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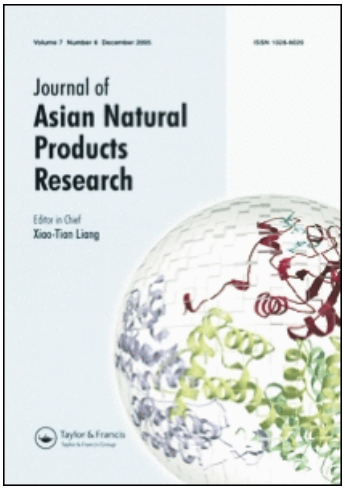

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A NEW BICOUMARIN FROM *STELLERA CHAMAEJASME* L.

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Thirteen compounds were isolated from roots of *Stellera chamaejasme* L. (Thymelaeaceae). They are β-sitosterol (**2**), simplexin (**3**), pimelea factor P2 (**4**), daucosterol (**5**), (+)-3-hydroxy-1,5-diphenyl-1-pentanone (**6**), 4-ethoxy-benzoic acid (**7**), 2,4,6-Trimethoxy-benzoic acid (**8**), (+)-afzelechin (**9**), fumaric acid (**10**), N,N-dimethyl-L-aspartic acid (**11**), umbelliferone (**12**), daphniretin (**13**) and a novel bicoumarin named bicoumastechamin (**1**). Among the known compounds, **7**, **8**, **9**, **10** and **11** were first isolated from this plant, and **6** was first isolated from the natural resources. Their structures have been elucidated on the basis of spectral data. *In vitro* bioassays showed that **4** inhibited cancer cell growth, **13** exhibited immunomodulatory activity, and **6** exhibited both immunomodulatory and anti-tumor activity.

Keywords: *Stellera chamaejasme* L.; Thymelaeaceae; Bicoumarins

INTRODUCTION

The roots of *Stellera chamaejasme* L. (Thymelaeaceae), known as "Lang Du" in Chinese traditional medicine, has been used for the clinical treatment of mange, stubborn skin ulcer, malignant tumor, chronic tracheitis and tuberculosis for many years in China [1, 2]. In the course of our continuous research on the active constituents of the roots, thirteen compounds were separated from petroleum ether and ether fractions of the ethanol extract of the roots. They are identified as β-sitosterol (**2**), simplexin (**3**) [3], pimelea factor P2 (**4**) [3], daucosterol (**5**) [4], (+)-3-hydroxy-1,5-diphenyl-1-pentanone (**6**) [5, 6], 4-ethoxy-benzoic acid (**7**) [7], 2,4,6-trimethoxy-benzoic

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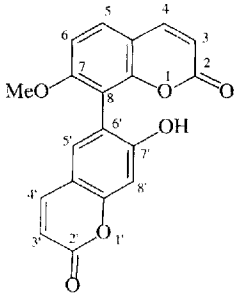
acid (**8**) [8], (+)-afzelechin (**9**) [9], fumaric acid (**10**) [10], N,N-dimethyl-L-aspartic acid (**11**), umbelliferone (**12**) [4], daphniretin (**13**) [11] and a novel bicoumarin named bicoumastechamin (**1**). Among the known compounds, **7**, **8**, **9**, **10** and **11** were first isolated from this plant, and **6** was first isolated from the natural resources. Their structures have been elucidated on the basis of spectral data and comparison with the reported information. *In vitro* bioassays showed that **4** inhibited human A549 cell growth by 96% and 36%, and **6** suppressed cultured P388 cell growth by 97% and 47% at the concentrations of 100 uM and 10 uM respectively. The results also showed that **6** and **13** had immunomodulatory activity. **6** increased the cell growth of B lymphocyte by 100% and 79%, and **13** increased the cell growth of T lymphocyte by 193% and 71% at the concentrations of 10 uM and 1 uM respectively. In this paper, we wish to report the isolation and structural identification of compound **1**, a novel bicoumarin.

