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PRENYLATED XANTHONES FROM CELL SUSPENSION CULTURES OF *HYPERICUM PATULUM**

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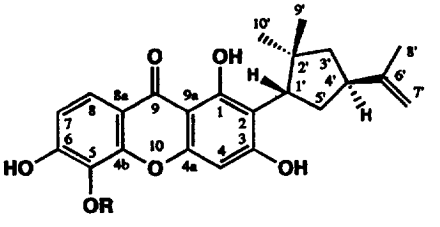
Key Word Index—*Hypericum patulum*; Guttiferae; xanthones; cell suspension culture; demethylpaxanthonin; tripteroside; padiaxanthone; 1,3,5,6-tetrahydroxy-2-(2′,2′-dimethyl-4′-isopropenyl)-cyclopentanyl-xanthen-9-one.

Abstract—A new xanthone named demethylpaxanthonin, 1,2,5,6,tetrahydroxy-2-2′,2′-dimethyl-4′-isopropenyl)-cyclopentanyl-xanthen-9-one, has been isolated from the callus tissues of *Hypericum patulum* together with the known compounds padiaxanthone and tripteroide. Their structures were elucidated by spectral techniques.

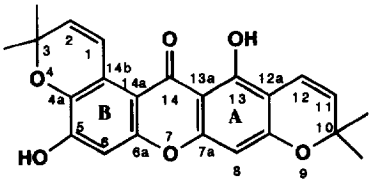
INTRODUCTION

Previously we have reported on the isolation and structural determination of eight prenylated xanthones from chloroform and methanol extracts of callus tissues of *Hypericum patulum* Thumb. [1–3].

Further investigation of the ethyl acetate-soluble parts of the methanol extract from the callus tissues has now led to the isolation of a new xanthone derivative, demethylpaxanthonin (**1**), and also padiaxanthone (**2**), which has been found for the first time from a natural source, together with a known tripteroside (**3**).



1 : R=H
4 : R=Me



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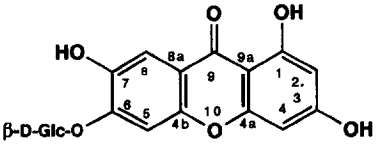

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RESULTS AND DISCUSSION

Compound **1** gave rise to a molecular ion peak at m/z 396 $[M]^+$ in its positive EI-mass spectrum. The IR spectrum suggested the presence of phenolic hydroxyl groups (3300 cm^{-1}) and a hydrogen bonded carbonyl (1630 cm^{-1}).

The ^1H NMR spectrum of **1** showed the presence of a hydrogen-bonded hydroxyl (δ 13.89) and three aromatic protons. One gave rise to a singlet at δ 6.53, while the other two appeared as a pair of *ortho*-coupled protons (δ 6.97 and 7.65).

The ^1H-, ^{13}C- and CH COSY-NMR spectra of **1** showed the presence of three tertiary methyl groups [δ 1.78 (H-8′), 1.11 (H-9′) and 0.97 (H-10′); δ 21.3, 30.7 and 25.3 (C-8′, C-9′ and C-10′), two quaternary carbons [δ 46.2 (C-2′) and 147.6 (C-6′)], an exo-methylene unit [δ 4.67 (H-7′b) and 4.77 (H-7′a); δ 108.2 (C-7′)], two methylenes [δ 1.61 (H-3′b) and 1.70 (H-3′a), and δ 1.70 (H-5′a) and 3.00 (H-5′b); δ 48.9 (C-3′) and 33.8 (C-5′)] and two methines [δ 3.70 (H-1′) and δ 3.08 (H-4′); δ 44.8 (C-1′) and 45.4 (C-4′)]. These data suggested it to be a compound with an irregular monoterpene skeleton which was very similar to paxanthonin (**4**), previously isolated from the chloroform extract of the same culture [1]. Thus the chemical-shift values and the coupling pattern of all the protons belonging to the monoterpene moiety of **1** were almost the same as **4**.

*Part 4 in the series 'Xanthones in Cell Suspension Cultures of *Hypericum patulum*'. For Part 3 see ref. [1].

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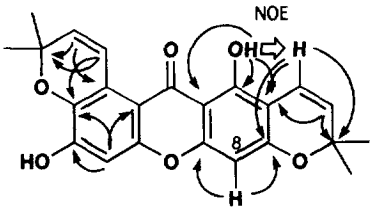

Fig. 1. Long-range correlations in the HMBC spectrum (→) and NOEs (⇒) of compound **2**.

Compound **1** has an extra hydroxyl group instead of the methoxyl group present in **4**. The higher chemical shift value (+0.2 ppm) for H-8 (*d* 7.65) of **1** than **4** supported the presence of a 5-OH group. Hence, compound **1** was confirmed to be 1,3,5,6-tetrahydroxy - 2 - (2′,2′ - dimethyl - 4′ - isopropenyl) - cyclapentanyl-xanthen-9-one, and was named demethylpaxanthonin. The absolute configuration has not yet been determined.

