Lactones in Autoxidized Vegetable Oils[1]

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Abstract

It was demonstrated that both *gamma* and *delta* saturated lactones are present in highly peroxidized vegetable oils. In the oils which were investigated the *gamma* isomers are predominant. Additional lactones also form when the hydroperoxides are reduced. Although no lactones were detectable in fresh, refined soybean oil, considerable amounts of both *gamma* and *delta* lactones were found to be present in highly peroxidized samples of cottonseed and soybean oils.

The lactones in the peroxidized oils were concentrated by column chromatography on silica gel and by vacuum distillation. Gas-liquid chromatography was used for separation and identification. This has been supplemented by thin-layer chromatography, infrared spectrophotometry, and nuclear-magnetic-resonance spectrometry.

Introduction

DURING THE PAST DECADE the attention of the food chemist has focused on the aliphatic saturated lactones (C_8–C_{12}) because of their aromatic properties. To date considerable work has been done on dairy products. Kenny, Patton, and co-workers identified *delta* deca- and dodecalactones as the coconut-like flavor in stored milk fat (1,2). Boldingh (3) and Jurriens (4) and their colleagues at the Unilever Research Laboratories have reported on the identification of lactones in butterfat. Recently they have examined, in detail, the methods for isolation and identification of flavor components of butterfat and reported saturated *delta* lactones with 6–16 carbon atoms (23). Urbach has used thin-layer chromatography to find *delta* lactones in Australian butter oil (5). Day and Libbey have combined GLC with mass spectrometry to show the presence of lactones in the neutral components of cheddar cheese flavor (6) and in the volatile components of *gamma*-irradiated milk fat (20). The results of all of these investigations emphasize that a) both *gamma* and *delta* lactones occur in milk fat, and the precursors of these are the corresponding hydroxy acids; b) lactones can exist both as "free" or as "bound" esterified hydroxy acids; and heating promotes lactone formation by freeing the bound hydroxy acids; and c) the *delta* lactones are present in much larger quantity than the *gamma* lactones.

Lactones have been found in other products also. Silverstein isolated caprolactone from pineapple (7), and the *gamma* C_7 and C_9 homologs have been identified in peach flavor (8). Allen found all of the even-numbered *delta* lactones from C_6 to C_{14} in coconut oil (9). Chang and his associates (10) demonstrated the presence of *gamma* hexa- and nonalactones in the volatiles from the hydrogenation of soybean oil. They also postulated a mechanism for the formation of *gamma*-hexalactone. The decomposition of the hydroperoxide of linolenic acid yields 3-*cis*-hexenoic acid, which by subsequent oxidation and hydrogenation gives the saturated lactone. Yet Boldingh and Taylor (3) maintain that lactones originate only from hydroxy acids which are already present in milk fat and not from autoxidation. One of the latest publications on this subject (24) shows that *delta* lactones are prevalent in the fat of various mammals. Here again, the lactones are said to arise from a nonoxidative process.

The perfume and flavor manufacturers are also concerned with lactones. *Gamma* valero-lactone is used to accent floral bouquets, and *gamma* octa- and nonalactones are suitable for the formulation of a coconut-like aroma (11). One whiff of *gamma*- undecalactone is enough to explain why it is used in peach-like flavor preparations. Coumarin and other aromatic lactones (13), β-propiolactone (12), and other α,β-unsaturated lactones (14) have been shown to possess undesirable physiological effects. Of these, the *gamma* lactone of 2-hexenoic acid is of particular interest because this compound has been tentatively identified in reverted soybean oil (21). Three unsaturated lactones have also been found in milk fat (3,25). In this phase of work on the oxidation products of fats vegetable oils were examined for the presence of lactones. The initial report focuses on the recovery and identification of the C_4 and C_{12} aliphatic lactones. Work now in progress will concentrate on the improvement of the recovery techniques and on the detection of the longer chain-length and unsaturated lactones.

