Differences in Essential Oil Composition of Basil (*Ocimum basilicum* L.) Italian Cultivars Related to Morphological Characteristics

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Ten Italian cultivars of basil were studied to establish a possible relation between morphological characteristics and essential oil composition. The morphological parameters were recorded at the beginning of the flowering stage and the essential oils, obtained by hydrodistillation, were analyzed by gas chromatography (GC) and GC/mass spectrometry (GC/MS). Among the cultivars, four phenotypes were distinguished on the basis of leaf size, shape, and color and plant height, weight, branching, and leafing. The composition of essential oils, all characterized by a high content of linalool, included three chemotypes: "linalool," "linalool and methylchavicol," and "linalool and eugenol". Two chemotypes each had their own suite of morphological characters, whereas two groups of cultivars, with different morphological parameters belonged to the same chemotype.

Keywords: *Basil cultivars; Ocimum basilicum L.; morphological characteristics; essential oil composition; chemotypes*

INTRODUCTION

The *Ocimum* genus belonging to the Lamiaceae family is characterized by a great variability of both morphology and chemotypes (Lawrence, 1988). The ease of cross-pollination leads to a large number of subspecies, varieties, and forms (Guenther, 1949). Among all the species, *Ocimum basilicum* (basil or sweet basil) has the most economic importance and is cultivated and utilized throughout the world. The aromatic leaves are used fresh or dried as a flavoring agent for foods, confectionery products, and beverages. Traditionally, the plant has been employed in folk medicine for its carminative, stimulant, and antispasmodic properties. The essential oil, mainly used in food industries and perfumery, also possesses antimicrobial activity (Prasad et al., 1985), and some of its components, such as 1,8-cineole, linalool, and camphor, are known to be biologically active (Morris et al., 1979). Camphor and 1,8-cineole seem also to be involved as agents in allelopatic reactions (Rice, 1979).

Basil oils have marked differences in composition, and some chemotypes from different geographical origins have been classified: the European chemotype, from Italy, France, Bulgaria, Egypt, and South Africa, is considered to have the finest flavor, and has linalool and methylchavicol as main components; the Reunion chemotype, from the Comoro Islands, Thailand, Madagascar, and Vietnam, is characterized by high concentrations of methylchavicol; the tropical chemotype, from India, Guatemala, and Pakistan, is rich in methyl cinnamate; and a chemotype from North Africa and the former USSR is rich in eugenol (Vernin, 1984). Morphological differences are evidenced in plant height, leaf color, leaf dimension, and leaf smoothness. Moreover, the aromatic and morphological characters, determined by genotype, are greatly influenced by environmental conditions and agronomic techniques (Piccaglia et al., 1991; Marotti et al., 1992).

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Table 1. List of Basil Cultivars

no.	cultivar	no.	cultivar
1	Blistered Lettuce Leaf	6	Giant Violet Leaf
2	Dwarf Violet	7	Lettuce Leaf
3	Genovese	8	Little Green
4	Genovese sel. Sanremo	9	Little Green Compact
5	Giant Genovese	10	Napoletano

The aim of this work was to evaluate the morphological and aromatic characteristics of 10 basil cultivars available in the Italian market to add to the knowledge of our local product and stimulate a more extensive use of Italian basil.

MATERIALS AND METHODS

Plant Material. Seeds of 10 cultivars of *Ocimum basilicum* L. (Table 1) collected from six different Italian suppliers were sown and grown in a greenhouse for 3 weeks. In May 1992, the plants were transplanted into a field in a randomized complete block design with four replications. The harvest of the whole plants was performed at the beginning of the flowering stage (in the first 10 days of August), and morphological characteristics and yield parameters were evaluated for each basil cultivar, a representative sample obtained from the four replications was used for essential oil extraction and analysis.

Essential Oil Extraction. Samples of fresh plant material were hydrodistilled (1 kg of material and 10 L of water) in a Clevenger-type apparatus for 2 h. The essential oils were dried over anhydrous sodium sulfate, stored in a dark glass bottle, and kept at 4 °C until analysis.

