近畿大農紀要 27：39～45 (1994)
Mem. Fac. Agr. Kinki Univ. 27：39～45 (1994)

Sesquiterpenoids of *Cyperus bulbosus*, *Cyperus tuberosus* and *Cyperus rotundus*

Koichiro KOMAI*, Mitsuru SHIMIZU**, C.S. TANG***,
Hikaru TSUTSUI****

Synopsys

Sesquiterpenoids in the tubers of three morphologically similar *Cyperus* species were compared. δ-Cadinene and α-copaene were the most abundant sesquiterpenoids in *C. tubersus*. In *C. bulbosus*, caryophyllene oxide and humulene oxide were the main sesquiterpenes. These chemotypes were different from the chemotype of *C. rotundus* (purple nutsedge), so chemotaxonomy can be used for the identification of these species. The biochemical differences of these species in relation to the environment are discussed.

Introduction

On the basis of the sesquiterpenoid composition of the essential oil of fresh mature tubers of purple nutsedge (*C. rotundus*), chemotypes H, M, and O have been identified[1]. The two most abundant sesquiterpenoids of type H are bicyclic (α-cyperone and β-selinene), and all of the nutsedge speciments collected in the Honshu rigion are of this type. Type H has been found in the Okinawa, Kyushu, and Shikoku, districts, as well. Type O plants have two abundant tricyclic sesquiterpenes (cyperotundone and cyperene), and plants of this chemotype has not been found as far north as Tanega Island. Type M contained all four of these sesquiterpenes, and this type has been found in southern Kyushu and Okinawa. Type K, with a large amount of sesquiterpene acetate, is the most common chemotype of purple nutsedge in Hawaii[2]. These sesquiterpenes are of interest because of their potential allelopathic activity[3]. The different sesquiterpenoid profiles of the plant may be useful in chemotaxonomy.

In 1991, details of the geographic distribution of purple nutsedge chemotypes in the Pacific Rim and Basin were published[3]. In that study, clones of *Cyperus bulbosus* and *Cyperus tuberosus* were collected. Several *Cyperus* species, including *C. tuberosus* and *C. bulbosus* have been missidentified as purple nutsedge[8], and a chemotaxonomic method may therefore be needed. In this study, we examined the sesquiterpene profiles of *C. tuberosus* and *C. bulbosus*, together with two chemotypes of purple nutsedge. The results allowed us to speculate about the biochemical pathways of these secondary metabolites.

* Lab. of Pesticide Chemistry, Dept. of Agricultural Chemistry, Kinki Univ., Nara, 631, Japan. (農芸化学科, 農薬化学研究室)
** Wakayama Agribio Reseach Center, Tukatuki Momoyama, Wakayama, 649-61 Japan. (和歌山アグリバイオ研究センター)
*** Dept. of Enviromental Biochem., Univ. of Hawaii, Honolulu, Hawaii, 96822, U.S.A.
**** Lab. of International Agricultural Development, Fac. of Agriculture, Kinki Univ., Nara, 631, Japan. (国際資源管理学科国際農業開発研究室)

Materials and Methods

Mature tubers of chemotypes H and K of purple nutsedge were collected from Miyazaki Japan and Oahu island, Hawaii, respectively. *C. tuberosus* and *C. bulbosus* tubers were collected from Bangkok and Chaing Mai in Thailand. Tubers were planted in pots and plants were grown for 3 month in greenhouse. The foliar parts and inflorescence were used for species identification. Essential oils of each species were prepared by homogenization of tubers with hexane. The extracts were filtered and concentrated under a stream of N_2. Three sesquiterpenoids, sugetriol triacetate, caryophyllene oxide and humulene oxide, were isolated and purified and purified by repeated column, chromatograph, gas chromatography and mass spectrometer (GC-MS), infrared spectrophotometer (IR), and nuclear magnetic resonance spectrometer (^{13}C-NMR). Data from GC-MS alone were insufficient for the identification of the sesquiterpenes.

The essential oil of purple nutsedge was chromatographed on activated alumina with benzene as the elutent. Following ketone fractionation, acetate fractions were eluted, combined and rechromatographed on silica gel. A crystallized substance was obtained after evaporation of the benzene, and sugetriol triacetate[4] was crystallized from light petroleum as colorless needles, mp 132°C. MS fragments, m/z : 378(M^+), 318(M-AcOH), 276, 258(M-2AcOH), 216, 198(M-3AcOH), 183, 173. IR(KBr)cm^- : 1736, 1235(acetoxyl).

