McWeney, D. J.; Bates, M. L. *J. Food Technol.* **1980,** *15,* 407.

Nydal, R.; Lovseth, K. *J. Geophys. Res.* **1983,** *88,* 3621.

Volpe, P.; Campi, E.; Mentasti, E. *Anal. Chem. Acta* **1982,** *141,* 345.

Weast, R. C., Ed. *Handbook Chemistry and Physics,* 61st ed.; CRC Press: Boca Raton, FL, 1980.

Whelan, T.; Sachett, W. M.; Benedict, C. R. *Biochem. Biophys. Res. Commun.* **1970,** *41,* 1205.

Byproduct Identification in the Carbodiimide-Assisted Synthesis of Fatty Acid Anilides Related to Spanish Toxic Oil Syndrome

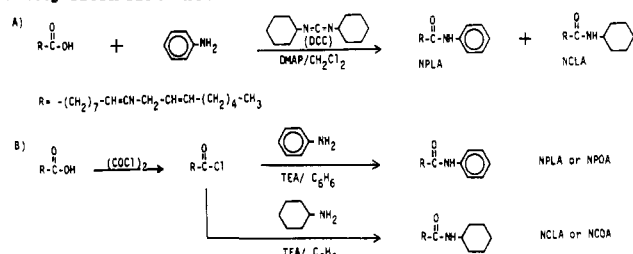Ramon Freixa, Josefina Casas, Angel Messeguer, Joan Roselló, and Emilio Gelpi*

Results are presented on the comprehensive analytical identification of the N-cyclohexylamides obtained as unexpected reaction byproducts in the synthesis of N-phenyloleamide (NPOA) and N-phenyllinoleamide (NPLA) by reaction of anilide with either oleic or linoleic acid. The data presented prove that these N-cyclohexylamide impurities (5–25%) are due to the N,N'-dicyclohexylcarbodiimide used as dehydrating agent. The synthesis of both phenylamides was required for toxicological testing in relation to the Spanish Toxic Oil Syndrome. Purification of the reaction crudes by silica gel column chromatography followed by subsequent TLC and HPLC assay of the presumably pure amides did not eliminate the cyclohexylamides, which had not been detected by either technique. Unequivocal identifications were achieved by a combined analytical approach involving packed and capillary GC and GC–MS, chemical ionization MS, IR and NMR spectroscopy, and elemental analysis. On the basis of the above results, an alternative procedure is suggested, leading to the desired amides in high purity and good yields.

INTRODUCTION

The anilides of oleic and linoleic acid have recently attracted much interest for their presumed implication in the mechanism(s) of toxicity underlying a new epidemic disease that has become known as the Spanish Toxic Oil Syndrome (TOS) (Tabuenca, 1981; Pestaña and Muñoz, 1982; Toxic Epidemic Syndrome Study Group, 1982; Gelpi, 1985). These compounds—which are formed by reaction of aniline with the free fatty acids in edible oils—were found in significant amounts in rapeseed oil batches. Rapeseed oil for industrial uses had been originally denatured with 2% aniline, but later it was subjected to a fraudulent and unsuccesful refining process with the aim of making it suitable for human consumption (Granjean and Tarkowski, 1984). For this purpose it was also mixed with other varieties of food oils and animal fats. The abatement of the epidemic disease caused by these illegal oils coincided with their withdrawal from the market by the Spanish authorities as soon as a reasonable epidemiological link was established (Granjean and Tarkowski, 1984).

Thus, from the beginning, the anilides of oleic and linoleic acids were the most likely candidates for detailed analytical and toxicological studies in the search of the ethiopathogenesis of TOS. The anilides of oleic and linoleic acids have been referred to either collectively as "oleoanilides" or specifically as oleyl- and linoleylanilides in the literature related to the TOS. In compliance with accepted nomenclature, these compounds will be desig-

Departments of Neurochemistry (R.F., J.R., E.G.) and Biological Organic Chemistry (J.C., A.M.), CSIC, 08034 Barcelona, Spain.

