Pergamon

PII: S0031-9422(98)00126-5

Phytochemistry, Vol. 49, No. 3, pp. 853–857, 1998

Printed in Great Britain
0031-9422/98/$—see front matter

GARSUBELLINS, POLYISOPRENYLATED PHLOROGLUCINOL DERIVATIVES FROM *GARCINIA SUBELLIPTICA*

YOSHIYASU FUKUYAMA*, HIROYUKI MINAMI and ATSUKO KUWAYAMA

Institute of Pharmacognosy, Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770, Japan

(*Received in revised form* 26 *January* 1998)

Key Word Index—*Garcinia subelliptica*; Guttiferae; wood; garsubellins A–E; phloroglucinol; bicyclo[3,3,1]nonane.

Abstract—Four new phloroglucinol derivatives, named garsubellins B ~ E, have been isolated from the wood of *Garcinia subelliptica*. Their structures have been elucidated by extensive analysis of spectroscopic data and comparison with those of previously known garsubellin A. Garsubellins which represent novel polyisoprenylated phloroglucinols having a hydrofuran ring are regarded as chemical components characteristic of the title species.

INTRODUCTION

Garcinia subelliptica is cultivated as a windbreak in the Okinawa islands, Japan. Its bark had been utilized as a source of yellow coloured dye and, thus, the chemical components of *G. subelliptica* are known to contain a number of xanthones with a variety of substituents [1–3], like other *Garcinia* species [4]. In preceding papers, we reported the isolation and structural elucidation not only of several prenylated xanthones which exhibited neurotrophic and antioxidant activity [5–8], but also of garsubellin A (**1**), a unique polyisoprenylated phloroglucinol derivative, which increased choline acetyltransferase activity in P10 rat septal neuron cultures [9]. In pursuit of biologically active substances, we have continued to examine the chemical components of the wood of *G. subelliptica* and now report on the isolation and characterization of four new isoprenylated phloroglucino derivatives **2**–**5** from this source.

RESULTS AND DISCUSSION

The wood was extracted with methanol and the methanol extract partitioned between ethyl acetate and water. The ethyl acetate-soluble portion was fractionated by repeated CC on silica gel and Sephadex LH-20 to give four new isoprenylated phloroglucino derivatives, named garsubellin B (**2**), garsubellin C (**3**), garsubellin D (**4**) and garsubellin E (**5**).

Garsubellin B (**2**) had the molecular formula ($C_{31}H_{46}O_5$) (HR-FAB-mass spectrum *m/z* 499.3395 $[M+1]^+$) and its ^1H and ^{13}C NMR spectra (Tables 1 and 2) resembled that of garsubellin A (**1**) [9], except for the presence of an ethyl group [δ_H 0.79 (3H, *dd*, J = 7.4, 7.4 Hz), 2.33 (1H, *ddd*, J = 13.7, 7.4, 2.4 Hz) and 1.69 (1H, *ddd*, J = 13.7, 9.5, 7.4 Hz); δ_C 11.7 and 27.0] instead of the methyl group at the C-30 position in **1**. Its IR and UV spectra also showed absorptions identical to those of **1**. Additionally, the analyses of H-H COSY and HMQC of **2** indicated the presence of a *sec*-butyl group (Fig. 1), which was not involved in the structure of **1**. These spectral data suggest that **2** is another phloroglucinol derivative, in which the C-30 methyl group of **1** is replaced by the ethyl group. This partial unit was clarified to bond to the C-27 carbonyl carbon on the basis of the HMBC correlations of the C-27 signal [δ_C 208.1] with the H_3-29 and the H-28 signals. Garsubellin B was concluded to have the same relative stereochemistry as that of garsubellin A (**1**) based on the results of 2 D NOESY experiments. Thus, the structure of garsubellin B was represented as **2**.

