Aldehydes From Rancid Corn and Avocado Oils

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SWIFT *et al.* (1) have recently validated the premise that: "when fats oxidize, Δ^2-unsaturated aldehydes, such as Δ^2-undecenal and its analogs and homologs, should be among the constituents of oxidizing fat systems," and they demonstrated the presence of $\Delta^{2,4}$-decadienal, Δ^2-octenal, and hexanal in autoxidized cottonseed oil. The purpose of this paper is to report a procedure in many respects simpler, clearly substantiating the general findings of Swift *et al.*, but indicative however to a much greater extent of complexity in the breakdown of natural oils. In the present case avocado oil, and for comparative purposes, the very similar olive oil, and the less saturated corn oil were examined.

Experimental

Materials. Commercial samples of corn and olive oil were purchased locally. Avocado oil was obtained by hydraulic expression after steam treatment. The unbleached oil was dark green after refining with alkali and steam deodorization. The bleached oil was in addition decolorized with "Darco" and was a clear golden yellow, of iodine number 88 to 92.

Methods. The oils were rancidified under accelerated testing conditions. Quantities varying from 10 to 500 grams were placed in stoppered tubes or flasks and aerated in an oil bath maintained at 100°C. Samples were periodically withdrawn for determination of peroxide number and absorption spectra.

Absorption spectra were determined for the range 400 to 220 mμ. on the Beckman DU spectrophotometer. Solvents used were: absolute ethanol for the oils, water for the steam distillates, and 95% ethanol for the dinitrophenylhydrazones (DNPHs).

In the earlier work 1-gram samples of fresh and rancid oils of known peroxide value were steam-distilled, and u.v. measurements were made directly on the distillates. In the later experiments 50 grams of the oil were placed in a micro-Kjeldahl distillation assembly to which an external steam-generating flask was directly attached. The micro-Kjeldahl all-glass still has definite advantages in steam distillation since the air can rapidly be displaced by steam before appreciable heating of the sample occurs. After a vigorous jet of steam emerges from the outlet of the external jacket, the system is closed and a negligible amount of air remains to be displaced. This enabled collection of 500 ml. distillate with ease. Fifty ml. 2,4-dinitrophenylhydrazine (saturated, filtered solution in 2 *N* HCl) was placed in the receiving flask prior to distillation. The DNPHs from rancid corn and bleached rancid avocado were thus prepared from two samples of each, in separate runs. They separated quickly and were reddish in color. After standing overnight, the precipitates were filtered, washed with 2 *N* HCl, and dried. As much as 5 mg. of derivative might thus be collected per distillation. This has proved in all cases tested to be a complex mixture.

The DNPHs were chromotographed by procedures described by F. H. Stadtman (2). In the present samples the derivatives were first fractionated into two groups (after filtration, washing and drying), on the basis of solubility in petroleum ether. A residue was found, after exhaustive extraction with petroleum ether, to be readily soluble in benzene. The adsorbent used was Baker's anhydrous $MgSO_4$, in columns 10 cm. long, by 2.5 cm. diameter. The colored zones were collected separately, as they passed through the column, in petroleum ether. (As repeatedly noted, all solvents used on the column must be checked for impurities which show absorption in the range 200 to 250 mμ. Redistillation is normally adequate in the case of paraffin base petroleum ether.) Each zone in solution was then evaporated to dryness.

Absorption spectra were determined in 95% ethanol and mixed chromatograms were made of apparently comparable zones from different oils.

Results. Fresh corn oil (peroxide value 7) had an absorption spectrum almost identical with that reported by Beadle (3) for a deodorized sample (Fig. 5, p. 143) though the absorption coefficients were somewhat greater. In the triene conjugation region (*ca* 280 mμ.) the specific absorption coefficient was 0.51, and at 230 mμ., 0.65. Fresh olive oil (P. V. 34) and avocado oils (P. V. 30, 35) gave the same general picture except that in the 270 to 280 mμ. region the triene bands, though quite distinct, gave lower coefficients, 0.03 for olive, 0.05 and 0.14 for unbleached and bleached avocado oils, respectively. The coefficients at 230 mμ. were approximately one-half those of corn.

