Phytochemistry, 1980, Vol. 19, pp. 1799–1803. © Pergamon Press Ltd. Printed in England. 0031-9422/80/0801-1799 $02.00/0

FOUR NEW SACCULATANE-TYPE DITERPENOIDS FROM *TRICHOCOLEOPSIS SACCULATA* AND *PELLIA ENDIVIAEFOLIA*

YOSHINORI ASAKAWA, MASAO TOYOTA and TSUNEMATSU TAKEMOTO

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

(*Revised received* 21 *December* 1979)

Key Word Index—*Trichocoleopsis sacculata*; *Pellia endiviaefolia*; Hepaticae; 18-hydroxysacculatal; 19-hydroxysacculatal; 3-hydroxyisosacculatal; 3-hydroxysacculatanolide; sacculatanes; diterpene dialdehyde; diterpene lactone; pungency.

Abstract—The characteristic pungency of the liverwort, *Trichocoleopsis sacculata* is due to the mixture of three sacculatane-type diterpene dialdehydes. Two have new structures, 18-hydroxysacculatal and 19-hydroxysacculatal, and the third is the previously known sacculatal. The absolute configurations of the new diterpene dialdehydes were tentatively shown by the co-occurrence of sacculatal and isosacculatal. Two new non-pungent sacculatanes, 3-hydroxyisosacculatal and 3-hydroxysacculatanolide have been isolated from the liverwort *Pellia endiviaefolia* together with sacculatal and isosacculatal. The position of the hydroxyl group at C-3 in the new diterpenes was also assumed on biogenetic considerations and a structural analogy with perrottetianal B, a previously known sacculatane-type diterpene dialdehyde isolated from the liverwort.

INTRODUCTION

Some liverworts, e.g. *Porella*, *Plagiochila*, *Chiloscyphus*, *Diplophyllum*, *Wiesnerella* and *Conocephalum* species, contain characteristic pungent substances. Recently, we reported that the sesquiterpene dialdehyde (**14**), *ent*-2,3-secoaromadendrane-type sesquiterpene hemiacetal and sesquiterpene lactones are responsible for the pungency and these terpenoids do not indicate plant growth activity [1–10]. Pungent substances are also present in the liverworts *Trichocoleopsis sacculata* and *Pellia endiviaefolia* from which two unique diterpene dialdehydes (**2** and **5**) have been isolated and their structures established [11, 12]. Further investigation of the pungent extract of *T. sacculata* resulted in the isolation of the two new pungent sacculatane-type diterpene dialdehydes, 18-hydroxysacculatal (**3**) and 19-hydroxysacculatal (**1**), together with the previously known pinguisane-type sesquiterpenes (**16** and **17**). Two new non-pungent sacculatane-type diterpene dialdehydes, 3-hydroxyisosacculatal (**4**) and the sacculatane-type diterpene lactone, 3-hydroxysacculatanolide (**6**) have been isolated from *Pellia endiviaefolia*. We now report the chemical structures of four new sacculatane-type diterpenes.

RESULTS AND DISCUSSION

The crude extract of *T. sacculata* showed the intense pungency and produced five 2,4-DNP positive spots on examination by TLC. Column chromatographies on Si gel and on Sephadex LH-20 of the extract resulted in the isolation of two new labile pungent diterpene dialdehydes, named 18-hydroxysacculatal (**3**) and 19-hydroxysacculatal (**1**), together with the previously known pungent sacculatal (**2**), non-pungent isosacculatal (**5**) and pinguisane-type sesquiterpenes (**15** and **16**). The viscous pungent extract of *P. endiviaefolia* was also chromatographed on Si gel to afford 3-hydroxyisosacculatal (**4**) and 3-hydroxysacculatanolide (**6**), along with sacculatal and isosacculatal.

