on fractionated extracts of abalone meat, Konosu and Hashimoto (1964) demonstrated a similar key interrelationship to a meaty character between glutamic acid and adenosine 5′-monophosphate. Komato (1964) obtained similar results with "Uni", the unripe gonad of the sea urchin. Of the amino acid fraction in combination with mononucleotides, he found glycine, valine, alanine, glutamic acid, and, particularly, methionine to be important. The elimination of glycine resulted in an increase in bitterness and a decrease in sweetness.

Such findings tend to confirm the suggestions that amino acids are likely to be important nonodoriferous contributors to the overall flavor of fish sauce.

Experimental evidence obtained by chemical and sensory analyses has shown that mixtures of NaCl and KCl (NaCl:KCl = 50:50) could provide as a possible replacement for common salt (NaCl) generally used in fish sauce fermentation.

**Registry No.** Na, 7440-23-5; NaCl, 7647-14-5; KCl, 7447-40-7; ammonia, 7664-41-7; nitrogen, 7727-37-9; butanol, 123-72-8; octanal, 124-13-0; 2,4-decadienal, 2363-88-4; 2-undecenal, 2463-77-6; tetradecenal, 54264-02-7; hexadecanal, 629-80-1; octadecanal, 638-66-4; octadecenal, 71873-66-0; octadecadienal, 28982-40-3; monomethylamine, 74-89-5; dimethylamine, 124-40-3; trimethylamine, 75-50-3.

LITERATURE CITED


Amano, K.; Bito, M. *Bull. Jpn. Soc. Sci. Fish.* **1951**, *16* (12), 10.
AOAC "Official Methods of Analysis"; Association of Official Analytical Chemists: Washington, DC, 1975.
Baxter, R. M.; Gibbons, N. E. *Biotechnol. Bioeng.* **1956**, *8*, 237.
Block, R. J.; Weiss, K. W. "Amino Acid Handbook"; Charles C. Thomas: Springfield, IL, 1956; p 74.
Chayovan, S.; Rao, R. M.; Liuzzo, J. A.; Khan, M. A. *J. Agric. Food Chem.* **1983**, *31*, 14–17.
Dahl, L. K. *Nutr. Rev.* **1972**, *18*, 97.
Diemair, W., F.A.O. Symposium on the Significance of Fundamental Research in the Utilization of Fish, Husum, Germany, 1964, WP/4/9.
Diemair, W.; Schams, F. *Z. Anal. Chem.* **1962**, 189.
Forss, D. A. *J. Dairy Sci.* **1963**, *46*, 614.
Frank, R. L.; Mickelson, O. *Am. J. Clin. Nutr.* **1969**, *22*, 464.
Fugimaki, M. S.; Kirigaya, A. N.; Sakurai, Y. *Agric. Biol. Chem.* **1965**, *29* (9), 855.
Gaddis, A. M.; Ellis, R.; Currie, G. T. *Food Rev.* **1958**, *23*, 283.
Hashimoto, Y., F.A.O. Symposium on the Significance of Fundamental Research in the Utilization of Fish, Husum, Germany, 1964, WP/2/6.
Hughes, R. B. *Nature (London)* **1958**, *181*, 1281.
Ingram, M. J. *J. Appl. Bacteriol.* **1966**, *29* (2), 217.
Institute of Food Technologists *Food Technol. (Chicago)* **1980**, *34*, 85.
Jones, N. R.; Murray, J. *Physiol. Rev.* **1961**, *44*, 174.
Kabot, F. J.; Ettre, L. S. *J. Gas Chromatogr.* **1963**, *1* (10), 7.
Kasemsarn, B. Master's Thesis, University of Washington, Seattle, WA, 1963.
Kempner, W. *Am. J. Med.* **1948**, *4*, 345.
Komata, Y., F.A.O. Symposium on the Significance of Fundamental Research in the Utilization of Fish, Husum, Germany, 1964, WP/2/6.
Konosu, S.; Hashimoto, Y., F.A.O. Symposium on the Significance of Fundamental Research in the Utilization of Fish, Husum, Germany, 1964, W/2/6.
Konosu, S.; Maeda, Y.; Fugita, T. *Bull. Jpn. Soc. Sci. Fish.* **1960**, *26*, 45.
Meneely, G. R.; Ball, C. O. T. *Am. J. Med.* **1958**, *25*, 713.
Meneely, G. R.; Gall, C. O. T.; Youmans, J. B. *Ann. Intern. Med.* **1957**, *47*, 263.
Michelsen, O.; Makdani, D.; Gill, J. L.; Frank, H. L. *Am. J. Clin. Nutr.* **1977**, *30*, 2033.
Preston, S. T.; Spreckelmeyer, S. "A Guide to the Analysis of Fatty Acids and Their Esters by Gas Chromatography"; PolyScience Corp.: Evanston, IL, 1971; pp 48–50.
Saisithi, P. B.; Kasemsarn, B.; Liston, J.; Dollar, A. M. *J. Food Sci.* **1965**, *31*, 105.
Simidu, W.; Hibiki, S.; Sibata, S.; Takeda, K. *Bull. Jpn. Soc. Sci. Fish* **1953a**, *19*, 871.
Simidu, W.; Korokawa, Y.; Ikeda, S. *Bull. Res. Inst. Food Sci., Kyoto Univ.* **1953b**, *12*, 40.
Snedecor, G. W. "Statistical Methods"; Iowa State College Press: Ames, IA, 1959.
Tobian, L. *Physiol. Rev.* **1960**, *40*, 280.
Tressler, D. K.; Lemon, J. M. W. "Marine Products of Commerce"; Reinhold: New York, 1951.
Tucker, R. G.; Ball, C. O. T.; Darby, W. J.; Early, W. R.; Kory, R. C.; Youmans, J. B.; Meneely, G. R. *J. Gerontol.* **1957**, *12*, 182.
Uyenco, V.; Lawas, I.; Briones, P. R.; Taruc, R. S. *Proc. Int. Conf. Fish., 4th* **1952**, 210.
Vanderstoep, J. *Can. Inst. Food Sci. Technol. J.* **1978**, *11* (2), A32.
Velankar, N. K. *J. Sci. Ind. Res.* **1952**, *113*, 310.
Weurman, G.; Rooji, C. *J. Food Sci.* **1961**, *2*, 239.


