Tam et al., 1982). It thus appears that elevated levels of arsenic as arsenobetaine or arsenocholine in food fish do not pose a serious hazard to human health. However, the metabolism and possible long-term effects on humans are largely unknown and require further study. The arsenic compounds in freshwater fish and salmon merit further research because of their unknown chemical structure and biological activity.

ACKNOWLEDGMENT

G. Lacroix is thanked for performing the total arsenic analyses. Dr. P. Y. Lau is thanked for arranging for mass spectrometric analyses and interpreting the data.

Registry No. As, 7440-38-2; arsenobetaine, 64436-13-1; arsenocholine, 39895-81-3.

LITERATURE CITED

Brinckman, F. E.; Blair, W. R.; Jewett, K. L.; Iverson, W. P. *J. Chromatogr. Sci.* **1977**, *15*, 493.
Cannon, J. R.; Edmonds, J. S.; Francesconi, K.; Raston, C.; Saunders, J.; Skelton, B.; White, A. H. *Aust. J. Chem.* **1981**, *34*, 787.
Cannon, J. R.; Saunders, J. B.; Toia, J. R. *Sci. Total Environ.* **1983**, *31*, 181.
Dabeka, R. W., accepted for publication in *Analyst*.
Edmonds, J. S.; Francesconi, K.; Cannon, J. R.; Raston, G. L.; Skelton, B.; White, A. H. *Tetrahedron. Lett.* **1977**, *18*, 543.
Food Chemical News **1984**, *25* (47), 13.
Kennedy, V. S. *J. Fish Res. Board Can.* **1976**, *33*, 1388.
Koizumi, H.; Hadeishi, T.; Maclaughlin, R. *Anal. Chem.* **1978**, *50*, 1700.
Kurosawa, S.; Yasuda, K.; Taguchi, M.; Yamazaki, T. S.; Morita, M.; Uehiro, T.; Fuwa, K. *Agric. Biol. Chem.* **1980**, *44*, 1993.
Lunde, G. *Acta Chem. Scand.* **1973**, *27*, 1586.
Lunde, G. *J. Sci. Food Agric.* **1975**, *26*, 1257.
Lunde, G. *Environ. Health Perspect.* **1977**, *19*, 47.
Luten, J. B.; Riekwel-Booy, G.; Rauchbaar, A. *Environ. Health Perspect.* **1982**, *45*, 165.
Norin, H.; Ryaage, R.; Christakopolous, A.; Sandstrom, M. *Chemsphere* **1983**, *12*, 277.
Marafante, E.; Vahter, M.; Dencker, L. *Sci. Total Environ.* **1984**, *34*, 223.
Penrose, W. R. *CRC Crit. Rev. Environ. Control* **1974**, *4*, 465.
Penrose, W. R. *J. Fish Res. Board Can.* **1975**, *32*, 2385.
Penrose, W. R.; Conacher, H. B. S.; Black, R.; Meranger, J. C.; Miles, W.; Cunningham, H. M.; Squires, W. R. *Environ. Health Perspect.* **1977**, *19*, 53.
Sen, N. P.; Tessier, L.; Seaman, S. W. *J. Agric. Food Chem.* **1983**, *31*, 1033.
Stockton, R. A.; Irgolic, K. J. *Int. J. Environ. Anal. Chem.* **1979**, *6*, 313.
Tam, G. K. H.; Charbonneau, S. M.; Bryce, F.; Sandi, E. *Bull. Environ. Contam. Toxicol.* **1982**, *28*, 669.
Vahter, M.; Marafante, E.; Dencker, L. *Sci. Total Environ.* **1983**, *30*, 197.
Weinstein, G. L. *Job Safety Health* **1978**, *6*, 15.
Zook, E. G.; Powell, J. J.; Hackley, B. M.; Emmerson, J. R.; Brooker, J. R.; Knobl, G. M. *J. Agric. Food Chem.* **1976**, *24*, 47.

Received for review June 21, 1985. Revised manuscript received November 18, 1985. Accepted November 26, 1985.

Chemical Composition of Chesnut Honey: Analysis of the Hydrocarbon Fraction

Giorgio Bonaga,* Angelo G. Giumanini,[1] and Grazia Gliozzi[2]

The hydrocarbon fraction of chesnut honey (*Castanea sativa*) was investigated by combination of physical, chemical, and instrumental techniques. The quantitative analysis and the identification of hydrocarbons isolated from honey were carried out by gas chromatography with short capillary column and by combined gas chromatography/mass spectrometry. Linear hydrocarbons, saturated and unsaturated, at even and odd number of carbon atoms, from C_{10} to C_{37}, were found in chesnut honey. *n*-Heptacosane, *n*-nonacosane, *n*-tricosane, *n*-pentacosane, and *n*-hentriacontane were the largest gas chromatographic peaks in *n*-alkane fraction (ca. 36.0 area %), whereas *n*-tritriacontene and *n*-hentriacontene predominated in the unsaturated portion (ca. 64.0 area %). The positional and geometrical isomerism of the double bond in *n*-alkenes was investigated by the study of their epoxides. At least 50% of unsaturated material is made up of Δ^{10}-alkenes (mainly C_{31} and C_{33}). A new observation allowed us to assign a prevailing cis configuration about C_{10}–C_{11} unsaturation on the basis of the mass spectral pattern of the corresponding epoxides. The position of the unsaturation varies with continuity from C_8 to C_{16}. The hydrocarbon content of honey reproduced to a great extent that of beewax composition.

INTRODUCTION

Honey is a complex natural product, made mainly of carbohydrates and water, but containing a large number of minor components of which only a fraction is known. The present knowledge of honey components may be defined as good for the carbohydrates, inorganic salts, and a number of specific classes of compounds like amino acids, vitamins, and so on, but the identification of other honey components, as hydrocarbons and some other aroma and flavor compounds, is not satisfactory. Downing et al. (1961), Callow et al. (1964), Streibl et al. (1966), Tulloch (1980), and Lercker et al. (1981) investigated the hydrocarbon fraction of beewax and royal jelli, but not many literature entrees refer to the hydrocarbons of honey. On the other hand the hydrocarbons are the largest single class of compounds contained in *n*-hexane extract of honey, and the quantitative and/or qualitative fluctuations of hy-

Dipartimento di Protezione e Valorizzazione Agroalimentare, Sezione di Chimica e Tecnologia degli Alimenti, Università di Bologna, 40126 Bologna, Italy.
[1]Present address: Dipartimento di Chimica, Università di Udine, 30133 Udine, Italy.
[2]Student for the Course of Chimica e Tecnologia Farmaceutiche, Università di Bologna, 40126 Bologna, Italy.