19-*Hydroxysacculatal* (**1**)

The spectral data of the minor component (**1**), $C_{20}H_{30}O_3$ (M^+ *m/e* 318), indicated the presence of a hydroxyl group (3400 cm^{-1}), an α,β-unsaturated aldehyde group [1680 cm^{-1}; δ 9.38 (*s*)], a saturated aldehyde group [1710 cm^{-1}; δ 9.50 (*d*, J = 5 Hz)]. The ^1H NMR spectrum (Table 1) also contained the signals of two tertiary methyl groups, a vinylic proton conjugated with an aldehyde group, one vinylic methyl group and a vinylic proton. The ^1H NMR spectrum of **1** was similar to that of sacculatal (**2**), except for the presence of a broad singlet signal at δ 3.42 (2H), assignable to a hydroxy methyl group in place of one of two vinylic methyl groups, indicating that a hydroxyl group might be located at C-19 or C-20 of sacculatal. Since the original dialdehyde was labile, it was converted into the acetate. Reduction of **1** with $LiAlH_4$ gave a triol (**8**) [3360 cm^{-1}; 3.77–4.40 (4H), 3.40 (2H)], followed by acetylation to afford a triacetate (**9**) [1745 cm^{-1}; 1.97 (3H), 2.07 ppm (6H)]. The location of the hydroxyl group of **1** was confirmed by ^{13}C NMR (Table 2) of **9**. The ^{13}C NMR spectrum of **9** was similar to that of the diacetate (**10**) of sacculatal, except for the absence of a quartet signal at 25.7 ppm, indicating that a hydroxyl group of **9** was located at C-19 of sacculatal. Thus, the gross structure of the new diterpene dialdehyde was represented as **1**. The absolute configuration was assumed on the structural analogy with that of sacculatal (**2**).

Table 1. ^{1}H NMR chemical shifts (ppm from internal TMS) of sacculatanes

	1	3	4	6	8	9	12
H-3			3.50 (*t*, *J* = 7)	3.45 (*t*, *J* = 7)			
H-7	7.03 (*m*)	7.03 (*m*)	6.93 (*m*)	6.70 (*m*)	5.70 (*bs*)	5.81 (*m*)	5.85 (*m*)
H-9			3.60 (*bs*)				
H-11	9.50 (*d*, *J* = 5)	9.50 (*d*, *J* = 5)	9.70 (*d*, *J* = 2)	4.30 (*m*)	3.77 ~ 4.40 (*m*)	4.20 (*m*) 4.50 (*bs*)	4.20 (*m*) 4.50 (*bd*)
H-12	9.38 (*s*)	9.37 (*s*)	9.30 (*s*)				
H-13	1.00 (*s*)	1.00 (*s*)	0.96 (*s*)		0.83 (*s*)	0.87 (*s*)	0.90 (*s*)
H-14	0.93 (*s*)	0.93 (*s*)	0.93 (*s*)	0.87 (*s*)	0.77 (*s*)	0.72 (*s*)	0.83 (*s*)
H-16		5.53 (*s*)					5.56 (*s*)
H-17	5.30 (*t*, *J* = 7)		5.00 (*t*, *J* = 7)	5.30 (*t*, *J* = 7)	5.53 (*t*, *J* = 7)	5.02 (*t*, *J* = 7)	
H-19	3.42 (*bs*)	1.33 (*s*)	1.58 (*bs*)	1.53 (*bs*)	3.40 (*bs*)	4.85 (*bs*)	1.30 (*s*)
H-20	1.70 (*s*)		1.63 (*bs*)	1.63 (*bs*)	1.68 (*bs*)	1.67 (*bs*)	
Ac						1.97 (*s*)	2.00 (*s*)
						2.07 (*s*)	2.03 (*s*)
						2.07 (*s*)	

