aglycone was passed through a column containing Dowex 3 (20–50 mesh) underneath and Dowex 50 (100 mesh) on top. The Dowex 3 had been washed with 20% NaOH solution, followed by thorough washing with distilled water to neutrality. The Dowex 50 had been washed with 5% H_2SO_4 solution, then with distilled water. Each filtrate was passed through the resin separately. Each effluent solution was concentrated under reduced pressure to 1 ml. Identification was accomplished on paper chromatograms by comparing the unknowns with known samples of sugars, using as the solvent system n-butyl alcohol-pyridine-benzene-water (5:3:1:3 v./v.) and using the phosphate salt of 2-aminodiphenyl in glacial acetic acid as a chromogenic spray (7).

In the hydrolysate from compound "2A-2" (rutin), glucose and rhamnose were present. In the hydrolysate from compound "3A" (isoquercitrin), glucose was identified as the only sugar present.

For determination of the ratio of each sugar to the aglycone present per molecule of glycoside, the hydrolysate was concentrated *in vacuo*, and the remaining suspension was set in the refrigerator over-night. It was then filtered on a sintered glass funnel. The filtrate was passed through the column containing the Dowex resins, as previously described, and concentrated to about 3 ml. under reduced pressure, then the concentrate was diluted to 5 ml. in a volumetric flask. An aliquot (0.4 ml.) was streaked on Whatman No. 1 paper and analyzed quantitatively by a method based on the procedure reported by Timell *et al.* (7).

The precipitate from the hydrolysate was dissolved in 95% ethyl alcohol, and the solution was contrated *in vacuo*. The solution volume was then adjusted to 5 ml. An aliquot (0.5 ml.) was chromatographed, using the n-butyl alcohlo-acetic acid-water system. The quercetin zone was cut out and eluted with ethyl alcohol-water (4:1) for 24 hr. The eluate was diluted to 10 ml. A blank strip was run in the same manner except that no quercetin was present. The absorbance of each quercetin solution was measured at 375 mµ, using a 1-cm. silica cell and a Beckman spectrophotometer, Model DU. The quantity of quercetin present was determined from a standard quercetin curve.

To obtain the standard quercetin curve, five standard samples of different, but known, concentrations of quercetin were processed through exactly the same procedure as already described for quercetin in the glycoside hydrolysate. A straight-line standard curve was obtained by plotting the absorbance against micrograms of quercetin originally streaked at the beginning of its paper chromatography.

By these procedures, compound "2A-2" (rutin) was found to have a ratio, within experimental error, of one quercetin to one glucose to one rhamnose whereas compound "3A" (isoquercitrin) had a ratio, within experimental error, of one quercetin to one glucose.

Summary

The flavonol glycosides, rutin and isoquercitrin, have been separated from crushed, delinted cottonseed (kernel and hull) by extensive use of paper chromatography. The identification of these flavonoid pigments has been achieved through paper chromatography, ultraviolet and infrared spectrophotometry, and qualitative and quantitative analysis of their hydrolysis products. Details of the separation and identification have been described.

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[Received February 2, 1959]

# Reactions of Dienophiles with Vegetable Oils. I. Reactions of Maleic Esters with Sulfur Dioxide Catalyst[1]

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PREVIOUS WORK in these laboratories (2, 8) has been concerned with the Diels-Alder addition of dienophiles to conjugated fatty acids. In that work linoleic acid was isomerized to produce *cis,trans*-conjugated acid. After elaidinization to the *trans,trans* form these acids were treated with various dienophiles

to give adducts of the type I. These adducts and their derivatives have potential utility as plasticizers (2). This potential may be limited however by the cost of obtaining the *trans,trans*-conjugated acids from vegetable oils. Further work (6) showed that *cis,trans*-acids could be used in these reactions if selenium were present as a catalyst. The next logical step is the preparation of adducts having structure I directly from vegetable oils. Adducts of the whole oil might have properties superior to those of the simple ad-

$$CH_3(CH_2)_{5\ or\ 4}—CH \underset{CH———CH}{\overset{CH===CH}{\diagup\diagdown}} CH-(CH_2)_{7\ or\ 8}—CO_2R.$$
$$R''\quad R'$$