0021-8561/86/1434-0319$01.50/0

Table I. Quantitative Analysis of Total Hydrocarbons in Chesnut Honey (*Castanea sativa*)

peak	ret time, min	area %[a] A	area %[a] B	mol wt	formula	compd	basis for identifcn[b,c]
1	2.91	0.1		142	$C_{10}H_{22}$	*n*-decane	GC, MS
2	4.49	0.1		156	$C_{11}H_{24}$	*n*-hendecane	MS
3	6.17	0.2		170	$C_{12}H_{26}$	*n*-dodecane	GC, MS
4	7.85	0.2		184	$C_{13}H_{28}$	*n*-tridecane	GC, MS
5	9.47	0.3		198	$C_{14}H_{30}$	*n*-tetradecane	GC, MS
6	11.03	0.2		212	$C_{15}H_{32}$	*n*-pentadecane	GC, MS
7	12.51	0.2		226	$C_{16}H_{34}$	*n*-hexadecane	GC, MS
8	13.91	0.4		240	$C_{17}H_{36}$	*n*-heptadecane	GC, MS
9	15.26	0.3		254	$C_{18}H_{38}$	*n*-octadecane	GC, MS
10	16.54	0.5	0.2	268	$C_{19}H_{40}$	*n*-nonadecane	GC, MS
11	17.79	0.3	0.1	282	$C_{20}H_{42}$	*n*-eicosane	GC, MS
12	18.97	0.9	0.9	296	$C_{21}H_{44}$	*n*-heneicosane	GC, MS
13	20.11	0.3	0.3	310	$C_{22}H_{46}$	*n*-docosane	GC, MS
14	21.02	1.0	0.8	322	$C_{23}H_{46}$	*n*-tricosene	GC, MS
15	21.21	5.7	6.2	324	$C_{23}H_{48}$	*n*-tricosane	GC, MS
16	22.26	0.4	0.4	338	$C_{24}H_{50}$	*n*-tetracosane	GC, MS
17	23.10	1.5	1.3	350	$C_{25}H_{50}$	*n*-pentacosene	MS
18	23.28	5.6	6.0	352	$C_{25}H_{52}$	*n*-pentacosane	MS
19	24.25	0.4	0.4	366	$C_{26}H_{54}$	*n*-hexacosane	GC, MS
20	25.03	1.1	1.0	378	$C_{27}H_{54}$	*n*-heptacosene	MS
21	25.21	7.5	8.2	380	$C_{27}H_{56}$	*n*-heptacosane	MS
22	26.13	0.2	0.2	394	$C_{28}H_{58}$	*n*-octacosane	GC, MS
23	26.87	1.0	0.8	406	$C_{29}H_{58}$	*n*-nonacosene	MS
24	27.03	5.8	6.4	408	$C_{29}H_{60}$	*n*-nonacosane	MS
25	27.28	0.3	0.1	420	$C_{30}H_{60}$	*n*-triacontene	MS
26	27.28	0.3	0.2	422	$C_{30}H_{62}$	*n*-triacontane	GC, MS
27	28.49	20.0	20.6	434	$C_{31}H_{62}$	*n*-hentriacontene	MS
28	28.65	3.9	4.2	436	$C_{31}H_{64}$	*n*-hentriacontane	MS
29	29.32	0.6	0.3	448	$C_{32}H_{64}$	*n*-dotriacontene	MS
30	29.32	0.6	0.3	450	$C_{32}H_{66}$	*n*-dotriacontane	GC, MS
31	30.14	37.1	37.0	462	$C_{33}H_{66}$	*n*-tritriacontene	MS
32	30.28	0.8	0.8	464	$C_{33}H_{68}$	*n*-tritriacontane	MS
33	31.44	1.9	1.7	476	$C_{35}H_{70}$	*n*-pentatriacontene	MS
34	31.62	0.7	0.7	478	$C_{35}H_{72}$	*n*-pentatriacontane	MS
35	32.88	0.5	0.2	518	$C_{37}H_{74}$	*n*-heptatriacontene	MS
36	32.88	0.5	0.1	520	$C_{37}H_{76}$	*n*-heptatriacontane	MS
		100.0	100.0				

satd hydrocarbons = 35.8%
monounsatd hydrocarbons = 64.2%

[a] Key: A, values are obtained from total hydrocarbons chromatogram (Figure 1); B, values are calculated on the basis of the percentages (Tables II and III) obtained from saturated hydrocarbon and epoxide chromatograms (Figures 2 and 3). [b] GC, gas chromatographic data. [c] MS, mass spectral data.

drocarbon fraction components might yield helpful information to correlate the chemical composition of honey with geographical, climatic, and botanical parameters.

High-resolution gas chromatography (HRGC) offers advantages that make the technique particularly attractive for the separation of complex mixtures, and combined gas chromatography/mass spectrometry (GC/MS) analysis may help in the rapid preliminary identification of components of these mixtures.

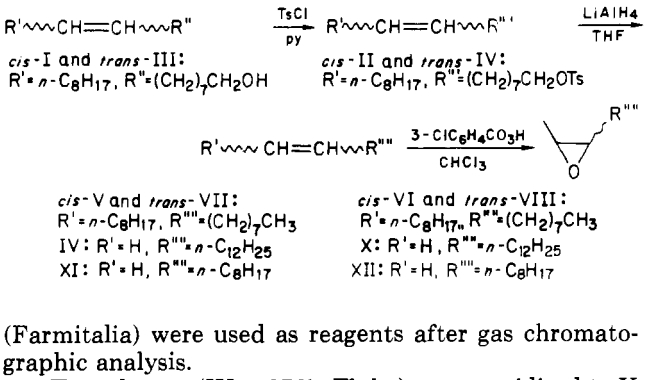
These instrumental techniques were used in this work to determine the hydrocarbon composition in honey, in conjunction with chemical transformations and other analytical techniques also in order to determine the geometrical and positional isomerism of double bond in *n*-alkenes.

MATERIALS AND METHODS

Natural Material. Chesnut honey samples were collected carefully (avoiding heating) from beehives kept from areas as free as possible of industrial contaminants and pesticides in Northeastern Italy (Friuli). The honey-depositing bees (*Apis mellifera* var. carnica-liburnica) were perfectly healthy, and the pollen analysis showed it to be better than 98% monofloral (*Castanea sativa*).

Reagents. All solvents were of analytical grade and were redistilled before use and checked by gas chromatography. 3-Chloroperbenzoic acid (85%, Fluka), periodic acid dihydrate (Fluka), and potassium permanganate

Scheme I. Preparation of Authentic Oxiranes VI, VIII, X, and XII



(Farmitalia) were used as reagents after gas chromatographic analysis.

1-Tetradecene (IX, >97%, Fluka) was epoxidized to X and XII according to a standard procedure (Swern, 1965) (see Scheme I below); *cis*- and *trans*-9-octadecen-1-ol (I and III, 85%, Aldrich Chemical Co.) were used as purchased after gas chromatographic analysis.

