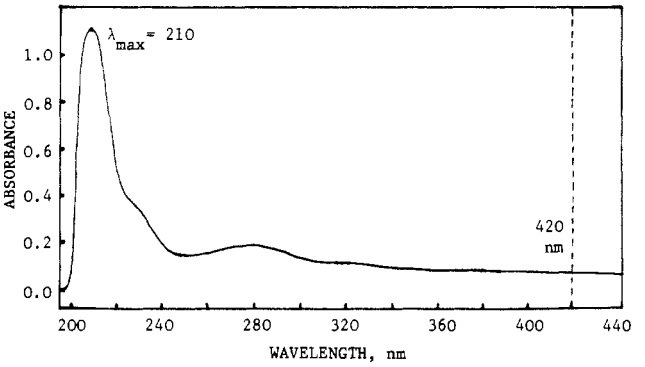

Figure 1. UV spectrum of the colored band (B band) recovered from fraction III by silica gel column chromatography (19 mg/L in distilled water).

some phenolic standards permitted us to recognize their structure. Peak 1 has been identified as *p*-hydroxyphenol (hydroquinone), whose β-glucoside, the arbutine, is a well-known derivative, already detected in plants (Pigman and Horton, 1970). Peak 2 has only one hydroxy group: the mass spectra and GLC retention time agree with 3,4-dimethoxyphenol. This compound is the major component of the aglycon mixture. We explain its high concentration due to the poor retention by the anionic-exchange reins used in processing of molasses, in consideration of the lack of free phenolic OHs; this structural feature increases also the chemical stability against oxidation. Peak 3 has a methoxy and two hydroxy groups asymmetrically disposed; peak 4 has two hydroxy and two methoxy groups; the structures proposed for these two compounds have been 3-methoxy-4-hydroxyphenol (peak 3) and 3,5-dimethoxy-4-hydroxy (peak 4).

Natural substances with similar ring substitution have already been isolated from cane and cane sugars: vanillic, veratric, *p*-hydroxybenzoic, and syringic acids have been recovered by solvent extraction (Farber and Carpenter, 1971). These products probably originate from microbial and chemical degradation of lignins during processing of cane and cane juice, even if some are already present in natural cane. The coloring characteristics of the phenolic compounds isolated was considered. We found the color in liquid sugar from cane molasses increased with time owing to the autoxidation of the phenolic glucosides. We could verify their oxidation on silica gel TLC chromatograms of the raw colored material: when exposed to air, the color intensity of the B band region increased in a few hours, changing from yellow-orange to orange-brown. We may conclude that phenolic glucosides and their oxidation products greatly contribute to the coloring of the liquid sugars examined, even if they represent only the 10–15% by weight of the isolated nonsugar components.

Other minor aglycons have been detected in the GLC profile (Scheme I), but their low concentration did not allow their identification. Our studies are now aimed at the identification of the compounds of the fraction II and at the characterization of other noncolored components, to obtain in this way a detailed and exhaustive composition of the nonsugars of this kind of syrup.

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Deuteration of Mutagenic Aromatic Nitrogen Heterocycles Derived from Protein and Amino Acid Pyrolyzates

The results are described of applications of two methods (platinum catalysis and acid catalysis) for deuterium exchange of three polycyclic aromatic amines of the type isolated from amino acid pyrolyzates. The results indicate that both methods lead to comparable total amounts of exchange when reactions are conducted for similar periods. However, the deuterium label is incorporated into several positions by the platinum catalysis method, which are unaffected by the acid catalysis method.

