Synthesis and Insecticidal Activity of Novel 4β-Halogenated Benzoylamino Podophyllotoxins against *Pieris rapae* LINNAEUS

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Twelve new 4β-halogenated benzoylamino compounds (7.1—7.12) of podophyllotoxin have been synthesized, and their structures were confirmed by IR, ^{1}H-NMR, MS spectra as well as CHN elemental analysis. These compounds showed delayed insecticidal activity against 5th instar larvae of *Pieris rapae* LINNAEUS *in vivo*, when tested by a leaf-dipping method at a concentration of 250 ppm. By preliminary qualitative structure–activity relationship analysis, we found the following results: 1) Compounds 7.2, 7.5—7.9 were more potent than the nature parent product in the mortality after 15 d against *P. rapae in vivo.* Especially compounds 7.5 and 7.6 bearing *meta*- and *para*-chlorobenzene substituents respectively, were the most potent of these compounds; 2) Substitution on the benzene ring moiety of 4β-benzoylamino podophyllotoxin (PPT) with Cl, Br, I at the *para* or at the *meta* position yielded compounds which were as potent or more potent than those containing the corresponding substituting group at the *ortho* position. 3) Substitution on the benzene ring moiety of 4β-benzoylamino podophyllotoxin with I either at the *ortho*, *meta* or *para* position yielded less potent compounds (7.10—7.12) when compared with PPT.

Key words podophyllotoxin; botanical pesticide; synthesis; *Pieris rapae*; delayed insecticidal activity

Podophyllotoxin (PPT, **1**) is a phenyltetralin-type of lignan which is widely distributed in higher plants. As a result of the development of etoposide (VP-16, **2**) and teniposide (VM-26, **3**) as anticancer drugs, semi-synthetic analogues of the naturally occurring PPT have drawn much renewed interest in recent years. It is believed that such analogues of 4′-demethylepipodophyllotoxin exert their cytotoxic, antitumor activity through stabilization of a cleavable complex between DNA and type II DNA topoisomerase. This leads ultimately to inhibition of DNA catenation activity and produces single and double strand breaks.[1—3] Meanwhile, It has been reported that deoxypodophyllotoxin (DPT, **4**) has insecticidal,[4—6] phytogrowth inhibitory and ichthytoxic activities.[7] As regards the insecticidal activity, it was confirmed that the symptoms caused by DPT developed slowly; namely, DPT is a delayed toxicant.[4,8,9] This conclusion was supported by the finding that the action of DPT on the 5th instar larvae of silkworm, *Bombyx mori* LINNE, involved severe damage to the epidermal cells accompanied with coagulation of the chromatin.[6] Meanwhile, it was reported that the *trans*-lactone and the 4′-OCH_3 groups in the PPT derivatives were essential to keep the insecticidal activity.[4,10] But up to the present, there has been few reports that deal with insecticidal activity and structure-insecticidal activity relationship studies of PPT analogues.[11—13] According to the known structure–activity relationship on the medical research and our previous studies,[14—20] we found that replacement of the 4β-*O*-glucosidic substituent of compound **2** with 4β-halogenated anilino moiety yielded a number of compounds which were as potent or more potent than **2** in inhibiting the human DNA topoisomerase II and causing cellular protein-linked DNA breakage because the basic nitrogen and the aromatic ring might cause a bond delocalization to generate a charged 4β-N atom. Considering 4β-halogenated benzoylamino moiety might cause a bond delocalization to generate a more stable benzyl onium ion than 4β-halogenated anilino moiety, thereby allowing the alkylation of the target enzyme to occur and causing protein linked DNA breakage, so we herein, in order to find the more potent compounds than PPT, introduced halogenated benzoylamino moiety into PPT at the C-4β position and have synthesized twelve novel 4β-halogenated benzoylamino compounds **7.1**—**7.12**. The insecticidal activity of these new compounds was tested *in vivo* against the 5th instar larvae of *Pieris rapae* LINNAEUS.

