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Note

Thermal decomposition of pectic substances

AKIO OHNISHI ERIKO TAKAGI AND KUNIO KATŌ

Central Research Institute The Japan Tobacco & Salt Public Corporation 6-2 Umegaoka Midori-ku Yokohama 227 (Japan)

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Despite the great importance of pectic substances in the food industry, little is known about their behavior on thermal decomposition except for the pyrolytic decarboxylation first reported by Perlin[1]

Our previous papers[2–4] reported some results of thermal decomposition of neutral polysaccharides by c p p–g l c (Curie-point pyrolysis, gas–liquid chromatography) This paper deals with the pyrolysis of pectic substances isolated from tobacco leaves these substances are rich in uronic acids and their methyl esters as sugar residues

RESULTS AND DISCUSSION

Isolation and characterization of pectic substances — Pectic substances were isolated from tobacco leaves by oxalate extraction followed by fractionation on DEAE-cellulose (Table I) The PA3 fraction which is the major component, was used as a pyrolysis sample in the following experiments

Dynamic thermal analysis — The result of d t a of PA3 in helium carrier-gas is shown in Fig 1 The t g (thermogravimetric) curve shows a weight loss over the temperature range 200–300° followed by a slow constant weight-loss The d t g (derivative thermogravimetric) curve shows a peak at 257°, corresponding to major decomposition and subsequent volatilization In contrast, the d s c (differential-scanning calorimetric) curve shows no remarkable change between 130–500°, except for a very small, broad endotherm peak near 247° These patterns are very similar to those exhibited by xylans[4,5] but the maximum decomposition-temperature is lower than that of xylans by ~42°, although the decomposition starts at about the same temperature (~200°) Also, PA3 left more charred residue (35% at 400°) than did the xylans [27 (ref 4) and ~16 (ref 5) % at 400°]

The t g , d t g , and d s c curves of PA3 that had been de-esterified with sodium hydroxide, denoted as PA3-DE, changed as shown in Fig 2 the decomposition shifted toward lower temperature (the d t g peak is at 248°) and the yield of charred residue increased (43% at 400°) This earlier initiation of decomposition and in-

TABLE I

ANALYTICAL RESULTS OF FRACTIONS FROM DEAE-CELLULOSE COLUMN CHROMATOGRAPHY

Fraction	*Eluted by*	*Yield*[a] (%)	$[\alpha]_D^{23}$[b] (°)	*Sugar component* (%)						*Ds*[d] (%)	*N* (%)
				GalA	*Rha*	*Ara*	*Gal*	*Xyl*	*Glc*		
PA1	water	trace	+128	nd[c]	nd	nd	nd	nd	nd	nd	nd
PA2	0 25M buffer (pH 5 5)	14 8	+225	76 0	1 1	trace	1 9	trace	trace	62 0	0 00
PA3	0 50M buffer (pH 5 5)	60 5	+239	91 9	1 8	trace	1 2	trace	trace	41 2	0 00
PA4	0 1M sodium hydroxide	21 3	+192	98 7	trace	trace	trace	trace	trace	—	0 00
PA5	0 5M sodium hydroxide	1 8	+138	35 4	trace	trace	2 2	trace	trace	—	0 55

[a]Based on the weight of material used [b]Measured in water (*c* 0 5) [c]Not determined [d]Degree of methyl-esterification of D-galactopyranuronic acid residues in the polysaccharide molecule

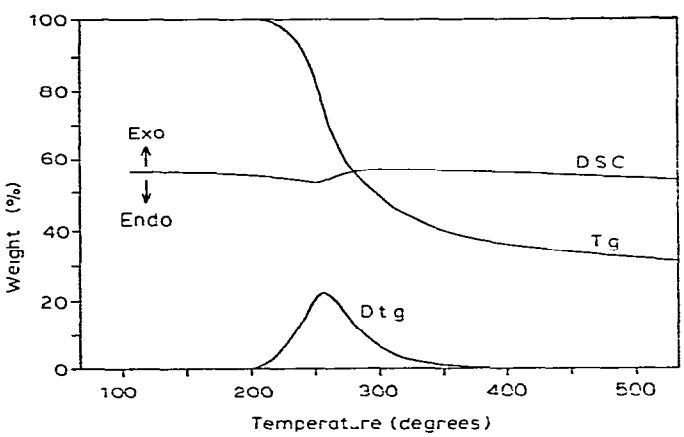


Fig 1 Thermogram of fraction PA3

creased charring could be caused by small proportions of sodium ion remaining in the substrate The sodium content (0 052%) could not be decreased further by conventional procedures The presence of such inorganic ions is known to influence pyrolytic reactions[6]

