tively small amounts of material separated. This limitation results from the high solvent to solute and the high adsorbent to solute ratios. Also, as currently applied, adsorption analysis has the disadvantages inherent in a batch process.

In the fractionation of soybean oil by adsorption analysis reported here, no evidence has been obtained for the occurrence of glycerides more unsaturated than dilinoleo-monolinolenin or for the occurrence of di- or trisaturated glycerides. These observations are in accordance with the theory of even distribution of fat acids.

The immediate application anticipated in these fundamental studies is the preparation of soybean oil fractions of both high and low linolenic acid content and a study of these fractions in regard to the "linolenic acid" theory of flavor instability (15). Consequently, the next step is the preparation of high- and low-iodine-value fractions in quantity using the naturally occurring carotenoid pigments as internal indicators for the location of glyceride fractions.

Acknowledgment

The authors are indebted to J. C. Cowan for his advice and encouragement throughout the course of the work.

Summary

A rapid method of fractionating soybean glycerides and their ethyl esters by adsorption analysis on an aluminum oxide column is described. It compared favorably with other methods of fractionation. In the adsorption analysis of crude soybean oil, the iodine values of fractions ranged from 104 to 173, a spread of 69 units. However, under other conditions of adsorption, iodine values as high as 200 were obtained. In the analysis of ethyl esters of soybean oil, iodine values ranged from 61 to 185, a spread of 124 units. The fat acid composition of each fraction was calculated from spectrophotometric and iodine value determinations. Recoveries from the columns were 82.4% in the case of the glycerides, and 90.0% in the case of the esters.

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Adsorption Analysis of Lipids. III. Synthetic Mixtures of Ethyl Stearate, Oleate, Linoleate, and Linolenate*

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THE separation of esters derived from naturally occurring fats is commonly achieved by distillation. Esters of saturated fat acids which differ in length by two carbon atoms may be separated satisfactorily for analytical purposes (1, 2) by the use of modern, efficient columns or by the amplified distillation technique. Mixtures of fat acid esters of the same carbon chain length but with varying degrees of unsaturation, however, can be only partially fractionated, if at all. Norris and Terry (3), using a Podbielniak column, recovered fractions containing 95% methyl oleate from a mixture of equal parts of methyl stearate and methyl oleate; however, with a mixture of equal parts of methyl oleate and methyl linoleate, no appreciable fractionation resulted. Little success was anticipated for the separation of other members of the homologous series of unsaturated fat acids, presumably because of "association of the acids."

Fractional crystallization and countercurrent extraction methods appear more promising for the separation of unsaturated esters of the same carbon chain length than do distillation procedures. However, since no data comparable to that presented herein have been published, comparison is limited in this paper between adsorption analysis and the less-efficient distillation procedure. In the previous paper of this series comparisons were made of the fractionation of soybean glycerides accomplished by adsorption analysis, molecular distillation, countercurrent extraction, and fractional crystallization (4).

The feasibility of applying chromatographic adsorption analysis to the separation and preparation of individual fat acids has been studied qualitatively by several workers (5, 6, 7, 8), but few systematic investigations which give quantitative information as to column performance have been reported. Cassidy (9) has studied quantitatively the completeness of separation in the homologous series of saturated fat acids on carbon columns by the use of conventional chromatographic development and elution techniques. In connection with the "frontal analysis" technique Claesson (10) has made a remarkable contribution by developing the theory and obtaining data which permit the analytical determination of mixtures of saturated fatty acids and a few unsaturated fat acids and

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their esters. This technique, however, is not applicable to preparative problems. Kaufmann (11) appears to have been the first to explore systematically the efficacy of the adsorption analysis technique for the separation of an homologous series of unsaturated fat acids. While the iodine values indicate some fractionation occurred, data were not given with which to evaluate the column performance, such as weight and composition of the fractions and the percentage recovery.

In the first paper of this series (12) the separation of a mixture of equal parts of stearic and oleic acids upon carbon was reported. By judicious choice of fractions 63% of the oleic was recovered with a purity of 99%, and 53% of the stearic with a purity of over 90%. In the second paper of the series (4) the utility of adsorption analysis was demonstrated for the separation of glycerides and ethyl esters of soybean fat acids.

The purpose of the present paper is to report the adsorption analysis of binary mixtures of an homologous series in unsaturation, consisting of ethyl stearate, ethyl oleate, ethyl linoleate, and ethyl linolenate. Measures of the performance of the adsorption technique have also been obtained.

