trans-Polyunsaturated Fatty Acids in French Edible Rapeseed and Soybean Oils

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The fatty acid compositions of rapeseed and soybean oils marketed in France have been determined by gas liquid chromatography on a fused-silica capillary column coated with a 100% cyanopropyl polysiloxane stationary phase. Under the operating conditions employed, methyl esters of linolenic acid geometrical isomers could be separated and quantitated easily without any other complementary technique. With only one exception, all samples under study (eight salad oils and five food samples) contain geometrical isomers of linolenic acid in measurable, although variable, amounts. Total *trans*-18:3 acids may account for up to 3% of total fatty acids. This value corresponds to a degree of isomerization (percentage of *trans* isomers relative to total octadecatrienoic acids) of 30%. Examination of our data indicates that the distribution pattern of linolenic acid geometrical isomers does not depend on the degree of isomerization. The two main isomers always have the *c,c,t* and the *t,c,c* configurations. These isomers occur in the almost invariable relative proportions of 47.8 ± 1.7% and 41.1 ± 1.0%, respectively. The third mono-*trans* isomer is present in lower amounts—6.5 ± 0.7%. The only di-*trans* isomer that can be quantitated with sufficient accuracy is the *t,c,t* isomer (4.9 ± 1.5%). Mono-*trans* isomers of linoleic acid are also present in these oils. However, their maximum percentages are lower than those determined for linolenic acid geometrical isomers. In the oils showing the highest degrees of isomerization, *trans* isomers of linoleic acid account for 0.5% (rapeseed oils) and 1% (soybean oils) of total fatty acids. Taking into account all data, it would appear that the probability of isomerization of linolenic acid is about 13–14 times that of linoleic acid.

KEY WORDS: Fatty acid composition, linoleic acid, linolenic acid, rapeseed oil, soybean oil, *trans* isomers.

Deodorization is an important step in rapeseed and soybean oil refining. This operation is realized at high temperature (optimum range of 245–250°C) under vacuum (absolute pressure of 1–6 mm Hg) for 15 min to several hours in the presence of steam (1). However, such conditions have been shown to induce geometrical isomerization of linoleic (*cis*-9,*cis*-12 18:2, further referred to in the text as the *c,c* or all-*cis* isomer) and linolenic (*cis*-9,*cis*-12,*cis*-15 18:3, further referred to in the text as the *c,c,c* or all-*cis* isomer) acids (2,3). Refined soybean and rapeseed oils marketed in North America may contain up to 25% of their total 18:3 acids as geometrical isomers (2). The main isomers that accumulate have the *c,c,t* and the *t,c,c* structures. These artefacts are accompanied by smaller amounts of the *c,t,c* and *t,c,t* isomers (2).

The same isomers have been recently identified in the fat from low-calorie spreads marketed in France (4). These products are mixtures of vegetable oils and/or dairy fats. In some instances, they contain rapeseed or soybean oil, which are responsible for the presence of linolenic acid geometrical isomers (4). As the degree of isomerization of linolenic acid in these spreads may reach 28% (4), it was of interest to extend the study to edible rapeseed and soybean oils in France. In the present study, we have analyzed most of the commercial samples of rapeseed and soybean oils available in France. We also have analyzed the oil from some foods believed to contain rapeseed or soybean oils. Generally, the labels on these foods are vague about the exact nature of the oil. Our attention was focused on the detection of linolenic acid geometrical isomers in these oils.

EXPERIMENTAL PROCEDURES

Samples and chemicals. Salad oils (rapeseed and soybean oils or mixtures of both) and foods (mayonnaise, canned tuna fish in oil, tartar and mayonnaise sauces) were purchased from local supermarkets in January and February, 1991, and immediately analyzed. Linolenic and linoleic acid geometrical isomers were prepared by elaidination of linseed oil essentially as described by Grandgirard *et al.* (5). Identification of individual isomers in edible oils was realized as described elsewhere (6) using elaidinized linseed oil fatty acid methyl esters as reference compounds.

