GC–IRMS in the Authenticity Control of the Essential Oil of *Coriandrum sativum* L.

Claudia Frank, Armin Dietrich, Ulrike Kremer, and Armin Mosandl*

Institut für Lebensmittelchemie, Johann Wolfgang Goethe-Universität Frankfurt, Marie-Curie-Strasse 9, 60439 Frankfurt/Main, Germany

The essential oils of 10 authentic coriander samples of different origin were analyzed. Capillary gas chromatography, on-line coupled with isotope ratio mass spectrometry (cGC-IRMS) using Stabilwax as the stationary phase, was applied. Alternatively, heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin in PS 268 was used as the chiral stationary phase. The enantiomeric ratio and the $\delta^{13}C_{PDB}$ values of some characteristic compounds were compared with those of commercially available spices and essential oils. The influence of isotopic effects on the δ values during the primary CO_2 fixation was eliminated by definition of a suitable *i*nternal *i*sotopic *st*andard (i-IST). The isotopic effects among genuine monoterpenes are, therefore, exclusively determined by the influence of enzymatic reactions during secondary biogenetic pathways.

Keywords: *GC–IRMS; authenticity control; essential oil; Coriandrum sativum*

INTRODUCTION

Essential oils are commonly defined as complex mixtures of fragrance and flavor substances originated from plants. Nowadays, by applying sophisticated methods in structural analysis as well as chromatographic techniques, comprehensive data on essential oil constituents are known. Nevertheless, reliable assessments of the naturalness of essential oils remain rather difficult, as synthetic analogues of essential oil constituents are more and more commercially available. In this respect, specific methods for the determination of the origin of essential oils are of fundamental interest.

The analysis of enantiomeric purity is well-known as an efficient possibility for the authenticity control of chiral compounds in essential oils (Mosandl, 1992).

Although enantioselective analysis has proved to be a powerful tool in the authenticity control of genuine flavor and fragrance compounds, this method fails in the case of (i) natural racemates, (ii) liability of chiral compounds to be analyzed and, of course, (iii) nonchiral aroma active compounds. Alternatively, the natural isotopic distribution analyzed by isotopic ratio mass spectrometry is used in the origin evaluation of flavors and essential oils (Braunsdorf, 1994).

This paper deals with the merits of enantioselective capillary GC and/or isotope ratio mass spectrometry (IRMS), coupled on-line with capillary GC, in the authenticity assessment of genuine compounds of coriander oil.

MATERIALS AND METHODS

Plant Material. Dried fruits of *Coriandrum sativum* L. of defined variations were cultivated in 1990 and kindly provided by the Bayerische Landesanstalt für Bodenkultur und Pflanzenbau, Freising, Germany, and used as authentic samples. The samples were stored as whole fruits, ground and extracted prior to isolation of the essential oils.

Sample Preparation. Air-dried fruits of coriander (20 g samples) are ground under liquid N_2. The essential oils are obtained by steam distillation according to the DAB 10 method (*Deutsches Arzneibuch*, 1991).

These oils are separated from the water phase by liquid–liquid extraction, three times with 50 mL portions of *n*-pentane/diethyl ether 1/1 (v/v). The organic phase is dried with Na_2SO_4 and concentrated with a Vigreux column to about 10 mL.

Depletion of the Main Compound Linalol. Linalol (**d**), the main component of coriander oils (Figure 1), is directly measured by GC–IRMS. To increase the concentration of minor compounds, the main compound linalol (**d**) has to be depleted by PHS-C (**p**reparative **h**igh resolution **s**egment) **c**hromatography (Kraft and Strömmer, 1989).

For separation of linalol, 8 mL of the solution was fractionated by PHS-C (column Baker "prep200" with eight segments, height of one segment 19 mm; i.d. 20 mm). The column was filled with silica gel 30 μm (Baker) as the sorbent; *n*-pentane/diethyl ether 1/1 (v/v) was used as the eluent. The segments were separated and the sorbent was extracted with 50 mL of diethyl ether. After concentration (Vigreux column) to about 0.5 mL, the solution was ready to use for GC and GC–IRMS.

