Short Communications

Goitrin – a nitrosatable constituent of plant foodstuffs

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Summary. N-nitrosamides are known as direct-acting carcinogens at the site of their formation; they do not need any metabolic activation in vivo. The conditions leading to their formation in the stomach, and also their genotoxicity, have been thoroughly studied with some model compounds[1,2]. Several reports link this type of compound to the induction of gastric cancer in human[3,4]. However, only limited data are presently available about possible precursors of N-nitrosamides in foods. In the present study we found that goitrin – a naturally occurring compound in cruciferous vegetables and rape – could be easily nitrosated by treatment with nitrite under stomach conditions, yielding with loss of sulfur the N-nitroso-oxazolidone **4** (fig.). This product has a mutagenicity pattern and potency similar to that of N-nitroso-N-methyl-N′-nitroguanidine (MNNG) in the Ames Salmonella/mammalian microsome test.

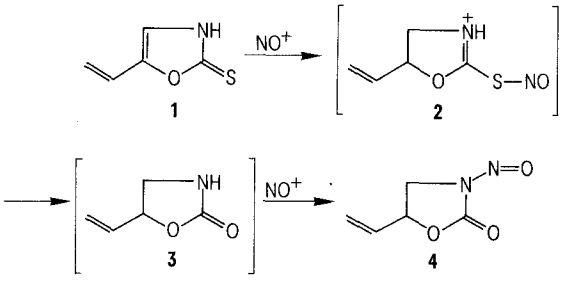
^{35}S- and ^{14}C-labelled (S)-5-vinyl-oxazolidin-2-thione (goitrin, **1** in figure) were isolated from rape seedlings *(Brassica napus)* after feeding ^{35}S-methionine and (2-^{14}C)-acetate, according to the method of Chisholm & Wetter[5] with the modification proposed by Elfving[6], and further purified by repeated recrystallization from ether. A radiochemical purity higher than 96% was thus obtained and all spectral data were in agreement with the literature data for goitrin[6].

The reaction of 3 mg ^{14}C-goitrin with 6.4 mg sodium nitrite in 3 ml buffer at pH 3 and 37 °C was followed by applying aliquots on TLC (silicagel, chloroform as eluent). After 5 min, besides the educt (RF = 0.5) a further spot with RF = 0.7 appeared on TLC, and gave the pink color typical for N-nitroso-compounds when sprayed with sulfanilic acid/α-naphthylamine[7]. After 30 min the reaction mixture was extracted with chloroform. A radio-TLC revealed that most of the extractable C-14-activity consisted of unchanged goitrin; about 5% of the radioactivity showed the same TLC-behavior as the unknown N-nitroso compound. In a second experiment 3 mg ^{35}S-goitrin were treated with sodium nitrite under the same conditions. The nitroso compound formed proved to be inactive which was consistent with a loss of sulfur during the nitrosation reaction. In a preparative trial 50 mg of the N-nitroso-compound could be isolated as a yellow oil; it was further purified by chromatography on silicagel. The spectral data, ms:m/z = 142 (M^+); UV ($CHCl_3$): 423, 405, 389, 238 nm; IR ($CHCl_3$): 3020, 1810, 1470, 1150, 980 cm^{-1}; and NMR: 3.59 (1 H, q, J = 6Hz), 4.18 (1 H, q, J = 8), 5.15 (1 H, m), 5.50 (2 H, m), 5.90 (1 H, m) were in agreement with the structure for N-nitroso-5-vinyl-2-oxazolidone (fig., structure **4**). The reaction probably proceeds via the S-nitroso intermediate **2** and the oxo-analogue **3** in analogy with the recently found desulfuration and nitrosation of 2-pyrrolidinethione[8].

