琉球大学学術リポジトリ

Glucosides from the Leaves of Cynanchum liukiuense (2)

メタデータ	言語:
	出版者: 琉球大学理学部
	公開日: 2010-07-06
	キーワード (Ja):
	キーワード (En):
	作成者: 荻原, 和仁, 鈴鹿, 俊雅, 比嘉, 松武, 與儀, 誠一
	メールアドレス:
	所属:
URL	http://hdl.handle.net/20.500.12000/17408

Glucosides from the Leaves of *Cynanchum liukiuense* (II)[1]

Kazuhito Ogihara,† Ryouko Chinen,† Toshimasa Suzuka,† Matsutake Higa,† and Seiichi Yogi†

†Department of Chemistry, Biology, and Marine Science, Faculty of Science, University of the Ryukyus, 1 Senbaru, Nishihara-cho, Okinawa 903-0213, Japan

Abstract

Three glucosides were isolated from an *n*-BuOH soluble fraction in a methanol extract of the leaves of *Cynanchum liukiuense* Werb. These compounds were identified as (*1R*, *6R*)-3-oxo-6-hydroxy-α-ionol 11-*O*-β-D-glucopyranoside (**9**), quercetin 3-*O*-β-D-glucopyranoside (**10**), and kaempferol 3-*O*-β-D-glucopyranoside (**11**), respectively, by spectroscopic methods and chemical evidences.

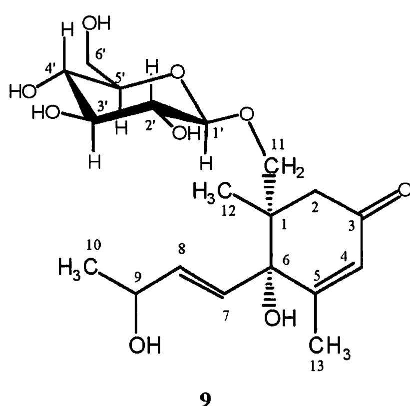
Introduction

Cynanchum liukiuense is a perennial liana and grows in the Sakishima-Islands of Japan. This plant is known as food plant of danaid buttefly, *Salatura genutia*. In connection with a study on the available constituents from food plants of some butterflies in Okinawa, we have previously reported isolation of five triterpenoids (**1-5**), methyl stearate (**6**), and phytosterols (**7a-c**) and phytosterol glycosides (**8a-c**) from the chroloform soluble fraction in the methanol extract of the leaves of *C. liukiuense*. [1)] We continuously examined constituents in the methanol extract of the leaves of *C. liukiuense* and isolated three glucosides from the 1-butanol soluble fraction in the methanol extract.

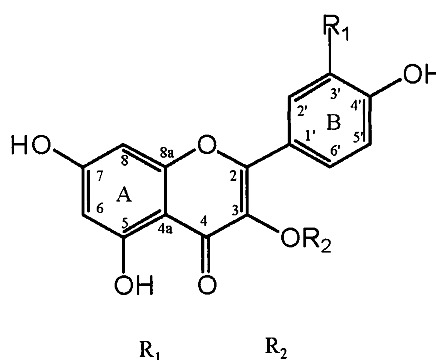
Herein, we describe the separation and identification of these constituents.

Results and Discussion

A 1-butanol soluble fraction from a methanol extract of the leaves of *C. liukiuense* was subjected to column chromatography on silica gel to give **9-11**.



9



	R_1	R_2
10	OH	β-D-glucopyranosyl
10a	OH	H
11	H	β-D-glucopyranosyl
11a	H	H

Structures

Received: January 8, 2010

[1] Part I: K. Ogihara et al., Bull. Fac. Sci. Univ. Ryukyu, **70**, 83 (2000).

Compound **9** was obtained as colorless oil. ^{1}H and ^{13}C NMR spectra of **9** showed 25 proton and 19 carbon signals, respectively. The SIMS showed of a quasi-molecular ion peak at *m/z* 425 due to $[M+Na]^{+}$ and a fragment ion peak at *m/z* 223 due to $[M-180+H]^{+}$, which indicated **9** to be a glycoside with a sugar moiety. The IR spectrum of **9** showed wide bands at 3700-3100 (OH) and 1200-950 cm^{-1} (C-O), which supported **9** to be a glycoside. The IR spectrum also showed a band characteristic to α, β-unsaturated carbonyl group at 1650 cm^{-1}. ^{1}H and ^{13}C NMR signals were completely assigned by means of DEPT, HMQC, and ^{1}H-^{1}H COSY spectroscopic techniques, elucidating **9** to have seven partial structures (**a-g**) shown in Fig. 1. The partial structures were mainly determined by ^{1}H and ^{13}C NMR

