

related precursor is utilized for pheromone biosynthesis. In fact, studies of the male boll weevil, *Anthonomus grandis* Boheman, show that ^{14}C is readily incorporated into all four of the pheromones from common metabolic precursors such as mevalonate, glucose, and acetate (Mitlin and Hedin, 1974). Nevertheless, pecan weevils may either require terpenoids or be attracted to them.

LITERATURE CITED

- Hirose, Y., *Shitsuryo Bunseki* 15, 162 (1967).
 Kováts, E., *Anal. Chem.* 181, 351 (1961).
 Mitlin, N., Hedin, P. A., *J. Insect Physiol.* 20, 1825 (1974).
 Mody, N. V., Miles, D. H., Neel, W. W., Hedin, P. A., Thompson, A. C., Guedner, R. C., *J. Insect Physiol.* 19, 2063 (1973).
 Neel, W. W., *Miss. Farm Res.* 33, 3 (1970).

- Raney, H. G., Eikenbary, R. D., Flora, N. W., *J. Econ. Entomol.* 63, 697 (1970).
 Ryhage, R., von Sydow, E., *Acta Chem. Scand.* 17, 2025 (1963).
 Stenhagen, E., Abrahamsson, S., McLafferty, F. W., Ed., "Atlas of Mass Spectral Data", Vol. 1, 2, and 3, Interscience, New York, N.Y., 1969.
 von Sydow, E., *Acta Chem. Scand.* 17, 2504 (1963).
 von Sydow, E., *Acta Chem. Scand.* 18, 1099 (1964).
 Wang, P. S., Odell, G. V., *J. Agric. Food Chem.* 20, 206 (1972).

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Pyrolysis of Chitin, a Potential Tobacco Extender

William S. Schlotzhauer, Orestes T. Chortyk,* and Paul R. Austin¹

Chitin and tobacco were pyrolyzed separately and in admixture under identical conditions. The pyrolyzates were fractionated and analyzed for neutrals, bases, phenols, and carboxylic acids. Except for acidic products, chitin produces much smaller quantities of pyrolytic products than tobacco and in different ratios. Chitosan was also pyrolyzed to determine the effect of deacetylation on the carboxylic acid profile of the chitin pyrolyzate. It was concluded that both chitin and chitosan could become suitable tobacco extenders.

Current increases in tobacco leaf prices coupled with the smoking and health controversy have led numerous investigators and tobacco companies in search of tobacco extenders or "synthetic tobaccos". These extenders are to be mixed into cigarettes, replacing some tobacco and reducing the harmful tar and nicotine contents. Difficulties encountered in developing acceptable modified smoking materials have included unsatisfactory rate and continuity of burn, smoke temperature, ash retention, and more subtle problems of aroma, flavor, and biological response. Natural cellulose or modified cellulose products have been of recent interest as tobacco fillers or substitutes, accounting for 10–50% of cigarette composition ("Tobacco Situation", 1973; *Tab.-J. Int.*, 1974; *World Tob.*, 1974; *Tob. Reporter*, 1975). In addition to the substitute materials currently in use, two natural carbohydrate polymers, chitin and chitosan, are also of interest as potential tobacco extenders ("Pacific Northwest Sea", 1973; Pariser and Bock, 1972).

Chitin, a mucopolysaccharide, poly(*N*-acetyl-D-glucosamine) [(C₈H₁₂O₅N)_n], occurs widely in nature, for example, in the cell walls of fungi and shells of insects and crustaceans. Chitin is recovered from "crab meal" after alternate hydrolyses with mild alkali and acid which remove proteineous and calcereous constituents. Chitosan (2-deoxy-2-aminoglucose polymer), a deacetylated chitin, is normally prepared by strong alkaline hydrolysis of chitin.

The product is then subsequently neutralized and isolated ("Pacific Northwest Sea", 1973; Tracey, 1957). Deacetylated chitin has been utilized as an adhesive for tobacco particles in reconstituted tobacco sheet formulation (Moshy and Germino, 1966). A low tar yield in smoke of chitin led to its trial as a tobacco extender (Austin, 1975) and prompted this study of chitin pyrolysis.

