A new salicyl lactone was detected from the unidentified *Oulenzia* sp. and its chemical structure was elucidated as 7-hydroxyphthalide (7-hydroxy-3*H*-isobenzofuran-1-one), based on its GC/MS and GC/FT-IR data and SiO$_2$ column behavior. The compound was synthesized by NaBH$_4$ reduction of 3-hydroxyphthalic anhydride. The GC/MS and GC/FT-IR spectra of the natural compound were consistent with those of the synthetic product. Although the compound is known as a medical material, this is the first example of its presence in nature.

Key words: *Oulenzia* sp.; 7-hydroxy-3*H*-isobenzofuran-1-one; 7-hydroxyphthalide; Winterschmidtiae

The unidentified *Oulenzia* sp. (Astigmata: Winterschmidtiae), whose ITS portion is composed of a 290-base sequence, is a line originating from the deteriorated banana that was found in June 1997. The species, which is smaller in body size and more lively moving than *Tyrophagus putrescentiae*, possesses a pair of well-developed opisthonotal glands visible under a binocular microscope as brown circles, like the case of *Carpoglyphus lactis*. A total of 67 compounds, composed of monoterpenes, hydrocarbons and aromatics, have so far been identified in the gland secretion from 52 species belonging to 9 families, and part of them function as a semiochemical$^1$ (alarm, aggregation and sex pheromones) to each corresponding species or as antifungal substances.$^2$

Among them, at least 12 compounds are novel as natural products.

In order to accumulate more knowledge on mite components, we raised *Oulenzia* sp. by feeding on dry yeast at r.t. under 70% RH, and found a novel aromatic lactone (1, $t_R$: 11.40 min), apart from the following known compounds, in a 3-min hexane rinse of the species: neral ($t_R$: 9.53 min), geraniol ($t_R$: 9.90 min), dodecane ($t_R$: 8.85 min), tridecane ($t_R$: 10.30 min), 3-hydroxybenzene-1,2-dicarbaldehyde (2, $t_R$: 10.20 min), Z-7-pentadecane ($t_R$: 12.78 min) and pentadecane ($t_R$: 12.85 min) (see Fig. 1 for the relative abundances). We report here the structural elucidation and synthesis of this new natural lactone (1).

All efforts to isolate 1 by SiO$_2$ column chromatography from the mites extract were unsuccessful, like the case of 2, for unknown reasons, possibly loss by chelation due to metallic impurity in SiO$_2$. GC/MS analysis of the peak (1, $t_R$: 11.38 min) gave M$^+$ at $m/z$ 150 (69%) and the base ion at $m/z$ 121, along with other diagnostic ions (Fig. 2). An aromatic structure with -CHO group or -OH substitutions was suggested, based on the following facts that the spectrum was similar to that of 2, and that the intensities of the M$^+$ (92%) ion at $m/z$ 150 and M$^+ - 1$ (9%) ion at $m/z$ 149 were relatively higher than usual. The base ion at $m/z$ 121 was also presumed to be a product of CO elimination from M$^+ - 1$, indicating an aldehydic or esteristic structure.

The GC/FT-IR spectrum of 1 obtained from the hexane rinse of mites (32 mg) indicates a C=O stretching band in the unusually high region of 1769 cm$^{-1}$, together with C–O–C stretching bands at 1197 and 1061 cm$^{-1}$, suggestive of the presence of a five-membered lactone in the molecule of 1, while C=C stretching bands at 1622 and 1473 cm$^{-1}$ indicated the presence of an aromatic ring structure with 1,2,3 substitution (776 and 684 cm$^{-1}$). The presence of a -CHO group was dismissed, because two C–H stretching bands characteristic of an aldehyde were

\[ \begin{align*}
\text{OH} & \quad \text{O} \\
\text{CHO} & \quad \text{CHO}
\end{align*} \]

Scheme 1

\[ \begin{align*}
\text{1} \\
\text{2}
\end{align*} \]

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not apparent at around 2700 and 2800 cm\(^{-1}\). The stretching band around 3454 cm\(^{-1}\) suggests the presence of an \(-\text{OH}\) group in the molecule (see Fig. 3). Based on the GC/MS and GC/FT-IR spectra, we postulate the structure of 1 to be a 7-hydroxyphthalalide.

Following the reported procedure,\(^3\) compound 1 was prepared by reducing 3-hydroxyphthalic anhydride with sodium borohydride (NaBH\(_4\)) in \(N,\) \(N\)-dimethylformamide (DMF). Two reduction products, compound 1 (as the major product, 47% yield) and 4-hydroxyphthalalide (as the minor product, 13% yield), were obtained and separated from each other in an SiO\(_2\) column, using a hexane-ethyl acetate mixture as the eluent.