Materials

Although starting materials of highest purity were unnecessary for this work, the spectroscopic absorption constants of the ethyl linoleate and linolenate used were equal to published values. The ethyl oleate preparation used contained linoleate as its principal impurity. The ethyl stearate was of commercial grade (Eastman Kodak No. 1600, m.p. 30°-31°C., iodine value 1.62) and presumably contained some ethyl palmitate.

Ethyl oleate was prepared from the ethyl esters of commercial oleic acid by low temperature crystallization from acetone. The general procedure of Swern, Knight, and Findley was followed (13). The ethyl oleate fraction used for these experiments had an iodine value of 87.2 (theory 81.45). Spectrophotometric analysis showed that it contained 0.3% of linolenic acid and 6.8% of linoleic acid.

Both ethyl linoleate and ethyl linolenate were prepared from their corresponding bromostearic acids using the procedure (14) described in *Organic Synthesis*, Vol. 22, with minor variations. A saturated solution of hydrogen chloride in absolute ethanol was used to speed the esterification reactions. The esters were extracted from the alcoholic solution with petroleum ether before washing and drying. Solvent was then removed and the esters purified further by vacuum distillation. Spectrophotometric analysis of the ethyl linoleate showed that it contained 1.00% (diene 0.99, triene 0.01) original conjugation. The absorption coefficient expressed as liters/gm. cms. of the isomerized ester at 2340 A was 82.3. Ethyl linolenate similarly prepared had an iodine number of 248.3 (theory 248.47) and diene conjugation of 0.11%. The absorption coefficient of isomerized esters at 2680 A was 48.2.

Experimental Procedure

The apparatus and procedure used for this series of experiments were nearly the same as that for the adsorption analysis of soybean glycerides and ethyl esters described in the previous paper (4). A mixture of equal parts of the individual esters (total charge 20 gms.) was adsorbed on a column containing approximately 700 gms. of alumina (Harshaw's A1-2).[2] The column was developed with a solvent consisting of 1.75% diethyl ether in petroleum ether. Nitrogen pressure was used to speed percolation and to reduce oxidation of esters on the columns.

Except for one instance when the spectrophotometric method was applied, the composition of the fractions from binary mixtures was calculated from the determined iodine number of the mixture and the theoretical iodine values for the pure components. Six adsorptions were carried out, this being the number of possible combinations of the four esters as binary mixtures.

Results

The effectiveness of adsorption analysis in separating the six binary combinations of the four ethyl esters is shown graphically in Fig. 1. In the lower section of the graph for each binary combination is the concentration of the fractions eluted in centigrams per 100 ml. and the corresponding iodine values, both plotted against the volume of eluate. The upper section for each binary mixture gives the percentage composition of each component, also plotted against the corresponding volume of eluate.

From inspection of the three graphs in the left column (ethyl stearate in combination with ethyl oleate, ethyl linoleate, and ethyl linolenate) it can be seen that the greater the difference in degree of unsaturation of the pair, the more complete is the fractionation. Specifically, the best separation occurred in the stearate-linolenate system. This and other conclusions, however, can best be drawn by consulting Table I.

In order to obtain indices for comparing the effectiveness of adsorption analysis in the separation of the six binary systems, the eluted esters from each column were divided into two fractions, the division coinciding with the cross-over point (50%) of the composition curves. The average per cent composition for a specific component in each of these two large fractions was calculated from the compositions of the smaller fractions and is expressed as the per cent by weight of that component in the total weight of ester present in the large fraction. Average per cent composition for component A=

$$\frac{\Sigma \text{ per cent compositions A} \times \text{portion weights}}{\Sigma \text{ portion weights}}$$

From the columns of Table I, giving the percentage of component A in the first fraction and the percentage of component B in the second fraction, it can be seen that the best fractionation occurred between the components of the stearate-linolenate system, corresponding to a difference in molecular structure of three double bonds. Less complete fractionation occurred in the stearate-linoleate and oleate-linolenate systems between which a difference of two double bonds exists. The poorest fractionation was found in mixtures of stearate-oleate, oleate-linoleate, and linoleate-linolenate where a difference of only one double bond exists. It will also be noted that, in accordance with theory (15), the first component to be eluted (component A) is obtained in higher average purity than the second component (B), in the second portion.