In the initial trials, it was determined that chitin and chitosan could be added in substantial amounts to tobacco blends without altering significantly such physical properties as packing ability, burning rate, and ash retention, or grossly affecting organoleptic attributes such as aroma, taste, or mildness. Since identification of the major pyrolytic products of chitin would provide additional evaluation of this material as a tobacco extender, chitin and tobacco were pyrolyzed separately and in admixture under identical conditions; the pyrolyzates were analyzed for gas-liquid chromatographically volatile, ether-soluble neutrals, bases, phenols, and carboxylic acids. Chitosan was also pyrolyzed to determine the effect of deacetylation on the carboxylic acid profile of the chitin pyrolyzate.

EXPERIMENTAL SECTION

Preparation of Materials for Analysis. Chitin and chitosan, obtained from the Food, Chemical, and Research Laboratories (Seattle, Wash.), were used without further chemical purification. The tobacco was North Carolina, flue-cured, whole leaf (provided by the Tobacco Research Laboratory, Agricultural Research Service, USDA, Oxford, N.C.). It was ground to 32 mesh in a ball mill to promote homogeneity upon admixture with chitin. Prior to pyrolysis, all samples were equilibrated over 76% glycerol in water for 48 hr to standardize moisture at 12.0% in

¹Tobacco Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Athens, Georgia 30604.

*Present address: College of Marine Studies, University of Delaware, Newark, Delaware.

tobacco, 7.0% in chitin, and 10.0% in chitosan. To prepare the admixture, equal weights of humidified tobacco and chitin were mechanically agitated in a shaker apparatus until homogeneous in appearance.

Pyrolyses. Pyrolyses were performed in a 48-in. Vycor tube positioned in a Lindberg Hevi-Duty tube furnace, with an inlet for dry nitrogen gas (200 ml/min) and an exit connected to a series of cold traps, immersed in ice-water and in dry ice-acetone in succession. A gas-washing bottle containing diethyl ether-0.5 N NaOH (aqueous) served as a final trap for more volatile effluents. Samples were positioned in the middle one-third of the tube, the hot zone of the oven, and the oven was brought from ambient to 900°C, at a rate of 10°C/min, through a variable transformer. When pyrolysis was completed, the oven was cooled, and pyrolyzate was recovered from the train of traps by back-flushing with the diethyl ether-aqueous base from the gas-washing bottle. The combined washings were partitioned (separatory funnel). By use of appropriate solvent extractions and pH adjustments, as previously described (Higman et al., 1970), ether solutions of neutral, basic, phenolic, and acidic products, suitable for gas chromatographic analysis, were obtained.

Product Analyses. Gas chromatography (GC) was performed on a Varian Model 2100 instrument, equipped with dual, U-shaped, 6 ft × 1/8 in. i.d. glass columns and dual flame ionization detectors. In all analyses, helium and hydrogen flows were maintained at 32 ml/min, and air flow was maintained at 250 ml/min. The pyrolyzate fractions were analyzed under the following conditions.

Neutrals. The column was 5% Dexsil 300 GC on 80-100 Chromosorb W; injector and detector temperatures were 275 and 325°C, respectively; column temperature was held at 40°C for 3 min post-injection, then programmed to 292°C at 6°C/min.

Bases. The column was 10% Carbowax 20M-2% KOH on 80-100 Chromosorb W-AW; injector and detector temperatures were 200°C; column temperature was held at 60°C for 3 min post-injection, then programmed to 180°C at 4°C/min.

Phenols. The column was 10% SP-2401 on 100-120 mesh Supelcoport; injector and detector temperatures were 225°C; column temperature was held at 40°C for 3 min post-injection, then programmed to 200°C at 4°C/min.