RESULTS AND DISCUSSION

Compound **1** was assigned the molecular formula $C_{19}H_{12}O_6$ (HRMS, $[M^-]$ = m/z 336.0612, calculated 336.0630). The IR absorption at 3420,



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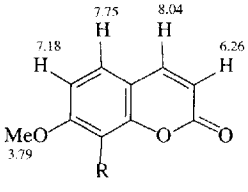

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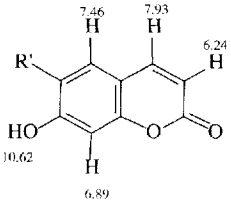

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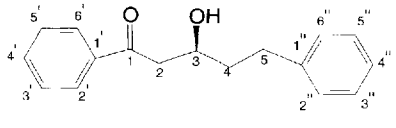

6

FIGURE 1 Compounds from *Stellera chamaejasme* L.

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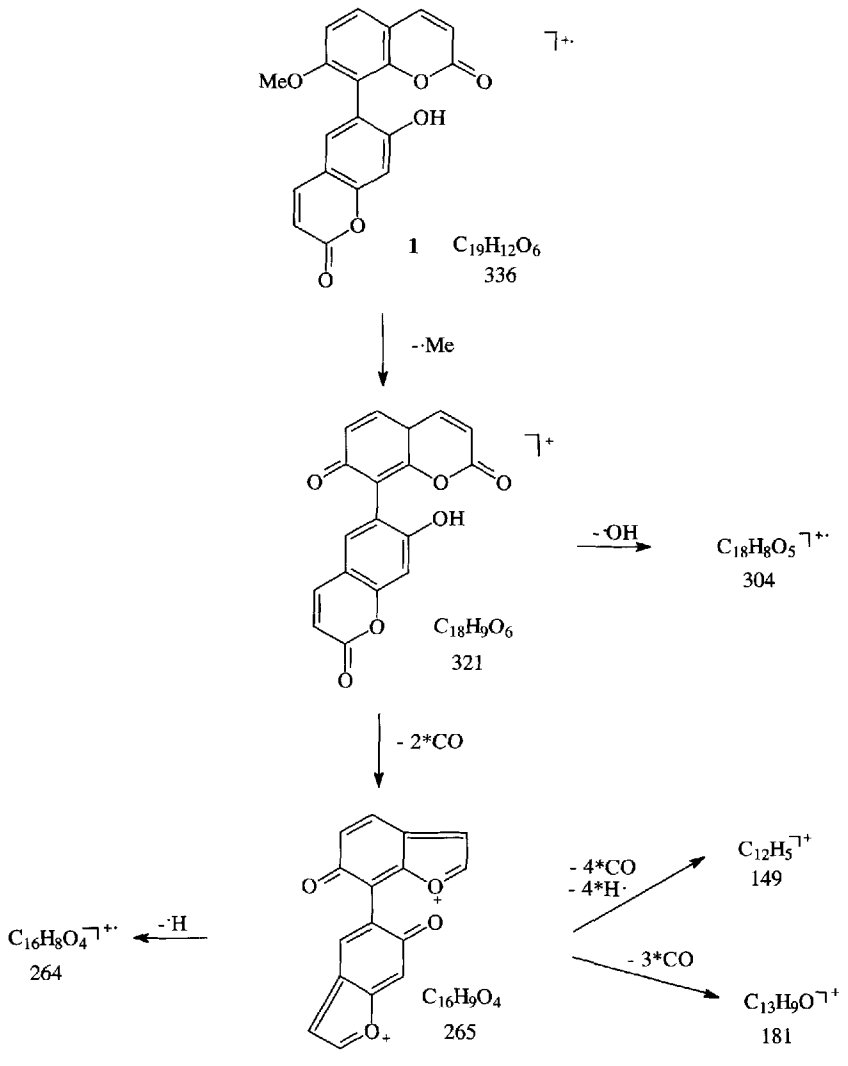

FIGURE 2 EI-MS Fragments of Compound **1**.

