Three New Triterpenoids from *Lycopodium japonicum* THUNB

by **Jian Yan**[a])[b]), **Xiang-Ming Zhang**[a]), **Zhong-Rong Li**[a]), **Lin Zhou**[a]), **Jian-Chao Chen**[a])[b]), **Li-Rong Sun**[a])[b]), and **Ming-Hua Qiu***[a])[b])

[a]) Kunming Institute of Botany, the Chinese Academy of Sciences, Kunming, 650204, P.R. China (phone: +86-871-5223255; fax: +86-871-5150227; e-mail: mhchiu@public.km.yn.cn or mhchiu@mail.kib.ac.cn)

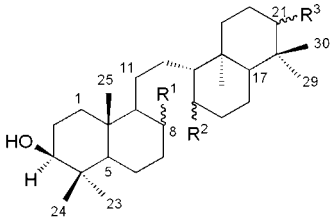
[b]) Graduate School of the Chinese Academy of Sciences, Beijing, 100039, P.R. China

Three new triterpenoids, (3β,8β,14α,21α)-26,27-dinoronocerane-3,8,14,21-tetrol (**1**), (3β,8β,14α,21β)-26,27-dinoronocerane-3,8,14,21-tetrol (**2**), and lycopodiin A (**3**), together with four known compounds, lycoclavanol (**4**), lycoclaninol (**5**), α-onocerin (**6**), and 3-epilycoclavanol (**7**), were isolated from *Lycopodium japonicum* THUNB (Lycopodiaceae). Their structures were established by means of spectroscopic analyses. Compounds **3** and **7** showed moderate antitumor activity. Compounds **4** and **6** exhibited acetylcholinesterase inhibition activity.

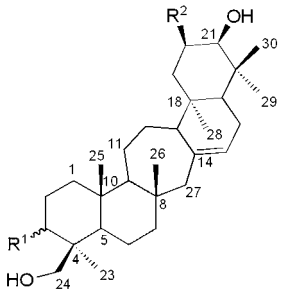
1. Introduction. – The chemical constituents of the genus *Lycopodium* have been investigated previously [1–3]. Plants of the genus characteristically contain triterpenoids of the serratene group or derivatives of its biogenetic precursor, α-onocerin [4]. Serratenes constitute a group of naturally occurring pentacyclic triterpenoids possessing seven tertiary Me groups and a seven-membered ring C, usually with a C=C bond between C(14) and C(15) and *O*-functionalities at C(3) and C(21) [5]. *L. japonicum* was shown to occur in the Chinese provinces Guangdong, Guangxi, Yunnan, and Guizhou [6]. This plant is one of the most commonly encountered traditional Chinese herbal medicines for treatments of arthritic pain, quadriplegia, dysmenorrhea, and contusion [7][8]. In the search of its biologically active constituents, seven compounds were isolated from *L. japonicum*. In this paper, we describe the isolation and identification of three new triterpenoids, compounds **1**–**3**.

2. Results and Discussion. – Compound **1** possesses the molecular formula $C_{28}H_{50}O_4$ as shown by HR-FAB-MS (*m/z* 449.3619 ($[M-H]^-$)), which is consistent with its NMR data (*Table 1*). The IR bands showed the presence of OH groups (3440 cm^{-1}). The structure of **1** was elucidated as (3β,8β,14α,21α)-26,27-dinoronocerane-3,8,14,21-tetrol after detailed spectral analysis.

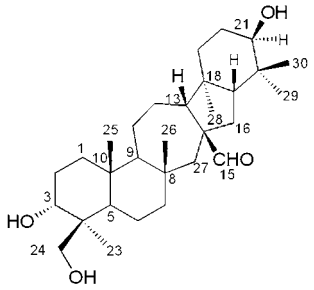
The ^{1}H-NMR spectrum of **1** displayed signals for three Me groups at δ(H) 1.15 (*s*), 1.28 (*s*), and 1.37 (*s*), two coinciding axial protons of oxygenated methine groups (δ(H) 3.52 (*d*, *J* = 11.3, H_α–C(3) and H_β–C(21)), and two equal protons of oxygenated methine groups (δ(H) 4.36 (br. *s*, H_α–C(8) and H_β–C(14)), and no signals due to a C=C bond. The ^{13}C-NMR and DEPT spectra showed only 14 C-signals (3 Me, 5 CH_2, 2 OCH, 2 CH, and 2 C), suggesting the presence of either a C_2 axis or a plane of symmetry within this molecule. As **1** is optically active, the latter possibility can be ruled out. The spectral data of **1** were very similar to tetracyclic dinortriterpenoids having OH and 6 tertiary Me groups such as lyclavatol [9] and α-onocerin [10][11]. In the ^{1}H,^{1}H-COSY plot, cross-peaks at δ 3.52 (H–C(3))/1.92 (H–C(2)), and 1.11 (H_α–C(5)) were observed. The