Included also in Table I are data on the overall loss of esters placed on the column. Thus, in the stearate-linolenate system, for example, the loss was 12.5%. One fraction calculated as described was obtained, comprising 47.48% esters recovered and contained 82.77% stearate, while another fraction was

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[2] The mention of this product does not imply that it is endorsed or recommended by the Department of Agriculture over others of a similar nature not mentioned.

TABLE I

Comparison of Fractionations

Components		Percentage loss on column	First fraction			Second fraction			Percentage recovery of components	
A—least adsorbed	B—more strongly adsorbed		Per cent of recovered esters	Average per cent component A	Highest per cent component A	Per cent of recovered esters	Average per cent component B	Highest per cent component B	A	B
Stearate	Oleate	21.4	63.4	68.0	88.9	36.6	63.5	83.7	88.7	68.5
Stearic acid	Oleic acid (12)	8.0	49.6	87.0	92.3	50.4	94.0	99.7	85.0	99.0
Stearate	Linoleate	23.3	55.4	81.7	98.5	44.6	69.0	94.8	90.7	62.7
Stearate	Linolenate	12.5	47.5	82.8	99.7	52.5	75.7	100.0	91.2	83.8
Oleate	Linoleate	28.2	43.9	67.8	91.2	56.1	65.0	78.2	70.9	72.7
Oleate	Linolenate	22.3	59.5	73.1	92.9	40.5	76.4	93.8	82.5	72.9
Linoleate	Linolenate	27.0	59.9	68.6	83.5	40.1	46.82	58.5	91.1	54.9
(Spectrophotometric)										
Linoleate	Linolenate	27.0	59.9	68.7	83.9	40.1	61.25	74.5	82.8	63.2

obtained comprising 52.52% of the esters recovered and contained 75.5% ethyl linolenate. In actual preparative work intermediate portions included in the fractions described would be withheld and subsequently readsorbed. The purity of these fractions would thus be correspondingly higher than the calculated average values. Figures for the highest purity fractions obtained indicate that relatively high-purity esters may be recovered in certain fractions from a single adsorption analysis.

Erratic iodine values and, hence, erratic composition figures were obtained in the linoleate-linolenate system. This behavior is probably due to peroxide development in the original components and the segregation of the oxidized esters by the column. Although precautions of low temperature and inert gas storage were taken, the peroxide value of the linolenate rose to 106.1 mg. of oxygen/kg. over the 3-month period in which the work was carried out. The peroxide value of the linoleate meanwhile rose to 391.8. The linolenate-linoleate system was the last to be studied. Spectrophotometric analyses for ethyl linolenate were performed on the fractions of this system. Agreement between the compositions calculated from spectrophotometric and iodine value data is considered satisfactory on the first half of the fractions. In the latter half, which presumably contained the oxidized acids, the compositions calculated from spectrophotometric data appear less erratic than do those from the iodine values, as these peroxides appear to affect the iodine values more than they affect the spectral properties of the esters.

The percentage recovery of each of the components originally placed on the column is shown in the last two columns of the table. In general, the highest per cent recovery was obtained for the first component to be eluted from the alumina, the more strongly adsorbed component suffering the greatest loss.

The data for the analysis of stearic and oleic acid on carbon black calculated from data given in a previous paper are also given in Table I (12). A better fractionation was achieved under those conditions than in the results currently reported. On carbon black the more unsaturated component is eluted first, the reverse order of adsorption from that on aluminum oxide. This accounts for the average composition of component B in the second fraction being higher than component A in the first fraction.

Discussion

Binary mixtures of fat acid ethyl esters considered inseparable by distillation techniques have been fractionated upon adsorption columns. Separation by distillation and adsorption appears to involve different molecular characteristics. The success of the distillation process in separating members of an homologous series depends on the difference in their vapor pressures which in turn is a function of their molecular weights. Although theory relating molecular structure and adsorption properties is inadequate, empirical observations on homologous series of polyene pigments (16, 17) have shown that the number of double bonds the pigments contain influences their rate of movement on the chromatographic column. It is apparent that a difference in adsorption properties between a triene and diene may represent a large difference compared to the difference in molecular weight or vapor pressure. This large difference may partly explain the success of the adsorption analysis technique with the unsaturated mixtures studied.

In order that comparisons between columns might be justified the adsorption analyses described here have been performed under uniform but not necessarily optimal conditions. Among the variables which influence the completeness of separation of solutes are length of column, the specific character of the adsorbent, qualitative and quantitative compositions of the developing solvent, the rate of flow of the developing solvent through the column; and the nature, concentration, and number of solutes. While some exploratory investigations of these variables were performed to establish the standard conditions used, even better separations should be achieved by a more careful study of conditions for the specific binary mixture.