GC and GC/MS Analysis. The GC analyses were performed with a Carlo Erba HRGC 5160 Mega gas chromatograph equipped with a flame ionization detector (FID) and an Hitachi 2000 integrator. A SPB-5 fused silica capillary column (Supelco) (30 m × 0.32 mm i.d.; film thickness, 0.25 μm) was employed. An on-column injection was utilized, and the oven temperature was programmed from 60 to 200 °C at 3 °C/min, and the final temperature was held for 10 min. The detector temperature was 250 °C, and the carrier gas (helium) had a flow rate of 1 mL/min.

The MS analyses were run on a Finnigan Mat Ion Trap detector (model 800) set at 70 eV and equipped with software version 3.0. The chromatographic conditions adopted for the GC/MS analyses were the same as already described for the analytical GC. The identification of components was based

S0021-8561(96)00106-9 CCC: $12.00

Table 2. Morphological and Yield Parameters

no.	leaf size	leaf shape	leaf margin	leaf color	plant height (cm)	plant weight[a] (g)	plant branches (no.)	leaf mean weight[a] (g)	leaves/ plant (%)	oil content[a] (% v/w)
1	large	ovate	serrate	green	39.6	173.2	15.9	0.65	61.5	0.6
2	medium	ovate	undulate	dark violet	31.3	71.6	13.1	0.27	70.2	0.8
3	medium-large	ovate	entire	pale green	51.1	153.9	17.4	0.43	60.1	0.6
4	medium-large	ovate	serrate	pale green	44.4	136.2	17.2	0.49	65.0	0.5
5	medium-large	ovate	serrate	pale green	46.3	145.5	17.7	0.45	63.3	0.6
6	medium	ovate	undulate	scarlet red	33.0	87.7	13.2	0.26	71.3	0.6
7	large	ovate	serrate	pale green	36.6	140.5	13.8	0.51	61.6	0.4
8	small	lanceolate	entire	pale green	37.7	125.5	15.7	0.05	56.9	0.3
9	small	lanceolate	serrate	pale green	40.2	129.4	12.1	0.07	58.2	0.4
10	large	ovate-roundish	undulate	dark green	33.9	129.7	17.2	0.52	64.8	0.7
LSD[b] ($p \leq 0.05$)					5.9	49.4	1.3	0.14	7.4	
LSD[b] ($p \leq 0.01$)					7.9	66.5	1.7	0.16	9.9	

[a] On fresh weight basis. [b] Least significant differences.