Scheme I. Synthesis Procedures for the Preparation of Fatty Acid Anilides

R = $-(CH_2)_7-CH=CH-CH_2-CH=CH-(CH_2)_4-CH_3$ or $-(CH_2)_7-CH=CH-(CH_2)_7-CH_3$

NPLA: N-phenyllinoleamide; NPOA: N-phenyloleamide; NCLA: N-cyclohexyllinoleamide; NCOA: N-cyclohexyloleamide

nated herein as indicated in Scheme I.

Different samples of oils presumably related to clinical case histories have been screened for the presence and content of these anilides by both chromatographic and mass spectrometric techniques (Artigas et al., 1983; Granjean and Tarkoski, 1984). Also they were subjected to various in vitro and in vivo toxicological testing procedures (Granjean and Tarkowski, 1984). All of this work has created the need for the availability of the pure anilides as reference compounds, as well as for relatively large amounts of the same compounds that are needed in animal model testing.

Consequently, several laboratories, including the Instituto Nacional de Toxicología, Madrid, attempted the synthesis of pure ole- and linoleanilides. Some of these syntheses have been reported in the literature (Casals et al., 1983; Fernandez-Alvarez, 1983). However, the laboratories that prepared these anilides, either for their own

use or for distribution to other research centers, were for the most part unaware that the chosen synthesis could lead to unexpected reaction byproducts. Such compounds are potentially capable of creating confusion in the interpretation of animal toxicity tests since the toxicity of these reaction byproducts is unknown.

Although we have not been able to check the purity of the various synthetic anilide batches used at the different laboratories conducting TOS studies, a batch independently analyzed in our laboratory was found to contain an additional unidentified synthesis product. This particular batch has been used in toxicity tests on mice (De Vera et al., 1983).

We describe in this study the application of refined analytical techniques for the unequivocal identification of these byproducts as well as present an alternative route for the synthesis of anilides having sufficiently high purity for toxicological research.

MATERIALS AND METHODS

Reagents. All compounds utilized in this study were from commercial sources, and they were used without further purification unless otherwise stated. Hexane and ethyl acetate (p.a. from Scharlau-Ferosa, Barcelona, Spain) were distilled before use. Linoleic acid (puriss., 99%), oleic acid (puriss., 99%), aniline (puriss. p.a.), triethylamine (TEA) (puriss. p.a.), N,N'-dicyclohexylcarbodiimide (DCC) (purum), and 4-(dimethylamino)pyridine (DMAP) (purum) were from Fluka A.G. Cyclohexylamine (purum, from Fluka A.G.) was distilled before use.

Synthesis Procedures. *Method A.* A dichloromethane solution containing equimolecular amounts of linoleic acid (LA, 0.5 mmol), aniline, and N,N'-dicyclohexylcarbodiimide (DCC) was allowed to react in the presence of 4-(dimethylamino)pyridine (DMAP, 0.05 mmol) at room temperature (Scheme IA). When the reaction was completed, as monitored by TLC, the crude reaction mixture was filtered to remove the N,N'-dicyclohexylurea. The filtrate was washed with 1 N HCl, 1 N NaOH, and water and dried over magnesium sulfate. The resultant residue was purified by silica gel column chromatography with a final recovery of 77% for N-phenyllinoleamide (NPLA).

Method B. A mixture containing the corresponding acid and a 100% molar excess of oxalyl chloride (Scheme IB) was allowed to react in a dry atmosphere for 20 h at room temperature. The residue that was obtained after careful removal of unreacted oxalyl chloride under reduced pressure was diluted with benzene, washed with cold water, and dried over magnesium sulfate. The crude acyl chloride solution was concentrated to half of its original volume under vacuum, and equimolecular amounts of triethylamine and of the corresponding amine (aniline, cyclohexylamine) relative to the starting acid were added with external cooling. The reaction mixture was stirred for 3 h at room temperature, diluted with diethyl ether, and washed with 1 N HCl, and the ethereal fraction dried over magnesium sulphate.

