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Nitro- *para*- and *meta*-Substituted 2-Phenylindolizines as Potential Antimicrobial Agents

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Abstract □ Some *para*- and *meta*-substituted nitro-2-phenylindolizines were prepared and tested as potential antimicrobial agents. The syntheses were accomplished *via* the Chichibabin–Stepanow synthesis, using the properly substituted α-picoline and phenacyl bromides followed by direct nitration.

Keyphrases □ Antimicrobial activity—potential, nitro *para*- and *meta*-substituted 2-phenylindolizines □ 2-Phenylindolizines—potential antimicrobials, nitro *para*- and *meta*-substituted, synthesis □ Heterocycles—*para*- and *meta*-substituted 2-phenylindolizines, preparation, potential antimicrobials

Earlier reports (1–4) have generated considerable interest in the fundamental chemistry of the indolizine heterocyclic system (I). However, there have been few reports of the biological activity of indolizines (5–7), and no systematic study has been reported.

BACKGROUND

One report (8) considered 2-(4-fluoro-2-methylphenyl)indolizine and 2-(4-fluoro-2-methylphenyl)-7-methylindolizine as carcinogens, but failed to mention whether these compounds were actually tested for carcinogenic properties. 2-(4-Cyclohexylphenyl)indolizine was reported to be noncarcinogenic when painted on the skin of experimental animals (9).

Another report (10) considered 1-indolizinealanine to be a tryptophan antimetabolite. Preliminary tests (11) reported that indolizine-1-acetic acid, the structural analog of indole-3-acetic acid(heteroauxin), showed some auxinlike activity.

1-Diethylaminomethyl-3-methyl-2-phenylindolizine reportedly possessed central nervous system (CNS) depressant activity (12). No useful activity was found for some 1-aminoalkyl-2-phenylindolizines, which were screened for their effects on the CNS in mice and in cats (13). The compounds were stimulants at low doses, depressants at higher doses, and caused death by convulsions.

The 2,3-bis(*p*-methoxyphenyl)indolizines were reported to possess antiexudative activity (14). It was previously reported (15) that 2-(4-fluoro-3-methylphenyl)indolizine decreased the duration of paralysis caused by the drug zoxazolamine when tested in rats. No anti-inflammatory activity for 2-(*p*-methylphenyl)-1-phenylindolizine and 2-(*p*-bromophenyl)-1-(*p*-methoxyphenyl)indolizine was found (16) relative to the reference indoxole.

Rosseels *et al.* (17) found that 3-acetyl-2-alkyl-1-nicotinoylindolizines showed anti-inflammatory activities equivalent to acetylsalicylic acid, and 2-ethyl and 2-*n*-propyl-1-nicotinoylindolizines possessed analgesic activities greater than that of antipyrine.

Two earlier reports (18, 19) stated 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 antiserotonin antihis-

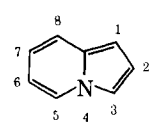

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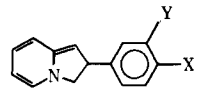
Table I—Indolizines



Compound Number	X	Y	Melting Point	Percent Yield	Formula	Analysis Calc.	Analysis Found
XI*a*	H	H	214–215°	80	$C_{14}H_{11}N$	—	—[a]
XI*b*	Br	H	253–254°	86	$C_{14}H_{10}NBr$	—	—[b]
XI*c*	H	Br	175–176°	81	$C_{14}H_{10}NBr$[c]	271.000	271.000
XI*d*	Cl	H	245–246°	88	$C_{14}H_{10}NCl$	—	—[b]
XI*e*	H	Cl	160–161°	96	$C_{14}H_{10}NCl$[c]	227.050	227.050
XI*f*	OCH_3	H	227–228°	28	$C_{15}H_{13}NO$	—	—[d]
XI*g*	H	OCH_3	125–126°	89	$C_{15}H_{13}NO$[c]	223.100	223.099
XI*h*	CH_3	H	215–216°	85	$C_{15}H_{13}N$	—	—[b]
XI*i*	H	CH_3	143–145°	60	$C_{15}H_{13}N$[c]	207.105	207.105

[a] Reference 36. [b] Reference 43. [c] Empirical formula confirmed by high resolution mass spectrometry[11]. [d] Reference 44.

tamine and antiacetylcholine properties with some CNS activity.

Some reports (21, 22) considered 2-alkyl-3- and 2-alkyl-1-(4-dialkylaminoalkoxybenzoyl)indolizine derivatives as antianginal agents. Other groups (23, 24) reported that 2- and 3-benzoylindolizine derivatives showed hemodynamic characteristics similar to those of amiodarone with noncompetitive antiadrenergic properties.

