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CONSTITUENTS OF THE ESSENTIAL OILS FROM THREE TETRAPLOID SPECIES OF *CHRYSANTHEMUM*

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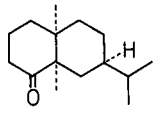
Key Word Index—*Chrysanthemum indicum*; *C. yoshinaganthum*; *C. cuneifolium*; Compositae; essential oil; mono- and sesquiterpenoids; valeranone; estafiatin; spiroketalenolether polyynes; chemotaxonomic aspects.

Abstract—The chemical constituents of the volatile oils from *Chrysanthemum indicum*, *C. yoshinaganthum* and *C. cuneifolium*, three botanically related tetraploid species, are described. By spectroscopic methods, 42 compounds were identified, including 22 monoterpenoids, 17 sesquiterpenoids and 3 acetylenic compounds. The sesquiterpenoids estafiatin (*C. yoshinaganthum*) and valeranone (*C. indicum*) have been found for the first time in *Chrysanthemum* species.

INTRODUCTION

The genus *Chrysanthemum* comprises di-, tetra-, hexa-, octa- and deca-ploid species with $2n = 18$, 36, 54, 72 and 90, respectively. In a program devoted to chemosystematic investigations of the *ca* 20 species of *Chrysanthemum* native to Japan, we have already reported the composition of the essential oils of *C. boreale* ($2n = 18$), *C. makinoi* ($2n = 18$), *C. vulgare* ($2n = 18$), *C. shiwogiku* ($2n = 54$), *C. japonense* ($2n = 54$) and *C. japonense* var. *debile* ($2n = 54$) [1–5]. In the present paper we report the volatile constituents of *C. indicum* L., *C. yoshinaganthum* Makino *ex* Kitam. and *C. cuneifolium* Kitam., which are all tetraploid. *C. cuneifolium* is assumed by Tanaka [6] to be a natural hybrid between the two first-mentioned species as a result of morphological and cytological studies.



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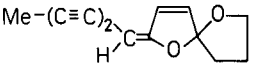

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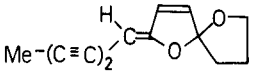

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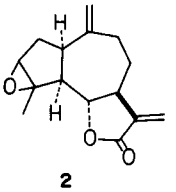

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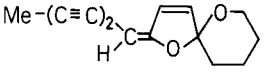

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RESULTS AND DISCUSSION

The plants used in this study were collected in the region of the Naka River, Tokushima, and grown in the experimental garden at Hiroshima University. The essential oil, obtained by steam distillation from the powdered plant material, was separated by vacuum distillation into three fractions; (a) monoterpene hydrocarbons, oxygenated monoterpenoids and sesquiterpene hydrocarbons; (b) oxygenated sesquiterpenoids; and (c) more highly oxygenated compounds. The second fraction was further divided into the sesquiterpene hydrocarbons and oxygenated monoterpenes on a Si gel column eluting with petrol and then ether. Each mono- and sesquiterpene hydrocarbon fraction was subjected to prep. GLC. The oxygenated monoterpenoid, oxygenated sesquiterpenoid and oxygenated compounds were separated by a combination of column chromatography, preparative TLC and preparative GLC. The isolated compounds were identified by comparison of IR, ^{1}H NMR and mass spectra with those of authentic spectra [4, 7], and the relative percentage of the constituents found in three *Chrysanthemum* species are listed in Table 1, where trace constituents (less than 0.1%) were determined by GC/MS.