Chromatographic Methods. Thin-layer chromatography (TLC) analyses were carried out on silica gel 60 precoated plates with fluorescence label (Merck, Darmstadt, W. Germany). The plates were run with hexane-ethyl acetate (3:1) and were developed by spraying with 10% sulfuric acid in methanol.

Final purification of the synthesized products was performed by "flash column chromatography" (Clark-Sill et al., 1978), using appropriate columns packed with silica gel (230–400 mesh, Merck, Darmstadt, W. Germany). Elution with hexane–ethyl acetate from 95:5 to 85:15 yielded the corresponding amide.

High-performance liquid chromatography (HPLC) determinations were carried out on 30 cm × 0.39 cm i.d. columns repacked with Spherisorb ODS-2, 10 μm (Tracer Analitica, S.A., Barcelona, Spain). The mobile phase was acetonitrile–water (9:1) at 1.5 mL/min. Elution was monitored on a Kontron Uvikon Model 722LC detector (Kontron A.G.) and a Waters Model 481 UV detector (Waters Associates). Both detectors were operated at either a 254- or 215-nm wavelength.

Packed-column GC analyses were performed with a 180 cm × 0.2 cm i.d. glass column packed with 3% OV-101 (Applied Science Laboratories Inc., State College, PA) on 80–100 mesh silanized Chromosorb W (Carlo Erba, Milano, Italy) mounted in a Carlo Erba Model 4300 gas chromatograph equipped with a flame ionization detector (FID). The isothermal separations were obtained at 280 and 300 °C, respectively. The capillary GC runs were carried out on a Hewlett-Packard 5790 equipped with an split–splitless injector and FID. The column in this case was a 15 m × 0.32 mm i.d. glass capillary coated with SE-30. The injector and detector temperatures were set at 280 and 300 °C, respectively, and the column temperature was programmed from 220 to 270 °C at a rate of 5°/min. Helium was used as carrier gas at the optimum flow.

Other Instrumental and Analytical Methods. The gas chromatographic and mass spectrometric (GC–MS) determinations were carried out in a Hewlett-Packard 5995 GC–MS system fitted with a 22 m × 0.35 mm i.d. glass capillary OV-101 bonded-phase column. The capillary injector temperature was set at 60 °C with the ramp rate at 15 units and 0.5-min purge-off time. The injector port temperature was 280 °C. The column was programmed from 180 to 290 °C at a rate of 6°/min. The transfer line, ion source, and analyzer temperature were kept at 280, 148, and 180 °C, respectively.

The chemical ionization (CI) mass spectra of the purified synthesis products were obtained on an updated AEI MS-902 high-resolution mass spectrometer with a PDP 11/24 computer for data processing. Mass spectra were obtained with ammonia as the reagent gas. The ion source was maintained at 210 °C. The current trap was 0.5 mA and the accelerating voltage 8 kV. Samples were introduced into the ion source through the solids probe.

Melting points (uncorrected) were determined on a Reichert-Kofler apparatus (Wien, Austria). Elemental analyses were performed with a Carlo Erba Model 1106 instrument (Milano, Italy). The IR spectra were recorded using a Perkin-Elmer 399 B spectrometer (Norwalk, CT). The ^{1}H NMR spectra were recorded with a Bruker WP 80 SY apparatus (Karlsruhe, W. Germany) operating at 80.13 MHz in the Fourier transform mode. All chemical shifts are given in ppm downfield from tetramethylsilane.

