saturated acids of the same chain length (6). Such monoenoic acids are absent from *Zelkova* oil, as shown by the iodine values of zero or nearly zero for fractions 1–9 (Table I). The saturated acids of *Zelkova* are nearly all C_8–C_{12} while the unsaturated acids apparently occur entirely in the C_{18} chain length. This finding lends support to the view of Hilditch (7) that saturated and unsaturated acids are formed in the plant by separate mechanisms.

Summary

Seeds of the tree *Zelkova serrata,* family *Ulmaceae,* were found to contain 21.7% of glyceride oil having iodine value 12.9, saponification value 292, and glycerol yield 13.6%. The oil was converted to methyl esters and examined by gas chromatography, followed by fractional distillation of the esters and identification of the individual acids. The percentage composition of the acids is estimated as follows: caprylic 8, capric 73, lauric 3, myristic 1, palmitic 2, stearic 1, oleic 3, linoleic 3, undetermined 6. The content of capric acid is higher than has been found in any natural oil or fat. Comparing it with other genera of *Ulmaceae,* the oil of *Zelkova* is seen to resemble closely that of *Ulmus* sp. but to be quite different from the oils of *Celtis* and *Trema.*

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Labelling Fatty Acids by Exposure to Tritium Gas. I. Saturated Methyl Esters[1]

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A SIMPLE PROCESS for the labelling of organic compounds by exposure to tritium gas has been described by Wilzbach (4); it has been applied to a variety of compounds including n-heptane and other hydrocarbons (5). While the tritium incorporated in these aliphatic compounds was not labile, the production of radiation decomposition products necessitated the application of rigorous purification procedures to obtain the desired substituted hydrocarbons. No attempts have been reported to date in which fatty acids have been labelled by the gas-exposure technique.[2]

The present paper describes the application of this procedure to the labelling of methyl esters of saturated fatty acids in which the anticipated substitution of hydrogen by tritium is found to take place. More complicated reactions, principally addition, occur in the labelling of unsaturated fatty methyl esters and will be the subject of a subsequent paper.

Experimental

a) Methyl esters of stearic and palmitic acids were obtained from the Hormel Institute. Methyl laurate and methyl myristate were prepared from coconut oil and separated by distillation through a Podbielniak 13-mm. diameter column with 4 ft. of "Heligrid" packing.[3]

b) Irradiations on gram amounts of methyl esters of palmitic and stearic acids were carried out at room temperature in the solid state, deposited as thin layers by solvent evaporation on the inside walls of a 1×10-cm. reaction tube, using a source of approximately 1 curie.

Methyl laurate and methyl myristate are liquid at room temperature and require the use of sealed ampoules equipped with break seals. The ampoules were rotated to provide continuously renewed thin films and were irradiated by a 5-curie source.

c) Purification of the tritiated methyl ester includes saponification and extraction of unsaponifiables (1), exchange of labile tritium by distillation of 1.5 liters of anhydrous ethanol from the soaps in 50-ml. batches, and acidification and extraction of the free acids with diethyl ether.

d) Chromatographic methods, both partition and gas-liquid, were used to establish freedom of the chemically purified fatty acids from radiation decomposition products and from exchangeable tritium. The Nijkamp (2) procedure was used for the separation of monobasic acids. Alternate 1-ml. fractions of eluate were: a) titrated in a nitrogen atmosphere with 0.04 N NaOH to a thymol blue end-point, using a Gilmont micro-buret and b) diluted with 15 ml. of scintillation solution for radioactive assay with an automatic "Tri-Carb" scintillation spectrometer. Quenching of fluorescence by the fatty acids and by the chromatographic solvent was negligible.

Gas chromatography of methyl esters before purification and of purified fatty acids, after methylation with diazomethane, was carried out in "Aerograph" equipment at 205°C. on a 5-ft. Resoflex 296 column. Simultaneously with the recording of thermal conductivity, an ion chamber electrometer system recorded radioactivity (ion currents) on the gas stream issuing from the thermal conductivity cell. Alterna-

[1] Presented at fall meeting, American Oil Chemists' Society, Chicago, Ill., October 20–22, 1958.
[2] Rosenthal and Kritchevsky in a publication of the Radiation Laboratory (UCRL-1331) report the production of tritiated stearic acid through a catalyzed exchange reaction with tritiated water.
[3] Mention of commercial equipment or products does not constitute endorsement by the U. S. Department of Agriculture over those of other manufacturers.

tively radioactivity was determined by directly trapping methyl esters from the effluent gas stream in vials containing 15 ml. of scintillation solution for specified periods, *e.g.*, 1 min., and by subsequent assay in the scintillation spectrometer.

Results

Summarized in Table I are the results of irradiation of four saturated fatty acid esters. From the data shown for the first three fatty acid esters, the tritium incorporated is roughly related to the time of exposure and intensity of the source. The tritium was removed from the myristate, as noted, in the frozen state by evacuation; it probably carried along dissolved tritium into the pentane-hexane used as diluting solvent. If so, probably the tritiated preparations of palmitate, stearate, and laurate evacuated at room temperature may also have contained small amounts of tritium gas which would have been assayed in the "unsaponifiable" and labile fractions.

