indicating that these parameters had reached a plateau.

Although the liver copper level was found to decrease, as a result of RPC feeding for 4 weeks (Shah et al., 1979), a similar effect was not observed after a feeding period of 8 or 16 weeks. The liver manganese content was not affected, and the changes in the levels of zinc and iron were not consistent. Thus the adverse effect of dietary zinc on liver copper stores reported by O'Dell et al. (1976) in rats and by Omole and Bowland (1974) and Ivan and Grieve (1975) in Holstein calves was not observed by us, either in the rats fed casein or RPC with a zinc supplement of 150 μg/g diet, in the presence of about 6 μg/g of added copper. Anderson et al. (1976) suggested that feeding a zinc supplemented diet containing rapeseed flour had an adverse effect on the metabolism of iron and manganese. Although the level of iron in the liver and testes was less than the controls at 8 weeks, a similar difference was not seen at 16 weeks. There was no difference in the liver manganese stores at either time. This could indeed be explained by the observation of Ivan and Grieve (1976), that zinc supplementation did not affect net absorption of manganese in Holstein calves.

The changes in zinc, iron, and copper levels in the testes of the rats, due to RPC feeding, were not consistent. The slight adverse effect of dietary zinc on the copper content of the testes reported by O'Dell et al. (1976) was also not observed by us.

From 8 to 16 weeks the iron levels in liver and testes increased significantly in all the groups and the copper content of the testes showed a similar trend. Interestingly, the magnesium content of the femur decreased slightly but significantly from 8 to 16 weeks, whereas the levels of zinc and calcium did not change appreciably.

Zinc supplementation of the diet containing casein affected calcium and magnesium level in serum and iron in testes at 8 weeks, but at 16 weeks no significant differences were noted.

Thus a zinc supplement of 150 μg/g to a rat diet containing 20% protein from RPC was sufficient to completely overcome the symptoms of zinc deficiency and did not have any adverse effects on the tissue levels of zinc, iron, copper, manganese, calcium, and magnesium. Whether the enlargement of thyroids would persist when Tower RPC with reduced glucosinolates is included in the diet remains to be determined.

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Compounds Produced by the Reaction of 2-Hydroxy-3-methyl-2-cyclopenten-1-one with Ammonia and Hydrogen Sulfide

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The compounds produced from three model browning systems [2-hydroxy-3-methyl-2-cyclopenten-1-one (cyclotene)/NH_3, cyclotene/H_2S, and cyclotene/NH_3/H_2S] were isolated and identified. The main constituents of these model browning systems were sulfur- and nitrogen-containing heterocyclic compounds, which included a thiol, pyrazines, cyclic ketones, and cyclic methylene polysulfides.

The browning reactions play an important role in determining the acceptance of processed and stored foods (Lea, 1950; Shallenberger et al., 1959; Pomeranz et al., 1962). Compounds formed during heat treatment of food constituents have been determined either using browning model systems to elucidate the formation mechanisms (Rizzi, 1972, 1974; Shibamoto and Bernhard, 1977) or through analyses of cooked foods to determine the conditions of formation (Stoll et al., 1967; Watanabe and Sato, 1971; Walradt et al., 1971). The browning reactions produce tremendous numbers of chemicals which range from highly volatile compounds (e.g., formaldehyde, methanol,

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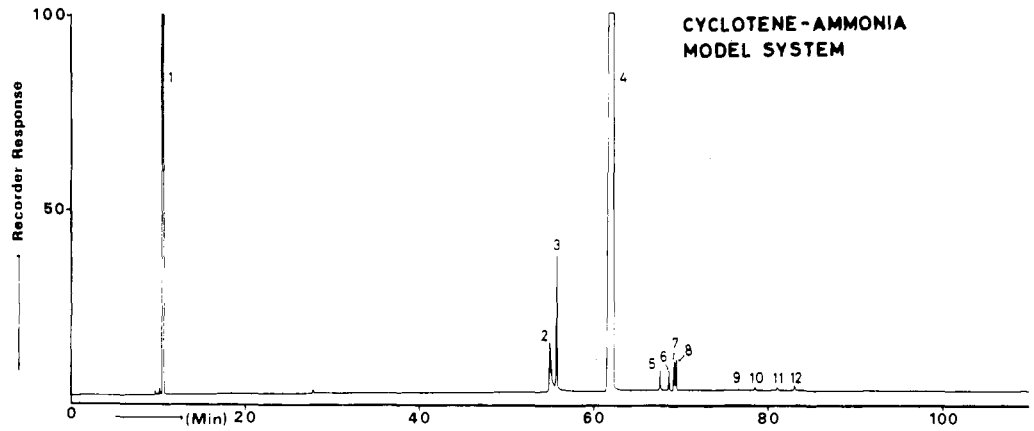

