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POLYGONOLIDE, AN ISOCOUMARIN FROM *POLYGONUM HYDROPIPER* POSSESSING ANTI-INFLAMMATORY ACTIVITY

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Key Word Index—*Polygonum hydropiper*; Polygonaceae; polygonolide; 3,4-dimethyl-6-methoxy-8-hydroxyisocoumarin; synthesis; anti-inflammatory reaction.

Abstract—A new isocoumarin polygonolide which inhibits the reversed passive Arthus reaction has been isolated from the methanol extract of the root of *Polygonum hydropiper*. The structure of polygonolide has been elucidated on the basis of spectroscopic data and confirmed to be 3,4-dimethyl-6-methoxy-8-hydroxyisocoumarin by total synthesis.

INTRODUCTION

The folk medicinal plant, *Polygonum hydropiper* (Polygonaceae) elaborates the intense pungent sesquiterpene dialdehyde, polygodial, which shows potent antifeedant, antimicrobial, plant growth inhibitory, cytotoxic, piscicidial and anticomplement activities, and the related drimane-type sesquiterpenoids in its leaves and seeds [1–4]. Recently, we reported the isolation of hydropiperoside, a novel tri-*p*-coumaryl glycoside from the roots of this plant [5]. Our continuing study of the biologically active substances from the roots of the plant led to the isolation of a new isocoumarin (**1**) named polygonolide, which shows inhibitory effect on the reversed passive Arthus reaction (RPAR) [6]. This paper reports the structural elucidation and a total synthesis of polygonolide, and the effects of its derivatives on the RPAR in the rat.

RESULTS AND DISCUSSION

Polygonolide (**1**), mp 142°, had the molecular formula $C_{12}H_{12}O_4$ ($[M]^+$ *m/z* 220.0733). Its IR spectrum revealed the presence of a hydroxy group (3425 cm^{-1}), conjugated carbonyl (1673 cm^{-1}) and olefinic (1625 cm^{-1}) moieties. The ^{13}C NMR spectrum (Table 1) of **1** showed the presence of 12 carbons comprised of three methyl groups including one methyl group bearing an oxygen atom, two non-substituted aromatic carbons, four substituted aromatic carbons including two aromatic carbons bearing an oxygen atom, two quaternary vinyl carbons and one carbonyl group. The ^1H NMR spectrum of **1** exhibited signals due to two olefinic methyl groups [δ2.09, 2.30 (each 3H, *q*, J = 0.7 Hz)] showing homoallylic coupling to each other, a methoxy group [δ3.89 (3H, *s*)], *meta*-coupled aromatic protons [δ6.40, 6.46 (each 1H, *d*, J = 2.4 Hz)], and a hydrogen-bonded hydroxy proton appeared in lowfield (δ11.46). The above spectral data disclosed that **1** could be assignable to a 6-methoxy-8-hydroxyisocoumarin bearing 3,4-dimethyl groups. The mass spectrum of **1** showed prominent peaks at *m/z* 177 $[M-Ac]^+$, 149 $[M-Ac-CO]^+$, suggesting that **1** is a 3,4-dimethyl substituted isocoumarin. In addition, this fact was strongly supported by the observation of the NOE for H-5 (9%) upon irradiation at H-10. Thus, the structure of polygonolide was elucidated as **1**. In order to confirm the new structure as well as evaluate biological properties, the total synthesis of **1** was carried out starting from 1,3-dimethoxybenzene (Scheme 1). On Friedel–Crafts reaction between 1,3-dimethoxybenzene and tigloyl chloride using stannic chloride, **3** was obtained in 84.6% yield. Intramolecular cyclization of **3** was completed by polyphosphoric acid (PPA) to yield the indanone (**4**), which gave the enolacetate (**5**) after refluxing with isopropenyl acetate in the presence of acid [7]. Ozonolysis of **5** afforded the lactol (**6**) in 79% yield followed by reductive treatment with dimethylsulphide [8]. On acid treatment of **6**, 6,8-dimethoxyisocoumarin (**2**) was obtained quantitatively. Selective demethylation of **2** using boron trichloride [9] at −70° gave 8-hydroxy-

Table 1. ^{13}C NMR data of **1** (50 MHz, $CDCl_3$, TMS int. standard)

Carbon	**1**
1	167.1 *s*
3	150.1 *s*
4	140.8 *s*
4a	108.9 *s*
5	99.7 *d*
6	166.3 *s*
7	99.6 *d*
8	164.5 *s*
8a	100.2 *s*
9	17.1 *q*
10	12.4 *q*
OMe	55.7 *q*