TABLE I

Tritium Incorporation and Distribution

Methyl ester	Exposure		Tritium incorporated, MC	"Unsaponifiables"	Labile	Acid aqueous phase	Fatty acid
	Source (curies approx.)	Days					
Palmitate	1	4	1.8	24.0	14.9	7.5	53.6
Stearate	1	13	7.1	15.3	7.9	0	76.8
Laurate	5	7	47.8	0 [a]	21.4	0	78.6
Myristate	5	8	134.0 [b]	.....	.....	.....	.....

[a] "Unsaponifiables" of methyl laurate were measured after exchange of labile tritium (see text).
[b] Tritium pumped from sample frozen in liquid nitrogen.

It is apparent that labile tritium, rapidly exchanging with ethanol, would also appear in the "unsaponifiable" extract layer. To confirm indications that the unsaponifiable fraction was largely ethanol, containing exchanged tritium, the ethanol was distilled from the sodium laurate prior to extraction of unsaponifiables. Insignificant radioactive material remained in the unsaponifiable fraction. The saponification step was omitted in the purification of methyl myristate, and exchange by distillation was the only treatment applied. Results of gas chromatography led to the conclusion that this simple treatment was effective in removing detectable chemical and radiochemical impurities.

Figures 1, 2, and 3 present the progress of purification of tritiated methyl palmitate as determined by three methods of analysis. Figure 1 depicts gas chromatography of methyl palmitate before purification. The method of condensing radioactivity in the scintillation solution was used. Figure 2 shows the gas chromatogram after chemical purification and partial exchange of labile tritium. The ion chamber was used for radioactivity assay. Figure 3 presents liquid-partition chromatographic analysis after completion of alcohol exchange. Titrimetric analyses and scintillation counting of the fatty acids were employed.

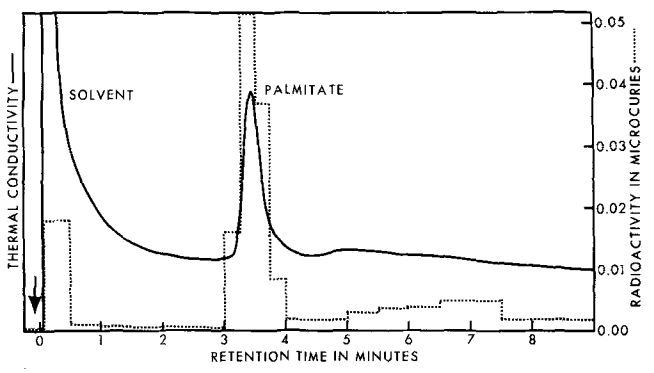

FIG. 1. Gas chromatogram of crude tritiated methyl palmitate.

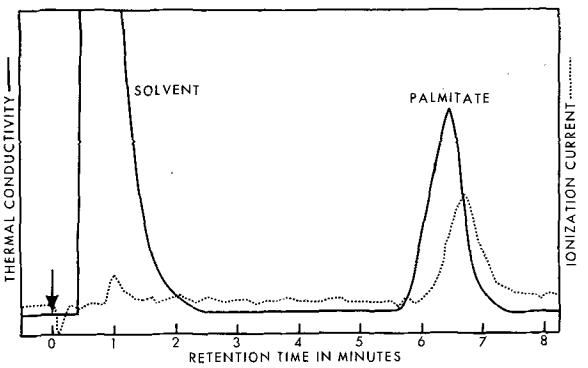

FIG. 2. Gas chromatogram with ion chamber for purified methyl palmitate.

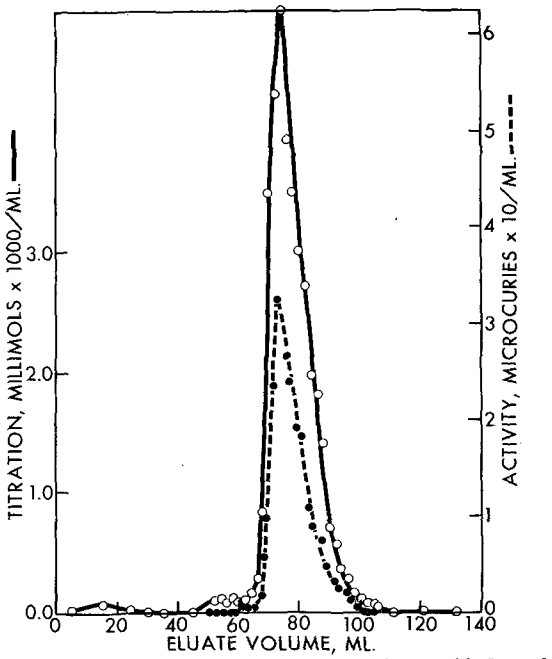

FIG. 3. Liquid-partition chromatogram for purified methyl palmitate.