The genotoxic activity of the N-nitroso-compound **4** was tested in the Ames Salmonella/mammalian microsome assay[9] (table). The strongest direct-acting mutagenic effect was detected with TA1535 followed by TA100, indicating a specifity for base pair substitutions. A check for the number of surviving bacteria revealed a strong bacteriotoxicity even at low concentrations. The strains sensitive for frameshift mutagens were not induced (TA1538) or the mutagenic activity was very weak or questionable (TA98, TA1537). The addition of S-9 (rat liver homogenate) reduced the mutagenicity observed with strains TA1535 and TA100 (data not shown). These results mean that **4** has a similar mutagenicity pattern to MNNG or MNU, which are both direct-acting mutagens with a specifity for basepair substitution.

Knowledge concerning the chemistry and toxicity of Nitroso-oxazolidones is scarce. Miyahara et al. tested several alkyl derivatives of N-nitroso-2-oxazolidones with 4-(p-nitrobenzyl)pyridine (NBP reagent) for their alkylating activities, and found generally a strongly positive response[10]. Hassner and Reuss[11] studied the base-catalyzed decomposition of N-nitroso-2-oxazolidones and report that this class of compounds can react with 2 equivalents of nucleophiles with nitrogen and carbonate as leaving groups and thus may act as bifunctional agent.

Goitrin and the precursor 2-hydroxy-3-butenyl-glucosinolate are constitutents of the vegetables cabbage, Brussel sprouts, turnip, rutabaga and rape seed meal; the latter is being increasingly used as an animal food[12]. Most of these plants contain also considerable amounts of nitrate (range 100–1000 mg/kg) and nitrite (ca. 1 mg/kg)[13]. On the other hand, from an epidemiological viewpoint the consumption of vegetables and fresh



Number of revertants[a] in the Salmonella/microsome mutagenicity assay induced by the goitrin-nitrosation product N-nitroso-5-vinyl-2-oxazolidone in the Salmonella strains TA98, TA100, TA1535, TA1537, TA1538 without metabolic activation with rat liver S-9

Strain	Dose of N-nitroso-5-vinyl-oxazolidone/plate (μg)								Positive controls	
	Control	1.6	3.1	6.2	12.5	25	50	100		
TA1535	15 ± 5	76 ± 11	138 ± 11	234 ± 13	358 ± 18	1004 ± 72	22 ± 3[b]	3 ± 0[c,b]	∞[d]	(10 μg MNNG)
TA1537	4 ± 1	4 ± 1	4 ± 1	6 ± 2	5 ± 2	10 ± 3	5 ± 3[c]	16 ± 4[c]	∞[d]	(100 μg 9-AA)
TA1538	10 ± 2	10 ± 3	9 ± 1	8 ± 2	11 ± 4	13 ± 5[c]	3 ± 2[c]	8 ± 3[c]	1229	(7.5 μg NF)
TA98	14 ± 2	14 ± 5	16 ± 3	23 ± 1	25 ± 7	25 ± 6	26 ± 2	12 ± 4[b]	877	(7.5 μg NF)
TA100	104 ± 8	142 ± 7	432 ± 22	358 ± 34	505 ± 17	829 ± 16	525 ± 39[b]	19 ± 9[c,b]	1053	(2.0 μg MNNG)

[a] Mean value and standard deviation of triplicates; [b] Bactericidal effects: Total number of surviving cells significantly reduced; [c] Inhibition of the background growth; [d] Many small colonies which were only partially detected by the electronic colony counter.

fruits is negatively associated with the incidence of intestinal metaplasia and atrophic gastritis in a high risk area for gastric cancer[14]. A conclusive risk estimate will require further studies on the in vivo formation of N-nitroso-5-vinyl-2-oxazolidone after consumption of goitrin-containing vegetables and on the genotoxic potential of this compound.

