

## Bilirubin: E-/Z-, But Not Easy

by

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Erin and Angelo listened closely to their guest seminar speaker, Professor Rita Barth, as she concluded her talk. She had certainly lived up to her advanced billing. Her explanation of the importance of understanding medicine at a molecular level was filled with interesting and insightful examples. Together, Erin and Angelo had taken both organic chemistry and biochemistry, and Professor Barth's talk helped them to see how important the concepts from these courses were to understanding the chemistry and biochemistry that underlies medicine at a molecular level. Since they both planned to pursue careers in medicine, her presentation was of great interest to them.

At the conclusion of her talk, Professor Barth offered to linger for a while so that she could speak with anyone in the audience who had questions or concerns that she could address. In addition to being extremely knowledgeable, she seemed warm, friendly, and approachable, so the two students decided to speak with her about their future plans and their interest in her work.

Erin approached Professor Barth and said, "Hello, Professor Barth, my name is Erin and this is my friend Angelo. We enjoyed your talk a lot. We are both interested in chemistry and want to attend medical school. We were wondering if you might have a problem that we could help you with. We have both taken organic chemistry and biochemistry and would love to put some of the ideas from those courses to work on a real-life problem."

"It's a pleasure to meet you, Erin and Angelo. I am so glad you enjoyed my talk. I have to try to keep myself in check when I discuss that topic. I love it so much that I have to be careful not to get carried away and rant on and on."

"Well, you certainly didn't do that today," said Angelo. "Your examples were great. They showed us how lots of the ideas from our chemistry and biochemistry courses are useful in medicine."

"So, you two are interested in applying some of the concepts that you have learned in class to a real-life medical problem. That's great. I do have a problem that I'd love to have you look into, and your background should allow you to do so. Would it be possible for you to meet me in my office at the University Hospital at nine o'clock on Saturday morning so that we could discuss it further?"

Erin and Angelo eagerly agreed.

When the students arrived at the hospital on Saturday morning Professor Barth greeted them warmly. Once they were seated in her office, each sipping a cup of coffee, she started to explain the problem she had mentioned.

“You are probably aware,” she began, “that newborn infants are often jaundiced and require phototherapy treatment with visible light in order to overcome that condition and obtain their normal skin color.”

“I’ve heard about that,” said Erin, “but I don’t know anything about the cause of the jaundice or what the light treatment does to clear it up.”

“I’m not surprised that you don’t, Erin. Let me try to explain. The jaundice arises from the fact that hemoglobin present in the infant’s body undergoes decomposition to form a yellow-orange compound called bilirubin. If the newborn’s liver is immature, it can’t process and remove the bilirubin from its body as a fully developed liver would. The build-up of bilirubin in the infant’s body causes the yellow skin color that we call jaundice. If the bilirubin is not removed and becomes too concentrated, it can do serious, life-long-lasting harm to the infant.”

“How does the light treatment allow the body to get rid of the bilirubin?” asked Angelo.

“You are familiar with geometric isomers, aren’t you?”

“Sure,” said Erin. “We called them cis/trans and Z/E isomers too.”

“Right, ‘cis/trans isomers’ is the term used when there are like groups present on the adjacent carbon atoms of a double bond or ring, and ‘Z/E isomers’ is used in more complex situations—when like groups aren’t present, and the simple cis/trans method can’t be used.”

“I remember that we had to assign priorities to the groups attached to the double bond so we could decide whether a compound was a Z- or E-isomer,” said Angelo.

“Well, geometric isomerism has a lot to do with the answer to your question, Angelo. Radiating an infant with visible light (we call it phototherapy) provides the energy needed to allow the naturally occurring bilirubin isomer formed by the degradation of hemoglobin to change into a different geometric isomer. The second isomer is more soluble in bile, urine, and feces than the first, so it is more easily eliminated from the infant’s body.”

“I see,” said Erin. “Changing the bilirubin that forms in the infant’s body into a more soluble isomer allows it to be removed from the body without the liver having to do the whole job.”

