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BIBENZYLS FROM *RADULA TOKIENSIS* AND *R. JAPONICA*

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Key Word Index—*Radula tokiensis*; *R. japonica*; Jungermanniales; Hepaticae; bibenzyls; dihydrooxepin ring; chemosystematics.

Abstract—A new bibenzyl having a dihydrooxepin ring was isolated from the acetone extract of the liverwort *Radula tokiensis*, together with the previously known 5 bibenzyls and 3 sesquiterpenes, *trans-β*-farnesene, cuparene and δ-cuparenol. Two known bibenzyls were isolated from *R. japonica*. The bibenzyl derivatives are significant chemosystematic markers of the Radulaceae.

INTRODUCTION

A number of *Radula* species, belonging to the liverwort order Jungermanniales, contain various bibenzyl derivatives. Some *Radula* spp. cause allergenic contact dermatitis [1] and their crude extracts show antimicrobial activity at low concentrations (Asakawa, Y., Matsuda, R. and Takemoto, T., unpublished results). Recently, we reported the isolation of 11 new bibenzyls and a new dihydrochalcone from Japanese and European *Radula* sp. [2, 3]. As part of our systematic investigation of biologically active substances of the Hepaticae, we have now studied the chemical constituents of Japanese *Radula* species. In the present communication, we report the isolation and distribution of bibenzyl derivatives and sesquiterpenoids in *R. tokiensis* and *R. japonica*.

RESULTS AND DISCUSSION

Air-dried *R. tokiensis* was extracted with Me_2CO and the crude viscous oil was directly chromatographed on silica gel to give a new bibenzyl (**2**) and the 5 previously known bibenzyls (**1, 3, 4, 5** and **6**), and 3 sesquiterpenes (**7, 8** and **9**). *R. japonica* was treated in the same manner to afford the 3 bibenzyls (**1, 3** and **4**).

Bibenzyl 2

The less polar component, $C_{20}H_{22}O_2$ (M^+ *m/e* 296), showed the presence of a benzyl group [δ 7.28, (*s*); *m/e* 91 (base)] and a OMe group (δ 3.82, *s*). The ^1H NMR spectrum also contained the signals for one vinyl Me group (δ 1.58, *bs*), one vinylic proton (δ 5.63, *m*), one methylene (δ 4.45, *bs*) located between an ether oxygen and a double bond, two equivalent methylenes (δ 2.88, *bs*), characteristic of benzylic methylenes, two protons (δ 6.50 and 6.62, each *bs*) located in a *meta* position and one methylene group (δ 3.47, *bd*) placed between a double bond and an aromatic ring. The above spectrum was similar to that of the bibenzyl (**1**) isolated from *R. variabilis* [2] and *R. complanata* [3], except for the presence of a OMe group, indicating that the new bibenzyl (**2**) might be a methoxy derivative of **1**. Methylation of **1** with MeI gave a mono methyl ether whose spectral data and chromatographic behavior were identical to those of **2**.

Bibenzyls 1, 3, 4, 5 *and* 6 *and sesquiterpenoids* 7, 8 *and* 9

The chemical, spectral and chromatographic properties of the bibenzyls **1, 3, 4, 5** and **6** were identical to those of the bibenzyls isolated from *R. variabilis* [2] and *R. complanata* [3], respectively. The spectral data and optical rotation of the sesquiterpenoids **7, 8** and **9** were in good agreement with those of authentic samples.

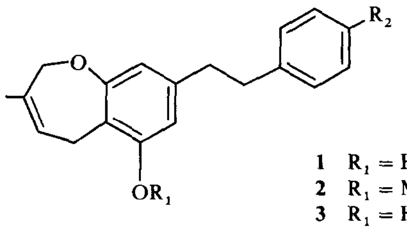
The major components of *R. tokiensis* and *R. japonica* are the bibenzyls **1** and **4**, respectively. The bibenzyls **5** and **6** have not been isolated from *R. japonica*. *R. tokiensis* is chemically quite similar to *R. variabilis* and *R. complanata*.

Most liverworts, belonging to the Jungermanniales, generally elaborate various terpenoids as the major components [4]. *Radula* species are chemically very specific since they contain a large quality of bibenzyl derivatives having a rare dihydrooxepin ring [5]. Chemically speaking, it is suggested that the Radulaceae are a distinct family of the Jungermanniales, just like the Isotachiaceaea [6].

