The presence of a secondary OH group was confirmed by acetylation of **IV** which afforded **V**, ν_{max} 1740 cm^{-1}; $\delta(CDCl_3)$ 2.12 (3H, s, CH_3CO), 4.69 (t, 1H, J 3.5Hz, HC-12).

Treatment of **IV** with pyridinium-chlorochromate in the presence of sodium acetate caused the oxidation of the secondary alcoholic group and the elimination of HBr to give the α,β-unsaturated ketone **II** (50%) identified by comparison ($[\alpha]_D$, m.m.p., IR, NMR, MS) with an authentic sample. This allowed the location of OH and Br at C-12 and C-14 respectively, taking into account the multiplicity of the signals of C*H*OH and C*H*Br groups in the NMR-spectrum of **IV**.

As a consequence, the stereostructure **IV** was assigned to the compound under investigation apart from the chiralities of C-12 and C-14. These were deduced from the NMR spectra of **IV** and **V** which indicated that HC-12 (t, J 3.5Hz) and HC-14 (dd, J 3 and 13Hz) must be equatorial and axial respectively.

The co-occurrence of the compounds **I** and **IV** in *Sphaerococcus coronopifolius* could be of biogenetic interest. We can now suppose that **IV** represents an intermediate in the transformation of bromosphaerol (**I**) into sphaerococcenol A (**II**).

1 This work was carried out under the auspices of 'Progetto Finalizzato per l'Oceanografia e i Fondi Marini', CNR, Rome.
2 E. Fattorusso, S. Magno, C. Santacroce, D. Sica, B. Di Blasio, C. Pedone, G. Impellizzeri, S. Mangiafico, G. Oriente, M. Piattelli and S. Sciuto, Gazz. chim. ital. *106*, 779 (1976).
3 W. Fenical, J. Finer and J. Clardy, Tetrahedron Lett. *1976*, 731.
4 F. Cafieri, L. De Napoli, E. Fattorusso, G. Impellizzeri, M. Piattelli and S. Sciuto, Experientia *33*, 1549 (1977).
5 E.J. Corey and J.W. Suggs, Tetrahedron Lett. *1975*, 2547.

Deuterium isotope effects in the ninhydrin reaction of primary amines

P.H. Yu and B.A. Davis[1]

Psychiatric Research Division, University Hospital, Saskatoon (Saskatchewan, Canada S7N OXO), 4 June 1981

Summary. The rate of development of Ruhemann's purple in the ninhydrin reaction of two deuterated primary amines, $\alpha\alpha$-d_2-p-tyramine and $\alpha\alpha$-d_2-β-phenylethylamine, is significantly reduced. It appears to be a primary isotope effect and indicates that the cleavage of the carbon-hydrogen bond at the α-position is involved in the rate-determining step of the color reaction.

Ninhydrin, 2,2-dihydroxy-1,3-indandione, has been widely used for the quantitative determination of amino acids and amines for many years. Several reaction mechanisms have been proposed for the color development[2,3]. Recently during our study on the kinetic isotope effect on the enzymatic deamination of trace amines [4], the ninhydrin reaction was observed to be unsuitable for measurement of the deuterated amines, and it is now clear that this is due to a deuterium isotope effect.

The estimation of the rate of ninhydrin reaction was adopted according to Moore's method[5]. The reagent was prepared by dissolving ninhydrin (0.11 M) and hydrindantin (2,2-dihydroxy (2,2-biindan)-1,1',3,3'-tetrone) (0.002 M) in dimethylsulfoxide buffered with lithium acetate (0.5 M) at pH 5.2. The different deuterated amine analogs were synthesized as previously described[4]. Both the chemical and isotopic purities of these compounds were over 95% in all cases[4]. An aqueous solution (0.5 ml) of the deuterated amines or the non-deuterated amines (25 µg) was mixed with an equal volume of ninhydrin reagent and incubated at 55 °C. The optical density at a wavelength of 525 nm was measured at different time intervals. As can be

