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A POTENT CYTOTOXIC WARBURGANAL AND RELATED DRIMANE-TYPE SESQUITERPENOIDS FROM *POLYGONUM HYDROPIPER*

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Key Word Index—*Polygonum hydropiper*; Polygonaceae; warburganal; polygodial; isopolygodial; polygonal; isodrimeninol; drimenol; confertifolin; drimane-type sesqui- and norsesquiterpenoids; pungency; anticomplement activity; cytotoxicity.

Abstract—Warburganal, a drimane-type sesquiterpene dialdehyde which has potent cytotoxic, antifeedant, antibiotic and molluscicidal activities was isolated from the leaf of *Polygonum hydropiper* together with its related drimane-type sesquiterpenes, polygodial, isopolygodial, isodrimeninol, drimenol and confertifolin, and a nor-sesquiterpene monoaldehyde, polygonal. Polygodial showed anticomplement activity (10.5 μg/ml). The distribution of drimane-type sesquiterpenoids in the plants of taxonomically different levels is discussed.

INTRODUCTION

The folk medicinal plant, *Polygonum hydropiper* L. contains intense pungent substances in leaf and seed and is used against cancer [1]. The young shoot is used as a spice with raw fish in Japan. On the other hand, it is known that this plant is toxic to fish [2, 3], pigs and sheep [3] and that the water-soluble fraction shows hemolytic properties [3]. The major component of the lipophilic materials of the leaf is a pungent polygodial (**2**) [4, 5], a drimane-type sesquiterpene dialdehyde which shows intense antifeedant and plant growth inhibitory activities [6, 7]. Previously, we reported the isolation of polygodial (**2**) and the related isopolygodial (**3**) [5], polygonal (**6**), isodrimeninol (**8**) and confertifolin (**10**) [5] from the seed of *P. hydropiper* [8]. During the course of the investigation of the anticomplement, piscicidal, hemolytic and antitumor active substances of *P. hydropiper*, we have now isolated an additional potent pungent sesquiterpene dialdehyde, warburganal (**1**).

RESULTS AND DISCUSSION

A combination of column and prep. TLC of the crude extracts of the seed and leaf resulted in the isolation of a potent pungent drimane-type sesquiterpene dialdehyde, warburganal (**1**), along with the new norsesquiterpene monoaldehyde, polygonal (**6**), isodrimeninol (**8**) and the previously known polygodial (**2**), isopolygodial (**3**), drimenol (**7**) and confertifolin (**10**).

Warburganal (**1**)

The compound (**1**) showed the following spectral data: IR ν_{max} 3460 (OH), 2850 (CHO), 1723 (CHO), 1687 (C=C–CHO); ^{1}H NMR (400 MHz): δ 9.73 (1H, *s*), 9.41 (1H, *s*), 7.24 (1H, *dd*, $J = 4.9$, 2.8 Hz), 4.11 (1H, *br s*, OH), 2.85 (1H, *ddd*, $J = 21.1$, 4.9, 4.9 Hz, H-6α), 2.35 (1H, *ddd*, $J = 21.1$, 11.9, 2.8 Hz, H-6β), 1.89 (1H, *dd*, $J = 11.9$, 4.9 Hz, H-5), 1.09, 0.99 and 0.95 (each 3H, *s*). The above spectral data, ^{13}C NMR and mass spectra were in good agreement with those of warburganal (**1**), recently isolated from *Warburgia ugandensis* (Canellaceae) [6, 9–11].

Polygonal (**6**)

