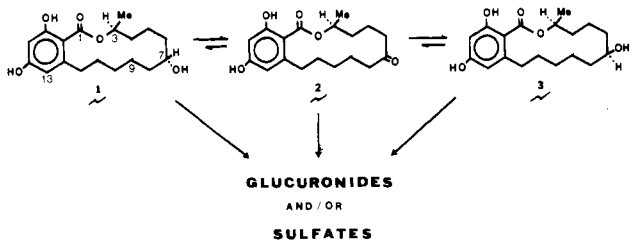

Figure 5. Structures and a possible biotransformation of zeranol (1).

spectively. The ratio of zeranol (1), zearalanone (2), and taleranol (3) has been reported (Migdalof et al., 1983) to be 30.1, 26.8, and 14.8% of the total urinary radioactivity following oral administration of [^{3}H]zeranol to a rabbit. It is conceivable that zeranol (1) is oxidized to zearalanone (2), in vivo, and then reduced to both zeranol (1) and taleranol (3) as proposed previously (Figure 5).

The analysis of urinary extracts by HPLC or TLC alone could be misleading unless the presence of the assumed metabolites is confirmed by spectral methods. A peak corresponding to taleranol in a fraction separated from urine sample 1 (which was collected from a bull treated with zeranol intraperitoneally) was detected by HPLC, but the identity could not be established by GC–MS. In addition, a fluorescent fraction separated from another extract by repeated preparative TLC did not contain either zeranol or taleranol by GC–MS.

Registry No. 1, 26538-44-3; 2, 5975-78-0; 3, 42422-68-4.

LITERATURE CITED

Bennet, G.; Beaumont, W. H.; Brown, P. M. R. *Vet. Rec.* **1974**, *94*, 235.
Cole, N. A.; Hutchson, D. P.; McLaren, J. B.; Phillips, W. A. *J. Anim. Sci.* **1984**, *58*, 527.
Ingerowski, G. H.; Hohls, F. W.; Stan, H.-J. *J. Dtsch. Lebensmittel-Rdsch.* **1976**, *72*, 126.
Ingerowski, G. H.; Stan, H.-J. *J. Environ. Pathol. Toxicol.* **1979**, *2*, 1173.
Migdalof, B. H.; Dugger, H. A.; Heider, J. G.; Coombs, R. A.; Terry, M. K. *Xenobiotica* **1983**, *13*, 209.
Sharp, G. D.; Dyer, I. A. *J. Anim. Sci.* **1972**, *34*, 176.

Received for review August 5, 1985. Accepted November 11, 1985. This was supported, in part, by the International Minerals and Chemical Corporation, Terre Haute, IN, and the Texas Agricultural Experiment Station, Texas A&M University System, Project No. H-6255.

Identification of Arsenobetaine and Arsenocholine in Canadian Fish and Shellfish by High-Performance Liquid Chromatography with Atomic Absorption Detection and Confirmation by Fast Atom Bombardment Mass Spectrometry

James F. Lawrence,* Paula Michalik,[1] Gerard Tam,[2] and Henry B. S. Conacher

The major organic forms of arsenic in fresh marine fish such as haddock, halibut, cod, herring, mackeral, sole, lobster, scallops, and shrimp obtained throughout Canada were identified as arsenobetaine and, in shrimp only, arsenocholine. Freshwater fish including pike, bass, carp, pickeral, whitefish, yellow perch, and striped perch contained no arsenobetaine or arsenocholine but did contain methanol-extractable arsenic, which has not yet been identified. Salmon obtained from British Columbia contained arsenobetaine and an unknown arsenic compound that eluted later from the reversed-phase HPLC system employed. The arsenobetaine levels for marine fish ranged from 0.15 to 15.8 μg/g of fresh weight. The sample extraction included freeze drying the tissue and then Soxhlet extracting with chloroform (which was discarded) and then with methanol. The methanolic extract containing the organoarsenic compounds was purified by alumina and ion-exchange chromatography then subjected to reversed-phase high-performance liquid chromatography with off-line graphite furnace atomic absorption detection. The compounds were confirmed by mass spectrometry using fast atom bombardment. Arsenobetaine was also confirmed by derivatization to the ethyl ester with further characterization by HPLC and mass spectrometry.