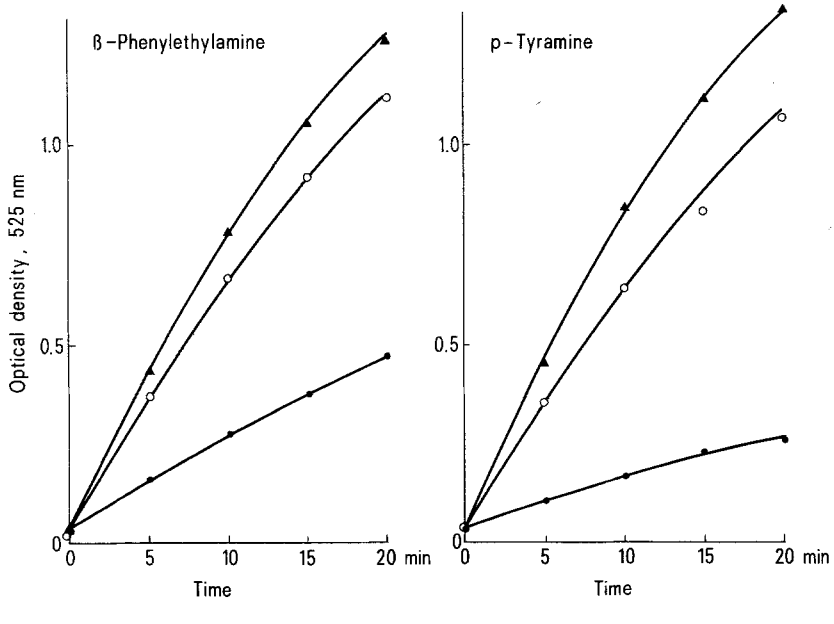


Figure 1. Relative rates of color development of primary amines in ninhydrin reaction. Protonated (○——○) phenylethylamine (25 µg) (left) and p-tyramine (25 µg) (right) and their α,α-deuterated (●——●) and β,β-deuterated (▲——▲) analogs were incubated with ninhydrin reagent at 55 °C. OD at 525 nm wavelength are plotted against time. Values are average of 4 experiments.

