Phytochemistry, Vol. 24, No 7, pp 1521–1524, 1985.
Printed in Great Britain

0031–9422/85 $3.00+0.00

DRIMANE-TYPE SESQUI- AND NORSESQUITERPENOIDS FROM *POLYGONUM HYDROPIPER*

YOSHIYASU FUKUYAMA, TSUNEO SATO, IWAO MIURA and YOSHINORI ASAKAWA*†

Laboratories of Natural Product Chemistry, Otsuka Pharmaceutical Co. Ltd., Kawauchi-cho, Tokushima 771-01, Japan; *Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770, Japan

(*Received* 26 *September* 1984)

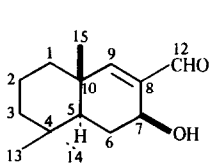
Key Word Index—*Polygonum hydropiper*; Polygonaceae; isopolygodial; polygonic acid; polygonone; 11-ethoxycinnamolide; polygodial acetal; valdiviolide; fuegin; drimane-type sesqui- and norsesquiterpenoids; anticomplement activity

Abstract—Polygonic acid, which shows weak anticomplement activity, has been isolated from leaves of *Polygonum hydropiper* together with four drimane-type sesquiterpenes, 11-ethoxycinnamolide, polygodial acetal, valdiviolide and fuegin and two drimane-type norsesquiterpenes, isopolygonal and polygonone.

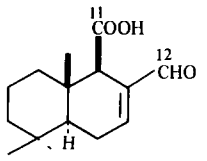
INTRODUCTION

The folk medicinal plant, *Polygonum hydropiper* L. belonging to the Polygonaceae elaborates the intense pungent sesquiterpene dialdehydes, polygodial (**5**) and warburganal (**7**), which show potent antifeedant, antimicrobial, plant growth inhibitory, cytotoxic and piscicidal activities, together with several related drimane-type sesquiterpenes [1–3]. Recently, we reported the isolation of a unique tri-*p*-coumaryl glucoside from the root of *P. hydropiper* [4]. Our continuing study of the chemical constituents of *P. hydropiper* leaves led to the isolation of three new drimane-type sesquiterpenes, polygonic acid (**2**), 11-ethoxycinnamolide (**3**) and polygodial acetals (**4a** and **4b**), together with the previously known valdiviolide (**10**) and fuegin (**11**) [5], and two new drimane-type norsesquiterpenes, isopolygonal (**1**) and polygonone (**9**). This paper reports on the isolation and structural elucidation of the novel sesquiterpenoids and their anticomplement activity.

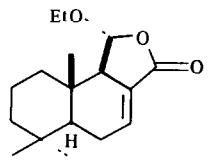
†To whom correspondence should be addressed.



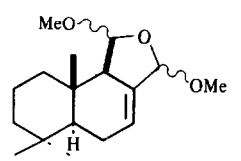
1



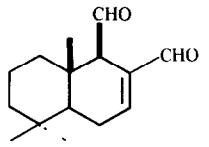
2



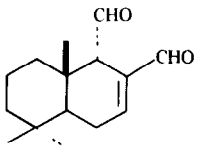
3



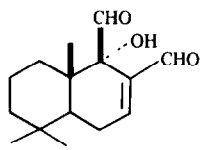
4a, **4b**



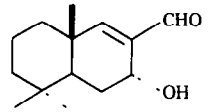
5



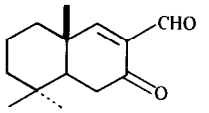
6



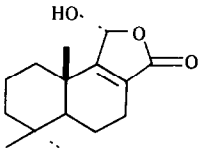
7



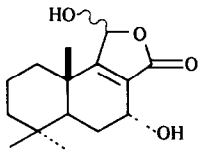
8



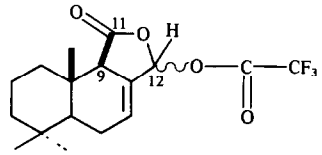
9



10



11



12

RESULTS AND DISCUSSION

The diethyl ether extract of the leaf of *P. hydropiper* was chromatographed on silica gel, followed by HPLC or CC to give four novel drimanes, isopolygonal (**1**), polygonic acid (**2**), 11-ethoxycinnamolide (**3**), and polygonone (**9**), together with the previously known drimanes, valdiviolide (**10**) and fuegin (**11**) [5]. Chromatography of the methanol extract on silica gel resulted in the isolation of the polygodial acetals **4a** and **4b**.