Acids. The column was 10% SP-2100-1% H₃PO₄ on 80-100 mesh Chromosorb W-AW; injector and detector temperatures were 180°C; column temperature was held at 40°C for 3 min post-injection, then programmed to 140°C at 4°C/min.

Individual components within the fractions were initially identified by retention data and by co-injection with authentic standards. Some of these components and other GC peaks, unidentifiable by the above procedure, were collected and analyzed by ultraviolet spectroscopy (Perkin-Elmer Model 257) and/or infrared spectrometry (Perkin-Elmer Model 457). Product compositions of the fractions were calculated from peak areas determined with an Infotronics Model CRS-204 digital integrator. Constituents of the basic fraction were also characterized by GC-mass spectral methods.

RESULTS AND DISCUSSION

Since the objective of the study was to learn what probable thermal decomposition products chitin would yield in a burning cigarette, the pyrolyses were conducted at a temperature range similar to that (up to 900°C) behind the burning cone of a cigarette (Touey and Mumpower, 1957). These conditions would allow distillation of the more volatile constituents and thermal decomposition of

Table I. Yields of Ether-Soluble Pyrolyzate Fractions of Tobacco, Chitin, and Tobacco-Chitin Mixture

Fraction	Tobacco ^a	Chitin ^a	Ratio tobacco ^b /chitin	Tobacco-chitin mixture ^a	
				Theor.	Exptl.
Neutrals	7.07	0.65	10.87	3.77	3.95
Bases	2.47	0.58	4.25	1.50	1.96
Phenols	2.97	0.91	3.26	1.91	1.74
Acids	0.51	0.53	0.96	0.52	0.70
Total	13.02	2.67	4.89	7.71	8.35
ether sols.					

^a Percent yield based on moisture corrected weight of material pyrolyzed. ^b Yield of fractions from tobacco pyrolysis divided by corresponding fractions from chitin pyrolysis.

Table II. Components Identified in Neutral Pyrolyzate Fractions

Compound	Product distribution, % GC volatile ^a		
	Tobacco	Chitin	Tobacco-chitin mixture
Toluene	6.67	8.50	6.80
Ethylbenzene	0.71	<1	1.09
<i>m,p</i> -Xylenes	5.10	<1	5.35
<i>o</i> -Xylene	3.07	6.08	2.19
Mesitylene	12.62		8.59
Dipentene	27.02		24.88
Naphthalene	8.00	<1	5.39
Unidentified components ^b	36.81	~85	45.71

^a Determined by integration of GC peaks and assumption of unitary detector response. ^b Sum of numerous unidentified components generally present in individual amounts less than 1% of total GC volatiles.

other components. The samples were pyrolyzed in nitrogen which approximates the essentially nonoxidative atmosphere occurring behind the burning cone of a cigarette (Newsome and Keith, 1965). Yields of the ether-soluble pyrolyzate fractions obtained from tobacco, chitin, and the tobacco-chitin mixture are compared in Table I. Chitin was found to produce substantially less total condensable products than did tobacco; only the yields of the acid fractions did not differ significantly. "Theoretical" yields for the mixture were determined with the assumption that chemical interactions or synergistic effects were absent during copyrolysis of tobacco and chitin. Overall, the theoretical and experimental distributions of the pyrolyzate fractions did not differ markedly. However, the greater than expected experimental yields of nitrogen bases (+31%) and acids (+34%) may have resulted from pyrolytic interactions.

GC-volatile components of the neutral fractions from chitin and tobacco and their relative distributions are presented in Table II. Tobacco pyrolyzate neutrals consisted mainly of alkylbenzenes, xylenes, and naphthalene, which are typical products of high temperature pyrolyses. Dipentene (*p*-mentha-1,8-diene), a major cigarette smoke component, which reportedly derives from pyrolytic breakdown of tobacco leaf terpenoids, especially solanesol (Grossman et al., 1962), was detected for tobacco but not for chitin. Compared to tobacco, chitin produced only 9% as much neutrals, which included small amounts of toluene and xylene and a variety of minor components in amounts too minute for ready characterization. Evidently, chitin would contribute little to the neutral products of cigarette smoke, as data for the admixture further indicate.

