isolated double bonds leads to incomplete absorption, and the increase in concentration and reaction period gives desirable results in the presence of mercuric acetate catalyst. A 1-hr. reaction period with 0.3 N HOCl reagent in the presence of 2.5% solution of the catalyst is recommended. A sample size varying between 0.07-0.1 g. and a 300-400% excess reagent should be employed to obtain reliable results. This procedure can be effectively used for determining the total unsaturation of tung oil, isomerized fatty acids, and dehydrated castor oil and can be employed for detecting the adulteration in commercial samples of tung oil, which cannot be ordinarily detected by the determination of the partial iodine number with the help of conventional procedures.

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Carbonyl Compounds in Rancid Corn Oil

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IT WAS NOTED by Brekke and Mackinney (1) that steam distillates of rancid corn and avocado oils, collected in 2,4-dinitrophenylhydrazine solution, yielded a complex mixture of hydrazones (DNPHs). These were separated on the basis of solubility in petroleum ether, in which the common dicarbonyl and short chain alkyl derivatives are virtually insoluble, followed by chromatography on anhydrous magnesium sulfate. Fractions with absorption spectra corresponding to saturated aldehydes and to 2-enals and 2,4-dienals [cf. Braude and Jones (2)] were thus isolated. It was concluded that the corn oil DNPH mixture was comprised of at least five components soluble in petroleum ether, together with an uninvestigated petroleum ether-insoluble (benzene-soluble) fraction.

A more intensive study has now been made of the DNPHs obtained from rancid corn oil by the procedures already described, and 14 components have been separated in the petroleum ether-soluble fraction, as well as 16 from the P.E.-insoluble fraction. Two components have been isolated in quantity sufficient for elementary analyses and mixed melting point determinations with known compounds.

The data illustrate the advantages and definite disadvantages of techniques for separating dinitrophenylhydrazine derivatives in a really complex mixture.

Experimental

A commercial refined corn oil was subjected to accelerated rancidification by heating and aeration. It was then steam-distilled, and the carbonyls in the distillate were converted to 2,4-dinitrophenylhydrazones, which were separated by chromatography and their properties studied.

Methods

Rancidification. The corn oil, in three lots of a pint each, was held in a one-liter round bottom flask for 10 days at temperatures between 78° and 85° with air drawn through by suction from an aspirator at a rate of 40 to 60 bubbles per minute. The progress of rancidification was followed by determinations of the peroxide value of the oil.

Steam Distillation. Steam was generated in a two-liter flask short-coupled to the oil flask, admitting the steam through a glass tube extending to the bottom. To reduce condensation, the oil flask was kept in a boiling water bath during distillation. A water-cooled condenser received the distillate; its tip led below the surface of reagent in a flask.

Conversion of Carbonyl Breakdown Products to DNPHs. A saturated solution of 2,4-dinitrophenylhydrazine in 2 *N* HCl was employed in the receiving flask, precipitating red-orange derivatives. Successive 25-ml. portions of the reagent in 125-ml. Erlenmeyer flasks were used, substituting a fresh flask while there was still an excess of unreacted reagent in the supernatant of the previous flask. Initially about 25 ml. of distillate were collected with each portion, but after the greater part of the carbonyl compounds had been distilled, quantities up to 60 ml. were collected each time. From each pint lot of rancidified oil a total of 2,500 to 3,000 ml. of distillate was obtained. Precipitate was still forming in the receiving flask in small amount when distillation was stopped.

The precipitated DNPHs were washed repeatedly by decantation, first with hot 2 *N* HCl to remove unreacted DNP hydrazine, then with distilled water to neutrality. After drying under vacuum at temperatures not exceeding 40°C., the material was dissolved in benzene, yielding a deep red solution.

Preliminary Separation. To remove the DNPHs soluble in light petroleum ether (P.E.), 10 to 15 volumes of P.E. were added to successive portions of the benzene solution, and the precipitated P.E.-insolubles were filtered with Whatman No. 1 paper previously washed with benzene and P.E. The filter paper con-

TABLE I

Adsorption, Spectrophotometric, and Color Reaction Data on Chromatographic Bands of 2,4-Dinitrophenylhydrazones from Rancid Corn Oil

