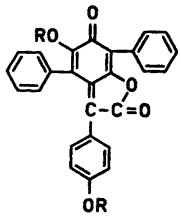

I: R = $COCH_2Br$
II: R = H

that xylerythrin is also closely related to polyporic acid (2,5-diphenyl-3,6-dihydroxy-1,4-benzoquinone) another fungus pigment.[6] Its structure can, at least formally, be constructed from one molecule of polyporic acid and one molecule of *p*-hydroxyphenylacetic acid.

Of the other pigments of the fungus, pigment A has the composition $C_{27}H_{18}O_5$ with one methoxyl group. Methylation gave a monomethyl ether, identical with xylerythrin dimethyl ether. Pigment A is thus one of the two possible monomethyl ethers of xylerythrin.

Pigment C has the composition $C_{26}H_{16}O_6$ and its spectral properties suggest that it is a hydroxy derivative of xylerythrin. Pigment D has not yet been completely characterised, but its optical properties are similar to those of the other pigments, suggesting a close relationship to them.

It is hoped to publish a full account of the isolation of the pigments, the chemical reactions of xylerythrin and the determination of the complete structure of the other pigments at a later occasion.

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1. Abrahamsson, S. and Innes, M. *Acta Chem. Scand.* **19** (1965) 2246.
2. Brown, J.P., Robertson, A., Whalley, W.B. and Cartwright, N.J. *J. Chem. Soc.* **1949** 867.
3. Roberts, J.C. and Warren, C.W.H. *J. Chem. Soc.* **1955** 2992.
4. Barton, D.H.R. and Hendrickson, J.B. *J. Chem. Soc.* **1956** 1028.
5. McOmie, J.F.W., Tute, M.S., Turner, A.B. and Kullimore, B.K. *Chem. Ind. (London)* **1963** 1689.
6. Kögl, F. *Ann.* **447** (1926) 78.

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# A Study of the Ninhydrin-Positive Components Derived from Cystine during the Cyanide-Nitroprusside Test

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The cyanide-nitroprusside test, or Brand's test,[1] has frequently been used for the detection of an increased amount of cystine in the urine. The test depends upon the fact that cystine (CSSC) is reduced by sodium cyanide (NaCN) to its sulphydryl derivative, cysteine (CSH), and the latter reacts with sodium nitroprusside (NaNP) to give a magenta red colour in slightly alkaline medium. In practice the procedure has the disadvantage of involving a poison, NaCN, and in laboratories without adequate ventilation systems it is potentially hazardous to carry out large series of Brand's tests, as in screening examinations for the detection of cystinuria.[2-5]

In order to minimize the hazard, sodium hydridoborate was tested instead of NaCN as a reducing agent for CSSC. Sodium hydridoborate is known to have a low toxicity and its water solutions are capable of reducing CSSC into two moles of CSH. The reduction of CSSC by NaCN, however, has been reported to produce only one mole of CSH per mole of CSSC, the other half of the CSSC molecule being converted *via* α-amino-β-rhodanpropionic acid into a cyclic compound, 2-aminothiazoline-4-carboxylic acid or its tautomer, 4-carboxythiazolidon-2-imide,[6,7] which do not take part in the colour reaction with NaNP. It was found that the reduction of CSSC took place rapidly when sodium hydridoborate was used and the nitroprusside reaction yielded about the same colour intensity by visual comparison, as when NaCN was used as reducing agent. However, the colour produced when sodium hydridoborate was used faded very quickly. This is in agreement with the finding that fading of the colour developed by the nitroprusside reaction can be inhibited by addition of cyanide.[8,9] In order to obtain some qualitative and quantitative data regarding the ninhydrin-positive components produced during the reaction be-

tween CSSC, NaCN and NaNP, the following study was carried out by means of an automatic amino acid analyzer.