1 R^1= β-OH R^2 = α-OH R^3 = α-OH
2 R^1= β-OH R^2 = α-OH R^3 = β-OH
6 R^1 = =CH_2 R^2 = =CH_2 R^3 = α-OH



4 R^1= α-OH R^2 = H
5 R^1= α-OH R^2 = OH
7 R^1= β-OH R^2 = H



3

relative configuration of the substituents was revealed by an analysis of the ROESY plot. The correlations H–C(3)/H_α–C(5) and H_α–C(1) were observed but no correlation H–C(3)/Me(25), which suggested that H–C(3) was α-configured. The same type of argument showed that H–C(8) possessed α-configuration. Finally, the proposed structure of **1** was consistent with the EI-MS data. Besides the loss of two H_2O and one Me group, the primary cleavage of the molecular ion (*m/z* 450) occurred between C(9) (or C(13)) and C(11) (or C(12)) creating a series of major fragment ions at *m/z* 193 (A/B ring system; 63), 175 (A/B-H_2O, 100), and three ions due to the subsequent loss of CH_2 groups.

Compounds **2** and **1** were obtained initially as a mixture after column (silica gel) chromatography and were subsequently separated by HPLC. Compound **2** had the same molecular formula ($C_{28}H_{50}O_4$) according to the HR-FAB-MS (*m/z* 473.3599 ($[M+Na]^+$). The IR bands showed the presence of OH groups (3440 cm^{-1}). Comparison of the NMR spectra of **1** and **2** (*Table*) disclosed the similarity of these two compounds. Finally, **2** was identified as the 21-epimer of **1**, *i.e.*, (3β,8β,14α,21β)-26,27-dinoronocerane-3,8,14,21-tetrol.

In the ^{1}H-NMR spectrum of **2** appeared six Me peaks at δ(H) 0.99 (*s*), 1.13 (*s*), 1.25 (*s*), 1.28 (*s*), 1.32 (*s*), 1.39 (*s*), and two axial protons of oxygenated methine groups (δ(H) 3.53 (*d*, J = 11.3, H_α–C(3), and br. *s*, of H_α–C(21)), two axial protons and one proton of an oxygenated methine group (δ(H) 4.35 (br. *s*, H_α–C(8) and H_β–C(14)). Two oxygenated methine protons demonstrated the presence of corresponding geminal OH groups (2 br. *s*, at 5.80 and 5.52 for OH–C(3) and OH–C(8), resp.). The ^{13}C-NMR and DEPT spectra of **2** displayed 28 C-signals (6 Me, 10 CH_2, 4 OCH, 4 CH, and 4 C), demonstrating that the symmetry element present in **1** is absent in **2**.

Table 1. *^{13}C- and ^{1}H-NMR Data* (C_5D_5N) *of* **1**–**3** δ in ppm, J in Hz.