Isopolygonal (**1**) had the molecular formula $C_{14}H_{22}O_2$ (HRMS: $[M]^+$ 222.1643; calc. 222.1620). Its spectral data showed the presence of an α,β-unsaturated aldehyde group [IR ν_{max} cm^{-1}: 1675, 1635; UV λ_{max} nm: 226; ^{1}H NMR: δ9.39 (1H, *s*), 196.2 (*d*)], a hydroxyl group (3540 cm^{-1}), three tertiary methyl groups (δ0.90, 0.93, 1.15) and an olefinic proton (δ6.49, *s*). The ^{1}H NMR spectrum of **1** was quite similar to that of polygonal (**8**), previously isolated from the seed of *P. hydropiper* [1, 2] except for the signal δ4.66 (*dd*, J = 9.7, 7.3 Hz) due to H-7. The ^{13}C NMR spectrum indicated a drimane-type norsesquiterpenoid feature (Table 1). These spectral data suggested a close structural relationship between **1** and **8**. Oxidation of **1** with pyridium chlorochromate [2] yielded polygonone (**9**), which was also isolated from the same diethyl ether extract and its spectral data were in agreement with those of **9** obtained from **8** by the same method [2]. This chemical conversion unambiguously verified that **1** has the same plane structure as **8** except for the configuration of the secondary hydroxyl group attached at C-7. The β-configuration of this hydroxyl group was evident from the J value (9.7 Hz) for H-7 compared with that ($W_{1/2}$ = 7 Hz) in **8**. Thus, the structure of isopolygonal was established to be **1**.

Polygonic acid (**2**) had the molecular formula $C_{15}H_{22}O_3$ ($[M]^+$ 250). Its spectral data revealed the presence of a carboxylic acid group [IR ν_{max} cm^{-1}: 3150, 1705; ^{13}C NMR: δ176.6 (*s*)], an α,β-unsaturated aldehyde group [IR ν_{max} cm^{-1}: 1685, 1645; UV λ_{max} nm: 225; ^{1}H NMR: δ9.38 (*s*); ^{13}C NMR: 192.6 (*d*)], three tertiary methyl groups (δ0.92, 0.95, 0.97) which were similar to a drimane-type sesquiterpenoid. The ^{1}H NMR and ^{13}C NMR spectra of **2** were found to be very similar to those of polygodial (**5**), the main pungent substance of *P. hydropiper* [1, 2] but, significantly, the secondary aldehyde group at C-9 in **5** was absent. These facts suggested that the carboxylic group in **2** might be placed at C-9. Treatment of **2** by trifluoroacetic anhydride gave a lactol (**12**). The direct ^{1}H NMR measurement of **12** indicated the presence of a singlet methine bearing a trifluoroacetate at δ6.80 assignable to H-12 and an olefinic proton at δ6.34 due to H-7 which was shifted to a higher field since the conjugation was broken, suggesting that the carboxylic acid group in **2** should be placed at C-9. The orientation of the carboxylic acid group at C-9 was established by comparison of the ^{13}C NMR chemical shifts with those of polygodial (**5**) and isopolygodial (**6**) (Table 1). Namely, the chemical shifts of **2** were almost identical with those of **5**, except for C-9 and C-11 which were shifted due to the effect of the different functional groups on each other, whereas the signals (δ49.5, 15.2) due to C-5 and C-15 in **2** were found to be different from those in **6** (δ44.5, 21.5), thus suggesting that the carboxylic acid at C-9 had to be in the β-configuration as in **5**. Thus, the structure of polygonic acid was established to be **2**.

11-Ethoxycinnamolide (**3**) showed the presence of a conjugated γ-lactone moiety (IR ν_{max} cm^{-1}: 1755, 1685; UV λ_{max} nm: 218). The ^{1}H NMR spectrum revealed the presence of three tertiary methyl groups (δ0.83, 0.91, 0.93), an olefinic proton [δ6.84 (*ddd*, J = 4.7, 3.9, 3.6 Hz)] assignable to a β-proton of an α,β-conjugated double bond, a methine [δ5.32 (*d*, J = 6.1 Hz)] attached to an acetal carbon [δ104.0 (*d*)] and also an ethoxy group [δ1.27 (3H, *t*, J = 7.2), 2.95 and 3.69 Hz (each 1H, *m*)] attached to an asymmetrical carbon. The ^{13}C NMR spectrum (Table 1) and the extensive selective decoupling experiments of the ^{1}H NMR spectrum (see Experimental) of **3** disclosed that it is a drimane-type sesquiterpene.