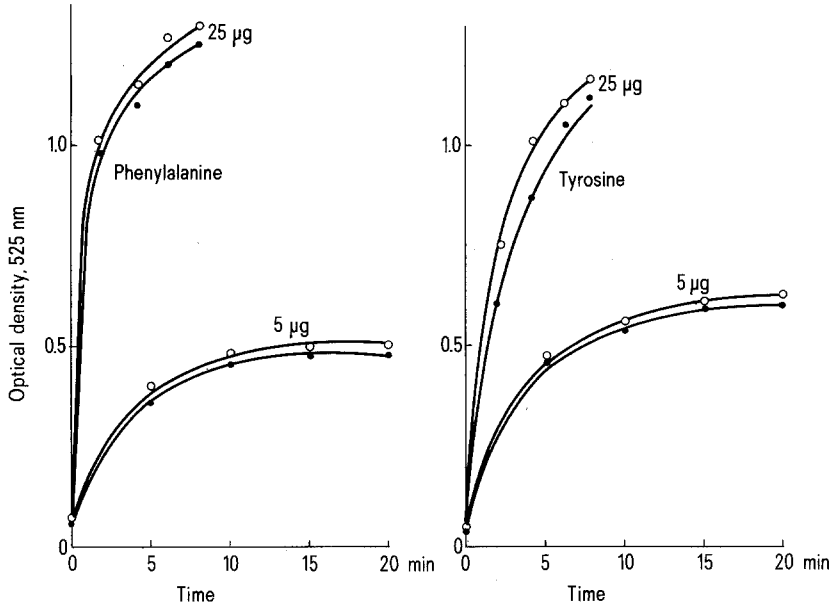


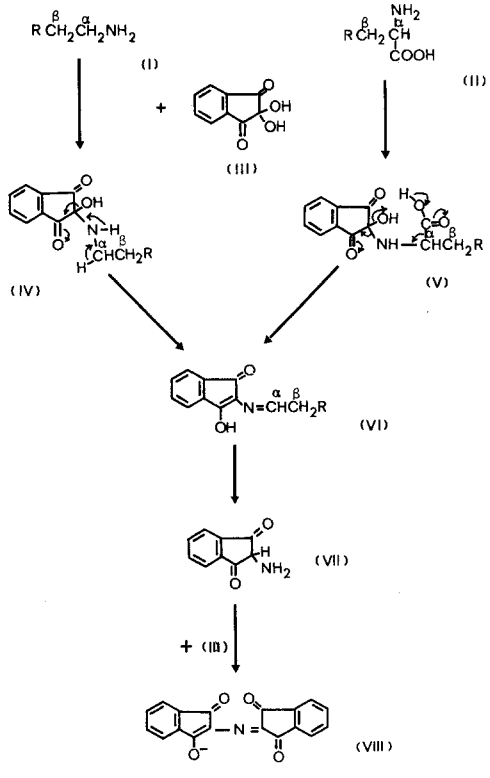
Figure 2. Relative rates of color development of amino acids in ninhydrin reaction. Protonated (○——○) phenylalanine (25 µg and 5 µg) (left) and p-tyrosine (25 µg and 5 µg) (right) and their α,β-deuterated (●——●) were estimated as described in legend of figure 1.

seen in figure 1, substitution of deuterium at the α-position of the amines significantly reduced the rate formation of the Ruhemann's purple, diketohydrindylidene-diketohydrindamine. The k_H/k_D ratios are 4.1 and 2.6 with respect to deuterated p-tyramine and β-phenylethylamine. Replacement of deuterium at the β-position, however, slightly enhanced the rate of the color reaction.

The mechanism of the ninhydrin reaction of primary amines has been proposed previously[3,6].

During the formation of compound **VI** from **IV**, it is proposed that 1 hydrogen is cleaved from the α carbon. The profound isotope effect induced by a replacement of both α hydrogens by deuterium makes it appear the cleavage of this bond is involved in the rate-determining step of the ninhydrin reaction with the primary amines. The enhanced rate of the color reaction with substitution of deuterium at the β-position may perhaps be attributed to a reduced hyperconjugation of the β-C-D bond which in turn results in an enhancement of the polarity of the α-C-H (or α-C-D) bond and therefore an enhancement of its rate of cleavage.

The reaction of ninhydrin with primary amines has been considered as a special case of the general ninhydrin reaction for the amino acids[3]. We have, therefore, also analyzed the ninhydrin reactions of phenylalanine and p-tyrosine and their deuterated analogs. The rate of the color development of the non-deuterated phenylalanine and tyrosine with ninhydrin was found to be about 4 times faster than that of the corresponding primary amines, i.e. β-phenylethylamine and p-tyramine, under the identical experimental conditions. This suggests that the carboxyl group of the amino acid participates in a concerted electronic mechanism[6] (see scheme) in the initial reaction of ninhydrin with the amino acid and therefore facilitates a faster formation of compound **VI** from **V**. No significant difference was observed between the color reaction of deuterated and non-deuterated amino acids (fig. 2), since cleavage of the C-H bond at the α position is not involved in this reaction. Thus the reaction of ninhydrin with amino acids can be viewed as a special case of the general ninhydrin reaction of primary amines in which a hydrogen atom has been replaced with a substituent, the carboxyl group, whose electronic characteristics are more favorable for cleavage.



1 Acknowledgments. We thank Dr A.A. Boulton for his advice and encouragement and the Canadian Medical Research Council and Saskatchewan Health for their continuing financial support.

2 S.J. Ruhemann, J. chem. Soc. *99*, 1486 (1911).
3 D.J. McCaldin, Chem. Rev. *60*, 39 (1960).
4 P.H. Yu, S. Barclay, B.A. Davis and A.A. Boulton, Biochem. Pharmac. *30*, 3089 (1981).
5 S. Moore, J. biol. Chem. *243*, 6281 (1968).
6 S. Moore and W.H. Stein, J. biol. Chem. *176*, 367 (1948).