Stabilization of Tocopherol by Three Components Synergism Involving Tocopherol, Phospholipid and Amino Compound

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ABSTRACT

The effects of minor-component phospholipids, sterols, trimethylamine oxide (TMAO) and tri-n-octylamine (TOA) on the stabilization of γ-tocopherol (γ-Toc) were investigated during the autoxidation of methyl linoleate (ML). On autoxidation of lard containing γ-Toc and TMAO, γ-Toc was rapidly oxidized and decreased to the 50-60% level in the initial stage of reaction. After that, the total amounts of γ-Toc and its reducing dimers, γ-Toc diphenyl ether dimer, γ-Toc biphenyl dimer (H), and γ-Toc biphenyl dimer (L), formed from oxidized γ-Toc, were maintained at a higher level for a longer time. These results indicate the presence of minor components contribution to the stability of γ-Toc. Therefore, three-component synergism involving γ-Toc, TMAO and each phospholipid was tested using ML as substrate. Accordingly, phosphatidyl ethanolamine (PE), phosphatidyl serine (PS), phosphatidyl inositol and phosphatidic acid showed a marked synergistic effect: PS especially inhibited the oxidation of γ-Toc. More than 0.02% of PE was found to keep a constant level of the residual amount of γ-Toc and to retared the formation of γ-Toc biphenyl dimers, which are perferential in the presence of TMAO. Phosphatidyl choline (PC), phosphatidyl glycerol and sterols did not show such an effect. However, PC synergized with TOA for stabilizing γ-Toc in autoxidizing ML.

INTRODUCTION

Tocopherol (Toc) in a higher concentration synergized with trimethylamine oxide (TMAO) in inhibiting the autoxidation of methyl linoleate (ML) (1,2). During the autoxidation of ML containing γ-Toc and TMAO, γ-Toc was oxidized to form γ-Toc diphenyl ether dimer (γ-TED) and 2 atropisomers, γ-Toc biphenyl dimers (γ-TBD[H] and γ-TBD[L]) (2). They are believed to play an important role in synergism between γ-Toc and TMAO. Recently, public attention has been paid to the biological function of Toc in relation to human health and nutrition (3-8). If edible oils and fats contribute greatly to the supply of physiologically active Toc monomer, then the inhibition of its oxidation will be very important for us. Toc is an excellent antioxidant, and augmenting the effectiveness of Toc by using potent synergists is reasonable. Oil in cuttlefish fried with soybean oil was very stable (9) and the effective constitutent for promoting its shelf life has been found to be TMAO (10). TMAO is widely present in fish. As TMAO itself acts as a prooxidant, γ-Toc in a lower concentration did not always synergize with TMAO in autoxidizing ML (11). However, TMAO always synergized with Toc, even in this lower concentration, when lard was used as a substrate (12,13). These facts suggest that natural oils and fats, e.g., lard, contain some minor components that can repress the negative activity of TMAO and draw forth its positive activity as a synergist.

The future developments in antioxidation probably lie in the direction of multicomponent synergism. Therfore, in this paper various phospholipids and sterols were used as minor components. Whether they can contribute to the stabilization of γ-Toc by synergizing with γ-Toc and TMAO was investigated during the autoxidation of ML.

EXPERIMENTAL PROCEDURES

Materials

γ-Tocopherol was prepared from natural Toc mixture (Eisai Co., Tokyo, Japan) (14). ML of a commercial product (Tokyo Kasei Co., Tokyo, Japan) was passed through a silica gel column equilibrated with n-hexane to remove peroxides. Lard with no antioxidants except citric acid was supplied by Kanegafuchi Chemicals Co. Phospholipids used were all palmitoyl ester unless otherwise noted. Phosphatidyl ethanolamine (PE) and phosphatidyl choline (PC) were products of Fluka AG., Buchs, Switzerland. Lysophosphatidyl ethanolamine (LPE), phosphatidic acid (PA), phosphatidyl glycerol (PG), 2-phosphatidyl ethanolamine (2-PE), phosphatidyl serine (PS), phosphatidyl inositol (PI), bis-phosphatidic acid, phosphatidyl-N,N-dimethyl ethanolamine and phosphatidyl choline dilinoleoyl were purchased from Serdary Research Labs., London, Ontario, Canada. Cholesterol was a product of Eastman Kodak, Rochester, NY and stigmasterol and β-sitosterol were products of Nakarai Chemicals, Kyoto, Japan.

