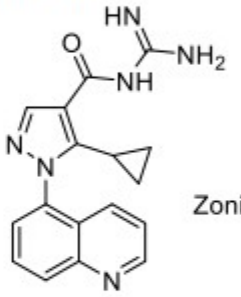
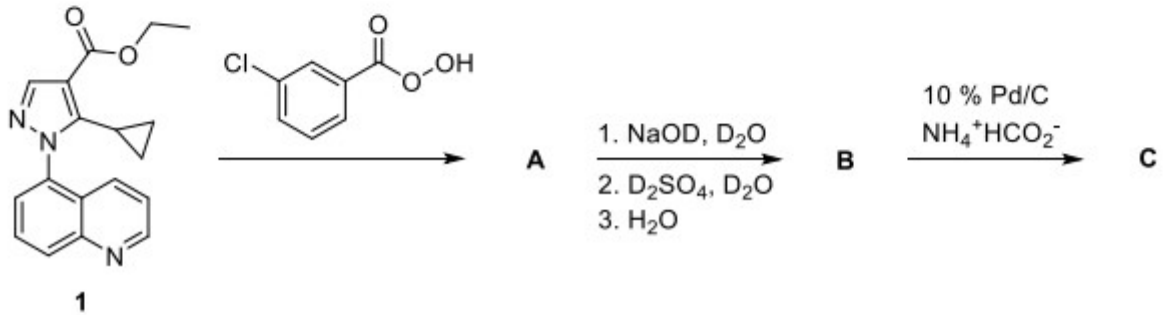
Problem 27. Zoniporide

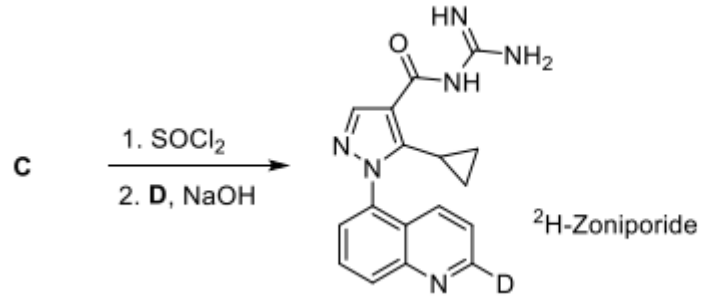
An emerging strategy to improve the pharmacokinetics (PK) of drugs takes advantage of the kinetic isotope effect. Molecules containing non-radioactive heavy isotopes in metabolically relevant positions may be cleared more slowly from the body. Zoniporide, a cardioprotective inhibitor of the Na^+/H^+ antiporter 1 protein, was considered a candidate for improved PK upon deuteration, since the major metabolic pathway of zoniporide involves the oxidation by aldehyde oxidase in position 2 of the quinoline core.

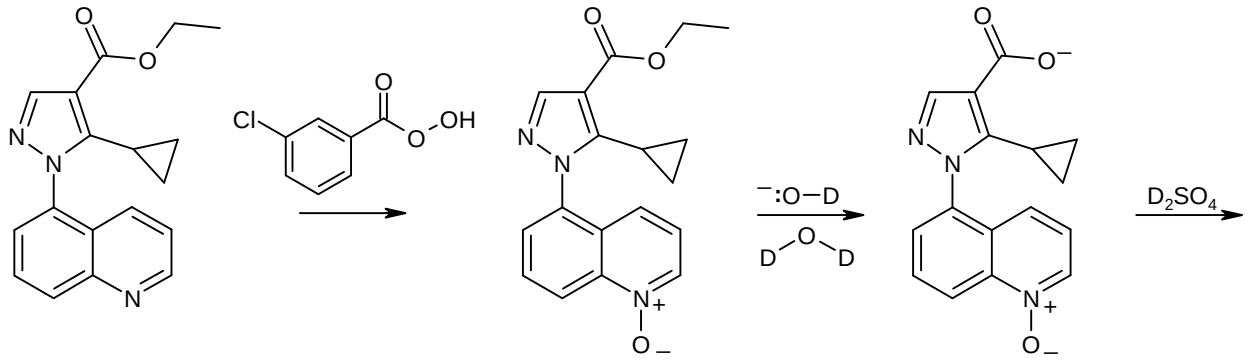


Zoniporide

^{2}H-Zoniporide, deuterated in position 2 of the quinoline core is synthesized from ester **1** by the following sequence of reactions:







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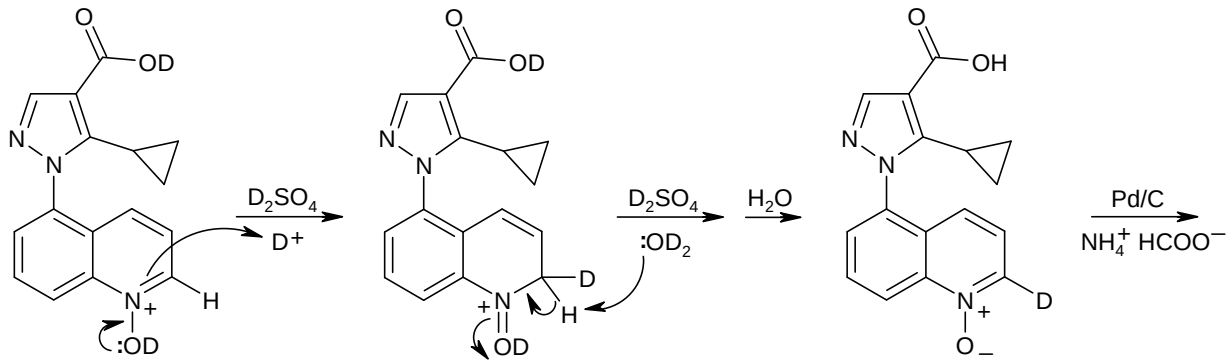
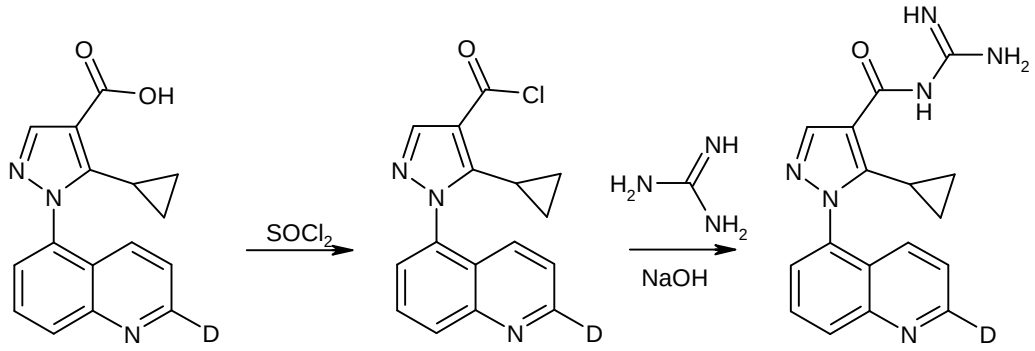


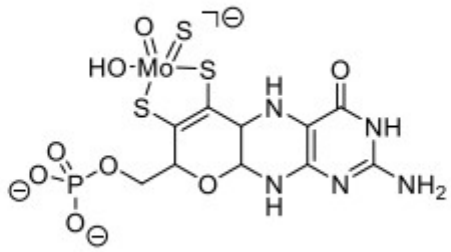


C D

27.2 Ammonium formate decomposes upon mild heating in the presence of palladium on charcoal (transformation **B** → **C**) into three gaseous products, one of which is the reducing agent required for the aforementioned transformation. Draw the structures of these compounds.