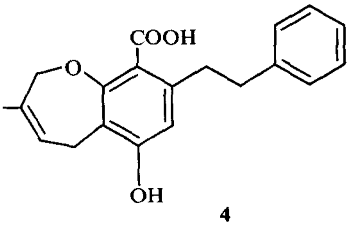
EXPERIMENTAL

Spectra were measured in $CDCl_3$ (60 MHz, ^1H NMR), 95 % EtOH (UV), liquid film (IR) and direct inlet system at 70 eV (MS) and column SE-30 1 %, temp. 50–270° at 5°/min, He 30 ml/min at 70 eV (GC–MS). TLC and PLC were on precoated Si gel (0.25 mesh) F_{254}, spots were detected by spraying with 30 % H_2SO_4 and viewing in UV light (254 nm).

Plant material. *Radula* species identified by Dr. S. Hattori and Dr. T. Kodama are deposited in the Herbarium, The Hattori Botanical Laboratory, Miyazaki, Japan, and Institute of Pharmacognosy, Tokushima Bunri University.



1 $R_1 = H, R_2 = H$
2 $R_1 = Me, R_2 = H$
3 $R_1 = H, R_2 = OH$



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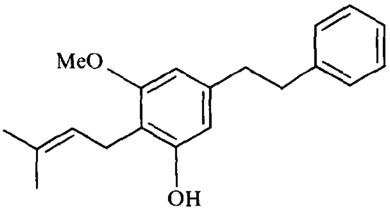

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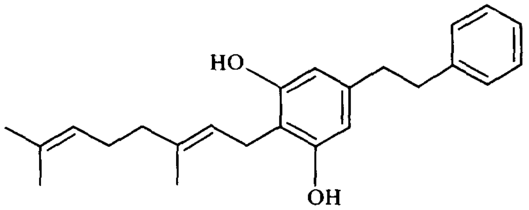

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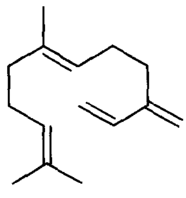

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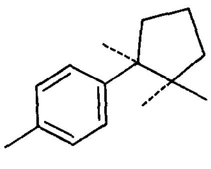

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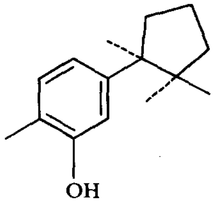

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Extraction and isolation. R. tokiensis Steph. (360 g) collected in Kyoto in October 1978 was air-dried for 5 days and then extracted with Me_2CO for 10 days. The crude extract (3.89 g) was directly chromatographed on Si gel using gradient elution with C_6H_6–EtOAc. The first fraction eluted by C_6H_6 contained a mixture of sesquiterpenes (58 mg) in which calamenene, farnesene, cuparene and δ-elemene were detected by GC–MS. The mixture was rechromatographed on Si gel using *n*-hexane to afford *trans*-β-farnesene (**7**) (17 mg) and (−)-cuparene (**8**) (7 mg). The second fraction (C_6H_6–EtOAc, 19:1) gave a mixture of carotenoids (120 mg). The third fraction (4:1) contained blue fluorescent compounds (940 mg) which were rechromatographed on Si gel using C_6H_6–EtOAc gradient to afford the bibenzyls **1** (580 mg) [2, 3], **2** (120 mg) and **3** (15 mg) [3]. All spectral data of compound **2** were identical to those of the Me ether derived from **1** by methylation [2]. The fourth fraction (3:1) gave a mixture of bibenzyl derivatives and phytosterols (1.531 g) which was rechromatographed on Si gel using a C_6H_6–EtOAc gradient to afford (−)-δ-cuparenol (**9**) (53 mg) $[\alpha]_D - 64°$ [7, 8], the bibenzyl **5** (15 mg) [3], 3,5-dihydroxy-4-(3,7-dimethyl-2,6-octadienyl)-bibenzyl (**6**) (65 mg) [2], phytosterols (campesterol–stigmasterol–sitosterol; 1:2:1 on GC–MS) (59 mg) and unidentified bibenzyls (320 mg). The fifth fraction (1:1) gave a pure bibenzyl (**6**) (226 mg). The sixth fraction (1:3) gave a mixture of acidic bibenzyls which were rechromatographed on Si gel using C_6H_6–EtOAc to afford the bibenzyl **4** (475 mg) [2, 3].

R. japonica Gott. ex Steph. (180 g) collected in Tokushima prefecture in May 1979 was treated as described above. The crude extract was directly analysed by GC–MS and the presence of calamenene, cupranene (**8**) and δ-cuprarenol (**9**) was confirmed. The crude extract (780 mg) was chromatographed on Si gel using C_6H_6–EtOAc and each fraction was purified by PLC to afford the bibenzyls **1** (150 mg), **3** (10 mg) and **4** (82 mg), respectively.

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