Reaction of *o*-Phthalaldehyde and Thiols with Primary Amines: Formation of 1-Alkyl(and aryl)thio-2-alkylisoindoles

S. Stoney Simons, Jr.,* and David F. Johnson

Laboratory of Chemistry, National Institutes of Arthritis, Metabolism and Digestive Diseases, Bethesda, Maryland 20014

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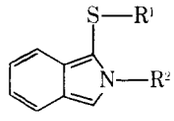
The fluorogenic reaction of *o*-phthalaldehyde (OPTA) and β-mercaptoethanol (MERC) with primary amino acids gives a 1-alkylthio-2-alkylisoindole as the product. The structure of this group of previously unknown isoindoles was determined (1) from an in situ analysis of the adducts formed in solution from OPTA, MERC or ethanethiol (ET), and *n*-propylamine, (2) from the characterization of solid derivatives of these MERC and ET adducts, and (3) from the studies of two isolable isoindoles (an OPTA/*tert*-butylthiol/*n*-propylamine adduct and a dimeric adduct formed from OPTA, ethanedithiol, and *n*-propylamine). The reaction is found to be quite general as OPTA and numerous thiols rapidly react with *n*-propylamine or leucine to give isoindoles in excellent yield. Most adducts were not isolated, but their physical properties in solution were qualitatively identical with those of the *tert*-butyl and dimeric ethanedithiol adducts. Analyses of the chemical shifts of the isoindole alkyl substituents in the NMR spectra support the previous conclusions that these heterocycles have relatively low levels of aromatic character. The addition sequence of the reaction is important; the best results are obtained by mixing OPTA and thiol before adding the amine. With some thiols, this procedure results in the initial formation of a 1-alkylthio-3-hydroxy-1,3-dihydroisobenzofuran. However, these 1:1 adducts do not appear to be obligatory intermediates in the subsequent reaction with primary amines to form 1-thio-substituted isoindoles.

The fluorogenic reaction of *o*-phthalaldehyde (OPTA) and β-mercaptoethanol (MERC) with amines[1,2] and amino acids and proteins[3-9] has recently attracted much attention due to the high sensitivity of the assay which can be conducted in aqueous solutions. Thus, picomole quantities of amino acids can be readily detected. In recent preliminary communications we deduced that these intensely fluorescent OPTA reaction products are 1-alkylthio-2-alkyl-substituted isoindoles **1**.[2,10]



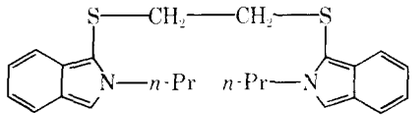
1. R^1 = alkyl or aryl; R^2 = alkyl

Isoindoles in general are quite reactive and eluded isolation until 1951.[11,12] In spite of their 10-π-electron apparently aromatic structure, N-substitution and especially halogen substitution[13-15] are required to increase the stability of isoindoles not conjugated with other unsaturated functional groups. Very few oxygen-[13,16] or nitrogen-substituted[17] isoindoles have been reported, and the effect of such substitution on isoindole stability is not yet clear. For this reason, thio-substituted isoindoles **1** are an important new class of hetero-substituted isoindoles which should be useful in further defining the physical and chemical properties of these interesting 10-π-electron bicyclic heterocycles. In this paper we present the details of our preliminary communications,[2,10] describe the preparation and physical properties of several new 1-alkyl(and aryl)thio-2-alkylisoindoles, and assess the scope and mechanism of the OPTA reaction. A discussion of the fluorescence properties of these compounds can be found elsewhere.[18]

Results and Discussion

1-(*tert*-Butylthio)-2-*n*-propylisoindole. In a modification of our earlier procedure,[10] addition of 1 equiv of *n*-propylamine to an equal molar amount of OPTA and *tert*-butylthiol caused an exothermic reaction, after which **1** (R^1 = *t*-Bu, R^2 = *n*-Pr) soon crystallized out of solution in 86% yield. After recrystallization this material was identical with the previously characterized *tert*-butyl adduct.[10] An analytical sample remelted with very little change in the melting point, and it could be stored at −20 °C for six months. In solution this isoindole was less stable, and complete destruction of the adduct (5×10^{-3} M in isooctane) occurs after <40 h of exposure to room lighting. Thus, this *tert*-butyl adduct still possesses the high reactivity characteristic of simple isoindoles.

Ethanedithiol Dimer Adduct 2. Analytically pure **2**



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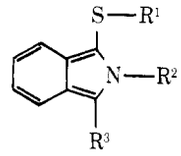
crystallized out of a reaction solution of 0.5 equiv of ethanedithiol and 1 equiv each of OPTA and *n*-propylamine in nearly quantitative yield. While the solid adduct is reasonably stable in air at room temperature, it is discolored by room light and decomposes in solution. Rapid, low temperature, light-shielded recrystallizations consistently gave less pure solids. Thus, like the *tert*-butyl adduct, the isoindole **2** is only relatively more stable than other simple isoindoles.

β-Mercaptoethanol and Ethanethiol Adducts. As was reported earlier,[2,19] neither of these adducts was readily isolable. However, as seen for the *tert*-butyl and dimeric ethanedithiol adducts directly, NMR and TLC analyses of the reaction solutions revealed that the MERC and ET adducts could be formed in >90% yield and purity. Thus, the inability to isolate these latter adducts did not preclude their characterization. The IR spectra of these adducts and the *tert*-butyl and ethanedithiol dimer adducts each display numerous prominent bands that are undoubtedly characteristic of this isoindole ring system. Several of these bands (i.e., those at 3030, ~2900, 1460, and ~746 cm^{-1}) and the lack of aromatic bands in the region of 1600–1500 cm^{-1} have also been observed in the IR spectrum of isoindole itself.[20] Likewise, the MERC, ET, *tert*-butyl, and ethanedithiol dimer adducts have virtually superimposable UV spectra (Table I).

