Synthesis and Reactions of Heterocyclic 2,3-Epoxypropionitriles with Pyrrolidine

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The Darzen's reaction of 2-(3-)pyridinecarboxaldehydes **5** with chloroacetonitrile yielded a mixture of stereoisomers *cis*-**6** and *trans*-3-(pyridinyl)-2,3-epoxypropionitriles **7** in a ratio of approximately 1:1. Oxidation of *cis*-**6** and *trans*-**7** afforded the corresponding *cis*-**8** and *trans*-3-(1-oxidopyridinyl)-2,3-epoxypropionitriles **9** in good yield. The reaction of **8a** and **9a** with pyrrolidine at 25° gave the respective *threo*-**10** and *erthyro*-2-(1-pyrrolidino)-3-hydroxy-3-(1-oxido-2-pyridinyl)propionitrile (**11**). A number of selected compounds (**7-9a-b**) were found to be inactive in the P388 Lymphocyctic screen.

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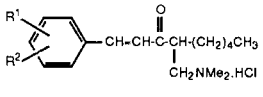
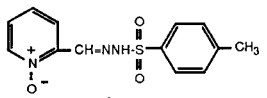
There has recently been a considerable degree of pharmacological interest in Mannich bases **1** [1] and arylsulfonylhydrazones of 2-formylpyridine *N*-oxide **2** [2] as antineoplastic agents. The antineoplastic activity of **2** has been attributed to the potent alkylating species 1-oxidopyridin-2-yldiazomethane **3** resulting from intramolecular abstraction of the nitrogen proton by the *N*-oxide group followed by release of the arylsulfinic acid [3]. It has been reported that the β-carbon of α,β-epoxy sulfoxides **4** is highly reactive towards nucleophiles yielding dialkyl ketones or aldehydes in high yields under mild conditions [4]. In an earlier report, we described the synthesis of 1-[1-oxido-2-(3-,4-)pyridinyl]-2-methyloxiranes and their reactions with sulfur, oxygen and nitrogen nucleophiles [5]. It was therefore of interest to extend this study to include activated oxiranes, which may react with cellular thiols, for evaluation as antitumor agents. We now describe the synthesis of some 3-(pyridinyl)-2,3-epoxypropionitriles and their reaction with the nitrogen nucleophile pyrrolidine.



1, $R^1 = R^2 = H$, Cl



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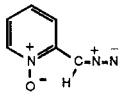

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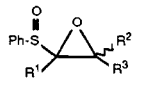


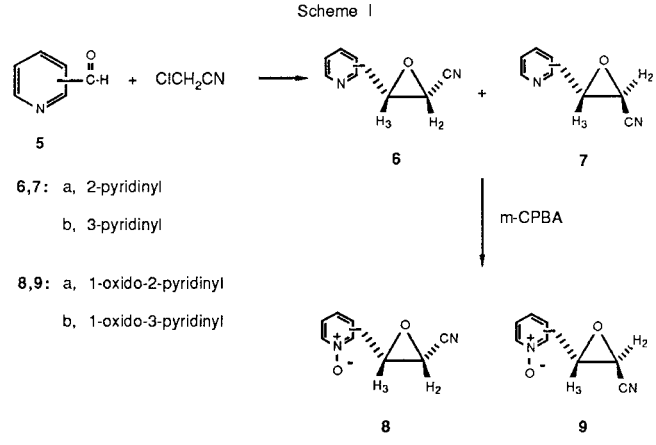
4, R^1 = alkyl, arylalkyl

R^2 = alkyl, aryl

R^3 = hydrogen, methyl

The Darzen's reaction of 2-pyridinecarboxaldehyde (**5a**) with chloroacetonitrile in the presence of potassium *t*-butoxide in *t*-butyl alcohol afforded a mixture of stereoisomers *cis*-**6a** (23%) and *trans*-3-(2-pyridinyl)-2,3-epoxypropionitrile (**7a**, 27%) (Scheme I). A similar reaction of **5b** yielded *cis*-**6b** (17%) and *trans*-**7b** (20%). Oxidation of the 2,3-epoxypropionitriles **6** and **7** with *m*-chloroperbenzoic acid gave the respective *cis*-**8** and *trans*-**9** *N*-oxide analogs which were routinely purified by elution from a neutral alumina column to remove excess *m*-chloroperbenzoic acid and *m*-chorobenzoic acid.