Garsubellin D (**4**) was found to have the same molecular formula ($C_{30}H_{44}O_5$) (HR-FAB-mass spectrum, *m/z* 507.3087 $[M+Na]^+$) as that of garsubellin A (**1**) and its NMR data (Tables 1 and 2) were very similar to those of **1**, indicating that **4** was another phloroglucinol derivative which was closely related to garsubellin A. The ^1H-^1H COSY and HMQC spectra showed the presence of four structural fragments **A** ~ **D** and a dimethyl carbinol group (δ_H 0.79 and 0.90; δ_C 24.0, 24.7 and 71.4) (Fig. 2), which garsubellin A is also made up of, as well as of a tetrasubstituted

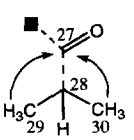
* Author to whom correspondence should be addressed.

Table 1. ^{1}H NMR data of compounds **1**–**5** (400 MHz, δ in C_6D_6)

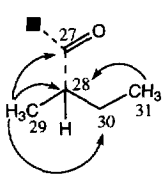
H	1	2	3	4	5
7	1.30 *dd* (13.6, 7.3)*	1.29 *dd* (13.4, 5.6)	1.37 *dd* (14.7, 13.7)	1.32 *dd* (13.5, 13.5)	1.31 *dd* (13.4, 13.2)
	1.93 *dd* (13.6, 4.5)	1.92 *dd* (13.4, 4.6)	1.97 *dd* (13.7, 4.4)	1.84 *dd* (13.5, 3.9)	1.84 *dd* (13.4, 3.9)
8	1.73 *m*	1.75 *m*	1.72 *dddd* (14.7, 13.9, 4.4, 2.5)	1.54 *m*	1.55 *m*
10	1.24 *s*	1.25 *s*	1.20 *s*	1.18 *s*	1.19 *s*
11	1.60 *s*	1.61 *s*	1.60 *s*	1.54 *s*	1.56 *s*
12	1.58 *m*	1.54 *m*	1.58 *ddd* (13.9, 7.6, 2.5)	1.45 *m*	1.45 *m*
	2.09 *ddd* (13.4, 7.1, 3.6)	2.06 *brdd* (13.7, 7.2)	2.10 *ddd* (13.9, 13.9, 7.6)	2.02 *m*	2.02 *m*
13	4.96 *dd* (7.1, 7.1)	4.95 *dd* (7.2, 7.2)	5.03 *dd* (7.6, 7.6)	4.94 *dd* (6.6, 6.6)	4.94 *dd* (7.6, 6.8)
15	1.45 *s*	1.44 *s*	1.43 *s*	1.40 *s*	1.40 *s*
16	1.58 *s*	1.57 *s*	1.58 *s*	1.61 *s*	1.60 *s*
17	1.30 *dd* (12.9, 5.9)	1.23 *dd* (12.9, 5.9)	2.66 *dd* (14.9, 10.7)	2.59 *dd* (15.1, 10.5)	2.57 *dd* (15.1, 10.5)
	2.73 *dd* (12.9, 10.7)	2.71 *dd* (12.9, 10.5)	2.84 *dd* (14.9, 7.6)	2.73 *dd* (15.1, 7.8)	2.72 *dd* (15.1, 8.1)
18	3.92 *dd* (10.7, 5.9)	3.90 *dd* (10.5, 5.9)	4.01 *dd* (10.7, 7.6)	4.20 *dd* (10.5, 7.8)	4.18 *dd* (10.5, 8.1)
20	0.94 *s*	0.92 *s*	0.92 *s*	0.90 *s*	0.89 *s*
21	0.77 *s*	0.76 *s*	0.76 *s*	0.79 *s*	0.78 *s*
22	3.21 *dd* (14.2, 7.3)	3.20 *dd* (14.2, 7.7)	2.41 *dd* (16.8, 4.9)	2.46 *dd* (14.9, 8.3)	2.43 *dd* (13.7, 7.8)
	3.39 *dd* (14.2, 7.1)	3.37 *dd* (14.2, 7.3)	2.61 *dd* (16.8, 4.9)	2.64 *dd* (14.9, 6.6)	2.65 *dd* (13.7, 6.3)
23	5.40 *dd* (7.3, 7.1)	5.40 *dd* (7.7, 7.3)	5.37 *dd* (4.9, 4.9)	5.48 *dd* (8.3, 6.6)	5.45 *dd* (7.8, 6.3)
25	1.70 *s*	1.69 *s*	1.53 *s*	1.64 *s*	1.66 *s*
26	1.61 *s*	1.60 *s*	1.57 *s*	1.66 *s*	1.68 *s*
28	2.26 *qq* (6.6, 6.6)	2.05 *ddq* (9.5, 6.6, 2.4)	2.40 *qq* (6.6, 6.6)	2.45 *qq* (6.6, 6.6)	2.23 *m*
29	1.30 *d* (6.6)	1.32 *d* (6.6)	1.31 *d* (6.6)	1.36 *d* (6.6)	1.41 *d* (6.6)
30	1.37 *d* (6.6)	1.69 *ddd* (13.7, 9.5, 7.4)	1.40 *d* (6.6)	1.45 *d* (6.6)	1.71 *m*
		2.33 *ddd* (13.7, 7.4, 2.4)			2.43 *m*
31		0.79 *dd* (7.4, 7.4)			0.90 *dd* (7.6, 7.6)