RESULTS AND DISCUSSION

The discovery of a quantitatively significant impurity in batches of synthesized NPOA (Figure 1), which had been used in toxicology tests (De Vera et al., 1983) as received, led to a detailed analytical study of the identity and origin of this compound. Our early attempts to synthesize the anilide of linoleic acid or N-phenyllinoleamide (NPLA) by a previously described procedure (Fernandez-Alvarez, 1983) (method A in Materials and Methods; Scheme IA) resulted in clear experimental evidence to the fact that the final product could contain as much as a 25% of an unknown compound that was not readily detectable by TLC or HPLC assays. For example, the TLC R_f values of the starting and final products of the synthesis were as follows: DMAP and N,N'-dicyclohexylurea, 0; aniline, 0.30; linoleic acid, 0.34; unknown contaminant, 0.53; NPLA,

Figure 1. Gas chromatographic profile of a batch of *N*-phenyloleylamide (NPOA) obtained according to method A. Separation was achieved on a glass capillary SE-30 column, as described in the text. The NPOA peak eluting at 16.96 min represents a 78.9% of the total integrator computed area under the peaks. The peak marked with the star, eluting at 16.08 min, represents 21.0% of the total area.
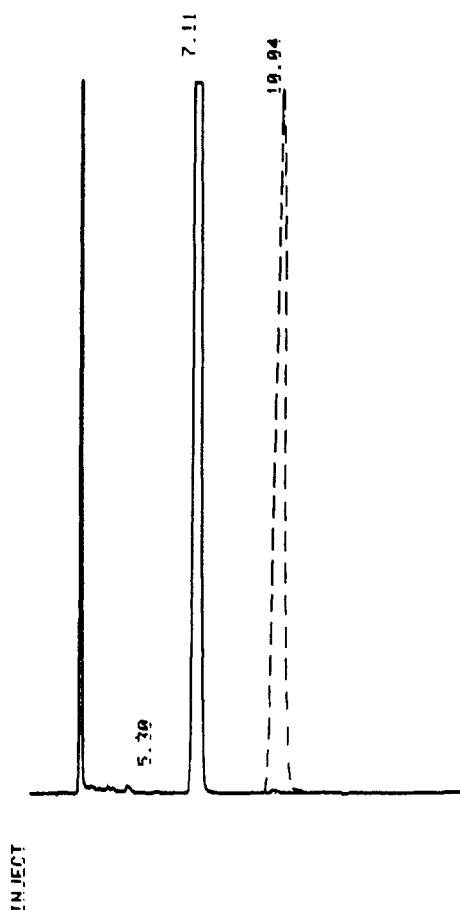

Figure 2. HPLC profile of NPLA (peak at 7.11 min) synthesized according to method A. The dashed peak indicates the position where the contaminant elutes (peak at 10.04 min). This component of the sample would not be detected by direct analysis of the reaction crude due to its very low response at 254 nm in the UV detector.

Figure 3. Gas chromatograms of the reaction crudes obtained from aniline and either oleic or linoleic acids according to the conditions of method A. The separations were obtained on a packed column as described in the text. (A) Products obtained according to method A using aniline and oleic acid (see Scheme I). (b) Coinjection with pure NCOA obtained according to method B (see Scheme I). (C) Pure NCOA as obtained by method B. (D) Products synthesized by method A using aniline and linoleic acid. (E) Coinjection of NCLA obtained according to method B. (F) Pure NCLA as obtained by Method B.

means, as described below. In other words, because of its lack of sufficient unsaturation centers, the contaminant shows very limited absorbance at the usual 254-nm UV detection wavelength, as illustrated in the chromatogram of Figure 2 where no sizable peak other than that of the NPLA analyte can be inferred. As it is known, however, that the detectability of organic compounds in general can be increased at lower wavelengths, the HPLC elution profile of NPLA was also monitored at 215 nm. In this case, the coinjection of a quantity of the contaminant (NCLA) amounting to a 25% of the level of NPLA in the sample results in an HPLC profile showing a peak that is only a 10% of the major NPLA peak at 7.11 min in Figure 2. Under the HPLC conditions described, the retention times of NPLA and its unknown contaminant were 7.11 and 10.04 min, respectively.

