and 119, which could readily be assigned as shown in Scheme 1.

Derivatives of anthranilic acid have not so far been reported from the Compositae.

EXPERIMENTAL

Dried aerial parts (leaves and stems 3 kg) of *Inula oculus-christi* collected in the flowering season, were extracted with MeOH and after evaporation the residue was chromatographed on a Si gel column. Elution with petrol–$CHCl_3$ (1 : 4) gave crude crystals of gaillardin (70 mg), and with $CHCl_3$ gave pulchellin C (50mg). Their structures were established by comparing the ^1H NMR spectra with those of authentic material. Further elution with $CHCl_3$–MeOH (23 : 2) gave crude crystals of **1** (40 mg) which were recrystallized from Me_2CO–petrol, mp 91–93°. IR ν_{max}^{KBr} cm^{-1}: 1600, 1490, 1450 and 750 (*ortho* substituted benzene ring), 3350, 1707 and 1690 ($-CO_2H$ and $-CON\langle$), MS (70 eV): 459.371 $[M]^+$, (1) ($C_{29}H_{49}O_3N$), 441.359 $[M-H_2O]$ (12), 179 (**2**, 20%), 161 (**3**, 100%), 137 (**4**, 71%) and 119 (**5**, 15%).

REFERENCES

1. Kupchan, S. M., Cassady, J. M., Kelsey, J. E., Schnoes, N. K., Smith, D. H. and Burlingame, A. L. (1966) *J. Am. Chem. Soc.* **88**, 5292.
2. Kiseleva, E. Y., Cheitchenko, V. I., Ribalko, K. S., Chreter, A. I. and Pakaln, D. A. (1969) *Khim. Prir. Soedin.* **5**, 444.
3. Currie, M. and Sim, G. A. (1973) *J. Chem. Soc. Perkin Trans.* 2, 400.

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MONO- AND SESQUITERPENOIDS FROM *HYDROCOTYLE* AND *CENTELLA* SPECIES

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Key Word Index—*Hydrocotyle sibthorpioides*; *H. maritima*; *Centella asiatica*; Umbelliferae; monoterpenoids; sesquiterpenoids; allelopathy; chemosystematics.

Abstract—*Hydrocotyle sibthorpioides*, *H. maritima* and *Centella asiatica* were investigated for their terpenoid constituents. The major component of *H. sibthorpioides* and *H. maritima* is *trans*-β-farnesene. The latter species also elaborates α-terpinene and thymol methyl ether in respectable amount. The sesquiterpenoid constitution of *C. asiatica* is rather similar to *H. maritima*. Possible allelopathy between *H. sibthorpioides* and a liverwort is suggested.

INTRODUCTION

Hydrocotyle and *Centella* species (Umbelliferae) produce characteristic essential oils throughout the whole plant. It is known that *H. sibthorpioides* Lam. and *H. maritima* Honda have hemostatic and antitumor activities[1]. The latter species contains a flavonoid glycoside, hyperin[2]. *Centella asiatica* Urb. (*H. asiatica* L.) which is morphologically close to *Hydrocotyle* species produces biologically active triterpenoids with possible therapeutical use in ulcerations, extensive wounds and eczemas etc.[3–10].

Whenever *H. sibthorpioides* grows near the liverwort, *Marchantia polymorpha*, in the greenhouse the liverwort gradually dies. It is suggested that *H. sibthorpioides* and its related species may produce chemicals which inhibit the growth of liverworts. As part of a systematic study of biologically active terpenoids of plants, we have studied the chemical constituents of *H. sibthorpioides*, *H. maritima* and *C. asiatica*. In the present paper, we wish to report the distribution of mono- and sesquiterpenoids in the above three species.