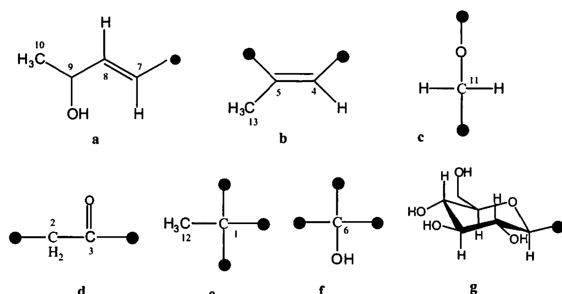


Fig. 1. Partial structures of **9**

spectra and ^{1}H-^{1}H COSY spectra as described below. In the ^{1}H NMR spectrum, each doublet of doublets at δ_H 5.73 (1H, J = 0.5,15.5 Hz) and 5.82 (1H, J = 5.5,15.5 Hz) were assigned as *trans*-olefin protons at H-7 and H-8. In the ^{1}H-^{1}H COSY spectra, a multiplet at δ_H 4.32 showed a cross peak with signal due to H-8 at δ_H 5.82, which indicated this signal to be assigned to H-9 which connected to carbon atom bonded to a oxygen atom. A doublet at δ_H 1.23 showed a cross peak with signal due to H-9 at δ_H 4.32 in the ^{1}H-^{1}H COSY spectra and was assigned to H-10. These results indicated the presence of a 3-hydroxy-1-butenyl moiety as the partial structure **a**. A signal due to H-4 olefinic proton at δ_H 5.89 showed a cross peak with methyl proton signal at δ_H1.90 in the ^{1}H-^{1}H COSY spectra and the signal at δ_H1.90 was assigned to H-13. These facts indicated the presence of a methylvinylene moiety as the partial structure **b**. An AB quartet due to a methylene group bonded to an oxygen atom at δ_H 3.76 was assigned to H-11, which indicated the presence of an oxymethylene group bonded to a quaternary carbon atom as the partial structure **c**. Another AB quartet due to a methylene group bonded to a carbonyl group at δ_H 2.49 was assigned to H-2, which indicated the presence of an oxomethylene group as the partial structure **d**. In the ^{1}H-^{1}H COSY spectra, no relation between a singlet due to a methyl group at δ_H 1.06 and any signals was observed, which indicated the presence of a methyl group bonded to a quaternary carbon atom as the partial structure **e**. In the ^{13}C NMR spectrum, signal at δ_C 79.3 was assigned to the quaternary carbon atom bonded to an oxygen atom, which indicated the presence of a hydroxyl group bonded the quaternary carbon atom as the partial structure **f**. Signals at δ_C 104.6, 75.1, 78.0, 71.5, 77.9, and 62.6 indicated the presence of a sugar moiety which was assigned to glucose by comparison of these data with those described in reference [2] as the partial structure **g**.

Connections of these partial structures were established by means of its HMBC spectral analyses shown in Fig. 2. Observation of a cross peak between

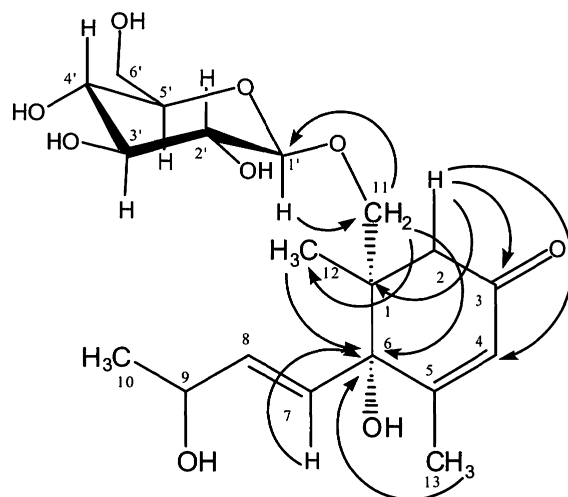


Fig. 2. H-C Interaction observed by HMBC spectra of **9**.