	1		**2**		**3**	
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)
CH_2 (1)	36.9 (CH_2)	2.23 (*m*)	36.9 (CH_2)	2.23 (*m*)	34.1 (CH_2)	1.34, 1.29 (2*m*)
CH_2 (2)	28.5 (CH_2)	1.92 (*m*)	28.2 (CH_2)	1.86 (*m*)	27.8 (CH_2)	1.95 (*m*)
H–C(3)	78.4 (CH)	3.52 (*d*, $J = 11.3$)	78.5 (CH)	3.53 (*d*, $J = 11.3$)	70.1 (CH)	4.41 (br. *s*)
C(4)	38.3 (C)	–	38.3 (C)	–	44.3 (C)	–
H–C(5)	55.9 (CH)	1.11 (*m*)	55.9 (CH)	1.06 (*m*)	49.8 (CH)	1.75 (*m*)
CH_2 (6)	17.8 (CH_2)	1.28 (*m*)	17.8 (CH_2)	1.28 (*m*)	19.2 (CH_2)	1.52, 1.62, (2*m*)
CH_2 (7)	38.5 (CH_2)	1.52,2.16 (2*m*)	38.5 (CH_2)	1.54, 2.19 (2*m*)	45.3 (CH_2)	1.23 (*m*)
H–C(8) or C(8)	66.3 (CH)	4.36 (br. *s*)	66.3 (CH)	4.35 (br. *s*)	39.5 (C)	–
H–C(9)	55.0 (CH)	1.12 (*m*)	54.9 (CH)	1.09 (*m*)	66.3 (CH)	0.89 (*m*)
C(10)	39.8 (C)	–	39.8 (C)	–	38.7 (C)	–
CH_2 (11)	23.2 (CH_2)	1.99 (*m*)	23.2 (CH_2)	1.93 (*m*)	21.9 (CH_2)	0.91 (*m*)
CH_2 (12)	23.2 (CH_2)	1.99 (*m*)	26.4 (CH_2)	1.99 (*m*)	29.5 (CH_2)	1.20 (*m*)
H–C(13)	55.0 (CH)	1.12 (*m*)	54.9 (CH)	1.13 (*m*)	50.6 (CH)	2.75 (*dd*, $J = 13.0, 6.3$)
H–C(14) or C(14)	66.3 (CH)	4.36 (br. *s*)	66.7 (CH)	4.35 (br. *s*)	59.3 (C)	–
CH_2 (15) or H–C(15)	38.5 (CH_2)	1.52,2.16 (2*m*)	38.3 (CH_2)	1.52, 2.16 (2*m*)	204.3 (CH)	9.68 *s*)
CH_2 (16)	17.8 (CH_2)	1.28 (*m*)	17.6 (CH_2)	1.28 (*m*)	35.3 (CH_2)	1.42 (*m*)
H–C(17)	55.9 (CH)	1.11 (*m*)	49.7 (CH)	1.06 (*m*)	46.0 (CH)	1.38 (*m*)
C(18)	39.8 (C)	–	39.8 (C)	–	45.1 (C)	–
CH_2 (19)	36.9 (CH_2)	2.23 (*m*)	33.1(CH_2)	2.23 (*m*)	33.7 (CH_2)	1.56 (*m*)
CH_2 (20)	28.5 (CH_2)	1.92 (*m*)	30.1(CH_2)	1.89 (*m*)	26.7 (CH_2)	1.59 (*m*)
H–C(21)	78.4 (CH)	3.52 (*d*, $J = 11.3$)	75.5 (CH)	3.53 (br. *s*)	74.3 (CH)	3.63 (br. *s*)
C(22)	38.3 (C)	–	38.3 (C)	–	37.4 (C)	–
Me (23)	29.2 (Me)	1.28 (*s*)	29.2 (Me)	1.28 (*s*)	23.7 (Me)	0.85 (*s*)
Me (24) or CH_2 (24)	16.8 (Me)	1.37 (*s*)	16.8 (Me)	1.32 (*s*)	65.6 (CH_2)	4.00 (*d*, $J = 10.7$); 3.91 (*d*, $J = 11.1$)
Me (25)	16.7 (Me)	1.15 (*s*)	23.1 (Me)	1.13 (*s*)	17.2 (Me)	0.91 (*s*)
Me (26)	–	–	–	–	22.8 (Me)	0.94 (*s*)
CH_2 (27)	–	–	–	–	54.8 (CH_2)	1.55, 1.75 (2*d*, each $J = 14.7$)
Me (28)	16.7 (Me)	1.15 (*s*)	22.9(Me)	1.25 (*s*)	16.7 (Me)	0.75 (*s*)
Me (29)	16.8 (Me)	1.37 (*s*)	16.8 (Me)	1.39 (*s*)	29.3 (Me)	1.07 (*s*)
Me (30)	29.2 (Me)	1.28 (*s*)	29.7 (Me)	0.99 (*s*)	22.8 (Me)	0.94 (*s*)

Compound **3** was shown to possess the molecular formula $C_{30}H_{50}O_4$ from the HR-FAB-MS (*m/z* 473.3623 ($[M-1]^-$), consistent with the analysis of its NMR (*Table*) data. The IR spetrum showed OH bands (3441 cm^{-1}) and the presence of a carbonyl group (1707 cm^{-1}). The structure of **3** is formulated as (3α,13*R*,14*S*,21β)-3,21,24-trihydroxy-16(15 → 14)-abeoserratan-15-al.

