

Mass Spectral and Pyrolytic Behavior of the Two Main Products of Phenylbutazone Degradation: Simulation of Unusual Mass Spectral Fragmentation

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Abstract □ The two major routes of degradation of phenylbutazone (I) are oxidation and hydrolysis. Hydrolysis gives rise to *n*-butylmalonic acid mono-(*N,N'*-diphenyl)hydrazide (II). Oxidation at the C-4 position yields 1,2-diphenyl-4-*n*-butyl-4-hydroxypyrazolidine-3,5-dione (III), which is readily hydrolyzed to *n*-butyltartronic acid mono-(*N,N'*-diphenyl)hydrazide (IV). Whereas the mass spectra of I, III, and the methyl esters of carboxylic acids II and IV all demonstrate major peaks corresponding to their respective molecular ions, the mass spectrum of II is essentially identical with that of I, suggesting facile dehydration. In the mass spectrum of IV, the peak of highest mass is found at *m/e* 205; no peak could be perceived corresponding to the molecular ion or to loss of water, carbon dioxide, or both of these elements from the molecular ion. Under normal pyrolytic conditions, II is decarboxylated to *N*-caproylhydrazobenzene (V) and IV is readily dehydrated to yield III. The mass spectral fragmentation of IV was successfully simulated in the laboratory to give an excellent yield of aniline and α -keto-*N*-caproylaniline (VI) (mol. wt. 205). The probable course of this unusual transformation was elucidated from studies of the accelerated decomposition of IV and derivatives considered as possible intermediates in the degradation process.

Keyphrases □ Phenylbutazone—mass spectral and pyrolytic behavior of products of degradation by oxidation and hydrolysis □ Mass spectrometry—determination of products of phenylbutazone degradation by oxidation and hydrolysis □ Pyrolysis—behavior of products of phenylbutazone degradation by oxidation and hydrolysis

Qualitative and preparative TLC procedures were developed which allow unequivocal identification, as well as semiquantitative estimation, of the degradation products of phenylbutazone (I).

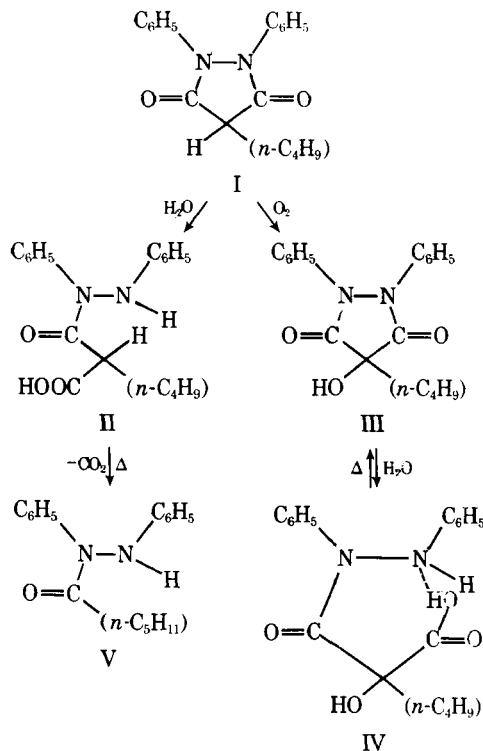
The utilization of mass spectrometry in the identification of material isolated by preparative TLC affords a considerable saving of time and effort in view of the small quantities of sample normally required for satisfactory analysis. However, while the mass spectrometric behavior of I and the majority of its derivatives is unexceptional, the fragmentations of the two carboxylic acids, the major products of phenylbutazone degradation, are unusual and essentially different.

Accordingly, to standardize identification procedures and to gain a better understanding of the chemical behavior of these two acids, a thorough examination of their thermal degradative and mass spectrometric behavior was undertaken.

