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Title: INDOLIZINES AS POTENTIAL ANTIMICROBIAL AGENTS

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In an effort to explore nitroindolizines as potential medicinal agents, some para and meta-substituted nitro-2-phenylindolizines were prepared. These compounds were designed to be investigated for possible antimicrobial activity. The syntheses were accomplished via the Chichibabin-Stepanow synthesis, using the properly substituted α-picoline and phenacyl bromides followed by direct nitration.

In addition, an ultraviolet spectroscopic investigation of several indolizine derivatives in the range 200-400 nm was reported. The spectra of most neutral indolizines consisted of three main absorption bands: high intensity absorption in the 220-250 nm range (Band I), and two medium intensity bands in the 260-320 (Band II) and 330-370 (Band III) nm regions. The spectra of

indolizine cations were found to be quite different from those of the neutral indolizine species. The effects of several substituents at different positions on the indolizine molecule were discussed.

The ionization constants of a series of *para* and *meta* substituted 2-phenylindolizines were measured spectrophotometrically in 20 percent methanolic aqueous hydrochloric acid. The pKa values ranged from 1.10 to 3.31. They were correlated with the Hammett substituent values (σ) yielding two parallel lines: one for the *meta* and another for the *para* substituents. Better Hammett correlations were found using the *para* substituents.

Finally, the partition coefficient (log P) of indolizine nucleus was calculated using fragment method and measured rapidly by high-pressure liquid chromatography (HPLC) on bonded octadecylsilane supports. The log P for indolizine was compared with the π and fragment values for the indolizinyl substituents. Good agreement was obtained for all three values.

Indolizines As Potential Antimicrobial Agents

by

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CHAPTER I

NITRO- *p*- AND *m*- SUBSTITUTED 2-PHENYLINDOLIZINES AS POTENTIAL ANTIMICROBIAL AGENTS

INTRODUCTION

Considerable interest in the fundamental chemistry of the indolizine heterocyclic system (Figure 1) has been generated by the publications of Galbraith et al. (1), Boekelheide and Miller (2), Boekelheide and Windgassen (3), and Windgassen et al. (4). In contrast to the study of this aspect of the indolizine system, there have been only scattered reports of the biological activity of indolizines, and no systematic study has been reported (5-7). Buu-Hoi and Xuong (8) considered 2-(4-fluoro-2-methylphenyl)indolizine and 2-(4-fluoro-2-methylphenyl)-7-methylindolizine as carcinogens, but they failed to mention whether these compounds were actually tested for carcinogenic properties. Buu-Hoi et al. (9) reported that 2-(4-cyclohexylphenyl)indolizine was non-carcinogenic when painted on the skin of experimental animals.

Carbon and Brehm (10) considered 1-indolizinealanine to be a tryptophan antimetabolite. Cardellini et al. (11) reported that, in preliminary tests, indolizine-1-acetic acid, the structural analog of indole-3-acetic acid (heteroauxin), showed some auxinlike activity. Harrell and Doerge reported that 1-diethylaminomethyl-3-methyl-2-phenylindolizine possessed central nervous system (CNS) depressant activity (12).

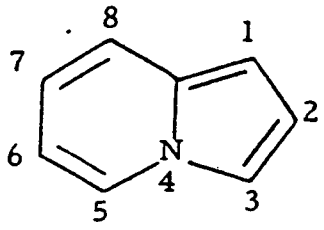


Figure 1 Structure of Indolizine

Walter and Margolis (13) found no useful activity for some 1-aminoalkyl-2-phenylindolizines, which were screened for their effects on the CNS in mice and, in some instances, in cats. The compounds were stimulants at low doses, were depressants at higher doses, and caused death by convulsions. The 2,3-bis(p-methoxyphenyl)-indolizines were reported to possess antiexudative activity (14). In 1968, Buu-Hoi and Hien (15) reported that, when tested in rats, 2-(4-fluoro-3-methylphenyl)-indolizine decreased the duration of paralysis caused by the drug zoxazolamine. Kallay and Doerge (16) in 1972 found no antiinflammatory activity for the compounds 2-(p-methylphenyl)-1-phenylindolizine and 2-(p-bromophenyl)-1-(p-methoxyphenyl)indolizine relative to the reference indoxole.

Rosseels et al. (17) found in 1975 that 3-acetyl-2-alkyl-1-nicotinoylindolizines showed antiinflammatory activities equivalent to acetylsalicylic acid and 2-ethyl and 2-n-propyl-1-nicotinoylindolizines possessed analgesic activities greater than that of antipyrine. In 1977, Cardellini et al. (18) and Claude et al. (19) reported that N^1-substituted hydrazides of indolizine-2-carboxylic acid were more active than iproniazid in the inhibition of monoamine oxidase. Antonini et al. (20) also found that 3-(3-aminopropyl)-2-methylindolizine possessed anti-5-hydroxytryptamine, antihistamine and

antiacetylcholine properties with some CNS activity.

Gubin et al. (21,22) in 1977 considered 1-benzoyl-(4-dialkylamino-alkoxy)- and 3-benzoyl-(4-dialkylamine-alkoxy)indolizine derivatives as antianginal agents. Charlier et al. (23) and Olster and Charlier (24) reported that 2- and 3-benzoylindolizine derivatives showed hemodynamic characteristics similar to those of amidozone with noncompetitive antiadrenergic properties.

Muro et al. (25) reported that 2-[4-(2-indolizinyl)-phenyl]propionitrile and propionic acid derivatives have analgesic, antipyretic, and antiinflammatory activities.

In 1978, Bondeau et al. (26) found that the indolizine analog of pindolol has nonselective β-adrenergic blocking activity.

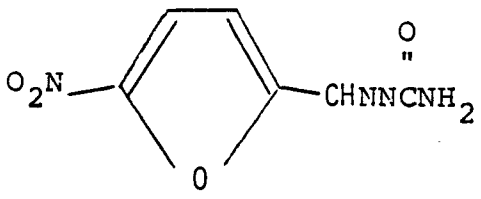
Rational For Drug Design

The chemotherapeutic effectiveness of numerous heterocyclic compounds containing a nitro moiety (Figure 2) has been well documented. Nitrofurazone (I) (Furacin^R) is used in urinary tract infections and is effective against both gram-positive and gram-negative organisms (27,28). Metronidazole (II) (Flagyl^R) is an effective trichomonacidal agent used in vaginal infections (29). Outside of the United States, ipronidazole (III) and tinidazole (IV) are used for their antimicrobial action (30,31). Niridazole (V) and the open chain analog, nithiazide (VI), are known to have antibacterial activities (32,33).

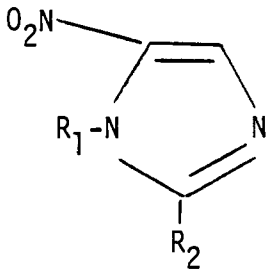
There is a general need for the development of new antimicrobial agents because of resistance developed by the microbial organisms, toxic side effects, limited spectrum of activity, and inability to concentrate in desired tissues using the compounds now available. The antimicrobial properties of indolizines have not been studied. For this reason, a preliminary investigation of the antimicrobial action of substituted phenylindolizines with and without the nitro group was conducted.

DISCUSSION AND RESULTS

All the compounds prepared were *para* and *meta* substituted 2-phenylindolizines Table I, [Compounds X(a-i)].



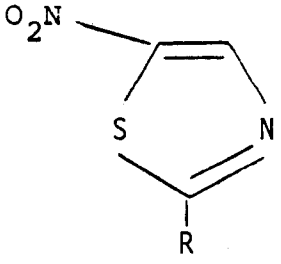
(I)

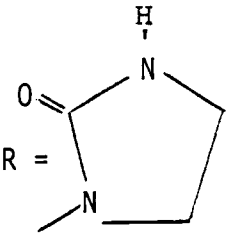


(II) $R_1 = CH_2CH_2CH_2OH$, $R_2 = CH_3$

(III) $R_1 = CH_3$, $R_2 = -CH(CH_3)_2$

(IV) $R_1 = CH_2CH_2SO_2CH_2CH_3$, $R_2 = CH_3$



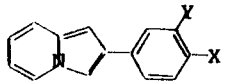


(V) R =

(VI) R = $-NHCONHCH_2CH_3$

Figure 2 Heterocyclic chemotherapeutic agents with a nitro moiety.

TABLE I. INDOLIZINES



Compound Number	X	Y	Melting Point, °C	Percent Yield	Formula	Analysis (MW) Calc. Found
Xa	H	H	214 -215	80	$C_{14}H_{11}N$	_____a
Xb	Br	H	253 -254	86	$C_{14}H_{10}NBr$	_____b
Xc	H	Br	175 -176	81	$C_{14}H_{10}NBr$[d]	271.000 271.000
Xd	H	Cl	160 -161	96	$C_{14}H_{10}NCl$[d]	227.050 227.050
Ie	Cl	H	245 -246	88	$C_{14}H_{10}NCl$	_____b
Xf	OCH_3	H	227 -228	28	$C_{15}H_{13}NO$	_____c
Xg	H	OCH_3	125 -126	89	$C_{15}H_{13}NO$[d]	223.100 223.099
Xh	CH_3	H	215 -216	85	$C_{15}H_{13}N$	_____b
Xi	H	CH_3	143 -145	60	$C_{15}H_{13}N$[d]	207.105 207.105

a - Reference (36)

b - Reference (43)

c - Reference (44)

d - Empirical formula confirmed by high resolution mass spectrometry[10].

They were prepared from 2-methylpyridine (VII) phenacyl bromides (VIII) via the Chichibabin-Stepanow syntheses (34) (Figure 3). The 2-methylpyridine and phenacyl bromides were combined, resulting in the formation of a pyridinium salt (IX) [Table II, Compounds IX(a-i)] which was cyclized in refluxing aqueous sodium bicarbonate to the corresponding indolizine. The indolizines in solution were unstable in light, especially when in chloroform.

A mechanism for the Chichibabin-Stepanow syntheses was postulated by Bragg and Wibberly (35). Nitration of 2-phenylindolizine derivatives (X) was accomplished with nitric acid (d=1.4) in the presence of concentrated sulfuric acid at 0°C (36). The mechanism by which 2-phenyl-indolizines are nitrated has been recently discussed in the literature (37). The orientation of the nitro group appears to depend on the reagent and experimental conditions. Nitration of indolizines in a mixture of nitric and sulfuric acid occurs at the 1-position (36), but nitration in acetic anhydride occurs at the 3-position (38). Thus, with acetic anhydride it is presumably the free indolizine which is nitrated at the position commonly susceptible to electrophilic attack (the 3-position). With nitric or sulfuric acid as the solvent, 1-nitration predominates because the attached species is a 3-protonated indolizine. UV studies show that indolizines are almost completely protonated at the 3-position (39).