Several highly mutagenic polycyclic aromatic amines have been isolated from pyrolyzates of proteins and amino acids (Yamamoto et al., 1978; Yoshida et al., 1978; Kosuge et al., 1978; Wakabayashi et al., 1978). Preparation of radiolabeled derivatives of these compounds is desirable to facilitate the many chemical and biological studies anticipated for these substances. A method for deuterium and tritium labeling of a product of tryptophan pyrolysis has been described (Hashimoto et al., 1978). As models for their tritiation reactions we report here the application of two simple methods of deuteration of a soybean globulin pyrolyzate product (Yoshida et al., 1978), 9*H*-1,9-diazafluoren-2-amine (compound **2**), the corresponding parent compound, 9*H*-1,9-diazafluorene (compound **1**), and a

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Table I. Deuterium Content Based on Mass Spectroscopy

compound	method	reaction time, h	estimated total deuterium content, %
1	A[a]	7	6
		22	28
	B[b]	7	10
		22	29
2	A	5	11
		17	25
		17[c]	16
	B	7	>15[d]
		22	26
3	A	2	0
		20[e]	11
		44[f]	20

[a] D_2SO_4 catalyzed. [b] Platinum catalyzed. [c] A 17-h deuterium exchange reaction followed by a 2-h reflux in pH 1.2 HCl (aqueous). [d] Deuterium content based on NMR peak areas. [e] The 2-h reaction product treated for an additional 18 h in 4 M D_2SO_4. [f] The 20-h reaction product treated for an additional 24 h in 7 M D_2SO_4.

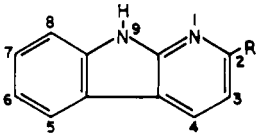
compound present in glutamic acid pyrolyzates, 8-methyl-4,4b,9-triazafluoren-3-amine (compound **3**). The structures and synonyms for these compounds are presented in Figure 1.

[^{2}H]Sulfuric acid is used in the first method as a homogeneous catalyst under aqueous reaction conditions. The second method uses powdered platinum metal in a sealed heterogeneous system containing deuterium oxide and deuterated acetic acid as cosolvents. The extent and positions of deuterium–hydrogen exchange were determined by ^{1}H NMR and mass spectroscopy.

EXPERIMENTAL SECTION

The soybean globulin pyrolyzate product (compound **2**) (Stephenson and Warburton, 1970; Matsumoto et al., 1979) and the glutamic acid pyrolyzate product (compound **3**) (Takeda et al., 1978) were prepared following published syntheses. Compound **1** is a precursor to **2** in the synthetic sequence used.

NMR data (Varian EM-390) (measured at 90 MHz with tetramethylsilane as the internal standard) were as follows: for compound **1** (CD_3COOD), δ 7–7.5 (m, 4, C_3H, C_6H, C_7H, and C_8H), 7.8 (d, 1, $J_{5,6}$ = 7 Hz, C_5H), 8.2 (d, 1, $J_{2,3}$ = 6 Hz, C_2H), 8.3 (d, 1, $J_{3,4}$ = 8 Hz, C_4H); for compound **2** (CD_3COOD), δ 6.4 (d, 1, $J_{3,4}$ = 9 Hz, CH_3), 7–7.3 (m, 2, C_6H and C_7H), 7.5 (dd, 1, $J_{8,7}$ = 6 Hz, $J_{8,6}$ = 2 Hz, C_8H, 7.7 (dd, 1, $J_{5,6}$ = 6 Hz, $J_{5,7}$ 2 Hz, C_5H), 8.2 (d, 1, $J_{4,3}$ = 8 Hz, C_4H); for compound **3** (HCl salt in CD_3OD), δ 2.6 (s, 3, C_3H), 6.9 (d, 1, $J_{2,1}$ = 9 Hz, C_2H), 7.4 (dd, 1, $J_{6,7}$ = $J_{6,5}$ = 7 Hz, C_6H), 7.8 (m, 2, C_1H and C_7H), 8.6 (d, 1, $J_{5,6}$ = 7 Hz, C_5H); for compound **3** (CD_3OD), δ 6.4–6.7 (m, 2, C_6H and C_2H), 6.9 (d, 1, $J_{7,6}$ = 7 Hz, C_7H), 7.7 (d, 1, $J_{1,2}$ = 8 Hz, C_1H).