Hydrocarbon gas chromatographic retention times were checked against those of authentic samples, if available (see Table I for the list; suppliers were K & K Laboratories and EGA-Chemie). Kieselgel F_{254} plates (Merck) were used for thin-layer chromatography (TLC). A packed gas chromatography column was made by packing a 3.0 m × 0.6 mm i.d. glass tube with 3% SE 30 supported on silanized,

Table II. Quantitative Analysis of Saturated Hydrocarbon Fraction in Chesnut Honey (*Castanea sativa*)

peak	ret time, min	area %	mol wt	formula	compd	basis for identificn
10	16.53	0.5	268	$C_{19}H_{40}$	*n*-nonadecane	GC
11	17.88	0.2	282	$C_{20}H_{42}$	*n*-eicosane	GC
12	18.97	2.6	296	$C_{21}H_{44}$	*n*-heneicosane	GC
13	20.12	0.9	310	$C_{22}H_{46}$	*n*-docosane	GC
15	21.20	17.4	324	$C_{23}H_{48}$	*n*-tricosane	GC
16	22.25	1.2	338	$C_{24}H_{50}$	*n*-tetracosane	GC
18	23.28	17.0	352	$C_{25}H_{52}$	*n*-pentacosane	GC
19	24.27	1.2	366	$C_{26}H_{54}$	*n*-hexacosane	GC
21	25.21	23.0	380	$C_{27}H_{56}$	*n*-heptacosane	GC
22	26.12	0.8	394	$C_{28}H_{58}$	*n*-octacosane	GC
24	27.01	18.1	408	$C_{29}H_{60}$	*n*-nonacosane	GC
26	27.30	0.2	422	$C_{30}H_{62}$	*n*-triacontane	GC
28	28.63	11.8	436	$C_{31}H_{64}$	*n*-hentriacontane	GC
30	29.34	0.4	450	$C_{32}H_{66}$	*n*-dotriacontane	GC
32	30.29	2.4	464	$C_{33}H_{68}$	*n*-tritriacontane	GC
34	31.61	2.1	492	$C_{35}H_{72}$	*n*-pentatriacontane	GC
36	32.90	0.2	520	$C_{37}H_{76}$	*n*-heptatriacontane	GC
		100.0				

Table III. Quantitative Analysis of Epoxidation Products Isolated by TLC

peak	ret time, min	area %	mol wt	formula	compd	basis for identificn
1	23.90	1.2	338	$C_{23}H_{46}O$	epoxytriacosane	GC
2	25.45	2.0	366	$C_{25}H_{50}O$	epoxypentacosane	GC
3	26.86	1.6	394	$C_{27}H_{54}O$	epoxyheptacosane	GC
4	28.21	1.2	422	$C_{29}H_{58}O$	epoxynonacosane	GC
5	28.85	0.1	436	$C_{30}H_{60}O$	epoxytriacontane	GC
6	29.45	32.0	450	$C_{31}H_{62}O$	epoxyhentriacontane	GC, MS
7	30.17	0.5	464	$C_{32}H_{64}O$	epoxydotriacontane	GC
8	30.75	58.4	478	$C_{33}H_{66}O$	epoxytritriacontane	GC, MS
9	31.71	2.7	506	$C_{35}H_{70}O$	epoxypentatriacontane	GC
10	32.80	0.3	534	$C_{37}H_{74}O$	epoxyheptatriacontane	GC

Table IV. Quantitative Analysis of Carboxylic Acid Methyl Esters

peak	ret time, min	area %	mol wt	formula	compd	basis for identificn
1	4.95	1.9	158	$C_9H_{18}O_2$	octanoic acid methyl ester	GC
2	7.11	2.1	172	$C_{10}H_{20}O_2$	nonanoic acid methyl ester	GC
3	9.49	16.8	186	$C_{11}H_{22}O_2$	decanoic acid methyl ester	GC
4	10.17	0.6	200	$C_{12}H_{24}O_2$	hendecanoic acid methyl ester	GC
5	14.47	3.5	214	$C_{13}H_{26}O_2$	dodecanoic acid methyl ester	GC
6	16.97	0.3	228	$C_{14}H_{28}O_2$	tridecanoic acid methyl ester	GC
7	19.27	1.5	242	$C_{15}H_{30}O_2$	tetradecanoic acid methyl ester	GC
8	21.29	0.6	256	$C_{16}H_{32}O_2$	pentadecanoic acid methyl ester	GC
9	23.71	2.8	270	$C_{17}H_{34}O_2$	hexadecanoic acid methyl ester	GC
10	25.74	0.5	284	$C_{18}H_{36}O_2$	heptadecanoic acid methyl ester	GC
11	27.78	2.5	298	$C_{19}H_{38}O_2$	octadecanoic acid methyl ester	GC
12	29.73	1.2	312	$C_{20}H_{40}O_2$	nonadecanoic acid methyl ester	GC
13	31.54	1.3	326	$C_{21}H_{42}O_2$	eicosenoic acid methyl ester	GC
14	33.36	15.1	340	$C_{22}H_{44}O_2$	heneicosenoic acid methyl ester	GC
15	35.06	2.1	354	$C_{23}H_{46}O_2$	docosanoic acid methyl ester	GC
16	36.88	43.1	368	$C_{24}H_{48}O_2$	tricosanoic acid methyl ester	GC
17	38.32	0.6	382	$C_{25}H_{50}O_2$	tetracosanoic acid methyl ester	GC
18	39.92	3.0	396	$C_{26}H_{52}O_2$	pentacosanoic acid methyl ester	GC
19	41.68	0.3	410	$C_{27}H_{54}O_2$	hexacosanoic acid methyl ester	GC
20	42.59	0.1	424	$C_{28}H_{56}O_2$	heptacosanoic acid methyl ester	GC
21	44.03	0.1	438	$C_{29}H_{58}O_2$	octacosanoic acid methyl ester	GC
		100.0				

acid-washed Gas Chrom P, 100/120 mesh (Supelco, Inc.).

Apparatus. All GC analysis of hydrocarbons were performed with a Carlo Erba Model 4160 gas chromatograph equipped with flame ionization detector and using a WCOT 10 m × 0.3 mm i.d. glass capillary column coated with SE 52 (film thickness 0.15 μm). Helium was used as carrier gas at flow rate of 2.2 mL/min. Column temperature was programmed from 30 to 360 °C at 10 °C/min; injector and detector temperatures were kept at 350 °C. The injection mode was on-column. A Spectra-Physics Model 4100 printer/plotter integrator was used to determine the peak areas reported in Tables I–III. Gas chromatographic analysis of carboxylic acid methyl esters was performed with a Varian Model 3700 gas chromatograph equipped with flame ionization detector and using a homemade packed column (see Materials). Nitrogen was used as carrier gas at flow rate of 35 mL/min. Column temperature was programmed from 55 to 250 °C at 5 °C/min. The injector and detector temperature was kept at 280 °C. A Varian Model CDS 111 printer/plotter integrator was used to determine the peak areas reported in Table IV. Positive-ion, electron impact (EI) mass spectra were recorded with an LKB Model 9000 gas chromatograph/mass spectrometer, operating at 70 eV, by introducing the samples via gas chromatographic or the direct inlet.