Received for review September 28, 1982. Accepted March 28, 1983. This paper is based on the work performed by S.C. toward his Ph.D. dissertation, "Isolation of Flavor Constituents and Organoleptic Acceptance of a Dietary Sodium-Potassium Fish Sauce".

# Indole Glucosinolates in Swede (*Brassica napobrassica* L. Mill)

Roger J. W. Truscott,* Patricia K. Johnstone, Ian R. Minchinton, and Joseph P. Sang

A new high-performance liquid chromatography (HPLC) procedure for glucosinolate analysis has been used to reinvestigate the glucosinolate content of swede (*Brassica napobrassica* L. Mill), also known as rutabaga. Swede rind was shown to contain four distinct indole glucosinolates: glucobrassicin, 4-hydroxyglucobrassicin, 4-methoxyglucobrassicin, and neoglucobrassicin. The desulfo derivative of neoglucobrassicin was purified by HPLC and the 1-methoxyglucobrassicin structure confirmed by a combination of ultraviolet spectroscopy, nuclear magnetic resonance spectroscopy, and mass spectrometry techniques. Thus, two methoxyglucobrassicin isomers, 4-methoxy and 1-methoxy, coexist in the same plant tissue.

The glucosinolate content of cruciferous plants is important in view of their role as flavoring constituents. Once cellular disruption occurs glucosinolates undergo enzymic hydrolysis with endogenous myrosinase, releasing isothiocyanates and other products which contribute to the flavor of brassica vegetables. The presence of glucosinolates in *Brassica* species is also associated with toxic and

Division of Agricultural Chemistry, Department of Agriculture, East Melbourne, Victoria, Australia 3002.

0021-8561/83/1431-0863$01.50/0 

growth inhibitory properties.

The glucosinolate content of turnip and swede root has been investigated by a number of workers (VanEtten et al., 1969; Mullin et al., 1980; Hak-Yoon Ju et al., 1980; Carlson et al., 1981). However, in all cases, the indole glucosinolates were estimated as a group by determination of total thiocyanate ion formed by myrosinase digestion. Until the advent of new GC (Heaney and Fenwick, 1980) and HPLC techniques (Helboe et al., 1980; Minchinton et al., 1982), it has not been possible to measure the amount of individual indole glucosinolates.

