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## Free Radicals in Some Reactions of Ninhydrin

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The similarity between ninhydrin and alloxan has been stressed by many authors<sup>1</sup>. Both substances give rise to coloured products in reactions with amino acids. The characteristic product obtained from ninhydrin and amino acids, *i.e.* "Ruhemann's Purple", is considered to have a structure analogous to that of the alloxan derivative murexide. Alloxantin, a substance derived from two molecules of alloxan, has its counterpart in hydrindantin formed by two molecules of ninhydrin.

By the technique of electron spin resonance (ESR) the present authors<sup>2</sup> have recently shown that free radicals are formed when alloxan is reduced by thiol compounds such as glutathione or cysteine. In view of this result and the analogy between alloxan and ninhydrin, a search has been made for free radicals in the reactions of the latter substance. This note describes some experiments in which free radicals derived from ninhydrin were found.

*Experimental.* The radical spectra were obtained by a Varian 100 kc spectrometer. All runs were performed at ambient room temperature with the samples contained in a flat aqueous solution cell. The magnetic field was calibrated by the hyperfine splitting field (13.0 gauss) of the radicals derived from peroxyaminodisulphonate. Standard barbiturate and phosphate buffers were used in all experiments. pH of the solutions was measured by standard technique. All reactions with ninhydrin were performed at room temperature.

*Results.* (i) Free radicals were obtained in buffer solutions of ninhydrin and amino acids. The radical concentration was found to be high, especially in an alkaline medium. No radicals could be detected below pH 7.

(ii) There seems to be no correlation between the radical concentration and the intensity of the purple colour developed in the reaction between ninhydrin and amino acids. No radicals could be detected in an aqueous solution of "Ruhemann's Purple" synthesized as described by MacFadyen<sup>3</sup>.

(iii) When an equimolar amount of sodium dithionite\* was added to a solution of ninhydrin, a high yield of free radicals was obtained. As in the reactions with amino acids, the radical content was higher in an alkaline medium than in a neutral one. No radicals could be detected in a reaction mixture of a pH value lower than 6.5.

\* When dithionite is used for the production of free radicals, an excess of this substance should be avoided, as radicals derived from dithionite are sometimes obtained under such a condition. The ESR-spectrum of the dithionite radicals exhibits a very narrow single line. Free radicals from sodium dithionite have been described by Hodgson *et al.*<sup>4</sup>, but the possible occurrence of "false" radicals in reaction mixtures containing dithionite seems not always to have been considered.

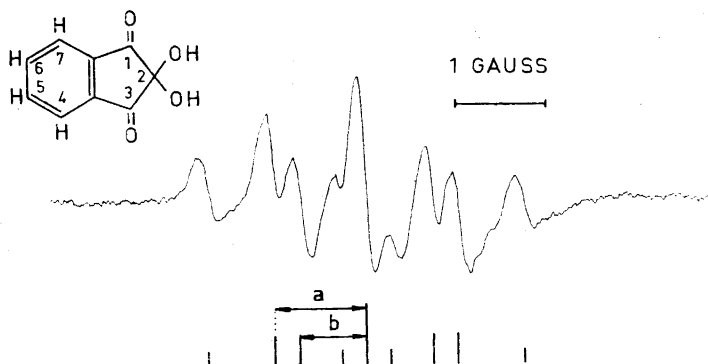


Fig. 1. ESR-spectrum of the free radicals obtained from ninhydrin (0.02 M) and glycine (0.02 M) dissolved in a barbiturate buffer of pH 8.6. The suggested origin of the spectrum is schematically given below the recorded spectrum.

(iv) A high yield of free radicals was obtained when glyoxal was added to a slightly alkaline solution of ninhydrin. No radicals could be detected after the addition of acetaldehyde.

(v) The solutions in which radicals had been produced by dithionite and glyoxal respectively, exhibited a reddish-brown colour. When shaken in an open vessel, the colour of these solutions faded, and at the same time it was noted that the radical concentration had decreased. When kept in a stoppered sample cell, however, the solutions were found to contain a rather high concentration of free radicals for more than two days after filling with a freshly prepared reaction mixture.

(vi) No radicals could be detected when the solutions contained only ninhydrin.

(vii) Identical ESR-spectra of the radicals were obtained in the reactions of ninhydrin with amino acids, dithionite and glyoxal respectively. These spectra exhibited a nine-line structure (Fig. 1). In order to achieve maximum resolution, a rather low microwave power (15 db. down) had to be used. The resolution was not improved by degassing of the reaction mixtures.

(viii) When the reduction of ninhydrin by dithionite was performed in  $D_2O$  instead of  $H_2O$  an identical spectrum was obtained.

