Detection and thin-layer chromatography of derivatives of ethyleneimine

I. N-Carbamoyl aziridines

Ethyleneimine and its derivatives (substituted aziridines) have extensive industrial and biomedical application. For example, N-substituted[1,2], N-carbamoyl[3], sulfur[4,5] and phosphorus[6–10] containing aziridines have been utilized as insect chemosterilants. Other areas of utility of aziridines include neoplasm[11–14] and monoamine oxidase inhibitors[15], ion-exchange copolymers[16], wear and water proofing[17], textile[18–20] and paper-improving agents[21,22].

The analysis of aziridines has been effected *via* colorimetric techniques utilizing γ-(4-nitrobenzyl)-pyridine[23] and 1,2-naphthoquinone-4-sulfonate[24,25], and direct[26,27] and potentiometric titrations[28]. Gas chromatography of aziridines has been limited to *cis*- and *trans*-alkyl derivatives[29]. Thin-layer chromatography has been utilized by BEROZA AND BOŇKOVEC[30] for the investigation of the stability of Tepa, Metepa and Tretamine.

The present study relates to an investigation of the utility of both thin-layer chromatographic techniques and detecting reagents (primarily pi-electron acceptors) for the separation and identification of aryl-N-carbamoyl aziridines and to concomittantly elaborate the effect of structure on their chromatographic behavior in several solvent systems.

Experimental

Thin-layer chromatography. The silicic acid chromatoplates were prepared according to the method of MORLEY AND CHIBA[31]. Silica-gel DF-5* was applied on 8 × 8 in. plates to a thickness of 280 μ. After air-drying, the plates were activated in an oven for 30 min. Acetone solutions (1–2 μl containing 1–10 μg/μl) of test substance were applied along a line 2.5 cm from the lower end of the plate and developed by the ascending method. After evaporation of the solvent, the spots were located by U.V. detection, then sprayed with one of the chromogenic reagents and the initial color development as well as subsequent color changes noted. The sprayed plates were then exposed briefly to ammonia vapors with the results described in Table I. The developing solvent systems utilized in this work were:

(A) Acetone–methanol–water (20:10:70)[32]

(B) Benzene–ethyl acetate (80:20)[32]

(C) 2.5 % Acetone in benzene

Detecting reagents. The following were used:

(1) DDQ reagent: 2 % 2,3-dichloro-5,6-dicyano-1,4-benzoquinoneimine in benzene.

(2) TCNE reagent: 2 % tetracyanoethylene in benzene.

(3) Gibbs reagent: 2 % 2,6-dibromo-N-chloro-*p*-benzoquinoneimine in benzene.

(4) NPB reagent: 5 % γ-(4-nitrobenzyl)-pyridine in acetone.

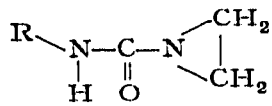
(5) Rhodamine-B: 0.5 % rhodamine-B in acetone.

Materials. The N-carbamoyl aziridines and methyl aziridines were prepared *via* the reaction of ethyleneimine or propyleneimine with the appropriate isocyanate and

* Obtained from Camag, Muttenz, Switzerland.

TABLE I

SPOT COLORS OF N-CARBAMOYL AZIRIDINES ON SILICA GEL DF-5



Colors developed at room temperature: B = blue; Bg = beige; Bl = black; Bn = brown; C = crimson; G = green; Gr = grey; L = lilac; M = magenta; Ma = maroon; O = orange; Ol = olive; P = purple; Pk = pink; T = tan; Y = yellow; V = violet.