The existence of two major indole glucosinolates, glucobrassicin (Gmelin et al., 1960) and neoglucobrassicin (Gmelin and Virtanen, 1962), has been known for some time. On the basis of chemical degradation studies, Gmelin and Virtanen proposed that neoglucobrassicin should be 1-methoxyglucobrassicin. Recently, two additional indole glucosinolates, 4-methoxyglucobrassicin (Truscott et al., 1982b) and 4-hydroxyglucobrassicin (Truscott et al., 1982a), have been discovered. Following the identification of 4-methoxyglucobrassicin, it seemed important to reexamine the structure of neoglucobrassicin to determine if two structural isomers of methoxyglucobrassicin exist or if the original assignment of neoglucobrassicin as a 1-methoxy compound was incorrect.

In this paper, a combination of paper and ion-exchange chromatography together with HPLC has been used to investigate the glucosinolate profile of swede rind with particular reference to the indole glucosinolates.

## EXPERIMENTAL SECTION

**Reagents.** DEAE-Sephadex A-25 was purchased from Pharmacia (Uppsala, Sweden); arylsulphatase Type H1 was from Sigma (St. Louis, MO); pyridine, BSTFA, and TriSil concentrate were from Pierce (Rockford, IL). All organic solvents were analytical or HPLC grade. Swede plants (*Brassica napobrassica* L. cv. Champion Purple Top) were grown at Knoxfield, Victoria, Australia.

**Analytical Procedures.** Swede roots were peeled and the rind (approximately 1 mm thick) was immediately immersed in liquid $N_2$. The frozen sample was pulverised and extracted with boiling methanol and the protein precipitated by using lead/barium acetate as described by Truscott et al. (1982b).

Preparative descending paper chromatography was carried out on 3MM paper (Whatman) by using 1-butanol/acetic acid/water (4/1/2) as the solvent. Thin-layer chromatography (TLC) was carried out on cellulose $F_{254}$ and silica gel $60F_{254}$ plates (Merck) with solvent systems II, III, IV, VI, and VIII of Matsuo (1970). The chromatograms were visualized by using (dimethylamino)-cinnamaldehyde (DMAC).

Purification of the intact glucosinolates from the methanol extract and isolation of the indole desulfo (DS) glucosinolates was performed as described by Truscott et al. (1982c). Briefly, total glucosinolates were adsorbed onto DEAE-Sephadex A-25. Non-indole glucosinolates were eluted with 0.125 M pyridine/acetate buffer and subsequently indole glucosinolates were eluted with 0.25 M pyridine/acetate buffer. Aliquots of both these eluates were diluted to a final concentration of 0.025 M pyridine/acetate prior to on-column desulfation with arylsulphatase (Minchinton et al., 1982).

HPLC was performed by using a Spectra Physics S-P8000 ternary gradient instrument coupled to a Spectromonitor III (Laboratory Data Control) ultraviolet detector set at 227.5 nm.

Two solvent programs were used: the original solvent program described by Minchinton et al. (1982) and a short program using a linear gradient of 0–25% $CH_3CN$ over 30 min. The modified HPLC program was used to monitor the bands obtained by preparative paper chromatography. UV spectra were recorded on a Shimadzu UV-240 ultraviolet spectrophotometer by using water as the solvent. NMR spectra were obtained on a Bruker HX-270 spectrometer operated at 270 MHz.

**Mass Spectrometry.** DS glucosinolates were collected from the HPLC and examined as silyl derivatives using GC/MS (Minchinton et al., 1982). Underivatized DS glucosinolates were examined by direct probe mass spectrometry using ammonia (0.4 torr) as the chemical ionization reagent gas. The ion source temperature was 200 °C and the probe was temperature programmed from ambient to 200 °C over a period of 6 min.

## RESULTS

Initially preparative paper chromatography was used to investigate the indole glucosinolate content of swede rind. Staining with the indole reagent DMAC revealed the presence of two major components ($R_f$ 0.39 and 0.32) and one minor component ($R_f$ 0.22). The two major species were tentatively identified as neoglucobrassicin ($R_f$ 0.39) and glucobrassicin ($R_f$ 0.32) on the basis of their mobilities and color reactions by reference to those documented by Butcher et al. (1974). As reported by these authors, the neoglucobrassicin did not stain immediately with the DMAC reagent, but after 20 min a brown color was observed which changed to a reddish purple after 1 h.

The minor component ($R_f$ 0.22) was identified as 4-hydroxyglucobrassicin on the basis of staining with diazotized sulfanilic acid and HPLC retention time following desulfation (Truscott et al., 1982a).

The total glucosinolate profile of swede root was obtained by HPLC analysis of the corresponding DS glucosinolates obtained from on-column desulfation with arylsulphatase (Minchinton et al., 1982). On-column desulfation was first described by Thies (1979). All the major glucosinolates previously described in swede roots were found to be present (Figure 1a). These were collected from the HPLC, dried, and identified by GC/MS as silyl derivatives.

