TG release, or may act indirectly in the intact animal via some mechanism, as stimulation of another endocrine organ. E and NE, in addition, inhibited the uptake of nonesterified fatty acid by the liver, and E also appeared to inhibit the uptake of TG from a neutral fat emulsion.

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• *Letters to the Editor*

Preparation of *a*-Monoglycerides

THE METHOD OF PREPARING *a*-monoglycerides based on the use of isopropylidene glycerol (1) has become a generally accepted procedure. The isopropylidene derivative is usually prepared by esterification of isopropylidene glycerol with fatty acid chlorides (2). Hartman (3) greatly reduced the time and work involved in the preparation of high purity monoglycerides by use of a method based on the esterification of isopropylidene glycerol with a free fatty acid.

This letter reports a further simplification of the technique for obtaining acyl isopropylidene glycerol utilizing standard laboratory apparatus. Instead of utilizing the aqueous phase collection apparatus suggested by Hartman (3), a medium sized Soxhlet extraction apparatus is used. The extraction thimble contains an appropriate amt of anhydrous magnesium sulfate, which is used to absorb the water formed during the esterification reaction (4). The reactants are placed in the boiling flask and refluxed for the required time. Isopropylidene glycerol can be obtained either by preparing it in the present apparatus by the condensation of acetone and glycerol catalyzed by *p*-toluene sulfonic acid by the method of Hartman (3), or by purchasing commercially available material (Aldrich Chemical Co.). Esterification is carried out by condensation of isopropylidene glycerol with the required free fatty acid using *p*-toluene sulfonic acid as a catalyst with alcohol-free chloroform or preferably with benzene as the carrier in order to remove the water formed during the reaction. For example, *a*-palmitoyl isopropylidene glycerol was prepared by refluxing a mixture of 0.14 mole palmitic acid, 0.44 mole isopropylidene glycerol and 0.02 mole *p*-toluene sulfonic acid dissolved in 300 ml benzene. The extraction thimble of the Soxhlet apparatus contained 0.3 mole of anhydrous $MgSO_4$. The reaction was virtually complete after four hr under reflux; its course was monitored with the aid of TLC on silica Gel G coated glass plates. The isopropylidene glycerol, free fatty acid, and isopropylidene ester were completely separated by a solvent system which was composed of

TABLE I

Fatty acid[a]	Time required for complete acylation of isopropylidene glycerol	Solvent	Yield of monoglyceride[b]	Purity of monoglyceride[c]
	(hr)		(%)	(%)
Lauric	3.5	benzene	98.5	98.9
Lauric	13	chloroform	63.5	97.4
Palmitic	4.5	benzene	87.0	98.0
Palmitic	16–21	chloroform	69.0	98.9
Stearic	3.5	benzene	80.0	98.9
Stearic	12	chloroform	76.0	97.4
Oleic	4.5	benzene	78.5	98.0
Oleic	7	chloroform	70.5	98.9
Linoleic	11	benzene	85.7	98.0
Linoleic	16	chloroform	78.0	98.0

[a] The purity of fatty acid employed here was 98% or greater.
[b] Yields presented are those obtained after recrystallization of the product. Yields prior to recrystallization were in the 90–98% range.
[c] Determined by the periodic acid method. Mehlenbacher, V. C., "The Analysis of Fats and Oils," Garrard Press, Champaign, Ill., 1960, p. 492.

diethyl ether, ethyl acetate, iso-octane, hexane and glacial acetic acid (10:5:35:50:1). The spots were visualized by spraying the plates with 50% aqueous H_2SO_4 saturated with potassium dichromate and charring. The procedure of Hartman (3) was employed for the cleavage of the isopropylidene glycerol ester and the isolation of α-monopalmitin. α-Monoglycerides of lauric, palmitic, stearic, oleic and linoleic acid were prepared using this method (Table I). The α-monoglyceride of linoleic acid darkened somewhat during preparation, but was easily decolorized with the aid of activated carbon.