Experimental Procedure and Data

Preparation of Oxidized Oils

Refined soybean oil (SBO) was saponified according to the AOCS procedure (16) except that the sodium hydroxide was substituted for the KOH. The harder, easier-to-handle sodium soaps were washed three times with ethyl ether to remove unsaponifiables. The fatty acids were liberated by the addition of 6N HCl to pH 2.0 and extracted with ether; the extract was dried over anhydrous $MgSO_4$. To promote the migration of double bonds toward the carboxyl group (17) so as to favor lactone formation (22), the fatty acids were heated for one hour at 180C with 0.2% of Harshaw nickel catalyst in a nitrogen atmosphere. This isomerization step was carried out with Sample I (Table I) but was omitted with Sample II. Prior to oxidation the fatty acids were methylated by refluxing for one hour with excess methanol containing 1% concentrated H_2SO_4 (v/v). The methanol was evaporated, and the esters were taken up in ether, washed with water, and dried over anhydrous $MgSO_4$. The dried esters were oxidized by bubbling oxygen through the oil at elevated temperature by using a sintered glass bubbler. The introduction of the gas was continued at a slow but undetermined rate until the sample was removed for analysis. Upon termination of the oxidation the samples were analyzed for peroxide value (18), free fatty acid content (19), and saponification value (16), as shown in Table I. Sample III was a refined cottonseed oil (CSO), which had been heated in an open beaker with gentle stirring for 42 days at 80C. It was subsequently stored for 20 months at room temperature prior to analysis.

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TABLE I
Oxidation Data

Sample		Time	T (°C)	Mode of oxidation	PV	FFA
I	(SBO)	64 hrs	75	Bubbling O_2	664	27.8
II	(SBO)	69 hrs	80	Same	364	35.2
III	(CSO)	42 days	80	Air	268	0.55

Isolation of Lactones

The oxidized methyl esters were dissolved in ethyl ether and washed with 5% Na_2CO_3 to remove the free fatty acids. Unfortunately, as was later found, this also removes appreciable amounts of the shorter-chain lactones (25). The acid-free esters were then chromatographed on a 60 × 5 cm. silica gel column. The column was prepared by slurrying 50 g of silica gel (Grace Davison Chemicals, grade 923, 100–200 mesh) in 100 ml of petroleum ether (bp 30–60C) containing three ml of methanol (for partial inactivation). For each 40 g of sample approximately 200 g of dry silica gel were used. The oxidized esters, dissolved in petroleum ether, were added to the column and eluted with solvents in the following sequence: petroleum ether, ethyl ether, and methanol. The elution patterns and the corresponding quantitative results which were obtained with the oxidized soybean oil methyl esters (Samples I and II) are shown in Table II. Fraction B, containing hydroperoxy, epoxy, and monohydroxy esters was then treated with methanolic NaOH to saponify the esters and simultaneously to reduce the hydroperoxides. The resulting soaps were acidified, and the fatty acids were taken up in ether. Subsequent drying over anhydrous $MgSO_4$ facilitated lactonization of the 4 and 5-hydroxy acids. The lactones were then recovered by vacuum distillation for three hours at 100–110C and at a pressure of 5–10 microns of Hg. Trapping was achieved by using a cold finger filled with liquid nitrogen.

The heated cottonseed oil (Sample III) was first subjected to vacuum distillation to remove any volatile free lactones present; the oil was then saponified, acidified, and redistilled to obtain the "bound" lactone fraction. When a fresh sample of soybean oil (PV 1.6 meq/kg) was analyzed by this scheme, no lactones could be detected.

Analysis of the Distillates

Thin-layer chromatography (TLC) was carried out by using commercially available (Analtech Inc.) 250 microns silica gel G plates. Figure I is a photograph of a plate developed with petroleum ether/ethyl ether/acetic acid, 75/25/1. For visualization it was sprayed with 50% sulfuric acid and subsequently heated for 5 min at 135C. Lanes 1 and 11 represent a lactone mixture containing C_4–C_{11} and C_{18} *gamma* lactones as well as C_8–C_{12} *delta* lactones. Lane 2 is a mixture of C_4–C_{18} straight-chain fatty acids. Lane 3 (barely visible) is sebacic acid, and Lanes 4 and 5 are *cis*-epoxystearic and 12-keto-oleic acids respectively. Lane 6 is a mixture of ricinoleic and other fatty acids from castor oil. Lane 7 contains 9,10 dihydroxystearic acid, and Lanes 8–10 show *gamma* stearolactone, *gamma* C_5 and C_6 lactones, and the *delta* C_9–C_{12} lactones respectively. The lower spots visible in the lactone lanes (1,9–11) represent the 4- and 5-hydroxy acids.