Since experience in radiochemistry generally has shown that the employment of several criteria of purity is desirable, if not mandatory, use of both liquid-partition columns and gas-partition chromatography is offered to support the conclusion that palmitic acid labelled with tritium is homogeneous and that alcohol exchange is effective in removing labile tritium.

Use of the three chromatographic methods of establishing purity permits certain general observations and comparisons. While liquid-partition chromatography with combined titration and scintillation counting is the most quantitative in regard to sample introduction and recovery, gas-liquid chromatography and scintillation counting are more readily performed. Obviously the simultaneous recording of ion current and thermal conductivity has the unmatched advantage of speed and ease of performance.

The comparative absence of radiation damage and radiation decomposition products in the tritiation of saturated fatty acid esters was quite unexpected in view of the complexity of radiochemical reaction products reported by Wilzbach (5) for hexane and cyclohexane.

It is concluded that saturated fatty esters may be easily labelled with tritium in high specific activity by the Wilzbach procedure of gas exposure and that both the standard procedures for fatty acid purification and the alcohol distillation for removal of exchangeable tritium are effective.

Acknowledgment

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Current Status of the Toxic Principle Causing the Chick Edema Syndrome

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THE BROILER INDUSTRY was faced with a new malady, which appeared in epidemic proportions during the fall of 1957. Reports of the syndrome had been made earlier in the year, but the number of birds involved was quite small and the condition apparently disappeared spontaneously.

The losses of birds by broiler raisers reached some thousands of birds per day at the peak of the trouble. The birds began to die at three to four weeks of age, and post-mortem examination revealed the pericardial sac surrounding the heart almost always distended with fluid. As the condition progressed, the abdominal cavity quite often was filled with ascitic fluid, which led to the common term "water belly" as a name for the condition (Figure 1).

In the young chick the symptoms of this trouble included gasping, poor weight gain, paleness, and a waddling duck-like gait as the abdomen began to fill with fluid. Sudden deaths occurred in the affected flocks at three to four weeks of age.

The symptoms have not been observed to be as severe in the adult chicken. Laying hens suffered from a drop in egg production and fertility of the eggs. The typical condition of edema has not been produced in turkey poults and ducks when they were fed diets containing the toxic material. Laboratory rats, calves, and pigs did not exhibit the symptoms of edema when kept on diets containing the toxic material. White rats fed a broiler ration which was toxic to chickens did show some depression in growth.

Sanger *et al.* (1) reported that the fluids collected from the abdomen and pericardial cavity of birds suffering from the edema symptoms were sterile. No organism of a significant nature was recovered from cultures of liver, heart, and lung. Intestinal washings and fluid from the heart and abdomen appeared to be nontoxic when injected into white mice. Whole intestinal tracts homogenized and fed to white mice and four-week-old broilers for several days had no toxic effect. The condition was apparently not of an infectious nature. The various medications and additives commonly found in feeds did not cause the disease.

The accumulation of fluid in the pericardial sac is the characteristic gross lesion of the condition called chick edema or "water belly." As much as 20 ml. of fluid have been collected from the pericardial sac of broilers in advanced stages of the condition. In most cases of field outbreaks of this condition the affected broilers had from 100–500 ml. of liquid in the abdominal cavity (1).

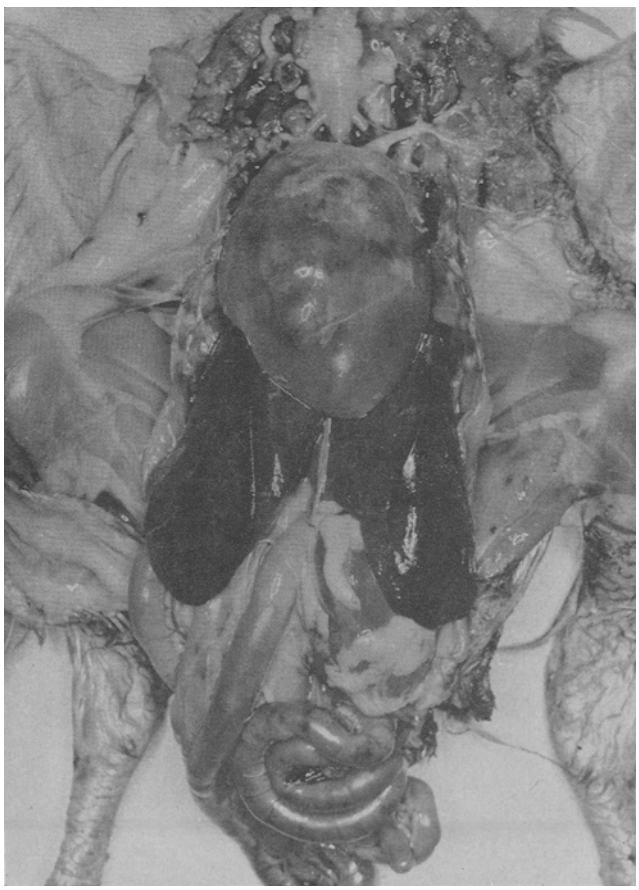

FIG. 1. Hydropericardial condition in broiler suffering from "chick edema."