We were unable to prepare α-monoglycerides(as the isopropylidene derivatives) in the time periods suggested by Hartman (3). In order to obtain complete reaction of the fatty acids employed here, considerably longer periods of time were required when chloroform was used as the solvent. Benzene proved to be superior to chloroform as a carrier solvent. The removal of the water formed during the reaction is most efficiently accomplished by the use of an adsorbent such as anhydrous magnesium sulfate.

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Stabilization of Linoleic Acid by Arginine and Lysine Against Oxidation[1]

THE REACTION of fatty acid anions with protein materials has long been known and since amino acids have proved to be at least potential antioxidants, we are prompted to report the stability of linoleic acid salts of amino acids toward oxidation by air.

The results illustrated in Tables I and II, clearly indicate that the linoleic acid salts with the basic amino acids have unusual stability toward oxidation. Triethanolamine also showed a stabilizing effect. The basic nitrogen compounds appear to stabilize the unsaturated site of linoleic acid and eliminate oxidative rancidity as evidenced by the fact that the iodine value and apparent linoleic acid content remained almost unchanged.

While it is recognized that linoleate content by spectral analysis may not be an accurate measure of the amt of unchanged linoleate in an oxidized linoleate, the gross decreases in the control compared to the very slight changes in the linoleate salts is certainly an indication of autoxidation stabilization in the salts. The oxidized controls were grossly rancid, while the linoleate salts were essentially non-rancid. Also, the linolenate salt of arginine, which showed no stability by analyses, was quite rancid.

The mechanism of stabilization of the unsaturated sites of the linoleic acid by basic amino acids is not fully understood. However, we speculate that these basic nitrogen compounds may either form a complex or may act as free radical chain terminators or oxygen scavengers. It is thought that C-11 (the α-methylene group) of linoleate tends to form free radicals in the presence of oxygen. This leads to the formation of hydroperoxides which undergo secondary reactions causing rancidity. The failure of basic amino acids to stabilize linolenic acid (in contrast to the linoleate) indicates that salt-complex formation is more important than free radical termination. Perhaps the free amino group of the basic amino acid forms a salt with the carboxylic acid group of the fatty acid and the α-amino carboxylic acid portion of the basic amino acid is then in a favorable position to affect the double bonds or the doubly activated α-methylene group at C-11 in a manner which stabilizes it to attack by oxygen or free radicals.

The fact that *linolenic* salts of basic amino acids are *not* stable may be explained by the presence of two active methylenes and only one available α-amino carboxylic acid group.

TABLE I

Room Temp Storage Tests

	Iodine value after exposure, days[a]		
	0	16	49
Safflower fatty acid	149	133.3	102.1
L-Arginine safflate	92.1	94.1	93.1
L-Lysine safflate	90.6	87.2	87.3
L-Lysine linoleate	116.8	113.9	114.2
Linolenic acid	268.9	142.0 (14 days)	
Arginine linolenate	161.7	83.3 (14 days)	

[a] Evidence of gross loss of linoleate in the linoleic acid control and very little loss in the linoleate salts was also obtained by spectral measurements of diene content.

TABLE II

Storage Test of Salts at 60C, Hot Air Circulated Oven[a]

	Iodine value after exposure, days					
	0	10	25	53	54	103
Safflower fatty acid	149	115.4	113.8	.....	89.1	.....
Linolenic acid	181	97.1	67.4	.....	60	.....
Arginine safflate	92.1	92.8	90.6	.....	86.4	.....
Lysine safflate	90.6	92.9	93.1	93.6	.....	92.1
Lysine linoleate	116.8	114.8	114.5	114.8	.....	113.3

[a] In this series, spectral determination of diene content indicated gross loss of linoleate in the control acids and very little loss in the salts.

The fact that *linolenic* salts of basic amino acids are *not* stable may be explained by the presence of two active methylenes and only one available α-amino carboxylic acid group.

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