After 5 hours, with P. V.'s varying from 70 to 150, the characteristic triene bands were replaced by a

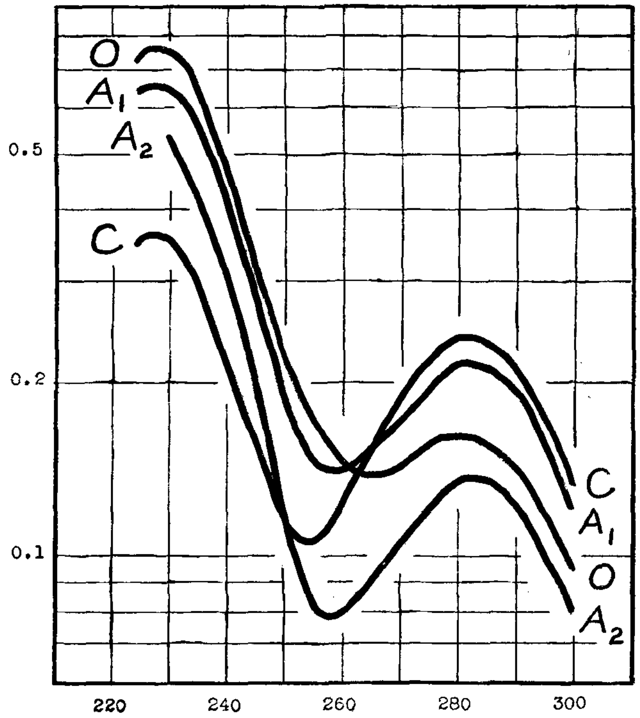

FIG. 1. Absorption spectra of steam distillates.
A_1, Avocado oil unbleached. A_2, Avocado oil bleached.
C. Corn oil. O, Olive oil.
Ordinate: optical density. Abscissa: wave length in mμ.

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single peak, between 275 and 280 mμ., the coefficients showing little change at this wave length but doubling *ca* 230 mμ. After 15 to 21 hours, close to maximum peroxide accumulation, P. V. 463 and 488 for unbleached and bleached avocado, 584 for corn, and 523 for olive, well-defined maxima *ca* 280 and 230 mμ. were apparent, and the values of the coefficients for avocado and olive approached more closely that of the corn.

The specific coefficients ranged from 0.22 to 1.0 at 280 mμ. and from 2.5 to 6.0 at 230 mμ. for unbleached avocado and corn, respectively. This summarizes results of 24 curves on different samples of these three oils.

It is of particular interest that steam distillates of the rancid oils (P. V. 463 to 584) show such marked similarity in their absorption from 300 to 220 mμ. (Fig. 1). The values for the ordinate (in arbitrary units) are strictly comparable, 1 gram of rancid oil being exhaustively steam-distilled, the distillate then being made to volume. Measurements on the rancid oil, prior to distillation, indicate that about 10% of the material responsible for the increase in absorption at 280 mμ. of rancid corn oil appears in the absorption of the steam distillate. By contrast, the steam distillates from fresh samples showed negligible absorption under these conditions (optical densities of 0.02 to 0.03 being recorded for the most concentrated samples), and tests for carbonyl compounds were negative. Samples with P. V.'s of 70 to 150 were intermediate, with respect to the ultraviolet absorption, and in the amount of precipitate obtained with 2,4-dinitrophenylhydrazine.

In this paper only the fractions soluble in petroleum ether are discussed. To the precipitate on a sintered glass filter were added successive 5-ml. aliquots of petroleum ether. The fourth addition resulted in a colorless filtrate, and the residue gave no further color to this solvent when warmed. The combined colored filtrates were then chromatographed. Excellent separation of 3 zones was obtained from the corn preparation, and 4 from the avocado. The 3 zones from the corn (I, II, III, in order of increasing adsorbability) appeared to be identical in behavior and appearance with corresponding Zones I, II, III of the bleached avocado. Zone IV of the avocado was however clearly unique to this oil. The corn chromatogram was repeated three times with increasing quantities of added derivative, but in no case could a fourth zone be detected from the corn.

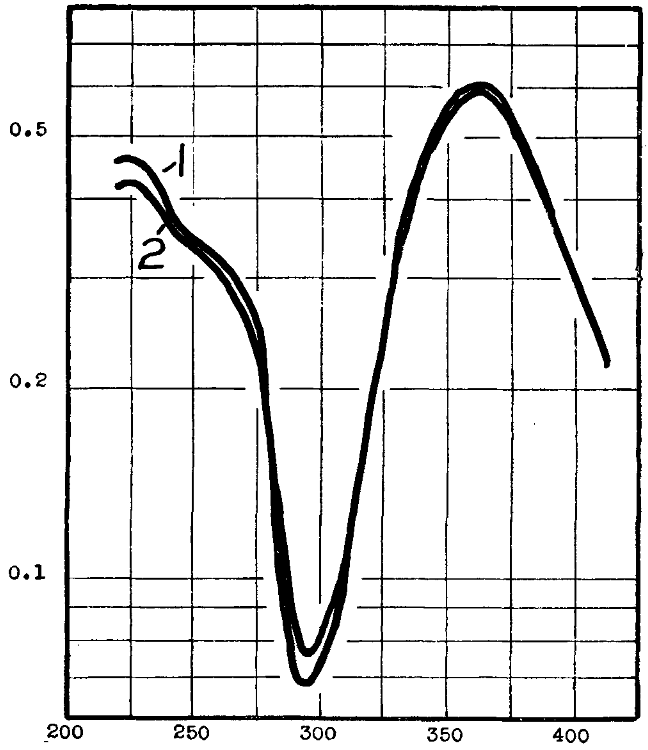

Fig. 2. Absorption spectra of DNPH, Zone I.
1. Avocado. 2. Corn.
Ordinate: optical density. Abscissa: wave length in mμ.

