solved in water, decolorized and recrystallized; m. p. 127°, mixed m. p. with authentic sample, no depression.

Sulfur Analyses of Acetylated, Mercaptalated Products. —The sulfur analyses were performed by the Parr bomb method, employing a total sample of approximately 1 g., on a moisture-free basis, in the manner previously described.[13]

In Table I are recorded the sulfur analytical data and the corresponding average degrees of polymerization calculated from them. The degree of polymerization (D. P.) may be calculated from the sulfur content by the equation

$$\text{D. P.} = 2 + \left(\frac{6412}{\%S \times C_{12}H_{16}O_8}\right) - 2\left(\frac{C_{14}H_{19}O_9 + C_2H_5S}{C_{12}H_{16}O_8}\right)$$

$$\text{or D. P.} = \frac{22.25}{\%S} - 0.72$$

where $C_{14}H_{19}O_9$ is the molecular weight of the end structural units and $C_{12}H_{16}O_8$ is the molecular weight of the intervening units.

TABLE II

HYDROLYSIS OF β-d-GLUCOPYRANOSE 1-PHOSPHATE WITH POTATO PHOSPHORYLASE

Enzymic digest contained 28.5 mg. of the dipotassium salt of β-d-glucopyranose 1-phosphate prepared from the crystalline dibrucine salt,[5b] 3 cc. of enzyme, and 2 cc. of 0.5 M citrate buffer of pH 6.0; total volume, 10 cc.

Time, min.	0	30	60	100
Free P, mg. per 100 cc. of digest	0.3	1.2	1.4	1.8
Free + ester P[a]	2.6	2.6	2.6	2.6

[a] Ester P is the phosphorus liberated as phosphate after three minutes of hydrolysis with N perchloric acid at 100°. All phosphorus determinations were made by the method of Allen.[21]

The assistance in the laboratory of Mr. Irving Auerbach is acknowledged.

(21) R. J. L. Allen, Biochem. J., **34**, 858 (1940).

Summary

1. Synthetic starch prepared by the action of potato phosphorylase on the Cori ester *in vitro* has been hydrolyzed with a solution of concentrated hydrochloric acid at 0° in the presence of an excess of ethyl mercaptan. The resulting mercaptalated mixtures of hydrolyzed products were isolated as their acetates at various time intervals.

2. Sulfur analytical data indicated that the average degree of polymerization of the mercaptalated products varied from 24 glucose units after 0.4 hour to 2.8 glucose units after thirteen hours.

3. The course of the hydrolytic reaction (without mercaptalation) at 0° was followed by optical rotation measurements, and was found to be similar to that of natural potato starch.

4. A graphic analysis of the data yielded a value of 3.2×10^{-2} for the specific rate constant (hours^{-1}) of the rate of change of the degree of polymerization in concentrated hydrochloric acid at 0°, a value similar to the k for natural potato starch.

5. By graphic analysis the value 32 ± 1 glucose units was obtained for the initial average degree of polymerization of the synthetic potato starch.

6. β-d-Glucopyranose 1-phosphate did not undergo polysaccharide formation with potato phosphorylase.

Studies on the Chemistry of the Fatty Acids. XI. The Isolation of Linoleic Acid from Vegetable Oils by Low Temperature Crystallization

BY JEROME S. FRANKEL, WESLEY STONEBURNER AND J. B. BROWN

In 1941 we reported the isolation of pure linoleic acid from corn oil by a low temperature crystallization procedure.[1] This was the first time this acid had been prepared pure, as evaluated from the iodine number, by a method other than the classical bromination–debromination technique of Rollett.[2] In the present work we have applied the crystallization method to four additional oils of high linoleic acid content: sesame, cottonseed, grapeseed and poppyseed, and in addition to olive oil which contains comparatively

small amounts of linoleic acid. Preparations of linoleic acid, 97–100% pure, were obtained from the first four oils mentioned. Purities, calculated from the iodine numbers as binary mixtures of oleic and linoleic acids, were compared to those calculated from the tetrabromide numbers, based on the factor 102.9 for pure linoleic acid.[3] The latter values were 2–6% lower. These differences may be due to the presence of small amounts of octadecadienoic acids which do not yield petroleum ether insoluble tetrabromides. We feel, however, that with the present evidence, this state-

(1) Frankel and Brown, THIS JOURNAL, **63**, 1483 (1941).

