# Rebuttal on Comparison of Methods for Determining Myrosinase Activity

*Sir:* In their letter Wilkinson and co-workers essentially make three points, to which we reply as follows.

(1) *Linearity of the enzymatic reaction*: In their first paper Wilkinson et al. (1984) show a linear response of the assay up to an enzyme concentration of ca. 8 U (U expressed in nmol/min) only. At the end of this paper, they state that "the rate of hydrolysis of sinigrin against protein concentration showed non-linearity ... This observation is being further investigated". We repeated their experiment and confirmed the nonlinearity. It is easy to show, as we did, that this feature, which of course greatly impairs the reliability of the assay, is simply due to an insufficient concentration of the auxiliary enzyme system (HK-G6PDH) in the MYR-(HK-G6PDH) coupled-enzyme assay (SCEA) so that the reaction rate of (HK-G6PDH) rather than MYR (myrosinase) becomes rate limiting. This finding is not "speculation" on our part but is the result of a direct experimental observation (Palmieri et al., 1987) coupled with a simple stoichiometric calculation based on eq II-76 of Segel (1975).

(2) *Preequilibration of the reaction mixture*: It is well-known that all coupled-enzyme reactions have a lag phase (e.g. Fig. II-29 in Segel (1975)). In our case the lag was a few minutes (observed also with our PCA coupled-enzyme method) in both the presence and absence of ascorbate. From this, two observations can be made: First, it is operationally improper, and in fact senseless, to measure the reaction rate immediately after time zero. One should measure the rate after the lag phase; since we are under zero-order conditions, it does not matter if the first few minutes are lost. Second, we are unable to understand how Wilkinson and co-workers are able to make meaningful measurements "over the initial 20 second period..." when, moreover, the HK-G6PDH units of their proposed assay (SCEA) are much less than those indicated also by Kunst et al. (1984) for glucose kinetic determination.

(3) *Ascorbate*: We made our point clearly enough in our paper (Palmieri et al., 1987). We only add that, generally speaking, an assay should work without an activator whenever possible. Furthermore, in this specific case the system is already very complex and the activation mechanism is still poorly understood (although it has been much studied in the past decade). Actually the systematic use of ascorbate is not only unnecessary, but even dangerous, since the enzyme activity might be spuriously modified in an unknown manner. For example, we have recently shown with the DSA method that myrosinases from different sources are activated differently by ascorbate (Iori et al., 1987). It is perhaps convenient to use ascorbate when the activity is particularly low, as in the crude extracts of cruciferous stems and leaves used by Wilkinson et al. (1984), but it is certainly not a necessary feature of general myrosinase assay.

**Registry No.** Myrosinase, 9025-38-1; L-ascorbic acid, 50-81-7.

## Literature Cited

Iori, R.; Fontana, F.; Palmieri, S. 7th International Rapeseed Congress, Poznan, Poland, 11–14 May 1987.

Kunst, A.; Draeger, B.; Ziegenhorn, J. In *Methods of Enzymatic Analysis: Metabolites 1: Carbohydrates*; Bergmeyer, H. U., Ed.; Verlag Chemie: Weinheim, 1984; Vol. VI, pp 163–172.

Palmieri, S.; Iori, R.; Leoni, O. *J. Agric. Food Chem.* **1987**, *35*, 617.

Segel, I. H. "Kinetics of Coupled Assay". In *Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady-State Enzyme Systems*; Wiley: New York, 1975; pp 85–89.

Wilkinson, A. P.; Rhodes, M. J. C.; Fenwich, G. R. *J. Sci. Food Agric.* **1984**, *35*, 543.

**Sandro Palmieri**
**Renato Iori**
**Onofrio Leoni**

*Instituto Sperimentale per le Colture Industriali*
*Italian Ministry of Agriculture*
*40129 Bologna, Italy*

Received for review April 8, 1988. Accepted April 25, 1988.

# Comments on Photohydrolysis of Ethylene Dibromide

*Sir:* A paper in this journal (Castro and Belser, 1985) described the photochemical hydrolysis of ethylene dibromide and gave figures for the rate constants of two conversion steps, ethylene bromide to 2-bromoethanol and 2-bromoethanol to ethylene oxide. However, while photochemical conversion processes may be expected under certain circumstances to exhibit pseudo-first-order kinetics, the observed rate constant and half-life will both depend on the experimental parameters, in particular on the flux of light entering the reaction vessel that is effective in causing the photoreactions. Thus, neither can be regarded as a fundamental measurement of the efficiency of the reaction.

Let us first consider the hypothetical conversions

$$B \xrightarrow{h\nu} C \qquad C \xrightarrow{h\nu} D$$

where the eventual product D is totally transparent to the photolyzing light, which for simplicity we will assume to be monochromatic. If within the path length $l$ of the reaction vessel, the absorbance, at the wavelength of the photolyzing light, due to both B and C, i.e. $A = (\epsilon_B[B] + \epsilon_C[C])l$, is much less than unity, then the fraction of the incident light of this wavelength absorbed within the cell is given by (1). Of this, some is absorbed by B and some

$$f = 1 - 10^{-A} \tag{1}$$

by C. The fraction of the total incident light absorbed by B is given by (2). Similarly the fraction of the incident

$$\begin{aligned} f_B &= \frac{[B]\epsilon_B}{[B]\epsilon_B + [C]\epsilon_C}(1 - 10^{-A}) \\ &= \frac{[B]\epsilon_B}{[B]\epsilon_B + [C]\epsilon_C}(1 - e^{-2.303A}) \\ &= \frac{[B]\epsilon_B}{[B]\epsilon_B + [C]\epsilon_C}(1 - 1 + 2.303A - \ldots) \\ &\approx \frac{[B]\epsilon_B}{[B]\epsilon_B + [C]\epsilon_C}2.303(\epsilon_B[B] + \epsilon_C[C])l \\ &= 2.303\epsilon_B[B]l \end{aligned} \tag{2}$$

0021-8561/88/1436-0872$01.50/0 