The effect of silicic acid and alkali concentration on the saponification of lipids

As part of a study on intramuscular lipids thin-layer chromatography (TLC) on silicic acid was used to separate the triglycerides and the phospholipids from the total lipid extract. After separation, these classes were scraped from the thin-layer plate but were not extracted from the silicic acid. Instead, the lipid fractions still on the silicic acid adsorbent were saponified with ethanolic potassium hydroxide. The free fatty acids were then extracted and methylated. Surprisingly, only small amounts of fatty acid methyl esters were found by gas–liquid chromatography (GLC) despite the fact that TLC separations were made on milligram amounts of lipid. The effect of silicic acid and of alkali concentration on saponification was therefore further investigated.

Experimental

Lipids were extracted from ground beef muscle using the prodecure of BLIGH AND DYER[1]. Twelve milligrams of the extracted lipids were separated, under nitrogen, by TLC on silicic acid. The thin-layer plates were 8 in. × 8 in., the silicic acid thickness 500 μ, and the developing solvent was hexane–ethyl ether–acetic acid (75:25:1). Phospholipids remained at the origin, and the triglyceride band, because of its high concentration, could be observed by viewing the plate by transmitted light. The phospholipid and triglyceride fractions plus the approximately 0.5 g of silicic acid on which each was absorbed were scraped from the plate and heated in a glass stoppered test tube with 4 ml of 0.5 *N* potassium hydroxide in 95% ethanol at 75° under nitrogen. Triglycerides were heated for 30 min, phospholipids for 45 min. The clear ethanol solution was cooled, diluted with an equal volume of water and acidified. The fatty acids plus ethyl esters formed by transesterification, and any residual triglycerides and neutral substances were extracted into the hexane. The fatty acids were in turn removed from the hexane solution by adsorbing the acids on a basic ion-exchange resin[2]. After the adsorption of the free fatty acids on the ion-exchange resin the hexane solution was concentrated and an aliquot analyzed for ethyl esters by GLC. The acids on the ion-exchange resin were methylated with 5% anhydrous HCL–methanol directly on the resin. The methyl esters were then extracted into hexane, and the solvent volume reduced to 1 ml. A 1–3 μl portion was analyzed by GLC. The instrument was an F & M Model 810 gas chromatograph using a flame ionization detector. The column was a 6 ft. × 1/8 in. O.D. stainless steel tube packed with 10% DEGS on 60–80 mesh chromosorb W, acid washed and silanized. Operating conditions were: isothermal at 185°; nitrogen carrier gas, flow rate 20 ml/min; hydrogen flow rate to detector, 20 ml/min; air flow to detector, 300 ml/min.

Results and discussion

The recoveries of methyl esters from phospholipids and triglycerides in the presence of silicic acid showed similar anomalies. For convenience, this discussion is therefore limited to the triglycerides. Heating the triglycerides with 0.5 *N* ethanolic potassium hydroxide in the presence of silicic acid produced very low yields of free fatty acids and considerable amounts of ethyl esters indicating that transesterification rather than hydrolysis was taking place. To see if the silicic acid was in fact responsible for these results, the same amount of lipid (6 mg) was spotted on each half of an 8 in. ×

TABLE I

CONVERSION OF FATTY ACID PORTIONS OF INTRAMUSCULAR TRIGLYCERIDES TO THE FREE ACIDS AND TO ETHYL ESTERS USING STANDARD SAPONIFICATION CONDITIONS

(a) In the presence of silicic acid; (b) in the absence of silicic acid. Free fatty acids FFA are determined by GLC analysis of their methyl esters.

