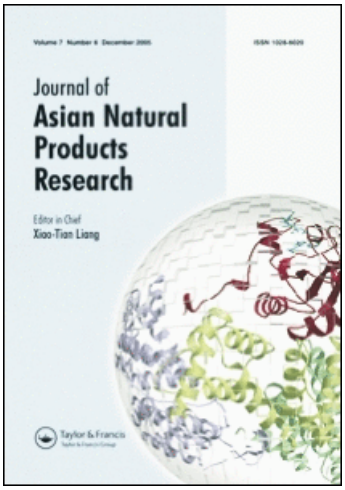


Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Bioactive triterpenoids from *Symplocos chinensis*

Xi-Hao Li[a]; Dian-Dian Shen[a]; Nan Li[a]; Shi-Shan Yu[a]
[a] Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

Online publication date: 09 September 2010

To cite this Article Li, Xi-Hao , Shen, Dian-Dian , Li, Nan and Yu, Shi-Shan(2003) 'Bioactive triterpenoids from *Symplocos chinensis*', Journal of Asian Natural Products Research, 5: 1, 49 — 56
To link to this Article: DOI: 10.1080/1028602031000080469
URL: http://dx.doi.org/10.1080/1028602031000080469

Journal of Asian Natural Products Research, 2003 VOL. 5 (1), pp. 49–56

BIOACTIVE TRITERPENOIDS FROM *SYMPLOCOS CHINENSIS*

XI-HAO LI, DIAN-DIAN SHEN, NAN LI and SHI-SHAN YU*

Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

(Received 8 March 2002; Revised 1 April 2002; In final form 16 July 2002)

A new triterpenoid, 2β,3β,19α,24-tetrahydroxy-23-norurs-12-en-28-oic acid (**4**), together with three known triterpenoids 3-oxo-19α,23,24-trihydroxyurs-12-en-28-oic acid (**1**), 2α,3β,19α,23-tetrahydroxyurs-12-en-28-oic acid (**2**), 2α,3α,19α,23-tetrahydroxyurs-12-en-28-oic acid (**3**), was isolated from the roots of *Symplocos chinensis*. The new triterpenoid shows significant cytotoxic activity against B16 and BGC-823 cells.

Keywords: *Symplocos chinensis*; Triterpenoid; X-ray crystallography; Anticancer activity; B16 cells; BGC-823 cells

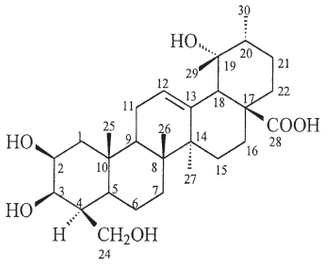
INTRODUCTION

Symplocos chinensis is a toxic herb found in Guangxi province, South China. It has been used as a folk medicine to treat malaria, tumefaction, enteritis, nephritis and snake bite.

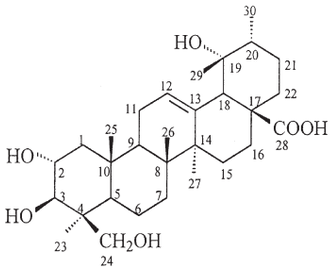
Besides some triterpenoids [1], triterpenoid saponins [2], flavanol glycosides [3–5], iridoid glycosides [6] and lignan glycosides [7] isolated from *Symplocos* genus, no other compounds have been reported which could explain the ethnomedical use of this plant. In our investigations, the EtOAc-soluble extract exhibited significant cytotoxic activities and we isolated four triterpenoids (**1**–**4**) from this fraction. We report here the isolation and structural determination of the new compound **4** by means of chromatographic and spectroscopic techniques.