*Experimental.* The analysis of the ninhydrin-positive compounds was performed according to the method described,[10] by means of an automatic amino acid analyzer.[11] The results of the chromatographic runs were evaluated according to the height-times-width method [10] and the readings were taken at 440 mμ and 570 mμ wavelength. As reference solutions 0.01 M of CSH-hydrochloride and CSSC were used. For CSH the correlation coefficient between the area and micromoles could only be determined indirectly since CSH in the solution applied to the column had already been oxidised to CSSC to some extent. The test mixtures contained 0.1 mmole of CSH and CSSC, respectively. NaCN was added in equimolar amounts (0.1 mmole) and NaNP 0.02 mmole in order to meet the conditions in the Brand's test.[1] The mixture was made slightly alkaline by the addition of 0.1 ml of concentrated ammonia and the final volume of the test mixtures was adjusted to 10 ml with distilled water. Aliquots of 0.5–1.0 ml were applied to the 150 cm column of the automatic amino acid analyzer and the analyses were run using two different 0.067 M sodium citrate buffers of pH 3.25 and 4.25, respectively.[11] Table 1 gives the composition of the samples analysed through experiments Nos. 1 to 6. The time interval from mixing the components until an aliquot was applied on the column was 15 min. A duplicate of sample No. 6 was also analysed after 24 h. CSSC and CSH were obtained from Merck Co. Rahway, N.J. The chemicals for the automatic amino acid analyzer were obtained from Pierce Chemical Co., Rockford, Ill.

As seen in Table 1 (Expt. No. 1) it is clear that if freshly prepared solutions of commercial CSH are used, two peaks are always obtained. The variation of the peaks seems to indicate that before the chromatographic separation some oxidation of CSH to CSSC had occurred. Experiment No. 2 was designed to show that on the addition of NaCN to CSH no additional peaks were formed. However, a new peak ($\gamma$) did appear, which was also present when CSSC was treated with NaCN (Expts. Nos. 5 and 6). This finding might be explained by partial reoxidation of CSH into CSSC in Expt. No. 2, and a subsequent cleavage of CSSC thus formed into CSH and α-amino-β-rhodan-propionic acid, which in turn cyclizes to thiazolidon-2-imide and 2-aminothiazoline derivatives.[6] This seems to be confirmed in Expts. Nos. 5 and 6 where pure CSSC was used and the corresponding compounds were found at $\gamma$ and $\delta$ peaks. No other ninhydrin-positive peaks were found in the chromatograms after the $\delta$ peak, except the ammonia peak which appears about 24 h after the delta peak.

It can be seen in Expt. No. 2 that total recovery was approximately 80 % for the amino acids in question, the rest probably being converted to the $\gamma$ peak assuming 100 % recovery. Quantitative evaluation of the $\gamma$ peak was not possible since the correlation coefficient for this compound could not be accurately determined. Support for a cyclic derivative is seen in the absorbance readings, where the $\gamma$ peak gives the highest optical

*Table 1.* Absorbance readings of the peaks and recovery of CSH and CSSC in the samples analysed.

| Expt. No. | Components | Absorbance readings | | | | | | μmoles recovered | |
|---|---|---|---|---|---|---|---|---|---|
| | | $\alpha^a$ | $\beta^a$ | $\gamma^b$ | CSH[a] | CSSC[a] | $\delta^a$ | CSH | CSSC |
| 1 | CSH a. | — | — | — | 26.2 | 5.9 | — | 9.4 | 0.25 |
| | b. | — | — | — | 22.0 | 24.9 | — | 7.4 | 1.3 |
| 2 | CSH + NaCN + $NH_3$ | — | trace | 19.2 | 19.8 | 11.8 | trace | 6.9 | 0.6 |
| 3 | CSH + NaNP + $NH_3$ | 7.0 | 15.6 | trace | 26.5 | 19.4 | — | 9.2 | 1.0 |
| 4 | CSSC + $NH_3$ a. | — | — | — | — | 190 | — | — | 9.9 |
| | b. | — | — | — | — | 194 | — | — | 10.1 |
| 5 | CSSC + NaCN + $NH_3$ | — | trace | 16.2 | 6.3 | 134.5 | 3.8 | 2.2 | 7.1 |
| 6 | CSSC + NaCN + NaNP + $NH_3$ | | | | | | | | |
| | a. after 15 min | — | 26.8 | 28.0 | 3.3 | 148.2 | 3.4 | 1.1 | 7.8 |
| | b. after 24 h | — | 25.0 | 8.1 | 2.8 | 83.1 | 8.5 | 1.0 | 4.4 |

[a] Absorbance readings at 570 mμ wavelength; [b] absorbance readings at 440 mμ wavelength.