The essential oil of *C. bulbosus* was chromatographed on silica gel. After percolation of the hydrocarbon fraction with chloroform, repeated elution with the same solvent gave epoxide fractions which were combined and rechromatographed on silica gel with hexane as the elutent. Caryophyllene oxide was obtained first, followed by humulene oxide. Physical properties were : caryophyllene oxide[5] : mp 64°C ; IR(KBr)cm^- : 2960(-CH_3), 1450(>C=C<), 1261, 915, 873, 855, 765(-O-). GC-MS m/z : 220(M^+), 161, 138, 121, 107. ^{13}C-NMR in $CDCl_3$ (δ ppm) : 17.0, 24.7, 27.3, 29.9, 30.5, 34.0, 39.2, 39.8, 48.8, 50.7, 57.8, 63.7, 80.4, 112.7, 151.4. Humulene-1,2-epoxide[6,7] : GC-MS m/z : 220(M^+), 138, 123, 109. IR (liq. film) cm^- : 2960(-CH_3), 1450, 1380, 1360(gem-dimethyl), 1235, 972, 915, 875, 822, 785(-O-). ^{13}C-NMR in $CDCl_3$(δ ppm) : 15.1, 17.3, 24.8, 25.6, 27.0, 36.5, 36.6, 40.3, 42.6, 62.0, 63.2, 122.1, 125.7, 131.7, 143.1.

GC was done with a gas chromatograph (Spectra-Physics SP-7100 gas) equipped with a flame ionization detector and DB-5 fused silica capillary column (20 m×0.25 mm ID). The oven temperature was programmed to increase from 100 to 250°C at 4°C/min. For GC-MS, a model HP-5730 apparatus for GC-MS was used. The conditions for GC in GC-MS were similar to those for GC alone. For MS, voltage of 70 eV and electron energy of 300 mA were used. The ion source temperature was 200°C.

IR spectra were obtained with an IR spectrophotometer (model IR-435, Shimazu). Nuclear magnetic resonance spectra were obtained with a Fourier transform NMR spectrometer (model 60Q, Jeol) Fourier in $CDCl_3$ with Me_4 Si as an internal standard.

Results and Discussion

The morphological characteristics of the three species studied are list in Table 1. When grown under the same conditions, these *Cyperus* species seemed morphologically distinct. Purple nutsedge was distinguishable from *C. Tuberosus* by plant hight, infloresence type, glume color and number of lateral veins, and from *C. bulbosus* by plant height, inflorescence type, tuber form and glume color. Parker[8] point out that these speceis are often misidentified when only morphological features are used. Cooke[9] indicated that *C. tuberosus* is not distinguishable from purple nutsedge by any of its characteristic, and *C. tuberosus* has often been referred as a

Table 1. Morphologicac characteristics of *Cyperus rotundus*, *C. tuberosus* and *C. bulbosus* grown in greenhouse

	C. rotundus	*C. tuberosus*	*C. bulbosus*
Tuber	Blackish Globose ovoid 0.30 g/tuber	Bright brown Corm like 0.25 g/tuber	Blackish Bulb like 0.84 g/tuber
Stem height (cm)	35	50	55
Inflorescence	Compound 8-15 cm Top : ≥25 cm	Simple Imperfect Top : ≥10 cm	Simple Imperfect Top : ≥10 cm
Spikelets	2-3 mm wide 2-3 mm long	2 mm wide 2-3 mm long	2 mm wide 1-2 mm long
Glumes	Purple Ovate to elliptic 3 mm long	Brown Ovate to oval 12-20 mm long	Green Ovate to oblong 2-4 mm long
Lateral veins	2 or 3	4 or 5	2 or 3

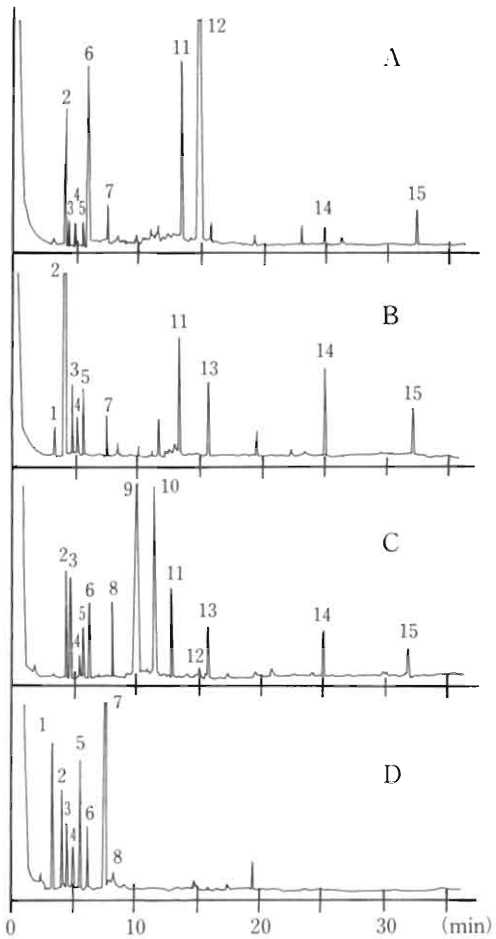

Fig. 1. Gas chromatograms of essential oils from mature tubers of Cyperus species.
See Table 2 for names of compounds assigned to the numbered peak. A: Type M *C. rotundus*; B: Type K *C. rotundus*; C: *C. bulbosus*; D: *C. tuberosus*.

