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# A PHLOROGLUCINOL DERIVATIVE FROM CELL SUSPENSION CULTURES OF *HYPERICUM PATULUM**

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**Key Word Index**—*Hypericum patulum*; Guttiferae; xanthones; cell suspension cultures; 3-[2,2-dimethyl-4-(1-methylvinyl)cyclopentyl]-2,4,6-trihydroxyphenyl phenyl ketone.

**Abstract**—A new phloroglucinol derivative named paglucinol, 3-[2,2-dimethyl-4-(1-methylvinyl) cyclopentyl]-2,4,6-trihydroxyphenyl phenyl ketone, has been isolated from cell suspension cultures of *Hypericum patulum*, together with the known compounds oleanolic acid and β-sitosterol. Their structures were elucidated by spectral techniques. © 1998 Elsevier Science Ltd. All rights reserved

## INTRODUCTION

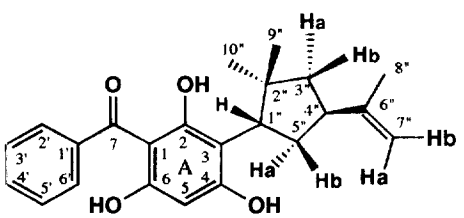
Plants of the genus *Hypericum* have been used as traditional medicinal plants in various parts of the world [1]. Recently, antifungal [2], antibiotic [3], antiviral [4] and anticancer [5] compounds have been isolated from these species.

*Hypericum patulum* has been used in Chinese herbal medicine for the treatment of hepatitis, bacterial diseases and nasal haemorrhage [6]. Previously, as part of a continuing study of *Hypericum* [3], we reported the isolation and structural determination of 13 prenylated xanthones and epicatechin from chloroform or methanol extracts of cell suspension cultures of *H. patulum* [7–11] and discussed the possible biosynthetic relationship between them [9]. Further investigation of the chloroform extract has now led to the isolation of a new phloroglucinol derivative which was named paglucinol, together with the known oleanolic acid and β-sitosterol.

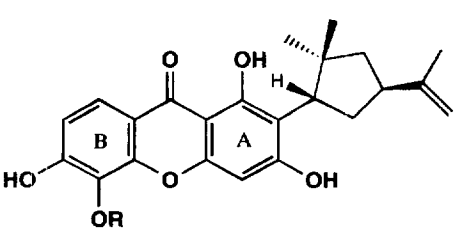
## RESULTS AND DISCUSSION

Suspension cells of *H. patulum* maintained in Linsmaier–Skoog medium [12] containing 2,4-D and kinetin at 27° in the dark were harvested at 2–3 week intervals (see Experimental). Harvested cells were dried and extracted with chloroform. The chloroform extract was subjected to silica and Sephadex LH 20 chromatography to give paglucinol (**1**), oleanolic acid and β-sitosterol.

Paglucinol (**1**) was a yellow powder whose molecular formula, $C_{23}H_{26}O_4$, was established by positive EI mass spectrometry in conjunction with NMR data. The IR spectrum suggested the presence of a phenolic hydroxyl group at 3300 $cm^{-1}$ and a conjugated carbonyl group at 1620 $cm^{-1}$, while the UV spectrum had $\lambda_{max}$ at 211, 255 sh and 313 nm. The $^1H$ NMR spectrum of **1** showed the presence of a hydrogen-bonded hydroxyl proton at $\delta$ 12.61 and two hydroxyl




**1**




**2** : R=Me

**3** : R=H

* Part 6 in the series 'Prenylated xanthones from Cell Suspension Cultures of *Hypericum patulum*'. For Part 5 see ref. [7].

