Most selectivity ratio data published in the literature have been obtained by comparing the potential measured in 0.1*M* NaCl with that measured in 0.1*M* KCl (corresponding to $X_1 = 0$ in our measurements). For NAS-11-18 electrodes in dilute solutions at pH = 7, the selectivity ratio has been reported to be between 0.003 (*1*, *18*) and 0.001 (*16*). For the LAS-10-23 electrodes, K has been reported to be as small as $10^{-5}$, but depends strongly on glass composition (*1*, *18*).

We have calculated values of selectivity ratio K from Equation 3 using our potential measurements together with activity coefficient values based on isopiestic data. The empirical equations of Rush (*8*), fitted to the experimental isopiestic data of Robinson (*14*, *15*) were evaluated to obtain $\gamma_{12}$ and $\gamma_{21}$. The selectivity ratios obtained by solving Equation 3 are listed in Table I. Note that values of K obtained for $X_1 > 0.01$ are unreliable, since they tend to reflect small differences between the potential and isopiestic measurements.

Our values of K (for $X_1 < 0.01$) obtained at high ionic strengths (1*m* and 3*m*) are of the order of 0.001, qualitatively confirming the low ionic strength results. However, since the exact glass compositions were not known; and only one sample of electrode C and two samples of electrode B were used, these measurements cannot be considered a definitive study of selectivity at high ionic strengths. Nevertheless, K seems to be somewhat smaller for electrode C than for electrode B, and this leads to the tentative conclusion that the NAS-11-18 glass is not only more stable than the LAS-10-23 glass at high ionic strengths, but is also more selective. The differences, however, are not large.


### ACKNOWLEDGMENT

The authors thank R. M. Rush and R. A. Robinson for providing the results of the latest calculations on their isopiestic data.

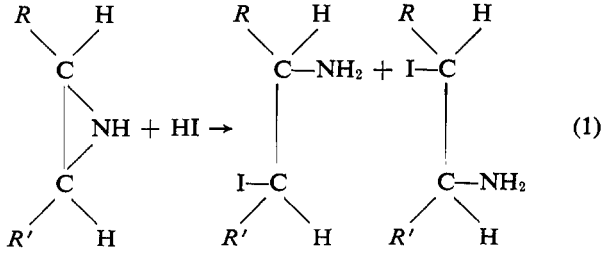
RECEIVED for review May 26, 1969. Accepted July 28, 1969. This work was supported by the U. S. Department of the Interior, Office of Saline Water.


# Determination of 2,3-Dialkylaziridines by Direct Titration with Hydrogen Iodide


**Gerhard Maerker, E. T. Haeberer, L. M. Gregory, and T. A. Foglia**

*Eastern Utilization Research & Development Division, Philadelphia, Pa. 19118*


THE CHEMISTRY of 2,3-dialkylaziridines unsubstituted on nitrogen, has recently aroused considerable interest and is being studied in this laboratory. No suitable direct titrimetric method has been reported for the determination of aziridines of this type, most of which are insoluble in aqueous media. Jay (*1*) described the direct titration of epoxides and aziridines with perchloric acid in the presence of quaternary ammonium halides. In this procedure the active agent is the hydrogen halide generated *in situ* (HBr for epoxides, HI for aziridines). This method, developed for application to aziridines bearing electron-withdrawing substituents on nitrogen, is unsatisfactory for compounds of type I, because the iodoamines formed (Equation 1) interfere with the determination of the end point.




(1)

The following procedure, a modification of the Jay method, permits the direct titration of 2,3-dialkylaziridines unsubstituted on nitrogen. Prior to titration the sample is treated with acetic anhydride to form the *N*-acyl derivative which is not isolated. The anhydride is usually used as the solvent in which the titration is carried out, but may be used as reagent in conjunction with another, inert, solvent.

(1) R. R. Jay, ANAL. CHEM., **36**, 667 (1964).