The ^{1}H- and ^{13}C-NMR spectra of **3** (*Table*) exhibited six tertiary Me, ten CH_2, and four CH groups, six quaternary C-atoms, a secondary OH group (δ(H) 3.63 (br. *s*, 1 H); δ(C) 74.3 (*d*)), an other secondary OH group (δ(H) 4.41 (br. *s*, 1 H); δ(C) 70.1 (*d*)), a CH_2OH group (δ(H) 4.00 (*d*, $J = 10.7$, 1 H) and 3.91 (*d*, $J = 11.1$, 1 H); δ(C) 65.6 (*t*)), and an aldehyde group (δ(H) 9.68 (*s*, 1 H); δ(C) 207.3 (*d*)). Apart from a slightly different

substituent pattern at C(3) (OH instead of MeO) and C(24) (CH_2OH instead of Me), the new metabolite **3** is very similar to (3β,13*R*,14*S*,21β)-21-hydroxy-3-methoxy-16(15 → 14)-abeoserratan-15-al and (3α,13*R*,14*S*,21β)-21-hydroxy-3-methoxy-16(15 → 14)-abeoserratan-15-al, which were found recently identified [12] (DEPT and HMQC spectra evidence). The HMBC spectrum of **3** (*Fig.*) showed the correlations δ 4.41(H–(C(3))/34.1 (C(1)), 27.8 (C(2)), 44.3 (C(4)), 49.8 (C(5)), 23.7 (C(23)), and 65.6 (C(24)) and δ 3.63 (H–C(21))/46.0 (C(17)), 33.7 (C(19)), 26.7 (C(20)), 37.4 (C(22)), 29.3 (C(29)), and 22.8 (C(30)). The correlations δ 0.85 (Me(23)/65.6 (C(24)) (CH(24)/OH: δ 4.00 and 3.82) demonstrated the 1,3-relationship of the corresponding C-atoms. In the NOESY plot, significant NOEs were observed between the aldehyde proton and H_β–C(16), H_β–C(27), and Me(26), which suggested the β-position of the aldehyde group. NOEs were not observed for H–C(3)/Me(23) and H_α–C(5), indicating that H–C(3) has the axial β-configuration. Finally, the EI-MS of **3** showed eight predominant fragment-ion peaks due to cleavage of the C- and D-ring at *m/z* 409, 231, 203, 223, 205, 154, 136, and 121.

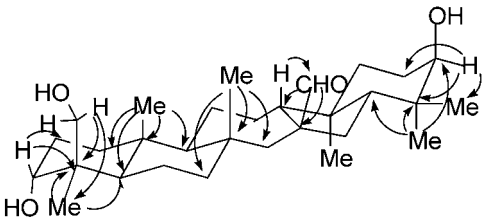


Figure. *Key HMBC correlations of* **3**

This work was supported by the *NKIP Foundation* of the Chinese Academy of Sciences (KSCZX-SW-301-08) and the *Foundation of Key State Lab of Phytochemistry.* The authors are grateful to the analytical group of the Phytochemistry Laboratory, Kunming Institute of Botany, the Chinese Academy of Sciences, for spectral measurements.

Experimental Part

General. Column chromatography (CC): Silica gel (200–300 mesh; *Qingdao Marine Chemical*, China), *Lichroprep RP-18* (40–63 um; *Merck*, Darmstadt, German), and *Sephadex LH-20* (*Pharmacia Fine Chemical Co. Ltd.*). TLC: detection by spraying with 10% H_2SO_4 soln. followed by heating. M.p.: *Yanaco MP-52* apparatus; uncorrected. Optical rotations: *Horiba SEAP-300* spectropolarimeter. IR Spectra: *Shimadzu IR-450* instrument; in cm^{-1}; KBr pellets. NMR Specta: *Bruker AM-400*, or *DRX-500* instruments; chemical shifts δ in ppm; $SiMe_4$ as internal standard; *J* in Hz. FAB-MS and HR-EI-MS: *VG Autospec-3000* spectrometer; in *m/z* (rel. int. in % of the base peak).

Plant Material. The whole plants of *L. japonicum* THUNB were obtained from the Chinese herbal market. It was identified by Prof. *Su-Gong Wu*. A voucher specimen (KUN No. 001143) was deposited at the Laboratory of Phytochemistry, Kunming Institute of Botany.