The ^1H NMR spectra of the MERC,[2] ET, *tert*-butyl, and ethanedithiol dimer adducts are almost identical (Table II) and thus, with the above compared UV data, firmly establish the isoindole ring structure for the unisolated MERC and ET adducts. Theoretical[21] and experimental ^1H NMR spectra of isoindole[14,20] and a detailed analysis of the spectrum of *N*-methylisoindole[22] give a pattern and assignment[21,22] of the aromatic region protons similar to that of these adducts. A

Table I. Effect of Substituents and Solvents on the UV Spectral Properties of Isoindoles 1[a]



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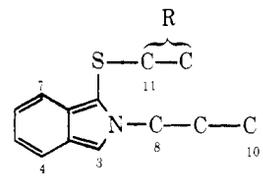
				95% EtOH			Isooctane		
R^1	R^2	R^3	Registry no.	λ_{max} (nm)	$\lambda_{shoulder}$ (nm)	$\epsilon \times 10^{-3}$	λ_{max} (nm)	$\lambda_{shoulder}$ (nm)	$\epsilon \times 10^{-3}$
Et	*n*-Pr	H	61214-22-0	333	~345	7.5	331, 346	~318	8.5
$-CH_2CH_2OH$	*n*-Pr	H	61214-21-9	332	~345	7.6	330, 345	~317	8.4
t-Bu	*n*-Pr	H	64807-91-6				333, 348	~320	9.3
Ph	*n*-Pr	H	66161-39-5	330	~343	7.1			
$-CH_2COOMe$	*n*-Pr	H	66161-40-8	332	~345	8.6			
$-CH_2CH_2SH$	*n*-Pr	H	66161-41-9	332	~345	7.4			
$-CH_2\rightarrow_2$	*n*-Pr	H	66161-42-0	333	~346	14.4			
$-CH_2CHOHCH-OHCH_2SH$	*n*-Pr	H	66161-43-1	333	~345	7.1			
$-CH_2CHOH\rightarrow_2$	*n*-Pr	H	66161-44-2	332	~345	14.4			
t-Bu	*n*-Pr	-S-*t*-Bu	66161-45-3				344, 361	~330	17.0
$-CH_2CH_2OH$	-CH(*i*-Bu)COOH	H	66161-46-4	336	~350	5.7			

[a] All adducts were formed as described in the Experimental Section. The solid adducts were diluted directly into isooctane (for the two *tert*-butyl adducts) or dissolved in EtOAc and then diluted 1:400 with 95% EtOH (for the ethanedithiol dimer). The MERC and ET adducts formed in 95% EtOH were diluted 1:333 with isooctane or 95% EtOH. All other solutions containing the initially formed adducts were diluted about $1:10^3$ with 95% EtOH. Of the two λ_{max} peaks in isooctane, the lower wavelength peak is always the more intense; the listed ϵ corresponds to this more intense band.

Table II. Effect of Substituents on the ^1H NMR Spectra of Isoindoles and Assessment of Isoindole Aromaticity[a]



	tert-Butyl adduct (R = *t*-Bu)		Ethanedithiol dimer adduct (R = $CH_2\rightarrow_2$)		ET adduct (R = Et)		MERC adduct (R = CH_2CH_2OH)		Di-*tert*-butyl adduct (R = *t*-Bu; C_3-H = C_3-S-*t*-Bu)	
Proton	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$
C_3	7.28 (broad singlet)		7.21 ($J \sim 0.75$ Hz)		7.32 ($J \sim 0.75$ Hz)		7.31 ($J \sim 0.75$ Hz)			
C_4, C_7	7.4–7.8		7.3–7.8		7.4–7.7		7.4–7.7		7.63–7.84	
C_5, C_6	6.8–7.1		6.8–7.1		6.8–7.1		6.8–7.1		6.95–7.16	
C_8	4.33 (J = 7.3 Hz)	−1.69	4.13 ($J \simeq$ 7.4 Hz)	−1.49	4.30 (J = 7.3 Hz)	−1.66	4.30 (J = 7.5 Hz)	−1.66	4.66 (J = 7.5 Hz)	−2.02
C_9	1.83 ($J \simeq J' \simeq$ 7.3 Hz)	−0.38	1.69 ($J \simeq J' \simeq$ 7.4 Hz)	−0.24	1.84 ($J \simeq J' \simeq$ 7.3 Hz)	−0.39	1.80 ($J \simeq J' \simeq$ 7.5 Hz)	−0.35	1.67 ($J \simeq J' \simeq$ 7.5 Hz)	−0.22
C_{10}	0.85 (J' = 7.3 Hz)	+0.05	0.78 ($J' \simeq$ 7.2 Hz)	+0.12	0.85 (J' = 7.3 Hz)	+0.05	0.81 (J' = 7.5 Hz)	+0.09	0.83 (J' = 7.5 Hz)	+0.07
C_{11}			2.63	+0.05	2.53 (J'' = 7.3 Hz)	0.00	2.68 (J'' = 7.0 Hz)	+0.01		
C_{12}	1.22	+0.18			1.04 (J'' = 7.3 Hz)	+0.31	3.50 (J'' = 7.0 Hz)	+0.20	1.23	+0.17
C_{12}-OH							2.9–3.7			