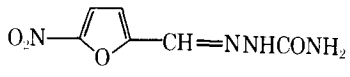
It was reported (25) that 2-[4-(2-indolizinyl)phenyl]propionitrile and propionic acid derivatives have analgesic, antipyretic, and anti-inflammatory activities. The indolizine analog of pindolol was found to have nonselective β-adrenergic blocking activity (26).

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

There is a 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.

RESULTS AND DISCUSSION

Preparation of Compounds—All the compounds prepared were *para*- and *meta*-substituted 2-phenylindolizines (Table I, compounds XI*a–i*). They were prepared from α-picoline (VIII) and phenacylbromides (IX) *via* the Chichibabin–Stepanow synthesis (34) (Scheme I). The α-picoline and phenacyl bromides were combined to form a pyridinium salt (X) (Table II, compounds Xa–i), which was cyclized in refluxing aqueous sodium bicarbonate to the corresponding indolizine (XI). The indolizines in solution were unstable in light, especially when in chloroform.



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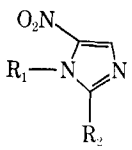
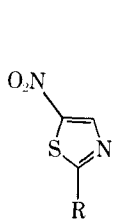
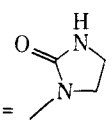


III: $R_1 = -CH_2CH_2OH$, $R_2 = -CH_3$
IV: $R_1 = -CH_3$, $R_2 = -CH(CH_3)_2$
V: $R_1 = -CH_2CH_2SO_2CH_2CH_3$, $R_2 = -CH_3$

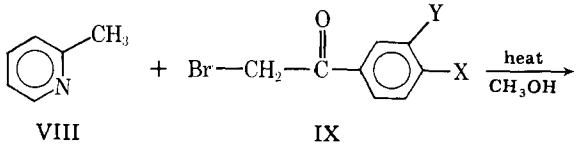




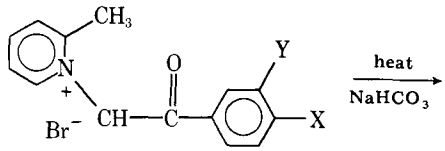
VI: R =
VII: R = $-NHCONHCH_2CH_3$

A mechanism for the Chichibabin–Stepanow synthesis was postulated earlier (35). Nitration of 2-phenylindolizine derivatives (XI) was accomplished with nitric acid (d = 1.4) in the presence of concentrated sulfuric acid at 0° (36). The mechanism by which 2-phenylindolizines are nitrated was recently discussed (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, in acetic anhydride, it is presumably the free indolizine which is nitrated at the position commonly susceptible to electrophilic attack. 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).

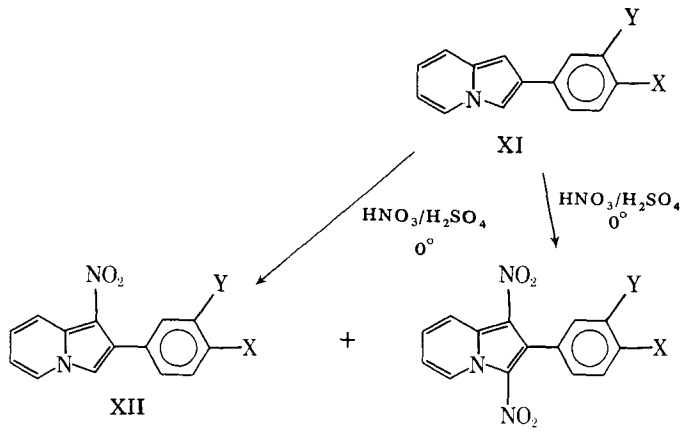
The first step of the proposed mechanism (Scheme II) for the nitration of indolizines using a nitric and sulfuric acid mixture is protonated at the 3-position (XIV). Protonation blocks position 3 from further electrophilic attack. The second step is the nitronium ion attack at the 1-position, forming 1-nitroindolizinium cation (XV). In the presence of a base, proton removal occurs, yielding compound XVII. With excess nitronium ions,



VIII IX



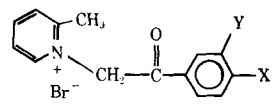
X



XII XIII

Scheme I—Synthesis of nitro-2-phenylindolizine.

Table II—Pyridinium Bromides



Compound Number	X	Y	Melting Point	Percent Yield	Formula
X*a*	H	H	215–216°	70	$C_{14}H_{14}BrNO$
X*b*	Br	H	185–186°	27	$C_{14}H_{13}Br_2NO$
X*c*	H	Br	250–251°	62	$C_{14}H_{13}Br_2NO$
X*d*	Cl	H	145–148°	33	$C_{14}H_{13}BrClNO$
X*e*	H	Cl	227–228°	58	$C_{14}H_{13}BrClNO$
X*f*	OCH_3	H	140–141°	84	$C_{15}H_{16}BrNO_2$
X*g*	H	OCH_3	185–186°	75	$C_{15}H_{16}BrNO_2$
X*h*	CH_3	H	183–185°	54	$C_{15}H_{16}BrNO$
X*i*	H	CH_3	195–197°	60	$C_{15}H_{16}BrNO$

further nitration can occur at position 3, forming 1,3-dinitroindolizinium cation (XVI). In a basic environment, proton removal occurs giving compound XVIII.