The presence of a significant impurity in the final NPLA product, as in the case of NPOA (Figure 1), is readily apparent in the chromatograms of Figure 3. The GC FID analyses of the crude synthesis products generated by the reaction of oleic and linoleic acids with aniline (according to method A) show peaks eluting earlier than those corresponding to pure NPOA (Figure 3A) and pure NPLA (Figure 3D). In the case depicted in Figure 3A the computed area of the minor NCOA peak eluting at 10.4 min amounts to a 12.9% of the major NPOA peak at 12.1 min. The corresponding NCLA peak in Figure 3D eluting at 10.3 min amounts to a 23.3% of the major NPLA peak eluting at 12.0 min. The same result was obtained by glass

0.54; DCC, 0.66. Thus, monitoring the synthesis by TLC would not give any indication of an unsuspected impurity since it would coelute with NPLA.

Likewise, the HPLC elution of the crude reaction products monitored at 254 nm did not reveal the presence of any unknown contaminant to the synthesized NPOA, even though we had the evidence previously obtained by capillary GC analyses (Figure 1). The reason became clear after the contaminant itself had been identified by other
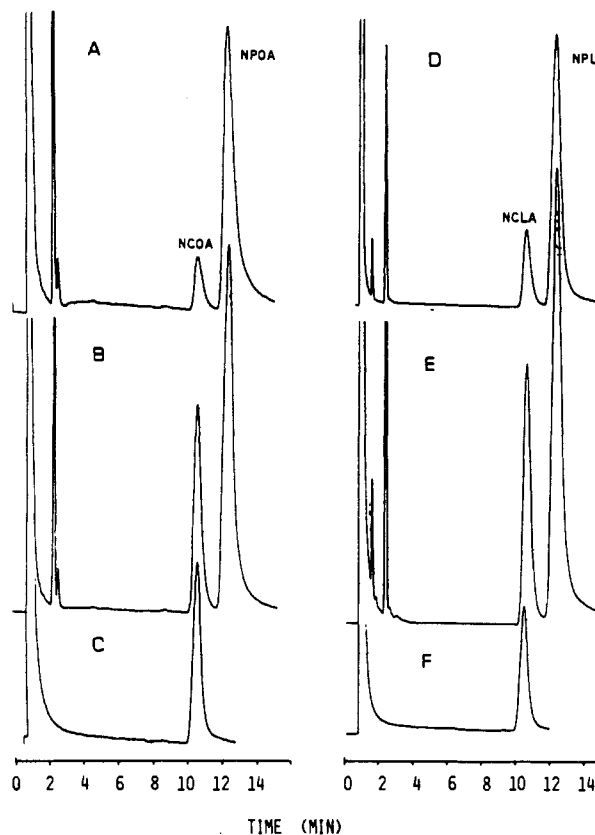
Table I. Formation of *N*-Cyclohexylamide of Linoleic Acid (NCLA) under Different Reaction Conditions

reagents	conditions	solvent	% NCLA
LA, DCC, DMAP (10%)	4 h at rt, darkness	dichloromethane	24.5
LA, DCC, DMAP (10%)	slow addn of DCC	dichloromethane	24.5
LA, DCC, DMAP (10%)	4 h at rt	diethyl ether	26
LA, DCC, DMAP (10%)	4 h at 0 °C	dichloromethane	24
LA, DCC, DMAP (10%)	4 h at -10 °C	dichloromethane	3.6
LA, DCC	4 h at rt	hexane	5.6

Table II. Ammonia CI Mass Spectra of *N*-Phenyl- (NP) and *N*-Cyclohexylamides (NC) of Oleic (OA) and Linoleic (LA) Acids[a]

	NP			NC		
	mol wt	M + H ion	m/z 93	mol wt	M + H ion	m/z 141
OA	357	358 (100)	(40)	363	364 (100)	(11)
LA	355	356 (100)	(24)	361	362 (100)	(5)

[a] Relative abundances given in parentheses.

capillary GC with retention times on SE-30 of 16.08 min for the minor NCLA peak and 16.96 min for the major peak (NPLA).