Gas Chromatography. The GC analyses (compounds of the oil samples, PHS-C fraction control) were performed with a DANI 6500 GC equipped with a flame ionization detector connected to a Shimadzu Chromatopac C-R3A integrator. A modified polyethylene glycol fused silica capillary column (Stabilwax, 35 m × 0.32 mm i.d., 0.25 μm film thickness, Restek) was used for the analyses. For the enantioselective analysis a heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin in PS 268, 40 m × 0.32 mm i.d., 0.32 μm film thickness (Dietrich et al., 1994) was used as the chiral stationary phase. Conditions: 0.1 μL split injection; split ratio, 1:13; flow rate of carrier gas (helium), 1.6 mL/min; injector temperature, 220 °C. Temperature program: (a, nonchiral analysis) the column was held at 60 °C for 2 min and then programmed at 5 °C/min to 220 °C, which was held for 20 min; (b, chiral analysis) the column was held at 70 °C for 10 min and then programmed at 1.5 °C/min to 220 °C, which was held for 20 min.

Gas Chromatography–Mass Spectrometry. The identification of the compounds was performed with a Fisons GC 8000 series equipped with a mass selective detector (Fisons MD 800) connected to the Fisons lab-base data handling system. As stationary phase was used a modified polyethylene glycol fused silica capillary column (Stabilwax, 35 m × 0.32 mm i.d., 0.25 μm film thickness, Restek). Conditions: 0.1 μL split injection; split ratio, 1:30; flow rate of carrier gas (helium),

* Author to whom correspondence should be addressed.

0021-8561/95/1443-1634$09.00/0

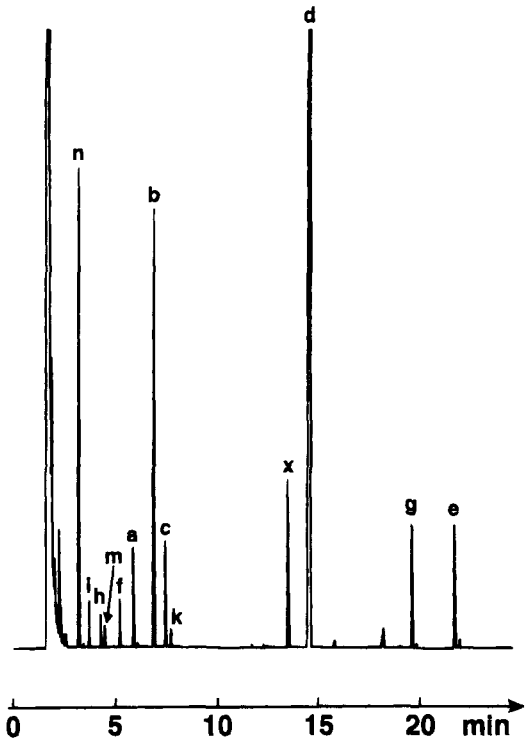


Figure 1. GC analysis on Stabilwax of coriander oil, self-prepared (for further conditions, see Materials and Methods): (x) camphor, GC–MS detected, but not GC–IRMS evaluated until now.

1.4 mL/min; injector temperature, 220 °C; interface temperature, 250 °C; ion source temperature, 220 °C. Temperature program: the column was held at 40 °C for 5 min and then programmed at 3 °C/min to 220 °C, which was held for 20 min.