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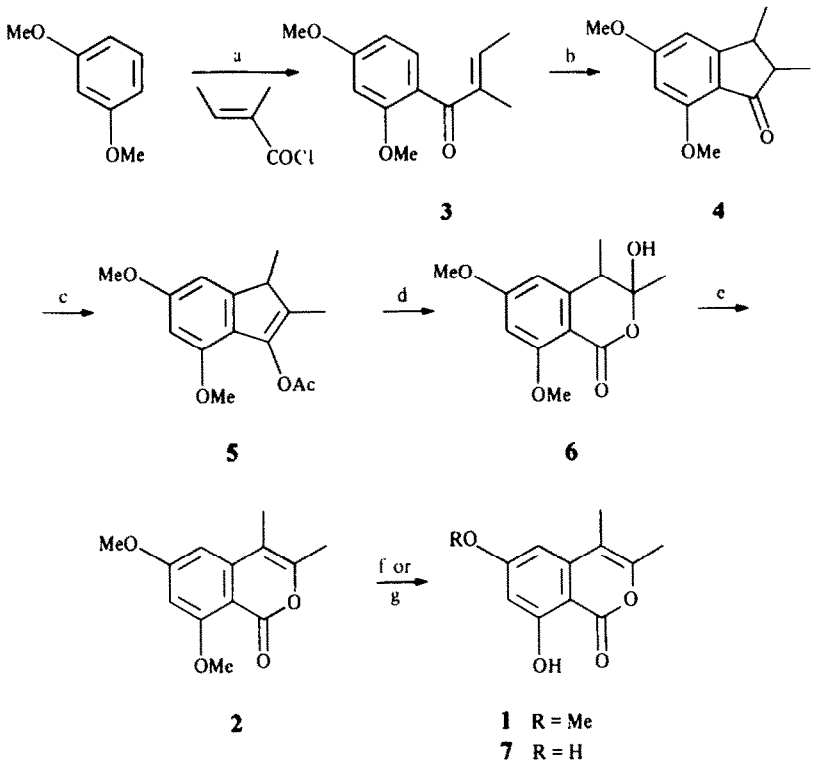
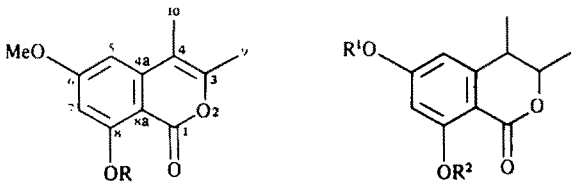


Scheme 1. Synthetic scheme of polygonolide (1).

6-methoxy-3,4-dimethylisocoumarin, the spectral data (NMR, mass spectrum and IR) of which were superimposable with those of polygonolide (1); mmp determination of both samples showed no depression. On the other hand, 6,8-dihydroxy-3,4-dimethylisocoumarin (7) was obtained quantitatively by the use of boron tribromide [10] at −78°. Dihydroisocoumarins **8** and **9** were readily available from usual hydrogenation of **2** and **1**, respectively.

Garg *et al.* [11, 12] reported that an ethanolic extract of the root of *P. hydropiper* showed antifertility activity against female albino rats. Through our extensive study on chemical constituents in this plant, polygonolide (1) might be implicated as a fertility regulatory principle in the light of Garg's publications. General pharmacological examination on polygonolide (1) found that **1** inhibited the reversed passive Arthus reaction (RPAR) in rats *via* oral administration, which represents an acute model of immune complex induced inflammation [13, 14]. The effects of **1** and its derivatives on the RPAP test at a dose of 100 mg/kg p.o. are summarized in Table 2. Although the inhibitory effects on the RPAR are moderate in comparison with that of hydrocortisone, **1** seems to have the potential of becoming a prototype for developing a new antiinflammatory agent due to its simple structure,



1 R = H
2 R = Me

8 $R^1 = R^2 = Me$
9 $R^1 = Me$, $R^2 = H$

Table 2. Effect of polygonolide (1) and its derivatives on rat RPAR

Compound	[Inhibition %*]	
1	39.2	$P < 0.05$
2	36.8	$P < 0.05$
6	16.8	
7	34.9	$P < 0.05$
8	54.4	$P < 0.01$
9	NA†	

*Represents the inhibition % at a dose of 100 mg/kg oral administration 1 hr before eliciting the RPAR.
†No significant activity obtained.

which does not correspond to any of the previously known anti-inflammatory agents including the steroidal ones. The detailed pharmacological properties of **1** and its derivatives will be published elsewhere.