I
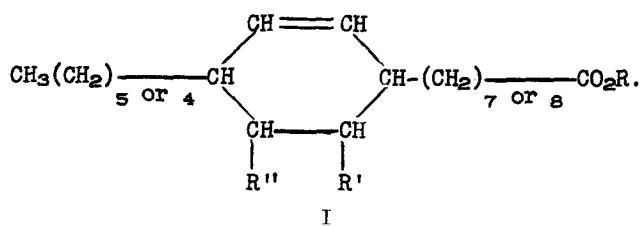
ducts. These in turn should be readily available by saponification or hydrolysis.

Safflower oil was chosen for these experiments because of its high linoleate content. Di-n-butyl maleate was used in the initial experiments because its reported high boiling-point permitted reaction at atmospheric pressure. An improved technique later permitted successful use of the lower-boiling dimethyl maleate.

Use of an isomerization-elaidinization catalyst is necessary to transform the linoleate into a suitable configuration for the desired reaction. Sulfur dioxide appeared to be a most promising catalyst because of its great versatility. Dependent on conditions, sulfur dioxide has been shown to isomerize, elaidinize, or polymerize oils (5, 9). Consideration was given anthraquinone, which yields conjugated oils with a high proportion of *trans,trans* isomer (1, 3). Preliminary experiments with this catalyst were not very successful, largely because of the difficulty in separating anthraquinone from the reaction mixture.

Experimental

Reaction of Maleic Ester with Safflower Oil. Two typical experiments are described to illustrate the methods used.

A. Safflower oil (120.5 g., equivalent to 0.1 mole of trilinolein) and 68.5 g. (0.3 mole) of di-n-butyl maleate were charged to a 500-ml., 3-necked flask fitted with a thermometer, a reflux condenser, and a gas delivery tube. The gas delivery tube was connected to a manifold which permitted introduction of nitrogen, sulfur dioxide, or both. The flask was heated with an electric mantle, and the contents were stirred magnetically. The mixture was heated to 290° and swept with nitrogen for 15 min. Sulfur dioxide was then turned on, and the nitrogen was turned off. These conditions were maintained for 1.25 hrs. Nitrogen was then turned on, and the sulfur dioxide and heat were turned off. The cooled mixture had a refractive index $n^{30/D}$ 1.4746 as compared with 1.4609 before the reaction. During the reaction the mixture lost 4.8 g. Heating the mixture under vacuum with carbon dioxide ebullition resulted in the distillation of 2.7 g. of colorless liquid, $n^{30/D}$ 1.4372, and in a further loss in weight of 1.0 g. The final product was a viscous, rather cloudy, yellow liquid, $n^{30/D}$ 1.4752, I.V. 56.3.

B. A 250-ml. flask, fitted with a reflux condenser, gas delivery tube, and dropping funnel, was charged with 100 g. (0.083 mole trilinolein) of safflower oil. The oil was swept with nitrogen, and the flask was lowered into a Wood's metal bath at 220°. When the bath temperature reached 294°, in about 0.5 hr., sulfur dioxide was turned on and the nitrogen was turned off. After 0.5 hr. of sweeping with sulfur dioxide at this temperature, 36 g. (0.25 mole) of dimethyl maleate were added dropwise over a period of 0.5 hr. The temperature of the bath was held as steady as possible during this addition. After all the maleate had been added, the heating and sulfur dioxide sweep were continued for 0.5 hr., after which nitrogen was turned on and the mixture was allowed to cool. Loss during reaction was 5.4 g. Distillation under reduced pressure (*ca.* 75 mm.) recovered 10.8 g. of ester, mainly in the form of dimethyl fumarate. The product had $n^{30/D}$ 1.4819 and I.V. 69.5.