INTRODUCTION

Arsenic is of concern as an environmental pollutant because it is known to give rise to adverse health effects involving respiratory, gastrointestinal, cardiovascular, and nervous systems. Effects of arsenic exposure range from reversible to cancer and acute death (Weinstein, 1978).

The World Health Organization has recently lowered the maximum tolerable daily intake of arsenic from 50 μg/kg of body weight to 2 μg/kg of body weight with the recommendation that more work be done to elucidate the nature of arsenical compounds that occur in food and in particular in seafood, where arsenic levels are generally high (Food Chemical News, 1984). In Canadian fish, levels of total arsenic have been observed in the range of 0.1–90 mg/kg and vary with species and location (Kennedy, 1976; Zook et al., 1976).

The concentrations of arsenic found in fish and shellfish are higher than in the surrounding water. Unpolluted fresh water generally contains up to 1 μg/kg of arsenic, while

Food Research Division, Food Directorate, Health Protection Branch, Ottawa, Ontario, Canada K1A 0L2.
[1] Present address: University College of Cape Breton, Sydney, Nova Scotia B1P 6L2, Canada.
[2] Deceased.

0021-8561/86/1434-0315$01.50/0

seawater contains from 1 to 8 μg/kg. Since arsenic is removed from seawater by sedimentation, it is present in sediments at higher levels, in the order of 2–20 mg/kg (Penrose, 1974). Levels of arsenic in pelagic fish are in the order of 0.3–3 mg/kg while bottom feeders and shellfish generally contain from 1 to 55 mg/kg of arsenic (Penrose et al., 1977).

The toxicity of arsenic depends on the chemical form in which it exists. Toxicities of various arsenic compounds in decreasing order are as follows: arsines > arsenite (inorganic) > arsenoxides > arsenate (inorganic pentavalent arsenicals (Penrose, 1974). Fish and shellfish are known to accumulate both inorganic and organic arsenic compounds (Lunde, 1973; Lunde, 1975; Penrose, 1975). Previous workers have shown that the arsenic compounds that exist in fish are mainly quaternary arsonium compounds. There has been some dispute as to the exact nature of these compounds and it is not unlikely that the compounds vary from place to place and between species. However, it appears that the major arsenicals in fish are arsenocholine and arsenobetaine (Edmonds, et al., 1977; Kurosawa et al., 1980; Norin et al., 1983; Cannon et al., 1981, 1983), although it has been shown that compounds other than these are present in certain marine and fresh water species (Penrose, 1975; Lunde, 1977). Numerous methods for the determination of inorganic and organic arsenic in fish tissue have been developed. Ideally, the experimental procedure should be simple, sensitive and selective. Several workers have shown that atomic absorption (AA) spectrometry is a useful detector for organometallic compounds after separation by high-performance liquid chromatography (HPLC) (Brinckman et al., 1977; Koizuma et al., 1978). An on-line HPLC/AA system has been developed for the separation of organic and inorganic arsenic compounds (Stockton and Irgolic, 1979). The present paper describes results of our research to chemically identify the organoarsenical compounds present in fish and shellfish commonly eaten in Canada. The method employs HPLC with atomic absorption detection for speciation while mass spectrometry and chemical derivatization are used for confirmation. As far as we are aware this is the first conclusive evidence for the presence of arsenobetaine and arsenocholine in Canadian and perhaps North American coastal fish.

MATERIALS AND METHODS

Samples of various species of fish in quantities sufficient to yield about 1 kg of edible muscle were obtained from various regions of Canada. Fish were shipped fresh frozen on dry ice, either whole or filleted. On arrival, unfilleted finfish were filleted and skinned. Scallops were obtained shucked, and shrimps had been peeled and deveined prior to shipment. Lobsters were shipped whole, and the edible muscle was removed on arrival.