RESULTS AND DISCUSSION

Whole plants of *H. sibthorpioides* and *C. asiatica* were extracted with diethyl ether after being air-dried and ground. *H. maritima* was divided into leaf, stem and flower and each part was treated in the same

Table 1. Terpenoids of *Hydrocotyle sibthorpioides* and *H. maritima*

Peak no.	Terpenoids	*H. sibthorpioides* T*(%)‡	Y†(%)	*H. maritima* Leaf (%)	Stem (%)	Flower (%)
1	α-Pinene	3.0	4.2	1.3	3.9	4.0
2	Camphene	1.0	0.8	Trace	0.6	0.7
3	β-Pinene	5.1	7.2	Trace	1.6	8.2
4	Ocimene	0.4	0.6	—	—	—
5	Myrcene	—	—	Trace	1.6	1.3
6	Limonene	—	—	Trace	0.8	0.7
7	α-Terpinene	—	—	7.3	16.4	14.0
8	Thymol methyl ether	—	—	10.0	23.0	11.2
9	β-Caryophyllene	2.3	2.8	6.4	3.0	4.7
10	α-Humulene	1.8	2.3	—	—	—
11	*trans*-β-Farnesene	24.6	32.5	20.9	13.0	31.0
12	Germacrene D	—	—	1.4	1.4	0.2
13	M^+ ? (base 55)	—	—	—	0.4	0.5
14	M^+ ? (base 55)	16.9	13.2	—	—	—
15	Phytol	0.8	0.3	—	0.6	0.3
16	M^+ 384 (base 131)	—	—	—	—	0.3
17	M^+ ? (55)	0.8	0.3	—	—	—
18	M^+ ? (83)	—	—	—	—	0.5
19	M^+ ? (43)	1.4	1.5	—	—	—
20	M^+ ? (57)	0.2	0.4	—	—	—
21	M^+ ? (131)	0.5	0.5	1.1	Trace	2.2
22	M^+ ? (131)	2.5	1.4	1.5	Trace	1.0
23	M^+ 354 (149)	—	—	—	—	1.0
24	Stigmasterol	13.5	6.6	—	—	2.8
25	Stigmasterol isomer	7.4	3.5	3.6	4.0	0.7

*Collected in Tokushima.
†Collected in Yamaguchi.
‡Relative abundance estimated by GC equipped with a computer.

Table 2. Terpenoids of *Centella asiatica*

Peak no.	Terpenoids	%*
1	α-Pinene	0.2
2	β-Pinene	0.2
3	Myrcene	0.6
4	γ-Terpinene	0.4
5	Bornyl acetate	0.2
6	α-Copaene	0.9
7	β-Elemene	3.0
8	β-Caryophyllene	12.5
9	*trans*-β-Farnesene	17.7
10	Germacrene-D	16.0
11	M^+ 204 (base 93)	1.3
12	Bicycloelemene	2.3
13	M^+ 236 (123)	0.2
14	M^+ 274 (157)	36.4
15	M^+ 274 (129)	0.5
16	M^+ 274 (43)	0.5
17	M^+ 410 (69)	Trace
18	M^+ ? (57)	0.3
19	Campesterol	0.2
20	Stigmasterol	0.2
21	Sitosterol	0.2

*Relative abundance estimated by GC equipped with computer.

manner as above. Each extract was filtered and the filtrate monitored by TLC and GC before analysis by computerized GC/MS. The components were identified by GC coinjection of standard compounds and direct comparison of mass spectra with those of authentic samples. The major components were further isolated by prep. GC and their structures were confirmed by spectral evidences. Tables 1 and 2 show the compounds detected in each species and in each part of *H. maritima*.

The three species all contained mono- and sesquiterpene hydrocarbons. The major components of *H. sibthorpioides* are *trans*-β-farnesene and an unidentified sesquiterpene (peak 14). *H. maritima* produces *trans*-β-farnesene, α-terpinene and thymol methyl ether as the major components. The latter two components have not been detected in *H. sibthorpioides* and *C. asiatica*. *H. sibthorpioides* and *H. maritima* produce the same unidentified aromatic compounds (peaks 21 and 22) and an isomer of stigmasterol (peak 24). Thus, *H. sibthorpioides* is chemically close to *H. maritima* except for the presence of α-terpinene and thymol methyl ether. There is no chemical difference between samples of *H. sibthorpioides* collected in different localities. The leaf, stem and flower of *H. maritima* contain the same major terpenoids. The major component of *C. asiatica* is an unidentified terpenic acetate [peak 14; M^+ 274 (base

157)]. β-Caryophyllene, *trans*-β-farnesene and germacrene D have been detected in *C. asiatica* in respectable amount. Although *C. asiatica* and *H. maritima* produce three common sesquiterpene hydrocarbons, the chemistry of the two species differs considerably. Previously, *C. asiatica* was included in the *Hydrocotyle*. Phytochemistry supports the classification of *C. asiatica* (*H. asiatica*) in a separate genus, *Centella*.

