Stability of Aqueous Solutions of Mibolerone

M. I. AMIN ˣ, K. T. KOSHY, and J. T. BRYAN

Abstract □ The kinetics and mechanism of degradation of mibolerone were studied in aqueous buffered solutions in the pH range of 1–8 at 67.5°. Mibolerone showed maximum stability between pH 5.5 and 6.4. At pH 1–2, the major degradative pathway was dehydration followed by migration of the 18-methyl group to form 7α,17,17-trimethylgona-4,13-dien-3-one. While there was only one degradation product at pH 1–2, the degradation at pH 7–8 was complex. As many as 12 degradation products were detected by GLC. Mass spectral data indicated that the majority of these products were either oxidation products or isomers. At pH 7.6, the apparent first-order rate constants exhibited marked dependency on buffer concentration. Incorporation of a sequestering agent into the solutions eliminated this dependency, suggesting that trace metal impurities from the buffer reagents were catalyzing the degradation. This was confirmed by degradation studies of solutions in water for injection containing 5 ppm of trace metal ions. Sn^{+2}, Cu^{+2}, and Fe^{+2} accelerated the degradation, with Fe^{+2} having the most catalytic effect. The temperature dependence of the rate of degradation was studied in 0.05 *M* phosphate buffer at pH 6.4. The activation energy was 19.6 ± 1.63 kcal/mole.

Keyphrases □ Mibolerone—aqueous solutions, kinetics and mechanism of degradation, GLC evaluation □ Stability—mibolerone aqueous solutions, kinetics and mechanism of degradation, GLC evaluation □ GLC—evaluation, kinetics and mechanism of degradation of mibolerone aqueous solutions □ Anabolic–androgenic agents—mibolerone, aqueous solutions, kinetics and mechanism of degradation, GLC evaluation

Mibolerone[1] (7α,17α-dimethyl-19-nortestosterone) (I) is an androgenic–anabolic nonprogestational steroid. Given orally on a continuous basis, it effectively inhibits estrus in a high percentage of bitches and queens (1).

The purpose of this study was to investigate the factors affecting its degradation in aqueous solutions and the nature of its degradation products so that liquid and semisolid dosage forms could be formulated.

EXPERIMENTAL

Kinetic Studies—*pH–Rate Studies*—Solutions of 0.2 *M* KH_2PO_4 were adjusted to pH 1–8 using either hydrochloric acid or 0.2 *M* sodium hydroxide and were diluted with water for injection to 0.05 *M* phosphate concentration. To 99 ml of the buffer solution at each pH, 1 ml of a 0.2% mibolerone solution in alcohol was added and the contents were mixed. About 11 ml of the solutions was poured into ampuls, sealed, and placed in a 67.5° constant-temperature oven. At appropriate times, two samples were withdrawn; the pH of one was measured, and the other was stored at 4° until GLC analysis. During the study, the pH did not vary by more than 0.15 pH unit.

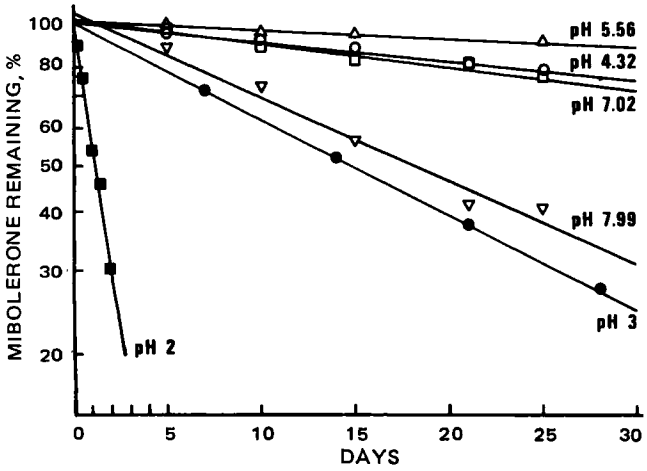


Figure 1—*Apparent first-order disappearance of mibolerone in 0.05 M phosphate buffers at 67.5°.*

Table I—Apparent First-Order Rate Constants of Degradation of Mibolerone in 0.05 *M* Phosphate Buffer at 67.5°

pH	k, days^{-1} × 10^3
1.00	10,219
2.00	6,302
3.00	46.46
4.32	9.22
5.56	3.73
6.40	4.28
7.02	10.40
7.99	39.97

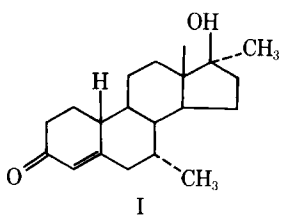
Table II—GLC–Mass Spectral Data for Mibolerone, pH 7.95, 67.5°, 28 Days