A large portion of the essential oil of *Chrysanthemum indicum* is sesquiterpenoid (*ca* 62% of total oil), whereas the *C. yoshinaganthum* and *C. cuneifolium* monoterpenoid constituents amount to *ca* 65 and 66% of their respective oils. It may be also noted that *C. yoshinaganthum* contains a very large amount of myrtenol (54.8%), whereas *C. cuneifolium* contains α-pinene (5.7%), sabinene (6.4%), 1.8-cineole (23.0%) and camphor (14.7%), and *C. indicum* myrcene (6.0%), 1,8-cineole (6.0%) and bornyl acetate (7.5%) as major monoterpenoid components. All three species contain the sesquiterpene hydrocarbons α-copaene, β-elemene, β-caryophyllene, β-farnesene, β-humulene and germacrene-D, the last of which is present as the common main component. Furthermore, α-selinene, *ar*-curcumene, calamenene, γ-cadinene and calacorene were identified in the oil of *C. indicum*, whereas these hydrocarbons were not found in the other two species. The oxygenated sesquiterpenoid, T-muurolol which is absent from the oil of *C. yoshinaganthum* is prominent in *C. indicum* and *C. cuneifolium*. Varelanone (**1**) in *C. cuneifolium* and estafiatin (**2**) in *C. yoshinaganthum* have never been isolated previously from

Table 1. Constituents of the essential oils from three tetraploid species of *Chrysanthemum*

Compound	*C. indicum*	*C. yoshinaganthum*	*C. cuneifolium*
α-Pinene	0.9	1.0	5.7
Camphene	2.0	trace*	2.2
Sabinene	1.1	0.6	6.4
β-Pinene	0.1	0.1	0.3
Myrcene	6.0	0.2	2.2
α-Terpinene	1.6	—	—
Limonene	—	trace*	—
p-Cymene	0.9	trace*	trace*
1,8-Cineole	6.0	6.8	23.0
1-Octen-3-ol	—	0.1	0.3
Linalol	0.7	—	—
α-Thujone	1.5	—	—
Chrysanthenone	1.6	—	—
Camphor	0.6	trace*	14.7
trans-Pinocarveol	trace*	—	0.3
Borneol	3.0	—	2.5
Terpinen-4-ol	—	0.3	2.1
Myrtenal	—	0.2	—
Myrtenol	—	54.8	trace*
α-Terpineol	—	—	3.0
Linalyl acetate	1.5	—	—
Bornyl acetate	7.5	—	1.1
Other monoterpenoids†	1.8	2.5	2.9
Total monoterpenoids	37.4	65.1	66.2
α-Copaene	0.3	trace*	trace*
β-Elemene	0.4	0.8	0.6
β-Caryophyllene	0.8	2.0	3.8
β-Farnesene	5.0	1.7	2.6
β-Humulene	trace*	trace*	0.2
Germacrene-D	8.5	10.6	7.7
α-Selinene	8.0	—	—
ar-curcumene	1.0	—	—
Calamenene	0.6	—	—
γ-Cadinene	5.1	—	—
Calacolene	3.9	—	—
Nerolidol	2.8	—	—
Caryophyllene oxide	3.8	1.5	2.5
T-Muurolol	5.3	—	6.8
α-Cadinol	4.5	—	—
Valeranone (**1**)	—	—	2.1
Estafiatin (**2**)	—	0.4	—
Other sesquiterpenoids†	11.6	5.4	6.8
Total sesquiterpenoids	61.6	22.4	33.4
Acetylenic compound (**3**)	—	5.7	trace*
Acetylenic compound (**4**)	—	2.5	—
Acetylenic compound (**5**)	—	0.8	—

*Trace: <0.1%.

†Several oxygenated mono- and sesquiterpenoids unidentified.

Chrysanthemum species. Several oxygenated non-terpene compounds were identified in the oil of *C. yoshinaganthum* as the spiroketalenolether polyynes (**3**), (**4**), and (**5**), which are widespread in members of the Compositae [8], by comparing the IR, ^{1}H NMR and mass spectra with those of reported data. *C. cuneifolium* is closely related botanically to *C. indicum* and *C. yoshinaganthum* [6]. It is interesting to note that the three species show mutually different patterns with regard to the oil constituents.

