Regioselective glucosylation of oxyresveratrol by cell suspension cultures of *Solanum mammosum*

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The glucosylation of oxyresveratrol by cell suspension cultures of *Solanum mammosum* has been investigated.

Keywords: oxyresveratrol, oxyresveratrol 4-*O*-β-D-glucopyranoside, cell suspension cultures, *Solanum mammosum*

trans-Oxyresveratrol (**1**), 3,4,3',5'-tetrahydroxy-*trans*-stilbene, is a major metabolite of *Morus macroura*.[1,2] It has antiinflammatory,[3] antioxidant,[4] free radical scavenger,[4,5] and hepatoprotective activity.[5] It is a more powerful inhibitor of tyrosinase than kojic acid.[6] In order to increase its water solubility, its glycoside derivative(s) was prepared. Recently cell suspension cultures of *Solanum mammosum* have been developed which have the ability to convert aromatic acid substrates into their mono- and diglucosides derivatives.[7,8] We now describe the conversion of **1** into *cis*- (**2a**) and *trans*-oxyresveratrol 4-*O*-β-D-glucopyranoside (**2b**) by cultures of *S. mammosum*.

Compound **1** was incubated with cultures of *S. mammosum* for 6 days under continuous light. The combined EtOAc fraction obtained from both the medium and the biomass, was fractionated using silica gel chromatography. The fraction rich in phenolic compounds which was more polar than **1** was repeatedly purified by radial chromatography to give compound **2** as an inseparable mixture of **2a** and **2b** (1:2 from NMR data). Compound **2** was obtained as a brownish-yellow solid and exhibited a $[M+H]^+$ ion at *m/z* 407.1346 from its positive HRFABMS spectrum, corresponding to a molecular formula $C_{20}H_{22}O_9$ (calcd. 407.1342). The UV spectrum of **2** (see Experimental) showed absorption maxima characteristic of the presence of a stilbene chromophore. The IR spectrum of **2** exhibited absorptions for hydroxyl and aromatic functionalities. The NMR data (Table 1) displayed two pairs of doublet signals in the ^{1}H NMR spectrum at δ_H 6.87 and 7.17 (J = 16.5 Hz) and δ_H 6.28 and 6.47 (J = 12.4 Hz), as well as signals assignable to a pair of 2,4-dihydroxyphenyl and 3,5-dihydroxyphenyl groups, indicating that **2** is a mixture of *cis*- and *trans*-oxyresveratrol derivatives in a ratio of 1:2 from the integration of the signals. The NMR data also showed signals characteristic to a pair of glycosyl moieties. These were assigned to β-D-glucopyranosyl groups by comparison of their ^{13}C NMR signals with those of reported data[9] and the coupling constants of the anomeric proton signals at δ_H 4.75 (J = 7.7 Hz, **2a**) and 4.79 (J = 7.3 Hz, **2b**). In addition, the ^{1}H NMR spectrum also revealed both phenolic and aliphatic hydroxyl signals (see Table 1). The attachment of the glucosyl groups was shown to be at C-4 in both **2a** and **2b** from HMQC and HMBC spectra. The HMBC spectrum, in particular, exhibited long range correlations between the anomeric proton signals with carbon signals at δ_C 158.3 (C-4 of **2a**) and 158.4 (C-4 of **2b**), respectively. These carbon signals did not showed correlations with one of the vinylic proton signals at δ_H 6.47 (C-α of **2a**) and 7.17 (C-α of **2b**), respectively. Other selective HMBC correlations in support for structure **2** is shown Fig. 1. Accordingly, compound **2** was assigned as a mixture of *cis*- (**2a**) and *trans*-oxyresveratrol 4-*O*-β-D-glucopyranoside (**2b**).

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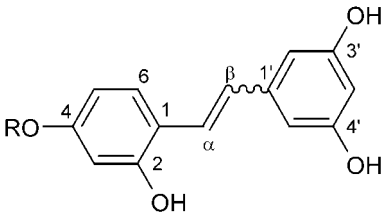
Table 1 NMR data of compounds **2a** and **2b** [δ_C values (ppm)][a]

Carbon	δ_H (*multiplicity*, J in Hz)		δ_C	
	2a	**2b**	**2a**	**2b**
1	–	–	118.1	118.5
2	–	–	156.6	156.3
3	6.36 (*d*, 2.2)	6.54 (*d*, 2.6)	103.9	104.5
4		-	158.3	158.4
5	6.31 (*dd*, 8.2, 2.2)	6.52 (*dd*, 8.5, 2.6)	107.0	108.1
6	6.97 (*d*, 8.2)	7.45 (*d*, 8.5)	130.4	127.6
α	6.47 (*d*, 12.4)	7.17 (*d*, 16.5)	125.6	123.2
β	6.28 (*d*, 12.4)	6.87 (*d*, 16.5)	128.7	126.8
1'	–	–	139.6	140.3
2'/6'	6.12 (*d*, 2.0)	6.36 (*d*, 2.2)	107.0	104.7
3'/5'	–	–	158.7	159.1
4'	6.03 (*t*, 2.0)	6.09 (*t*, 2.2)	102.0	102.2
1"	4.75 (*d*, 7.7)	4.79 (*d*, 7.3)	100.9	101.0
2"	3.20 (*m*)	3.20 (*m*)	73.7	73.7
3"	3.30 (*m*)	3.30 (*m*)	77.2	77.2
4"	3.20 (*m*)	3.20 (*m*)	70.1	70.1
5"	3.30 (*m*)	3.30 (*m*)	77.5	77.6
6"	3.69 (*m*)	3.50 (*m*)	61.1	61.2
2-OH	9.56 (*s*)	9.77 (*s*)		
3'/5'-OH	9.06 (*s*)	9.15 (*s*)		
2"-OH	5.25 (*d*, 5.2)	5.27 (*d*, 5.0)		
3"-OH	5.03 (*d*, 5.0)	5.05 (*d*, 4.7)		
4"-OH	4.96 (*d*, 5.2)	4.98 (*d*, 5.2)		
6"-OH	4.49 (*t*, 5.8)	4.52 (*t*, 5.8)		