The nitro derivatives XI*j*, XVII*a–f*, and XVIII*a–f* used in this study were purified by alumina-column chromatography and preparative TLC. They rapidly turned green if not protected from air and sunlight. In some situations the reaction mixture contained both the 1-nitro (XII) and 1,3-dinitro indolizine (XIII); in others only the 1,3-dinitroindolizine (XIII) could be isolated. Because the compounds did not exhibit the desired biological activity, no attempt was made to improve yields.

Biological Results—Compounds XI*j*, XVII*a–f*, and XVIII*a–f* were evaluated *in vitro* for antibacterial activity against Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* bacteria. A preliminary disk-agar diffusion test was done using nitrofurazone[1] and chloramphenicol[1] as standards. None of the test compounds exhibited any antimicrobial activity against the organisms tested. Negative results were also obtained when the indolizine was placed directly onto the test agar.

EXPERIMENTAL

Antimicrobial Evaluation—The bacteria used were Gram-negative *E. coli* and Gram-positive *S. aureus*[2]. Before antimicrobial testing, the compounds in solution were sterilized using a fritted-glass filtering disk[3] (40).

The disk-agar diffusion method following the 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 a special agar for susceptibility testing of microorganisms[4] and a standard inoculum applied in a potency for each of the chemotherapeutic agents being tested.

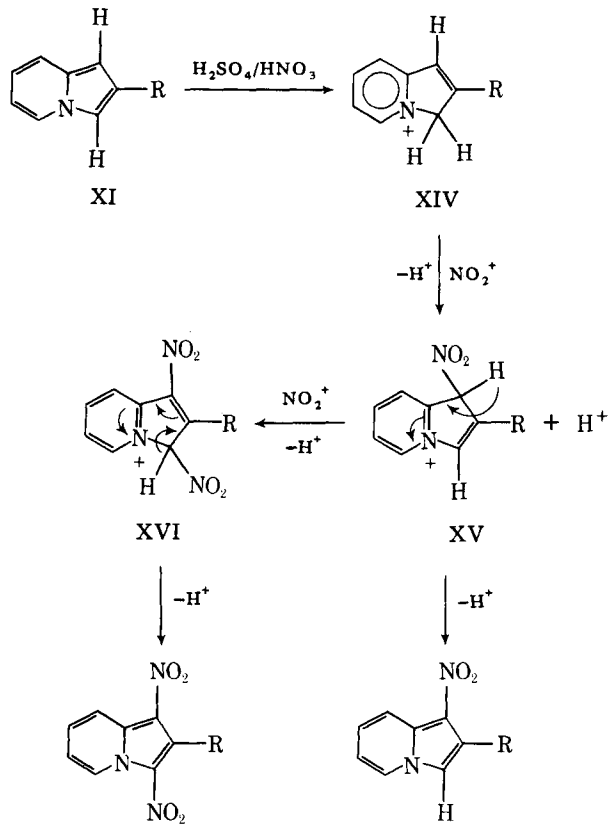
The experimental disks[5] were placed in a methanol solution containing varying amounts of the indolizine and then were air dried and placed on the top of the agar. The pure compounds (5 mg) were also placed directly on the top of the agar. Disks previously soaked in methanol and then air dried were used as blanks.

Chemistry—Melting point values were determined using an open capillary melting point apparatus[6] and are uncorrected. The NMR spectrometer[7] was used with tetramethylsilane as an internal standard and deuterated chloroform as a solvent. IR[8] and UV[9] 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[10]. Elemental analyses were performed by high-resolution mass spectrometry[11].

Analytical TLC plates were precoated with silica gel[12]. The preparative TLC plates were coated with silica gel[13] (20 × 20 cm, 2 mm). The three methylene chloride–hexane developing systems used were A (1:3), B (1:1), and C (3:1). Spots were visualized with UV light at 254 nm. The purity of each compound was confirmed in each of the TLC systems and in a 40% acetonitrile high-pressure liquid chromatography (HPLC) system[14] using a persilated octadecylsilane column[15]. All compounds gave spectral data consistent with the proposed structure.