TABLE II
Fractionation of Oxidized Soybean Oil Methyl Esters on Silica Gel

Fraction	Eluent	Elution Vol. (ml) I	Elution Vol. (ml) II	Recovered Wt. (%) I	Recovered Wt. (%) II	Ester Description
A	Pet ether	300	600	31.5	13.6	Unoxidized
B	Ethyl ether	600	600	61.8	73.5	Hydroperoxy epoxy monohydroxy lactones
C	Methanol	400	600	6.2	10.5	Polyhydroxy

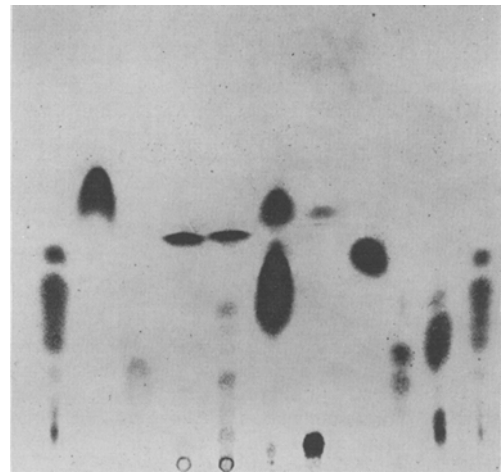


FIG. 1. Lactones, fatty acids, and other oxidation products.

Figure 2 shows the results which were obtained with the lactone distillates. The developing solvent was petroleum ether/ethyl ether/acetic acid-65/35/2. Again, the first and last lanes are the known lactone mixtures. Lane 2 is the distillate from Sample I (Table I), Lane 3 is the distillate from Sample II, and Lane 4 is the same distillate after esterification with diazomethane. Lanes 5 and 6 are from the oxidized cottonseed oil (Sample III), representing the free and "bound" lactones respectively. Lane 7 contains the volatiles from unoxidized cottonseed oil (PV 2.4 meq/kg) which were distilled for one hour at 165–170C and at a pressure of 5–10 microns of Hg.

Most of the gas-liquid chromatography (GLC) on the distillates was carried out by using an F&M 810 Gas Chromatograph with a flame ionization detector. The columns used were: 6 ft, ⅛ in., 3% QF-1 on 100/120 mesh Gas-Chrom Q and 6 ft, ⅛ in., 3%

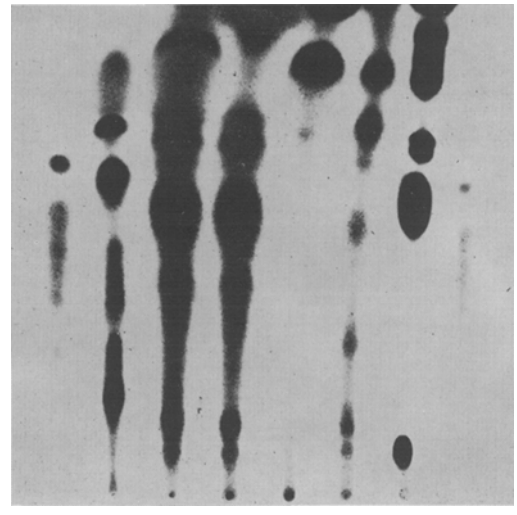


FIG. 2. Lactone concentrates from oxidized oils.

HIEFF-8BP on 100/120 mesh Gas Chrom Q (both from Applied Science Laboratories Inc.); 10 ft., ⅛ in., 12% stabilized DEGS on 70/80 mesh Anakron ABS. The helium flow rate was usually 75 ml/min. Both isothermal (120C) and temperature-programmed runs were made. The other instrument used was a Research Specialties Company Gas Chromatograph, equipped with a *beta* ionization detector. This instrument permits the organoleptic characterization of the compounds which are eluted from the column. The column was 6 ft, ¼ in., 12% Carbowax 20 M, on 80/100 mesh Anakron ABS. Manual temperature-programming from 150–200C was used. Argon at 15–20 psi was the carrier gas. The data in Table III was obtained by using this chromatograph.