## EXPERIMENTAL

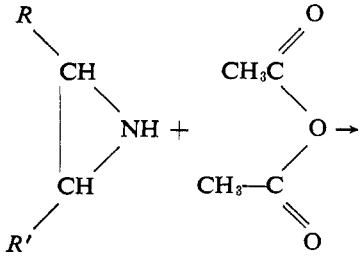
**Reagents.** Standard 0.02*N* $HClO_4$ in glacial acetic acid. Add 1.7 ml of 72% $HClO_4$ and 4 ml of acetic anhydride to 300 ml of glacial acetic acid. Dilute to 1 liter with glacial acetic acid and allow to stand overnight. Standardize against potassium acid phthalate dissolved in 10 ml of glacial acetic acid using crystal violet indicator (1% solution in glacial acetic acid).

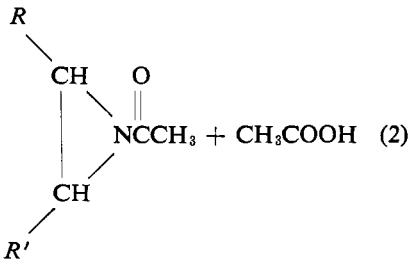
Tetra-*n*-butylammonium iodide reagent, 10% in chloroform. Prepared by the method of Jay (*1*). The reagent should be prepared fresh daily.

**Procedure.** A sample containing 0.2 to 0.4 meq of titratable function is weighed into a 50-ml Erlenmeyer flask, dissolved in 10 ml of acetic anhydride, and allowed to stand in the stoppered flask for 15 min. Solution may be aided by slight warming, but the solution must be returned to room temperature before proceeding further. Five milliliters of tetra-*n*-butylammonium iodide solution and one drop of crystal violet indicator solution are added, and the magnetically stirred mixture is titrated rapidly with standard $HClO_4$ from a 25-ml buret to the first true blue end point. The reagent blank should not be in excess of 0.2 to 0.4 ml.

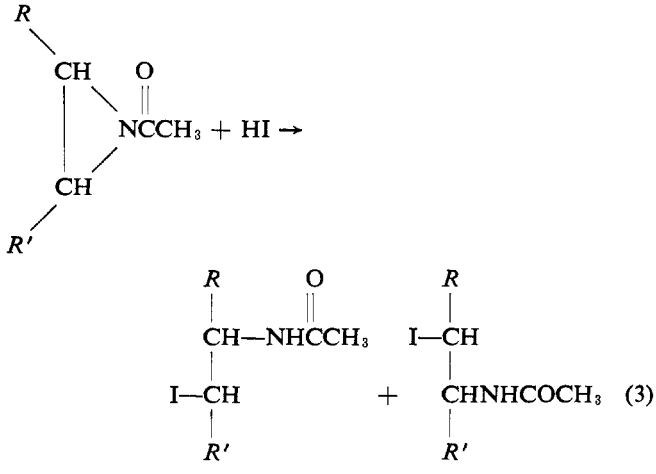
## DISCUSSION AND RESULTS

Aziridines which are unsubstituted on nitrogen react with acetic anhydride according to Equation 2. The acylated





aziridine slowly reacts with the acetic acid formed as byproduct, and care should be taken to avoid letting the acylating solution stand for much longer than 15 min. Reaction of the acylated aziridine with hydrogen iodide formed *in situ* leads to the iodoamide (Equation 3) which is not basic and therefore does not react further with HI or with $HClO_4$. The rate of reaction of the hydrogen halide with *N*-acylaziridines



depends on the nucleophilicity of the anion, and HBr and HCl react too slowly for satisfactory titration.

Basic functional groups, such as primary and secondary amines, if present as impurities, do not interfere in this procedure because they are also acylated by acetic anhydride, and the amides formed do not react with HI or $HClO_4$. Tertiary amines, however, do interfere and must be absent. Other functional groups which react with HI—*e.g.*, epoxides and $\alpha,\beta$-unsaturated ketones, interfere, as do groups which exchange iodide for less nucleophilic anions.

The use of HBr in acetic acid for the determination of alkali metal salts of carboxylic acids was described in an earlier report (*2*). Salts of carboxylic acids are also basic toward HI, and if they are present as impurities they will interfere with the determination.

