Guanabenz Degradation Products and Stability Assay

CHARLES M. SHEARER ˣ **and NICHOLAS J. DeANGELIS**

Received October 20, 1977, from the *Pharmacy Research and Development Division, Wyeth Laboratories, Inc., Radnor, PA 19087.* Accepted for publication February 9, 1979.

Abstract □ Guanabenz, [(2,6-dichlorobenzylidene)amino]guanidine, acetate was shown to be the *E*-isomer. It decomposed to form the *Z*-isomer, 2,6-dichlorobenzaldehyde, aminoguanidine, and 2,6-dichlorobenzaldehyde semicarbazone. A stability-indicating assay for the intact drug in the presence of all of its decomposition products by the use of UV spectroscopy is presented.

Keyphrases □ Guanabenz—degradation products, stability, UV spectroscopic analysis □ Antihypertensive agents—guanabenz, degradation products, stability, UV spectroscopic analysis □ Spectroscopy, UV—analysis, guanabenz degradation products □ Stability—guanabenz, degradation products

Guanabenz, [(2,6-dichlorobenzylidene)amino]guanidine, acetate is being evaluated as an antihypertensive agent. To develop a stability-indicating assay, the drug was decomposed by all meaningful modes, *i.e.*, hydrolytic, thermal (dry heat), and photolytic, and all degradation products were isolated and identified. This paper describes the isolation and identification of these degradation products and the development of a rapid spectrophotometric assay specific for guanabenz in their presence.

EXPERIMENTAL

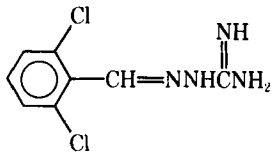
Guanabenz Degradation—Sufficient degradation products for characterization were obtained by using accelerated conditions. The hydrolysates were prepared by refluxing a guanabenz acetate solution in equal volumes of 2 *N* NaOH and ethanol for 8 hr or in 1 *N* HCl for 5 days. These products were separated from the intact drug by a biphase extraction into chloroform from aqueous acid.

Thermal degradation was studied by storing the drug substance at 100 and 75° for several weeks.

The photolytic degradation product was formed by exposing a 1% guanabenz solution in ethanol to UV light in a closed quartz vessel[1] for 1 week. The photolytic product was separated from guanabenz by column chromatography using silica gel[2] as the adsorbent and increasing methanol concentrations in chloroform containing 1% ammonium hydroxide as the eluent.

TLC—TLC was used to determine the purity of the isolated degradation products and to help establish their identity. Silica gel TLC plates with a fluorescent indicator[3] and an eluting solvent of chloroform–methanol–ammonium hydroxide (48.5:50:1.5) were used. Visualization was by shortwave UV light.

For quantitative TLC, 100-μg quantities were applied to the plate; after elution, the silica gel containing the intact guanabenz was scraped off and extracted from the silica gel with 10 ml of 10% 0.1 *N* NaOH in ethanol. The guanabenz concentration was determined by comparing the absorbance of the resulting solution at 305 nm to the absorbance of a standard carried through the same procedure.



I

Spectrophotometric Analysis—UV spectra[4] were obtained using 1-cm path length silica cells and the described solvents. To obtain IR spectra[5], samples were presented in the form of potassium bromide pellets. NMR spectra[6] were obtained by preparing solutions of the samples in deuterated dimethyl sulfoxide containing tetramethylsilane as the internal marker. Mass spectra[7] were obtained using an energy beam of 70 ev with the sample probe at 150°.

Stability-Indicating Assay—*Solvents*—For the pH 4.5 buffer, a 0.2 *M* aqueous monobasic sodium phosphate solution was diluted with an equal volume of methanol.

For the pH 10.8 buffer, a 0.1 *M* aqueous dibasic sodium phosphate solution was adjusted to pH 10.8 with 5 *N* NaOH. Two volumes of this buffer were diluted with one volume of methanol.

Procedure—The sample was quantitatively weighed or diluted into each solvent to obtain a concentration of ~0.01 mg of guanabenz/ml. The method was applicable in the range of 0.004–0.015 mg of guanabenz/ml. If the addition of the sample to the buffer solvent changed the pH by >0.1 unit, the sample was neutralized with acid or base as necessary.