The indole glucosinolates were purified from the total glucosinolate extract by using ion-exchange chromatography (Truscott et al., 1982c) and the individual components separated as the desulfo derivatives by HPLC (Figure 1b). An examination of the profile showed that three major indole containing DS glucosinolates were present. Two of these were identified as DS glucobrassicin (peak 5) and DS 4-methoxyglucobrassicin (peak 7). The third peak (peak 8) was thought to be due to DS neoglucobrassicin.

Since paper chromatography had shown two major indole-containing compounds whereas HPLC had indicated that three were present, the bands at $R_f$ 0.32 and 0.39 were cut out and the paper strips eluted with water. The eluates were applied to DEAE-Sephadex and desulfated prior to analysis by the modified HPLC program. Figure 2b shows that the band at $R_f$ 0.39 consists of only one major component corresponding to peak 8 in Figure 1. The band at $R_f$ 0.32, however, was found to contain both glucobrassicin and 4-methoxyglucobrassicin. These were present in approximately the same ratio as that found in the original extract (Figure 1a). We were unable to achieve adequate resolution of glucobrassicin and 4-methoxyglucobrassicin by thin-layer chromatography on either silica or cellulose in any of the solvent systems described.

**Identification of Desulfo Indole Glucosinolates.** The three major indole glucosinolates present in swede rind glucobrassicin (peak 5), 4-methoxyglucobrassicin

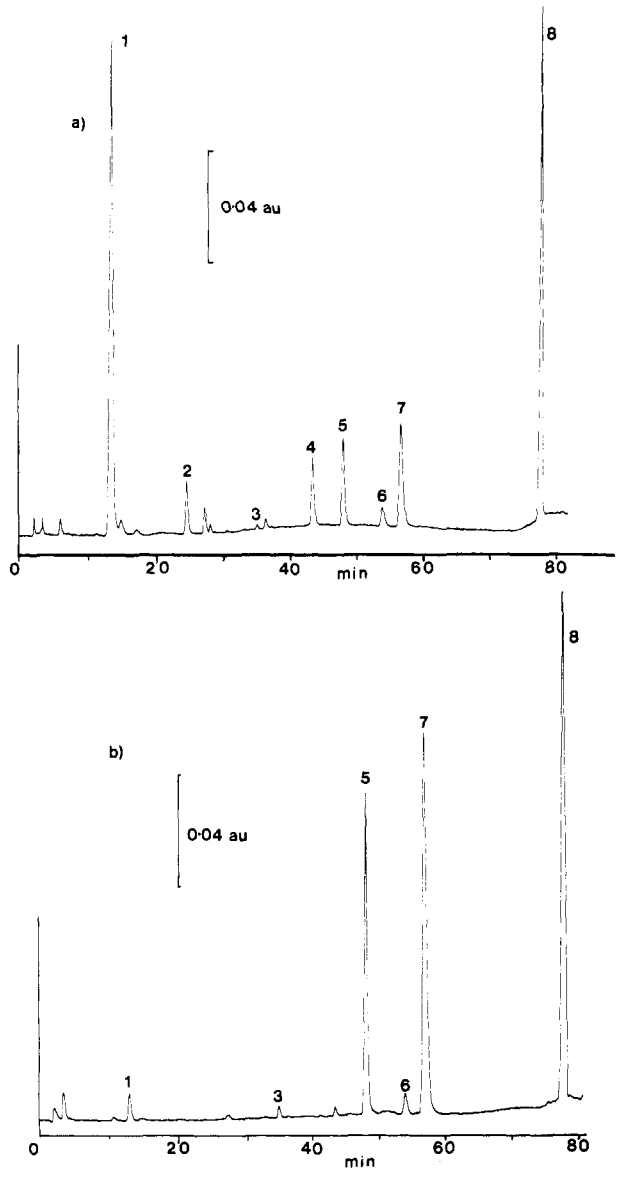



**Figure 1.** (a) HPLC profile of total DS glucosinolates obtained from an extract of swede root. (b) HPLC profile of indole DS glucosinolates purified by ion-exchange chromatography. DS glucosinolate peaks were identified as follows: 1, 2-hydroxybut-3-enyl (progoitrin); 2, 4-(methylsulfinyl)butyl (glucoraphanin); 3, (4-hydroxy-3-indolyl)methyl (4-hydroxyglucobrassicin); 4, 4-(methylthio)butyl (glucoerucin); 5, 3-indolylmethyl (glucobrassicin); 6, phenylethyl (gluconasturtin) plus 5-(methylthio)pentyl; 7, (4-methoxy-3-indolyl)methyl (4-methoxyglucobrassicin); 8, neoglucobrassicin.

