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PHENOLIC COMPOUNDS FROM *PEPEROMIA OBTUSIFOLIA*

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Key Word Index—*Peperomia obtusifolia*; Piperaceae; piperogalin; peperobtusins A–B; isopeperobtusin A, 2′-hydroxydihydrochalcone.

Abstract—From the aerial parts of *Pepermia obtusifolia*, five phenolic compounds bearing a methyl, an isoprenyl and a geranyl group on a benzene ring core have been isolated. The structures were determined by the spectroscopic analysis including 2D NMR techniques and synthesis. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Some *Peperomia* species have been used as folk medicine, e.g. *P. japonica* Makino has been used for the treatment of malignant tumors [1]. Compared with the genus *Piper*, few phytochemical studies of *Peperomia* have been reported [2–6]. Nevertheless, some structurally attractive compounds have been isolated, for example, unique secolignans (peperomins A–D) from *P. japonica* Makino [2] and *P. glabella* (Sw.) A. Dieter [3] and prenylated phenols with the antiparasite activity from *P. galioides* H.B.K [4, 5]. In this paper we describe the isolation and structural determination of phenolic compounds in aerial parts of *P. obtusifolia*. A. Dieter which grows from Mexico to the northern parts of South America and is well known as a popular foliage plant.

RESULTS AND DISCUSSION

Fresh aerial parts of *P. obtusifolia* were homogenized in MeOH and left at room temperature. After filtration, the residues were further extracted with MeOH. The first MeOH solution and the second solution were combined and concentrated *in vacuo* to give a dark greenish extract. The EtOAc soluble portion of the extract after partition was chromatographed on silica gel eluted with a *n*-hexane-Me_2CO mixture to give five compounds (**1**–**5**) after further purification with silica gel or Sephadex LH20 column chromatography, and preparative TLC.

Compound **1**, a yellow oil, gave M^+ at *m/z* 328.2395 in the high resolution EIMS (HR-EIMS) which corresponds to $C_{22}H_{32}O_2$. The UV spectrum indicated that **1** had a benzene ring. The ^1H and ^{13}C NMR spectrum showed the presence of a geranyl, an isoprenyl, a methyl and two phenolic hydroxyl groups. By NMR spectral analysis (HH long range COSY, CH COSY, COLOC and NOE experiments), **1** was identified as piperogalin which has been isolated from *P. galioides* [3].

Compound **2**, a pale yellow oil, gave M^+ at *m/z* 326.2257 in the HR-EIMS which corresponds to $C_{22}H_{30}O_2$. Compound **2** gave a monomethyl ether (**2a**) by the usual methylation. The UV spectral data suggested that **2** was a benzene derivative. The ^1H NMR spectrum showed the presence of a methyl (δ 2.20), an isoprenyl [δ 1.66, 1.77 (3H each, *br s*, Me), 3.24, 3.36 (1H each, *dd*, J = 15, 7 Hz, CH_2), 5.15 (1H, *t like m*, CH=)], a phenolic hydroxyl (δ 4.75) and an aromatic proton in a singlet (δ 6.15). The ^1H NMR spectrum also exhibited the presence of mutually coupled two methylenes [δ 1.70 (1H, *m*), 1.88 (1H, *ddd*, J = 14, 5, 5 Hz) and 2.42 (1H, *ddd*, J = 17, 5, 5 Hz), 2.61 (1H, *ddd*, J = 17, 5, 5 Hz)], three further olefinic protons [5.45 (*d*, J = 16 Hz), 5.76 (*br d*, J = 11 Hz) and 6.33 (*dd*, J = 16, 11 Hz)], two vinyl methyls [1.66, 1.73 (3H each, *br s*)] and a tertiary methyl group [1.41 (3H, *s*)]. HH correlations in the HH long range COSY spectrum were observed as in Fig. 1 and NOEs as in Fig. 2. Thus, **2** had a C_{10} alkyl chain composed of an *E*-diene unit drawn as a bold line in Fig. 1. The NOE experiments indicated that *ortho*-positions of the aromatic proton were substituted with the phenolic OH (OCH_3 in the case of **2a**) and the methyl group. The isoprenyl group was attached at the *ortho*-position to the methyl group. Therefore, the structure of this compound is either **2** or **2′**. In the ^{13}C NMR spectrum, quaternary carbons bearing an oxygen were observed at δ 152.57 and 153.43, indicating that **2** had a resorcinol, not a catechol oxygenation. The isoprenyl meth-

