it does not tautomerize, hydrolyze, or polymerize when treated with NaOD–D_2O in acetone-d_6 for several days.[9]

Acknowledgment. We express our gratitude to the National Science Foundation for financial support (GP-20099) and to Professor Ray Jesaitis for informative discussions.

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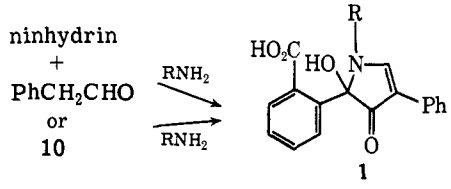
Received May 17, 1972

A Novel Reagent for the Fluorometric Assay of Primary Amines

Sir:

The formation of fluorescent pyrrolinones (**1**) from ninhydrin, phenylacetaldehyde, and primary amines provides the basis for a novel assay, which is of particular value in peptide analysis.[1,2] The conditions which are required to impel the "fluorogenic ninhydrin reaction" are severe enough to often impede its wider utility. Frequently, the oxidizing properties of ninhydrin are the cause of limiting side reactions. Therefore, we sought to replace ninhydrin and phenylacetaldehyde by a single reagent which would react with peptides and other primary amines of biological importance, to afford the same, or closely related fluorophors.

We report here the design and synthesis of the novel reagent **10**, which now supersedes the fluorogenic ninhydrin reaction.

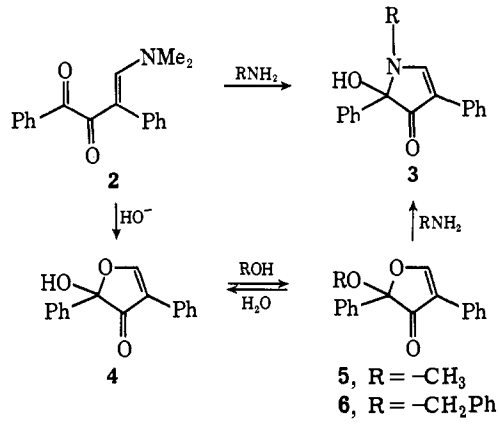


1

We had previously observed[2] that 1-dimethylamino-2,4-diphenyl-1-butene-3,4-dione (**2**) reacts with primary amines to give fluorescent pyrrolinones of structure **3** (Scheme I). However, the use of this reagent is restricted to nonaqueous systems, since it is rapidly destroyed by hydrolysis.

When the enamine **2** was subjected to alkaline hydrolysis, it was converted to 2-hydroxy-2,4-diphenyl-3-(2*H*)-furanone (**4**) [90%; mp 125°; uv max (MeOH) 244 (ε 18,400) and 292 nm (6,250); nmr ($CDCl_3$) δ 8.59 (s, =C*H*O)].[3] Heating of **4** in methanol afforded the methyl ether **5** [83%; mp 93°; uv max (MeOH) 241 (ε 18,750) and 307 nm (3,500); nmr ($CDCl_3$) δ 8.69 (s, =C*H*O), 3.43 (CH_3O)]. Reaction of **5** with benzyl alcohol at 100° furnished the benzyloxy compound **6** [52%; mp 140°; uv max (MeOH) 240 (ε 21,650) and 300 nm (5,100); nmr (C_6H_6-d_6) δ 4.48 and 4.58 (AB, J = 12 Hz, PhCH_2O)]. The cyclic nature of **4**, **5**, and **6**

Scheme I



2 3 4 5, R = –CH3 6, R = –CH2Ph

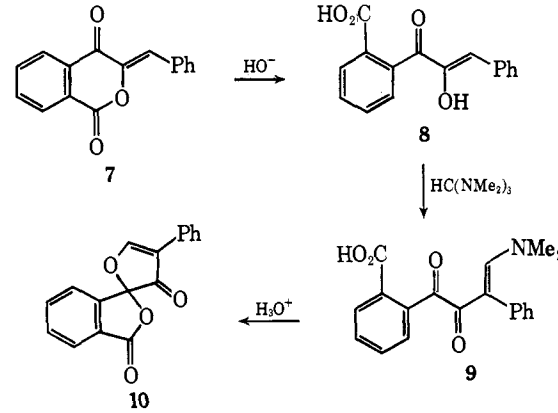
is strongly supported by the nmr spectrum of **6**, which shows that the benzylic methylene protons are nonequivalent and hence in proximity to a chiral center.

The methoxyfuranone **5** reacts rapidly with primary amines in nonaqueous solution to yield fluorescent pyrrolinones. For example, with ethylamine in acetonitrile, it gives the fluorophor **3** [R = $-C_2H_5$,[2] 85%]. In aqueous systems, however, the methoxyfuranone **5** readily reverts to the unreactive hydroxy compound **4**, and is thus unsuited as a reagent for assay purposes.

