Effects of Structure on Pyrolysis Gases from Amino Acids

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A variety of α-amino acids (alanine, α-aminobutyric acid, glutamic acid, leucine, lysine, phenylalanine, proline, serine, tryptophan, and valine) was pyrolyzed at 850 °C in a helium atmosphere, and the weights of carbon monoxide, carbon dioxide, hydrogen cyanide, ammonia, methane, ethylene, and ethane produced were determined by using a combi nation of wet techniques and gas chromatographic analysis. Alanine, α-aminobutyric acid, leucine, and valine yield relatively large amounts of CO, presumably via diketopiperazine and subsequent HNCO formation. Proline yields very little CO but yields large amounts of CO_2 by decarboxylation. Glutamic acid undergoes only monodecarboxylation and the other carboxyl is incorporated into a pyrrolidinone ring with subsequent formation of pyrrole. Proline and glutamic acid give relatively large amounts of HCN via N-heterocycle formation. The small, simple amino acids are almost totally converted to gases (alanine, 95%; α-aminobutyric acid, 94%). Larger or more complex amino acids and those which can form aromatic products give lower conversions to gases.

The thermal behavior of α-amino acids has been extensively studied by several researchers under different conditions (Chatelus, 1964, 1965; Fox, 1960; Hurd, 1929; Masuda et al., 1965; Merritt and Robertson, 1967; Patterson et al., 1969, 1973; Simon and Giacobbo, 1965; Smith et al., 1975; Vollumin et al., 1966; Waser, 1925; Winter and Albro, 1964). Neutral, basic, and acidic species produced from these pyrolyses have been identified and quantitatively determined. The gases produced, however, were only treated in a qualitative manner and only hydrogen cyanide has had some share of quantitative consideration (Johnson and Kang, 1971). The study offers the first comprehensive quantitative determination of the gases evolved from the thermolysis of some α-amino acids found in tobacco at a temperature approximating that of the burning cigarette, 850 °C. The study also proposes initial breakdown mechanisms for the different amino acids as suggested from the yields of the gases produced.

EXPERIMENTAL SECTION

Materials. DL-Alanine, DL-α-aminobutyric acid, DL-glutamic acid, DL-leucine, DL-lysine hydrochloride, DL-phenylalanine, DL-proline, DL-serine, DL-tryptophan, and DL-valine (Nutritional Biochemicals, Cleveland, OH) were used as received.

Methods. *Pyrolysis and Collection of Gases.* The pyrolyses were carried out at 850 °C in the apparatus previously described (Patterson et al., 1968) using 10 mL of Berl Saddles, a helium flow of 100 mL/min, and a rotating screw device for the introduction of the samples into the pyrolysis tube.

The gases produced in the pyrolysis were passed through an empty trap which was connected in series to two traps containing 2 × 125 mL of an aqueous solution of (2 M) KOH (decarbonated water), followed by an empty trap and a trap containing 100 mL of a 3.5 M H_2SO_4 solution. Gas samples were collected after the first empty trap via a 100-mm IR gas cell and used for the gas chromatographic analyses.

Gas Chromatographic Analysis. The gas samples collected in the IR cell were injected in the gas chromatograph by using a 3-mL vacuum syringe, and a 4 ft × 0.125 in. silica gel (100/120) column kept at 25 °C. Before each run, the column was heated at 100 °C for 15 min and then left to cool to 25 °C. The areas of the peaks of individual gas components were measured by using an Infratronics electronic integrator. Carbon dioxide, whose weight could be determined independently by wet techniques, was used as an internal standard. Amounts of individual gases produced in the pyrolyses are reported in Tables I and II. No gases other than those reported in the tables could be detected by gas chromatography or by infrared spectra.