Summary

The adsorption analysis of six binary mixtures composed of ethyl stearate, ethyl oleate, ethyl linoleate, and ethyl linolenate has been studied on alumina columns. Distillation methods have been shown by other workers to fractionate the stearate-oleate system but not the oleate-linoleate system. Application of the adsorption analysis technique resulted in fractionation of all six systems including the oleate-linoleate mixture.

Measures of the performance of the columns were obtained by dividing the eluate into two portions at the point in the fractionation at which the starting composition was reached. The first component to be eluted from each of the six pairs was obtained in the first fraction with an average purity of between 68 and 83%. The second component was obtained in the second fraction with average purity of 61 to 76%. The first component to be eluted was obtained in higher purity than the second. The efficiency of separation of pairs appeared to depend on the difference in degree of unsaturation of the components. Overall

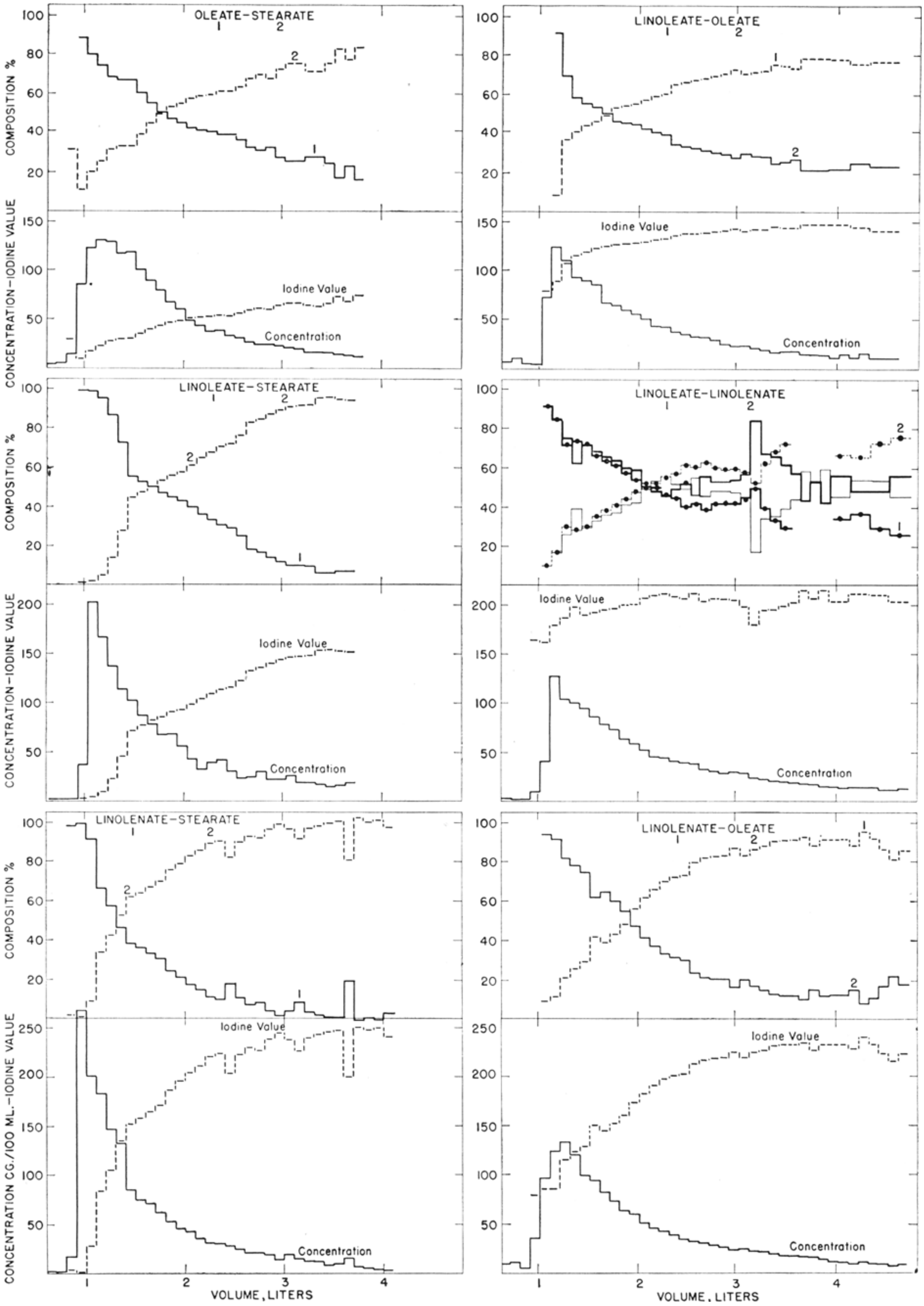

FIG. 1. Adsorption analysis of binary mixtures of ethyl stearate, ethyl oleate, ethyl linoleate, and ethyl linolenate. In the linoleate-linolenate system solid lines are calculated from iodine value data. The solid circles designate spectrophotometrically determined compositions.

recoveries of esters from the columns ranged from 71.8 to 87.5%.

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A Suggested Modification of the Bauer Mill Used for Grinding Small Samples of Soybeans

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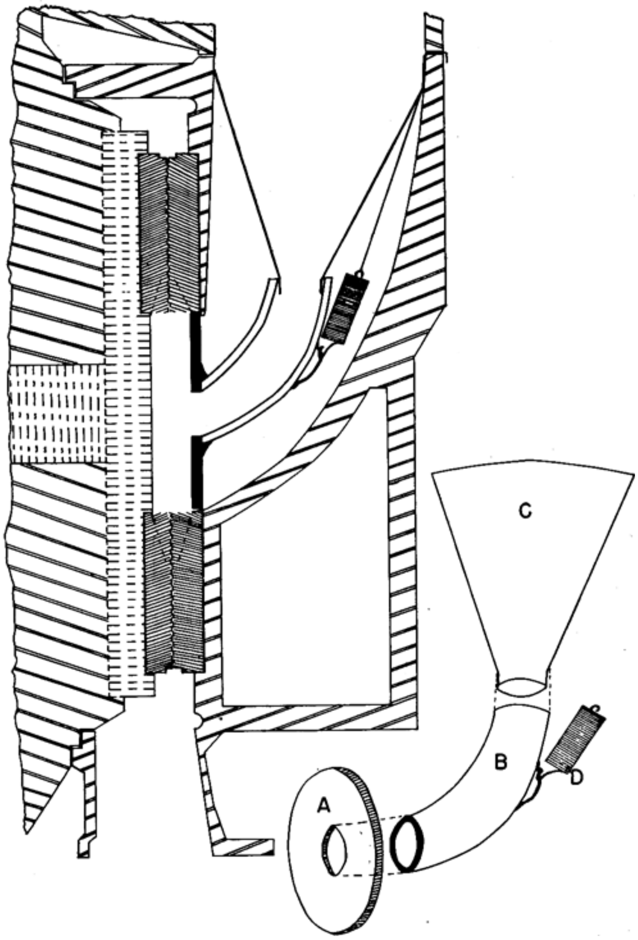