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

Synthesis of *m*-Substituted 2-Phenylindolizines (Table I)—A typical synthesis will be described for 2-(*m*-methylphenyl)indolizine (XI*i*). 3′-Methylacetophenone (3.4 g, 0.025 mole) was diluted with 10 ml of anhydrous methanol in a 25-ml triple-necked flask. Bromine (4.0 g, 0.025 mole) diluted in 2.5 ml of anhydrous methanol was added dropwise for 10 min. If the reaction mixture became clear, more bromine was added until a dark red color persisted. After 30 min of stirring, the mixture was poured into a beaker containing chipped ice. The resulting crystals were washed several times with cold water. (When the substituted phenacyl



Scheme II—Mechanism of nitration for indolizines.

[1] Sensi-Disc, microbial susceptibility test discs, BBL, Cockeysville, Md.
[2] From a collection maintained by Oregon State University, Departments of Microbiology and Food Science and Technology.
[3] Pyrex brand chemical glass #774, UF Porosity, Corning Glass Works, Corning, N.Y.
[4] Mueller-Hinton agar, Difco Laboratories, Detroit, Mich.
[5] No. 740-E, Schleicher & Schuell, Keene, N.H.
[6] Thomas-Hoover.
[7] Varian Anaspect EM 360.
[8] Perkin-Elmer model 727.
[9] Beckman model DB-GT.
[10] System Industries 150.
[11] High resolution mass spectrometer CEC-21B-110. Instrument set at 70 eV. Analyses were done at the Department of Chemistry, University of Oregon, Eugene, Ore.
[12] Silica gel 60 F-254, EM reagents.
[13] Silica gel PF-254 + 366, EM reagents.
[14] Model ALC/GPC 201 liquid chromatograph, model M 6000A pump, model U-6K injector, Waters Associates, Milford, Mass.
[15] Corasil C_{18}, Waters Associates, Milford, Mass.

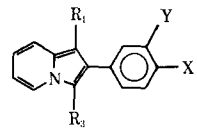


Table III—Nitroindolizines

Compound	X	Y	R_1	R_3	M.P.	Percent Yield	Formula	Analysis Calc.	Analysis Found
XVIII*a*	H	H	NO_2	NO_2	245–246°	14	$C_{14}H_9N_3O_4$	—	—[a]
XI*j*	NO_2	H	H	H	236–237°	50	$C_{14}H_{10}N_2O_2$	—	—[a]
XVII*a*	NO_2	H	NO_2	H	235–236°	3	$C_{14}H_9N_3O_4$	—	—[a]
XVII*b*	Br	H	NO_2	H	153–155°	3	$C_{14}H_9BrN_2O_2$[b]	315.985	315.985
XVIII*b*	Br	H	NO_2	NO_2	265–266°	15	$C_{14}H_8BrN_3O_4$[b]	360.970	360.972
XVII*c*	H	Br	NO_2	H	174–175°	14	$C_{14}H_9BrN_2O_2$[b]	315.985	315.982
XVIII*c*	Cl	H	NO_2	NO_2	215–216°	3	$C_{14}H_8ClN_3O_4$[b]	317.020	317.022
XVII*d*	H	Cl	NO_2	H	190–191°	18	$C_{14}H_9ClN_2O_2$[b]	272.035	272.033
XVIII*d*	OCH_3	H	NO_2	NO_2	268–269°	2	$C_{15}H_{11}N_3O_5$[b]	313.070	313.067
XVIII*e*	H	OCH_3	NO_2	NO_2	183–184°	17	$C_{15}H_{11}N_3O_5$[b]	313.070	313.070
XVII*e*	CH_3	H	NO_2	H	178–179°	5	$C_{15}H_{12}N_2O_2$[b]	252.090	252.088
XVIII*f*	CH_3	H	NO_2	NO_2	235–236°	12	$C_{15}H_{11}N_3O_4$[b]	297.075	297.075
XVII*f*	H	CH_3	NO_2	H	179–180°	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[11].

bromide was a liquid it was washed in a separator.) The crystalline material was transferred to a 50-ml flask and α-picoline (2.25 g, 0.025 mole) in 25 ml of anhydrous methanol was added. The solution was refluxed for 30 min. A precipitate formed, which was filtered and washed with cold water and recrystallized from hot ethyl acetate or ether. The white crystalline solid was then placed in a 50-ml round-bottom flask containing sodium bicarbonate (2.10 g, 0.025 mole) in 25 ml of water. The mixture was refluxed for 10 min. The resulting yellow precipitate was filtered, washed with water, and recrystallized from ethyl acetate. Yields and analytical data are found in Table I.

Synthesis of Nitroindolizines (Table III)—*1,3-Dinitro-2-phenylindolizine* (*XVIII*a)—2-Phenylindolizine (XI*a*) (2.0 g, 10.4 mmoles) dissolved in 10 ml (0.155 mmole) of nitric acid (d = 1.4) was warmed according to Borrows *et al.* (36) to form green-orange crystals (mp 233–238°). The crystals were recrystallized from acetic acid to give 0.40 g (14%) of compound XI*a* as a dark yellow solid: mp 245–246°. Compound XI*a* appeared as one spot when analyzed by TLC (solvent system C, R_f 0.43). UV (CH_3OH): 220 (log 4.27), 252 (4.04), 300 (3.85), and 355 (4.25) nm; mass spectrum: *m/z* 283 (M+, 100%).