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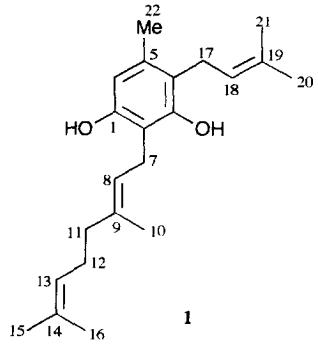

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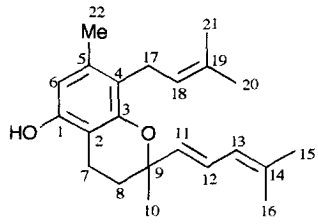

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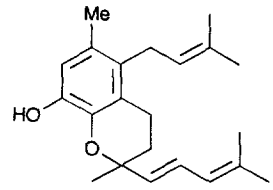

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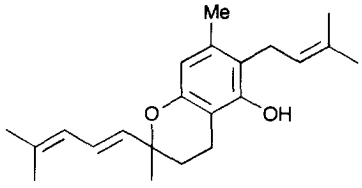

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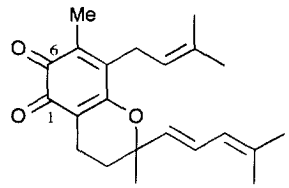

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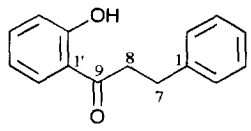


5

ylene carbon at δ 25.65 suggested that both *ortho*-positions of the isoprenyl group were substituted with a *C*- or *O*-substitution [6]. These results showed that the structure was **2**, and named peperobtusin A, which was substantiated by the analysis of the COLOC spectrum (Fig. 3). The quaternary carbon (δ 153.43) was correlated with the isoprenyl methylene protons through ^3J.

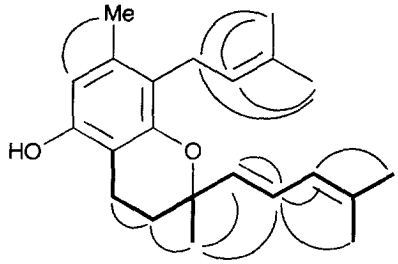
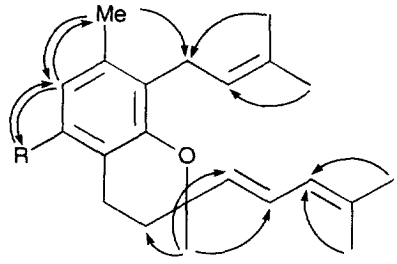


Fig. 1. HH correlations in HH long range COSY (PI1 = 100 ms) of **2**.

Compound **3**, a pale yellow oil, gave M^+ at *m/z* 326.2264 in the HR-EIMS. All spectral data of **3** were very similar to those of **2**. However **2** reacted with Gibbs reagent to become purple, **3** did not. Hence, a *para*-position of the hydroxyl group in **3** was not substituted. The structure of **3** is an isomer of **2**, in which the C_{10} alkyl chain was cyclized with the other hydroxyl group at C-1. The structure of **3**, named isopeperobtusin A, was confirmed by the results of COLOC spectrum and NOE experiments.

Compound **4**, a yellow oil, gave M^+ at *m/z* 340.2055 in the HR-EIMS, which indicated the empirical formula $C_{22}H_{28}O_3$. In the ^1H NMR spectrum, the presence of an isoprenyl, a methyl and a C_{10} alkyl chain was exhibited as in **2** and **3**. However the aromatic proton and phenolic hydroxyl group observed in **2** and **3** were lost in **4**. The ^{13}C NMR spectrum showed



R = OH (**2**) and OCH3 (**2a**)

Fig. 2. NOE interactions in DIFNOE of **2** and **2a**.

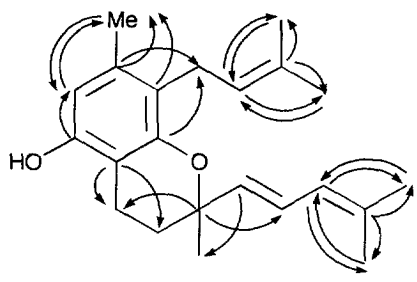


Fig. 3. CH long range correlations in COLOC spectrum (J = 10 Hz) of **2**.

the presence of two carbonyl groups at δ 182.60 and 186.44, which suggested that **4** was a *para-* or *ortho*-benzoquinone derivative of **2** or **3**. NOEs were observed between the methyl group and the methylene protons of isoprenyl group, and between the methyl group on a pyran ring (δ 1.49) and the same methylene protons. These results indicated that the substitution pattern of the methyl, the isoprenyl and the C_{10} alkyl chain unit was the same as **2**. Therefore, the structure of **4** was the *ortho*-benzoquinone derivative of **2**, which was confirmed by the analysis of the COLOC spectrum, and named peperobtusin B.

