

Conjugated Unsaturation in Fatty Acids of Immature White Potatoes

A polyunsaturated acid present in fatty acid extracts of immature Kennebec potato tubers was found to resemble dimorphecolic acid (9-hydroxy-*trans,trans*-10,12-octadecadienoic acid) in the peak patterns of conjugated trienes produced from it by gas chromatography and in the ultraviolet and infrared spectra of these trienes. Spectra of unchromatographed material indicated conjugated

unsaturation due mainly to a *trans-trans* diene. Spectroscopic examination of methyl esters produced under acidic conditions showed further resemblances; both ether- and ester-formation occurred if the reaction was carried out at room temperature, but at higher temperatures a triene was formed. The possibility of similar results being given by epoxydienes or by ene-yne is discussed.

Although polyunsaturated acids containing conjugated double bonds have been reported in numerous oil seeds (Hopkins and Chisholm, 1968; Wolff, 1966), there has been little mention of similar acids from other plant organs. Nichols and James (1964) found small amounts of conjugated octadecadienoic acid in the fatty acids of daffodil and narcissus bulbs. Although Highlands *et al.* (1954) found traces of "preformed conjugation" in ultraviolet spectrograms of potato fatty acids prior to their alkaline isomerization, Buttery *et al.* (1961), Mondy *et al.* (1963), Fricker (1969), and others who studied the fatty acid composition of potato lipids apparently did not specifically look for this type of acid and did not report its presence.

In a study of changes in the fatty acid content of Kennebec and Red Pontiac potato tubers during growth, maturation, and storage (Schwartz *et al.*, 1968), gas chromatography of fatty acid esters prepared from lipids of immature tubers yielded an unidentified peak which, on the basis of peak size, appeared to represent up to 35% of the acids. By harvest time it had decreased to trace quantities. Mass spectrometry of material collected from several chromatographic runs indicated a mixture with the major parent peak of the spectrogram corresponding to an 18-carbon acid with three double bonds. The present work gives evidence that this material was an artifact derived from a conjugated diene which, in several ways, resembles the conjugated hydroxy diene, dimorphecolic acid (9-hydroxy-*trans,trans*-10,12-octadecadienoic acid).

EXPERIMENTAL

Apparatus. For gas chromatography an F&M Model 810 chromatograph with a thermal conductivity detector, a 4-ft by $\frac{3}{8}$ -in. aluminum preparative column packed with 20% diethylene glycol succinate (DEGS) on 70 to 80-mesh Gas Chrom RZ, and an 8-ft by $\frac{1}{8}$ -in. stainless steel analytical column packed with 15% DEGS on 60 to 80-mesh Gas Chrom Z was used. Ultraviolet analyses were made with a Cary Model 14 spectrophotometer; free acids were run in *n*-hexane, and esters in 2,2,4-trimethylpentane. For infrared analysis, a Beckman IR-7 spectrophotometer with a beam condenser was used. A potato fatty acid ester preparation that had not been subjected to gas chromatography was analyzed both in the neat state (oil film pressed thin between salt plates) and in CS₂ solution in a 1-mm microcell; the other samples were run in the neat state only.

Gas Chromatography. The method used for preparing esters to be separated on the gas chromatograph was the same as described in the previous publication, except that quantities were doubled. Glass tubes held to the exit port of the chromatograph were used to collect the unidentified material from repeated separations of the methyl esters on the preparative column (column oven 218° C, helium flow 220 ml per min). The collected esters were rechromatographed on

the preparative column, collected again, and put through the analytical column (oven 180° C, helium flow 80 ml per min). The latter resolved the unidentified peak into two overlapping peaks resembling, in position and shape, those obtained by Morris *et al.* (1960) by gas chromatography of methyl dimorphecolate or autoxidized methyl linoleate. The small, unsymmetrical peak reported by Morris to occur before these peaks was also noted. Ultraviolet spectrograms of the two major peaks showed, for each, the characteristic three absorption maxima of a conjugated triene; infrared spectra also showed conjugated unsaturation. Both spectra were similar to those of Morris *et al.* (1960), (ultraviolet maxima for the two peaks, in order of elution, 269.9 and 267.4 nm, Morris *et al.*, 269.5 and 267.3 nm.; infrared absorption maxima at 992 and 996 cm⁻¹, Morris *et al.*, 998 cm⁻¹ for the second peak, a slightly smaller value for the first).

