of the same chemical composition as the model system. Of course, if the drug being investigated exists as different polymorphs, and these are sufficiently different energetically, it is important to identify the crystal form which may separate from solid solution and to use this polymorph in constructing the standard curve.

CONCLUSIONS

Although the method developed in this study has its obvious drawbacks and limitations, it has been shown to be useful for estimating the degree of crystallinity in two diverse systems of known chemical composition. In the design of these experiments, it was necessary to choose conditions under which (*a*) the observed dissolution rate was directly proportional to the surface area and (*b*) a reasonably large difference existed between the dissolution rate of the physical mixtures and their corresponding solid solutions. In the case of the indomethacin–PEG system, for example, 0.01 *M*, pH 7.2 phosphate buffer could not be used since this difference was small and could easily lead to erroneous results. Data published by Goldberg *et al.* (4) appears to substantiate the usefulness of the method. Studying the dissolution characteristics of a griseofulvin–succinic acid system they reported, "Although it may be fortuitous, the eutectic mixture which consists of 60% solid solution shows a rate at 3 min. which is just 60% that of the solid solution." This result is not surprising in view of the fact that the dissolution rate of the physical mixture is much slower than that of the solid solution.

Various other applications of the method come to mind. It may be interesting to determine the dissolution rate of drug-carrier systems which form solid solutions as a function of drug concentration. In this way it may be possible to use this technique to construct phase diagrams in regions of solid-solid equilibria. It is expected that more data will be forthcoming when this and other applications have been investigated.

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5(or 4)-[3,3-Bis(2-chloroethyl)-1-triazeno]imidazole-4(or 5)-carboxamide: A Titrimetric Determination of Its *v*-Triazolinium Transformation Product and Studies of Its Stability

RUBY H. JAMES, PAUL D. STERNGLANZ, and Y. FULMER SHEALY

Abstract □ The transformation product, a *v*-triazolinium salt, of 5(or 4)-[3,3-bis(2-chloroethyl)-1-triazeno]imidazole-4(or 5)-carboxamide (I, NSC-82196) is sufficiently acidic to be titrated with standard base. Titrations of typical specimens of the triazene (I) indicate that they contain 2.5–4.2% of the transformation product. The titration method was used to estimate the rate of change of I to its transformation product in methanol and aqueous methanol solutions; for example, in 60% methanol at 25° the half-life of I is estimated to be about 25 min.

Keyphrases □ Triazenoimidazoles □ *v*-Triazolinium salts—analysis □ 5(or 4)-[3,3-Bis(2-chloroethyl)-1-triazeno]imidazole-4(or 5)-carboxamide—stability □ Titrimetry—analysis □ IR spectrophotometry—identity

5(or 4)-[3,3-Bis(2-chloroethyl)-1-triazeno]imidazole-4-(or 5)-carboxamide (I, NSC-82196) has demonstrated interesting antineoplastic activity in animal tumor systems (1–4). The triazene undergoes a change in solution and, very slowly, in the solid state at room temperature to a transformation product (II) containing ionic chloride (1, 5). A *v*-triazolinium salt structure (6) was considered (1, 5) to be one of the likely candidates for the structure of II, and this structure has recently been assigned to II on the basis of an X-ray crystal structure analysis[1] (7). Once a pure specimen of II had been obtained, the quality of specimens of the triazene (I) could be estimated qualitatively from distinctive differences in the IR spectra of I and II (5). A titrimetric method for the determination of II—and, indirectly, of I—is now reported together with additional information on the stability of the triazene (I). A colorimetric (10) and a microbiological (11) method of assaying I were recently reported. The possible formation of II from I was not mentioned in those reports, and it is not clear whether the material being assayed was I, II, or a mixture.

[1] Good chemical evidence for the formation of *v*-triazolinium salts in the benzenoid series has been reported by Mohr and Hertel (6). This type of structure has also been assigned (8, 9) to other phenyl derivatives, but evidence that would distinguish the *v*-triazolinium structure from alternative structures was not presented.

Table I—Titration of I, II, and Synthetic Mixtures

Calcd. % of II in Synthetic Mixture[a]	% of II Found
No II added	2.5–4.2[b, c]
3.8	4.7
5.0	6.3
7.3	8.4
9.5	10.7
10.4	10.3
11.7	11.6[d]
25.7	24.9[d]
35.9	35.3[d]
37.0	38.0[e]
46.8	46.4[d]
100[f]	100 ± 2[c]
17.9[g] lactose	18.3[h]
19.6[i] present	20.2[h]

[a] Calculated from milligrams of II actually added + milligrams of II (found by acidimetric titration) introduced as an impurity when I was added to the synthetic mixture. [b] Typical values for different specimens of NSC-82196 were 2.5, 3.2, 3.3, 3.4, 4.1, 4.2%. [c] Solvent for specimens was either absolute methanol or 75% methanol. [d] Solvent for mixture was absolute methanol. [e] Titration time = 33 min. [f] Specimens of pure II. [g] 4.16 mg. of II added + 25 mg. of I containing 4.2% of II + 330 mg. of lactose; 4.16 mg. + 1.05 mg. = 5.21 mg. of II. Percent = 5.21 × 100/29.16 = 17.9%. [h] Reversed procedure. [i] 4.8 mg of II + 25 mg. of I containing 4.2% of II + 330 mg. of lactose; 19.6% of II calculated as in *g*.

