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A CHEMOTYPE OF *CYPERUS ROTUNDUS* IN HAWAII

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Key Word Index—*Cyperus rotundus*; Cyperaceae; chemotypes; ecotypes; sesquiterpenes; essential oils; capillary GC-MS.

Abstract—A new chemotype of *Cyperus rotundus* was found in Hawaii based on the sesquiterpene composition of the mature tubers. The K-type has higher concentrations of patchoulenyl acetate and sugeonyl acetate than the three known Asian chemotypes. Biochemical and evolutionary aspects of these findings are discussed.

INTRODUCTION

Purple nutsedge (*Cyperus rotundus*), also known as nutsedge or nutgrass, is a weed native to India. It is difficult to control by either hand weeding or herbicides [1], and it has been considered as the worst weed in the world [2, 3]. The problem is most persistent in the tropical and subtropical regions. In the temperate zone *C. rotundus* has been less successful mainly because of low winter temperatures [4]. *Cyperus rotundus* is adapted to a wide range of soil conditions, which represent different soil types, moisture, pH, temperature, and microbial populations. Its ability to adapt suggests the existence of ecotypes [5].

As early as 1925, Ranade and Burns [6] in India described and distinguished the ecotypes of *C. rotundus* by variations in glume colour. Later, Claver [7] recognized different types in Argentina by their growth and development patterns. More recently, Komai *et al.* reported the presence of geographical variation in sesquiterpene components of the essential oils from fresh, mature tubers [8]. Different chemotypes have been collected and characterized in Japan, Southern China, Taiwan, the Philippines and Vietnam [9].

The present study shows that in Hawaiian Islands, a new chemotype characterizes the *C. rotundus* population. The accumulation of data on chemical variation as it relates to geological distribution will be helpful in understanding the principle of biochemical adaptation under various environmental conditions. Information on the distribution of chemotypes could also offer clues to the history of spreading of this important weed species.

RESULTS AND DISCUSSION

Banthorpe *et al.* [10] proposed that the major components of an essential oil are the most appropriate compounds in the chemotaxonomical characterization of higher plants because they are the likely end products of biosynthetic pathways. In the case of *C. rotundus*, tuber sesquiterpenes have been successfully used to distinguish chemotypes from different parts of Asia [9]. Three chemotypes of nutsedge have been reported, the H-type in Japan, the M-type in China, Hong Kong, Japan, Taiwan, and Vietnam, and the O-type in Japan, Taiwan, Thailand and the Philippines.

In the present work, analysis of the major group of clones collected in Hawaiian Islands indicated that they belonged to a new chemotype since distinctive chemical differences existed when they were compared with those of Asian *C. rotundus* (Fig. 1). This new, Hawaiian chemotype was designated the K-type because the first GC evidence was obtained from a Kauian clone. Out of the 29 clones randomly collected from the islands of Oahu, Hawaii, Maui, and Kauai, 19 were of the K-type, five were similar to the O-type widely distributed in Asia, and five collected from the Kalapana Black Sand Beach area on the Island of Hawaii, unexpectedly, were similar to the H-type, which was previously found only in Japan [9].

Distribution of the 13 sesquiterpenes in the different chemotypes of nutsedge are listed in Table 1. The O-type found in Hawaii has minor differences in comparison to those found in Asia. The former has a higher content of the oxygenated sesquiterpene, cyperotundone (13% vs 25%). Similarly, the H-type found in the Black Sand Beach area on the Island of Hawaii also showed minor quantitative differences when compared with the Japanese H-type. These differences, however, were not related to immediate environmental factors because both Japanese O-type and H-type clones grown in Hawaii retained their original tuber sesquiterpene distributions.

The O- and K-type differ from the H- and M-type in their high content of cyperene (20–30% vs 0–7%) and the lack of β-selinene, α-cyperone and cyperol [9]. However, the K-type is unique in its marked enhancement in contents of patchoulenyl acetate and sugeonyl acetate, which were not detected or present only as traces in other chemotypes (Fig. 1). An overall comparison leads to the conclusion that the K-type is closely related to the O-type. A possible biochemical pathway for sesquiterpene synthesis in *Cyperus* spp. has been proposed by Komai *et al.* [9]. In both the O- and K-types, the lack of α-cyperone, cyperol and β-selinene indicates the absence of bicyclic compounds derived from germacrane. Instead, a

