Antioxidant Activity of Glycerylphosphatides* and Their Hydrolysis Products. IV. Reaction of Methyl Oleate Hydroperoxide Concentrate with Phosphoric Acid and β-Glycerylphosphoric Acid and the Isolation of a Phosphorus-containing, Oil-soluble Product from Autoxidized Methyl Oleate in the Presence of β-Glycerylphosphoric Acid

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Phosphate ester as well as phosphatides have tentatively been classified as decomposers of peroxides,(1) but no significant evidence has been presented. Therefore, such a generalization must be subjected to further tests. In connection with this, it has been sugested that a secondary phosphorus-containing, oil-soluble product formed as the result of a reaction of glycerylphosphoric acid with methyl oleate or its oxidation products in the early stages of autoxidatation might be responsible for its antioxidant activity.(2,3)

It was observed that β-glycerylphosphoric acid did not decompose the methyl oleate hydroperoxide concentrate, much as is shown

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* The "lecithin" used in the previous papers(2,3) will be changed to this term in future publications.

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1) K. U. Ingold, *Chem. Revs.*, **61**, 574, 581 (1961).

2) C. Urakami, Y. Kakutani and H. Okura, This Bulletin, **29**, 921 (1956).

3) C. Urakami, H. Kameyama and M. Okada, ibid., **32**, 36 (1959).

TABLE I. ANALYSIS OF THE ETHER-SOLUBLE FRACTION OF THE REACTION PRODUCTS OF THE METHYL OLEATE HYDROPEROXIDE CONCENTRATE WITH PHOSPHORIC ACID AND WITH β-GLYCERYLPHOSPHORIC ACID AT 45°C IN A NITROGEN STREAM

Reactants	Mol. ratio[a]	Time, hr.	Peroxide value[b]	Iodine value	R_f[c]	Phosphorus[d]
P. C.		0	261	61.2		
P. C. : H_3PO_4	1 : 1	1/3	21.8	36.2	0—0.51	+
					0.92	+
P. C. : β-GP[e]	1 : 0.018	1/2	234	59.8		
P. C. : β-GP[e]	1 : 1	2	245	66.5	1.00	−
P. C. : β-GP[e]	1 : 0.7	5	239	67.1	1.00	−

P. C. stands for the methyl oleate hydroperoxide concentrate[4] and β-GP for β-glycerylphosphoric acid.[2]

a) Based on hydroperoxide oxygen.

b) Expressed in mmol./100 g.

c) Developed with *n*-propanol - water (8 : 1, v/v) and sprayed with bromothymol blue.

d) The sections corresponding to the R_f values were incinerated for phosphorus determination along with a blank, another section of the same size: + indicates the presence of a significant amount, and −, the absence of phosphorus.

e) Glass beads and *n*-heptane were added, and the entire contents were mixed a motor-driven stirrer. For analysis, each of the reaction mixtures was diluted with dry ether, washed with water, dried over anhydrous sodium sulfate, and concentrated.

TABLE II. THE R_f VALUES OF THE ISOLATED COMPOUND AND ITS DECOMPOSITION AND HYDROLYSIS PRODUCTS

Sample	Days[a]	System	R_f			
			P	Rh-6G	$(AcO)_2Hg$	Fuchsine
I	11	I	0.49	0.51	0	
II	26	I	0.48	– (?)		
III	45	I	0.80			
			0.06			
IV	46	II	0.92[b]		0.86	0.91
			0.26[c]		—	—
V = IV + β-GP	47	II	0.91			0.90
			0.08			—
VI = Hyd. prod. of IV	47	II	—		0.91	0.91
			0.26[b]		—	0.21
			0.05[c]		—	—
β-GP		II	0.07		—	
H_3PO_4		II	0.19			

System I: Toyo No. 51 paper impregnated with silicic acid with a solvent system of diisobutyl ketone - acetic acid - water (40 : 25 : 5 by vol.). System II: Toyo No. 50 paper with a solvent system of *n*-propanol - acetic acid - water (8 : 1 : 1 by vol.). The R_f values are an average of several runs. β-GP stands for β-glycerylphosphoric acid. The − sign indicates a negative reaction with the reagent for the spot on the corresponding row. (?) For some unknown reason the reaction was negative; it should have been positive, as for sample I. a) A number of days elapsed since the end of autoxidation. The relative intensity of the color of the spots was b>c.

in Table I, in comparison with the hydroperoxide decomposing property of orthophosphoric acid, but it gave rise to a very unstable phosphorus-containing, oil-soluble compound in the early stages of the autoxidation of the methyl oleate containing it. The compound, initially isolated, sample II in Table II, was found to be very readily soluble in a mixture of chloroform and methanol and carbon tetrachloride, somewhat soluble in chloroform and methanol, slightly soluble in acetone, and insoluble in isopropanol. Its ultraviolet spectrum showed a $\lambda_{max\ 1}$ at a wavelength shorter than 240 mμ and a $\lambda_{max\ 2}$ at 276 mμ, with a shoulder at 282 mμ. The infrared absorption bands that appear to be characteristic of the initially-isolated compound (sample II) are as follows: 1730 cm^{-1} for a carbonyl group, the doublet at 1575 and 1595 cm^{-1} for a conjugated double-bond system, 1385 cm^{-1} for the methyl group (presumably the terminal group of a long alkyl

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4) J. E. Coleman, H. B. Knight and D. Swern, *J. Am. Chem. Soc.*, **74**, 4886 (1952).

chain), and 1285 cm^{-1} for the P=O stretching vibration. All these bands are absent, however, in the spectrum of β-glycerylphosphoric acid. The presence of the P-O-C(alkyl) in the molecule is indicated by the absorption bands at 1170, 1190, and possibly 1040 cm^{-1}, and at others in the lower frequencies.

Experimental

Isolation of a Phosphorus-containing, Oil-soluble Compound. — In a reaction tube were placed 16.493 g. of methyl oleate (b. p. 152—156°C/0.9 mmHg., iodine number 89.3, and saponification number 185.3) and an ethanol solution containing 5.207 mg. of β-glycerylphosphoric acid (0.03% in the methyl oleate) ; the solvent was removed at a temperature not exceeding 50°C. The tube was placed in a bath maintained at 50°C, and the mixture was autoxidized for 192 hr. by passing dry air through a sintered glass gas bubbler ; its peroxide value was 50 ± 2.4. After adding a small amount of a 0.05% ethanol solution of hydroquinone, the reaction mixture was dissolved in 200 ml. of freshly-distilled dry ether, and the solution washed successively with 50, 25, and 10 ml. of distilled water to remove unreacted water-soluble β-glycerylphosphoric acid ; 1% of the initially-added phosphorus was removed in the combined water washings. After drying the ether solution over anhydrous sodium sulfate, it was diluted further with an equal volume of dry ether and the solution applied to a column (10 mm. × 100 mm.) packed with 5.5 g. of a mixture of silicic acid and filter aid (2 : 1 by wt.), followed by elution with fresh dry ether ; 10% of the initially added phosphorus was found in the eluate. The adsorbent in the upper half of the column was extracted three times with chloroform-methanol (4 : 1 by vol.). The combined extract (sample I in Table II) was concentrated under mild conditions, and the residue treated with dry carbon tetrachloride. An insoluble fraction was found to be a colored wax and to contain phosphorus. The carbon tetrachloride - soluble fraction was concentrated in the usual manner, and the colored oil thus obtained (sample II in Table II) was subjected to various analyses. A small amount of this oil was hydrolyzed with 5% solution of ethanolic sodium hydroxide, and the reaction mixture deionized with IRC-50 (H^+) (sample VI in Table II).

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