The absorbance of each solution was determined at 265 and 305 nm in a 1-cm silica cell *versus* a solvent blank. The guanabenz concentration (c) in the final solutions was calculated using the following equation:

$$c = \frac{(A_2^{S1} - A_2^{S2}) - 1.32(A_1^{S1} - A_1^{S2})}{78.9} \qquad \text{(Eq. 1)}$$

where A_2^{S1} is the absorbance in pH 4.5 buffer at 265 nm, A_2^{S2} is the absorbance in pH 10.8 buffer at 265 nm, A_1^{S1} is the absorbance in pH 4.5 buffer at 305 nm, and A_1^{S2} is the absorbance in pH 10.8 buffer at 305 nm.

RESULTS AND DISCUSSION

Degradation Product Identification—Guanabenz was synthesized (1) as the acetate by the condensation reaction of 2,6-dichlorobenzaldehyde with aminoguanidine in the presence of acetic acid. Possible degradation hydrolysis routes were: (*a*) the reverse reaction of the synthesis, and (*b*) the replacement of the amino group on the guanidine by oxygen to give 2,6-dichlorobenzaldehyde semicarbazone. Both reactions occurred under reflux conditions in strong acid or base, based on a comparison of TLC R_f values of the degradation products to those of the known compounds.

For further proof of the reactions, the semicarbazone was isolated from the refluxed basic solution and identified by comparison of its IR spectrum with that of an authentic sample prepared from 2,6-dichlorobenzaldehyde and semicarbazide hydrochloride using the classical method (2). The 2,6-dichlorobenzaldehyde was similarly obtained in appreciable quantities from the refluxed acidic solution. No attempt was made to isolate and characterize the aminoguanidine found along with the 2,6-dichlorobenzaldehyde. TLC of the guanabenz-containing layers of the biphase extractions in both refluxed solutions showed a small amount of an additional degradation product, characterized as *Z*-2,6-dichlorophenylmethyleneaminoguanidine and later shown by TLC to be the same as the photoproduct.

In the thermal study, no degradation product was observed by TLC for samples of guanabenz acetate stored at 75 or 100° for 4 weeks. The sample stored at 100° did lose a portion of the acetic acid associated with it, as verified by titrimetric analysis (3) in 50% methanol–water.

The photolytic degradation product was not as readily identified as were the hydrolytic products. Guanabenz in mildly acidic, neutral, or mildly basic solutions undergoes a reversible reaction in the presence of light. The reaction reversibility was established by starting with the photoproduct and allowing it to convert to guanabenz. The same reversible reaction occurred in the dark but only in acidic solution.

[1] A 10-cm pathlength spectrophotometric cell.
[2] Silica gel grade 923, mesh 100–200, Grace & Co., Davison Chemical Division, Baltimore, Md.
[3] Silica gel GF, Analtech Inc., Newark, Del.
[4] Cary model 14.
[5] Perkin-Elmer model 537.
[6] Varian model XL-100.
[7] AE1-MS-902.

0022-3549/79/0800-1010$01.00/0

Table I—Absorptivities of Guanabenz *E*- and *Z*-Isomers

Wavelength, nm	*E*-Isomer		*Z*-Isomer	
	pH 10.8	pH 4.5	pH 10.8	pH 4.5
305	55.2	14.8	18.2	0.2
265	28.3	53.9	40.1	16.2

The reaction reversibility immediately suggested that the two compounds were isomers. The photoproduct structure proved to be like that of the intact guanabenz by many classical structure elucidation methods. The mass spectra of the two compounds were the same (*m/e* 230), as were the elemental analyses (guanabenz: C, 41.29; H, 3.61; Cl, 30.84; N, 23.99; photoproduct: C, 41.57; H, 3.60; Cl, 30.39; N, 24.35). The equivalent weights, as determined by nonaqueous titrations, were the same and were in agreement with the mass spectrometric molecular weight. The ionization constants were 8.3. These were determined spectrophotometrically, thus assuring that changes in the spectra were due to ionization and not to interconversion of guanabenz and the photoproduct. These data and the reversibility of the reaction support the contention that the two compounds are isomers.

IR spectra of guanabenz and the photoproduct were distinctly different, but no useful information could be derived from these differences. NMR spectra showed four nonexchangeable protons. Three of these protons were aromatic and, although the spectra of the two compounds in the aromatic region were slightly different, these differences are not readily interpreted. The singlet peak for the other proton associated with the imino carbon was at 8.22 ppm for guanabenz and at 7.21 ppm for the photoproduct.