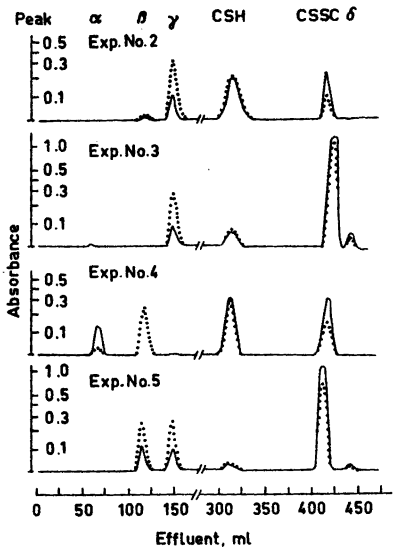


*Fig. 1.* Recording of the ninhydrin-positive peaks from different experiments. ··· Absorbance at 440 mμ wavelength, — absorbance at 570 mμ wavelength.

absorbance reading at 440 mμ wavelength, thus indicating a colour similar to that obtained between proline or hydroxyproline, and ninhydrin. The δ peak, on the other hand, gives the highest optical density at 570 mμ, as the amino acids usually do with ninhydrin (Fig. 1). These findings support the view that the δ peak represents the substance 2-aminothiazoline-4-carboxylic acid and the γ peak the substance 4-carboxy-thiazolidon-2-imide. The position of the peaks in the chromatograms are also in agreement with this hypothesis.

From Expt. No. 3 it is evident that the γ peak is absent when CSH is treated with NaNP only, and a β peak appears. This peak must be due to NaNP, as is the similar situation recorded in Expt. No. 6.

The total recovery in Expt. No. 3 was approximately 100 % for CSH and CSSC, further indicating that the α and β peaks are derived from the reagent. CSSC gives only one peak, as seen in Expt. No. 4. By the addition of NaCN to CSSC the cleavage described in the literature [6,7] is demonstrated (Expts. Nos. 5 and 6) by the formation of the γ and δ peaks. Since the total recovery of CSH and CSSC was approximately 85 % of the original CSSC, this may be due to the fact that when equimolar amounts of NaCN and CSSC are mixed only 15 % of CSSC is reduced. This is in agreement with the findings of several semi-quantitative studies [12,13] and may be the main reason why in Brand's test a large excess of NaCN is required.

Although using entirely different methods of analyses, the present results confirm in principle the earlier findings in respect to the components produced during the cyanide-nitroprusside test. Due to the complexity of the reaction, similar studies under the influence of several other reducing agents will be necessary in order to establish the superiority of cyanide in this splitting reaction.

1. Brand, E., Harris, M. M. and Biloon, S. *J. Biol. Chem.* **86** (1930) 315.
2. Lewis, H. B. *Ann. Internal Med.* **6** (1932) 183.
3. Dent, C. E. and Harris, H. *Ann. Eugen. (London)* **16** (1951) 60.
4. Boström, H. and Tottie, K. *Acta Paediat.* **48** (1959) 345.
5. Boström, H. and Hambraeus, L. *Acta Med. Scand.* (1964) *Suppl.* 411.
6. Schöberl, A., Kawohl, M. and Hamm, R. *Chem. Ber.* **84** (1951) 571.
7. Catsimpoolas, N. *Diss. Abstr.* **25** (1965) 6924.
8. Fujita, A. and Iwatake, D. *Biochem. Z.* **277** (1935) 284.
9. Herrmann, H. and Moses, S. G. *J. Biol. Chem.* **158** (1945) 33.
10. Spackman, D. H., Stein, W. H. and Moore, S. *Anal. Chem.* **30** (1958) 1190.
11. Hambraeus, L. *Svensk Kem. Tidskr.* **75** (1963) 638.
12. Hata, T. and Matsushita, S. *Mem. Res. Inst. Food Sci. Kyoto Univ.* **9** (1955) 30.
13. Grunert, R. R. and Phillips, P. H. *Arch. Biochem. Biophys.* **30** (1951) 217.

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