When the pectic acid, PA3-DE, was re-esterified with diazomethane, the decomposition of the resultant material, PA3-RE, shifted again toward higher temperature (the d t g -peak is at 278°) and the proportion of charred residue decreased (35% at 400°), as shown in Fig 3

Composition of the pyrolyzate — Preliminary analysis[3] of the Curie-point, pyrolysis product gave the results summarized in Table II As may be seen, the

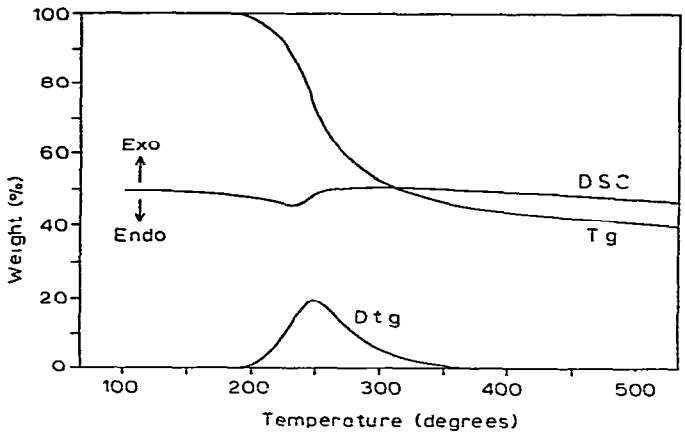


Fig 2 Thermogram of fraction PA3-DE

TABLE II

COMPOSITION OF PYROLYSATES FROM THE CURIE-POINT PYROLYSIS OF SAMPLES

Sample	*Temperature* (°)	*Component* (%)			
		Volatile	*Tar*[a]	*Residue*	*(Gas)*[b]
PA3	386	17	32	16	35
PA3-DE	386	26	35	10	29
PA3-RE	386	25	27	14	34
Cellulose	460	16	57	12	15
Xylan	423	23	55	11	11

[a]The tar component contained water produced by thermal decomposition [b]The gas content was obtained indirectly by the following calculation (Gas) = 100 − (Volatile + Tar + Residue)

proportion of gaseous component from PA3 is considerably higher than that from cellulose or xylan Both PA3-DE and PA3-RE also gave large proportions of a gaseous fraction (Table II)

Gaseous product — For analysis of gaseous decomposition-products a gas–liquid chromatograph having a Carbosieve-B column and a thermal-conductivity detector was combined with a Curie-point pyrolyzer via a cold trap at −72° The c p p–g l c chromatogram of PA3 showed that carbon dioxide was the only major compound in the gaseous fraction of the pyrolyzate (Table III) These results suggest that decarboxylation is one of the most characteristic primary reactions during the pyrolysis of pectic substances

Volatile products — For the analysis of volatile pyrolysis-products, another gas–liquid chromatograph having a Carbowax-20M column and a hydrogen flame-ionization detector was combined directly with the pyrolyzer as previously described[2] The c p p–g l c chromatograms of PA3 and PA3-DE are shown in Fig 4 The