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groups at $\delta$ 8.77 and 9.10. One of the six aromatic protons gave rise to a singlet at $\delta$ 6.05, while the other five appeared as mono-substituted benzene protons at $\delta$ 7.39, 7.47 and 7.58. The signal pattern at $\delta$ 1–5 was very similar to that of the irregular 5-membered ring monoterpene moiety found in paxanthonin (**2**) [10] and demethylpaxanthonin (**3**) [11], previously isolated from the same culture. The chemical shifts and coupling pattern of all the protons—three singlet methyl groups at $\delta$ 0.97, 1.09 and 1.76, one exomethylene unit at $\delta$ 4.75 and 4.86, two methylene groups and two methine groups—belonging to the monoterpene moiety of **1** were almost the same as **2** and **3**. These data suggested **1** to be a compound with an irregular 5-membered ring monoterpene moiety.

From comparison of $^{13}C$ NMR and CH-COSY with those of **2** and **3**, **1** has an aromatic ring (ring A) having the same substitution pattern as ring A of compounds **2** and **3**. The chemical shifts of ring A of **1** is consistent with that of **2** in acetone-$d_6$ and similar to the reported shifts for the closely related 2-(3-hydroxy-3-methylbutyl)-1,3,5,6-tetrahydroxyxanthone from *Calophyllum ionphyllum* [13] or calabaxanthone from *C. calaba* [14]. Therefore, ring A of **1** has the same substitution pattern as **2**.

The CH-COSY and $^{13}C$ NMR spectra of **1** also indicated the presence of a benzoyl group, in addition to the signals corresponding to ring A and the monoterpene moiety of **2**. The lower shift (*ca* 20 ppm) than that of compound **2**, which appears at $\delta$ 181.0, supported the presence of an unfused benzoyl carbonyl group for compound **1**. A phenolic proton at $\delta$ 12.61 in the $^1H$ NMR suggested the formation of a hydrogen bond with the benzoyl carbonyl oxygen. A fragment (*m/z* 105) observed in the mass spectrum of **1** also supported the presence of the benzoyl group.

The structure was finally confirmed by assignment of all the carbons and the bonding pattern by the HMBC methods as shown in Fig. 1. Thus, paglucinol was established to be 3-[2,2-dimethyl-4-(1-methylvinyl)cyclopentyl]-2,4,6-trihydroxyphenyl phenyl ketone (**1**). The absolute configuration has not yet been determined.

The isolation of paglucinol is of biosynthetic interest since it suggests that the irregular 5-membered ring monoterpene moiety of compounds **2** and **3** is introduced before ring formation of the xanthone. It has been suggested that prenylation in xanthones usually occurs at the xanthone stage but not at the benzophenone stage [15]. However, here we demonstrate the irregular 5-membered ring monoterpene moiety found in the benzophenone stage.

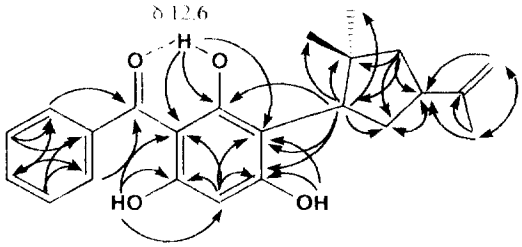


Fig. 1. Long-range correlations in the HMBC spectrum of paglucinol (**1**).

Bennett *et al.* [16] have reported that 2-prenylation is most common but is only found in the presence of 1,3-dioxygenation, suggesting perhaps that the prenyl group inhibits reduction of the 3-hydroxy, which is very often absent in simple xanthones [16]. The irregular 5-membered ring monoterpene moiety in *H. patulum* is present at the 2-position in the presence of 1,3-dioxygenation, suggesting that the monoterpene group inhibits reduction of the 3-hydroxy.

Oleanolic acid and $\beta$-sitosterol were identical with authentic samples by comparing the spectral data. However, this is the first time these compounds as well as paglucinol have been isolated from the cell suspension culture of *H. patulum*.

## EXPERIMENTAL

*Plant material.* Flowers of *H. patulum* Thunb. were planted and grown in our university medicinal plant garden and verified by Dr G. Yoneda (Faculty of Pharmaceutical Sciences, Osaka University). A voucher specimen is kept in our laboratory.

