# New Tests for the *Nicotiana* Alkaloids, Nornicotine and Anabasine[1]

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We wish to give preliminary accounts of two new color tests for *Nicotiana* alkaloids, one for nornicotine and anabasine, and another for nornicotine. We find that nicotine fails to give similar color reactions in both tests.

A solution of quinhydrone (.5 g in 100 ml of ethyl alcohol) will react with nornicotine and anabasine at a pH of 7 (phosphate buffer) to give a cherry-red solution. Five-hundredths g of either nornicotine or anabasine in 10 ml of buffer solution and 10 ml of quinhydrone solution will give immediately a deep cherry-red solution. A similar quantity of nicotine will give almost no color change in the original quinhydrone solution.

The second test (for nornicotine) uses as color-producing reagents 1,3-di-ketohydrindene, *p*-hydroxybenzoic acid, acetone, and di-isopropyl ketone. Nornicotine reacts with these reagents to give a violet color, which has a maximum absorption peak at approximately 540 m$\mu$. This test is extremely sensitive. Three hundred sixty-three $\gamma$ of nornicotine in 5 ml of acetone, plus 15 ml of di-isopropyl ketone, 2 ml of 2% *p*-hydroxybenzoic acid in di-isopropyl ketone, and 2 ml of .3% solution of 1,3-di-ketohydrindene in di-isopropyl ketone gives a reading of 384 at 540 m$\mu$ on the Klett-Summerson colorimeter within 60 min after the start of the reaction. The same quantity of nicotine or anabasine does not give this violet color under the same conditions.

We are continuing work on these two tests.

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[1] Report of a study under the Research and Marketing Act of 1946.

# Mechanism of the Dakin and West Reaction

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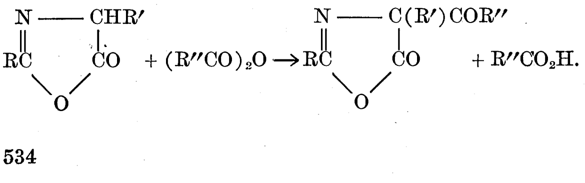
Dakin and West (*2*) showed that many $\alpha$-amino acids give $\alpha$-acetamidoalkyl methyl ketones and carbon dioxide when heated with pyridine and acetic anhydride at 100° C:

$$\mathrm{H_2NCHRCO_2H} + 2(\mathrm{CH_3CO})_2\mathrm{O} \rightarrow \mathrm{CH_3CONHCHRCOCH_3} + 2\mathrm{CH_3CO_2H} + \mathrm{CO_2}$$

It is our view that in this synthesis the new carbon-carbon link is formed by the base-catalyzed acylation of an oxazolone:



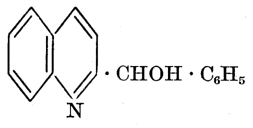
This reaction mechanism is cited in full detail by Wiley (*5*), who nevertheless rejects it in favor of an initial base-catalyzed decarboxylation, the carbanion then reacting with the acetic anhydride or other carbonyl component to give the final product:

$$\mathrm{RCONHCHR'CO_2H} \xrightarrow{\text{base}} \mathrm{CO_2} + \underset{\ominus}{\mathrm{RCONHCHR'}} \xrightarrow{(\mathrm{CH_3CO})_2\mathrm{O}} \mathrm{RCONHCHR'COCH_3}.$$

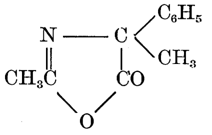
Wiley's rejection of the oxazolone mechanism is based on his observation that *N*-acetylsarcosine, which he says cannot form an oxazolone, can be converted under the usual conditions to *N*-methylacetamidoacetone.

It should first of all be mentioned that the idea of "decarboxylation as a source of reactive carbanions" is not novel, having been put forward some time ago (*4*) to account for the behavior of quinaldinic acid when decarboxylated in the presence of a carbonyl compound: the product with benzaldehyde, for example, is phenyl 2-quinolyl carbinol (I).

We find no decarboxylation of acetylglycine to occur on boiling with pyridine (or with a mixture of pyridine and benzaldehyde). This fact alone makes Wiley's mechanism unacceptable without modification. In addition, Dakin and West (*2*) found that $\alpha$-aminohydratropic acid affords no carbon dioxide (and no ketone) when heated with acetic anhydride and pyridine. This is readily understood on the basis of the oxazolone mechanism, for no hydrogen atom replaceable by acetyl is available in the oxazolone (II); but the fact is not accommodated by Wiley's scheme, as *N*-acetamidohydratropic acid could give a carbanion by decarboxylation.

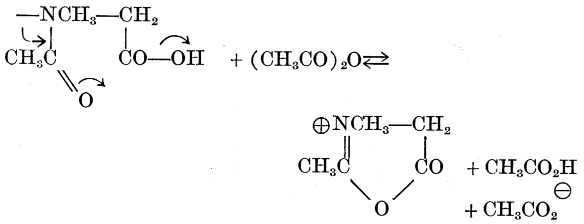


I



II

Wiley's result with acetylsarcosine can be explained by assuming the transitory formation of an oxazolonium cation:



Acetylation, ring-opening, and decarboxylation are then assumed to follow in the usual way. We believe that the condensation of *N*-benzoylsarcosine with benzaldehyde to give $\alpha$-(*N*-methylbenzamido)cinnamic acid (*3*) proceeds by way of an analogous intermediate.

It is interesting that Dakin and West (*2*) reported that sarcosine itself gave little carbon dioxide and no