Table 2. Comparison of sesquiterpenoids in essential oil from tuber of *Cyperus rotunds*, *C. tuberosus*, and *C. bulbosus*

Peak no.*	Sesquiterpenoid	% of total peak area				Methods**
		I	II	III	IV	
1	α-Copaene	t	0.7	t	18.2	1, 2
2	Cyperene	8.0	31.3	5.8	7.1	1, 2, 3
3	β-Elemene	2.5	5.2	4.7	5.0	1, 2
4	β-Caryophylline	3.0	3.8	1.5	3.8	1, 2, 3
5	α-Humulene	2.2	4.1	2.0	10.5	1, 2, 3
6	β-Selinene	16.0	n	2.6	5.2	1, 2, 3
7	δ-Cadinene	3.1	3.0	t	30.4	1, 2, 3
8	Calamenene	t	1.5	3.5	0.8	1, 2
9	Caryophyllene oxide	n	n	26.3	n	1, 2, 4, 5
10	Humulene oxide	n	n	24.1	n	1, 2, 4, 5
11	Cyperotundone	19.5	12.0	5.6	n	1, 2, 3
12	α-Cyperone	31.4	n	0.7	t	1, 2, 3
13	Patchoulenyl acetate	t	9.1	5.4	n	1, 2, 3
14	Sugeonyl acetate	0.5	8.5	3.3	n	1, 2, 3
15	Sugetriol triacetate	2.5	4.5	1.5	n	1, 2, 4, 5

I=type M *C. rotundus*; II=type K *C. rotundus*; III=*C. bulbosus*; IV=*C. tuberosus*.

* Order based on GC retention time. See Fig. 1 for chromatograms and Fig. 2 for chemical structures.

** Methods used for identification: 1=retention time on GC; 2=GC-MS; 3=co-chromatography; 4=IR; 5=^{13}C NMR

t: Trace amound; n: Not detected

subspecies of purple nutsedge[10]. However, *C. tuberosus* was added as a separate species to the flora of the Ryukyus by S. Tawada[10]. *C. bulbosus* may be easily confused with purple nutsedge, especially in the absence of their characteristic tubers, which are not included in herbarium specimens. The two species can be further distinguished by their inflorescence, bracts, and glumes[11]. Unfortunately, these organs sometimes do not develop if environmental factors such as soil temperature, nutrients, moisture, and day length are unsuitable.

The essential oil of *C. bulbosus* has a distinctive aroma different from that of purple nutsedge or *C. tuberosus*. Figure 1 and Table 2 summarize the qualitative and quantitative data obtained from the hexane extracts. The differences in sesquiterpenoid composition offer a reliable method for identification by chemotaxonomy. The composition of the essential oil of *C. tuberosus* was simple, and the main sesquiiterpene hydrocarbons were α-copaene (18.2%) and δ-cadinene (30.4%). Oxygenated sesquiterpenes were not abundant; only a trace amount of α-cyperone was detected. *C. bulbosus* contained mainly caryophyllene oxide (26.3%) and humulene oxide (24.1%). There were similarities in the sesquiterpenoid composition of *C. bulbosus* and type M purple nutsedge. The major difference was the absence of sesquiterpene oxides in type M purple nutsedge.

We have reported elsewhere that the essential oil from six wetland or paddy *Cyperus* species, *Cyperus difformis* L., *Cyperus haspan* L., *Cyperua polystachyos* Rottb., *Cyperus globosus* Aublet, *Cyperus sanginolentus* Vahl., and *Cyperus brevifolius* Hask., were rich in wax. We later compared the essential oil of *Cyperus previfolius* with that *Cyperus kyllingia* Endl., a morphologically similar dryland species[12]. The latter had a large amount of terpenoid, but little wax, and we speculated