Table III. Components Identified in Basic Pyrolyzate Fractions

Compound	Product distribution, % GC volatiles ^a		
	Tobacco	Chitin	Tobacco-chitin mixture
Pyridine	20.76	0.91	75.79
Picoline(s)	0.90	16.87	1.79
Pyrazine	0.72	15.49	1.03
Quinoline	1.09	8.53	1.05
Nicotine	43.63		13.89
Unidentified components ^b	32.90	49.20	6.45

^a Determined by integration of GC peaks and assumption of unitary detector response. ^b Sum of numerous unidentified components generally present in individual amounts less than 1% of total GC-volatile materials.

Table IV. Components Identified in Phenolic Pyrolyzate Fractions

Compound	Product distribution, % GC volatiles ^a		
	Tobacco	Chitin	Tobacco-chitin mixture
Phenol	51.90	59.62	57.80
<i>o</i> -Cresol	12.10	10.45	6.40
<i>m,p</i> -Cresols ^b	7.32	13.60	11.52
2,6-Xylenol	2.36	4.12	4.38
<i>p</i> -Ethylphenol	6.47	3.06	7.81
3,5-Xylenol	10.32		2.55
Unidentified components ^c	9.33	7.13	9.64

^a Determined by integration of GC peaks and assumption of unitary detector response. ^b Not resolvable under GC conditions used. ^c Sum of unidentified components generally present in individual amounts less than 1% of total GC volatile materials.

The nitrogen-containing bases identified in the pyrolyzates of tobacco, chitin, and the tobacco-chitin mixture are shown in Table III. GC-MS examination of the basic fraction showed no evidence of arylamines among the numerous, small (<1%) components. The major basic components of tobacco pyrolysis were pyridine and nicotine, while those from chitin were picolines and pyrazine. The presence of pyrazine is of special interest since pyrolysis of glucosamine yields pyrazines, which are reportedly responsible for the "roasted" aroma of certain cooked foods (Wang and Odell, 1973). Enhancement of pyrazines content of cigarette smoke might result in altered flavor and aroma. Co-pyrolysis could not have significantly enhanced pyrazine yield, however, since the contribution of chitin-derived bases to the total bases was theoretically only 23% of the total (Table I). Surprisingly, however, the basic pyrolyzate from the mixture contained a substantially higher concentration of pyridine than that from either tobacco or chitin. The high concentration suggests that some chemical interactions of N-containing intermediates occurred during pyrolysis. Increased yields of pyridine in the smoke from chitin-tobacco cigarettes would be expected to alter the flavor characteristics of smoke. The effects of such alterations would have to be examined by flavor panel tests.

Yields of phenolic products from tobacco, chitin, and the mixture are presented in Table IV. Phenols are important in tobacco smoke chemistry because they affect organoleptic properties and are tumor promoters and cilia-toxins (Wynder et al., 1963). The distributions of volatile phenols in tobacco and chitin pyrolyzates were markedly similar. However, since the total yield of phenols from tobacco was more than triple that of chitin (Table

Table V. Components of the Acidic Pyrolyzate Fractions

Compound	Product distribution, % GC volatiles ^a			
	Tobacco	Chitin	Tobacco-chitin mixture	Chitosan
Formic acid-acetic acid ^b	11.86	92.10 ^c	71.52	41.34
Propionic acid	14.74	3.84	7.03	4.25
Isobutyric acid	2.98	0.33	0.98	<1
Butyric acid	3.58	0.28	1.57	19.88
Isovaleric acid	7.65	0.16	1.74	2.99
Valeric acid	1.93	<1	0.58	<1
Isocaproic acid	13.88	1.03	3.04	<1
Caproic acid	7.52	0.52	1.89	<1
Unidentified components ^d	35.86	1.74	11.65	31.53

^a Determined by integration of GC peaks and assumption of unitary detector response. ^b Not resolvable by GLC method used. ^c Infrared spectra of collected peak effluent indicated preponderance of acetic acid over formic acid. ^d Sum of unidentified components generally present in individual amounts less than 1% of total GC-volatile material.

