**Note**

Oviposition Stimulant Activity of Tryptamine Analogs on a Rutaceae-feeding Swallowtail Butterfly, *Papilio xuthus*

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The specific oviposition stimulants of a Rutaceae-feeding swallowtail butterfly, *Papilio xuthus* L., have been characterized as a mixture of vicenin-2, narirutin, hesperidin, rutin, adenosine and 5-hydroxy-N-methyltryptamine (1). However, it was suggested that the host plant, *Citrus unshiu*, contains several additional components that contribute to the induction of oviposition by the female butterflies. Since tryptamine analog 1 has been shown to be one of the key factors synergizing the activity of the flavonoids (FM: vicenin-2 + narirutin + hesperidin + rutin), we searched for other related compounds in the *Citrus*-leaf extracts. As a result, two other tryptamine bases, bufotenine (2) and its 5-O-glucoside (3), were isolated from the water-soluble fraction. Compound 2 was found to be a potent synergist for FM. In contrast, compound 3 was entirely inactive in spite of the presence of a tryptamine structure. Here, we describe the identification of these two components isolated from the leaves of *C. unshiu*. We also report the activity of several other related tryptamine analogs.

A methanolic extract of *C. unshiu* leaves was fractionated as described in previous reports. Besides compound 1, fraction CA was found to contain two other tryptamine analogs (2 and 3). Compound 2 exhibited its molecular ion peak at *m/z* 204, which is 14 mass units higher than that of 1. The proton and carbon-13 NMR (PMR and CMR) spectra of compound 2 were practically identical to those of 1, but exhibited an N-methyl single signal equivalent to two methyl groups, indicating the presence of an N,N-dimethyl moiety. Thus, compound 2 was identified as bufotenine (5-hydroxy-N,N-dimethyltryptamine).

Compound 3 showed negative optical rotation ([α]_D = −58°, in water). The molecular weight of 3 was determined to be 366 from its field desorption mass spectrum (FD-MS). This compound appeared to be a mono-glucoside of bufotenine, since compound 3 gave 2 and glucose upon acid-hydrolysis, and the PMR spectra exhibited characteristic signals both of 2 and of the glucosyl moiety. The CMR data of compounds 2, 3 and phenyl β-D-glucopyranoside ([α]_D = −72°, in water) are listed in Table I. Signals from the bufotenine and glucosyl portions of 3 closely resembled the corresponding values seen in 2 and the glucosyl portion of phenyl β-D-glucopyranoside, respectively. Some upfield shifts of H-4 (δ 7.15) and H-6 (δ 6.99) were observed in the PMR spectrum of 3, when compared with that of bufotenine (H-4, δ 6.87; H-6, δ 6.80). The anomic proton (1') of compound 3 exhibited a broad doublet (J = 6.6 Hz), indicating a β-configuration for the glucosidic linkage. The non-decoupled CMR spectra of both 3 and phenyl glucoside also showed similar anomic C-H coupling values (J = 161.13 and 162.36 Hz, respectively). Thus, compound 3 was verified as bufotenine 5-O-β-D-glucopyranoside.

The yields of compound 1 and 2 in *C. unshiu* leaves were both estimated to be approximately 8–10 µg/gram leaf, while that of bufotenine glucoside (3) was estimated to be

**Table 1. CMR CHEMICAL SHIFTS OF BUFOTENINE (2), BUFOTENINE β-D-GLUCOSIDE (3) AND PHENYL β-D-GLUCOSIDE**

<table>
<thead>
<tr>
<th>Position</th>
<th>Compound 2</th>
<th>Compound 3</th>
<th>Phenyl glucoside</th>
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<tr>
<td>2</td>
<td>127.53</td>
<td>127.91</td>
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</tr>
<tr>
<td>3</td>
<td>110.19</td>
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<tr>
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<td>9</td>
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<tr>
<td>2CH$_3$N</td>
<td>45.13</td>
<td>45.24</td>
<td></td>
</tr>
</tbody>
</table>

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12 μg/gram leaf. No glucoside of compound 1 was detected.

Unlike compound 2, its glucoside 3 showed no oviposition activity in combination with FM (Table II). Several other available tryptamine analogs, in combination with FM, were offered to swallowtail females in oviposition bioassays, all except N-acetylsertotonin being as active as oviposition synergists as was 1 (Table II). Serotonin was not found in detectable quantities in the C. unshiu leaves, in spite of its significant synergistic activity. The 5-hydroxy moiety did not seem to be necessary for activity since N’-methyltryptamine was significantly active (Table II).

Some of these simple tryptamine bases are known to act as potent neurotransmitters in arthropods. These compounds might have been evolved originally as chemical barriers against attack by various herbivores. Tryptamines occur in several families of higher plants including the Gramineae, Leguminosae, and Rutaceae. However, the distribution of these simple indole alkaloids in the plant kingdom is not well known. It is especially interesting that such tryptamine-type bases act as oviposition synergists, since we have recently characterized a phenethylamine-type neurotransmitter analog, synephrine, as one of the oviposition stimulant components for P. xuthus from C. unshiu leaves, where it occurs in relatively high concentrations. Details of the multi-component oviposition stimulant system of P. xuthus will be discussed separately.