The compound (**6**), $C_{14}H_{22}O_2$ (M^+ 222), mp 116–117°, was isolated from the seed as colorless needles. It formed a 2,4-DNP, mp 139–140°. The spectral data showed the presence of an α,β-unsaturated aldehyde group [IR ν_{max} 2740, 1675 cm^{-1}; UV λ_{max} 223 nm (log ϵ 4.14); δ 9.50 (1H, *s*)], a hydroxyl group (3400 cm^{-1}), a vinylic proton (δ 6.53, *s*), a carbinyl proton (δ 4.66, *br s*) and three tertiary methyl groups (δ 0.93, 1.00, 1.06, *s*), two of which were assigned to a gem-dimethyl group by the presence of well-separated IR bands at 1375 and 1385 cm^{-1}. Acetylation of **6** with acetic anhydride–pyridine gave a monoacetate (**16**), [$C_{16}H_{24}O_3$ (*m/z* 204 [M − 60]$^+$); 1730, 1245 cm^{-1}; 2.00 (3H, *s*)] and a small amount of a dehydrated product (**17**), $C_{14}H_{20}O$ (M^+ 204), indicating that **6** possessed a secondary hydroxyl group. The above spectral and chemical data coupled with the molecular formula indicated that **6** is a bicyclic norsesquiterpene monoaldehyde. Oxidation of **6** with chromate–pyridine afforded an α,β-unsaturated ketoaldehyde (**18**), [$C_{14}H_{20}O_2$ (M^+ 220); δ 10.03 (1H, *s*)], indicating the presence of a secondary alcohol at C-7. The α-configuration of the secondary alcohol was confirmed by the broad singlet signal ($W_{1/2} = 7$ Hz) of the carbinyl proton at C-7 and the facile dehydration of **6**. The negative Cotton effect of **6**, together with the

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co-occurrence of drimane-type sesquiterpenoids (**2**, **3**, **7**, **8**, **10**) and the biogenetic consideration of **6** via **19** shown by an arrow led to structure (**6**) for (−)-polygonal [8]. (±)-Polygonal has also been obtained during the course of the synthesis of warburganal (**1**) and the spectral data of the natural polygonal are identical to those of the synthetic sample.

Isodrimeninol (**8**)

The new compound (**8**), $C_{15}H_{24}O_2$ (M^+ 236), showed the presence of a hydroxyl group (3400 cm^{-1}), a vinylic proton (δ 5.26, *br s*), three tertiary methyl groups (δ 0.80, 0.86, 0.93, each *s*), and an isolated methylene group (δ 4.15, 4.50, each 1H, *d*, $J = 11$ Hz) in an asymmetrical environment attached to an oxygen atom and a carbinyl proton (δ 5.26, *d*, $J = 4$ Hz), which collapsed to a singlet on irradiation of the allylic methine proton (δ 2.13, *m*, H-9). Acetylation of **8** afforded a labile monoacetate (**20**), [$C_{17}H_{26}O_3$ (M^+ 278); 1742 cm^{-1}; δ 2.02 (3H, *s*)], along with a small amount of a β,β'-disubstituted furano compound (**21**), [$C_{15}H_{22}O_2$ (M^+ 218)] [12, 13]. Lithium aluminium hydride reduction of **8** gave the known drimanediol (**22**) [13, 14]. Oxidation of **8** with Collins reagent gave isodrimenin (**23**) [14]. The above spectral data and chemical conversion indicated that **8** is a drimane-type sesquiterpene hemiacetal with the same absolute configuration as polygodial and its structure was assigned as **8** or **9**. The hemiacetal (**9**) has been isolated from the pungent liverwort *Porella vernicosa* complex [13]. The chromatographic behavior of the new hemiacetal was different from that of **9**, although the spectral data of **8** and **9** were almost identical. Thus, the structure of isodrimeninol can be represented as **8**.

Kubo *et al.* [9–11] reported that warburganal (**1**) showed potent cytotoxicity (0.01 μg/ml against KB), had antifeedant effect against African armyworm (0.1 ppm/cm^2) and also had antibiotic and molluscicidal activities. *P. hydropiper* shows anticomplement activity. One of the active substances is polygodial (**2**) (10.5 μg/ml). However, warburganal (**1**) and isopolygodial (**3**) do not show anticomplement activity (> 100 μg/ml).

Drimane-type sesquiterpenoids are present in several plants. Table 1 summarizes the distribution of drimane-type sesquiterpenoids in three pungent plants, *P. hydropiper*, *Warburgia ugandensis*, *W. stuhlmanni* and the *Porella vernicosa* complex which are in taxonomically quite unrelated; the former two species belong to the Dicotyledoneae and the latter to