Determination of the Weight of Ammonia. The solution from the H_2SO_4 trap was rinsed into a 200-mL volumetric flask and diluted with water to the mark. A 50-mL aliquot was placed in a 500-mL three-necked flask, mixed with 50 mL of NaOH solution, and steam distilled for 30 min into a 400-mL aliquot of a 4.8% boric acid solution. When the steam distillation was complete, the solution was diluted to 1 L and a 200-mL aliquot was titrated with standardized HCl to the pH of a 2:5 dilution of the stock boric acid solution to water. Results obtained by this method may be high because any water steam-distillable bases are included along with ammonia.

Determination of the Weights of Carbon Dioxide and Hydrogen Cyanide. The combined KOH solutions from the two traps were washed into a 500-mL volumetric flask and diluted to the mark with decarbonated water. To a 200-mL aliquot enough 8% $Ba(NO_3)_2$ solution was added to precipitate all the carbonate. The precipitate was filtered and washed through a Gooch crucible, and the weight of the $BaCO_3$ was found by difference.

The filtrate from the carbonate determination was diluted to 500 mL, and to each 100-mL aliquot was added 0.2 g of KI and 6–8 mL of 6 M NH_4OH. This was then titrated with an aqueous solution of $AgNO_3$ of known concentration to permanent cloudiness (Kolthoff et al., 1969).

RESULTS AND DISCUSSION

The results indicate that the structure of the amino acid has a significant effect on the percent transformation of the acid to gaseous products upon pyrolysis. It appears that the smaller the amino acid and the less encumbered it is with extra functional groups, the more thoroughly it is converted to gases as is indicated by the almost quantitative conversion of alanine and α-aminobutyric acid to gases (95.0 and 93.5%, respectively). As the molecule of the neutral amino acid is lengthened or branched, the amount of gases produced decreases as in the cases of valine (88.3%) and leucine (89.4%). The incorporation of an extra functional group (capable of hydrogen bonding)

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Table I. Concentration of Gaseous Components Produced in the Pyrolysis of the Amino Acids at 850 °C[a]

amino acid	concn, mol/mol						
	CO_2	CO	HCN	NH_3	CH_4	C_2H_4	C_2H_6
alanine	0.02[b]	2.30[b]	0.01	0.05	1.10[b]	0.04	0.01
α-aminobutyric acid	0.02[b]	2.14[b]	0.01	0.03	0.36[b]	0.71[b]	0.13[b]
glutamic acid	0.30	0.33	0.33	0.01	0.14	0.11	0.01
leucine	0.03[b]	2.40[b]	0.01	0.03	1.68[b]	0.54[b]	0.03[b]
lysine	0.14	0.13	0.39	0.10	0.20	0.19	0.03
phenylalanine	0.06	0.99	0.17	0.003	0.12	0.05	0.002
proline	0.31	0.05	0.31	0.02	0.18	0.28	0.01
serine	0.18	0.25	0.20	0.01	0.14	0.03	0.003
tryptophan	0.18	0.37	0.45	0.01	0.18	0.01	0.0003
valine	0.04[b]	1.88[b]	0.03	0.05	0.86[b]	0.58[b]	0.04[b]

[a] Values are reported as moles of gas per mole of amino acid pyrolyzed. All pyrolyses were run at 850 °C in helium. Acetylene was identified in the IR of all the gas samples but was not determined quantitatively. [b] The use of carbon dioxide as an internal standard in the GC analysis of amino acids that give very low yields of carbon dioxide renders the values obtained for CO, CH_4, C_2H_4, and C_2H_6 inaccurate. Table II reports grams of gas per mole of amino acid pyrolyzed relative to carbon dioxide = 1 as obtained from calculation of the relative areas of the gas chromatographic peaks of the gases.