EXPERIMENTAL

Mps are uncorr. Solvents used for spectral determinations were TMS–$CDCl_3$ [^{1}H NMR (200 MHz); ^{13}C NMR (50 MHz)], EtOH (UV). MS: 70 eV; CC: silica gel (Merck 70–230 mesh), TLC: precoated silica gel F_{254} (Merck, 0.25 mm). Spots were visualized under UV (254 nm) and after spraying with 40% $CeSO_4$–H_2SO_4.

Plant material. Polygonum hydropiper L. identified by Y.A. is deposited in the Herbarium of the Institute of Pharmacogonosy, Tokushima Bunri University.

Extraction and isolation. A MeOH extract (450 g) of roots of *P. hydropiper*, collected in Oct. 1978, was partitioned between EtOAc and H_2O to obtain an EtOAc soluble portion (90 g). This fraction (30 g) was chromatographed on silica gel using *n*-hexane–C_6H_6–$CHCl_3$–MeOH and divided into six fractions. The second fraction (3.3 g) eluted with C_6H_6 was rechromatographed on silica gel (C_6H_6–EtOAc, 1:1) to give polygonolide (**1**, 215 mg) as colourless prisms, mp 142°; UV λ_{max} nm(ε): 242 (13 000), 248 (16 000), 260 (3000), 280 (1900), 298 (1100), 330 (1800); IR ν_{max}^{KBr} cm^{-1}: 3425 (OH), 1673 (C=O), 1625 (C=C), 1580 (aromatic ring); MS *m/z* (rel. int): 220.0733 $[M]^+$ (100, $C_{12}H_{12}O_4$), 205 $[M-Me]^+$ (20), 191 (30), 177 (80), 149 (50); ^{1}H NMR: δ2.09 (3H, *q*, *J* = 0.7 Hz, 9-H), 2.30 (3H, *q*, *J* = 0.7 Hz, 10-H), 3.89 (3H, *s*, OMe), 6.40 (1H, *d*, *J* = 2.5 Hz, 7-H), 6.46 (1H, *d*, *J* = 2.5 Hz, 5-H, 11.46 (1H, *s*, OH); ^{13}C NMR: Table 1.

Synthesis of polygonolide (*1,3-dimethoxy-6-tigloylbenzene*, **3**). To a soln of 1,3-dimethoxybenzene (3.0 g, 21.7 mmol) and tigloyl chloride (2.57 g, 21.7 mmol) in 10 ml of CH_2Cl_2 at 0° was added dropwise $SnCl_4$ (2.75 ml, 23.9 mmol) over 15 min. After stirring at room temp. overnight, the reaction was terminated by the addition of 2 N HCl (50 ml) and then extracted with Et_2O. The Et_2O soln was washed sequentially with satd $NaHCO_3$ soln, satd NaCl soln, then dried (Na_2SO_4). Removal of solvent *in vacuo* gave an oil. Distillation at 130° (0.5 mmHg) yielded **3** (4.05 g, 84.6%); IR ν_{max}^{film} cm^{-1}: 1638, 1605, 1580, 1505; ^{1}H NMR: δ1.83 (3H, *d*, *J* = 7.0 Hz), 1.91 (3H, *s*) 3.77 (3H, *s*), 3.83 (3H, *s*), 6.36 (1H, *q*, *J* = 7.0 Hz), 6.46 (2H, *m*), 7.14 (1H, *d*, J = 8.9 Hz); MS *m/z*: 220 $[M]^+$, 205, 165. (Calc. $C_{13}H_{16}O_3$: C, 70.88; H, 7.32. Found: C, 71.06; H, 7.22.) *2,3-Dimethyl-5,7-dimethoxyindanone* (**4**). A mixture of **3** (11.22 g, 50.9 mmol) and PPA (100 g) was stirred at 90–100° for 2.5 hr. After cooling at 0°, ice-H_2O was added. The reaction mixture was then extracted with EtOAc. The EtOAc soln was sequentially washed with satd $NaHCO_3$ soln, satd NaCl soln, then dried (Na_2SO_4). Removal of solvent *in vacuo* left a crude oil, which was distilled at 144–147° (0.5 mmHg) to give **4** (9.75 g, 86.9%); IR ν_{max}^{film} cm^{-1}: 1692, 1600, 1590; ^{1}H NMR: δ1.16 and 1.20 (total 3H, each *d*, *J* = 7.5 Hz), 1.26 and 1.38 (total 3H, each *d*, *J* = 7.3, 7.0 Hz), 2.19 (1H, *m*), 2.77 and 3.35 (total 1H, *m*), 3.88 (3H, *s*), 3.90 (3H, *s*), 6.30 (1H, *d*, *J* = 1.9 Hz), 6.48 (1H, *d*, *J* = 1.9 Hz); MS *m/z*: 220 $[M]^+$ 205, 191, 183, 180. (Calc. $C_{13}H_{16}O_3$: C, 70.88; H, 7.32. Found: C, 70.64; H, 7.33.)

