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THIOPHILIC RING-OPENING AND REARRANGEMENT REACTIONS OF EPOXYKETONE NATURAL PRODUCTS

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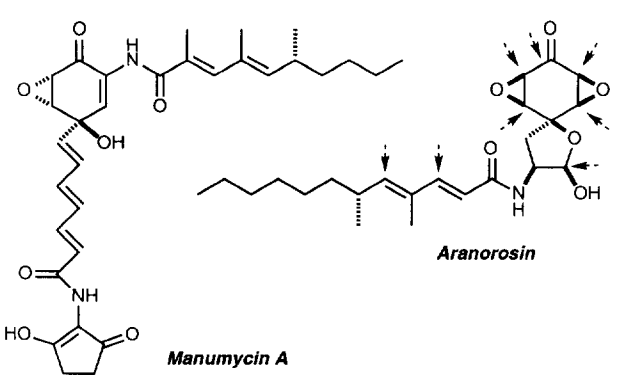
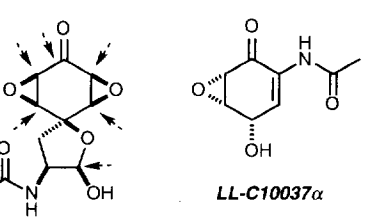
Abstract: Thiol additions to the highly functionalized core structures of aranorosin- and manumycin-type antibiotics reveal the general reactivity patterns of epoxyketone natural products. Rapid hemiacetal and hydrate formations decrease the reactivity of the epoxyketone moiety in aqueous media toward the cellular scavenger glutathione, and secondary 1,2-shift, elimination, aromatization and intramolecular aldol reactions provide novel reaction pathways. In a hydrophobic environment, the thiol-capture function of the ketone moiety facilitates electrophilic attack.

Due to their powerful, yet selective alkylating capabilities, epoxides have an extraordinary potential to interact with proteins, nucleic acids, and biosynthetic intermediates. The presence of both oxygenases and epoxide hydrolases in biological tissue makes it difficult to assess the health risks associated with this functional group. Not surprisingly therefore, the toxicological profile of epoxides has been a reason for considerable concern in environmental legislation.[1] Whereas the reactivity profiles of enediyne antibiotics[2] and the nitrogen mustard family[3] have been addressed extensively in recent years, research on multifunctional epoxides has mainly focused on elucidation of biosynthetic pathways.[4,5] As part of our efforts toward the total syntheses of the epoxyketone natural products aranorosin,[6] LL-C10037α,[7] manumycin,[8] and diepoxin σ,[9] we were interested in investigating the fundamental alkylating properties of these antitumor antibiotics. Many members of the structurally loosely defined class of epoxyketone natural products present formidable arrays of electrophilic functionalities. The fungal metabolite aranorosin,[10] for example, has at least eight reactive sites for the covalent interaction with nucleophilic biomolecules.