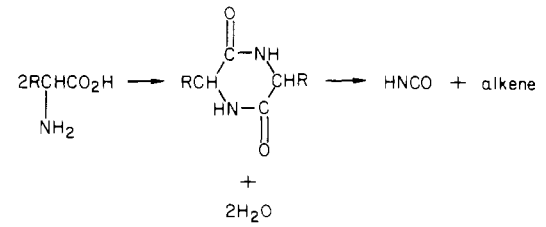
Table II. Relative Concentrations of CO_2, CO, CH_4, C_2H_4, and C_2H_6 in the Pyrolysis of Alanine, α-Aminobutyric Acid, Leucine, and Valine at 850 °C[a]

amino acid	rel concn, g/mol				
	CO_2	CO	CH_4	C_2H_4	C_2H_6
alanine	1	72.59	19.46	1.21	0.29
α-aminobutyric acid	1	83.32	7.94	27.69	5.57
leucine	1	47.52	19.09	10.74	0.57
valine	1	32.68	8.57	10.18	0.76

[a] Values are reported as grams of gas per mole of amino acid relative to CO_2 = 1. Duplicate pyrolyses of glutamic acid gave results in which HCN, CO_2, and NH_3 quantities agreed to ±5% of the value reported (on a gram per mole basis).

seems to sharply curtail the breakdown of the amino acid to gaseous products; thus, only 71.5% of serine and 66.0% of lysine are transformed to gaseous products. Amino acids with structures which may easily form a stable aromatic nucleus give yields of gases which are considerably smaller than those obtained from other amino acids which are not capable of such transformations; hence proline and glutamic acid which are known to give high yields of pyrrole at this temperature (Johnson and Kang, 1971; Patterson et al., 1970; Smith et al., 1975) produce 81–82% of their total weights in gases. Phenylalanine and tryptophan which already contain an aromatic ring yield only 27.7 and 40.6% gases, respectively. Earlier studies support the fact that compounds containing aromatic nuclei are less easily broken down at 850 °C (Badger, 1965).

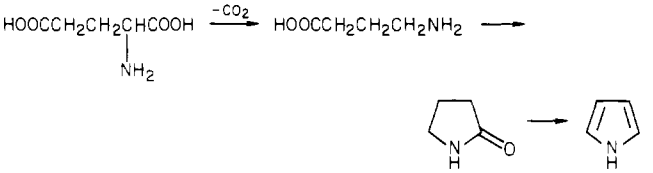
Alanine, α-aminobutyric acid, leucine, and valine yield high values for carbon monoxide. This is due to the initial formation of their corresponding diketopiperazines which eventually break down to isocyanic acid which is the source of carbon monoxide (Back and Childs, 1968; Clough et al., 1973):



$$2HNCO \rightarrow {}^2/_3NH_3 + {}^2/_3N_2 + 2CO$$

The formation of diketopiperzines from α-amino acids is frequently reported in the literature (Fox, 1960; Hurd, 1929; Waser, 1925), and the pyrolysis of diketopiperazines is reported to produce carbon monoxide and carbon dioxide (Patterson et al., 1973). Introduction of carbon dioxide into a tube containing the carbonized residue from the pyrolysis of valine at 850 °C did not yield carbon monoxide, thus excluding the possibility of carbon monoxide formation from the reduction of carbon dioxide by carbon.

In direct contrast to the behavior of alanine, α-aminobutyric acid, leucine, and valine is the behavior of proline which gives relatively high amounts of carbon dioxide but almost negligible amounts of carbon monoxide. This may indicate that proline undergoes initial decarboxylation quite easily whereas the aforementioned amino acids break down via the diketopiperazine route. The yield of carbon dioxide from proline is almost equal to that from glutamic acid which would be expected to give higher yields of carbon dioxide if both carboxyl groups are lost as carbon dioxide. The production of pyrrole from the pyrolysis of glutamic acid (Patterson et al., 1970), however, clearly indicates that only one carboxyl is lost as carbon dioxide whereas the second carboxyl group is incorporated into a pyrrolidinone ring before changing to pyrrole:



It should be noted that the amino acids capable of forming a relatively stable nitrogen-containing aromatic ring in the early steps of the thermolysis yield large amounts of hydrogen cyanide as seen in the cases of proline and glutamic acid which form pyrrole (Smith et al., 1975; Patterson et al., 1970) and phenylalanine and tryptophan which yield indole (Patterson et al., 1969, 1973). Nitrogen-containing rings are known to break down at high temperatures to give high levels of hydrogen cyanide (Johnson and Kang, 1971; Patterson et al., 1968). The high level of hydrogen cyanide detected in the pyrolysis of lysine could be due to the availability of more nitrogen in the molecule or to the breakdown of pyridine or piperidine which could theoretically be formed from lysine but was undetected in this study.