Peak	*m/e*	Remarks
1	249	Not likely to be the molecular ion
2		Very weak spectrum
3	279	Not likely to be the molecular ion
4	304	Two more mass units than mibolerone; reduction product?
5	302, 304	An isomer and a reduction product?
6	302	Another isomer of mibolerone?
7		Did not scan
8	302	Mibolerone
9	316	An epoxide?
10	316, 318	Oxidation products
11	314, 318	Oxidation products
12	316, 318	Oxidation products
13		Did not scan

Temperature–Rate Studies—The effect of temperature on the rate of mibolerone degradation was studied in 0.05 *M* KH_2PO_4 buffer, pH 6.4, at 40, 47, 56, 80, and 95°. At appropriate intervals, samples were withdrawn and stored at 4° until GLC analysis.

Assay Procedure: GLC—The samples stored at 4° were brought to room temperature. Ten milliliters of the sample was pipetted into a 50-ml glass-stoppered centrifuge tube. Exactly 10 ml of a chloroform solution containing 15.0 μg/ml of stanolone (17β-hydroxy-5α-androstan-3-one) was added as the internal standard. The tube was shaken, the layers were allowed to separate, and the aqueous layer was removed by aspiration.

The chloroform was removed by a stream of nitrogen, and the residue was redissolved in 0.5 ml of chloroform and transferred to a 1-ml tube. Then 1.0 μl of solution was injected into a gas chromatograph[2]



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[1] The Upjohn Co.

[2] Mikrotek MT-220, Tracor Inc., Austin, Tex.

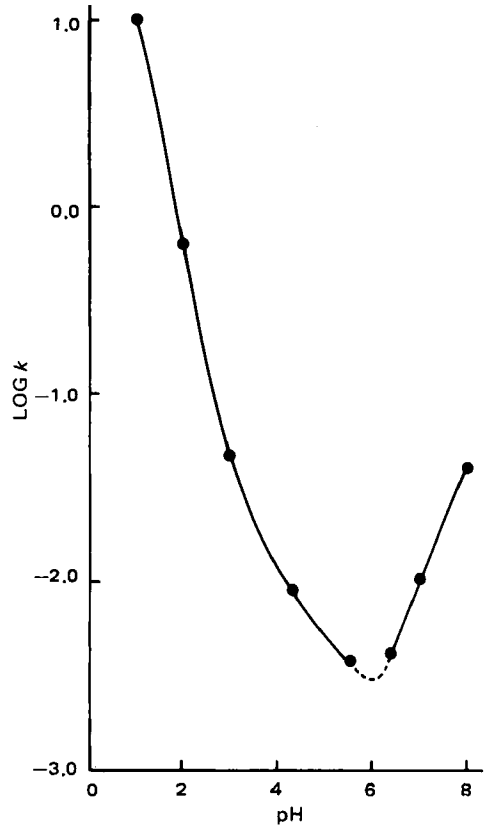


Figure 2—*The pH–rate profile of mibolerone degradation at 67.5°.*

under the following conditions. The column used was 1% QF-1 on Gas Chrom Q (100–120 mesh) in a 3-mm i.d. glass tube, 0.46 m (18 in.). The temperatures were: column, 190°; inlet, 230°; and flame-ionization detector, 260°. The gas flow rates were: helium, 70 ml/min; hydrogen, 40 ml/min; and air, 400 ml/min.

The retention times for stanolone and mibolerone were 2.5 and 4 min, respectively. The concentration of mibolerone in each sample was calculated by comparing mibolerone–internal standard peak height ratios to the peak height ratios of a standard solution of mibolerone treated in the same manner as the sample.

RESULTS AND DISCUSSION

Kinetic Studies—*pH–Rate Studies*—At constant pH and temperature, the degradation of mibolerone followed an apparent first-order process (Fig. 1). The apparent first-order rate constants were calculated from the slopes of log percent residual concentration–time data by means of a computer-programmed least-squares regression analysis (Table I).

The pH–rate relationship for mibolerone is shown in Fig. 2, and the maximum stability was observed between pH 5.5 and 6.4 (the dotted line).

