Phytochemistry, 1966, Vol. 5, pp. 693 to 697. Pergamon Press Ltd. Printed in England

THE *n*-ALKANES OF CABBAGE (VAR. *COPENHAGEN*) AND SAUERKRAUT*

ADA S. HILL and LEONARD R. MATTICK

Department of Food Science and Technology, New York State Agricultural Experiment Station, Geneva, New York

(*Received* 29 *September* 1965)

Abstract—The hydrocarbons of cabbage (var. *Copenhagen*), normal sauerkraut, and "soft" sauerkraut have been analyzed using gas chromatography. They consist of a complete homologous series of *n*-alkanes from about C_{21} to C_{33}, among which C_{29} (67 per cent), C_{31} (27 per cent), and C_{30} (3 per cent) predominate. No major difference between the hydrocarbons of cabbage and either of the sauerkrauts was found. Significant differences in the *n*-alkane mixtures exist between the *Copenhagen* variety of cabbage we analyzed and the *Winnigstadt* variety analyzed by Purdy and Truter, who found C_{29} and C_{31} to be present in the ratio of 93:3 respectively. About 90 per cent of the cabbage hydrocarbons are located on the leaf surface.

INTRODUCTION

OUR interest in the possible relationship between sauerkraut quality and metabolism of hydrocarbons during fermentation of cabbage to sauerkraut gave rise to the present work.

A number of other groups have studied cabbage hydrocarbons. Chibnall and his co-workers isolated hydrocarbons[1] from ether extracts of expressed cabbage leaf fluids[2] and reported their identification as *n*-C_{29}[1] and *n*-C_{31}[3] in the approximate ratio of 95:5 respectively.[3] Purdy and Truter reported the composition of the *n*-alkane mixture isolated from the surface of cabbage (var. *Winnigstadt*) leaves to be C_{29} (93 per cent), C_{31} (3 per cent), C_{30} (1 per cent), C_{27} (1 per cent), C_{28} (1 per cent), and the remainder (1 per cent) to be a mixture of C_{22}, C_{23}, C_{24}, C_{25}, and C_{26}.[4] Kolattukudy has reported similar results with an unspecified variety of cabbage.[5] Analyses of hydrocarbons from other varieties of cabbage or from sauerkraut have not been reported.

RESULTS AND DISCUSSION

The hydrocarbons isolated by silicic acid column chromatography of the chloroform extractable materials from various samples of cabbage (var. *Copenhagen*), normal sauerkraut, and "soft" sauerkraut (all made from the *Copenhagen* variety of cabbage) had m.p. 55–64° and comprised 0·005 per cent of the whole vegetable or 0·05 per cent of the dehydrated vegetable.[6] Gas chromatography separated the hydrocarbon mixtures into components

* This investigation was supported in part by Public Health Service Research Grant EF 00031-03 from the Division of Environmental Engineering and Food Protection.

Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 1452, September 7, 1965.

[1] H. J. CHANNON and A. C. CHIBNALL, *Biochem. J.* **23**, 168 (1929).
[2] A. C. CHIBNALL and H. J. CHANNON, *Biochem. J.* **21**, 225 (1927).
[3] A. C. CHIBNALL, S. H. PIPER, A. POLLARD, E. F. WILLIAMS and P. N. SAHAI, *Biochem. J.* **28**, 2189 (1934).
[4] S. JEAN PURDY and E. V. TRUTER, *Proc. Roy. Soc. (London) Ser.* **B 158** (973), 536, 553 (1963).
[5] P. E. KOLATTUKUDY, *Biochem.* **4**, 1844 (1965).
[6] A. S. HILL, L. R. MATTICK and C. S. PEDERSON, To be published.

belonging to one homologous series readily identified as *n*-alkanes by the usual criteria.[7] The highest detectable member of the series was C_{33} and the lowest was C_{14}. Peaks comprising $<0{\cdot}1$ per cent of the sample and having fractional apparent carbon numbers of about 15–25 occurred in some of the sauerkraut samples. These peaks always appeared only in the last of the hydrocarbon fractions to elute from the silicic acid columns, which suggests that these peaks are probably due to unsaturated hydrocarbons. Their sporadic appearance among the sauerkraut hydrocarbons also suggests they may be due to contaminants. Purdy and Truter reported that unsaturated and branched chain hydrocarbons were absent from their samples of cabbage (var. *Winnigstadt*) hydrocarbons.[4] Another component that sometimes appeared among the hydrocarbons of both cabbage and sauerkraut had a gas chromatographic retention time between those of *n*-C_{29} and *n*-C_{30}. It was a minor but not a trace component, the identity of which remains uncertain. A branched chain structure is considered likely as it elutes from silicic acid at the same rate as the *n*-alkanes and thus it is probably saturated. Branched and cyclic hydrocarbons of relatively high molecular weight have been reported to occur in some plants.[8]