The preliminary GC-MS identification of these peaks provided mass spectral patterns consistent with the structures of the anilides of oleic and linoleic acid for the major components in the chromatograms of Figure 3 parts A and D, respectively. In both cases, the base peak was at m/z 93 (aniline fragment) and this was accompanied by the characteristic McLafferty rearrangement ion at m/z 135 as well as by the ion at m/z 148 arising by simple β cleavage of the fatty acid chain. As expected, the molecular ion of the NPLA was at m/z 355 and that of NPOA was at m/z 357. On the other hand, the mass spectral patterns of the two minor peaks labeled NCLA and NCOA in Figure 3 parts A and D, showed a common base peak at m/z 141 with molecular ions at m/z 361 and 363. There was no m/z 93 fragment indicative of the aniline moiety. An evaluation of these data and the possible byproducts of the synthesis suggested a structure consistent with the *N*-cyclohexylamide of the fatty acids. In this case the cyclohexylamide moiety would be derived from the DCC used as the dehydrating agent in these reactions (Scheme IA). This was in agreement with the mass spectrometric features, the m/z 141 ion being equivalent to that appearing at m/z 135 in the mass spectra of the fatty acid anilides. Also, the β cleavage ion at m/z 148 would be shifted to m/z 154 as seen in the mass spectra of the two synthesis impurities (NCOA and NCLA in Figure 3). Although it was evident from these data that the impurities

containing the cyclohexyl moiety were due to the presence of DCC in the reaction mixture, this hypothesis was further confirmed under different reaction conditions, as summarized in Table I. The *N*-cyclohexylamide of linoleic acid was formed under all of the conditions quoted in Table I, though at variable conversion yields. These results indicated that previous reports (Fernandez-Alvarez, 1983) on preparation of the ole- and linoleanilides by the procedure described in method A in Materials and Methods should be questioned with regard to the purity of the final product.

In spite of the wide use of DCC as a condensation agent in the preparation of amides (particularly in the peptide field), to our knowledge there have been no previous reports on the isolation of *N*-cyclohexylamides as byproducts in this type of reaction. DCC was verified not to contain any detectable amount of cyclohexylamine prior to its addition to the reaction mixture. We speculate that the *O*-acylurea—postulated as the primary reaction product from the interactions of the carboxylic acid and the diimide (Hegarty at al., 1977)—rearranges to an *N*-acylurea, which in turn can be cleaved by the nucleophilic species of the medium, affording either the normal acyl derivative or the *N*-cyclohexylamide depending on the reaction conditions (see also Avramovici-Grisaru and Sarel, 1982). In this context, work is in progress to clarify the origins and scope of these processes.

Finally, independent syntheses, either for the *N*-phenyl- or *N*-cyclohexylamides of both fatty acids, were carried out by a different approach involving the formation of an intermediate acyl chloride that subsequently was allowed to react with the corresponding amine (aniline or cyclohexylamine in Scheme IB). This procedure, which has also been reported for the preparation of fatty acid anilides (Mancha, 1982), constitutes a more convenient route to the isolation of the expected *N*-phenyl- or *N*-cyclohexylamides in their pure forms. The reactions are clean, are easy to perform, and afford good overall yields as illustrated in Table III. Chromatographically, the pure *N*-cyclohexylamides (Figure 3, parts C and F) coeluted with the impurity peaks of NPOA (Figure 3B) and NPLA (Figure 3E). Additional mass spectrometric confirmation of structures was derived from direct-probe positive-CI analyis of the individual solids (Table II). A summary of additional spectroscopic and analytical data supporting the above identifications is given in Table III.