Preparation of Adduct Esters. The products of the reactions of maleic ester with safflower oil either were saponified with ethanolic potassium hydroxide and the resulting acids esterified or were transesterified with alcohol containing hydrogen chloride. In either procedure the alcohol used was the same as that of the maleic ester. The esters so obtained were finally distilled under vacuum. Distillation of the butyl esters was invariably accompanied by decomposition, evidenced by evolution of uncondensable fumes, increasing pressure, large amounts of residue, and over-all loss in weight. Distillation of the methyl esters was much easier and cleaner. Losses and residue were generally nominal although some decomposition was always observed.

The entire quantity of volatile material boiling higher than the simple unreacted esters was considered to be "adduct fraction." Yields were calculated as the percentage of linoleate converted to adduct fraction. Because of their volatility and physical properties adducts containing 1 mole of maleate per mole of linoleate were assumed.

Results and Discussion

Original reaction conditions were chosen such that the sulfur dioxide would activate the oil for polymerization (9). A configuration of the oil suitable for polymerization should be equally suitable for Diels-Alder addition. Under conditions of Experiment A a 51.8% yield of adduct fraction was obtained. Variation of conditions gave no improvement in yield. At 200° there was no reaction, but at 250° a 24.0% yield was obtained. Doubling the reaction time at 250° raised the yield to 38% but increased the residue by a like amount. Omission of the sulfur dioxide greatly reduced the yield.

Use of excess maleate did not improve the yield, but use of one-third less than the stoichiometric amount did not lower it. The fact that no unreacted maleate could be recovered even when an excess was used, together with the high loss and residue, indicates the extent of side reactions experienced with dibutyl maleate. Use of the n-butyl esters of safflower fatty acids in place of the oil or of di-n-butyl fumarate in place of the maleate had no material effect on the reaction.

To avoid the decomposition and side reactions of the dibutyl maleate reactions, attention was directed to the use of dimethyl maleate. With this lower-boiling ester the dropwise addition technique of Experiment B was utilized. Variation of conditions had the same effect on the results as had been observed with dibutyl maleate. Sweeping the oil with sulfur dioxide prior to the addition of the maleate proved to have significant effect on the yield of the adduct fraction. Using the procedure of Experiment B, a ess was conducted at 100°F.(38°C.) with furfural containing 2.5% of water. The cost of conducting the separation on a large scale is believed to be favorable enough to make the process commercially attractive.

67.2% yield of adduct fraction was obtained on trans-esterification and distillation. Another run under the same conditions gave a 70.3% yield.

To determine the nature of the adduct fractions, a sample from the dibutyl maleate reactions was carefully purified by passage through a charcoal-alumina column and redistilled. Table I gives constants for

TABLE I
Comparison of Properties of Adduct n-Butyl Esters

Property	From 9,12-cis,cis acid	From 9,11-trans,trans acid	Theory
$n^{30/D}$	1.4650	1.4661	....
I.V.	55.5	43.9	44.9
S.E.	190.6	....	188.3
C	71.62	72.16	72.29
H	10.53	10.58	10.70

this sample together with those for the known Diels-Alder adduct of trans-trans-9,11-linoleic acid. There are two significant points. The iodine value of this purified adduct fraction is higher than that of the Diels-Alder adduct. It is close to that for the acrylic adduct, 54.7. The saponification equivalent however is in good agreement with the theoretical value for the Diels-Alder adduct, but triesters of this type have repeatedly been shown to give unreliable saponification equivalents (2).

Table II gives similar data for a sample of adduct fraction from dimethyl maleate (Adduct B3). As with the butyl esters, the iodine value is near that for the acrylic adduct, 66.7. The saponification equivalent however does not agree with the theory for di- or triester. A larger sample was distilled. Constants for the main fractions are also shown in Table II (Fractions 3A, 3E, 3F). Fraction 3A, which made up about 60% of the distillate, appears to be Diels-Alder adduct. Fraction 3E, 34%, is very similar to Adduct B3. Fraction 3F does not correspond to any other adduct or adduct fraction.

