Synthesis and Characterization of Some 3-Nitroso-2-arylimidazo(1,2-*a*)pyrimidines

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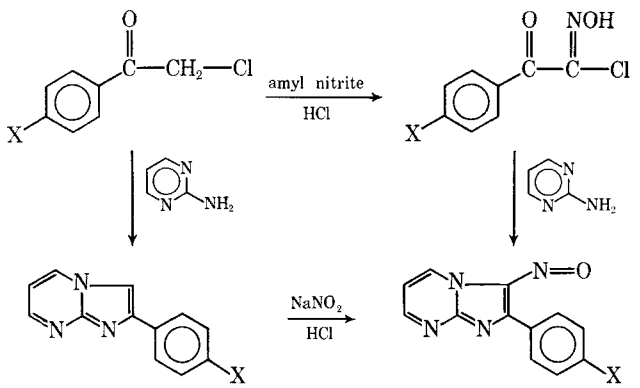
Abstract □ While attempting to prepare amides derived from 2-aminopyrimidine and arylglyoxylohydroxamyl chlorides, deeply colored, highly insoluble products were obtained. Both IR and NMR data proved that the desired amides had not been obtained. This manuscript details the information used to identify the products as 3-nitroso-2-arylimidazo(1,2-*a*)pyrimidines.

Keyphrases □ 3-Nitroso-2-arylimidazo(1,2-*a*)pyrimidines—synthesis □ Antimicrobial activity—3-nitroso-2-arylimidazo(1,2-*a*)-pyrimidines □ NMR spectroscopy—structure □ IR spectrophotometry—structure

While attempting to condense arylglyoxylohydroxamyl chlorides with 2-aminopyrimidine to obtain the corresponding *N*-(2-pyrimidinyl)arylglyoxylohydroxamamides, nearly insoluble, highly colored compounds were obtained instead which, according to data detailed in this report, were identified as 3-nitroso-2-arylimidazo(1,2-*a*)pyrimidines.

The route for the synthesis of the title compounds and an alternative route involving direct nitrosation of 2-phenylimidazo(1,2-*a*)pyrimidine are given in Scheme I.



Scheme I

EXPERIMENTAL[1]

The arylglyoxylohydroxamyl chlorides required for this project were synthesized according to previously described procedures (1, 2). The α-chloroketones, which served as starting materials, were obtained from Distillation Products Industries as was 2-aminopyrimidine. All melting points were taken on a Thomas–Hoover melting-point apparatus and are uncorrected.

Synthesis of 3-Nitroso-2-phenylimidazo(1,2-*a*)pyrimidine (Compound 1, Table I)—*Method A: Ring Closure*—Phenylglyoxylohydroxamyl chloride (1.8 g., 0.01 mole) was dissolved in 50 ml. of anhydrous ether and treated with 1.9 g. (0.02 mole) of 2-aminopyrimidine. Since the aminopyrimidine is only sparingly soluble in dry ether, it was added in small portions with stirring. The reaction progressed slowly and, when all the amine had been added, the flask was stoppered tightly and stirred continuously at room temperature for 2 days. The product and amine hydrochloride coprecipitated as a chalky green solid. After filtration, the solid was washed with ether to remove unreacted starting materials and then with water to remove the by-product amine hydrochloride salt. The crude product was recrystallized twice from boiling isopropyl alcohol (175 ml./g. of crude product) and gave 1.17 g. (52.1% yield) of fine, emerald-green needles melting at 223.5–224.5°.

Anal.—Calcd. for $C_{12}H_8N_4O$: C, 64.27; H, 3.59; N, 24.99. Found: C, 64.20; H, 3.70; N, 24.59.

NMR and IR spectral data are given and discussed in context with structural proof in the *Discussion* section.

Method B: Nitrosation of 2-Phenylimidazo(1,2-a)pyrimidine—1. *Synthesis of 2-Phenylimidazo(1,2-a)pyrimidine (Compound 5, Table I)*—Preparation of this intermediate was accomplished by a modification of the procedures of Buu-Hoi and Xuong (3) and Almirante *et al.* (4).

Thus, phenacyl chloride (2-chloroacetophenone) (0.05 mole, 773 g.) and 2-aminopyrimidine (0.05 mole, 476 g.) in 50 ml. of absolute ethanol in a round-bottom flask was heated at 60° with stirring (magnetic stirring apparatus) for 3 hr.,[2] followed by 18 hr. of stirring at room temperature. The reaction mixture was chilled and filtered. The resultant salt was recrystallized once from hot 10% hydrochloric acid solution, collected on a sintered-glass funnel, suspended in 100 ml. of water, and neutralized with 10% sodium hydroxide. The free base was then extracted repeatedly with 50-ml. portions of chloroform[3] until no further base was obtained. The combined chloroform extracts were freed from chloroform in a rotary evaporator, and the solid base recrystallized (charcoal treatment) from isopropyl alcohol. Yields varied from about 40 to 60% theory on repeated runs. The product appeared as broad, flat needles melting at 200.5–201.5° [lit. (3) m.p. 202°].

