Determination of Dialkyltriazenoimidazoles by Nonaqueous Titration

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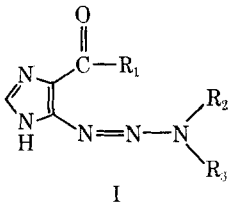
Abstract □ An analytical method for dialkyltriazenoimidazoles has been developed. It serves as a reliable purity check on these anticancer drugs whose effectiveness is a function of their purity. The method consists of titration in nonaqueous media with perchloric acid. The titrant, perchloric acid, is dissolved in a nitroethane–chlorobenzene mixture. Samples are dissolved in glacial acetic acid or in acetonitrile. Poor solubility of some of the compounds is overcome by the selection of an appropriate solvent system, chosen to give also optimum titration end-points. The electrode system consists of conventional glass and calomel electrodes. The method is directly applicable to the unstable 5(or 4)-[3,3-bis(2-chloroethyl)-1-triazeno]imidazole-4(or 5)-carboxamide, without interference by its transformation product, 1-(2-chloroethyl)-3-[5(or 4)-carbamoylimidazol-4(or 5)-yl]-*v*-triazolinium chloride, usually present in small amounts.

Keyphrases □ Dialkyltriazenoimidazoles—analysis □ Anticancer drugs—purity determination □ Potentiometric nonaqueous titration—analysis

Dialkyltriazenoimidazoles (1, 2) (I) exemplified by II–IV have shown much promise as useful chemotherapeutic drugs (3–7). 5(or 4)-(3,3-Dimethyl-1-triazeno)imidazole-4(or 5)-carboxamide (II) is receiving clinical trial as an anticancer agent (8, 9). 5(or 4)-[3,3-Bis(2-chloroethyl)-1-triazeno]imidazole-4(or 5)-carboxamide (IV) is highly active against experimental leukemia (4, 7), and methyl 5(or 4)-(3,3-dimethyl-1-triazeno)-imidazole-4(or 5)-carboxylate (III) has shown activity as both an antileukemic and an antimicrobial agent (5). For these reasons, a simple assay method that could be applied equally well to all three triazenoimidazole derivatives, as well as to other derivatives of the general structure of I, would be potentially very useful. In addition, Compound IV is unstable at room temperature, but it can be preserved for months at −15°. Its transformation product (V) has been assigned (10) the structure of a *v*-triazolinium salt formed by internal alkylation. Since the transformation product V has no demonstrated biological activity, it is important that its presence does not influence the assay of IV.

A colorimetric method for dialkyltriazenoimidazoles in plasma and urine has been reported recently (11). However, this method may not distinguish between IV and V and is less suitable as an assay method of solid samples. In contrast, the proposed method is applicable equally well to the three imidazole derivatives II, III, or IV; neither the transformation product V nor lactose, a formulating agent for oral administration, interferes. The method is based on a modification (12) of the nonaqueous titration of weak bases with perchloric acid.

Solutions of Compounds III and IV are prepared in acetonitrile and of Compound II in glacial acetic acid, respectively, the choice being dictated by solubility. The titrant is perchloric acid dissolved in acetic acid or in a nitroethane–chlorobenzene mixture. Either titrant can be used for the titration of Compounds II and III. However, perchloric acid must be dissolved in a nitroethane–chlorobenzene mixture for the titration of Compound IV. This titrant, recently recommended by Huber (12), has not been applied to any extent in nonaqueous titrimetry. The special titrant was chosen because the presence of glacial acetic acid causes poor end-points in titrations of Compound IV and mixtures of IV and V.



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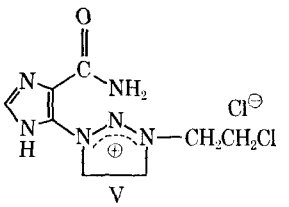

V

II, $R_1 = NH_2$, $R_2 = R_3 = CH_3$
III, $R_1 = OCH_3$, $R_2 = R_3 = CH_3$
IV, $R_1 = NH_2$, $R_2 = R_3 = CH_2CH_2Cl$

EXPERIMENTAL

Apparatus—A Corning model 12 research pH meter (with expanded scale) was used with a Sargent/Jena combination electrode (E. H. Sargent, item S-30072-15 or miniature S-30070-10). The aqueous KCl in the reference electrode compartment was replaced by 0.1 *M* $LiClO_4$ in isopropanol.

Preparation of Titrant (12)—Place about 500 ml. of nitroethane in a 1000-ml. volumetric flask; cool in an ice bath; and add 2.1 ml. perchloric acid (72% w/w) and then, dropwise, 5 ml. acetic anhydride. Fill to the mark with chlorobenzene. Let stand overnight. Standardize against either potassium acid phthalate (National Bureau of Standards Sample No. 84) or against 1,3-diphenylguanidine (Standard Grade, G. F. Smith Chemical Co., Columbus, Ohio).

Procedure—Use 0.05 to 0.07-mmole sample; dissolve III or IV in 15 to 20 ml. acetonitrile and II in 15 to 20 ml. glacial acetic acid. The compounds are only partially soluble but will dissolve completely during titration under magnetic stirring. Titrate with standard 0.025 *N* perchloric acid to the potentiometric end-point.

The titrant is introduced from a 5-ml. buret with 0.01-ml. subdivisions.

If mixtures of Compound IV and lactose are analyzed, the sample size should be adjusted so that the lactose will not exceed 350 mg. Although the lactose is insoluble in acetonitrile and is not expected to interfere, amounts exceeding 350 mg. have been found to affect the electrode response adversely.