THE Bauer mill, specified for grinding samples of soybeans and cottonseed for oil determinations (A.O.C.S. Official Method Aa 4-38 and AC 3-44) (1) has a feed throat approximately 2½ inches in diameter. During the grinding of a sample coarsely ground material is repeatedly thrown back into the throat, and a small fraction of the sample is thrown out of the mill. The throat usually retains from 3-7 grams of coarsely ground material. When the mill door is opened to remove the ground sample, this partially ground portion is usually brushed into the rest of the sample. For soybeans, Collins and Krober (2) show that coarse material in the sample may cause low values in the oil percentage when oil is determined on the sample.

The device shown in the sketch eliminates essentially all of this coarse material from the ground sample, prevents loss of the sample thrown back through the throat, and simplifies cleaning the mill between samples. The device consists of four parts: A, a circular plate of brass or iron ⅛ inch in thickness turned to fit the throat opening of the mill and with a ¾ inch hole drilled in its center; B, a curved piece of metal tubing 3 to 3½ inches long and with ¾ inch inside diameter shaped to fit the mill throat curvature and welded or soldered to the plate "A"; C, a sheet metal funnel made to fit the mill throat with the small end fitting loosely inside the tube and extending a short distance into the tube; D, a short spring with a 5- to 10-pound tension installed to hold plate "A" in position.

The sketch shows the assembled parts of the device installed in a Bauer mill of the water-cooled type. The tension spring holds the attachment described above firmly in place but also allows the mill operator to remove it quickly.



SKETCH OF MODIFIED THROAT FOR BAUER MILL

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