Procedures. *Extraction and Separation of Honey Hydrocarbons.* Anhydrous sodium sulfate (4.0 g) was added to 40 g of honey, and the mixture was extracted three times with *n*-hexane (50 mL) in a Vibromixer op-

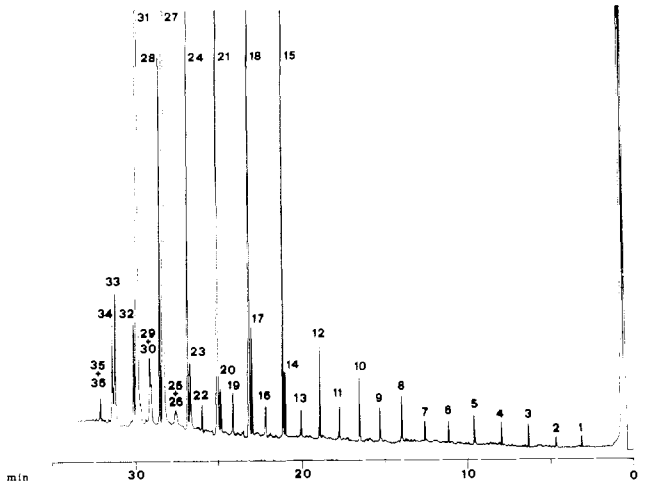


Figure 1. Chromatogram of total hydrocarbons in chesnut honey, on a 10 m × 0.3 mm WCOT glass capillary column coated with SE 52. The column temperature was programmed from 30 to 360 °C at 10 °C/min. The injector and detector temperature was 350 °C. For peak identification, see Table I.

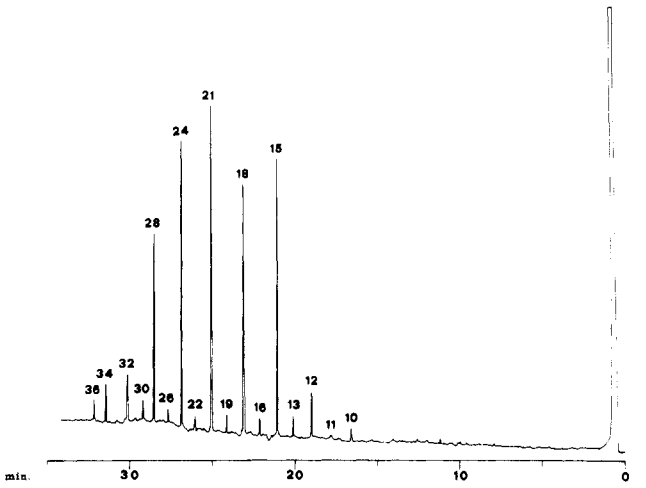


Figure 2. Chromatogram of saturated hydrocarbons, at the same conditions of Figure 1. For peak identification, see Table II.

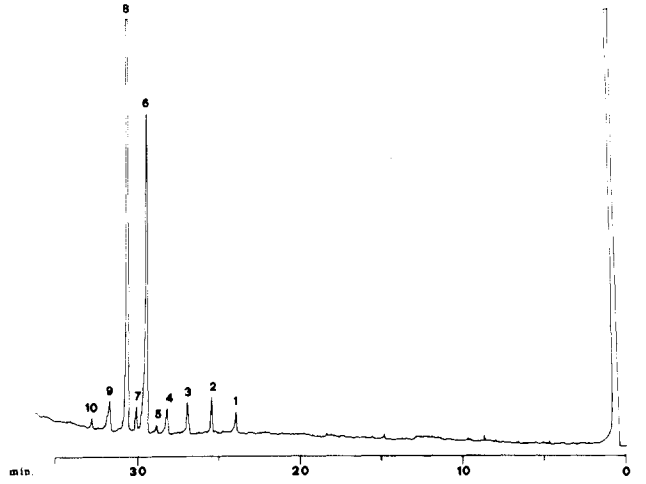


Figure 3. Chromatogram of the epoxides, at the same conditions of Figure 1. For peak identification, see Table III.

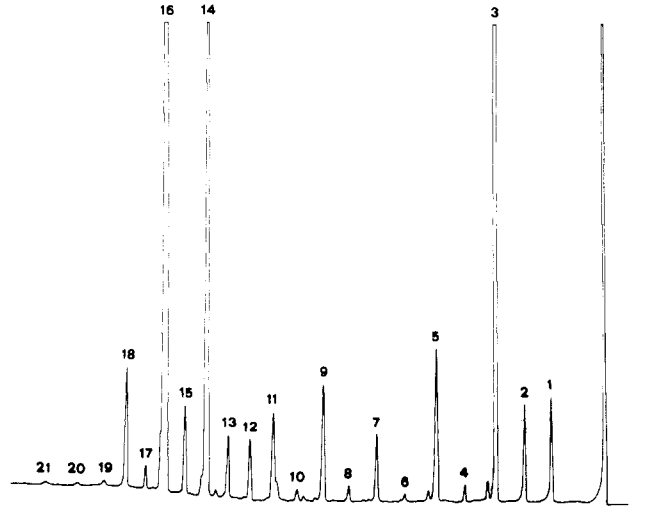


Figure 4. Chromatogram of the carboxylic acid methyl esters, on a 3 m × 0.6 mm column packed with SE 30. The column temperature was programmed from 55 to 250 °C at 5 °C/min. The injector and detector temperature was 260 °C. For peak identification, see Table IV.

erating at 100 rpm, for 5 min. The combined extracts were dried over anhydrous sodium sulfate, and the solvent was evaporated in vacuo (15 torr) on a water bath (maximum temperature 60 °C) by means of a rotary evaporator. About 10 mg of residue was usually obtained. The hydrocarbons were separated from other components by preparative TLC by elution with dry *n*-hexane/ethyl ether (95/5, v/v) at room temperature and reaching as high as 16 cm with the solvent front. Band location was evidenced by spraying the layer with an ethanol solution of 2,7′-dichlorofluorescein sodium salt (0.2%) and ultraviolet detection. The hydrocarbon band (R_f 0.91–0.98) was scraped off, extracted three times with dry ethyl ether, dried over anhydrous sodium sulfate, concentrated in vacuo (15 torr), and then analyzed by GC and GC/MS. The gas chromatographic profile of total hydrocarbons is reported in Figure 1; Table I give the identification and relative percentage of single peaks.

Epoxidation of Olefinic Components. Total hydrocarbons (60 mg) were dissolved in dry, methanol-free, freshly distilled chloroform (4.0 mL), and the resultant mixture was added to a solution of 3-chloroperbenzoic acid (1.5 mmol) in the same solvent (8.0 mL). The reaction was carried out for 12 h at 52 °C; then, to the mixture was added ethyl ether (10 mL) and an aqueous solution of sodium sulfite (5%) and sodium hydrogen carbonate (5%) to neutrality. The mixture was then washed with distilled water, dried, and concentrated in vacuo (15 torr). Unreacted saturated hydrocarbons were separated from the epoxides by TLC as described above and analyzed by GC. The GC profiles of saturated hydrocarbons and epoxides are reported in Figures 2 and 3. Tables II and III give the identification and the relative percentage of single peaks.

Cleavage of Epoxides and Synthesis of Their Derivatives. Epoxides (40 mg) were dissolved in 1.0 mL of ethyl ether distilled from lithium aluminum hydride and added to 38.4 mg (0.17 mmol) of periodic acid dihydrate in ethyl ether (10 mL). The resulting solution was stirred at room temperature for 2 h, and then 5.0 mL of an aqueous solution of 0.01 mol m^{-3} sulfuric acid and 50 mL (in small quantities) of an aqueous solution of 0.002 mol m^{-3} of potassium permanganate were added. The resulting carboxylic acids were extracted, dried, and concentrated; their purification was performed by TLC, eluting them with *n*-hexane/ethyl ether (90/10, v/v). The carboxylic acid band (R_f 0.43) was scraped off, extracted, and dried, and the solvent was distilled in vacuo (15 torr). The residue was derivatized with an ether solution of diazomethane at room temperature. The carboxylic acid methyl esters were analyzed by GC. The GC profile is reported in Figure 4.