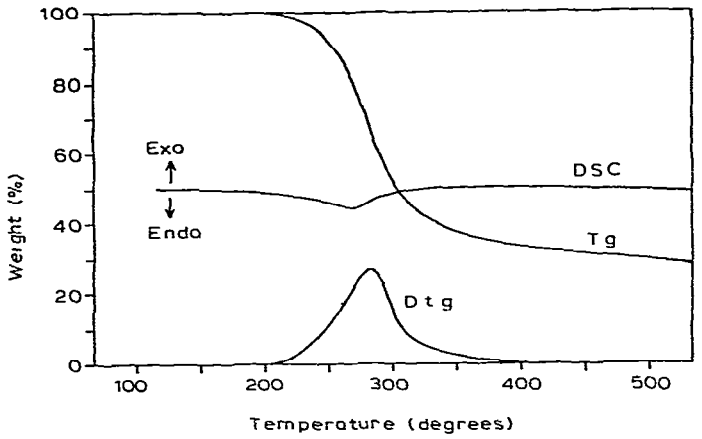


Fig 3 Thermogram of fraction PA3-RE

TABLE III

CARBON DIOXIDE FROM THE CURIE-POINT PYROLYSIS

Sample	*Temperature* (°)	CO_2 (%)
PA3	386	12 0
PA3-DE	386	12 4
PA3-RE	386	10 0
Cellulose	460	1 4
Xylan	423	2 0

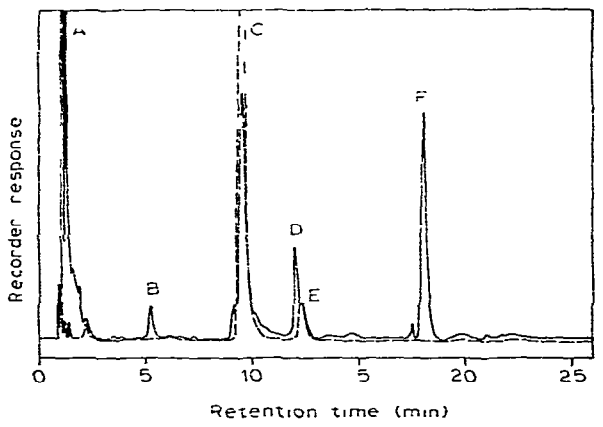
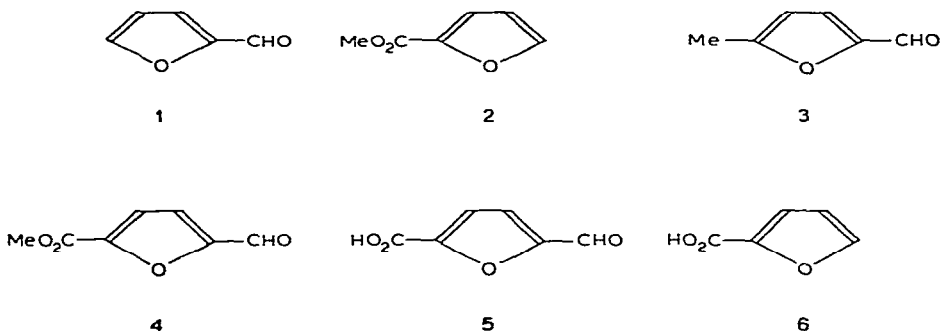


Fig 4 C p p –g l c chromatogram of fractions PA3 (solid line) and PA3-DE (dashed line)

PA3-RE fraction showed essentially the same chromatogram as that of PA3 By direct combination of a mass spectrometer to the c p p –g l c apparatus, the peak A component in Fig 4 was recognized as a mixture of furan, 2-methylfuran, and other compounds of high volatility The peak B, C, D, and E components were identified as methyl pyruvate, 2-furaldehyde (**1**), methyl 2-furoate (**2**), and 5-methyl 2-furaldehyde (**3**) by comparing their mass-fragmentation patterns and retention times with those of authentic compounds

The peak F component was collected as pale-yellow crystals by preparative c p p –g l c [2] and was subjected to spectrometric analysis From its mass-spectral and ^{1}H-n m r data and its melting point (91°, after recrystallization from water), it was identified as methyl 5-formyl-2-furoate (**4**) An authentic sample of **4** showed the same signals



As may be seen in Fig 4, the major products from PA3 (solid line) in the volatile fraction are **1**, **4**, and **2**, whereas the product from PA3-DE (dashed line) is entirely **1** The de-esterification of PA3, therefore, seemed to decrease the formation of methyl furoates and also of the peak A component