$NH_4^+\ HCOO^- \xrightarrow{Pd/C} NH_3 + H_2 + CO_2$

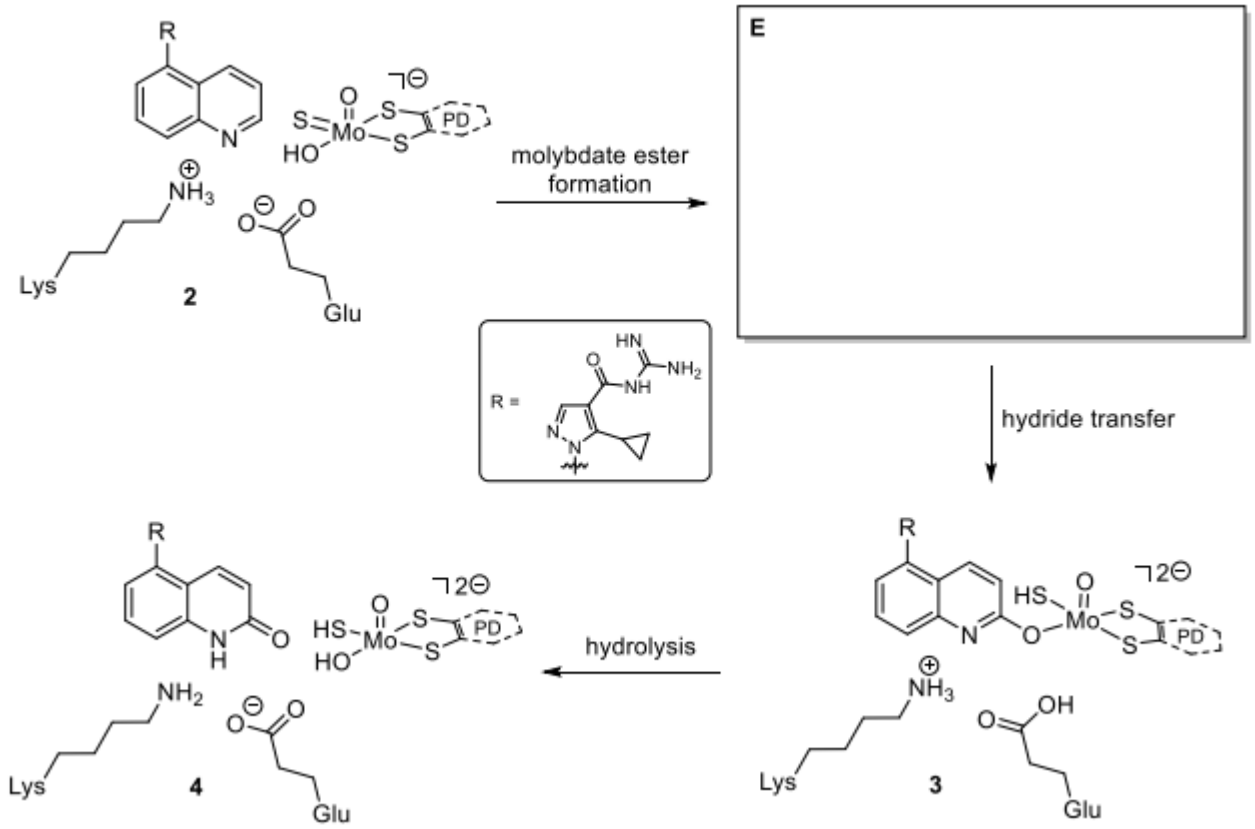
The active site of the aldehyde oxidase enzyme contains a molybdenum(VI) cofactor chelated by pyranopterin dithiolate (PD).



Molybdenum(VI) cofactor chelated by pyranopterin dithiolate (PD)