Scheme I



5 **6** **7**

6,7: a, 2-pyridinyl

b, 3-pyridinyl

8,9: a, 1-oxido-2-pyridinyl

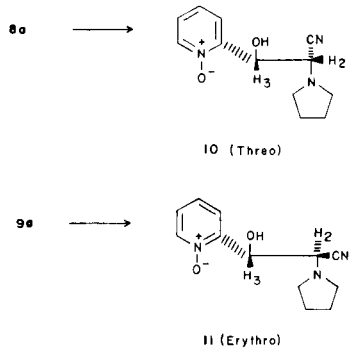
b, 1-oxido-3-pyridinyl

8 **9**

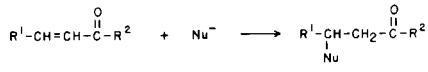
The stereospecific reaction of *cis*-**8a** and *trans*-3-(1-oxido-2-pyridinyl)-2,3-epoxypropionitrile (**9a**) with nucleophiles as amines afforded the respective *threo* (2*R*,3*R*/2*S*,3*S*) and *erthyro* (2*R*,3*S*/2*S*,3*R*) β-aminoalcohol diastereoisomers [5,6] (Scheme II). Thus reaction of *cis*-**8a** with pyrrolidine at 25° gave *threo*-2-(1-pyrrolidino)-3-hydroxy-3-(1-oxido-2-pyridinyl)propionitrile (**10**, 86%) whereas reaction of *trans*-**9a** gave rise to *erthyro*-**11** (82%). The amination reaction was regiospecific since no product arising from attack by pyrrolidine at C-3 of **8a** or **9a** was detected [5].

The antineoplastic activity of compounds containing the α,β-unsaturated structural moiety has been attributed to their reaction with cellular nucleophiles [7-9]. Nucleophilic attack by anions on α,β-unsaturated carbonyl compounds is known as the Michael reaction and has been studied extensively primarily due to its synthetic usefulness [1]. It was expected that oxiranes having elec-

Scheme II



tron attracting cyano and pyridinyl (**6-7**) or 1-oxidopyridinyl (**8-9**) substituents would be highly activated toward



attack by cellular nucleophiles. On this basis, activated oxiranes may be useful antineoplastic agents. Although the activated oxiranes *cis*-**8a** and *trans*-**9a** reacted rapidly with pyrrolidine at 25° to yield *threo*-**10** and *erthyro*-**11** respectively, the 2,3-epoxypropionitriles **6-9** were inactive in the P388 Lymphocyctic Leukemia screen. The lack of tumor inhibiting activity for activated oxiranes **6-9** is likely due to their inability to act as biological alkylating agents.

EXPERIMENTAL

Melting points were determined with a Büchi capillary apparatus and are uncorrected. Nuclear magnetic resonance spectra were determined in deuteriochloroform (unless otherwise noted) with TMS as internal standard with a Varian EM-360A spectrometer. Infrared spectra (potassium bromide unless otherwise noted) were taken on a Unicam SP-1000 or Perkin-Elmer 267 spectrometer. Mass spectra were measured with an AEI-MS-12 mass spectrometer. Preparative high pressure liquid chromatography was performed using a Water's Prep LC/System 500A using Prep Pak-500 silica cartridges.

Cis-3-(2-Pyridinyl)-2,3-epoxypropionitrile (**6a**) and *Trans*-3-(2-Pyridinyl)-2,3-epoxypropionitrile (**7a**). Procedure A.