Table 3. Chemical Composition (%) of Basil Cultivar Essential Oils

no.	compound	cultivar no. 1	2	3	4	5	6	7	8	9	10	identification method[a]
1	α-pinene	0.06	0.38	0.16	0.05	0.18	0.37	0.14	0.15	nd[b]	tr[c]	RT GC MS
2	camphene	tr	0.09	0.40	tr	0.04	0.75	tr	0.03	nd	0.09	RT GC MS
3	sabinene	0.09	0.37	0.25	0.10	0.26	0.38	0.15	0.10	nd	0.09	RT GC MS
4	*β*-pinene	0.22	0.86	0.58	0.24	0.67	0.87	0.37	0.28	nd	2.00	RT GC MS
5	myrcene	0.21	0.91	0.40	tr	0.49	0.94	0.23	0.19	nd	tr	RT GC MS
6	α-terpinene	nd	0.05	tr	tr	tr	0.05	tr	tr	nd	tr	RT GC MS
7	*p*-cymene	nd	tr	tr	tr	tr	tr	tr	tr	nd	tr	RT*GC MS
8	limonene	tr	0.43	0.23	tr	0.27	0.58	0.16	0.13	tr	nd	RT GC MS
9	1,8-cineole	5.85	12.25	11.45	8.04	9.90	12.91	6.10	5.00	0.94	2.00	RT GC MS
10	*cis*-ocimene	tr	tr	tr	tr	tr	tr	tr	tr	nd	tr	RT* MS
11	*trans*-ocimene	0.27	tr	1.02	0.44	0.83	tr	0.56	0.69	0.13	nd	RT* MS
12	*γ*-terpinene	tr	0.06	0.05	tr	0.06	0.08	0.06	0.18	nd	0.06	RT GC MS
13	menth-2-en-1-ol	0.19	0.27	0.32	0.25	0.25	0.30	0.33	0.41	0.47	0.11	RT* MS
14	terpinolene	0.09	1.34	0.25	0.08	0.12	1.47	0.61	0.16	tr	tr	RT GC MS
15	linalool	47.85	69.40	60.76	69.06	64.14	69.86	59.54	76.20	69.41	41.17	RT GC MS
16	fenchol	nd	0.30	tr	tr	nd	0.30	nd	nd	nd	0.30	RT GC MS
17	camphor	0.32	0.83	0.80	0.34	0.38	0.77	0.21	0.54	0.39	0.10	RT GC MS
18	borneol	0.47	0.95	0.79	0.83	1.00	1.00	1.21	0.85	1.12	0.21	RT GC MS
19	4-terpineol	tr	0.11	0.72	tr	0.75	tr	1.20	0.59	3.14	tr	RT GC MS
20	α-terpineol	0.61	0.67	1.14	1.00	0.92	0.69	0.42	0.36	0.56	0.23	RT GC MS
21	methylchavicol	32.21	nd	nd	nd	nd	nd	18.01	nd	nd	41.40	RT GC MS
22	fenchyl acetate	nd	0.56	0.03	nd	nd	0.49	nd	nd	nd	nd	RT GC MS
23	bornyl acetate	0.55	0.23	0.63	1.02	0.92	0.20	0.29	1.03	0.99	0.18	RT GC MS
24	*δ*-elemene	tr	nd	tr	tr	tr	nd	nd	nd	tr	nd	RT* MS
25	eugenol	nd	nd	2.22	tr	3.89	nd	nd	1.16	1.99	nd	RT GC MS
26	α-copaene	0.17	0.04	0.04	0.04	0.04	nd	nd	tr	0.16	0.04	RT* MS
27	*β*-elemene	0.12	0.40	0.37	0.41	0.30	0.35	0.10	0.15	0.66	0.29	RT* MS
28	α-*cis*-bergamotene	tr	nd	0.03	tr	tr	nd	nd	tr	0.16	nd	RT* MS
29	caryophyllene	0.09	0.80	0.30	0.20	0.10	0.70	0.18	0.10	0.23	0.10	RT GC MS
30	α-*trans*-bergamotene	1.68	nd	3.37	2.86	3.20	nd	1.03	0.95	1.33	1.94	RT* MS
31	α-guaiene	tr	nd	0.01	tr	0.09	nd	nd	tr	0.27	nd	RT* MS
32	α-cadinene	0.06	nd	0.06	0.12	0.05	nd	0.05	0.05	0.36	nd	RT* MS
33	α-humulene	0.38	0.27	0.58	0.46	0.41	0.30	0.40	0.26	0.82	0.50	RT GC MS
34	*γ*-muurolene	0.15	0.09	0.21	0.21	0.14	0.04	0.11	0.07	0.43	0.18	RT MS
35	germacrene D	1.21	1.25	2.11	1.17	1.40	0.84	1.17	0.72	1.80	1.72	RT* MS
36	germacrene B	0.77	0.89	1.30	1.12	0.81	0.85	0.70	0.50	1.18	0.96	RT* MS
37	α-farnesene	0.26	0.80	0.70	0.91	0.09	0.58	0.41	0.43	0.86	0.46	RT* MS
38	*γ*-cadinene	0.93	0.51	1.30	1.37	1.04	0.38	0.79	0.48	1.12	1.19	RT* MS
39	calamenene	0.12	0.09	0.21	0.18	0.19	0.04	0.80	0.07	0.15	0.14	RT* MS
40	*τ*-cadinol	3.96	2.06	5.38	6.63	5.17	1.76	3.82	3.90	7.55	5.12	RT* MS

[a] RT, comparison with pure standard retention time; GC, gas chromatographic coelution with pure standard; MS, mass spectrometry; RT*, comparison of the relative retention time with those reported by Adams, 1988. [b] Not detected. [c] Traces.

on comparison of their relative retention times with those of authentic standards, by coelution and MS analysis. For the components, mostly sesquiterpenes, for which reference substances were not available, the identification was performed by matching their mass spectra with those of the ITD library and those reported by Adams (1988) and by comparing their relative retention times with those reported by Adams. Quantitative data were obtained from normalized area values, and each analysis was repeated twice.