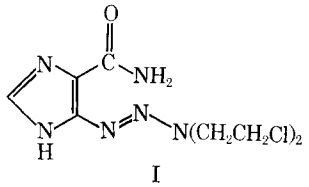
The change of I to II results in the formation of a more acidic compound, pKa′ = 7.4, that can be titrated with standard base. The endpoint in a methanol–water mixture (3:1) occurs at an apparent pH of about 9; pure specimens of II gave assay values of 100 ± 2%. The method can be used to determine the percentage of II in specimens of I, but the preparation of the solution of I and the titration should be performed rapidly and at 5–10° in order to minimize the error that may be introduced by the generation of additional II during the determination. Excessive speed, however, may also introduce an error due to the failure of the solution or the electrodes to reach equilibrium. Typical specimens of I that were shown by their IR spectra (especially at 1515 cm.$^{-1}$) to contain only small amounts of II (*e.g.*, Fig. 3, *Reference* 5) gave values of 2.5–4.2% of II by acidimetric titration in methanol–water (3:1) at ice-bath temperatures. Results of the titration of synthetic mixtures were in reasonably good agreement with the calculated composition (Table I). These results, including those from one experiment in which the titration time was deliberately prolonged, indicated that very little II is formed under the conditions used for the determination. As shown in Table I, the presence of lactose, a potential formulating agent for oral administration, does not interfere with the titration of II.

Table II—Decomposition of I in the Solid State at 21°

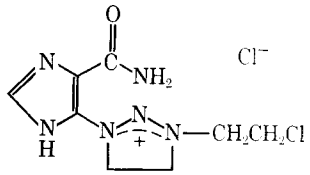
Storage Time, Days	% II Found
Zero	4.9
4	5.5
8	7.0
11	8.8
14	7.7
18	10.0
35	20.7
56	49.1
126	95.0

Table III—Stability of I in Methanol–Water Mixtures

Solvent Mixture Methanol, %	Solvent Mixture Water, %	Temp., °C.	Approximate Half-Life
100	0	25	4 ± 0.2 hr.
75	25	25	58 ± 10 min.
60	40	25	26 ± 5 min.
60	40	0	17 ± 3 hr.
40	60	25	16 ± 5 min.



I



II

Previously, the formation of II from I in the solid state at room temperature had been observed by IR spectroscopy (5). This change has been followed by acidimetric titration of portions of a specimen of I stored at 21° during a period of about 4 months. The titrimetric data are summarized in Table II and indicate approximately 20 and 95% conversion after 35 and 126 days, respectively. IR spectra, determined each time a portion was titrated, also revealed the increasing concentration of II and were consistent with the titration data. In absolute methanol solution at 0°, Compound I was essentially unchanged after 3 hr.; the initial and final percentages of II found were 2.5 and 3.4, respectively. At 25°, the half-life in methanol, as determined by acidimetric titration, is approximately 4 ± 0.2 hr. Information on the stability of I in aqueous media is of greater relevance to biological or clinical studies, but the solubility of I in pure water is too slight to permit a meaningful determination. Application of the titrimetric method to aqueous methanol solutions of I yielded the approximate half-lives recorded in Table III. These data suggest that the triazene (I) would be rapidly inactivated if it were administered at ordinary temperatures in a homogeneous aqueous vehicle. (In animals, it has been administered as a suspension.)

NMR determinations showed that Compound I was also converted to II in DMSO-d_6 and in acetone-d_6-D_2O, a solvent combination chosen because of evidence (5) of considerable solubility of I in acetone-H_2O. However, in anhydrous trifluoroacetic acid, the NMR spectrum of I (the recording of which was started within 3 min. of the addition of the solvent) was different from that of II and consistent with the triazene structure (I). The spectrum was unchanged after 4 hr. (temperature about 37°), an observation indicating a stabilizing effect of protonation on I. In the less acidic medium provided by acetic acid (deuterated or glacial), the change of I to II could be observed by means of the chemical shifts of the 2-imidazole protons and proceeded somewhat more slowly than it does in the aqueous methanol solutions studied.[2]

[2] The rate of change in acetonitrile, DMSO, and an unspecified solvent of a bis(2-chloroethyl)triazene of the phenyl series to a transformation product, presumably a *v*-triazolinium salt (see *Footnote* 1), has been estimated from NMR data (9).