In this and in the previous work (Schwartz *et al.*, 1968) it is not likely that the conjugated unsaturation was derived from autoxidized linoleate since, of many potato samples treated in the same way, only those of immature tubers had shown more than traces of conjugated unsaturation. It was felt important, nevertheless, to demonstrate the presence of conjugation in a preparation which had not been subjected to gas chromatography.

Preparation and Analysis of Free Acids. The conjugated acid had appeared in 1966 and 1967 Kennebec and Red Pontiac potatoes. Further work was done with 1968 Kennebecs. The potatoes were extracted by a more gentle method than previously used, and the fatty acids were protected with nitrogen as much as possible during this process and subsequent treatments. The immature tubers were put in an abrasive peeler for 15 sec, cut into 8-mm thick strips, and frozen. For extraction, the still-cold strips were ground in a food blender with a volume of water equal to one-sixth their weight and filtered through a paper-lined basket centrifuge. The moist cake was extracted 5 hr at room temperature (occasional swirling) with a volume equal to 2.3 times its weight of 3 to 1 ethanol-ethyl ether. The mixture was then filtered, and the residue was allowed to stand with the same amount of solvent for 2 days at 8° C. The combined extracts were evaporated to a small volume *in vacuo* (rotary evaporator, bath temperature 36° C) in order to remove nonaqueous solvent, and the concentrate was extracted with hexane. The hexane extract was washed with water, dried, concentrated to a few milliliters, and refluxed 30 min under a nitrogen flow with 5% KOH in ethanol. After the unsaponifiables were removed by extraction, the reaction mixture was acidified, and the free acids were extracted with hexane.

An ultraviolet spectrogram of the free acids showed a large absorption peak at 234 nm and a much smaller one at 266 nm—close to the expected positions for a conjugated diene and a conjugated triene, respectively. The conjugated

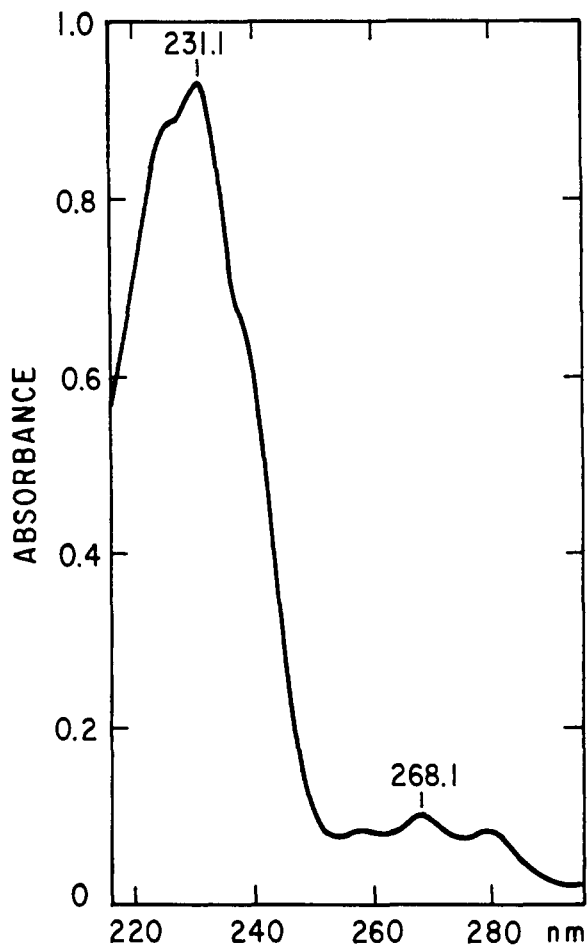


Figure 1. Ultraviolet spectrum of fatty acid methyl esters

diene comprised approximately 23% of the fatty acids according to calculations based on an extinction coefficient [$E_{1\text{cm}}^{1\%}$] of 1160—an average of the more recent values quoted by Pitt and Morton (1957) for 18-carbon *trans-trans* dienes.