Significant yields of ammonia are observed only from the pyrolysis of lysine, which by virtue of its having two amino functions is expected to produce a considerably higher yield of ammonia than the other amino acids.

In interpreting these results as they might apply to tobacco smoking, it should be noted that some authors

(Jenkins et al., 1970, 1975; Schmeltz et al., 1979) have questioned the extrapolation from classical pyrolysis data to cigarette smoking.

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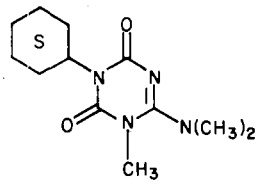
Determination of Hexazinone and Metabolite Residues Using Nitrogen-Selective Gas Chromatography

Richard F. Holt

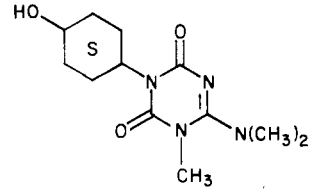
Residues of the weed killer 3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione (hexazinone) and two primary metabolites (A and B) in plants, animal tissues, and soil are determined by initial extraction with chloroform, cleanup by liquid/liquid partitioning techniques, and measurement by nitrogen-selective gas chromatography after reaction of the metabolites with trifluoroacetic anhydride. Additionally, by modification of the extraction scheme, two minor metabolites (D and E) can also be detected. A fifth metabolite (C) can be determined through a separate isolation procedure. Relative to a 25-g sample, method sensitivity is 0.04 ppm for the parent compound and metabolites A and B, 0.1 ppm for metabolites D and E, and 0.2 ppm for metabolite C.

Hexazinone is the approved common name for 3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4-(1*H*,3*H*)-dione. This material, which was formerly known as DPX-3674, is the active compound in Du Pont's Velpar weed killer.

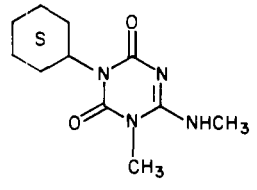
Sensitive analytical procedures are described for the determination of the parent compound and five metabolites: 3-(4-hydroxycyclohexyl)-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione (metabolite A); 3-cyclohexyl-6-(methylamino)-1-methyl-1,3,5-triazine-2,4-(1*H*,3*H*)-dione (metabolite B); 3-(4-hydroxycyclohexyl)-6-(methylamino)-1-methyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione (metabolite C); 3-cyclohexyl-1-methyl-1,3,5-triazine-2,4,6-(1*H*,3*H*,5*H*)-trione (metabolite D); 3-(4-hydroxycyclohexyl)-1-methyl-1,3,5-triazine-2,4,6(1*H*,3*H*,5*H*)-trione (metabolite E). These metabolites were detected in [^{14}C]hexazinone studies with alfalfa and sugarcane. Metabolites A and B were detected in [^{14}C]hexazinone soil studies (Rhodes, 1980a). Metabolites A and C were detected in the urine and feces of rats preconditioned on hexazinone and given a single dose of [^{14}C]hexazinone (Rhodes, 1980b).



hexazinone



metabolite A



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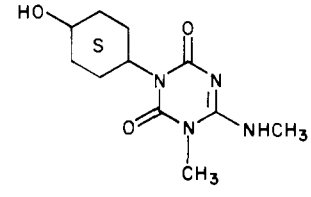
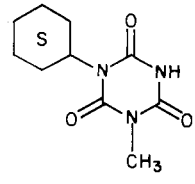
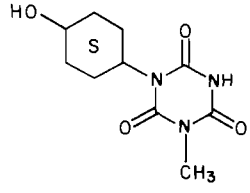


metabolite C



metabolite D



metabolite E

These analytical procedures are based on selective nitrogen-sensitive gas chromatographic measurement after

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