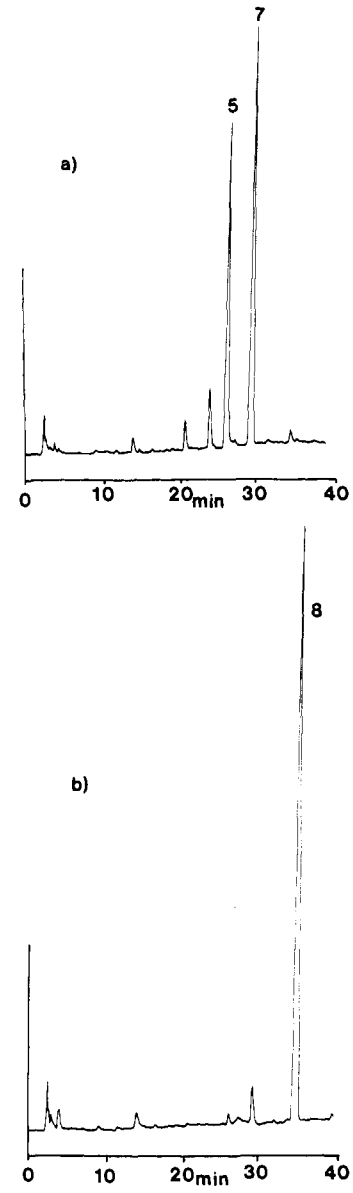



**Figure 2.** HPLC profiles (short program) of DS glucosinolates obtained after separation of intact glucosinolates by paper chromatography. Indole glucosinolates were detected with DMAC reagent. (a) $R_f$ 0.32 band; (b) $R_f$ 0.39 band. DS glucosinolate peak numbers as in Figure 1.

(peak 7), and neoglucobrassicin (peak 8) were collected in the desulfo form from the HPLC column and subjected to analysis by UV and NMR spectroscopy as well as GC/MS.

The UV spectra of desulfo derivatives of glucobrassicin, 4-methoxyglucobrassicin, and neoglucobrassicin were distinctly different (Figure 3). The spectrum of DS neoglucobrassicin is similar to that reported for neoglucobrassicin by Gmelin and Virtanen (1962). A comparison of the NMR spectra (Figure 4) shows the aromatic proton pattern to be very similar for DS glucobrassicin and DS neoglucobrassicin. Substitution of the benzene ring has a marked effect on the pattern (Figure 4c). The singlet peak in DS glucobrassicin ($\delta$ 7.17, Figure 4a), presumably due to the 2-proton, is shifted downfield ($\delta$ 7.46) in the case of DS neoglucobrassicin.

Both DS glucobrassicin and DS 4-methoxyglucobrassicin showed an indole N–H signal at $\delta$ 10.8 in hexadeuteriodimethyl sulfoxide. This signal disappeared on addition of $H_2O$. DS neoglucobrassicin lacked a peak in this region, indicating that the indole N–H was absent.

A peak at $\delta$ 4.03 (3 H) suggested that the substituent could be an $-OCH_3$ group, since the $-OCH_3$ signal of 1-methoxy-*N*,*N*-dimethyltryptamine in $CCl_4$ has been reported at $\delta$ 4.0 (Morimoto and Oshio, 1965). This supposition was confirmed by direct insertion mass spectrometry of underivatized DS neoglucobrassicin, DS 4-methoxyglucobrassicin, and DS glucobrassicin (Table I).

Both DS 4-methoxyglucobrassicin and DS neoglucobrassicin fragmented to produce ions at $m/z$ 160 and 204; each ion 30 amu higher than the corresponding two most abundant ions present in the mass spectrum of DS glucobrassicin. The probable cleavages involved are depicted in Table I.