Cluster Analysis. Quantitative data of the main essential oil components were used to compute a similarity distance matrix. The data were transformed with the STAND procedure from NTSYS-pc (Rohlf, 1990). In this transformation, the mean is subtracted from the original value and divided by standard deviation. The standardization values were used in the SIMINT subroutine of NTSYS-pc (Rohlf, 1990) to compute a matrix of distances among all pairs of cultivars with the average taxonomic distance. Clustering of cultivars were obtained by the Unweighted Pair-Group Mean Average (UPGMA) clustering method.

RESULTS AND DISCUSSION

Morphological Characteristics. The examined basil cultivars showed a great variability for each observed characteristic (Table 2). The leaves showed different shapes, sizes, colors, and weights, and the

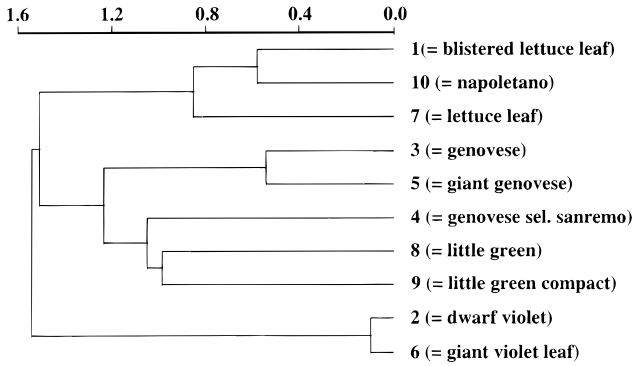

Figure 1. Clustering of the basil cultivars based on their main essential oil components.

plants differed in height, weight, branching, and leafing. Considering all the collected data it was possible to identify cultivars with similar phenotypic characters that could be considered as a homogeneous group. In this way, four groups were distinguished: group A including cultivars 1, 7, and 10, which were characterized by large leaves with a blistered surface and by the highest leaf mean weight (0.65, 0.51, and 0.52 g, respectively); group B including cultivars 3, 4, and 5, which had medium-large leaves with ovate shape, the tallest plants in comparison with those of the other cultivars, and, on average, the highest number of branches (17.5); group C including cultivars 8 and 9, which were characterized by small and lanceolate leaves with the significantly lowest mean weight (0.05 and 0.07 g, respectively), scantily leafed plants (56.9 and 58.2%, respectively), and the lowest oil contents; group D including cultivars 2 and 6, which typically had red-violet leaves of medium size and ovate shape, the smallest plants with the lowest weight, and the highest percentage of leaves (>70%).

Chemical Characterization. The essential oil composition of the 10 basil cultivars, along with the quantitative data and the identification methods, are listed in Table 3. Forty compounds, representing ~98% of the GC profile, were identified. The majority of compounds (24) were monoterpenes (12 hydrocarbons and 12 oxygenated), but sesquiterpenes were also present, with τ-cadinol, germacrene D, and α-*trans*-bergamotene as the main constituents.

Comparison of the analytical data of the oils revealed marked differences in qualitative and quantitative composition. Considering the main components, all the cultivars were characterized by high contents of linalool, (41−76%) and relatively abundant amounts of 1,8-cineole (1−12%), τ-cadinol (2−8%), and α-*trans*-bergamotene (absent in cultivars 2 and 6, and ranging from 1 to 3% in the other ones). Important differences were

