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CUPARANE- AND ISOCUPARANE-TYPE SESQUITERPENOIDS IN LIVERWORTS OF THE GENUS *HERBERTUS**

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Key Word Index—*Herbertus aduncus*; *H. sakuraii*; *H. subdentatus*; Jungermanniales; Hepaticae; cuparene; 2-hydroxycuparene; 3-hydroxycuparene; 2,3-dihydroxycuparene; herbertene; 2-hydroxyisocuparene; 4-hydroxyisocuparene; α-cuparenone; ent-sesquiterpenoids; chemosystematics.

Abstract—Canadian and Japanese *Herbertus aduncus*, Japanese *H. sakuraii* and Colombian *H. subdentatus* belonging to the Jungermanniales (Hepaticae) were chemically investigated. The previously known ent-cuparane- and novel isocuparane-type sesquiterpenoids are the major components which are of importance as chemical markers in the Herbertaceae.

INTRODUCTION

Most liverworts have oil bodies which contain mainly mono-, sesqui- and diterpenoids and/or lipophilic aromatic compounds. These substances are present as major components and thus we can apply them to a chemosystematic study of the Hepaticae [1–13].

Herbertus species belonging to the suborder Herbertineae are rather primitive liverworts in the Jungermanniales. In previous papers [2, 5, 8, 9], we noted that *H. aduncus* (Dicks.) S. Gray, *H. sakuraii* (Warnst.) Hatt. and *H. subdentatus* (Steph.) Fulf. produced cuparane- and isocuparane-type sesquiterpenoids. More recently, Matsuo *et al.* [14] reported the isolation of herbertene (isocuparene) (**6**) from *H. aduncus*. The present communication is concerned with the distribution of ent-cuparane- and ent-isocuparane-type sesquiterpenoids in three *Herbertus* species, *H. aduncus*, *H. sakuraii* and *H. subdentatus*, collected in Canada, Colombia and Japan.

RESULTS AND DISCUSSION

Air-dried and ground material of each species was extracted with diethyl ether. The extracts were checked by TLC and GC and further analysed by GC/MS equipped with a computer. Chemical components were identified by GC co-injection of standard compounds, analysis of each mass spectrum and direct comparison of mass spectra with those of authentic samples. The major components were further isolated by prep. TLC and GC, and their structures confirmed by spectral evidence and chemical degradations. The species, sampling locations, date of collection and compounds detected in each *Herbertus* species were listed in Table 1.

The major components of *H. aduncus* (sample 1) are new isocuparane-type sesquiterpene alcohols, 2-hydroxyisocuparene [*m*-(1, 2, 2-trimethylcyclopentyl)-*o*-cresol] (**8**), 4-hydroxyisocuparene [*m*-(1, 2, 2-

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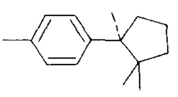
*Part 11 in the series"Chemosystematics of Bryophytes." For Part 10, see (1981) *Phytochemistry* **20**, 2187.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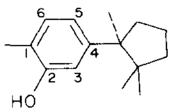

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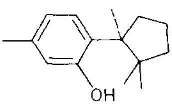

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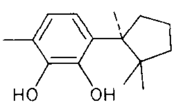

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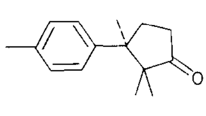
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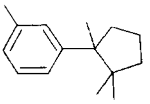
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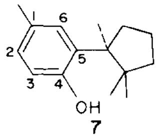

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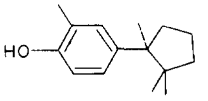

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Table 1. Cuparane- and isocuparane-type sesquiterpenoids of *Herbertus* species

Sample no	Species	Site and date of collection	Compounds detected								
			1	2	3	4	5	6	7	8	Sterols‡
1	*Herbertus aduncus*	Mt Fujikura, Imajo-cho, Nanjo-gun, Fukui, Japan Oct. 1978	+*	+	—	+++++	—	++	+++++	++	+
2	*H. aduncus*	Komagadake, Hakone, Kanagawa, Japan Aug. 1980	+	+	—	—	—	++	+++++	+	+
3	*H. aduncus*†	Bridal Veil Falls, Popkum, Fraser River Valley, British Columbia, Canada May 1981	+	+	—	++	+	++	+++	+++	+
4	*H. sakuraii*	Yaku Island, Kagoshima, Japan Dec. 1979	—	+	—	—	—	+	++++	—	+
5	*H. subdentatus*	Paramo de Guasca, Columbia Aug. 1980	+	+	+	+++++	—	+	+++++	+++	+

*The symbols, +, ++, +++ etc are the relative concentrations estimated by GC/MS.