Scheme II. Procedure Employed in the Present Work

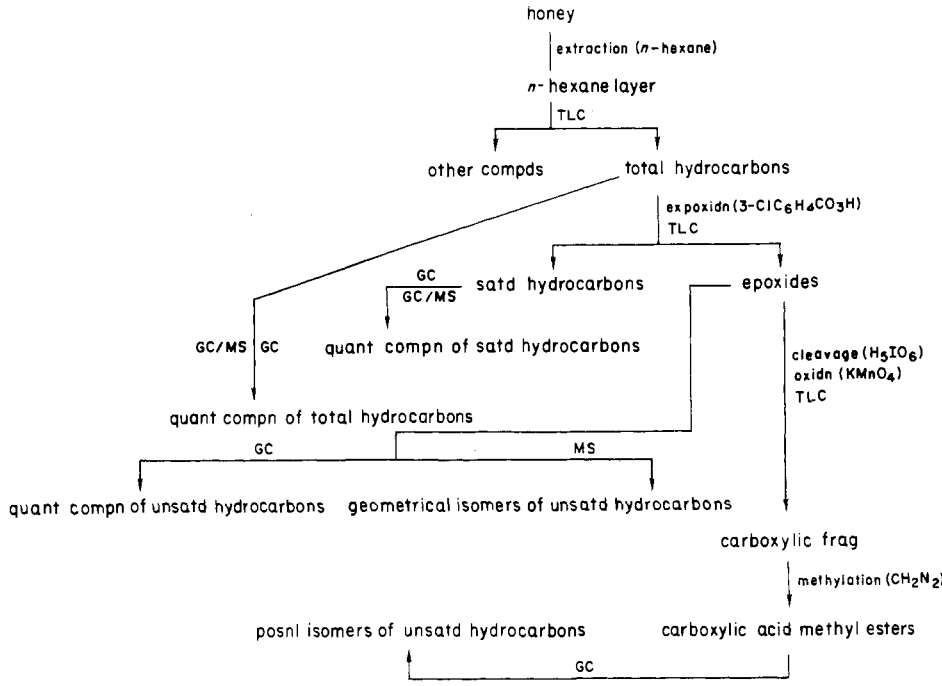


Table IV give the identification and the relative percentage of single peaks.

Syntheses. 9-Octadecenes V and VII were prepared from the corresponding 9-octadecen-1-ols I and III via tosylated II and IV according to a described procedure (Dyen et al., 1966), and their epoxides VI and VIII were obtained by reaction with 3-chloroperbenzoic acid (Scheme I).

The EI mass spectra of the olefins V and VII at 20 and 70 eV are practically identical, even in the fine details of the metastable peaks: (20 eV) *m/e* 252 (100), 253 (20); (70 eV) *m/e* 57 (100), 83 (98), 70 (88), 97 (79), 55 (71), 56 (69), 71 (66), 43 (65), 72 (44), 109 (41). Their IR spectra differ mainly for a sharp peak (medium) present only in the trans isomer at 970 cm^{-1} due to a bending mode of the alkene hydrogen atoms. Interestingly, the IR spectra of the precursor alcohols I and III and tosylates II and IV are identical.

RESULTS AND DISCUSSION

The analytical procedure for the identification of honey hydrocarbons is reported in Scheme II.

The results of quantitative analysis of total hydrocarbons in chesnut honey are reported in Table I. Peak number (column 1 on the left side) shows the elution order on the 10-m SE 52 column (Figure 1). Retention time (column 2), area percent (column 3), molecular weight (column 4), empirical formula (column 5), systematic name (column 6), and the basis for identification (column 7) are also reported for every listed compound.

Epoxidation of the TLC-separated total hydrocarbon fraction followed by TLC separation gave a mixture of epoxides of the olefinic material as a mixture of saturated hydrocarbons; the corresponding GC traces are reported in Figures 2 and 3; the relative peak areas are given in Tables II and III.

The identification of single hydrocarbons was performed by GC/MS analysis and also by comparison of the retention time of single components with those of authentic samples. The saturated hydrocarbons (35.8 area %) are linear, at even and odd numbers of carbon atoms, from C_{10} to C_{37}, but *n*-C_{23}, *n*-C_{25}, *n*-C_{27}, *n*-C_{29}, and *n*-C_{31} are the major *n*-alkanes (ca. 90% of total *n*-paraffins). The monounsaturated hydrocarbons (64.2 area %) are *n*-C_{23}, *n*-C_{25}, *n*-C_{27}, *n*-C_{29}, *n*-C_{31}, *n*-C_{33}, *n*-C_{35}, and *n*-C_{37}, but the *n*-C_{31} and *n*-C_{33} are by far the major components (91% of total *n*-olefins). The epoxides were cleaved with periodic acid dihydrate (H_5IO_6) in ether solution; the resulting aldehydic fragments are oxidized with potassium permanganate in acid solution to the corresponding carboxylic acids. The acids thus obtained were purified by preparative TLC, then derivatized with diazomethane, and last analyzed by GC using a packed column. Figure 4 shows the gas chromatogram and Table IV reports the quantitative analysis of carboxylic acid methyl esters.

In this work we have identified the major components of the hydrocarbon fraction of a honey of a reasonably well-identified floral origin, collected with due care, well preserved and elaborated by *Apis mellifera* var. carnica-liburnica.

To our knowledge, this is the first report of hydrocarbons in honey, although scattered works have found specific hydrocarbons [Hoopen, 1963; Callow, 1963; and Graddon et al., 1979]. The GC profiles (Figures 1 and 2) are dominated by high molecular weight odd *n*-alkanes and *n*-alkanes in the range C_{23}–C_{33}, with minor contributions from the ranges at either ends. Although beewax is usually carefully separated from honey, it is unavoidable that it may be contaminated by some amounts of this material, which is what its container is made of. It was of interest, therefore, to observe whether the hydrocarbon content of honey reproduced to a great extent beewax composition. On the other hand, in particular cases, wax may be transported from bees from some parts of visited plants. Although even as recently as in the work by Letcoer (1984), a typical analysis of beewax is reported with a hydrocarbon fraction containing as much as 18% of melene, apparently an *n*-alk-1-ene ($C_{28}H_{57}CH{=}CH_2$), already cast into doubt by Downing et al. (1961). The fundamental work by Streibl et al. (1966) established a hydrocarbon pattern for beewax very similar to what we now have found for hydrocarbons in honey. The latter authors also found that the unsaturated fraction was mainly cis in character by infrared spectroscopy. We reach the same conclusions on the basis of a feature of the mass spectra of the epoxides that seems very sensitive to this geometrical isomerism.