Table 1. ^{13}C NMR spectral data of compounds **1**–**6** (100.61 MHz, $CDCl_3$ TMS as int standard)

C No	1	2	3	4	5	6
1	38.0 (*t*)	40.1 (*t*)	39.3 (*t*)	39.9 (*t*)	39.7 (*t*)	37.2 (*t*)
2	18.5 (*t*)	18 6 (*t*)	18.5 (*t*)	18.5 (*t*)	18.0 (*t*)	18.5 (*t*)
3	41.5 (*t*)	42.0 (*t*)	42 3 (*t*)	42 0 (*t*)	41.9 (*t*)	42.2 (*t*)
4	33 0 (*s*)	33.2 (*s*)	33.1 (*s*)	33 3 (*s*)	29.6 (*s*)	32.9 (*t*)
5	48.6 (*d*)	49.5 (*d*)	49.7 *d*)	49.9 (*d*)	49.2 (*d*)	44.5 (*d*)
6	27 0 (*t*)	24.8 (*t*)	25.1 (*t*)	23.7 (*t*)	25.1 (*t*)	25 7 (*t*)
7	67 7 (*d*)	151.0 (*d*)	136.0 (*d*)	121.2 (*d*)	154.2 (*d*)	153.1 (*d*)
8	139.1 (*s*)	139 1 (*s*)	128.2 (*s*)	137.7 (*s*)	139.0 (*s*)	137.6 (*s*)
9	164.5 (*d*)	55.3 (*d*)	57.6 (*d*)	58.0 (*d*)	60 6 (*d*)	58.8 (*d*)
10	37 7 (*s*)	35.7 (*s*)	34.1 (*s*)	33.4 (*s*)	36 9 (*s*)	37 7 (*s*)
11	—	176.6 (*s*)	104.3 (*d*)	104.0 (*d*)	202 1 (*d*)	201 7 (*d*)
12	196.2 (*d*)	192.6 (*d*)	167.8 (*s*)	107.0 (*d*)	193 6 (*d*)	192 6 (*d*)
13	21.3 (*q*)	22.1 (*q*)	21.4 (*q*)	21 5 (*q*)	21.8 (*q*)	21.9 (*q*)
14	32 7 (*q*)	33.3 (*q*)	33 2 (*q*)	33.1 (*q*)	33 0 (*q*)	32.7 (*q*)
15	20 1 (*q*)	15 2 (*q*)	15.3 (*q*)	14.1 (*q*)	15.1 (*q*)	21 5 (*q*)
OMe	—	—	—	55.7 (*q*)	—	—
				54.0 (*q*)		
OCH_2Me	—	—	14.7 (*q*)	—	—	—
OCH_2Me	—	—	66 4 (*t*)	—	—	—

Although the molecular ion in the mass spectrum was absent, a prominent peak $[M-OEt]^+$ at m/z 233.1522 ($C_{15}H_{21}O_2$) was detected, indicating that the ethoxy group could be incorporated into the acetal function. It was verified that the ethoxy group was bonded to C-11 since the methine proton [δ5.32 (*d*)] attached to the acetal carbon collapsed to a singlet on irradiation at H-9 (δ2.53). On consideration of the drimane-type sesquiterpene skeleton, coupled with the spectral data mentioned above, compound **3** might be a cinnamolide bearing the ethoxy group at C-11. Although there is no proof to support the assigned stereochemistry of the ethoxy group at C-11 at present, it was considered to be in the α-orientation which is the thermodynamically stable form [5].