[a]Measured in [^{6}H]DMSO.



1 α,β-*trans*, R = H
2a α,β-*cis*, R = β-D-glucopyranoside
2b α,β-*trans*, R = β-D-glucopyranoside

To our knowledge compounds **2a** and **2b** are new compounds. Previous reports described the isolation of oxyresveratrol 2-*O*-glucoside from *Schoenocaulon officinale*,[9] oxyresveratrol 3'-*O*-glucoside from *Veratrum grandiflorum*,[10] and oxyresveratrol 3',4-*O*-diglucoside (mulberroside A) from *S. officinale* and mulberry plants.[9,11,12] However, the presence of *cis* isomer of **2** in the EtOAc fraction of the culture may arise as an artifact from continuous illumination during the course of incubation. Study on the incubation of **2** in the cultures of *S. mammosum* without continuous light and optimisation of its biotransformation capacity are in progress.

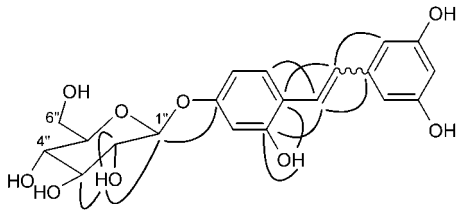


Fig. 1 Selected important HMBC correlations in **2a** and **2b**.

Experimental

UV and IR spectra were measured with UV/VIS Varian Cary 100 Conc and Perkin-Elmer One spectrophotometers, respectively. ^{1}H and ^{13}C NMR spectra were recorded with JEOL JNM ECA600 spectrometer, operating at 600.2 MHz (^{1}H) and 150.9 MHz (^{13}C) using residual and deuterated solvent peaks as reference standards. High resolution mass spectrum was obtained with a JEOL JMS-AM20 mass spectrometer, using the FAB mode. Vacuum liquid (VLC) and column chromatography were carried out using Merck silica gel 60 GF_{254} and silica gel G60 35-70 mesh. For TLC analysis, precoated silica gel plates (Merck Kieselgel 60 GF_{254}, 0.25 mm) were used.

Cell suspension cultures and biotransformation conditions: Cell suspension cultures of *S. mammosum* were cultivated in Erlenmeyer flasks (300 ml) containing 50 ml of modified MS medium,[13] supplemented with sucrose (3%, w/v), kinetin (2.5 mg/l), 1-napthylacetic acid (0.5 mg/l) and casein hydrolysate (1 g/l) on a gyrotary shaker (100 rpm) at 25 ± 1 °C under continuous light (*ca* 1500 lux; Philips TL 54/40 W) as previously reported.[7] Biotransformation experiments were carried out using 16 Erlenmeyer flasks. Cells (10 g fresh weight) were added to the medium (50 ml) containing **1**[1,2] (500 mg/l) and incubated for 6 days.

Isolation: The medium was partitionated into EtOAc, while the biomass was extracted with MeOH and the concentrated MeOH was also partitionated into EtOAc. The combined EtOAc fractions (420 mg) was fractionated by radial chromatography (silica gel, 5-25% MeOH in $CHCl_3$) into ten fractions A-J. On TLC analysis, fraction B (100 mg) contained **1**. The major fraction more polar than **1**, fraction H (70 mg), was purified by radial chromatography (silica gel, EtOAc) to give compound **2** (40 mg). This compound showed one compact spot on TLC analysis using three different solvent systems.

Compound **2** was obtained as a brownish-yellow solid, $[\alpha]_D$ – 59 (MeOH, *c* 0.1); ν_{max}/cm^{-1} (KBr) 3459, 2927, 1622, 1515, 1270, 836; λ_{max}/nm (log ε) (MeOH) 216 (4.31), 300 (4.14), 324 (4.23); (MeOH+NaOH) 204 (4.85), 295 (4.05), 345 (4.09); δ_H (600 MHz, [^{6}H]DMSO) see Table 1; δ_C (150 MHz, [^{6}H]DMSO) see Table 1; *m/z* found: $[M+H]^+$ 407.1346. $C_{20}H_{22}O_9$ requires M_r+1 407.1342.

We thank the Project of Competitive B Batch 1 Year 2004, Ministry of National Education, Republic of Indonesia, for financial support.

Received 4 August 2004; accepted 30 August 2004
Paper 04/2702

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