The Preparation of Purified Methyl Linoleate By Chromatography[1, 2]

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The use of Twsett's chromatographic technique in separating and purifying the fatty constituents and investigating the mixed glycerides of vegetable seeds and animal fats provides a promising approach to the solution of some of the problems of fat chemistry. The investigations of Kaufmann (1) (2) who separated pure myristic and stearic acids from a mixture of the two acids and fractionated oleic and linoleic acids, and of Walker and Mills (3) who fractionated the glycerides of linseed oil have demonstrated the usefulness of the chromatographic technique in the fractionation of mixtures. Cassidy (4) (5) (6) investigated the adsorption isotherms of fatty acids in studying the mechanism of chromatographic separations.

Walker and Mills found that mixtures of linseed oil fatty acids were not readily separated chromatographically using alumina, because association and ionization interfered with fractional adsorption; the glycerides of the oil, however, were readily separated with excellent fractionation and good recovery of the oil fractions. The successful application of chromatography in this separation of the glycerol esters of fatty acids suggests that the separation of the methyl esters may be more easily effected than separation of the acids themselves and, in the present investigation, chromatography of the methyl esters of cottonseed oil fatty acids has been used in preparing purified methyl linoleate; this technique offers a worthwhile method for the preparation of highly purified natural methyl linoleate that does not involve the Rollett-bromination-debromination procedure (7) which yields a synthetic product and which, in convenience and efficiency, has advantages over Brown and Frankel's low temperature crystallization method (8) (9) by which the highly purified natural acid (97-100 percent pure) may be obtained in low yield.

Experimental

In applying chromatography to the preparation of purified methyl linoleate in the present work, the use of appropriate, reproducible, experimentally-determined conditions has been employed to remove the complication introduced by the lack of visual means of differentiating between the adsorbed bands. The results of initial experiments indicated that the activity of the alumina adsorbent was the most critical single factor, the use of highly active alumina being attended with large losses of unrecoverable methyl esters as well as poor separations and the use of slightly active alumina being ineffective. The chief problem, therefore, was the selection of an alumina of suitable activity; afterwards, the determination of the proper proportions of alumina, methyl esters, and of the developing and eluting solvents was readily accomplished.

The alumina used in all chromatographic procedures was Alorco, chromatographic grade, grade A, minus 80 mesh, which is prepared by the Aluminum Ore Company of East St. Louis, Illinois. A series of alumina samples of varying degrees of activity was prepared by exposing strongly activated alumina to moist air and, using Brockmann's dye test method (10) to determine the activity of the various preparations in conjunction with tests of the effectiveness of the various specimens, an activity corresponding to that of IV in Brockmann's series was found to be the most active alumina which permitted good recovery of the esters. In these experiments 3 gms. of the methyl esters of cottonseed oil fatty acids, dissolved in 50 ml. of petroleum ether (35-60°), was adsorbed on a column of alumina (200 gms. in a chromatograph tube 1½-in. diameter) and the chromatogram was developed with 300 ml. of the petroleum ether. The alumina was removed from the tube with pressure as described by Turkevich (11); the column was cut in sections which were extracted with ether and acetone in continuous extractors and the iodine values and weights of the extracted and eluted fractions were determined.

When alumina of activity IV was used, 90-95 percent of the methyl esters were recovered and of this percentage slightly over 25 percent was separated in fractions having an iodine value of 145-155. On using 400 gms. of alumina (IV), adsorbing 4 gms. of methyl esters dissolved in 50 ml. of petroleum ether on the alumina, and developing with 600 ml. of petroleum ether, approximately 35 percent of the recovered esters were obtained in fractions of 145-160 iodine value. In later work a larger ratio of alumina to methyl esters was preferred in order to increase the yield of purified methyl linoleate and, as will be noted, a ratio of 200 to 1 was finally used in preparing the methyl linoleate of high purity.