After derivatization with silylating reagents and examination by GC/MS DS neoglucobrassicin was found to behave in an identical fashion to DS glucobrassicin. This observation suggests the N–$OCH_3$ bond is heat sensitive,

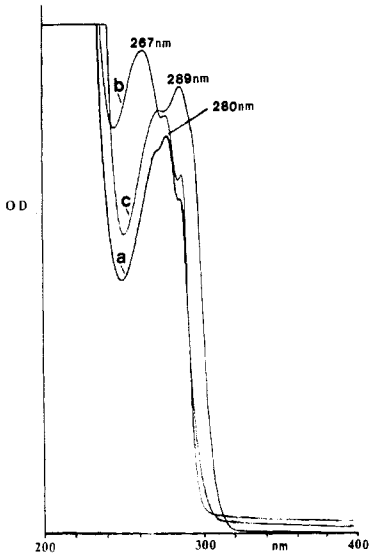
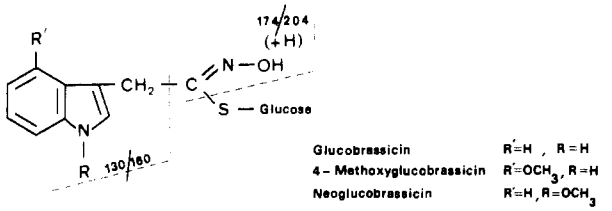



**Figure 3.** UV spectra of (a) DS glucobrassicin, (b) DS 4-methoxyglucobrassicin, and (c) DS neoglucobrassicin.

Table I. Solid Probe Mass Spectrometry of Underivatized DS Glucosinolates




two most abundant ions, $m/z$ (%)

| glucobrassicin | 4-methoxyglucobrassicin | neoglucobrassicin |
|---|---|---|
| 130 (100) | 160 (36) | 160 (100) |
| 174 (36) | 204 (100) | 204 (48) |

being cleaved either during derivatization (120 °C, 30 min) or in the GC injection port (280 °C). The similar behavior of glucobrassicin and neoglucobrassicin derivatives under GC/MS conditions has been noted previously (Heaney and Fenwick, 1982).

## DISCUSSION

The structure of neoglucobrassicin as 1-methoxy-3-indolylmethyl glucosinolate has been confirmed by using a combination of spectroscopic techniques following purification of the desulfo derivative by HPLC.

Comparison of the properties of this compound with those of the desulfo derivative of 4-methoxyglucobrassicin showed that the two compounds were clearly different. Thus, swede rind contains high levels of two structural isomers: 1-methoxyglucobrassicin and 4-methoxyglucobrassicin.

Following the suggestion of Ettlinger and Kjaer (1968) that the unusual 1-methoxyglucobrassicin structure originally proposed (Gmelin and Virtanen, 1962) should be reinvestigated, Elliott and Stowe (1971) attempted to purify neoglucobrassicin and compare its structure with that of glucobrassicin using NMR. Unfortunately, the nature of their NMR spectra made it difficult to draw definitive conclusions. Their data did, however, support the idea that the indole nitrogen of neoglucobrassicin was substituted. The nature of the substituent was not studied in detail.

It would seem from the results shown in this paper that a problem in their study may have been that the glucobrassicin used for NMR was purified by preparative paper chromatography and thus could have been contaminated with the neoglucobrassicin isomer, 4-methoxyglucobrassicin. We have found that woad leaves contain significant amounts of 4-methoxyglucobrassicin. In the present study this problem was overcome by using the corresponding desulfo derivatives, which can easily be separated by HPLC and collected in a highly purified state. These derivatives are also more stable than the parent glucosinolates.