TABLE II
Comparison of Properties of Adduct Methyl Esters

| Product	$n^{30/D}$	I.V.	S.E.	C	H
From 9,12-cis,cis acid					
Adduct B3	1.4755	65.4	169.2	70.55	9.82
Fraction 3A	1.4709	61.5	155.6	68.58	9.62
Fraction 3E	1.4768	60.5	167.8	70.08	9.86
Fraction 3F	1.4806	70.9	120.4	73.46	10.41
From 9,11-trans,trans acid	1.4718	59.1	....	68.36	9.31
Theory	....	57.5	146.2	68.46	9.6

That two preparations of adduct fraction from the same reactants give different major fractions (Adduct B3 and Fraction 3A) can most reasonably be ascribed to relatively slight variations in reaction conditions. The most probable cause for the general differences between the present adducts and the Diels-Alder adducts of trans,trans-9,11-linoleic acid is the formation of isomers.

Reaction of a symmetrical dienophile of stabilized configuration, such as maleic anhydride, with trans,trans-conjugated acid could result in only two isomers, depending on whether the anhydride ring is oriented on the same or the opposite side of the cyclohexene ring as are the two side chains. Formation of the triester from either such anhydride adduct should give a single isomer. The more vigorous reaction conditions required for reaction of maleic esters with a trans,trans-acid may be such as to promote the isom-

erization of the maleic ester to a fumaric ester. The still more vigorous conditions, together with the presence of the isomerization catalyst, required for reaction of a maleic ester with the cis,cis-unconjugated portions of the glyceride virtually assure the maleic/fumaric isomerization. Recovery of dimethyl fumarate from maleate reaction mixtures is further confirmation. This isomerization leads to more isomeric possibilities. The ester groups may be on the same or opposite sides of the ring from the side chains, and either ester group may occupy either position. Comparison of the adduct trimethyl esters prepared from maleic anhydride, dimethyl maleate, and dimethyl fumarate shows a small but interesting gradation in refractive index, from $n^{30/D}$ 1.4718 for the adduct from maleic anhydride to 1.4687 for that from dimethyl fumarate. From these considerations the adduct from dimethyl maleate would be assumed to be a mixture of maleic and fumaric adducts, and indeed it has an intermediate refractive index of 1.4693.

When the reaction is carried out with unconjugated oil, eight geometric isomers are theoretically possible, each having four asymmetric carbon atoms. The double bonds which determine the location of the reaction could be shifted to virtually any position along the carbon chain.

In addition to these possibilities, other reactions are conceivable. In transesterification a transannular lactone, II (4), might be formed, or a decarboxylation might take place to give an acrylic derivative. Substituted succinates (7) may also be formed.

II
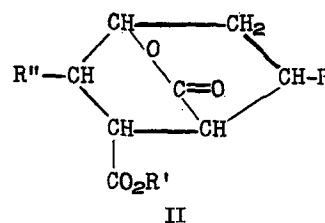
Summary

Direct reactions of dibutyl and dimethyl maleate with safflower oil were investigated. When transformed into esters and distilled, the products are generally different from the Diels-Alder adducts obtained from trans,trans-9,11-linoleic acid. The differences most probably result from the formation of one or more isomers of the known product, of the substituted succinate, of a transannular lactone, or of a mixture of these and other isomers. No definite assignments of structure can yet be made. In one experiment the Diels-Alder adduct was obtained as the major product from the reaction of dimethyl maleate with safflower oil.

Reactions are best carried out by heating safflower oil to 290° while sweeping it with sulfur dioxide. The maleic ester is then added dropwise, and the product is transesterified and distilled. By this procedure a distillable adduct fraction is obtained in yields up to 70%, based on linoleate. Fumaric esters give similar results. Omission of the sulfur dioxide catalyst greatly reduces the yield.

Acknowledgment

ultraviolet and infrared spectra; and to Mrs. C. E. McGrew for microanalyses.