The \(^1\)H-NMR spectrum of synthsized 1 indicated the following partial structures: a 1,2,3-trisubstituted benzene structure \(\delta 77.54\) (1H, dd, \(J=8.07\) and 7.62 Hz), \(\delta 66.99\) (1H, d, \(J=7.60\) Hz), \(\delta 66.87\) (1H, d, \(J=8.10\) Hz), a hydrogen-bonded hydroxy group at \(\delta 10.45\) (1H, s, Ar.-OH), and an oxygen-substituted methylene at \(\delta 52.29\) (2H, s, =C-CH\(_2\)-O-). The \(^13\)C-NMR spectrum gave all chemical shifts corresponding to eight carbons: two carbons indicating the lactone moiety at 172.6 ppm (C=O) and 70.9 ppm (-CH\(_2\)-O-), along with 6 aromatic carbons at 112.4, 114.0, 116.4, 137.6, 150.5, and 158.4 ppm. The HR-MS spectrum revealed the M\(^+\) ion at \(m/z\) 150.0326 (C\(_9\)H\(_{16}\)O\(_2\), calc. as 150.0317).

The structure of synthetic 1 was therefore confirmed to be 7-hydroxyphthalalide (7-hydroxy-3H-isobenzofuran-1-one), whose GC \(R\) value (11.38 min), GC/MS data (see Fig. 2) and GC/FT-IR spectra (see Fig. 3) were identical to those of natural 1. Although compound 1 is known to be effective against antigenic diseases like rheumatism,\(^6\) this is the first example as its natural product possibly der-
ived from an intramolecular Cannizzaro reaction from 3-hydroxybenzene-1,2-dicarbaldehyde (2) that is distributed widely among astigmatid mites (Sakata et al., personal communication).

The semichemical function of present compound 1 is obscure for Oulenia sp. at the moment. It is interesting that the compound available for curing human sickness was accidentally discovered from mites. Most of the aromatic compounds identified so far from acarid mites, including the present compound, have been salicylaldehyde analogs with 1,2,3-substitutions.13 These facts indicate that the same biosynthetic pathway is applied among acarid mites to produce biologically active compounds such as the alarm pheromone, sex pheromone, and antifungal agents.

Experimental

Mites collected from a culture medium by the conventional saturated saline flotation method were extracted by dipping into hexane for 3 min as previously reported.7 GC was performed with a Hewlett Packard 5890 Plus gas chromatograph equipped with an HP-5 column (30 m × 0.25 mm; 0.25 μm) operated in the splitless mode at 60°C for 2 min and then programmed at 10°C/min to 290°C and held at this temperature for 5 min. Mass spectra were measured with a Hewlett Packard HP 5989B mass spectrometer operated at 75 eV with the same capillary column and under the same conditions as those just described. IR spectra were recorded with a Bio-Rad FT-IRD instrument coupled to a Hewlett Packard 5890 Plus gas chromatograph. Both the 1H-NMR and 13C-NMR spectra were measured by a Bruker AC 300 MHz spectrometer in CDCl3/OD solutions.

4-Hydroxyphthalaldehyde (20 mg, 13%), mp 251°C, GC \( t_R = 15.39 \) min; MS m/z (%): 150 (M+, 66), 149 (14), 122 (8), 121 (100), 105 (2), 94 (3), 93 (28), 92 (5), 77 (2), 76 (2), 74 (3), 65 (16) and 51 (5); 1H-NMR (300 MHz) \( \delta = 7.37 \) (1H, d, 7.71, 7.37 Hz), 7.30 (1H, d, 7.35 Hz), 7.06 (1H, d, 7.73 Hz), 5.29 (2H, s, \(-\text{CH}_2-)\); 13C-NMR (75 MHz) \( \delta = 173.7 \) (C = O), 153.8 (C =), 134.8 (C =), 131.7 (C =), 128.2 (C =), 121.0 (C =), 116.8 (C =) and 69.7 (C =).

Acknowledgments

This study was partly supported by grant-aid for scientific research from the Ministry of Education, Science, Sports and Culture of Japan (nos. 08406010, 09556010, 09876091 and 8956), and also by research funding from Daily Foods Corporation (Tokyo). We thank Dr. N. Akimoto in Faculty of Pharmaceutical Sciences at Kyoto University for measuring the HRMS data.

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