

Note

Isolation of *N,N*-Dimethyl and *N*-Methylserotonin 5-*O*- β -Glucosides from Immature *Zanthoxylum piperitum* Seeds

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Two serotonin derivatives, *N,N*-dimethylserotonin 5-*O*- β -glucoside (1a**) and *N*-methylserotonin 5-*O*- β -glucoside (**1b**) were isolated from immature seeds of *Zanthoxylum piperitum*. Their structures were determined by multi-step conversion reactions and spectroscopic analyses. Immature seeds of *Z. piperitum* contained extremely high levels of compounds **1a** and **1b** of approximately 0.29% and 0.15% (w/w), respectively.**

Key words: *Zanthoxylum piperitum*; Japanese pepper; *N,N*-dimethylserotonin 5-*O*- β -glucoside; *N*-methylserotonin 5-*O*- β -glucoside

Zanthoxylum piperitum (Japanese pepper) is a plant native to Japan and other parts of East Asia. It bears a tiny red fruit between August and September. The fresh young leaves of the plant as well as the fruit pericarp are used as spices which provide a fresh and hot flavor in Japanese cuisine. Whole immature fruits that include seeds are used as a spice for tsukudani which contains traditionally preserved sea foods. Only the pericarp of the mature fruit of *Zanthoxylum bungeanum* is used in China as a stomach medicine, an analgesic, and a spice. The shiny black seeds are discarded or ignored.

The most well-known *Zanthoxylum* cultivars in Japan are Asakura-sansho, located in Kyoto and Hyogo, and Budo-sansho, in Wakayama. A wild species called Kogen-sansho is cultivated in the Hida area of Gifu prefecture and is considered to produce a better aroma than the others.

It is known that the main pungent components in this fruit are shanshool and shanshoolamide^{1–3)} and that the flavor components are limonene, geraniol, *etc.*^{4,5)} However, not much is known about the constituents of *Zanthoxylum* seeds. We therefore attempted to elucidate the components present in immature seeds of *Zanthoxylum* that were cultivated in the Hida area of Gifu prefecture. These seeds were collected between late July and August in 2007.

The seeds (860 g) were extracted twice with ethyl acetate in order to remove the non-polar components, and then extracted twice with methanol, affording an ethyl acetate extract and a methanol extract (13.5 g).

A reversed-phase HPLC analysis of the methanol extract indicated that it contained no pungent components (*e.g.*, α -sanshool), but did contain two highly polar components with unique UV absorptions.

The methanolic extract was purified by silica gel column chromatography [MeOH–CHCl₃–28% aq. NH₃–

H₂O, 50:42:1:7 (v/v) and then 80:0:4:16 (v/v)] to afford crude **1a** (4.90 g) and crude **1b** (2.41 g). An analysis by HPLC suggested that these samples were almost pure, but that they still contained some mineral salt. The samples were therefore adsorbed in a Diaion HP-20SS column which was eluted with methanol after washing with water. This process removed the mineral salt, and then compounds **1a** (2.97 g, purity >99%) and **1b** (0.53 g, purity >99%) were obtained as colorless solids.

UV spectra showed that compounds **1a** and **1b** contained the indole skeleton in the molecule. In addition, ¹H-NMR spectra showed that compounds **1a** and **1b** both had an indole ring with a glycoside unit at the 5- or 6-position. These results and the mass spectra suggested the structures of compounds **1a** and **1b** to be as shown in Fig. 1.

The ¹H-NMR spectrum of compound **1a** was similar to that of *N,N*-dimethylserotonin 5-*O*- β -glucoside which has been synthesized by Krasavina *et al.*⁶⁾ and isolated from the leaves of *Citrus unshiu* in the study by Nishida *et al.* on the oviposition-stimulating activity of tryptamine analogs of the Rutaceae-feeding swallowtail butterfly.^{7,8)} However, our data are slightly different from their spectral data. Although *N,N*-dimethylserotonin 5-*O*- β -glucoside and its *N*-oxide have been isolated from the same rutaceous plant, no spectral data were reported.⁹⁾ We therefore synthesized their acetyl derivatives to confirm these structures (Fig. 1).