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Linoleic Acid from Safflower Oil by Liquid-Liquid Extraction

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SEPARATION of mixed fatty acids according to their degree of unsaturation is accomplished by numerous methods. Three of the methods are used commercially, namely, low-temperature crystallization, urea-complex formation, and liquid-liquid extraction. The first is used to separate saturated from unsaturated acids; the second and third, for the treatment of lubricating oils. The third, from a cost standpoint, appears particularly suitable in separating linoleic acid from mixed fatty acids derived from safflower oil. Furthermore the efficiency of liquid-liquid extraction has already been shown on other materials, and the techniques and equipment needed to conduct the separation or purification have been developed.

Linoleic acid of high purity, if it were available in quantity, has several uses that are potentially important. Because of its ability to impart film hardness without appreciable film yellowing, linoleic acid appears to be suitable particularly for alkyd resin manufacture. According to Moore (8), the hardness of alkyd resin films after six weeks of aging is proportional to the polyunsaturated acid content of the fatty acids used in making the resin even though drying time of the alkyds appears to be decreased only slightly when unsaturation is above 70%. In the production of dibasic, "dimer," acids by the thermal polymerization of fatty acids from drying and semidrying oils, the data of Goebel (5, 6) indicate that a high linoleic acid content in the original fatty acids is important in obtaining a high ratio of dimeric to trimeric acids. The molecular weight of polyester or polyamide condensation polymers made with these acids is directly related to this ratio. Therefore a high linoleic acid content of the original acids is important to obtaining polymers having high tensile strength and good elastomer properties. Kadesch (7) reviewed other uses for the dimer acids. Recently Teeter et al. (10) described a method for making a new type of difunctional fatty acid derivative by a Diels-Alder reaction between alkali-conjugated linoleic acid and a dienophile. These materials have potential uses for making plasticizers, coatings, elastomers, fibers, and other items of commercial interest.

Liquid-liquid extraction of mixed fatty acids to obtain separations based on iodine value differences may be performed with a number of solvent systems. Both Freeman (3) and Gloyer (4) conducted work on the separation of mixed fatty acids, using furfural

and a hydrocarbon solvent; however they reported no results with safflower fatty acids. From preliminary laboratory studies and from practical considerations, the system furfural-water-hexane-fatty acids was selected for pilot-plant investigations. The present tests were conducted in a stainless-steel, Podbielniak, centrifugal extractor, "double-pup" model. Some operating characteristics of the "pup" model have been described by Barson and Beyer (2).

Equipment, Materials, and Procedure

Figure 1 is a simplified flow diagram, showing the general method of operating the extraction system in the experiments reported. The furfural used in the extractor was freshly distilled under vacuum before use and was protected with nitrogen. The solvent was used as long as the color was not darker than a light straw-yellow. Moisture content of the furfural was determined by the Karl Fischer method and was adjusted to the desired level, indicated later, by the addition of distilled water. The hexane used in the tests was a commercial extraction grade.
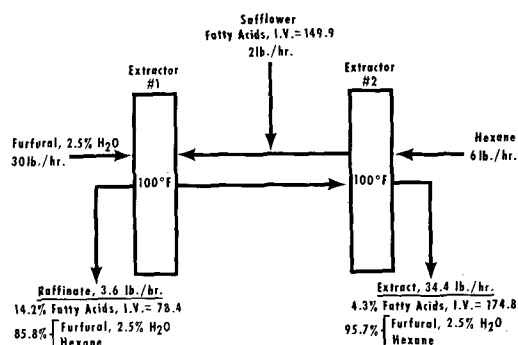

FIG. 1. Fractionation of fatty acids by liquid-liquid extraction—simplified flow diagram.

Fatty acids were hydrolyzed from nonbreak safflower oil by the method of Sutton and Moore (9) using water, a cation exchange resin in the hydrogen form (Dowex 50×4, 200–400 mesh), and an emulsifier (Petrosul No. 742) in the proportions: 75 lb. of oil. 80 lb. of water, 60 g. of emulsifier, 3 lb. of resin. The reactants were heated to 240°F. in a stainless-steel pressure vessel and were agitated until the free fatty acid content reached a constant value. They were then cooled, a small amount of sulphuric acid was added