Contamination from various sources during preparation and handling of the hydrocarbon samples was a possibility that we investigated. The following likely sources of contaminants were extracted and analyzed in the same manner as were the vegetable samples: polyethylene films, Teflon-covered bar magnets, parts of the rotary evaporator and the Waring blendor used in the preparation of extracts, Lubriseal, human skin oils, a lanolin preparation* used as a hand lotion, and residues left on evaporation of freshly distilled *n*-hexane. The results of these studies ruled out any detectable contamination from all but the polyethylene, which contained hydrocarbons with apparent carbon numbers of 18·8, 20·7, 22·8, and lesser amounts of 24·9 and 26·9, and the hexane. The values found for the trace sauerkraut hydrocarbons not corresponding to *n*-alkanes are 16·6, 17·6, 18·6, 19·6, 20·6, 21·6, 22·5, 23·6, 24·4, and 25·6, the most abundant being 23·6, indicating that these hydrocarbons originated from sources other than polyethylene. *n*-Hexane residues yielded *n*-alkanes with 14–19 carbons, with the lower ones predominant. Consequently only those *n*-alkanes with twenty or more carbons are considered to have originated unequivocally from the vegetables. However, the consistent appearance of *n*-alkanes with eighteen and nineteen carbons in all samples except II (Table 1) indicates that these too may have originated from cabbage and sauerkraut. Alkanes with less than eighteen carbons were not evident in many samples.

The results presented in Table 1 show that no major changes occurred in the *n*-alkanes of cabbage either during normal or during the one abnormal type of fermentation studied. The occurrence of hydrocarbons other than *n*-alkanes was sporadic among the sauerkraut samples making it unlikely that they were a direct or normal result of the fermentation process. Some of the lower *n*-alkanes showed changes in abundance (Table 1) in a few samples, such as C_{23} in sample XI, but these changes also lacked the consistency required to relate them unequivocally to the fermentation process.

The predominant *n*-alkanes were C_{29} and C_{31}, which occurred in the approximate ratio of 2·4:1 respectively. Together they comprised about 94 per cent of the total hydrocarbon mixture. Purdy and Truter found these two *n*-alkanes to occur in the ratio of 93:3 respectively in the cuticle wax of cabbage (var. *Winnigstadt*).[4] Others have reported ratios of 95:5 for

* Nivea creme, made in Canada by Nivea Pharmaceuticals, Ltd., Montreal.

[7] (*a*) R. P. W. SCOTT, *Gas Chromatography* (Proceedings of a Symposium), p. 300. Butterworths, Washington (1960); (*b*) E. BAYER, *Gas Chromatography*, p. 70. Elsevier, Amsterdam (1961).

[8] A. KUKSIS, *Biochem.* 3, 1086 (1964).

TABLE 1. *n*-ALKANES OF CABBAGE AND SAUERKRAUT, PRESENTED AS AREA RATIOS TO *n*-TRIACONTANE

Source*	C_{18}	C_{19}	C_{20}	C_{21}	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	"iso C_{30}"	C_{30}	C_{31}	C_{32}	C_{33}	C_{29}/C_{31}
I, fresh cabbage	0·008	0·008	0·009	0·026	0·053	0·14	0·105	0·109	0·057	0·38	0·26	20·1	—	1	9·3	0·14	0·11	2·24
II, leaf surface of fresh cabbage	—	—	—	0·009	—	0·011	0·006	0·018	0·008	0·29	0·18	20·9	0·19	1	8·8	—	—	2·35
III, freeze-dried cabbage	0·008	0·009	0·010	0·040	0·062	0·19	0·061	0·093	0·053	0·30	0·17	20·8	—	1	11·4	—	—	1·88
IV, unsaponifiables of III	0·008	0·012	0·017	0·053	0·092	0·22	0·099	0·116	0·062	0·34	0·18	25·3	—	1	11·9	0·10	—	2·15
V, freeze-dried cabbage	0·008	0·003	0·003	0·020	0·050	0·10	0·031	0·035	0·011	0·34	0·20	28·4	0·02	1	10·5	—	—	2·75
VI, unsaponifiables of V	0·001	0·003	0·003	0·011	0·006	0·089	0·009	0·043	0·007	0·34	0·17	24·8	—	1	9·0	0·065	0·056	2·77
Average of all values	0·008	0·007	0·008	0·027	0·051	0·125	0·052	0·069	0·033	0·33	0·19	23·4	0·10	1	10·1	0·10	0·083	2·36
VII, fresh sauerkraut	0·004	0·004	0·003	0·026	0·019	0·066	0·022	0·026	0·015	0·37	0·23	28·0	—	1	11·6	0·12	—	2·43
VIII, freeze-dried sauerkraut	0·010	0·006	0·007	0·029	0·034	0·12	0·15	0·051	0·034	0·29	0·22	25·6	0·26	1	9·8	0·10	0·14	2·40
IX, unsaponifiables of VIII	0·009	0·009	0·010	0·076	0·050	0·18	0·060	0·074	0·046	0·34	0·25	23·0	—	1	9·7	—	—	2·46
Average of all values	0·008	0·006	0·007	0·044	0·034	0·12	0·077	0·050	0·032	0·33	0·23	25·5	0·26	1	10·3	0·11	0·14	2·43
X, fresh "soft" sauerkraut	0·002	0·020	0·020	0·014	0·018	0·026	0·012	0·017	0·008	0·29	0·16	28·1	0·24	1	10·9	—	—	2·30
XI, freeze-dried "soft" sauerkraut	0·027	0·026	0·024	0·031	0·028	1·26	0·042	0·49	0·018	0·40	0·20	35·5	—	1	11·3	0·18	—	3·20
XII, leaf surface of fresh cabbage	0·002	0·003	0·003	0·002	0·004	0·004	0·007	0·01	0·030	0·47	0·27	30·5	—	1	10·5	—	—	3·05
XIII, fresh cabbage, after removal of leaf surface waxes	0·060	0·070	0·040	0·030	0·040	0·030	0·030	0·05	0·030	0·42	0·28	28·5	—	1	8·5	—	—	2·90