2-(p-Nitrophenyl)indolizine (*XI*j)—Nitric acid (d = 1.4; 0.35 ml, 5.43 mmoles) was added dropwise over a 5-min period to an ice-cold stirred solution of 0.50 g (2.59 mmoles) 2-phenylindolizine (XI*a*) in concentrated sulfuric acid (36). A yellow-brown precipitate (0.40 g) was formed and recrystallized first from acetonitrile, and then acetone–charcoal yielding 0.30 g (50%) 2-(*p*-nitrophenyl)indolizine (XI*b*); mp 236–237°, which appeared as one spot when analyzed by TLC (solvent system B, R_f 0.43). UV (CH_3OH): 240 (log 3.52), 300 (2.93), and 340 (3.15) nm; mass spectrum: *m/z* 238 (M+, 100%).

1-Nitro-2-(p-nitrophenyl)indolizine (*XVII*a)—Nitric acid (d = 1.4; 0.8 ml, 12.4 mmoles) was added dropwise over 5 min to an ice-cold stirred solution containing 2.0 g (8.40 mmoles) of 2-(*p*-nitrophenyl)indolizine (XI*j*) in 2.0 ml of concentrated sulfuric acid (36). A green–yellow precipitate was formed and recrystallized from acetonitrile and then from acetone with charcoal, yielding 0.07 g (3%) of XVII*a* as yellow needles (mp 235–236° dec.). Compound XVII*a* appeared as one spot when analyzed by TLC (solvent system C, R_f 0.43). UV (CH_3OH): 220 (log 4.50), and 275 (4.33) nm; mass spectrum: *m/z* 283 (M+, 100%).

1-Nitro-2-(p-bromophenyl)indolizine (*XVII*b) *and 1,3-Dinitro-2-p-bromophenyl)indolizine* (*XVIII*b)—Nitric acid (d = 1.4; 0.1 ml, 1.55 mmoles) was added dropwise over 5 min to an ice-cold stirred solution containing 0.35 g (1.29 mmoles) 2-(*p*-bromophenyl)indolizine (XI*b*) in 2.0 ml of concentrated sulfuric acid in a 50-ml round-bottom flask equipped with a magnetic stirring bar. The dark red solution was stirred for 38 min at 0°. The mixture was poured into a beaker containing 20 g of crushed ice and yielded 0.30 g of a dark green solid; mp 150–165°. The precipitate was placed in an alumina column, and compound XVIII*b* was eluted using a mixture of 1:1 hexane–methylene chloride as the eluant. The eluate was evaporated under vacuum and the residue was recrystallized from acetone, yielding 0.012 g (3%) of a dark yellow precipitate (mp 153–155° dec.). Compound XVII*b* appeared as one spot when analyzed by TLC (solvent system C, R_f 0.70). UV (CH_3OH): 214 (log 4.23), 260 (4.52), and 286 (4.32) nm; mass spectrum: *m/z* 316 (M+, 95%) and 318 (M + 2, 100%).

Changing the solvent to methylene chloride–hexane (3:1) caused the elution of compound XVIII*b*. The eluate was evaporated under vacuum and yielded 0.05 g (15%) of a light yellow precipitate (mp 265–266° dec.). Compound XVIII*b* appeared as one spot when analyzed by TLC (solvent system C, R_f 0.45). UV (CH_3OH): 210 (log 4.23) and 236 (4.50) nm; mass spectrum: *m/z* 361 (M+, 89%) and 363 (M + 2, 100%).

1-Nitro-2-(m-bromophenyl)indolizine (*XVII*c)—Nitric acid (d = 1.4; 0.175 ml, 2.72 mmoles) was added dropwise over 5 min to an ice-cold stirred solution of 0.75 g (2.76 mmoles) of 2-(*m*-bromophenyl)indolizine (XI*c*) in 10 ml of concentrated sulfuric acid in a 50-ml round-bottom flask equipped with a magnetic stirring bar. The light yellow solution was stirred for 38 min at 0°. The mixture was poured into a beaker containing 20 g of crushed ice and filtered under vacuum using a sintered-filter funnel with medium porosity. The red-yellow precipitate was recrystallized from acetonitrile and yielded 0.12 g (14%) yellow needles (mp 174–175° dec.). Compound XVII*c* appears as one spot when analyzed by TLC (solvent system C, R_f 0.73). UV (CH_3OH): 215 (log 4.30), 242 (4.48), and 295 (4.17) nm; mass spectrum: *m/z* 191 (100%), 316 (M+, 48%), and 318 (M + 2, 52%).

