Pergamon

PII: S0031-9422(96)00513-4

Phytochemistry, Vol. 43, No. 6, pp. 1249–1253, 1996
Copyright © 1996 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0031-9422/96 $15.00 + 0.00

PINGUISANE AND SACCULATANE TERPENOIDS FROM THE LIVERWORT *PORELLA PLATYPHYLLA*

MALCOLM S. BUCHANAN, JOSEPH D. CONNOLLY and DAVID S. RYCROFT*

Department of Chemistry, University of Glasgow, Glasgow G12 8QQ, Scotland, U.K.

(*Received* 7 *June* 1996)

Key Word Index—*Porella platyphylla*; Porellaceae; Jungermanniales; Hepaticae; liverwort; pinguisane sesquiterpenoids; sacculatane diterpenoids; ^{1}H and ^{13}C NMR parameters.

Abstract—A new pinguisanoic acid sesquiterpenoid derivative, methyl 2α-hydroxy-6-oxo-11-pinguisanoate and a new sacculatane diterpenoid hemiacetal, (5*S*,9*S*,10*R*,13*S*)-11,13-epoxy-8(12),17-sacculatadiene-13β,15ξ-diol [(13*S*)-15ξ-hydroxysacculaporellin], have been isolated from the liverwort *Porella platyphylla* together with three known pinguisanes and the known sacculatane perrottetianal B. Their structures were elucidated from their spectroscopic properties. The stereochemistry of one of the known pinguisanes, β-pinguisenediol, has been revised at C-2 and assigned at C-7. It is suggested that the configuration at C-13 in sacculaporellin, a sacculatane hemiacetal reported previously from *P. perrottetiana*, should be revised from 13*R* to 13*S*. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The liverwort genus *Porella* is a rich source of drimane, pinguisane, guaiane, pseudoguaiane, germacrane, aromadendrane and striatane sesquiterpenoids [1, 2]. The genus has been divided into two major chemotypes: pungent (referring to taste rather than smell) and nonpungent [3]. The former type produces the intensely pungent drimane polygodial and related compounds; in addition it usually also produces aromadendranes and pinguisanes. The nonpungent type does not produce drimanes but usually produces large amounts of pinguisanes. *P. platyphylla* (L.) Pfeiff. belongs to the family Porellaceae of the order Jungermanniales and investigations to date on *P. platyphylla* indicate that it belongs to the nonpungent type of *Porella* species. *P. platyphylla* produces a wide range of terpenoid metabolites including mono-, sesqui-, di- and triterpenoids [1–10]. All the sesquiterpenoids and diterpenoids produced by this liverwort are pinguisanes [1–6] and sacculatanes [7–9], respectively; these carbon skeletons remain unique to liverworts. The structures originally assigned to pinguisanin [3, 6] and perrottetianal B [9, 11] have had to be revised.

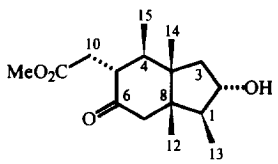
In this paper, we report on the isolation and characterization of pinguisanes and sacculatanes from *P. platyphylla* collected in the Yorkshire Dales, a limestone area of England. In addition, revisions to two structures already in the literature are proposed.

*Author to whom correspondence should be addressed.

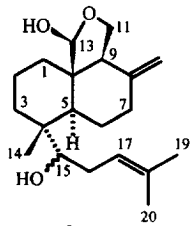
RESULTS AND DISCUSSION

From a methanol extract of *P. platyphylla* a new pinguisane (**1**) and a new sacculatane (**2**) were isolated along with the known compounds pinguisanin (**3**) [6], β-pinguisenediol (**4**) [3], porellapinguisanolide (**5**) [12] and perrottetianal B (**6**) [9, 11] which were identified by comparison of their spectroscopic properties with the published data.