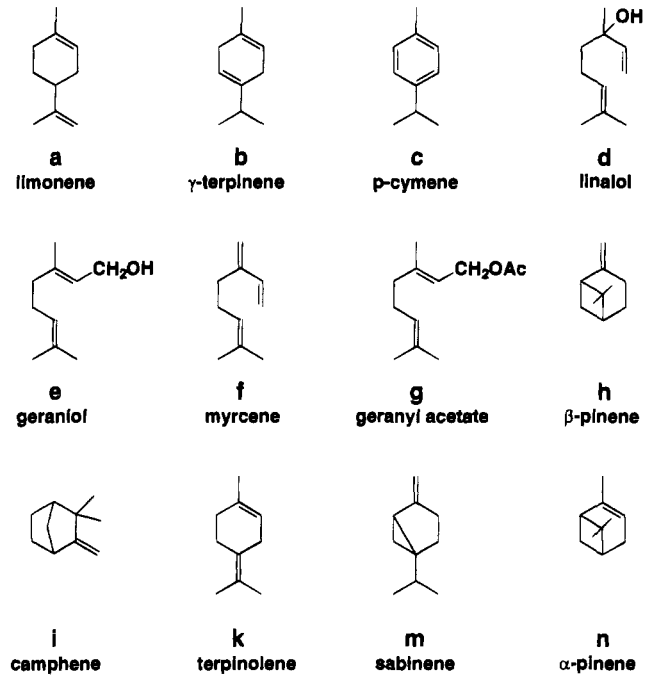
Gas Chromatography–Isotope Ratio Mass Spectrometry. Isotope ratio measurements were performed with a Finnigan MAT delta S isotope mass spectrometer, on-line coupled to a Varian 3400 GC via a combustion interface. The GC was equipped with a modified polyethylene glycol fused silica capillary column (Stabilwax, 35 m × 0.32 mm i.d., 0.25 μm film thickness, Restek). For the enantioselective analysis heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin in PS 268, 40 m × 0.32 mm i.d., 0.32 μm film thickness, was used as the chiral stationary phase. Conditions: (a, nonchiral analysis) 0.1 μL split injection (split ratio 1:13); flow rate of carrier gas (helium), 1.6 mL/min; injector temperature, 220 °C; the column was held at 50 °C for 5 min and then programmed at 5 °C/min to 120 °C followed by a 3 °C/min increase to 220 °C, which was held for 20 min; to prevent the combustion furnace from deactivation, the backflush mode was held for 500 s; (b, chiral analysis) 0.1 μL split injection (split ratio 1:13); flow rate of carrier gas (helium), 1.6 mL/min; injector temperature, 220 °C; the column was held 70 °C for 10 min and then programmed at 1.5 °C/min to 220 °C, which was held for 20 min; to prevent the combustion furnace from deactivation, the backflush mode was held for 500 s.

Isotope ratios are expressed as δ values (‰) versus a standard.

$$\delta^{13}C = \frac{(^{13}C/^{12}C)\text{sample} - (^{13}C/^{12}C)\text{standard}}{(^{13}C/^{12}C)\text{standard}} \times 1000$$

The isotope mass spectrometer was calibrated against CO_2 gas with a defined $^{13}C/^{12}C$ content relative to the PDB standard defined as the $^{13}C/^{12}C$ isotope ratio for CO_2 gas yielded by the reaction of *Belemnitilla americana*, from the Peedee Formation of South Carolina with 100% phosphoric acid; it is commonly used for $^{13}C/^{12}C$ IRMS (Craig, 1957). Furthermore, the system performance was checked by introducing a mixture of references with well-known $\delta^{13}C_{PDB}$ values: 5-nonanone (−28.05‰); γ-octalactone (−23.17‰); γ-decalactone (−30.12‰) (Braunsdorf et al., 1993a).

Chart 1. Main Compounds of Coriander Essential Oil Investigated



RESULTS AND DISCUSSION

Enantioselective multidimensional gas chromatography (enantio-MDGC), using the combination of a nonchiral precolumn and a chiral main column, has proved to be a powerful tool in the systematic evaluation of origin-specific enantiomeric ratios and for differentiating natural flavor or fragrance compounds from those of synthetic origin (Mosandl, 1992, and literature cited therein).

Also linalol, the chiral main compound of coriander, was reported with fruit-specific enantiomeric distribution, favoring the *S*-enantiomer (>80%) (Schubert and Mosandl, 1991; Derbesy and Uzio, 1993).

Nevertheless, this method fails in principle in the case of the fraudulent addition of synthetic compounds such as γ-terpinene (**b**) or any other nonchiral substance.

This paper, therefore, reports on the authenticity assessment of the essential oil of *C. sativum* L. by GC–IRMS analysis.

Coriander oil is obtained by steam distillation of the ripe fruits of *C. sativum* L. It is used in seasoning mixtures and in perfume compositions. Monoterpene hydrocarbons, alcohols and esters are the most important constituents of coriander oil (Chart 1; Figure 1).

In Table 1 the composition of the main compounds of the analyzed coriander oils is outlined. The named compounds are identified by GC–MS. Ten self-prepared authentic samples were rather similar according to the literature (Derbesy and Uzio, 1993), while two commercial spices and three commercial oils contained more linalol (**d**) and contained all of the other compounds in trace amounts. This fact may be explained by a considerable volatility of monoterpenes, e.g. α-pinene (**n**) or limonene (**a**), and by the unknown duration of storage in the case of these commercial samples (Frank, 1995).