Compound **1a** was acetylated with acetic anhydride in pyridine to give tetra-acetate **2a** in an 87% yield. Although compound **2a** had already been synthesized and analyzed by ¹H-NMR,⁶⁾ our ¹H-NMR data for tetraacetate **2a** differed from theirs.

The acetylation of **1b** with acetic anhydride/pyridine gave a pentaacetylated derivative of **2b** in addition to the four acetyl groups on the glycoside portion. The two conformers of **2b** arising from the presence of the amide group enabled its structure to be confirmed by the mass and ¹H-NMR spectral data at 120 °C.

The acid-hydrolysis of compound **1a** or **1b** in 2 M HCl at 70 °C for 1 h respectively gave dimethylserotonin **3a** (a 74% yield from **1a**) or monomethylserotonin **3b** (a 57% yield from **1b**). Their spectral data were identical to those of synthetic **3a** or **3b** prepared from serotonin by methylation, although they were not identical to those reported in the literature.^{10,11)} The discrepancy between our data and the literature data was due to the strong basicity of these compounds, the compounds reported in the literature being considered to be the carbonates of

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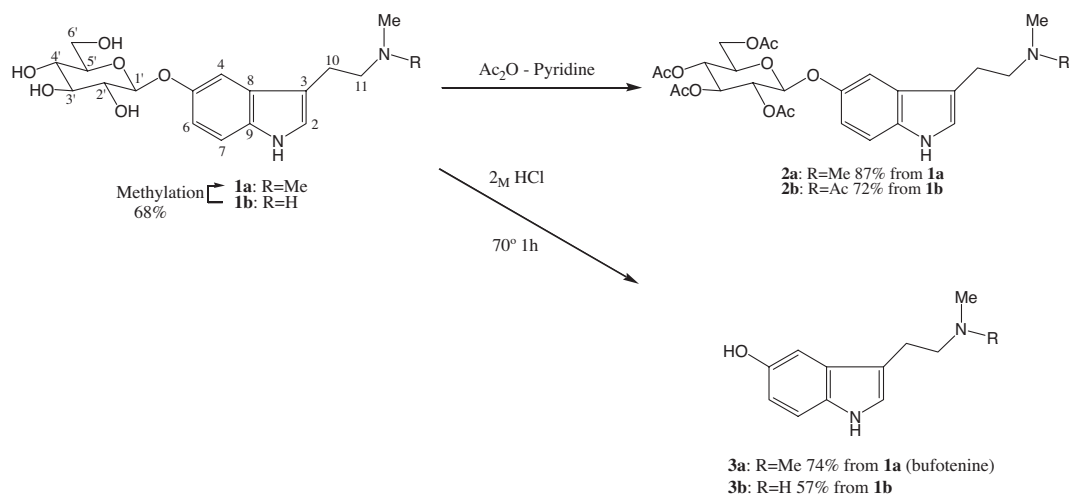


Fig. 1. Transformation of Serotonin Analogs **1a** and **1b**.

amines rather than the free amine. Indeed, **3a** and **3b** afforded $^1\text{H-NMR}$ data similar to those reported in the literature upon the addition of carbon dioxide or acetic acid.

The structures of compounds **1a** and **1b** were therefore respectively determined to be *N,N*-dimethylserotonin 5-*O*- β -glucoside and *N*-methylserotonin 5-*O*- β -glucoside. Furthermore, compound **1b** could be converted to **1a** by *N*-methylation which proceeded in a 68.2% yield. The respective contents of **1a** and **1b** in immature seeds of *Z. piperitum* were estimated to be 0.29% and 0.15% (w/w) by an HPLC analysis, and neither of these compounds nor their aglycons **3a** and **3b** were detected in the pericarps.

In summary, two serotonin analogs were isolated from the seeds of *Z. piperitum*, and their structures were determined to be *N,N*-dimethylserotonin 5-*O*- β -glucoside **1a** and *N*-methylserotonin 5-*O*- β -glucoside **1b**. Dimethyl derivative **1a** was isolated from *Z. piperitum* for the first time together with novel monomethyl derivative **1b**. The levels of **1a** and **1b** in the seeds were significantly high enough to suggest that they may have some unknown important functions in this plant. We are currently studying the relationship between the growth stage and endogenous levels of these compounds to further elucidate their biological significance.