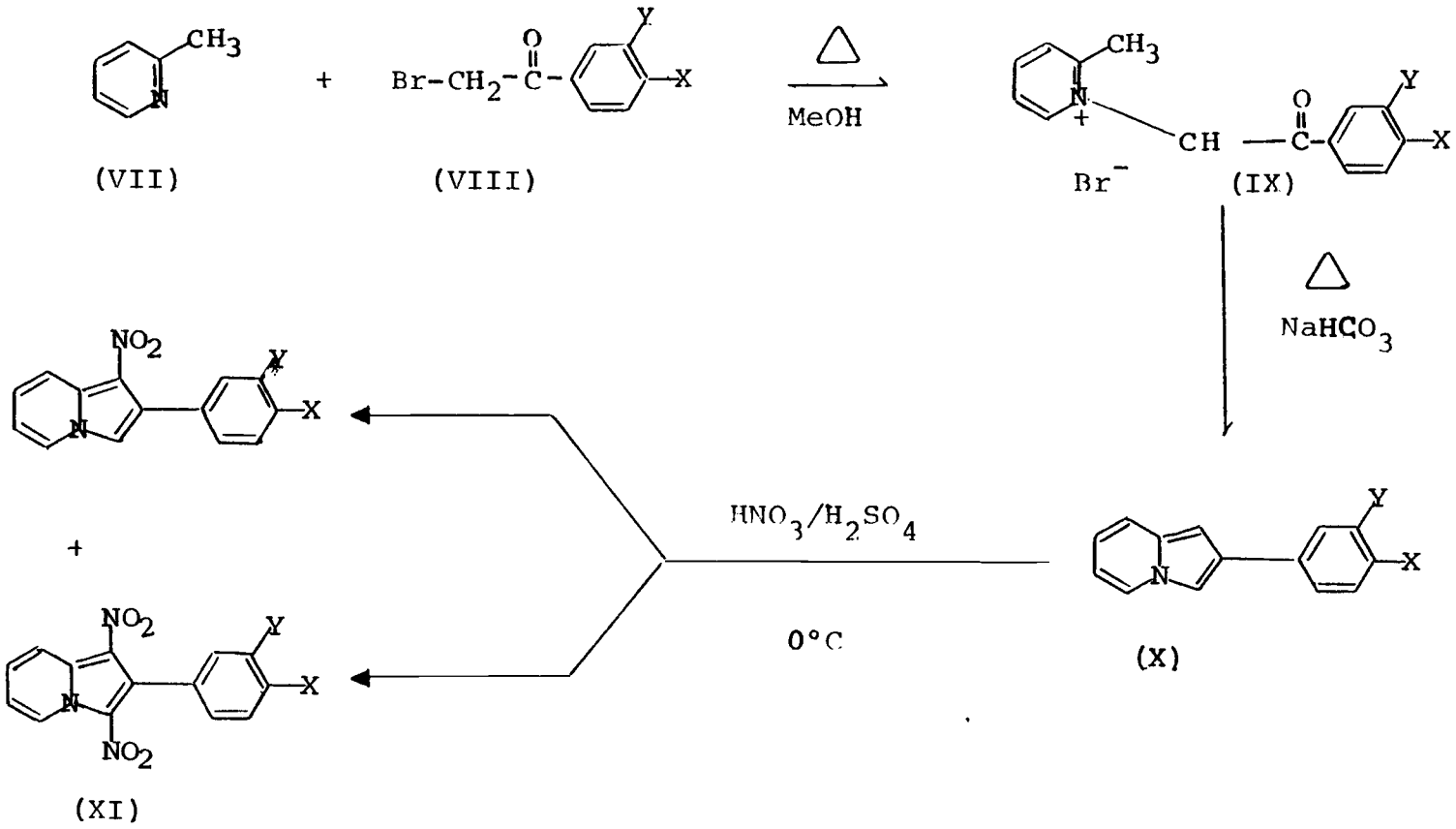
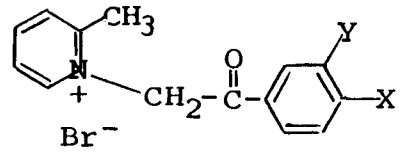


Figure 3 Synthesis of nitro-2-phenylindolizine

TABLE II. PYRIDINIUM BROMIDES



Compound Number	X	Y	Melting Point, °C	Percent Yield	Formula
IXa	H	H	215 -216	70	$C_{14}H_{14}BrNO$
IXb	Br	H	185 -186	27	$C_{14}H_{13}Br_2NO$
IXc	H	Br	250 -251	62	$C_{14}H_{13}Br_2NO$
IXd	Cl	H	145 -148	33	$C_{14}H_{13}BrClNO$
IXe	H	Cl	227 -228	58	$C_{14}H_{13}BrClNO$
IXf	OCH_3	H	140 -141	84	$C_{15}H_{16}BrNO_2$
IXg	H	OCH_3	185 -186	75	$C_{15}H_{16}BrNO_2$
IXh	CH_3	H	183 -185	54	$C_{15}H_{16}BrNO$
IXi	H	CH_3	195 -197	60	$C_{15}H_{16}BrNO$

The first step of the proposed mechanism (Figure 4) of the nitration of indolizines using the nitric and sulfuric acid mixture is protonation at the 3-position (XII). This protonation blocks position 3 from further electrophilic attack. The second step is the nitronium ion attack at the 1-position, forming 1-nitroindolizinium cation (XIII). In the presence of a base, elimination occurs yielding compound XV. With excess of nitronium ions, further nitration can occur at position 3 forming 1,3-dinitroindolizinium cation (XIV). In a basic environment, elimination occurs giving compound XVI.

The nitro derivatives XI used in this study [Table III, compounds (XI(a-m)] were purified by alumina column chromotography and preparative thin layer chromatography (TLC). They rapidly turned green if not protected from air and sunlight.

Biological Results

Compounds X(a-i) and XI(a-m) were evaluated *in vitro* for antibacterial activity against gram-negative *E. coli* and gram-positive *S. aureus* bacteria. A preliminary Disc -Agar diffusion test was done using nitrofurazone[1] and chloramphenicol[1] as standards. None of the test compounds exhibited any antimicrobial activity against these organisms. Negative results also were obtained when the indolizine was placed directly onto the test agar.

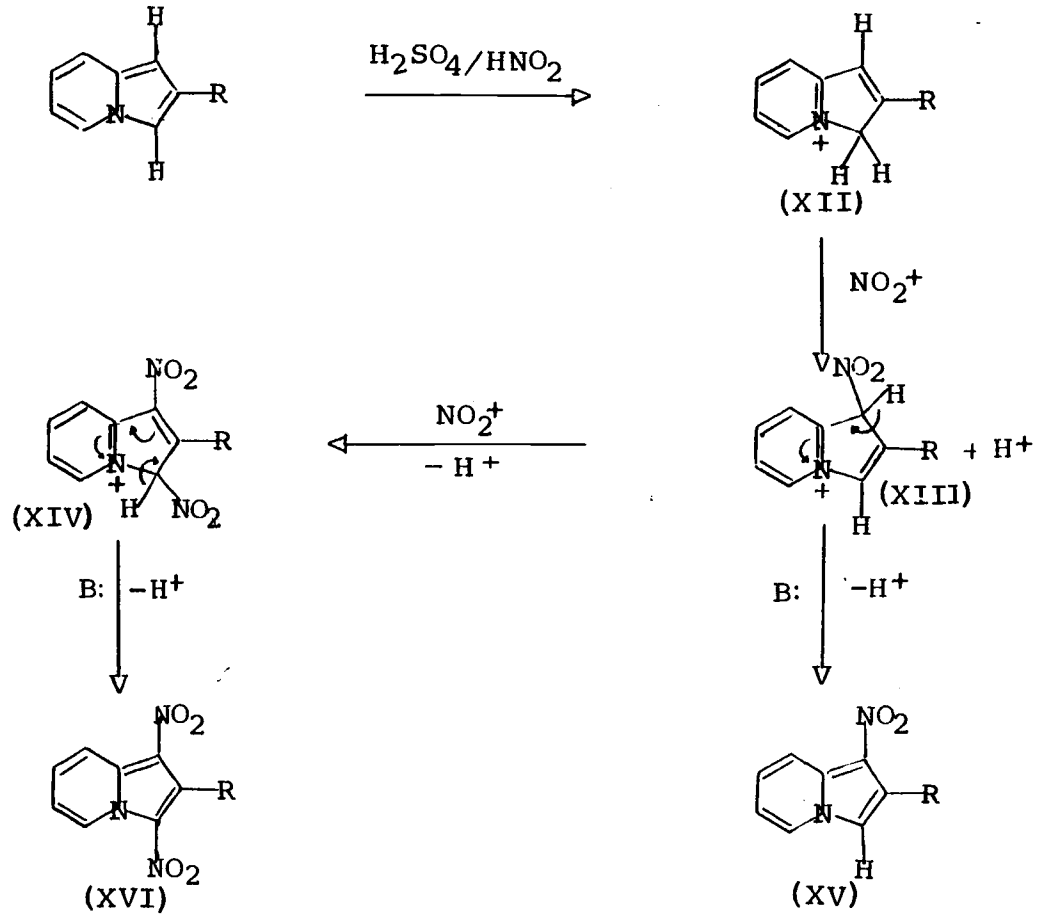
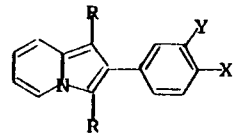


Figure 4 Mechanism of nitration for indolizines

TABLE III. NITROINDOLIZINES



Compound	X	Y	R_1	R_3	M.P., °C	Percent Yield	Formula	Analysis (MW) Calc.	Found
XIa	H	H	NO_2	NO_2	245 to 246	14	$C_{14}H_9N_3O_4$	————	a
XIb	NO_2	H	H	H	245 to 246	50	$C_{14}H_{10}N_2O_2$	————	a
XIc	NO_2	H	NO_2	H	235 to 236	3	$C_{14}H_9N_3O_4$	————	a
XId	Br	H	NO_2	H	153 to 155	3	$C_{14}H_9BrN_2O_2$ [b]	315.985	315.985
XIe	Br	H	NO_2	NO_2	265 to 266	15	$C_{14}H_8BrN_3O_4$ [b]	362.968	362.970
XIf	H	Br	No_2	H	174 to 175	14	$C_{14}H_9BrN_2O_2$ [b]	317.983	317.982
XIg	Cl	H	NO_2	NO_2	215 to 216	10	$C_{14}H_9ClN_3O_4$ [b]	317.020	317.022
XIh	H	Cl	NO_2	H	190 to 191	18	$C_{14}H_9ClN_2O_2$ [b]	272.035	272.033
XIi	OCH_3	H	NO_2	NO_2	268 to 269	3	$C_{15}H_{11}N_3O_5$ [b]	313.070	313.067
XIj	H	OCH_3	NO_2	NO_2	183 to 184	20	$C_{15}H_{11}N_3O_5$ [b]	313.070	313.070
XIk	CH_3	H	NO_2	H	178 to 179	5	$C_{15}H_{12}N_2O_2$ [b]	252.080	252.088
XIl	CH_3	H	NO_2	NO_2	235 to 236	13	$C_{15}H_{11}N_3O_4$ [b]	297.075	297.074
XIm	H	CH_3	NO_2	H	179 to 180	5	$C_{15}H_{12}N_2O_2$ [b]	252.090	252.090

a - Reference (36)

b - Empirical formula confirmed by high resolution mass spectrometry[10].

EXPERIMENTAL

Antimicrobial Evaluation

The bacteria used were gram-negative E. coli and gram-positive S. aureus which were obtained from a collection maintained by the Oregon State University's Departments of Microbiology and Food Science Technology. Before any antimicrobial testing, the compounds in solution were sterilized using a fritted glass filtering disc[2] (40).

The Disc Agar Diffusion method following Kirk-Bauer procedure (41) was used for preliminary testing. The basic concept is that the size of the zone of inhibition correlates with the antimicrobial activity of the agent being tested.

The standard procedure requires the use of Mueller-Hinton agar[3] (a special agar for susceptibility testing of micro-organisms), a standard inoculum applied in a potency for each of the chemotherapeutic agents being tested.

The experimental discs were prepared by placing them in a methanol solution containing 200 µg/ml of indolizine and placed on the top of the Mueller-Hinton agar. Also, the pure compounds (~5mg) were placed directly on the top of the agar. Discs[3] previously soaked in methanol were used as blanks.