Autoxidation

ML (850 mg) containing γ-Toc and other reagents was placed in a petri dish, 45 mm i.d., and kept in the dark at 50 C. An induction period was measured by the weight-gain method and defined as the days required to increase the weight of the substrate by 0.5%.

Lard (150 mg) and ML (150 mg) containing γ-Toc and other reagents were placed in a vial with a flat bottom, 18 mm i.d., and autoxidized in the dark at 60 C and 50 C, respectively. Samples in duplicate, taken out at varied time intervals, were dissolved in 5 mL of n-hexane and an aliquot applied on high pressure liquid chromatography (HPLC) for the determination of γ-Toc and its reducing dimers (14).

RESULTS AND DISCUSSION

Synergism of γ-Toc and TMAO in Autoxidizing Lard

Toc synergized with TMAO in inhibiting autoxidation of lard (12,13). In order to help clarify the mechanism, time-course changes in the amounts of γ-Toc and its dimers were followed during the autoxidation of lard containing γ-Toc (0.1%) and TMAO (0.02%, 0.1%). As Figure 1 shows, γ-Toc was rapidly oxidized in the absence of TMAO. The presence of TMAO brought about a drastic decrease in the amount of γ-Toc in the initial stage of autoxidation. However, γ-Toc dimers were rapidly formed and the total amount of γ-Toc and its dimers was maintained at a higher level within the period tested. These results are of interest in connection with synergism between γ-Toc and TMAO during the autoxidation of ML (2). In ML substrate, γ-Toc disappeared in the presence of TMAO and γ-Toc dimers played an important role in synergism. On the other hand, a large amount of γ-Toc remained in lard substrate. The difference between these results is probably attributable to the presence of minor components in lard.

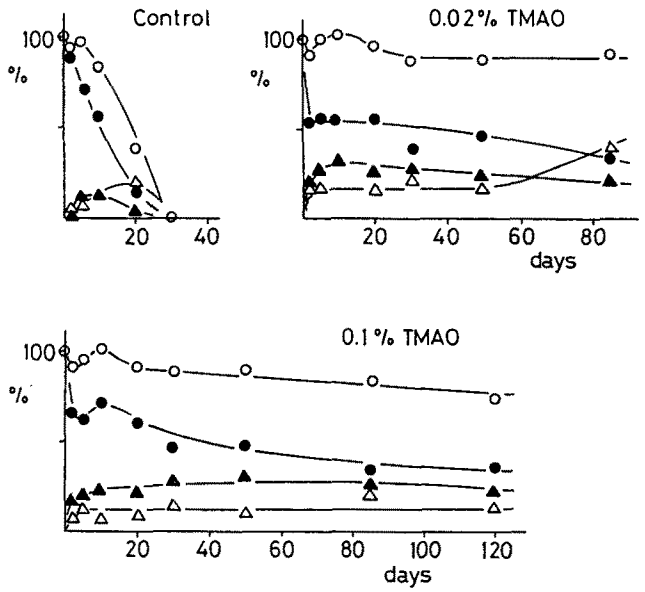

FIG. 1. Changes in the amounts of γ-Toc and its reducing dimers during the autoxidation of lard. The initial concentration of γ-Toc was 0.1% (○ total, ● γ-Toc, △ γ-TED, ▲ γ-TBD).

TABLE I

Synergism of γ-Toc and Minor Components During the Autoxidation of Methyl Linoleate

	None	TOA	TMAO	PC	PE	CH	SI	ST
None	28	42	22	28	32	25	25	27
TOA	42	–	41	51	60	47	47	51
TMAO	22	41	–	36	60	36	22	–

	PC+CH	PC+SI	PC+ST	PE+CH	PE+SI	PE+ST
TOA	48	47	51	74	56	57
TMAO	39	25	31	44	67	67

Figure show induction periods (day) set by the weight method (50 C). The initial concentrations of γ-Toc and each reagent were 0.1% and 0.05%. CH, SI and ST are cholesterol, β-sitosterol and stigmasterol.