A solution of potassium *t*-butoxide, prepared by dissolution of potassium (3.0 g) in dry *t*-butyl alcohol (75 ml), was added dropwise during 90 minutes to a solution of 2-pyridinecarboxaldehyde (**5a**, 8.02 g, 75 mmoles) and chloroacetonitrile (5.62 g, 75 mmoles) with stirring and cooling at such a rate that the reaction temperature did not exceed 10°, under a nitrogen atmosphere. The reaction was allowed to proceed for an additional 1 hour, at which time tlc indicated the absence of **5a**. The *t*-butyl alcohol was removed *in vacuo* and 75 ml water was added to the residue. Extraction with ether (4 x 50 ml), drying (sodium sulfate) and removal of the solvent *in vacuo* gave a solid (6.5 g) which was partially purified by elution from a 5 x 30 cm silica gel column with ether as eluant to afford a mixture (6 g) of **6a** and **7a**. Preparative hplc using hexane-ether (1:9 v/v) as eluant at a flow rate of 250 ml min^{-1} gave **7a** (3 g, 27%) in the 1100-1500 ml fraction and **6a** (2.5 g, 23%) in the 1700-2500 ml fraction. Stereoisomer **6a** (oil) had ir (neat): 2280 (CN), 1225 (oxirane) cm^{-1}; ^1H nmr: δ 3.94 (d, $J_{2,3}$ = 4 Hz, 1H, H_2), 4.2 (d, $J_{2,3}$ = 4 Hz, 1H, H_3), 7.25-7.65 (m, 2H, pyridinyl H_3, H_5), 7.8 (d, $J_{4,5}$ = 8.5 Hz of d, $J_{3,4}$ = 7 Hz of d, $J_{4,6}$ = 2 Hz, 1H, H_4), 8.68 (d, $J_{5,6}$ = 5 Hz of d, $J_{4,6}$ = 2 Hz, 1H, H_6). Stereoisomer **7a** had mp 54°; ir: 2240 (CN), 1200 (oxirane) cm^{-1}: ^1H nmr: δ 3.92 (d, $J_{2,3}$ = 2 Hz, 1H, H_2), 4.4 (d, $J_{2,3}$ = 2 Hz, 1H, H_3), 7.1-7.45 (m, 2H, H_3, H_5), 7.76 (d, $J_{4,5}$ = 8.5 Hz of d, $J_{3,4}$ = 7 Hz of d, $J_{4,6}$ = 2 Hz, 1H, H_4), 8.6 (d, $J_{5,6}$ = 5 Hz of d, $J_{4,6}$ = 2 Hz, 1H, H_6).

Anal. Calcd. for $C_8H_6N_2O$: C, 65.75; H, 4.10; N, 19.17. Found: C, 65.51; H, 4.30; N, 18.99.

Cis-3-(Pyridinyl)-2,3-epoxypropionitrile (**6a**) and *Trans*-3-(3-Pyridinyl)-2,3-epoxypropionitrile (**7b**).

Reaction of 3-pyridinecarboxaldehyde (**5b**, 10.7 g, 100 mmoles) with chloroacetonitrile (7.0 g, 100 mmoles) in the presence of potassium *t*-butoxide (11.2 g, 100 mmoles) as described in Procedure A afforded a mixture of **6b** and **7b** (3.25 g). Preparative hplc using hexane-ether (3:20 v/v) as eluant at a flow rate of 250 ml min^{-1} afforded **7b** (2.9 g, 20%) in the 1200-1550 ml fraction and **6b** (2.5 g, 17%) in the 1800-2500 ml fraction. Stereoisomer **6b** (oil) had ir (neat): 2180 (CN), 1230 (oxirane) cm^{-1}; ^1H nmr: δ 3.98 (d, $J_{2,3}$ = 4 Hz, 1H, H_2), 4.4 (d, $J_{2,3}$ = 4 Hz, 1H, H_3), 7.5 (d, $J_{4,5}$ = 8.5 Hz of d, $J_{5,6}$ = 5 Hz, 1H, H_5), 7.84 (d, $J_{4,5}$ = 8.5 Hz of d, $J_{4,6}$ = 2 Hz of d, $J_{2,4}$ = 2 Hz, 1H, H_4), 8.76 (d, $J_{5,6}$ = 5 Hz, of d, $J_{4,6}$ = 2 Hz, 1H, H_6), 8.94 (d, $J_{2,4}$ = 2 Hz, 1H, H_2).

Anal. Calcd. for $C_8H_6N_2O$: C, 65.75; H, 4.10; N, 19.17. Found: C, 65.89; H, 4.22; N, 19.17.