3-Acetoxy-1,2-dimethyl-4,6-dimethoxyindene (**5**). A mixture of **4** (1.38 g, 6.26 mmol), isopropenylacetate (20 ml) and three crystals of *p*-toluenesulphonic acid was stirred at 100° under an N_2 atmosphere for 20 hr. After cooling to room temp., the reaction mixture was concd *in vacuo* and then diluted with Et_2O. The Et_2O soln was washed sequentially with satd $NaHCO_3$ soln, satd NaCl soln, then dried (K_2CO_3). Removal of solvent *in vacuo* left an oil, which was distilled at 220° (0.5 mmHg) to give **5** (1.6 g, 97.5%); IR ν_{max}^{film} cm^{-1}: 1757, 1652, 1590; ^{1}H NMR: δ1.28 (3H, *d*, *J* = 7.6 Hz), 1.83 (3H, *d*, *J* = 0.6 Hz), 2.27 (3H, *s*), 3.21 (1H, *q*, *J* = 7.6 Hz), 3.77 (3H, *s*) 3.80 (3H, *s*), 6.34 (1H, *d*, *J* = 1.8 Hz), 6.57 (1H, *d*, *J* = 1.8 Hz); MS *m/z*: 262 $[M]^+$, 210, 205, 189.

3,4-Dihydro-3,4-dimethyl-6,8-dimethoxy-3-hydroxyisocoumarin (**6**). A soln of **5** (1.38 g, 6.26 mmol) in dried MeOH (60 ml) and dried CH_2Cl_2 (28 ml) was cooled to −78°. Into this soln was passed O_3 for 1.5 hr. After removal of excess O_3 by bubbling N_2, dimethylsulphide (6 ml) was added. The reaction mixture was warmed to room temp. and stirring was continued overnight. Satd Na_2CO_3 soln (50 ml) was added and then stirring was continued for 2 hr. The aq. layer was washed with EtOAc and then acidified with 3 N HCl. The acidic aq. layer was extracted with EtOAc. The EtOAc soln was then washed with satd NaCl soln and dried (Na_2SO_4). Removal of solvent *in vacuo* afforded a crystalline solid, which was recrystallized from CH_2Cl_2 to give **6** (3.72 g, 79%) as colourless prisms, mp 112–113°; IR ν_{max}^{KBr} cm^{-1}: 3360, 1683, 1598, 1579, 1456; ^{1}H NMR: δ1.31 and 1.40 (total 3H, each *d*, *J* = 7.0 Hz), 1.64 (3H, *s*), 3.05 (1H, *m*), 3.87 (3H, *s*), 3.92 (3H, *s*), 6.40 (2H, *m*); MS *m/z*: 252 $[M]^+$, 234, 210, 193, 163. (Calc. $C_{13}H_{16}O_5$: C, 61.89; H, 6.39. Found: C, 61.94; H, 6.36.)

3,4-Dimethyl-6-8-dimethoxyisocoumarin (**2**). A soln (19.5 ml) prepared from 70% $HClO_4$ (0.05 ml) and Ac_2O (4.8 ml) in 46 ml of EtOAc was added to **6** (195 mg, 0.772 mmol). The reaction mixture was stirred at room temp under N_2 for 30 min. Cold (0°) satd $NaHCO_3$ soln was added and the reaction mixture extracted with EtOAc. The EtOAc soln was washed sequentially with satd $NaHCO_3$ soln, satd NaCl soln, then dried (Na_2SO_4). Removal of solvent *in vacuo* gave a crystalline solid, which was recrystallized from CH_2Cl_2–*n*–hexane to afford **2** (180 mg, 99.6%) as colourless prisms, mp 181.5°; IR ν_{max}^{KBr} cm^{-1}: 3090, 1715, 1664, 1602, 1572; ^{1}H NMR: δ2.10 (3H, *q*, *J* = 0.7 Hz), 2.28 (3H, *q*, *J* = 0.7 Hz), 3.93 (3H, *s*), 3.98 (3H, *s*), 6.44 (1H, *d*, *J* = 2.3 Hz), 6.47 (1H, *d*, *J* = 2.3 Hz); MS *m/z*: 234 $[M]^+$, 205, 191, 163, 161. (Calc. $C_{13}H_{14}O_4$: C, 66.65; H, 6.02. Found: C, 67.17; H, 6.13.)