TABLE II

Three-Component Synergism of γ-Toc, Phospholipid and Amino Compound During the Autoxidation of Methyl Linoleate

	None	PS	PI	PND	Bis-PA	PA
None	24	36	32	22	23	24
TOA	35	73	59	45	9	65
TMAO	20	75	44	29	4	59

	2-PE	LPE	PG	PC-L	PE	PC
None	38	33	6	22	33	22
TOA	77	66	7	33	75	45
TMAO	60	44	4	28	53	32

Reaction conditions were the same as in Table I. PND, Bis-PA and PC-L are phosphatidyl-N,N-dimethyl ethanolamine, bis-phosphatidic acid and phosphatidyl choline dilinoleoyl.

Synergism of γ-Toc and Minor Components

In order to find effective minor components assumed to be present in edible oil and fat, ML containing γ-Toc (0.1%) and each substance (0.05%) was autoxidized at 50 C according to the weight-gain method. Tri-n-octylamine (TOA) has been reported to act as an antioxidant (15) and a potent synergist for Toc (16). Therefore, TOA was compared with TMAO. As Table I shows, autoxidation of ML was scarcely affected by the minor components tested, but PE synergized with γ-Toc in the presence of TMAO or TOA.

Table II shows the effects of various phospholipids on synergism between γ-Toc and TMAO (or TOA) under the same conditions as in Table I. Phospholipids, e.g., PS, PI, PA synergized with TMAO and TOA as PE analogs did. However, PG and bis-phosphatidic acid showed negative activity.

Synergism of γ-Toc, TOA and Minor Component

Time-course changes in the amount of γ-Toc and its dimers during the autoxidation of ML containing γ-Toc (0.1%), TOA (0.15%) and some minor components (0.05%) were followed and the results are shown in Table III.

Minor components tested without TOA did not show any effect, and ML completely deteriorated at 40 days. In the case of the coexistence of TOA, PE and PC synergized with γ-Toc, and its oxidation was remarkably retarded. The presence of cholesterol and stigmasterol promoted the oxidation of γ-Toc.

TABLE III

Changes in the Amounts of γ-Toc and its Reducing Dimers During the Autoxidation of Methyl Linoleate Containing TOA and Minor Component

Time treated (days)		Without TOA					With TOA (0.15%)				
		None	PC	PE	CH	ST	None	PC	PE	CH	ST
5	γ-Toc	81.8	81.6	76.3	87.4	87.5	49.1	74.0	73.5	66.7	56.3
	γ-TED	1.2	1.0	4.9	1.2	1.0	29.6	8.2	11.3	21.4	23.7
	γ-TBD	0	0	0	0	0	0	0	0	0	0
10	Toc	80.5	80.0	79.1	76.5	73.6	40.0	78.0	78.0	50.1	51.9
	TED	3.3	2.5	3.5	3.8	3.3	40.0	10.6	14.1	36.5	26.2
	TBD	2.4	0	2.4	0	0	6.6	1.3	1.4	3.7	0.8
20	Toc	31.8	25.5	24.8	36.1	38.6	36.1	64.4	60.6	26.0	31.5
	TED	14.5	31.4	28.3	15.6	15.3	37.0	15.4	16.2	40.6	38.6
	TBD	3.3	2.4	2.6	2.6	3.8	7.2	2.0	3.9	6.9	6.8
40	Toc						30.2	70.6	62.1	6.7	13.5
	TED						40.8	13.6	18.1	39.4	45.1
	TBD						4.4	1.3	1.9	2.4	6.2

The amount of each compound was expressed in its weight proportion (%) to the initial weight of γ-Toc (0.1%).

Synergism of γ-Toc, TMAO and Minor Component

Effects of TMAO and minor components, e.g., PC, cholesterol and stigmasterol (0.01%, 0.05%), on the changes in the amounts of γ-Toc and its dimers were compared with the case of TOA. As Table IV shows, PC showing synergism with TOA was not effective with TMAO. γ-Toc was rapidly oxidized by TMAO to preferentially form γ-TBD in autoxidizing ML (2,14). However, PC, cholesterol and stigmasterol did not inhibit such an effect of TMAO.