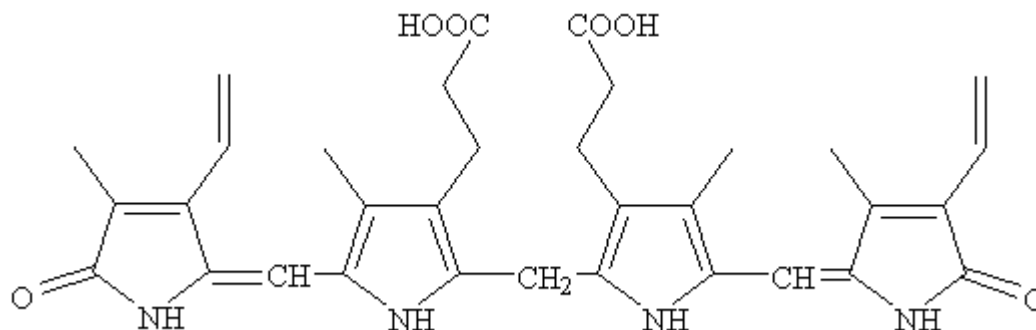
“You’ve got it, and that brings us to the problem that you two could give me a hand with.”

“Sure, that’s what we’re here for. What can we do?” asked Angelo, eagerly.

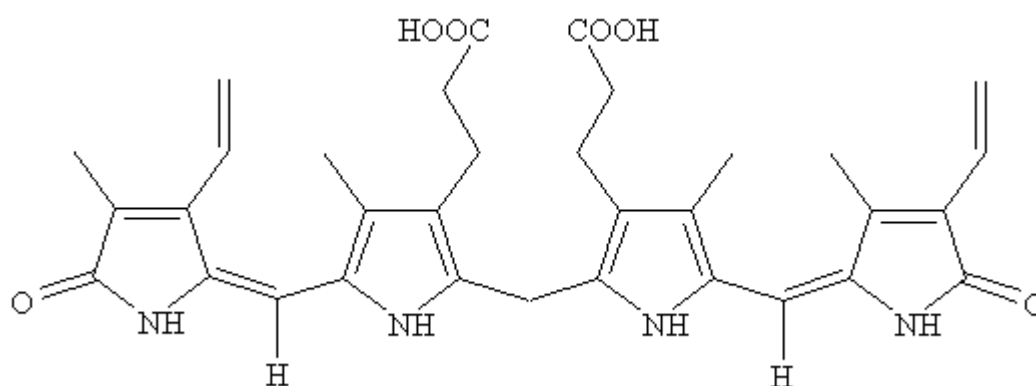
“You can help sort out a lot of confusion that exists in the biochemical literature and in some of the best biochemistry textbooks about the structure of bilirubin, and the geometry and nomenclature of its two geometric forms; the one formed from the degradation of hemoglobin, and the more soluble isomer that forms after phototherapy.”

Professor Barth went to a filing cabinet, pulled out a folder, and withdrew several sheets of paper from it. The students could see drawings of the structures of a number of heterocyclic organic molecules on the pages.

Pointing to the structures, Professor Barth said, “The structure of bilirubin was originally established by a Noble Laureate, Hans Fischer, in 1942. He knew that bilirubin could exist in geometric isomeric forms, and since he didn’t know whether the bilirubin that he had isolated existed as the Z,Z- or E,E- form or, perhaps, in some combination of these forms, he always drew it in a linear form like this, one that didn’t indicate the geometry about the double bonds.”



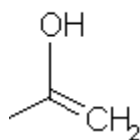
“Years later, one of Fischer’s students, Rudolph Lemberg, studied the naturally occurring form of bilirubin and claimed that it exists in the Z,Z- form. If you look in, for example, the widely used biochemistry text authored by Lenninger, or in a very important recent paper in the biochemistry literature that establishes bilirubin as a key molecule in a biochemical cycle that protects us against free radicals, you will find this structure drawn for bilirubin. It implies that bilirubin exists in the E,E- isomeric form.” Professor Barth pointed to the structure shown below:



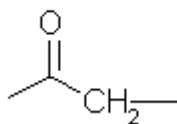
“And there’s also another problem with bilirubin’s representation in reference sources. This one concerns tautomerism in the two terminal pyrrole rings of bilirubin. Here, let me show you what I mean.”

Professor Barth pointed to some additional structural formulas and said, “I’m sure that you are familiar with enol-keto tautomerism in carbonyl compounds, but you may be less aware that tautomers also exist for amide carbonyl groups. Amide tautomers involve the nitrogen atom and are called lactim and lactam forms. They look like these structures.”