(2) Rollett, Z. physiol. Chem., **62**, 410 (1909).

(3) Matthews, Brode and Brown, THIS JOURNAL, **63**, 1064 (1941).

ment should be made only as a possibility. Melting points of the several preparations, −5.2 to −5.8°, were close to the observed melting point of the pure acid, −5.2°.[3]

Application of the crystallization method to olive oil fatty acids resulted in a number of preparations of somewhat lower purity, maximum purity being 95.8% although repeated attempts were made to increase this value. Melting points of these were very low, −9.0 to −17.4°. The tetrabromide numbers of these preparations were also very low. For example, two such products were 95.2 and 95.8% pure by the iodine number, but only 76 and 56% pure, respectively, by the tetrabromide number. The only reasonable explanation for these results is that olive oil linoleic acid is a mixture of two or more isomeric octadecadienoic acids, linoleic acid itself (cis, cis-9,12-octadecadienoic acid) being present in somewhat larger amounts than the others. No further attempt has yet been made to separate this mixture and to ascertain the nature of the isomeric acids.

Our results with reference to the first four semi-drying oils and corn oil are in essential agreement with previous conclusions[4,5] from this Laboratory and with the results obtained by the entirely different method of Kass and co-workers[6] on corn, cottonseed and poppyseed oils, but they do not agree with these workers in their conclusions on olive oil. They reported 13.9% linoleic acid in olive oil by the thiocyanogen number–iodine number calculation, and only 3.0% by the tetrabromide number determination. In other words, olive oil fatty acids showed a much lower ratio of alpha-linoleic to calculated linoleic acid than the several other oils studied, a finding which is due to an abnormally low tetrabromide number and is explained by our results.

Experimental

Description of Low Temperature Crystallization Procedure.—Although the method has been described previously[1] its details are briefly summarized here. The principal steps are as follows.

1. The mixed fatty acids of the oil are dissolved in acetone (75 g./liter solvent) and cooled to −20 to −25°. This is done in a cold room at this temperature, or the operation may be carried out in a dry-ice alcohol bath with slow stirring. The crystal fraction is removed by inverted suction filtration.

2. The filtrate from 1 is further cooled to −50° with slow stirring and is filtered, as before.

(4) Brown and Stoner, THIS JOURNAL, **59**, 3 (1937).

(5) Brown and Frankel, *ibid.*, **60**, 54 (1938).

(6) Kass, Lundberg and Burr, *Oil and Soap*, **17**, 50 (1940).

3. The filtrate from 2 is cooled to −70°. The crystal fraction is again removed. Calculated from the iodine number as a binary mixture of oleic and linoleic acids, this crystal fraction is about 90% linoleic acid.

4. The crystal fraction from 3 is dissolved in 30–60° petroleum ether, 65 g./l. and cooled to −48°. The crystals at this temperature are usually 95% linoleic acid. At −48° the solubilities of oleic and linoleic acids are more nearly equal in petroleum ether than in acetone, so that relatively larger amounts of the oleic acid remain in solution.