This result suggests that these methyl furoates arise without decarboxylation from methyl D-galactopyranosyluronate residues in the polysaccharide molecule On the other hand, thermal decomposition of D-galactopyranosyluronic acid residues afford exclusively **1** by dehydration, decarboxylation, and molecular rearrangement

The formation of **3** (peak E in Fig 4) is observed with both PA3 and PA3-DE Therefore, it is not caused by D-galactopyranosyluronic acid residues or by methyl D-galactopyranuronate residues, but by L-rhamnopyranosyl residues in the polysaccharide molecules The pyrolytic formation of **3** from L-rhamnose was also clearly confirmed by c p p –g l c

Tarry product — The yield of carbon dioxide from PA3-DE by thermal decarboxylation was 12 4% (Table III), which is considerably lower than the theoretical value (25 0% from D-galactopyranosyluronic acid residues) As the major, volatile product from PA3-DE was merely **1** (Fig 4), those products arising from the residues without decarboxylation must have been in the tarry fraction of the pyrolyzate Accordingly, the tarry fraction was analyzed by g l c with a Carbowax-

20M column, after treatment of the vacuum-pyrolyzate with an ethereal solution of diazomethane The chromatogram obtained from the PA3-DE pyrolyzate after this treatment showed that significant amounts of **4** and **2** were generated by the reaction with diazomethane However, these methyl furoates have never been found directly in the c p p –g l chromatogram of PA3-DE (dashed line in Fig 4) This result strongly suggests that 5-formyl-2-furoic acid (**5**) and 2-furoic acid (**6**) were actually produced during the pyrolysis of PA3-DE, despite the presence of small amounts of sodium ion These furoic acids should arise from D-galactopyranosyluronic acid residues of the polysaccharide molecule without decarboxylation These acids are retained in the tarry fraction of the pyrolyzate because of their low volatility

Pathway of formation — The foregoing results suggest that the heating of pectic substances affords such primary decomposition-products as **5**, **6**, carbon dioxide, and **1** from D-galactopyranosyluronic acid residues, whereas the methyl D-galactopyranuronate residues give **4** and **2**, in addition to carbon dioxide and **1** On the other hand, the L-rhamnopyranosyl residues characteristically present[7] in pectic substances afford **3**

3-Deoxyaldos-2-uloses have been considered as intermediates in the pyrolytic formation of 2-furaldehydes from cellulose and other polysaccharides[8,9]. According to these proposals, it would be predicted that the present 2-furoic acids and their methyl esters are produced via such intermediates as 3-deoxy-D-*threo*-hex-2-ulosuronic acid and its methyl ester, respectively, during the pyrolysis of pectic substances

Neither the formation of anhydro sugars, nor the formation of lactones, observed in the pyrolysis of cellulose[2] and xylan[3], respectively, was encountered in this study

EXPERIMENTAL

Materials — Tobacco-leaf pectic substances were isolated from fresh leaves of *Nicotiana tabacum* cv BY-4 harvested after the flowering stage, essentially according to Sabir *et al*[10], with subsequent fractionation on a column (70 mm diameter, 350 mm) of DEAE-cellulose (Brown Co, New Hampshire, U S A, phosphate form)[11] with stepwise elution by water, 0 25, and 0 50M potassium phosphate buffer, and 0 1 and 0 5M sodium hydroxide solution Each polysaccharide sample was finally obtained as a white, amorphous substance after dialysis against de-ionized water and freeze-drying

As may be seen in Table I, the content of D-galacturonic acid residues is highest in the fraction PA4, which was eluted by 0 1M sodium hydroxide, but this fraction evidently had the disadvantage of containing some products of β-elimination The major fraction PA3 (yield 60 5%), eluted by 0 50M potassium phosphate buffer, contained 91 9% of D-galacturonic acid residues It also contained 1 2% of L-rhamnose and small proportions of other neutral sugars However, some aldobiouronic acids were detected (papergram) in its acid hydrolyzate[11], and thus the content of uronic acids and neutral sugars are considered to be somewhat higher than the

values recorded in Table I The nitrogen analysis of the polysaccharide (Table I) indicates that the fraction PA5 contained some protein