1,3-Dinitro-2-(p-chlorophenyl)indolizine (*XVIII*c)—Nitric acid (d = 1.4; 0.175 ml, 2.72 mmoles) was added dropwise over 10 min to an ice-cold stirred solution containing 0.65 g (2.86 mmoles) of 2-(*p*-chlorophenyl)indolizine (XI*d*) in 10 ml of concentrated sulfuric acid in a 50-ml round-bottom flask equipped with a magnetic stirring bar. The mixture turned light yellow immediately and turned dark red after the addition of the final 0.05 ml of nitric acid.

The solution was stirred for 38 min, keeping the temperature at 0°. The mixture was poured into a sintered-filter funnel containing 20 g of crushed ice. After washing the filter with cold water several times, 0.30 g of a green-yellow precipitate (mp 130–145°) was recovered. The solid was placed in an alumina column and eluted with a mixture of 3:1 methylene chloride–hexane. The eluate was evaporated under vacuum. The green-yellow precipitate was recrystallized from acetonitrile and yielded 0.030 g (3%) yellow crystals (mp 215–216° dec.). Compound XVIII*c* appeared as one spot when analyzed by TLC (solvent system C, R_f 0.43). UV (CH_3OH): 235 (log 4.37) nm; mass spectrum: *m/z* 272 (100%) and 317 (M+, 25.2%).

1-Nitro-2-(m-chlorophenyl)indolizine (*XVII*d)—Nitric acid (d = 1.4; 0.08 ml, 1.24 mmoles) was added dropwise over 5 min to an ice-cold stirred solution containing 0.30 g (1.32 mmoles) 2-(*m*-chlorophenyl)-indolizine (XI*e*) dissolved in 2.0 ml of concentrated sulfuric acid. The light yellow solution was stirred for 38 min at 0°. The mixture was poured in a beaker containing 20 g of crushed ice. A red precipitate formed at once. The suspension was adjusted to pH 10.5 by adding 20% KOH solution. A bright yellow precipitate (0.3 g, mp 150–165° dec.) was recovered. The precipitate was recrystallized from acetonitrile and yielded 0.07 g (18%) of yellow needles (mp 190–191° dec.). Compound XVII*d* appeared as one spot when analyzed by TLC (solvent system B, R_f 0.49). UV (CH_3OH): 215 (log 4.29), 242 (4.46), and 295 (4.15) nm; mass spectrum: *m/z* 227 (100%) and 272 (M+, 25%).

1,3-Dinitro-2-(p-methoxyphenyl)indolizine (*XVIII*d)—Nitric acid (d = 1.4; 0.1 ml, 1.55 mmoles) was added dropwise over 5 min to an ice-cold stirred solution containing 0.33 g (1.48 mmoles) of 2-(*p*-methoxyphenyl)indolizine (XI*f*) dissolved in 2.0 ml of concentrated sulfuric acid in a 50-ml round-bottom flask equipped with a magnetic stirring bar. The reddish-brown mixture was stirred for 38 min at 0°. The solution was poured into a beaker containing 20 g of crushed ice and basified to pH

10.5 by adding 20% KOH solution. A yellow precipitate (0.40 g, mp 160–165°) was recovered. The precipitate was placed in an alumina oxide column and eluted with a mixture of methylene chloride–hexane (3:1). The eluate was evaporated under vacuum apparatus and yielded 0.010 g (2.2%) yellow crystals (mp 268–269°). Compound XVIII*d* appeared as one spot when analyzed by TLC (solvent system C, R_f 0.43). UV (CH_3OH): 210 (log 3.43) and 234 (3.72) nm; mass spectrum: *m/z* 313 (M+, 100%).

1,3-Dinitro-2-(m-*methoxyphenyl*)*indolizine* (*XVIIIe*)—Nitric acid (*d* = 1.4; 0.1 ml, 1.55 mmoles) was added dropwise over 5 min to an ice-cold stirred solution containing 0.33 g (1.4 mmoles) 2-(*m*-methoxyphenyl)indolizine (XI*g*) dissolved in 2.0 ml of concentrated sulfuric acid in a 50-ml round-bottom flask equipped with a magnetic stirring bar. The orange-yellow solution was stirred for 38 min at 0°. The mixture was poured into a beaker containing 20 g of crushed ice and basified with 20% KOH solution until pH 10.5. The yellow precipitate (0.14 g, mp 130–145° dec.) was recrystallized from acetonitrile and yielded 0.08 g (17%) rust-red needles(mp 183–184° dec.). Compound XI*j* appeared as one spot when analyzed by TLC (solvent system C, R_f 0.66). UV (CH_3OH): 220 (log 4.25), 236 (4.39), 290 (4.07) nm; mass spectrum: *m/z* 69 (100%) and 314 (M+, 95.5%).