5. The 95% acid is dissolved in petroleum ether, 6.25 g./l., and cooled to −60 to −62°. This time the crystal fraction is practically pure linoleic acid. The objective of this step is to use enough solvent to keep all of the oleic acid in solution, and thus to crystallize pure linoleic from the solution. The success of the operation depends on the fact that the starting material is a 19/1 linoleic/oleic acid mixture, while at −60° the ratio of solubilities is very much less. Very low concentrations also tend to minimize mixed crystal formation.
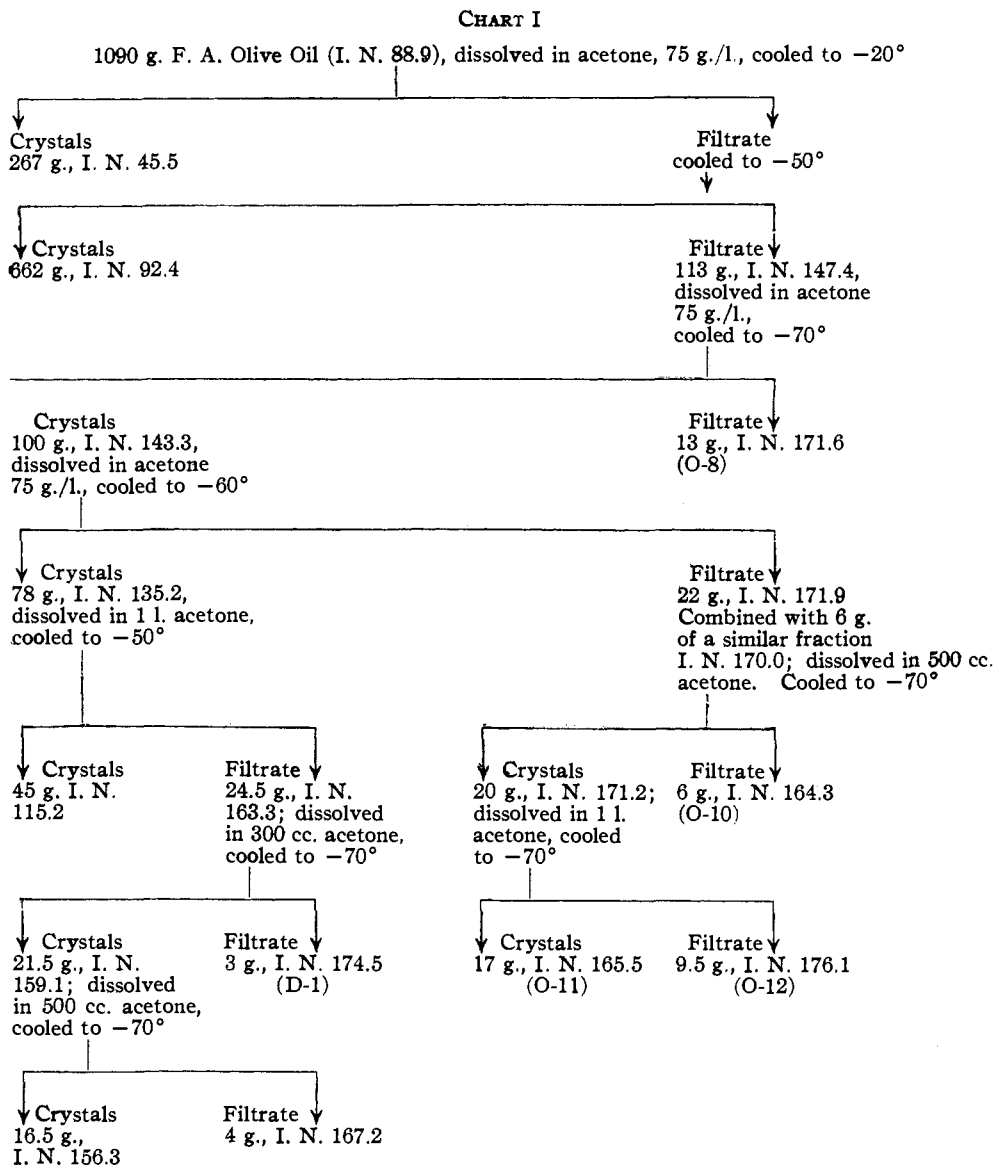
Application of Method to Sesame, Cottonseed, Grapeseed and Poppyseed Oils.—The previous method was applied to cottonseed, sesame, grapeseed, poppyseed and olive oils, in addition to the corn oil previously reported. In certain instances, it was found advisable to further crystallize the final product. In the case of corn oil, it has already been noted that traces of linoleic acid were removed by this additional crystallization. The product from poppyseed oil was considerably improved by recrystallization. The method was successful, except with olive oil, the results appearing in Table I.