Oil extraction and fatty acid methyl esters (FAME) preparation. Oil was extracted from foods (with the exception of oil from canned tuna fish, which was directly pipetted and treated like salad oil) as follows. An aliquot of food is weighed in a Teflon beaker and dispersed in isopropanol (10 mL) with an Ultra-Turrax T25 homogenizer (Janke & Kunkel GmbH & Co. KG, Staufen, Germany) equipped with an S 25 N 10 G shaft. Sufficient anhydrous Na_2SO_4 is added to the suspension in order to remove water. Hexane (15 mL) is poured in the beaker and the suspension is homogenized a second time. A portion of the suspension (2.5 mL) is withdrawn with an all-glass syringe and filtered through a single-use filter unit (Millex-GV; Millipore, Molsheim, France) into a Teflon-lined screw-capped tube. Solvents and volatile substances are then removed under a stream of N_2 in a water bath at 45°C and 1.5 mL of a 12% (w/v) solution of BF_3 in methanol (Fluka, Buchs, Switzerland) is added to the fat extract (or to 2 drops of oil). The resulting suspension is homogenized with benzene (7). The reaction is performed in an oil bath at 95°C for 45 min (7). After cooling the tube, hexane (2.5 mL) and water (1.5 mL) are added successively. FAME are extracted a second time with hexane (2.5 mL). The pooled extracts are stored at −20°C. In some instances, FAME have been fractionated by thin-layer chromatography (TLC) on silica gel plates impregnated with $AgNO_3$ ($AgNO_3$-TLC) as described in detail elsewhere (4).

Gas-liquid chromatography (GLC). Analyses of FAME by GLC were carried out on a Carlo Erba 4130 chromatograph equipped with a flame ionization detector and a split injector (Carlo Erba, Milano, Italy). A fus-