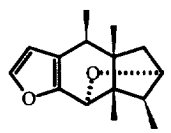
The published structure [3] of β-pinguisenediol is **4a**; the configuration of C-7 could not be assigned because of signal overlap involving H-7. The arguments used to relate the ^{1}H NMR coupling pattern observed for H-2 in the diacetate to the configuration reported for C-2 were not presented, but are unsustainable in the light of evidence derived from NOE difference experiments we have performed (at 360 MHz). The complete assignment of the 200 MHz ^{1}H NMR data of β-pinguisenediol is given in the Experimental along with the ^{13}C NMR data. The ^{1}H signals for the tertiary methyls at the ring junction can be assigned because of NOEs observed between the olefinic methyl Me-15 and Me-14, and between the secondary methyl Me-13 and Me-12. H-7 receives an NOE from both Me-12 and Me-14, and is therefore β. Irradiation of Me-12 produces an enhancement of H-2 as well as of H-7; H-2 is therefore β. Consequently, the structure of β-pinguisenediol is revised to **4b**. In this structure the configuration of the oxygen substituents at C-2 and C-7 is now the same as in the corrected structure of pinguisanin (**3**) [6], and the 2-α-hydroxyl group is analogous to that in the labile alcohol we have reported previously from *P. platyphylla* [5].



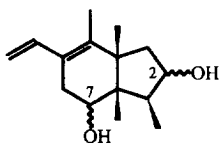
1



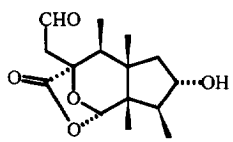
2



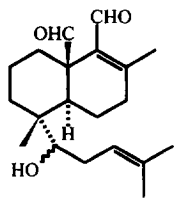
3



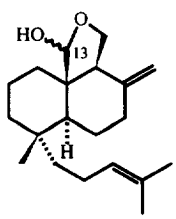
4a 2β-OH, 7ξ-OH
4b 2α-OH, 7α-OH



5



6



7a 13α-OH (13*R*)
7b 13β-OH (13*S*)

The coupling constants between H-1 and H-2 that are reported by Toyota *et al.* [12] for porellapinguisanolide (**5**) (13.9 Hz) and two other pinguisane derivatives are anomalously large for vicinal H–H interactions in these situations and presumably are derived from misinterpretation of the multiplet structures. Our observed value of the coupling between H-1 and H-2 in compound **5** (8.8 Hz) is in line with those observed in similar compounds (compare compounds **4b** and **1**). Porellapinguisanolide has not been observed previously in *P. platyphylla*.

Compound **1** has the molecular formula $C_{16}H_{26}O_4$ ($[M]^+$ at *m/z* 282.1829). The IR spectrum shows the presence of a hydroxyl (ν_{max} 3467 cm^{-1}), a six-membered ketone (ν_{max} 1717 cm^{-1}) and a saturated ester (ν_{max} 1740 cm^{-1}). The ^1H NMR spectrum has signals for two tertiary methyls, two secondary methyls, a methoxyl and an oxygen-bearing methine. The ^{13}C NMR spectrum confirmed the presence of a ketone (δ_C 210.4 (*s*)), a methyl ester (δ_C 173.6 (*s*), 51.7 (*q*)) and a secondary alcohol (δ_C 77.9 (*d*)). It contained further signals for four methyls, three methylenes, three methines and two quaternaries.

The molecule is therefore bicarbocyclic and the data are consistent with a pinguisane skeleton. The five-membered ring portion of compound **1** was easily identified as the ^1H NMR spectrum signal pattern is similar to that of other pinguisanes that contain this structural unit [12]. Once this portion was identified the remainder of the pinguisane skeleton was easily constructed by careful analysis of the NMR data. Further evidence for the methyl acetate side chain came from mass spectrometry which shows a peak at *m/z* 208 arising from loss of $H_2C{=}C(OH)(OMe)$ in a McLafferty rearrangement.

The stereochemistry at C-5 was confirmed by NOE difference experiments. Thus, NOEs were observed between H-5 and H_3-14 indicating that H-5 and H-4 are *trans* diaxial and the methyl acetate side chain is α-orientated. The normal β-configuration of all the methyls was confirmed by the NOEs observed between (i) H_3-12 and H_3-13, (ii) H_3-12, and H_3-14 (iii) H-5 and H_3-14, and (iv) H-5 and H_3-15. The NOEs observed between H_3-12 and both C-7 protons indicate that H_3-12 must be equatorial and *gauche* to both an axial H-7β and an equatorial H-7α, with the six-membered ring of compound **1** in a chair conformation. The NOE observed between H-4 and H-1 implies that H-4 is axial. From the preceeding spectral data, compound **1** was identified as methyl 2α-hydroxy-6-oxo-11-pinguisanoate. It is possible that compound **1** was formed from the corresponding acid by acid-catalysed esterification with methanol, although methyl esters of other pinguisane acids do occur naturally [1]. The present pinguisanoic acid is a new natural product.

