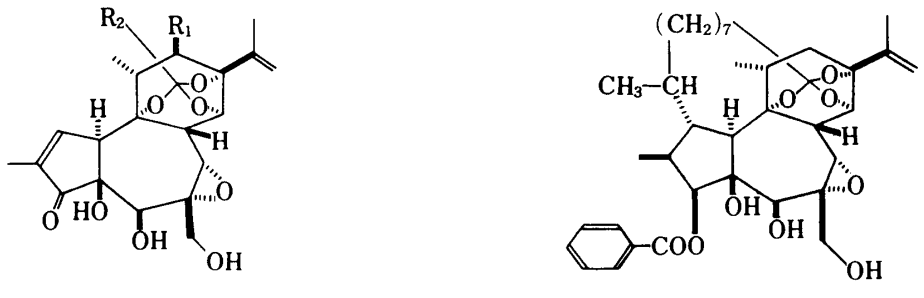
The roots of *Stellera chamaejasme* L. are known as "lang du" ("rou doku" in Japanese) in Chinese medicine.[1] Previous chemical studies on this drug resulted in the isolation of carbohydrates,[2] coumarins[3] and flavones.[4,5]

In the course of studies on the co-carcinogenic constituents of Euphorbiaceae and Thymelaeaceae plants, four piscicidal diterpenes have been isolated from the roots of *Stellera chamaejasme* L. This paper describes the isolation and identification of the four daphnane type diterpenes responsible for this activity.

Results and Discussion

The dried roots of the plant collected in Sichnan Province (China) were extracted with methanol at room temperature. The isolation of bioactive substances was monitored by testing for piscicidal activity on killifish ("himedaka" in Japanese),[6] because many diterpenes possessing co-carcinogenic activity also have piscicidal activity.[7]

The hexane-soluble fraction of the methanolic extract was roughly separated by column chromatography on silica gel (chloroform–methanol), and then rechromatographed on silica gel (chloroform–acetone). Two active fractions were further separated by a combination of preparative thin layer chromatography (TLC) and high performance liquid chromatography



pimelea factor P_2

huratoxin : $R_1=H$, $R_2=CH_3(CH_2)_8CH=CH-CH=CH-$
subtoxin A : $R_1=OAc$, $R_2=CH_3(CH_2)_8CH=CH-CH=CH-$
simplexin : $R_1=H$, $R_2=CH_3(CH_2)_8-$

Fig. 1

Table I. Piscicidal Activity to Killifish

Concentration (ppb)	Number of test fish surviving after 24 h			
	Huratoxin	Subtoxin A	Simplexin	Pimelea factor P_2
Control	5	5	5	5
5	0	0	0	0
3	1	2	0	0
1	5	5	1	0
0.5	5	5	5	1
0.4			5	5

(HPLC) to afford four daphnane type diterpenes (huratoxin,[8] subtoxin A,[9] simplexin[10] and pimelea factor P_2[9]) in 0.00133, 0.00043, 0.00035 and 0.00038% yields from the dried material, respectively. The identification of these isolated compounds was done by detailed comparison of the spectral data with the reported values.[8-10] The piscicidal activities of these diterpenes towards killifish are shown in Table I.

Further studies of the piscicidal fractions soluble in other solvents are in progress.

Experimental

Optical rotations were measured with a JASCO DIP-181 digital polarimeter using 99.5% C_2H_5OH as the solvent (cell length; 100 mm). Ultraviolet (UV) spectra were obtained on a JASCO UVIDEC-610 photometer using 99.5% C_2H_5OH as the solvent. Infrared (IR) spectra were recorded neat on a JASCO IR A-2 spectrophotometer. Nuclear magnetic resonance (NMR) spectra were taken on a JEOL FX-100 using $CDCl_3$ as the solvent and the chemical shifts are given in ppm relative to internal tetramethylsilane (TMS). Mass (MS) spectra were obtained on a JEOL JMS-D300 spectrometer operating at an ionization energy of 70 eV.

Column chromatography was carried out on Mallinckrodt silica gel 100 mesh and preparative TLC was carried out on Whatman precoated plates PLK 5F (1 mm, 20 × 20 cm). Preparative HPLC was performed with a JASCO TRIROTAR III [column; Megapak SIL C-18, 7.2 × 250 mm (Jasco) or Cosmosil C-18, 4.6 × 150 mm (Nakarai Chemicals)].

Isolation——The roots of the plant (418 g) were extracted with MeOH (2.5 l) for 3 weeks at room temperature. Evaporation of the MeOH under reduced pressure afforded a brownish residue (38 g), which was partitioned 3 times between hexane (150 ml) and 50% aq. MeOH (500 ml). The hexane phase was separated and concentrated to afford a residue (3.74 g) having piscicidal activity. The residue (3.6 g) was chromatographed on silica gel (60 g) and eluted successively with $CHCl_3$ (0.5 l), $CHCl_3$–MeOH (85: 15) (0.4 l) and $CHCl_3$–MeOH (50: 50) (0.4 l). The highest activity was found in the fraction (0.77 g) eluted with $CHCl_3$–MeOH (85: 15). This fraction (0.70 g) was rechromatographed on silica gel (30 g) using $CHCl_3$ containing an increasing ratio of acetone to give two active fractions.

One fraction (60 mg) eluted with $CHCl_3$–acetone (100: 1) was further separated by preparative TLC (silica gel, $CHCl_3$–MeOH (100: 1)) to afford crude subtoxin A (3.4 mg) and a mixture of two compounds (14 mg), and the mixture was separated by preparative HPLC (ODS, MeOH–H_2O (90: 10)) to give huratoxin (6.5 mg) and simplexin (2.1 mg).