Experimental

EI (electron impact, 70 eV) and FD-MS (5.5 kV using carbon emitters) spectra were measured with a Hitachi M-80 mass spectrometer. Optical rotation was measured with a JASCO ORD Model J-5 spectropolarimeter. UV spectra were measured with a Waters 990 J photodiode array detector in a mixture of acetonitrile, acetic acid and water (4 : 0.3 : 95.7). PMR and CMR spectra were measured with a JEOL JNM FX-90Q spectrometer (90 MHz) using DSS as a standard. The letters s, d and m represent singlet, doublet and multiplet, respectively. Bioassay with P. xuthus females, 3-10 days old, followed the procedures described in previous reports. Standard samples of phenyl β-d-glucopyranoside, 5-hydroxy-N’-methyltryptamine oxalate and N’-methyltryptamine were purchased from Aldrich Chemical Co. Inc., and serotonin oxalate and N’-acetylserotonin were purchased from Sigma Chemical Co. The oxalates were converted to their corresponding acetates before the bioassay.

Isolation of compounds 2 and 3. Fresh leaves of Citrus unshiu were extracted with methanol and fractionated into ether, ethyl acetate, butanol and water layers by solvent extraction. The water layer was chromatographed using reverse-phase columns, and fraction CA was obtained as a syrup (yield 0.2 g) from 1 kg of the fresh leaves, as described previously.

Fraction CA (1 kg leaf equivalent) was then subjected to chromatography on a reverse-phase open column (10 g of ODS-W, microbead silica gel 5D, 100-200 mesh, Fuji-Davison Chemical Ltd.). Fraction 1, eluted with 5% methanol in water (40 ml), was rich in compound 3. Fraction 2, which was rich in compounds 1, and 2, was then eluted with 40 ml of a mixture of methanol, water and acetic acid (10 : 90 : 1). Fraction 2 was next chromatographed on an HPLC column (Asahipak GS-220, 500 mm x 7.6 mm i.d.), eluting with 1% acetic acid in water.
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(1 ml). An eluate (t_R = 17–20 min), which was rich in both 1 and 2, was collected (18 mg yield in total) and rechromatographed on a reverse-phase HPLC column (YMC-pack, S-5 ODS, 5 μm, 300 mm x 8 mm i.d.), eluting with a mixture of methanol, water and acetic acid (10:90:1) at 2 ml/min. Compound 1 was eluted at t_R = 8.6 min (8 mg/kg of leaf yield), and compound 2 at t_R = 9.5 min (10 mg/kg of leaf yield). Compound 3 was isolated from fraction 1 by reverse-phase HPLC (Chempak ODS, 300 mm x 7.5 mm i.d.), eluting with 5% methanol and 1% acetic acid in water at a rate of 2.5 ml/min. Compound 3 gave t_R = 17.5 min (12 mg/kg of leaf yield).

Compound 2 (bufotenine). EI-MS m/z (%): 204 (15), 160 (4), 146 (13), 117 (4), 91 (6), 58 (100), 42 (31). UV λ_max (nm): 222, 276, 297 (shoulder). PMR (D_2O) δ: 7.30 (1H, d, J = 8.5 Hz), 7.13 (1H, s), 6.87 (1H, d, J = 1.5 Hz), 6.80 (1H, double d, J = 1.5 and 8.5 Hz), 3.21 (2H, m), 3.02 (2H, m), 3.02 (6H, d). CMR: listed in Table I.

Compound 3 (bufotenine 5-O-β-D-glucopyranoside) FD-MS m/z (%): 367 (41), 205 (24), 204 (100). [α] D = -58° (c = 0.5, in water). UV λ_max (nm): 224, 275. PMR (D_2O) δ: 7.38 (1H, d, J = 8.5 Hz), 7.15 (1H, d, J = 1.5 Hz), 7.12 (1H, s), 6.99 (1H, double d, J = 1.5 and 8.5 Hz), 4.99 (1H, broad d, J = 6.6 Hz), 4.0–3.4 (6H, m), 3.33 (2H, m), 3.03 (2H, m), 2.80 (6H, s). CMR: listed in Table I.

Hydrolysis of compound 3. Compound 3 (1 mg) was dissolved in 2N HCl (0.5 ml) and held at 70°C for 1 hr. After removing the solvent in vacuo, the reaction mixture was passed through a Sep-pak C_18 cartridge (Waters Associates) with water (2 ml), and then with 30% methanol in water (4 ml). Glucose and bufotenine were recovered from the water and 30% methanol eluates, respectively.

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References
9) L. E. Fellows and E. A. Bell, Phytochemistry, 10, 2083 (1971).