Experimental

All melting points were taken on a Kofler melting point apparatus and uncorrected. IR spectra were obtained on a NIC-5DX spectrophotometer. ^{1}H-NMR spectra were obtained by using either a Bruker AM-400 or AC-80 NMR spectrometer. In all cases, samples were dissolved in deuterochloroform and all chemical shifts were reported in ppm from tetramethylsilane (TMS). The elemental analyses were determined on a Carlo Elba 1106 instrument, mass spectral analyses were taken on a VG ZAB-HS instrument at 70 eV and optical rotations were determined on a J-20C spectropolarimeter. Podophyllotoxin (**1**), mp 175—176 °C, $[\alpha]_D^{25}$ −109° (c=1.0, $CHCl_3$), isolated from a Chinese medicinal herb *Podophyllum emodi.* Wall *var.* Chinesis sprague, was used as the starting material. The synthesis route of target compounds was shown in Fig. 2.

4β-Azido Podophyllotoxin (5) PPT (2.0 g, 4.8 mmol) and hydrazoic acid (4.8 ml, C=1.04 M, benzene) were suspended in anhydrous dichloromethane (70 ml) and cooled to below −10 °C and stirred. $BF_3 \cdot Et_2O$ (0.9 ml) was added dropwise so as to keep temperature below −10 °C. After TLC

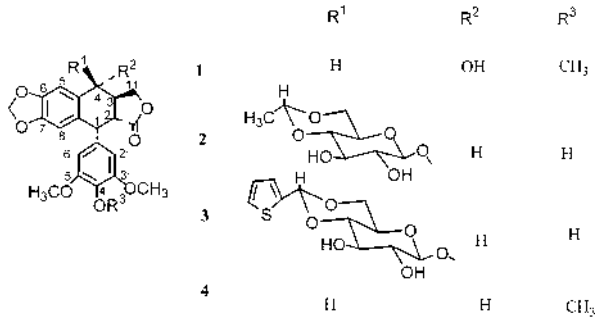

Fig. 1. The Chemical Structures of PPT and Its Analogues

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analysis showed that most of the starting material had been consumed, pyridine (0.9 ml) and distilled water (20 ml) were added. The organic phase was seperated from the mixture, washed by distilled water, 5% hydrochloric acid, distilled water, 2% sodium bicarbonate, distilled water, dried over anhydrous sodium sulfate, and then filtered and evaporated under reduced pressure. The residue was recrystallized in acetone–methanol to produce a white solid.Yield 84.0%, mp 192—194 °C, $[\alpha]_D^{25}$ −80.8° (c=0.5, $CHCl_3$); MS (electron impact (EI)): 439 (M^+, 51%); ^{1}H-NMR δ: ppm: 6.82 (s, 1H, H-5), 6.60 (s, 1H, H-8), 6.28 (s, 2H, H-2′, H-6′), 6.02 (s, 2H, OCH_2O), 4.78 (d, J=3.2 Hz, 1H, H-4), 4.60 (d, J=4.6 Hz, 1H, H-1), 4.34 (d, J=8.0 Hz, 2H, H-11), 3.82 (s, 3H, 4′-OCH_3), 3.70 (s, 6H, 3′, 5′-OCH_3), 3.10 (m, 2H, H-2, H-3); IR (KBr) cm^{-1}: 2102 (N_3), 1768 (γ-lactone), 1580, 1507 and 1484 (aromatic, C=C).