Table 2. ^{13}C NMR chemical shifts of sacculatanes (ppm from internal TMS)*

	2		9		10	
C-1	44.2	*t*	44.2	*t*	39.0	*t*
C-2	20.6†	*t*	20.7†	*t*	20.7†	*t*
C-3	39.4	*t*	39.1	*t*	39.0	*t*
C-4	36.6	*s*	35.9‡	*s*	35.9‡	*s*
C-5	46.9	*d*	47.2	*d*	47.4	*d*
C-6	21.8†	*t*	21.0†	*t*	21.1†	*t*
C-7	154.3	*d*	130.4	*d*	130.1	*d*
C-8	138.3	*s*	131.1	*s*	131.7	*s*
C-9	60.5	*d*	51.2	*d*	51.2	*d*
C-10	35.9	*s*	35.4‡	*s*	35.1‡	*s*
C-11	201.9	*d*	67.8	*t*	67.7	*t*
C-12	193.3	*d*	62.9	*t*	62.9	*t*
C-13	15.7	*q*	14.9	*q*	14.9	*q*
C-14	17.8‡	*q*	18.4	*q*	20.6	*q*
C-15	37.3	*t*	37.4	*t*	37.6	*t*
C-16	25.0	*t*	26.0	*t*	26.2	*t*
C-17	124.4	*d*	125.1	*d*	113.1	*d*
C-18	131.4	*s*	131.7	*s*	143.0	*s*
C-19	17.6‡	*q*	17.6	*q*	18.1	*q*
C-20	25.7	*q*	25.7	*q*	78.1	*t*
Ac			170.9	*s*	170.4	*s*
			171.1	*s*	170.9	*s*
					171.1	*s*

* The spectra were obtained at 22.6 MHz in Fourier transform mode in $CDCl_3$ solutions.

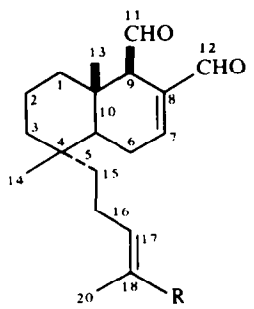
†‡ Values within any vertical column may be reversed.

18-*Hydroxysacculatal* (**3**)

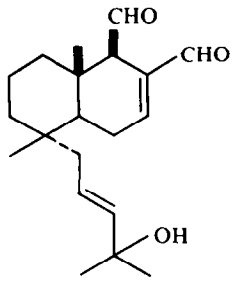
The pungent unstable aldehyde **3**, $C_{20}H_{30}O_3$ (M^+ *m/e* 318), showed the presence of a hydroxyl group (3400 cm^{-1}), an α,β-unsaturated aldehyde group [1680 cm^{-1}; δ 9.37 (*s*)] and a saturated aldehyde group [1710 cm^{-1}; 9.50 (*d*, *J* = 5 Hz, 1 H)]. The ^{1}H NMR spectrum (Table 1) also contained the signals of four tertiary methyl groups, two of which might be assigned to a hydroxy dimethyl group on the basis of a sharp singlet signal at 1.33 (6 H) [13, 14], a vinylic proton conjugated with an aldehyde group and two vinylic protons appeared as the singlet. Reduction of **3** with $LiAlH_4$, followed by acetylation gave a diacetate (**12**), $C_{24}H_{38}O_5$ (M^+ *m/e* 406), [3500, 1740 cm^{-1}; δ 2.00, 2.03 (each 3 H, *s*)] with a hydroxyl group, showing that the hydroxyl group might be tertiary. The above spectral and chemical evidence together with the molecular formula of **3** and **12** indicated that **3** might be a bicyclic diterpene dialdehyde with a tertiary hydroxyl group. The ^{1}H NMR spectra of **3** and its diacetate (**12**) were quite similar to those of sacculatal (**2**) and its acetate (**10**), except for the presence of two vinylic protons at 5.53 and a hydroxy dimethyl group, instead of the signals of a dimethylallyl group. These facts indicated that the new compound (**3**) possessed the same sacculatane-type diterpene dialdehyde as **1** and a hydroxyl group might be located at C-18 and a double bond at C-15 or C-16 in the side chain of sacculatal (**2**). The natural product, yomogialcohol A [13, 14] possesses the same partial structure as the formula **3** proposed for the second new diterpene dialdehyde. The chemical shifts of the singlet signal of the *trans*-ethylenic protons and of the hydroxy dimethyl group of yomogialcohol A were almost identical to those of the side chain of **3** and **12**. The above identification and the higher chemical shift of a quarternary methyl group at C-14, along with the biogenetic consideration show that the gross structure of the new sacculatane-type diterpene dialdehyde is most favourably represented by the formula **3**. The absolute configuration was also tentatively shown by structural analogy to that of sacculatal (**2**).