Melting point values determined using an open capillary melting point apparatus[5] were recorded uncorrected. The Nuclear Magnetic Resonance (NMR) spectrometer[6] was used with tetramethylsilane (TMS) as an internal standard and deuterated chloroform as a solvent. Infrared [7] and ultraviolet[8] (UV) spectra were recorded on suitable double beam spectrophotometers. Mass spectra were obtained with a single focusing magnetic mass spectrophotometer equipped with a data recording system[9]. Elemental analyses were performed by high resolution mass spectrometry[10] at the Department of Chemistry, University of Oregon, Eugene, Oregon.

Analytical TLC plates were precoated with silica gel[11]. The preparative TLC plates were coated with silica gel[12] (20x20cm, 2mm). Three developing systems were used: A, methylene chloride-hexane (1:3); B, methylene chloride-hexane (1:1); C, methylene chloride-hexane (3:1). Spots were visualized with UV light at 254nm. The purity of each compound was confirmed in each of the TLC systems and in a 40 percent acetonitrile High Pressure Liquid Chromatography (HPLC) system[13] using a persilated octadecylsilane column[14]. All compounds gave spectra data consistent with the proposed structure.

The necessary phenacyl bromides were prepared by bromination of the corresponding acetophenone in methanol (42).

Synthesis of Nitroindolizines (Table III)

1,3-Dinitro-2-phenylindolizine(XIa)

2-Phenylindolizine 2.0 g (10.4 mmole) dissolved in 10 ml (0.155 mole) nitric acid (d=1.4) was warmed according to Borrows *et al.* (36) to form greenish-orange crystals (m.p. 233° to 238°). The crystals were recrystallized from acetic acid to give 0.40 g (14 percent) of compound XIa as a dark yellow solid, m.p. 245° to 246°C. Compound XIa appeared as one spot when analyzed by TLC (System C, Rf 0.43). UV (CH_3OH): 220 (log ε 4.27), 252 (4.04), 300 (3.85) and 355 (4.25) nm; mass spectrum:m/e 283 (M +,100%).

2-(p-Nitrophenyl)indolizine (XIb)

Nitric acid (d=1.4; 0.35 ml, 5.4 mmole) was added dropwise over a five-minute period to an ice-cold, stirred solution of 0.50 g (2.6 mmole) 2-phenylindolizine (Xa) in concentrated sulfuric acid according to Borrows *et al.* (36). A yellow-brown precipitate (0.40 g) was formed and recrystallized first from acetonitrile, and then acetone-charcoal yielding 0.30 g (50 percent) 2-(p-nitrophenyl)indolizine (XIb) which appeared as one spot when analyzed by TLC (System B, Rf 0.43). UV (CH_3OH): 240(log ε3.52), 300 (2.93) and 340 (3.15) nm; mass spectrum: m/e 238 (M +, 100%).

1-Nitro-2-(p-nitrophenyl)indolizine (XIc)

Nitric acid (d=1.4; 0.8 ml, 1.0 mmole) was added dropwise over five minutes to an ice-cold stirred solution containing 2.0 g (8.4 mmole) of 2-(p-nitrophenyl) indolizine (XIb) in 2.0 ml concentrated sulfuric acid according to Borrows et al. (36). A greenish-yellow precipitate was formed and recrystallized from acetonitrile and then from acetone with charcoal, yielding 0.07 g (3 percent) of XIc as yellow needles (m.p. 235° to 236°C dec.). Compound XIc appeared as one spot when analyzed by TLC (System C, Rf 0.43). UV (CH_3OH):220 (log ε 4.50) and 275 (4.33) nm; mass spectrum:m/e 283 (M +, 100%).

1-Nitro-2-(p-bromophenyl)indolizine:(XId)

Nitric acid (d=1.4; 0.1 ml, 1.56 mmole) was added dropwise over five minutes to an ice-cold stirred solution containing 0.35 g (1.28 mmole) 2-(p-bromophenyl)indolizine (Xb) in 2.0 ml concentrated sulfuric acid in a 50 ml round-bottom flask equipped with a magnetic stirring bar. The dark red solution was stirred for 38 minutes at 0°C. The mixture was poured into a beaker containing 20 g crushed ice and yielded 0.30 g of a dark green solid with m.p. 150° to 165°C. The precipitate was placed in an alumina column and compound (XId) was eluted using a mixture of 1:1 hexane and methylene chloride as the eluant.

The eluate was evaporated under vacuum and the residue recrystallized from acetone yielding 0.012 g (3 percent) of a dark yellow precipitate (m.p. 153° to 155°C dec.). Compound XId appeared as one spot when analyzed by TLC (System C, Rf 0.70). UV (CH_3OH); 214 (log ε 4.23), 260 (4.52) and 286 (4.32) nm; mass spectrum:m/e 316 (M +, 95) and 318 (M + 2, 100%).

1,3-Dinitro-2-(p-bromophenyl)indolizine (XIe)

Nitric acid (d=1.4; 0.1 ml - 1.56 mmole) was added dropwise over five minutes to an ice-cold stirred solution containing 0.35 g (1.28 mmole) 2-(p-bromophenyl)indolizine (Xb) in 2.0 ml concentrated sulfuric acid in a 50 ml round - bottom flask equipped with a magnetic stirring bar. The dark red solution was stirred for 38 minutes at 0°C. The mixture was poured into a beaker with crushed ice and yielded 0.30 g dark green precipitate (m.p. 150° to 165°C). The precipitate was placed in alumina column and compound (XIe) eluted using a mixture of 3:1 methylene chloride and hexane as eluant. The eluate was evaporated under vacuum and yielded 0.05 g (15 percent) of a light yellow precipitate (m.p. 265° to 266°C dec.). Compound (XIe) appeared as one spot when analyzed by TLC (system C, Rf 0.45). UV (CH_3OH): 210 (log ε 4.23) and 236 (4.50) nm; mass spectrum:m/e 361 (M +, 89%) and 363 (M + 2, 100%).

1-Nitro-2-(m-bromophenyl)indolizine (XIf)

Nitric acid (d=1.4; 0.95 ml, 0.77 mmole) was added dropwise over five minutes to an ice-cold stirred solution of 1.05 g (3.8 mmole) of 2-(m-bromophenyl)indolizine (Xc) in 2.0 ml concentrated sulfuric acid in a 50 ml round-bottom flask equipped with a magnetic stirring bar. The light yellow solution was stirred for 38 minutes at 0°C. The mixture was poured into a beaker containing 20 g crushed ice and filtered under vacuum using a sintered filter funnel with medium porosity. The reddish-yellow precipitate was recrystallized from acetonitrile and yielded 0.12 g (14 percent) yellow needles (m.p. 174° to 175°C dec.). Compound (XIf) appears as one spot when analyzed by TLC (System C, Rf 0.73). UV (CH_3OH):215 (log ε 4.30), 242 (4.48) and 295 (4.17) nm; mass spectrum:m/e 191 (100%), 316 (M +, 48%), and 318 (M + 2, 52%).

1,3-Dinitro-2-(p-chlorophenyl)indolizine (XIg)

Nitric acid (d=1.4; 0.175 ml, 2.72 mmole) was added dropwise over ten minutes to an ice-cold stirred solution containing 0.65 g (2.9 mmole) of 2-(p-chlorophenyl)indolizine (Xe) in 10 ml concentrated sulfuric acid in a 50 ml round-bottom flask equipped with a magnetic stirring bar. The mixture turned light yellow immediately and after the addition of the final 0.05 ml of nitric acid changed dark red. The solution was stirred for 38 minutes keeping the temperature at 0°C. The mixture was poured into a sintered filter

funnel containing 20 g crushed ice. After washing the filter with cold water several times, 0.30 g of a greenish-yellow precipitate (m.p. 130° to 145°C) was recovered. The solid was placed in an alumina column and eluted with a mixture of 3:1 methylene chloride and hexane. The eluate was evaporated under vacuum. The greenish-yellow precipitate was recrystallized from acetonitrile and yielded 0.030 g (10 percent) yellow crystals (m.p. 215° to 216°C dec.). Compound XIg appeared as one spot when analyzed by TLC (System C, Rf 0.43). UV (CH_3OH): 235 (logε 4.37) nm; mass spectrum:m/e 272 (100%) and 317 (m +, 25.2%).

1-Nitro-2-(m-chlorophenyl)indolizine (XIh)

Nitric acid (d=1.4; 0.08 ml, 1.24 mmole) was added dropwise over five minutes to an ice-cold stirred solution containing 0.30 g (1.3 mmole) 2-(m-chlorophenyl)indolizine (Xd) dissolved in 2.0 ml concentrated sulfuric acid. The light yellow solution was stirred for 38 minutes at 0°C. The mixture was poured in a beaker containing 20 g crushed ice. A red precipitate formed at once. The suspension was adjusted to pH 10.5 by adding 20 percent KOH solution. A bright yellow precipitate (0.3 g, m.p. 150° to 165°C dec.) was recovered. The precipitate was recrystallized from acetonitrile and yielded 0.07 g (18 percent) of yellow needles (m.p. 190° to 191°C dec.). Compound XIh appeared as one spot when analyzed by TLC (System B, Rf 0.49). UV (CH_3OH): 215 (logε 4.29), 242 (4.46) and 295 (4.15) nm; mass spectrum:m/e 227 (100%) and 272 (M +, 25%).

1,3-Dinitro-2-(p-methoxyphenyl)indolizine (XIi)

Nitric acid (d=1.4; 0.1 ml, 1.5 mmole) was added dropwise over five minutes to an ice-cold stirred solution containing 0.33 g (1.5 mmole) of 2-(p-methoxyphenyl)indolizine (Xf) dissolved in 2.0 ml concentrated sulfuric acid in a 50 ml round-bottom flask equipped with a magnetic stirring bar. The reddish-brown mixture was stirred for 38 minutes at 0°C. The solution was poured into a beaker containing 20 g crushed ice and basified to pH 10.5 by adding 20 percent KOH solution. A yellow precipitate (0.40 g, m.p. 160° to 165°C) was recovered. The precipitate was placed in an alumina oxide column and eluted with a mixture of 3:1 methylene chloride and hexane. The eluate was evaporated under vacuum apparatus and yielded 0.10 g (3 percent) yellow crystals (m.p. 268° to 269°C). Compound (XIi) appeared as one spot when analyzed by TLC (System C, Rf 0.43). UV (CH_3OH): 210 (logε 3.43) and 234 (3.72) nm; mass spectrum:m/e 313 (M +, 100%).

1,3-Dinitro-2-(m-methoxyphenyl)indolizine (XIj)

Nitric acid (d=1.4; 0.1 ml, 1.5 mmole) was added dropwise over five minutes to an ice-cold stirred solution containing 0.33 g (1.5 mmole) 2-(m-methoxyphenyl)indolizine (Xg) dissolved in 2.0 ml concentrated sulfuric acid in a 50 ml round-bottom flask equipped with a magnetic stirring bar. The orange-yellow solution was stirred for 38 minutes at 0°C. The mixture was poured into a beaker containing

20 g crushed ice and basified with 20 percent KOH solution until pH 10.5. The yellow precipitate (0.14 g; m.p. 130° to 145°C dec.) was recrystallized from acetonitrile and yielded 0.08 g (20 percent) rust-red needles (m.p. 183° to 184°C dec.) Compound XIj appeared as one spot when analyzed by TLC (System C, Rf 0.66). UV (CH_3OH): 220 (logε 4.25), 236(4.39), 290 (4.07) nm; mass spectrum:m/e 69 (100%) and 314 (M +, 95.5%).