Compound **2** is crystalline and has the molecular formula $C_{20}H_{32}O_3$ ($[M]^+$ at *m/z* 320.2332). Its IR spectrum showed hydroxyl absorptions. The ^1H NMR spectrum (Table 1) contained signals for a tertiary methyl, an exomethylene, an oxygenated methylene forming the AB part of an ABX system, two oxygenated methines, one of which (δ_H 5.31; *d*, $J = 10.0$ Hz) is coupled to a hydroxyl proton at δ_H 2.42 (*d*, $J = 10.0$ Hz) and is associated with a hemiacetal, and a dimethylallyl group. The ^{13}C NMR spectrum (Table 1) showed the presence of 20 carbons: three methyls, six methylenes, two methines, two quaternaries, an exomethylene, a trisubstituted double bond, an oxygenated methylene, a hemiacetal (δ_C 102.4 (*d*)) and an oxygenated methine. Correlation of carbons and protons is based on a two-dimensional direct δ_C/δ_H correlation experiment, which also located protons which were obscured by overlap in the one-dimensional ^1H spectrum.

A sacculatane diterpenoid hemiacetal was indicated for compound **2** as its ^1H and ^{13}C NMR data are similar to those of sacculaporellin (**7**), isolated from the liverwort *P. perrottetiana* [13], except for the replacement of a methylene by an oxygen-bearing methine (δ_H 3.44; *ddd*, $J = 7.6$, 4.8, 2.8 Hz; δ_C 75.5 (*d*)); the 2.8 Hz splitting was not always observed in the signal of this methine and is attributed to coupling to a hydroxyl proton which is in fast exchange on the NMR time-scale in some solutions. The position of this secondary alcohol was determined as follows. In separate

Table 1. ^1H and ^{13}C NMR data* for compound **2**

Site	δ_C and mult.	δ_H and mult.	J_{HH} (Hz)
1α	37.0 *t*	1.15 *m*	
1β		1.78 *m*	
2	19.0 *t*	1.60 *m*, 2H	
3	30.9 *t*	1.4-1.6 *m*, 2H	
4	39.9 *s*		
5	45.3 *d*	2.23 *dd*	12.0, 4.7
6	23.5 *t*	1.82 *m*, 2H	
7α	35.3 *t*	2.14 *td*†	12, 6
7β		2.55 *br dt*	13.2, 4.0
8	149.7 *s*		
9	51.5 *d*	2.29 *dd*	6.1, 1.6
10	51.1 *s*		
11α	64.7 *t*	3.88 *dd*	9.2, 6.1
11β		4.30 *dd*	9.2, 1.6
12*Z*	106.8 *t*	5.00 *br s*	
12*E*		4.86 *br s*	
13	102.4 *d*	5.31 *d*	10.0
14	18.3 *q*	0.93 *s*	
15	75.5 *d*	3.44 *ddd*	7.6, 4.8, 2.8
16	29.6 *t*	2.12 *m*, 2H	
17	121.7 *d*	5.17 *t* sept	7.5, 1.4
18	136.0 *s*		
19	26.0 *q*	1.74 *br s*	
20	17.9 *q*	1.64 *br s*	
13-OH		2.42 *d*	10.0

*Location of signals and assignments were helped by homonuclear decoupling, two-dimensional direct δ_C/δ_H correlation, and by comparison with published data [11, 15, 16].

†Observed in NOE difference spectrum.

homonuclear decoupling experiments, irradiations at δ_H 3.44 and the olefinic proton H-17 (δ_H 5.17; *t sept*, J = 7.5, 1.4 Hz) affected the same multiplet at δ_H 2.12, associated with H_2-16. The two-dimensional direct δ_C/δ_H correlation spectrum confirmed that H_2-16 are the only protons resonating around δ_H 2.1 apart from H-7α, the presence of which does not affect the argument as decoupling showed that the C-7 methylene is allylic to the exomethylene and the splitting pattern of H-7β (δ_H 2.55; *br dt*, J = 13.2, 4.0 Hz) shows that it is also vicinal to a methylene. Therefore the signal at δ_H 3.44 arises from H-15. In the reverse experiment, irradiation at δ_H 2.12 (H_2-16) caused collapse of H-15 into a broad singlet and of H-17 into a broad septet, and also simplified the methyl signal at δ_H 1.64 (*br s*, H_3-20) by removal of a homoallylic coupling. NOEs in difference experiments were observed between (i) H-15 and H-17, (ii) H-15 and H-5, (iii) H-15 and H_2-16, (iv) H-15 and H_2-6, and (v) H-15 and H_3-14. These results indicate attachment of the isopentenyl moiety to the α-oriented carbon substituent at C-4 and that the secondary alcohol is at C-15.