4β-NH_2-podophyllotoxin (6) To a solution of the 4β-azido-podophyllotoxin (2.0 g, 4.6 mmol) in EtOAC (40 ml) was added 10% palladium on carbon (400 mg). This mixture was shaken under 5 atm of H_2 and at 40 °C for 24 h. The reaction mixture was filtered and the filtrate evaporated in vacuum. The residue was chromatographed on a silica gel column and eluted with acetone–petroleum ether to get a white powder. Yield 64.4%, mp 174—176 °C, $[\alpha]_D^{25}$ −18.6° (c=0.5, $CHCl_3$); MS (EI): 413 (M^+, 79%); ^{1}H-NMR δ: ppm: 6.86 (s, 1H, H-5), 6.52 (s, 1H, H-8), 6.28 (s, 2H, H-2′, H-6′), 5.98 (s, 2H, OCH_2O), 4.60 (d, J=4.0 Hz, 1H, H-4), 4.32 (m, 3H, H-11, H-1), 3.85 (s, 3H, 4′-OCH_3), 3.75 (s, 6H, 3′, 5′-OCH_3), 3.40 (dd, 1H, H-2), 2.88 (m, 1H, H-3): IR (KBr) cm^{-1}: 3323 (NH), 1771 (γ-lactone), 1587, 1508 and 1483 (aromatic, C=C).

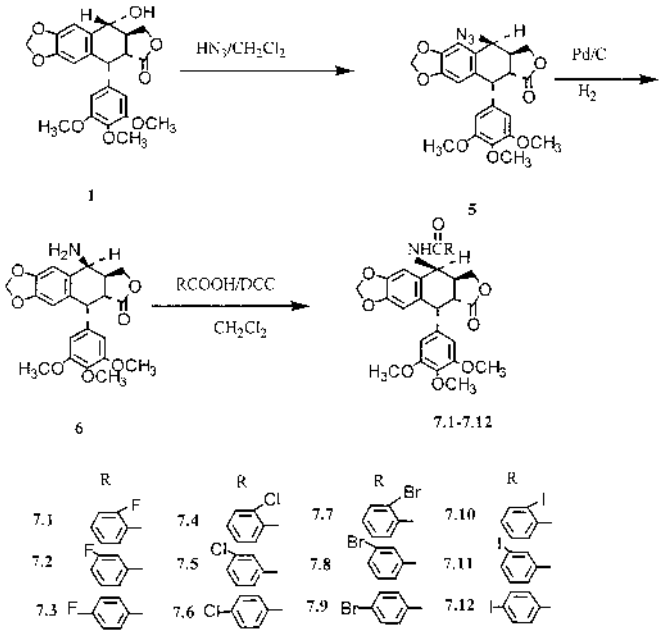

Fig. 2. The Synthesis Route of Compounds **7.1**—**7.12**

General Procedure of Synthesizing Compounds (7.1—7.12) To a solution of compound **6** (2.5 mmol) in dry dichloromethane (40 ml) was added corresponding acid (2.5 mmol) and dicyclohexylcarbodiimide (DCC, 0.65 g). The mixture was stirred at 0 °C until TLC indicated that the reaction was complete. Several drops of glacial acetic acid was added. Then the mixture was filtered, and the filtrate was washed successively by 5 N ammonia liquor, distilled water twice, then dried over anhydrous sodium sulfate. The solvent was evaporated off in vacuum. The residue was purified by silica gel column chromatography using chloroform–methanol as eluent and recrystallized in acetone to produce a pure solid. The analytical data and spectra data were illustrated in Tables 1 and 2, respectively.

Bioassay Leaf-dipping method was used.[21] The 5th instar larvae of *P. rapae* of the same size, health and starvation for 2 h were used, which were gathered from field and reared in room. For each compound, 30 larvae (10 larvae per group) were used. Acetone solutions of compounds **7.1**—**7.12**, PPT, **6** and Parathion (used as a standard) were prepared at a concentration of 250 ppm. Fresh wild cabbage leaves were dipped into the solution for 3 s, then taken out and dried in a room. Leaves treated with acetone alone were used as a control group. Several treated leaves were kept in each dish, and every 10 larvae was raised in it. If the treated leaves were consumed, the corresponding ones were added to the dish. The entire experiment was repricated three times. Every experiment was carried out at 25±2 °C, relative humidity (RH) 65—80% and on 12 h/12 h (light/dark) photoperiod.

The number of deaths was observed at intervals of 5 d for 15 d until emerging adults of *P. rapae.* The final mortality of these compounds against *P. rapae* and control group examined after 5, 10 and 15 d were shown in Table 3.