1-Nitro-2-(p-methylphenyl)indolizine (XIk)

Nitric acid (d=1.4; 0.1 ml - 1.5 mmole) was added dropwise over five minutes to an ice-cold stirring solution containing 0.30 gm (1.5 mmole) 2-(p-methylphenyl)indolizine (Xh) dissolved in 2.0 ml concentrated sulfuric acid in a 50 ml round-bottom flask equipped with a magnetic stirring bar. The dark yellow solution was stirred for 38 minutes at 0°C. The mixture was poured into a beaker contain 20 g crushed ice and adjusted to pH 10.5 by adding 20 percent KOH solution. The yellow suspension was filtered using a sintered glass funnel and the yellow precipitate was dried overnight in a vacuum desiccator (0.36 g; m.p. 130° to 145°C dec.). The precipitate was recrystallized from acetonitrile and then acetone. A greenish-yellow precipitate (0.12 g, m.p. 160° to 195°C) was recovered. The solid was purified in an alumina oxide column using a 1:3 mixture of methylene chloride-hexane.

The filtrate was evaporated under vacuum. The thin layer chromatogram using system C showed two spots. Starting material Compound (Xh) Rf 0.80 and Compound (XIk) RF 0.67. The nitroindolizine (XIk) was separated by preparative thin layer chromatography with system B. The yellow band (Rf 0.53) was scraped off the plate and extracted with acetone. Yellow crystals were covered yielding 0.02 g (5 percent) with m.p. 178° to 179°C dec. UV (CH_3OH): 210 (log ε 4.08), 255 (4.56) and 280 (4.22) nm; mass spectrum; m/e 252 (M + 100%).

1,3-Dinitro-2-(p-methylphenyl)indolizine (XIl)

Nitric acid (d=1.4; 0.1 ml, 1.5 mmole) was added dropwise over five minutes onto an ice-cold stirred solution of 0.30 g (1.4 mmole) 2-(p-methylphenyl)indolizine (Xh) dissolved in 2.0 ml concentrated sulfuric acid in a 50 ml round-bottom flask equipped with a magnetic stirring bar. The brownish yellow mixture was stirred for 38 minutes at 0°C. The solution was poured into a beaker containing 20 g crushed ice and adjusted to pH 10.5 by using 20 percent KOH solution. The yellow precipitate was filtered under vacuum using a sintered filter funnel with medium porosity and dried overnight under a vacuum desiccator. The precipitate (0.36 g; m.p. 130° to 145°C dec.) was recrystallized from acetonitrile and then from acetone. A greenish-yellow precipitate (0.12 g; m.p. 160° to 195°C dec.) was recovered. The solid was purified by alumina

column using a mixture (methylene chloride-hexane 1:1) initially and then changing to a more polar system, methylene chloride-hexane, (3:1). The filtrate was evaporated under vacuum and yielded 0.05 g (13 percent) of a bright yellow compound (m.p. 235° to 236°C dec.). Compound (XIl) appeared as one spot when analyzed by TLC (System C, Rf 0.38). UV (CH_3OH): 235 (log ε 4.40) and 265 (4.04) nm; mass spectrum:m/e 297 (M+, 100%).

1-Nitro-2-(m-methylphenyl)indolizine (XIm)

Nitric acid (d=1.4; 0.1 ml - 1.5 mmole) was added dropwise over five minutes to an ice-cold stirred solution containing 0.30 g (1.4 mmole) of 2-(m-methylphenyl) indolizine (Xi) dissolved in 2.0 ml concentrated sulfuric acid in a 50 ml round-bottom flask containing a magnetic stirring bar. The light yellow mixture was stirred for 38 minutes at 0°C and poured into a beaker containing 20 g crushed ice. The solution was adjusted to pH 10.5 by adding 20 percent KOH. The yellow suspension was filtered using a sintered filter funnel. The yellow precipitate was dried overnight under vacuum. The compound (0.33 g: m.p. 100° to 125°C dec.) was recrystallized from acetonitrile and then acetone, yielding 0.03 g (m.p. 160° to 165°C dec.) greenish yellow crystals. The thin layer chromatograph using system C showed two spots: one from the starting material (Xi) Rf. 0.86 and the other from compound (XIm) Rf 0.69. The nitro derivative was purified

by preparative thin layer chromatography using system B. The yellow band (Rf 0.66) was scraped off from the plate and placed in a Hirsch funnel. The silica gel was washed several times with acetone until all the yellow color disappeared. The filtrate was concentrated under vacuum yielding 0.02 g (5 percent) sparkling yellow crystals (m.p. 179° to 180°C dec.) which turned light green immediately in contact with air. UV (CH_3OH): 210 (log ε 4.07), 240 (4.39), 294 (4.06) and 350 (4.07); mass spectrum:m/e 252 (M +, 100%).

Footnotes

1. Sensi-Disc, Microbial Susceptibility Test Discs, BBL, Cockeysville, Maryland.

2. "Pyrex" Brand Chemical Glass #774, UF Porosity, Corning Glass Works, Corning, N.Y.

3. Difco Laboratories, Detroit, Michigan.

4. No. 740-E, Schleicher & Schuell, Inc., Keene New Hampshire.

5. Thomas-Hoover.

6. Varian Anaspect EM 360.

7. Perkin-Elmer Model 727.

8. Beckman Model DB-GT.

9. System Industries 150.

10. High Resolution Mass Spectrometer CEC-21B-110 Instrument Set at 70 EV.

11. Silica Gel 60 F-254, EM Reagents.

12. Silica Gel PF-254 + 366, EM Reagents.

13. Model ALC/6PC 201 Liquid Chromatograph, Model M 6000A Pump, Model U-GK Injector, Waters Associates, Milford, Massachusetts.

14. Corasil C_{18}, Waters Associates, Milford, Massachusetts.

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CHAPTER II

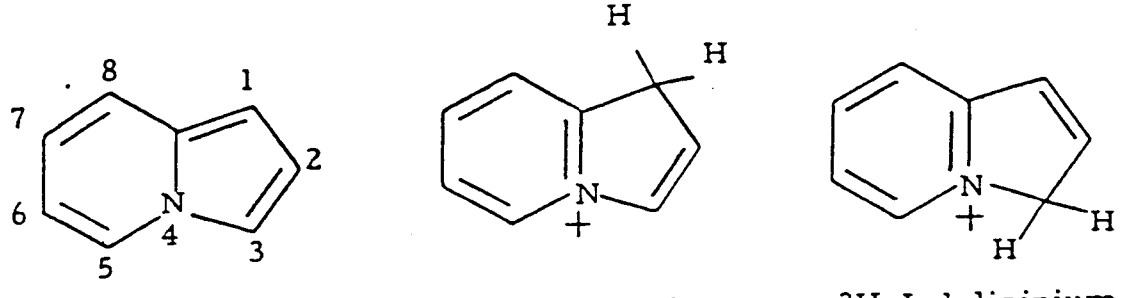
AN ULTRAVIOLET SPECTROSCOPIC INVESTIGATION OF SEVERAL INDOLIZINE DERIVATIVES

INTRODUCTION

The ultraviolet spectra of indolizines (Figure 1) have been reported by numerous investigators (1-8). For the most part, these studies have given the spectral information as part of the usual physico-chemical data reported for newly synthesized compounds. In none of these studies was there any attempt to correlate substituents, either as to type or location on the indolizine ring, with the direction of changes in peak location or intensity. The purpose of this study was to investigate how substituents on the indolizine ring affect the ultraviolet spectra. In addition to the compounds already reported in the literature, ultraviolet spectra were obtained for a series of substituted 2-phenylindolizines. These spectra were compared with the ones already reported.

RESULTS AND DISCUSSION

Based on studies to date, the spectra of most neutral indolizines consist of three main absorption bands: high intensity absorption at 220-250 nm (Band I) and two medium intensity bands at 260-250 nm (Band II) and 330-370 nm (Band III) (Tables I-IV). In these studies no attempt was made to classify these three bands, although it is known that the spectra of heteroaromatic compounds have three well-defined absorption bands that strongly resemble the ^1L_b, ^1L_a, and ^1B bands of naphthalene (9). According to Platt(10), these bands originate from $\pi \rightarrow \pi^*$ trasitions.



Indolizine
(I)

1H-Indolizinium ion
(Ia)

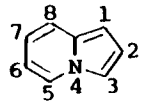
3H-Indolizinium ion
(Ib)

Figure 1 Structures of neutral and cationic forms of indolizine.

The intense band near 230 nm results from an allowed transition, whereas the two medium bands (near 260 and 360 nm) result from forbidden transitions. Heterocyclic aromatic compounds also possess $n \rightarrow \pi^*$ absorptions associated with the transfer of one of the non-bonding electrons on the heteroatom to an antibonding orbital of the π system (11). In case of methylindolizines (1,-16, Table I), the $n \rightarrow \pi^*$ transition (Band II) is believed to occur near 280 nm because this band is not seen in the spectra of indolizinium cations.

Changes in solvent polarity have little or no effect on unsubstituted neutral indolizine as evidenced by the fact that the spectra of indolizine in water and in cyclohexane are very similar (1 and 2). As pointed out above, the spectra of the cations are significantly different from those of the neutral species. The spectrum of indolizinium cation consists of two main bands (Band I and III). Band II is absent because of the change of the aromatic delocalization energy of the non-bonding electrons of the nitrogen causing inhibition of $n \rightarrow \pi^*$ transitions. Band I shifts bathochromically (2-40 nm) except in compounds where the methyl is at the 6 or 7 positions (7 and 8). Band III exhibits a hypsochromic shift (15-58 nm) and a hyperchromic effect (Δ log ε 0.12-0.53) with the exception of the 3-methyl-(5) and 3,7-dimethyl (13) compounds. These display a hypochromic effect of Δ log ε of 0.48 and 0.29, respectively.

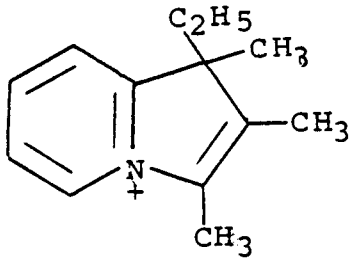
Table I Ultraviolet Spectra of Methylindolizines



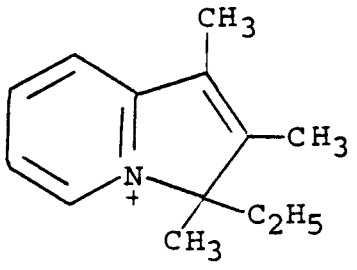
Spectrum Number	Indolizine	NEUTRAL λ max nm (log ε) Bands			CATION λ max nm (log ε) Bands			Solvent	Ref.
		I	II	III	I	II	III		
1	unsubstituted	238(4.51)	283(3.45)	336(3.23)	--	--	--	cyclohexane	1
2	unsubstituted	232(4.47)	281(3.49)	337(3.26)	234(3.79)	--	305(3.76)	water	6
3	2-methyl	235(4.50)	285(3.61)	335(3.75)	225(4.40)	--	300(3.75)	ethanol	2
4	2-methyl	238(4.50)	287(3.71)	338(3.63)	240(3.97)	--	317(3.75)	water	6
5	3-methyl	231(4.89)	281(3.57)	348(3.28)	270(3.86)	--	316(2.80)	water	6
6	5-methyl	223(4.54)	282(3.62)	330(3.39)	232(3.85)	--	305(3.87)	water	6
7	6-methyl	235(4.54)	284(3.50)	335(3.38)	211(4.38)	--	311(3.78)	water	6
8	7-methyl	234(4.54)	283(3.62)	337(3.25)	213(4.50)	--	300(3.78)	water	6
9	1,2-dimethyl	239(4.44)	292(3.36)	349(3.28)	245(4.06)	--	325(3.73)	water	6
10	2,3-dimethyl	235(4.45)	285(3.48)	348(3.33)	241(3.78)	--	290(3.72)	water	6
11	2,5-dimethyl	235(4.48)	287(3.53)	331(3.40)	240(4.04)	--	316(3.89)	water	6
12	3,5-dimethyl	232(4.48)	286(3.64)	343(3.39)	236(3.82)	--	320(3.75)	water	6
13	3,7-dimethyl	234(4.54)	283(3.69)	348(3.24)	272(3.88)	--	312(2.95)	water	6
14	1,2,3-trimethyl	240(4.46)	291(3.41)	360(3.31)	246(4.05)	--	325(3.71)	water	6
15	1,3,5,7-tetramethyl	227(4.30)	300(3.75)	365(3.25)	--	--	--	ethanol	5
16	1,3,5,8-tetramethyl	227(4.20)	300(3.75)	360(3.85)	250(3.40)	--	325(3.80)	ethanol	5

Holland and Nayler(2) pointed out that it should be possible to distinguish cations (Ia) and (Ib) (Figure 1) by comparing their ultraviolet spectra. They observed that 1-ethyl-1,2,3-trimethylindolizinium cation (IIa) has a band of approximately 275 nm compared to the 3-ethyl -1,2,3-trimethylindolizinium cation (IIb) which absorbs at approximately 250 and 330 nm (Figure 2). Consistent with these results, most methylindolizine cations show protonation predominately on C-3 position. An exception to this is shown by spectra 5 and 13 which indicate protonation at the C-1 position because of the pronounced bathochromic shift of Band I.