*Coupling constants (J in Hz) given in parentheses.

double bond (δ_C 176.3 and 118.7). The HMBC of **4** disclosed long-range correlations similar to those of **1** (Fig. 2), except for the following differences. The H-22 in the partial unit **B** correlated to the C-2 quaternary carbon (δ_C 55.1) and the C-1 carbonyl carbon (δ_C 206.4), whereas the H-7 [δ_H 1.32 (*dd*, J = 13.5, 13.5 Hz) and 1.84 (*dd*, J = 13.5, 3.9 Hz)] showed a long-range correlation to the C-1 and C-2 signals. Thereby, a bicyclo-ring in **4** was indicated at the C-2 and C-6 positions, unlike being at the C-4 and C-6 positions, in the case of **1** and **2**. The respective HMBC correlations of the H-18 [δ_H 4.20 (*dd*, J = 10.5, 7.8 Hz)] and H-17 [δ_H 2.59 (*dd*, J = 15.1, 10.5 Hz) and 2.73 (*dd*, J = 15.1, 7.8 Hz)] with the C-3 quaternary olefinic carbon and the C-3 and C-4 carbons (Fig. 2) indicated



Garsubellins A (**1**), C (**3**), D (**4**)



Garsubellins B (**2**), E (**5**)

Fig. 1. Isopropyl and *sec*-butyl groups characteristic of garsubellins A (**1**)–E (**5**) confirmed by the HMBC correlation indicated by arrows between the proton (tail) and carbons (heads).

that the sole tetrasubstituted double bond was involved in the dihydrofuran ring, at the C-18 position on which the dimethyl carbinol group turned out to be bonded by the distinct HMBC correlation between H-18 and C-19 (δ_C 71.4). Although the C-6 quaternary carbon had no other cross-peaks in the HMBC, it should be adjacent to the three carbonyl groups (C-1, C-5 and C-27) to satisfy its low chemical shift (δ_C 83.6). Considering the above spectral evidence and the molecular formula, the planar structure of **4** was proposed (Fig. 2). The relative configuration at C-2 and C-6 are fixed by the bicyclo[3,3,1]nonane framework. Thus, the relative stereochemistry of the chiral carbons at C-8 and C-18 must be clarified. Judging from the large J values (13.5 Hz) for the H-7 methylene vicinal to H-8 (Table 1), an isoprepenyl group involved in the partial unit **A** should take an equatorial orientation at the C-8 position (8*S**). In a NOESY experiment, H-8 showed cross-peaks to the H_3-20 and H_3-21 signals (Fig. 3), thereby assigning C-18 to the *R** configuration. Accordingly, the relative stereostructure of garsubellin D (**4**) was elucidated as shown in Fig. 3.