*Corresponding author. Tel.: +86-10-63165324. Fax: +86-10-63017757. E-mail: yushishan@imm.ac.cn

ISSN 1028-6020 print/ISSN 1477-2213 online
DOI: 10.1080/1028602031000080469



Compound **4**

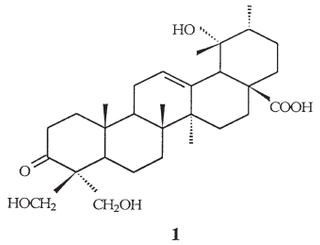


24-Hydroxytormentic acid

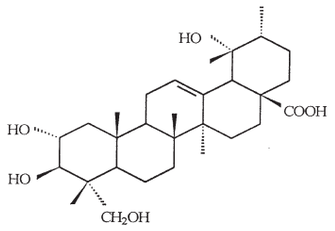
RESULTS AND DISCUSSION

From the EtOAc-soluble fraction of the EtOH extract of the roots of *Symplocos chinensis*, four triterpenoids 3-oxo-19α,23,24-trihydroxyurs-12-en-28-oic acid (**1**) [8], 2α,3β,19α, 23-tetrahydroxyurs-12-en-28-oic acid (**2**) [9], 2α,3α,19α,23-tetrahydroxyurs-12-en-28-oic acid (**3**) [9], and 2β,3β,19α,24-tetrahydroxy-23-norurs-12-en-28-oic acid (**4**) were purified by repeated chromatography on silica gel. The chemical structures of compounds **1**–**3** were identified by comparing the melting points and spectroscopic data (^{1}H NMR, ^{13}C NMR and MS) to literature values. Compound **4** appeared to be new and its structure elucidation is the subject of this study.

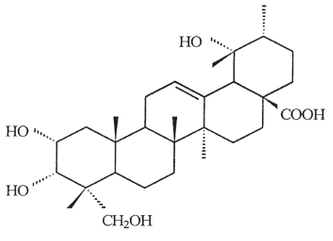
Compound **4** was obtained as colorless crystals. The molecular formula was established as $C_{29}H_{46}O_6$ on the basis of HR-FABMS data. The 29 carbons were characterized by DEPT experiments and showed the presence of five methyls, nine methylenes, eight methines and seven quaternary carbons in the molecule. The positive Liebermann–Burchard test and the ^{13}C NMR spectrum suggested a triterpenoid with a basic structure similar to 24-hydroxytormentic acid [9] (Table I).



1



2



3

TABLE I ^{13}C NMR spectral data of compound **4**

C	*Compound* **4**	*24-Hydroxytormentic acid**
1	45.4	47.8
2	72.4	68.7
3	76.4	85.8
4	49.5	44.0
5	49.2	56.6
6	24.8	19.4
7	33.6	33.8
8	41.4	40.4
9	47.7	47.9
10	37.0	38.3
11	25.1	24.4
12	128.6	127.9
13	140.4	140.0
14	42.78[†]	42.1
15	29.7	29.3
16	26.9	26.9
17	48.8	48.3
18	55.2	54.6
19	73.1	72.7
20	42.84[†]	42.4
21	27.4	26.4
22	38.9	38.5
23	–	24.2
24	62.9	65.7
25	17.3	17.3
26	17.6	17.1
27	25.2	24.6
28	181.2	180.7
29	27.6	27.1
30	17.0	16.8

* The spectrum of 24-hydroxytormentic acid was run in C_5D_5N at 125 MHz.
[†] The values are interchangeable.

Compared to the 24-hydroxytormentic acid skeleton ($C_{30}H_{48}O_6$, $M = 504$), which has 30 carbons, compound **4** has one methyl group less, while one quaternary carbon was changed into a methine and the chemical shifts of C-1 to C-6 were changed. This suggested that one methyl in the 24-hydroxytormentic acid skeleton was substituted by a proton.

The EI mass spectrum of compound **4** showed fragment ions at *m*/*z* 264, 218 and 146. The fragment ion at *m*/*z* 264 which had a formula $C_{16}H_{24}O_3$ would be formed by a retro-Diels–Alder fragmentation between C-9 and C-11, and between C-8 and C-14, as shown in Fig. 1. This kind of cleavage is characteristic of pentacyclic triterpenoids having a double bond at C-12 such as a urs-12-ene skeleton [10]. Formation of the other fragment ions might be explained by fragmentation of this ion. Furthermore, the fragment ion at *m*/*z* 264 revealed that the missing methyl was not located in ring C, D and E, consequently it should be on ring A or B. The position of the missing methyl and the hydroxyl groups were established by HMBC and HMQC analysis.