N,N-Dimethylserotonin 5-*O*- β -glucoside **1a**. Amorphous; $^1\text{H-NMR}$ δ_{H} (600 MHz, D_2O): 2.34 (6H, s, N-Me), 2.74 (2H, t, $J = 7.8$, H-10), 2.94 (2H, t, $J = 7.8$, H-11), 3.49 (1H, t, $J = 9.4$, H-4'), 3.55–3.66 (3H, m, H-2', 3', 5'), 3.75 (1H, dd, $J = 6.0$, 12.4, H-6'), 3.94 (1H, dd, $J = 2.3$, 12.4, H-6'), 5.07 (1H, d, $J = 7.3$, H-1'), 7.04 (1H, dd, $J = 2.3$, 8.7, H-6), 7.24 (1H, s, H-2), 7.37 (1H, d, $J = 2.3$, H-4), 7.44 (1H, d, $J = 8.7$, H-7); $^{13}\text{C-NMR}$ δ_{C} (150 MHz, D_2O): 24.8 (C-10), 46.4 (N-C), 61.5 (C-11), 63.4 (C-6'), 72.3 (C-4'), 75.9 (C-2'), 78.4 (C-3'), 78.9 (C-5'), 104.7 (C-1'), 108.2 (C-4), 115.3 (C-3), 115.3 (C-7), 116.0 (C-6), 127.1 (C-2), 129.7 (C-8), 135.6 (C-9), 153.2 (C-5); UV λ_{max} (MeOH) nm (ϵ): 278 (3,680), 220 (16,300); IR ν_{max} (KBr) cm^{-1} : 2924, 2863, 1627, 1482, 1200, 1075, 1043; HR-ESI-MS m/z : calcd. for $\text{C}_{18}\text{H}_{27}\text{N}_2\text{O}_6$, 367.1869; found, 367.1873; $[\alpha]_{\text{D}}^{25} -32.07$ (c1.0, H_2O).

N-Methylserotonin 5-*O*- β -glucoside **1b**. Amorphous; $^1\text{H-NMR}$ δ_{H} (400 MHz, D_2O): 2.49 (3H, s, N-Me), 3.04

(2H, br.d, $J = 5.0$, H-10), 3.08 (2H, br.d, $J = 5.0$, H-11), 3.48 (1H, t, $J = 9.4$, H-4'), 3.56–3.61 (3H, m, H-2', 3', 5'), 3.74 (1H, dd, $J = 6.4$, 12.4, H-6'), 3.94 (1H, dd, $J = 1.8$, 12.4, H-6'), 5.09 (1H, d, $J = 7.8$, H-1'), 7.06 (1H, dd, $J = 2.3$, 9.2, H-6), 7.28 (1H, s, H-2), 7.37 (1H, d, $J = 2.3$, H-4), 7.45 (1H, d, $J = 8.7$, H-7); $^{13}\text{C-NMR}$ δ_{C} (150 MHz, D_2O): 25.5 (C-10), 36.3 (N-C), 52.6 (C-11), 63.5 (C-6'), 72.4 (C-4'), 75.9 (C-2'), 78.4 (C-3'), 78.9 (C-5'), 104.6 (C-1'), 108.1 (C-4), 113.4 (C-3), 115.4 (C-7), 116.1 (C-6), 127.9 (C-2), 129.6 (C-8), 135.7 (C-9), 153.3 (C-5); UV λ_{max} (MeOH) nm (ϵ): 278 (4,700), 220 (21,400); IR ν_{max} (KBr) cm^{-1} : 2924, 1627, 1483, 1199, 1075, 1042; HR-ESI-MS m/z : calcd. for $\text{C}_{17}\text{H}_{25}\text{N}_2\text{O}_6$, 353.1713; found, 353.1719; $[\alpha]_{\text{D}}^{25} -30.63$ (c1.0, H_2O).

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