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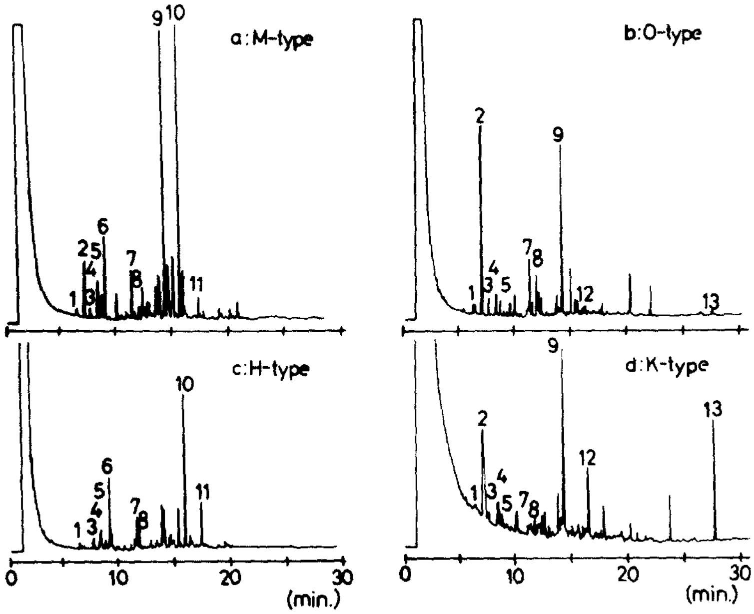

Fig. 1. Capillary gas chromatograms of essential oils in the *n*-hexane extract of *Cyperus rotundus* tubers. See Table 2 for the name of compound assigned to each number.

Table 1. Comparison of essential oils from different chemotypes of *C. rotundus*; distribution of the major sesquiterpenoids in tubers

Peak no.*	Sesquiterpenoid	% of total peak area†				
		I	II	III	IV	V
1	α-Copaene	t	2.0	t	3.6	t
2	Cyperene	n	30.8	7.2	28.7	20.7
3	β-Elemene	0.8	5.2	2.5	6.5	2.6
4	Caryophyllene	6.2	3.8	3.3	5.0	3.2
5	α-Humulene	4.5	4.0	1.7	3.9	4.7
6	β-Selinene	18.5	t	17.8	n	n
7	δ-Cadinene	1.0	3.5	2.0	3.8	3.4
8	Calamenene	3.9	2.6	t	1.5	1.7
9	Cyperotundone	n	13.1	19.4	8.8	25.0
10	α-Cyperone‡	38.6	n	30.7	n	n
11	Cyperol	7.4	n	5.6	n	n
12	Petchoulenyl acetate	n	t	t	8.0	t
13	Sugeonyl acetate	n	t	t	6.9	t

*Sequence based on R_t, see Fig. 1 for chromatograms and Fig. 2 for chemical structures. Data taken from representative samples.

†I: H-type, II: O-type, III: M-type, IV: K-type, V: Hawaiian O-type. n = none detected; t = trace.

‡A minor peak following α-cyperone was identified by GC-MS as patchoulenone in samples I and III.

pathway from guaiene leading to tricyclic sesquiterpenes is shared by these two chemotypes.

Dominance of the new K-type in the geologically segregated Hawaiian Islands suggests that it has evolved biochemically from the O-type since both patchoulenyl acetate and sugeonyl acetate require additional enzymes for hydroxylation and acetylation in their biosynthesis. Figure 2 is a proposed scheme of the biosynthetic pathway. The environmental factors and the number of generations required for these evolutionary changes, however, are subjects for future research. On the other hand, the unexpected finding of H-type clones in the Black Sand Beach area, a region covered with more recent volcanic soils and frequented by tourists, suggests

Table 2. Spectral data of the major sesquiterpenoids in the *n*-hexane extract of *C. rotundus* tubers

