ing way. If, for a given time-interval, the measured increase in iodine absorbed by the oil in 100 seeds is divided by the appropriate increase in weight of oil, the quotient can be considered the iodine value of the new oil. When the safflower data were tested in this way, the iodine value of the new oil ranged from 133 to 207 with a mean of 155 for 14 items and with the highest values occurring between the thirtieth and fortieth days. An iodine value greater than 181, in the presence of only dienoic fatty acids, suggests the operation of an alternative to *de novo* synthesis. The iodine value of 207 was however an isolated instance. With flax, the iodine value of the new oil, calculated in this manner, ranged from 176 to 255, with a mean of 190 for 18 items. Again the highest values occurred toward the mid-point of development. The presence of linolenic acid in flax obviates the necessity of invoking the desaturation hypothesis.

The dynamic state of lipids in plant tissue means that any observed change in unsaturation is, of necessity, the result of concurrent anabolism and catabolism. It is therefore incorrect to speculate on the occurrence of desaturation or to attribute changes in iodine value to *de novo* synthesis without knowledge of the turnover numbers of the various fatty acids at progressive stages of development.

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Reactions of Ozone. V.[1] A New Method of Determining Unsaturation Values of Fatty Acids and Oils by Ozone[2]

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FOR YEARS a simple, reliable, quantitative method for determining olefinic unsaturation has been sought. Existing methods such as bromination, iodination, catalytic hydrogenation, and peroxide reactions have their specific limitations as discussed by numerous investigators (1,2,5,6). However, for many olefinic compounds, ozone is an excellent titrimetric reagent (5). Boer and Kooyman (3) have shown that ozonization could well supplant these other methods. Their procedure can be simplified by using commercially-available apparatus. The analysis is based on determining the time required to titrate a sample of olefin with a stream of ozonized air containing a known amount of ozone. Three things are necessary: a constant stream of ozonized air, complete absorption of the ozone, and precise recognition of an end-point.

Experimental

A schematic diagram of the equipment is shown in Figure 1. Clean dry air is supplied to the Model T-23 laboratory ozonator.[3] Using 0.02 c.f.m. of air, the applied voltage[4] is adjusted to *ca.* 90 V., to give 15 to 20 mg. of ozone per minute. Precise control of gas flow is assured with the stainless steel needle[5] valve and the external flow meter.[6]

Ozone production is determined by absorbing the ozone in neutral 2% KI solution for a measured 3 min. After acidification, the liberated iodine is titrated with 0.1 N thiosulfate. The ozone rate is calculated in mg./min. The ozone production should be rechecked periodically, before and after each series of determinations. At this low production rate it is not necessary to have a glass frit on the gas-washing bottle, but a tube drawn to *ca.* 1 mm. i.d. capillary disperses the gas sufficiently to give complete absorption. There is very little back pressure in the system without a frit, making it easier to maintain the same flow rate for determining the ozone production and for determining the unsaturation in the sample. Complete absorption of ozone for a particular modified dispersing tube can be checked by placing a second regular absorber in series.

Complete absorption of ozone is obtained in a "Mini Lab"[7] gas-liquid reactor. The stirring speed (*ca.* 2,500 r.p.m.) is adjusted to raise the liquid level to 1 cm. from the bottom of the glass joint while the flask is cooled to −50°C. Care should be taken to avoid forcing liquid into the ground glass joint at the top of the reactor.

To carry out a determination, a sample containing 6 to 7 milli-equivalents of double bond is weighed accurately into the reaction flask and dissolved in 35 ml. of chloroform. The solution is held at −45 to −55°C., using a dry-ice acetone bath. After the ozone production rate is determined, the ozone stream

is passed into the stirred reaction vessel and the time is simultaneously recorded. The end-point is indicated by the appearance of a distinct yellow color of the KI solution, and simultaneously by the appearance of a blue color in the reactor because of excess ozone. The end-point was found consistently to occur 0.4 of a minute early. This correction should be determined on a material of known high purity and applied to the readings of the unknown sample.

Results

The results given in Table I are expressed as "Ozone Value," i.e., the number of grams of ozone absorbed per 100 g. of materials.

All the compounds listed in Table I are better than 99% pure, as analyzed by gas phase chromatography. The diethylene glycol succinate column separates the methyl esters of mixed fatty acids except for cis,trans isomers which do not affect the unsaturation content anyway.