The content of D-galacturonic acid residues in the polysaccharide was determined by the carbazole method as modified by Knutson *et al* [12] The neutral-sugar composition of the polysaccharide was determined by g l c of the derived alditol acetates[13], after removal of the uronic acid fraction by Dowex 1-X2 (acetate) resin from the acid hydrolyzate according to Eda *et al* [14] The degree of esterification of the pectic substances was determined by analysis for the methoxyl content[15]

The de-esterification of PA3 was performed in an aqueous solution of sodium hydroxide (pH 12) for 30 min at 15°, according to Ozawa *et al*[16] The resultant solution was acidified (to pH 1) with M hydrochloric acid and the resultant precipitate was washed successively with M hydrochloric acid, 80% ethanol, 95% ethanol, abs ethanol, and ether The degree of esterification was decreased to zero by this treatment The sodium content of PA3-DE was estimated by atomic-absorption measurement after ashing for 20 h at 500°

Esterification of PA3-DE with diazomethane was performed as previously described[17] This treatment gave a degree of esterification of 73 0%

Microcrystalline cellulose powder (Avicel, Asahi Chemical Industry Co , Osaka, Japan) and tobacco-stalk xylan[3] were used as the reference samples for pyrolysis

L-Rhamnose, methyl 2-furoate 2-furaldehyde, and 5-methyl 2-furaldehyde of reagent grade were purchased from Tokyo Kasei Kogyo Co (Tokyo, Japan)

The authentic methyl 5-formyl-2-furoate was prepared from calcium D-*xylo*-5-hexulosonate (calcium ' 5-keto-D-gluconate), kindly donated by Kyowa Hakko Kogyo Co (Tokyo, Japan), by the procedure of Feather *et al* [18] m p 92° (lit [18] 91–92°), m s (70 eV) *m/e* 123 (100), 39 (60), 154 (57, M^+), 95 (46), 38 (40) and 28 (31), ^{1}H-n m r (carbon tetrachloride, 100 MHz) δ 3 96 (s 3 H), 7 23 (s, 2 H), and 9 80 (s, 1 H)

Dynamic thermal analysis — The t g d t g , and d s c curves were obtained as described in the previous paper[4]

Analysis of the pyrolyzate — The composition of the pyrolyzate was analyzed as described in the previous paper[3]

C p p–g l c — The c p p–g l c was performed as described in the previous paper[2] For analysis of the gaseous products, a cold trap at −72° was installed between the pyrolyzer (Shimadzu model PYR-10) and the gas–liquid chromatograph (Hitachi model 063) having a 2-m stainless-steel column of Carbosieve-B (Supelco Inc , Pennsylvania, U S A , 100–120 mesh) Helium as carrier-gas (30 ml min) and a thermal conductivity detector were used The column oven was kept at 95° A standard gas-mixture (Takachiho Chemical Industry Co , Tokyo, Japan) was used for calibration of carbon dioxide

Analysis of tarry products — A ferromagnetic conductor foil holding a 5-mg sample of PA3-DE was put into a quartz tube (10 mm diameter, 120 mm) having a stopcock at the end The tube was then fitted perpendicularly at the middle of the

induction-coil of a JAI model JHP-3 Curie-point pyrolyzer, and was evacuated to 2 torr After pyrolysis of the sample, an ethereal solution of diazomethane was introduced from the stopcock, and the tube was shaken throughly for 5 min An aliquot sample of the resultant mixture was subjected to the same gas–liquid chromatography as used in c p p –g l c for the analysis of volatile products

Instrumentation — The optical rotations (in water) of polysaccharides were measured in a JASCO model J-20 spectropolarimeter equipped with a 5-cm quartz cell Nitrogen analyses were conducted with a Yanaco model MT-2 analyzer The mass and ^{1}H-n m r spectra were recorded with Hitachi model RM-50GC and JEOL model JNM-PS-100 spectrometers, respectively The atomic absorption measurement was carried out with a Hitachi model 207 spectrometer

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