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

Louis Feinstein and Edward T. McCabe

Bureau of Entomology and Plant Quarantine, ARA,
U. S. Department of Agriculture, Beltsville, Maryland

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 mμ. This test is extremely sensitive. Three hundred sixty-three γ 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 mμ 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.

---

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

Mechanism of the Dakin and West Reaction

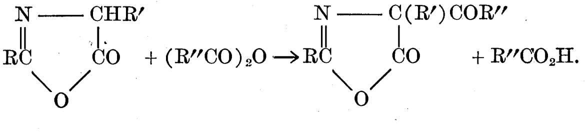
J. W. Cornforth and D. F. Elliott

National Institute for Medical Research,
London, England

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

$$H_2NCHRCO_2H + 2(CH_3CO)_2O \rightarrow CH_3CONHCHRCOCH_3 + 2CH_3CO_2H + CO_2$$

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



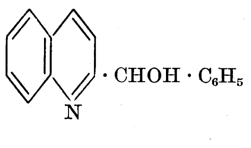
This reaction mechanism is cited in full detail by Wiley (*5*), who nevertheless rejects it in favor of an initial base-catalyzed decarboxylation, the carbanion then reacting with the acetic anhydride or other carbonyl component to give the final product:

$$RCONHCHR'CO_2H \xrightarrow{\text{base}} CO_2 + \underset{\ominus}{RCONHCHR'} \xrightarrow{(CH_3CO)_2O} RCONHCHR'COCH_3.$$

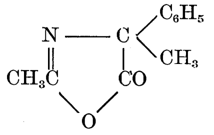
Wiley's rejection of the oxazolone mechanism is based on his observation that *N*-acetylsarcosine, which he says cannot form an oxazolone, can be converted under the usual conditions to *N*-methylacetamidoacetone.

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 α-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.

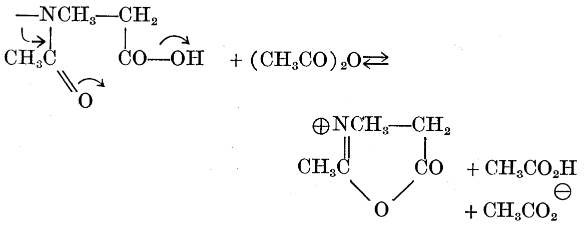


I



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 α-(*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 identifiable ketone on heating with pyridine and acetic anhydride. The contrast between this and the result with *N*-acetylsarcosine is perhaps due to the presence of additional acetic acid (arising from the initial acetylation) in the reaction mixture from sarcosine. Acetic acid has been shown (*1*) to have a deleterious effect on the condensation of hippuric acid with acetic anhydride in the presence of pyridine.

We regard the Dakin and West reaction as a special case of the Erlenmeyer synthesis of oxazolones; we hope to have shown here that Wiley's observation is consistent with this view and does not require a separate hypothesis.

References

1. Attenburrow, J., Elliott, D. F., and Penny, G. F. *J. Chem. Soc.*, 1948, 310.
2. Dakin, H. D., and West, R. *J. biol. Chem.*, 1928, **78**, 91, 757.
3. Deulofeu, V. *Ber.*, 1934, **67**, 1542.
4. Dyson, P., and Hammick, D. Ll. *J. Chem. Soc.*, 1937, 1724.
5. Wiley, R. *Science*, 1950, **111**, 259.

Electronic Radiography by Transmission Using Radioactive Monolayers

D. E. Beischer

U. S. Naval School of Aviation Medicine,
Pensacola, Florida

Herein is described a method of electronic radiography by transmission which may be compared with the photographic printing process, with the difference that electrons, or α particles, replace the light. The specimen is placed between, and in intimate contact with, a uniformly radiating source on one side and a photographic plate on the other. This method was made possible by the preparation of homogeneously radiating sources in the form of mono- or multilayers of molecules tagged with radioactive isotopes (*1*). The layers were prepared by using the technique developed by Langmuir and Blodgett (*2*) for "built-up" films.

A β-emitting source can be prepared by spreading C^{14} labeled stearic acid on the surface of an aqueous solution (10^{-4} *M* $CaCl_2$, 10^{-3} *M* $KHCO_3$) and transferring the monomolecular film of calcium stearate to a solid substratum by dipping a plate once or several times through the surface. In this way a mica plate of a few square centimeters in area can be covered on both sides homogeneously with one or several monolayers of radioactive calcium stearate. By cleaving the mica sheet, two sources buttered with radioactive material on only one side can be prepared. It is obvious that this method can be used for the preparation of homogeneous sources of α, β, or γ radiation, with wide variation in penetrating power, by incorporating various radioactive elements in the monolayers—for instance, by the use of radium, Sr^{90}, Ca^{45} stearate.

The picture of a thin specimen in the β light of C^{14} can be prepared by enclosing the specimen in a printing frame between the radioactive layer and a photographic emulsion (no-screen x-ray film or industrial x-ray film, Type M, from Eastman Kodak Company), as demonstrated in Fig. 1. An example of the results of this method is given in the picture of an unscaled wing of a butterfly reproduced in Fig. 2.

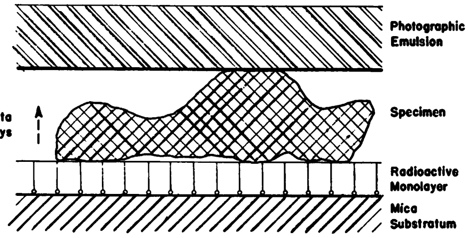


FIG. 1. Diagram of arrangement for electronic radiography by transmission (arbitrary measure).

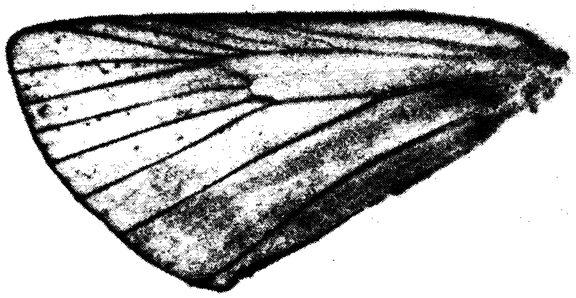


FIG. 2. Unscaled wing of butterfly depicted by electrons emitted from C^{14} (× 5).

These electron radiographs can be explained in the same way as images obtained with the electron microscope. In fact, the mean velocity of the electrons emitted from C^{14} corresponds to the velocity of the electrons in the usual electron microscopes (50 kv). The shades in the picture are determined by the product of density and thickness of the specimen. When this product surpasses a certain value for a given electron velocity, only the outlines of the specimen can be made visible.

Compared with the electron microscope, the contact method is limited in resolving power and in magnification. The electrons are emitted from the source in all directions with different velocities. Under these conditions the resolving power is a complex function with a variety of factors. Some of these are the distribution of the electron velocity in the β spectrum, the thickness in g/cm^2 of the specimen and the photographic emulsion, and the grain size of the photographic material. From the separation of two points in Fig. 2 a resolving power of about 10–20 μ was estimated. Under these conditions the useful magnification cannot surpass the factor 10.

The contact method, however, has some marked peculiarities that may recommend its use in special cases. The wide variety of electron velocities from 0.015 to some million ev, which can easily be applied by changing the radiating element in the source, offers an inexpensive possibility (no machine) for studies of transmission of electrons over a considerable range. In this regard beta-