[a] NMR spectra of ~2 M solutions of MERC and ET adducts formed from 1 equiv of each reagent (see Experimental Section) in CD_3CN were determined at 100 and 60 MHz, respectively. Recrystallized samples of the *tert*-butyl and ethanedithiol dimer adducts (60 MHz spectra) and the di-*tert*-butyl adduct (100 MHz spectrum) were examined in $CDCl_3$. The change in chemical shift of the alkyl substituent protons is expressed as $\Delta\delta$ = (δ in starting material) − (δ in **1**).

comparison of the chemical shifts of the alkyl substituent protons of **1** (Table II) vs. the starting compounds reveals three important features. (1) The magnitude of deshielding of the C_8 methylene protons is indicative of appreciable ring current and aromaticity in the isoindole system. However, this ring current is less than that of benzene, as witnessed by the position of the C_{11} methylene protons vs. the CH_2 protons of *S*-ethylthiophenol at δ 3.00.[23] This result confirms previous theoretical calculations[12] and ^1H NMR studies of the isoindole ring protons.[22] (2) The deshielding effect of the ring current

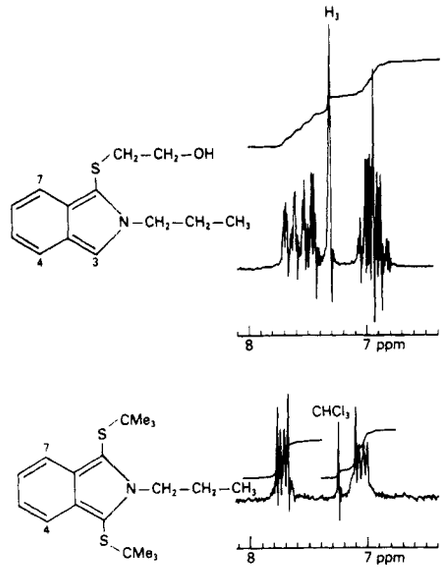
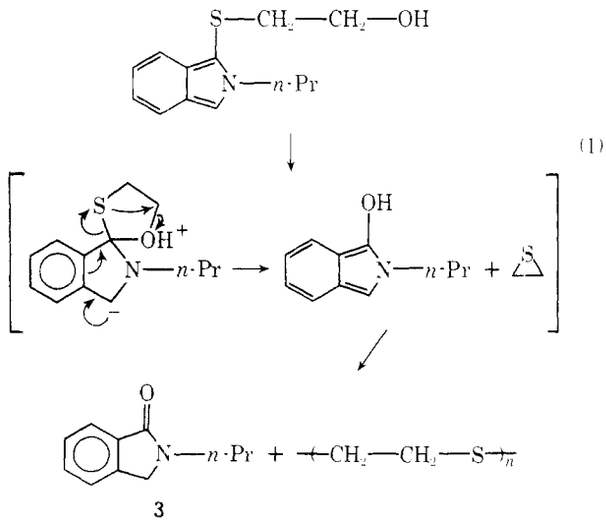


Figure 1. 100-MHz proton NMR spectra of MERC (top) and di-*tert*-butyl (bottom) adducts. Spectra showing the aromatic region for the MERC adduct (the 5 protons of C_3–C_7) in CD_3CN and the di-*tert*-butyl adduct (the 4 protons of C_4–C_7) in $CDCl_3$ are compared.

falls off rapidly with increasing distance from the ring. (3) Those protons δ to the ring system (i.e., C_{10} and C_{12} protons) are shielded. This shielding effect is most noticeable for the thiol substituents and is largest with the ET adduct. In contrast, the chemical shift of the CH_3 group of *S*-ethylthiophenol[23] is unchanged from that of ethanethiol at δ 1.35.

Derivatives of MERC and ET Adducts. The MERC adduct at 0.3 M in 95% EtOH slowly decays at room temperature to give, inter alia, the 2,3-dihydro-1*H*-isoindol-1-one **3** and an insoluble solid which was identified as polyethylene sulfide.[2] This reaction was not appreciably accelerated by the presence of additional water. Under the same conditions, the ET adduct yields a different decomposition product (see below) and no **3**. These results suggest that an intramolecular *nucleophilic attack*, as shown in eq 1, leads to the observed products. A



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major peak at *m/e* 175 (42% of the base peak) in the exact mass spectrum of the MERC adduct that was uniquely identified as $C_{11}H_{13}NO$ (obsd = calcd = 175.0996) further supports this hypothesis. This species, which is the base peak or a major peak in CI mass spectra, could arise from intermolecular attack by the water generated as a reaction byproduct. However, the CI mass spectra of the ET adduct exhibit no such peak even though there is just as much water present.

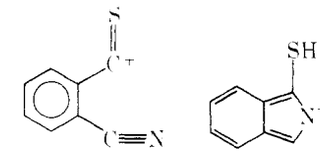
Weakly coppered zinc in acetic acid is efficient in reducing isoindoles to isoindolines.[14,24] When this procedure was applied to the ET adduct, a rapid reaction ensued with the evolution of ethanethiol to give *n*-propylisoindoline. This product presumably was formed by reduction, elimination of ethanethiol with the formation of a cyclic iminium salt, and further reduction.