RESULTS AND DISCUSSION

As illustrated in Scheme I, the two main routes of degradation of phenylbutazone (1,2-diphenyl-4-*n*-butylpyrazolidine-3,5-dione) (I) are oxidation and hydrolysis (1). Hydrolysis of I gives rise to *n*-butylmalonic acid mono-(*N,N'*-diphenyl)hydrazide (II). Oxidation at the C-4 position of I produces 1,2-diphenyl-4-*n*-butyl-4-hydroxypyrazolidine-3,5-dione (III), which is readily hydrolyzed to *n*-butyltartronic acid mono-(*N,N'*-diphenyl)hydrazide (IV).

Whereas hydrolysis of III to IV is a reaction that can be reversed

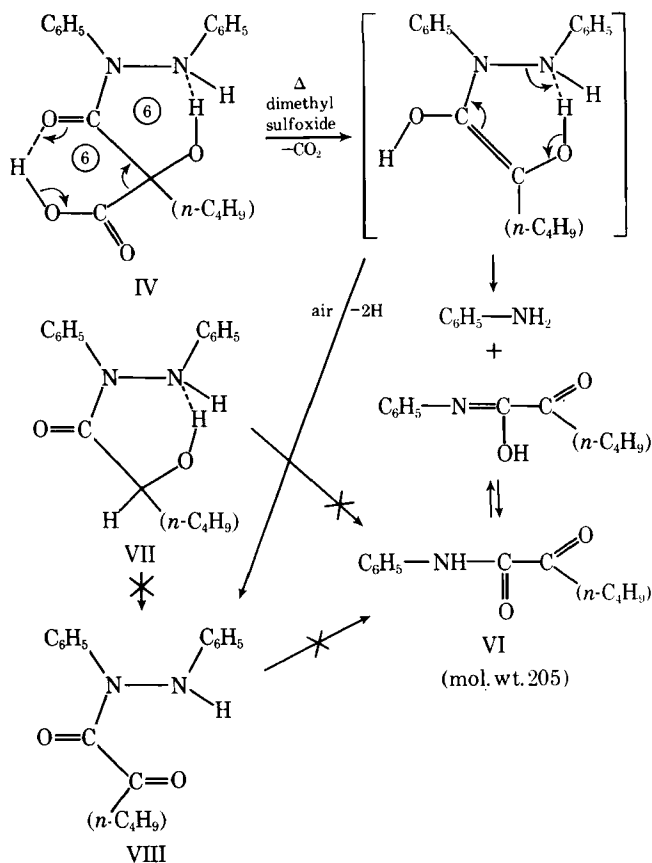


easily by heating the acid neat or in solution in most solvents, pyrolysis of II is an effective method of decarboxylation and yields *N*-caproylhydrazobenzene (V).

Since incorporation of electron-withdrawing substituents alpha to the carboxylic acid grouping normally facilitates decarboxylation of β -keto acids (2), the dramatic difference in the pyrolytic behavior of acids II and IV would seem to be attributable to a difference in the conformational disposition of the carboxylic acid group. The facile dehydration of IV could reflect a close proximity of the carboxylic acid grouping to the terminal nitrogen atom. Association between these two structural elements would also discourage the attainment of the cyclic transition state supported for decarboxylation of β -keto acids (3) (IV in Scheme II).

The mass spectra of I, III, and the methyl esters of II and IV all demonstrate major peaks corresponding to their respective molecular ions. The mass spectrum of II is essentially identical with that of I, indicating facile dehydration with ring closure under the mass spectral conditions employed. No peak corresponding to its molecular ion or to loss of water, carbon dioxide, or both of these elements from its parent molecule could be perceived in the mass spectrum of IV, the highest mass peak being found at *m/e* 205 (48%) and the base peak occurring at *m/e* 93. Scheme III illustrates the probable structures of the main fragments observed for I and its derivatives.

The preference of II for dehydration in the mass spectrometer, rather than decarboxylation as observed under normal pyrolytic conditions, can be rationalized on the basis that decomposition in the mass spectrometer occurs in the gaseous state which would favor the existence of unassociated molecules; the course of normal pyrolysis is directed by the conformation of intermolecularly associated molecules. An acceptable internal hydrogen bond can un-



Scheme II—Degradation of IV

doubtedly be formed between the carboxylic hydroxyl group and the terminal nitrogen atom¹, as illustrated in Fig. 1.