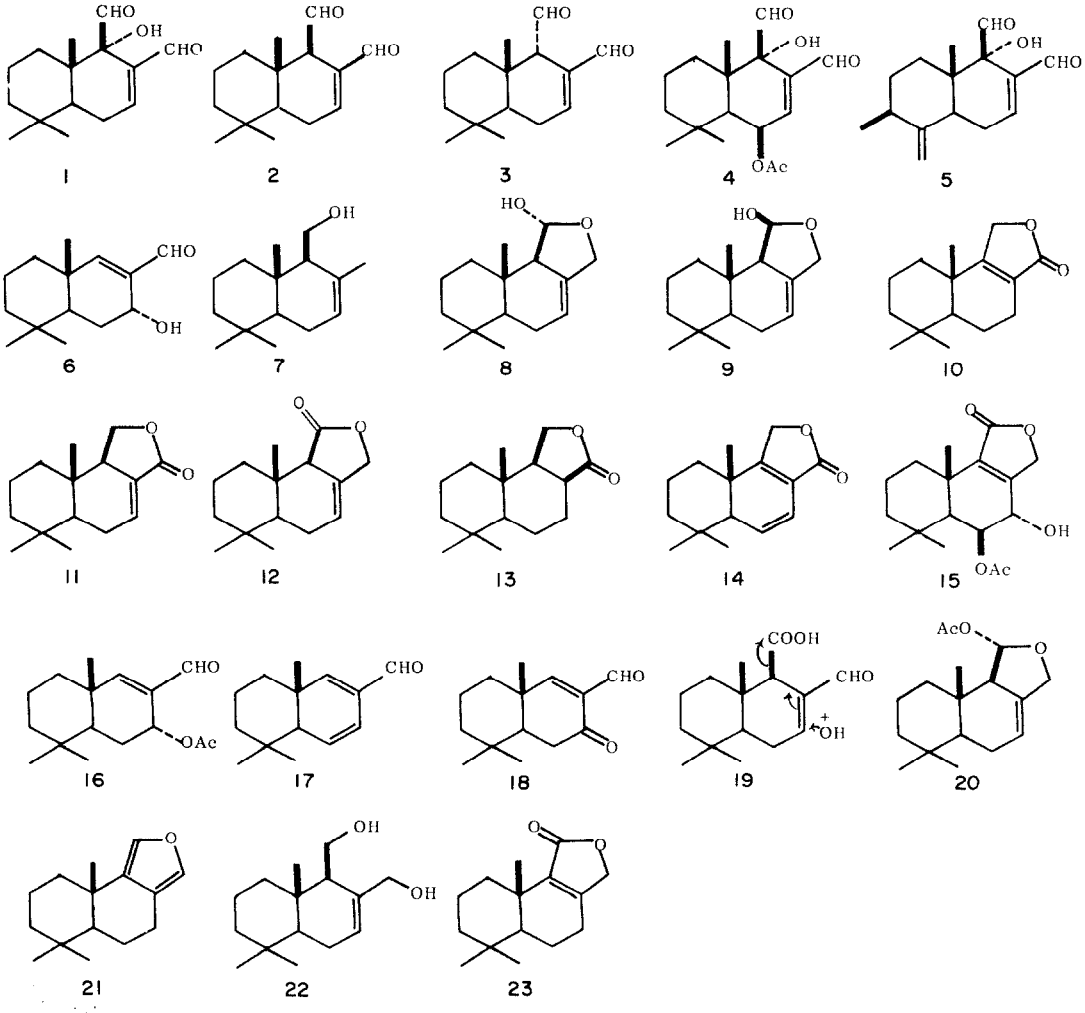

Table 1. Distribution of drimane-type sesquiterpenoids in *P. hydropiper* and two taxonomically unrelated plants

Species	Compounds														
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Dicotyledoneae															
Polygonaceae															
Polygonum hydropiper	+	+	+	—	—	+	+	+	—	+	—	—	—	—	—
Canellaceae															
Warburgia stuhlmanni [9–11]	—	+	—	—	—	—	—	—	—	—	+	—	—	+	—
W. ugandensis [6, 9–11, 17]	+	—	—	+	+	—	—	—	—	—	+	—	—	+	+
Hepaticeae															
Porellaceae															
Porella vernicosa complex [13, 15, 16]	—	+	—	—	—	—	+	—	+	—	—	+	+	+[20]	—

the Hepaticae. These three plants produce hot tasting sesquiterpene dialdehydes; polygodial (**2**) in *P. hydropiper*, *W. stuhlmanni* [9–11] and the *P. vernicosa* complex [15, 16], and warburganal (**1**) in *W. ugandensis* [6, 9–11] and *P. hydropiper*. *W. ugandensis* elaborates two additional pungent dialdehydes, ugandensidal (= cinnamodial) (**4**) and muzigadial (**5**) [6, 9–11, 17]. These compounds have not been detected in the seed and leaf of *P. hydropiper* or in the *P. vernicosa* complex. On the other hand, the sesquiterpene hemiacetals (**8**, **9**) which may be the possible precursors of drimanolides (**10–15**) have been isolated from *P. hydropiper* and the *P. vernicosa* complex, but not from *Warburgia* species.