3-*Hydroxyisosacculatal* (**4**) *and* 3-*hydroxysacculatanolide* (**6**)

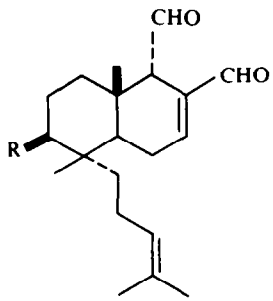
The two new diterpenes **4** and **6** were isolated from *P. endiviaefolia* as the minor component. The structures of **4** and **6** have been confirmed only by spectrometry. The non-pungent dialdehyde, named 3-hydroxyisosacculatal, $C_{20}H_{30}O_3$ (M^+ *m/e* 318), exhibited the presence of an α,β-unsaturated aldehyde group [1680 cm^{-1}; δ 9.30 (1 H, *s*)], a saturated aldehyde group [1720 cm^{-1}; 9.70 (1 H, *d*, *J* = 2 Hz)] and a dimethylallyl group [1.58, 1.63 (each 3 H), 5.00 (1 H, *t*, *J* = 7 Hz)] and a hydroxyl group (3400 cm^{-1}). The presence of an equatorial hydroxyl group was confirmed by the triplet signal at 3.50 (*J* = 7 Hz). The ^{1}H NMR spectrum of **4** was almost identical to that of isosacculatal (**5**) isolated from the same plant, except for the presence of a carbinyl proton at δ 3.50



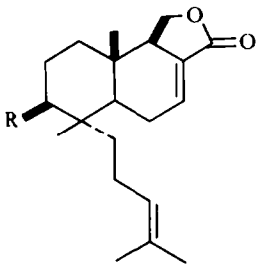
1 R = CH_2OH
2 R = Me



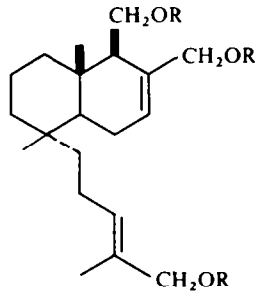
3



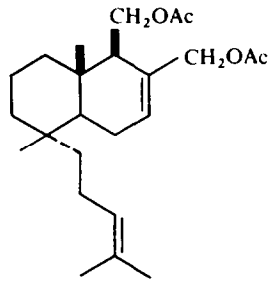
4 R = OH
5 R = H



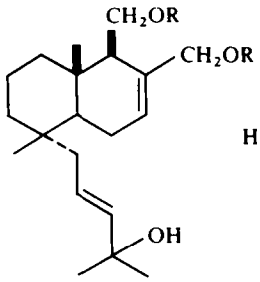
6 R = OH
7 R = H



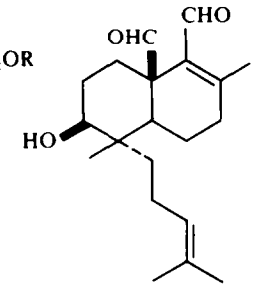
8 R = H
9 R = Ac



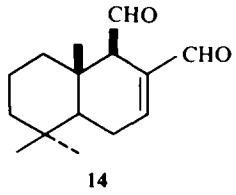
10



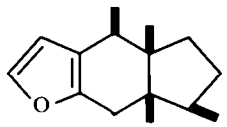
11 R = H
12 R = Ac



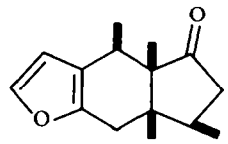
13



14



15



16

showing that this third new diterpene dialdehyde was isosacculatal with a secondary hydroxyl group at C-1, C-3 or C-15. Further chemical reactions were not carried out because of the small amount of the original material. However, the equatorial hydroxyl group was tentatively located at C-3 by biogenetic considerations and the presence of 3-hydroxysacculatane-type diterpene dialdehydes, such as perrottetianal B (**13**), in the liverwort, *Porella perrottetiana* [15].