The fish muscle tissue was homogenized in a Waring blender, freeze-dried, and blended to a fine homogeneous powder. About 50 g of sample was placed in a Soxhlet apparatus and extracted for 24 h with 500 mL of ethanol-free, dry chloroform, which was discarded. The samples were then extracted for 24 h with 500 mL of methanol, which removed the compounds of interest. The methanol was rotary flash evaporated at 30 °C to about 2 mL and the residue taken up in 100 mL of chloroform. A 10-mL aliquot was then applied to a column containing 25 g of basic alumina and eluted with 100 mL of chloroform that was discarded. The column was then eluted sequentially with 10 mL each of solutions 1, 3, 5, 7, 10, and 15% methanol in chloroform that were discarded and 20 mL each of solutions of 20, 40, 60, 80, and 100% methanol in chloroform. The last five fractions, containing the arsenic, were combined and flash evaporated to about 0.2 mL, at 30 °C, the residue taken up in 10 mL of chloroform and applied to a second alumina column, and the cleanup procedure repeated twice more. The final collected fractions were combined and flash evaporated, and the residue was dissolved in 50 mL of methanol for HPLC. For mass spectrometric analysis the residue was dissolved in 5 mL of water and applied to a column containing 10 mL of AG 50WX-8 cation-exchange resin that had been previously washed with water. The column was eluted with 40 mL of water, which was discarded. The arsenic compounds were eluted with 110 mL of 5% ammonia solution that was neutralized with 1 N HCl and rotary evaporated to dryness at 30 °C. The residue was submitted directly to mass spectrometric analysis.

Esterification of arsenobetaine was carried out exactly as described by Sen et al. (1983) for the esterification of nitrososarcosine. A 1-mL portion of boron trifluoride–ethanol reagent was added to the residue obtained from evaporation of an aliquot of fish extract and the mixture heated at 60–70 °C for 30 min. The sample was cooled to room temperature and mixed with 4 mL of water. A 1-mL portion of dichloromethane was added and the test tube shaken. The dichloromethane layer was evaporated just to dryness and the residue analyzed by HPLC and mass spectrometry.

High-pressure liquid chromatographic conditions were optimized against standards of arsenobetaine, arsenocholine, and arsenobetaine ethyl ester. The compounds were separated on a Waters 30 cm × 4.6 mm i.d. μBondapak C18 column. The mobile phase consisted of 10% methanol in distilled water (v/v) adjusted to pH 3.5 with glacial acetic acid. The flow rate was 1.5 mL/min. Fractions (0.5 mL) were collected in plastic sample cups and manually transferred to the autosampler of a Varian AA 875 atomic absorption spectrometer equipped with a GTA-95 graphite furnace. Suitable standards of arsenobetaine and arsenocholine were used to quantitate the arsenic present in each fraction. Portions (15 μL) of each solution plus 5 μL of a 1000 ppm nickel solution in 1% HNO_3 were used for each analysis. The purified fish extracts and derivatives were analyzed under identical conditions as the standards. Identification was achieved by comparison of retention volumes (i.e., fraction numbers).

Mass spectrometric analyses of standards and purified fish tissue extracts were carried out with a VG 7070EQ hybrid MS/MS (EBQQ configuration) with a fast atom bombardment (FAB) source using xenon as a target gas at 5 kV energy. The samples and standards were suspended in ethylene glycol for analysis.

Total arsenic determinations were carried out by using a dry ashing coprecipitation method (Dabeka, 1985). Recovery studies were performed on several fish samples after the approximate organoarsenic content was determined. Portions of the dry fish tissue samples were spiked with appropriate amounts of arsenobetaine and arsenocholine standards, extracted, and analyzed as described above.

RESULTS AND DISCUSSION

The methodology developed in this work was directed toward isolating enough of the organoarsenic species so that FAB mass spectrometric analyses could be performed. For this reason large samples (50 g of freeze-dried material) were taken for the determinations, and in several cases the procedure was repeated and extracts were combined. The HPLC–AA speciation procedure proved to be extremely sensitive and selective, enabling the detection of 1 ng/g