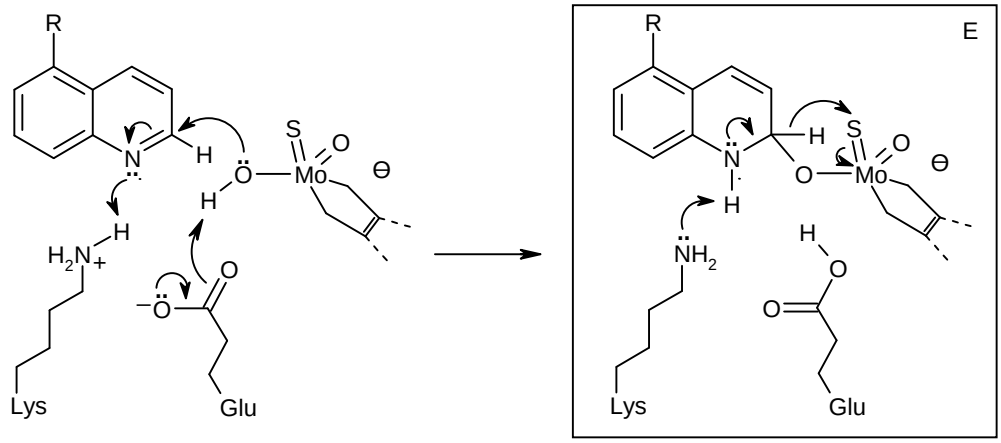
The structure of zoniporide bound in the active site of aldehyde oxidase (**2**) is shown below. Two mechanisms have been proposed for the oxidation of the drug in position 2 of the quinoline core. **Mechanism 1** involves three major individual steps: formation of a molybdate ester, a hydride transfer and a final hydrolytic step.

Mechanism 1



27.3 Draw the intermediate active site structure **E** involved in the proposed **Mechanism 1** of zoniporide oxidation.

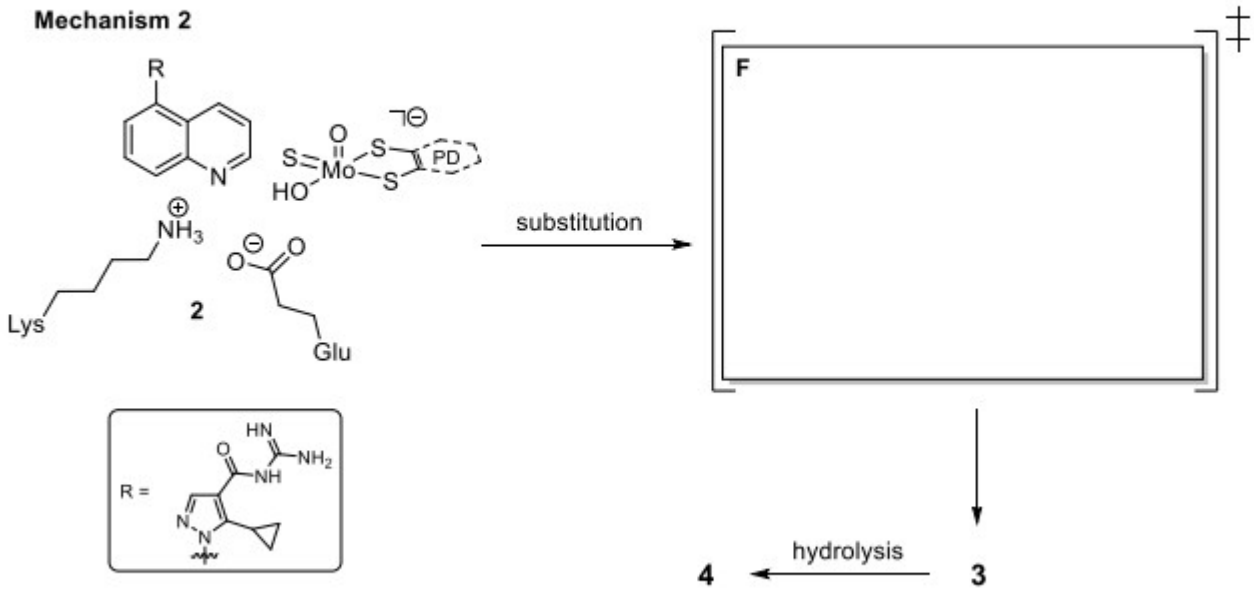
27.4 Give the oxidation state of molybdenum in each of the intermediate structures **E**, **3** and **4**.



