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Hydrolytic behavior of dantrolene in acidic media at body temperature

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Dantrolene has been widely used as a muscle relaxant. Tomonaga et al. (1979) studied the degradation of dantrolene in alkaline media at room temperature and found the degradation product, to be an open-ring derivative of the hydantoin ring, but no report has been published on a possible reaction of dantrolene in neutral or acidic media at the body temperature.

Reversible ring-opening reactions of various benzodiazepine derivatives and nitrofurantoin at azomethine bonds in acidic solutions at 37°C have been examined recently (Inotsume and Nakano, 1980, 1981). Such reactions of the acid-labile drugs in acidic media when studied by means of non-specific methods such as simple spectrophotometry may cause errors in the measurement of dissolution rates in acidic media due to chemical degradation of the drug following its dissolution from capsules. In order to determine if such a problem might exist, the rate and extent of hydrolytic reactions of dantrolene were examined.

The kinetic studies on the hydrolysis of dantrolene (from Yamanouchi Pharmaceuticals, Tokyo) were carried out spectrophotometrically. Detailed procedures were described previously (Nakano et al., 1979). The spectral change of dantrolene with time in 0.1 N HCl is shown in Fig. 1. The reaction reached equilibrium after 10 h, and the spectra exhibited two isosbestic points at 311 and 370 nm indicating little accumulation of possible intermediates. The fact that the first-order differential spectrum of dantrolene of 5 nm of differential width at time zero had a negative peak whose amplitude was reduced with time. No derivative band was observed in the equilibrated solution at about 419 nm. These data indicate that the equilibrated solution contained a negligible amount of unreacted dantrolene. The spectrum of the equilibrated solution was thus attributed to the degraded products, 1-aminohydantoin and 5-(*p*-nitrophenyl)furfural, as was the case with nitrofurantoin (Inotsume and Nakano, 1981). Since 1-aminohydantoin (from

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Norwich-Eaton Pharmaceuticals, Norwich, NY) was found to show negligible absorbance beyond 250 nm, absorbance at 383 nm was attributed solely to 5-(*p*-nitrophenyl)furfural. Molar absorptivity of the latter product, was calculated to be 13,400 at 383 nm from the spectrum of the equilibrated solution in 0.1 N HCl which showed no further spectral change with time.

Although the parent drugs were regenerated from acidic solutions by adjusting the pH value to 7.4 in the cases of benzodiazepines (Nakano et al., 1979) and nitrofurantoin (Inotsume and Nakano, 1981), such was not the case with the dantrolene system. The spectrum of the equilibrated dantrolene solution in 0.1 N HCl (which differed markedly from the spectrum of dantrolene itself in 0.1 M phosphate buffer, pH 7.4) did not change even after adjusting the pH value of the solution to 7.4.

The NMR spectral change (JEOL FX-200, Tokyo) of dantrolene in the DMSO-d_6-DCl-D_2O (Merck, Japan) system at 52°C was recorded with time. At time zero, the phenyl proton (4H, 8.01–8.33 ppm, double doublet), azomethine proton (1H, 7.80 ppm, singlet), and furan proton (2H, 7.09–7.34, double doublet) were observed. A new singlet peak appeared at 9.66 ppm and its intensity was increased with time whereas the intensity of the singlet peak due to the azomethine proton was decreased with time. Other peaks of the phenyl and furan protons were shifted to a lower field. The singlet peak at 9.66 ppm was assigned to the aldehyde proton (of 5-(*p*-nitrophenyl)furfural) which is characteristic in this region.

In the case of nitrofurantoin, a phenylhydrazine formed from a reaction of phenylhydrazine with the degraded compound in the equilibrated solution was found to be identical with the authentic 5-nitrofurfural phenylhydrazone. It may be considered that the bond cleavage of dantrolene is similar to that of nitrofurantoin