The MERC and ET adducts react with dienophiles, and a solid 1:1 substitution product of ET adduct and dimethyl acetylenedicarboxylate (DMAC) has been previously reported.[2] These reactions and a detailed examination of the ET adduct–DMAC product are discussed elsewhere.[25]

Other Isoindoles Formed in the Reaction of OPTA and Thiols with Amines. So far, this reaction has proved to be completely general. As anticipated from the results of the *tert*-butyl, ethanedithiol dimer, MERC, and ET adducts, a 1:1:1 ratio of OPTA/thiol/amine gave excellent yields of adducts, as determined by TLC. Development of the TLC plates with I_2 characteristically produced an array of intense, specifically colored spots. Most likely these colors are due to the formation of isoindole–I_2 charge transfer complexes, which is consistent with the π excessive nature of these heterocycles. With dithiol reagents, the above proportions were varied in order to select for the monomer or dimer (e.g., **2**) adduct. All of the adducts were somewhat unstable in solution at room temperature.

Most of the adducts prepared here were not isolated. However, a comparison of the UV spectral properties of the fully characterized *tert*-butyl and ethanedithiol dimer adducts with those of the other adducts in solution established the presence of the isoindole chromophore in each case (Table I). These spectra are quite similar to, but contain much less fine structure than, those of isoindole[20] and *N*-methylisoindole.[26] Of the 1-thio-substituted 2-alkylisoindoles, only the *S*-phenyl and α-carboxyl *N*-alkyl groups cause any noticeable shifts in the λ_{max} wavelengths. The extinction coefficients of the dimeric isoindoles formed with ethanedithiol and dithiothreitol are quite large, but, per isoindole ring, all of the isoindoles examined have much the same ε values.

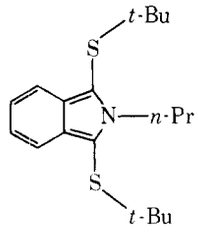
The isoindole structure of each adduct (except for the OPTA/MERC/leucine adduct, which was not examined) was confirmed by the mass spectral molecular weight and fragmentation patterns. In every case, the EI and/or CI[27] mass spectra contained a major peak at *m/e* 146 and/or *m/e* 148. The assigned structures of these ions (**4** and **5**) were supported



	4	5
calcd exact mass:	146.0064	148.0221
obsd exact mass in MERC adduct spectrum:	146.0063	148.0226

by an exact mass determination and appear to be characteristic of isoindoles **1**.

1,3-Dithio-Substituted 2-Alkylisoindoles. Upon standing in solution at room temperature or during attempted recrystallizations, the *tert*-butyl adduct decomposed to give the considerably more stable di-*tert*-butyl adduct **6**. Other isomeric structures were eliminated on the basis of the ^1H NMR spectrum (Figure 1). The absence of a signal attributable to H_3 (or H_1) and the relatively simple and symmetrical two groups of aromatic signals would seem to be diagnostic of the substitution indicated in **6**. As seen for the 1,2-disubstituted isoindoles **1**, the 1,2,3-trisubstituted isoindole **6** also possesses a relatively weak aromatic ring current and causes shielding of the C_{10} and C_{12} protons (Table II). A red shift in the UV



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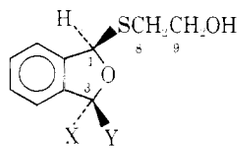
maxima and an increase in the λ_{max} ϵ for the di-*tert*-butyl adduct, compared to the *tert*-butyl adduct (Table I), appear to be characteristic of this substitution pattern. Similar changes have previously been observed for 1,3-diphenyl- vs. 1-phenylisoindoles.[28]

The formation of **6** does not occur by reaction of *tert*-butylthiol with the *tert*-butyl adduct since an excess of *tert*-butylthiol during the production of *tert*-butyl adduct[10] yields virtually no di-*tert*-butyl adduct. Di-*tert*-butyl sulfide, possibly formed during the decomposition of *tert*-butyl adduct, could undergo electrophilic attack on the remaining *tert*-butyl adduct to give **6**. However, when 1.5 equiv of di-*tert*-butyl sulfide was added to almost pure *tert*-butyl adduct, no increased rate of production of **6** was observed. Thus, the di-*tert*-butyl adduct may arise from a disproportionation, or autoxidation,[29] reaction of the *tert*-butyl adduct.

MERC and thiophenol adducts, and especially the ET adduct,[18] are observed to slowly give unisolated decomposition products that behave similarly to the di-*tert*-butyl adduct. Whether they are the analogous 1,3-dithio-substituted isoindoles remains to be established.

Formation of 1,3-Disubstituted 1,3-Dihydroisobenzofurans as Intermediates in the OPTA Reaction. The first operational step in the reaction of OPTA and thiols with primary amines is to combine OPTA and thiol. Since all of the thiols we have examined gave an excellent yield of the isoindole **1**, we expected that each thiol would follow the same basic course of reaction with OPTA. In fact, the results were highly variable. When each thiol was combined with 1 equiv of OPTA, some adduct was always observed by TLC with those thiols carrying functional groups capable of hydrogen bonding while the alkyl- and arylthiols gave no apparent reaction. All attempts to isolate the OPTA/MERC reaction product failed.