R	*Detecting reagents*									
	Before NH_3 exposure					*After NH_3 exposure*				
	1	*2*	*3*	*4***	*5*	*1*	*2*	*3*	*4*	*5*
Methyl	Bn	Bn→Y	B→Pk	B-V	M/O*	Bg	Y-O	Y-G	B	M
Ethyl	Bn	T→Y	B→Pk	B-V	M/O*	Bg	Y-O	Y-G	B	M
n-Propyl	Bn	T→Y	B→Pk	B-V	M/O*	Bg	Y-O	Y-G	B	M
Isopropyl	Bn	T	B→Pk	B-V	M/O*	Bg	Y-O	Y-G	B	M
n-Butyl	Bn	T→Y	B→Pk	B-V	M/O*	Bg	Y-O	Y-G	B	M
Cyclohexyl	B-Bn	T	Pk	P	M/O*	O-C	Y-G	T	B	M
Phenyl	B	Ol	O	P	M/O*	O-C	Y	Bn	B	M
p-Bromophenyl	L-B	Ol	O-C	P	M/P*	O-C	Y	T	B	M
o-Chlorophenyl	Ol	Y-T	O	P	M/O*	O-C	Y-G	Bn	B	M
m-Chlorophenyl	Gr	Ma	O	P	M/O*	O-C	Y-G	Bn	B	M
p-Chlorophenyl	B-G	P-T	O	P	M/O*	O-C	Y-G	Bn	B	M
2,5-Dichlorophenyl	O	Y-O	Y	P	M/O*	O-C	Y	Bn	B	M
3,4-Dichlorophenyl	B-Gr	Y	Y	P	M/O*	O-C	Y-G	T	B	M
p-Fluorophenyl	B	Y-G	O	P	M/O*	O-C	Y	Bn	B	M
o-Methoxyphenyl	B	G-Gr	O-C	P	M/P*	O-C	Y	Bn	B	M
p-Methoxyphenyl	G	G-B	O-C	P	M/V-B*	O-C	Y	Bn	B	M
o-Nitrophenyl	Y	Y	Y	P	M/B-Pk*	O-C	Y-G	Y	B	M
m-Nitrophenyl	O-T	Y-O	Y	P	M/P*	O-C	Y-G	Y	B	M
p-Nitrophenyl	O	Y-O	Y	P	M/Ol-B*	O-C	Y-G	Y	B	M
p-Cyanophenyl	O	Y	Y	P	M/O*	O-C	Y	Bn	B	M
o-Tolyl	P	Y	O	P	M/O*	O-C	Y	Bn	B	M
m-Tolyl	B	B-Bl	O-C	P	M/O*	O-C	Y	Bn	B	M
p-Tolyl	B-G	T	Y-O	P	M/O*	O-C	Y	Bn	B	M
α-Naphthyl	B-G	Y-G	O-T	P	M/P*	O-C	Y	Bn	B	M

* Fluorescence after spraying.
** Color development after 2 min at 85°.

recrystallized from benzene or anhydrous ether according to the procedure of BOŘKOVEC AND WOODS[3].

Results and discussion

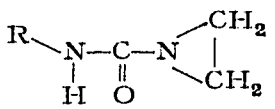
Table I depicts the spot colors of alkyl and aryl-N-carbamoyl aziridines on silica gel DF-5 plates obtained with five detecting reagents before and after exposure to ammonia vapors. Tables II en III depict the R_F values of N-carbamoyl aziridines and methyl aziridines respectively.

A number of cogent observations can be made in respect to the chromogenic behavior of alkyl and isomeric aryl-N-carbamoyl aziridines, as well as to the general utility of pi-electron detecting reagents utilized, *viz.*:

(1) The isomeric chlorophenyl, dichlorophenyl, tolyl and methoxyphenyl de-

TABLE II

R_F VALUES × 100 OF ALKYL AND ARYL-N-CARBAMOYL AZIRIDINES



R	M.p. or b.p. (°C/mm)	Solvents		
		A	B	C
Methyl	38– 39 (10)	33	9	6
Ethyl	54– 56 (10)	39	14	10
n-Propyl	65– 66 (10)	43	18	15
Isopropyl	50– 52	40	15	11
n-Butyl	85– 87 (4)	47	20	18
Cyclohexyl	68– 70	54	25	21
Phenyl	81– 82	57	28	23
p-Bromophenyl	140–141	58	35	31
o-Chlorophenyl	52– 53	65	41	35
m-Chlorophenyl	93– 95	58	36	29
p-Chlorophenyl	133–135	53	30	25
2,5-Dichlorophenyl	84– 85	69	50	39
3,4-Dichlorophenyl	104–105	64	46	32
p-Fluorophenyl	73– 74	45	18	9
o-Methoxyphenyl	64– 65	61	38	35
p-Methoxyphenyl	114–116	56	34	30
o-Nitrophenyl	227–230	72	51	44
m-Nitrophenyl	135–137	66	46	38
p-Nitrophenyl	171–172	61	42	32
p-Cyanophenyl	131–133	65	44	34
o-Tolyl	74– 76	59	37	28
m-Tolyl	71– 72	61	35	32
p-Tolyl	98–100	55	30	27
α-Naphthyl	108–109	59	37	28

rivatives can be distinguished from one another utilizing preferentially the DDQ reagent or secondarily the TCNE reagent.