Barret(3) compared the absorption spectra of several substituted 3-acetylindolizines with the analogous substituted indolizine derivatives lacking the 3-acetyl substituent (Table II and III). The acetyl group at the 3-position of indolizine exhibits a hypsochromic shift at Band I (1-19 nm) and Band II (33-58 nm) and a bathochromic shift at Band III (3-15 nm). Band I exhibits a hypochromic effect of Δ log ε 0.07-0.29, and Bands II and III exhibit a hyperchromic effect of Δ log ε 0.23-0.48 and Δ log ε 0.36-0.65 respectively. (18→17, 20→19, 22→21, 24→23, 26→25, 32→31, 33→34). Moreover, the effect of a phenyl group at the 1-position (17) compared with the unsubstituted indolizine (2) is a bathochromic shift of Band II (24 nm) and Band III (25 nm) with a hyperchromic



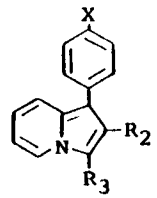
(II a)



(II b)

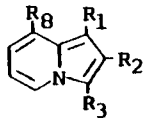
Figure 2 Substituted indolizinium cations

Table II Ultraviolet Spectra of 1-Phenylindolizines



Spectrum Number	X	R_2	R_3	λ max nm (log ε) Bands			Solvent	Ref.
				I	II	III		
17	H	H	H	236(4.36)	305(4.10)	362(3.53)	ethanol	3
18	H	H	Ac	235(4.15)	267(4.48)	370(4.18)	ethanol	3
19	Cl	H	H	239(4.51)	313(4.18)	360(3.78)	ethanol	3
20	Cl	H	Ac	220(4.25)	265(4.41)	369(4.18)	ethanol	3
21	H	Me	H	242(4.38)	310(3.90)	347(3.46)	ethanol	3
22	H	Me	Ac	225(4.11)	274(4.36)	355(3.95)	ethanol	3
23	Cl	Me	H	242(4.40)	315(4.04)	360(3.65)	ethanol	3
24	Cl	Me	Ac	225(4.33)	273(4.41)	363(4.16)	ethanol	3
25	H	Ph	H	250(4.50)	305(3.96)	360(3.75)	ethanol	3
26	H	Ph	Ac	--	270(4.40)	373(4.11)	ethanol	3
27	Cl	Ph	H	248(4.58)	307(4.06)	357(3.71)	ethanol	3
28	H	H	Me	239(4.51)	312(4.08)	373(3.65)	ethanol	3
29	Cl	H	Me	243(4.30)	320(4.11)	385(3.68)	ethanol	3
30	H	H	Ph	238(4.45)	309(4.29)	336(3.60)	ethanol	3

Table III Ultraviolet Spectra of Multisubstituted Indolizines



Spectrum Number	R_1	R_2	R_3	R_8	λ max nm (log ε) Bands I	II	III	Solvent	Ref.
31	p-$MeOC_6H_4$	H	H	H	240(4.41)	305(4.10)	367(3.63)	ethanol	3
32	p-$MeOC_6H_4$	H	Ac	H	239(4.27)	272(4.54)	366(4.14)	ethanol	3
33	Ph	H	H	Me	235(4.45)	303(3.92)	350(3.60)	ethanol	3
34	Ph	H	Ac	Me	224(4.16)	270(4.40)	365(4.16)	ethanol	3
35	p-MeC_6H_4	H	Ac	H	236(4.16)	269(4.48)	373(4.15)	ethanol	3
36	o-MeC_6H_4	H	Ac	H	220(4.08)	266(4.31)	367(3.98)	ethanol	3
37	p-ClC_6H_4	H	Ac	Me	223(4.34)	264(4.45)	370(4.18)	ethanol	3
38	5-Cl-2-thienyl	H	Ac	H	226(4.30)	265(4.25)	361(4.17)	ethanol	3
39	2-pyridyl	H	Ac	H	248(4.17)	263(4.34)	360(4.23)	ethanol	3
40	Me	H	Ac	H	227(4.21)	266(4.39)	370(4.20)	ethanol	4
41	$CH_2CH_2NMe_2$	H	Ac	H	225(4.20)	262(4.28)	364(4.04)	ethanol	4
42	Et	Me	Ac	H	231(4.34)	275(4.40)	370(4.14)	ethanol	4
43	$CH_2CH(CH_3)NMe_2$	Me	Ac	H	230(4.32)	276(4.34)	370(4.11)	ethanol	4
44	Pr	Et	Ac	H	232(4.36)	276(4.36)	373(4.15)	ethanol	4
45	Me	Me	Ac	H	230(4.30)	275(4.38)	372(4.14)	ethanol	4
46	CMe_3	H	Ac	H	236(4.24)	264(4.38)	370(4.20)	ethanol	4
47	H	Ph	H	H	--	254(4.62)	350(3.45)	cyclohexane	1
48	H	Ph	H	H	--	256(4.60)	350(3.50)	methanol	7

effect of Δ log ε 0.61 and 0.27, respectively. The hyperchromic effect indicated an increase of conjugation of the chromophore with indolizine. Electron donating substituents (chloro and methoxy) located in the para position of the phenyl ring (19 and 31) cause a bathochromic shift of Band I (3-4 nm). The introduction of a methyl at the 2-position causes a bathochromic shift of Band II (2-7 nm) (17→21) 18→22, 19→23). Placement of a methyl at position 3 of indolizine results in a shift in Band I (3-8 nm), Band II (7 nm), and Band III (11-25 nm) (17→28, 19→29). These shifts are more dramatic than what is observed for 2-methyl and 3-methylindolizine (2→4 and 2→5). Adding an extra phenyl group at the 2-position of indolizine causes a bathochromic shift at Band I (4 nm), Band II (7 nm), and Band III (25 nm) (29→19). In contrast, the phenyl group at the 3-position causes a hypsochromic shift at Band III (26 nm) (30→17).

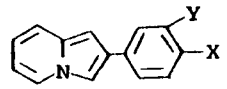
Barret and Chambers(4) reported the absorption spectra of several 3-acetylindolizine derivatives having different substituents at 1- and 2-positions (Table III). By replacing the phenyl moiety with a methyl group at the 1-position, a hypsochromic shift of Band I (8 nm) (40→18) is observed. There is no significant change in the spectra when the methyl is replaced by a 2-dimethylaminoethyl group (41). When the methyl is added at the 2-position of the molecule, there is a bathochromic shift at both Band I (4-5 nm) and Band II (9-10 nm) (42, 43, 45→40).

The same effect is noticeable when propyl is added at the 2-position and ethyl at the 3-position (44). Adding a t-butyl group at the 1-position (46) causes a bathochromic shift at Band I (9 nm).

The ultraviolet absorption spectrum of the unsubstituted 2-phenylindolizine has been reported (1,7). Consistent with what was observed for unsubstituted indolizine, there is no significant change in the spectrum by increasing the polarity of the solvent from cyclohexane to methanol (47,48). This indicates no interaction of the molecule with the solvent. Two main bands were reported using methanol as a solvent: a high intensity band at 256 nm and a medium intensity band at 350 nm. However, the complete absorption spectrum of 2-phenylindolizine (including Band I) has not been reported. Also, the effects of different substituents in the phenyl ring of 2-phenylindolizines have not been examined.

Examination of spectra 49-58 (Table IV) show that all three bands are present, but Band I appears at significantly lower wavelength when compared to other substituted indolizines. The spectra of neutral substituted 2-phenylindolizine derivatives have a medium intensity absorption of 212-230 nm (log ε 4.13-4.73) for Band I, a high intensity of 256-265 nm (log ε 4.45-4.91) for Band II, and a low intensity of 344-363 nm (log ε 3.38-3.80) for Band III. Comparison with unsubstituted 2-phenylindolizine (49) shows a consistent bathochromic shift at

Table IV Ultraviolet Spectra of 2-Phenylindolizines



Spectrum Number	X	Y	NEUTRAL λ max nm (log€) methanol Bands I	II	III	CATION λ max nm (log€)[a] Bands I	II	III
49	H	H	212(4.17)	256(4.61)	352(3.38)	210(4.16)	278(4.23)	350(4.17)
50	Br	H	214(4.24)	265(4.64)	360(3.43)	210(4.21)	282(4.25)	350(4.25)
51	H	Br	223(4.73)	260(4.91)	350(3.80)	214(4.64)	274(4.57)	350(4.55)
52	Cl	H	216(4.26)	264(4.66)	360(3.53)	210(4.19)	277(4.29)	352(4.26)
53	H	Cl	220(4.39)	260(4.63)	363(3.45)	214(4.38)	274(4.30)	347(4.27)
54	Me	H	214(4.21)	257(4.60)	350(3.41)	208(4.13)	284(4.18)	358(4.18)
55	H	Me	216(4.32)	257(4.61)	360(3.41)	210(4.26)	279(4.23)	350(4.19)
56	OMe	H	214(4.16)	262(4.45)	344(3.75)	208(4.17)	292(4.08)	372(4.21)
57	H	OMe	230(4.39)	258(4.67)	350(3.49)	213(4.32)	276(4.24)	350(4.19)

a) 20% (v/v) methanolic 0.1 N hydrochloric acid

Band I (2-18 nm) and Band II (1-9 nm) from the introduction of substituents in the phenyl group. The extent of the difference between the influence of a meta and a para substituent on the spectrum becomes more pronounced with an increase in the polarizability of the substituents. Thus, greater differences are seen for m-bromo (51) versus p-bromo (50) and m-methoxy (57) versus p-methoxy (56) than are observed for m-chloro (53) versus p-chloro (52) and m-methyl (55) versus p-methyl (54).

The spectra of 2-phenylindolizine cations in 0.08 N aqueous-hydrochloric acid consist of three main bands of approximately equal intensity: Band I at 208-214 nm (log ε 4.13-4.64), Band II at 274-292 nm (log ε 4.08-4.57) and Band III at 347-352 nm (log ε 4.17-4.55). The cations show quite different spectra from those of neutral species with a consistent hypsochromic shift at Band I (2-17 nm) and bathochromic shift at Band II (13-30 nm). There is a hypochromic effect at Band II (Δ log ε 0.28-0.43) and a pronounced hyperchromic effect at Band III (Δ log ε 0.46-0.82). In contrast with other spectra reported for methylindolizine cations, substituted 2-phenylindolizinium species show a "Band II". This middle band may be part of the normal aromatic absorption pattern from 2-phenyl substituent which remains in conjugation with the indolizinium ion. Thus this "Band II" may be the result of a $\pi \rightarrow \pi^*$ transition, and not a $n \rightarrow \pi^*$ transition as previously postulated for Band II.