We then envisaged the structurally modified reagent **10**. This molecule, which retains the structural features of **5** responsible for fluorogenicity, but also possesses a more reactive leaving group, was anticipated to react with primary amines to yield fluorophors, identical with those of the fluorogenic ninhydrin reaction.

The synthesis of the spirolactone **10** is outlined in Scheme II. Alkaline hydrolysis of 3-benzylidene-1,4-

Scheme II



7 8 9 10

isochromanedione (**7**)[4] gave *o*-(α-hydroxycinnamoyl)-benzoic acid **8** [75%; mp 106–115° dec; uv max (Et_2O) 315 nm (ε 23,300)]. Formylation of **8** with tris(dimethylamino)methane[5,6] in *N*,*N*-dimethylformamide proceeded to the dimethylaminomethylene derivative

(1) (a) K. Samejima, W. Dairman, and S. Udenfriend, *Anal. Biochem.*, 42, 222 (1971); (b) K. Samejima, W. Dairman, J. Stone, and S. Udenfriend, *ibid.*, 42, 237 (1971).

(2) M. Weigele, J. F. Blount, J. P. Tengi, R. C. Czajkowski, and W. Leimgruber, *J. Amer. Chem. Soc.*, 94, 4052 (1972).

(3) All new compounds gave satisfactory elemental analyses. Melting points are uncorrected.

(4) J. N. Chatterjea, B. K. Banerjee, and H. C. Jha, *J. Indian Chem. Soc.*, 44, 911 (1967); E. B. Knott, *J. Chem. Soc.*, 402 (1963).

(5) (a) H. Weingarten and W. A. White, *J. Org. Chem.*, 31, 2874 (1966); (b) H. Bredereck, F. Effenberger, T. Brendle, and H. Muffler, *Chem. Ber.*, 101, 1885 (1968).

(6) In connection with an unrelated problem, we observed that this reagent is a powerful formylating agent whose reactivity is similar to that of aminal esters (A. Wick and W. Leimgruber, unpublished results); *cf.* H. Bredereck, F. Effenberger, and H. Botsch, *Chem. Ber.*, 97, 3397 (1964), and H. Bredereck, G. Simchen, and R. Wahl, *ibid.*, 101, 4048 (1968).

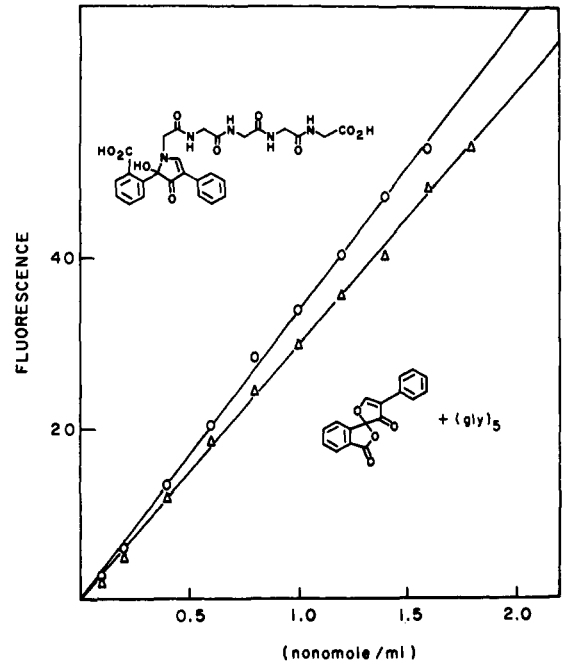


Figure 1. Relative fluorescence of **1** [R = $CH_2(CONHCH_2)_4CO_2H$] (O) and of the reaction of pentaglycine with **10** (Δ). Fluorescence intensities are given in arbitrary units (excitation max, 390 nm; emission max, 475 nm). The data are corrected for blank.

9,[7] which upon acidification (pH 3.5) was directly converted to the desired 4-phenylspiro[furan-2(3*H*),1′-phthalan]-3,3′-dione **10** [63% from **8**; mp 154°; uv max (Et_2O) 235 (ε 25,900), 276 (3,950), 284 (4,100), and 306 nm (3,800); ir ($CHCl_3$) 1810, 1745, 1722 cm^{-1}; nmr ($CDCl_3$) δ 8.71 (s, OC*H*=)].

As expected, the spirolactone **10** reacts with primary amines to yield intensely fluorescent substances of the general structure **1**. Most importantly for practical purposes, fluorescence is also produced efficiently in aqueous media at room temperature. Optimal reaction has been found to occur when the solution to be assayed is at pH 8–9 and an excess of the reagent is added in a water-miscible, nonhydroxylic solvent, such as acetone, dioxane, or acetonitrile.