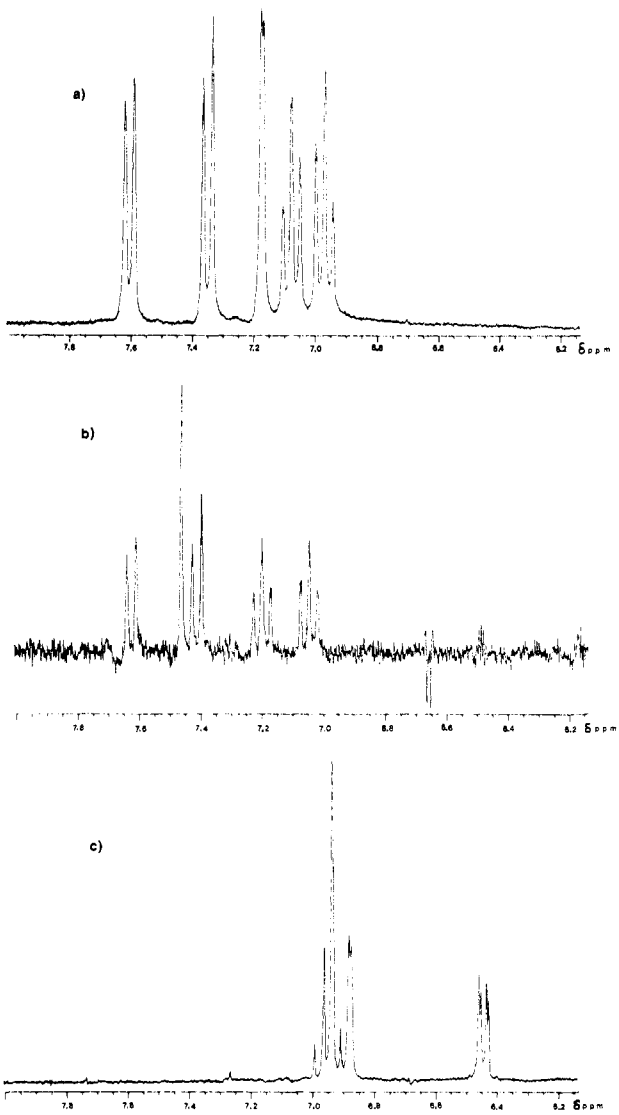



**Figure 4.** NMR spectra of (a) DS glucobrassicin, (b) DS neoglucobrassicin, and (c) DS 4-methoxyglucobrassicin.

All four indole glucosinolates, glucobrassicin, 4-methoxyglucobrassicin, 4-hydroxyglucobrassicin, and 1-methoxyglucobrassicin, were found in the swede root extract. 1-Sulfoglucobrassicin (Elliott and Stowe, 1970) was not investigated in this study. Neoglucobrassicin was the most abundant indole glucosinolate in swede root (Figure 1) but was found to be present at much lower levels in leaf and seed tissue. A high root but low leaf concentration for neoglucobrassicin has been noted previously (Kutacek, 1964). In swede leaf, glucobrassicin was the most abundant whereas in swede seed 4-hydroxyglucobrassicin was present in the highest levels. Interestingly, the indole glucosinolate profile varies greatly between seed, root, and leaf tissue of all Cruciferous plants so far examined. This aspect is being studied at present and will be detailed in a future publication.

**Registry No.** Neoglucobrassicin, 5187-84-8; glucobrassicin, 4356-52-9; 4-hydroxyglucobrassicin, 83327-20-2; 4-methoxyglucobrassicin, 83327-21-3; DS glucobrassicin, 43110-92-5; DS neoglucobrassicin, 85505-04-0; DS 4-methoxyglucobrassicin, 85422-08-8.

LITERATURE CITED

Butcher, D. N.; El-Tigani, S.; Ingram, D. S. *Physiol. Plant Pathol.* **1974**, *4*, 127–140.
Carlson, D. G.; Daxenbichler, M. E.; VanEtten, C. H.; Tookey, H. L.; Williams, P. H. *J. Agric. Food. Chem.* **1981**, *29*, 1235–1239.
Elliott, M. C.; Stowe, B. B. *Phytochemistry* **1970**, 1629–1632.
Elliott, M. C.; Stowe, B. B. *Plant Physiol.* **1971**, *47*, 366–372.
Ettlinger, M. G.; Kjaer, A. "Recent Advances in Phytochemistry"; Appleton-Century-Crofts: New York, 1968; Vol. 1, pp 89–144.
Gmelin, R.; Saarivirta, M.; Virtanen, A. I. *Suom. Kemistil. B* **1960**, *33*, 172.
Gmelin, R.; Virtanen, A. I. *Acta Chem. Scand.* **1962**, *16*, 1378–1384.
Hak-Yoon Ju; Chong, C.; Bible, B. B.; Mullin, W. J. *Can. J. Plant Sci.* **1980**, *60*, 1295–1302.
Heaney, R. K.; Fenwick, G. R. *J. Sci. Food Agric.* **1980**, *31*, 593–599.
Heaney, R. K.; Fenwick, G. R. *J. Sci. Food Agric.* **1982**, *33*, 68–70.
Helboe, P.; Olsen, O.; Sorensen, H. *J. Chromatogr.* **1980**, *197*, 199–205.
Kutacek, M. *Biol. Plant.* **1964**, *6*, 38–39.
Matsuo, M. *J. Chromatogr.* **1970**, *49*, 323–324.
Minchinton, I.; Sang, J.; Burke, D.; Truscott, R. J. W. *J. Chromatogr.* **1982**, *247*, 141–148.
Morimoto, H.; Oshio, H. *Justus Liebigs Ann. Chem.* **1965**, *682*, 212–218.
Mullin, W. J.; Proudfoot, K. G.; Collins, M. J. *Can. J. Plant Sci.* **1980**, *60*, 605–612.
Thies, W. *Naturwissenschaften* **1979**, *66*, 364–365.
Truscott, R. J. W.; Burke, D. G.; Minchinton, I. R. *Biochem. Biophys. Res. Commun.* **1982a**, *107*, 1258–1264.
Truscott, R. J. W.; Minchinton, I. R.; Burke, D. G.; Sang, J. P. *Biochem. Biophys. Res. Commun.* **1982b**, *107*, 1368–1375.
Truscott, R. J. W.; Minchinton, I. R.; Sang, J. *J. Sci. Food Agric.* **1982c**, in press.
VanEtten, C. H.; Daxenbichler, M. E.; Wolff, I. A. *J. Agric. Food Chem.* **1969**, *17*, 483–491.