The molecular formula and the spectral data of garsubellin C (**3**) were very similar to those of garsubellin D (**4**). The planar structure of **3** which was derived by the extensive analyses of various 2 D NMR spectra was identical to that of **4**. The H-18 signal

Table 2. ^{13}C NMR data of compounds **1**–**5** (100 MHz, δ in C_6D_6)

C	1	2	3	4	5
1	192.9	193.0	206.5	206.4	206.5
2	116.7	116.7	54.8	55.1	55.2
3	173.2	173.0	175.9	176.3	176.2
4	59.8	59.8	119.5	118.7	118.7
5	204.7	204.7	186.8	187.1	187.1
6	82.6	82.6	83.6	83.6	83.7
7	39.0	39.2	39.3	38.6	38.8
8	43.0	43.0	43.8	43.8	43.8
9	46.6	46.7	46.5	46.4	46.6
10	16.5	16.5	16.0	16.1	16.0
11	23.1	23.1	23.7	23.5	23.6
12	27.0	27.0	27.3	26.9	26.9
13	123.2	123.2	123.3	123.4	123.4
14	133.2	133.1	133.2	133.1	133.1
15	17.8	17.8	17.9	17.7	18.3
16	25.9	25.9	25.8	25.8	25.8
17	30.3	30.3	27.3	27.6	27.6
18	90.1	90.1	93.2	92.4	92.4
19	70.2	70.3	71.2	71.4	71.4
20	26.3	26.2	25.9	24.7	24.6
21	24.4	24.3	23.5	24.0	23.9
22	22.6	22.6	29.8	29.6	29.6
23	122.0	122.0	120.9	119.4	119.4
24	132.4	132.4	134.2	134.7	134.0
25	17.9	17.9	18.1	18.1	18.3
26	25.7	25.7	25.9	26.1	26.2
27	208.5	208.1	208.5	208.6	208.2
28	42.7	49.6	43.1	43.0	50.0
29	21.9	18.0	22.0	21.9	17.6
30	20.9	27.0	21.0	20.7	27.3
31		11.7		11.9	

Assignments confirmed by HMQC and HMBC.

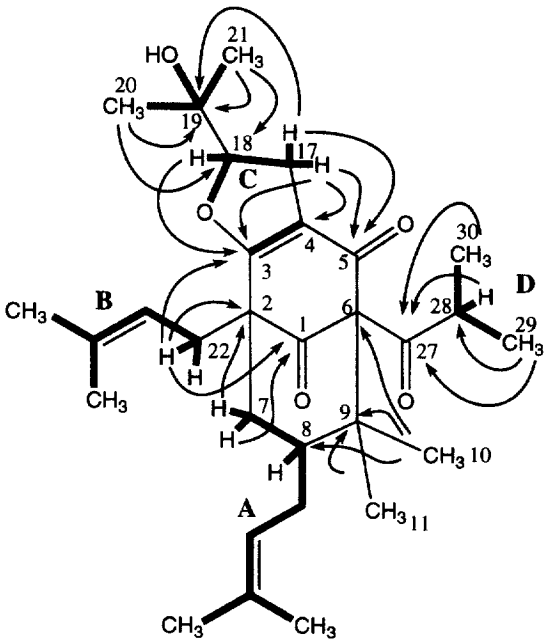


Fig. 2. Bold lines indicate the partial structures (A–D) for garsubellin D (**4**) inferred from H-H COSY and HMQC. Arrows denote the HMBC ($J_{C\cdots H}$ = 8.1 Hz) correlation between the proton (tail) and carbons (heads).

appeared at a higher field (δ_H 4.01) than **4** (Table 1), suggesting that **3** was an epimer with regard to C-18 in **4**. Contrary to the case of **4**, no NOE interaction was observed between the axial H-8 and H_3-20 or H_3-21. Thus, the structure of garsubellin C (**3**) was assigned as the stereoisomer with the 18*S** configuration of garsubellin D (**4**).

Garsubellin E (**5**) was found to have the same molecular formula ($C_{31}H_{46}O_5$) (HR-FAB-mass spectrum, *m/z* 521.3259 $[M+Na]^+$) as that of **2** and its NMR data (Tables 1 and 2) showed the presence of a *sec*-butyl group (Fig. 1). Additionally, all the 2 D NMR data for **5** were consistent with the planar structure which substituted the C-30 methyl group in **4** with an ethyl group. The relative stereochemistry for **5** was assigned to be the same as that of **4** on the basis of the presence of cross-peaks between the H-8 (δ_H 1.55) and the H_3-20, 21 (δ_H 0.78 and 0.89) signals in the NOESY. Thus, the structure of garsubellin E (**5**) was represented as the ethyl derivative at C-30 of garsubellin D (**4**).