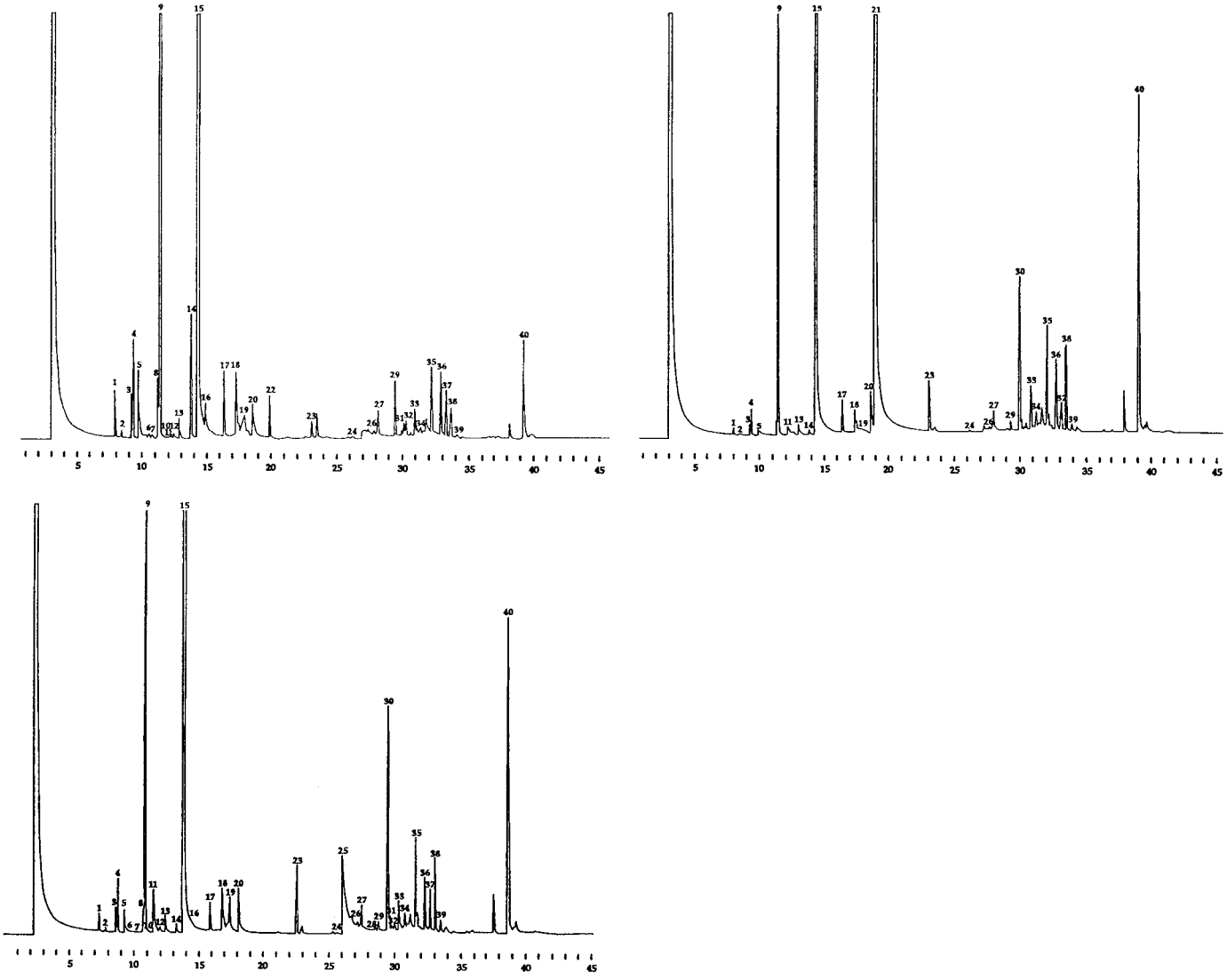

Figure 2. Typical GC profiles of essential oils from three chemotypes: cultivar 6, "linalool chemotype" (top left); cultivar 1, "linalool and methylchavicol chemotype" (top right); and cultivar 5, "linalool and eugenol chemotype" (bottom).

determined by the presence or absence of methylchavicol and eugenol.