The infrared spectra were obtained from a Perkin-Elmer Model 521 Grating Infrared Spectrophotometer, and the nuclear-magnetic-resonance (NMR) analysis was performed with a Varian A-60 Spectrometer. The NMR spectra are recorded in ppm relative to tetramethylsilane (TMS).

Discussion

TLC has proven useful in the isolation of lactones in butterfat (4). Figure I illustrates that the problem is more complex with mixtures obtained from oxidized oils. When an acidic eluting solvent is used, the aliphatic monocarboxylic fatty acids are easily separated from the lactones. But dicarboxylic acids, epoxy, keto, mono, and dihydroxy acids, all known products of fat oxidation, will interfere.

GLC was found to be most useful for the identification of individual lactones. The lactones in Table III were identified by their retention time and confirmed, whenever possible, organoleptically. All of the lactones in the C_8–C_{12} region have a low perception threshold and can be recognized by their characteristic aroma.

The lactones appearing in Table IV were identified by the comparison of their elution characteristics with those of their respective known standard on, at least, two columns. The lower-load, medium-polarity columns (QF-1 and HIEFF-8BP) were found useful in this work because they allowed linear temperature programming throughout the entire elution range of C_4–C_{18} lactones with relatively low column bleed. This can be of help in identification where standards are not available (26). As shown by Figure 3 however, these columns do not resolve the lower homologs even though they separate the higher-chain length lactones. A column with a heavier load, and more polar substrate separates the lower lactones but tends to push the lower *delta* isomer (cross-hatched peaks in Figure 4) too close to the next higher *gamma* isomer hence a run on each type of column was considered best for this study.

TABLE III

Lactones in Oxidized Soybean Oil[a]

Lactone	Retention time (min)[b]	Estimated amounts in the oil (ppm)[c]
γ-C_5	6.2	1.7
δ-C_5	6.9	2.4
γ-C_6	7.9	18.3
δ-C_6	9.1	2.8
γ-C_7	10.6	10[d]
δ-C_7	12.6	9[d]
γ-C_8	14.0	44.7
δ-C_8	15.8	14.2
γ-C_9	18.3	100.3
δ-C_9	20.8	22.3
γ-C_{10}	24.4	15.8
δ-C_{10}	27.7	29.2
γ-C_{11}	31.9	18.3
δ-C_{11}	34.4	47.5

[a] Sample 1.
[b] Carbowax 20 M column.
[c] Values are approximate; computed from peak heights x attenuation.
[d] Shoulder peaks; values uncertain.

TABLE IV

Lactones in Oxidized Oils[a]

Lactone	Oxidized Soybean Oil I	Oxidized Soybean Oil II	Oxidized Cottonseed Oil IIIA Volatiles[b]	Oxidized Cottonseed Oil IIIB Residue[c]
γ-C_5	L	?	S	L
δ-C_5	NIL	M?	S	M
γ-C_6	VL	VL?	S	M
δ-C_6	S	M?	?	VS?
γ-C_7	L	VL	L	S
δ-C_7	VS	L?	S	S
γ-C_8	L	VL?	S	S
δ-C_8	M	M	VS	S
γ-C_9	VL	VL?	S?	L
δ-C_9	L	M	S	S
γ-C_{10}	?	M	M	S
δ-C_{10}	S	M	S	S?
γ-C_{11}	S	L	NIL	S
δ-C_{11}	?	S?	M	?
γ-C_{12}	S	S	?	S
δ-C_{12}	S	?	NIL	VS

[a] The symbols VL, L, M, S, and VS stand for very large, large, medium, small, and very small respectively.
[b] Free lactones present in the oil.
[c] "Bound" lactones and/or those formed by reduction of hydroperoxides.

As used in Table IV, the symbols very large (VL), large (L), medium (M), small (S), and very small (VS) stand for a total scale response of 100%, 50–99%, 10–49%, 1–9.9%, and less than 1% for an approximately equal volume of concentrated distillate. The ? after a peak size indicates that the retention time is close but not identical to the one expected for the standard. These peak shifts could be attributable to the effect of impurities, e.g., fatty acids and branched or unsaturated lactones. A ? alone indicates that the presence of a particular component cannot be affirmed or denied because of a technical difficulty, e.g., proximity to a large peak, or that a signal is very small and cannot be clearly distinguished from the noise.