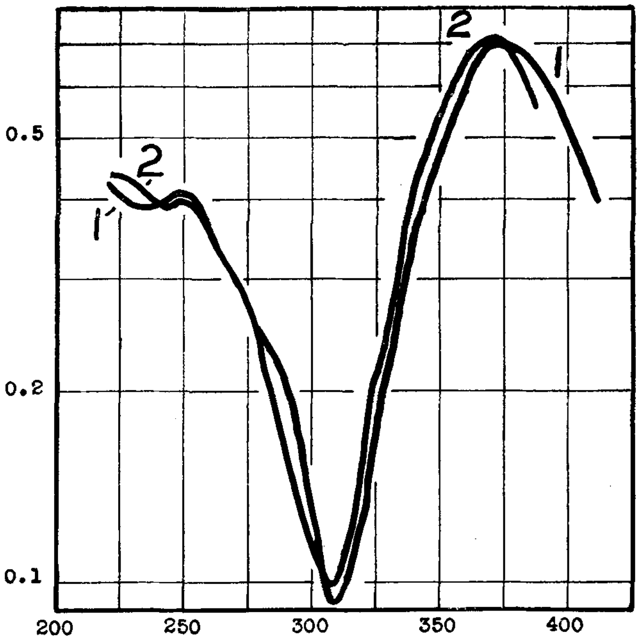

Fig. 3. Absorption spectra of DNPH, Zone II.
1. Avocado. 2. Corn.
Ordinate: optical density. Abscissa: wave length in mμ.

The absorption spectra for corn I and avocado I DNPHs (Fig. 2) are clearly identical and are typical of these derivatives of saturated aldehydes (4). The spectra for corn II and avocado II DNPHs are displaced 10 to 15 mμ. to the red from those of Zone I. They represent typical Δ^2-enal DNPH curves but are clearly not identical. Zones III (Fig. 4) show still greater displacement, and the structure of their spectra is indicative of $\Delta^{2,4}$-dienals. Avocado IV does not differ markedly from avocado III DNPH (Fig. 4) except in its extremely slow rate of movement on the column in petroleum ether. The position of the main maximum for DNPHs of saturated aldehydes is given as 3,560 to 3,600 Å; of -enals as 3,730; and of dienals as 3,790 to 3,950 by Braude and Jones (4), cf. also Roberts and Green (5).

Mixed chromatograms were made of Zones I, II, and III from avocado and corn without obtaining separation. This was definitely disturbing in the case of Zone II (Fig. 3) where the two samples are obviously not identical. Now the curves shown in Figures 2 to 4 represent almost the whole of the zone, after elution from the column. It therefore became necessary to repeat the individual chromatograms, examining the first few and last few drops of each zone to determine whether it was homogeneous, and it was possible to

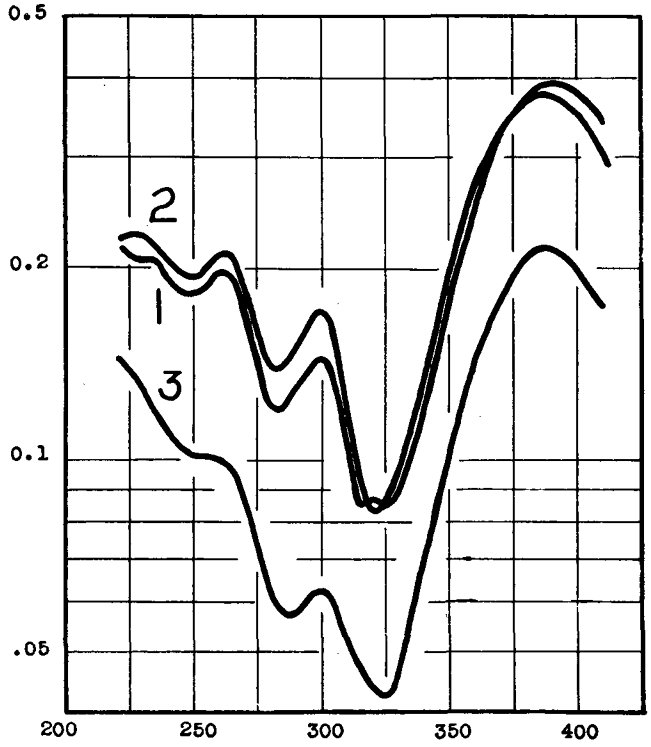

FIG. 4. Absorption spectra of DNPH, Zones III and IV.
1. Avocado III. 2. Corn III. 3. Avocado IV.
Ordinate: optical density. Abscissa: wave length in mμ.

show that this was not the case. Zones I and II (and probably also III) of both corn and avocado are mixtures, with components having maxima and minima as shown in Table I.

