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1. Vodrazka, Z. *Chem. listy* **46** (1952) 208.
2. Wannagat, U. and Pfeiffenschneider, R. *Z. anorg. Chem.* **297** (1958) 151.
3. Berlin, A. Ya. *J. Gen. Chem. (U.S.S.R.)* **17** (1947) 95; *Chem. Abstr.* **42** (1948) 51.

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Electroorganic Preparations X. Polarography and Reduction of α-Naphthoic Acid

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Comparatively few compounds containing an ester or an amide group are polarographically reducible. Derivatives of the phthalic acids and of the pyridine mono- and dicarboxylic acids have been investigated polarographically and it has been found that the methyl esters of phthalic and terephthalic acid are reduced to phthalide [1] and *p*-hydroxymethylbenzoic acid methyl ester [2], respectively, and that isonicotinic amide at pH 3 is reduced to γ-pyridyl carbinol [3].

α-Naphthoic amide was found to be polarographically reducible and a controlled potential reduction was made in order to establish the nature of the reduction.

α-Naphthoic acid yields a polarographic wave only in unbuffered or alkaline solution as the wave in acid solution is masked by the hydrogen wave. Only solutions containing lithium or tetraalkylammonium ions as supporting electrolyte can be used. In unbuffered 0.2 M tetraethylammonium bromide solution containing 40 % alcohol the half-wave potential is 1.88_5 V *vs* S.C.E.

By a controlled potential reduction it was shown that the reduction consumed 2 electrons per molecule and a product with m.p. 150° and an analysis corresponding to $C_{11}H_{11}NO$ was obtained. The elementary analysis suggested a reduction in the naphthalene ring rather than in the amide group, the product thus being a dihydronaphthoic amide. As the reduction most probably would take place in the ring to which the amide group was bonded and the melting points of 1,2-dihydronaphthoic amide-1, m.p. 200° [4], and 3,4-dihydronaphthoic amide-1, m.p. 185° [4] or 128° [5], differed considerably from that of the isolated compound, the product might probably be 1,4-dihydronaphthoic amide-1 which apparently has not been described before.

In order to prove that, 1,4-dihydronaphthoic acid-1, m.p. 88°, prepared according to Baeyer [6], was treated with thionyl chloride and the resulting chloride in benzene solution treated with concentrated aqueous ammonia. The compound formed had m.p. 150° and an infrared spectrum identical with that of the product obtained from the electrolytical reduction.

The UV-spectrum of the supposed 1,4-dihydronaphthoic amide, max. at 267 mμ and 273 mμ with ε_{267} 520 and ε_{273} 570, confirmed that no migration of the double bonds had taken place during the preparation from the acid, as 1,2- and 3,4-dihydronaphthoic amide would be expected to show a strong absorption (ε about 10^4) around 250 mμ similar to that of styrene.

The polarographic reduction of α-naphthoic amide in alkaline solution is thus a 1,4-addition to the naphthalene ring. The reduction with sodium amalgam often ressembles the reduction at a mercury electrode, but a reduction of α-naphthoic amide with sodium amalgam in acid solution yields α-naphthyl carbinol [7]. The reduction thus takes place at different positions in acid and alkaline solution. This is also found in the polarographic reduction of isonicotinic amide which in acid solution is reduced in a four electron reduction to γ-pyridyl carbinol [3] and in alkaline solution yields a two-electron wave indicating the formation of a dihydropyridine derivative.

Experimental. The polarograph was a pen-recording polarograph Radiometer Polariter PO4d. The potentiostat was an electromechanical potentiostat of the Lingane-Jones type. The melting point determinations were made with a Leitz "Mikroskop-heiztisch 350". The infrared spectra were recorded at a Perkin-Elmer Infracord and the UV-spectra with a Perkin-Elmer UV-137.