Figure 1. Gas chromatogram of volatiles formed by cyclotene/ammonia model system. For chromatographic conditions see Experimental Section. For peak identification see Table I.

acetone, etc.) to polymolecular compounds (e.g., complex brown pigments). Recently, the products formed by browning reactions have attracted much attention from the viewpoint of flavor chemistry. Certain compounds produced from browning reactions have already been used as flavor additives (pyrazines, thiazoles, pyrroles, etc.). In order to learn more about browning products, we studied some reaction products of cyclotene/ammonia/hydrogen sulfide browning model systems.

EXPERIMENTAL SECTION

Experiment 1: Reaction of 2-Hydroxy-3-methyl-2-cyclopenten-1-one (I) with Ammonia. Compound I (0.1 mol) was dissolved into 50 mL of deionized water in a Kjeldahl flask. Ammonium hydroxide solution (0.5 mol as NH_3) was then added. The neck of the flask was flame-sealed and the flask placed in an oven at 90 °C for 5 h.

Experiment 2: Reaction of I with Hydrogen Sulfide. Compound I (0.1 mol) was dissolved into 100 mL of deionized water in a Kjeldahl flask and hydrogen sulfide gas was bubbled through this solution at 0 °C for 10 min (ca. 0.02 mol of H_2S). The neck of the flask was flame-sealed and the flask placed in an oven at 90 °C for 5 h.

Experiment 3: Reaction of I with Ammonia and Hydrogen Sulfide. Compound I (0.1 mol) was dissolved into 50 mL of deionized water and hydrogen sulfide gas was bubbled through this solution at 0 °C for 10 min (ca. 0.01 mol of H_2S). Ammonium hydroxide solution (0.5 mol as NH_3) was then added to the above solution. The neck of the flask was flame-sealed and the flask placed in an oven at 90 °C for 5 h.

Sample Preparations for GC/MS Analysis from the Three Reaction Mixtures. The reaction solutions from the above three experiments were each extracted with 200 mL of methylene chloride using a liquid–liquid continuous extractor for 16 h. The methylene chloride solutions were dried over anhydrous magnesium sulfate and concentrated to yield 0.5 g each of brown liquid. The identification of products was conducted with GC/MS technique.

Analysis of Reaction Products. Identification of gas chromatographic peaks of reaction mixtures was made by comparison of their mass spectra and gas chromatographic retention indices to those of authentic compounds. A Hewlett-Packard Model 5710-A gas chromatograph equipped with a flame ionization detector, modified for capillary analyses, and 50 m × 0.28 mm i.d. glass capillary column coated with Carbowax 20M was used. The column temperature was programmed from 80 to 200 °C at 2 °C/min. The gas chromatograph was fitted with an all-glass injector splitter of our own design to avoid any contact with metal surfaces and was operated with an injector split ratio of 100:1. The injector temperature was 250 °C. Peak areas were integrated using a Hewlett-Packard Model 3385-A automation system combined with the above gas chromatograph. A Hitachi Model RMU-6M combination mass spectrometer–gas chromatograph (Hitachi Model M-5201) equipped with Hitachi Model M-6010 and 10 II/A data system was used under the following conditions: ionization voltage, 70 eV; emission current, 80 μA; ion accel voltage, 3100 V; ion source temperature, 200 °C. The gas chromatographic column and oven conditions were as described for the Hewlett-Packard instrument.

Some major peaks which could not be identified by the above method were trapped in semicapillary tubes (20 cm × 1 mm i.d.) cooled with dry ice using a Perkin-Elmer Model 900 gas chromatograph equipped with a thermal conductivity detector and a glass column (2 m × 4 mm i.d.) packed with 5% Carbowax 20M on Chromosorb W (60/80). The column temperature was programmed from 80 to 200 °C at 3 °C/min. The trapped materials were identified by comparison of their IR and NMR spectra, in conjunction with their mass spectra and GC retention indices. The IR spectra were obtained with a Hitachi Model EPI-G3 grating infrared spectrophotometer. The NMR spectra were obtained with a Hitachi Model R-20A magnetic resonance spectrometer (60 MHz) in deuteriochloroform with tetramethylsilane as an internal standard.