Stereoisomer **7b** had mp 69°; ir: 2180 (CN), 1230 (oxirane) cm^{-1}; ^1H nmr: δ 3.58 (d, $J_{2,3}$ = 2 Hz, 1H, H_2), 4.43 (d, $J_{2,3}$ = 2 Hz, 1H, H_3), 7.4 (d, $J_{4,5}$ = 8.5 Hz of d, $J_{5,6}$ = 5 Hz, 1H, H_5), 7.65 (d, $J_{4,5}$ = 8.5 Hz of d, $J_{2,4}$ = 2 Hz, 1H, H_4), 8.72 (d, $J_{2,4}$ = 2 Hz, 1H, H_2), 8.76 (d, $J_{5,6}$ = 5 Hz of d, $J_{4,6}$ = 2 Hz, 1H, H_6).

Cis-3-(1-Oxido-2-pyridinyl)-2,3-epoxypropionitrile (**8a**). Procedure B.

A solution of *m*-chloroperbenzoic acid (2.76 g of 85%, 137 mmoles) in methylene choride was added dropwise to a solution of **6a** (1.82 g, 125 mmoles) in methylene chloride (15 ml) at 0° with stirring. The reaction mixture was stirred at 0° for 30 minutes, 1 hour at 25° followed by heating at reflux for 24 hours. The volume was reduced by 50% and the reaction mixture was cooled and filtered. Removal of the solvent from the filtrate gave a residue which was purified by elution from a neutral alumina column using methanol-chloroform (1:19 v/v) as eluant. The product (1.9 g) was recrystallized from chloroform-ether (1:4 v/v) to yield **8a** (1.7 g, 85%), mp 114°; ir: 2250 (CN), 1260 (*N*-oxide), 1230 (oxirane) cm^{-1}; ^1H nmr: δ 4.1 (d, $J_{2,3}$ = 4 Hz, 1H, H_2), 4.95 (d, $J_{2,3}$ = 4 Hz, 1H, H_3), 7.25-7.62 (m, 3H, 1-oxido-2-pyridinyl H_3, H_4, H_5), 8.4 (m, 1H, 1-oxido-2-pyridinyl H_6).

Anal. Calcd. for $C_8H_6N_2O_2$: C, 59.25; H, 3.70; N, 17.28. Found: C, 59.09; H, 3.74; N, 17.46.

Trans-3-(1-Oxido-2-pyridinyl)-2,3-epoxypropionitrile (**9a**).

Oxidation of **7a** (1.46 g, 100 mmoles) with *m*-chloroperbenzoic acid (2.77 g of 85%, 110 mmoles) as described by Procedure B afforded **9a** (1.25 g, 77%), mp 153°; ir: 2260 (CN), 1260 (*N*-oxide), 1210 (oxirane) cm^{-1}; ^1H nmr: δ 3.55 (d, $J_{2,3}$ = 2 Hz, 1H, H_2), 4.98 (d, $J_{2,3}$ = 2 Hz, 1H, H_3), 7.2-7.6 (m, 3H, 1-oxido-2-pyridinyl H_3, H_4, H_5), 8.28-8.52 (m, 1H, 1-oxido-2-pyridinyl H_6).

Anal. Calcd. for $C_8H_6N_2O_2$: C, 59.25; N, 3.70; N, 17.28. Found: C, 58.88; H, 3.76; N, 17.12.

Cis-3-(1-Oxido-3-pyridinyl)-2,3-epoxypropionitrile (**8b**).

Oxidation of **6b** (1.46 g, 100 mmoles) with *m*-chloroperbenzoic acid (2.22 g of 85%, 110 mmoles) as described by Procedure B afforded **8b** (1.2 g, 74%), mp 125°; ^1H nmr: δ 3.92 (d, $J_{2,3}$ = 4 Hz, 1H, H_2), 4.3 (d, $J_{2,3}$ = 4 Hz, 1H, H_3), 7.3-7.5 (m, 2H, 1-oxido-3-pyridinyl H_4, H_5), 8.15-8.5 (m, 2H, 1-oxido-3-pyridinyl H_2, H_6).

Anal. Calcd. for $C_8H_6N_2O_2$: C, 59.25; H, 3.70; N, 17.28. Found: 58.89; H, 3.86; N, 17.16.

Trans-3-(1-Oxido-3-pyridinyl)-2,3-epoxypropionitrile (**9b**).