EXPERIMENTAL

The solvents used for spectral determinations were TMS–$CDCl_3$ [^{1}H NMR (400 and 60 MHz)]; $CHCl_3$ (IR and $[\alpha]_D$); EtOH (UV); MeOH (CD), unless otherwise stated. TLC (analytical and prep.): precoated Si gel (0.25 mm) F_{254}, *n*-hexane–EtOAc (4:1) and C_6H_6–EtOAc (4:1) as solvents. Spots were visualized by UV light (254 nm) and spraying with 30% H_2SO_4 or 2,4-DNP. GC/MS: 70 eV, 5% OV-17 3 m × 2 mm glass column, temp. programme: 50–270° at 5°/min, He 30 ml/min.

Plant materials. Polygonum hydropiper L. identified by Y. A. and T. T. were deposited in the Herbarium of the Institute of Pharmacognosy, Tokushima Bunri University.

Screening of anticomplement activity [18]. Serial dilutions of the test sample were made in GVB^{2+} (isotonic Veronal-buffered saline containing 0.1% gelatin, 0.15 mM $CaCl_2$, and 1.0 mM $MgCl_2$, pH 7.3 and $u = 0.147$) in Microtiter plates. The solns were mixed with sensitized sheep erythrocytes (ET) at a final concn of 2×10^7 cells/ml and 1/600 guinea pig serum (complement) in GVB^{2+}, and incubated at 37° on vibrators. Inhibition was scored at the dilution of each test sample giving 50% hemolysis. Polygodial (**2**) caused 50% inhibition of hemolysis at a concn of 10.5 μg/ml.

Extraction and isolation. Polygonum hydropiper L. was collected in Tokushima in Oct. 1977 (sample A), 1978 (sample B) and 1980 (sample C). The seeds were separated from samples A and B and crushed mechanically. The ground material (50 g) was extracted with Et_2O for 2 months. The leaves of sample B were separated from the whole plants and air-dried for 1 week and then mechanically crushed. The fragrant powder (3.380 kg) was extracted with Et_2O for 2 months. The crude extract (12.0 g) from the seeds was directly chromatographed on Si gel using an *n*-hexane–EtOAc gradient to divide it into eight fractions. The first fraction (*n*-hexane 100%) gave the mixtures of sesquiterpene hydrocarbons and paraffins (260 mg). The crude oil obtained from the second fraction was rechromatographed on Si gel (*n*-hexane–EtOAc gradient) to give triglycerides (3.350 g) and confertifolin (**10**) (260 mg) [5]. The third fraction (4:1) and the fourth fraction (7:3) were combined and the pungent oil was rechromatographed on Si gel (C_6H_6–EtOAc gradient) to afford isopolygodial (**3**) (660 mg), polygodial (**2**) (2.20 g) and phytosterols (60 mg). Warburganal (**1**) was not isolated from this fraction (4:1 and 7:3); however, the presence of **1** was detected by GC/MS and TLC. The sixth (3:2) and seventh (1:1) fractions were combined and viscous oil was rechromatographed on Si gel (C_6H_6–EtOAc gradient) to afford polygodial (**2**) (70 mg), polygonal (**6**) (190 mg) and isodrimeninol (**8**) (260 mg). **6**: mp 116–117°; $[\alpha]_D$ −7.3° (*c* 7.4); $C_{14}H_{22}O_2$; UV λ_{max} nm (log ϵ): 223 (4.14); IR ν_{max} cm^{-1}: 3400 (OH), 2740, 1675 (C=C–CHO), 1630 (C=C), 1385, 1375 (gem-dimethyl), 1285, 1240, 1215, 1159, 1165, 1120, 1070, 1030, 1015, 960, 915, 885, 870; $\Delta\epsilon_{235\,nm}$ − 9.96, $\Delta\epsilon_{330\,nm}$ − 2.52; MS *m/z* (rel. int.) 222 $[M]^+$ (1), 204 $[M-18]^+$ (71), 189 (100), 175 (42), 161 (43), 147 (30), 133 (43), 119 (51), 109 (81), 105 (65), 91 (62). 2,4-DNP derivative: mp 139–140°; UV λ_{max} nm (log ϵ): 255 (4.00), 370 (4.27). **8**: $[\alpha]_D$ −37° (*c* 1.5); $C_{15}H_{24}O_2$; IR ν_{max} cm^{-1}: 3400, 1015 (OH), 1380, 1370 (gem-dimethyl); 1310, 1220, 1165, 1145, 1120, 1090, 970, 920, 895, 865, 825; MS *m/z* (rel. int.) 236 $[M]^+$ (1), 204 (35), 189 (61), 175 (32), 161 (25), 147 (21), 133 (30), 119 (45), 109 (100), 105 (69), 91 (59). The eighth fraction (2:3) was also rechromatographed on Si gel (C_6H_6–EtOAc) to give drimenol (**7**) [13, 19]. The crude pungent extract (250 g) from the leaf was chromatographed on Si gel using an *n*-hexane–EtOAc gradient to divide into eight fractions. The first fraction (*n*-hexane, 100%) contained the mixtures of sesquiterpene hydrocarbons and paraffins (2.50 g). The second