2. *Nitrosation of 2-Phenylimidazo(1,2-a)pyrimidine*—Nitrosation of 2-phenylimidazo(1,2-*a*)pyrimidine has not previously been reported. Since procedures have been reported for nitrosation of 2-phenylimidazo(1,2-*a*)pyridine (5–7) and 2-phenylindolizine (2-phenylpyrrocoline) (8, 9), the probability of success was considered favorable. Although some difficulties were encountered,[4] fine emerald-green needles were obtained according to the following procedure, a modification of the procedure of Pentimalli and Bozzini (7).

Thus, 1.54 g. (0.0075 mole) of 2-phenylimidazo(1,2-*a*)pyrimidine was warmed with 6 ml. of glacial acetic acid until dissolved. Then, 9 ml. of distilled water was added, the solution was quickly cooled to about 5°, and 0.75 g. (0.19 mole) of sodium nitrite was added portionwise with cooling and stirring. The formation of a green coloration followed by precipitation of a green solid rapidly ensued.[4] After 24 hr. of periodic stirring, 1.6 g. (95.2% of theory) of crude, green product was obtained by filtration. Recrystallization once

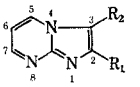
[1] Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn.

[2] The red coloration which resulted during the heating period proved to be very difficult to remove except by direct charcoal treatment of the hydrochloride salt.

[3] Due to the poor solubility, a large quantity of chloroform is required. The procedure is not satisfactory for obtaining sizable quantities of this compound but it is more satisfactory than other procedures (3, 4) for obtaining a pure product. Direct recrystallization of the free base from isopropyl alcohol also proved superior to literature procedures.

[4] Neutralization of the reaction mixture with base, as would be customary when aqueous hydrochloric acid serves as the reaction solvent, resulted in loss of the nitroso group in early experiments. The use of aqueous acetic acid as the reaction solvent circumvents the necessity of adding base prior to filtration.

Table I—NMR Data of H-3, H-5, H-6, H-7, and Aromatic Protons [2-Aryl-3-nitrosoimidazo(1,2-*a*)pyrimidines and 2-Phenylimidazo(1,2-*a*)pyrimidine][a-c]



Compound No.	R_1	R_2	Chemical Shift, p.p.m. H-3	H-5	H-6	H-7	Aromatic[c]		Coupling Constant, Hz. $H_{5,6}$	$H_{5,7}$	$H_{6,7}$
1	Phenyl	−N = O	—	9.98	7.28	8.92	7.6(3)	8.8(2)	6.9	2.1	4.3
2	4-Methoxyphenyl[d]	−N = O	—	(10.0)	(7.5)	(9.1)	(7.0(2))	(8.8(2))	(6.5)	(2.0)	(4.5)
3	4-Chlorophenyl	−N = O	—	9.96	7.28	8.93	7.5(2)	8.7(2)	6.5	2.5	4.5
4	4-Methylphenyl	−N = O	—	10.01	7.3[e]	8.90	7.4(2)	8.7(2)	7.0	2.0	4.0
5	Phenyl	−H	8.41	9.1	7.07	8.59	7.5(3)	8.3(2)	6.8	2.0	4.2

[a] Similar spectra have been reported (12, 13). [b] Solutions were in $CDCl_3$ (saturated) and generally were less than 1%; Compounds 2 and 5 were run in DMSO-d_6 (saturated). TMS served as the reference compound. [c] Aromatic protons for these compounds appeared as two multiplets. These data are given in the table with the number of protons, in parentheses, immediately following. [d] Resolution and sensitivity were inadequate, due to low concentration, for more than rough approximation of chemical shifts and coupling constants. For this compound in saturated solution, resolution required settings of 1000 sec. sweep time, sensitivity of 400, time constant of 1 sec., and power of 5×10^3. [e] A portion of the aromatic proton multiplet overlapped this pattern. The H-6 multiplet, in part, appeared as shoulders on the aromatic proton pattern.

from isopropyl alcohol gave 1.25 g. (74.4% of theory) of fine emerald-green needles melting at 223.5–224.5°. The IR and NMR spectra were identical in every regard to those obtained for the same compound prepared by reaction of phenylglyoxylohydroxamyl chloride with 2-aminopyrimidine.