The following experiment illustrates the potential of **10** as a fluorometric peptide reagent. Reaction of equimolar amounts of **10**, pentaglycine, and triethylamine in aqueous acetonitrile afforded the triethylammonium salt of the fluorophor **1** [R = $CH_2(CONHCH_2)_4CO_2H$; 72%; mp 105° dec; uv max (H_2O) 269 (ε 15,000), 380 nm (5,450)]. The fluorescence of this compound was measured in aqueous buffer solutions of pH 8 and compared to the fluorescence generated *in situ* by the addition, at room temperature, of the reagent **10** in dioxane (0.5 μmol/ml) to aqueous solutions containing 0.1–2 nmol/ml of pentaglycine. The results, recorded in Figure 1, demonstrate that the fluorogenic reaction of the spirolactone **10** with peptides may proceed to near completion (*ca.* 90%) in aqueous systems.

Efficient fluorogenic reaction has also been observed with a large variety of other aliphatic and aromatic primary amines, including amino acids, catecholamines, sulfonamides, and antibiotics. It is therefore felt that the spirolactone **10** will find many uses as a powerful fluorometric reagent in varied areas of biochemical research.[8]

(7) Attempts to isolate this intermediate have failed. Its structure is presumed by analogy with the synthesis of **2**.[2]

Acknowledgment. We thank Mrs. Karin Manhart and Dr. V. Toome for fluorescence measurements.

(8) Reports on specific applications of this reagent are forthcoming (S. Udenfriend, private communication).

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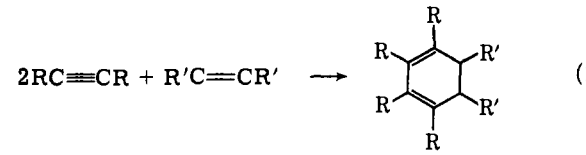
Catalysis of the Cyclotrimerization of Acetylenes with N-Substituted Maleimides

Sir:

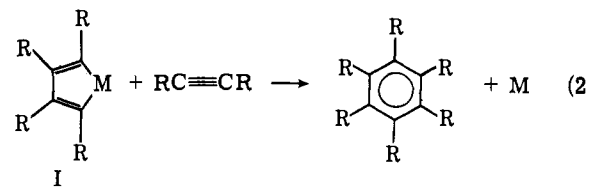
The catalysis of the cyclotrimerization of acetylenes by transition metal complexes has been extensively studied but very few examples of the cyclotrimerization of acetylenes with olefins as in eq 1 have been noted.[1]



(1)

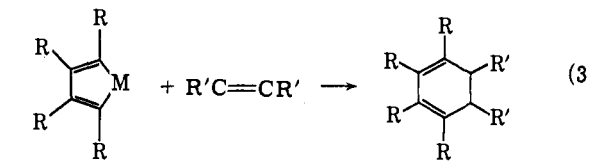
One of the better known examples, the cyclotrimerization of ethyl acrylate catalyzed by $Ni(CO)_2(PPh_3)_2$, appears in fact to proceed *via* a linear intermediate.[2]

This is surprising since among the mechanisms suggested for the catalysis of acetylene cyclotrimerization is one which involves the intermediacy of a metallocyclopentadiene complex I. Complexes such as I have been shown to react with acetylenes to give substituted benzenes as in eq 2.[3,4] In the presence of an



I (2)

olefin bearing electronegative substituents one might hope to intercept I with a Diels–Alder reaction as in eq 3 and thereby catalyze eq 1.



(3)

Our interest in finding conditions for the catalysis of eq 1 concerns its potential for the synthesis of linear polyphenylenes when a bifunctional acetylene is substituted for the monofunctional acetylene and the resulting polymer is aromatized. A related synthesis of branched polyphenylenes has recently been disclosed based on the cotrimerization of bifunctional and monofunctional acetylenes.[5]

(1) C. W. Bird, "Transition Metal Intermediates in Organic Synthesis," Logos, London, England, 1967, p 30.
(2) T. L. Cairns, V. A. Engelhardt, H. L. Jackson, G. H. Kalb, and J. C. Sauer, *J. Amer. Chem. Soc.*, 74, 5636 (1952).
(3) K. Moseley and P. M. Maitlis, *Chem. Commun.*, 1604 (1971).
(4) J. P. Collman, J. W. Kang, W. F. Little, and M. F. Sullivan, *Inorg. Chem.*, 7, 1298 (1968).
(5) A. J. Chalk and A. R. Gilbert, *J. Polym. Sci., Part A-1*, in press.