1, R = H
2, R = NH_2

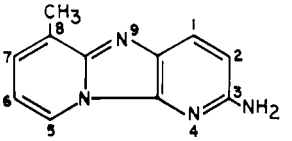


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Figure 1. Structures of compunds: **1**, 9*H*-1,9-diazafluorene (synonyms: 9*H*-pyrido[2,3-*b*]indole, α-carboline); **2**, 9*H*-1,9-diazafluoren-2-amine (synonyms: 9*H*-pyrido[2,3-*b*]indol-2-amine, α-carbolin-2-amine); **3**, 8-methyl-4,4b,9-triazafluoren-3-amine (synonyms: 6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazol-2-amine, Glu-P-1).

Mass spectral data, *m/z* (rel intensity), were as follows: for compound **1**, 168 (100); for compound **2**, 183 (100), 156 (20), 155 (19), 129 (11); for compound **3** (HCl salt), 198 (53), 144 (44), 74 (70).

Method A. Typically, 30 mg of compound is dissolved in D_2SO_4–D_2O (3 mol/L) and heated at reflux (about 120 °C) for 18–24 h. After being cooled, the solution is made basic with concentrated NaOH and the precipitate is collected. The precipitate may be washed with dilute HCl to remove labile δ.

Method B. Following the procedure of Calf and Garnett (1964), the platinum catalyst (typically 8 mg) is activated by slowly adding sodium borohydride (30 mg) to a suspension of $PtO_2 \cdot 2H_2O$ in D_2O (1 mL), followed by warming to 70 °C, washing with D_2O to remove salts, and decanting. To the fine precipitate of active catalyst (typically 8 mg) in a vacuum hydrolysis tube (Kontes No. K-896860), 20 mg of compound is added, dissolved in D_2O–CD_3COOD (1:1 v/v). The final reaction volume is about 1 mL. The solution is degassed by three freeze–thaw cycles at about 130 Pa, and the vacuum hydrolysis tube is sealed and heated at about 150 °C for 20–24 h. After the solution is cooled, excess dilute HCl (aqueous) is added to the tube and the catalyst is filtered off. Acetic acid rinses are combined with the reaction solution, and the solvent is removed under rotary evaporation.

RESULTS AND DISCUSSION

The total deuterium content measured after different reaction times is presented in Table I. The deuterium content and distribution in the labeled products are indicated in Table II. Also shown is the order of positions

Table II. Deuterium Content and Distribution and Sites of Deuteration

compound	method	reaction time, h	deuterium distribution, %[a]							decreasing order of positions which were preferably exchanged[b]
			d_0	d_1	d_2	d_3	d_4	d_5	d_6	
1	A[c]	7	60	33	7	0.5				(6, 8)
		22		6	67	24	3			(6, 8) (3)
	B[d]	7	45	35	15	4	1			(2) (3, 6)
		22	6.5	19	29	28	16	2	0.1	(2) (3, 6, 7, 8)
2	A	5	14	75	10	0.3				(3)
		17	3	17	41	31	8	1		(3) (6)
		17[e]	11	44	35	9	1			(3) (back-exchange)
	B	7								(3) (6, 7)
		22	2	13	42	33	9			(3) (5, 6, 7)
3	A	20[f]								(2) (5)
		44[g]								(2) (5, 6)

[a] Calculated from the MS relative peak abundances for the molecular ions corrected for contributions due to naturally occurring isotopes. [b] Figures grouped into parentheses show approximately equal amounts of exchange as indicated by NMR spectroscopy. [c] D_2SO_4 catalyzed. [d] Platinum catalyzed. [e] See footnote *c* in Table I. [f] See footnote *e* in Table I. [g] See footnote *f* in Table I.

preferably exchanged as determined by the ^1H NMR coupling patterns.