In ternary synergism of γ-Toc, TMAO and PE, more than 0.02% of PE kept a higher level of γ-Toc in the initial stage of reaction. PE retarded the formation of γ-Toc dimers, especially γ-TBD. In the later stage, however, γ-TED, which was preferentially formed in the absence of TMAO, accumulated characteristically (Table V).

The residual amount of γ-Toc in autoxidizing ML was found to be greatly affected by the kinds of phospholipids used. Therefore, the effects of phospholipids other than PE and PC on time-course changes in the amount of γ-Toc and its dimers were investigated using ML containing γ-Toc (0.1%), TMAO (0.05%) and each phospholipid (0.04%). The results are shown in Table VI.

The addition of PS along with TMAO resulted most effectively in the inhibition of oxidation of γ-Toc, and a very small amount of γ-Toc dimers was formed. In such a ternary synergism, original γ-Toc might be regenerated from γ-Toc of radical types, which were formed when γ-Toc had been consumed during the autoxidation of ML. Therefore, γ-Toc apparently remained in a higher level. In the case of phospholipids, e.g., PI, LPE, 2-PE and PA, γ-Toc was oxidized gradually but the total amounts of γ-Toc and its dimers formed were maintained at a higher level, as in the case of PE. On the contrary, phospholipids, e.g., PG, phosphatidyl-N,N-dimethyl ethanolamine and phosphatidyl choline dilinoleoy, did not retared the oxidation of γ-Toc. Bis-phosphatidic acid especially, acted to accelerate the prooxidative activity of TMAO.

Lard contains ca. 0.05% phospholipids (17). The stabilization of vegetable oil by the addition of TMAO (18), however, does not always depend on the effects of phospholipids for the following reasons. Only 0.002-0.004% of phospholipids exist in highly refined vegetable oils (17). The composition of commercially available lecithin makes us firm a lower content of the effective phospholipids (19). Lecithin showed the protective effect on thermal oxidation of Toc (20,21). Hudson and Mahgoub (22) reported that α-Toc and phospholipids in leaf synergized and remarkably retarded the autoxidation of lard. Therefore, phospholipids are important in protecting edible oil and fat from oxidative deterioration.

TABLE IV

Changes in the Amounts of γ-Toc and its Reducing Dimers During the Autoxidation of Methyl Linoleate Containing TMAO and Minor Components

Time treated (days)		None	PC 0.01%	PC 0.05%	CH 0.01%	CH 0.05%	CH 0.01%	CH 0.05%
5	γ-Toc	12.2	10.8	9.9	3.8	4.5	14.9	16.7
	γ-TED	8.9	6.9	9.0	9.1	7.8	6.0	5.7
	γ-TBD	52.2	51.9	51.3	45.4	47.2	55.9	52.7
10	Toc	10.0	5.0	5.5	5.4	6.4	7.3	9.8
	TED	7.3	7.1	8.1	7.4	7.0	6.4	6.9
	TBD	47.0	48.4	48.7	47.9	50.3	49.6	48.9
20	Toc	0.4	1.9	7.5	9.8	1.4	5.1	9.6
	TED	2.7	3.3	8.7	11.8	5.0	4.1	14.9
	TBD	6.0	36.1	47.0	32.1	33.3	20.9	35.4

Figures show the amount of each compound as in Table III.

TABLE V

Changes in the Amounts of γ-Toc and its Reducing Dimers During the Autoxidation of Methyl Linoleate Containing TMAO and Phosphatidyl Ethanolamine

Time treated (days)		Concentration of PE (%) 0	0.01	0.02	0.03	0.05	0.1
2	γ-Toc	14.1	69.6	78.8	80.7	80.5	88.9
	γ-TED	6.2	1.5	0.8	1.6	1.5	0.6
	γ-TBD	52.4	17.2	13.7	15.8	13.7	6.9
10	Toc	16.0	62.3	66.3	68.7	60.7	72.4
	TED	8.6	4.1	4.4	3.8	8.2	2.1
	TBD	48.7	24.7	18.8	15.1	19.5	17.2
21	Toc		37.2	50.6	21.6	60.6	64.1
	TED		18.2	8.3	20.3	10.0	6.7
	TBD(H)		23.4	22.2	30.0	16.6	13.8
30	Toc			47.5	43.1	48.3	43.4
	TED			17.0	19.0	19.4	17.7
	TBD			22.4	19.6	17.5	14.9
50	Toc		4.1	8.6	11.7	5.8	11.9
	TED		23.1	31.5	35.8	29.3	32.1
	TBD		9.0	18.4	16.1	14.1	13.3

Figures show the amount of each compound as in Table III.