Petroleum Ether-Soluble Fractions			Spectral Absorption Characteristics as Wavelengths (mμ) of *Maxima*, Minima and (Inflections)	Color in Alcoholic NaOH	Type of Carbonyl Compound Indicated
Band No.	Developing Solvent	Times Chromatographed			
1	PE[a]	3×	*360*, 293, (250), 225	Red	Saturated
2	PE	3×	*375*, 310, (283), *247*, 228	Red	2-enal
3	PE	3×	*374*, 312, (250)	Red	2-enal
4	PE	3×	*392*, 325, *300*, 283, *264*, 253	Red	2,4-dienal
5	10Bz[a]:90PE	3×	*364*, 301, (250)	Red	Saturated
6	10Bz:90PE	2×	*375*, 311, (283), (243)	Red	2-enal
7	10Bz:90PE	3×	*373*, 309, (250)	Red	2-enal
8	20Bz:80PE	3×	*369*, 315, (285), *258*, 245	Red	2-enal
9	20Bz:80PE	3×	*378*, 314, (255)	Red	2,4-dienal
10	20Bz:80PE	3×	*388*, 325, *255*, 248	Purplish	Dicarbonyl[b]
11	20Bz:80PE	3×	*392*, 309, (252)	Purplish	Dicarbonyl[b]
12	40Bz:60PE	3×	*355–390*, 318, (252)	Purplish	Dicarbonyl[b]
13	40Bz:60PE	3×	*380–395*, 310, *255*, 250, (232)	Purplish	Dicarbonyl[b]
14	50Bz:50PE	3×	*372*, 309, (287)	Red	2-enal
15	40Bz:60PE	2×	*376*, 313, (280), (250)	Red	2-enal
16	40Bz:60PE	2×	*371*, 306, (250)	Red	2-enal
17	40Bz:60PE	2×	*367*, 296, (253)	Red	2-enal
18	40Bz:60PE	2×	*365*, 297, (255)	Red	Saturated
19	40Bz:60PE	2×	*393*, 308, (255)	Blue	Dicarbonyl[b] 2,4-dienal
20	40Bz:60PE	2×	*370*, 307, (255)	Purplish	2-enal
21	50Bz:50PE	2×	(430), *405*, 305, (255)	Purplish	2,4-dienal
22	50Bz:50PE	2×	395–435, 320, (295), (252)	Blue	Dicarbonyl[b]
23	50Bz:50PE	3×	(400), *355*, 300, (250)	Red	2,4-dienal
24	50Bz:50PE	5×	*380*, 309, (280), *252*, 245	Red	Saturated
25	50Bz:50PE	5×	*395–430*, 318, (250)	Blue	2,4-dienal Dicarbonyl[b]
26	55Bz:45PE	4×	(425), *400*, 312, (300), (252)	Blue	2,4-dienal
27	55Bz:45PE	4×	(420), *400*, 318, (295), (252)	Blue	
28	55Bz:45PE	4×	(430), *393*, 315, (295), (250)	Blue	2,4-dienal 2,4-dienal
29	55Bz:45PE	4×	(435), 392, 312, (255)	Blue	2,4-dienal
30	Bz	5×	(400), *352*, 308, 261, 254	Red	Saturated

[a] PE: petroleum ether; Bz: benzene.
[b] Dicarbonyls or other complex carbonyls.

taining the precipitate was then extracted in an intermittent type Soxhlet apparatus with P.E. boiling below 42°C., continuing the operation until there was no perceptible color in the solvent returning to the flask. Different lots required from 15 to 24 hrs. The P.E.-insoluble DNPHs remaining were dried under vacuum and redissolved in benzene. Yields averaging 400 mg. of DNPHs were obtained per pint of oil, of which approximately 40% were P.E.-insoluble.

Chromatographic Purification. Purification of the crude fractions was conducted on $MgSO_4$ columns with benzene-P.E. combinations as developing solvents, greater proportions of benzene being needed to develop the more strongly adsorbed fractions. Among P.E. insolubles were some (not investigated further) so strongly adsorbed that benzene alone would not develop them. Ethanol or ethyl acetate mixtures were required for elution. Individual fractions were chromatographed two to five times in efforts to remove extraneous material. In Table I the properties of 14 P.E.-soluble and 16 P.E.-insoluble components are listed, together with the number of times they were chromatographed to ensure homogeneity. Of these 30 compounds two (Bands I and II) were sufficiently abundant to warrant crystallization and more detailed examination.

As shown in Table II, it was surmised that Band I might be the DNPH of n-undecanal and Band II of n-undecenal. The derivatives of these aldehydes were therefore prepared by steam-distillation of the products of chromic acid oxidation of the pure alcohols, chromatographed on $MgSO_4$, and re-crystallized.

TABLE II

Elementary Analyses of Dinitrophenylhydrazones

	n-Decanal DNPH, Calc.	Band 1 Found	n-Undecanal DNPH, Calc.	n-Decenal DNPH, Calc.	Band 2 Found	n-Undecanal DNPH, Calc.
	%	%	%	%	%	%
Carbon	57.20	57.34	58.30	57.50	57.86	58.60
Hydrogen	7.14	7.27	7.43	6.59	6.70	6.90
Nitrogen	16.66	16.11	16.00	16.76	16.30	16.10
Molecular Weight	336	347[a]	350	334	343[a]	348

[a] Calc. from *N* content.

Melting points of the prepared derivatives, while in agreement with the literature, were widely divergent from those found for Bands I and II [see Table III (3), (4)]. As a first approximation it was assumed that the analyses of Table II represent a C_{11} fragment, accompanied by a C_{10}—or slightly smaller contaminant.