Polygodial acetals (**4a** and **4b**) were isolated from the methanol extract. Their IR and ^{13}C NMR spectra did not show any absorptions attributable to carbonyl groups. The ^1H NMR spectrum showed a drimane-type sesquiterpene with, in particular, two methoxy groups (δ3.40, 3.47 for **4a** and 3.44, 3.49 for **4b**) and two different methines [δ4.90 (*d*, J = 4.0 Hz), 5.11 (*s*) for **4a**; 4.90 (*d*, J = 4.0 Hz), 5.39 (*s*) for **4b**] attached to an acetal carbon. Polygodial (**5**) was treated with methanol in the presence of *p*-toluenesulphonic acid to afford **4a** and **4b**, the formation of which was confirmed on TLC. Thus, **4a** and **4b** were the methanol adducts of polygodial (**5**). It is noteworthy that **4b** was easily isomerized to **4a** even during the measurement of the NMR spectra in deuterochloroform and this resulted in a mixture of **4a** and **4b** being formed. The stereochemistry of **4a** and **4b** remain to be clarified. It is considered that the acetals **4a** and **4b** may be artefacts because they were isolated from the methanol extract.

Table 2 shows the anticomplement activity of the isolated drimanes. Polygonic acid (**2**) possessed moderate activity among the new drimane-type sesqui- and nor-sesquiterpenoids.

EXPERIMENTAL

Mps: uncorr. The solvents used for spectral determinations were $CDCl_3$ [^1H NMR (400 or 200 MHz); ^{13}C NMR (100.61 MHz)], $CHCl_3$ ([α]$_D$ and IR), unless otherwise stated, EtOH (UV). MS: 70 eV; CC: silica gel (Merck 70–230 mesh); TLC: precoated silica gel plates F_{254} (Merck, 0.25 mm). Spots were visualized by UV (254 nm) and 40% $CeSO_4$–H_2SO_4 or 2,4-dinitrophenylhydrazine HPLC: LS-410 KG 20 × 300 mm (flow rate 9.9 ml/min), μ-Porasil 8 × 300 mm (3.5 ml/min), detected at 230 or 254 nm.

Table 2 Anticomplement activity*

Compounds	IC_{50} (μg/ml)
Isopolygonal (**1**)	>500
Polygonic acid (**2**)	250
11-Ethoxycinnamolide (**3**)	>1000
Polygodial acetals (**4a** and **4b**)	>1000
Polygodial (**5**) [2]	10.5
Isopolygodial (**6**) [2]	>500
Warburganal (**7**) [2]	>500
Polygonal (**8**) [2]	>500

*EA: 1×10^8 cells/ml; 1/180 GPC in GVB^{2+} [2] EA, Sensitized sheep erythrocytes; GPC, guinea pig serum (complement); 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

Plant material. Polygonum hydropiper was identified by one of us (Y.A.) and a voucher specimen has been deposited in the Herbarium of the Institute of Pharmacognosy, Tokushima Bunri University.

Extraction and isolation. Polygonum hydropiper was collected in Tokushima in October 1978. The Et_2O extract (250 g) of the leaves was chromatographed on silica gel using an *n*-hexane–EtOAc gradient to produce eight fractions. Fraction 4 (*n*-hexane–EtOAc, 4:1) (15.3 g) was rechromatographed on silica gel using a C_6H_6–EtOAc gradient and divided into eight fractions. Fraction 4-2 (420 mg) was purified with Bio-Beads S-X8 using C_6H_6 followed by HPLC (LS-410 KG, MeCN–H_2O, 7:3) to give 11-ethoxycinnamolide (**3**) (22.6 mg). Fraction 4-5 (930 mg) was purified with Bio-Beads S-X8 using C_6H_6 to obtain isopolygonal (**1**) (61 mg) and polygonone (**9**) (1.4 mg) whose spectral data were identical to those of the compound previously obtained from **8** by pyridium chlorochromate (PCC) oxidation [1, 2]. Fraction 4-6 (1.37 g) was further chromatographed on silica gel using C_6H_6 and divided into 20 fractions. Fraction 4-6-4 was purified by HPLC (LS-410 KG, MeCN–H_2O, 1:1) to give valdiviolide (**10**) (10.2 mg) [5]. Fraction 4-6-5 was purified by HPLC (μ-Porasil, *n*-hexane–EtOAc, 9:1) to obtain polygonal (21 mg) previously isolated from the seeds of *P. hydropiper* [1, 2]. Fraction 4-8 (1.1 g) was purified with Bio-Beads S-X8 using C_6H_6 followed by HPLC (LS-410 KG, MeCN–H_2O, 1:1) to give polygonic acid (**2**) (73.1 mg) and fuegin (**11**) (33 mg) [5].

