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CHARACTERIZATION OF CABBAGE LEAF LIPIDS: *n*-ALKANES, KETONE, AND FATTY ACIDS*

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Abstract—The major components of the lipid fraction of the leaves of cabbage have been identified by means of gas chromatography and combined gas chromatography–mass spectrometry. The saturated hydrocarbons ranged predominantly from C_{27} to C_{31} with C_{29} comprising the vast majority (92 per cent) of the *n*-heptane fraction examined. Mass spectra information is presented for the first time which provides confirming evidence for the structure of a symmetrical ketone containing 29 carbon atoms. A number of unsaturated and saturated fatty acids were also identified; the major components being linolenic, linoleic, and palmitic, which together constituted more than 50 per cent of the total fatty acids present.

INTRODUCTION

SEVERAL studies have provided indirect evidence for the chemical nature of the total lipid fraction[1] and the surface lipids[2] of cabbage (*Brassica oleracea*) leaves. More recently Hill and Mattick[3] have used gas chromatography to study the *n*-alkanes present. Kolattukudy,[4] Robinson,[5] and Purdy and Truter,[2] have all reported on proposed mechanisms for the biosynthesis of several lipid components in cabbage.

The data thus far reported suggest that the major portion of cabbage lipids consists of C_{27} to C_{31} carbon-numbered compounds, except for fatty acids which are predominantly C_{14} to C_{18}. By the use of thin-layer chromatography and chemical analysis of the compounds resolved, Purdy and Truter[2] were able to classify a large number of compounds from the surface of cabbage leaves. They found saturated hydrocarbons comprised approximately 36 per cent by weight, ketones 13·8 per cent and fatty acids 9·2 per cent, the remainder being a complex mixture of ketols, esters, primary and secondary alcohols. Of particular interest was the suggestion of the presence of large quantities of a C_{29} ketone. Kolattukudy[4] reported considerable C^{14} incorporation into both paraffins and a long-chained ketone compound when carbon labeled fatty acids were supplied as precursors to cabbage leaves. The exact nature of this ketone therefore becomes important not only because of the quantities present but also its metabolic relationship to cabbage lipid formation.

It has been suggested[2,4,5] that fatty acids serve as starting materials for the formation of more complex lipids in higher plants. Cabbage and related species have been used in such

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[1] A. C. CHIBNALL and H. J. CHANNON, *Biochem. J.* **21**, 225 (1927); **21**, 233 (1927); **21**, 479 (1927); **21**, 1112 (1927); **23**, 168 (1929); **23**, 176 (1929).
[2] S. J. PURDY and E. V. TRUTER, *Proc. Roy. Soc. London* **B158**, 536, 544, 553 (1963).
[3] A. S. HILL and L. R. MATTICK, *Phytochem.* **5**, 693 (1966).
[4] P. E. KOLATTUKUDY, *Biochemistry* **4**, 1844 (1966); **5**, 2265 (1966).
[5] R. ROBINSON, *Nature* **124**, 262 (1967).

studies but it seemed that additional structural evidence for the nature of the more metabolically significant compounds involved in wax biosynthesis was necessary. Therefore we undertook to determine the chemical characteristics of selected lipid compounds from cabbage leaves by use of gas chromatography and mass spectrometry.

RESULTS AND DISCUSSION

The heptane fraction was found to contain, as determined by gas-chromatographic retention times and internal standards, normal paraffins C_{27} (2 per cent), C_{28} (< 1 per cent) C_{29} (92 per cent), C_{30} (< 1 per cent) and C_{31} (5 per cent) with only trace quantities of lower molecular weight paraffins present. Mass spectra confirmed that all major compounds were straight-chain saturated hydrocarbons having the following respective parent ion peaks: C_{27} (*m/e* of 380); C_{29} (408); C_{31} (436).

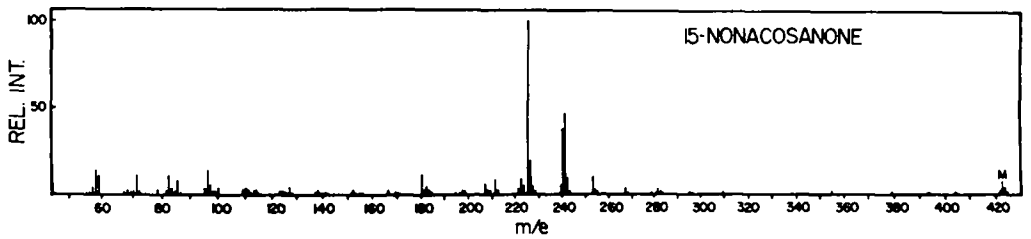

FIG. 1. MASS SPECTRA *m/e* OF THE MAJOR COMPONENT IN THE BENZENE FRACTION OF EXTRACTS FROM *Brassica oleracea* LEAVES.

Compound was separated from gas chromatography on a 0·64 cm × 1·8 m glass column packed with chromosorb Q coated with 1 per cent SE-30 and then ionized at 20 eV and scanned from 0·0 to 450 *m/e* in an LKB 9000 Gas Chromatograph–Mass Spectrometer. Separation was achieved by programming the column at 6° per min from 200 to 300° The spectrum was recorded by means of an oscillograph recorder set at a chart speed of 10 cm per sec. The component was scanned for a 10 sec interval. E. Gelpi was intrumental in interpreting the above mass spectrum.

The benzene fraction was found to contain one major component in very high concentration as shown by gas chromatography. Five other components were present in much lower concentrations. Thin-layer chromatography in two different solvents confirmed the presence of a major 2,4-dinitrophenylhydrazone-forming compound. The mass spectra of this compound is shown in Fig. 1. The parent ion peak is at *m/e* 422; the major peak is at *m/e* 225.