*Discussion.* It is believed that the radicals obtained in the reactions here described are derived from ninhydrin. The ESR-spectra are tentatively connected to the interaction of the unpaired electron with

the four protons attached to the benzene ring of ninhydrin. It is suggested that the two equivalent protons in the positions 4 and 7 (see Fig. 1) split the original single line into three with a hyperfine splitting field of 1.0 gauss ( $a$  in Fig. 1). These three lines are each split into three to a total number of nine lines with a splitting field of 0.75 gauss ( $b$  in Fig. 1) by the two equivalent protons in the positions 5 and 6. As an identical spectrum was obtained when the reaction was performed in  $D_2O$  instead of  $H_2O$  and provided that a rapid equilibrium exchange of the hydroxyl protons takes place, it is evident that these protons do not contribute to the splitting.

As evidenced from the reactions with dithionite and glyoxal, the radicals seem to be formed by an one-electron reduction of ninhydrin. It is well known that reducing substances such as aldehydes are formed in the reaction of ninhydrin with amino acids. Also, the production of glyoxal derivatives of the corresponding amino acids have been postulated in the ninhydrin reaction<sup>5</sup>. It may be suggested that such a substance, an aldehyde or a glyoxal derivative, is responsible for the reduction of ninhydrin to a free radical in the reaction with amino acids.

Considering the coloured substances produced in the reactions of ninhydrin, there is no evidence that the radicals here found are involved in the formation of "Ruehmann's Purple", since this substance is formed at a pH value much lower than that

necessary for production of the radicals (also *cf.* (ii)). However, the reddish-brown colour described above (v) seems in some way to be connected with the ninhydrin radicals, but it is not clear if the coloured substances is identical with the radicals or not. Evidently, both of them are destroyed by molecular oxygen.

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## The Absolute Configuration of Cleomin\*

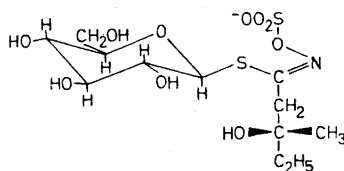
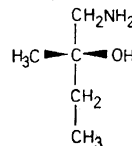
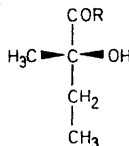
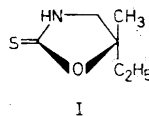
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Glucocleomin is a thioglucoside occurring in *Cleome spinosa* Jacq. and other species of the family Capparidaceae.<sup>1</sup> Upon enzymic hydrolysis, glucocleomin produces cleomin, previously identified in this laboratory as (-)-5-ethyl-5-methyl-2-oxazolidinethione.<sup>1</sup> We now report that cleomin possesses the absolute configuration depicted in (I).

On reaction with liquid ammonia at 100° for 42 h, levorotatory methyl 2-hydroxy-2-methylbutyrate, to which we recently assigned the (*R*)-configuration (II, R = OCH<sub>3</sub>),<sup>2</sup> was converted into (*R*)-2-hydroxy-2-methylbutyramide (II, R = NH<sub>2</sub>), m.p. 55° (stable) or 46° (dimorphism), [α]<sub>D</sub><sup>22</sup> + 18.8° (*c* 4.2, 96 % ethanol). (Found: C 51.25; H 9.54; N 11.93. Calc. for C<sub>5</sub>H<sub>11</sub>NO<sub>2</sub>: C 51.26; H 9.47; N 11.96). By reduction with lithium aluminium hydride in ether, the

\* Part XLVII of a series of papers on isothiocyanates (part XLVI: *Acta Chem. Scand.* **16** (1962) 2065).



amide was transformed into (*R*)-1-amino-2-methyl-2-butanol (III) which was isolated as the dextrorotatory neutral oxalate, m.p. 204° (decomp.), [α]<sub>D</sub><sup>23</sup> + 3.5° (*c* 2.4, H<sub>2</sub>O). (Found: C 48.53; H 9.58; N 9.52. Calc. for C<sub>12</sub>H<sub>28</sub>N<sub>2</sub>O<sub>6</sub>: C 48.63; H 9.52; N 9.46). On critical comparison with the previously synthesized specimen of the oxalate, that was formerly transformed into cleomin,<sup>1</sup> the two preparations proved to be identical,\* except for their optical rotations which were equal in magnitude within the experimental error *but opposite in sign*. Consequently, the naturally derived, levorotatory cleomin possesses the (*S*)-configuration (I).

2-Oxazolidinethiones arise from spontaneous cyclization of 2-hydroxy-substituted isothiocyanates, which are initial products of the enzymic hydrolysis of the corresponding glucosidic progenitors (*cf.* Ref.<sup>3</sup>). Consequently, glucocleomin possesses the absolute configuration shown in (IV).

The work is part of investigations supported by *The Carlsberg Foundation* and *Kai Hansen's Fond*.

\* The previously reported<sup>1</sup> m.p. 192° for the levorotatory oxalate appears to be too low. When determined under the present conditions (oil bath, capillary tube, rate 2°/min), the same sample now decomposed at 204°.