TABLE I

ANALYSES OF CRYSTALLIZATION LINOLEIC ACIDS FROM VEGETABLE OILS

Oil	Iodine no.	Tetra-bromide no.	M. p., °C.	n^{20}	Purity from I. N.[a]	T. N.[b]
Corn	180.8	100.6	−5.4	1.4699	100	98
Sesame-I	178.4	95.1	−5.8	1.4692	97	92
Sesame-II	178.2	96.0	−5.2	1.4692	97	93
Cottonseed	179.9	99.4	−5.8	1.4697	99	97
Grapeseed	180.0	97.5	−5.8	1.4692	99	95
Poppyseed	179.4	95.2	−5.8	1.4697	98	92
Poppyseed, 4X recrystallized	180.8	97.8	−5.2	1.4697	100	95
Linoleic by debromination	181.0	102.9	−5.2	1.4699	100	100

[a] Calculated from iodine number; therefore, total octadecadienoic acid. [b] On the basis of the tetrabromide number, using the factor 102.9.

Application of Method to Olive Oil.—Linoleic acid is one of the lesser components of olive oil, usually amounting to 8–13% of the total acids. As a preliminary step, therefore, it is necessary to prepare a concentrate of the acid. This may be done by crystallizing either the methyl esters or the acids two or three times at −60° from methyl alcohol or acetone, the filtrates containing 50 to 87% linoleic acid, depending on the conditions. The product is then treated by the general procedure described above. This, however, had to be considerably modified to secure the desired results. A characteristic series of crystallizations is described in Chart I.

CHART I

1090 g. F. A. Olive Oil (I. N. 88.9), dissolved in acetone, 75 g./l., cooled to −20°

Crystals
267 g., I. N. 45.5

Filtrate
cooled to −50°

Crystals
662 g., I. N. 92.4

Filtrate
113 g., I. N. 147.4,
dissolved in acetone
75 g./l.,
cooled to −70°

Crystals
100 g., I. N. 143.3,
dissolved in acetone
75 g./l., cooled to −60°

Filtrate
13 g., I. N. 171.6
(O-8)

Crystals
78 g., I. N. 135.2,
dissolved in 1 l. acetone,
cooled to −50°

Filtrate
22 g., I. N. 171.9
Combined with 6 g.
of a similar fraction
I. N. 170.0; dissolved in 500 cc.
acetone. Cooled to −70°

Crystals
45 g. I. N.
115.2

Filtrate
24.5 g., I. N.
163.3; dissolved
in 300 cc. acetone,
cooled to −70°

Crystals
20 g., I. N. 171.2;
dissolved in 1 l.
acetone, cooled
to −70°

Filtrate
6 g., I. N. 164.3
(O-10)

Crystals
21.5 g., I. N.
159.1; dissolved
in 500 cc. acetone,
cooled to −70°

Filtrate
3 g., I. N. 174.5
(D-1)

Crystals
17 g., I. N. 165.5
(O-11)

Filtrate
9.5 g., I. N. 176.1
(O-12)

Crystals
16.5 g.,
I. N. 156.3

Filtrate
4 g., I. N. 167.2

In the course of this work several preparations were obtained which were 81–95% pure on the basis of the iodine number. For purposes of comparison, three of the products from Chart 1 and three others are compared in Table II.

TABLE II

ANALYTICAL DATA ON LINOLEIC ACID PREPARATIONS FROM OLIVE OIL

Specimen no.	Iodine no.	Tetra-bromide no.	M. p., °C.	% Linoleic from I. N.	% Linoleic from T. N.
D-1	174.5	63.6	−12.4	92.5	62
O-10	164.3	52.6	−17.4	81.5	51
O-11	165.5	..	− 9.0	82.7	..
O-12	176.1	62.5	−11.4	94.2	61
O-4	177.1	78.6	....	95.2	76
O-6	177.5	57.4	....	95.8	56

It is to be noted that the preparations in Table II had very low melting points and tetrabromide numbers. The differences in purities as calculated from the iodine and tetrabromide numbers are interpreted as being due to the presence of 19–40% octadecadienoic acids other than linoleic acid.