†β-Pinene and 1-octen-3-ol were detected in this species as minor components.

‡Campesterol, stigmasterol and sitosterol.

trimethycyclopentyl)-*p*-cresol] (**7**) and a new sesquiterpene diol, 2, 3-dihydroxycuparene (**4**) in which **7** is the most abundant. Cuparene (**1**), herbertene (isocuparene) (**6**) and 2-hydroxycuparene (**2**) were also detected as minor components. *H. aduncus* was further collected in two different localities, Kanagawa (Japan) (sample 2) and British Columbia (Canada) (sample 3). The gas chromatogram of the crude extract (sample 2) was simpler than that of sample 1. The major component of sample 2 was 4-hydroxyisocuparene (**7**). However, 2, 3-dihydroxycuparene (**4**) was not detected in sample 2, even in GC/MS analysis. It is not clear from the present results whether the presence or absence of 2, 3-dihydroxycuparene in the two Japanese *H. aduncus* is caused by seasonal or geographical variation. The chemical constitution of Canadian *H. aduncus* is quite similar to that of the same species in Japan (sample 1), except for the presence of an additional cuparane-type sesquiterpene ketone, α-cuparenone (**5**), as a minor component.

H. sakuraii synthesized 4-hydroxyisocuparene (**7**) and 2-hydroxyisocuparene (**8**), together with herbetene (**6**). Thus, *H. sakuraii* is chemically quite close to *H. aduncus*. Colombian *H. subdentatus* also produced the same cuparane- and isocuparane-type sesquiterpenoids as *H. aduncus* and *H. sakuraii* and their chemical profiles quite resemble that of *H. aduncus* (sample 1). In addition to the above components, *H. subdentatus* contains 3-hydroxycuparene (**3**) whose structure was tentatively assigned by GC/MS analysis.

In previous communications [2, 5, 8, 9], we reported that 2-hydroxycuparene (**2**) was the major component of *Herbertus* species. Further chemical and spectral data of each of the isolated cuparene- and isocuparene-type sesquiterpenes showed that the major constituents were hydroxyisocuparenes (**7, 8**) whose mass spectra were quite similar to those of 2-hydroxycuparene (**2**).

From the above results, it is clear that isocuparane- and cuparane-type sesquiterpenoids are characteristic constituent of *Herbertus* species. Recently, we reported that the chemical markers of *Trichocolea tomentella*, which is referred to the suborder Herbertineae in the modern classification of the Hepaticae [15], are isoprenyl benzoates [13]. It is apparent therefore, that there is no chemical affinity between *Herbertus* and *Trichocolea* species. The present cuparane- and isocuparane-type sesquiterpenoids belong to the ent-series and are thus characteristically distinctive in stereochemistry from the sesquiterpenoids of higher plants.

EXPERIMENTAL

GC/MS spectra were obtained under the following conditions: electrical energy 70 eV; trap current 60 μA; temp. 270°. GC column: 5% SE-30, 3m × 2 mm glass column: temp. 50–270° at 5°/min, inject temp. 260°, He 30 ml/min. Analytical and prep. GC: 5% SE-30, 3m × 2 mm and 5% DEGS glass column, temp. programme 50–270° at 5°/min, inject temp. 260°, N_2 30 ml/min. TLC and prep. TLC: pre-coated Si gel (0.25 mesh) F_{254}, *n*-hexane-EtOAc or C_6H_6–EtOAc (4:1) as solvents. Compounds were detected by I_2 vapor, 30% H_2SO_4 or UV light (254 nm).

Plant materials. Herbertus species identified by W. B. S., S. R. G., Drs. S. Hattori and H. Inoue were deposited in the Herbaria of Institute of Pharmacognosy, Tokushima Bunri University, Universities of Bogota and Utrecht.

Extraction and isolation. Each *Herbertus* species sample was air-dried for 5 days, ground mechanically and the powder (250 mg) extracted with Et_2O for 5 days. The extracts were filtered through a short glass column packed with Si gel (230–400 mesh) and the filtrates directly analysed by TLC, GC and GC/MS equipped with a computer, after removal of the solvent. The remaining materials were also extracted with Et_2O for 1 month and then re-extracted with MeOH for 2 weeks. Each extract was combined and the crude oils were chromatographed on Si gel using a *n*-hexane–EtOAc or C_6H_6–EtOAc gradient. The chemical structures of the isolated compounds by prep. TLC and GC were confirmed by IR, ^{1}H NMR, and MS analyses and some chemical degradations. The structure determination of the new isocuparenes and 2,3-dihydroxycuparene from *Herbertus* species will be reported elsewhere.

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