In conclusion, a closely related and thus previously unsuspected impurity has been identified in the crude synthetic products of the reaction of aniline with either linoleic or oleic acids. The presence of such impurities in the preparations used in tests of the toxicity of these anilides (e.g., cell culture work or solutions applied to live animals) could explain, unless demonstrated otherwise, the conflicting reports and discrepancies in the data thus reported previously (Granjean and Tarkowski, 1984). This work

Table III. Analytical and Spectroscopic Data of *N*-Phenyl- (NP) and *N*-Cyclohexylamides (NC) of Linoleic Acid (LA) and Oleic Acid (OA) Prepared According to Method B (Figure 1)

compd	overall yield, %	mp, °C	mol formula[a] (mol wt)	IR (CCl₄): ν, cm⁻¹	¹H NMR (CDCl₃): δ
NCLA	80	oil	$C_{24}H_{43}NO$ (361.6)	3440, 3300, 1700	0.90 (t, 3 H, J = 6 Hz), 1.1–2.2 (complex abs, 33 H), 2.78 (m, 2 H), 4.75 (br, 1 H), 5.20–5.45 (complex abs, 4 H)
NCOA	81	48	$C_{24}H_{45}NO$ (363.3)	3450, 3310, 1700	0.90 (t, 3 H, J = 6 Hz), 1.1–2.2 (complex abs, 39 H), 4.75 (br, 1 H), 5.15–5.30 (2 H)
NPLA	75	oil	$C_{24}H_{37}NO$ (355.5)	3290, 1665, 1600	0.88 (t, 3 H, J = 6 Hz), 1.1–1.6 (16 H), 1.6–2.4 (complex abs, 6 H), 2.76 (m, 2 H), 5.35 (m, 4 H), 7.0–7.6 (complex abs, 5 H)
NPOA	72	43	$C_{24}H_{39}NO$ (357.5)	3300, 1665, 1600	0.90 (t, 3 H, J = 6 Hz), 1.1–1.6 (22 H), 1.6–2.4 (complex abs, 6 H), 5.32 (t, H, J = 5 Hz), 7.0–7.6 (complex abs, 5 H)

[a] All products gave satisfactory microanalyses: C, ±0.21; H, ±0.37; N, ±0.07.

demonstrates the necessary role of modern high-perform-ance analytical techniques in assessing xenobiotic product purity for toxicological studies. In these cases, verification of toxicant purity must be approached rigorously; otherwise, the results of associated toxicological studies may be in error.

LITERATURE CITED

Artigas, F.; Martínez, E.; Rosellô, J.; Suñol, C.; Tusell, J. M.; Gelpi, E. In *Collected Papers for Programme on the Study of Spanish Toxic Oil Syndrome*; Pestaña, A., Ed.; CSIC: Madrid, 1983; Vol. 1, p 100.
Avramovici-Grisaru, S.; Sarel, S. *Nouv. J. Chim.* **1982,** *6,* 455.
Casals, C.; Garcia-Barreno, P.; Municio, A. M. *Biochem. J.* **1983,** *212,* 339.
Clark-Still, W.; Kahn, M.; Mitra, S. *J. Org. Chem.* **1978,** *43,* 2923.
De Vera, N.; Cristofol, R. M.; Camón, L.; Planas, A.; Rodriguez-Farré, E. In *Collected Papers* for *Programme on the study of Spanish Toxic Oil Syndrome*; Pestaña, A., Ed.; CSIC: Madrid, 1983; Vol. 2; p 201.
Fernández-Alvarez, E. In *Collected Papers for Programme on the Study of Spanish Toxic Oil Syndrome*; Pestaña, A., Ed.; CSIC: Madrid, 1983; Vol. 2, p 11.
Gelpi, E. *Trends Anal. Chem.* **1985,** *4,* vi.
Granjean, P.; Tarkowski, S. *The Toxic Oil Syndrome*, Report on WHO Meeting of Working Group on Denatured Rapeseed Oil Toxicology Syndrome, Madrid, March 21–23, 1983; WHO: Copenhagen, 1984.
Hegarty, A. F.; McCormack, M. T.; Ferguson, G.; Roberts, P. J. *J. Am. Chem. Soc.* **1977,** *99,* 2015.
Mancha, M. *Grasas Aceites* **1982,** *33,* 85.
Pestaña, A.; Muñoz, E. *Nature* **1982,** *298,* 608.
Tabuenca, J. M. *Lancet* **1981,** *2,* 567.
Toxic Epidemic Syndrome Study Group. Toxic Epidemic Syndrome, Spain 1981. *Lancet* **1982,** *2,* 697.