Temperature–Rate Studies—The temperature dependence of the rate of degradation was studied in 0.05 *M* phosphate buffer at pH 6.4. This pH was selected because the semisolid dosage form had a pH of 6.4. The Arrhenius plot is shown in Fig. 3. From the slope of this line, the activation energy was calculated as 19.6 ± 1.63 kcal/mole, from

Table III—Apparent First-Order Rate Constants of Degradation of Mibolerone in Phosphate Buffers in the Presence and Absence of Edetate Disodium at 80°, pH 7.6, and Ionic Strength 0.4

Buffer Concentration	k, Days^{-1} × 10^3 With Edetate Disodium	k, Days^{-1} × 10^3 Without Edetate Disodium
0.1 *M*	3.52	56.19
0.2 *M*	5.88	67.69

Table IV—Apparent First-Order Rate Constants of Degradation of Mibolerone in Water for Injection at 95° and with Different Trace Metal Ions at 5-ppm Concentration

Trace Metal Ions Salt	k, days^{-1} × 10^3
None	15.65
Stannous sulfate	21.49
Cupric sulfate pentahydrate	31.64
Ferrous sulfate heptahydrate	122.88

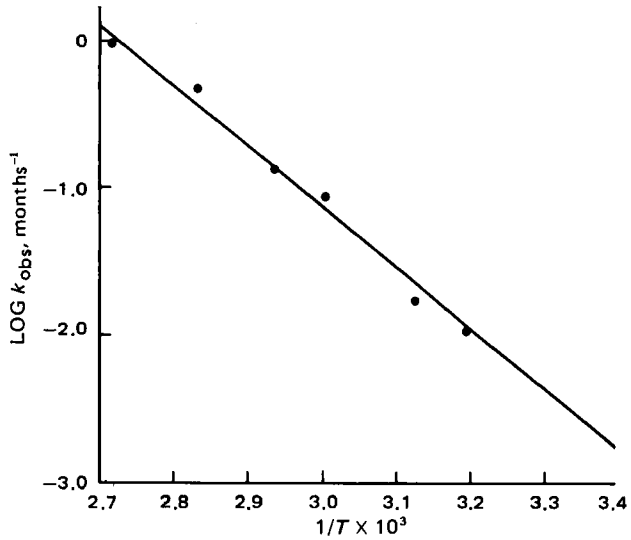


Figure 3—*Arrhenius plot for degradation of mibolerone at pH 6.4.*

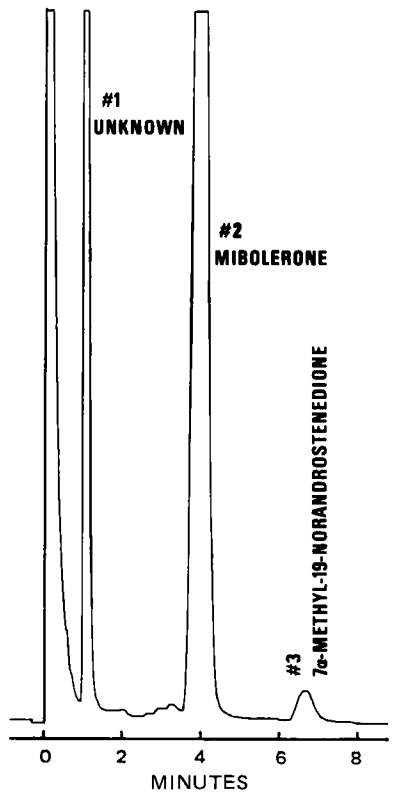


Figure 4—*Gas–liquid chromatogram of a chloroform extract of a buffered aqueous solution of mibolerone, pH 2, heated for 72 hr at 67.5°.*

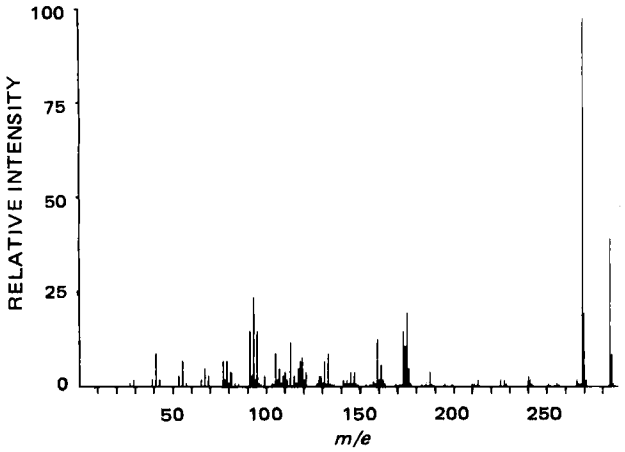


Figure 5—*Mass spectrum of degradation compounds isolated from a buffered aqueous solution of mibolerone, pH 2, heated for 72 hr at 67.5°.*