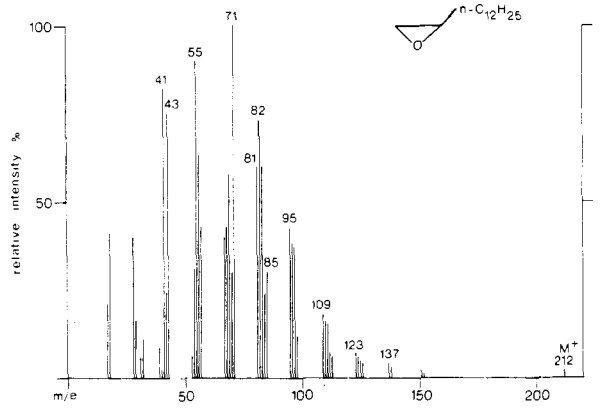


Figure 5. Mass spectrum of 1,2-epoxytetradecane (70 eV).

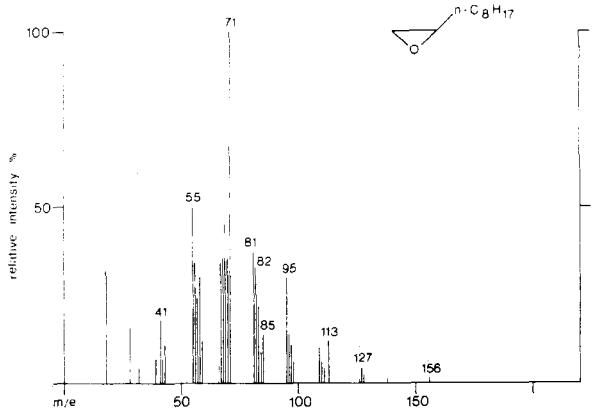


Figure 6. Mass spectrum of 1,2-epoxydecane (70 eV).

It is, of course, well-known that the epoxides are formed with retention of the stereochemical configuration of the original olefin (March, 1977). Alkene mass spectra do not yield patterns characteristic of the molecular structure especially for long-chain compounds because of fast rearrangement reaction proceeding on the parent ion before breakdown (Budzikiewicz et al., 1967). We wanted to see whether mass spectra of epoxides derived from long-chain unsaturated hydrocarbons of different geometry could possibly be diagnostic for locating the position of the unsaturation and identifying the geometry of the oxirane ring. To this end we prepared authentic samples from the geometric isomers of an internal olefin, namely *cis*-9,10-epoxyoctadecane (VI) and *trans*-9,10-epoxyoctadecane (VIII), and two terminal alkenes, namely 1,2-epoxytetradecane (X) and 1,2-epoxydecane (XII) (Scheme I).

As already observed (Budzikiewicz et al., 1967), EI mass spectra of terminal epoxides X and XII are hardly of any diagnostic usefulness, exhibiting very weak parent ions and undiscriminating features with clusters of intense ions below m/e 100 (Figures 5 and 6).

On the other hand, the internal oxiranes VI and VIII yielded EI mass spectra at 20 eV (Figures 7 and 8) observable molecular (M^+) and quasi-molecular ($M + 1^+$) peaks, a peak for water loss from the parent ions, an intense peak for a substituent loss ($M^+ - C_8H_{17}$), and a rearrangement peak ($M^+ - C_9H_{19}$) that can be easily correlated to the original structure. In addition, the peak for the chain (C_8H_{17}) loss was prominent for both isomers and peaks for β and γ cleavage were also observed at 70 eV. Interestingly, the cross section for self-protonation of these ions appears to be high, because the quasi-molecular ions were twice as intense as the parent ions and seemed to generate their own spectral pattern (Figures 9 and 10).

The spectra of geometric isomers VI and VIII are distinctly different at both 20 and 70 eV. Decay of the mo-

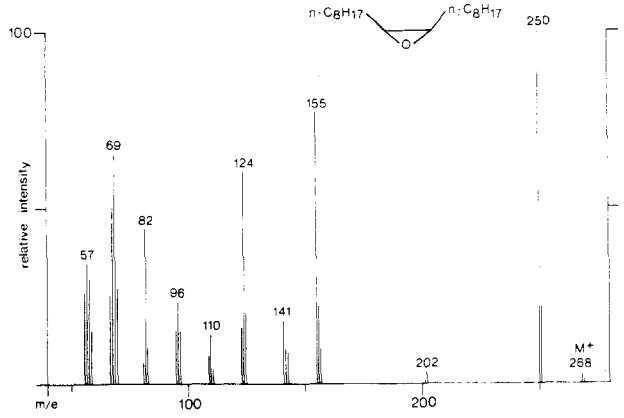


Figure 7. Mass spectrum of *cis*-9,10-epoxyoctadecane (20 eV).

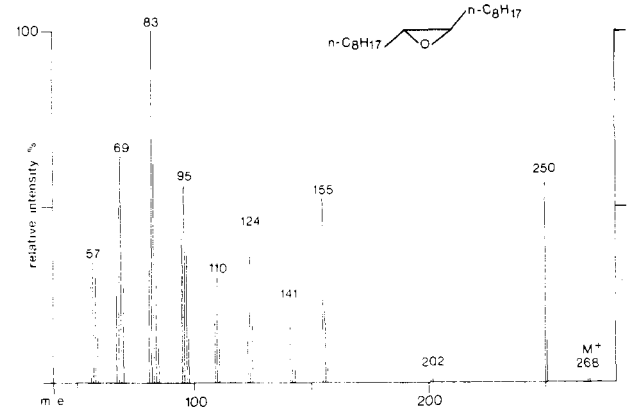


Figure 8. Mass spectrum of *trans*-9,10-epoxyoctadecane (20 eV).

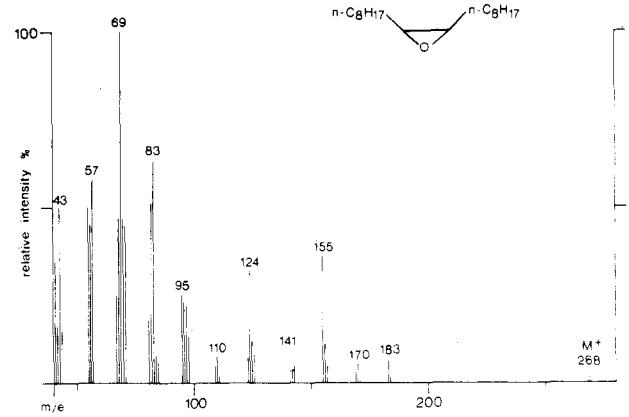


Figure 9. Mass spectrum of *cis*-9,10-epoxyoctadecane (70 eV).

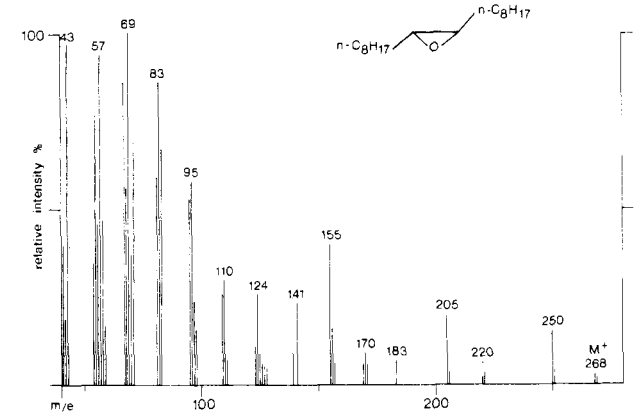


Figure 10. Mass spectrum of *trans*-9,10-epoxyoctadecane (70 eV).