Reaction of **7b** (1.09 g, 75 mmoles) with *m*-chloroperbenzoic acid (1.65 g of 85%, 82 mmoles) as described by Procedure B yielded **9b** (0.7 g, 58%), mp 118°; ir: 2250 (CN), 1280 (*N*-oxide) cm^{-1}; ^1H nmr (perdeuteriomethanol): δ 3.72 (d, $J_{2,3}$ = 2 Hz, 1H, H_2), 4.38 (d, $J_{2,3}$ = 2 Hz, 1H, H_3), 7.2-7.36 (m, 2H, 1-oxido-3-pyridinyl H_4, H_5), 7.93-8.15 (m, 2H, 1-oxido-3-pyridinyl H_2, H_6).

Anal. Calcd. for $C_8H_6N_2O_2$: C, 59.25; H, 3.70; N, 17.28. Found: C, 58.92; H, 3.84; N, 16.88.

Threo-2-(1-Pyrrolidino)-3-hydroxy-3-(1-oxido-2-pyridinyl)propionitrile (**10**).

Reaction of *cis*-**8a** (0.324 g, 2 mmoles) with pyrrolidine (0.426 g, 6 mmoles) at 25° for 15 minutes and removal of excess pyrrolidine *in vacuo* gave a semi-solid. Recrystallization from acetone gave **10** (0.40 g, 86%), mp 138°; ir: 2260 (CN), 1255 (*N*-oxide) cm^{-1}; ^1H nmr (deuteriochloroform + DMSO-d_6): δ 1.45-2.0 (m, 4H, pyrrolidino H_3, H_4), 2.5-3.0 (m, 4H, pyrrolidino H_2, H_5), 5.0 (d, $J_{2,3}$ = 6 Hz, 1H, H_2), 5.4 (d, $J_{2,3}$ = 6 Hz, 1H, H_3), 6.3-6.7 (br s, 1H, OH, exchanges with deuterium oxide), 7.3-7.9 (m, 3H, 1-oxido-2-pyridinyl H_3, H_4, H_5), 8.32 (d, $J_{5,6}$ = 6 Hz of d, $J_{4,6}$ = 2 Hz, 1H, 1-oxido-2-pyridinyl H_6).

Anal. Calcd. for $C_{12}H_{15}N_3O_2$: C, 61.80; H, 6.43; N, 18.02. Found: C, 61.80; H, 6.43; N, 18.07.

Erythro-2-(1-Pyrrolidino)-3-hydroxy-3-(1-oxido-2-pyridinyl)propionitrile (**11**).

Reaction of **9a** (0.324 g, 2 mmoles) with pyrrolidine (0.426 g, 6 mmoles) at 25° for 15 minutes and removal of the excess pyrrolidine *in vacuo* gave a semi-solid which on recrystallation from acetone gave **11** (0.38 g, 82%, mp 142°; ir: 2265 (CN), 1255 (*N*-oxide) cm^{-1}; ^1H nmr (deuteriochloroform + DMSO-d_6): δ 1.64-1.95 (m, 4H, pyrrolidino H_3, H_4), 2.65-3.0 (m, 4H, pyrrolidino H_2, H_5), 4.54 (d, $J_{2,3}$ = 4 Hz, 1H, H_2), 5.4 (d, $J_{2,3}$ = 4 Hz, 1H, H_3), 6.5 (br s, 1H, OH, exchanges with deuterium oxide), 7.3-7.85 (m, 3H, 1-oxido-2-pyridinyl H_3, H_4, H_5), 8.3 (d, $J_{5,6}$ = 6 Hz of d, $J_{4,6}$ = 2 Hz, 1H, 1-oxido-2-pyridinyl H_6).

Anal. Calcd. for $C_{12}H_{15}N_3O_2$: C, 61.80; H, 6.43; N, 18.02. Found: C, 61.67; H, 6.40; N, 17.98.

Antitumor Screening.

The screening was performed by the Drug Evaluation Branch of the NCI using the P388 Lymphocytic screen. A once daily maximum nontoxic dose (12.5-400 mg/kg range) in saline was administered by ip injection to mice for a total of nine doses. The % T/C (% T/C = treated/control x 100) was calculated for the different doses administered. Compounds **7a** (12.5 mg/kg), **7b** (400 mg/kg), **8a** (25 mg/kg), **8b** (50 mg/kg), **9a** (12.5 mg/kg) and **9b** (125 mg/kg) were considered to be inactive since the % T/C remained close to 100 for all compounds tested.

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