H-24 showed correlations with three methine carbons (δ 49.2, δ 49.5 and δ 76.4) through three bonds and two bonds, respectively, in the HMBC spectrum. One methine carbon at δ 49.2 was assigned to C-5. The methine proton at δ 49.5, which was assigned to C-4, suggested that the missing methyl carbon was C-23 in the 24-hydroxytormentic acid skeleton. Another methine carbon at δ 76.4 was assigned to C-3 (Fig. 2).

In the $^1H-^1H$ COSY spectrum, H-2 (δ 4.45,m) was correlated with H-3 (δ 4.09, m). H-3 was correlated with H-4 (δ 2.57, m), and H-4 was correlated with H-24 (δ 4.11, δ 5.14, m).

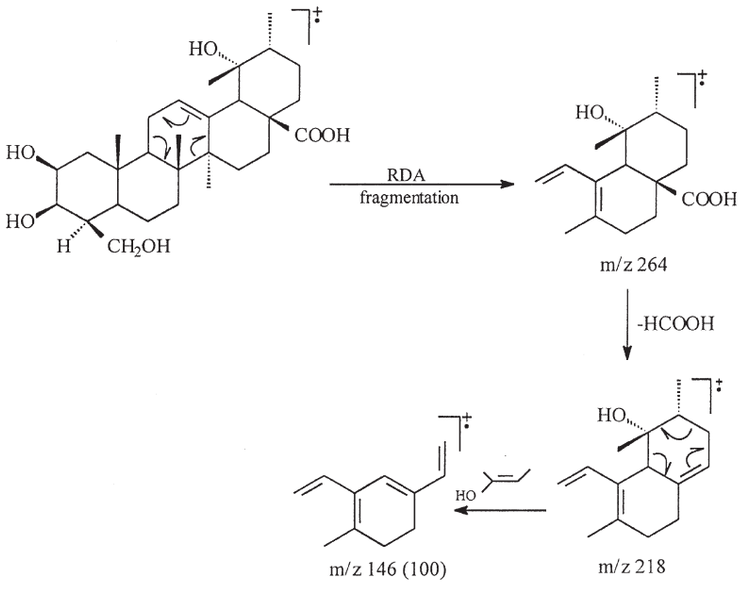


FIGURE 1 Proposed EI mass fragmentation pattern of compound **4**.

The β-configurations for hydroxyls on C-2 and C-3 were revealed by an X-ray experiment, which at the same time confirmed the suggested conformation of compound **4** (Fig. 3).

The cytotoxic activity of compound **4** was evaluated against a panel of cancer cell lines (Table II). After the cells had been treated for 120 h, the cell growth was measured with an MTT assay procedure, and the IC_{50} values calculated from a dose-dependent curve on B16 cells and BGC-823 cells were 0.068 and 0.029 μM, respectively, which showed a strong activity. The values of B16-BL6 (IC_{50} 0.26 μM) and Ketr-3 (IC_{50} 0.35 μM) also displayed relatively weaker activity.

The structures of compounds **1**–**3** were determined by comparison of their physical and spectral data with references and were further confirmed by their 2D NMR spectra.

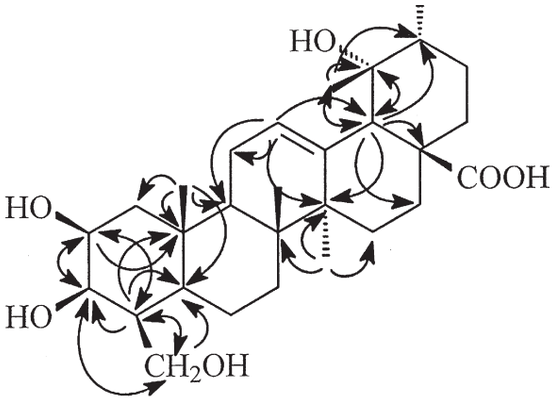


FIGURE 2 HMBC correlations of compound **4**.