The other stereochemical assignments of compound **2** are also based largely on NOE difference experiments. The methyl attached to C-4 and the hemiacetal must be on the same side of the decalin system because of NOEs between H_3-14 and H-13. In addition NOEs involving the hemiacetal and the exomethylene moieties (between H-12*Z* and H-11β, between H-12*E* and H-7β, and between H-11α and H-1β) are consistent with a *trans* decalin in a chair–chair conformation. NOEs observed between H-13 and H-1β and between H-13 and H-2β demonstrate that the hydroxyl group on the hemiacetal carbon must be β-orientated. The configuration at C-15 remains undetermined. On the basis of these findings compound **2** was formulated as (5*S*,9*S*,10*R*,13*S*)-11,13-epoxy-8(12),17-sacculatadiene-13β,15ξ-diol (13*S*)-15ξ-hydroxysacculaporellin] (the normal sacculatane absolute configuration is assumed). The oxygenation pattern of compound **2** is the same as that in the revised structure of perrottetianal B (**6**), but was deduced independently [14].

The reported structure of sacculaporellin is **7a** [13]; the hemiacetal hydroxyl group at C-13 was assigned the opposite configuration from that in compound **2** on the basis of an NOE observed between H-13 and a signal assigned as H-6β. The close similarity of δ_C of carbons of compound **2** and sacculaporellin in the neighbourhood of the hemiacetal is incompatible with different configurations at C-13, because differences in the γ-interactions would produce a large difference in the ^{13}C shift of C-1 in particular. Another striking point is the remarkable similarity of the hemiacetal hydroxyl proton resonance in compound **2** and sacculaporellin, both in chemical shift and in the large coupling constant to the hemiacetal methine proton. Two-dimensional direct δ_C/δ_H correlation experiments were not performed with sacculaporellin [13] and a possible explanation of the discrepancy is that the enhanced signal assigned as H-6β (either δ_H 1.72 or 1.80 as configurational assignments of H-6 were not reported by Asakawa *et al.* [13]) in fact arises from H-1β, unassigned by Asakawa *et al.* [13] but expected to be around δ_H 1.7–1.8 as in compound **2**. However, in the NOE difference experiments performed with compound **2**, the signal enhanced by irradiation of H-13 and assigned to H-2β cannot be mistaken for a proton in ring B which would require the C-13 configuration to be changed. It therefore seems likely that in sacculaporellin the 13*R* configuration shown in structure **7a** should be revised to 13*S* as in **7b**.

Another discrepancy between compounds **2** and **7** involves the exomethylene protons. The *Z* and *E* assignments of H_2-12 in sacculaporellin are not stated explicitly by Asakawa *et al.* [13], but strict interpretation of the *a* and *b* labels used and the NOEs reported leads to the unlikely result that the assignments are reversed between compounds **2** and **7**.

This sample of *P. platyphylla* produced large amounts of pinguisane sesquiterpenoids along with sacculatane diterpenoids. No polygodial or related compounds were detected. Thus, this sample of *P. platyphylla* belongs to the nonpungent type of *Porella* species.

EXPERIMENTAL

General. TLC: Over Merck precoated silica gel 60

F_{254} and visualised under UV light (254 nm) and by spraying with 25% H_2SO_4 and heating. Flash CC and prep. TLC: Silica gel GF_{254}. Mps uncorr. NMR spectra (^1H, 200MHz; ^{13}C, 50 MHz) were recorded for $CDCl_3$ solutions relative to $CHCl_3$ at δ_H 7.25 and $CDCl_3$ at δ_C 77.0. Assignment of ^1H NMR signals was aided by homonuclear decoupling and NOE difference experiments. Multiplicities were determined by DEPT experiments. The 2D direct δ_C/δ_H correlation experiment used a standard pulse sequence incorporating a BIRD decoupling pulse with a composite 90°–240°–90° ^{13}C inversion pulse in the middle of the incremented delay. IR spectra were measured for CCl_4 and $CHCl_3$ solutions. EI-MS were measured at 70 ev.