Preparation and Analysis of Methyl Esters. A portion of the free acids was heated for 2 min at 65° C in a closed vial with 4% methanolic sulfuric acid (Luddy *et al.*, 1968). Ultraviolet analysis of the product indicated that this esterification method had resulted in an increase in unsaturation, since the triene absorption peak was much greater than that for diene, contrary to the results which had been obtained by spectroscopy of the free acids. When a second portion of acids was converted to methyl esters by allowing it to stand 45 min at room temperature with 5% methanolic sulfuric acid, an ultraviolet spectrogram of the extracted ester mixture (Figure 1) was similar to that of the free acids. The wavelength of the major absorption peak (231 nm) indicated that the diene is mainly *trans-trans*. Literature values (Chipault and Hawkins, 1959; Pitt and Morton, 1957) are 231 for the *trans-trans* isomer and approximately 233 for the *cis-trans* and 235 for the *cis-cis*.

The infrared spectrum of the esters was similar to that obtained from a sample of methyl *trans,trans*-9,11-octadecadienoate. Although the latter had been run only in the neat state, the potato acid esters were run both in the neat state and in CS_2 and, as to be expected for esters, there was no significant change in peak positions for the two methods. The most significant part of each spectrum is shown in Figure 2; the two curves were displaced in a vertical direction so

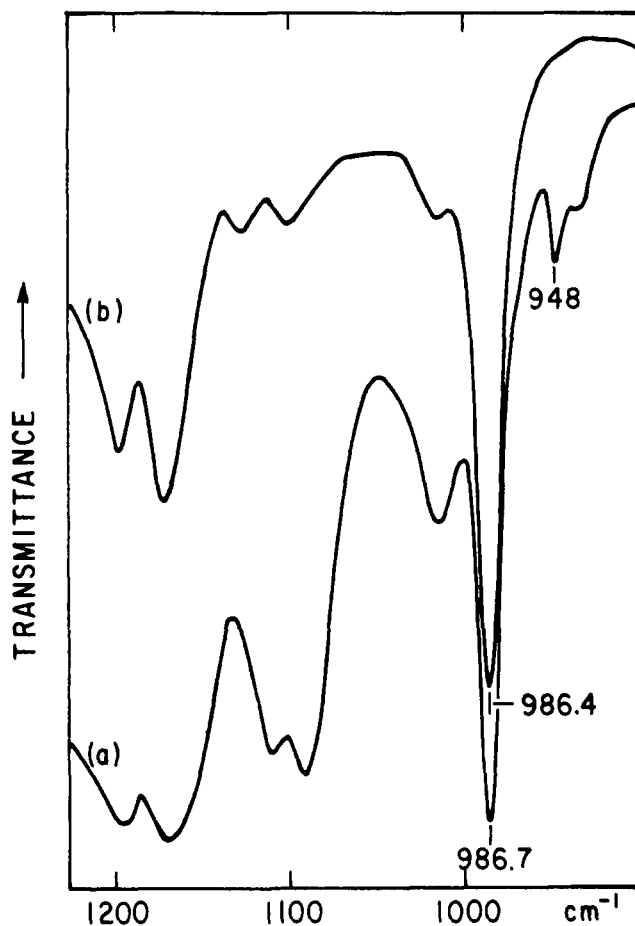


Figure 2. Comparison of infrared spectra of potato fatty acid methyl esters (a, in CS_2) and methyl *trans,trans*-9,11-octadecadienoate (b, in neat state)