Reduction of α-naphthoic amide. 2.0 g of α-naphthoic amide was suspended in 180 ml 50 % methanol containing 5 g of lithium chloride and 2 g of tetramethylammonium hydroxide and reduced at −1.90 V *vs* S.C.E. The compound dissolved during the reduction which required two electrons per molecule. After completion of the reduction the solution was made slightly acid with acetic acid and the main part of the methanol was removed in vacuo. The product was extracted from

the aqueous layer with a 3:1 mixture of chloroform and isopropyl alcohol which was evaporated. The residue was dissolved in a small volume of methanol and on addition of water a precipitate, 1.6 g, was obtained. After two recrystallizations from benzene the compound, 1.3 g, had m.p. 149.5°–150°. (Found: C 76.17; H 6.58; N 8.09. Calc. for $C_{11}H_{11}NO$: C 76.25; H 6.42; N 8.19).

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1. Whitnack, G. C., Reinhart, J. and Gantz, E. St. C. *Anal. Chem.* **27** (1955) 359.
2. Ono, S. and Nakaya, J. *J. Chem. Soc. Japan.* **74** (1953) 907. *Chem. Abstr.* **48** (1954) 8082.
3. Lund, H. *Collection Czechoslov. Chem. Communs.* **25** (1960) 3313.
4. Ranedo, J. and Leon, A. *Anales soc. espan. fis. quim. Madrid* **25** (1927) 421; *Chem. Abstr.* **22** (1928) 777.
5. Birch, A. J. and Robinson, R. *J. Chem. Soc.* **1944** 503.
6. Baeyer, A. *Ann.* **266** (1891) 169.
7. West, B. L. *J. Am. Chem. Soc.* **42** (1920) 1656.

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The C-Terminal Amino Acids of Three Variants of Horse Myoglobin

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In previous communications [1,2] the isolation and analysis of three different horse myoglobins have been reported. The almost identical analytical data for the three components, including the amino acid composition, indicated this to be a case of microheterogeneity, where the difference between the three components consists of an exchange of one amino acid for another or a different distribution of amino acids or charged groups within the molecule. As myoglobin has only one N-terminal amino-acid group [3,4], it is fairly certain to be built up of a single polypeptide chain and identification of the end groups in the three variants would in this case be of singular interest. The N-terminal amino acid has been determined in all three myoglobins and found to be the same [3], while the C-terminal amino acids have been determined only in unseparated horse myoglobin, where one of the components accounts for about 80 % of the material. By following the rate of liberation of amino acids from the apomyoglobin on hydrolysis with carboxypeptidase A the sequence of four C-terminal amino acids was determined [5]. This paper reports the determination of four C-terminal amino acids in the three different myoglobins, using the same method, but with a somewhat different result.

Experimental. The three myoglobin components, designated MbI, II_1 and II_2, were prepared as described previously.[2] Carboxy peptidase A * (CP A) was a commercial product and was treated with diisopropylfluorophosphate (DFP) before use. As the rate of action of CP A on the native as well as denatured myoglobins is extremely slow, the apoproteins were used for the degradation experiments. The latter were prepared by splitting the myoglobins with acid acetone in the cold as described earlier [1]. The native apoproteins, however, proved almost as resistant to attack by the enzyme as unsplit myoglobin. To render them accessible to carboxypeptidase action, the apoproteins were denatured by heating in saltfree solution at pH 8.5 and 90°C for 4 min. Under these conditions a uniform suspension of a finely divided precipitate was obtained, which did not aggregate visibly during the time of hydrolysis. The DFP-treated CP A was dissolved in a 10 % LiCl solution immediately before use.

The hydrolyses were carried out in an unbuffered medium at 37°C and pH 8.5 under continuous stirring. The weight ratio of enzyme to apoprotein was 1:20. The pH was kept constant by addition of 0.1 M NaOH and at suitable intervals 1 ml samples were withdrawn, transferred to centrifuge tubes containing 1 ml of trichloroacetic acid and immediately centrifuged. The supernatant was then analyzed for amino acids in an automatic amino acid analyzer according to Spackman *et al.* [6]. As asparagine and glutamine under these conditions appear exactly coincident with the serine peak on the chromatogram, while aspartic and glutamic acids are well resolved, it was necessary to analyze the supernatant before as well

* Carboxypeptidase, which is equivalent to carboxypeptidase A, was obtained from Worthington Biochemicals, Freehold, New Jersey, as a water suspension of three times recrystallized material.