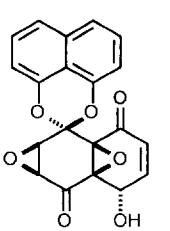


Manumycin A



Aranorosin

LL-C10037α



Diepoxin σ

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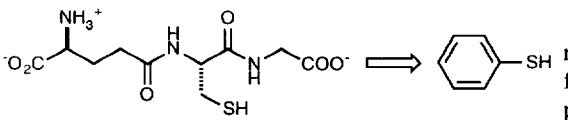
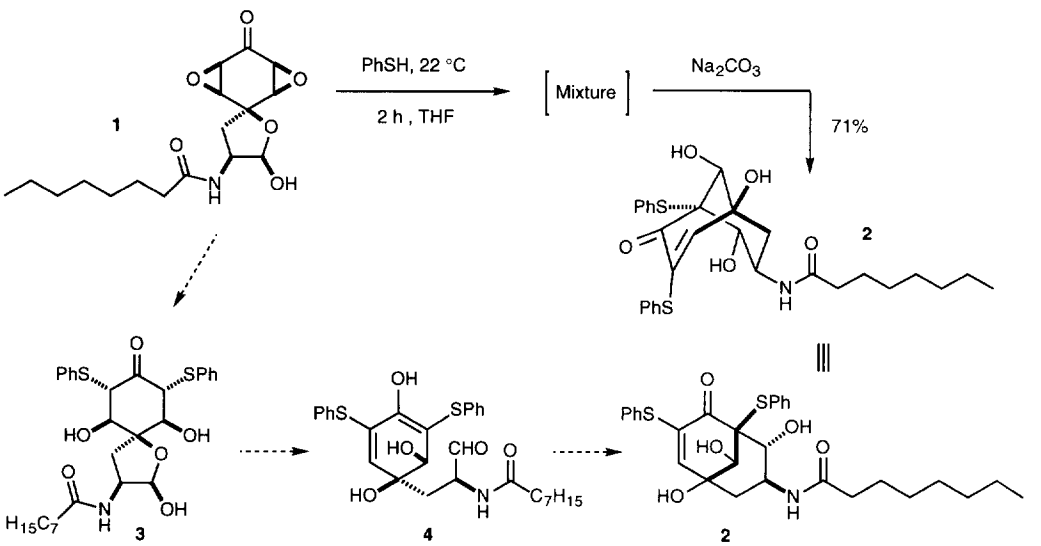


Glutathione (GSH)

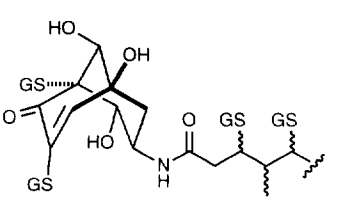
We chose thiophenol as a model for the sulfur nucleophile glutathione, which is abundant in cells and forms the first line of defense against electrophiles passing cell membrane or cell wall.[11]

Treatment of the aranorosin core **1**[6a] with thiophenol immediately revealed an unexpected and novel reaction pathway. In the presence of 5 equiv of thiophenol in THF, a complex mixture of products was rapidly formed and was converted upon addition of solid sodium carbonate into a major compound, bicyclo[3.3.1]nonane **2** in 71% yield. A hypothesis for the reaction pathway is shown in Scheme 1. Sequential opening of the epoxide rings and aldol dehydration provided **4**, which underwent a base-catalyzed intramolecular aldol reaction to the lactol/aldehyde function. The new C,C-bond was formed stereoselectively, and only the thermodynamically more favored diastereomer **2** was isolated.[12]