that the absorption peaks at 987 cm^{-1} could be shown separately. For dienes, a peak at this wavelength is characteristic of either *trans-trans* or *cis-trans* conjugation, but the peak at 948 cm^{-1} is produced by *cis-trans* only. Chipault and Hawkins (1959) have obtained spectra of mixtures containing various proportions of *cis-trans* and *trans-trans* isomers. The ratio of the peak height at 948 cm^{-1} to that at 987 cm^{-1} is similar to the ratio obtained by these authors for a mixture containing 75% of the *trans-trans* isomer. Because of the small amount of conjugated triene present, these percentages must be taken as rough estimates only. The spectroscopy was run with sufficient resolution to rule out the possibility of a coincident peak at 968 cm^{-1} due to the presence of an isolated *trans* double bond.

DISCUSSION

Ultraviolet spectrograms of free acids (and of esters prepared at room temperature) had shown the presence of conjugated triene, but in quantities too small to account for the relatively large amounts that had been found in eluates from gas chromatography. This apparent discrepancy can be explained if all or part of the conjugated diene consists of dimorphecolic acid or a similar hydroxy diene which could be converted to triene by dehydration. Preparation of esters by heating the free acids with acidic methanol had converted most of the diene to triene. Smith *et al.* (1960) found that dimorphecolate could be dehydrated by refluxing with glacial acetic. Powell *et al.* (1967) reported that dimorphecolate was rapidly etherified at room temperature with 0.1*N* methanolic

sulfuric acid, but that on subsequent heating the major product was triene.

The infrared spectrum of unchromatographed esters prepared at room temperature, as expected, contained no peak in the hydroxyl region, but it did have strong overlapping peaks in the ether region at 1092 and 1112 cm^{-1} (Figure 2a). Literature values for the resulting ether infrared band in dimorphecolate methyl ethers are 9.18 μ (Kleiman *et al.*, 1969), equivalent to 1089 cm^{-1} , and "a broad maximum at 8.9 to 9.2 μ " (Powell *et al.*, 1967), equivalent to 1087 to 1123 cm^{-1} . The infrared spectrum of the chromatographed esters did not show the ether band. Since these esters had been prepared by heating with an acid catalyst, it is possible that all or part of their conversion to triene had taken place before they were chromatographed.

Although the conjugated material resembles dimorphecolic acid in several ways, it may be that, in addition to conjugated double bonds, it contains a functional group such as epoxide, which would behave somewhat like the hydroxyl group. There is also some possibility that conjugation was produced, during saponification of the potato extract, from a fatty acid containing a double and a triple bond. Mikolajczak *et al.* (1965) showed that methyl crepenynate (methyl *cis*-9-octadecen-12-ynoate) rearranges at room temperature under basic conditions to give a conjugated ene-allene, which can be converted by heat to a conjugated triene. Although crepenynic acid itself and the ene-allene derived from it are somewhat different in behavior and spectral properties from the acid being studied, other positional or geometric isomers involving a double and triple bond might resemble it more closely. However, they could not account for the presence of the ether band in infrared spectra of the esters.

Gas chromatographic evidence had indicated that the other fatty acids present in immature tubers in more than trace quantities are the same as those in mature tubers and were not such as to interfere with the spectroscopic analyses. Insufficient material was available to isolate and characterize the unidentified acid. However, the information here presented should be of physiological interest, since it appears that the acid may be an intermediate in some biochemical pathway of the growing tuber.

ACKNOWLEDGMENT

We thank S. F. Herb and Heino Susi of this laboratory for helpful advice. We are also indebted to S. F. Herb for supplying the sample of *trans,trans*-octadecadienoate and to Roy L. Shaw, Jr., of the Red River Valley Potato Processing Laboratory, East Grand Forks, Minn., for shipments of immature tubers.

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Received for review March 30, 1970. Accepted May 21, 1970.
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