A 1:1 OPTA/MERC adduct was suggested by CI mass spectra. Further analysis of the reaction in situ led to the assignment of the diastereomeric 1,3-dihydroisobenzofurans **7** to the unstable OPTA/MERC adducts. The presence of two



7a, X = H; Y = OH
b, X = OH; Y = H

hydroxyl groups in the OPTA/MERC adduct was deduced from the observation of two single proton ^{1}H NMR signals with temperature dependent chemical shifts ($\Delta\delta$ = 0.99 and 0.80 Hz/°C). The existence of an unequal mixture of the diastereomers **7a** and **7b** was most readily seen by the two sets of ^{1}H NMR signals of unequal intensity assigned to the C_3–OH and C_8 methylene protons and the C_9 methylene signals at 56 °C. The major component of this diastereomeric mixture is postulated to be **7a**, which is capable of being stabilized via intramolecular hydrogen bonding, on the basis of a lower OH group frequency in the IR spectrum of the MERC adduct (~3340 cm^{-1}) as compared to the ET adduct (~3400 cm^{-1}). The ^{1}H NMR spectrum of the OPTA/ET adduct exhibits signals which can be assigned to a structure analogous to **7**. However, analysis of the spectrum revealed that only ~20% of the OPTA/ET mixture was present as the 1,3-dihydroisobenzofurans vs. ~85% in the OPTA/MERC solution. A reversible increase in the residual OPTA aldehyde signal at δ 10.1 with increasing temperature indicates that the MERC adducts are in equilibrium with the starting materials.

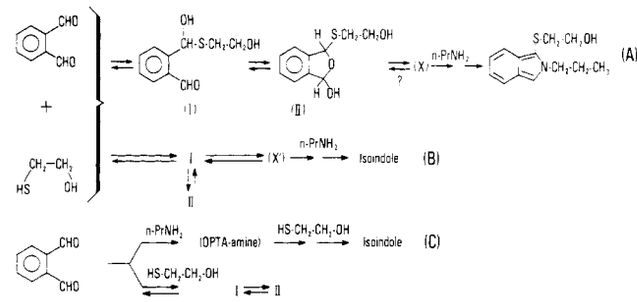


Figure 2. Possible mechanistic pathways for the reaction of OPTA and MERC (or thiol) with *n*-propylamines (or primary amines) to give 1-alkyl(and aryl)thio-2-alkylisoindoles.

Mechanism of the Reaction of OPTA and Thiol with Primary Amines. Two general observations indicate that a 1,3-dihydroisobenzofuran does not react directly with added primary amine. First, while **7** could easily give rise to reactive structures, the intact **7** should be totally unreactive toward added primary amine. Second, the amount of dihydroisobenzofuran formed is very sensitive to the structure of the thiol added to OPTA, and yet all OPTA/thiol solutions react extremely rapidly, even at 0 °C, to give the appropriate isoindole.

Of the three basic schemes (Figure 2) considered for the complete OPTA reaction mechanism, initial reaction of the amine with OPTA, where the formation of II is a nonproductive side reaction (pathway C), was eliminated for two reasons. First, when OPTA and *n*-PrNH$_2$ were mixed under the same conditions used to form isoindoles **1**, a rapid reaction ensued where the color of the solution turned yellow and finally a greenish black within 4 min at 0 °C. In contrast, addition of *n*-PrNH$_2$ to OPTA/thiol solutions gave colorless or light yellow solutions after 10 min at room temperature. These colored products may be related to the unstable colored pigments formed in the reaction of *o*-acetylbenzophenone with primary amines.[30] Second, the amount of isoindole formed is severely reduced by adding the amine to OPTA before adding the thiol. Such addition sequences are also reported to decrease the yield of fluorescent product.[3] Addition of OPTA to a MERC/*n*-propylamine solution at 0 °C or room temperature gave essentially normal yields of the MERC adduct.

Conceivably a reactive intermediate X, or X′, might not form spontaneously in OPTA/thiol solutions but rather could be generated by the presence of added amine. However, the addition of up to 2 equiv of triethylamine caused no perceptible change in the TLC behavior of the OPTA/MERC solution. On the basis of these results, the predicted unreactivity of **7** and the insensitivity of yields of isoindoles **1** to variations in the relative amounts of dihydroisobenzofurans vs. OPTA + thiol, we presently favor pathway B and the scheme in Figure 3. The ability of thiol, when added with amine to OPTA, to eliminate virtually all of the color formed in the reaction of amine with OPTA is consistent with a more rapid addition of thiol than amine to OPTA. Intramolecular hydrogen bonding in the OPTA/thiol addition product, as has been observed with the closely related *o*-(dimethylaminomethyl)benzyl alcohol,[31] would make the affected carbonyl more susceptible to attack by amine than the carbonyls of OPTA. Protonation of OH vs. SR in the imine intermediate

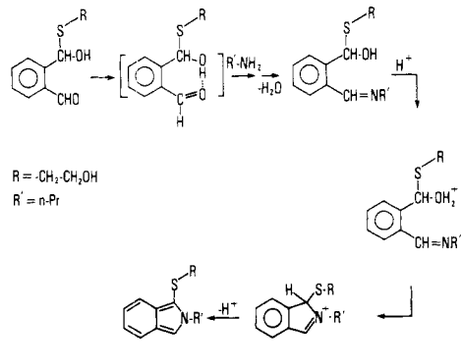


Figure 3. Proposed mechanism for OPTA reaction to give isoindoles.

would be kinetically controlled by the greater basicity of OH to give, after a partially S_N1-like intramolecular reaction, the protonated isoindole and finally **1**. This mechanistic scheme will also account for the lack of fluorogenic reaction with secondary amines[3,6] and primary amines in low pH solutions.[3,4]

Conclusions

We have described a new method of entry into the isoindole ring system and, in particular, the preparation of previously unknown 1-alkyl(and aryl)thio-2-alkylisoindoles. Most of these isoindoles **1** were not isolated but were identified in solution on the strength of spectral data and comparisons with two fully characterized, isolated adducts. The formation of **1** proceeds rapidly under mild, stoichiometric conditions and in very high yield, so there is little need for purification before studying the physical properties or performing further chemistry. This approach is directly supported by the isolation of analytically pure ethanedithiol dimer adduct **2** in 93% yield from the initial reaction solution. The procedure reported here is exceedingly simple and appears to be quite general. In fact, we were surprised that lowering the nucleophilic character of the thiol (i.e., thiophenol) or increasing the steric bulk α to the thiol (i.e., *tert*-butylthiol) had no obvious effect on the rate or yield of the overall reaction.

