Roth M. Fluorescence reaction for amino acids. *Anal. Chem.* 43:880-2, 1971.
[Laboratoire Central, Hôpital Universitaire, Genève, Switzerland]

o-Phthalaldehyde reacts with amino acids in alkaline medium in the presence of 2-mercaptoethanol by giving rise to strongly fluorescing compounds. This allows for a much more sensitive assay of amino acids than with ninhydrin. The reaction is well suited to the automatic determination of amino acids after ion exchange fractionation. [The *SCI*® indicates that this paper has been cited in more than 530 publications.]

Improving the Sensitivity of Amino Acid Analysis

Marc Roth
Laboratoire Central de Chimie Clinique
Hôpital Cantonal Universitaire
CH-1211 Geneva 4
Switzerland

July 10, 1990

Fluorescence, besides its usefulness as an analytical tool, has an intrinsic beauty that many people find fascinating. As an analytical chemist, I often had to use fluorimeters, but whenever I just had to control whether a solution was fluorescing or not, I preferred to use the ultraviolet lamp as a simpler and more aesthetic way of observation.

In the late 1960s, amino acid analyzers almost exclusively depended on ninhydrin as the reagent and colorimetry as the detection method. There was an obvious lack of a good fluorimetric method to improve the sensitivity of the technique. For this reason I became interested in an article by G. Hillmann,[1] who had been able to produce a fluorescence upon reaction of o-diacetylbenzene with proteins and peptones. I ordered that compound; and on a late afternoon, having finished my more serious work, I took a few test tubes, went to the dark room, and observed under the ultraviolet lamp what happened if solutions of alanine and diacetylbenzene were mixed. The result was poor. I then tried to add a reducing substance, sodium borohydride, and enjoyed one of the most exciting moments of my biochemist's life: The tube with alanine exhibited a bright blue fluorescence, whereas the control was completely dark.

Further investigations showed that diacetylbenzene was advantageously replaced by the parent compound o-phthalaldehyde, and borohydride, by mercaptoethanol. The reaction turned out to be a general one for primary amines. This led to the development of a highly sensitive method of assay for α-amino acids and primary amines that soon found various applications in liquid chromatography.[2,3] The reagent is still widely used today, and one of its most recent applications has been the precolumn derivatization of amino acids and other primary amines, owing to the fact that every amine yields a distinct product and that a mixture of such products can be separated by high pressure liquid chromatography.[4] Apart from fluorimetry, electrochemical detection can be used as a sensitive way of determining the reaction products.[5] The above developments explain why the seminal article is so often cited.

Looking back at the story of this reagent, I am amazed by the fact that over the years I have run a number of investigations based on detailed research programs, supported by research grants and for which I enjoyed the assistance of collaborators. But that particular discovery, which led to the most-cited article I ever wrote, was done without any systematic program, research grant, or personnel. The only material was an ultraviolet lamp, a few test tubes, and the reacting substances. The idea came from the reading of an article written some 27 years before by Hillmann, a distinguished German clinical chemist. I sent him a letter together with a reprint of my article, and in his reply he told me how nicely he was surprised to see his earlier work eventually having such fruitful consequences.

1. **Hillmann G.** Über die Fluorescenzreaktion des o-Diacetylbenzol mit Eiweiss und Eiweissabbauprodukten und ihre Anwendung auf die Abderhaldensche Abwehrfermentreaktion (On the fluorescence reaction of o-diacetylbenzol with albumin and albumin reduction product and its utilization with the Abderhalden reaction to protective ferments). *Hoppe Seyler's Z. Physiol. Chem.* 277:222-32, 1942.
2. **Roth M & Hampai A.** Column chromatography of amino acids with fluorescence detection. *J. Chromatogr.* 83:353-6, 1973. (Cited 225 times.)
3. **Benson J R & Hare P E.** o-Phthalaldehyde: fluorogenic detection of primary amines in the picomole range. Comparison with fluorescamine and ninhydrin. *Proc. Nat. Acad. Sci. USA* 72:619-22, 1975. (Cited 640 times.)
4. **Qureshi G A & Qureshi R.** Determination of free amino acids in biological samples: problems of quantitation. *J. Chromatogr.* 491:281-9, 1989.
5. **Allison L A, Mayer G S & Shoup R E.** o-Phthalaldehyde derivatives of amines for high-speed liquid chromatography/electrochemistry. *Anal. Chem.* 184:1089-96, 1984. (Cited 85 times.)