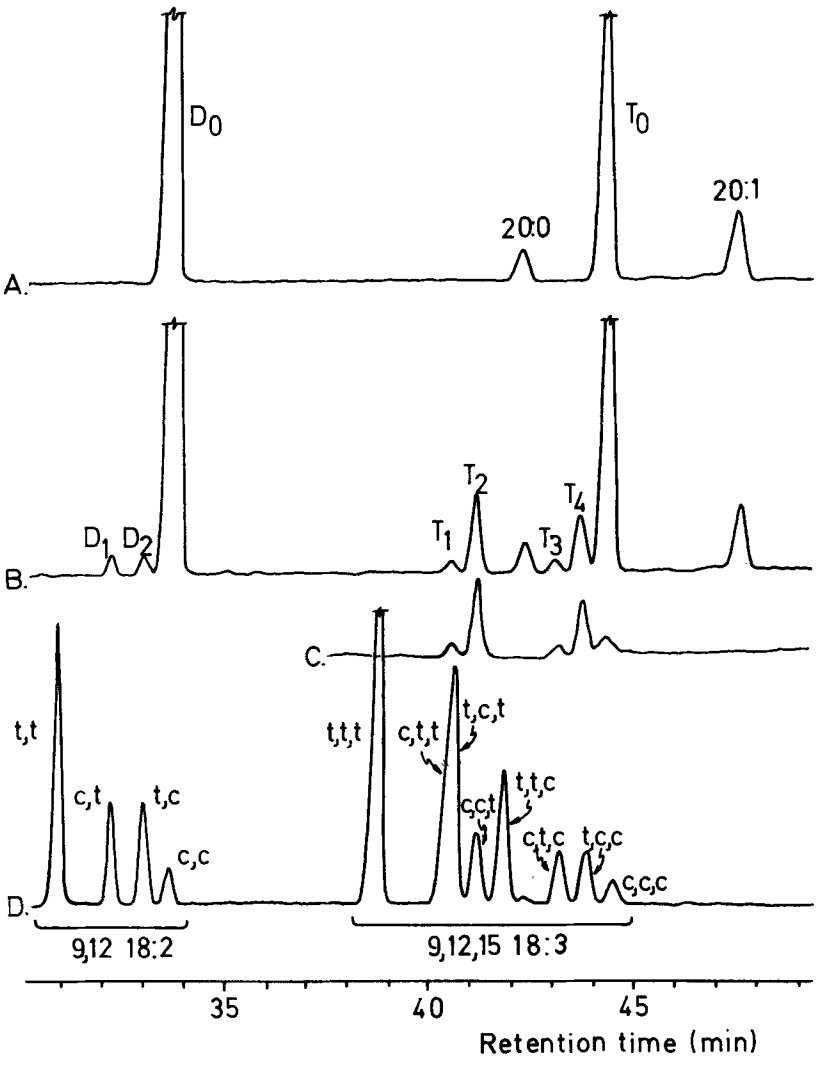

FIG. 1. Partial chromatograms of FAME prepared with: A, oil extracted in the laboratory from rapeseed; B, commercial rapeseed oil; C, commercial rapeseed oil after $AgNO_3$-TLC fractionation; and D, elaidinized linseed oil. Peaks D_0 and T_0 are the all-*cis* isomers of 18:2 and 18:3 acids, respectively. Peaks D_1 and D_2 are the *c,t* and *t,c* isomers of linoleic acid, respectively. Peaks T_1, T_2, T_3 and T_4 correspond to geometrical isomers of linolenic acid having the structures *t,c,t, c,c,t, c,t,c* and *t,c,c*, respectively. Analyses on a CP™Sil 88 fused-silica capillary column.

ed silica capillary column coated with 100% cyanopropyl polysiloxane (CP™Sil 88, 50 m × 0.33 mm i.d., 0.24 μm film; Chrompack, Middelburg, Holland) was used with helium as carrier gas (inlet pressure, 0.8 kg/cm²). The column was operated isothermally at 150°C for 50 min. The temperature was then increased at a rate of 7.5°C/min up to 195°C and held at this temperature until completion of the analysis. The injection port and the detector were maintained at 250°C. Quantitative analyses were performed with an SP 4290 integrator (Spectra Physics, San Jose, CA).

RESULTS AND DISCUSSION

A partial chromatogram of FAME prepared with rapeseed oil is shown in Figure 1. This chromatogram is compared with a chromatogram of FAME prepared with oil extracted from rapeseed in the laboratory, and with a chromatogram of FAME prepared with elaidinized linseed oil. It is clear that the extra peaks found in the commercial oil are due to the refining process. They correspond to linoleic and linolenic acid geometrical isomers. This last assignment is confirmed by analysis (Fig. 1) of a fraction migrating between all-*cis* linolenic acid and all-*cis* linoleic acid after $AgNO_3$-TLC separation of total FAME prepared with rapeseed oil. In addition, it is known that heat treatments (deodorization or deep frying) of rapeseed or soybean oils do not induce any significant positional isomerization of the ethylenic bonds (2,5). The two main artefacts correspond to the *c,c,t* (peak T_2) and to the *t,c,c* (peak T_4) isomers of linolenic acid. The two other small peaks correspond to isomers having the *t,c,t* (peak T_1)

and the *c,t,c* (peak T_3) configurations. In those oils showing a high degree of isomerization, two other components can be observed when the *trans*-octadecatrienoic acid fraction is analyzed by GLC at high loads. These components have equivalent chainlength values identical to those of the two other di-*trans* isomers (*c,t,t* and *t,t,c*) (results not shown). However, these isomers were too low to be quantitated (less than 0.01 to 0.02% of total fatty acids in those oils showing the highest degree of isomerization), and are not included in this study. The all-*trans* isomer was not detected. Analyses by GLC of FAME in the conditions described in the Experimental Procedures section allow an almost base-line resolution of the four major isomers of linolenic acid, without interference from either 20:0 or 20:1 acids. Therefore, quantitation of these isomers is immediate and does not require any complementary technique. However, the time of analysis is relatively long—the retention time of the all-*cis* isomer is about 45 min.