The data in Tables I and II require no comment except perhaps a further word of explanation of the calculations of purity. These are based on assumptions which seem to the writers to be warranted. In the calculation of purity from the iodine numbers, it is assumed that the only fatty acids present are oleic and octadecadienoic acids, the latter being exclusively those acids which give theoretical iodine numbers for two double bonds. Conjugated dienoic acids and any possible isomers with double bonds close to the carboxyl are neglected in the calculation. Further, the calculation is slightly in error because, admittedly, very small amounts of saturated acids are present in the mixtures. In so far as this is true the values for per cent.

purity are slightly low. The other calculation of purity is
based on the tetrabromide number, in which the value of
102.9 for the tetrabromide number twelve-times recrystal-
lized alpha-linoleic acid (see Matthews, Brode and Brown)[3]
is taken as the standard for pure *cis,cis*-9-12-octadeca-
dienoic acid. The values by this calculation are obviously
lower than those by the iodine number. If the differences
are small, we are very cautious in concluding the presence
of isomeric acids, which do not yield insoluble tetrabro-
mides. On the other hand, when the differences are large
we believe they represent definite indications of the pres-
ence of octadecadienoic acids which do not yield these
characteristic bromides (m. p. 114–115°). In view of the
work of Kass and Burr[7] it is likely that the isomeric acid is
either the *cis–trans* or the *trans–cis* modification, and that
it is definitely not the *trans–trans* form.

The difficulties in applying the crystallization method
to the isolation of linoleic acid from olive oil are due, no
doubt, to the presence in this oil of isomeric linoleic acids,
solubilities of which prevent clean-cut separations.

(7) Kass and Burr, THIS JOURNAL, **61**, 1062 (1939).

Summary

1. Linoleic acid has been prepared from
sesame, cottonseed, grapeseed and poppyseed oils
by low temperature crystallization.

2. The linoleic acid from these oils, isolated by
crystallization, is essentially identical with corn
oil linoleic acid prepared by this method and with
recrystallized alpha-linoleic acid, prepared by
reduction of tetrabromostearic acid.

3. The crystallization method was applied
somewhat less successfully in the isolation of
linoleic acid from olive oil.

4. Analytical data on a number of fractions
from olive oil were interpreted as meaning that
the linoleic acid of this oil is a mixture of octa-
decadienoic acids, of which linoleic is the principal
component.

Antispasmodics. I. Basic Esters of Some Arylacetic Acids[1]

BY ROBERT R. BURTNER AND JOHN W. CUSIC

Antispasmodics may be divided into two classes,
those which act to prevent or abolish the action
of stimulation of autonomic nerves, and those
which are not thus related to innervation. The
latter class may be referred to as musculotropic
and the former neurotropic. The neurotropic
drugs may be still further divided into sympa-
tholytic and parasympatholytic, depending upon
whether the action is on the sympathetic or para-
sympathetic system. Of greatest practical im-
portance are the drugs belonging to the para-
sympatholytic group (the prototype of which is
atropine) and to the group "not related to inner-
vation" (whose chief representatives are papa-
verine and the nitrites).

The drugs of both groups, however, have a
number of disadvantages. Thus papaverine has
little effect in abolishing spasms induced by
neural excitation, while on the other hand atropine
is almost ineffective against spasms of entirely
muscular origin brought about by such sub-
stances as histamine. In addition, papaverine
relaxes all the smooth muscles equally, thus when
relaxation of the intestinal tract is required,

there results also a prolonged and undesirable
fall in arterial blood pressure. The nitrites are
rarely used to combat intestinal spasms because
of their transient action and undesired side-
effects on the circulation. Furthermore, the
parasympathetic inhibition of atropine occurs in
all organs activated by nerves of the autonomic
system, thus causing three undesired side-effects,
namely, cyclopegia, dryness in the mouth,
tachycardia and sometimes a rise in arterial
pressure.

For these reasons attempts were made to syn-
thesize substances which have both a neuro-
tropic and musculotropic action in one molecule,
and in which the neurotropic or atropine-like
action is somewhat differently directed; in other
words, compounds which have a selective atro-
pine-like action on the smooth muscles of the
hollow viscera, and no, or only slight, effect, on
the pupils, salivary glands and circulation.

In the present work our efforts have been
directed toward modifying the structure of
atropine to attain this end. Consequently a
series of esters were prepared from acids which
might substitute for the acid fraction of atropine
and various amino alcohols corresponding to the