Compound **2** gave a peak at *m/z* 392 in its positive EI mass spectrum. The UV spectrum showed striking similarities to those of xanthones with an extended chromophore, such as rheediaxanthone [4], paxanthone and paxanthone B [2, 3].

The ^{1}H NMR spectrum of **2** showed the presence of signals for two 2,2-dimethyl-2H-pyran rings, and of one-proton singlets at δ 13.8 (13-OH), 6.83 (6-H) and 6.27 (8-H). The angular fusion to the B ring to one of the pyran rings (δ 1.46, 5.94, 8.03) was deduced from the lowfield shift of H-1 (δ 8.03) being located in the deshielding area of the carbonyl group [5]. The linear fusion to the A ring to the other pyran ring (δ 1.46, 5.71, 6.69) was deduced from the NOED experiment. Irradiation of the hydroxyl proton (δ 13.8) enhanced the signal of H-12 in a slight but significant manner. Additional support was provided by the HMBC correlation (Fig. 1).

The HMQC and HMBC allowed also the assignment of all the carbons and confirmed the structure. Although compound **2**, 5,13 - dihydroxy - 3,3,10,10 - tetramethyl - 3H,10H,14H-dipyrano[3,2-a:2′,3′-i] xanthen-14-one, has been synthesized by Sen *et al.* by the oxidative cyclization of garcinone B with DDQ [6], this is the first report of its isolation from a cell suspension culture. Thus we named this compound padiaxanthone.

Compound **3** was found to be identical with tripteroside (1,3,7-trihydroxyxanthone-6-β-D-glucoside) by comparison of its spectral data. It was isolated from the methanol extract of the fresh herb *Tripterospermum taiwanense* (Gentianaceae) [7], and has been isolated from the cell suspension culture of *H. patulum* for the first time.

EXPERIMENTAL

Plant material. Hypericum patulum Thumb. was 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. Callus tissue cultures were established from the flower of *H. patulum* and they were cultured in the dark on Linsmeier–Skoog medium containing 10^{-5} M, 2,4-D and 10^{-7} M kinetin.

Extraction and isolation. Dried callus tissues (dry wt. 526 g) were extracted with MeOH. The MeOH extract (186 g) was dissolved in the least amount of water and was partitioned with EtOAc and water, respectively. Compounds **2** (4.4 mg) and **3** (21.4 mg) were isolated from the EtOAc fraction (11.6 g) by flash chromatography on silica gel using a $CHCl_3$–MeOH gradient system and benzene–EtOAc gradient system followed by crystallization from benzene–Me_2CO or MeOH.

Separately, newly dried callus tissues (dry wt. 1210 g) were extracted with MeOH. The MeOH extract (283.3 g) was dissolved in the least amount of water and was partitioned with EtOAc and water. Compound **1** (9.9 mg) was isolated from the EtOAc fraction (17.9 g) by flash chromatography on silica gel using a $CHCl_3$–MeOH gradient system followed by LH 20 with MeOH.

Demethylpaxantonin (**1**). Yellow powder, mp 183–186° $[\alpha]_D$ − 262° (*c* 0.26, MeOH); positive EI-MS: *m/z* (rel. int.): 396 $[M]^+$ (90), 381 (32), 353 (97), 339 (27), 325 (14), 299 (11), 285 (39), 273 (100); IR ν_{max}^{KBr} cm^{-1}: 3300, 1630, 1610, 1580, 1530; UV λ_{max}^{MeOH} nm (log ε): 253 (4.59), 284 (3.89), 327 (4.29); ^{1}H NMR (500 MHz, $(CD_3)_2CO$); δ 0.97 (3H, *s*, 10′-Me), 1.11 (3H, *s*, 9′-Me), 1.61 (1H, *t*, J = 11.6 Hz, 3′-H_b), 1.70 (1H, *m*, 5′-H_a), 1.70 (1H, *t*, 11.6, 3′-H_a), 1.78 (3H, *s*, 8′-Me), 3.00 (1H, *m*, 5′-H_b), 3.08 (1H, *m*, 4′-H), 3.70 (1H, *dd*, $J_{1',5B}$ = 7.94, $J_{1'5'a}$ = 11.0 Hz, 1′-H), 4.67 (1H, *br s*, 7′-H_a), 4.77 (1H, *br s*, 7′-H_b), 6.53 (1H, *s*, 4H), 6.97 (1H, *d*, J = 9.15 Hz, 7-H), 7.65 (1H, *d*, J = 8.54 Hz, 8-H), 13.89 (1H, *s*, 1-OH); ^{13}C NMR (500 MHz, CD_3OD): δ 21.3 (*q*, C-8′), 25.3 (*q*, C-10′), 30.7 (*q*, C-9′), 33.8 (*t*, C-5′), 44.8 (*d*, C-1′), 45.4 (*d*, C-4′), 46.2 (*s*, C-2′), 48.9 (*t*, C-3′), 94.7 (*d*, C-4), 103.1 (*s*, C-9_a), 108.2 (*t*, C-7′), 112.0 (*s*, C-2), 113.6 (*d*, C-7), 115.2 (*s*, C-8_a), 117.7 (*d*, C-8), 133.6 (*s*, C-5), 147.6 (*s*, C-6′), 150.9 (*s*, C-4_b), 152.9 (*s*, C-4_a), 157.2 (*s*, C-6), 163.5 (*s*, C-3), 165.9 (*s*, C-1), 182.0 (*s*, C-9).