Isolation of 1,5(or 7)-Dimethyl-1,2,3,5,6,7-hexahydrodicyclopentapyrazine. The brown material obtained from experiment 1 by the method described in the section "Sample Preparations for GC/MS Analysis" was entirely dried to a solid under reduced pressure. The dark-brown solid obtained was recrystallized from petroleum ether. A pale-yellow solid was obtained and identified as 5-imino-2-methylcyclopenten-1-one: NMR ($CDCl_3$) δ 1.90 (3 H, s, $-CH_3$), 2.33 (4 H, s, $-CH_2$), 3.35 (2 H, broad s, –OH and =NH); IR 3425, 3330, 2920, 2837, 1695, 1595, 1400, 1360, 1313, 1258, 1210, 1120 cm^{-1}; MS 112 (14), 111 (100), 110 (18), 96 (21), 83 (53), 82 (95), 69 (12), 68 (57), 67 (7), 66 (11), 57 (7), 56 (18), 55 (83), 54 (38), 43 (33), 42 (35), 41 (10).

The petroleum ether filtrate was evaporated and pale-brown material was obtained. The material was purified by TLC (Meric silica gel, solvent: hexane/ethyl acetate, 3:1, R_f ca. 0.3) and identified as the two pairs of diastereomers; 1,5(or 7)-dimethyl-1,2,3,5,6,7-hexahydrodicyclopentapyrazine: NMR ($CDCl_3$) δ 1.35 (6 H, d, J = 7 Hz, $-CH_3$), 2.10 (4 H, m, CH–CH_2), 2.98 (4 H, t, C–CH_2), 3.2 (2 H, m, –CH); IR 2962, 2925, 2860, 1465, 1458, 1437, 1375, 1330, 1293, 1225, 1180, 1155, 1070 cm^{-1}; MS 189 (9), 188

Table I. Compounds Identified from Cyclotene, NH_3 or H_2S, and NH_3 and H_2S Model Browning Systems

compound	I_u[a]	I_k[b]	experiment 3 (Figure 3) peak no.	area %	experiment 1 (Figure 1) peak no.	area %	experiment 2 (Figure 2) peak no.	area %	odor description	MS references
solvent (CH_2Cl_2)			1		1		1			
2-methylcyclopentanone	1174	1177	2	0.24			2	0.30	roasted beef	Stenhagen et al. (1974a)
3-methylcyclopentanone	1197	1194	3	0.05			3	c	roasted beef	Stenhagen et al. (1974b)
1,2,4-trithiolane	1687	1695	4	0.11			4	0.64	roasted beef	Morita and Kobayashi (1967)
s-trithiane	2115	2123	16	0.20			7	c	sulfurous	Stenhagen et al. (1974c)
1,2,4,6-tetrathiepane	2360	2368					8	0.85	onion-like	Morita and Kobayashi (1967)
thioformaldine (tentative)	1876		9	0.07					rubber-like	
2-hydroxy-3-methyl-2-cyclopenten-1-one	1750	1750	5	6.64	2	2.22	5	96.14	sweet burnt	Flament et al. (1976)
5-imino-4-methylcyclopenten-1-ol	1751	1756	6	0.86	3	1.92			rice cracker	Flament et al. (1976)
5-imino-2-methylcyclopenten-1-ol	1866	1870	8	85.57	4	94.27			cooked rice	Flament et al. (1976)
1-mercapto-2-methylcyclopenten-5-one	1760	1766	7	0.07			6	0.31	seaweed-like	
1,5(or 7)-dimethyl-1,2,3,5,6,7-hexahydrodicyclopentapyrazine	1962		10	1.04	5	0.26			roasted bean	
	1980		11	1.01	6	0.26			roasted bean	
	1994		12	1.48	7	0.41			roasted bean	
	1998		13	1.52	8	0.42			roasted bean	
dimethyltetrahydrodicyclopentapyrazine (tentative)	2106		14	0.10	9	0.01			roasted bean	
	2125		15	0.38	10	0.05			roasted bean	
	2156		17	0.03	11	0.06			roasted bean	
	2181		18	0.30	12	0.08			roasted bean	

[a] Kovats Index of unknowns. [b] Kovats Index of authentic sample. [c] Peak area percent was less than 0.01.