The reasonableness of this assumption is shown in Table IV, where the specific and molar extinction coefficients are listed for these preparations.

Discussion

Where a complex mixture is present, as in an oil, the foregoing procedures clearly require supplementary techniques, whether further fractionation (*e.g.*, by counter-current partition) or analyses of infrared spectra, as made by Ross (5). The possibility of polymorphism in the crystals and the existence of meta-

TABLE III

Melting Point Determinations on Prepared 2,4-Dinitrophenylhydrazones of 7-, 10-, and 11-Carbon *N* Aldehydes and Compounds Comprising Bands 1 and 2

	Determined Melting Point (corr.)	Reported Values
	°C.	°C.
n-Heptanal DNPH	106.3	106.0 (3)
n-Decanal DNPH	103.5	104.0 (3)
n-Undecanal DNPH	104.0	104.0 (3)
Band 1 DNPH	87.0	......
Mixed Decanal (25%) and Undecanal (75%) DNPHs	84.0	......
Mixed Heptanal (50%) and Undecanal (50%) DNPHs	75.0	......
Mixed Band 1 and Undecanal (approx. 50%:50%) DNPHs	81.0	......
Band 2 DNPH	97.0–97.5	......
2-Decenal DNPH	......	128.8 (4)
2-Undecenal DNPH	......	122.6 (4)

stable states, as discussed by Braddock *et al.* (6) must also be reckoned with here. However, apart from melting point variations and transition points, discussed in some detail by Wahhab (7), we have never observed changes in absorption spectra of our derivatives after crystallization by different methods and subsequent dissolution. Stadtman's spectrum of furfural DNPH (8) is readily reproducible and would be described as the *trans*-form as judged by a transmittance curve given by Braddock *et al.* (6). This is clearly the stable form and the only one repeatedly encountered on the chromatogram under our conditions. It is unlikely therefore that the number of components isolated in this work will be greatly reduced by duplication as metastable forms. However the method obviously may be of great utility, as Kawahara, Dutton, and Cowan (9) have shown, in isolating fractions from a single source such as methyl linolenate.

Summary

Fourteen petroleum ether-soluble and 16 insoluble DNPH derivatives have been isolated from rancid corn oil and their properties discussed. Evidence suggests that n-undecanal and n-undecenal are among the components of the rancid oil.

TABLE IV

Extinction Coefficients of Prepared 2,4-Dinitrophenylhydrazones and of Unknown Bands 1 and 2

	Heptanal DNPH	Decanal DNPH	Undecanal DNPH	Band 1 DNPH
Specific Ext. Coeff.:				
357 mμ	70.30	66.90	58.10	58.85
250 mμ	38.90	34.15	30.30	32.05
225 mμ	50.10	46.25	40.10	43.40
Molecular Weight of DNPH	294	336	350	347[a]
Molecular Ext. Coeff.:				
357 mμ	20,700	22,490	20,340	20,450
250 mμ	11,440	11,470	10,610	11,130
225 mμ	14,740	15,560	14,050	15,080

	Band 2 DNPH			2-Decenal DNPH (38)	2-Undecenal DNPH (4)
Specific Ext. Coeff.:					
376 mμ	78.5	78.5	78.5	86.3	81.7
247 mμ	47.3	......	......	......	......
Molecular Weight of DNPH	343[a]	334[b]	348[c]	334	348
Molecular Ext. Coeff.:					
376 mμ	26,930	26,250	27,360	28,850	28,440

[a] Assumed on basis of *N* content.
[b] Assumed on basis of 2-decenal.
[c] Assumed on basis of 2-undecenal.

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Solubility of Monoglycerides in Oil and Its Relation to the Production of Global Edible Spread[1]

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THIS PAPER reports the solubility of monoglycerides in vegetable oil and the influence of tempering monoglyceride suspensions on solubility behavior. Plastic mixtures of saturated monoglycerides and vegetable oil exhibit uniform consistency over a wide range of temperature and, as formulated for edible use, have been proposd as a global spread suitable for use in extremes of temperature. Provided it has been adequately tempered after solidification in manufacture (9), the spread leaves the mouth without a tallowy sensation even though it does not melt at body temperature. Physical studies have been made to reveal the basis for changes caused by tempering, and in a previous report (5) it was established by X-ray diffraction and microscopic observations that monoglycerides in spreads recrystallized during tempering. The low solubility of monoglycerides found in the present investigation explains the small temperature dependence of global spread consistency between 32° and 100°F. (0°-38°C.). Solubility data also confirm that the monoglyceride precipitated by rapid chilling in the production of global spread is present in an unstable state, which is stabilized by tempering.

Experimental

Materials. To reveal the influence of fatty acid composition on solubility, samples of essentially pure stearic and palmitic acid monoglycerides were investigated. Four molecularly distilled monoglycerides were prepared by Distillation Products Inc.,[2] from the following materials: a) commercial high-purity stearic acid, b) commercial palmitic acid more than 90% pure, c) triple-pressed stearic acid containing

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