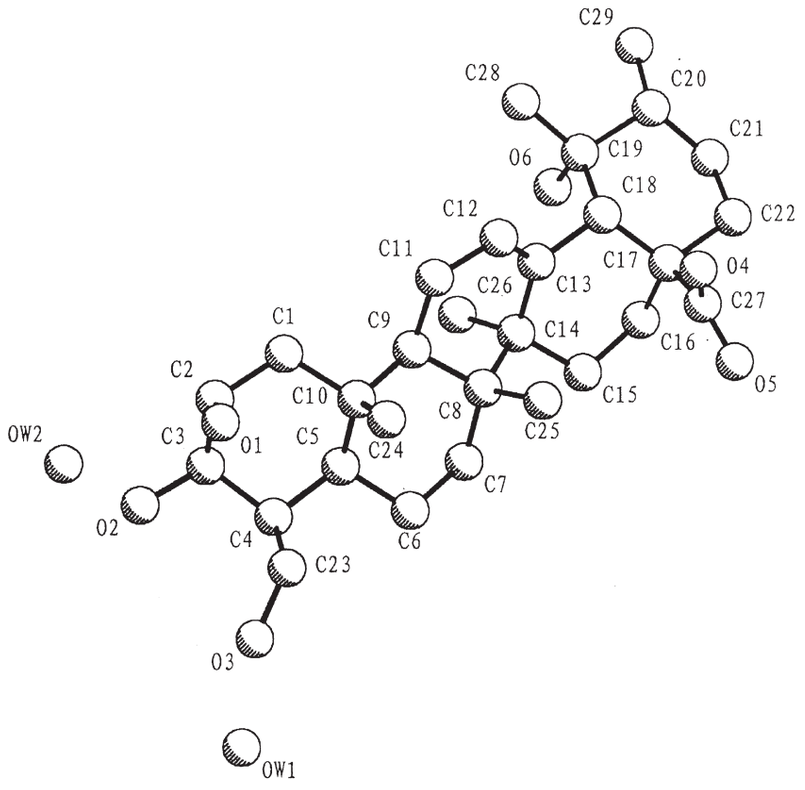


FIGURE 3 X-ray crystal structure for compound **4**.

EXPERIMENTAL SECTION

General Experimental Procedures

Mps were determined on an X_4 micromelting point apparatus and are uncorrected. Optical rotations were measured on a PE-241 MC polarimeter. IR spectra were recorded as KBr discs on a Perkin–Elmer 683 IR spectrometer. ^{1}H NMR and ^{13}C NMR spectra as well as 2D NMR experiments were taken with TMS as internal standard on an Inova 500 FT-NMR spectrometer. High resolution mass spectra were measured on an Autospec-ultima TOF (Micromass) spectrometer and low resolution mass spectra were measured on VG ZAB-2f spectrometer. X-ray structural analysis: MAC DIP 2030 K diffractometer. Silica GF254 for TLC and silica gel (200–300 mesh) for CC were produced by Qingdao Marine

TABLE II Evaluation of the anticancer potential of compound **1**

*Cell line**	*KB*	*B16*	*B16-BL6*	*Ketr-3*	*BGC-823*
$IC_{50}(\mu M)$	1.2	0.068	0.26	0.35	0.025
Evaluation[†]	+	+++	++	++	+++

* KB = human oral epidermoid carcinoma; B16 = C57/BL mouse melanoma; B16-BL6 = C57/BL mouse high-metastatic melanoma; Ketr-3 = human renal carcinoma; BGC-823 = human stomach carcinoma.

[†] [b] +++ = strongly active ($IC_{50} < 0.1\,\mu M$); ++ = active ($0.1\,\mu M < IC_{50} < 1\,\mu M$); + = weakly active ($1\,\mu M < IC_{50} < 10\,\mu M$); inactive ($IC_{50} > 10\,\mu M$).

Chemical Company, Qingdao, China. Solvents and chemicals were of analytical grade and purchased from Beijing Chemical Company, Beijing, China.