**Table I. Determination of 2,3-Dialkylaziridines**

| Compound | Purity by acylation–titration (%) | Estimated purity (%) |
|---|---|---|
| *cis*-9,10-Epiminooctadecane | 98.0, 97.7 | 98.0[a] |
| *trans*-9,10-Epiminooctadecane | 99.2, 100.3 | >99.0[a] |
| Methyl *cis*-9,10-epimino-octadecanoate | 99.3, 99.2 | 99.0[b] |
| Methyl *trans*-9,10-epimino-octadecanoate | 99.5, 99.1 | 99.1[b] |
| *cis*-9,10-Epimino-1-octadecanol | 99.4, 99.4 | >99.0[a] |
| *cis*-2,3-Epiminohexane | 95.0, 94.4 | 92.8[b] |
| *trans*-2,3-Epiminohexane | 93.3, 93.5 | 94.3[b] |

[a] Estimated by thin-layer chromatography.
[b] Estimated by gas–liquid chromatography.

**Table II. Analysis of Alkylaziridines Substituted on Nitrogen**

| Compound | Acylation–titration % purity | Estimated % purity |
|---|---|---|
| *cis*-3,4-(*N*-Benzenesulfonyl epimino)hexane | 98.7, 98.2 | >99[a] |
| *trans*-3,4-(*N*-Benzenesulfonyl-epimino)hexane | 101.0, 100.7 | >99[a] |
| Methyl *cis*-9,10-(*N*-benzene-sulfonylepimino) octadecanoate | 99.2, 98.7 | >99[a] |
| 1,2-(*N*-Acetylepimino)octadecane | 97.4, 97.9 | >99[a] |

[a] Homogeneous to thin-layer chromatography.

The assay of a series of internal aliphatic aziridines is shown in Table I. The synthesis of these compounds was carried out by the iodine isocyanate route (*3*) and will be reported elsewhere. The purity values obtained by titration compare well with independent estimates of purity obtained by thin-layer or gas–liquid chromatography. Also analyzing correctly, but not listed in Table I was a sample of *cis*-2,3-diphenylaziridine obtained from D. Swern (Chemistry Department, Temple University, Philadelphia, Pa.). The procedure failed to give satisfactory results with four terminal aziridines (2-hexyl-, 2-octyl-, 2-decyl-, and 2-hexadecylaziridine) all of which gave titration purities which were about 15% lower than expected from independent purity determinations. It is believed that the failure of the terminal aziridines to give correct analyses stems from the interference by a side reaction, perhaps ring-opening by acetate anion, during the acylation step. Support for this view is found in the fact the 1-acetyl-

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(3) C. A. Gebelein, G. Swift, and D. Swern, *J. Org. Chem.*, **32**, 3314 (1967).

**Table III. Determination Carboxylic Acid Salts**

| Compound | Acylation–titration % Purity | Acylation–titration Color of endpoint | Elemental anal. % Metal by ash | Elemental anal. % N |
|---|---|---|---|---|
| Potassium palmitate | 100.5, 100.7, 100.0 | blue | 101.1 | ... |
| Sodium stearate | 99.1, 98.8 | blue-green | 99.6 | ... |
| Potassium 9,10-epiminooctadecanoate | 99.2, 99.6 | blue | 102.3 | 96.9 |
| Sodium 9,10-epiminooctadecanoate | 98.2, 98.2 | blue-green | 98.1 | 98.9 |
| Lithium 9,10-epiminooctadecanoate | 96.8, 96.9 | blue | 101.3 | 99.8 |
| Barium 9,10-epiminooctadecanoate | 94.4, 94.5 | blue | 99.5 | 101.8 |

2-hexadecylaziridine, the purified acetylation product of 2-hexadecylaziridine, gave correct results (see Table II).

Alkylaziridines bearing electron withdrawing substituents on nitrogen are expected to be assayed satisfactorily by the present method as well as by the method of Jay. Because these compounds contain no replaceable hydrogen on nitrogen, they should not, and in fact do not, react with acetic anhydride. Table II shows that such types of aziridines can be determined by the present method.