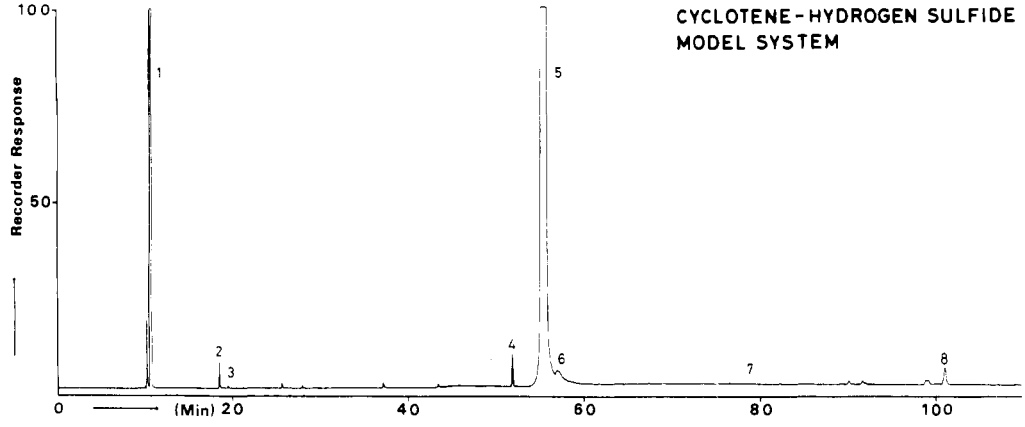

Figure 2. Gas chromatogram of volatiles formed by cyclotene/hydrogen sulfide model system. For chromatographic conditions see Experimental Section. For peak identification see Table I.

(52), 187 (8), 174 (24), 173 (100), 160 (7), 158 (10), 76 (6), 65 (13).

Isolation of 1-Mercapto-2-methylcyclopenten-5-one. The pH of the reaction solution obtained from experiment 2 was adjusted to 1 with hydrochloric acid. This acidic solution was extracted with 500 mL of diethyl ether, which was then evaporated off under reduced pressure. The brown solid obtained was recrystallized from petroleum ether. The recrystallization was repeated several times and white needles were obtained. This material was identified as 1-mercapto-2-methylcyclopenten-5-one: NMR ($CDCl_3$) δ 2.11 (3 H, s, $-CH_3$), 2.55 (4 H, s, $-CH_2$), 3.60 (1 H, s, SH); IR 2960, 2900, 2520, 1698, 1613, 1431, 1410, 1378, 1310, 1285, 1200, 1162, 980, 855, 812, 665, 603 cm^{-1}; MS 130 (5), 129 (9), 128 (100), 113 (13), 100 (30), 99 (23), 95 (9), 87 (6), 86 (9), 85 (81), 72 (20), 71 (17), 67 (26), 66 (7), 65 (9), 59 (8), 55 (9), 45 (8), 41 (9).

RESULTS AND DISCUSSION

The compounds identified from the model systems consisting of 2-hydroxy-3-methyl-2-cyclopenten-1-one (cyclotene), ammonia or hydrogen sulfide, and ammonia and hydrogen sulfide and their odor descriptions are listed in Table I. Their gas chromatograms are shown in Figures 1–3. Cyclotene has been known as a product of sugar degradation or caramelization (Hodge, 1967). It is also

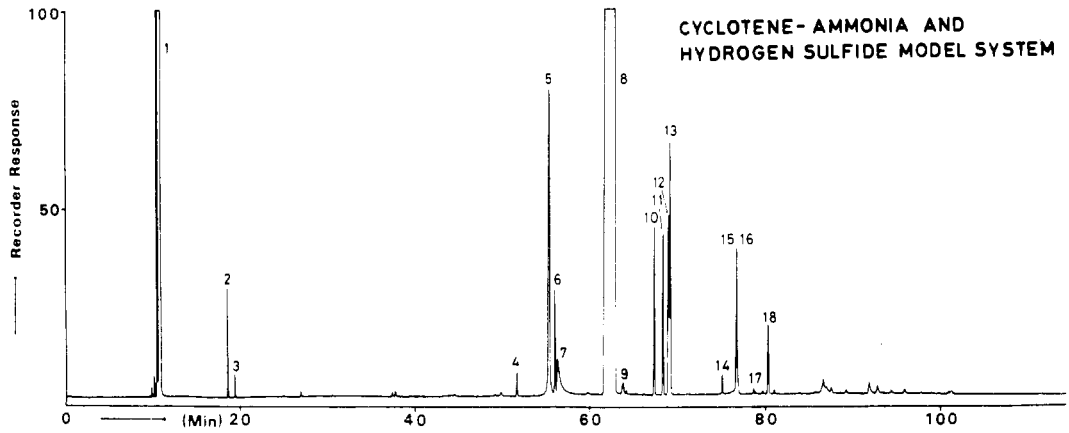

Figure 3. Gas chromatogram of volatiles formed by cyclotene/ammonia/hydrogen sulfide model system. For chromatographic conditions see Experimental Section. For peak identification see Table I.