Discussion and Conclusion

The assignment of the configuration at C-4 position for compounds **5**, **6** and **7.1**—**7.12** was based on $J_{3,4}$ coupling constants. According to Karplus dihedral angle rule in six-membered rings, the C-4 β-substituted compounds have $J_{3,4}\approx$4.0 Hz, due to a cis relationship between H-3 and H-4. While the C-4 α-substituted compounds have $J_{3,4}\geqq$10.0 Hz, as H-3 is trans to H-4.[14]

The insecticidal activity of compounds **7.1**—**7.12**, PPT and **6** against *P. rapae in vivo* at a concentration of 250 ppm was examined by the leaf-dipping method. As shown in Table 3, we found that, in contrast to parathion, the mortalities caused by these compounds were far higher after 15 d than those after 10 and 5 d. That is, these compounds showed delayed insecticidal activity. Meanwhile, we noticed that spontaneous movement in the insects treated by these compounds was little different from that of control insects in 24 h after treatment. But after 48 h some of the treated groups were paralyzed, lost body liquid and were becoming immobilized. Immobilization was increased with the passage of

Table 1. The Analytical Data of Compounds **7.1**—**7.12**

Compd.	Yield (%)	mp (°C)	$[\alpha]_D^{25}$ (°) (c=0.5, $CHCl_3$)	Formula	Calculated/Found (%)		
					C	H	N
7.1	38.6	120—124	−29.2	$C_{29}H_{26}O_8NF\cdot 1/2H_2O$	63.97/64.13	4.96/5.08	2.57/2.82
7.2	36.7	124—127	−82	$C_{29}H_{26}O_8NF\cdot H_2O$	62.93/63.14	5.06/5.18	2.53/2.72
7.3	38.6	138—140	−38	$C_{29}H_{26}O_8NF\cdot H_2O$	62.93/62.86	5.06/4.93	2.53/2.31
7.4	41.2	132—136	−83.6	$C_{29}H_{26}O_8NCl\cdot 1/2H_2O$	62.14/62.47	4.82/4.71	2.50/2.78
7.5	43.1	118—120	−48	$C_{29}H_{26}O_8NCl\cdot H_2O$	61.16/61.29	4.92/4.77	2.46/2.66
7.6	57.9	152—154	−24.8	$C_{29}H_{26}O_8NCl\cdot H_2O$	61.16/61.25	4.92/4.74	2.46/2.71
7.7	52.0	158—160	−96.4	$C_{29}H_{26}O_8NBr\cdot 1/2H_2O$	57.61/57.31	4.47/4.28	2.32/2.54
7.8	52.0	148—150	−48	$C_{29}H_{26}O_8NBr$	58.49/58.24	4.37/4.21	2.35/2.58
7.9	43.3	158—160	−176.8	$C_{29}H_{26}O_8NBr$	58.49/58.41	4.37/4.19	2.35/2.48
7.10	59.4	118—120	−65.6	$C_{29}H_{26}O_8NI\cdot 1/2H_2O$	53.37/53.15	4.14/4.10	2.15/2.11
7.11	72.3	138—140	−56.4	$C_{29}H_{26}O_8NI\cdot 1/2H_2O$	53.37/53.24	4.14/4.01	2.15/2.19
7.12	56.2	161—164	−106.8	$C_{29}H_{26}O_8NI\cdot 1/2H_2O$	53.37/53.18	4.14/4.09	2.15/2.12