Scheme 1



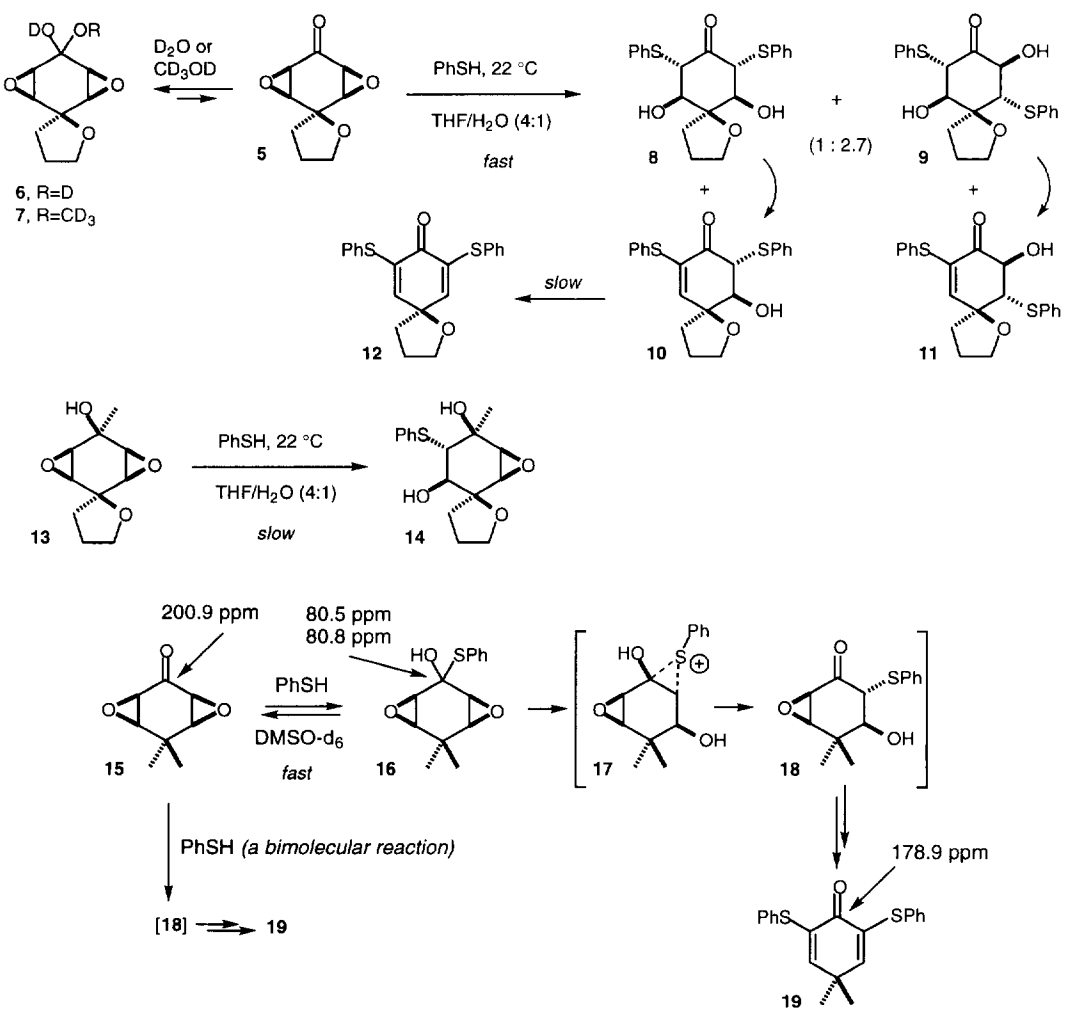
^{1}H NMR studies of the reaction of aranorosin and glutathione revealed a similar reaction pathway. In a D_2O/CD_3OD mixture, the bisunsaturated side chains reacted rapidly with two equiv of GSH to give a mixture of diastereomers. A slower addition-rearrangement process converted the spirocycle into the bicyclo[3.3.1] system. A total of 4 equiv of GSH were added to the natural product within ca. 2 h at 22 °C.



NMR studies of these reactions revealed that the ^{13}C carbonyl signal of core model **1**, which is clearly visible in aprotic solvents in the absence of S-nucleophiles, rapidly disappeared in the presence of thiophenol or in CD_3OD/D_2O-containing media, and new resonances that are indicative of hemiacetal or hydrate structures appeared. Since the simplistic idea of an inductive acceleration of the oxirane S_N2-thiolysis by the carbonyl function was clearly discredited by these data, we became interested in gaining a better mechanistic understanding of this process.

Treatment of diepoxyketone **5**[13] with 5 equiv of thiophenol in THF/H_2O resulted in the formation of a 1:2.7 ratio of the α,α'-addition product **8** and the α,β'-addition product **9** (Scheme 2). The pseudo first order half-life $t_{1/2}$ of this process was ca. 1 h. Diols **8** and **9** were slowly ($t_{1/2}$ = 6 h) dehydrated to **10** and **11**; **10** was further converted ($t_{1/2}$ = 12 h, low yield) to the diene **12**. However, upon addition of a sample of **5** to D_2O or CD_3OD, the ^{13}C carbonyl signal at 200.5 ppm again disappeared within seconds and a quaternary carbon appeared in the acetal region at 89.5 ppm, indicating a >95% conversion into the hydrate **6** or hemiacetal **7**, respectively. This hydration should significantly reduce the ease of solvolysis of the reactive diepoxyketone functionalities in **5** and, in analogy, aranorosin in aqueous media. Oxirane ring opening of the diepoxyalcohol **13**, for example, requires several days. Furthermore, it is possible that the initial site of thiophenol attack on diepoxyketones is the carbonyl group rather than the α- or β-position of the epoxide, and the actual ring-opening occurs *intramolecularly* via 1,2-shift as shown in **15**→**18**.