EXPERIMENTAL

The 2-phenylindolizines were prepared according to the literature (12). Ultraviolet spectra were measured using a Beckman DB-GT spectrophotometer. The ultraviolet spectra of neutral 2-phenylindolizines were measured in methanol. The cations were measured in a solvent consisting of 1 part methanol and 4 parts aqueous 0.1 N hydrochloric acid.

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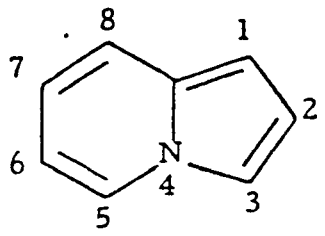
CHAPTER III

IONIZATION CONSTANTS: DETERMINATION AND APPLICATION OF THE HAMMETT EQUATION TO *para* AND *meta* SUBSTITUTED 2-PHENYLINDOLIZINES

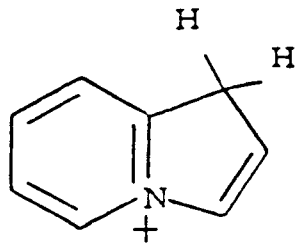
INTRODUCTION

Indolizine is a weakly basic heterocyclic compound (pKa 3.94)(1) with the nitrogen at the bridge head position (Figure 1). It has been shown that a number of electrophilic reactions (e.g. methylation and formylation) take place predominantly at C-3(2). It was also reported(3) that reactions of 1,2,3,-trimethylindolizine with alkyl iodides occurred predominantly at the 3-position with a minor occurrence at the 1-position. A study of the ionization and ultraviolet spectra of methylindolizines showed that protonation at the C-3 position (Ib) when hydrogen was located at this position. However, when a methyl group was situated at the 3-position (i.e. 3 - methyl-, 2,3-dimethyl and 3,7-dimethylindolizines) protonation occurred at C-1 instead of C-3(1). Later, it was shown by nuclear magnetic resonance spectroscopy that 3-methyl substituted indolizines yield a mixture of C-1(Ia) and C-3(Ib) protonated cations in aqueous acid solution(4). Similar studies were done using 2-phenyl-, 1-methyl-2-phenyl, and 5-methyl-2-phenylindolizine. In trifluoroacetic acid protonation occurred exclusively at the 3-position(Ib)(5). Theoretical calculations using the free electron molecular orbital (FEMO) model predicted greater electrophilicity of position 3 over position 1 of 2-phenylindolizine(6). This suggests that protonation of 2-phenylindolizines is at the 3-position.



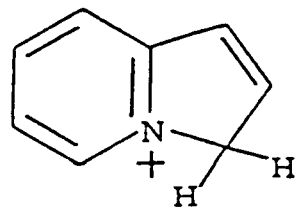
Indolizine

(I)



1H-Indolizinium ion

(Ia)



3H-Indolizinium ion

(Ib)

Figure 1 Structures of neutral and cationic forms of indolizine.

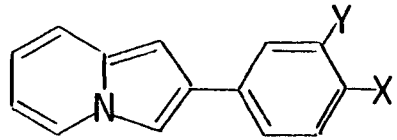
RESULTS AND DISCUSSION

Ionization Constants of 2-Phenylindolizines

A series of *para* and *meta* substituted 2-phenylindolizines was synthesized(7) and their ionization constants determined spectrophotometrically(8).

The *meta* substituted 2-phenylindolizines have a higher pKa than the *para* substituted (Table I). In most cases, the methyl substituent shows greater electron donating properties at the *para* rather than at the *meta* position(9). In contrast, the methyl substituent decrease the acidity of 2-phenylindolizine by 0.88 pKa units when in the *meta* position. A similar effect is noticed with the methoxy group. The *meta* methoxy outweighs the electron donating properties of the *para* methoxy by 0.65 pKa units. Halogens also behave in an unusual way. They decrease the basic strength of 2-phenylindolizine because of their electron withdrawing properties. The *meta* chloro substituent exhibits a smaller electron withdrawing effect (0.16) than the *para* (0.93 pKa units). The *para* and the *meta* bromo behave in a similar manner, increasing the acidic strength by 1.23 and 0.57 pKa units, respectively. This seems to indicate that the inductive electron withdrawing properties of halogens at the *para* position are stronger than at the *meta*.

TABLE I: Ionization Constants of 2-Phenylindolizines



Compound Number	X	Y	pK_a^a	log(Ka/KH)	Concentration ($\times 10^{-3}$M)	λ max, nm
1	H	H	2.33	0	.414	278
2	Br	H	1.10	+1.23	.368	282
3	H	Br	1.76	+ .57	.221	274
4	Cl	H	1.26	+1.07	.352	277
5	H	Cl	2.17	+ .16	.352	274
6	CH_3	H	2.35	- .02	.386	284
7	H	CH_3	3.19	- .86	.386	279
8	OCH_3	H	2.66	- .33	.359	292
9	H	OCH_3	3.31	- .98	.448	276

a) 20% (v/v) methanolic aqueous hydrochloric acid.

Linear Free-Energy Relationships

The ionization constants of substituted 2-phenylindolizines were correlated with the Hammett (σ) substituents(10).

The reaction constant (ρ) for the ionization of 2-phenylindolizines in 20 percent aqueous methanol at 25°C was calculated to be 1.765 by a least square treatment of $\log(Ka/K_H)$ for the ionization of several 2-phenylindolizine derivatives (p-OCH_3, p-CH_3, p-Cl, p-Br, m-OCH_3, m-CH_3, m-Cl, and m-Br) versus the corresponding Hammett substituent constants (σ).

This correlation is represented by equation 1:

$$\log(Ka/K_H) = 1.765(\pm 1.15)\sigma - 0.0781(\pm 0.291) \quad \text{(Eq. 1)}$$

$$n = 8 \quad s = 0.752 \quad r^2 = 0.2830 \quad F_{1,6} = 2.36 \quad (p>0.10)$$

The values in the parentheses are the standard deviations, n = number of compounds, s = standard deviation of correlation, r^2 = coefficient of determination and F is the overall significance parameter.

Figure 2 is a plot of $\log (Ka/K_H)$ for the ionization of substituted 2-phenylindolizines versus the available values of the Hammett substituent constants (σ). Superimposed on the plot are the straight lines obtained by regression analysis performed separately on the para and meta 2-phenylindolizines. The plots for the 95 percent confidence limits for each regression line show

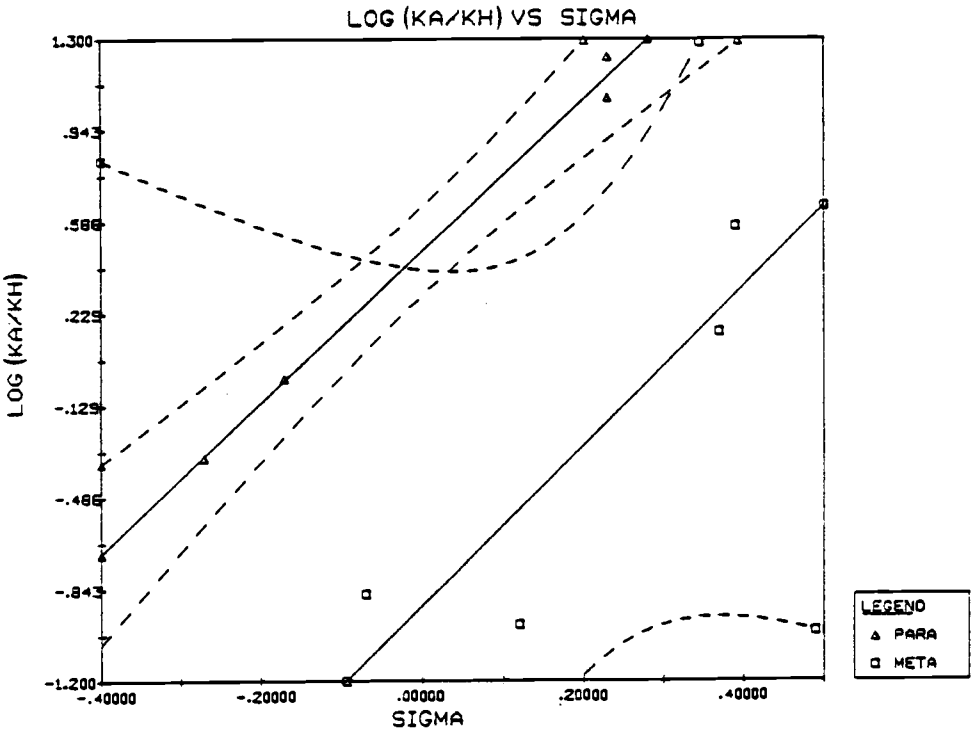


Figure 2. Log ($Ka/K_{[]}$) versus Sigma (σ) showing separate regression lines. The dashed lines are the 95 percent confidence limits for each regression line.

that the para substituted compounds follow a Hammett relationship (Eq. 2). In contrast the meta substituted compounds don't seem to follow a Hammett relationship as well (Eq. 3). To test whether or not these two lines are parallel, the para and meta substituents of 2-phenylindolizines were analyzed separately. The $\log(Ka/K_H)$ values for the 2-phenylindolizine were correlated with Hammett para constants (Eq. 2). Least squares treatment of the four points (OCH_3, CH_3, Cl and Br) leads to a different value for the reaction constant of $\rho = 2.948$.

$$\log Ka/K_H = 2.948(\pm 0.176)\sigma p + 0.472(\pm 0.0402) \quad \text{(Eq. 2)}$$
$$n = 4 \quad s = 0.0836 \quad r^2 = 0.9929 \quad F_{1,2} = 279.2 \quad (p < 0.001)$$

The $\log (Ka/K_H)$ values for the 2-phenylindolizines were then correlated with Hammett meta constants. Least squares treatment of the four points (OCH_3, CH_3, Cl and Br) leads to the reaction constant $\rho = 3.111$ as shown in Equation 3.

$$\log(Ka/K_H) = 3.111(\pm 1.095)\sigma m \; 0.9075(\pm 0.304) \quad \text{(Eq. 3)}$$
$$n = 4 \quad s = 0.4158 \quad r^2 = 0.8015 \quad F_{1,2} = 8.07 \quad (p > 0.10)$$

To test whether or not the slopes of these two regression lines are significantly different, a general approach was used by constructing a confidence interval for the difference in the slopes, $\beta_{12} - \beta_{13}$ (11). β_{12} represents the slope for equation 2 and β_{13} for equation 3. The difference in the slopes is -0.163. The 95 percent confidence limits for $\beta_{12} - \beta_{13}$ are the following:

$$-8.629 \geq \beta_{12} - \beta_{13} \leq 8.303$$

These results suggest that the two slopes are the same within the 95 percent confidence limits. In a similar manner, the intercepts can be checked whether or not they are significantly different. The difference in the intercept is +1.38. The 95 percent confidence limits for β_{02} and β_{03} are the following:

$$.528 \leq \beta_{02} - \beta_{03} \leq -2.23$$

These results indicate that the intercepts are different within 95 percent confidence limits.