Table 2. Spectral Data of Compounds **7.1**—**7.12**

Compd.	IR (KBr)/C cm^{-1}	MS (EI)/*m*/*z*	^{1}H-NMR δ ppm
7.1	3306 (NH), 1777 (γ-lactone), 1655 (NHCO), 1505, 1483 (Aromatic, C=C)	535 (M^+, 8%), 396 (44%), 168 (16%)	7.24—8.06 (4H, aromatic ring H), 6.82 (1H, H-5), 6.58 (1H, H-8), 5.42 (1H, NH)
7.2	3298 (NH), 1776 (γ-lactone), 1651 (NHCO), 1587, 1506, 1483 (Aromatic, C=C)	535 (M^+, 12%), 396 (62%), 168 (32%)	7.20—7.56 (4H, aromatic ring H), 6.82 (1H, H-5), 6.58 (1H, H-8), 5.42 (1H, NH)
7.3	3327 (NH), 1777 (γ-lactone), 1657 (NHCO), 1590, 1532, 1500 (Aromatic, C=C)	535 (M^+, 5%), 396 (31%), 168 (17%)	7.06—7.84 (4H, aromatic ring H), 6.84 (1H, H-5), 6.58 (1H, H-8), 5.42 (1H, NH)
7.4	3284 (NH), 1776 (γ-lactone), 1649 (NHCO), 1590, 1505, 1483 (Aromatic, C=C)	553 (M^+, 2%), 551 (M^+, 6%), 396 (75%), 168 (19%)	7.24—7.72 (4H, aromatic ring H), 6.90 (1H, H-5), 6.52 (1H, H-8), 5.46 (1H, NH)
7.5	3226 (NH), 1777 (γ-lactone), 1640 (NHCO), 1590, 1505, 1483 (Aromatic, C=C)	553 (M^+, 2%), 551 (M^+, 7%), 396 (80%), 168 (35%)	7.28—8.06 (4H, aromatic ring H), 6.82 (1H, H-5), 6.52 (1H, H-8), 5.46 (1H, NH)
7.6	3324 (NH), 1776 (γ-lactone), 1658 (NHCO), 1591, 1505, 1482 (Aromatic, C=C)	553 (M^+, 2%), 551 (M^+, 5%), 396 (52%), 168 (27%)	7.36—7.82 (4H, aromatic ring H), 6.82 (1H, H-5), 6.52 (1H, H-8), 5.40 (1H, NH)
7.7	3260 (NH), 1776 (γ-lactone), 1649 (NHCO), 1588, 1505, 1482 (Aromatic, C=C)	597 (M^+, 5%), 595 (M^+, 5%), 396 (100%), 168 (40%)	7.22—7.59 (4H, aromatic ring H), 6.92 (1H, H-5), 6.56 (1H, H-8), 5.44 (1H, NH)
7.8	3325 (NH), 1776 (γ-lactone), 1659 (NHCO), 1588, 1504, 1484 (Aromatic, C=C)	597 (M^+, 10%), 595 (M^+, 10%), 396 (96%), 168 (46%)	7.24—7.96 (4H, aromatic ring H), 6.84 (1H, H-5), 6.60 (1H, H-8), 5.44 (1H, NH)
7.9	3328 (NH), 1777 (γ-lactone), 1657 (NHCO), 1589, 1504, 1482 (Aromatic, C=C)	597 (M^+, 11%), 595 (M^+, 11%), 396 (100%), 168 (43%)	7.38—7.64 (4H, aromatic ring H), 6.84 (1H, H-5), 6.68 (1H, H-8), 5.44 (1H, NH)
7.10	3271 (NH), 1775 (γ-lactone), 1642 (NHCO), 1585, 1504, 1483 (Aromatic, C=C)	643 (M^+, 7%), 396 (70%), 168 (23%)	7.10—7.94 (4H, aromatic ring H), 6.96 (1H, H-5), 6.56 (1H, H-8), 5.42 (1H, NH)
7.11	3326 (NH), 1775 (γ-lactone), 1628 (NHCO), 1586, 1504, 1484 (Aromatic, C=C)	643 (M^+, 13%), 396 (100%), 168 (47%)	7.20—8.10 (4H, aromatic ring H), 6.80 (1H, H-5), 6.48 (1H, H-8), 5.42 (1H, NH)
7.12	3323 (NH), 1776 (γ-lactone), 1658 (NHCO), 1586, 1504, 1481 (Aromatic, C=C)	643 (M^+, 12%), 396 (72%), 168 (34%)	7.44—7.86 (4H, aromatic ring H), 6.82 (1H, H-5), 6.52 (1H, H-8), 5.42 (1H, NH)