Compound **5**, colorless oil, gave M^+ at *m/z* 226.1008 in the HR-EIMS corresponds to $C_{15}H_{14}O_2$. In the ^1H NMR spectrum, characteristic signals based on dihydrochalcone were observed as well as a monosubstituted and a 1,2-disubstituted benzene ring, suggesting the structure of **5** was 2′-hydroxydihydrochalcone. A hydrogenated product of 2′-hydroxychalcone prepared by condensation of benzaldehyde and 2-hydroxyacetophenone was identified with **5**. Dihydrochalcones have been isolated from some Piperaceaeous plants such as *Piper aduncum* L. [7], but **5** has a very simple and unusual substitution for a naturally occurring dihydrochalcone.

EXPERIMENTAL

Plant material

Plant material of *Peperomia obtusifolia* A. Dieter horticulturally cultivated at Nagoya City, Japan were purchased in March, 1997. The voucher specimens are deposited at the Herbarium of Gifu Pharmaceutical University.

Extraction and isolation

Fresh aerial parts (8 kg) of *P. obtusifolia* homogenized with a mixer were added to MeOH (15 l). After filtration, the residues were again left in MeOH (15 l) at room temp. Both MeOHs were combined and concentrated *in vacuo* to give a greenish extract (227 g). The extract was partitioned with EtOAc to give an EtOAc soluble extract (102 g). The EtOAc extract was chromatographed on silica gel eluted with *n*-hexane–Me_2CO mixture. The *n*-hexane–Me_2CO (8:1) fraction was chromatographed on Sephadex LH20 (solvent, Me_2CO). Crude phenolic compounds were further chromatographed on silica gel column, and purified by preparative TLC [solvent systems: $CHCl_3$; benzene; cyclohexane–Me_2CO (20:1); cyclohexane–benzene (5:1) and benzene–Me_2CO (5:1)] repeatedly to give **1** (120 mg), **2** (56 mg), **3** (48 mg), **4** (6 mg) and **5** (12 mg), respectively.

Compound **1** (*piperogalin*). A yellow oil. HREIMS M^+ *m/z* 328.2395 (Calcd. 328.2502 for $C_{22}H_{32}O_2$), EIMS *m/z* (rel. int.): 328 (30, M^+), 313 (3), 243 (20), 205 (41), 203 (45), 189 (23), 177 (17), 162 (17), 162 (18), 149 (100), UV λ_{max}^{MeOH}: 237, 282 nm, ^1H NMR (400 MHz, $CDCl_3$): δ 1.59 (3H, *br s*, Me, H-20), 1.67 (3H, *br s*, Me, H-21), 1.72 (3H, *br d*, *J* = 1 Hz, Me, H-15), 1.79 (6H, *br s*, Me × 2, H-10 and 16), 2.05 (2H, *t* like *m*, CH_2, H-11), 2.08 (2H, *t* like *m*, CH_2, H-12), 2.20 (3H, *br s*, Me, H-22), 3.27 (2H, *br d*, *J* = 7 Hz, CH_2, H-17), 3.39 (2H, *br d*, *J* = 7 Hz, CH_2, H-7), 5.03 (1H, *br s*, C-1-OH), 5.05 (1H, *t* like *m*, CH=, H-13), 5.13 (1H, *t* like *m*, CH=, H-18), 5.25 (1H, *t* like *m*, CH=, H-8), 5.35 (1H, *br s*, C-3-OH), 6.25 (1H, *s*, H-6).