Extraction and Isolation. The dried, milled whole plant of *L. japonicum* (19.0 kg) was exhaustively extracted with 90% MeOH (3 × 10 l) under reflux. The MeOH extract was evaporated to yield a syrup (910 g). The MeOH extract was suspended in H_2O/MeOH 9:1 (1500 ml) and extracted successively with AcOEt (3 × 2000 ml) and BuOH (3 × 2000 ml) to give AcOEt-soluble (410 g) and BuOH-soluble fractions (101 g). The AcOEt extract was adsorbed on silica gel (600 g) and fractionated by CC (silica gel (1.5 kg), $CHCl_3$/Me_2CO 10:0, 9:1, 8:2, 7:3, 6:4, and 0:10): *Fractions 1* (oil), *2* (104 g), *3* (119 g), *4* (64 g), *5* (49 g), and *6* (22 g). *Fr.* 2 (104 g) was subjected to CC (silica gel, $CHCl_3$/MeOH 40:1, 30:1, and 20:1): *Fr. 2.1–2.4. Fr. 2.1* was further purified by repeated CC (silica gel, $CHCl_3$/MeOH 60:1 and 50:1): **6** (20 g). *Fr. 2.3* was dissolved in $CHCl_3$ and yielded crystalline needles of **4** (10.0 g). *Fr. 2.4* was further purified by CC (silica gel, $CHCl_3$/MeOH 35:1): **7** (8 g). *Fr. 3* (119 g) was purified by repeated CC (silica gel, $CHCl_3$/MeOH 25:1, 15:1, and 10:1): *Fr. 3.1–3.5. Fr. 3.3* (200.0 mg), a mixture **1**/**2**, was repeatedly subjected to HPLC (MeOH/H_2O 70:30): **1** (50.0 mg) and **2** (40.0 mg). *Fr. 4* (64 g) was subjected to CC (silica gel, $CHCl_3$/MeOH 15:1 and 10:1): *Fr. 4.1–4.5. Fr. 4.1* was purified by repeated CC (silica gel, $CHCl_3$/MeOH 10:1): **5** (6.0 g). *Fr. 5* (49 g) was subjected to CC (silica gel,

$CHCl_3$/MeOH 10:1, 8:1, and 5:1): *Fr. 5.1–5.4. Fr. 5.2* was further separated into *Fr. 5.21–5.24. Fr. 5.2.1* was subjected to CC (*RP-18*, MeOH/H_2O 65:35) to give **3** (100 mg), which was further purified by CC (*Sephadex LH-20*, $Me_2CO/CHCl_3$ 9:1).

*(1*R*,1′*R*,2*S*,2′*S*,4a*R*,4′a*R*,6*S*,6′*S*,8a*S*,8′a*S*)-1,1′-(Ethane-1,2-diyl)bis[decahydro-5,5,8a-trimethylnaphthalene-2,6-diol]* (**1**): White powder. M.p. 167–169°. $[\alpha]_D^{23} = +14.29$ ($c = 0.7$, MeOH). IR (KBr): 3440*s* (br.), 2934, 2871, 1630, 1457, 1386, 1164, 1047, 1004, 577. ^{1}H- and ^{13}C-NMR: *Table.* EI-MS: 450 (1, M^+), 432(5), 417 (30), 399 (13), 193 (68, $C_{13}H_{21}O^+$ (A/B ring)), 175 (100), 161 ($[C_{13}H_{21}O-H_2O-CH_2]^+$), 147 ($[C_{13}H_{21}O-H_2O-2\ CH_2]^+$), 133 ($[C_{13}H_{21}O-H_2O-3\ CH_2]^+$). HR-FAB-MS: 449.3619 ($[M-H]^-$, $C_{28}H_{49}O_4^-$; calc. 449.3630).

*(1*R*,1′*R*,2*S*,2′*S*,4a*R*,4′a*R*,6*R*,6′*S*,8a*S*,8′a*S*)-1,1′-(Ethane-1,2-diyl)bis[decahydro-5,5,8a-trimethylnaphthalene-2,6-diol]* (**2**): White powder. M.p. 140–142°. $[\alpha]_D^{23} = +36.36$ ($c = 1.1$, MeOH). IR (KBr): 3440*s* (br.), 2934, 2855, 1634, 1456, 1387, 1367, 1181, 1048, 1004, 985, 955, 581. ^{1}H- and ^{13}C-NMR: *Table.* EI-MS: 450 (1, M^+), 432 (16), 417 (50), 399 (16), 193 (63, $C_{13}H_{21}O^+$ (A/B ring)), 175 (100), 161 ($[C_{13}H_{21}O-H_2O-CH_2]^+$), 147 ($[C_{13}H_{21}O-H_2O-2\ CH_2]^+$), 133 ($[C_{13}H_{21}O-H_2O-3\ CH_2]^+$). FAB-MS: 473 ($[M+Na]^+$). HR-FAB-MS: 473.3599 ($C_{28}H_{50}O_4Na^+$; calc. 473.3606).