These six compounds were considered for a cluster analysis to identify possible chemotypes. The resulting dendrogram (Figure 1) showed the existence of three main clusters of different size. The first group was formed by cultivars 2 and 6, which could be identified as "linalool" chemotypes. Their oils, having very little variation in quantitative composition, showed high percentages of linalool (70%), the absence of methylchavicol and eugenol, and a relatively high amount of 1,8-cineole (13%). A second group included cultivars 1, 10, and 7, all ascribing to chemotypes "linalool and methylchavicol" and characterized by high contents of linalool (41−60%) and methylchavicol (18−41%) but not eugenol, and moderate amounts of 1,8-cineole (2−6%). Inside this cluster, cultivar 7 seemed somehow differentiated, having a lower amount of methylchavicol and higher content of linalool than the other two cultivars. The third group is the largest one and consisted of cultivars 3, 5, 4, 8, and 9, which belonged to the same chemotype "linalool and eugenol". All their oils were characterized by a linalool content ranging from 61 to 76%, eugenol percentages from traces to 4%, the absence of methylchavicol, and very variable amounts of 1,8-cineole (1−11%). This last group was composed of two subclusters: one of these was formed by cultivars 3 and 5 and was characterized by higher levels of eugenol and relatively lower amounts of linalool compared with the other three cultivars 4, 8, and 9. Our findings are in good agreement with those reported by Mariani et al. (1991) who found in the cultivar Genovese a high content of linalool and a lower amount of eugenol and in the cultivar Napoletano considerable amounts both of linalool and methylchavicol.

Among the chemotypes, interesting quantitative differences were also observed in the sesquiterpene fractions, the total amount of which varied from 14% in the "linalool and eugenol" to 11% in "linalool and methylchavicol" type and 7% in "linalool" type. This latter type is characterized by the absence of α-*trans*-bergamotene, which is one of the more abundant constituents of the sesquiterpene fraction in the other chemotypes.

The typical GC profiles of the three chemotypes are reported in Figure 2 which shows the compositions of the essential oil from cultivar 6 of the "linalool chemotype" (top) in which methylchavicol and eugenol were absent; the composition of cultivar 1 of the "linalool and methylchavicol chemotype" (middle) that is very rich in methylchavicol, and the composition cultivar 5 of the "linalool and eugenol chemotype" (bottom) that is characterized by the presence of eugenol. The oils belonging to the second and third chemotypes, characterized by the simultaneous presence of linalool and methylchavicol or linalool and eugenol, respectively, can be ascribed to those plants in which essential oil constituents are produced by two different biosynthetic pathways. In contrast, only one biosynthetic pathway is operating for the oils of the first chemotype. In fact, methylchavicol and eugenol have a common biosynthesis originating from the same precursors (L-phenylalanine and cinnamic acid), whereas linalool follows another biogenetic pathway from mevalonic acid via geranyl pyrophosphate (Nikänen, 1989). It is interesting to note that in some cases the oils ascribing to the same chemotype were obtained from cultivars with similar morphological characteristics, as has been found for plants of the A and the D groups (chemotype "linalool and methylchavicol" and "linalool", respectively). The cultivars of groups B and C, which were very different in morphological characteristics, were found to be the same chemotype "linalool and eugenol".

Table 4. Major Components of Essential Oils from the Four Morphological Groups of Basil Cultivars

basil group	linalool	methylchavicol	eugenol	1,8-cineole
A	50[a]	30	−	4
B	65	−	2	10
C	73	−	2	3
D	70	−	−	13

[a] Mean value %.

The distribution of the main compounds and characterization of the essential oils from the four morphological groups are summarized in Table 4. These results suggest that it is not always possible to establish a strict correlation between morphological characteristics and chemotype, but in some cases, these correlations seem evident. The high content of linalool in all the essential oils indicates that the examined cultivars could be considered as originating from the European chemotype with some influences of the North Africa chemotype when eugenol is present.

ACKNOWLEDGMENT

We are grateful to Dr. Enrico Noli for the help with the statistical analysis.

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Received for review February 15, 1996. Accepted September 19, 1996.[⊗]

JF9601067

[⊗] Abstract published in *Advance ACS Abstracts*, November 15, 1996.