Previous investigators (4–9) extensively studied the NMR spectra of *Z*- and *E*-isomers about imino and similar bonds. In all cases, the signal from the *E*-isomer was downfield from that of the *Z*-isomer. A comparison of these data with those for guanabenz and the photoproduct indicated that the photoproduct is the *Z*-isomer and that guanabenz is the *E*-isomer. However, only aliphatic compounds were studied previously (4–9).

UV spectrophotometry was most important in proving the structure of the two compounds. Other investigators (10) found that phenyl ketone semicarbazones were categorized into two types by their UV spectra. One group had a maximum absorbance around 280 nm, and the second group showed a maximum absorbance only at lower wavelengths, if at all. Stenberg *et al.* (11) reported that *Z*-phenyl acetophenone semicarbazone had no peak in its UV spectrum above 230 nm while the *E*-isomer had a maximum at about 270 nm. When the UV spectra for guanabenz and the photoproduct (Fig. 1) were compared with these observations, it became evident that guanabenz was the *E*-isomer and that the photoproduct was the *Z*-isomer.

Differences in the UV spectra of the two isomers can be explained on the basis of steric hindrance. In an examination of a molecular model, a restricted rotation of the dichlorophenyl group in the *Z*-isomer is apparent. The π orbital of the imino bond is nearly perpendicular to the π orbital of the phenyl ring. The π orbital system for the *E*-isomer is nearly parallel throughout the molecule. Thus, the *E*-isomer, with a longer conjugated π electron system, will have a maximum absorbance at a longer wavelength than the *Z*-isomer.

Well-documented *cis–trans* isomerizations of semicarbazones and similar molecules occur under photolytic conditions as well as acidic conditions. Previous investigators (11) reported the photoisomerization of semicarbazones and gave evidence that the photoproduct of acetophenone semicarbazone, when dissolved in an ether solution, isomerized to the starting semicarbazone on exposure to anhydrous hydrogen chloride. Earlier work (12, 13) showed that *Z*- and *E*-isomers of semicarbazones could be interconverted by UV light.

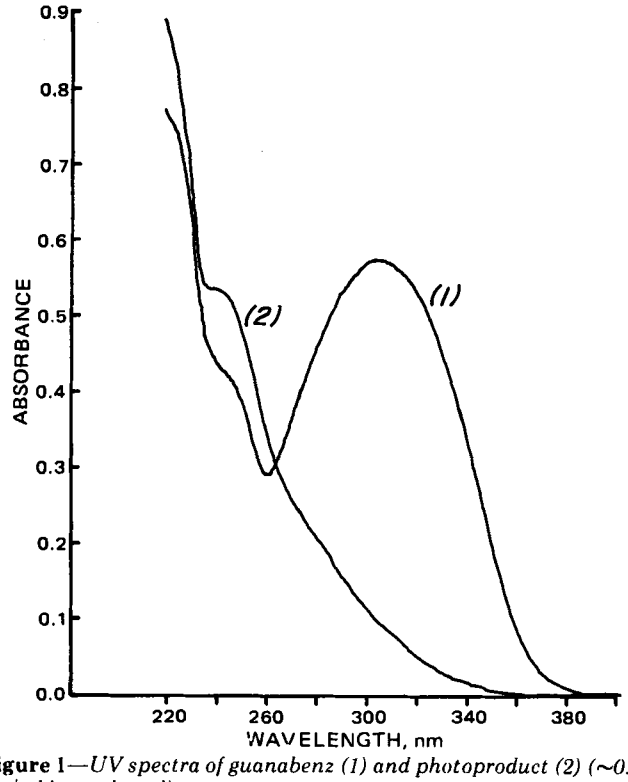


Figure 1—*UV spectra of guanabenz (1) and photoproduct (2) (~0.01 mg/ml in methanol).*

The *cis–trans* photoisomerization of aromatic Schiff bases has been studied (14). Aldehyde 2,4-dinitrophenylhydrazones underwent isomer equilibration in acid solution (15). Methylene bromide solutions of acetaldehyde 2,4-dinitrophenylhydrazone did not reach equilibrium after standing at room temperature for 10 days, while the addition of traces of sulfuric acid effected equilibration in less than a day. Another report (7) gave evidence for acid-catalyzed isomerization of several phenylhydrazones, semicarbazones, and thiosemicarbazones. 2,4-Dinitrophenylhydrazones of α-bromoacetophenone and α-bromobutyrophenone underwent *cis–trans* isomerization when refluxed in an organic solvent in the presence of a trace of hydrochloric acid (16).