All samples under study contained geometrical isomers of linolenic acid (Table 1), from trace amounts up to 3% of total fatty acids. One sample (rapeseed oil from canned tuna fish in vegetable oil, not included in Table 1) showed two tiny peaks corresponding to the *c,c,t* and *t,c,c* isomers that could not be taken into account by the integrator. The degree of isomerization varies from less than 2% to almost 30%. As demonstrated by a few authors (2,3), geometrical isomerization of linolenic acid during deodorization increases with temperature and heating time. This would indicate that the conditions used during the deodorization step also vary widely from one producer to another. A sample of rapeseed oil from Poland that we also analyzed had a *trans*-18:3 acid content of 0.86 ± 0.03%, corresponding to a degree of isomerization equal to 8.8 ± 0.1% (mean ± S.D. of five analyses; values not included in Table 1). A similar degree of isomerization (8.3%) can be calculated from published analytical data concerning an Israeli brand of soybean oil (8). This suggests that isomerization of linolenic acid during deodorization is of more general relevance than previously believed. In the case of rapeseed oils, the sum of all linolenic acid geometrical isomers (including the natural all-*cis* isomer) is practically constant. Values obtained in this way vary from 8.7 to 10.2% of total fatty acids (Table 1). This exactly corresponds to the linolenic acid content of oils extracted in the laboratory from seven different varieties of rapeseed cultivated in France (unpublished results), which varies from 8.9 to 10.4%. This means that the major effect of deodorization of rapeseed oil on linolenic acid is apparently limited to geometrical isomerization. This probably also holds true for soybean oil. The distribution pattern of linolenic acid geometrical isomers is almost the same for all samples (Table 1), independent of their origin and degree of isomerization. The main isomers always have the *c,c,t* and *t,c,c* structures, and they occur in the almost invariable relative proportions 47.6 ± 1.3 and 41.1 ± 1.0%, respectively. They are always accompanied by minor amounts of the *c,t,c* (6.5 ± 0.7%) and *t,c,t* (4.9 ± 1.5%) isomers. These values are quite close to values previously reported for low-calorie spreads (4). In this study, the *t,c,t* plus *c,c,t* isomers accounted for 52.0–54.5% of total *trans*-18:3 isomers. Values for the *c,t,c* and *t,c,c* isomers were in the ranges 4.4–5.7% and 41.1–42.3%, respectively. Therefore, it can be deduced that the ethylenic bond in position 9 is slightly more sensitive to geometrical isomerization than is the ethylenic bond in position 15. On the other hand, it appears that the double bond in position 12 is considerably more resistant than the two other double bonds. Similar observations were reported in previous studies (2,4). Simultaneous isomerization of double bonds in positions 9 and 15 (which leads to the *t,c,t* isomer) is lower than can be predicted from the probabilities of isomerization of individual double bonds. This would indicate that isomerization of one of the two double bonds diminishes the probability of isomerization of the second double bond in the same molecule. The phenomena that occur during deodorization under vacuum are apparently different from those that take place during heating of the oils in the presence of air. Although this treatment leads to the same geometrical isomers of linolenic acid, there is an overall decrease of total octadecatrienoic acids with temperature and heating time (9,10). Possibly, oxidation, cyclization and polymerization compete with or follow geometrical isomerization. Consequently, the relative proportions of individual *trans* isomers of linolenic acid determined under such conditions are different from those presented in this study (9,10).

Small amounts of mono-*trans*-isomers of linoleic acid were also detected in the oils (peaks D_1 and D_2 in Fig. 1). However, their levels (maximum values: 0.5% and 1% in rapeseed and soybean oils, respectively) are generally lower than those determined for linolenic acid geometrical isomers (Table 1). In this instance too, the ethylenic bond near the carboxylic end (position 9) appears to be slightly more susceptible to *cis-trans* isomerization than the ethylenic bond on the methyl side (position 12) of the molecule (*ca.* 55% *vs.* 45% of the *c,t* and *t,c* isomers, respectively) (Table 1). The ratio between the degrees of isomerization calculated for 18:3 and 18:2 acids (D.I. 18:3 / D.I. 18:2) seems to be constant (Table 1). This ratio equals to 13.4 ± 1.4 (mean ± S.D. of ratios calculated for all samples with the exception of sample M). It indicates that the probability for a 18:3 molecule to be isomerized during deodorization is 13–14 times higher than that for a 18:2 molecule. The exception to this rule in Table 1 (sample M, last column) may correspond to a selectively hydrogenated soybean oil or to a mixture of oils deodorized under different conditions. Non-methylene-interrupted dienes were not observed in the chromatographic zone where these isomers are expected to elute from cyanoalkyl polysiloxane-coated capillary columns (11–15).