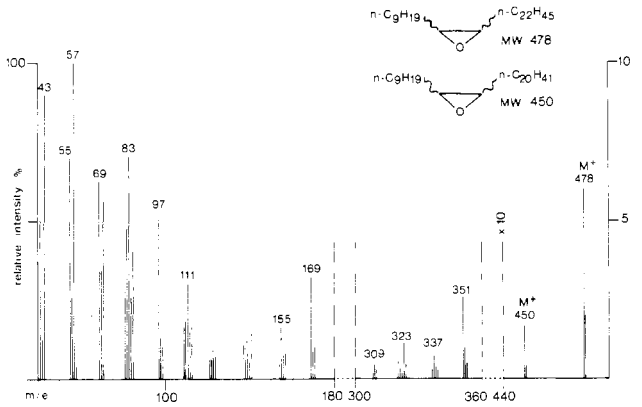


Figure 11. Mass spectrum of mixture of the epoxides from unsaturated hydrocarbons (70 eV).

lecular structure of the trans isomer is more pronounced than of the cis isomer at 20 eV. But, one interesting feature is the ratio of peak intensities for the analogue fragmentation on side chain ($M^+ - C_8H_{17}$) and rearrangement–cleavage ($M^+ - C_9H_{19}$) that came out to be, respectively, 4.10 for the cis isomer and 1.75 for the trans isomer. An attractive rationale for this observation may be a steric compression for the cis compound to precede the usually more favored γ cleavage. This pattern was observed in the epoxides (compounds **6** and **8**, Table III) of the C_{31} and C_{33} alkenes derived from honey. The unsaturation being originally between C_{10} and C_{11}, they are unsymmetrical oxiranes and therefore showed different fragmentations. The C_{31} oxirane lost either the larger substituent ($C_{20}H_{41}$) or the smaller (C_9H_{19}) accompanied by the corresponding rearrangement–cleavage peaks (losses of $C_{21}H_{43}$ and $C_{10}H_{21}$). The C_{33} oxirane yielded identical ions (m/e 169 and 155) when the largest substituent was involved in the fragmentation, but ions at different masses (m/e 351 and 337) when the smaller substituent was involved. All these structural breakdowns of the parent ions were observed in the actual spectra (Figure 11).

The internal consistency of the following ratio was remarkable:

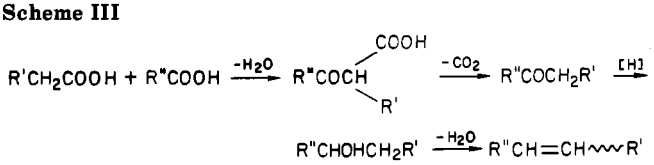
large substituent involved: $i_{169}/i_{155} = 3.00$

smaller substituent involved: $i_{351}/i_{337} = 3.90$

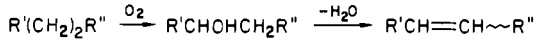
$i_{323}/i_{309} = 3.85$

These values are in fact quite close among themselves and compare well, just slightly on lower side, for those for the cis isomer of 9,10-epoxyoctadecane (VI). On this basis, we assume that the original olefins C_{31} and C_{33} should be prevalently in the cis configuration. The capillary GC analysis of the epoxide mixtures was the definitive evidence of the apparent simplicity of the mixture as it could be inferred from the direct-inlet EI mass spectral analysis of the whole mixture after TLC separation. In fact, further chemical elaboration (oxidative cleavage with $KMnO_4$ and methyl esterification) of the epoxide mixture yielded a gas chromatogram (Figure 4) showing methyl esters of carboxylic acids between octanoic through octacosanoic acid without interruption. Since the limit hydrocarbon was a C_{37} mixture (0.3–1.0%), but practically a C_{35} mixture (1.5%), we can safely conclude that at least 50% of total unsaturation is due to $C_{31}H_{62}$ and $C_{33}H_{66}$ (both Δ^{10}). The remnant unsaturation is scattered (mostly in C_{33} and C_{31} monounsaturated hydrocarbons) rather evenly between Δ^8 and Δ^{16}. Since the dodecanoic acid methyl ester was the third most intense gas chromatographic peak and the pentacosanoic acid methyl ester the fourth most intense peak, we might well add that the Δ^{12} and Δ^{15} unsaturation

Scheme III



Scheme IV



may be strongly represented in C_{31} and C_{33} olefins, although the hydrocarbon GC profile never brought this fact to light. Since alkene isomerization postfactum introduced by artifacts may be safely ruled out, one must conclude that olefin biosynthesis in bee is a flexible tool, able either to use a number of substrates or to activate different positions. Alkene biosynthesis, in fact, may be assumed to follow either a condensation–decarboxylation–reduction–elimination mechanism (Kolattukudi et al., 1976) (Scheme III) or a distinct oxidation–elimination mechanism, where atmospheric oxygen is brought to react with an alkane methylene group enzymatically (Blomquist et al., 1975). The uninterrupted continuity of double-bond position between C_8 and C_{16}, although peaking sharply at C_{10}, would then require mixed biosynthesis from very uncommon and unlikely precursor acids (Callow et al., 1964) in most instances according to the former mechanism; in fact, decanoic, docosanoic, and tetracosanoic acids, necessary to build up the most abundant alkenes observed, are themselves uncommon at all.

Most probably, oxidation of alkanes produced *sec*-alcohols with random distribution from (higher) alkenes, which might not be esterified as quickly (Blomquist et al., 1973) as lower molecular weight *sec*-alcohols and, therefore, be amenable to enzymatic dehydration to olefins (Scheme IV).

Somewhere in the biochemical sequence some regioselectivity becomes enacted to produce a large amount of C_{10} unsaturation. We feel that our present findings are of a preliminary nature for two reasons: there are obvious procedural and instrumental improvements to the basic ones outlined here, and a better definition of results is possible. On the other hand, the analytical setup may be very suitable for the hydrocarbon analysis in waxes of both plant and animal origin, where the matrix complexity might have generated rough approximation and misrepresentation in the past, owing to inadequate separation–identification techniques.

A better picture may yield surprising biochemical hints and, perhaps, few handles to cope with some foods and wax adulteration, as well as technical insights in the field of pesticide action.

ACKNOWLEDGMENT

This work was supported by a grant from the Consiglio Nazionale delle Ricerche of Italy (CT. 83.01949.03) and a financial contribution from the Istituto Nazionale di Apicoltura of Bologna. We thank Dr. Anna Sabatini for pollen analysis and useful assistance.