A number of polyisoprenylated phloroglucinol derivatives have been isolated from *G. subelliptica* and other *Garcinia* species [4, 11]. Most of them belong to a benzophenone-type which is regarded as the precursor of biosynthesis of xanthones [12], whereas garsubellins A ~ E and subellinone (**6**) [13], having an isopropyl- or a *sec*-butyl group, are considered to be closely related to humulone and lupulone, phloroglucinol derivatives occurring in *Humulus lupulus* [14, 15]. It should be noted that phloroglucinol derivatives like garsubellins were isolated for the first time from *Garcinia* plants which are rich in xanthones.

EXPERIMENTAL

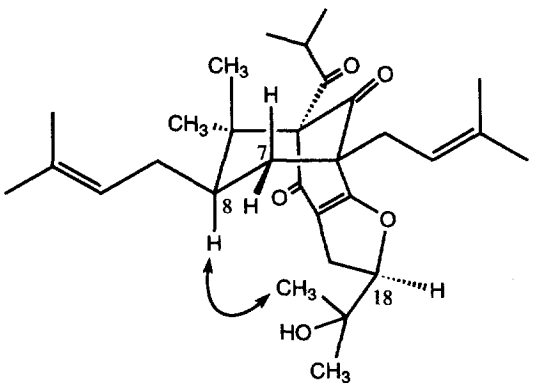
^1H and ^{13}C NMR: TMS as int. standard. CC: silica gel (Merck, 230 ~ 400 mesh and Wakogel C-300) and Sephadex LH-20 (Pharmacia). TLC: precoated silica gel F254 (Merck). Spots were visualized by UV (254 nm) and 10% $CeSO_4$–H_2SO_4.

Plant material

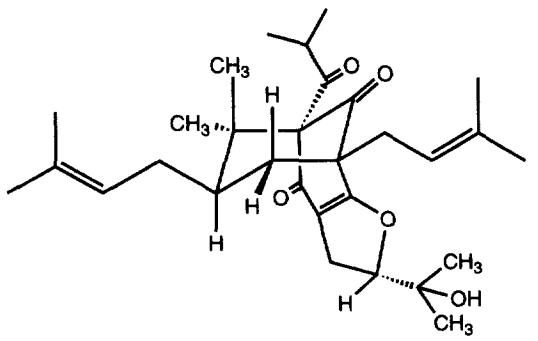
Garcinia subelliptica Merr. was collected in Ishigaki Island, Japan, and identified by Dr Hiroyuki Murata (Ibusuki, Kagoshima, Japan). A voucher specimen is deposited at the Institute of Pharmacognosy.

Extraction and isolation

The MeOH extract was partitioned between EtOAc and H_2O. The EtOAc-sol. portion (150 g) was mixed with Celite (150 g) and the solvent was completely removed *in vacua* to give solids, which were pulverized.



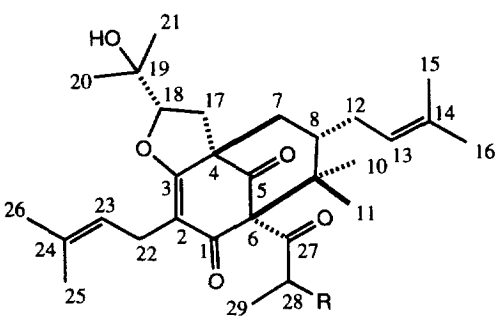
4



3

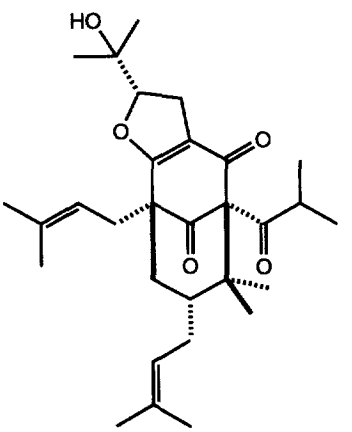
Fig. 3. Relative stereochemistry for garsubellin D (**4**) and garsubellin C (**3**) based on NOESY (arrow) and *J* values of H-8.