The hydrogen-bonding arrangement suggested for unassociated IV would account for its failure to undergo dehydration in the mass spectrometer, yielding III which, as noted earlier, exhibits a molecular ion (m/e 324). In the solid state (and liquid melt), IV would then be extensively involved in intermolecular hydrogen bonding utilizing the 4-hydroxy and 3-keto functions, with the carboxylic hydroxyl group internally hydrogen bonded to the terminal nitrogen. In the solid state and in solution, II is probably not significantly involved in intramolecular hydrogen bonding².

It was felt that pyrolytic studies in a solvent possessing the ability to disrupt the hydrogen bonding proposed for IV in solid and molten state (as well as in solution in "inert" solvents) might test the preceding rationale. It was hoped that it would be possible to simulate the mass spectral fragmentation and to observe intermediates in this transformation. In particular, it was expected that disruption of the internal hydrogen bond involving the carboxylic acid group would make the decarboxylation step more favorable; it appeared to be the most likely initial phase of the fragmentation.

Accordingly, IV was pyrolyzed in dimethyl sulfoxide. Within 10 min at 160°, IV was totally decomposed and an excellent yield of α -keto-*N*-caproylaniline (VI) (mol. wt. 205) was realized. It was readily apparent that conversion to VI and aniline was close to quantitative, since a similar pyrolysis performed in dimethyl sulfoxide- d_6 yielded a product whose NMR spectrum was superimposable with that of an artificial mixture of authentic samples of the two compounds in equivalent proportions. The mass spectrum of IV was essentially a summation of the mass spectra of VI (m/e 205, 61%) and of aniline (m/e 93, 100%). The mass spectrum of deuterated IV showed peaks at m/e 206 and 95, corresponding to deuterated VI and dideuterated aniline, respectively.

¹ Spectroscopic data (4) and deuterium oxide solvent isotope effects (5) support the generalization that OH—NH bonds are stronger than NH—O bonds.

² Due to lack of solubility, a thorough IR investigation of the character of hydrogen bonding in II and IV could not be pursued. IR spectra of the solids also did not allow any meaningful conclusions to be drawn.

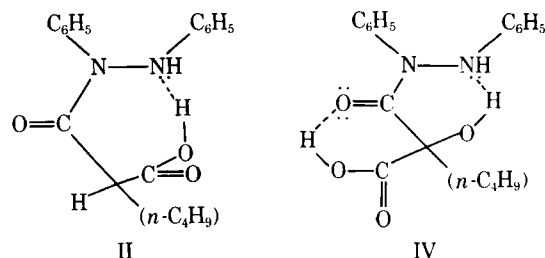
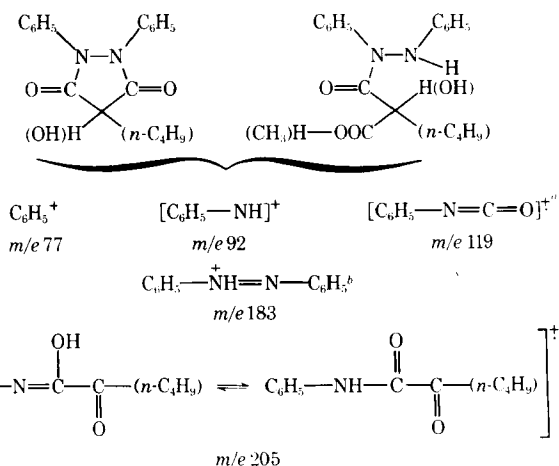


Figure 1—Suggested hydrogen bond formation in unassociated molecules of II and IV.

To gain evidence for the proposed mechanism of decomposition of IV, its pyrolysis under varying conditions was followed by TLC. Chromatography of the product of decomposition in dimethyl sulfoxide at 160° indicated the presence of two other compounds in addition to VI and aniline. Comparison of R_f values and reactions to spray reagents (1) indicated that the major product was VIII and the minor product was III, the product of dehydration of IV. The identity of VIII was confirmed by preparative TLC separation, followed by mass spectrometric and IR spectroscopic analysis; its spectra were identical to those of an authentic sample. Semi-quantitative estimation (1) of the solid precipitated by aqueous dilution of the dimethyl sulfoxide solution indicated a ratio of 91:6:3 (VI:VIII:III). The aqueous filtrate contained aniline and only traces of the other compounds.