Another approach to test for parallelism between regression lines is to use an indicator variable(11). In equation 4, I = 1 when the compound is <u>para</u> substituted and I = 0 when the compound is <u>meta</u> substituted.

$$\log(Ka/K_H) = 3.015(\pm 0.453)\sigma + 1.360(\pm 0.210)I - 0.888(\pm 0.163) \quad \text{(Eq. 4)}$$

$$n = 8 \quad s = .269 \quad r^2 = 0.9236 \quad F_{2,5} = 30.2 \quad (p<0.005)$$

In effect, the use of the indicator variable brings the two separate regression lines together. The ρ value for equation 4 lies between the ρ values for equation 2 and 3. Similarly the intercept value for equation 4 lies between the intercept term for equation 2 and 3.

Because the <u>meta</u> substituted compounds showed a poor Hammett relationship, the Yukawa-Tsuno equation which utilizes both σ and $\sigma+$ constants was tried. The results were poorer and inferior to the use of an indicator variable (Eq. 4).

In summary, the statistical analyses of the results provides evidence that the ρ values for the meta and para 2-phenylindolizines are not significantly different. Therefore, the meta and para show similar effects on the ionization of 2-phenylindolizines.

EXPERIMENTAL

The synthesis of 2-phenylindolizines is described in literature(7).

Physical properties - Ultraviolet spectra were measured with a Beckman DB-GT spectrophotometer. Ionization constants of 2-phenylindolizine derivatives were measured in 20% methanol using spectrophotometric techniques(8). The optical densities were measured within one minute of mixing neutral species in acid solution, during which time decomposition of the neutral species was negligible. All measurements were made at constant ionic strength. Statistical analyses were performed using Oregon State University Statistical Interactive Programing System (SIPS).

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CHAPTER IV

DETERMINATION OF OCTANOL-WATER EQUIVALENT PARTITION COEFFICIENTS OF INDOLIZINE AND SUBSTITUTED 2-PHENYLINDOLIZINES BY REVERSED-PHASE HIGH-PRESSURE LIQUID CHROMATOGRAPHY AND FRAGMENTATION VALUES

INTRODUCTION

There is considerable interest in partition coefficient determination in the emerging area of rational drug design (1). The partition coefficient, log P, represents the distribution of a substance between an organic and aqueous phase. Several methods may be used to determine partition coefficients including the shake-flask method, liquid-liquid chromatography on lipid impregnated plate technique, and high pressure liquid chromatography (HPLC).

No studies have been done on the log P of indolizines. Indolizine is a 10 π aromatic heterocyclic compound with the nitrogen at the bridge head position (Figure 1). It is a nearly electrically neutral and weakly basic compound with a pka value of 3.94 (2).

For the normal shake-flask method, octanol and water have been used as the biological lipid and aqueous phases, respectively, in partition determination (3). Due to the instability of the indolizine nucleus, measuring log P values of this compound by this technique has proven difficult. The shake-flask procedure (4) is a tedious process, time consuming, and subject to problems in purity, stability, and mass balance from the compounds being measured.

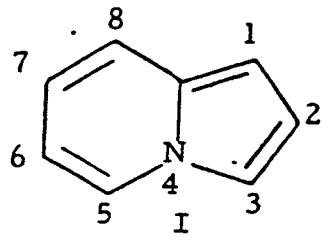

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Figure 1 Indolizine

Liquid chromatography on paper or lipid impregnated plates has been used as an alternative to octanol-water partition. Martin (5) derived Equation 1 for thin layer or paper chromatography (TLC).

$$\log P = \log K + Rm \qquad \text{(Eq. 1)}$$

where K = a constant for the system

$Rm = \log [(1/Rf)-1]$

$$Rf = \frac{\text{distance traveled by the solute}}{\text{distance traveled by the solvent front}}$$

Reversed-phase HPLC has been used to measure lipophilicity of several compounds. In HPLC, the capacity factor k' replaces Rm and is defined as

$$k' = \frac{(t_r - t_o)}{t_o} = \frac{(V_r - V_o)}{V_o} = \frac{(d_r - d_o)}{d_o} \qquad \text{(Eq. 2)}$$

where

t_r = retention time of the compound

t_o = retention time of the solvent

V_r = retention volume of the compound

V_o = retention volume of the solvent or dead volume

d_r = distance between the peak of the compound and peak of the solvent front

d_o = distance between the point of injection and the peak of the solvent front.

Haggerty and Murrill (6) measured log P values for a family of nitrosoureas using a column packed with octa-

decylsilane bonded to silica[1] eluted with 30 percent acetonitrile in pH 7.41 buffer solution. Carlson et al. (7) obtained good correlations between log k' and log P of some substituted phenol and aniline derivatives using bonded[2] columns with various mole percentages of distilled water and acetone as the eluent. McCall (8) found that exhaustive silylation of octadecylsilane reversed-phase columns gave a better correlation between k' and log P than the untreated packing material. The rationale is that exhaustive silylation eliminates absorption phenomena due to free SiOH sites.

Hulshoff and Perkin (9) determined the lipophilicities of 1,4-benzodiazepine derivaties using oleyl alcohol and supported on a porous silica[3] as an HPLC procedure and compared their results with reversed-phase TLC techniques. Similar work was done by Mirrlees et al. (10) using 1-octanol supported on diatomaceous earth[4] with octanol-saturated water as the eluent.

Henry et al. (11) compared various HPLC techniques using three different columns: one reversed-phase[1], one unmodified absorption system[5], and one nonbonded porous silica[6] coated with 1-octanol or squalene. They reported several good correlations between log k' and log of biological activity and warned investigators using these techniques to be careful in interpreting these correlations.

Unger et al. (12) had near perfect agreement between shake-flask and reversed-phase HPLC procedures over a log P range of 3.5 units, using octanol-saturated pH 7.00 (0.01M) phosphate buffer as the mobile phase, and silylated octadecyl bonded silica[1] as the stationary phase.

For this study, the latter HPLC procedure (12) was used to determine the log P of unstable indolizine. Then, a modified HPLC method was developed to measure partition coefficients of compounds having a log P value in the range of 3.5 to 5.0. Using this method, the π and fragment values for the indolizinyl substituents were also determined.

EXPERIMENTAL

Solvents were of analytical reagent quality, and the 1-octanol for chromatographic purposes was purified according to reported procedures (13). Standards were obtained from commercial sources. Indolizine itself, and 2-phenylindolizines were synthesized by the Boekelheide (14) method and via the Tschitschibabin-Stepanow (15) route, respectively.

Samples were dissolved in water-saturated octanol and/or a minimal amount of methanol. Sodium nitrate in octanol-saturated water was used (a suitable nonretained compound) to define dead volume, t_o. Sample concentrations

were adjusted so that the relative peak areas remain approximately constant.

The high-pressure liquid chromatograph[7] consisted of a pump[8] and injector[9]. A UV-visible spectrophotometer[10] equipped with low dead volume flow cells was used as detector. Standards and indolizines were analyzed at 260 nm. Peaks were measured on a 25 cm dual pen recorder[11]. The column packing of silica particles (37-50μ) with an octadecylsilane-bonded coating[1] was persilated by McCall's method (8). Stainless steel columns (2 mm i.d.) in lengths of 5,10,30 and 60 cm were packed using published "Tap-fill" procedures (16), and then mounted onto the liquid chromatograph.

Two methods were used to determine log P. One system followed Unger's procedure (12). The second procedure was patterned after reported methods (6-8) and used persilated octadecylsilane columns[1] with 40 percent (V/V) acetonitrile in water as the mobile phase to determine large log P's (range 3.5 to 5.0) of standards and 2-phenylindolizines. Samples were dissolved in acetonitrile and/or a minimal amount of methanol. For both procedures, all solutions containing solute were first filtered[12] to reduce contamination or column clogging. All experiments were performed at ambient temperature ($25 \pm 1^{o}C$).

Log P values for unknowns obtained from HPLC data were determined by calculation using regression analysis derived from literature Log P values (17) versus their log k' obtained experimentally. Daily standards were run with excellent reproducibility. Statistical analyses were performed using a statistical package.[13]

RESULTS AND DISCUSSIONS

Log P Determination of Indolizine by HPLC

Due to the lack of stability of indolizine and the difficulty in measuring log P by the normal shake-flask technique, the log P value of this compound was estimated using HPLC techniques. A group of compounds possessing physical properties similar to those of indolizine was selected. These reference compounds were electrically neutral and had partition coefficients close to the estimated value for indolizine (log P 2.29; calculated by the fragment method; see below). These compounds were chromatographed on a silylated column[1] coated with octanol. The mobile phase was octanol-saturated water using a 10 cm column. A linear relationship found between log P values and their log k' values (Figure 2) is given by Equation 3:

$$\log P = 1.516\ (\pm 0.059) + 1.094\ (\pm 0.057)\ \log k' \quad \text{(Eq.3)}$$

$$n = 6 \quad s = 0.0453 \quad r^2 = 0.9893 \quad F_{1,4} = 370$$

The values in parentheses are the standard deviations, n = number of compounds, s = standard deviation of correla-

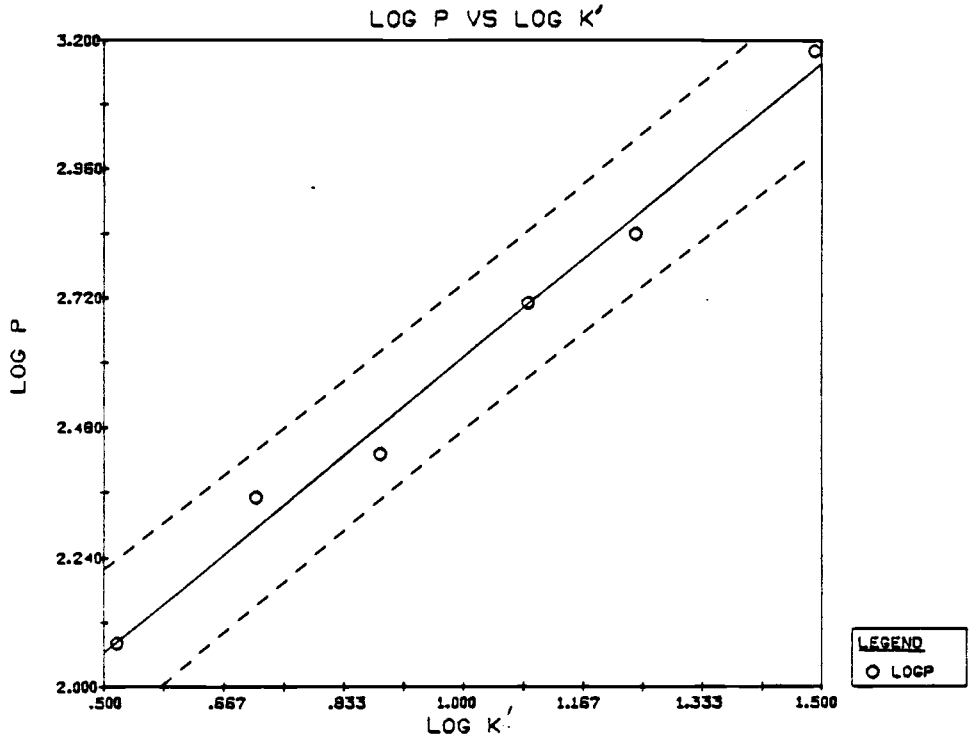


Figure 2. Literature log P values versus log k' determined by octanol-coated octadecylsilyl-bonded support with octanol-saturated water as mobile phase. Dashed lines indicate the 95% confidence limit of the predicted line

tion, r^2 = coefficient of determination, and F is the overall significance parameter.

The log k' value for indolizine (0.894) was measured under the same conditions used for the standards (Table I) and a log P value of 2.49 was calculated using Equation 3.

Determination of π Value of the Indolizinyl Moiety by HPLC.