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Simple integrated rate equations for reversible bimolecular reactions

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Summary. If the complete rate equations for reversible, one-step, bimolecular reactions are written with $P_e - P$ as the concentration variable (where P_e is the equilibrium, and P is the instantaneous, product concentration), the 3 possible stoichiometries can be reduced to a single straightforward differential equation. This can be solved very economically. For each stoichiometry,

$$\mathrm{A} \rightleftharpoons \mathrm{P} + \mathrm{Q}: \qquad k_1(-1/K_e)Dt = -\ln\left(1 - \frac{\Delta P}{P_e - P_o}\right) + \ln\left(1 - \frac{\Delta P}{D + P_e - P_o}\right)$$

$$\mathrm{A} + \mathrm{B} \rightleftharpoons \mathrm{P}: \qquad k_1 Dt = -\ln\left(1 - \frac{\Delta P}{P_e - P_o}\right) + \ln\left(1 - \frac{\Delta P}{D + P_e - P_o}\right)$$

$$\mathrm{A} + \mathrm{B} \rightleftharpoons \mathrm{P} + \mathrm{Q}: \qquad k_1(1 - 1/K_e)Dt = -\ln\left(1 - \frac{\Delta P}{P_e - P_o}\right) + \ln\left(1 - \frac{\Delta P}{D + P_e - P_o}\right)$$

where t is time, k_1 is the forward rate constant, K_e is the equilibrium constant, and ΔP is $P - P_o$. The terms $P_e - P_o$ and $D + P_e - P_o$ are the physically possible and physically impossible roots of the quadratic equation for $P_e - P_o$ in terms of the initial concentrations and K_e. D is the discriminant in this equation. All 3 quantities can be calculated if the equilibrium constant is known. A plot of t against $\ln\{[1 - \Delta P/(D + P_e - P_o)]/[1 - \Delta P/(P_e - P_o)]\}$ should be a straight line for any second order reaction. For each stoichiometry, $P_e - P_o$ approaches A_o, the initial concentration of the first reactant, as the equilibrium constant increases. When a second reactant is present, $D + P_e - P_o$ approaches B_o. The limiting equation is then that of an irreversible bimolecular reaction. For $\mathrm{A} \rightleftharpoons \mathrm{P} + \mathrm{Q}$, D approaches $-K_e$ as the equilibrium constant becomes large, and the value of the second logarithmic term in the integrated equation approaches zero. The limiting equation is that of an irreversible, unimolecular reaction.

Rates of reaction are ordinarily calculated from measurements of product (or reactant) versus time. The usefulness of integrated rate equations, which express the quantity actually measured, has therefore long been recognized. However, even for reactions as common and simple as 1-step bimolecular processes, the standard textbook derivations become painfully cumbersome as soon as anything more complex than an irreversible reaction is considered.

To circumvent this, bimolecular reactions, and reactions that display second-order kinetics, have usually been treated as a series of separate special cases: $\mathrm{A} + \mathrm{B} \rightarrow \mathrm{P}$, where the reaction is irreversible and $A_o = B_o$, or $\mathrm{A} + \mathrm{B} \rightarrow \mathrm{P} + \mathrm{Q}$, where $A_o \gg B_o$, or etc.[2]. The result is a series of equations that are difficult to reconcile and have little intuitive appeal, and plotting methods that apply only in special cases. An additional very serious consequence is that integrated equations for enzyme-catalyzed reactions, whose potential utility is widely recognized, have either been impossible to obtain or too complex to be of practical value.

It is possible, by choosing the right concentration variable, to solve the differential equations for $\mathrm{A} \rightleftharpoons \mathrm{P} + \mathrm{Q}$, $\mathrm{A} + \mathrm{B} \rightleftharpoons \mathrm{P}$, and $\mathrm{A} + \mathrm{B} \rightleftharpoons \mathrm{P} + \mathrm{Q}$ in a simple and uniform way. With a single exception, where the mathematical limit is not trivial, the resulting equation covers all possible initial conditions. The equation is straightforward, has a simple mathematical meaning, can easily be analyzed in terms of the shape of the product versus time curve, and can be used to make a simple logarithmic plot of any second-order reaction. This approach leads to an intuitive appreciation of uncatalyzed second-order processes, and is directly applicable to enzyme-catalyzed reactions.

Derivation. Examples of more-or-less standard textbook derivations can be found in Moore[3], Frost and Pearson[4], Hammes[5], etc. Szabo[6] obtains an involved result that is applicable to all initial conditions. In all these derivations, the reaction