TABLE VI

Changes in the Amounts of γ-Toc and its Dimers During the Autoxidation of Methyl Linoleate Containing TMAO and Phospholipid

Time treated (days)		Without TMAO								With TMAO (0.05%)							
		LPE	PA	PG	2-PE	PI	PND	PS	Bis-PA	PC-L	LPE	PA	PG	2-PE	PI	PND	PS	Bis-PA	PC-L
2	γ-Toc	94.5	96.8	92.5	97.3	96.4	64.7	98.9	86.4	70.5	64.9	52.9	0	59.4	91.8	19.2	92.2	2.0	49.8
	γ-TED	2.7	1.3	2.7	2.2	0	14.5	0	10.4	17.9	7.3	8.5	9.6	7.1	0.7	9.4	0.6	7.9	5.8
	γ-TBD	0	0	0	0	0	0	0	0	0	20.1	28.2	32.4	25.7	3.6	58.5	3.6	53.5	39.4
7	Toc	71.4	87.1	71.6	84.7	93.6	63.1	93.5	74.1	73.3	62.6	51.5	0	47.4	76.7	6.2	99.0	0.1	27.1
	TED	10.9	5.8	3.9	7.1	1.2	18.5	1.6	19.3	11.1	8.0	12.7	8.3	10.8	1.1	11.2	1.1	3.5	10.4
	TBD	0	0	0	0	0	0	0	0	0	22.6	27.6	19.9	31.4	1.7	60.1	1.7	31.9	60.6
20	Toc	0	7.7	0	0	10.1	14.3	45.4	19.7	22.4	50.0	22.1	0	33.4	39.8	1.2	86.4	0	7.2
	TED	0	23.9	0	0	17.4	18.8	31.1	23.8	13.1	21.0	31.2	0	21.6	7.6	7.9	2.6	0	18.8
	TBD	0	0	0	0	1.2	0	0.5	0	0	21.2	25.1	0	26.8	46.8	38.5	5.7	0	46.7

Figures show the amount of each compound as in Table III. PND, Bis-PA and PC-L were phosphatidyl N,N-dimethyl ethanolamine, bis-phosphatidic acid and phosphatidyl choline dilinoleoyl.

Though many studies have been done on binary synergism between Toc and synergist, most of them were unconsciously investigated on multicomponent synergism using natural oil and fat (23-28). Kawashima et al. (29) and Pongracz (27) reported on ternary synergism, including Toc as at least one component, without elucidating its mechanism.

Synergism of γ-Toc and Phosphoric Compound

The synergistic effects of phosphorus substitutes of amino compounds on γ-Toc were tested using ML (50 C) and lard (60 C). Phosphoric compounds tested showed no synergistic and prooxidative activities. TOA synergized with γ-Toc when lard was used as substrate (13). However, tri-n-octylphosphine, a phosphorus substitute of TOA, did not synergize with γ-Toc. The reasons for this are assumed to be as follows: (a) steric hindrance by P atom larger than N atom; (b) difference in electron donating ability; (c) whether tri-n-octylphosphine can form its analogs, e.g., di-n-octylhydroxylamine (30), which is formed during the oxidation of TOA and acts as antioxidant.

What kinds of residues in phospholipids are required to synergize with Toc? The fact that PA is effective suggests the participation of the phosphate group. As phospholipids easily form hydrogen bonds, even in organic solvents (31-34), the participation of the amino group must be considered. In any event, the rate of interaction of TMAO with the phospholipids inhibiting the oxidation of γ-Toc was much faster than that of TMAO with ML. In order to help clarify the mechanism of ternary synergism for stabilizing γ-Toc during the autoxidation of ML, characteristic oxidation products from the substrate ML and interactions, especially between phospholipids and TMAO, must be further investigated in relation to time-course changes of γ-Toc and its reducing dimers.