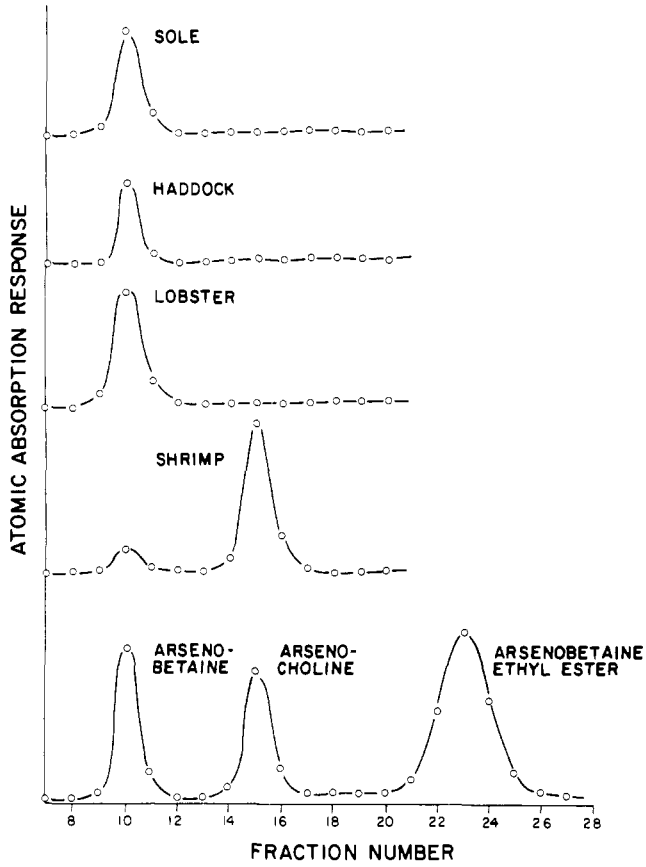

Figure 1. Chromatograms of four fish extracts. The bottom chromatogram represents standards. Conditions as described in the text.

or less of arsenic in the samples. Method blanks showed no arsenic above the detection limit.

The prime purpose of this work was to identify the unknown organoarsenic compounds in fish and not to develop a simple routine method. Thus, for routine analyses by HPLC–AA several modifications to simplify the method might be made including a reduction in sample size to about 5 g with only one passage through the alumina column using one or two eluting solvent combinations. It is also possible that the ion-exchange column cleanup could be eliminated for certain samples.

It is interesting to note that although arsenobetaine and arsenocholine in a reasonably pure form are slightly soluble in chloroform, they were not extracted at all from the freeze-dried fish tissue with that solvent during the Soxhlet extraction. It appears that the compounds are weakly bound and that a very polar solvent such as methanol is required to release them.

Because of the off-line method of detection, chromatograms were constructed by plotting peak height vs. fraction number. Typical results for standards and fish extracts are shown in Figure 1. It can be seen that sole, haddock, and lobster contain a single organoarsenic compound that elutes in the same fractions as arsenobetaine. The shrimp extract contains two substances containing arsenic that correspond to arsenobetaine and arsenocholine. Arsenocholine has already been identified in commercial boiled shrimp in Sweden (Norin et al., 1983) although no arsenobetaine was found.

The identity of arsenobetaine in the fish extracts was confirmed by treating the extracts with boron trifluoride in ethanol. Under these conditions arsenobetaine is converted to the corresponding ethyl ester that elutes from the HPLC column much later than arsenobetaine, as

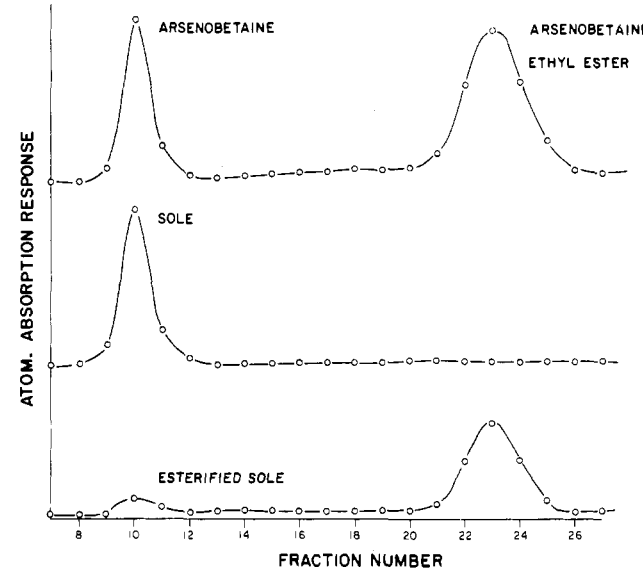

Figure 2. Confirmation of arsenobetaine in sole by conversion to arsenobetaine ethyl ester. Top chromatogram represents standard compounds. The middle chromatogram is a sole extract, and the bottom is the same extract after esterification. Conditions as described in the text.