It is known that ketones frequently undergo rearrangements through a six-membered ring intermediate, followed by fragmentation with retention of the positive charge on the oxygen-containing fragment. In such a case, one would expect a peak at *m/e* 240, and then by double rearrangement a peak also at *m/e* 182 as observed in the spectra in Fig. 1. Such a spectra could only correspond to a symmetrical ketone having 29 carbon atoms, specifically 15-nonacosanone.

The methanol fraction, following preparation of methyl esters, was also characterized by gas chromatography which produced major peaks corresponding to the methyl esters of palmitic acid, linoleic acid, and linolenic acid. Several other components were also present, such as the odd carbon-numbered fatty acids, but only as minor components. Mass spectra of the major gas chromatographic peaks confirmed the above findings with the following parent ion *m/e* values: palmitic acid, 270 *m/e*, linoleic acid, 294 *m/e*, and linolenic acid,

292 *m/e*. Spectra were identical to those reported earlier from this laboratory for the methyl esters of fatty acids derived from microbial systems.[6]

Palmitic acid was the major saturated fatty acid which comprised 12 per cent of the methanol fraction. The predominant components in the methanol fraction were the unsaturated fatty acids, linoleic acid, 18 per cent, and linolenic acid, 22 per cent.

Later reports from this laboratory will deal with the metabolic relationship between the various lipids in cabbage leaves. Label incorporation studies point to the conclusion that nonacosane and 15-nonacosanone are formed from a common fatty acid precursor but follow independent pathways.

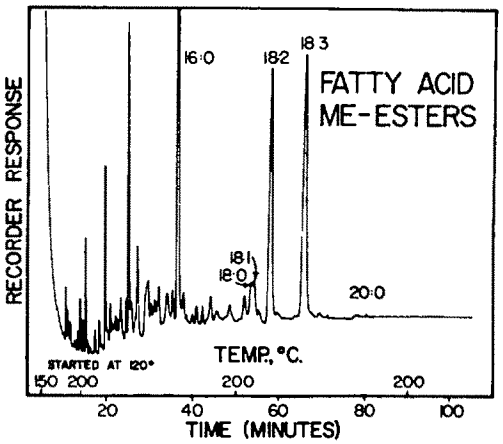

FIG. 2. GAS CHROMATOGRAPHIC SEPARATION OF THE ESTERIFIED METHANOL FRACTION FROM 3·5 gm OF *Brassica oleracea* LEAVES WITH NITROGEN CARRIER GAS AT 2430 g/cm AND A HYDROGEN FLAME IONIZATION DETECTOR.

No split was used. Programmed at approximately 6° per min from 120 to 200°, and held isothermally at 200°. Column used was a 0·076 cm × 155 m stainless-steel capillary column coated with Igepal CO-990. Attenuation 100×, Range 1.

EXPERIMENTAL

Leaves were obtained from 6- to 8-week-old cabbage plants (*Brassica oleracea*; var. Round Dutch) grown in flats provided with soil. The leaves were then washed with distilled water and dried over $CaSO_4$ until they attained a constant weight. From 3–5 g of dry leaves were cut into 1 cm × 1 cm squares and immersed in solvents for 30 min with frequent stirring at 50°. The first extraction was with 50 ml *n*-heptane followed by two extractions with 50 ml benzene–methanol mixture (3:1). All three extracts were combined and evaporated to dryness at 50° under a stream of prepurified N_2.

The total lipid extracts were then taken up in 5-ml portions of *n*-heptane and separated as reported earlier[7] on a silica gel column (1 × 20 cm) which has been previously washed with 3 vol. of *n*-heptane and activated at 425° for 10 hr immediately prior to use. Each of the three solvent fractions collected (*n*-heptane, benzene and methanol) were taken to dryness under a stream of prepurified nitrogen.

In order to prepare methyl esters, the methanol fraction was first saponified in 50 ml of methanol:NaOH (1:1) by refluxing for 3 hr. The saponified fraction was taken to dryness under N_2 at 40°, following adjustment of pH to 1·0 with HCl, and extracted three times with *n*-heptane. The residue was then taken up in 50 ml of methanol containing 2·5 ml 2,2-dimethoxypropane and 0·25 ml H_2SO_4 and refluxed for 2 hr. 5 ml of H_2O was added and the methyl esters extracted with *n*-heptane and taken to dryness at 40° under N_2.

Fractions collected from the silica gel column were analyzed by gas chromatography with an F & M Model 810 gas chromatograph equipped with 0·025 cm × 30 m stainless-steel capillary column coated with Apiezon-L

[6] J. ORÓ, T. G. TORNABENE, D. W. NOONER and E. GELPI, *J. Bacteriol.* **93**, 1811 (1967).
[7] J. ORÓ, J. L. LASETER and D. J. WEBER, *Science* **154**, 399 (1966).

for the *n*-heptane and benzene fractions. Stainless-steel capillary columns, 0·076 × 155 cm, coated with CO-990 were used for the separation of the fatty acid methyl esters. Mass spectra of selected compounds were obtained as they emerged from the gas chromatographic column by use of an LKB-9000 Gas Chromatograph–Mass Spectrometer combination[8] equipped with a 0·64 cm × 1·8 m glass column packed with Chromosorb Q and coated with 1 per cent SE-30.

[8] R. RYHAGE, *Anal. Chem.* 36, 759 (1964); R. RYHAGE, S. WIKSTRÖM and G. R. WALLER, *Anal. Chem.* 37, 435 (1965).