1725, 1600, and 1500 cm^{-1} suggested the presence of hydroxyl, carboxyl (lactone) and benzyl groups. The UV spectrum absorption at 212 nm (lgε 4.44), 275 nm (w), and 325 nm (lgε 4.37) showed the characteristics of 7-oxygen-substituted coumarin [12]. The ^{1}HNMR (in DMSO-d_6) indicated one methoxyl signal (δ 3.79), eight aromatic proton signals (δ 6.24 ~ 8.05), and a hydroxyl proton signal (δ 10.62). The two pairs of typical H-3 and H-4

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signals at δ 6.24 (1H, d, J = 9.7 Hz), 7.93 (1H, d, J = 9.7 Hz), and δ 6.26 (1H, d, J = 9.5 Hz), 8.04 (1H, d, J = 9.5 Hz) predicted that the molecule was composed of two C-3 and C-4 unsubstituted coumarin moieties. This also matched the fourteen degrees of unsaturation required by its molecular formula. The positions of the two correlated protons (δ 7.75, 1H, d, J = 8.7 Hz, and δ 7.18, 1H, d, J = 8.8 Hz), two single signals (δ 6.89, 1H, s, and δ 7.46, 1H, s), together with the connection of the two moieties were determined by its NOESY experiment. In its NOESY spectrum, the correlations between H-4′ (δ 7.93) and H-5′ (δ 7.46), H-8′ (δ 6.89) and OH (δ 10.62), H-4 (δ 8.04) and H-5 (δ 7.75), together with H-6 (δ 7.18) and OMe (δ 3.79) were observed. It is obvious that the compound consisted of two parts, a and b, as shown in Figure 1. The structure is also deduced by its ^{13}C NMR data and EI-MS decomposition fragments shown in Figure 2. So the structure of compound **1** was determined as 7-methoxy-7′-hydroxy-[8,6′] bichromenyl-2,2′-dione named bicoumastechamin.

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EXPERIMENTAL SECTION

General Experimental Procedures

Melting points were determined with a Kofler apparatus and were uncorrected; The IR spectra were run on a Perkin-Elmer 599B spectrometer and UV spectra obtained on a Shimatzu UV-250 spectrometer; ^{1}H and ^{13}CNMR were recorded on Bruker AM-300 or 400 instrument in DMSO-d_6 or $CDCl_3$; MS were performed with a Finnigan MAT-711 instrument.

Plant Material

The roots of *Stellera chamaejasme* L. were purchased from De-Yang Herb Corporation of Sichuan Province in August, 1993 and identified by Prof. Dao-Feng Chen. A voucher specimen (No. 19930801) was deposited in the Herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Extraction and Isolation

The air-dried ground plant roots (20 kg) were extracted with 95% EtOH and a portion of the extracts after concentration were successively partitioned with petroleum ether and ether. The petroleum ether extract

(830 g) and ether extract (1750 g) were found to exhibit inhibition against cultured P-388 cells. The petroleum ether extract was then subjected to repeated column chromatography over silica gel, using petroleum ether–acetone, and methanol of increasing polarity as eluate. **2** (20 mg) was obtained in petroleum ether–acetone (4 : 1) fractions by re-crystallization. The petroleum–acetone (7 : 3) fractions were collected and after further column chromatography over silica gel, using chloroform-methanol (9 : 1) as eluate yield **3** (14 mg) and **4** (16 mg). The methanol fractions gave white solid **5** (21 mg). Ether extract was subjected to repeated column chromatography over silica gel, using hexane and acetone mixtures of increasing polarity as eluate, **12** (26 mg) was obtained from hexane-acetone (4 : 1) fractions. The mother liquor was further chromatographed over silica gel, using petroleum ether-ethyl acetate as eluate to give **6** (167 mg), **7** (11 mg), and **8** (9 mg), respectively. The hexane-acetone (6 : 4) fractions gave **9** (11 mg) and **10** (8 mg). The hexane-acetone (1 : 2) fractions were collected and the solvent was evaporated to give fraction B. The water soluble portion of fraction B was subjected to Sephadex LH-20, using ethanol-water as eluate. The 20% EtOH fractions gave **11** (11 mg), and 50% EtOH fractions gave **13** (47 mg). The water insoluble portion of fraction B was further separated by RP-8 chromatography, using 40% EtOH as eluate to yield **1** (9 mg).