Assay Development—The difference in the UV spectra of guanabenz and the *Z*-isomer, as well as the change in spectra with pH as related to the determination of the ionization constant, has been discussed. Figures 2 and 3 present the spectra of the neutral and the protonated species of

Table II—Analysis of Synthetic Samples of Decomposed Guanabenz Acetate

Micrograms per Milliliter					
Z-Isomer	2,6-Dichlorobenzaldehyde Semicarbazone	2,6-Dichlorobenzaldehyde	Guanabenz Acetate	Guanabenz Acetate, mg/ml found	Recovery, %
0.0	0.0	0.0	0.0269	0.0270	100
0.0	0.0	0.0	0.0242	0.0243	100
0.0	0.0	0.0	0.0215	0.0217	101
0.0	0.0	0.0	0.0188	0.0188	100
0.0018	0.0	0.0	0.0242	0.0242	100
0.0	0.0021	0.0	0.0242	0.0244	101
0.0	0.0	0.0018	0.0252	0.0249	99
0.0007	0.0004	0.0004	0.0252	0.0255	101
0.0	0.0012	0.0	0.0252	0.0255	101
0.0007	0.0	0.0008	0.0252	0.0255	101
0.0	0.0	0.0	0.0269	0.0271	100
0.0006	0.0	0.0	0.0263	0.0262	100
0.0011	0.0	0.0	0.0258	0.0260	100
0.0021	0.0	0.0	0.0241	0.0246	100
0.0033	0.0	0.0	0.0236	0.0235	100
0.0054	0.0	0.0	0.0215	0.0217	101

Table III—Comparison of Stability Assay and Quantitative TLC for Decomposed Guanabenz Acetate

Condition for Degradation	Intact Guanabenz Acetate Found, %	
	Stability Assay	Quantitative TLC
5 weeks at 60° in water	89	92
5 weeks in direct sunlight in water	50	50
5 weeks at 60° in 0.1 *N* HCl	76	73
5 weeks in direct sunlight in 0.1 *N* HCl	80	76
5 weeks in direct shortwave UV light (solid)	99	98
3 days at 170° (solid)	72	69

guanabenz and the *Z*-isomer, respectively. Aminoguanidine has no appreciable UV spectrum. The spectra of the two UV-absorbing hydrolysates, 2,6-dichlorobenzaldehyde and 2,6-dichlorobenzaldehyde semicarbazone, do not change between pH 4.5 and 10.8. Thus, only guanabenz and the *Z*-isomer have spectral differences at these two pH values.

By utilizing these differences, a simple stability-indicating assay was developed. The procedure can be considered to be a two-component spectral method; but rather than using the predetermined absorptivities of the two species, this method uses the differences in the absorptivities (Table I) at two wavelengths and the two pH values. Thus, this method combines the concepts of $\Delta\epsilon$ values described by Aulin-Erdtman (17) and Vieroidt's method (18) for two-component mixtures.

Method Validation—To determine the accuracy and specificity of this method, solutions were prepared containing varying amounts of guanabenz and the degradation products (Table II). Solutions containing only small percentages (≤20%) of the decomposition products were prepared since this is the range of interest.

To evaluate this assay procedure further, guanabenz acetate was decomposed in solution and as a dry drug substance under various conditions. These samples were analyzed by the stability-indicating assay, and the results of these analyses were compared with the results of quantitative TLC. The conditions for the degradation and the results are given in Table III.

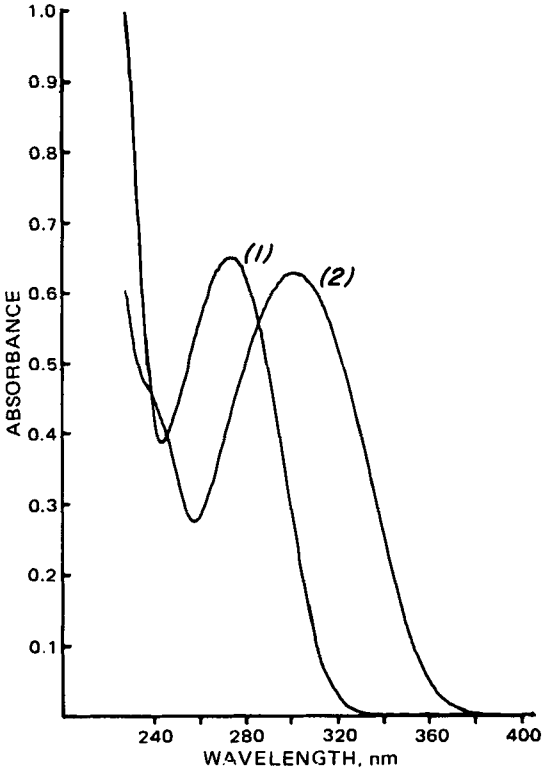


Figure 2—*UV spectra of guanabenz (E-isomer) in pH 4.4 buffer (1) and pH 10.8 buffer (2).*