The other more polar fraction (76 mg) eluted with $CHCl_3$–acetone (100: 3) was separated by preparative TLC and HPLC in the same manner as described above to give pimelea factor P_2 (2.1 mg).

Finally, huratoxin, subtoxin A, simplexin and pimelea factor P_2 were purified by preparative HPLC (ODS, CH_3CN–H_2O (75: 25)) to give yields of 5.6, 1.8, 1.5 and 1.6 mg, respectively.

Huratoxin: $C_{34}H_{48}O_8$: $[\alpha]_D$ +4° (c=0.12); MS m/z 584.3337 (Calcd 584.3347); UV λ_{max} 231 nm (ε 31700); IR ν 3430 br and 1685 cm^{-1}; NMR δ 0.87 (3H, t, J=6 Hz), 1.17 (3H, d, J=7 Hz), 1.80 (6H, near s), 2.94 (1H, d, J=3 Hz), 3.46 (1H, s), 3.82 (3H, t, J=2 Hz), 4.24 (1H, s), 4.44 (1H, d, J=3 Hz), 4.90 (1H, t, J=1 Hz), 5.02 (1H, s), 5.6—6.2 (3H, complex), 6.70 (1H, dd, J=10, 16 Hz) and 7.62 (1H, q, J=1 Hz).

Subtoxin A: $C_{36}H_{50}O_{10}$: $[\alpha]_D$ +39° (c=0.13); MS m/z 642.3389 (Calcd 642.3377); UV λ_{max} 232 nm (ε 23200); IR ν 3450 br, 1730 and 1685 cm^{-1}; NMR δ 0.88 (3H, t, J=6 Hz), 1.30 (3H, d, J=7 Hz), 1.80 (6H, m), 1.99 (3H, s), 2.38 (1H, q, J=7 Hz), 3.51 (1H, s), 3.52 (1H, d, J=3 Hz), 3.81 (3H, complex), 4.24 (1H, s), 4.76 (1H, d, J=3 Hz), 4.96 (1H, br s), 4.97 (1H, s), 4.99 (1H, near s), 5.5—6.2 (3H, complex), 6.68 (1H, dd, J=10, 16 Hz) and 7.56 (1H, q, J=1 Hz).

Simplexin: $C_{30}H_{44}O_8$: $[\alpha]_D$ +43° (c=0.51); MS m/z 532.3038 (Calcd 532.3036); UV λ_{max} 240 nm (ε 9000); IR ν 3440 br and 1685 cm^{-1}; NMR δ 0.86 (3H, t, J=6 Hz); 1.15 (3H, d, J=7 Hz), 1.77 (6H, br s), 2.90 (1H, d, $J=3$Hz), 3.43 (1H, s), 3.6—3.9 (3H, complex), 4.24 (1H, s), 4.36 (1H, d, $J=3$ Hz), 4.89 (1H, br s), 5.00 (1H, br s) and 7.60 (1H, q, $J=1$ Hz).

Pimelea Factor P_2: $C_{37}H_{50}O_9$: $[\alpha]_D$ +19° ($c=0.34$); MS m/z 638.3457 (Calcd 638.3455); UV λ_{max} 229 (ε 14200), 273 (ε 1240) and 282 nm (ε 1040); IR ν 3450 br, 1700 and 1600 cm^{-1}; NMR δ 0.83 (3H, d, $J=7$ Hz), 1.05 (3H, d, $J=7$ Hz), 1.44 (3H, d, $J=7$ Hz), 1.74 (3H, s), 2.89 (1H, d, $J=3$ Hz), 3.11 (1H, d, $J=11$ Hz), 3.35 (1H, s), 3.77 (1H, d, $J=14$ Hz), 3.91 (1H, d, $J=14$ Hz), 4.11 (1H, s), 4.28 (1H, d, $J=3$ Hz), 4.85 (1H, near s), 4.97 (1H, s), 5.08 (1H, d, $J=5$ Hz), 7.56 (3H, m) and 8.05 (2H, m).

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References

1) College of Jiang Su New Medicine ed., "Zhongyao Dacidian" (Chūyaku Daijiten in Japanese), Scientific and Technological Publishing House of Shanghai, China, 1977, p. 1898.
2) D. Ts. Tsybikova and E.B. Tykheeva, *Tr. Vost.-Sib. Tekhnol. Inst.*, 3, 22 (1970) [*Chem. Abstr.*, **77**, 79577*q* (1972)].
3) L.I. Tikhomirova, L.P. Markova, H. Tumbaa and G.A. Kuznetsova, *Khim. Prir. Soedin.*, **1974**, 402 [*Chem. Abstr.*, **81**, 117101*x* (1974)].
4) O.I. Rezanova and L.I. Bubeeva, *Rastit. Resur.*, **12**, 389 (1976) [*Chem. Abstr.*, **85**, 174253*e* (1976)].
5) W. Hwang and C. Chang, *K'o Hsueh T'ung Pao*, **24**, 24 (1979) [*Chem. Abstr.*, **90**, 135086*m* (1979)].
6) K. Sakata, K. Kawazu and T. Mitsui, *Agric. Biol. Chem.*, **35**, 1084 (1971).
7) H. Ohigashi, M. Hirota, A. Kobayashi, K. Koshimizu, H. Fujiki, M. Mori and T. Sugimura, The 23rd Symposium on the Chemistry of Natural Products (Nagoya, 1980), Symposium papers, p. 450.
8) K. Sakata, K. Kawazu and T. Mitsui, *Agric. Biol. Chem.*, **35**, 2113 (1971).
9) P.W. Freemann, E. Ritche and W.C. Taylar, *Aust. J. Chem.*, **32**, 2495 (1979).
10) S. Zayed, W. Adolf, A. Hafez and E. Hecker, *Tetrahedron Lett.*, **1977**, 3481.