Registry No. *n*-Decane, 124-18-5; *n*-hendecane, 1120-21-4; *n*-dodecane, 112-40-3; *n*-tridecane, 629-50-5; *n*-tetradecane, 629-59-4; *n*-pentadecane, 629-62-9; *n*-hexadecane, 544-76-3; *n*-heptadecane, 629-78-7; *n*-octadecane, 593-45-3; *n*-nonadecane, 629-92-5; *n*-eicosane, 112-95-8; *n*-heneicosane, 629-94-7; *n*-docosane, 629-97-0; *n*-tricosene, 56924-46-0; *n*-tricosane, 638-67-5; *n*-tetracosane, 646-31-1; *n*-pentacosene, 30551-31-6; *n*-pentacosane, 629-99-2; *n*-hexacosane, 630-01-3; *n*-heptacosene, 67537-80-8; *n*-heptacosane, 593-49-7; *n*-octacosane, 630-02-4; *n*-nonacosene, 77046-61-8; *n*-nonacosane, 630-03-5; *n*-triacontene, 36731-14-3;

n-triacontane, 638-68-6; n-hentriacontene, 77046-64-1; n-hentriacontane, 630-04-6; n-dotriacontene, 85792-05-8; n-dotriacontane, 544-85-4; n-tritriacontene, 85792-06-9; n-tritriacontane, 630-05-7; n-pentatriacontene, 69198-75-0; n-pentatriacontane, 630-07-9; n-heptatriacontene, 77046-53-8; n-heptatriacontane, 7194-84-5.

LITERATURE CITED

Blomquist, G. J.; Jackson, L. L. *Biohem. Biophys. Res. Commun.* **1973**, *53*, 703.
Blomquist, G. J.; McCain, D. C.; Jackson, L. L. *Lipids* **1975**, *10*, 303.
Budzikiewicz, H.; Djerassi, C.; Williams, D. H. "Mass Spectrometry of Organic Compounds"; Holden-Day, Inc.: San Francisco, 1967.
Callow, R. K. *Bee World* **1963**, *44*, 95.
Callow, R. K.; Chapman, J. R.; Paton, P. N. *J. Apic. Res.* **1964**, *3*, 77.
Downing, D. T.; Kranz, Z. H.; Lamberton, J. A.; Murray, K. E.; Redcliffe, A. H. *Aust. J. Chem.* **1961**, *14*, 253.
Dyen, M. E.; Hamman, H. C.; Swern, D. *J. Am. Oil Chem. Soc.* **1966**, *43*, 431.
Graddon, A. D.; Morrison, J. D.; Smith, J. F. *J. Agric. Food Chem.* **1979**, *27*, 431.
Hoopen, H. J. G. *Z. Lebensm. Forsch.* **1963**, *119*, 478.
Kolattukudi, P. E.; Croteau, R.; Buckner, J. S. "Chemistry and Biochemistry of Natural Waxes"; Kolattukudi, P. E., Ed.; Elsevier, Amsterdam, 1976; Chapter 8, p 294.
Letcoer, S. C. "Kirk-Othmer Encyclopedia of Chemical Technology", 3th ed.; Wiley: New York, 1984; Vol. 24, p 466.
Lercker, G.; Capella, P.; Conte, L. S.; Ruini, F. *Lipids* **1981**, *16*, 912.
March, J. "Advances in Organic Chemistry"; McGraw-Hill: New York, 1977.
Streibl, M.; Strânkŷ, K.; Šorm, F. *Fette Seifen Anstrich* **1966**, *68*, 799.
Swern, D. "Organic Reactions"; Wiley: New York, 1965; Vol. VII, p 378.
Tulloch, A. P. *Bee World* **1980**, *61*, 47.

Received for review September 13, 1985. Accepted December 18, 1985.

Nonstarchy Polysaccharides of *Phaseolus vulgaris*, *Lens esculenta*, and *Cicer arietinum* Seeds

Martine Champ,[1] Jean-Marc Brillouet,* and Xavier Rouau

Nonstarchy polysaccharides of three legume seeds (kidney bean, lentil, chickpea) have been isolated and analyzed. Trichloroacetic acid soluble materials represent respectively 7.1, 0.8, and 2.1% of kidney bean, lentil, and chickpea whole dry seeds. Arabinose is the major sugar of the three extracts. Their arabinose:galactose ratios are respectively 1:0.35, 1:0.77, and 1:0.57. Cotyledon cell walls were defatted and then treated with pronase and α-amylase. Dry matter ranged from 7.5% in lentil to 13.7% in chickpea. Cell walls from kidney bean, lentil, and chickpea contained respectively 67, 73, and 42% pectic polysaccharides associated with 16, 12, and 10% cellulose. Arabinose was the major pectic sugar of the three walls. Hulls were mainly composed of cellulose (29–41%) associated with hemicellulosic and pectic polymers. They had low lignin contents (1.2–1.7%). Kidney bean hulls contained the greatest percentage of the (xylose + glucose) pair whereas chickpea exhibited the greatest amount of pectic polysaccharides.

INTRODUCTION

Legume seeds have been extensively studied by biochemists and nutritionists mainly for their proteins that constitute more than 30% of the seed and are better balanced in amino acids than most other plant proteins. Moreover, several studies suggest that they seem to decrease postprandial hyperglycemia (Jenkins et al., 1980a,b) and cholesterolemia (Mathur et al., 1968) and increase transit rate (Saraswathi and Shurpalekar, 1983) in humans.

However diarrhea has been observed in chicken fed raw and cooked kidney beans whereas chickpea and lentil did not induce such effects (Mansouri et al., 1983). Nonstarchy polysaccharides may be implicated in this disorder; therefore, the present study was undertaken to provide detailed information on these compounds.

MATERIALS AND METHODS

Plant Material. Seeds of kidney bean (*Phaseolus vulgaris*, cv. Michelet), chickpea (*Cicer arietinum*, cv. Aïn Temouchent), and lentil (*Lens esculenta*, cv. Large Blonde du Chili) were provided by the National Agronomic Institute of El Harrach (Algeria). Seeds were hand dehulled and freed from germ. Cotyledons and hulls were ground in a cooled (0 °C) mill (Ika M20) respectively for 3 and 4 min to pass a 0.5-mm sieve.

Chemicals. α-Amylase from *Bacillus subtilis* (EC 3.2.1.1, type II-A 1000 U/mg) and pronase from *Streptomyces griseus* (6 U/mg) were respectively obtained from Sigma and Boehringer (Mannheim, Germany). All reagents were of analytical grade.

Preparation of Soluble Nonstarchy Polysaccharides. Whole seeds were grossly ground in a cooled (0 °C) IKA M20 mill (3 min) and then in a cooled ball grinder (Dangoumeau) to pass through a 0.5-mm sieve. A 100-g portion of flour was treated with 500 mL of 10% (w/v) trichloroacetic acid (TCA) for 16 h at 4 °C under continuous stirring and then centrifuged for 30 min at 10000*g* (0 °C). Extraction of soluble polysaccharides was performed with cold 10% trichloroacetic acid to prevent extensive solubilization of cytoplasmic proteins (Susheelamma and Rao, 1978). Cooling prevents any acid hydrolysis of labile α-L-arabinofuranosidic linkages in the polysaccharides. The insoluble residue was retreated twice with TCA as described above. The supernatant liquids

Laboratoire de Biochimie et Technologie des Glucides, Institut National de la Recherche Agronomique, 44072 Nantes Cedex, France.
[1] Present address: Laboratoire de Technologie des Aliments des Animaux, 44072 Nantes Cedex, France.

0021-8561/86/1434-0326$01.50/0