According to Ackman *et al.* (2), partial hydrogenation is "unlikely to generate methylene-interrupted geometrical isomers of linolenic acid in any high proportions of all-*cis* 18:3". Indeed, these isomers are hardly detectable in soybean oil samples that were hydrogenated with a sulfur-containing nickel catalyst (13). One cannot exclude that linolenic acid geometrical isomers that were detected in a U.S. sample of partially hydrogenated soybean oil (salad oil) (16) may have appeared during a deodorization step following partial hydrogenation. Although hydrogenation reduces the level of 18:3 acid, any further heat treatment, such as deodorization, can still produce some linolenic acid geometrical isomers. However, their amounts will be limited. On the other hand, linoleic acid geometrical isomers can appear during both partial hydrogenation and deodorization. Consequently, their level in partially hydrogenated soybean and rapeseed oils will be higher than that of linolenic acid geometrical isomers. This is not

TABLE 1

***trans*-Polyunsaturated Fatty Acid Content in Rapeseed and Soybean Oils and in Foods Containing These Oils**

	RSO 1[a]	RSO-SBO	SBO 1	RSO 2
Total *trans* 18:3	2.99 ± 0.05[b]	1.97 ± 0.02	1.91 ± 0.03	1.87 ± 0.02
All-*cis* 18:3	7.10 ± 0.06	8.30 ± 0.07	5.09 ± 0.06	8.30 ± 0.10
Total 18:3[c]	10.09 ± 0.08	10.27 ± 0.08	7.01 ± 0.09	10.17 ± 0.12
D.I. 18:3[d]	29.6 ± 0.3	19.2 ± 0.2	27.2 ± 0.4	18.4 ± 0.1
t,c,t[e]	7.1 ± 0.7	3.7 ± 0.3	7.1 ± 0.5	3.5 ± 0.3
c,c,t	45.9 ± 0.6	47.7 ± 0.6	46.0 ± 0.6	48.0 ± 0.3
c,t,c	6.8 ± 1.0	7.2 ± 0.7	6.5 ± 0.4	7.1 ± 0.5
t,c,c	40.1 ± 0.6	41.4 ± 0.5	40.4 ± 1.0	41.4 ± 0.3
Total *trans* 18:2	0.47 ± 0.03	0.34 ± 0.02	1.04 ± 0.05	0.30 ± 0.03
All-*cis* 18:2	21.27 ± 0.09	24.55 ± 0.17	52.12 ± 0.22	21.95 ± 0.22
Total 18:2	21.74 ± 0.11	24.89 ± 0.16	53.16 ± 0.27	22.25 ± 0.22
D.I. 18:2	2.2	1.4	2.0	1.4
c,t	52.8 ± 2.0	52.7 ± 1.7	53.6 ± 0.9	53.2 ± 1.3
t,c	47.2 ± 2.0	47.3 ± 1.7	46.4 ± 0.9	46.8 ± 1.3
D.I. 18:3/D.I. 18:2	13.5	13.7	13.6	13.1

	RSO 3	RSO4	SBO2	SBO3
Total *trans* 18:3	1.82 ± 0.03	1.38 ± 0.02	0.55 ± 0.02	0.16 ± 0.02
All-*cis* 18:3	7.58 ± 0.06	7.28 ± 0.02	6.00 ± 0.02	6.92 ± 0.10
Total 18:3	9.39 ± 0.08	8.65 ± 0.03	6.55 ± 0.03	7.06 ± 0.11
D.I. 18:3	19.4 ± 0.3	16.0 ± 0.2	8.4 ± 0.1	2.3 ± 0.3
t,c,t	4.8 ± 0.5	4.2 ± 0.2	5.9 ± 0.7	tr[E]
c,c,t	47.6 ± 0.5	48.6 ± 0.6	48.4 ± 1.2	51.6 ± 2.3
c,t,c	6.1 ± 0.5	5.4 ± 0.4	7.5 ± 0.2	
t,c,c	41.5 ± 0.4	41.8 ± 0.6	39.0 ± 1.3	48.4 ± 1.3
Total *trans* 18:2	0.33 ± 0.02	0.25 ± 0.01	0.27 ± 0.03	0.10 ± 0.02
All-*cis* 18:2	22.98 ± 0.14	21.33 ± 0.09	52.24 ± 0.25	53.04 ± 0.55
Total 18:2	23.31 ± 0.16	21.58 ± 0.09	52.51 ± 0.26	53.14 ± 0.55
D.I. 18:2	1.4	1.2	0.5	0.2
c,t	54.5 ± 1.5	56.2 ± 3.6	63.7 ± 3.6	49.7 ± 5.1
t,c	46.5 ± 1.5	46.8 ± 3.6	36.3 ± 3.6	50.3 ± 5.1
D.I. 18:3/D.I. 18:2	13.9	13.3	16.8	11.5