fraction (*n*-hexane–EtOAc, 9:1) (12.0 g) contained triglycerides and confertifolin (**10**). The third fraction (4:1) (31.0 g) contained isopolygodial (**3**) and polygodial (**2**). The fourth fraction (4:1) (15.3 g) was rechromatographed on a Si gel (C_6H_6–EtOAc) gradient to divide into eight fractions. The crude oil from fraction 4 was further chromatographed on Bio-Beads S-X8 using C_6H_6 to divide it into 30 fractions. Fractions 20–30 (630 mg) were purified by HPLC using *n*-hexane–EtOAc (93:7) to obtain the potent pungent dialdehyde (34.5 mg) whose spectral and chromatographic behavior were identical to those of warburganal (**1**) isolated from *Warburgia ugandensis* [9–11]. The sixth fraction (1:1) gave the viscous oil (56 g) which contained polygodial (**2**), polygonal (**6**), isodrimeninol (**8**) and unidentified drimane-type sesquiterpenoids. The seventh fraction (2:3) (44 g) contained fatty acids and drimenol (**7**). The presence of each drimane-type sesquiterpenoids in each fraction was confirmed by GC/MS. The structures of sesquiterpene hydrocarbons and the minor drimane-type sesquiterpenes found in the seeds and the leaves of *P. hydropiper* will be reported elsewhere.

Acetylation of **6**. The compound (**6**) (80 mg) was treated with Ac_2O–pyridine and left overnight. Work-up as usual gave the oil which showed two spots on TLC. Prep. TLC gave monoacetate (**16**) (59 mg) and a dehydrated product (**17**) (2 mg). **16**: $C_{16}H_{24}O_3$; IR ν_{max} cm^{-1}: 1730, 1245 (OAc), 1690 (C=C–CHO), 1645 (C=C), 1015, 960; ^1H NMR: δ 0.86 (6H, *s*), 1.00 (3H, *s*), 2.00 (3H, *s*), 5.57 (1H, *br s*), 6.63 (1H, *s*), 9.46 (1H, *s*); MS *m/z* (rel. int.) 221 $[M-43]^+$ (47), 204 $[M-60]^+$ (60), 189 (100), 175 (35), 135 (32), 133 (31), 119 (48), 109 (58), 105 (55), 91 (60). **17**: $C_{14}H_{20}O$; MS *m/z* (rel. int.) 204 $[M]^+$ (52), 189 (75), 161 (22), 133 (42), 119 (68), 105 (100), 91 (90).

Oxidation of **6**. **6** (40 mg) in CH_2Cl_2 was oxidized by CrO_3–pyridine and the reaction mixture was filtered through a short column packed with Si gel to give pale yellow oil, purified by prep. TLC to afford a ketoaldehyde (**18**) (13 mg). $C_{14}H_{20}O_2$; ^1H NMR: δ 1.07 (9H, *s*), 10.36 (1H, *s*); MS *m/z* (rel. int.) 220 $[M]^+$ (20), 215 (20), 192 (35), 124 (82), 109 (100).