Table 3. Insecticidal Activity of PPT and Its Derivatives against *Pieris rapae* (%)

Entry	Mortality		
	5 d	10 d	15 d
Control	0	6.67±1.56	10±1.22
PPT	34.02±2.44	53.36±2.83	74.74±3.23
6	21.69±2.36	36.43±4.17	49.48±2.86
7.1	22.68±3.47	46.41±1.66	68.42±2.66
7.2	18.57±2.59	49.46±3.65	86.70±2.30
7.3	11.11±1.92	44.74±2.55	66.76±1.70
7.4	4.54±1.33	36.36±3.18	65.56±1.28
7.5	16.56±1.87	54.25±2.75	94.73±1.48
7.6	36.84±2.53	72.08±4.48	94.95±2.86
7.7	4.35±1.02	44.78±1.77	74.74±1.56
7.8	46.15±3.74	69.23±3.15	85.42±0.98
7.9	14.81±2.15	51.85±2.43	85.97±1.98
7.10	12.62±1.95	48.36±1.99	69.69±2.26
7.11	8.35±2.65	40.71±1.64	69.69±3.62
7.12	20.73±3.61	48.28±2.08	73.87±1.88
Parathion	100±0	—	—

a) Temperature: 25±2 °C; RH: 65—80%; photoperiod: light/dark=12 h/12 h. *b*) Experimental size: 10 insects per group, 3 groups. *c*) Concentration: 250 ppm. *d*) Bioassay method: Leaf-dipping method. *e*) Each value represents the mean±S.D.

time. We also found that normal adults emerged from pupae in the control group and some adultoids (*i.e.* adults retaining pupal characteristics) resulting from the PPT and its derivatives treatment failed to reproduce. What is more, the stage of *P. rapae* treated by PPT and its derivatives from the larvae till it reached adulthood was longer than that of control group. In this work, preliminary qualitative analysis showed the relative relationship between the bioactivity and the substituting group. It was shown that hydroxy group at C-4 of PPT merely substituted by amino group (compound **6**) cannot lead to increasing the final mortality. Compounds **7.2**, **7.5**, **7.6**, **7.8** and **7.9** were more potent than PPT in the mortality after 15 d against *P. rapae*. Especially compounds **7.5** and **7.6**, whose final mortalities to *P. rapae* were 94.73% and 94.95% respectively at the concentration of 250 ppm, were the most active of these compounds, *i.e.* The introduction of Cl at the *meta* or *para* position on the benzene ring of 4β-benzoylamino podophyllotoxin will enhance the insecticidal activity to a great extent. We also found that substitution on the benzene ring of 4β-benzoylamino podophyllotoxin with Cl, Br, I at the *para* or at the *meta* position yielded compounds which were as potent or more potent than those containing the corresponding substituting group at the *ortho* position, substitution with F, Cl, I at the *ortho* position of 4β-benzoylamino podophyllotoxin afforded less active compounds (**7.1**, **7.4**, **7.10**), and substitution on the benzene ring moiety of 4β-benzoylamino podophyllotoxin with I either at the *ortho*, *meta* or *para* position yielded less potent compounds (**7.10**—**7.12**) when compared with PPT. These results demonstrate the possibility of further elaboration of the 4β-amino substitutent to optimize the structure of this class of insecticidal compounds. The nature of the difference in mechanism of action between PPT derivatives and conventional insecticide is not clear at present. Further study for insecticidal activity of these synthesized compounds is in progress.