Tocopherols and Tocotrienols in Finnish Foods: Vegetables, Fruits, and Berries

Vieno Piironen, Eeva-Liisa Syväoja, Pertti Varo, Kari Salminen, and Pekka Koivistoinen*

This study is part of a survey carried out to determine the tocopherol and tocotrienol contents of Finnish foods. Tocopherols and tocotrienols from 75 commodities of vegetables, fruits, berries, and their respective processed products and those of almond, peanut, and mushrooms were analyzed by using an HPLC method. α-Tocopherol was the predominant compound in all other samples except in pea, pea products, bean, cauliflower, raspberry, and potato chips where γ-tocopherol predominated. Small amounts of β- and δ-tocopherols as well as α- and γ-tocotrienols were also found. The proportions of non-α-tocopherols and tocotrienols were highest in berries. The highest α-tocopherol values (>1 mg/100 g, fresh weight) among the fresh vegetables analyzed were found in dark green leafy vegetables and in sweet pepper. The α-tocopherol content of fruits ranged from 0.06 to 0.96 mg/100 g and that of berries from 0.56 to 4.14 mg/100 g. No tocopherols were found in mushrooms. α-Tocopherol was found to be quite stable in freezing as well as in canning and marmalade and jam making, but only small amounts of α-tocopherol were detected in juices.

Plants synthesize tocopherols (α-, β-, γ-, δ-tocopherols) and tocotrienols (α-, β-, γ-, δ-tocotrienols). No chlorophyll *a* containing higher plant tissue has been proved devoid of tocopherols (Booth, 1963). α-Tocopherol is the major tocopherol in chlorophyll-containing tissue, and it is localized in chloroplasts (Booth, 1963; Bucke, 1968; Newton and Pennock, 1971; Janiszowska and Korczak, 1980). Non-α-tocopherols are situated mainly in nongreen tissues such as vegetable oils, nuts, fungi, and cereal grains (Newton and Pennock, 1971), and in chlorophyll-containing tissue they are localized mainly outside the chloroplast (Booth, 1963; Newton and Pennock, 1971; Janiszowska and Korczak, 1980). Tocotrienols have been found in carrots (McLaughlin and Weihrauch, 1979; Leth and Andersson,

1982), kale and broccoli (Leth and Andersson, 1982), mushrooms (McLaughlin and Weihrauch, 1979), and vegetable oils and cereal grains (McLaughlin and Weihrauch, 1979; Bauernfeind, 1980; Piironen et al., 1986; Syväoja et al., 1986). However, generally only α-tocopherol is determined, and knowledge of the tocopherol and tocotrienol composition of vegetables, fruits, and berries is poor.

α-Tocopherol concentrations have been shown to be high in dark green tissues, moderate in fast-growing leaves, light green tissues, and colored fruits, and low in roots, etiolated tissues, and colorless fruits (Booth and Bradford, 1963). α-Tocopherol content is high (1.8–14.5 mg/100 g, fresh weight) in for example dandelion leaves, mint leaves, nettle leaves, spinach, parsley, and asparagus (Booth and Bradford, 1963; Leth, 1975; McLaughlin and Weihrauch, 1979). Large amounts of α-tocopherol were also found in wild blackberries (Booth and Bradford, 1963) and in pepper fruit (Kanner et al., 1979).

Variation in the α-tocopherol values of vegetables, fruits, and berries is caused, in addition to variation between species, by many other factors. One factor is the variety

Department of Food Chemistry and Technology, University of Helsinki, SF-00710 Helsinki 71, Finland (V.P., P.V., P.K.), and Research and Development Department, Valio Finnish Co-operative Dairies' Association, Kalevankatu 56 B, SF-00180 Helsinki 18, Finland (E.-L.S., K.S.).