EXPERIMENTAL

Potentiometric Titration of II in I—A combination electrode is precooled in an ice bath to about 5°, and the pH meter is standardized with buffers precooled to the same temperature.[3] After standardization, the electrode is again immersed in the ice bath so that thermal equilibrium is maintained at all times. The sample (about 20 mg.) is dissolved quickly in 15 ml. of anhydrous methanol precooled to 5° and 5 ml. of precooled water is added. Titration is performed with standard 0.01 *N* NaOH as fast as possible. The end-point is found, as usual, at the point where the ratio $\Delta pH/\Delta ml.$ is the greatest and occurs at an apparent pH of about 9. Typical results of titrations of I, II, and synthetic mixtures are shown in Table I.

The sample dissolves faster in methanol than in methanol–water mixtures, but water is added because electrode response in methanol alone is slow. Nevertheless, titrations done in methanol alone agree well with those done in the 3:1 methanol–water mixture (*cf.* Table I). Care is taken that the temperature remains around 5° and under no circumstances rises above 10°. The titration should proceed as fast as the electrode response permits. Typical titration times were 5–8 min., but obviously less time is required when small amounts of II are present. Excessive speed in a titration might cause inaccuracy due to incomplete mixing and nonequilibrium condition of the electrodes. Since the time factor cannot be eliminated, some transformation product may be generated during the titration. Evidence that the error due to decomposition is relatively small is as follows: (*a*) when a synthetic mixture containing 37% (calculated) of II was determined by deliberately prolonging the titration time to 33 min., the value found was 38% (Table I); (*b*) the stability data for methanol or 60% methanol solutions at 0° indicate that the change of I to II is slow at the ice-bath temperature used for the titration; (*c*) the purity of a sample of I determined by a nonaqueous method[4] was 97 ± 1%, and the amount of II found by titration of the same sample in 75% methanol with standard base, as described above, was 3%.

Titration of mixtures of I (25 mg.) and lactose (330 mg.) necessitated a reversal of the order of adding solvents. First, 5 ml. of cold water is added to the sample and then 15 ml. of cold methanol, and the titration is carried out rapidly as described before. If the order of solvent addition is not reversed, too much undissolved lactose is present, and the electrodes respond poorly. The reversed procedure was also used to titrate I alone. Results obtained by the reversed procedure are almost identical with those from the regular procedure.

pKa′ Value of II—The pKa′ value was determined by titrating about 0.15 meq. of II with 0.06 *N* NaOH and taking pH readings when 1/4, 1/2, and 3/4 of II was neutralized. The pKa′ value, calculated from the three readings by using the Henderson-Hasselbach equation, was 7.4.

Stability Determinations—A crystalline specimen of I was stored in a sealed (screwcap) brown bottle in a constant-temperature room at 21 ± 1° and 65% relative humidity. Portions were removed at intervals for titration; results are shown in Table II.

The triazene (I) is too insoluble in water for a determination of the rate of change of I to II in completely aqueous media; the apparent (slow) dissolution of I in water is due chiefly to the conversion of I to II. However, determination of approximate rates of conversion of I to II in aqueous methanol is feasible because Compound I dissolves quickly in amounts adequate for titrimetric determination of II. A specimen of I (usually 25 mg./100 ml.) was dissolved at either 25 or 0° in methanol or the specified methanol–water mixture, aliquots were removed and rapidly adjusted to 5°, and the titration was quickly performed at 5–10° according to the procedure described above. At least three determinations were made for each solution. Plots of the logarithm of the concentration of undecomposed I *versus* time indicate that the decomposition follows first-order kinetics, at least through one to two half-lives. From these plots half-lives were estimated and are summarized in Table III. Obviously, the time factor becomes important for aqueous methanol solutions at 25°, especially for 40 and 60% methanol, because the reaction is relatively fast. The following modifications of the stability determinations in 40 and 60% methanol solutions were also tried: (*a*) the solutions were prepared by dissolving I in cold absolute methanol and adding cold water, the temperatures of the two solvents having been predetermined to give a temperature (due to heat of mixing) of 25° after mixing (zero time); (*b*) the aliquot solutions were converted quickly to 75% methanol by adding precooled methanol in order to make the titration conform to the standard procedure. The results were within the stated ranges (Table III). Despite the limitations of the titration method, approximate half-lives obtainable by titration can provide useful information, not previously available, on the stability of I in solution.

NMR—NMR spectra were determined with an NMR spectrometer (Varian model A-60A); chemical shifts are in parts per million (δ scale) downfield from tetramethylsilane, the internal standard. Chemical shift data for the triazene (I) in trifluoroacetic acid (not deuterated) were as follows: 4.19 (center of A_2B_2 multiplet, $—CH_2-CH_2Cl$), about 7.7 (broad, NH_2), 8.67 (singlet, imidazole CH). Chemical shifts of the protons of II in trifluoroacetic acid are as follows: 4.38 (center of A_2B_2 multiplet, $—CH_2CH_2Cl$), 5.13 (multiplet approaching a singlet, $—CH_2CH_2—$), about 7.9 (broad, NH_2), 8.87 (singlet, imidazole CH).

[3] A Beckman model 76 or a Corning model 12 pH meter and a Sargent macro or micro combination electrode were used.

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