The detection of VIII supports the suggestion that the first step of the degradation is decarboxylation (Scheme II). However, formation of VIII requires an oxidation process in addition to loss of carbon dioxide. In the hope of observing the precursor of VIII, the hydroxy acid IV was heated at a reduced temperature (steam bath) under a nitrogen atmosphere. In a control experiment, as in the pyrolyses at 160°, no attempt was made to exclude oxygen. Under a nitrogen atmosphere, no trace of VIII was observed. GC and NMR analysis indicated formation of VIII and VI in a ratio of roughly 1:1, with III being produced in only minor quantity. Compounds VIII and VI were completely stable under all conditions of pyrolysis employed.

Compound VII, which was viewed as a possible precursor to VIII (and which is the acyloin that would be expected to be derived from ketonization of the suggested enediol product of decarboxylation of IV) (Scheme II), was resistant to oxidation under all conditions of pyrolysis utilized, an observation that precludes the intermediacy of VII in the formation of VIII.



Scheme III—Postulated structures for salient mass spectral fragments of I and its derivatives. ^a This fragment does not appear in the mass spectrum of V. ^b This ion is not observed for III, presumably because C-4 hydrogen is essential to its formation in the absence of NH hydrogen. Its abundance in the spectrum of IV is only 10% of that of the base peak, indicative of the ascendancy of N—N fracture. ^c Except for IV and VI, as discussed in the text, only III demonstrates significant abundance (26%) of this fragment; for VII and VIII, the only other compounds in which it is detected, its abundances are 11 and 6%, respectively.

EXPERIMENTAL³

Materials—Reference samples of all compounds examined were used as obtained⁴, and periodic checks by TLC⁵ were made to ascertain their integrity. Additional quantities of IV were prepared by the method of Efimovsky (7).

Solvents were of analytical grade and were used as obtained commercially. Dimethyl sulfoxide was kept dry over molecular sieves.

Pyrolysis Procedures—*Neat*—Substrate (200 mg) was heated for 10 min in an oil bath preheated to 160°. The product was then subjected to TLC and spectroscopic analysis.

Dimethyl Sulfoxide—To 200 mg of substrate was added 2.0 ml of solvent, and the mixture was pyrolyzed in a preheated bath⁶. After cooling to room temperature, the reaction medium was diluted with approximately 8 ml of distilled water.

In the case of II, the cloudy aqueous dimethyl sulfoxide medium was extracted with 2 × 5-ml portions of chloroform, dried over anhydrous sodium sulfate, and then analyzed.

Aqueous dilution of the dimethyl sulfoxide solution of pyrolyzed IV resulted in precipitation of 0.9 g⁷ of yellowish solid. This solid was purified by preparative TLC and identified as VI, mp⁸ 84–85° (uncorrected); IR (mineral oil mull): 3322 (NH), 1720, and 1686 (C=O) cm⁻¹; UV (95% ethanol): λ_{max} 280 nm (ε 530); NMR (dimethyl sulfoxide-*d*₆): δ 10.40 (broad, 1H, amide proton), centered at 7.83 (pair of multiplets, 2H, phenyl protons *ortho* to amide grouping), centered at 7.32 (multiplet, 3H, phenyl protons *meta* and *para* to amide group), 2.92 (broadened triplet, *J* = 7 Hz, 2H, —CH₂—, α to carbonyl), centered at 1.45 (multiplet, 4H, —CH₂—CH₂—, β and γ to carbonyl), and 0.90 (triplet, *J* = 6 Hz, 3H, —CH₃).

*Anal.*⁹—Calc. for C₁₂H₁₅NO₂: C, 70.24; H, 7.31; N, 6.82. Found: C, 70.12; H, 7.46; N, 6.72.

