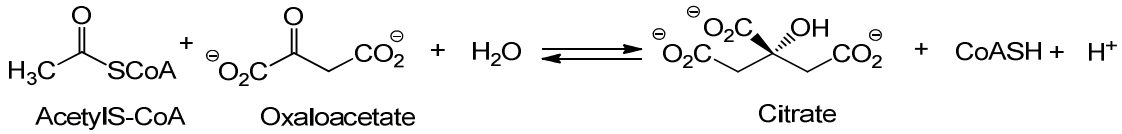
EXAM OF SCIENTIFIC CULTURE

CHEMISTRY

3.1. The Krebs cycle assures a large part of the energetic needs of the cell, thanks to the electrons present in the C-H and C-C bonds of acetylcoenzyme A (CH_3-CO-SCoA). Globally, this coenzyme is oxidized into carbon dioxide with the intervention of other coenzymes, such as FAD.

3.1.1 Complete the redox equation below and calculate the oxidation state of the carbon atoms of acetylcoenzyme A and carbon dioxide.

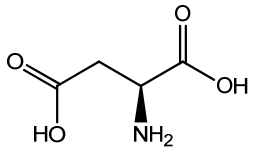
The first step in the Krebs cycle is catalyzed by *citrate synthetase*, as shown below:



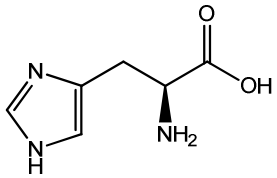
3.1.2 This step can be decomposed into three reactions: (a) enolisation of acetylS-CoA, (b) condensation of the formed enol onto oxaloacetate and (c) hydrolysis of the thioester function. Propose a mechanism for each reaction under acidic catalysis conditions.

Actually, an X-ray diffraction study of *citrate synthetase* has shown that two amino acids, the carboxylate of aspartate 375 and the imidazole of histidine 274, participate in the catalytic activity of the enzyme.

3.1.3 These residues taking part in the catalysis present lateral chains that can be ionized. The pKa values are: 2.1; 3.9 and 9.8 for aspartic acid and 1.7; 6.0 and 9.1 for the imidazole cycle of histidine. Assign these pKa values to the different acid-base functions of the amino acids shown below.



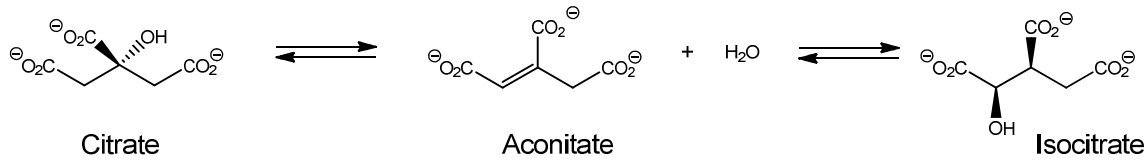
Aspartic acid



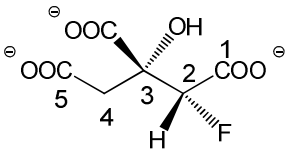
Histidine

3.1.4 Knowing that in the active site of the enzyme the lateral chains of aspartic acid and histidine are present as CO_2^- and ImH_2^+, respectively, and taking their pKa values as unchanged in the protein, indicate approximately at which pH the protein functions the best. Justify your answer.

Then the enzyme *aconitase* isomerizes citrate into isocitrate by forming aconitate as an intermediate:



One of the configuration isomers of 2-fluorocitrate, shown below, is an inhibitor of the enzyme aconitase.

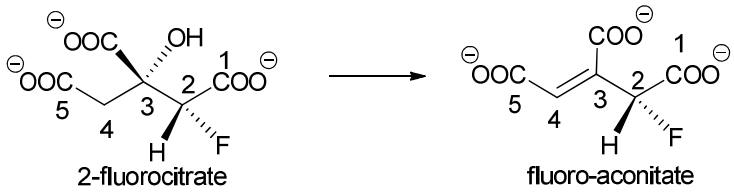


3.1.5 How many configuration stereoisomers exist of 2-fluorocitrate? Justify your answer.

3.1.6 This molecule presents three carboxylate groups. Which of the three functions is the less basic? Justify your answer.

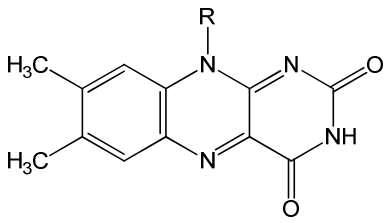
3.1.7 Indicate the absolute configuration of the asymmetric carbon atoms of 2-fluorocitrate. Justify your answer.

The 2-fluorocitrate undergoes several reactions until finally *in fine* a competitive inhibitor of *aconitase* is produced. The first reaction is a dehydration resulting in the formation of fluoro-aconitate.



3.1.8 What is the configuration of the double bond of fluoro-aconitate shown above? Justify your answer.

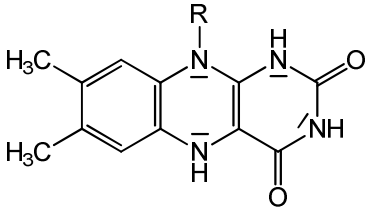
3.2 One of the important cofactors in the Krebs cycle is a flavine cofactor (FAD). Its structure is presented below:



3.2.1 Propose the hybridization state of the four nitrogen atoms of FAD and indicate the nature of the orbital containing the non-bonding electron pair.