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♣Derivatization of Keto Fatty Acids. III. Synthesis of Terminal Thiazole and Oxazole Derivatives from α-Bromoketones

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ABSTRACT

The synthesis of alkyl chain substituted thiazole and oxazole derivatives is described. The reaction of 11-bromo-10-oxoundecanoic acid with thiourea and acetamide yielded terminally located thiazole and oxazole derivatives, respectively. A similar treatment with urea produced the unexpected urea-substituted product, together with an unidentified product.

INTRODUCTION

Compounds containing thiazole and oxazole nuclei are known to possess pharmacoactive properties and are used as antibacterial agents (1), fungicides (2), surface and infiltration anaesthesia (3) and tranquilizers (4). Long-chain sulfur and oxygen-containing heterocycles have recently been an area of wide interest. Several thiazolidinones prepared from oxoesters were reported in a previous paper (5). Because fatty derivatives with terminally located heterocyclic functions are comparatively rare or little known, an attempt was made to synthesize these compounds from long-chain α-bromoketones. In this paper we report the use of thiourea, acetamide and urea in the synthesis of long-chain thiazole and oxazole derivatives from the bromoketones.

EXPERIMENTAL PROCEDURES

All melting points are uncorrected. Infrared (IR) spectra were obtained on samples in nujol (6) with a Perkin-Elmer 621 spectrophotometer. Nuclear magnetic resonance (NMR) spectra were run in $CDCl_3$ on a Varian A-60 spectrometer with tetramethylsilane (TMS) as the internal standard. The abbreviations s, d, m, q, br and t denote singlet, doublet, multiplet, quartet, broad and triplet. Mass spectra were measured with AEI MS-902 spectrometer coupled to a DS-55 mass data system at 70 eV. Thin layer chromatographic (TLC) plates were coated with silica gel G, and a mixture of petroleum ether/ether/acetic acid (80:20:1, v/v) was used as developing solvent. The spots were visualized by charring after spraying with a 20% aqueous solution of perchloric acid. Light petroleum refers to a fraction of b.p. 40-60 C.

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MATERIALS AND METHODS

10-Undecenoic acid (I, 1.84 g, 0.01 mol), when stirred with N-bromosuccinimide (NBS, 1.88 g, 0.01 mol) and water (4 mL) (7) for 1 hr yielded 11-bromo-10-hydroxyundecanoic acid (II) m.p. 48-49 C. The exclusive formation of II, is in accordance with Markovnikoff's rule, which on Jones oxidation (8) gave 11-bromo-10-oxoundecanoic acid (III). The Jones reagent was prepared by dissolving chromium trioxide (35 g) in water (100 mL), and added concentrated sulphuric acid (30 mL) by drops. The compound (III) m.p. 90-91 C (positive Beilstein test) was further characterized by elemental and spectral analysis (recorded in Results and Discussion section).

Reaction of Thiourea with III (9)

11-Bromo-10-oxoundecanoic acid (III, 2.0 g, 0.007 mol) was refluxed with thiourea (0.53 g, 0.007 mol) in alcohol (4 mL) for 2 hr. The reaction mixture was allowed to cool at room temperature and poured into ice-water (100 mL). To this solution ammonium hydroxide was added to make it just alkaline. The reaction mixture was extracted with ether, washed with water and dried over anhydrous sodium sulphate. Evaporation of ether gave a solid that on crystallization from alcohol at low temperature, yielded 2-amino-4-(8-carboethoxyoctyl) thiazole, IV (1.42 g, ca. 71%) m.p. 81-82 C (Scheme 1). Analysis: calculated for $C_{14}H_{24}N_2O_2S$: C, 56.25; H, 7.81; N, 10.93. Found: C, 55.81; H, 7.23; N, 10.12% (spectral values are recorded in the discussion part of this paper).

Reaction of Urea with III

A similar treatment as described above, α-bromoketone (III, 2.0 g, 0.007 mol) on refluxing with urea (0.42 g, 0.007 mol) in alcohol (4 mL) for 2 hr yielded a brown viscous oil (1.9 g), which showed 2 distinct spots on TLC plate.

A column of silica gel G (38 g), prepared in petroleum ether, was charged with total crude mixture, and the column was eluted with a mixture of petroleum ether/benzene (95:5, v/v) (fractions of 15 mL were collected). TLC-monitored eluates were combined to give product (V) (Scheme 1) as a yellow viscous oil (0.65 g, ca. 34%, not identified).