Compound **2** (*peperobtusin A*). A pale yellow oil. $[\alpha]_D$ +2.6° (*c* = 0.39, MeOH), HREIMS M^+ *m/z* 326.2257 (Calcd. 326.2354 for $C_{22}H_{30}O_2$), EIMS *m/z* (rel. int.): 326 (100, M^+), 311 (12), 283 (20), 270 (21), 25 (17), 243 (24), 227 (60), 205 (45), 189 (42), 161 (30), 149 (75), 122 (13), 107 (45), 91 (17). UV λ_{max}^{MeOH}: 239sh, 277sh, 283 nm, ^1H NMR (400 MHz, $CDCl_3$): δ 1.41 (3H, *s*, Me, H-10), 1.66 (6H, *br s*, Me × 2, H-16 and 20), 1.70 (1H, *m*, H-8), 1.73 (3H, *br s*, Me, H-15), 1.77 (3H, *br s*, Me, H-21), 1.88 (1H, *ddd*, *J* = 14, 4, 4 Hz, CH_2, H-8), 2.20 (3H, *br s*, Me, H-22), 2.42, 2.61 (1H each, *ddd*, *J* = 17, 5, 5 Hz, H-7), 3.24, 3.36 (1H each, *dd*, *J* = 15, 7 Hz, CH_2, H-17), 4.75 (1H, *br s*, C-1–OH), 5.15 (1H, *t* like *m*, CH=, H-17), 5.54 (1H, *d*, *J* = 16 Hz, CH=, H-11), 5.75 (1H, *br d*, *J* = 11 Hz, CH=, H-13), 6.15 (1H, *br s*, H-6), 6.33 (1H, *dd*, *J* = 16, 11 Hz, CH=, H-13).

Methylation of **2**. *Peperobtusin A* (**2**, 5 mg) was treated with CH_3I/K_2CO_3 in dry acetone under reflux. After filtration, the reaction mixture was condensed *in vacuo* and purified by preparative TLC (solvent: benzene) to give **2a** (3 mg). EIMS *m/z* (rel. int.): 340 (100, M^+). ^1H NMR (400 MHz, $CDCl_3$): δ 1.40 (3H, *s*, Me, H-10), 1.66 (6H, *br s*, Me × 2, H-16 and 20), 1.70 (1H, *m*, H-8), 1.73 (3H, *br s*, Me, H-15, 1.78 (3H, *br s*, Me, H-21), 1.90 (1H, *ddd*, *J* = 14, 5, 5 Hz, H-8), 2.27 (3H, *br s*, Me, H-22), 2.49, 2.62 (1H each, *ddd*, *J* = 16, 5, 5 Hz, CH_2, H-7), 3.28, 3.37 (1H each, *dd*, *J* = 14, 6 Hz, CH_2, H-17), 3.76 (3H, *s*, OCH_3), 5.14 (1H, *t* like *m*, CH=, H-18), 5.56 (1H, *d*, *J* = 16 Hz, CH=, H-11), 5.76 (1H, *br d*, *J* = 11 Hz, CH=, H-13), 6.22 (1H, *br s*, H-6), 6.38 (1H, *dd*, *J* = 16, 11 Hz, CH=, H-12).

Compound **3** (*isopeperobtusin A*). A pale yellow oil. $[\alpha]_D$ 0°. HREIMS M^+ *m/z* 326.2264 (Calcd. 326.2354 for $C_{22}H_{30}O_2$), EIMS *m/z* (rel. int.): 326 (100, M^+), 311 (12), 283 (16), 270 (18), 255 (17), 243 (25), 227 (55), 205 (53), 189 (32), 161 (20), 149 (95), 122 (20), 107 (75), 91 (38), UV λ_{max}^{MeOH}: 237, 276sh, 280 nm, ^1H NMR (400 MHz, $CDCl_3$): δ 1.41 (3H, *s*, Me, H-10), 1.69 (3H, *br s*, Me, H-16), 1.74 (6H, *br s*, Me × 2, H-15 and 20), 1.80 (1H, *m*, H-8), 1.81 (3H, *br s*, Me, H-21), 2.22 (3H, *br s*, H-22), 2.55, 2.61 (1H each, *ddd*, *J* = 16, 8, 6 Hz, CH_2, H-7), 3.28 (2H, *br d*, *J* = 7 Hz, CH_2, H-17), 5.15 (1H, *br s*, C-3–OH), 5.16 (1H, *t* like *m*, CH=, H-18), 5.59 (1H, *d*, *J* = 15 Hz, CH=, H-11), 5.76 (1H, *br d*, *J* = 11 Hz, CH=, H-13), 6.34 (1H, *br s*, H-6), 6.40 (1H, *dd*, *J* = 15, 11 Hz, CH=, H-12).