Received for review November 1, 1982. Accepted February 28, 1983. This work was supported in part by a grant from the Oilseeds Research Committee, Australia.

# Polycyclic Aromatic Hydrocarbons in Grilled Food

Bonny K. Larsson,* Greger P. Sahlberg, Anders T. Eriksson, and Leif Å. Busk

The levels of 22 polycyclic aromatic hydrocarbons (PAH) were determined in 63 samples of grilled meat and meat products by capillary gas chromatography. The results reveal that the PAH levels are strongly dependent on the method of cooking and type of heat source used. The grilling of frankfurters in the flames of a log fire resulted in extremely high PAH levels, up to 212 μg/kg benzo[a]pyrene (BaP). When the grilling was carried out over the embers, the average level of BaP was only 7.7 μg/kg. Relatively high PAH levels, an average of 17.6 μg of BaP/kg, were found in frankfurters grilled over smoldering spruce or pine cones. The BaP levels in charcoal-grilled frankfurters did not exceed 1 μg/kg, whereas charcoal-grilled whole meat samples contained 2.3–6.1 μg/kg. Frying or electric broiling of frankfurters did not lead to any appreciable increase of the original trace levels. Extracts from flame-grilled frankfurters were mutagenic to *Salmonella typhimurium TA 100* after metabolic activation.

The formation of mutagenic and carcinogenic substances during the cooking of food is currently the subject of intensive research (Sugimura and Nagao, 1979, 1982; Powrie et al., 1982). It is well-known that grilling food can lead to the production and uptake of polycyclic aromatic hydrocarbons (PAH), some of which are potent carcinogens. The presence of PAH in smoked food is also well documented [Toth and Blaas (1972a,b), Fritz and Soós (1977), Potthast (1978), Larsson (1982a), and many others]. PAH, particularly benzo[a]pyrene (BaP), have been suggested as one, among other, etiological factor in large bowel cancer (Hecht and La Voie, 1981) and stomach cancer (Soós, 1980). Many of the studies on PAH in grilled and smoked food have concentrated on the determination of BaP only. This substance has served as an arbitrary indicator of the possible presence of other PAH and has often been used as a quantitative index of chemical carcinogens in foods. In the Federal Republic of Germany a 1 μg/kg limit for BaP in smoked meat products has been in force since 1973.

Systematic investigations on PAH in grilled meat were first carried out by Lijinsky and Ross (1967). Their results showed that the levels of PAH in charcoal-grilled meat was dependent on the fat content and the closeness of the meat to the heat source. They explained their findings by the theory that melted fat from the heated meat drips onto the hot coals and is pyrolyzed, giving rise to the formation of PAH, which are then deposited on the meat surface as the smoke rises. Several reports (Fritz, 1973; Toth and Blaas, 1973; Doremire et al., 1979) confirm that charcoal-grilled lean meat products contain lower levels of PAH (less than 1 μg of BaP/kg) than products with a high fat content. Lijinsky and Ross (1967) and Toth and Blaas (1973) reported that charcoal grilling of meat in a vertical grill, where the dripping of fat onto the heat source is prevented, or broiling with electricity and gas, where the heat source is above the food, resulted in only minimum contamination by PAH.

Extremely high levels of PAH (maximum 140 μg of BaP/kg) were found by Fritz (1973) in meat and bratwurst grilled over burning pine cones. The fat content did not seem to influence the PAH level in this case. Fritz stated that the contamination was instead due to incomplete combustion of the fuel itself. Work by Toth and Blaas (1973) and Binnemann (1979) have confirmed that grilling over pine or spruce cones results in serious PAH contamination of the grilled product.

Food Laboratory and Toxicology Laboratory, National Food Administration, S-751 26 Uppsala, Sweden.

0021-8561/83/1431-0867$01.50/0 