RESULTS AND DISCUSSION

Preliminary experiments had shown that the solubility of the imidazole derivatives in solvents suitable for titration was poor; for example, less than 0.1% of Compound IV dissolves in acetonitrile. This seemed to be an obstacle for a titration method but, fortunately, the undissolved particles of the imidazole derivatives go into solution while the stirred suspension is being titrated.

Poorly soluble compounds are generally not assumed to be analyzable with the same precision and accuracy as those of good solubility because titration end-points are less distinct in dilute solu-

Table I—Assay of Triazenoimidazole Derivatives by Nonaqueous Titration

	% Purity Found	Standard Deviation	Number of Determinations
Compound II[a]	99.1	0.79	5
Compound III[a]	99.7	0.20	5
Compound IV[b]	96.8	0.55	5

[a] Absence of impurities in Compounds II and III was confirmed by elemental analysis and TLC. [b] Contamination of IV by V was found by direct determination of V; the amount of V ranged from 2.5 to 4.2% (13).

tion. However, Table I proves that the precision and accuracy of the proposed method are good.

In Table II the analyses of synthetic mixtures of IV with lactose, and of IV with V and lactose, are shown. Comparison of the amount of Compound IV actually present (indicated in Column 4) and found (indicated in Column 5) shows that neither lactose nor Compound V interferes in the analysis of IV. The weighed amount of IV (in Column 1) differs from the actual amount present (in Column 4) because IV contains an average amount of 3% transformation product V.

Compounds II, III, and IV are weaker bases than imidazole (pKa′ = 7.20 for imidazolium ion in water) (14). The base-weakening effect is attributed to the electronegativity of the triazeno and carboxamide or carboxylate groups. The bis(2-chloroethyl)triazeno group of Compound IV is much more base weakening than the dimethyltriazeno group of Compounds II and III.

Glacial acetic acid cannot be used in the titration of IV for two reasons: Compound IV is too weak a base and, therefore, poor titration breaks would be obtained and, furthermore, the transformation product V, present as an impurity in IV, interferes. Bases too weak to be titratable in acetic acid often become titratable in acetic anhydride. This is the case with Compound IV, and good titration breaks are obtained; but acetic anhydride cannot be used in this particular analysis either, because the transformation product V would be simultaneously titrated with IV.

In acetonitrile, the situation is different; Compound IV is sufficiently basic to be titratable (if acetic acid is absent) while the transformation product V remains neutral. Perchloric acid in acetonitrile would be a good titrant but it is unstable. Perchloric acid in acetic acid is very stable but causes poor end-points in the titration of IV. Perchloric acid in nitroethane–chlorobenzene gives the best results and is therefore used.

Table II—Titration of Mixtures of IV and V and Lactose[a]

% IV Weighed	% V Weighed	% V Actual[b]	% IV Actual[b]	% IV Found
100	0			97.3
100	0			97.0
85.6	14.4	17.0	83.0	82.5
69.4	30.6	32.7	67.3	66.8
49.4	50.6	52.1	47.9	48.0
21.0	79.0	79.6	20.4	20.4
14.4	85.6	86.0	14.0	14.4

[a] Lactose (330 mg.) was added to all six samples. [b] The values for "% V Actual" and "% IV Actual" are based on an average assay of 97% purity (or presence of 3% V in the starting material IV, see Table I).

REFERENCES

(1) Y. F. Shealy, C. A. Krauth, and J. A. Montgomery, *J. Org. Chem.*, **27**, 2150(1962).

(2) Y. F. Shealy, C. A. Krauth, S. J. Clayton, A. T. Shortnacy, and W. R. Laster, Jr., *J. Pharm. Sci.*, **57**, 1562(1968).

(3) Y. F. Shealy, J. A. Montgomery, and W. R. Laster, Jr., *Biochem. Pharmacol.*, **11**, 674(1962).

(4) Y. F. Shealy and C. A. Krauth, *Nature*, **210**, 208(1966).

(5) Y. F. Shealy, C. A. Krauth, R. F. Pittillo, and D. E. Hunt, *J. Pharm. Sci.*, **56**, 147(1967).

(6) I. Wodinsky, J. Swiniarski, and C. J. Kensler, *Cancer Chemother. Rep.*, **52**, 393(1968).

(7) G. Hoffman, I. Kline, M. Gang, D. D. Tyrer, J. M. Venditti, and A. Goldin, *ibid.*, **52** (Part 1), 715(1968).

(8) C. McDonald, N. Wollner, F. Ghavimi, and J. Zweig, *Proc. Amer. Ass. Cancer Res.*, **8**, 43(1967).

(9) T. L. Loo, E. A. Stasswender, J. A. Jardine, and E. Frei, III, *ibid.*, **8**, 42(1967).

(10) D. J. Abraham, J. S. Rutherford, and R. D. Rosenstein, *J. Med. Chem.*, **12**, 189(1969).

(11) T. L. Loo and E. A. Stasswender, *J. Pharm. Sci.*, **56**, 1016 (1967).

(12) W. Huber, *Z. Anal. Chem.*, **216**, 260(1966).

(13) R. H. James, P. D. Sternglanz, and Y. F. Shealy, *J. Pharm. Sci.*, **58**, 1193(1969).

(14) C. J. Hawkins and D. D. Perrin, *J. Chem. Soc.*, **1962**, 1351.

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