The fourth new compound (**6**), $C_{20}H_{30}O_3$ (M^+ *m/e* 318), showed IR bands at 3350 cm^{-1} (OH), and 1755 and 1690 cm^{-1} (α,β-unsaturated γ-lactone). The ^1H NMR spectrum (Table 1) contained the signals of two tertiary methyl groups, a dimethylally group, a vinylic proton conjugated with lactonic carbonyl group and a carbinyl proton appeared as a triplet signal at δ 3.45 (J = 7 Hz). This ^1H NMR spectrum was similar to that of sacculatanolide (**7**) derived from sacculatal by cross Cannizzaro reaction [11], except for the presence of a carbinyl proton, indicating that the new diterpene lactone was sacculatanolide with an equatorial hydroxyl group at C-1, C-3 or C-15. The location of the hydroxyl group at C-3 was tentatively assigned by biogenetic considerations of the sacculatals.

Sacculatane-type diterpenes are interesting from the biogenetic view point. The ring system is well known in drimane-type sesquiterpenes and an additional isoprene unit has been attached at C-15. The sacculatals and sacculatanolide might be derived from geranyl-geranyl pyrophosphate by a cyclization mechanism analogous to that operating for conversion of farnesyl pyrophosphate into the drimane-type sesquiterpenes. Like *Porella vernicosa* complex, *P. endiviaefolia* contains diterpene dialdehydes (**2**, **4**, **5**) and a related diterpene lactone (**6**). By contrast diterpene lactones **6** and **7** have not been found in *T. sacculata* even by GC MS analysis.

EXPERIMENTAL

The solvents used for spectral determinations were TMS–$CDCl_3$ (^1H NMR 90 and 60 MHz, ^{13}C NMR 22.6 MHz) and $CHCl_3$ (IR and $[\alpha]_D$), unless otherwise stated. TLC was on precoated Si gel (0.25 mesh) F_{254}, spots were detected by spraying with 2,4-DNP and 30% H_2SO_4 and viewing in UV light (254 nm). GC–MS was at 70 eV, column, SE-30 1%, 3m × 2mm, temp. programme, 50 270° at 5°/min, inject. temp. 260°, He 30 ml/min.

Extraction and isolation. Trichocoleopsis sacculata, collected in Ehime prefecture in August 1978, was ground after being air-dried for 5 days. The ground material (188.2 g) was extracted with Et_2O for 2 weeks. The pungent extract (4.90 g) was directly chromatographed on Si gel using the solvent system *n*-hexane–EtOAc gradient. The first fraction (*n*-hexane 100%) gave sesquiterpene hydrocarbons (50 mg) in which bicyclogermacrene and calamenene have been detected by GC MS [16]. The crude material from the second fraction (*n*-hexane–EtOAc, 19:1) was purified by PLC to give deoxopinguisone (**15**) (10 mg). The third fraction (9:1) gave the mixture (1.220 g) of carotenoids, unidentified sesquiterpenes and triglycerides. The fourth fraction (4:1) gave three 2,4-DNP positive substances which were purified by PLC to give pinguisone (**16**) (65 mg), sacculatal (265 mg) and isosacculatal (200 mg). The fifth fraction (7:3) afforded pure sacculatal (1.520 g). The sixth fraction (1:1) contained two intense 2,4-DNP positive substances displaying pungent taste. Since the crude pungent materials were very labile on Si gel, the materials (720 mg) were rechromatographed on Sephadex LH-20 using a $CHCl_3$–MeOH gradient, to afford 18-hydroxysacculatal (**3**) (23 mg) and 19-hydroxysacculatal (**1**) (90 mg).