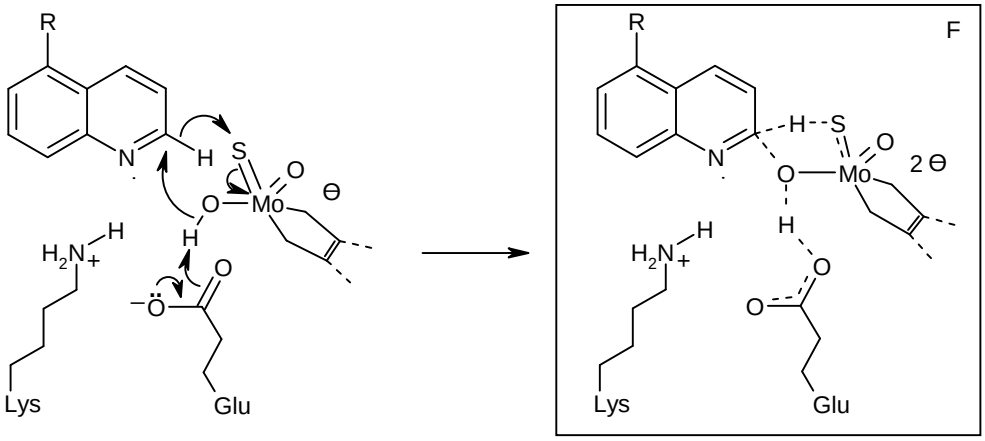
Il molibdeno in 2 e in E ha numero di ossidazione 6+, in 3 e in 4 ha n.o. 4+.

On the other hand, another proposed mechanism, **Mechanism 2**, involves a concerted substitution step yielding intermediate **3**, which is further hydrolyzed to **4** in the same fashion as in **Mechanism 1**.

Mechanism 2



27.5 Draw the transition state structure **F** for the concerted substitution step **2** → **3**. Use a dotted line for bonds which are being formed and cleaved.



The following experimental evidence was gathered to determine whether the transformation **2** → **3** in the mechanism of the oxidation of zoniporide (and related nitrogen heterocycles) by aldehyde oxidase is stepwise (**Mechanism 1**) or concerted (**Mechanism 2**):

a) The kinetic isotope effect, k_H / k_D, for zoniporide (deuterated in quinoline position 2) oxidation by aldehyde oxidase was 5.8 at 37 °C.

b) The introduction of electron withdrawing groups on the heterocycle core led to an increase in the reaction rate and a slight decrease in k_H / k_D.

27.6 Which of the two mechanisms (**Mechanism 1** or **Mechanism 2**) of quinoline oxidation by aldehyde oxidase is more plausible based on the aforementioned experimental evidence? Rationalize your answer.

Il meccanismo più probabile è quello concertato (2) perchè il forte effetto isotopico indica che lo strappo del deuterio avviene nel passaggio lento della reazione. Questo è il caso del Mecc 2, mentre nel Maccanismo 1 il passaggio lento è il primo nel quale vi è l'attacco nucleofilo all'anello che non coinvolge il deuterio.

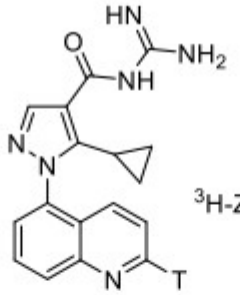
Inoltre l'introduzione di gruppi elettron-attrattori accelera l'attacco nucleofilo , che esiste in entrambi i meccanismi, ma diminuisce l'effetto isotopico. Nel Mecc 1 invece l'effetto isotopico dovrebbe aumentare perchè accelerare il passaggio lento dovrebbe rendere più importante il secondo passaggio che coinvolge il deuterio.

The molybdenum cofactor further needs to be reoxidized to its original state. The reducing equivalents from one reaction are transferred, via an iron sulfide cluster cofactor and a flavin cofactor, to a single molecule of oxygen as the stoichiometric oxidant.