the signal due to H-13 at δ_H 1.90 and that due to C-6 at δ_C 79.3 indicated that C-5 of the partial structure **b** was bonded to C-6 of the partial structure **f**. Observations of each cross peak between the signal due to H-2 at δ_H 2.49 and that due to C-4 at δ_C 127.7, between the signal due to H-2 at δ_H 2.49 and that due to C-1 at δ_C 46.2, and between the signal due to H-12 at δ_H 1.06 and that due to C-6 at δ_C 79.3 indicated that C-3 of the partial structure **d** was bonded to C-4 of the

partial structure **b**, C-2 of **d** to C-1 of **e**, and C-1 of **e** to C-6 of **f**, respectively. These facts indicated that a cyclohexenone skeleton was formed from the partial structures **b**, **d**, **e**, and **f**. Moreover, observations of each cross peak between the signal due to H-7 at δ_H 5.73 and that due to C-6 at δ_C 79.3 and between the signal due to H-11 at δ_H 3.76 and that due to C-1 at δ_C 46.2 suggested that C-7 of the partial structure **a** was bonded to C-6 of the partial structure **f**, and C-11 of the partial structure **c** to C-1 of the partial structure **e**, respectively. Observation of the cross peak between the signals due to anomeric proton (H-1') of the glucose moiety at δ_H 4.13 and that due to C-11 at δ_C 74.5 indicated that C-1' of glucose was bonded to C-11 of the partial structure **c**. The coupling constant of the anomeric proton of the glucose was observed with 7.5 Hz, which indicated that the glucose was bonded to the corresponding aglycone in β-configuration. The compound **9** was hydrolyzed with 0.1M H_2SO_4 to give the D-glucose. Thus, planar structure of **9** was elucidated to be 3-oxo-6-hydroxy-α-ionol 11-*O*-β-D-glucopyranoside (**9**).

In the NOESY spectra of **9**, observation of a cross peak between a signal due to H-12 at δ_H 1.06 and that due to H-7 at δ_H 5.73 and no observation a signal due to H-11 at δ_H 3.76 and that due to H-7 at δ_H 5.73 indicated the relative configuration between 12-CH_3 group and 3-hydroxy-1-butenyl group (partial structure **a**) as *cis*. The absolute configuration at C-6 position was established by application of the CD helicity rule of a cyclohexenone derivative [3]. In the CD spectrum of **9**, curve due to each R and K band of a carbonyl group showed negative and positive Cotton effect, respectively, indicating the absolute configurations of C-1 and 6 as *R* and *R*, respectively.

Thus, compound **9** was identified as (1*R*, 6*R*)-3-oxo-6-hydroxy-α-ionol 11-*O*-β-D-glucopyranoside (**9**). The physical and spectral data of **9** were in agreement with those described in reference [4]. It was already reported that **9** was isolated from a water-soluble fraction of leaves of this plant [4] and that a substance having the same planar structure as **9** was isolated from *Juniperus phoenicea* [5].

Table 1. ^{1}H NMR data and coupling constnts (Hz)* of compounds **9-11**

H	9			10			11		
2	2.49	AB*q*	(17.0)						
4	5.89	*s*							
6				6.34	*d*	(1.9)	6.36	*d*	(1.0)
7	5.73	*dd*	(0.5, 15.5)						
8	5.82	*dd*	(5.5, 15.5)	6.16	*d*	(1.9)	6.18	*d*	(1.0)
9	4.32	*m*							
10	1.23	*d*	(6.0)						
11	3.76	AB*q*	(10.0)						
12	1.06	*s*							
13	1.90	*d*	(1.0)						
1'	4.13	*d*	(7.5)						
2'	3.13	*t*	(8.5)	7.69	*d*	(1.9)	8.03	*d*	(8.9)
3'	3.32	*t*	(8.5)				6.88	*d*	(8.9)
4'	3.26	*t*	(8.5)						
5'	3.20	*m*		6.85	*d*	(8.6)	6.88	*d*	(8.9)
6'	3.64	*dd*	(5.5, 11.7)	7.57	*dd*	(1.9, 8.6)	8.03	*d*	(8.9)
	3.83	*dd*	(2.0, 11.7)						
1''				5.18	*d*	(7.3)	5.19	*d*	(7.3)
2''				3.47	*t*	(8.6)	3.28		
3''				3.41	*t*	(8.6)	|	*m*	
4''				3.35	*m*		3.44		
5''				3.20	*m*		3.21	*m*	
6''				3.56	*dd*	(5.4, 12.0)	3.51	*dd*	(5.1, 11.9)
				3.70	*dd*	(2.2, 12.0)	3.68	*dd*	(2.1, 11.9)

* Coupling constants are in parentheses.