The resultant powder was packed into a glass column and then eluted in turn with *n*-hexane (1.5 l), CH_2Cl_2 (1.5 l), EtOAc (1 l) and MeOH (1 l) giving 6 frs (1–6). Fr. 3 (70 g) was sepd by CC on silica gel (Merck) with CH_2Cl_2–MeOH (9 : 1) to give 10 frs (7–17). Fr. 8 (2.5 g) was again sepd by CC on silica gel (C-300) with CH_2Cl_2–EtOAc (25 : 1) to give 16 frs (18–24). Fr. 20 (1 g) was purified by repeated CC on silica gel (C-300)

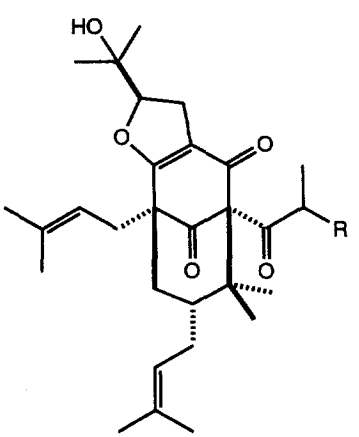


1 R = $^{30}CH_3$

2 R = $^{30}CH_2{}^{31}CH_3$

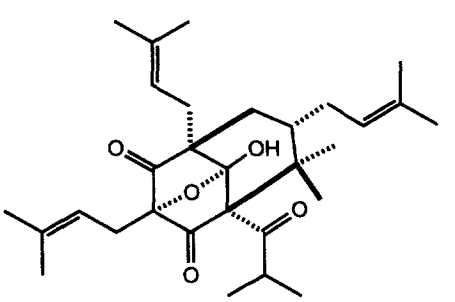


3



4 R = CH_3

5 R = CH_2CH_3



6

with CH_2Cl_2–EtOAc (3 : 1) followed by CC on LH-20 with MeOH to give compounds **1** (25 mg) and **2** (5 mg). Fr. 23 (350 mg) was sepd by CC on Cosmosil 75C_{18}-OPN with MeOH–MeCN–H_2O (4 : 1 : 1) to give 8 frs (25–33). Compound **5** (7 mg) was obtained from fr. 32. Fr. 26 (140 mg) was again sepd by CC on silica gel with CH_2Cl_2–Me_2CO (40 : 1) to give compounds **3** (4 mg) and **4** (10 mg).

Garsubellin B (**2**). Colourless oil. $[\alpha]_D^{24}$ −36 (*c* 0.6, EtOH). UV λ_{max}^{EtOH} nm: 269 (ε 12300). IR ν_{max}^{FT} cm^{-1}: 3437 (OH), 1730 (C=0), 1620. HR-FAB-MS: 499.3395 $[M+1]^+$ (calcd 499.3424 for $C_{31}H_{47}O_5$). EIMS *m/z* (rel. int.): 498 $[M]^+$ (12), 429 (8), 397 (10), 361 (100). ^1H and ^{13}C NMR: Tables 1 and 2.

Garsubellin C (**3**). Colourless oil. $[\alpha]_D^{24}$ +39 (*c* 0.4, EtOH). UV λ_{max}^{EtOH} nm: 278 (ε 13000). IR ν_{max}^{FT} cm^{-1}: 3441 (OH), 1725 (C=0), 1620. HR-FAB-MS: 507.3087 $[M+Na]^+$ (calcd 507.3087 for $C_{30}H_{44}O_5Na$). FAB-MS *m/z* (rel. int.): 507 $[M+Na]^+$ (25), 485 $[M+1]^+$ (58), 417 (50), 293 (80). ^1H and ^{13}C NMR: Tables 1 and 2.