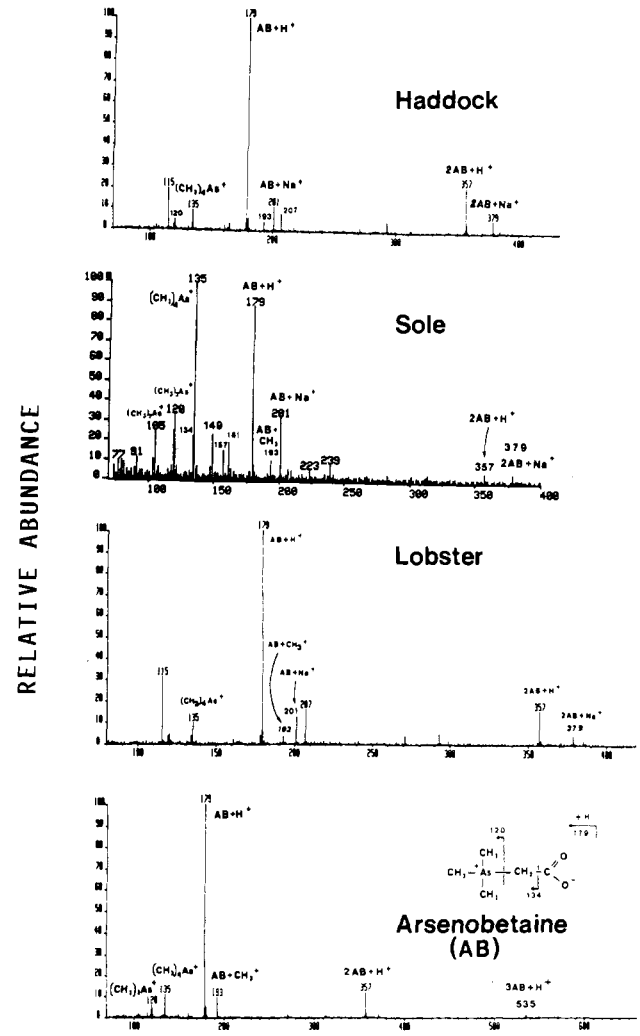

Figure 3. FAB mass spectra of arsenobetaine (AB) standard (bottom) and extracts of lobster, sole, and haddock. The mass number scale (abscissa) is different for each spectrum.

shown in Figure 2. It can also be seen in the figure that after the sole extract is esterified, the peak corresponding to arsenobetaine is substantially reduced and a new peak

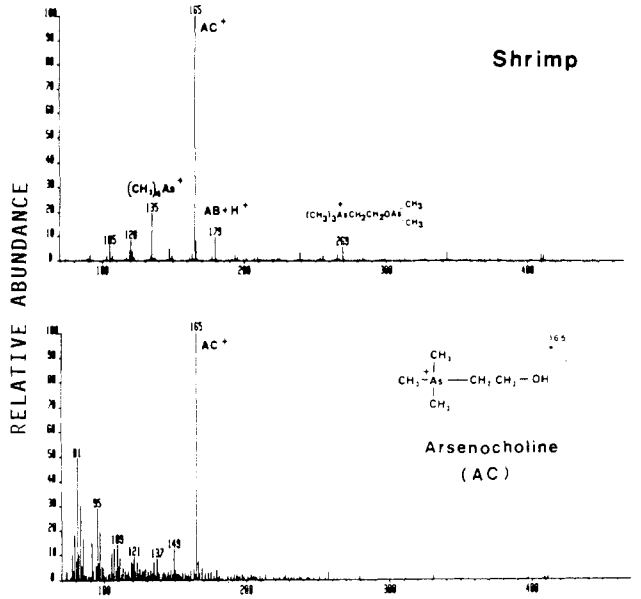

Figure 4. FAB mass spectra of arsenocholine standard and a shrimp extract containing both arsenocholine (AC) and arsenobetaine (AB).

Table I. Recoveries of Arsenobetaine (AB) and Arsenocholine (AC) Added to Selected Fish Samples

fish	AB added, μg/g fresh wt	AB detected, μg/g fresh wt: spiked sample	AB detected, μg/g fresh wt: unspiked sample	% rec
mackeral	0.25	0.45	0.25	80
cod	4.0	6.6	3.3	83
sole	10.0	18.1	9.7	84
shrimp	12.5	23.8	13.7	81
	5.0 (AC)	7.5 (AC)	3.4 (AC)	82
yellow perch	0.005	0.009	0.005	80

appears in exactly the same position as arsenobetaine ethyl ester.