Plant material. Porella platyphylla was collected from a lane-side wall near Ingleton, Yorkshire (England) on 2 August, 1992, and a voucher specimen is deposited in the herbarium of the Division of Environmental and Evolutionary Biology, Institute of Biomedical and Life Sciences, University of Glasgow.

Extraction and isolation. The ground material (336 g) was extracted with MeOH in a Soxhlet extractor to give 14 g; 8.2 g of this was divided into 10 frs by CC on silica gel using a petrol–Et_2O gradient. Prep. TLC of these frs on silica gel (petrol–Et_2O, CH_2Cl_2–Et_2O, *n*-hexane–CH_2Cl_2) yielded the following constituents in order of increasing polarity: pinguisanin (**3**) (300 mg), (5*S*,9*S*,10*R*,13*S*) - 11,13 - epoxy - 8(12),17 - sacculatadiene-13β,15ξ-diol (**2**) (30 mg), perrottetianal B (**6**) (5 mg), β-pinguisenediol (**4b**) (57 mg), porellapinguisanolide (**5**) (18 mg) and methyl 2α-hydroxy-6-oxo-11-pinguisanoate (**1**) (16 mg). It should be noted that six methoxylated pinguisane artefacts were also isolated (260 mg) (*cf.* ref. 5).

Methyl 2α-hydroxy-6-oxo-11-pinguisanoate (**1**). Oil. HR-MS: *m/z* 282.1829 $[M]^+$, $C_{16}H_{26}O_4$ requires 282.1831; EI-MS *m/z* (rel. int.): 282 $[M]^+$ (1), 263 (15), 250 (14), 232 (19), 209 (20), 208 (92), 190 (25), 176 (24), 148 (30), 134 (35), 108 (53), 69 (47), 55 (97), 41 (100); IR ν_{max} (CCl_4) cm^{-1}: 3467 (OH); 1740, 1717 (C=O); ^1H NMR: δ_H 3.85 (*ddd*, *J* = 9.1, 7.4, 2.8 Hz, H-2), 3.65 (*s*, 11-OMe), 2.74 (*dddd*, *J* = 12.2, 8.4, 4.0, 0.8 Hz, H-5), 2.60 (*dd*, *J* = 16.2, 8.4 Hz, H-10), 2.31 (*dd*, *J* = 13.8, 0.8 Hz, H-7), 2.29 (*dd*, *J* = 16.2, 4.0 Hz, H-10), 2.21 (*d*, *J* = 13.8 Hz, H-7), 2.10 (*dq*, *J* = 12.2, 6.7 Hz, H-4), 1.91 (*dd*, *J* = 14.6, 9.1 Hz, H-3β), 1.82 (*br quin*, *J* = 7.0 Hz, H-1), 1.69 (*dd*, *J* = 14.6, 2.8 Hz, H-3α), 1.01 (*s*, 3H-14), 0.96 (*d*, *J* = 6.7 Hz, 3H-15), 0.92 (*d*, *J* = 6.9 Hz, 3H-13), 0.76 (*s*, 3H-12), ^{13}C NMR: δ_C 210.4 (*s*), 173.6 (*s*), 77.9 (*d*), 51.7 (*q*), 51.2 (*s*), 48.8 (*d*), 48.0 (*d*), 47.1 (*t*), 46.8 (*s*), 44.7 (*t*) 42.3 (*d*), 31.9 (*t*) 19.8 (*q*), 15.4 (*q*), 14.6 (*q*), 11.9 (*q*).

(5S,9S,10R,13S) - 11,13 - *Epoxy* - 8(12),17 - *sacculatadiene*-13β,15ξ-*diol* (**2**). Crystals from MeOH, mp 130–135°. HRMS: *m/z* 320.2332 $[M]^+$, $C_{20}H_{32}O_3$ requires 320.2351. EI-MS *m/z* (rel. int.): 320 $[M]^+$ (1), 302 (11), 233 (42), 221 (10), 204 (11), 187 (59), 175 (100), 161 (42), 145 (23), 133 (27); IR ν_{max} ($CHCl_3$) cm^{-1}: 3600, 3500 (OH); 1700, 1600 (C=C); ^1H and ^{13}C NMR: Table 1.