Methylation of II and IV—To a magnetically stirred suspension of 500 mg of II or IV in 20 ml of anhydrous ether was added dropwise an ethereal alcoholic solution of diazomethane¹⁰. Immediate effervescence occurred in both cases, and addition was continued until the yellow color of diazomethane persisted. Stirring was continued for 15 min, and the solution was evaporated to dryness under reduced pressure.

For both reactions, TLC examination indicated complete consumption of II or IV. In the case of II, its methyl ester (IX) (*R_f* greater than that of II) was the exclusive product of the reaction; IR (film): 3330 (NH), 1750, and 1682 (C=O) cm⁻¹; NMR (deuteriochloroform): δ centered at 7.08 (multiplet, 11H, NH and phenyl protons), 3.90 (broadened triplet, *J* = 7 Hz, 1H, α to ester group

ing), 3.67 (singlet, 3H, COOCH₃), centered at 1.93 (multiplet, 2H, —CH₂—, β to carbonyl), centered at 1.28 (multiplet, 4H, —CH₂—CH₂—, γ and δ to carbonyl), and 0.87 [distorted triplet, *J* = 5 Hz, 3H, CH₂—(CH₃)].

The methyl ester (X) of IV was separated from traces of III¹¹ by preparative TLC; IR (potassium bromide): 3440 (OH), 3304 (NH), 1755, and 1690 (C=O) cm⁻¹; NMR (deuteriochloroform): δ centered at 7.17 (multiplet, 10H, phenyl protons), 4.47 (broad, 1H, OH), 3.43 (singlet, 3H, COOCH₃), 2.07 (broadened triplet, *J* = 6 Hz, 2H, —CH₂—, β to carbonyl), 1.33 (broad multiplet, 4H, —CH₂—CH₂—, γ and δ to carbonyl), and 0.87 [distorted triplet, *J* = 5 Hz, 3H, —CH₂—(CH₃)].

SUMMARY AND CONCLUSIONS

The evidence suggests that the initial decomposition product of IV in dimethyl sulfoxide and in the mass spectrometer is a highly reactive product of decarboxylation and readily degrades to VI and aniline at elevated temperatures. However, at lower temperatures in the presence of atmospheric oxygen, IV undergoes decarboxylation plus oxidation to yield VIII; the latter reaction gains importance comparable with that of N—N cleavage under such conditions. Facile oxidation of the proposed intermediate 1,2-enediol would be consistent with the establishment of such species as intermediates to explain the ease of oxidation of acyloins (8).

In dimethyl sulfoxide and in the mass spectrometer, dehydration of IV is an insignificant mode of degradation.

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³ NMR spectra were obtained with a Varian A-60A spectrometer, and chemical shifts are reported in parts per million downfield from tetramethylsilane as the internal standard. IR spectra were recorded on a Perkin-Elmer 621 spectrophotometer, and UV spectra were recorded on a Beckman Acta C-III spectrophotometer. Mass spectra were observed at 70-ev electron beam voltage with a Hitachi Perkin-Elmer RMU-6L spectrometer. IR and NMR spectra for all compounds but VI were previously reported and discussed (6).

⁴ Ciba-Geigy, Basel, Switzerland.

⁵ TLC procedures employed were as previously described (1).

⁶ Pyrolyses were conducted at 160° in an oil bath and at 100° on a steam bath, with care being taken to exclude moisture from the reaction flask. For pyrolyses in a deoxygenated atmosphere, dry nitrogen was bubbled through the reaction medium for 2 hr prior to heating and during the pyrolysis, which was followed by TLC.

⁷ TLC examination of the filtrate indicated only a trace amount of VIII in addition to VI and aniline.

⁸ Kofler micro hot stage melting-point apparatus.

⁹ H. Séguin, National Research Council of Canada Laboratories, Ottawa, Canada.

¹⁰ Diazald procedure of Aldrich Chemical Co.

¹¹ After prolonged storage, even at 0° in ether solution, X gave rise to appreciable amounts (by TLC) of III. In a control experiment, it was determined that the parent hydroxy acid (IV) was not an intermediate in this conversion since the acid underwent no detectable dehydration under conditions leading to formation of III from X.