Additional confirmation was carried out by analyzing standards, fish extracts, and derivatized fish extracts by FAB mass spectrometry. Figure 3 compares standard arsenobetaine with spectra obtained from extracts of locally purchased haddock, sole, and lobster after HPLC separation. The fragmentation patterns clearly indicate that the compound in the fish extracts is arsenobetaine, with $M + H^+ = 179$. Figure 4 compares the mass spectra of the shrimp extract (without HPLC separation) with standard arsenocholine. It can be seen that the major peak occurs at $M^+ = 165$, the molecular ion peak for the standard. The peak at mass 179 results from the presence of arsenobetaine in the extract. Assuming that the response factor is the same for both peaks, the ratio of arsenobetaine to arsenocholine is about 1:10, similar to that observed by HPLC–AA. The presence of arsenobetaine ethyl ester in derivatized fish extracts was also confirmed by mass spectrometry.

Table I lists the recoveries of arsenobetaine from five fish types over the spiking range of 0.005–12.5 μg/g (fresh weight) and of arsenocholine in shrimp at 5.0 μg/g (fresh weight). The results indicate that the two compounds are recovered through the analytical procedure consistently at about 82%.

Table II lists the results found in the samples analyzed. The total arsenic values were very low in the freshwater fish from Manitoba at 0.007–0.037 to 0.048–0.24 μg/g for Ontario compared to 0.31–20.8 μg/g for ocean fish. If the values obtained for arsenobetaine are corrected for recovery, the results would indicate that this compound

Table II. Total Arsenic and Organoarsenic Compounds Found in Seafood Samples

region/fish	recovery, μg/g: total arsenic[a]	recovery, μg/g: arsenic as arsenobetaine[c]	recovery, μg/g: other	% organic of total[c]
Atlantic				
herring	1.1	0.98		89
halibut	*b*	2.1		
haddock	6.0	4.7		78
cod	5.1	4.1		80
mackeral	0.55	0.44		80
sole	13.2	11.3		86
lobster	5.2	4.5		87
scallops	0.68	0.60		88
Pacific				
herring	1.0	0.86		86
salmon	0.31	0.15	0.13 (X)	90
sole	5.2	4.6		88
halibut	2.3	1.7		74
shrimp	20.8	15.8	3.2 (AC)[c]	91
cod	7.4	6.2		84
Freshwater, Ontario				
bass	0.12		0.087 (Y)	71
pike	0.048		0.034 (Y)	71
carp	0.18		0.13 (Y)	72
yellow perch	0.055		0.040 (Y)	73
striped perch	0.24		0.17 (Y)	71
Freshwater, Alberta				
yellow perch	0.007		0.006 (Y)	85
northern pike	0.023		0.020 (Y)	84
pickeral	0.037		0.031 (Y)	84
whitefish	0.024		0.019 (Y)	79
Locally Purchased, Ottawa				
haddock	3.5	3.0		86
sole	0.10	0.08		80
lobster	4.7	3.6		76
shrimp	7.2	0.58	5.2 (AC)	80

[a] Fresh weight basis, average of duplicates. [b] Result not reported because of poor replicate values (1.5–4 μg/g for four replicates). [c] Not corrected for recovery. AC = arsenocholine; X and Y = unknown.

represents essentially all of the arsenic present in the marine samples analyzed, with the exception of shrimp and salmon. it is interesting that the shrimp purchased in the Ottawa area displayed a ratio of arsenobetaine to arsenocholine of about 0.11 whereas it was about 5.1 for the Pacific shrimp. This variation appears to depend upon the type of shrimp and/or where it was harvested. Norin et al. (1983) for example found arsenocholine in commercial frozen boiled shrimp in Sweden but found no arsenobetaine. It has been suggested (Norin et al., 1983) that arsenocholine is a precursor of arsenobetaine and thus is likely to vary in concentration depending upon the metabolic rate of individual fish.

The freshwater fish samples contained no arsenobetaine or arsenocholine. However, methanol-extractable arsenic was detected and eluted unretained through the HPLC system, indicating that the unknown is more hydrophilic than arsenobetaine. It accounted for about 72% of the total in the Ontario samples and about 83% of the total arsenic in the Manitoba samples. This particular unknown arsenic was not observed in the ocean fish samples.

The present work has shown conclusively that arsenobetaine and arsenocholine are the major arsenic constituents of marine fish and shellfish species in Canadian coastal waters. It appears that these forms of arsenic do not increase urinary levels of inorganic arsenic. The organoarsenicals are rapidly excreted (Vahter et al., 1983; Marafante et al., 1984; Cannon, 1983; Luten et al., 1982;

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