The quantity of developing and eluting solvent necessary to remove the bulk of the esters of low iodine value was determined as follows: Four gms. of the methyl esters were dissolved in 150 ml. of petroleum ether and adsorbed on a column of alumina (400 gms. of alumina of activity IV in a tube 1¾-in. diameter); the chromatogram was developed and fractions were eluted with 3 liters of petroleum ether which permitted recovery of 3.6 gms. of the fractionated esters; the solvent was collected in ten portions containing fractions of methyl esters having the following weights and iodine values:

0.40 gm., I.V. 2.0; 0.39 gm., I.V. 28.0;
0.48 gm., I.V. 42.5; 0.39 gm., I.V. 80.5;
0.40 gm., I.V. 140.8; 0.48 gm., I.V. 142.0;
0.22 gm., I.V. 155.1; 0.20 gm., I.V. 158.9;
0.22 gm., I.V. 164.8; 0.45 gm., I.V. 171.2.

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The quantity of solvent necessary to remove all fractions of iodine value lower than 142 was found to be 1400 ml., the fractions of iodine value higher than 142 weighed 1.6 gms.; the Wijs iodine value, 171.2, of the last eluted and purest fraction indicated that the fraction was 98.6 percent methyl linoleate calculated from the iodine value, assuming the impurity to be methyl oleate.

The results of the previous experiments were used in outlining a procedure for separating methyl linoleate as follows: A column of alumina for chromatographic adsorption was prepared by alternately pouring alumina and petroleum ether (36-60°) in small portions into a chromatograph tube (400 gms. of alumina adjusted to activity of IV in a tube 1¾-in. diameter); a glass rod was used for stirring to free trapped air and packing, care being taken to maintain a layer of solvent over the adsorbent. The methyl esters of cottonseed oil fatty acids used in all of the experiments had been prepared using a procedure previously described (12); a portion, 4 gms., dissolved in 150 ml. of petroleum ether, was introduced into the column of alumina.

The chromatogram was developed with 1400 ml. of petroleum ether, which eluted a fraction of iodine value, 74.6, consisting of a mixture of the methyl esters of the saturated, oleic, and some linoleic acids. The methyl linoleate fraction was eluted by rapidly forcing an additional 2.5 liters of petroleum ether through with pressure. Previously a flow of 600-700 cc. per hour had been maintained during the development and elution without application of pressure to the top or vacuum to the bottom of the column; a nitrogen atmosphere was used to replace air over the column and the eluted esters.

Another 4-gm. portion of methyl esters was separated by the same procedure, using the slightly spent alumina. Two further 4-gm. portions were separated on a freshly prepared column. From a total of 16 gms. of methyl esters (Wijs iodine value 108.0, and thiocyanogen value, A.O.A.C., 63.1) containing 54.1 percent methyl linoleate, based on an empirical thiocyanogen value of 89.4 for methyl linoleate (13), 5.2 gms. of methyl linoleate, of iodine value 159.5, was obtained. The bulk of the impurities was removed as follows: Five gms. dissolved in 150 ml. of petroleum ether, was adsorbed on a column of alumina of the same activity (400 gms. in a 1¾-in. tube), and 700 ml. of solvent was used to remove the more saturated esters; 3.2 gms. of methyl linoleate was recovered from the next 2 liters of petroleum ether used for elution.

The specimen of methyl linoleate had the following characteristics: Wijs iodine value 170.4 (theory 172.4), thiocyanogen value, A.O.A.C. (14), 87.3, $n_D^{25} = 1.4594$. The product was distilled at 0.05 to 0.1 mm. pressure at 145-150° C.; the distilled ester had the following characteristics: Wijs iodine value 170.3, thiocyanogen value 88.1 and $n_D^{25} = 1.4594$. The specimen was 97.7 percent pure methyl linoleate calculated from the iodine value, assuming methyl oleate as the impurity, and 100.1 and 99.0 percent pure calculated from the iodine values and thiocyanogen values of the undistilled and distilled specimens, respectively, using equations based on empirical thiocyanogen value of 89.4 (13) for pure methyl linoleate.

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