3,4-Dihydro-3,4-dimethyl-6,8-dimethoxyisocoumarin (**8**). Usual hydrogenation of **2** (3.0 g, 12.8 mmol), 10% Pd–C (600 mg) in 5 ml of EtOH gave **8** quantitatively followed by silica gel CC ($CHCl_3$ eluent); IR ν_{max}^{film} cm^{-1}: 1710, 1600, 1577; ^{1}H NMR: δ1.21 (3H, *d*, *J* = 7.3 Hz), 1.39 (3H, *d*, *J* = 6.6 Hz), 2.79 (1H, *dt*, *J* = 2.9, 7.3 Hz), 3.87 (3H, *s*), 3.97 (3H, *s*), 4.59 (1H, *dt*, *J* = 2.9, 6.6 Hz), 6.32 (1H, *d*, *J* = 2.2 Hz), 6.81 (1H, *d*, *J* = 2.2 Hz); MS *m/z*: 236.1055 $[M]^+$ ($C_{13}H_{16}O_4$), 219, 203, 192, 175, 163.

6,8-Dihydroxy-3,4-dimethylisocoumarin (**7**). To a soln of **2** (2.4 g, 10.2 mmol) in 20 ml of dried CH_2Cl_2 was added 48.8 ml (49.9 mmol) of 0.838 M BBr_3 soln in CH_2Cl_2 at −70° over 15 min. The reaction mixture was stirred at room temp. overnight. H_2O (100 ml) was added and the ppt formed filtered and washed with H_2O. The filtrate was extracted with EtOAc. The EtOAc soln was washed with satd NaCl soln and then dried (Na_2SO_4). Removal of solvent *in vacuo* left a solid, which was sublimed under red. pres. to give quantitatively **7** as colourless crystals, mp 270°; IR ν_{max}^{KBr} cm^{-1}: 3200, 1662, 1621; ^{1}H NMR: δ2.07 (3H, *q*, *J* = 1.0 Hz), 2.29 (3H, *q*, *J* = 1.0 Hz), 6.38 (2H, *s*), 10.32 (1H, *s*), 11.33 (1H, *s*); MS *m/z*: 206 $[M]^+$, 163, 135, 107. (Calc. $C_{11}H_{10}O_4$: C, 64.07; H, 4.89. Found: C, 64.15; H, 4.92.)

Polygonolide (**1**). A soln of **2** (2.0 g, 8.54 mmol) in 20 ml of dried CH_2Cl_2 was added dropwise to 19.1 ml of 1.34 M BCl_3 in CH_2Cl_2 at −78° over 15 min. The reaction mixture was stirred at room temp. for 15 min and then poured onto ice-H_2O. Separated aq. layer was extracted with EtOAc. The combined organic layer was washed with satd NaCl soln and then dried (Na_2SO_4). Removal of solvent *in vacuo* afforded a crystalline solid, which was recrystallized from CH_2Cl_2–*n*-hexane to give **1** (1.62 g, 86.2%) as colourless prisms, mp 142° (142° in natural form); Calc. $C_{12}H_{12}O_4$: C, 65.44; H, 5.49. Found: C, 65.47; H, 5.55. The spectral data were identical to those of natural polygonolide.

3,4-Dihydro-3,4-dimethyl-6-methoxy-8-hydroxyisocoumarin (**9**). Usual hydrogenation of **1** (1.56 g, 7.08 mmol) using 10% Pd–C (500 mg) in 50 ml of EtOH gave **9** (1.56 g, 99%) as an oil; ^{1}H NMR: δ1.20 (3H, *d*, J = 7.4 Hz), 1.42 (3H, *d*, J = 6.6 Hz), 2.85 (1H, *dt*, J = 2.9, 7.4 Hz), 3.83 (3H, *s*), 4.72 (1H, *dt*, J = 2.9, 6.6 Hz), 6.27 (1H, *d*, J = 2.4 Hz), 6.37 (1H, *d*, J = 2.4 Hz); MS *m/z*: 222 $[M]^+$, 193, 178.

Reversed passive Arthus reaction (RPAR) test [15]. Reactions were induced by intradermal (id) injection of rabbit anti-ovalbumin serum in rats. Immediately thereafter, OVA (ovalbumin) was injected intravenously (IV). The drugs were administered orally (po) 1 hr before induction of Arthus reaction. After 5 hr, animals were sacrificed and the reaction sites of erythema and haemorrhage measured.

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