Acetylation of **8**. To pyridine soln of **8** (105 mg) was added Ac_2O and the soln left overnight. Work-up as usual gave the monoacetate (**20**) (70 mg) and a dehydrated product (**21**) (5 mg) [12, 13]. **20**: $[\alpha]_D$ −42.1° (*c* 3.8); $C_{17}H_{26}O_3$; ^1H NMR: δ 0.81, 0.88, 0.90 (each 3H, *s*), 2.02 (3H, *s*), 2.40 (*m*), 4.30 (2H, *br s*, 2H), 5.56 (1H, *m*), 6.17 (1H, *d*, J = 4 Hz); IR ν_{max}^{liq} cm^{-1}: 1742, 1230 (OAc), 1190, 1165, 1140, 1120, 1095, 1060, 1010, 990, 965, 950, 935, 925, 885, 825; MS *m/z* (rel. int.) 279 $[M+H]^+$ (0.5), 218 $[M-60]^+$, (100), 203 (45), 109 (40), 105 (25), 95 (15), 91 (20), 43 (15). **21**: $C_{15}H_{22}O$; ^1H NMR: δ 0.96, 0.98, 1.27 (each, 3H), 7.10 (2H, *br s*); MS *m/z* (rel. int.) 218 (100), 203 (35), 109 (55), 105 (32).

$LiAlH_4$ *reduction of* **8**. **8** (50 mg) in Et_2O was treated with $LiAlH_4$ to give a viscous oil (42 mg) whose spectral data were identical to those of drimanediol (**22**) [13, 14].

Oxidation of **8**. To a CH_2Cl_2 soln of a CrO_3–pyridine complex was added **8** (40 mg) in CH_2Cl_2. Work-up as usual gave a yellow oil, purified by prep. TLC to afford a lactone (12 mg) whose spectral data and physical constant were in good agreement of isodrimenin (**23**) [13, 14].

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REFERENCES

1. Hartwell, J. L. (1970) *Lloydia* **33**, 288.
2. Cribb, J. W. and Cribb, A. B. (1981) *Useful Wild Plants in Australia*, pp. 96–97. Collins, Sydney.
3. Akamatsu, K. (1970) *Wakanyaku*, p. 487. Ishiyakushuppan, Tokyo.
4. Barnes, C. S. and Loder, J. W. (1962) *Aust. J. Chem.* **15**, 322.
5. Osuka, A. (1963) *Nippon Kagaku Zasshi* **84**, 748.
6. Meinwald, J. M., Prestwich, G. D., Nakanishi, K. and Kubo, I. (1978) *Science* **199**, 1167.
7. Asakawa, Y. (1981) *J. Hattori Bot. Lab.* **50**, 123.
8. Asakawa, Y. and Takemoto, T. (1979) *Experientia* **35**, 1420.
9. Kubo, I., Lee, Y.-W., Pettei, M., Pilkiewicz, F. and Nakanishi, K. (1976) *J. Chem. Soc. Chem. Commun.* 1013.
10. Nakanishi, K. and Kubo, I. (1977) *Isr. J. Chem.* **16**, 28.
11. Kubo, I. and Nakanishi, K. (1977) in *Host Plant Resistance to Pests* (Hedin, P. A., ed.) Am. Chem. Soc. Symp. Ser. No. 62, p. 165. Am. Chem. Soc., Washington.
12. Cimino, G., De Stefano, S., Minale, L. and Trivellone, E. (1977) *J. Chem. Soc. Perkin Trans.* 1, 1587.
13. Asakawa, Y., Toyota, M. and Takemoto, T. (1978) *Phytochemistry* **17**, 457.
14. Appel, H. H., Connolly, J. D., Overton, K. H. and Bond, R. P. M. (1960) *J. Chem. Soc.* 4685.
15. Asakawa, Y., Totota, M., Uemoto, M. and Aratani, T. (1976) *Phytochemistry* **15**, 1929.
16. Asakawa, Y. and Aratani, T. (1976) *Bull. Soc. Chim. Fr.* 1469.
17. Kubo, I., Miura, I., Pettei, M. J., Lee, Y.-W., Pilkiewicz, F. and Nakanishi, K. (1977) *Tetrahedron Letters* 4553.
18. Miyazaki, W., Tamaoka, H., Shinohara, M., Kaise, H., Izawa, T., Nakano, Y., Kinoshita, T., Hong, K. and Inoue, K. (1980) *Microbiol. Immunol.* **24**, 1091.
19. Huneck, S. (1967) *Z. Naturforsch.* **22**, 462.
20. Huneck, S. and Schreiber, K. (1975) *J. Hattori Bot. Lab.* **39**, 215.