The isoindoles **1** are much more stable than isoindole itself.[20] Aryl,[24,28] halo,[13,15] and N substitution[32] are usually required for enhanced stability. Very few nonhalogen hetero-substituted isoindoles have reached our attention.[13,16,17] The 1-thio-substituted isoindoles prepared here appear more stable than the isologous 1-alkoxy derivatives.[16] The factors responsible for this stabilization are not understood but may be partially due to d-orbital overlap.

In spite of the aromatic character of isoindoles, they are hyperreactive at positions 1 and 3,[12] as seen by their reaction with dienophiles,[12,16] such as dimethyl acetylenedicarboxylate,[25] and acylating agents.[12,17] This apparent dichotomy has been ascribed to the relatively low energy difference between isoindole and the transient benzene derivative which is formed during electrophilic attack.[33] These same considerations may also explain how this π excessive aromatic heterocycle can undergo an apparent intramolecular nucleophilic reaction (i.e., eq 1). Further support for an intramolecular attack of the OH group of the MERC adduct derives from the observations that the MERC adduct decays faster than the ET adduct in aqueous buffers and that complexation of the OH group inhibits the decay of the MERC adduct.[18,19]

Experimental Section

Materials. OPTA (Aldrich) was recrystallized with a hot filtration from petroleum ether and stored at room temperature in the dark. MERC, ET, methyl mercaptoacetate (all from Eastman), *tert*-butylthiol, thiophenol, ethanedithiol, L-leucine, *n*-propylamine (all from Aldrich), and dithiothreitol (Calbiochem) were used as received. Isooctane (Aldrich gold label) and 95% ethanol (Pharmco) were found to be suitable for use without further purification. Hydro Services deionized water, which was subsequently distilled, was used to prepare 0.5 M $Na_2B_4O_7 \cdot 10H_2O$ (Allied Chemical). Silica gel (GF) and neutral alumina (GF) TLC plates were purchased from Analtech.

Instrumentation. Melting points were determined on a Fisher-Johns hot stage melting point apparatus and are uncorrected. Perkin-Elmer 237B grating infrared and Carey 14 spectrophotometers were used to record IR and UV spectra, respectively. NMR spectra were acquired at 60 (Varian A-60) or 100 MHz (Varian HA-100 spectrometer). Low-resolution mass spectra were obtained on Hitachi Perkin-Elmer RMU-6E (electron impact [EI] mode) or Finnigan 1015D (chemical ionization [CI] mode) spectrometers. A Jeol JMS-015G-2 spectrometer with an Ionomet photoplate was used for the high-resolution mass spectra. Analyses were performed by the Microanalytical Section of the Laboratory of Chemistry, NIAMDD, Bethesda, Md.

***tert*-Butyl Adduct.** Method A utilized 1 equiv of each reagent. A solution of OPTA (163 mg, 1.216 mmol) and *tert*-butylthiol (0.137 mL) in 1.2 mL of 95% EtOH was placed in ice for 10–15 min following 10 min at room temperature. The addition of *n*-propylamine (0.1 mL) gave an exothermic reaction, producing a yellow-orange solution. Brief (~5 s) cooling of the mixture in ice initiated crystallization which proceeded efficiently at room temperature. After cooling at 0 °C, an 86% yield (259 mg) of pale yellow, mica-like plates (mp 48.0–56.5 °C) was obtained. Three rapid recrystallizations from petroleum ether gave analytically pure *tert*-butyl adduct as almost colorless blocks: mp 58.3–59.0 °C; IR (Nujol) ~2900, 1460, 1364, 1317, 1162, 767, and 747 cm^{-1}. Conventional EI mass spectrometry gave peaks (% abundance in parentheses) at *m/e* 247 (M^+, 5.5), 191 (M − isobutylene, 63), 148 (191 − C_3H_7, 86), and 57 (Me_3C, 100). See Tables I and II for UV and ^1H NMR data. Anal. Calcd for $C_{15}H_{21}NS$: C, 72.82; H, 8.56; N, 5.66; S, 12.96. Found: C, 73.15; H, 8.46; N, 5.42; S, 12.56.

Method B involves the use of excess thiol. To 163 mg of OPTA (1.216 mmol) in 0.568 mL of 95% EtOH was added 0.548 mL of *tert*-butylthiol (4.861 mmol). After 15 min at room temperature and then again at 0 °C, *n*-propylamine (0.1 mL, 1.216 mmol) was added to produce an exothermic reaction and a bright yellow solution containing >90% of the *tert*-butyl adduct (by TLC; after I_2 visualization, the adduct was green-brown on silica gel and black on neutral alumina). After removing the volatile components under a stream of nitrogen, the residue was dissolved in a minimum amount of petroleum ether at room temperature to give a two-phase solution which, when cooled to 0 °C, yielded 199 mg (66%) of the *tert*-butyl adduct as clear, light yellow blocks (mp 58.5–59.5 °C).