Synthesis of 3-Nitroso-2-(4-methoxyphenyl)imidazo(1,2-*a*)pyrimidine (Compound 2, Table I)—4-Methoxyphenylglyoxylohydroxamyl chloride (2.0 g., 0.01 mole), prepared by previously described procedures (1, 2), was dissolved in 50 ml. of anhydrous ether and treated with 0.95 g. (0.01 mole) of 2-aminopyrimidine according to the previously described procedure (Method A). Thus, fine green crystals, melting at 247–248°, were obtained.

Anal.—Calcd. for $C_{13}H_{10}N_4O_2$: C, 61.40; H, 3.96; N, 22.04. Found: C, 60.78, 60.85; H, 4.22, 4.18; N, 21.87, 21.60.

Spectral data are summarized in the *Discussion* section.

Synthesis of 3-Nitroso-2-(4-chlorophenyl)imidazo(1,2-*a*)pyrimidine (Compound 3, Table I)—In a similar manner, 3.6 g. (0.02 mole) of 4-chlorophenylglyoxylohydroxamyl chloride and 1.90 g. (0.02 mole) of 2-aminopyrimidine gave 2.0 g. (41.7% of theory) of a green compound melting at 228°.

Anal.—Calcd. for $C_{12}H_7ClN_4O$: C, 55.71; H, 2.72; N, 21.66. Found: C, 56.32, 56.19; H, 2.90, 3.03; N, 21.63, 21.48.

Spectral data are given in the *Discussion* section.

Synthesis of 3-Nitroso-2-(4-methylphenyl)imidazo(1,2-*a*)pyrimidine (Compound 4, Table I)—In a similar manner, 3.58 g. (0.02 mole) of 4-methylphenylglyoxylohydroxyamyl chloride and 1.90 g. (0.02 mole) of 2-aminopyrimidine gave 1.5 g. (31.3% of theory) of green crystals melting at 253–254°.

Anal.—Calcd. for $C_{13}H_{10}N_4O$: C, 65.53; H, 4.23; N, 23.52. Found: C, 65.27, 65.08; H, 4.37; 4.17; N, 23.29, 23.48.

Spectral data are summarized in the *Discussion* section.

DISCUSSION

LaRocca and Gibson (10) reported the conversion of arylglyoxylohydroxamyl chlorides and *N,N*-bis-(2-chloroethyl)amine into the corresponding *N,N*-bis-(2-chloroethyl)arylglyoxylohydroxamamides. The present project was begun as an attempt to prepare the similar *N*-2-pyrimidinylarylglyoxylohydroxamamides from a condensation of arylglyoxylohydroxamyl chlorides with 2-aminopyrimidine. Instead of the expected products, highly colored, difficultly soluble products were obtained. The deep color and poor solubility in most solvents suggested the possible presence of a nitroso compound.

For example, the phenyl analog was prepared as described in Method A and was found to be a chalky green, amorphous powder upon initial recovery from the reaction mixture. After washing with water and recrystallizing the residue to constant melting point (185–189°) from toluene, the compound gave 23.13 and 23.50% *N* (semimicro Kjeldahl procedure) compared to a calculated value of 23.13% for the "amide," $C_{12}H_{10}N_4O_2$. On attempting to dissolve a sample of this product in deuterochloroform for NMR studies, however, the presence of two compounds was clearly indicated. TLC studies further verified this observation. A complete study by TLC further suggested that effective separation of the relatively abundant green compound from its colorless contaminant might be effected from 1- or 2-propanol, the only solvents in which the contaminant was more soluble (larger R_f value) than the principal product.

Recrystallization of a 1.0-g. sample of the crude, water-washed product required approximately 175 ml. of 2-propanol. After two recrystallizations, a pure product was obtained which amounted to 52.1% of theory and, based on recovery percentages, constituted about 80% by weight of the original, crude product. Similarly, about 20% of the original sample appeared to be composed of the white solid.

The IR spectra[5] of the crude product, when compared with that of the purified green compound, revealed that the impurity possessed a prominent amide carbonyl band at 1695 cm.$^{-1}$ and a strong C=N stretch frequency at 1655 cm.$^{-1}$. It cannot be stated that this colorless impurity is, indeed, the expected *N*-2-pyrimidinylphenylglyoxylohydroxamamide, since complete purification and characterization were not effected (11).

The purified principal product of this reaction was identified as 3-nitroso-2-phenylimidazo(1,2-*a*)pyrimidine in the following way. 2-Phenylimidazo(1,2-*a*)pyrimidine, whose synthesis previously was described (3), was prepared and nitrosated as described using Method B. The nitrosation would therefore have taken place at one of the unoccupied ring sites, *i.e.*, carbons 3, 5, 6, or 7. The NMR spectrum[6] showed three typical 1:1:1:1 quadruplet patterns with *J* values of >2.0 Hz., thus establishing the 3-nitroso isomer. Differences observed in the chemical shifts of the aromatic protons and loss of the one-proton singlet (H-3) on nitrosation gave further confirmation of the site of substitution. The H-3 singlet, observed at 8.4 p.p.m. in the spectrum of 2-phenylimidazo (1,2-*a*) pyrimidine, disappeared on nitrosation. Furthermore, the phenyl protons, which in the starting material appeared as two complex multiplets centered at about 7.5 and 8.3 p.p.m. and integrating for three and two protons, respectively, were found centered at 7.6 and 8.7 p.p.m. after nitrosation.