Garsubellin D (**4**). Colourless oil. $[\alpha]_D^{24}$ −12 (*c* 0.37, EtOH). UV λ_{max}^{EtOH} nm: 278 (ε 27000). IR ν_{max}^{FT} cm^{-1}: 3450 (OH), 1728 (C=0), 1622. HR-FAB-MS *m/z* (rel. int.): 507.3064 $[M+Na]^+$ (calcd 507.3087 for $C_{30}H_{44}O_5Na$). FAB-MS *m/z* (rel. int.): 507 $[M+Na]^+$ (25), 485 $[M+1]^+$ (58), 417 (50), 293 (80). ^1H and ^{13}C NMR: Tables 1 and 2.

Garsubellin E (**5**). Colourless oil. $[\alpha]_D^{24}$ −7 (*c* 0.44, EtOH). UV λ_{max}^{EtOH} nm: 278 (ε 14000). IR ν_{max}^{FT} cm^{-1}: 3488 (OH), 1728 (C=0), 1622. HR-FAB-MS *m/z* (rel. int.): 521.3259 $[M+Na]^+$ (calcd 521.3243 for $C_{31}H_{46}O_5Na$). FAB-MS *m/z* (rel. int.): 521 $[M+Na]^+$ (84), 499 $[M+1]^+$ (68), 431 (40), 397 (42), 307 (100). ^1H and ^{13}C NMR: Tables 1 and 2.

Acknowledgements—We are indebted to Miss Yasuko Okamoto (TBU) for MS measurements. This work was partially supported by a Grant-in-Aid for Scientific Research (No. 09680582) from the Ministry of Education, Science, Sports and Culture, Japan.

REFERENCES

1. Peres, V. and Nagem, T. J., *Phytochemistry*, 1997, **44**, 191.
2. Iinuma, M., Tosa, H., Tanaka, T., Shimano, R., Asai, F. and Yonemori, S., *Phytochemistry*, 1994, **35**, 1355.
3. Iinuma, M., Tosa, H., Tanaka, T., Asai, F. and Shimane, R., *Heterocycles*, 1995, **40**, 279.
4. Bennett, G. J. and Lee H.-H., *Phytochemistry*, 1989, **28**, 967.
5. Fukuyama, Y., Kamiyama, A., Mima, Y. and Kodama, M., *Phytochemistry*, 1991, **30**, 3433.
6. Minami, H., Kinoshita, M., Fukuyama, Y., Kodama, M., Yoshizawa, T., Sugiura, M., Nakagawa, K. and Tago, H., *Phytochemistry*, 1994, **36**, 501.
7. Minami, H., Takahashi, E., Fukuyama, Y., Kodama, M., Yoshizawa, T. and Nakagawa, K., *Chemical and Pharmaceutical Bulletin*, 1995, **43**, 347.
8. Minami, H., Kuwayama, A., Yoshizawa, T. and Fukuyama, Y., *Chemical and Pharmaceutical Bulletin*, 1996, **44**, 2103.
9. Fukuyama, Y., Kuwayama, A. and Minami, H., *Chemical and Pharmaceutical Bulletin*, 1997, **45**, 947.
10. Iinuma, M., Tosa, H., Tanaka, T., Kanamaru, S., Asai, F., Fobayashi, Y., Miyauchi, K. and Shimano, R., *Biological and Pharmaceutical Bulletin*, 1996, **19**, 311.
11. Sultanbawa, M. U. S., *Tetrahedron*, 1980, **36**, 1465.
12. Karanjgoakar, C. G., Rao, A. V. R., Venkataraman, K., Yemul, S. S. and Palmer, K. J., *Tetrahedron Letters*, 1973, **50**, 4977.
13. Fukuyama, Y., Kaneshi, A., Tani, N. and Kodama, M., *Phytochemistry*, 1993, **33**, 483.
14. Donnelly, W. J. G. and Shannon, P. V. R., *J. Chem. Soc.* (C), 1970, 524.
15. Wärtgen, K. and Wichtl, M., *Planta Med.*, 1989, **55**, 628.