Acids identification	*Silicic acid present*				*Silicic acid absent*		
	Fatty acids		*Ethyl esters*		*Fatty acids*		*Ethyl esters*
	Found (mg)	*Percentage of total FFA*	*Found (mg)*	*Percentage of total ethyl esters*	*Found (mg)*	*Percentage of total FFA*	*Found (mg)*
C^{0}_{14}	—	—	0.06	3.3	0.15	3.3	0
$C^{1=}_{14}$	—	—	0.02	1.1	0.04	0.8	0
C^{0}_{16}	0.06	28.6	0.48	26.3	1.20	26.1	0
$C^{1=}_{16}$	—	—	0.05	2.8	0.15	3.3	0
C^{0}_{18}	0.05	24.0	0.40	22.0	0.90	19.5	0
$C^{1=}_{18}$	0.10	47.4	0.76	41.7	1.97	42.8	0
$C^{2=}_{18}$	—	—	0.05	2.8	0.14	3.1	0
$C^{3=}_{18}$	—	—	—	—	0.05	1.1	0
	0.21	100.0	1.82	100.0	4.60	100.0	

8 in. TLC plate and separated. The triglyceride fraction from one half the plate was carried through the described procedure. The triglyceride fraction on the other half of the plate was extracted from the silicic acid with chloroform–methanol (1:1). The solvent was evaporated and the extracted triglycerides were saponified and esterified as described. In the presence of silicic acid only 0.21 mg of fatty acids were obtained from 4.8 mg of triglyceride, less than 5% of the expected yield (Table I). In addition, 1.82 mg of ethyl esters or 40 % of the triglycerides were converted to ethyl esters. The ratio of ester to acid formed on saponification was 8 to 1. On the other hand, no ethyl esters were found in the absence of silicic acid and, based on the weight of triglycerides, recovery of fatty acids was 96 % of that expected.

To see if transmethylation using sodium methoxide would also be affected by silicic acid, the lipids were separated into classes, and the triglycerides, with and without silicic acid, were heated at 60° for 30 min under nitrogen with 4 ml of 0.3 *N* sodium methoxide in anhydrous methanol. The products after dilution and acidification were extracted into hexane; acids and esters were separated as described. GLC analysis showed that 97 % transmethylation had taken place and less than 1 % of free acids was formed.

Silicic acid may have altered the nature of the reaction either directly or indirectly by removal of hydroxyl ion from the solution. To check the latter possibility, solutions containing equal amounts of tristearin and triolein were prepared and saponified with varying concentrations of potassium hydroxide in 95 % ethanol

TABLE II

EFFECT OF POTASSIUM HYDROXIDE CONCENTRATION IN 95% ETHANOL ON THE CONVERSION OF THE FATTY ACID PORTIONS OF EQUAL AMOUNTS OF TRISTEARIN AND TRIOLEIN TO FREE ACIDS AND TO ETHYL ESTERS

KOH normality	*Triglyceride saponified or transesterified* (%)	*Free acids formed* (%)	*Ethyl esters formed* (%)
0	0	0	0
0.01	64	17.57	82.43
0.05	75	24.45	75.55
0.10	80	36.57	63.43
0.25	92	89.94	10.06
0.50	98	100.00	0.00

(Table II). Despite the presence of 5% water in the ethanol as the concentration of potassium hydroxide was decreased, the reaction led to ethanolysis rather than to hydrolysis. In addition, the overall yield of combined ethyl ester plus free fatty acid decreased. The results parallel those obtained with 0.5 *N* potassium hydroxide in the presence of silicic acid and indicate that silicic acid acts to cut down the effective potassium hydroxide concentration. In fact, when the triglycerides were saponified in 0.01 *N* KOH in the presence of 0.5 g of silicic acid, splitting of the triglyceride was less than 8% and only ethyl esters were formed.

It is concluded that saponification of triglyceride should not be attempted in the presence of silicic acid and that any reaction mechanism that tends to lower the effective alkali concentration below 0.5 *N* will result in low yields of free fatty acids.

Market Quality Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Md. (U.S.A.)

I. HORNSTEIN
P. CROWE
B. RUCK

1 E. G. BLIGH AND W. J. DYER, *Can. J. Biochem. Physiol.*, 37 (1959) 912.
2 I. HORNSTEIN, A. ALFORD, L. E. ELLIOT AND P. F. CROWE, *Anal. Chem.*, 32 (1960) 540.

Received October 14th, 1966