As can be seen from Figure 1 as well as Table 1, linalol (**d**) is the characteristic main component of genuine coriander oils. So far, there is no problem in evaluating $\delta^{13}C_{PDB}$ values of component **d** by direct GC–IRMS measurements. However, an effective depletion

Table 1. Main Compounds of Coriander Essential Oil (Area Percent)

	as1–as10[a,d]	cs1[b,d]	cs2[b,d]	oil 1[c]	oil 2[c]	oil 3[c]
limonene (**a**)	1.3–1.8	2.0	0.9	3.1	6.3	6.3
γ-terpinene (**b**)	5.0–7.7	3.9	3.5	2.7	nn	nn
p-cymene (**c**)	0.6–1.4	1.0	1.3	2.6	5.9	3.0
linalol (**d**)	68.4–77.0	78.1	87.5	81.2	74.1	80.8
geraniol (**e**)	1.7–3.5	0.4	0.7	1.0	1.3	1.3
myrcene (**f**)	0.6–0.8	0.3	0.2	nn	nn	nn
geranyl acetate (**g**)	1.7–3.7	nn	nn	1.2	0.9	0.8
β-pinene (**h**)	>0.5	nn	nn	nn	nn	nn
camphene (**i**)	>0.5	nn	nn	nn	nn	nn
terpinolene (**k**)	>0.5	nn	nn	nn	nn	nn
sabinene (**m**)	>0.5	nn	nn	nn	nn	nn
α-pinene (**n**)	2.5–6.0	0.7	0.5	2.8	2.1	1.3

[a] authentic samples (as1–as10). [b] Commercial spices (cs1–cs2). [c] Commercial coriander oils (oils 1–3). [d] Samples as (cs) were self-prepared by steam distillation.

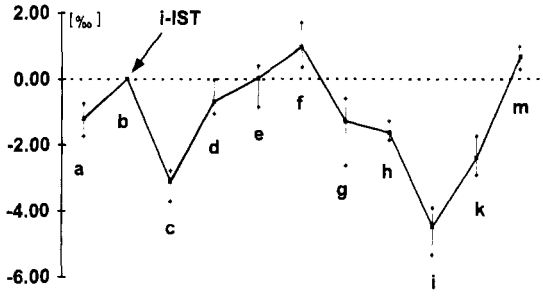


Figure 2. Isotope fingerprint of the essential oils from authentic samples ($n = 10$) of *C. sativum* L. with maximal and minimal values; γ-terpinene is the internal isotopic standard: limonene (a), γ-terpinene (b), *p*-cymene (c), linalol (d), geraniol (e), myrcene (f), geranyl acetate (g), β-pinene (h), camphene (i), terpinolene (k), sabinene (m).

of the main compound linalol (**d**) must be achieved by PHS-C to increase the concentration of further (minor) compounds to be analyzed. Only camphor (**x**) could not be separated from linalol (**d**) by this method. Therefore, $\delta^{13}C_{PDB}$ values of camphor (**x**) were not determined.

Although GC–IRMS analysis is of considerable importance in the origin assessment of flavor and fragrance compounds, this method seems to be seriously limited, as most plants cultivated for human nutrition belong to the group of C_3 plants (coriander included). Unfortunately, C_3 plants yield $\delta^{13}C_{PDB}$ values partially overlapping with those of synthetic substances from fossil sources.

To overcome this fundamental limitation, we proposed the comprehensive GC–IRMS analysis in the authenticity control of flavors and essential oils, using suitable internal isotopic standards (i-IST) (Braunsdorf et al., 1993b).

That means we pay exclusive attention to compounds of secondary biogenetic pathways (monoterpenes etc.), comparing their $\delta^{13}C_{PDB}$ levels in relation to each other compound of one and the same plant organism. Consequently, we obtain an isotopic "fingerprint" which is typical for this plant, as outlined in Figure 2.

Thus, the main influence of isotopic discrimination, caused by CO_2 fixation during photosynthesis, is eliminated. Isotopic effects among genuine compounds are, therefore, only limited by the influence of enzymatic reactions during secondary biogenetic pathways. As shown in Figure 3, the two curves nearly coincide when an internal isotopic standard is introduced.