Ethanedithiol Dimer Adduct 2. Reaction of 163 mg of OPTA (1.216 mmol), 51 μL of ethanedithiol (0.608 mmol), and 100 μL of *n*-propylamine in 2.28 mL of absolute EtOH was accomplished as for the preparation of the *tert*-butyl adduct (method A) to give a clear colorless solution which was allowed to warm to room temperature in the dark. Less than 1 min after adding the amine, the reaction mixture suddenly became a white emulsion from which solid soon began to crystallize. After 10 min at room temperature and 30 min at 0 °C, the crystalline product was isolated by centrifugation, washing with 10 mL of 95% EtOH at 0 °C, and recentrifugation to give, after drying under vacuum, 231 mg (93% yield) of small analytically pure, off-white needles (mp 124.5–130.5 °C). The adduct was purple-black on neutral alumina after I_2 visualization. Mass spectral[27] peaks were observed at *m/e* 408 (M^+, 2), 190 (100), and 148 (80); IR (Nujol) 3125, 3060, 1458, 1321, 1176, 769, and 752 cm^{-1}. See Tables I and II for UV and ^1H NMR data. Anal. Calcd. for $C_{24}H_{28}N_2S_2$: C, 70.54; H, 6.91; N, 6.86. Found: C, 70.12; H, 7.05; N, 6.60.

General Procedure for Preparation of Isoindoles 1. A 1 M solution of each reagent was prepared in 95% EtOH and stored at 0 °C. Some decomposition of 1 M OPTA was observed at 0 °C, but solutions could be stored for up to 6 months at −20 °C with almost no decomposition. While very concentrated solutions of the adducts were used to obtain the IR or NMR spectra (e.g., OPTA in enough benzene to affect dissolution followed by thiol, cooling, *n*-propylamine, and absorption of the generated water with 3A molecular sieves gave the IR sample), the usual reactions employed the above 1 M solutions. Thus, 100 μL each of 1 M OPTA and 1 M thiol were mixed at room temperature, and the mixture was allowed to stand for 10 min, cooled at 0 °C for 10 min, and then treated with 100 μL of 1 M *n*-propylamine. After 1 min at 0 °C followed by 10 min at room temperature, these ~0.33 M solutions of the various isoindoles were stored at 0 °C.

Exceptions to this general procedure include the preparations using leucine, where 50 μL of a 1:1 mixture of 1 M OPTA/1 M MERC at 0 °C was added to 0.25 mL of 0.1 M leucine in 0.05 M sodium tetrabo-

rate, and thiols such dithiothreitol and ethanedithiol which contain two SH groups. An eightfold excess of these thiols was used when the monomer adduct was desired; 0.5 equiv of the thiol yielded the bridged diisoindole or dimer adduct. When the ethanedithiol dimer was prepared by this method, a 95% EtOH insoluble white solid crystallized out of the reaction solution. This solid was dissolved immediately in EtOAc (final concentration, 2.0×10^{-2} M) and used for all subsequent UV and fluorescence[18] measurements. See Table I for the characteristic UV spectral properties of these isoindoles.

β-Mercaptoethanol Adduct. An exact mass determination and a full ^{1}H NMR spectrum of this adduct have been previously reported.[2] This adduct gave a red to red-brown color with I_2 staining on neutral alumina and silica gel, respectively: IR (~10 M in benzene) 3350, 3030, 1460, 1320, 1170, 759, and 747 cm^{-1}; EI mass spectral peaks at *m/e* 235 (M^+, 47), 190 ($M - C_2H_4OH$, 85), 175 ($M - C_2H_4S$, 42), 148 (**5**, 35), and 146 (**4**, 100). See Table II for ^{1}H NMR data.

Ethanethiol Adduct. On silica gel, or neutral alumina, this adduct turns brown, or blue-black, with I_2 staining: IR (~10 M in benzene) 3030, 1460, 1320, 1170, 758, and 746 cm^{-1}; CI[27] mass spectral peaks at *m/e* 219 (M^+, 44), 190 ($M - C_2H_5$, 100), and 148 (**5**, 81). See Table II for ^{1}H NMR data.

2,3-Dihydro-1*H*-isoindol-1-one 3. An approximately 1 M solution of the MERC adduct, prepared from 1 equiv (0.89 mmol) each of OPTA, MERC, and *n*-propylamine in 0.7 mL of acetonitrile and containing 7 equiv of water, was allowed to decompose for two weeks in the dark at room temperature. The insoluble solid that formed was removed by filtration (see below for characterization), and preparative TLC (3:1 benzene/ethyl acetate on neutral alumina) of the concentrated filtrate gave a 78% yield (123 mg) of the cyclic amide as an almost TLC pure yellow liquid. After two weeks at −20 °C, a low melting (~30 °C) solid was formed. Four recrystallizations from ~1:1 petroleum ether/ether (dissolved at room temperature and cooled to −50 °C in a $CHCl_3/N_2$ slush bath) gave colorless needles whose melting point (33.1–34.7 °C) was similar to that of the known *N*-ethyl derivative (44–45 °C).[34] The exact mass determination and IR and 60-MHz NMR spectra have been reported elsewhere.[2] The low-resolution mass spectrum (EI mode) gave peaks at *m/e* 175 (M^+, 28), 160 ($M - Me$, 2), 146 ($M - C_2H_5$, 100), and 91 (36).