18-Hydroxysacculatal (**3**). $C_{20}H_{30}O_3$. IR ν_{max}^{liq} cm^{-1}: 3400 (OH), 2700 (CHO), 1710 (CHO), 1680 (C=C–CHO), 1440, 1385, 1380, 1375, 905, 830. MS *m/e* (rel. int.): 318 (M^+, 5), 219 (58), 201 (65), 191 (79), 190 (75), 189 (62), 175 (50), 173 (62), 161 (53), 159 (50), 147 (70), 145 (55), 135 (50), 133 (63), 121 (60), 109 (70), 107 (60), 105 (100), 97 (58), 93 (56), 91 (88), 82 (66), 81 (52).

19-Hydroxysacculatal (**1**). $C_{20}H_{30}O_3$. IR ν_{max}^{liq} cm^{-1} 3400 (OH), 2720 (CHO), 1710 (CHO), 1680 (C=C–CHO), 1440, 1380, 980. MS *m/e* (rel. int.): 318 (M^+, 6), 257 (27), 203 (36), 201 (41), 191 (38), 190 (31), 189 (46), 188 (28), 187 (42), 173 (50), 163 (30), 161 (38), 151 (40), 147 (50), 145 (47), 133 (60), 121 (68), 119 (53), 107 (51), 105 (100), 93 (50), 91 (85), 81 (48), 79 (40), 69 (56), 55 (55), 41 (81).

P. endiviaefolia collected in Tokushima prefecture in May 1978 was treated in the same manner as described for the extraction of *T. sacculata*. The crude extract (8.20 g) of *P. endiviaefolia* (270 g) was directly chromatographed on Si gel using *n*-hexane EtOAc gradient. The first fraction (*n*-hexane, 100%) gave sesquiterpene mixtures in which α-elemene was detected by GC–MS. The second fraction (*n*-hexane–EtOAc, 9:1) contained carotenoids and unidentified sesquiterpenes (340 mg). The third fraction (4:1) gave two aldehydes which were purified by PLC to afford sacculatal (**2**) (502 mg) and isosacculatal (**5**) (170 mg). The crude material from the fourth fraction (3:1) was rechromatographed on Si gel using C_6H_6–EtOAc gradient to afford phytol (44 mg), 3-hydroxysacculatanolide (**6**) (8 mg) and 3-hydroxyisosacculatal (**4**) (16 mg).

3-Hydroxysacculatanolide (**6**). $[\alpha]_D$ − 39.4° (*c*, 0.57); $C_{20}H_{30}O_3$. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3350 (OH), 1755 (γ-lactone), 1690 (C=C). MS *m/e* (rel. int.): 318 (M^+, 23), 234 (36), 233 (39), 219 (44), 209 (96), 201 (27), 189 (57), 187 (78), 161 (53), 105 (58), 91 (55), 69 (100), 55 (50), 41 (54).

3-Hydroxyisosacculatal (**4**). $[\alpha]_D$ − 110° (*c*, 0.30); $C_{20}H_{30}O_3$. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH), 2710 (CHO), 1720 (CHO), 1680 (C=C–CHO). MS *m/e* (rel. int.): 318 (M^-, 5), 271 (32), 257 (25), 218 (50), 207 (50), 197 (53), 188 (81), 187 (74), 159 (52), 105 (59), 91 (50), 69 (100), 55 (50), 41 (54). The fifth fraction (3:1) gave phenolic compounds which were purified by PLC to afford δ-cuparenol (4 mg) [17–19].

Reduction of **1** *with* $LiAlH_4$. To $LiAlH_4$ (30 mg) in dry Et_2O (4 ml) was added **1** (55 mg) at 0° and stirring for 1 hr. Work-up yielded a viscous triol (**8**) (48 mg). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3360 (OH), 1440, 1385, 1040 (OH).