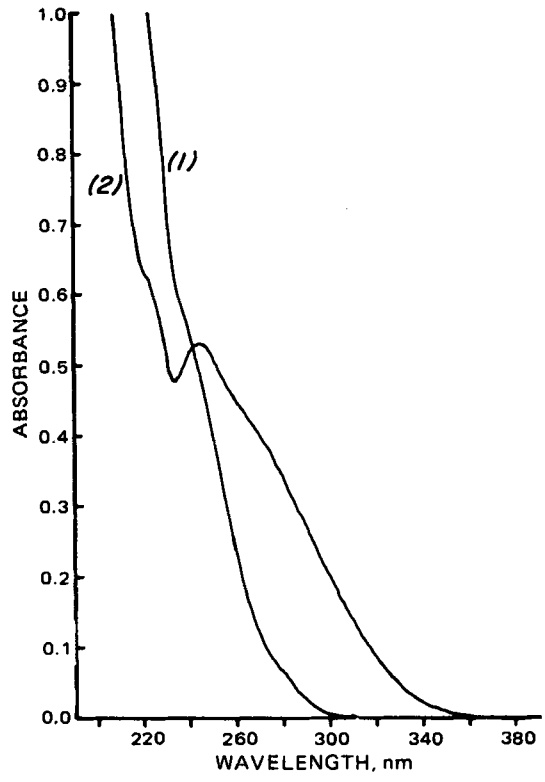


Figure 3—*UV spectra of photoproduct (Z-isomer) in pH 4.4 buffer (1) and pH 10.8 buffer (2).*

REFERENCES

(1) W. F. Bruce and T. Baum, Ger. Offen. 1,802,394.

(2) R. L. Shriner and R. C. Fuson, "The Systematic Identification of Organic Compounds," 3rd ed., Wiley, New York, N.Y., 1948, p. 170.

(3) T. V. Parke and W. W. Davis, *Anal. Chem.*, **26,** 642 (1954).

(4) G. J. Karabatsos and R. A. Taller, *Tetrahedron*, **24,** 3347 (1968).

(5) G. J. Karabatsos and C. E. Osborne, *ibid.*, **24,** 3361 (1968).

(6) G. J. Karabatsos and R. A. Taller, *J. Am. Chem. Soc.*, **85,** 3624 (1963).

(7) G. J. Karabatsos, F. M. Vane, R. A. Taller, and N. Hsi, *J. Am. Chem. Soc.*, **86,** 3351 (1964).

(8) G. J. Karabatsos and N. Hsi, *Tetrahedron*, **23,** 1079 (1967).

(9) G. J. Karabatsos and R. A. Taller, *ibid.*, **24,** 3557 (1968).

(10) M. Ramart-Lucas and M. Bruzau, *Bull. Soc. Chem. Fr.*, **1,** 119 (1934).

(11) V. I. Stenberg, P. A. Barks, D. Bays, D. D. Hammargren, and D. V. Rao, *J. Org. Chem.*, **33,** 4402 (1968).

(12) I. M. Heilbron and F. J. Wilson, *J. Chem. Soc.*, **101,** 1482 (1912).

(13) F. J. Wilson and R. M. Macaulay, *ibid.*, **125,** 841 (1925).

(14) G. Wettermark and E. Wallstrom, *Acta Chem. Scand.*, **22,** 675 (1968).

(15) G. J. Karabatsos, B. L. Shapiro, F. M. Vane, J. S. Fleming, and J. S. Ratka, *J. Am. Chem. Soc.*, **85,** 2784 (1963).

(16) F. Raimirez and A. F. Kirby, *ibid.*, **75,** 6026 (1953).

(17) G. Aulin-Erdtman, *Chem. Ind.*, **1955,** 581.

(18) A. E. Gillam and E. S. Stern, "An Introduction to Electronic Absorption Spectroscopy in Organic Chemistry," Edward Arnold Ltd., London, England, 1958, pp. 214–217.