*Suspension culture.* Callus tissues were induced from flowers on LS solid medium containing 3% sucrose, $10^{-5}$ M 2,4,-D, $10^{-7}$ M kinetin and 1.4% agar (pH 5.5) in the dark at 27°. Callus tissue cultures were transferred into liquid LS-medium [12] containing 3% glucose, $10^{-5}$ M 2,4-D and $10^{-7}$ M kinetin (pH 5.5) to initiate suspension culture and were subcultured every 2–3 weeks for one year. Suspension cultures were maintained at 27° in the dark on a rotary shaker (100 rpm).

*Extraction.* Cells were collected by filtering the suspension cultures maintained at 2–3 week intervals. Whole cells (1.2 kg), after being washed with $H_2O$ and dried under a flow of hot air at 60°, were extracted successively with $CHCl_3$ and MeOH.

*Isolation of constituents.* The $CHCl_3$ extract (27 g) was subjected to silica-gel flash CC using a step gradient of $CHCl_3$–MeOH to give 11 frs. Fr. 3 (4.95 g) was rechromatographed over silica gel using a step-gradient of $CHCl_3$–MeOH, followed by recrystallization from *n*-hexane to afford $\beta$-sitosterol (908 mg). Fr. 4 (1.7 g) was rechromatographed over silica gel using *n*-hexane–EtOAc, followed by purification on Sephadex LH 20 eluted with $Me_2CO$ and MeOH to give paglucinol (12.5 mg) and oleanolic acid (87 mg).

*Paglucinol* (**1**). Yellow powder, mp 64–66°. Positive EI-MS (20 eV) *m/z* (rel. int.): 366 $[M]^+$ (99), 323 $[M-C_3H_7]^+$ (43), 309 $[M-C_4H_9]^+$ (100), 295 $[M-C_5H_{11}]^+$ (16), 255 $[C_8H_{15}]^+$ (43), 243 $[M-C_9H_{15}]^+$ (89), 229 $[M-C_{10}H_{17}]^+$ (5), 105 [benzoyl] (58), 77 [phenyl] (34). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3300 (*br*, OH), 1620, 1595, 1510, 1175,

1130, 1070, 700. UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 211 (4.43), 255 sh (3.98), 313 (4.07). $^1$H NMR (500 MHz $(CD_3)_2CO$, TMS): $\delta$ 0.97 (3H, *s*, H-10″), 1.09 (3H, *s*, H-9″), 1.57 (1H, *t*, $J_{3''a,b} = J_{3''b,4''} = 11.6$ Hz, H-3″b), 1.67 (2H, *m*, H-5″a, H-3″a), 1.76 (3H, *s*, H-8″), 2.93 (1H, *m*, H-5″b), 3.04 (1H, *m*, H-4″), 3.63 (1H, *dd*, $J_{1'',5''b} = 7.9$ Hz, $J_{1'',5''a} = 11.0$ Hz, H-1″), 4.68 (1H, *br s*, H-7″a), 4.75 (1H, *br s*, H-7″b), 6.05 (1H, *s*, H-5), 7.39 (2H, *t*, $J = 7.2$ Hz, H-3″,5″), 7.47 (1H, *dt*, $J = 7.2$ and 1.8 Hz, H-4″), 7.58 (2H, *d*, $J = 7.2$ Hz, H-2″, 6″), 8.77 (1H, *s*, 6-OH), 9.10 (1H, *s*, 4-OH), 12.60 (1H, *s*, 2-OH). $^{13}$C NMR (125 MHz $(CD_3)_2CO$, TMS): $\delta$ 21.2 (Me, C-8″), 25.2 (Me, C-10″), 30.6 (Me, C-9″), 33.6 (C-5″), 44.3 (C-1″), 44.8 (C-4″), 45.6 (C-2″) 48.3 (C-3′), 95.9 (C-5), 105.1 (C-1), 108.0 (C-3), 108.1 (C-7″), 128.4 (C-3′, 5′), 129.0 (C-2′, 6′), 131.5 (C-4′), 143.2 (C-1′), 150.4 (C-6″), 159.6 (C-6), 164.7 (C-2), 165.2 (C-4), 200.4 (C-7).

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