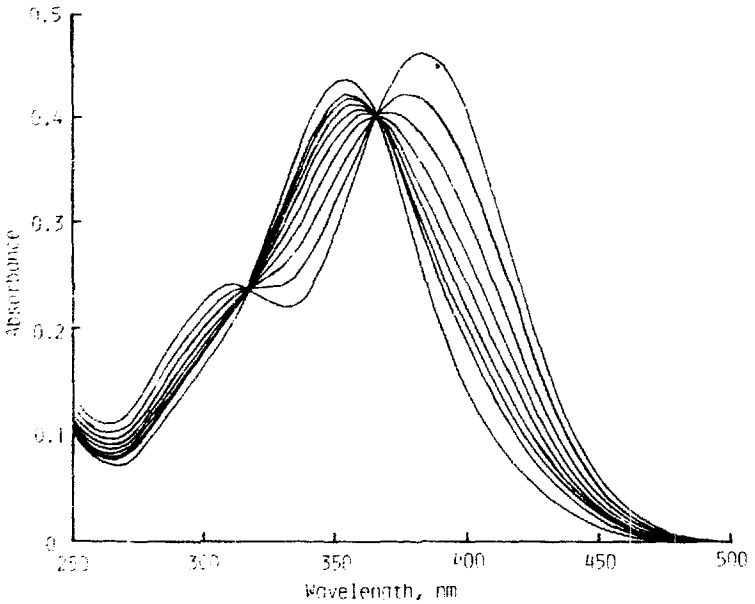

Fig. 1. Typical spectral changes for the hydrolysis of 1.80×10^{-5} M dantrolene in 0.1 N HCl at 37°C. Absorbance at 383 nm decreased with time (0, 20, 40, 60, 80, 100, 120, 140 and 600 (∞) min from the top.

because no other active functional group than an azomethine bond is contained in dantrolene. In the synthesis of dantrolene, 5-(*p*-nitrophenyl)furfural is added to 1-aminohydantoin hydrochloride in which hydrochloride plays a role of a catalyst to form the azomethine bond which is formed by a reversible reaction (Snyder et al., 1967).

In TLC experiments, the sample solution extracted with chloroform was spotted on a cellulose plate (Merck, Japan) repeatedly prior to development by methanol. Dantrolene solution in 0.1 N HCl at time zero gave one spot at $R_f = 0.24$, whereas the dantrolene solution in 0.1 N HCl, which was kept at 37°C for 2 days, gave no spot at $R_f = 0.24$. This observation supports the spectrophotometric data described earlier.

These data indicate that dantrolene degrades at the azomethine bond in acidic solutions to give 5-(*p*-nitrophenyl)furfural and 1-aminohydantoin and that a reversible reaction to regenerate dantrolene in the equilibrated solution is extremely slow. By adding 10 times as much 1-aminchydantoin as dantrolene to the equilibrated acidic solution, the spectra changed slightly in a reverse direction with time. This result indicates that reaction to regenerate dantrolene did occur but the reverse reaction was too slow to be followed quantitatively in the experimental period up to 1 h. When a 10-fold excess of 1-aminohydantoin was added to the incubated acidic solution and the pH value was adjusted to 7.4, no additional change in the spectrum was noted up to 3 h.

The linear relation was obtained in the plots according to the equation for the first-order reaction. The apparent first-order rate constant was calculated to be $0.347 \pm 0.018\ h^{-1}$ ($\pm$S.E.) (n = 4) ir 0.1 N HCl at 37°C. The rate constants at 30 and 42°C were calculated from spectral changes similarly to that at 37°C. An Arrhenius plot was linear and the activation energy was calculated to be 22.7 kcal/mol.

The preliminary studies indicated that spectral changes similar to those shown in Fig. 1 took place in the buffer solutions at 0.1 N HCl-0.1 M CH_3COOH buffer, pH 2.0 and 0.1 M CH_3COOH–0.1 M CH_3COONa buffer, pH 3.0. However, the changes in absorbance were smaller and slower than those in 0.1 N HCl, indicating that the reaction proceeded slower and/or the equilibria tended to favor intact dantrolene. A linear relationship was also obtained in plots based on the equation for the first-order reaction and rate constants were calculated to be 0.155 ± 0.00187 ($\pm$S.E., n = 3) h^{-1} at pH 2.0 and 0.0123 (mean, n = 2) h^{-1} at pH 3.0.

The result that the degradative reaction rate constant of dantrolene is greater than that of nitrofurantoin may be explained by the lower electron density of the furan ring of nitrofurantoin due to higher electron-withdrawing tendency of a nitro group in nitrofurantoin compared to that of a *p*-nitrophenyl group in dantrolene. The same mechanism may be applicable to the acidic hydrolysis of various benzodiazepines (Inotsume et al., 1980).

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