Plant Material

Roots of *Symplocos chinenesis* were collected in November 1998 from Guangxi Province, China. The sample was identified by Professor Liu Shouyang, Department of Pharmacognosy, Guangxi College of Chinese Traditional Medicine. The roots were harvested and air-dried at room temperature in darkness. A voucher specimen was deposited in the Herbarium of the Institute of Materia Medica, Chinese Academy of Medical Sciences.

Extraction and Isolation

The dried and chopped roots (22 kg) were extracted with EtOH and 2710 g of the extract were obtained, which was successively partitioned with EtOAc, BuOH and H_2O. The EtOAc extract (220 g) was chromatographed on a silica gel column eluted with petroleum ether–EtOAc (from 50:1 to 1:1), EtOAc, EtOAc–MeOH (1:1) and MeOH. Fraction 9 (43.3 g) eluted with EtOAc–MeOH (1:1) was purified by repeated silica gel column chromatography ($CHCl_3$–MeOH) to give compound **1** (2.1 g), **2** (130 mg), **3** (210 mg) and **4** (260 mg).

3-Oxo-19α,23,24-trihydroxyurs-12-en-28-oic Acid (1)

Amorphous powder (2.1 g). Mp 198–200°C. $[\alpha]_D^{18} + 32.5$ (MeOH, *c* 1.30), IR ν_{max} (KBr) cm^{-1}: 3448, 2935, 1697, 1045. FAB-MS $[M + 1]^+$ 503. ^1H NMR (500 MHz, DMSO-d_6): δ11.88 (*br*, 1H, COOH), 5.18 (1H, *t*, $J = 3.5$ Hz, H-12), 3.75, 3.21 (each 1H, d, $J = 10.5$ Hz H-24), 3.60, 3.41 (each 1H, d, $J = 11$ Hz H-23), 2.37 (1H, *s*, H-18), 0.83 (3H, d, $J = 6.5$ Hz, 30-Me), 0.75, 1.00, 1.07, 1.30 (each 3H, *s*). ^{13}C NMR (500 MHz, DMSO-d_6): δ 37.6 (C-1), 35.6 (C-2), 213.4 (C-3), 57.9 (C-4), 47.8 (C-5), 19.0 (C-6), 32.4 (C-7), 40.0 (C-8, Ref), 45.6 (C-9), 35.8 (C-10), 23.6 (C-11), 126.8 (C-12), 138.7 (C-13), 41.3 (C-14), 28.2 (C-15), 25.2 (C-16), 47.0 (C-17), 53.3 (C-18), 71.6 (C-19), 41.5 (C-20), 26.0 (C-21), 37.3 (C-22), 61.8 (C-23), 62.8 (C-24), 16.6 (C-25), 14.9 (C-26), 24.0 (C-27), 179.0 (C-28), 26.5 (C-29), 16.4 (C-30).

2α,3β,19α,23-Tetrahydroxyurs-12-en-28-oic Acid (2)

Amorphous powder (130 mg). Mp 282–284°C. $[\alpha]_D^{18} + 25.8$ (MeOH, *c* 1.05), IR ν_{max}(KBr) cm^{-1}: 3425, 2931, 1689, 1047. FAB-MS $[M + Na]^+$ 527. ^1H NMR (500 MHz, CD_3OD): δ5.24 (1H, *t*, $J = 3.5$ Hz, H-12), 3.63 (1H, ddd, $J = 11$, 10.0, 4.5 Hz, H-2), 3.44, 3.21 (each 1H, d, $J = 11$ Hz, H-23), 3.30 (1H, d, $J = 9.5$ Hz, H-3), 2.52 (1H, ddd, $J = 13.5$, 13.0, 4.5 Hz, H-16α), 2.44 (1H, *s*, H-18), 0.87 (3H, d, $J = 6.5$ Hz, 30-Me), 0.64, 0.74, 0.97, 1.14, 1.29 (each 3H, *s*). ^{13}C NMR (500 MHz, C_5D_5N): δ 47.8 (C-1), 68.8 (C-2), 78.3 (C-3), 43.6 (C-4), 48.2 (C-5), 18.6 (C-6), 33.1 (C-7), 40.4 (C-8), 47.9 (C-9), 38.3 (C-10), 24.1 (C-11), 128.0 (C-12), 140.0 (C-13), 42.1 (C-14), 29.2 (C-15), 26.3 (C-16), 48.2 (C-17), 54.5 (C-18), 72.6 (C-19), 42.3 (C-20), 27.0 (C-21), 38.4 (C-22), 66.5 (C-23), 14.2 (C-24), 17.2 (C-25), 17.3 (C-26), 24.6 (C-27), 180.7 (C-28), 26.8 (C-29), 16.7 (C-30).