Compound	*m/z* at 70 eV	IR(cm^{-1})	UV (λ_{max}) nm*
Cyperene	204 ([M]$^+$, 100), 189, 175, 161, 147, 119, 91, 41	1380, methylene	—
β-Selinene	204 ([M]$^+$, 100), 189, 175, 161, 147, 133, 119, 105	3040, 1650, 890, vinyliden olefin, C=C, C-H	—
Cyperotundone	218 ([M]$^+$, 100), 203, 175, 161, 147, 133, 119	1710, 1670, C=O, cyclopentenone	244.8
α-Cyperone	218 ([M]$^+$, 100), 203, 175, 161, 147, 136, 121, 91	3080, 1620, 890, vinyliden olefin, C=C, C-H. 1670; *α,β*-unsaturated ketone	248.0
Patchoulenyl acetate†	262 ([M]$^+$, 42), 231 [M − MeO]$^+$ 202 [M − MeCOOH]$^+$ 187, 161, 159, 147, 131, 119, 43 (100)	1733, 1243; acetoxyl 1704, 1671; cyclopentenone	246.0
Sugeonylacetate	276 ([M]$^+$, 15), + 233 [M − MeO]$^+$ 216 ([M − MeCOOH]$^+$ 47), 198 (100), 183, 159, 157, 43 (MeCO, 32)	1733, 1236; acetoxyl 1704, 1670; cyclopentene (KBr disc)	246.0

*In EtOH.

†~95% purity as indicated by GC.

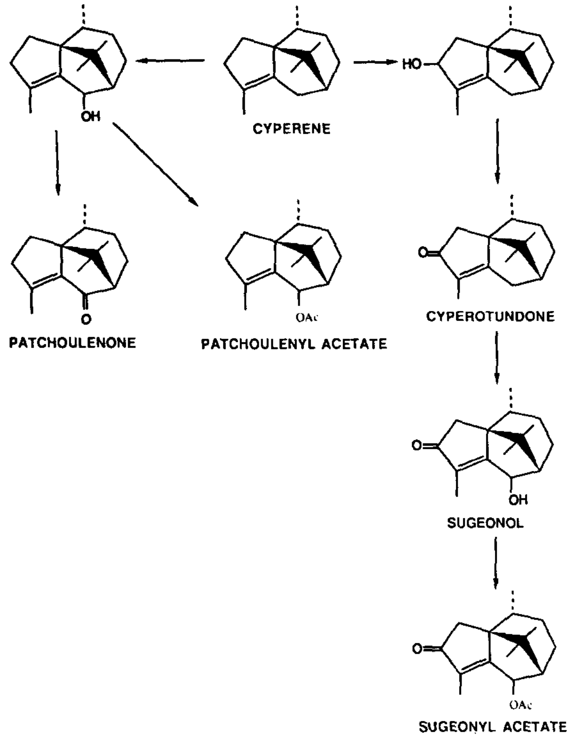

Fig. 2. Proposed biochemical pathway for the formation of sesquiterpenoids in the K-type *C. rotundus*.

that the Hawaiian H-type is a more recent introduction. Thus, the minor chemical differences between the Japanese and the Hawaiian H-types may have occurred in relatively short time. More detailed investigation is needed to verify this assumption.

Because of its world-wide distribution, the relatively simple and reproducible methods of analysis, and a collection of data on chemotyping already in existence, *C. rotundus* may serve as a model for the better understanding of the chemical evolution of weeds.

EXPERIMENTAL

Mature tubers of 24 clones of *Cyperus rotundus* L. were collected between September and December of 1987 from the Islands of Oahu, Maui, Kauai and Hawaii. Five additional clones were collected from the Black Sand Beach area in Kalapana, on the Island of Hawaii, March, 1988. Samples were prepared by homogenizing the fresh tubers in *n*-hexane using a mortar and pestle. The extract was filtered, concd under a stream of N_2, and used for instrumental analyses. GC was performed on a FID instrument using a 20 m × 0.25 mm id., DB-5 fused silica capillary column (J&W Scientific). He was used as carrier and the oven temp. was prog. from 90 to 250° at 4°/min. For GC-MS analysis conditions similar to those of the GC analysis on SP-7100 were used. MS were obtained at 70 eV and 300 mA; the ion-source temp was 200°.

Compounds were identified by comparison of GC R_t and MS with those of authentic compounds. Individual sesquiterpenes were prepd from 1 kg of mature tubers collected from a site on Oahu, where only the K-type *C. rotundus* was found. Compounds were isolated by CC on silica gel (10–40 μm, EM Laboratory, Darmstadt) using consecutive solvent elution. *n*-Hexane gave 8 non-oxygenated sesquiterpenes. Fractions 9, 10 and 11 were eluted with C_6H_6 and 12 and 13 by EtOH; the latter were oxygenated sesquiterpenes. Further purification was carried out by repeated CC on silica gel. Two crystalline compounds were obtained from the oxygenated fractions by repeated recrystallization from C_6H_6. Identification of these two compounds as patchoulenyl acetate and sugeonyl acetate were based on the comparison of their MS and IR spectra with published data [11–15].

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