The limited number of determinations given on octene-1 were made on four separate days and indi-
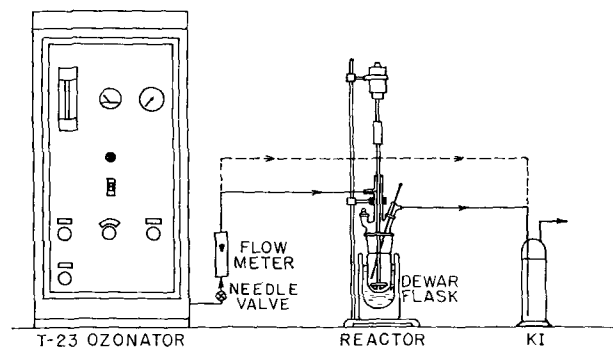

FIG. 1.

cate the reproducibility of this method. The standard deviation is 0.8%.

The compounds listed in Table II are typical oils, and actual ozone values are given beside the ozone values calculated from iodine values found, using the Wijs method.

The miscellaneous compounds listed in Table III were highly purified by repeated distillation and found by gas phase chromatography to be more than 98% pure.

Discussion

This proposed analytical determination eliminates most of the limitations and failings found in the

TABLE I

Compound	Ozone value theory	Ozone value found	% Unsat. of theory
Methyl oleate [a]	16.2	16.2, 16.4	100.6
Linoleic acid [b]	34.2	34.2, 34.4, 34.4, 34.5	100.5
Methyl linolenate [c]	49.2	49.2, 49.5	100.3
Octene-1 [d]	42.8	42.8, 42.4	99.6
Octene-1 [d]	42.8	42.4, 42.4	99.1
Octene-1 [d]	42.8	42.3, 42.9	99.5
Octene-1 [d]	42.8	43.1, 43.2	100.8

[a] Supplied by Applied Science Laboratories Inc.
[b] Supplied by Mann Research Laboratories Inc.
[c] Supplied by The Hormel Institute.
[d] Supplied by Phillips Petroleum Company. Since their Pure Grade is 99 mole % minimum and relatively inexpensive, it is recommended for standardizing and checking the procedure.

TABLE II

Substance	Ozone value calculated from I.V.	Ozone value found
Olive oil [a]	16.4	16.3, 16.5
Sesame oil [a]	21.3	21.7, 21.7
Safflower oil [a]	27.1	27.5, 27.5, 27.8
Tall oil [b] (FA, No. 2)	24.3	24.3, 24.5
Tall oil [c] (Pamak 1)	23.9	23.8

[a] Furnished by W.C. Ault, U.S.D.A.
[b] Furnished by Arizona Chemical Company.
[c] Furnished by Hercules Powder Company.

current bromine and iodine number procedures. The presence of tertiary hydrogens as in α-pinene does not cause the high varying results found in the bromine technique. The determination of olefinic unsaturation in the presence of an activated aromatic ring, as in isosafrole, is also shown to be feasible.

Samples containing small amounts of conjugated unsaturation, such as tall-oil fatty acids, can be satisfactorily determined. However, if a substantial por-

TABLE III

Compound	Ozone value theory	Ozone value found
Isosafrole [a]	29.5	29.0,[c] 29.7,[c] 29.1 [e]
α-Pinene [b]	35.3	36.1, 35.6

[a] Furnished by Shulton Inc.
[b] Furnished by Hercules Powder Company.
[e] The solution in the reactor does not turn blue because not enough of excess ozone forms in the reactor at the end-point. The end-point is indicated only by a change in color in the KI. The excess ozone reacts with the activated aromatic ring, but in appreciable quantities only after the side-chain double bond is consumed.

tion of the unsaturation is slow in reacting, as in the remaining double bond of a conjugated system such as 2,5 dimethyl, 2,4 hexadiene, or in sterically-hindered compounds (4), a slight loss of ozone into the KI solution occurs. With these slow-reacting compounds the end-point is obscured, and this method is not applicable. In such cases ozone concentration can be determined by using an ozone meter. Plotting ozone concentration versus time gives an end-point as a sharp break in the curve. The integration of the area under the curve gives the total absorbed ozone. A more complete discussion of the applicability of ozone titration to various unsaturated compounds is given by Boer and Kooyman (3).

The literature contains statements to the effect that all ozonides are dangerously unstable. However this is not true, particularly when the materials are in dilute solution and not evaporated to dryness. A few simple precautions assure safe handling of dilute ozonide in chloroform. Volatile peroxides from successive runs should not be permitted to accumulate and concentrate in a vapor trap. Furthermore the ozonolysis products are in dilute solutions and can be disposed of by means of a water drain. For a few compounds, such as butene, in which higher-oxygen-to-carbon ratios than pentene ozonide (O_3/C_5) are encountered, some special precautions should be employed, such as a laboratory protective shield.

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