Carboxylic acids react readily with 2,3-dialkylaziridines, causing the heterocyclic ring to open. For this reason aliphatic carboxylic acids containing aziridine rings have not yet been isolated as pure compounds. Salts of such acids, however, are synthesized fairly readily and a number of such salts have been prepared in this laboratory. The analysis of these salts, shown in Table III, presents a special analytical problem, because these compounds contain two different titratable groups. Three of the four different metal salts tested are assayed correctly using the blue end point of crystal violet. The sodium salt, however, requires the blue-green end point of this indicator as determined by potentiometric titration of sodium 9,10-epiminooctadecanoate and by titration of a sample of sodium stearate of known purity. Lithium 9,10-epiminooctadecanoate is poorly soluble in acetic anhydride but goes into solution during titration.

RECEIVED for review May 13, 1969. Accepted July 28, 1969.

# Gas–Liquid Chromatographic Determination of Oligogalacturonic Acids

**Wynn R. Raymond and Charles W. Nagel**

*Food Sciences Program, Department of Horticulture, Washington State University, Pullman, Wash. 99163*

WHEN INVESTIGATING enzyme-substrate reactions, much of the time spent in the study is often devoted to cumbersome analyses of end products. This is especially true of the study of enzymatic degradation of pectic acid. Present methods of analysis for oligohexuronic acids are time consuming, involving for example, qualitative identification by paper chromatography in conjunction with colorimetric analysis (*1–3*). None of the present colorimetric techniques can be suitably used to determine the full gamut of normal and unsaturated oligogalacturonic acids. The thiobarbituric acid method of Weissbach and Hurwitz (*3*) detects only unsaturated galacturonides and the carbazole method of McComb and McCready (*1*) detects all but the unsaturated monogalacturonic acid. In addition, there is, to date, no single method by which simultaneous qualitative and quantitative determinations of a mixture of oligogalacturonic acids can be made.

Gas–liquid chromatography, during the past decade, has come into its own as an analytical tool for the investigation of carbohydrates and related substances. McInnes *et al.* (*4*) were the first workers to apply gas chromatography to the separation of carbohydrates. More recently, Sweeley *et al.* (*5*) separated mono-, di-, and trisaccharides as their trimethylsilyl ethers by gas–liquid chromatography.

Literature concerning gas chromatographic analysis of hexuronic acids has been limited to monomeric forms. Tamura and Imanari (*6*) and Imanari and Tamura (*7*) have studied methyl, acetyl, and trimethylsilyl derivatives of O-glucuronides and have found the trimethylsilyl derivatives to have high volatility and heat stability. Lehtonen *et al.* (*8*) and Schmidt and Neukom (*9*) investigated the trimethylsilyl ethers of iduronic and galacturonic acids, respectively, by gas chromatography. Anomeric forms of hexuronic acid monomers have been demonstrated with the use of gas chromatographic techniques by Wiley *et al.* (*10*) and by Schmidt and Neukom (*9*). In addition to the above studies, a number of other workers (*11–13*) have utilized gas chromatography for studies of hexuronic acids.

The purpose of this study has been to develop a method by which gas chromatographic analysis can be extended to oligomeric forms of the normal and unsaturated galacturonic acids. A method is described by which samples may be taken directly from enzyme digestion mixtures and prepared for qualitative and quantitative analysis by gas chromatography.

## EXPERIMENTAL

**Apparatus.** A Varian Aerograph Model 1740 gas chromatograph equipped with dual flame ionization detectors was used for the bulk of the study. A Varian Aerograph HiFi 600-D gas chromatograph was used for the stability study.

The columns were of 4-ft × 1/8 in. stainless steel, packed with 0.5% SE-30 on acid-washed, silanized Chromosorb W (80–100 mesh). Both the liquid phase and solid support were obtained from Hewlett-Packard, Avondale, Pa. The carrier gas was $N_2$ at a flow rate of 25 ml/min. A Hamilton CR-700-20 syringe was used for sample injection.

**Standards.** Polygalacturonic acid was obtained from Sunkist Growers, Inc., Corona, Calif. (lot number, RE-28-349101). The individual mono-, and oligogalacturonic acids used as standards were purified by column chromatography (*14*). Hexamethyldisilazane and trimethylchlorosilane, spec-

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