* Samples I, III and IV were from cabbage harvested locally in the fall of 1962, and samples VII, VIII and IX were prepared from this harvest. Samples II, V and VI were from cabbage harvested locally in the fall of 1963, and samples X and XI were prepared from this harvest. Samples XII and XIII were from cabbage purchased in a local supermarket in the spring of 1965.

"cytoplasmic" hydrocarbons,[3] and approximately 9:1,[5] both for unspecified varieties of cabbage. These differences in *n*-alkane composition between the *Winnigstadt* and *Copenhagen* cabbages provides another example of the varietal specificity in hydrocarbon composition studied by Eglinton *et al.*,[9] which they have suggested can be used as a taxonomic criterion in many cases.[10]

The absence of some of the lower *n*-alkanes from sample II, the surface waxes, lead to an investigation of the distribution of *n*-alkanes on the leaf surface and within the leaf. The surface extract contained 20 per cent *n*-alkanes (sample XII) and the pulp extract contained 1·5 per cent of *n*-alkanes (sample XIII). Consequently about 90 per cent of the cabbage hydrocarbons were located in the external wax. Kolattukudy recently reported that 94–98 per cent of the hydrocarbons synthesized in a 4-hr period and incorporating [^{14}C]acetate fed to the cabbage leaf via its petiole during that time are located in the external wax.[5] The report by Channon and Chibnall that 12 per cent of the ether soluble "cytoplasmic" materials[1] are hydrocarbons is thus probably much too high.

Comparison of samples II, XII, and XIII shows that only minor differences in composition occur between those hydrocarbons located on the surface of the cabbage leaf and those located within the leaf and/or embedded in the cuticle: primarily that the lower hydrocarbons (26 carbons or less) are less abundant in the former than in the latter. Kolattukudy reported no difference in hydrocarbon composition at these two locations.[5] The exact location of the hydrocarbons not present on the leaf surface remains to be determined. Martin's group showed that for Bramley apple leaves a major proportion of these are occluded in the cuticle.[11]

EXPERIMENTAL

Preparation of Hydrocarbon Samples

Some of the vegetable samples were lyophilized in a Stokes freeze-drier. Most samples were either triturated in a large stainless steel Waring blendor or ground in a large porcelain mortar. All samples were extracted three times with redistilled chloroform, the extracts were combined and evaporated under reduced pressure. Whole leaves were extracted by dipping them into three successive portions of chloroform, a procedure known to extract only those materials located on the leaf surface.[11,12] The lipid mixtures were chromatographed on silicic acid columns using freshly distilled *n*-hexane as the eluant. The hydrocarbons appeared in the first fractions. Likely sources of contamination were extracted with chloroform and prepared for gas chromatography by the same method.

Gas Chromatography

Gas chromatography was carried out on a column of 5 per cent SE-30 on Chromosorb W, 60–80 mesh, 5 × ⅛ in. i.d., operated at 230° or 240° with N_2 as the carrier gas. An Aerograph Hy-F1 600-C gas chromatograph equipped with a hydrogen flame ionization detector was used.

Individual hydrocarbons were identified in the usual manner.[7] For samples I–IX (Table 1) peak areas were determined by three methods: triangulation, retention × peak height, and

[9] (*a*) G. EGLINTON, A. G. GONZALEZ, R. J. HAMILTON and R. A. RAPHAEL, *Phytochem.* **1**, 89 (1962); (*b*) G. EGLINTON, R. J. HAMILTON and M. MARTIN-SMITH, *Phytochem.* **1**, 137 (1962).

[10] G. EGLINTON and R. J. HAMILTON, *Chemical Plant Taxonomy* (Edited by T. SWAIN), p. 187. Academic Press, London (1963).

[11] A. M. SILVA FERNANDES, E. A. BAKER and J. T. MARTIN, *Ann. Appl. Biol.* **53**, 43 (1964).

[12] S. JEAN PURDY and E. V. TRUTER, *Nature* **190**, 554 (1961).

weight of paper circumscribed by the peak. For samples X–XIII only the third method was used. The value given for the relative area of each peak (Table 1) represents the average value for all chromatograms available for each sample. These values are given as areas relative to that of *n*-C_{30} as this hydrocarbon appeared in all the chromatograms at a level suitable for quantitation.

Acknowledgement—We are grateful to Mr. Robert M. Butts for technical assistance in the use of the gas chromatograph.