

Compounds identified in the secretions of *C. ceriferus* and *C. rubens*

Constituents	Content on GLC <i>C. ceriferus</i>	<i>C. rubens</i>	[ $\alpha$ ] <sub>D</sub> Obs. in CHCl <sub>3</sub> * <i>C. ceriferus</i>	<i>C. rubens</i>	Lit. value** (Ref.)
20. Cadina-1,4-diene	+	+			
21. Calamenene	+		+ 54°		— 67° <sup>10</sup>
22. C <sub>15</sub> H <sub>24</sub> O		+			
23. $\alpha$ -Calacorene	+				
24. $\beta$ -Calacorene	+				
25. Caryophyllene-oxide	+				
26. Humulene-epoxide-I	+				
27. Humulene-epoxide-II	+				
28. Cubenol	+	+	+ 37°		— 24.8° <sup>11</sup>
29. Epi-cubenol	+	+			
30. Neointermedeol	+	+	+ 3.4°		+ 8° <sup>12</sup>
31. Seli-11-en-4( $\alpha$ )-ol	+		+ 32.7°		— 18° <sup>13</sup>
Standards (see text)					
32. Dihydrobourbonene			— 7.7°		— 6.4° <sup>8</sup>
33. Elemene			+ 5°		— 4.8°
34. Selinane			— 20°		+ 12.5°

\* Since the optical rotations of the same compounds from a given insect on different host trees were nearly the same, the highest rotations were adopted. \*\* Unless otherwise referenced, the optical rotations are taken from Beilstein. \*\*\* Only the sign was observed at 589, 546, 436 and 365 nm, because of the limited sample size.

crene-D and  $\beta$ -bourbonene from *C. ceriferus* were of the enantiomeric series compared with those from common terrestrial plants<sup>4,6</sup>.

During the last decade, the presence of some cedrene-type sesquiterpenoids was reported in the scale insect *Laccifer lacca* Kerr<sup>7</sup>. As regards the origin of these substances, it seems very likely that they are metabolites of vegetable origin. Though the biosynthetic route to sesquiterpenoids in *C. ceriferus* and *C. rubens* is not yet known, it seems reason-

able to conclude from the present results that each insect possesses a different set of enzymes acting in the cyclization process to produce epimeric isopropyl groups. Therefore, these insects can presumably synthesize sesquiterpenoids at least from the stage of cyclization to produce a pair of 10-membered cyclic intermediates such as A and B in the hypothetical route shown in the scheme. Enzymatically controlled 1,3-hydride shift to the cationic center on the isopropyl group may then lead to the end products.

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Glutathione peroxidase in erythrocytes and plasma of rats during pregnancy and lactation<sup>1</sup>

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**Summary.** The glutathione peroxidase activity in the plasma of rats on the 20th day of pregnancy was found to be 50% lower than in nulliparous control animals. During lactation, the level rose again but was still significantly different from that of the control rats on the 20th day post partum. The erythrocyte enzyme activity remained unchanged.

The selenium metabolism of rats was found to be affected after mid-pregnancy. In the serum, the element content began to drop on the 12th day of gestation and reached its lowest value shortly before term<sup>4,5</sup>. During lactation, it rose again to its original level. When <sup>75</sup>Se-selenite was administered to rats, a significant decrease in the <sup>75</sup>Se-radioactivity in the erythrocytes was observed at the end of pregnancy as compared with nulliparous controls<sup>6</sup>. Because of the presence of selenium in glutathione peroxidase (GSH-Px)<sup>7,8</sup>, it

was of interest to investigate whether the metabolism of this enzyme is also influenced during the reproductive processes in female rats. Therefore the GSH-Px activity in the plasma and erythrocytes was measured during pregnancy and lactation.

**Experimental.** The study was carried out under the same conditions as described earlier<sup>5</sup>: 20 female 'Carworth' rats with body weights of 250 g were kept under standardized laboratory conditions and fed Altromin® rat pellets with a

selenium content of 0.3 ppm and tap water ad libitum; 10 rats served as controls. The other 10 were mated. When sperms were seen the next morning, this day was considered as day 1 of the experiment. After delivery on day 23 the litters were reduced to 8 young each.

On day 1, 10, 20, 27 (day 4 post partum) and on day 43 (day 20 post partum) samples of 1 ml of blood were taken from the orbital venous plexus under appropriate ether anesthesia and each mixed with 50  $\mu$ l of a 0.2 moles/l EDTA-solution. The erythrocytes and the plasma were separated by centrifugation.