Table 2. ^{13}C NMR spectral data of compounds **9-11**

C	9	10	10a	11	11a
1	46.2				
2	45.5	159.7	148.7	158.9	148.8
3	200.9	136.3	138.0	135.4	137.9
4	127.7	180.1	178.1	179.3	178.1
4a		106.2	105.3	105.4	105.2
5	167.1	163.6	163.3	163.0	163.2
6	79.3	100.7	100.0	100.2	100.1
7	129.6	166.9	166.3	166.9	166.5
8	137.2	95.5	95.1	94.9	95.2
8a		159.1	159	158.5	159.0
9	68.6				
10	23.8				
11	74.5				
12	20.1				
13	19.5				
1'	104.6	123.9	124.9	122.7	124.5
2'	75.1	116.7	116.7	132.2	131.4
3'	78.0	146.5	146.9	116.0	117.0
4'	71.5	150.5	149.5	161.5	161.3
5'	77.9	118.3	117.0	116.0	117.0
6'	62.6	123.7	122.4	132.2	131.4
1"		105.2		104.1	
2"		76.4		75.7	
3"		79.0		78.4	
4"		71.9		71.3	
5"		78.8		78.0	
6"		63.2		62.5	

Compound **10** gave positive UV/NH_3, aluminum chloride, Mg-HCl, and Zn-HCl reactions, which suggested **10** to be a flavonol 3-*O*-glycoside derivative [6]. The compound **10** was suggested to have a molecular formula of $C_{21}H_{20}O_{12}$ by observations of 5 proton and 21 carbon signals in its ^{1}H and ^{13}C NMR spectra, respectively, and of quasi-molecular ion peaks at *m/z* 465 and 487 due to $[M+H]^+$ and $[M+Na]^+$, respectively, in its SIMS and by the reason that **10** was a glycoside derivative. The IR spectrum of **10** showed characteristic bands due to an α, β-unsaturated carbonyl group, aryl alkyl ethers, and an aromatic ring at 1650, 1200 and 1010, and 1600 and 1500 cm^{-1}, respectively. The IR spectrum also showed characteristic bands due to a sugar moiety at 3650-3000 (OH) and 1300-950 cm^{-1} (C-O), which supported **10** to be a flavonol glycoside derivative. The ^{1}H NMR spectrum of **10** showed presences of a 1,2,3,5-tetrasubstituted aromatic ring [δ_H 6.34 (*d*, J = 1.9 Hz) and 6.16 (*d*, J = 1.9 Hz)] and a 1,3,4-trisubstituted aromatic ring [δ_H 7.69 (*d*, J = 1.9 Hz), 7.57 (*dd*, J = 1.9, 8.6 Hz), and 6.85 (*d*, J = 8.6 Hz)]. The ^{13}C NMR spectrum of **10** showed presences of two carbon atoms bonded oxygen atom at δ_C 166.9, 163.6 due to A ring, two carbon atoms bonded to oxygen atom at δ_C 150.5, 146.5 due to B ring, and a carbon atom bonded to oxygen atom at δ_C136.3 due to C-3 of γ-pyrone ring. These spectral data suggested **10** to be a quercetin glycoside.

The compound **10** was hydrolyzed with dil. HCl to give D-glucose and quercetin (**10a**). In the ^{1}H NMR spectrum, the coupling constant of an anomeric proton of the glucose moiety was observed with 7.3 Hz, which indicated the D-glucose was bonded to the corresponding aglycone in β-configuration. In comparison of ^{13}C NMR spectral data of **10** with those of quercetin (**10a**) from **10** by the hydrolysis, the signals due to C-2, 3, and 4 of **10** at δ_C 159.7, 136.3,

and 180.1 were shifted from those due to C-2, 3, and 4 of **10a** at δ_C 148.7, 138.0, and 178.1, which indicated that the D-glucose was located at C-3 position of **10a**.

These spectral data coincided with those described in reference[2]. Thus, compound **10** was identified as quercetin 3-*O*-β-D-glucopyranoside (**10**).