EXPERIMENTAL

The IR and ^{1}H NMR (60 MHz) spectra were recorded in CCl_4 soln. Analytical GLC was carried out on a GLC with FID detector fitted with three columns (each 2 m × 3 mm i.d.) of 3% PEG-6000, PEG-20M and SE-30 on Chromosorb AW (60–80 mesh), N_2 flow rate 20 ml/min, and prep. GLC with columns (each 2 m × 6 mm i.d.) of 15% PEG-6000, PEG-20M and SE-30 on Chromosorb AW (60–80 mesh) with TCD detector using a

flow rate of 30 ml/min. Mass spectra were determined at 70 eV and GC/MS were obtained at 20 eV on a single focusing instrument equipped with a Biemann–Watson He separator. CC was carried out on 70–230 mesh Si gel with monitoring by TLC. For prep. TLC Si gel (layer thickness, 0.50 mm) containing F_{254} was used. Spots and bands were detected by UV light, I_2 and spraying with 5% HNO_3–H_2SO_4.

Separation and fractionation of the essential oil. All materials used in the present experiment were collected from the region of the Naka River in Tokushima prefecture and grown in the experimental garden of the Botanical Institute at Hiroshima University. The ground parts to efflorescence of the plants were harvested in October, and then steam-distilled after air-drying for 1 day. Yields: 0.12–0.15%. Physical constants of the essential oils: *C. indicum*; $[\alpha]_D$ −6.4°, n_D 1.4841, d_4^{25} 0.8956, *C. yoshinaganthum*; $[\alpha]_D$ −31.1°, n_D 1.4889, d_4^{25} 0.9278, *C. cuneifolium*; $[\alpha]_D$ −27.1°, n_D 1.4560, d_4^{25} 0.8837. Each essential oil was fractionated by vacuum distillation into three fractions: bp 40–60°/16 mm Hg, 50–70°/3 mm Hg and 70° > 3 mm Hg. The first fraction contained monoterpene hydrocarbon mixtures. The second fraction was eluted on a Si gel column using petrol to remove sesquiterpene hydrocarbons, followed by Et_2O to remove oxygenated monoterpenoids. The mono- and sesquiterpene hydrocarbons mentioned above were separated by prep. GLC with a column of PEG-6000 at 75° and PEG-20M at 155°, respectively. Oxygenated monoterpenoids were chromatographed on a Si gel column using *n*-hexane–EtOAc (6:1) and purified by prep. GLC using PEG-20M at 125° and 150° from suitable fractions. The third fraction including oxygenated sesquiterpenoids and heavy oxygenated compounds were divided into smaller fractions on a Si gel column using a petrol–Et_2O gradient, from which the sesquiterpenoids were isolated pure by prep. GLC using a column of PEG-20M or SE-30 at 200° and PLC using petrol–Et_2O (3:1) and $CHCl_3$–Et_2O (5:1), and the acetylenic compounds were purified by PLC using C_6H_6–EtOAc (10:1).

Valeranone (**1**) [7]. MS *m/z* (rel. int.): 222 $[M]^+$, $C_{15}H_{26}O$ (42), 207 (10), 179 (21), 161 (21), 151 (20), 125 (83), 123 (40), 109 (55), 98 (100), 95 (56), 83 (29), 81 (43), 69 (52), 67 (37), 55 (43), 43 (23), 42 (64); IR ν_{max} cm^{-1}: 1700, 1383, 1372, 1365; ^1H NMR: δ 0.83 (3 H, *s*), 0.88 (6 H, *d*, *J* = 6.0 Hz), 1.02 (3 H, *s*).

Estafiatin (**2**) [9, 10]. Mp 106.0–106.5°; $[\alpha]_D$ −9.3°; MS *m/z* 246 $[M]^+$, $C_{15}H_{18}O_3$ (15), 231 (52), 228 (7), 203 (11), 175 (14), 162 (20), 152 (25), 124 (30), 105 (36), 97 (100), 95 (59), 91 (48), 69 (90), 67 (72), 55 (59), 53 (66); IR ν_{max} cm^{-1}: 1770, 1670, 1640, 905; ^1H NMR ($CDCl_3$): δ 1.61 (3 H, *s*), 3.37 (1 H, *s*), 4.07 (1 H, *dd*, *J* = 10.0, 8.5 Hz), 4.86 (1 H, *d*, *J* = 2.0 Hz), 4.96 (1 H, *d*, *J* = 2.0 Hz), 5.48 (1 H, *d*, *J* = 3.5 Hz), 6.20 (1 H, *d*, *J* = 3.5 Hz).