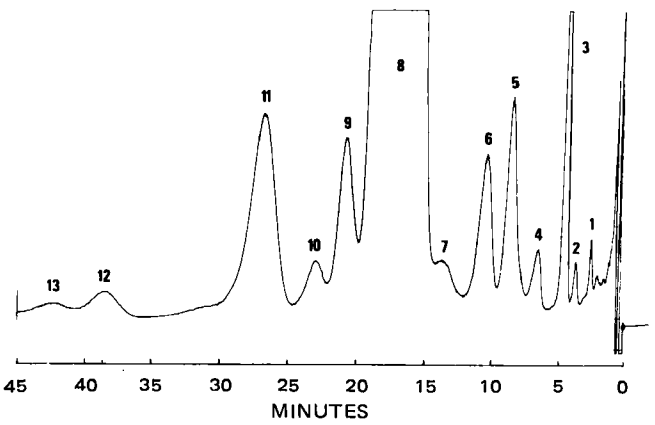


Figure 6—*Gas–liquid chromatogram of a chloroform extract (total ion current detector) of a buffered aqueous solution of mibolerone, pH 7.95, heated for 28 days at 67.5°.*

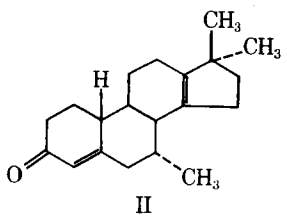
which the prediction was made that at pH 6.4 mibolerone will not degrade by more than 10% after 3 years at 25°.

Mechanism of Degradation and Identification of Degradation Products—A gas–liquid chromatogram of a chloroform extract of a degraded solution of mibolerone at pH 2 is shown in Fig. 4. A similar chromatogram was also obtained from a chloroform extract of a degraded solution of mibolerone at pH 1.

To determine the mechanism of degradation, the degradation product (peak 1) was isolated and identified. The extract of a degraded solution at pH 2 was subjected to TLC on silica gel plates (GF_{254}, 250 μm), using benzene–acetone (4:1) as the developing solvent. The unknown degradation product was nicely separated from mibolerone; the R_f values were 0.57 and 0.31, respectively. The band representing the unknown was scraped and eluted with ethanol, the ethanol was removed, and the residue was redissolved in chloroform. This extract was subjected to GLC–mass spectrometry[3] using 1% QF-1 on a Gas Chrom Q column [0.61 m (2 ft), 3 mm i.d.], and the response was monitored using a total ion current detector.

The mass spectrum is shown in Fig. 5. The molecular ion was *m/e* 284, 18 less than mibolerone. It was suspected that mibolerone had undergone Wagner–Meerwein rearrangement (2), which involves dehydration and migration of the 18-methyl group to form 7α,17,17-trimethylgona-4,13-dien-3-one (II). Authentic Compound II was synthesized by refluxing mibolerone with 3 *N* HCl for 5 hr in a nitrogen atmosphere. Crystals that separated on cooling were recrystallized from methanol (mp 97–99°). The structure was identified by elemental analysis and IR, NMR, and mass spectra. The unknown had TLC and GLC characteristics and a mass spectrum identical to those of the authentic compound.

At pH 7.95, mibolerone degraded more slowly than at pH 2, but the mechanism of degradation was more complex. A degraded buffered solution of mibolerone at pH 7.95 was extracted with chloroform, and the extract was directly subjected to GLC–mass spectrometry as already described [except that the column length was 0.9 m (3 ft)] (Fig. 6 and Table II). As many as 12 degradation products were found. Even though none of the degradation products was identified, it is evident that they were mostly oxidation products.



II

[3] LKB 9000 gas chromatograph–mass spectrometer.

Effect of Buffer Concentration—It was reported (3) that degradation of prednisolone in solutions is autoxidative and is catalyzed by trace metal impurities present in buffer reagents. Thus, the degradation of mibolerone was studied at pH 7.6 at two different buffer concentrations, but at a constant ionic strength, and in the presence and absence of a sequestering agent. In the absence of a sequestering agent, the apparent first-order rate constants showed a dependency on the buffer concentration, suggesting that trace metal impurities in the buffer reagents were catalyzing the degradation (Table III).

Effect of Specific Trace Metal Ions—The effect of specific trace metal ions on the degradation of mibolerone was confirmed by addition of known amounts of metal ions to a solution of mibolerone in water for injection. The amounts of salts used corresponded to 5 ppm of the specific metal ions. The data (Table IV) indicated that Sn^{+2}, Cu^{+2}, and Fe^{+2} accelerated the degradation, with Fe^{+2} having the most catalytic effect. Furthermore, when no metal ions were added, the apparent first-order rate constant was about 2000 times slower than the apparent first-order rate constant obtained from 0.05 *M*, pH 6.4 phosphate buffer at 95° (Fig. 3).

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