Table III. Fluorescence Intensity (Arbitrary Units) as a Function of the pH of Reaction[a]

	pH											
Compound	5.0	6.0	7.0	7.5	8.0 (phosphate)	8.0 (borate)	8.5	9.0	9.5	10.0	10.5	11.0	12
Alanine	0	2	24	36	43	52	51	51	51	53	52	53	53
Arginine	1	4	34	40	32	54	46	36	35	42	43	44	44
Cysteic acid	0	1	18	32	48	52	53	54	53	57	57	57	49
Glutamic acid	0	5	18	39	57	56	59	60	60	64	63	64	63
Glutamine	1	6	29	45	52	58	58	58	56	57	55	48	29
Histamine	12	30	6	2	1	7	10	12	18	26	30	30	32
Histidine	0	22	21	18	20	32	36	46	54	60	61	60	50
Lysine	1	10	10	3	2	3	2	1	1	1	2	2	2
Methionine	0	2	27	47	54	60	60	58	58	59	61	62	60
Serine	1	2	26	39	46	54	54	55	55	54	55	54	56
Taurine	2	9	30	23	25	48	47	46	46	47	50	50	51

[a] Buffers: acetate (pH 5–6), phosphate (pH 7–8), sodium borate (pH 8–11), and 0.01*N* NaOH (pH 12).

determinations, relative standard deviation was ±1.9% (alanine), ±2.5% (aspartic acid and methionine), and ±1.5% (arginine).

DISCUSSION

The new reaction described in this paper has a specificity resembling that of ninhydrin, except that the imino acids proline and hydroxyproline do not react. Cysteine gives a poor fluorescence, but can be oxidized to cysteic acid which reacts fairly well. Besides its high sensitivity, the method has the advantage over ninhydrin techniques of not requiring heating. These features make the reaction ideally suited to the automatic assay of amino acids fractionated by ion exchange chromatography. Investigations in progress in our laboratory show that it is now possible to use a fluorometric detection for amino acids separated by the classical technique of Spackman, Stein, and Moore (*3*) or similar procedures. The length of the tubing between column and detector may be enormously reduced, which provides much better peak resolution. The high sensitivity permits to reduce both column size and elution time.

At the present time, we have no idea of the nature of the chemical reaction involved. Some similarity with the ninhydrin reaction may exist, and it is interesting that ninhydrin is also often used in combination with a strongly reducing agent. It is possible that a single fluorescing reaction product is formed with any amino acid, since the excitation and fluorescence spectra were always the same. The order of addition of reagents is not of essential importance but the order given in the proposed method (amino acid added last) best minimizes unwanted side reactions.

A few years ago, Cohn and Lyle (*4*) presented a fluorometric assay of glutathione based on its reaction with *o*-phthalaldehyde at alkaline pH; this yields a product fluorescing maximally at λ_{ex} = 350 nm and λ_{fl} = 420 nm. No fluorescence is given by related compounds lacking a sulfhydryl group.

This reaction may well represent a particular case of the more general reaction described in the present paper. Glutathione would behave simultaneously as an amino compound and, by virtue of its sulfhydryl group, as a reducing agent, thus sharing the two functions otherwise assumed by distinct compounds.

Other investigators recently described fluorogenic reactions for amino acids. The fluorescence with 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (*5*), however, is less intense. The same is true for the Hantzsch condensation with acetylacetone and formaldehyde as proposed by Sawicki *et al.* (*6*). On the other hand, these two reactions may be used for the assay of amines, in which case they should prove superior to our phthalaldehyde reagent. Another fluorometric assay of amino acids has been presented by Guilbault and Hiesermann (*7*). It uses oxidation by the enzymes D- or L-amino acid oxidase. It is fairly sensitive, but is applicable only to those amino acids which are attacked by the enzymes. Interestingly, this permits the assay of proline, which is not detected by the present method (*8*).

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(8) *Note added in proof.* Since submission of this manuscript, I found that treatment of proline and hydroxyproline with sodium hypochlorite or chloramine T converts them to compounds giving the fluorescence reaction with *o*-phthalaldehyde and 2-mercaptoethanol.

Correction

Two Methods for Separation of Surface and Bulk Gases in Vacuum-Fusion Analysis of Metals

In this article by K. W. Guardipee [ANAL. CHEM., **42**, 469 1970)] the author neglected to mention a report of a method which was very similar to the multiple-sample method described in his paper. At the April 1967 meeting of Division I, Committee E-3, ASTM, Miss Virginia Horrigan of the Anaconda American Brass Co., Waterbury, Conn., reported such a method. Reference to this work should have been included in the above mentioned paper.