The NMR and IR absorption spectra for the phenyl analog prepared by nitrosation (Method B) and those for the same compound prepared by ring closure (Method A) were in every measurable respect identical. Table I gives the NMR data for the compounds prepared.

The IR spectra for all four of the nitroso-containing products were devoid of any absorption bands in the 1800 to 1650 cm.$^{-1}$ region where carbonyl and C=N frequencies normally are expected. Furthermore, all four of these compounds possess a moderately strong absorption at 1530 cm.$^{-1}$, which probably can be assigned as the C—N=O stretch frequency with reasonable confidence (11).

In conclusion, the products isolated from the condensation of 2-aminopyrimidine with arylglyoxylohydroxamyl chlorides have been identified as 3-nitroso-2-arylimidazo(1,2-*a*)pyrimidines.

PHARMACOLOGICAL RESULTS

Almirante *et al.* (4) investigated a series of 2-arylimidazo(1,2-*a*)-pyrimidine analogs for analgesic, anti-inflammatory, antipyretic,

[5] Perkin-Elmer model 237-B.
[6] Varian HA-100.

Table II—Antimicrobial Activity (*In Vitro*) of 3-Nitroso-2-arylimidazo(1,2-*a*)pyrimidines[a,b]

Compound	Organisms[c]			
	S. aureus[d]	*E. coli*	*C. albicans*	*C. neoformans*
2-Phenyl	1	1	1	2
2-*p*-Methylphenyl	1	0	1	1
2-*p*-Chlorophenyl	1	2	2	2
2-*p*-Methoxyphenyl	0	0	0	0

[a] Data for only four organisms are included. A total of 22 organisms was tested, however. [b] Activity ranges were as follows: 0, inactive at the 200 mcg./ml. level; 1, active in the 100–200 mcg./ml. level; and 2, active in the 40–99 mcg./ml. level. [c] *S. aureus* ranked fourth of four Gram-positive organisms tested in susceptibility, *i.e.*, poorest response; *E. coli* ranked third of four Gram-negative bacteria; *C. albicans* ranked third (tie) of five fungi tested; and *C. neoformans* ranked first of two yeasts tested. [d] Resistant strain.

and anticonvulsant properties. Their investigation revealed significant activity of the analgesic and anti-inflammatory types. Several of the compounds were active as antipyretic and hypothermic agents. One compound afforded protection against strychnine convulsions, while several of the compounds protected somewhat against electroshock and/or pentamethylenetetrazole seizures. Almirante *et al.* (4) concluded that while interesting pharmacological activity had been uncovered, no satisfactory structure–activity relationships could be formulated. Further, they noted that none of these compounds was as active as the previously reported imidazo(1,2-*a*)-pyridines (5).

Collins *et al.* (14) studied the hydrobromide salt of 2-phenylimidazo(1,2-*a*)pyrimidine (U-13,376B) as a strychnine antagonist. They reported that while this compound is a potent strychnine antagonist, it also shows strychninelike toxicity, albeit only 1/1000th as potent. This suggests that the compound functions as a partial agonist of the strychnine type (14).

The compounds prepared in this study were evaluated as potential antimicrobial and antiviral agents.[7] A total of 22 microbial agents, two helminths, and three viruses were used in the *in vitro* screening. All of the nitroso derivatives were inactive as anthelmintics and as antiviral agents. Using the results of tests *versus Staphylococcus aureus* (resistant strain), *Escherichia coli*, *Candida albicans*, and *Cryptococcus neoformans* as examples of Gram-positive bacteria, Gram-negative bacteria, fungi, and yeast, respectively, the spectrum of activity of these compounds was tabulated (Table II). 3-Nitroso-2-(4-chlorophenyl)imidazo(1,2-*a*)-pyrimidine was also examined *in vivo* using *Klebsiella pneumoniae*-infected Dierolf mice (18–20-g. males). A 20% survival at 250 mg./kg. *versus* 20% survival with kanamycin at 1 mg./kg. was observed. The compound was administered in water by the subcutaneous route. The general rank of activity seems remarkably parallel to the solubility of these compounds, all of which are difficultly soluble at best.

[7] Antimicrobial and antiviral screens were performed courtesy of Smith Kline & French Laboratories. The authors are indebted to Dr. Paul N. Craig of SKF for providing the screening data cited in this section.

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