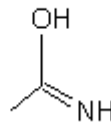
Enol form



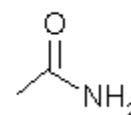
Keto form



Lactim form

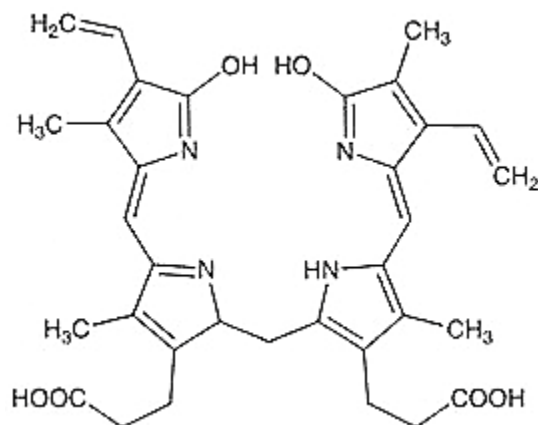


Lactam form



“The problem is,” she continued, “that some texts show the two terminal pyrrole rings of bilirubin in the lactim form and others show them in the lactam form. For example, the 12<sup>th</sup> edition of *The Merck Index* shows bilirubin’s pyrrole rings in the lactim form, and you have already seen that Lenninger’s textbook shows them in their lactam form.”

Professor Barth showed Angelo and Erin the following representation of bilirubin’s terminal pyrrole rings as they appear in the *Merck Index*:



Erin said, “I’m surprised that all of this confusion exists in the biochemical literature, but what would you like us to do?”

“Good question, Erin. I would like you two to double check the literature and find the correct structure for the bilirubin isomer that forms in the body when hemoglobin decomposes, and the structure of the bilirubin isomer that forms after phototherapy. Also, I’d like you to clear up the ambiguity about the lactim versus the lactam forms for the pyrrole rings present in bilirubin.”

“We’d like to give it a try,” said Angelo.

“Great,” said Professor Barth. “I’m glad that you’re interested. A couple of other things would also be helpful. When you have the structures figured out, please assign priorities to the substituents present on the bilirubin double bonds, and then draw correct structures for the two forms of bilirubin and label them as the Z,Z- and E,E-isomers.”

Erin asked, “When you say draw correct structures for bilirubin, do you mean structures that show the correct geometry about the double bonds?”

“Yes, Erin, that’s exactly what I mean. Also, be sure that you have the correct lactim or lactam form drawn for the terminal pyrrole rings.”

Angelo said, “We’ll get right at it, but could I ask what you’ll do with this information when we have gotten it all together?”

“When you have done these things I will ask you to write a brief paper that I can use with my first year medical students. By correctly explaining and identifying the bilirubin structures, your paper will be a big help to them. It will clear up the ambiguity that exists in the literature, and will help their understanding of the hemoglobin-bilirubin-phototherapy process.”

“We’ll do our best, Professor Barth, and we’ll get back to you as soon as possible.”

“That’s great,” Professor Barth replied. “I will be looking forward to your findings.”

## Erin and Angelo’s Findings and Your Assignment

After researching the primary literature, Erin and Angelo uncovered the following information about bilirubin:

1. The decomposition of hemoglobin in the body results in the formation of the *Z,Z*-isomer of bilirubin.
2. Phototherapy results in the conversion of the *Z,Z*-isomer of bilirubin into the *E,E*-isomer.
3. The two terminal rings of bilirubin exist in the lactam, not the lactim form.

Based on these findings, you should complete each of the following tasks:

1. Assign Cahn-Ingold-Prelog priorities to each of the substituent groups attached to the double bonds substituted on the terminal pyrrole rings of bilirubin.
2. Use these priorities to draw *geometrically correct* structures for the *Z,Z*-isomer, the *E,E*-isomer, and an *E,Z*-isomer of bilirubin and label each.
3. Draw both the lactim and lactam forms for each of the terminal pyrrole rings of bilirubin and properly label each. Pay attention to the placement of the double bonds in the rings.
4. Suggest a reasonable mechanism by which photolysis of the *Z,Z*-isomer of bilirubin allows it to be changed into the *E,E*-isomer, and offer an explanation of why this change may occur.
5. Write a brief note to the publishers of Lenninger’s biochemistry text and *The Merck Index* clearly indicating what is wrong with the representation(s) of bilirubin that appear in their publications and suggesting the corrections that should be made to subsequent editions of these books.

**Date Posted:** 10/17/03 nas

Originally published at <http://www.sciencecases.org/bilirubin/bilirubin.asp>

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