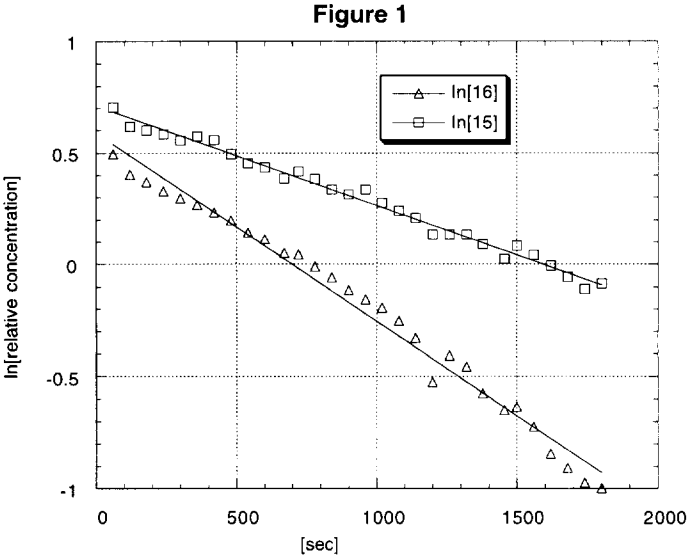
Scheme 2



Baldwin and coworkers have recently proposed this mechanism for thiol addition to the glucosamine synthetase inhibition anticapsin.[14] A carbonyl addition followed by a 1,2-shift has also been postulated for the overall conjugate addition of amines to quinones,[15] but we are not aware of any mechanistic studies of the reaction course of nucleophilic addition to α,β-epoxy ketones. The mechanism **15→16→18** is supported by the appearance of two peaks at 80 ppm and the disappearance of the carbonyl peak at 200.9 ppm in the presence of 3 equiv of thiophenol in $D_2O/CDCl_3/DMSO\text{-}d_6$. However, this spectroscopic evidence does not rigorously exclude reaction pathways where the thiol nucleophile adds directly to the epoxy ketone that is present in low concentrations, or to the thiohemiacetal intermediate. We anticipated that kinetic studies would be more conclusive for the selection between these alternative reaction mechanisms.