Compound **11** also showed positive UV/NH3, aluminum chloride, Mg-HCl, and Zn-HCl reactions, which suggested **11** to be a flavonol 3-*O*-glycoside derivative. The SIMS showed quasi-molecular ion peaks at *m/z* 449 due to $[M+H]^+$ and 471 due to $[M+Na]^+$ which were an oxygen atom less than **10**. The ^{1}H and ^{13}C NMR spectra of **11** coincided with those of **10**, except for the signals due to B ring. In the ^{1}H NMR spectrum, the signals due to 1,3,4-trisubstituted aromatic ring moiety for **10** disappeared and those due to 1,4-disubstituted aromatic ring moiety [δ_H 8.03 (2H, *d*, *J* = 8.9 Hz) and 6.88 (2H, *d*, *J* = 8.9 Hz)] newly appeared. In the ^{13}C NMR spectrum, a signal due to carbon atom bonded to an oxygen atom for **10** disappeared. Thus, compound **11** was suggested to be a kaempferol glycoside which lacks oxygen atom at B in **10**. The compound **11** was hydrolyzed with dil. HCl to give D-glucose and kaempferol (**11a**). In the ^{1}H NMR spectrum, the coupling constant of an anomeric proton of the glucose moiety was observed with 7.3 Hz, which indicated that the D-glucose was bonded to the corresponding aglycone in β-configuration. In comparison of ^{13}C NMR spectral data of **11** with those of kaempferol (**11a**) from **11** by the hydrolysis, the signals at δ_C 158.9, 135.4, and 179.3 due to C-2, 3, and 4 of **11** were shifted from those at δ_C 148.8, 137.9, and 178.1 due to C-2, 3, and 4 of **11a**, which indicated that the D-glucose was located at C-3 position of **11a**.

These spectral data coincided with those described in reference[2]. Thus, compound **11** was identified as kaempferol 3-*O*-β-D-glucopyranoside (**11**).

Experimental

Analytical TLC was carried out on Merck 60 F_{254} silica gel plate (thickness: 0.25 mm). HPLC analyses for D-glucose were performed on a Waters HPLC 600 instrument equipped with an ORD (OR-990, Jasco Co. Ltd) and a polyamide II column (4.6 I.D. x 300 mm, YMC Co. Ltd) with a solvent system of MeCN-H_2O (3:1) at a flow rate of 2 mL/min. ^{1}H (500 and 270 MHz) and ^{13}C NMR (125 and 67.5 MHz) spectra were taken on JEOL α 500 and EX-270 spectrometer in CD_3OD with TMS as int. standard. SIMS spectra were obtained on a Hitachi M-2500 double focusing mass spectrometer. CD spectrum was recorded on a Jasco J-720 spectropolarimeter.

Extraction and isolation. Fresh leaves (5.9 kg) of *C. liukiuense*, collected at Hateruma Island, Okinawa-prefecture in April, were ground in a mixer and immersed in MeOH for 1 month. The MeOH soln was concd *in vacuo* and the obtained concentrate (201.6 g) was suspended with H_2O. The suspension was partitioned succesively with Hexane, $CHCl_3$, EtOAc, and *n*-BuOH. The *n*-BuOH layer was concd *in vacuo* and the BuOH-soluble fraction (2.56 g) was subjected to column chromatography on silica gel developed with a solvent system of $CHCl_3$–MeOH-H_2O (7:3:0.5) to give 13 fractions. Fractions 7 and 8 were re-chromatographed on a C-18 column developed with MeOH-H_2O (1:4) to give **9** (33 mg). Each fraction 11 and 12 was individually subjected to gel-filtration on Sephadex LH-20 with MeOH to give **10** (34 mg) from fraction 11 and **11** (24 mg) from fraction 12, respectively.

(1S,6S)-3-Oxo-6-hydroxy-α-ionol 11-O-β-D-glucopyranoside (**9**). Colorless oil. $[\alpha]_D^{25}$ +44.2 (*c* 0.17, MeOH); CD (MeOH):λ_{ext} 400.0 ([θ]=0.0), 318.4 (-1900.8), 286.0 (0.0), 243.2 (23100), 205.0 (0.0); IR υ_{max}^{KBr} cm^{-1}: 3700-3100 (OH), 1650 (C=C-C=O), 1200-950 cm^{-1} (C-O); ^{1}H (500 MHz) and ^{13}C NMR (125 MHz): see Tables 1 and 2; SIMS *m/z* (rel. int.): 425 ($[M+Na]^+$, 23), 223 ($[M\text{-}glc+H]^+$, 5). The physical and spectral data coincided with those described in reference [4].