The liverwort, *Marchantia polymorpha* gradually dies in the presence of *H. sibthorpioides*. It is assumed that the volatile terpenoid constituents characterized in this study inhibit the growth of liverworts.

EXPERIMENTAL

GC/MS spectra were obtained under the following conditions: electrical energy 20 and 70 eV; trap current 60 μA; temp. 80–250°; GC column: 5% SE-30, 3 m × 2 mm glass column; temp. 50–270° at 5°/min, injection temp. 260°, He 30 ml/min. TLC and prep. TLC: precoated Si gel (0.25 mm) F_{254}, *n*-hexane, *n*-hexane–EtOAc (4:1) and C_6H_6–EtOAc (4:1) as solvents. Spots were detected by 30% H_2SO_4 at 120°, I_2 vapour and UV light (254 nm). Analytical and prep. GC: 5% SE-30, 3 m × 2 mm and 5% DEGS glass column, temp. 40–260° at 5°/min, injection temp. 250°, N_2 30 ml/min.

Plant materials. Hydrocotyle sibthorpioides Lam., *H. maritima* Honda and *Centella asiatica* Urb. identified by Y. A. and T. T. were deposited in the Herbarium of the Institute of Pharmacognosy, Tokushima Bunri University. The plant materials were collected in the following locations and months. *H. sibthorpioides*: grown in the greenhouse of the Botanical Garden, Tokushima Bunri University, May 1980, July 1981; Yanai-shi, Yamaguchi, Japan, October 1981. *H. maritima*: Botanical Garden of Tokushima Bunri University, June 1981; Okinoyama-cho, Tokushima, May–July 1981. *Centella asiatica*: Botanical Garden, Tokushima Bunri University, May 1981.

Extraction and isolation. H. sibthorpioides, *H. maritima* and *C. asiatica* (20 g) were extracted with Et_2O for 1 week, after being air-dried for 2 days and then ground. The extracts were filtered through a short column packed with Si gel and solvent was evaporated *in vacuo* at 25°. The oils were immediately diluted with Et_2O to a suitable concn and then analysed by TLC, GC and GC/MS equipped with a computer. The remaining materials were also extracted with Et_2O for 3 weeks in order to isolate terpenoids by prep. TLC and GC, after being chromatographed on Si gel (70–270 mesh) using a *n*-hexane–EtOAc gradient.

REFERENCES

1. Miyata, S. (1981) *Anticancer Crude Drugs and their Prescription* pp. 185–186. Kagakushoin, Tokyo (in Japanese).
2. Nakaoki, T. and Morita, N. (1960) *Yakugaku Zasshi* **80**, 1474.
3. Pasich, B., Kowalewski, Z. and Socha, A. (1968) *Diss. Pharm. Pharmacol.* **20**, 69.
4. Malhotra, C. L., Das, P. K., Sastry, M. S. and Dhalla, N. S. (1961) *Indian J. Pharm.* **23**, 106.
5. Boiteau, P. and Chanez, M. (1967) *C. R. Acad. Sci. Ser. D.* **264**, 407.
6. Dutta, T. and Basu, L. P. (1967) *Indian J. Chem.* **5**, 586.
7. Pinhas, H. and Bondiou, J. C. (1967) *Bull. Soc. Chim. Fr.* 1888.
8. Singh, B. and Rastogi, R. P. (1968) *Phytochemistry* **7**, 1385.
9. Dutta, T. and Basu, V. P. (1968) *Indian J. Chem.* **6**, 543.
10. Pinhas, H., Billet, D. and Heitz, S. (1970) *Bull. Soc. Chim. Fr.* 1890.