To choose a suitable i-IST, the following principal aspects must be taken into account:

(1) The substance selected as an i-IST should be a characteristic genuine compound of less importance in view of sensorial relevance.

(2) The compound must be available in sufficient amounts and free of isotopic discrimination during sample cleanup.

(3) The selected compound should be biogenetically related to the compounds under investigation.

(4) Chemical purity and inertness during storage and/or technical processes are mandatory.

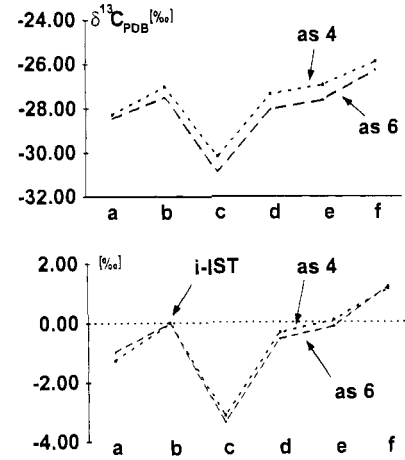


Figure 3. Isotope fingerprint of two authentic samples (as4, as6): (top) fingerprints using $\delta^{13}C_{PDB}$ values; (bottom) fingerprints calculated as $\delta^{13}C_{\gamma\text{-terpinene}}$ values; limonene (a), γ-terpinene (b), *p*-cymene (c), linalol (d), geraniol (e), myrcene (f).

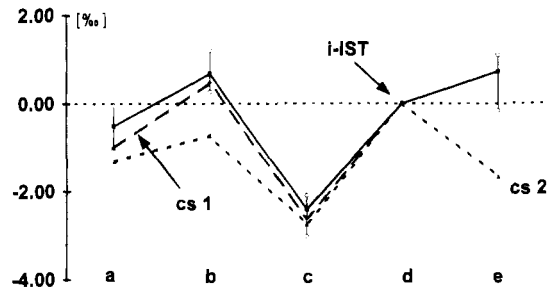


Figure 4. IRMS measurements of coriander oils: isotopic fingerprints of authentic oils (bold line; $n = 10$); commercial spices (cs1, cs2); linalol (i-IST); limonene (a), γ-terpinene (b), *p*-cymene (c), linalol (d), geraniol (e).

According to this method self-prepared authentic coriander oils as well as commercial samples were analyzed by GC–IRMS. The results are shown in Figure 4. Commercial spice sample cs1 is interpreted to be identical with the fingerprint of the authentic samples. Commercial spice sample cs2 is different from the isotopic fingerprint concerning the compounds limonene (**a**), γ-terpinene (**b**), and geraniol (**e**).

In this case linalol (**d**) was used as the internal isotopic standard (i-IST), because the $\delta^{13}C_{PDB}$ values of γ-terpinene (**b**) were detected to be not natural in the sample of cs1.

If an essential oil is natural, all $\delta^{13}C_{PDB}$ values have to be identical with the isotopic fingerprint, irrespective of the internal compound, which has been selected as an internal standard for origin evaluation.

In Table 2 the enantiomeric distribution of two characteristic compounds of coriander are shown. Limonene (**a**) in the authentic samples is found to be racemic, while in the commercial sample (cs2) and in three commercially available coriander oils (1–3) the *R*-configured enantiomer is enriched. In the case of oils 1–3 the enantiomeric ratio of linalol (**d**) differs significantly with respect to genuine oils (as1–as4).

Table 2. Enantiomeric Ratios of Limonene and Linalol in Coriander Oils

	limonene		linalol	
	(*S*)-(−)	(*R*)-(+)	(*R*)-(+)	(*S*)-(−)
as1−as4[a]	50	50	13	87
cs2[b]	24	76	9	91
oil 1[c]	38	62	33	67
oil 2[c]	12	88	27	73
oil 3[c]	7	93	34	66

[a] Authentic samples (as1−as4). [b] Commercial spice (cs2). [c] Commercial oils (oils 1−3).