***Hydrolysis of* 9**. According to the method described in reference [1], **9** was hydrolyzed. The compound **9** (3 mg) dissolved in *n*-BuOH and 0.1M H_2SO_4 (1:1) were heated at 80˚C for 2 hr. After hydrolysis, reaction mixture was shaken with $CHCl_3$ and H_2O. The H_2O layer was subjected to HPLC analysis and D-glucose was detected.

Quercetin 3-O-β-D-glucopyranoside **(10)**.

Yellow amorphous, mp 258°C (decomposed); IR υ_{max}^{KBr} cm^{-1}: 3650-3000 (OH), 1650 (C=C-CO), 1200 and 1010 (ph-o), 1600 and 1500 (aromatic ring), and 1300-950 cm^{-1} (C-O); ^{1}H (270 MHz) and ^{13}C NMR (67.5 MHz): see Tables 1 and 2; SIMS *m/z* (rel. int.): 487 ([M+Na]$^+$, 5), 465 ([M+H]$^+$, 15). The physical and spectral data coincided with those described in reference[2].

***Hydrolysis of* 10**. The compound **10** (17 mg) dissolved in 1.5 mL of 2M HCl were heated at 80°C for 1 hr. After hydrolysis, reaction mixture was shaken with $CHCl_3$ and H_2O. The $CHCl_3$ layer was washed with water and dried over anhydrous Na_2SO_4. The $CHCl_3$ layer, after concd, was subjected to a column chromatography on Si-gel to give quercetin (**10a**, 6 mg): light yellow powder, mp > 300°C ([lit. [2]: 314°C]); ^{13}C NMR (125 MHz): see Table 2. The H_2O layer was subjected to HPLC analysis and D-glucose was detected.

Kaempferol 3-O-β-D-glucopyranoside (**11**). Yellow amorphous, mp 255°C (decomposed); IR υ_{max}^{KBr} cm^{-1}: 3650-3000 (OH), 1650 (C=C-CO), 1200 and 1010 (ph-O), 1600 and 1500 (aromatic ring), and 1300-950 cm^{-1} (C-O); ^{1}H (270 MHz) and ^{13}C NMR (67.5 MHz): see Tables 1 and 2; SIMS *m/z* (rel. int.): 471 ([M+Na]$^+$, 30) and 449 ([M+H]$^+$, 10). The physical and spectral data coincided with those described in reference [2].

***Hydrolysis of* 11**. The compound **11** (12 mg) dissolved in 1.5 mL of 2M HCl were heated at 80°C for 4 hr. After hydrolysis, reaction mixture was shaken with *n*-BuOH and H_2O. The *n*-BuOH layer was washed with water and was subjected to a column chromatography on Si-gel to give kaempferol (**11a**, 4 mg): light yellow powder, mp. 259-264°C (lit. [2]: mp. 276-278°C); ^{13}C NMR (125 MHz): see Table 2. The H_2O layer was subjected to HPLC analysis and D-glucose was detected.

Acknowledgements---The authors thank Professor Tatsuo Higa, Faculty of Science, University of the Ryukyus, for the use of ^{1}H (500 and 270 MHz) and ^{13}C NMR (125 and 67.5 MHz) spectrometer, Dr. Yosikazu Hiraga, Faculty of Science, Hiroshima University, for the measurement of CD spectrum, and Professor Masayuki Kuniyoshi, Faculty of Science, University of the Ryukyus, for his helpful advices and useful comments on the manuscript.

References

1) K. Ogihara, S. Nishizato, M. Ishigaki, M. Higa and S. Yogi, *Bull. Fac. Sci. Univ. Ryukyus*, **70**, 83-92 (2000).

2) J.B. Harborne, T.J. Mabry, *The Flavonoids:Advances in Research*, Chapman and Hall, London, New York (1982).

3) In *Shin-jikkenkagaku-kouza*, 13 (II), p 783 , Maruzen, Tokyo (1977).

4) F. Abe, T. Yamauchi, K. Honda, and N Hayashi, *Chem. Pharm. Bull.*, **48** (7), 1090-1092 (2000).

5) Y. Champavier, G. Comte, J. Vercarteren, D.P. Allais, A. J. Chulia, *Phytochemistry*, **50**,1219-1223 (1999).

6) T. Murakami and T. Okamoto, in *Tennenbutsukagaku 2nd ed.*, pp 209-210, Hirokawa, Tokyo (1981).