	MS (RSO)	TS (SBO)	TFO (RSO)	M (UO)
Total *trans* 18:3	1.72 ± 0.02	1.52 ± 0.02	1.26	0.25 ± 0.02
All-*cis* 18:3	7.59 ± 0.05	5.19 ± 0.02	7.98	0.70 ± 0.01
Total 18:3	9.31 ± 0.06	6.71 ± 0.02	9.24	0.95 ± 0.02
D.I. 18:3	18.5 ± 0.2	22.7 ± 0.2	13.7	25.9 ± 1.0
t,c,t	3.6 ± 0.6	6.2 ± 0.4	3.2	5.7 ± 0.6
c,c,t	48.5 ± 0.3	45.8 ± 0.6	49.7	46.4 ± 2.3
c,t,c	6.4 ± 0.5	6.2 ± 0.4	5.6	
t,c,c	42.0 ± 1.0	41.7 ± 0.6	41.6	47.9 ± 2.8
Total *trans* 18:2	0.32 ± 0.02	0.90 ± 0.01	0.26	0.30 ± 0.04
All-*cis* 18:2	23.07 ± 0.08	52.00 ± 0.65	22.12	57.14 ± 0.06
Total 18:2	23.39 ± 0.10	52.90 ± 0.65	22.38	57.44 ± 0.06
D.I. 18:2	1.4	1.7	1.2	0.5
c,t	54.3 ± 2.1	53.3 ± 0.7	52.9	60.8 ± 3.1
t,c	45.7 ± 2.1	46.7 ± 0.7	47.1	39.2 ± 3.1
D.I. 18:3/D.I. 18:2	13.2	13.4	11.3	51.8

[a]RSO_x and SBO_x, samples of salad oils composed of pure rapeseed or soybean oil, respectively; RSO-SBO, salad oil made of a mixture of 90% RSO and 10% SBO; MS, vegetable oil from mayonnaise sauce; TS, vegetable oil from tartar sauce; TFO, vegetable oil from canned tuna fish in vegetable oil; M, vegetable oil from mayonnaise. Identification of the oils extracted from foods between parentheses; UO, unidentified oil.

[b]Percentages of total *trans* 18:3, all-*cis* 18:3 and total 18:3 acids are relative to total fatty acids and are means ± S.D. of 3–5 analyses by GLC, except for TFO (1 analysis). Same for 18:2 acids.

[c]Total 18:3 (or 18:2) is the sum of all 18:3 (or 18:2) isomers, including the all-*cis* isomer.

[d]Degree of isomerization for the indicated fatty acid–ratio of total *trans* 18:3 (or *trans* 18:2) on total 18:3 (or 18:2) times 100.

[e]Percentages of individual geometrical isomers are relative to their total. Configurations of ethylenic bonds (*c,cis; t, trans*) are given in the order 9, 12, 15 for 18:3 acids, and 9, 12 for 18:2 acids.

[E]tr, Trace.

the case for deodorized, unhydrogenated oils such as those analyzed in this study. This would explain why geometrical isomers of linolenic acid are so seldomly observed or mentioned, even in the most extensive studies of margarines, margarine-like foods and fat from margarine-containing foods, whereas isomers of linoleic acid are present in significant amounts (17,18).

It has recently been reported that some margarines in Canada also contain high levels of isomerized polyunsaturated fatty acids, including some geometrical isomers of linolenic acid (11,12). Although these isomers are generally low compared to linoleic acid isomers, they may occur in higher quantities than the all-*cis* linolenic acid itself (11). From the present knowledge, it is not possible to decide which parameters have to be taken into account for nutritional evaluation of linolenic acid geometrical isomers. If they compete with the natural and essential all-*cis* form, one should then consider their percentage relative to the sum of octadecatrienoic acids. From this study and others (2,4,8,11,16,19), it is clear that these artefacts are widespread and that their percentages relative to total 18:3 acids can be high—up to 30% in salad oils (2,16, this study) and low-calorie spreads (4); 36% in fried oils collected from restaurants (19); and up to 60% in tub margarines (11). These impressive values clearly show that more light should be rapidly cast out on the nutritional effects of linolenic acid geometrical isomers.

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[Received June 18, 1991; accepted October 23, 1991]