I), the latter was not expected to significantly alter the phenol content of the mixture pyrolyzate. In fact, aside from some differences in the proportions of the isomeric cresols, no drastic differences in the three pyrolyzate phenols were observed.

The identities and distributions of the major carboxylic acids in the pyrolyzates are shown in Table V. Although chitin in admixture contributed little to the neutral, basic, and phenolic fractions, Table V shows that chitin would significantly alter the carboxylic acid composition of smoke. On pyrolysis, tobacco yielded a series of lower fatty acids, where the C₂, C₃, and C₆ predominate. Chitin produced mostly acetic acid (92%). Chitosan was also pyrolyzed to determine its acidic products. In addition to C₂, C₃, and C₅ acids, chitosan yielded relatively large amounts of butyric acid. Chitosan yielded less than half of the acetic acid obtained from chitin, but more than three times that from tobacco. Chitosan also produced about 70 times as much butyric acid as chitin. Since tobacco and chitin yielded similar quantities of acids (0.5%) on pyrolysis, production of acetic acid by chitin was more than eightfold that of tobacco. This observation is reflected by the admixture pyrolyzate, in which acetic acid content increased to 71% of the fraction. Although the acid fraction comprised but 8% of the total pyrolyzate obtained from the mixture, a major increase in its acetic acid content might be expected to significantly affect smoke pH and flavor and aroma related properties.

Comparison of their pyrolytic products showed that chitin compared to tobacco produced (1) only one-tenth as much neutral products, (2) only one-fifth as much bases, which consisted mostly of picolines and pyrazines, (3) one-third as much phenols, similarly distributed, and (4) the same weight of acids, but with eight times as much acetic acid. Compared to chitin, chitosan yielded half as much acetic acid. Thus, both chitosan and chitin may be suitable extenders. However, organoleptic testing would be required to determine which extender, at a 25 to 50% concentration in cigarettes, would be preferable. Obviously, increased amounts of chitosan in tobacco extender formulations would be less likely to promote any undesirable effects arising from acetic acid production. Also, the acidic pyrolysis products of chitin and chitosan may blend with cigarette smoke which is generally mild and acidic, when compared to the harsher, basic smoke of cigars. Ultimately, the suitability and acceptability of

chitin and chitosan as tobacco extenders will have to be determined by analyses of smoke components of chitin-tobacco cigarettes and biological testing of resulting smoke condensates.

LITERATURE CITED

- Austin, P. R., application for U.S. Patent, Serial No. 555546, filed March 5, 1975.
- Grossman, J. D., Deszyck, E. J., Ikeda, R. M., Bavely, A., *Chem. Ind. (London)*, 1950 (1962).
- Higman, E. B., Schmeltz, I., Schlotzhauer, W. S., *J. Agric. Food Chem.* 18, 636 (1970).
- Moshy, R. J., Germino, F. J., U.S. Patent 3421519, Serial No. 567358, filed July 25, 1966.
- Newsome, J. R., Keith, C. H., *Tob. Sci.* 9, 65 (1965).
- "Pacific Northwest Sea", Vol. 6, No. 1, Oceanographic Commission of Washington, North Seattle, Wash., Winter 1973.
- Pariser, E. R., Bock, S., Report No. M.I.T.-SG 73-2, Cambridge, Mass., Oct 15, 1972.
- Tab.-J. Int.*, 309 (Oct 1974).
- Tob. Reporter* 102(4), 14 (1975).
- "Tobacco Situation", Economic Research Service, USDA, TS-145, Sept 1973, p 34.
- Touey, G. P., Mumpower, R. C., *Tob. Sci.* 1, 33 (1957).
- Tracey, M. W., *R. Aust. Chem. Inst. J. Proc.* 7(1), 1 (1957).
- Wang, P. S., Odell, G. V., *J. Agric. Food Chem.* 21, 868 (1973).
- World Tob.* 46, 44 (1974).
- Wynder, E. L., Kaiser, H. E., Goodman, D., Hoffmann, D., *Cancer* 16, 1222 (1963).