cis-*Spiroketalenolether polyyne* (**3**). MS *m/z*: 200 $[M]^+$, $C_{13}H_{12}O_2$ (base peak); IR ν_{max} cm^{-1}: 2230, 2125, 1630, 1580; ^1H NMR δ 1.98 (3 H, *d*, *J* = 1.0 Hz), 4.00 (2 H, *m*), 4.80 (1 H, *br s*), 6.12 (1 H, *dd*, *J* = 5.5, 1.5 Hz), 6.62 (1 H, *d*, *J* = 5.5 Hz).

trans-*Spiroketalenolether polyyne* (**4**). MS *m/z*: 200 $[M]^+$, $C_{13}H_{12}O_2$ (base peak); IR ν_{max} cm^{-1}: 2220, 2120, 1635, 1585; ^1H NMR: δ 2.00 (3 H, *d*, *J* = 1.0 Hz), 4.00 (2 H, *m*), 4.50 (1 H, *br s*), 6.10 (1 H, *dd*, *J* = 6.0, 0.5 Hz), 6.20 (1 H, *d*, *J* = 6.0 Hz).

cis-*Spiroketalenolether polyyne* (**5**). MS *m/z*: 214 $[M]^+$, $C_{14}H_{14}O_2$ (base peak); IR ν_{max}^{liquid} cm^{-1}: 2230, 2135, 1630, 1580; ^1H NMR: δ 2.00 (3 H, *d*, *J* = 1.5 Hz), 3.80 (2 H, *m*), 4.88 (1 H, *m*), 6.11 (1 H, *dd*, *J* = 6.0, 1.8 Hz), 6.63 (1 H, *d*, *J* = 6.0 Hz).

The IR and ^1H NMR spectra of the above three acetylenic compounds were in good agreement with those of reported data [11, 12] and authentic sample kindly supplied by Professor K. Yano [13].

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REFERENCES

1. Matsuo, A., Uchio, Y., Nakayama, M. and Hayashi, S. (1973) *Bull. Chem. Soc. Jpn* **46**, 1565.
2. Matsuo, A., Nakayama, M., Nakamoto, T., Uchio, Y. and Hayashi, S. (1973) *Agric. Biol. Chem.* **37**, 925.
3. Matsuo, A., Uchio, Y., Nakayama, M. and Hayashi, S. (1975) *Nippon Kagaku Kaishi* 2188.
4. Uchio, Y. (1978) *Bull. Chem. Soc. Jpn* **51**, 2342.
5. Uchio, Y. (1978) *Tetrahedron* **34**, 2893.
6. Tanaka, R. (1967) *Heredity (Tokyo)* **21**, 9.
7. Yukawa, Y. and Ito, S. (1973) *Spectral Atlas of Terpenes and the Related Compounds*. Hirokawa, Tokyo.
8. Bohlmann, F., Burkhardt, T. and Zdero, C. (1973) *Naturally Occurring Acetylenes*, p. 403. Academic Press, New York.
9. Sánchez-Viesca, F. and Romo, J. (1963) *Tetrahedron* **19**, 1285.
10. Yoshioka, H., Mabry, T. J. and Timmerman, B. N. (1973) *Sesquiterpene Lactones, NMR and Plant Distribution*, p. 219. University of Tokyo Press, Tokyo.
11. Bohlmann, F., Herbst, P., Arndt, C., Schönowsky, H. and Gleinig, H. (1961) *Chem. Ber.* **94**, 3193.
12. Bohlmann, F., Burkhardt, T. and Zdero, C. (1973) *Naturally Occurring Acetylenes*, pp. 53, 87. Academic Press, New York.
13. Yano, K., Takahashi, S. and Furukawa, T. (1972) *Phytochemistry* **11**, 2577.