27.7 What small molecule byproduct is formed by the reduction of O_2 in this process?

Il molibdeno deve cedere due elettroni per ossidarsi da 4+ a 6+. Questi due elettroni, passando per gruppi ferro-zolfo, giungono al FAD che infine li cede sotto forma di ione idruro ad O_2. Quindi O_2 viene ridotto ad acqua ossigenata H_2O_2. Questa è tossica per la cellula e deve essere ulteriormente degradata.

Deuterium is not the only heavy isotope of hydrogen. In theory, an even higher kinetic isotope effect would be expected using tritium. The isotope ^3H is not used in practice to slow down the metabolism of drugs due to economic and safety reasons but let us at least theoretically look at ^3H-zoniporide.



^3H-Zoniporide

27.8 Calculate the theoretical tritium (k_H / k_T) kinetic isotope effect for the oxidation of zoniporide by aldehyde oxidase at 37 °C. The deuterium kinetic isotope effect for the same reaction is 5.8. Consider the following approximations:

- The harmonic oscillator approximation
- Isotope exchange does not alter the rate determining step transition state structure
- The KIE is solely affected by the ^{12}C−H/D/T stretching vibration mode
- The KIE is solely determined by zero-point vibrational energies (the role of higher vibrational levels is negligible)

$m(^1H)$ = 1.0078 amu; $m(^2H)$ = 2.0141 amu; $m(^3H)$ = 3.0160 amu; $m(^{12}C)$ = 12.0000 amu

Hint: You need to calculate 1) the relevant reduced masses; and 2) the force constant for the C–H/D bond before you get to the final KIE calculation.

La massa ridotta per la coppia di atomi 1 e 2 si ricava dall'espressione $(m_1 m_2)/(m_1+m_2)$.
Per la coppia C-H si trova m_H = 0,92972 amu
Per la coppia C-D si trova m_D = 1,72463 amu
Per la coppia C-T si trova m_T = 2,41023 amu

La frequenza di stiramento del legame C-H è $\nu_H = \frac{1}{2\pi}\sqrt{\frac{k}{m_H}}$, l'energia del punto zero è $E^\circ_H = \frac{1}{2}h\nu_H$

L'equazione di Arrenius con l'idrogeno è $k_H = A\,e^{\frac{-E_H}{RT}}$ con il deuterio è $k_D = A\,e^{\frac{-E_D}{RT}}$

Il rapporto tra le due costanti di velocità per le reazioni con idrogeno e deuterio è quindi $\frac{k_H}{k_D} = e^{\frac{E_D - E_H}{RT}}$

Passando ai logaritmi $\ln\frac{k_H}{k_D}=\frac{E_D-E_H}{RT}=\frac{\Delta E_{DH}}{RT}$ dove E_D-E_H è la differenza tra le energie di attivazione.

Dato che l'energia dello stato di transizione E* è la stessa (in prima approssimazione) nei tre casi (con H, D e T), la differenza tra le energie di attivazione (E_D-E_H) è uguale all'inverso della differenza tra le energie del punto zero.
Infatti $(E_D-E_H)=(E^*\text{-}E^\circ_D)-(E^*\text{-}E^\circ_H)=(E^\circ_H-E^\circ_D)$
Quindi $\Delta E_{DH}=E^\circ_H-E^\circ_D=\Delta E^\circ_{HD}$
Il rapporto tra le costanti di velocità si può scrivere in funzione della differenza tra le energie del punto zero

$$\ln\frac{k_H}{k_D}=\frac{\Delta E^\circ_{HD}}{RT}$$

Per le due reazioni con H e D si ha $\Delta E^\circ_{HD}=\frac{1}{2}h\,\Delta\nu_{HD}$ dove $\Delta\nu_{HD}=\nu_H-\nu_D=\frac{\sqrt{k}}{2\pi}\left(\frac{1}{\sqrt{m_H}}-\frac{1}{\sqrt{m_D}}\right)$