Selected standards with known log P values (17) in the range estimated for substituted indolizines were chromatographed using several different systems. The systems consisted of a reversed-phase persilated column[1] with different column lengths and different concentrations of acetonitrile in water as the mobile phase. Various chromatographic systems were evaluated. Columns[1] varied in length from 5 cm to 10 cm and the solvent system consisted of acetonitrile concentrations in the range of 35-50 percent. Good linear relationship (Figure 3) was found in a system using 40 percent acetonitrile in a 10 cm column[1]. The results are displayed in Table II and represented by Equation 4:

$$\log P = 2.321\ (\pm\ 0.297) + 2.202(+\ 0.360)\ \log k' \quad \text{(Eq.4)}$$

$$n = 5 \quad s = 0.1411 \quad r^2 = 0.9256 \quad F_{1,3} = 37$$

A variety of 2-phenylindolizine analogs with widely different lipophilicities were prepared (Table III). These compounds

TABLE I. Estimated Log P Values on HPLC Using Octanol-Saturated Water as Mobil Phase.

	Log k'	Log P	
Compounds		Lit.[a]	HPLC[b]
Anisole	0.518	2.08	2.08
p-Chloroacetophenone	0.711	2.35	2.29
p-Bromoacetophenone	0.884	2.43	2.48
Toluene	1.09	2.71	2.71
Chlorobenzene	1.24	2.84	2.87
Benzophenone	1.49	3.18	3.15
Indolizine (Fig. 1)	0.894		2.49 ± 0.02[c]

a - Ref. (17)

b - Equation 3

c - Standard error estimate from Equation 3

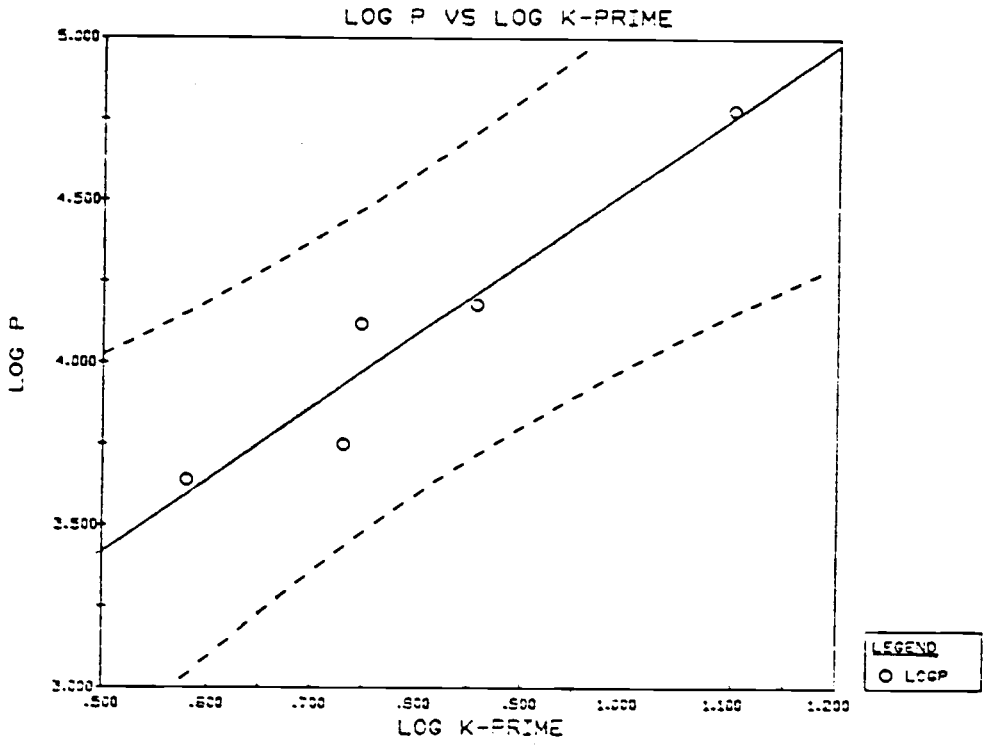


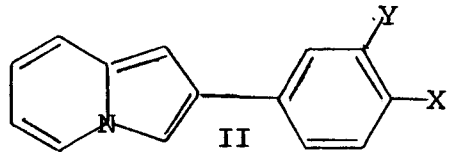
Figure 3. Literature log P value versus log k' determined by 10 cm column[1] using 40 percent (V/V) acetonitrile in water as the mobile phase. Dashed lines indicate the 95 percent confidence limits of the predicted line.

TABLE II. Estimated Log P Values on HPLC Using 40 Percent (V/V) Acetonitrile in Water

Compound	Log k'	Log P	
		Lit.[a]	HPLC[b]
o-Dibromobenzene	0.580	3.64	3.60
m-Dibromobenzene	0.732	3.75	3.93
Dibenzofuran	0.748	4.12	3.96
Fluorene	0.857	4.18	4.21
Diphenylacetylene	1.10	4.78	4.76

a - Ref.(17)
b - Equation 4

TABLE III. Estimated Log P on HPLC of 2-phenylindolizines



II

Compound	X	Y	log k'[a]	log P HPLC[b] (± SEM)
IIa	H	H	0.892	4.29(± 0.07)
IIb	OCH_3	H	0.851	4.20(± 0.07)
IIc	H	OCH_3	0.826	4.14(± 0.06)
IId	CH_3	H	1.19	4.95(± 0.15)
IIe	H	CH_3	1.15	4.86(± 0.14)
IIf	Cl	H	1.30	5.20(± 0.19)
IIg	H	Cl	1.27	5.14(± 0.18)
IIh	Br	H	1.40	5.43(± 0.22)
IIi	H	Br	1.35	5.31(± 0.21)

a - Values measured on a persilated column[1] (10cm) using 40% (V/V) CH_3CN as the eluant.

b - Equation 4

have different substituents in the para and meta positions on the 2-phenyl groups. These substituted 2-phenylindolizines were chromatographed under conditions identical to those in Table II. Their log k' values were used to calculate log P using Equation 4.

π Value Correlation from HPLC Data

An investigation to determine whether the retention times of substituted 2-phenylindolizines were proportional to Hansch π constants (18) using a persilated column[1] was conducted. The previously described mobile phase was 40 percent acetonitrile in water using a 10 cm column. A linear relationship was found between the π values and log k'. The results are represented by the following equation:

$$\pi = 0.536(\pm 0.0864) + 1.665\ (\pm\ 0.07474)\ \log k' \quad \text{(Eq. 5)}$$

$$n = 9;\ s = 0.0475;\ r^2 = 0.9861\quad F_{1,7} = 496.$$

This equation shows an excellent relationship between π and log k'.

The π indolizinyl values were calculated by subtracting the Hansch π constants (18) from the log P (Table IV) as shown in Equation 6:

$$\pi \text{ indolizinyl} = \log P - \pi \text{ substituents} \quad \text{(Eq. 6)}$$

The calculated π indolizinyl values vary from 2.20 to 2.61 with a mean of 2.41, a sample standard deviation of ±0.133, and a coefficient of variation of 5.5 percent.

TABLE IV. Estimated π Indolizinyl Values Using LogP HPLC

Compound	log k'[a]	π [b]	LogP HPLC[c]	π Indolizinyl[d]
IIa	0.892	1.96	4.29	2.33
IIb	0.851	1.94	4.20	2.26
IIc	0.826	1.94	4.14	2.20
IId	1.19	2.52	4.95	2.43
IIe	1.15	2.52	4.86	2.34
IIf	1.30	2.67	5.20	2.53
IIg	1.27	2.67	5.14	2.47
IIh	1.40	2.82	5.43	2.61
IIi	1.35	2.82	5.31	2.49

a - Values measured on a persilated column[1] (10cm) using 40% (V/V) CH_3CH as the eluent

b - Ref. (18)

c - Equation 4

d - Equation 6

The π indolizinyl average is within the range of the observed log P for indolizine (2.49±0.02) described in the early part of this study.

Log P of Indolizine Calculated by the Fragment Method

The log P of the indolizine nucleus can be also calculated by the fragment method using values from model systems (18).

For example, for the fused aromatic ring model, the aromatic carbon (fCH) has a value of 0.35, the ring fusion carbon ($f\dot{C}$) is 0.22 and the carbon next to a heteroatom ($f\overset{*}{C}$) is 0.44. For the nitrogen, several values can be used depending on the specific location of the atom in the molecule. According to Hansch and Leo (18), the fragment constant for the nitrogen in a ring system such as indole or pyrrole (-0.67) lies between the values of the nitrogen attached to one aromatic ring (-1.03), and two aromatic rings (-0.03). Indolizine is a special case because the nitrogen is located at the bridge head position of the molecule (Figure 1). The nitrogen in this system is more lipophilic than its isomer, indole, because the unshared electrons on the nitrogen resonate among all the atoms of the ring (19). Therefore, indolizine is not protonated at the nitrogen, but instead at the carbon on the 3-position of the ring (20), producing a pka of 3.94 (2). For

this reason, the fragment value of $f_{\overset{|}{N}-\phi} = -0.56$ was used as an approximation of the nitrogen constant for indolizine. This value represents a nitrogen in an aromatic system attached to an aromatic ring. The addition of all these fragment values is illustrated below in Equation 7:

$$\log P = 5\ f(CH) + f(\dot{C}) + 2\, f(\overset{*}{\dot{C}}) + f_{\overset{|}{N}-\phi} \quad \text{(Eq. 7)}$$

$$= 5(0.35) + (0.22) + 2(0.44) + (-0.56)$$

$$= 2.29$$

In a similar manner, the fragment substituents of 2-phenylindolizines were calculated by the fragment method (18) and listed in Table V. Indolizinyl fragment values were calculated by subtracting fragment substituent values from log P values (Equation 8).

$$\text{indolizinyl} = \log P - f \text{ substituents} \quad \text{(Eq. 8)}$$

The average for indolizinyl fragment values is 2.43 with a sample standard deviation of $\pm$ 0.165. The fragment values vary from 2.19 to 2.67 having a coefficient of variation of 6.8 percent.

In summary, the log P of the indolizine nucleus determined by the HPLC method shows good agreement with the approximate values of π indolizinyl measured by the additive method and f indolizinyl calculated by the fragment method.

TABLE V. Estimated *f*Indolizinyl Using Log P HPLC.

Compound	Log k'[a]	*f*Substituent[b]	Log P HPLC[c]	*f*Indolizinyl[d]
IIa	0.892	1.90	4.29	2.39
IIb	0.851	1.95	4.20	2.25
IIc	0.826	1.95	4.14	2.19
IId	1.19	2.56	4.95	2.39
IIe	1.15	2.56	4.85	2.30
IIf	1.30	2.61	5.20	2.59
IIg	1.27	2.61	5.14	2.53
IIh	1.40	2.76	5.43	2.67
IIi	1.35	2.76	5.31	2.55

a - Values measured on a persilated column[1] (10cm) using 40% (V/V) CH_3CN as the eluant

b - Ref. (18)

c - Equation 4

d - Equation 8

Footnotes

1. Corasil C18, Waters Associates.
2. Porasil B, Waters Associates.
3. Porasil C, Waters Associates.
4. Hyflosupercel, Johns-Manville.
5. Corasil II, Waters Associates.
6. Porasil A, Waters Associates.
7. Model ALC/6PC 201 Liquid Chromatograph, Waters Associates, Milford, Mass.
8. Model M6000A, Waters Associates, Milford, Mass.
9. Model U-GK, Waters Associates, Milford, Mass.
10. Varian Model 635.
11. Soltec
12. Fritted Disc, 10-15μ, Pyrex.
13. Oregon State University Statistical Interaction Programming System (SIPS).

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