(2) The isomeric chlorophenyl and dichlorophenyl derivatives can be differentiated from one another utilizing the DDQ reagent as the reagent of choice.

(3) The alkyl derivatives can be distinguished from the general class of aryl derivatives of both the aziridines and methyl aziridines utilizing primarily the DDQ reagent.

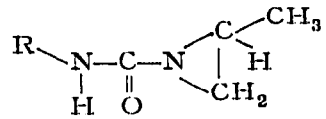
(4) For the pi-electron detectors, the order of decreasing utility for the detection and differentiation of the isomeric aryl derivatives of both N-carbamoyl aziridines and methyl aziridines: DDQ > TCNE >> Gibbs.

The general utility of pi-electron acceptors such as DDQ and TCNE for the detection and differentiation of thin-layer chromatograms of isomeric aryl derivatives has been demonstrated previously for sulfoxides, sulfones and sulfides[33] and chlorophenols[34].

(5) The utility of exposing the chromatoplates to ammonia vapors after spraying is most apparent for additional differentiation of the alkyl from the aryl derivatives of both the aziridines and methyl aziridines.

TABLE III

R_F VALUES × 100 OF ALKYL AND ARYL-N-CARBAMOYL METHYL AZIRIDINES



R	*M.p. or b.p.* (°C/mm)	*Solvents*		
		A	*B*	*C*
Methyl	43– 44 (10)	36	11	8
Ethyl	59– 61 (10)	41	15	12
n-Propyl	70– 71 (10)	47	19	17
Isopropyl	54– 56	44	15	12
n-Butyl	86– 87 (0.2)	50	23	22
Cyclohexyl	69– 71	51	25	24
Phenyl	63– 64	54	27	27
p-Bromophenyl	77– 79	58	38	38
o-Chlorophenyl	72– 74	62	43	46
m-Chlorophenyl	98– 100	56	39	41
p-Chlorophenyl	87– 89	51	35	35
2,5-Dichlorophenyl	56– 58	70	55	40
3,4-Dichlorophenyl	80– 82	66	50	36
p-Fluorophenyl	83– 85	39	31	30
o-Methoxyphenyl	43– 44	62	39	40
p-Methoxyphenyl	75– 77	56	34	37
o-Nitrophenyl	228–230	75	49	45
m-Nitrophenyl	99–101	70	44	40
p-Nitrophenyl	104–106	65	40	36
p-Cyanophenyl	93– 95	62	46	38
o-Tolyl	64– 67	57	43	45
m-Tolyl	48– 50	50	37	38
p-Tolyl	53– 55	45	32	33
α-Naphthyl	116–117	63	41	34

Correlation of R_F values with structure

In addition to the solvents utilized in this study (solvent systems A–C) the following solvent systems were evaluated and rejected because of their less overall utility in effecting separations with the majority of the aryl derivatives of both aziridines and methyl aziridines:

Benzene–acetic acid–water (125:72:3);

Hexane–ethyl ether (1:1);

Benzene–methanol–acetone (80:20:20).

The alkyl and isomeric aryl derivatives of N-carbamoyl aziridines and methyl aziridines have been best separated in this study on silica gel DF-5 plates utilizing solvent A (acetone–methanol–water (20:10:70) and secondarily solvent B (benzene–ethyl acetate (80:20). The order of R_F values for the chlorophenyl and tolyl derivatives of both series, in all the solvent systems tested, was $o>m>p$. The order of R_F values for the methoxyphenyl derivatives of both series was $o>p$ in all the solvent systems.

For the dichlorophenyl derivatives, in all solvent systems, 2,5>3,4.

The influence of functionality in the *para* position of N-aryl derivatives of both series is shown in the following order of R_F values: NO_2, CN>Br, OCH_3>Cl>CH_3>F.

It has not been found possible in this study to effect significant separations of members of the aziridine group from the analogous derivatives of the N-carbamoyl methyl aziridines.

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