Acetylation of **8**. To a Py soln of **8** (48 mg) was added Ac_2O and the soln allowed to stand overnight. Work-up as usual gave a triacetate (**9**) (54 mg). $[\alpha]_D$ + 14° (*c*, 1.7); $C_{26}H_{40}O_6$. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1745, 1245 (OAc), 1675, 1655 (C=C), 1445, 1385, 1370, 1030, 755. MS *m/e* (rel. int.): 448 (M^-, 1), 268 (56), 253 (100), 187 (56), 186 (25), 185 (26), 131 (32), 119 (30), 109 (44), 107 (28), 105 (32), 81 (28), 43 (66).

Reduction and acetylation of **3**. Compound **3** (23 mg) was reduced by the same manner as described above to give a viscous alcohol (**11**), followed by acetylation with Ac_2O–Py to give viscous oil, purified by PLC to afford a diacetate (**12**) (20 mg). $[\alpha]_D$ + 7° (*c*, 1.1); $C_{24}H_{38}O_5$. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500 (OH), 1740, 1250 (OAc), 1670 (C=C), 1445, 1400, 1380, 1030, 760. MS *m/e* (rel. int.): 406 (M^+, 2), 187 (100), 186 (25), 105 (21), 43 (27).

Acknowledgements We are grateful to Dr. S. Hattori (Hattori Botanical Laboratory, Miyazaki, Japan) and Dr. H. Inoue (National Museum of Tokyo) for their collection and identification of the liverwort species.

REFERENCES

1. Asakawa, Y. and Aratani, T. (1976) *Bull. Soc. Chim. Fr.* 1469.
2. Asakawa, Y., Toyota, M., Uemoto, M. and Aratani, T. (1976) *Phytochemistry* **15**, 1929.
3. Asakawa, Y., Toyota, M. and Takemoto, T. (1978) *Phytochemistry* **17**, 457.
4. Asakawa, Y., Huneck, S., Toyota, M., Takemoto, T. and Suire, C. (1979) *J. Hattori Bot. Lab.* **46**, 163.
5. Asakawa, Y., Toyota, M. and Takemoto, T. (1978) *Tetrahedron Letters* 1553.
6. Asakawa, Y., Toyota, M. and Takemoto, T. (1978) *Phytochemistry* **17**, 1794.
7. Asakawa, Y., Toyota, M., Takemoto, T. and Suire, C. (1979) *Phytochemistry* **18**, 1355.
8. Asakawa, Y., Toyota, M., Takemoto, T. and Suire, C. (1979) *Phytochemistry* **18**, 1007.
9. Asakawa, Y. and Takemoto, T. (1979) *Phytochemistry* **18**, 285.
10. Asakawa, Y., Matsuda, R. and Takemoto, T. (1980) *Phytochemistry* **19**, 965.
11. Asakawa, Y., Takemoto, T., Toyota, M. and Aratani, T. (1977) *Tetrahedron Letters* 1407.
12. Asakawa, Y. and Takemoto, T. (1978) *Phytochemistry* **17**, 153.

13. Willhalm, B. and Thomas, A. F. (1969) *J. Chem. Soc. Chem. Commun.* 1380.
14. Yano, K., Hayashi, S., Matsuura, T. and Burgstahler, A. W. (1970) *Experientia* **26,** 8.
15. Asakawa, Y., Toyota, M. and Takemoto, T. (1979) *Phytochemistry* **18,** 1681.
16. Asakawa, Y., Tokunaga, N., Toyota, M., Takemoto, T., Hattori, S., Mizutani, M. and Suire, C. (1979) *J. Hattori Bot. Lab.* **46,** 67.
17. Hopkins, B. J. and Perold, G. W. (1974) *J. Chem. Soc. Perkin Trans.* 1, 32.
18. Matsuo, A., Nakayama, M., Maeda, T., Noda, Y. and Hayashi, S. (1975) *Phytochemistry* **14,** 1037.
19. Asakawa, Y., Tokunaga, N., Toyota, M., Takemoto, T. and Suire, C. (1979) *J. Hattori Bot. Lab.* **45,** 395.