Quindi $\Delta E^\circ_{HD}=\frac{1}{2}h\frac{\sqrt{k}}{2\pi}\left(\frac{1}{\sqrt{m_H}}-\frac{1}{\sqrt{m_D}}\right)$

da cui si può ricavare $\sqrt{k}$

$$\sqrt{k}=\frac{4\pi\ \Delta E^\circ_{HD}}{h}\left(\frac{1}{\frac{1}{\sqrt{m_H}}-\frac{1}{\sqrt{m_D}}}\right)$$

Con ragionamento analogo si ricava l'espressione di $\ln\frac{k_H}{k_T}$ per l'effetto isotopico con il trizio

$\ln\frac{k_H}{k_T}=\frac{\Delta E^\circ_{HT}}{RT}$ dove la differenza tra le energie di punto zero vale

$\Delta E^\circ_{HT}=\frac{1}{2}h\frac{\sqrt{k}}{2\pi}\left(\frac{1}{\sqrt{m_H}}-\frac{1}{\sqrt{m_T}}\right)$ in cui posso sostituire $\sqrt{k}$ con il valore trovato sopra. Ottengo così:

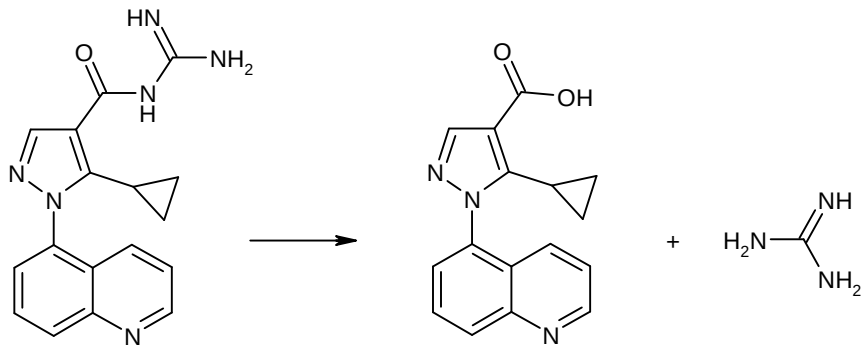
$$\Delta E^\circ_{HT}=\Delta E^\circ_{HD}\left(\frac{\left(\frac{1}{\sqrt{m_H}}-\frac{1}{\sqrt{m_T}}\right)}{\frac{1}{\sqrt{m_H}}-\frac{1}{\sqrt{m_D}}}\right)$$ dividendo entrambi i membri per RT ottengo:

$$\ln\frac{k_H}{k_T}=\ln\frac{k_H}{k_D}\left(\frac{\left(\frac{1}{\sqrt{m_H}}-\frac{1}{\sqrt{m_T}}\right)}{\frac{1}{\sqrt{m_H}}-\frac{1}{\sqrt{m_D}}}\right)$$ da cui posso ricavare il valore richiesto di effetto isotopico $\frac{k_H}{k_T}$

$$\ln\frac{k_H}{k_T}=\ln 5{,}8\left(\frac{\left(\frac{1}{\sqrt{0{,}92972}}-\frac{1}{\sqrt{2{,}41023}}\right)}{\frac{1}{\sqrt{0{,}92972}}-\frac{1}{\sqrt{1{,}72463}}}\right)$$ $\ln\frac{k_H}{k_T}=2{,}506198$ e quindi $\frac{k_H}{k_T}=12{,}3$

Unfortunately, the k_H/k_D kinetic isotope effect of 5.8 for the oxidation of zoniporide by aldehyde oxidase does not translate into a more complex system. The degradation rate of ^{2}H-zoniporide in human liver cells is only 1.9× lower than that of ^{1}H-zoniporide. This is because aldehyde oxidase is not the only enzyme involved in zoniporide catabolism. Nonspecific cellular hydrolases, as well as cytochrome P450 enzymes compete with aldehyde oxidase for the degradation of zoniporide.

27.9 Draw the two products of zoniporide hydrolysis by nonspecific cellular hydrolases.
Hint: Non-enzymatic aqueous alkaline hydrolysis under mild conditions would result in the same products.



Soluzione proposta da Mauro Tonellato – Padova