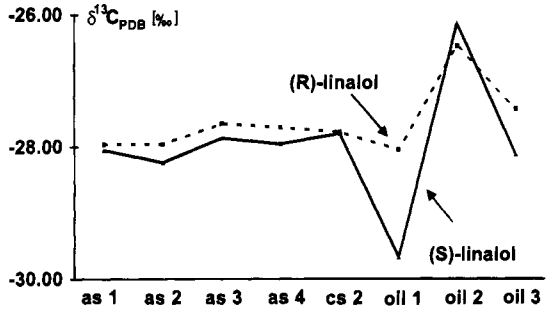


Figure 5. $\delta^{13}C_{PDB}$ values of (*R*)-(−)- and (*S*)-(+)-linalol: authentic samples (as1−as4); commercial spice (cs2); commercial oils (oils 1−3).

As the latest modification of the GC−IRMS analysis the main compound of coriander, linalol (**d**), was analyzed by enantio-GC−IRMS. The results are shown in Figure 5. While the $\delta^{13}C_{PDB}$ values the enantiomers are identical in the case of the authentic samples and samples cs2 and oil 2, the $\delta^{13}C_{PDB}$ values of the enantiomers in oils 1 and 3 are significantly different.

Enantioselective gas chromatography as well as enantio-GC−IRMS, as the latest technique, have been demonstrated as sophisticated methods in evaluating the authenticity of (chiral) flavor compounds. In the near future the analytical differentiation between naturally grown, fermentatively produced, and symmetrically synthesized aroma substances will require intensive and comprehensive basic investigations. It seems to be reasonable that the continuous approach in the analytical origin assignment of flavors and fragrances will be reflected in legal regulations as a consequence.

ACKNOWLEDGMENT

We thank Dr. T. Stahn, Bayerische Landesanstalt für Bodenkultur und Pflanzenbau, Freising, Germany, for providing authentic plant material and F. Dettmar for technical assistance.

LITERATURE CITED

Braunsdorf, R. Dissertation, Universitat Frankfurt/Main, 1994.

Braunsdorf, R.; Hener, U.; Prizibilla, G.; Piecha, S.; Mosandl, A. Analytical and technological influence of $^{13}C/^{12}C$ isotope ratio of orange oil components. *Z. Lebensm. Unters. Forsch.* **1993a**, *197*, 24−28.

Braunsdorf, R.; Hener, U.; Stein, S.; Mosandl, A. Comprehensive cGC-IRMS analysis in the authenticity control of flavours and essential oils. Part I: Lemon oil. *Z. Lebensm. Unters. Forsch.* **1993b**, *197*, 137−141.

Craig, H. Isotopic standards for carbon and oxygen and correction factors for mass-spectrometry analysis of carbon dioxide. *Geochim. Cosmochim. Acta* **1957**, *12*, 133−149.

Derbesy, M.; Uzio, R. Application of phase chiral chromatography to the control of the essential oil of coriander. *Ann. Falsif. Expert. Chim. Toxicol.* **1993**, *86* (923), 369−378.

Deutsches Arzneibuch, 10th ed.; Wissenschaftliche Verlagsgesellschaft: Stuttgart, 1991.

Dietrich, A.; Maas, B.; Mosandl, A. Diluted modified cyclodextrins as chiral cGC phases—The influence of the polysiloxane solvents to the enantioselectivity and column efficiency. *J. Microcolum. Sep.* **1994**, *6*, 33−42.

Frank, C. Dissertation, Universitat Frankfurt/Main, 1995, in preparation.

Kraft, P.; Strömmer, R. Segment columns in preparative chromatography. *GIT Suppl.* **1989**, *3*, 60−68.

Mosandl, A. Capillary gas chromatography in quality assessment of flavours and fragrances. *J. Chromatogr.* **1992**, *624*, 267−292.

Schubert, V.; Mosandl, A. Stereoisomeric flavour compounds LIII: chiral compounds of essential oils VIII; stereodifferentiation of linalol using multidimensional gas chromatography. *Phytochem. Anal.* **1991**, *2*, 171−174.

Received for review November 7, 1994. Revised manuscript received March 20, 1995. Accepted March 31, 1995.[⊗] Financial support by the Industrielle Gemeinschaftsforschung (Bundesministerium für Wirtschaft/AIF) and the Forschungskreis der Ernährungsindustrie (FEI) is gratefully acknowledged.

JF940624M

[⊗] Abstract published in *Advance ACS Abstracts,* May 15, 1995.