2α,3α,19α,23-Tetrahydroxyurs-12-en-28-oic Acid (3)

Amorphous powder (210 mg). Mp 255–257°C. $[\alpha]_D^{18}$ +19.1 (MeOH, *c* 1.10), IR ν_{max}(KBr) cm^{-1}: 3464, 2931, 1730, 1074, 1051. FAB-MS $[M + Na]^+$ 527. ^1H NMR (500 MHz, CD_3OD): δ5.33 (1H, *t*, $J = 3.5$ Hz, H-12), 3.91 (1H, ddd, $J = 12$, 3.0, 2.5 Hz, H-2), 3.63 (1H, d, $J = 2.5$ Hz, H-3), 3.56, 3.42 (each 1H, d, $J = 11$ Hz H-23), 2.61 (1H, ddd, $J = 13.5$, 13.0, 4.5 Hz, H-16α), 2.53 (1H, *s*, H-18), 0.96 (3H, d, $J = 7$ Hz, 30-Me), 0.81, 0.82, 1.05, 1.22, 1.38 (each 3H, *s*). ^{13}C NMR (500 MHz, C_5D_5N): δ 41.9 (C-1), 65.8 (C-2), 78.5 (C-3), 42.2 (C-4), 43.1 (C-5), 18.0 (C-6), 32.8 (C-7), 40.1 (C-8), 47.3 (C-9), 38.05 (C-10), 23.6 (C-11), 127.6 (C-12), 139.7 (C-13), 41.7 (C-14), 28.8 (C-15), 25.9 (C-16), 47.8 (C-17), 54.2 (C-18), 72.3 (C-19), 41.4 (C-20), 26.5 (C-21), 37.98 (C-22), 70.8 (C-23), 16.6 (C-24), 17.2 (C-25), 16.8 (C-26), 24.2 (C-27), 180.4 (C-28), 26.6 (C-29), 16.3 (C-30).

2β,3β,19α,24-Tetrahydroxy-23-norurs-12-en-28-oic Acid (4)

Colorless crystal (260 mg). Mp 301°C. $[\alpha]_D^{18}$ +78.2 (MeOH, *c* 1.10). HR-FABMS *m/z* 491.3372 $[M + 1]^+$, calcd. for $C_{29}H_{47}O_6$ 491.3372, Positive-ion FABMS *m/z* 583.5 $[M + H + G]^+$, 513.4 $[M + Na]^+$, 491.4 $[M + 1]^+$, 473.4 $[M + H\text{-}H_2O]^+$. EIMS *m/z*(rel.int): 490 $[M]^+$ ($C_{29}H_{46}O_6$) (0.5), 472 $[M\text{-}H_2O]^+$ ($C_{29}H_{44}O_5$) (0.5), 444 $[M\text{-}HCOOH]^+$ ($C_{28}H_{44}O_4$) (21), 264 [ring D, E]$^+$ ($C_{16}H_{24}O_3$) (6), 246 $[264\text{-}H_2O]^+$ ($C_{16}H_{22}O_2$) (15), 218 $[264\text{-}HCOOH]^+$ ($C_{15}H_{22}O$) (14), 146 $[218\text{-}C_4H_8O]^+$ ($C_{11}H_{14}$) (100). IR ν_{max}(KBr) cm^{-1}: 3438, 2920, 1682, 1153, 1016. ^1H NMR (500 MHz, C_5D_5): δ 5.59 (1H, *bs*, H-12), 5.14 (1H, m, 24-Ha), 4.45 (1H, m, H-2), 4.11 (1H, m, 24-Hb), 4.09 (1H, m, H-3), 3.11 (1H, ddd, $J = 13$, 13, 4 Hz, 16-Hα), 3.04 (1H, s, H-18), 2.57 (1H, m, H-4), 1.72 (3H, s, Me-27), 1.44 (3H, s, Me-29), 1.38 (3H, s, Me-25), 1.11 (3H, d, $J = 7$ Hz, Me-30), 1.098 (3H, s, Me-26).