1-Nitro-2-(p-*methylphenyl*)*indolizine* (*XVIIe*) *and 1,3-Dinitro-2-*(p-*methylphenyl*)*indolizine* (*XVIIIf*)—Nitric acid (*d* = 1.4; 0.1 ml, 1.55 mmoles) was added dropwise over 5 min to an ice-cold stirring solution containing 0.30 g (1.44 mmoles) 2-(*p*-methylphenyl)indolizine (XI*h*) dissolved in 2.0 ml of concentrated sulfuric acid in a 50-ml round-bottom flask equipped with a magnetic stirring bar. The dark yellow solution was stirred for 38 min at 0°. The mixture was poured into a beaker containing 20 g of crushed ice and adjusted to pH 10.5 by adding 20% 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, mp 130–145° dec.). The precipitate was recrystallized from acetonitrile and then acetone. A greenish-yellow precipitate (0.12 g, mp 160–195°) was recovered. The solid was purified in an alumina oxide column using 1:3 methylene chloride–hexane. The filtrate was evaporated under vacuum. TLC using solvent system C showed two spots: starting material XI*h* (R_f 0.80) and compound XVII*e* (R_f 0.67). The nitroindolizine XVII*e* was separated by preparative TLC with solvent system B. The yellow band (R_f 0.53) was scraped off the plate and extracted with acetone. Yellow crystals were recovered yielding 0.02 g (5%), mp 178–179° dec. UV (CH_3OH): 210 (log 4.08), 255 (4.56), and 280 (4.22) nm; mass spectrum: *m/z* 252 (M+, 100%).

Further elution of the column using 1:1 methylene chloride–hexane followed by a more polar 3:1 mixture yielded 0.05 g (12%) of a bright yellow compound (mp 235–236° dec.). Compound XVIII*f* appeared as one spot when analyzed by TLC (solvent system C, R_f 0.38). UV (CH_3OH): 235 (log 4.40) and 265 (4.04) nm; mass spectrum: *m/z* 297 (M+, 100%).

1-Nitro-2-(m-*methylphenyl*)*indolizine* (*XVIIf*)—Nitric acid (*d* = 1.4; 0.1 ml, 1.55 mmoles) was added dropwise over 5 min to an ice-cold stirred solution containing 0.30 g (1.44 mmoles) of 2-(*m*-methylphenyl)-indolizine (XI*i*) dissolved in 2.0 ml of concentrated sulfuric acid in a 50-ml round-bottom flask containing a magnetic stirring bar. The light yellow mixture was stirred for 38 min at 0° and poured into a beaker containing 20 g of crushed ice. The solution was adjusted to pH 10.5 by adding 20% KOH. The yellow suspension was filtered using a sintered-filter funnel. The yellow precipitate was dried overnight under vacuum. The compound (0.33 g, mp 100–125° dec.) was recrystallized from acetonitrile and then acetone, yielding 0.03 g (mp 160–165° dec.) greenish-yellow crystals. TLC using solvent system C showed two spots; one from the starting material, XI*i* (R_f 0.86) and the other from compound XVII*f* (R_f 0.69). The nitro derivative was purified by preparative TLC using solvent system B. The yellow band (R_f 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 yellow color disappeared. The filtrate was concentrated under vacuum yielding 0.02 g (5%) sparkling yellow crystals (mp 179–180° dec.), which turned light green immediately on contact with air. UV (CH_3OH): 210 (log 4.07), 240 (4.39), 294 (4.06), and 350 (4.07); mass spectrum: *m/z* 252 (M+, 100%).

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Synthesis and Bioevaluation of a Series of Alkyl Ethers of *p*-*N*,*N*-Bis(2-chloroethyl)aminophenol

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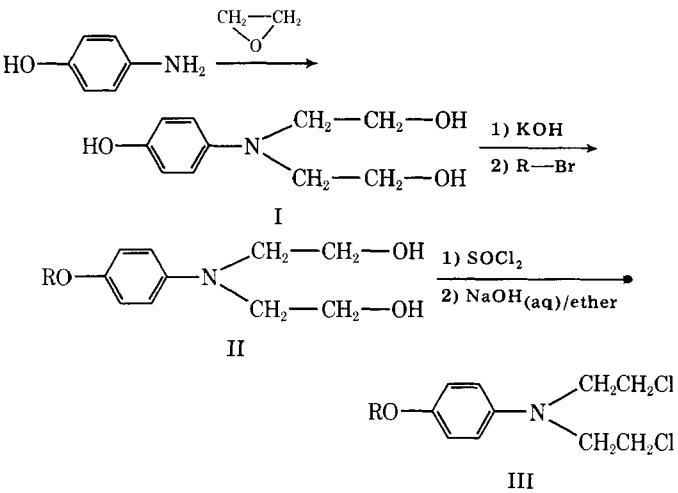
Abstract □ A series of even numbered normal alkyl ethers (C_2–C_{14}) of *p*-*N*,*N*-bis(2-chloroethyl)aminophenol were synthesized and evaluated as to acute toxicity in mice and effects on survival in L-1210 leukemic mice. All of the ether derivatives demonstrated significantly lower acute toxicity than the parent phenol mustard. Significant survival times (≥125%) were obtained with all compounds except the hexyl derivative. The decyl ether produced the greatest significant increase and the ethyl ether the lowest significant increase in mean survival time. Significant survival times were produced at four dosage levels for the butyl, decyl, and dodecyl derivatives, three dosage levels for the octyl and tetradecyl derivatives, and one dosage level for the ethyl derivative.