TABLE I

Spectral Characteristics of Zones
Maxima (underlined) and minima, in mμ.

I	a	$\underline{363}$	298	$\underline{225}$				
	b	$\underline{357}$	293	$\underline{225}$				
II	a	$\underline{375}$	310	$\underline{250}$				
	b	$\underline{368}$	298	(indef.)				
III	a	$\underline{385}$	320	$\underline{300}$	280	$\underline{263}$	250	$\underline{225}$
	b	$\underline{383}$	318	$\underline{298}$	280	$\underline{260}$	245	(......)
IV		$\underline{388}$	324	$\underline{298}$	283	$\underline{255}$		

The subscripts *a* and *b* to Zones I, II, and III refer to the upper fringe *a* and the lower fringe *b* for the respective zones. The difference in the case of III is slight.

It is clear therefore that each oil contains at least 5 components in the petroleum ether-soluble group and that for complete identification a more effective adsorbent must be employed. One lot of silicic acid has been used with success by other members of the Division on a different problem but not as yet reproducibly on other lots. We have tried the semicarbazone isolation procedure of Swift *et al.* (1). No crystals were obtained from rancid corn oil (50 gm.) though a turbid solution was obtained from avocado distillates, from which a slight precipitate could be centrifuged. Chloroform-extracted samples gave a major maximum at 229 mμ. (suggesting hexanal), a smaller peak at 265 mμ. (Δ^2-enal), and an inflection from 290 to 310 mμ. ($\Delta^{2,4}$-dienal).

Identification of the various aldehydes by Swift *et al.* (1) is based on two steps: first, fractionation by crystallization or solvent extraction of the semicarbazones, and secondly, for chain length, on conversion to the saturated, n-aliphatic dinitrophenylhydrazones. These latter derivatives are as nearly identical spectroscopically as are the unsaturated aldehydes, cf. 2-decenal and 2-undecenal for which Swift *et al.* report data (6). Unless therefore there is sufficient difference in chain length that the nitrogen content becomes a valid criterion, one is left with melting points which are not widely separated. In any event a few mg. of each component must be isolated.

It may legitimately be claimed that chromatography of the DNPHs admits of immediate classification into, and a rough estimate of the proportions of: saturated aldehyde, -enal, and di-enal. Zones II and III of both corn and avocado comprised over 75% of the total coloring matter, and Zone IV of the avocado less than 5%. With refinement in adsorbent and with some additional spectroscopic evidence, the sequence of events should be capable of close study at different P. V.'s admitting therefore of rate studies, by use of conveniently small samples of oil. In only one minor respect do we disagree with the findings of Swift *et al.* (6) where, discussing the absorption spectra of the aldehydes and their derivatives, which they state, explaining their Table III "exhibit single maxima having characteristic extinction coefficients for these types of compounds." We have elsewhere (7) quoted Lewis and Calvin (8) to the effect that "fundamental bands of the whole conjugated system may in simpler molecules be of such low intensity as to be frequently overlooked." Thus it can be predicted that 2-undecenal and 2-decenal will have second maxima *ca* 270 to 280 mμ., in which the molar extinction coefficient will be of the order of 16 to 30, contrasted with 16,000 at 221 to 222 mμ. This point is raised here because the steam distillates from rancid and fresh oils do show a significant difference at 270 to 280 mμ., and one cannot *a priori* eliminate the saturated aldehydes. This can however be effected by determining the contribution of each of the chromatographed zones to the total absorption, made possible by our procedure.

Summary

Steam distillates of rancid corn and avocado oils, collected in 2,4-dinitrophenylhydrazine solution, yield a complex mixture of DNPHs. These were readily separated by adsorption on anhydrous magnesium sulfate into derivatives of saturated aldehydes, -enals and dienals, fully confirming the findings of Swift *et al.* on autoxidized cottonseed oil. It is hoped that, with refinement of method, precise identification of each component may be made. The corn DNPH has at least five, the avocado DNPH at least six components soluble in petroleum ether, whose absorption spectra and chromatographic behavior are described.

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