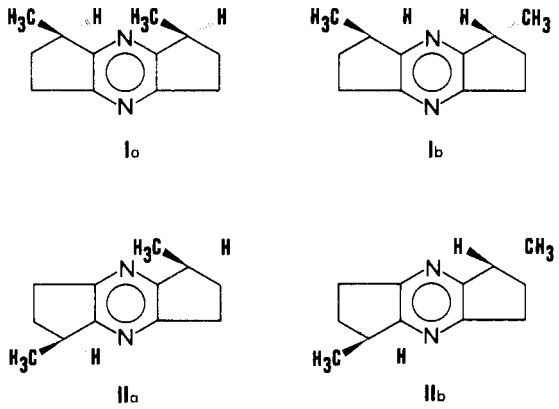

Figure 4. Possible structures of dimethyl-1,2,3,5,6,7-hexahydrodicyclopentapyrazine. Ia and Ib: diastereomer of 1,7-dimethyl-1,2,3,5,6,7-hexahydrodicyclopentapyrazine. IIa and IIb: diastereomer of 1,5-dimethyl-1,2,3,5,6,7-hexamethyldicyclopentapyrazine.

used for coffee flavor to give a roasted sweet taste. van den Ouweland and Peer (1975) obtained 15 sulfur-containing compounds from the reaction of 4-hydroxy-5-methyl-3(2*H*)-furanone, which was identified in natural beef broth (Tonsbeek et al., 1968), and hydrogen sulfide. Reaction products of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone [sugar degradation product and also isolated from pineapple; Rodin et al. (1965)]–hydrogen sulfide model system have been patented as a meat flavor (van den Ouweland and Peer, 1968). The reaction mixture obtained from the cyclotene–hydrogen sulfide model system gave a somewhat meaty aroma and contained some chemicals which gave a meatlike odor (refer to Table I). The formation of cyclic methylene polysulfides (1,2,4-trithiolane, *s*-trithiane, and 1,2,4,6-tetrathiepane) indicates that cyclotene fragmented into a small number of carbon units [formaldehyde; Yasumoto et al. (1971), Minor et al. (1965)] and reacted with hydrogen sulfide. The reaction of cyclotene and ammonia produced 5-imino-2-methylcyclopenten-1-ol in large amounts (gas chromatographic peak area percent: 94.27). Walradt et al. (1971) identified some 6,7-dihydro-5*H*-cyclopentapyrazines in roasted peanuts and postulated cyclotene as a precursor of this cyclopentapyrazine. Shibamoto et al. (1979) also identified 5-imino-2-methylcyclopenten-1-ol in their D-glucose–ammonia model system and suggested that cyclotene could be a precursor of cyclopentapyrazines. The above model system also produced some tricyclic pyrazines which were also formed in the reaction of cyclotene and DL-alanine (Rizzi, 1972) or ammonia and diacetyl (Flament et al., 1976). Four peaks at peak numbers 10, 11, 12, and 13 in Figure 3, or 5, 6, 7, and 8 in Figure 1 were identified as dimethyl-1,2,3,5,6,7-hexahydrodicyclopentapyrazines. Their possible structures are shown in Figure 4. The four peaks at peak number 9, 10, 11, and 12 in Figure 1 were tentatively identified as dimethyltetrahydrodicyclopentapyrazines. All those compounds give an interesting roasted beanlike odor.

The reaction system consisting of cyclotene, hydrogen sulfide, and ammonia produced more or less the same mixture of compounds produced in the two previous model systems. We could not, however, positively identify any nitrogen- and sulfur-containing compound in the same molecule. Thioformaldine, which is an analogue of thialdine [found in beef broth by Brinkman et al. (1972)] was tentatively identified. Unfortunately, an authentic sample of thioformaldine was not available to confirm the identity.