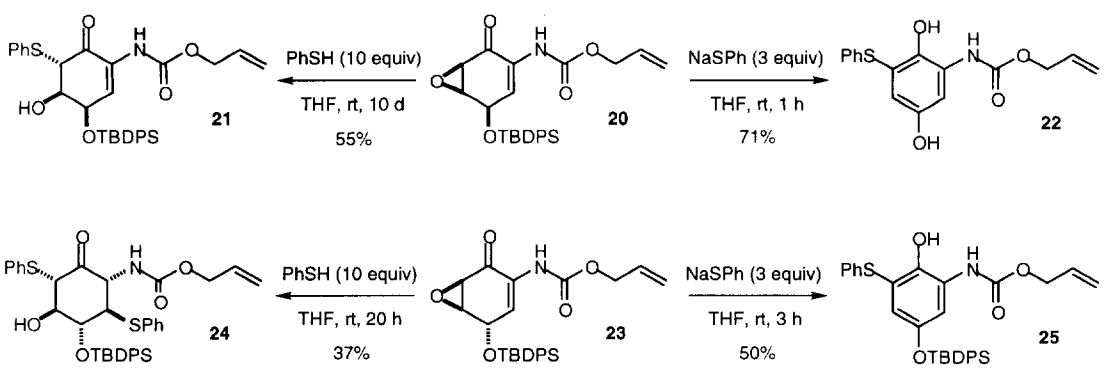
In the presence of 1.6 equiv of thiophenol, the rate of disappearance (^1H NMR) of the intermediate thiohemiacetal **16** as well as diepoxyketone **15** followed closely first order kinetics (Figure 1). Similar results were obtained with 2.0 and 5.2 equiv of thiophenol. Since the equilibrium between ketone **15** and mixed acetal **16** is undoubtedly fast, these data do not agree with a rate-limiting bimolecular attack of thiophenol to either species and provide further support for an intramolecular 1,2-shift in the ring-opening of the epoxide. Accordingly, whereas the strong preference of diepoxyketones for hydration of the carbonyl group decreases the electrophilicity of the adjacent epoxy rings, the thiol-capture function of the ketone provides an intriguing pathway for irreversible delivery of the nucleophile. It can only be speculated that the biological relevance of such a mechanism might lie in the selective alkylation of cysteine residues in hydrophobic environment.[16] This hypothesis is further substantiated by the ubiquitous presence of lipophilic side-chains in epoxy ketone natural products that increase their affinity toward membranes or lipophilic protein binding sites. Epoxyketones would therefore more easily bypass deactivation by cytosolic glutathione than simple epoxides and represent more site-specific alkylating agents.

Figure 1



Manumycins have recently been identified as potent and selective inhibitors of Ras farnesyltransferase,[17] and the epoxyquinol core and its aminoacyl side chain resembling a farnesyl group were proposed as pharmacophores. Accordingly, the interaction of the manumycin core with thiols was of particular interest. Model reactions with the epoxyquinols **20** and **23**[7] and thiophenol revealed a very similar reaction pattern to what we had observed with our aranorosin models. Under neutral conditions, α-opening of the epoxide gave **21** and was only in case of the 4α-stereochemistry in **23** followed by 1,4-addition to give **24** (Scheme 3). Under basic conditions, elimination after epoxide opening led to the aromatized hydroquinones **22** and **25**. Interestingly, desilylation of the C(4)-hydroxy group was only observed with the β-isomer **20**, and it can be argued that this difference in reactivity between the α- and β-silyl ethers is due to a neighboring group participation of the *syn*-hydroxyl substituent in the intermediate **21** which, under basic conditions, ultimately is eliminated to give the aromatic ring. The *trans*-disposition of the hydroxy group in the analogous intermediate derived from **23** would not permit the corresponding silyl group transfer before aromatization.

Scheme 3



In summary, our studies with aranorosin and manumycin model systems have revealed general reactivity patterns for the addition of sulfur nucleophiles to these highly functionalized natural products that are relevant for biological processes. Since epoxides are potent cellular toxins and are frequently used as enzyme suicide inhibitors and affinity labeling agents, information on the modulation of their activity by neighboring functional groups is important for rational design and risk assessment strategies. In aqueous environment, the intrinsic electrophilicity of enone epoxides is potentially lower than expected, because hemiacetal formation sterically and electronically decreases the rate of epoxide ring opening. The initial site of nucleophilic attack appears to be the carbonyl group, and in a subsequent, irreversible step 1,2-rearrangement leads to epoxide opening. This mechanistic hypothesis is supported by spectroscopic and kinetic studies. Secondary reactions due to enolate chemistry of aranorosin- and manumycin-type epoxyquinols include elimination, aromatization, and intramolecular aldol reactions that ultimately provide new scaffolds with very stable carbon-sulfur linkages. It is intriguing to speculate that the facile hydration of epoxy ketone moieties assists the natural products in bypassing premature deactivation by the cytosolic scavenger glutathione.

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