Besides data on oxidized soybean oil (Samples I and II described above) this table also lists the lactones found in an old sample of cottonseed oil oxidized in the presence of air at 80C (27). This had been heated for 42 days and, after standing at room temperature for 20 months, had a peroxide value of 268. A 365-g aliquot of this material was subjected to high vacuum distillation (5–10 μ Hg, 100C for three hours). Infrared spectrophotometry showed that the distillate contained lactones. The same was found to be true of the residue fraction after it had been sub-

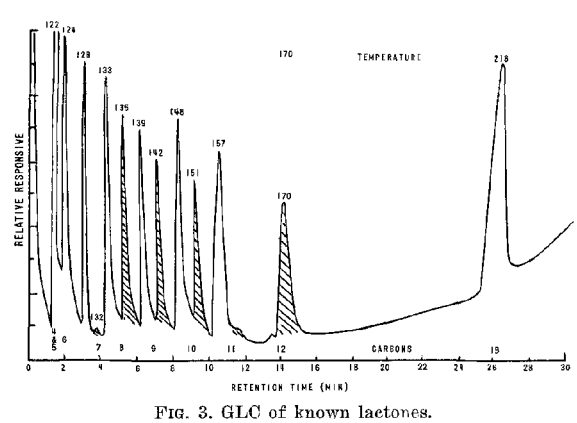


FIG. 3. GLC of known lactones.

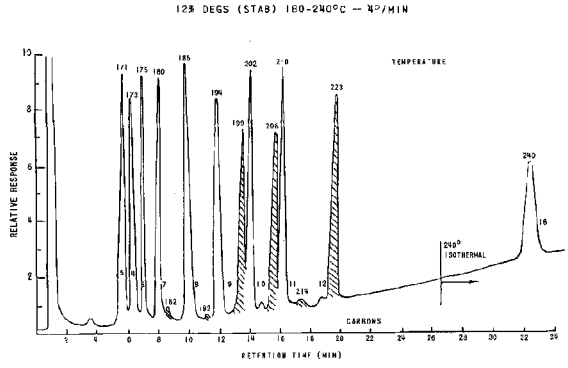


FIG. 4. GLC of known lactones.

jected to the saponification and lactonization procedure. As Table IV shows, this old oxidized sample contains a considerable number of free (III A) and "bound" (III B) lactones. "Bound" in this case also includes those 4- and 5-hydroxy fatty acids which were formed by the alkaline reduction of the hydroperoxides.

Perhaps the single most interesting fact that emerges from the data in Tables II and IV is that, in virtually all instances, the *gamma* is more abundant than the *delta* isomer. This is contrary to most of the work that has been reported on lactones heretofore (1–5,23,24). A notable exception to this can be found in the work of Chang and co-workers (10,21), where the formation of lactones is also attributable to oxidation.

The carbonyl stretching vibration of *gamma* lactones is at 1775 cm^{-1} whereas the C-C-O asymmetric stretching is at 1180 cm^{-1} (Figure 5). For *delta* lactones these peaks are at 1735 and 1240 cm^{-1} respectively. The absorption bands are characteristic and proved to be helpful for the detection of *gamma* and *delta* lactones in the distillate from oxidized oils. Figure 6 shows the spectrum of the distillate from Sample II, which also contained a substantial amount of normal and hydroxy fatty acids.

The NMR characteristics of *gamma*-decalactone appear in Figure 7. In addition to the terminal methyl hydrogens (a) and the ordinary-CH_2-hydrogens (b), the lactonic -CH_2- (c) and -CH- multiplets (d) are seen at 2.3 and 4.3 PPM respectively. The spectrum

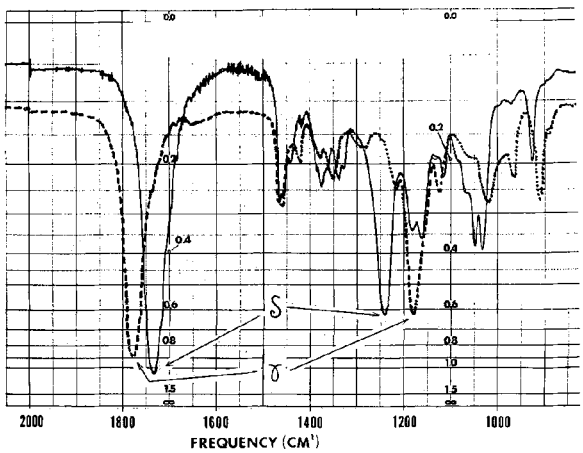


FIG. 5. Infrared spectra of C-10 lactones.