Padiaxanthone (**2**). Yellow needles, mp 229–232° (n-hexane–Me_2CO). Positive EI–MS *m/z* (rel. int.): 392 $[M]^+$ (37), 377 $[M-Me]^+$ (100), 359 $[M-Me-H_2O]^+$, (13), 347 (7), 181 (28), 149 (6); IR ν_{max}^{KBr} cm^{-1} 3490, 3300-3100, 1650, 1630, 1615, 1590; UV λ_{max}^{MeOH} nm (log ε) 254 (4.17), 289 (4.53), 300 (4.53), 330 (4.31), 383 (3.70); ^{1}H NMR (500 MHz, d_6-acetone: δ 1.46 (12H, *s*, 2Me), 5.71 (1H, *d*, J = 9.8, 11-H), 5.94 (1H, *d*, J = 9.8, 2-H), 6.27 (1H, *s*, 8-H), 6.69 (1H, *d*, J = 9.8, 8, 12-H), 6.83 (1H, *s*, 6-H), 8.03 (1H, *d*, J = 9.8, 1-H), 13.8 (1H, *s*, 13-OH); ^{13}C NMR (125 MHz, d_6-acetone); δ 27.2 (C-10 Me), 28.5 (C-3 Me), 76.9 (C-3), 78.9 (C-10), 94.8 (C-8), 103.6 (C-6), 104.4 (C-13a), 105.1 (C-12a), 108.4 (C-4a), 116.0 (C-12), 120.9 (C-14b), 121.5 (C-1), 128.5 (C-14), 133.7 (C-2), 139.1 (C-4a), 154.1 (C-5), 154.0 (C-6a), 157.4 (C-7a), 158.7 (C-13), 160.8 (C-9), 183.3 (C-14).

Tripteroside (**3**). Yellow needles, mp 260–263°

(MeOH–H_2O). Negative FAB-MS: *m/z* (rel. int.): 421 $[M-1]^+$; IR ν_{max}^{KBr} cm^{-1}: 3400, 3200, 2850, 1650, 1600, 1570, 1470; UV λ_{max}^{MeOH} nm (log ε): 239 (4.16), 256 (4.26), 309 (3.94), 364 (3.77); ^{13}C NMR (500 MHz, DMSO): δ 60.7 (*t*, glcC-6), 69.7 (*d*, glcC-4), 73.1 (*d*, glcC-2), 75.9 (*d*, glc-C3), 77.2 (*d*, glc-C5), 93.7 (*d*, C4), 97.9 (*d*, C2), 100.5 (*d*, glcC-1), 101.5 (*s*, C-9_a), 103.3 (*d*, C-5), 108.3 (*d*, C-8), 113.9 (*s*, C-9_b), 144.2 (*s*, C-7), 150.1 (*s*, C-4_b), 152.0 (*s*, C-6), 157.5 (*s*, C-4_a), 162.6 (*s*, C-1), 165.6 (*s*, C-3), 178.8 (*s*, C-9).

REFERENCES

1. Ishiguro, K., Nakajima, M., Fukumoto, H. and Isoi, K. (1995) *Phytochemistry* **39**, 903.
2. Ishiguro, K., Nakajima, M., Fukumoto, H. and Isoi, K. (1995) *Phytochemistry* **33**, 839.
3. Ishiguro, K., Nakajima, M., Fukumoto, H. and Isoi, K. (1995) *Phytochemistry* **38**, 867.
4. Monache, F. D., Botta, B., Nicoletti, M., Coelho, J. S. de and Lyra, F. D. de (1981) *J. Chem. Soc., Perkin Trans.* I, 484.
5. Goncalves De Oliveira, W., Gottlieb, O. R. and Lins Mesquita, A. A. (1972) *Phytochemistry* **11**, 3323.
6. Sen, A. K., Sarkar, K. K., Mazumder, P. C., Banerji, N., Uusvuori, R. and Hase, T. A. (1982) *Phytochemistry* **21**, 1747.
7. Lin, C., Chang, M., Arisawa, M., Shimizu, M. and Morita, N. (1982) *Phytochemistry* **21**, 948.