The MeOH extract (10.0 g) of the leaf was chromatographed on silica gel using *n*-hexane–EtOAc (9:1) and divided into nine fractions. Fraction 1-3 (1.1 g) was rechromatographed on silica gel using *n*-hexane–Et_2O (85:15) to give polygodial acetal (**4b**) (50 mg). Fraction 4 (400 mg) was further purified by CC on silica gel using the same solvent system to afford polygodial acetal (**4a**) (350 mg).

Isopolygonal (**1**). Colourless oil, $[\alpha]_D^{24}$ +73° (*c* 1.88); UV λ_{max} nm: 226 (ε9330); IR ν_{max} cm^{-1}: 3540–3130 (OH), 1675 (C=O), 1635 (C=C); ^1H NMR (200 MHz): δ0.90 and 0.93 (each 3H, *s*, H-13, H-14), 1.15 (3H, *s*, H-15), 1.15 (1H, *dd*, J = 13.0, 1.6 Hz, H-5), 1.52 (1H, *ddd*, J = 13.2, 13.0, 9.7 Hz, H-6β), 2.17 (1H, *ddd*, J = 13.2, 7.3, 1.6 Hz, H-6α), 4.66 (1H, *dd*, J = 9.7, 7.3 Hz, H-7), 5.20 (1H, *br s*, OH, disappeared on addition of D_2O), 6.49 (1H, *s*, H-9), 9.39 (1H, *s*, CHO); MS m/z (rel. int.): 222.1643 $[M]^+$ (calc. 222.1620 for $C_{14}H_{22}O_2$) (14), 204 (26), 189 (31), 161 (17), 126 (80), 109 (75), 41 (100).

Polygonic acid (**2**). Colourless needles, mp 96–97°, $[\alpha]_D^{23}$ −31° (*c*, 1.06); UV λ_{max} nm: 225 (ε8140); IR ν_{max} cm^{-1}: 3150 (OH), 1705, 1685 (C=O), 1645 (C=C); ^1H NMR (200 MHz): δ0.92, 0.95, 0.97 (each 3H, *s*, H-13–H-15), 1.27 (1H, *dd*, J = 11.3, 4.9 Hz, H-5), 2.25 (1H, *dddd*, J = 20.1, 11.3, 3.4, 2.4 Hz, H-6β), 2.41 (1H, *dddd*, J = 20.1, 5.2, 4.9, 2.1 Hz, H-6α), 3.06 (1H, *ddd*, J = 3.1, 2.4, 2.1 Hz, H-9), 6.96 (1H, *ddd*, J = 5.2, 3.4, 3.1 Hz, H-7), 9.38 (1H, *s*, CHO); MS m/z (rel. int.): 250 $[M]^+$ (8), 232 (6), 189 (9), 161 (6), 124 (68), 109 (100).

11-Ethoxycinnamolide (**3**). Colourless prisms, mp 45–47°; UV λ_{max} nm: 218 (ε8530); IR ν_{max}^{KBr} cm^{-1}: 1755 (C=O), 1685 (C=C); ^1H NMR (400 MHz): δ0.83 (3H, *s*, H-15), 0.91, 0.93 (each 3H, *s*, H-13, H-14), 1.27 (3H, *t*, J = 7.2, OCH_2Me), 1.35 (1H, *dd*, J = 11.6, 5.5 Hz, H-5), 2.03 (1H, *dddd*, J = 20.3, 5.5, 3.9, 3.9 Hz, H-6α), 2.40 (1H, *dddd*, J = 20.3, 11.6, 4.7, 4.5 Hz, H-6β), 2.53 (1H, *dddd*, J = 6.1, 4.5, 3.9, 3.6 Hz, H-9), 2.95, 3.69 (each 1H, *m*, OCH_2Me), 5.32 (1H, *d*, J = 6.1 Hz, H-11), 6.84 (1H, *ddd*, J = 4.7, 3.9, 3.6 Hz, H-7); MS m/z (rel. int.): 233.1522 $[M-OCH_2Me]^+$ (calc. 233.1520 for $C_{15}H_{21}O_2$) (3), 204 (100), 189 (45), 109 (59).