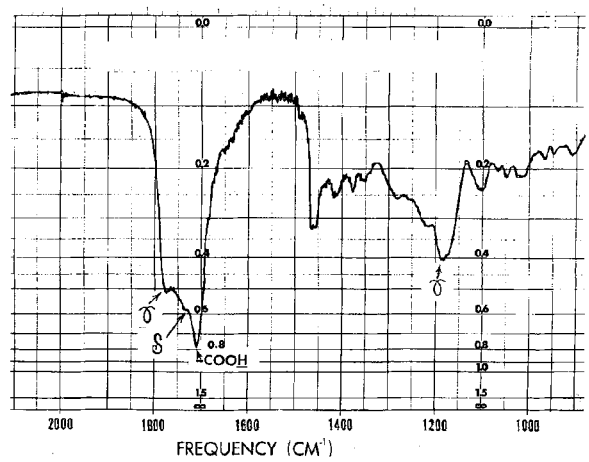


FIG. 6. Infrared spectrum of oxidized soybean oil distillate (Sample II).

for *delta*-decalactone is essentially the same except for the larger (c) peaks. These same peaks are visible in the spectrum of the distillate from oxidized soybean oil (Figure 8). Also shown here are the chemical shifts of the carboxyl, olefinic, and hydroxyl protons as well as uncharacterized peaks in the region 3.0–3.5 PPM.

The conclusions to be drawn from this preliminary report are that both *gamma* and *delta* saturated lactones are present in extensively oxidized vegetable oils. The *gamma* isomers were found to be predominant. None of the lactones could be found in freshly processed soybean oil. Reduction of hydroperoxides of heated oils promotes the formation of additional lactones. Isomerization with nickel catalyst to shift the double bond to a favorable position did not appear to increase the formation of lactone precursors during the oxidation of the oils.

From these results it seems pertinent to extend this work to include a search for lactones in mildly oxidized vegetable oils. Also included in future investigations will be a search for the higher homologs,

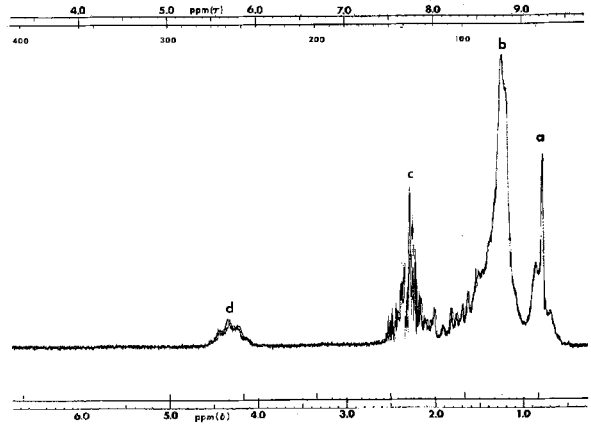
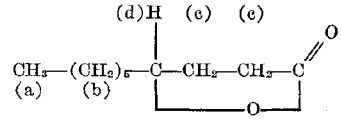


FIG. 7. NMR spectrum of gamma-decalactone.



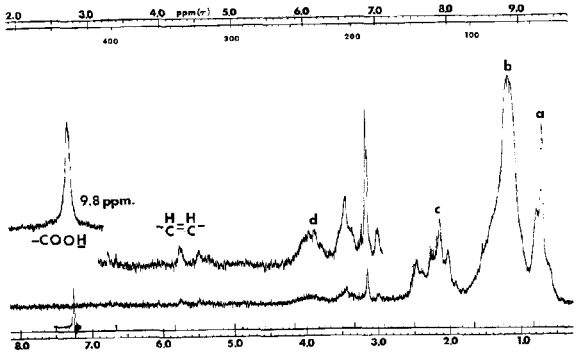


FIG. 8. NMR spectrum of oxidized soybean distillate (Sample I).

as well as the unsaturated lactones, which are of particular biological interest.

ACKNOWLEDGMENTS

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