Keyphrases □ Alkyl ethers—synthesis of alkyl ethers *p*-*N*,*N*-bis(2-chloroethyl)aminophenol □ Bioevaluation—alkyl ethers of *p*-*N*,*N*-bis(2-chloroethyl)aminophenol, toxicity in HA/ICR mice □ Antitumor activity—alkyl ethers of *p*-*N*,*N*-bis(2-chloroethyl)aminophenol, survival in L-1210 leukemic mice

Phenol mustard, *p*-[*N*,*N*-bis(2-chloroethyl)amino]-phenol, has demonstrated a low therapeutic index (1). Less toxic derivatives of phenol mustard have resulted from the synthesis of various esters of the phenol (1–3).



IIa, IIIa: R = CH_3—CH_2—
IIb, IIIb: R = CH_3—$(CH_2)_2$—CH_2—
IIc, IIIc: R = CH_3—$(CH_2)_4$—CH_2—
IId, IIId: R = CH_3—$(CH_2)_6$—CH_2—
IIe, IIIe: R = CH_3—$(CH_2)_8$—CH_2—
IIf, IIIf: R = CH_3—$(CH_2)_{10}$—CH_2—
IIg, IIIg: R = CH_3—$(CH_2)_{12}$—CH_2—

Scheme I

In an effort to develop latent derivatives of phenol mustard, a series of its substituted benzoate esters were studied (1). The results lent support to the hypothesis that hydrolysis of esters of *p*-[*N*,*N*-bis(2-chloroethyl)amino]-phenol to the free phenol mustard is a necessary step for antitumor activity (1). The effectiveness of aniline mustard, *N*,*N*-bis(2-chloroethyl)aniline, in the treatment of advanced plasma cell tumors has been demonstrated (4). The results indicated that aniline mustard was the optimally active compound in this system, and only those compounds that could be metabolized to a phenolic mustard demonstrated activity.

A series of alkyl ethers of phenol mustard should provide potentially latent nitrogen mustard derivatives with variable metabolic routes (5, 6). Such compounds would be expected to demonstrate antineoplastic activity with a reduction in host toxicity. In addition, lipophilicity would increase with the length of the alkyl chain. The specific objectives of this investigation were to: (*a*) synthesize a series of even-numbered, normal alkyl ethers (C_2–C_{14}) of *p*-[*N*,*N*-bis(2-chloroethyl)amino]phenol, (*b*) determine the acute toxicity as measured by the LD_{50} for each compound studied, and (*c*) determine the effect of each compound on the prolongation of life of L-1210 leukemic mice.

The compounds evaluated in this project were synthesized using Scheme I.

EXPERIMENTAL

Chemistry[1]—p-N,N-*Bis(2-hydroxyethyl)aminophenol* (*I*)—Twenty grams (0.18 mole) of *p*-aminophenol was added to a flask containing 200 ml of absolute methanol and equipped with a reflux condenser. The solution was stirred mechanically until dissolution occurred and then was cooled to 0°. Twenty grams (0.45 mole) of ethylene oxide was added to the cold reaction mixture. Stirring was continued and the reaction was allowed to reach room temperature. The resulting crystals were filtered and recrystallized from ethanol. The product had a melting point of 139.5–141° compared with the reported value of 140° (7).

[1] All IR spectral data were obtained from chloroform solutions of the derivatives on sodium chloride plates using a Beckman Model Acculab-4 spectrophotometer. Nuclear magnetic resonance spectra were obtained from deuterated chloroform solutions of the derivatives using a Hitachi-Perkin-Elmer model R-24 high resolution spectrometer with tetramethylsilane as the internal standard. The reported melting points were obtained using a Thomas Hoover capillary melting point apparatus and are uncorrected. The reported content of hydrogen, carbon, and nitrogen were obtained from analyses performed by Galbraith Laboratories, Knoxville, Tenn. All lyophilization was accomplished using a VirTis Model 10 freeze-dryer.

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