Polygodial acetal (**4a**). Yellow oil, IR ν_{max}^{liq} cm^{-1}: 2940, 1460,

1440, 1090, 960; ^{1}H NMR (400 MHz): δ0.77 (3H, *s*, H-15), 0.87, 0 91 (each 3H, *s*, H-13, H-14), 1.35 (1H, *dd*, J = 11.8, 5.2 Hz, H-5), 1.91 (1H, *dddd*, J = 18 4, 11 8, 3.7, 3.3 Hz, H-6β), 2.19 (1H, *dddd*, J = 18.4, 5 2, 3.3, 3.3 Hz, H-6α), 2 45 (1H, *dddd*, J = 4.0, 3.7, 3.3, 3.3 Hz, H-9), 3.40, 3.47 (each 3H, *s*, OMe), 4.90 (1H, *d*, J = 4.0 Hz, H-11), 5.11 (1H, *s*, H-12), 5 78 (1H, *ddd*, J = 3.3, 3.3, 3.3 Hz, H-7); MS m/z (rel. int.): 249.1855 $[M-OMe]^+$ (calc. 249.1855 for $C_{16}H_{25}O_2$) (30), 220 (100), 205 (100), 135 (70), 111 (85), 41 (50)

Polygodial acetal (**4b**). Yellow oil, IR ν_{max}^{liq} cm^{-1}: 2900, 1455, 1435, 1090; ^{1}H NMR (400 MHz): δ0.88 (3H, *s*, H-15), 0.86, 0.91 (each 3H, *s*, H-13, H-14), 1.20 (1H, *m*, H-5), 1.90 (1H, *m*, H-6β), 2.19 (1H, *m*, H-6α), 2.21 (1H, *m*, H-9), 3.49, 3.45 (each 3H, *s*, OMe), 4.90 (*d*, J = 4.0 Hz, H-11), 5.39 (1H, *s*, H-12), 5.79 (1H, *ddd*, J = 3 3, 3 3, 3.3 Hz, H-7); MS m/z (rel. int.): 249.1865 $[M-OMe]^+$ (calc 249.1855 for $C_{16}H_{25}O_2$) (30), 220 (100), 205 (100), 135 (75), 111 (85), 41 (60)

Trifluoroacetylation of **2**. A mixture of **2** (5 mg), $CDCl_3$–C_5D_5N (0 4–0.1 ml) and trifluoroacetic anhydride (20 μl) was allowed to stand at room temp. in an NMR tube. After the reaction was completed (calc 2 hr), the reaction mixture was subjected to ^{1}H NMR analysis. The ^{1}H NMR data indicated the formation of trifluoroacetyl lactol (**12**): ^{1}H NMR (400 MHz): δ0.84 (3H, *s*), 0 93 (6H, *s*), 3.03 (1H, *ddd*, J = 3.5, 3.5, 3.5 Hz, H-9), 6.34 (1H, *ddd*, J = 3 5, 3.5, 3.5 Hz, H-7), 6.80 (1H, *s*, H-12).

Oxidation of **1**. Compound **1** (20 mg) in CH_2Cl_2 (0.5 ml) was oxidized by pyridium chlorochromate (PCC) (80 mg) in CH_2Cl_2 (2 ml) at room temp. for 3 hr and the reaction mixture was filtered through a short column packed with silica gel to give polygonone (**9**) (8 mg) whose spectral data were identical with those of **9** obtained from the PCC oxidation of polygonal (**8**) [1, 2].

Preparation of polygodial acetals **4a** *and* **4b**. To a soln of polygodial (**5**) (2 mg) in MeOH (2 ml) was added one piece of *p*-toluenesulphonic acid and the mixture was stirred at room temp. overnight. TLC examination (*n*-hexane–Et_2O, 75:25) indicated the formation of **4a** and **4b** (R_f 0.41 for **4a** and 0.52 for **4b**).

Acknowledgement—This work was supported in part by a Grant-in-Aid for Cancer Research (to Y.A.) from the Ministry of Health and Welfare.

REFERENCES

1. Asakawa, Y. and Takemoto, T. (1979) *Experientia* **35**, 1420.
2. Fukuyama, Y., Sato, T., Asakawa, Y. and Takemoto, T. (1982) *Phytochemistry* **21**, 2895.
3. Asakawa, Y. (1984) *Rev. Latinoam. Quim.* **14**, 109.
4. Fukuyama, Y., Sato, T., Miura, I., Asakawa, Y. and Takemoto, T. (1983) *Phytochemistry* **22**, 549.
5. Appel, H. H., Bond, R. P. M. and Overton, K. H. (1963) *Tetrahedron* **19**, 635.