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Bicoumastechamin (1)

$C_{19}H_{12}O_6$, yellow square crystal, m.p. 264–265°C, IR ν_{max}^{KBr} cm^{-1}: 3420 (hydroxyl group), 1725 (carboxyl group), 1600, 1500 (benzyl group); UV λ_{max}^{MeOH}(nm): 212 (lgε 4.44), 257 (w), 325 (lgε 4.37); EI-HRMS m/z: 336.0612 (calcd for $C_{19}H_{12}O_6$, 336.0633), EI-MS m/z: 57, 71, 83, 116, 149 (base), 167, 181, 259, 264, 304, 321, 335, 336 (M^+); ^{1}HNMR (300 MHz, DMSO-d_6, ppm): δ 3.79 (3H, s, OCH_3), 6.24 (1H, d, J = 9.7 Hz, H-3′), 6.26 (1H, d, J = 9.5 Hz, H-3), 6.89 (1H, s, H-8′), 7.18 (1H, d, J = 8.8 Hz, H-6), 7.46 (1H, s, H-5′), 7.75 (1H, d, J = 8.7 Hz, H-5), 7.93 (1H, d, J = 9.7 Hz, H-4′), 8.04 (1H, d, J = 9.5 Hz, H-4), 10.62 (OH, s); ^{13}CNMR (75MHz, DMSO-d_6, ppm): δ 58.32 (q), 102.14 (d), 108.34 (d), 111.21 (s), 111.67 (d), 112.39 (d), 112.64 (s), 113.26 (s), 116.78 (s), 129.16 (d), 131.51 (d), 144.36 (d), 144.67 (d), 152.32 (s), 154.97 (s), 159.98 (s), 160.23 (s), 160.40 (s).

(+)-3-Hydroxy-1,5-diphenyl-1-pentanone (6)

$C_{17}H_{18}O_2$, yellow to brown oil, $[\alpha]_D^{18}$ + 4 (c = 0.275, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3460 (hydroxyl group), 1680 (carboxyl group), 1598, 1562,

1469 (benzyl group), 1450, 1215, 755, 694; UVλ^{EtOH}_{max}(nm): 212 (lgε 4.02), 245 (lgε 3.90, carboxyl group conjugated with benzyl ring); EI-HRMS m/z: 254.1296 (calcd for $C_{17}H_{18}O_2$, 254.1306), EI-MS m/z: 51, 77, 91, 105 (base), 120, 149, 179, 236 ($M^+ - H_2O$), 254 (M^+); ^{1}HNMR (400 MHz, $CDCl_3$, ppm): δ 1.17 and 1.94 (m, 2H, H-4), 2.75 and 2.88 (2H, m, H-5), 3.06 (1H, dd, J = 8.7, 17.8 Hz, H-2), 3.15 (1H, dd, J = 2.7, 17.8 Hz, H-2), 4.24 (1H, m, H-3), 7.16 ~ 7.32 (5H, m, H-1″ ~ 6″), 7.45 (2H, t, J = 7.8 Hz, H-3′, 5′), 7.57 (1H, t, J = 7.4 Hz, H-4′) 7.92 (2H, d, J = 7.8 Hz, H-2′, 6′); ^{13}CNMR (75 MHz, $CDCl_3$, ppm): δ 31.76(t), 38.05(t), 44.95(t), 67.02(d), 125.76(d), 127.98(d, 2C), 128.31(d, 2C), 128.40(d, 2C), 128.57(d, 2C), 133.45(d), 136.62(s), 141.82(s), 200.73(s).

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