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Sinalbin and Other Glucosinolates in Seeds of Double Low Rape Species and *Brassica napus* cv. Bronowski

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The content of glucosinolates in seeds of some double low rape cultivars has been investigated. Using a newly developed method, the glucosinolates have been isolated, separated, and determined semiquantitatively. The identification has been performed by chromatography, high-voltage electrophoresis, ^{1}H- and ^{13}C-NMR spectroscopy, and by gas chromatography–mass spectroscopy. Sinalbin (4-hydroxybenzyl glucosinolate) is one of the dominating glucosinolates in seeds of cv. Erglu rape, cv. Tower, cv. Candle, and some other double low rape species, whereas it is not found in appreciable amounts in seeds of other double low rape cultivars and *Brassica napus* cv. Bronowski. The presence of relatively high concentrations of sinalbin and indolylmethyl glucosinolates in seeds of some double low rape cultivars is previously unreported. This is briefly discussed in relation to the methods used for identification and semiquantitative estimation of glucosinolates and in relation to glucosinolate catabolism and the nutritional value of rape seeds.

The seeds of some cruciferous plants (e.g., rape) are economically important as they are used both as food and plant oil sources. High contents of erucic acid in the oil and of glucosinolates in the meal are the reason for some unfavorable qualities. Therefore, a lot of plant breeding efforts have been placed in the production of rape cultivars (double low cultivars), giving seeds with a relative low content of glucosinolates and erucic acid compared to that found in other varieties of rapeseed commonly used.

The problems concerning glucosinolates in food are related both to the total amount, to the type of glucosinolates present, and to the products produced from them by autolysis or other degradation. All glucosinolate-containing plants also seem to contain β-thioglucosidases, EC 3.2.3.1. (myrosinases), in a separate compartment (Bjørkman, 1976). When the plant is crushed the thioglucosides are hydrolyzed by the liberated enzymes, and during the autolysis process a Lossen-type rearrangement may occur with the formation of isothiocyanates (Ettlinger and Lundeen, 1956). Within some cruciferous plants autolysis leads to other products such as thiocyanates (Gmelin and Virtanen, 1962), cyanides and amines (Saarivirta, 1973), or oxazolidinethiones and cyanoepithiolkanes (Daxenbichler et al., 1977). In strong acid solution glucosinolates are hydrolyzed to the corresponding carboxylic acids (Ettlinger and Lundeen, 1956), and in strong alkaline solution some glucosinolates are transformed to amino acids in a Neber-type rearrangement (Friis et al., 1977) (Figure 1).

Although about 80 different glucosinolates have been identified, much is unknown about autolysis and glucosinolate catabolism. However, it is well known that some glucosinolates are more easily transformed to thiocyanates and the thiocyanate ion than others (Gmelin and Virtanen, 1962; Lüthy and Benn, 1977; Nielsen et al., 1979). If glucosinolates are transformed to the thiocyanate ion, they will escape detection by the methods commonly used for glucosinolate analysis since these methods are based on the identification of common hydrolysis products (Srivastava and Hill, 1975; Wetter and Youngs, 1976).

It is known that the thiocyanate ion is produced in rapeseed meal during thioglucosidase hydrolysis (Srivastava and Hill, 1975; McGregor, 1978), and it is a well-known fact that glucosinolates influence the nutritional value of rapeseed meal (Josefsson, 1975).

The present study is a continuation of our previous work on the isolation (Nielsen et al., 1979; Olsen and Sørensen, 1979) and catabolism of glucosinolates (Dalgaard et al., 1977). It forms part of a study on the nutritional value of different double low rape cultivars, and the purpose of this study is isolation of the total pool of glucosinolates from rapeseed meal, followed by identification and quantitative estimations of the isolated glucosinolates, including the previously unknown precursors of the thiocyanate ion, which is known to be the major glucosinolates in seeds of some double low rape species (McGregor, 1978).

EXPERIMENTAL SECTION

Plant Material. Seeds of *Brassica napus* L. cv. Bronowski were purchased from State Research Station, DK-4000 Roskilde, Denmark. Seeds of the common rape, *Brassica napus* L. cv. Gulle, and the double low rape cultivars, *Brassica napus* L. cv.: Erglu, DP 075, DP 076, DP 525, DP 540, DP 666, DP 724, DP 941, and DP 2/12 were obtained from Danish Plant Breeding, Boelshøj, DK-4660 St. Heddinge, Denmark. Seeds of *Sinapis alba* L. were obtained from Trifolium-Silo A/S, DK-2630 Tåstrup, Denmark. *Brassica napus* L. cv. Tower and *Brassica campestris* L. cv. Candle were obtained from Professor L. D. Campbell, Department of Animal Science, University of Manitoba, Winnipeg, Canada.

General Methods and Instrumentation. ^{1}H NMR spectra were determined in D_2O solution at 60 MHz and chemical shifts are given here in ppm downfield from sodium 2,2,3,3-tetradeuterio-3-(trimethylsilyl)propionate

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