β-Pinguisenediol (**4b**). ^1H NMR: δ_H 6.69 (*dd*, *J* = 17.3, 11.0 Hz, H-10), 5.13 (*dd*, *J* = 17.3, 1.4 Hz, H-11*Z*), 4.99 (*dd*, *J* = 11.0, 1.4 Hz, H-11*E*), 3.727 (*ddd*, *J* = 7.6, 7.2, 4.7 Hz, H-2β), 3.726 (*dd*, *J* = 10.4, 5.9 Hz, H-7β), 2.56 (*ddq*, *J* = 16.5, 5.9, 1.2 Hz, H-6β), 2.07 (*ddq*, *J* = 16.5, 10.4, 2.1 Hz, H-6α), 1.95 (*dq*, *J* = 7.2, 7.0 Hz, H-1α), 1.926 (*dd*, *J* = 14.6, 8.8 Hz, second-order ABX calculation, H-3β), 1.877 (*dd*, *J* = 14.6, 3.5 Hz, second-order ABX calculation, H-3α), 1.76 (*dd*, *J* = 2.1, 1.2 Hz, 3H-15), 1.09 (*d*, *J* = 7.0 Hz, 3H-13), 0.94 (*s*, 3H-14), 0.92 (*s*, 3H-12); ^{13}C NMR: δ_C 138.7 (*s*), 135.1 (*d*), 124.4 (*s*), 112.1 (*t*), 78.4 (*d*), 72.1 (*d*), 51.9 (*s*), 48.4 (*s*), 44.8 (*d*), 44.3 (*t*), 30.8 (*t*), 22.0 (*q*), 15.5 (*q*), 15.1 (*q*), 14.6 (*q*).

Acknowledgements—We thank the Loudon Trust and the Buchanan Society for a studentship and a bursary, respectively, (to M.S.B.), and Mr Guillaume Hoizey (Reims, France) for preparing the liverwort extract during a short summer visit to our laboratories in 1992.

REFERENCES

1. Asakawa, Y. (1995) *Progress in the Chemistry of Organic Natural Products* Vol. 65 (Herz, W., Kirby, G. W., Moore, R. E., Steglich, W. and Tamm, Ch., eds), p. 1. Springer, Wien, New York.
2. Asakawa, Y. (1982) *Progress in the Chemistry of Organic Natural Products* Vol. 42 (Herz, W., Grisebach, H. and Kirby, G. W., eds), p. 1. Springer, Wien, New York.
3. Asakawa, Y., Toyota, M., Takemoto, T. and Suire, C. (1979) *Phytochemistry* **18**, 1349.
4. Asakawa, Y. (1982) *J. Hattori Bot. Lab.* **53**, 283.
5. Connolly, J. D. (1990) in *Bryophytes: Their Chemistry and Chemical Taxonomy* (Zinsmeister H. D. and Mues, R., eds), pp. 41–58. Oxford University Press, Oxford.
6. Asakawa, Y., Connolly, J. D., Fakunle, C. O., Rycroft, D. S. and Toyota, M. (1987) *J. Chem. Res. (S)*, 82.
7. Asakawa, Y., Suire, C., Toyota, M., Tokunaga, N., Takemoto, T., Hattori, S. and Mizutani, M. (1980) *J. Hattori Bot. Lab.* **48**, 285.
8. Asakawa, Y., Toyota, M., Taira, Z., Takemoto, T., Kido, M. and Ichikawa, Y. (1980) *24th Symposium on Chemistry of Terpenes, Essential Oils and Aromatics*, p. 221. Symposium Papers, Koriyama, Japan.
9. Nagashima, F., Momosaki, S., Watanabe, Y., Toyota, M., Huneck, S. and Asakawa, Y. (1996) *Phytochemistry* **41**, 207.
10. Huneck, S. and Schreiber, K. (1975) *J. Hattori Bot. Lab.* **39**, 215.
11. Asakawa, Y., Toyota, M. and Takemoto, T. (1979) *Phytochemistry* **18**, 1681.
12. Toyota, M., Nagashima, F. and Asakawa, Y. (1989) *Phytochemistry* **28**, 3383.

13. Asakawa, Y., Toyota, M. and Ueda, A. (1990) *Phytochemistry* **29**, 2165.
14. Buchanan, M. S. (1994) Ph.D. Thesis, University of Glasgow.
15. Asakawa, Y., Toyota, M. and Takemoto, T. (1980) *Phytochemistry* **19**, 1799.
16. Hashimoto, T., Tori, M. and Asakawa, Y. (1987) *Tetrahedron Letters* **28**, 6293.