*(3*R*,4*S*,4a*R*,6a*S*,7a*S*,8a*R*,10*R*,12a*S*,12b*R*,14a*S*,14b*R*)-Eicosahydro-3,10-dihydroxy-4-(hydroxymethyl)-4,6a,9,9,12a,14b-hexamethylbenzo[*a*]naphth[2,1-f]azulene-7a(1*H*)-carboxaldehyde* (**3**): White powder. M.p. 229–231°. $[\alpha]_D^{23} = -19.35$ ($c = 3.1$, MeOH). IR (KBr): 3441*s* (br.), 2936, 2870, 1707, 1631, 1457, 1385, 1244, 1059, 1033, 995, 606. ^{1}H- and ^{13}C-NMR: *Table.* EI-MS: 474 (2, M^+), 409 (18), 231 (13), 203 (16), 223 (11), 205 (16), 154 (82), 136 (100), 121 (71). HR-FAB-MS: 473.3623 ($[M-1]^-$, $C_{30}H_{49}O_4$; calc. 473.3630).

Antitumor Activity. Human tumor A549 and K562 cell line assays were performed at Kunming Medical College by previously described bioassay methods [13–15]. The IC_{50} values for compounds **3** and **7** (10–100 μg/ml, against human-tumor A549 or K562 cells), indicated moderate antitumor activity.

Acetylcholinesterase Inhibition Activity. Acetylcholinesterase activity of these compounds were determined at 0.6 mg/ml concentration by the *Ellman* method [16][17]. Compounds **4** and **6** showed inhibition activity (20.0 and 39.0%, resp.). Galanthamine was used as the standard drug (inhibition 63.6%)

REFERENCES

[1] H. Zhou, C. H. Tan, S. H. Tan, D. Y. Zhu, *J. Nat. Prod.* **2003**, *66*, 1328.
[2] M. J. Kulshreshtha, D. K. Kushireshtha, R. P. Rastogi, *Phytochemistry* **1972**, *11*, 2369.
[3] P. Pant, R. P. Rastogi, *Phytochemistry* **1979**, *18*, 1095.
[4] Y. Tsuda, Y. Tabata, Y. Ichinohe, *Chem. Pharm. Bull*. **1980**, *28,* 3275.
[5] A. H. Conner, T. P. Haromy, M. Sundaralingam, *J. Org. Chem*. **1981**, *46*, 2987.
[6] C. Y. Yang, *Zhongyao Tongbao.* **1981**, *6*, 12.
[7] K. M. Dai, D. J. Pan, Z. H. Chen, X. Cai, *J. Plant Resource Environ.* **1992**, *1*, 36.
[8] Jingsu Institute of Botany, 'Outline of New China Herbal', Vol. III, The Shanghai Science and Technology Press, Shanghai, 1990, p. 625.
[9] T. Sano, T. Fujimoto, Y. Tsuda, *Chem. Pharm. Bull.* **1975**, *23*, 1784.
[10] X. Cai, D. J. Pan, *Acta Chim. Sin.* **1989**, *47*, 1025.
[11] F. P. Guido, *Planta Med.* **2000**, *66*, 299.
[12] T. Reika, T. Kazuhiro, I. Yasuka, I. Toshimasa, M. Shunyo, T. Harukuni, M. Osamu, *Tetrahedron* **2002**, *58*, 2505.
[13] T. Mosmman, *J. Immunol. Methods* **1983**, *65*, 55.
[14] M. C. Alley, D. A. Scudiero, A. Monks, *Cancer Res.***1988**, *48*, 589.
[15] J. J. Zhou, X. F. Yue, J. X. Han, *Chin. J. Pharm*. **1993**, *24*, 455.
[16] G. L. Ellman, *Arch. Biochem. Biophys.* **1958**, *74*, 443.
[17] G. L. Ellman, D. Courtney, V. Andres, *Biochem. Pharmacol.* **1961**, *7*, 88.

Received September 20, 2004