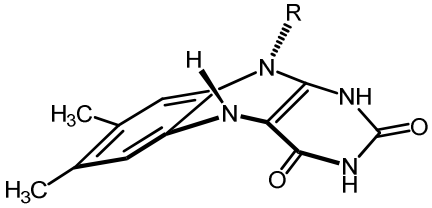
3.2.2 FAD is an aromatic compound. Justify this character by showing, among other possibilities, the number of electrons involved in the aromaticity.

FAD can be reduced into $FADH_2$ whose Lewis structure is shown below:

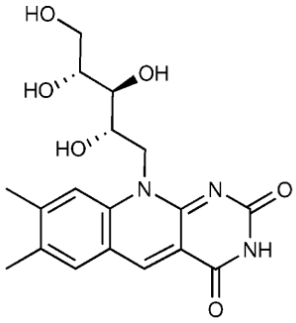


3.2.3 Assuming that the three cycles of $FADH_2$ are coplanar; would this tricyclic system be aromatic? Justify your answer.

3.2.4 Actually, the three cycles of $FADH_2$ are not coplanar. Indicate the state of hybridization of the four nitrogen atoms and the nature of the orbital containing the non-bonding electron pair.

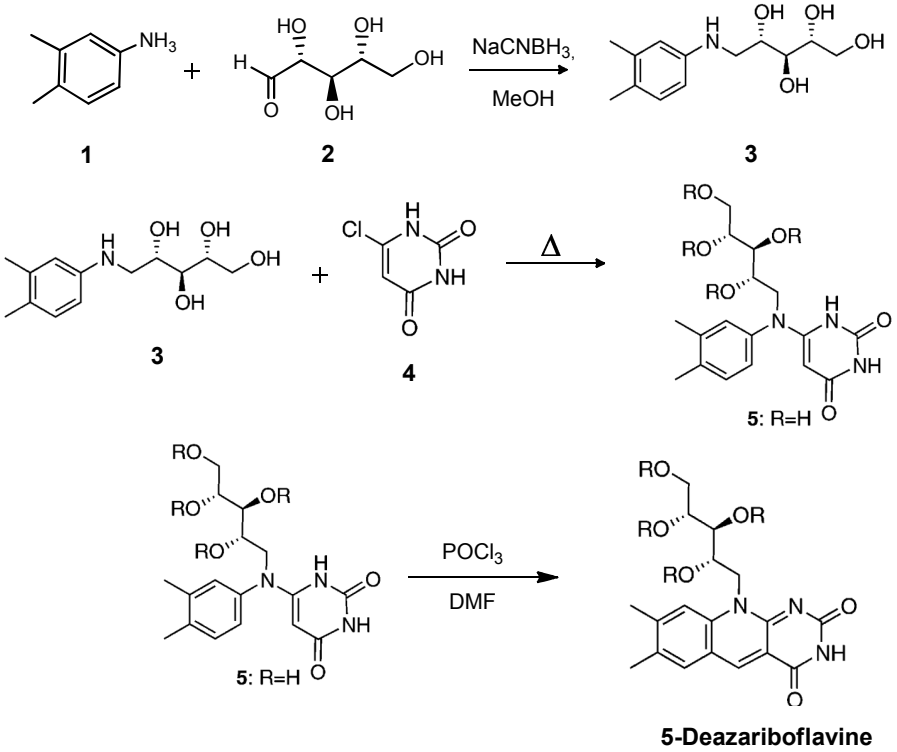


3.3 Flavin cofactors participate in catalysis *via* a large number of mechanisms. The synthesis of analogues of this molecule is therefore important for the study of these mechanisms. The 5-Deazariboflavine was synthesized with this aim:



5-Deazariboflavine

The general scheme of the synthesis of 5-Deazariboflavine is shown below:

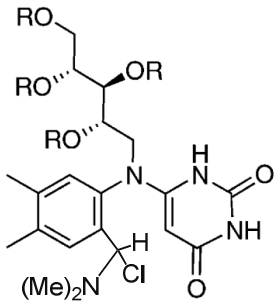


3.3.1 Write the mechanism of the transformation of **1** and **2** into **3**. What is the role of $NaCNBH_3$ in this reaction? Explain.

3.3.2 Identify the most electrophilic carbon in 6-chlorouracil **4**. Justify your answer.

3.3.3 Write the mechanism responsible for the coupling between **3** and **4** to form **5**.

In the last step, **5** is transformed into 5-Deazariboflavine. During this step an intermediate **I** is formed, shown below:



I

The reactive **I',** species responsible for the formation of this intermediate **I** through reaction with **5,** is formed *in situ* by reaction between DMF ($(CH_3)_2NCHO$) and $POCl_3$.

3.3.4 Write the mechanism of this reaction occurring between $P(=O)Cl_3$ and DMF giving **I'**.

3.3.5 Write the reaction between **I'** and **5** resulting in the formation of the intermediate **I**.

3.3.6 Propose a mechanism for the last step resulting in the formation of 5-Deazariboflavine.