The GSH-Px assay was performed using the coupled test procedure, which was developed by Paglia and Valentine<sup>9</sup> and improved by Günzler et al.<sup>10</sup>. In this method, the decrease in NADPH concentration which is proportional to the enzyme activity, is measured photometrically. The activity was expressed as  $U(37^\circ C) = 0.868 \times \Delta [NADPH] / [GSH]_0 \times \min^{11}$  which corresponds to  $U(37^\circ C) = \Delta \lg [GSH] / \min^{12}$ . For the determination of GSH-Px activity in the erythrocytes, the cells were hemolyzed in bidistilled water and the hemoglobin was transformed into cyanmethemoglobin. The enzyme activity in the plasma was measured after dilution with 25 mmol/l potassium phosphate buffer pH 7.0 without any further treatment. The concentration of hemoglobin in the hemolysate was determined by means of the cyanmethemoglobin method<sup>13</sup>.

**Results and discussion.** In the erythrocytes, the GSH-Px activity as calculated per volume of whole blood decreased after day 10 of pregnancy and rose again slowly after parturition (figure, b). However, when calculated for the amount of hemoglobin (figure, a) or for the hematocrit, no differences could be observed between the control and the mother animals. This means that the change was only

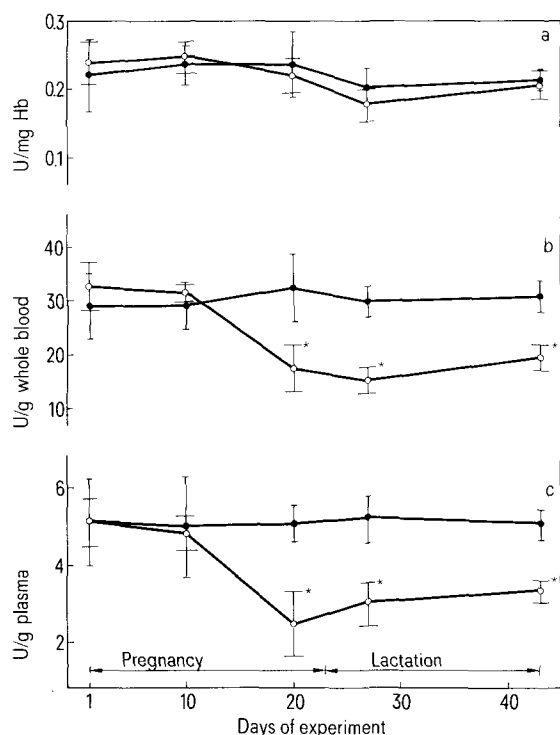
caused by the decrease in the hematocrit after mid-pregnancy and during lactation and that the GSH-Px concentration in the erythrocytes therefore remained constant.

In the plasma, the enzyme activity dropped after day 10 of pregnancy and was 50% lower on day 20 than that in the nulliparous control rats (figure, c). Although the activity increased again during lactation, it was still significantly reduced on day 20 post partum as compared with the control animals ( $p < 0.001$ ).

A similar effect as in the rat was also observed in man. In women in the last trimester of pregnancy, the plasma selenium level as well as the plasma GSH-Px activity was significantly decreased as compared with a nonpregnant control group<sup>14</sup>.

A positive correlation ( $r = 0.98$ ) was found in the rat during pregnancy and lactation between the erythrocyte GSH-Px per volume of whole blood and the enzyme activity in the plasma. Whether this finding reflects a relation between the total amount of the enzyme in the plasma and in the red blood cells remains to be seen.

The decrease in the serum selenium concentration from day 12 onwards coincides with the drop in the GSH-Px level in the plasma after day 10, but cannot be explained solely by this change. Assuming that the enzyme isolated from bovine blood (activity 500  $U_{37}/mg$  enzyme<sup>15</sup>) is identical with the enzyme in the erythrocytes (activity 0.25  $U_{37}/mg$  hemoglobin = 60  $U_{37}/g$  erythrocyte wet weight) and in the plasma (activity 5  $U_{37}/g$  plasma) of the rat, the amount of the GSH-Px in these fractions can be calculated via the activities and from that the amount of enzyme-bound selenium. It follows that in the red blood cells of the rat with a selenium content of 0.4  $\mu g/g$  wet weight nearly all of the element is contained in the GSH-Px, whereas in the plasma with likewise 0.4  $\mu g$  Se/g plasma only 10% of the selenium is bound to the enzyme. Consequently, a change in the GSH-Px level in the plasma only has a slight influence on the plasma selenium concentration. The decrease in the element content to 70% of the original level on day 20<sup>5</sup> therefore shows that not only the level of GSH-Px but also that of other selenium compounds in the plasma is affected after mid-pregnancy.



GSH-Px activity (mean  $\pm$  SD) in the erythrocytes and plasma of pregnant and lactating rats (○) and of nulliparous control animals (●). a Erythrocyte enzyme activity calculated per mg hemoglobin. b Erythrocyte enzyme activity calculated per volume of whole blood. c Plasma enzyme activity calculated per volume of plasma.

\* Mean significantly different from that of controls ( $p < 0.001$ ).

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