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Purification of Phosphoric Acid with Methanol and Ammonia

John F. McCullough* and Leland L. Frederick

In a laboratory study, wet-process phosphoric acid was lightly ammoniated and treated with methanol to precipitate most of the impurities as complex metal ammonium phosphates and fluorine compounds. The solids were separated and the methanol was distilled from the liquid phase for recycling. For rapid filtration and satisfactory purification of the acid, the initial mole ratio $\text{NH}_3:\text{H}_3\text{PO}_4$ should be about 0.175 and the acid should be treated with about 2.5 lb of CH_3OH per lb of H_3PO_4 . This treatment of the usual wet-process acid precipitated 90% or more of the Fe, Al, and F, up to 70% of the Mg, and 10% of the P_2O_5 . The precipitate contained about 5% N and 50% P_2O_5 , all of which was soluble in neutral ammonium citrate. Satisfactory filtration and settling required digestion of the methanol-acid mixture at 55°C or above for 30 to 50 min. The loss of methanol on distillation from 75% H_3PO_4 could be kept below 50 lb per ton of P_2O_5 , but there was some esterification to form monomethyl phosphate. This esterification increased with rising temperature of distillation and with increasing concentration of acid. It was minimized by distillation under vacuum and was negligible when the initial acid was only moderately concentrated.

Wet-process phosphoric acid contains metallic and fluorine impurities that form troublesome precipitates when the acid is converted to solution fertilizers (Frazier et al., 1972). Various purification processes have been developed to upgrade the acid to food, detergent, or solution fertilizer quality (Blumberg, 1971). Most of the processes are based on solvent extraction methods which divide the acid into purified and raffinate fractions. The methanol process is based on precipitation of impurities from lightly ammoniated wet-process phosphoric acid with methanol to produce acid suitable for solution fertilizer production.

Methanol alone does not precipitate impurities from the acid, but when alkali or ammonium ions are also present, most of the impurities are precipitated as complex alkali or ammonium metallic phosphates and fluorine compounds (Pulverfabrik Skodawerke-Wetzler A.-G., 1937). Alkali or ammonium ions can be added as any soluble source. Anhydrous ammonia was used in this study because no contaminating anions are thus introduced, and the nitrogen is recovered in the final fertilizer products. Other acid-miscible solvents also can be used (Pulverfabrik Skodawerke-Wetzler A.-G., 1937; Frankenfeld and

Götzmann, 1973; British Patent, 1974). Methanol, however, has the advantage of being cheaper than other potential solvents.

The methanol process consists of the following basic steps: recycled methanol, ammonia, and wet-process acid are brought together in a reactor to precipitate the impurities; precipitated impurities are separated from the methanol-acid mixture; and methanol and part of the water are distilled from the purified acid and separated in a fractionating column. Except for rectification, each of the steps was investigated in the laboratory for the purification of merchant-grade phosphoric acid. Esterification and ether formation during distillation also were investigated.

ANALYTICAL METHODS

Standard analytical procedures were used for determination of P_2O_5 and nitrogen (Association of Official Analytical Chemists, 1970). Low results for phosphorus were obtained when monomethyl phosphoric acid was present, presumably because the ester was not decomposed in the boiling HNO_3 solution normally used for digestion of the sample. Correct results were obtained by heating the sample with H_2SO_4 to fumes and then adding HNO_3 dropwise to oxidize the organic matter.

All metals were determined by atomic absorption spectrophotometry (Perkin-Elmer, 1971). Fluorine was

*Division of Chemical Development, Tennessee Valley Authority, Muscle Shoals, Alabama 35660.