The results in Tables I and II show that although the overall amounts of deuterium incorporated with either method are similar, the platinum-catalyzed exchange gives a distribution of labeling which is less specific than the acid-catalyzed reaction. Thus, higher levels of multiple exchange are observed with the platinum method than with the acid catalysis method. This may be an advantage when a labeled compound is to be used in biological experiments where the lability of the isotopic label is of concern. For example, under the back-exchange conditions noted in Table I (footnote *c*), the label at position 3 of compound **2** can be removed, thus eliminating the possibility of loss of label from this position under biological conditions. The additional deuterium introduced at positions 5 and 7 by the platinum-catalyzed exchange would thus somewhat compensate for the lost label at position 3. The platinum method is also useful in incorporating isotopic label at positions which are unaffected by acid catalyzed exchange conditions. Thus, positions 2 and 7 of compound 1 and positions 5 and 7 of compound **2** are exchanged by the platinum method but not under acid catalysis.

An additional consideration in comparing the two methods is the stability of the substrate toward the reaction conditions. In these experiments it was noted that the products from the more prolonged, sulfuric acid catalyzed reactions with compounds **2** and **3** showed some evidence of side reactions on the basis of the appearance of peaks of minor impurities in the NMR spectra. There was no evidence for the production of side products during the prolonged platinum catalysis reactions.

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Fractionation and Study of Chemistry of Pectic Polysaccharides

The heterogeneous nature of grapefruit (*Citrus paradisi*) pectin was investigated by employing ion-exchange chromatographic techniques. Four chemically distinct pectic polysaccharides were resolved. These polysaccharides eluted sequentially with 0.025, 0.1, 0.25, and 0.5 M sodium phosphate buffer, pH 6.0, and comprised 13, 5, 62, and 20%, respectively, of the total pectin subjected to ion-exchange chromatography. No striking differences in the galacturonic acid content, which ranged from approximately 70 to 80% of these pectic polysaccharides, were observed. However, the neutral sugar composition of these pectic polysaccharides was found to be different. These studies affirm the heterogeneous nature of pectin and suggest that meaningful evaluation of the dietary role of pectin could only be achieved by being aware of this heterogeneity.

Experimental findings of several investigators consistently suggest that pectin when supplemented in the diet causes lowering of the serum cholesterol level in man as well in several laboratory animals (Kay and Truswell, 1977; Mokady, 1973; Lin et al., 1957). In view of the heterogeneous nature of pectin, it is possible that, of a number of polysaccharides found in pectin, a single pectic polysaccharide may be uniquely responsible for the said nutritional role of dietary pectin (Baig and Cerda, 1980). It follows, therefore, that the fractionation of dietary pectin and subsequent nutritional studies on individually resolved pectic polysaccharides of known physicochemical characteristics may result in the elucidation of the elusive biochemical basis by which dietary pectin causes lowering of cholesterol levels. Described in this report are the fractionation and chemical characterization of several pectic polysaccharides found in commercially prepared grapefruit pectin. These studies serve as a stepping stone toward our understanding of the biochemical basis by which pectin and its various polysaccharidic components may cause lowering of cholesterol levels.

MATERIALS AND METHODS

Grapefruit pectin was a gift from Lykes-Pasco Co., Dade City, FL. This commercially available pectin was extracted from the grapefruit albedo by first working the albedo with deionized distilled water, followed by extraction of pectin from water-washed albedo with a hot (176 °F) solution of HCl, pH 1.6, for 45 min. The pectin thus extracted with acid was recovered by precipitation with 70% (v/v) isopropyl alcohol. Before use in experiments described in this report, this commercially available pectin was reprecipitated 3 times by using 70% (v/v) ethanol, dialyzed against deionized distilled water, and lyophilized.

DE-52 Diethylaminoethylcellulose Ion-Exchange Chromatography. A column (1.5 × 24 cm) was equilibrated with 0.025 M sodium phosphate buffer, pH 6.0, and 250 mg of pectin dissolved in 100 mL of equilibrating

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