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An Unusual Ring Contraction in the Formation of N-Nitrosohexamethyleneimine and N-Nitrosopiperidine from Tolazamide

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The previously reported reaction of tolazamide with nitrite, under physiological conditions, to form N-nitrosohexamethyleneimine and surprisingly, N-nitrosopiperidine was confirmed. By using the six-membered ring analogue of tolazamide, 1-(piperidyl)-3-(*p*-tolylsulfonyl)urea, which yields the corresponding N-nitrosopiperidine and N-nitrosopyrrolidine, the present study shows that an unusual ring contraction occurs, excising the carbon alpha to the nitrogen.

Key words: Tolazamide — N-Nitrosohexamethyleneimine — N-Nitrosopiperidine — N-Nitrosopyrrolidine

It has been reported by Sakai *et al.*[1] that tolazamide, (1-(hexahydro-1*H*-azepin-1-yl)-3-(*p*-tolylsulfonyl)-urea), a drug used in the treatment of diabetes,[2] produces N-nitrosohexamethyleneimine (NHM)[2] and N-nitrosopiperidine (NPIP) when reacted with excess sodium nitrite under acidic conditions. The formation of the latter is surprising since tolazamide contains a seven- but not a six-membered heterocycle (see Fig. 1). Similar results were obtained when the formulated product or pure tolazamide was used thus eliminating the possibility of an impurity in the formulated product.

Although the yields of each were low, NPIP was produced in larger amounts than NHM. From pure tolazamide the reported yield at 37°C, after one hour of nitrosation, was 1.9% for NPIP and that for NHM was 0.22%. The authors, understandably, were unable to offer an explanation for the formation of the six-membered ring compound. Although the production of NHM from tolazamide has been previously reported via nitrosation[3] and also by oxidation with hydrogen peroxide or oxygen[4] this was the first report of NPIP being formed from the drug. Since the formation of NPIP was unexpected, a study was undertaken to confirm the observation and to study the apparent ring contraction that could explain the formation of NHM and NPIP.

MATERIALS AND METHODS

All chemicals were of ACS reagent grade unless otherwise noted. Water was distilled and purified with a Barnstead NANO Pure II system. NHM, NPIP and N-nitrosopyrrolidine (NPYR) were obtained from the NCI Chemical Carcinogen Standard Reference Repository, Division of Cancer Etiology, Bethesda, MD. Tolazamide was obtained in pure form from a generic manufacturer. It was recrystallized twice from ethanol and checked for purity by elemental analysis; calculated: %C 54.00, %H 6.80, %O 15.41, %S 10.30, %N 13.49; found: %C 53.85, %H 6.62, %O 15.62, %S 10.56, %N 13.34. In addition, the melting point of the purified drug, 170–173°C, agreed with the literature value of 170–173°C.[2] N-Aminohexamethyleneimine (AHMI) was obtained from Aldrich Chem. Co., Milwaukee, WI. Cyclopentanol labeled at the one position with ^{13}C was obtained from Cambridge Isotopes Inc., Woburn, MA.

The six-membered ring analog of tolazamide, 1-(piperidyl)-3-(*p*-tolylsulfonyl)urea (PTU), was prepared by the following synthetic pathway. Cyclopentanol, labeled at the one position with ^{13}C, was converted to cyclopentanone (H_2CrO_4, acetone)[5] in 95% yield. The latter was subjected to the Schmidt expansion (NaN_3, conc. HCl)[6] to yield 2-piperidinone in 94% yield. This lactam was reduced to piperidine ($LiAlH_4$, dry benzene).[6] The yield of the latter was 95%. The piperidine was nitrosated ($NaNO_2$, HCl, water)[7] and reduced with $LiAlH_4$ in dry ether[2] to give a 92% yield of 1-aminopiperidine. The 1-aminopiperidine was reacted with *p*-toluenesulfoethylurethane[2] to give PTU containing ^{13}C in the position alpha to the nitrogen, in 98% yield. Unlabeled cyclopentanol was subjected to the same synthetic procedures. The products from the labeled and unlabeled material were compared by gas chromatography-mass spectroscopy (GC-MS) at each stage to insure that the ^{13}C was still present. In addition, the identity and purity of each compound was confirmed by GC-MS analysis.

The conditions for nitrosation were as previously described[1] (excess nitrite at pH 3 in acetate buffer). After nitrosation the nitrite was destroyed with sulfamic

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[2] Abbreviations used: NHM, N-nitrosohexamethyleneimine; NPIP, N-nitrosopiperidine; NPYR, N-nitrosopyrrolidine; PTU, 1-(piperidyl)-3-(*p*-tolylsulfonyl)urea; AHMI, aminohexamethyleneimine; GC-TEA, gas chromatography with a thermal energy analyzer detector; GC-MS, gas chromatography-mass spectroscopy.