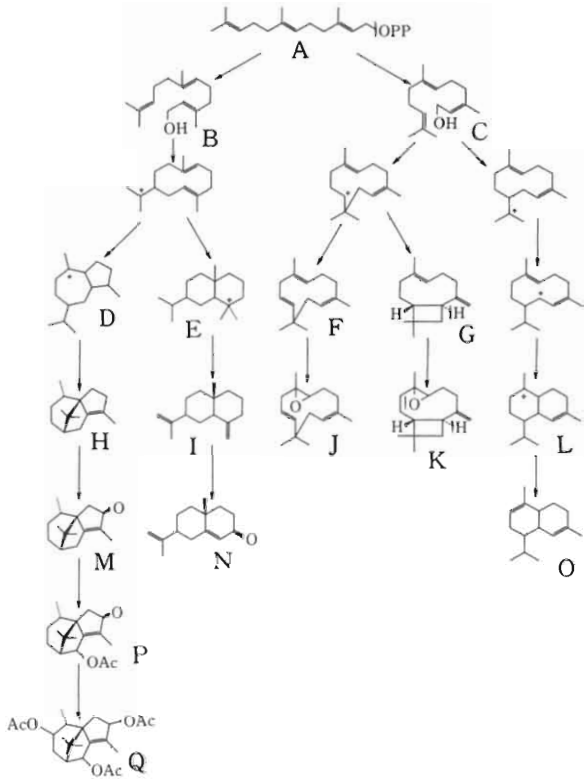

Fig. 2. Proposed biochemical pathway for the formation of sesquiterpenoids in *C. rotundus*, *C. bulbosus*, and *C. tuberosus*.
A=farnesol pyrophosphate; B=t,t-farnesol; C=t,c-farnesol; D=guaiane; E=eudesmane; F=α-humulene; G=β-caryophyllene; H=cyperene; I=β-selinene; J=humulene-1,2-oxide; K=caryophyllene-4,5-epoxide; L=cadinane; M=cyperotundone; N=α-cyperone; O=δ-cadinene; P=sugeonyl aceate; Q=sugetriol triacetate

that this difference was the result of adaptation to the different environments. In the present study, wax was not detected in *Cyperus bulbosus* which is generaiiy thought of a perennial weed in rice paddy fields in tropical regions. This lack suggests that although the wetland *Cyperus* species have a high wax content in general, this characteristic is not a necessary for survival. The large amount of oxygenated sesquiterpenoid in tubers of *Cyperus bulbosus* would contribute much to its allelopathic properties; since oxygenated sesquiterpenoids are more phytotoxic than non-oxygenated ones[3]. The abundance of oxygenated terpenes in *Cyperus bulbosus*, however, was an unexpected property since paddy conditions are anaerobic. therefore our biochemical observations in relation to environmental effects and adaptation are somewhat contradictory.

In addition to patchoulenyl acetate and sugeonyl acetate identified earlier in type K purple nutsedge[3], sugeonyl triacetate was isolated and identified. The triacetate is a terminal product of consecutive oxidation and acetylation through cyperene, cyperotundone, and sugeonyl acetate (Fig. 2). As expected, these acetates were absent in *Cyperus tuberosus* due to the lack of two of these precursors, but were found in small amount in type M purple nutsedge and in *Cyperus bulbosus*.

Refferences

1) K. KOMAI, K. OSAKI and K. UEKI : *Weed Sci.*, Japan, **23**, 10-14 (1978)
2) K. KOMAI and C.S. TANG : *Phytochem.*, **28**, 1883-1886 (1989)
3) K. KOMAI, C.S. TANG and R.K. NISHIMOTO : *J. Chem. Ecol.* **17**, 1-8 (1991)
4) H. HIKINO, K. AOTA and T. TAKEMOTO : *Chem. Pharm. Bull.*, **16**, 1900-1906 (1968)
5) N.P. DAMADARAN and S. DEV : *Tetrahedron*, **24**, 4113-4122 (1968)
6) N.P. DAMADARAN and S. DEV : *ibid.*, **24**, 4123-4132 (1968)
7) S.K. RAMASWAMI and S.C. BHATTACHARYYA : *ibid.*, **18**, 575-579 (1962)
8) C. PARKER : *Pans.*, **18**, 477-482 (1972)
9) T. COOKE : in "The Flora of The Presidency of Bombay" Vol. 2, 871-872 (1908)
10) T. KOYAMA : Classification of the family cyeraceae. Quart J. Taiwan Museum. Vol. 14, 179-171 (1961)
11) C.A. BSCKER and R.C. BAKHUIZEN : in "Flora of Java Vol. 3, Angiospermae Families, 191-238, Wolters-Noordhoff N.V. Groningen, The Netherland (1975)
12) K. KOMAI and C.S. TANG : *J. Chem. Ecol.*, **15**, 2171-2176 (1989)

Cyperus bolbosus, *Cyperus tuberosus* および *Cyperus rotundus* のセスキテルペノイド

駒井功一郎・冷水　充・C.S. TANG・筒井　暉

摘　　要

酷似した形態をもつカヤツリグサ属植物3種の塊茎中のセスキテルペノイドについて比較検討した。*C. tuberosus* には主成分として δ-cadinene と α-copaene を含んでいた。*C. bulbosus* では caryophyllene oxide と humulene oxide が主成分であった。これらのセスキテルペノイドの組成は，*C rotundus* の各 chemo-type のセスキテルペン組成とは異っており，これらの種における化学分類学の一手段になると考えられる。更にこれら草種の生化学的諸特性の差異と環境との関連性についても考察した。