However, there are much work to be done in the future. For example, to gain insight into the mechanism of delayed insecticidal action, compounds **7.2**, **7.5**, **7.6**, **7.8** and **7.9**, which were more active than PPT against *P. rapae*, should be further studied on toxicology. Moreover, as we all know that the activities of compounds are also determined by different insect pests, so other important agricultural insect pests should be added in bioassay later to extend the scope of study. Meanwhile, many compounds of PPT analogues with appropriately substituted group should be synthesized for screening and surveying quantitative structure-activity relationship as to find the biorational pesticide, the latter is being

done in our research group.

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References

1) Stahelin H. F., Wartburg A. V., *Cancer Res*., **51**, 5—15 (1991).
2) Stahelin H. F., *Eur. J. Cancer*, **6**, 303—311 (1970).
3) Loike J. D., Horwitz S. B., *Biochemistry*, **15**, 5435—5443 (1976).
4) Kozawa M., Baba K., Matsuyama Y., Kido T., Sakai M., Takemoto T., *Chem. Pharm. Bull*., **30**, 2885—2888 (1982).
5) Inamori Y., Kato Y., Kubo M., Baba K., Matsuyama Y., Sakai M., Kozawa M., *Chem. Pharm. Bull*., **31**, 4464—4468 (1983).
6) Inamori Y., Kato Y., Kubo M., Waku Y., Hayashiya K., Sakai M., Baba K., Kozawa M., *Chem. Pharm. Bull*., **32**, 2015—2019 (1984).
7) Inamori Y., Kato Y., Kubo M., Baba K., Ishida T., Nomoto K., Kozawa M., *Chem. Pharm. Bull*., **33**, 704—709 (1985).
8) Inamori Y., Kubo M., Tsujibo H., Oki S., Kodama Y., Ogawa K., *Chem. Pharm. Bull*., **34**, 2247—2250 (1986).
9) Inamori Y., Kubo M., Kato Y., Tsujibo H., Sakai M., Kozawa M., *Chem. Pharm. Bull*., **34**, 2542—2549 (1986).
10) Zhang X., Gao R., Tian X., Yu X.Y., *Acta Univ. Agri. Boreali-occidentalis*, **27**, 16—18 (1999).
11) Hansen H. F., Jensen R. B., Willumsen A. M., Norskov L., Ebbesen P., Nielsen P. E., Buchardt O., *Acta Chem. Scan*., **47**, 1190—1200 (1993).
12) Yazawa M., Fukuyama M., Yoshio K., Kato T., Ishikawa Y., *J. Agric. Food Chem*., **47**, 5108—5110 (1999).
13) Garcia E. S., Cabral M. M. O., Schaub G. A., Gottlieb O. R., Azambuja P., *Phytochemistry*, **55**, 611—616 (2000).
14) Lee K. H., Imakura Y., Haruna M., Beers S. A., Thurston L. S., Dai H. J., Chen C. H., Liu S. Y., Cheng Y. C., *J. Nat. Prod*., **52**, 606—613 (1989).
15) Hu H., Wang Z. Q., Liu S. Y., Cheng Y. C., Lee K. H., *J. Med. Chem*., **35**, 866—871 (1992).
16) Wang Z. Q., Hu H., Chen H. X., Cheng Y. C., Lee K. H., *J. Med. Chem*., **35**, 871—877 (1992).
17) Wang Z. Q., Kuo Y. H., Schnur D., Bowen J. P., Liu S. Y., Han F. S., Lee K. H., *J. Med. Chem*., **33**, 2660—2666 (1990).
18) Loike J. D., Brewer C. F., Sternlicht H., Gensler W. J., Horwitz S. B., *Cancer Res*., **38**, 2688—2693 (1978).
19) Dow L. W., Sinkule J. A., Look A. T., Horvath A., Evans W. E., *Cancer Res*., **43**, 5699—5706 (1983).
20) Tian X., Wang Y. G., Yang M. G., Chen Y. Z., *Life Science*, **60**, 511—517 (1997).
21) Take T., Inamori Y., Kato Y., Kubo M., Morimoto K., Morisaka K., Sakai M., Sawada Y., Taniyama H., *Chem. Pharm. Bull*., **28**, 2884—2991 (1980).