^{13}C NMR: Table I.

X-ray Crystallographic Analysis of (4)

A colorless transparent platelet was obtained from MeOH having a size of $0.4 \times 0.4 \times 0.05$ mm. The compound crystallized in space group $P2_12_12_1$, $a = 8.259(1)$ Å, $b = 10.769(1)$ Å, $c = 31.679(1)$ Å; $V = 2816.9(4)$ Å^3; $Z = 4$; $d_{calc} = 1.237$ g/cm^3; final $R_f = 0.083$. Data were collected on a MAC DIP 2030 K diffractometer using Mo-Kα radiation and a graphite monochromator. Image plate distance 100 mm, Φ rotation 0–180°, step $\Phi = 5°$, 2θ range 50°. A total of 2537 unique reflections were recorded of which 2443 were considered observed ($|F|^2 \geq 8\sigma|F|^2$). The crystal structure was solved by direct methods using the program SHELX-86 and refined by full-matrix least-squares techniques (Fig. 3).

Cell Culture and Assay for Cytotoxic Activity

Tumor cells (1×10^4 cells ml^{-1}) were seeded to RPMI 1640 medium supplemented with 10% heat-inactivated FBS and 0.1 mg/ml kanamin at 37°C in a humidified atmosphere containing 5% CO_2 for 24 h. Test compounds were added to this culture and incubated at 37°C for another 120 h without medium change. Then the cell viability was evaluated by the MTT-reducing test and compared with that of the control culture where the cells were treated without added test compounds. The IC_{50} values of **4** thus obtained were 1.2, 0.35, 0.26, 0.068 and 0.029 μM for KB, Ketr-3, B16-BL6, B16 and BGC-823 cell lines.

Acknowledgements

The project was supported by National Key Basic R&D (973) project No.G1998-051120. We are grateful to the Department of Analysis of the Institute of Materia Medica for performing the HR-FABMS, NMR and X-ray diffraction experiments. We thank the Department of Pharmacology of the Institute of Materia Medica for the cell cultivation and bioactivity tests.

References

[1] Ali, M., Bhutani, K.K. and Srivastava, T.N. (1992), *Phytochemistry* **29**, 3601–3604.
[2] Higuchi, R., Kawasaki, T., Momota, B., Pandey, V.B. and Dasgupta, B. (1982), *Phytochemistry* **21**, 907–910.
[3] Tiwari, R.D. and Tripathi, H.L. (1976), *Phytochemistry* **15**, 833–836.
[4] Tschesche, R., Braun, T.M. and Sassen, W.V. (1980), *Phytochemistry* **19**, 1825–1829.
[5] Tanaka, T., Kawamura, K., Kohda, H., Yamasaki, K. and Tanaka, O. (1982), *Chem. Pharm. Bull.* **30**, 2421–2426.
[6] Iida, I., Hayashi, M., Murata, T., Ono, M., Inoue, K. and Fujita, T. (1990), *J. Chromatogr.* **515**, 503–509.
[7] Inoryi, H., Takeda, Y. and Nishimuna, H. (1973), *Yakugaku Zasshi* **93**, 44–49.
[8] Sakakibara, J., Kaiya, T. and Fukuda, H. (1984), *Phytochemistry* **23**, 627–630.
[9] Seto, T., Tanaka, T., Tanaka, O. and Naruhashi, N. (1984), *Phytochemistry* **23**, 2829–2832.
[10] Wiedemann, B., Lerche, H., Lotter, H., Neszmelyi, A., Wagner, H. and Müller, A.A. (1999), *Phytochemistry* **52**, 333–337.