The above insoluble solid of the decomposed MERC adduct solution was washed with acetone to give 2.2 mg of a pale green solid. Similar preparations of polymer (mp 111.7–115.0 °C) gave CI (isobutane) mass spectral peaks at m/e $105 + n \times 60$, $123 + n \times 60$, $137 + n \times 60$, $139 + n \times 60$, and $153 + n \times 60$, where $n = 0$–4, were usually observed. "Authentic" polyethylene sulfide, formed from the boron trifluoride catalyzed reaction of ethylene sulfide in MeOH, decomposed at 180–187 °C and gave an IR spectrum almost identical with that of the above lower melting polymer. Sulfur analysis: calcd for $+CH_2CH_2-S+_n$, 53.34%; obsd for MERC adduct reaction product, 52.41%; obsd for "authentic" polymer, 51.74%.

***n*-Propylisoindoline.** ET adduct (0.25 mmol; formed in 0.1 mL of acetonitrile from 1 equiv of each reagent) was added to 2 mL of glacial acetic acid to give a bright green solution. Immediately, 0.17 g of fresh, weakly coppered zinc dust (~2.5 mmol) was added at room temperature during 5 min. After 20 min, 6 mL of H_2O was added to the red-brown solution, which smelled heavily of ethanethiol. After 2 h, filtration and washing with CH_2Cl_2 of the now yellow solution followed by addition of concentrated NH_4OH to pH >9, extraction (40 mL CH_2Cl_2), and removal of the dried ($MgSO_4$) solvent under reduced pressure gave a 68% yield (28.5 mg) of the crude amine as a light brown liquid. The IR spectrum (Nujol) was characteristic of 1,3-unsubstituted N-substituted isoindolines, i.e., no aromatic bands from 1600–1500 cm^{-1} and a band at ~2765 cm^{-1},[35] which was found at 2786 cm^{-1} here. Mass spectral peaks were observed at *m/e* 162 (MH^+, 100) in the CI mode with isobutane and at *m/e* 161 (M^+, 14), 160 ($M - H$, 13), 132 ($M - C_2H_5$, 100), 118 ($M - C_3H_7$, 24), and 105 ($M - C_3H_6N$, 34) in the EI mode.

Di-*tert*-butyl Adduct 6. *tert*-Butyl adduct (mp ~48–54 °C) gave, upon recrystallization from methanol or acetonitrile, a low yield of slightly impure di-*tert*-butyl adduct (mp 124–126 °C). Alternatively, the *tert*-butyl adduct in acetonitrile (± heat; ± added *tert*-butylthiol) for 1–2 weeks gave 15–40% yields of material after preparative TLC (3:1 petroleum ether/benzene on silica gel). A second preparative TLC treatment and two recrystallizations from acetonitrile gave analytically pure di-*tert*-butyl adduct **6**: mp 126.2–127.0 °C; IR (Nujol) ~2880, 1453, 1363, 1324, 1163, and 751 cm^{-1}. Mass spectral peaks were observed at *m/e* 336 (MH^+, 100) in the CI mode with isobutane and at *m/e* 335 (M^+, 5), 279 (M − isobutylene, 3), 223 (M − 2 isobutylene, 32), 146 (96), and 57 (Me_3C^+, 100) with the EI mode. See Tables I and II for UV and ^{1}H NMR data. Anal. Calcd. for $C_{19}H_{29}NS_2$: C, 68.00; H, 8.71; N, 4.17; S, 19.11. Found: C, 68.21; H, 8.76; N, 4.31; S, 18.88.

1,3-Dihydroisobenzofurans (e.g., 7). The same procedure that was used to form the isoindoles **1** was followed except that no amine was added. With MERC, CI[27] mass spectral peaks were observed at *m/e* 212 (M^+, 0.1), 194 ($M - H_2O$, 0.1), 167 ($M - C_3H_4OH$, 0.2), 135 ($M - SC_2H_4OH$, 72), 134 (38), and 77 (100). The 60-MHz ^{1}H NMR spectrum ($CDCl_3$ at 23.5 °C) of OPTA/MERC exhibited the following: aromatic H at δ 7.33 (4 H); 1-H and 3-H centered at δ 6.45 (2 H) as two pairs of unequal singlets; 3-OH as two broad, unequal signals at δ 5.85 and 5.98 (1 H); 9-OH at δ ~3.95 (broad) and 9-H at δ 3.67 (triplet, J = 5.5 Hz) (total of 3 H); and 8-H as two unequal triplets (J = 5.5 Hz) at δ 2.69 and 2.65 (2 H). A linear variation in the chemical shift of C_3–OH (0.99 Hz/°C) and C_9–OH (0.80 Hz/°C) signals was observed from −43 to +56 °C.

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Registry No.—**3**, 61214-23-1; **7a**, 66161-47-5; **7b**, 66161-37-3; HS–R (R = Et), 75-08-1; HS–R (R = CH_2CH_2OH), 60-24-2; HS–R (R = *t*-Bu), 75-66-1; HS–R (R = Ph), 108-98-5; HS–R (R = CH_2CH_2SH), 540-63-6; HS–R (R = *threo*-$CH_2CHOHCHOHCH_2SH$), 3483-12-3; HS–R (R = CH_2COOMe), 2365-48-2; L-leucine, 61-90-5; propylamine, 107-10-8; *o*-phthalaldehyde, 643-79-8; polyethylene sulfide, 24936-67-2; *N*-propylisoindoline, 66161-38-4.

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