Compound **4** (*peperobtusin B*). A yellow oil. $[\alpha]_D$ +3.3° (*c* = 0.06, MeOH), HREIMS M^+ *m/z* 340.2055 (Calcd. 340.2038 for $C_{22}H_{28}O_3$), EIMS *m/z* (rel. int.):

Table 1. ^{13}C NMR Spectral data of **1–4**

Carbon No.	1	2	3	4
1	152.57	152.16	152.57*	186.44
2	111.38	106.01	106.77	117.64
3	153.43	151.14	152.48*	152.13
4	118.13	120.01	115.86	138.05
5	135.21	134.96	135.28	143.26
6	109.72	107.78	110.65	182.60
7	22.54	17.03	17.09	16.36
8	121.95	31.52	31.96	31.01
9	137.44	75.98	75.72	79.36
10	16.14	27.35	26.82	26.85
11	39.67	134.17	134.15	131.96
12	26.38	125.00	125.39	126.09
13	123.82	124.59	124.50	124.06
14	133.32	135.10	135.32	133.55
15	25.70	25.78	25.76†	26.00
16	17.82	18.21	18.31	18.41
17	25.54	24.36	25.57	25.72
18	122.48	123.27	122.50	119.54
19	131.92	130.27	134.04	136.80
20	25.65	25.86	25.76†	25.72
21	17.66	17.95	17.84	17.97
22	19.74	19.47	19.99	11.69

Measured in $CDCl_3$ (100 MHz). * interchangeable, † overlapping.
All carbons were assigned by the aid of CH COSY and COLOC spectra.

340 (100, M^+), 325 (16), 297 (22), 285 (20), 272 (5), 220 (15), 219 (13), 205 (37), 192 (27), 177 (31), 165 (38), 120 (15), UV λ_{max}^{MeOH}: 236, 280, 360sh nm, ^1H NMR (400 MHz, $CDCl_3$): δ 1.49 (3H, *s*, Me, H-10), 1.68 (3H, *br s*, Me, H-20), 1.70 (1H, *m*, H-8), 1.71 (3H, *br s*, Me, H-16), 1.74 (3H, *br s*, Me, H-21), 1.75 (3H, *br s*, Me, H-15), 1.91 (1H, *ddd*, J = 13, 5, 5 Hz, H-8), 2.02 (3H, *s*, Me, H-22), 2.30, 2.47 (1H each, *ddd*, J = 18, 5, 5 Hz, CH_2, H-7), 3.17 (2H, *br d*, J = 7 Hz, CH_2, H-17), 4.94 (1H, *t* like *m*, CH=, H-18), 5.52 (1H, *d*, J = 15 Hz, CH=, H-11), 5.76 (1H, *br d*, J = 10 Hz, CH=, H-13), 6.35 (1H, *dd*, J = 15, 10 Hz, CH=, H-12).

Compound **5** (*2′-hydroxydihydrochalcone*). A colourless oil. HREIMS M^+ m/z 226.1008 (Calcd. 226.0994 for $C_{15}H_{14}O_2$), EIMS m/z (rel. int.): 226 (45, M^+), 208 (20), 207 (2), 121 (100), 91 (16). UV λ_{max}^{MeOH}: 215, 252, 324 nm, ^1H NMR (400 MHz, $CDCl_3$): δ 3.06 (2H, *t*, J = 8 Hz, CH_2, H-7), 3.47 (2H, *t*, J = 8 Hz, CH_2, H-8), 6.80 (1H, *ddd*, J = 9, 8, 2 Hz, H-5′), 6.93 (1H, *dd*, J = 8, 2 Hz, H-3′), 7.18 (1H, *m*, H-4), 7.20 (2H, *d* like *m*, H-2 and 6), 7.27 (2H, *t* like *m*, H-3 and 5), 7.37 (1H, *ddd*, J = 8, 8, 2 Hz, H-4′), 7.54 (1H, *dd*, J = 9, 2 Hz, H-6′), ^{13}C NMR (100 MHz, $CDCl_3$): δ 140.57 (C-1), 128.43 (C-2 and 6), 128.24 (C-3 and 5), 126.14 (C-4), 29.76 (C-7), 39.89 (C-8), 205.14 (C-9), 162.28 (C-2′), 118.72 (C-3′), 136.13 (C-4′), 118.33 (C-5′), 129.65 (C-6′).

Synthesis of 2′-hydroxydihydrochalcone (**5**). 2′-Hydroxychalcone (10 mg) which was prepared by the alkali (KOH-80% MeOH) condensation of 2′-hydroxyacetonephenone and benzaldehyde was hydrogenated with H_2-Pd/C in EtOAc. The resulting compound was identical with **5** (TLC, NMR and EIMS).

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