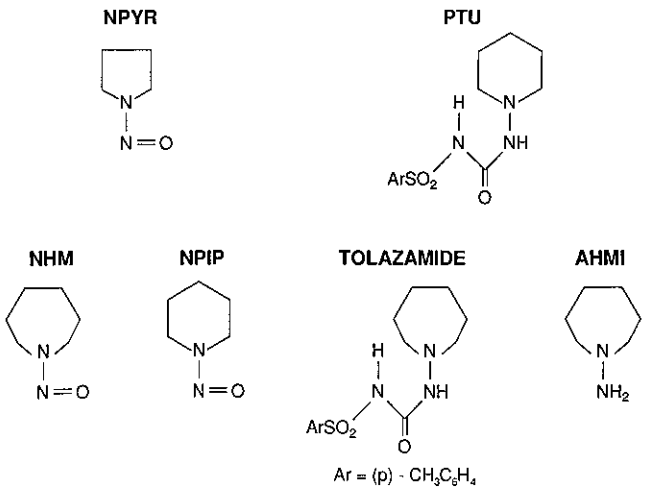

Fig. 1. Compounds used in this study.

acid and the nitrosamines were extracted three times with methylene chloride, concentrated in a Kuderna-Danish evaporator and analyzed by gas chromatography with a Thermal Energy Analyzer detector (GC-TEA). The GC-TEA conditions have been described elsewhere.[8] Confirmation was obtained by GC-MS using a Hewlett-Packard model 5995 mass spectrometer equipped with a Hewlett-Packard high-performance capillary column. The column was a 25 m crosslinked methyl silicone column with a 0.5 μm film. The temperature was programmed from 120 to 190°C at a rate of 5°C per minute. The injection port temperature was 200°C. Source temperature was 250°C. The ionization mode was electron impact with a voltage of 70 eV. Samples for GC-MS analysis were prepared as described previously.[4] The mass spectra obtained for the NHM, NPIP and NPYR prepared in this study were identical to those of the authentic compounds.

Tolazamide and *p*-toluenesulfonamide were determined by HPLC using a Technicon Fast-LC pump with a ISCO model V^4 variable-wavelength UV detector. The detector was set at 254 nm. The column was a reverse-phase one, no. 8005, from Alltech. The mobile phase consisted of 30% acetonitrile/sodium acetate buffer, pH 4. The flow rate was 0.3 ml/min. The injection loop was 20 μl.

RESULTS AND DISCUSSION

Essentially identical results were obtained when the previously reported nitrosation experiments[1] were repeated using the pure drug. At 37°C and after one hour

Table I. Amount of N-Nitrosamines Formed

Compound	Conditions (°C/h)	NHM (ppm)[a]	NPIP (ppm)[a]
AHMI	40/48	2115	17
NHM	50/3	—	48
NHM	70/3	—	1050
Tolazamide	30/7	522	1800
Tolazamide	25/5 days	9585	17,262

a) ppm based on 2–5 mg of starting material.

Sakai *et al.* reported 49 ppm of NPIP and 22 ppm of NHM. Under identical experimental conditions we obtained 65 ppm NPIP and 19 ppm NHM. The only other products found were AHMI and *p*-toluenesulfonamide. No unreacted tolazamide was found. In order for NHM and NPIP to form, both hydrolysis of tolazamide (to form NHM) and ring contraction (to form piperidine which can then be nitrosated by the excess nitrite) are needed.

Although unsaturated azepines have been reported to undergo ring contraction on treatment with acid, ring contraction in saturated azepines in without precedent.[9] Thus, two other simpler azepines, analogous to tolazamide, were treated with excess nitrite under acidic conditions. The two compounds were NHM itself and AHMI. These were selected to determine if the sulfonylurea group plays a key role in the ring contraction. The results are shown in Table I. As can be seen from the table, some of the compounds had to be nitrosated at slightly elevated temperatures and for prolonged times to obtain significant yields. The reaction is quite slow at room temperature. It can be seen from the table that NPIP is formed from each compound, although in very low yields compared to those obtained from tolazamide.

In order to further confirm that ring contraction actually occurred and to determine which carbon is lost, PTU was labeled with ^{13}C at the position alpha to the nitrogen. The synthetic pathway to this material was given above. This compound is an analog of tolazamide containing a six-membered ring rather than a seven-membered ring. ^{13}C-Labeled tolazamide was not prepared because the necessary starting materials were not available. It was reasoned, however, that if ring contraction occurred with the seven-membered ring, a similar contraction should occur with the six-membered ring resulting in the formation of N-nitrosopyrrolidine.

^{13}C-Labeled PTU and unlabeled PTU were subjected to the same nitrosation conditions as those used for tolazamide. The products in this case were NPIP and NPYR. The yields were similar to those found for NHM and NPIP from tolazamide.

Table II. Mass Ion Ratios

Compound	Molecular weight	$M^+/(M^+ + 1)$
N-Nitrosamines from labeled material		
N-Nitrosopyrrolidine	100, 101	1.01+/−0.03
N-Nitrosopiperidine	115	19.16+/−2.68
N-Nitrosamines from unlabeled material		
N-Nitrosopyrrolidine	100	14.66+/−1.81
N-Nitrosopiperidine	114	16.69+/−0.90

Table II shows the mass ion ratios found for the products from the labeled and unlabeled material. It can be seen from the table that unlabeled material yielded NPIP and NPYR which had the $M^+/(M^+ + 1)$ ratios one would expect from unlabeled material based upon the natural abundances of stable isotopes. However, the labeled material gave an $M^+/(M^+ + 1)$ ratio for NPYR of 1.01. This is exactly the ratio one would expect if the carbon alpha to the nitrogen was lost in the ring contraction. This is because the alpha carbons are equivalent and have an equal probability of excision. Therefore the NPYR formed should be 50% labeled and 50% unlabeled.

Thus it would appear that when the sulfonylurea group is attached to the nitrogen of a saturated azepine, reaction of that compound with acidic nitrite results in a most unusual ring contraction. Because of the low yields the reactions are obviously not a useful synthetic route to NHM, NPIP or NPYR. However, since the reaction conditions are similar to those present in the human stomach and the carcinogenic action, in animals, of all these nitrosamines is well established,[10] caution should be exercised in the use of the drug tolazamide.

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