Preparation of a Stable, Fluorescent 1-Alkylthio-2-alkylisoindole

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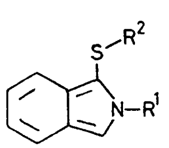
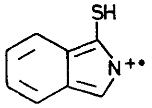
Summary The first thio-substituted isoindole, formed in the fluorogenic reaction of *o*-phthalaldehyde and thiols with primary amines, has been isolated and characterized.

Various isoindoles have been synthesized[1] but very few heterosubstituted isoindoles have yet been isolated.[2] Interest in sulphur-substituted isoindoles has been stimulated by the recent report that the reaction of *o*-phthalaldehyde and β-mercaptoethanol with primary amines[3] gives 1-alkylthio-2-alkylisoindoles [*e.g.*, (I)] as the intensely fluorescent products.[4] This reaction appears to be the most sensitive method presently available for the detection of amino-acids.[3] The structure of (I) was originally arrived at indirectly after examining solutions of (Ia) and (Ib), both of which resisted isolation.[4] We now describe the preparation and first reported isolation of a thio-substituted isoindole [*i.e.*, (Ic)], and present direct proof of structure of the fluorescent *o*-phthalaldehyde adducts.

In a modification of the usual fluorogenic reaction conditions,[3] *o*-phthalaldehyde, Bu^tSH, and Pr^nNH_2 were combined (1:4:1) at 0 °C in 95% EtOH to give a 1 M solution of the t-butyl adduct (Ic) in excellent yield, as determined by t.l.c. Removal of volatile material under a stream of nitrogen gave the crude, somewhat unstable solid adduct. Several recrystallizations from light petroleum gave analytically pure crystals of (Ic), m.p. 58·3—59·0 °C, ν (Nujol) 1460, 1162, 766, and 746 cm^{-1}. Mass spectral analysis of (Ic) (M^+ 247) revealed peaks at *m/e* 57 and 148 (relative abundance 100% and 86%, respectively), which were assigned to Me_3C^+ and the resonance-stabilized ion (II),



(I)



(II)

a; $R^1 = Pr^n, R^2 = CH_2CH_2OH$
b; $R^1 = Pr^n, R^2 = Et$
c; $R^1 = Pr^n, R^2 = Bu^t$

and the n.m.r. spectrum (60 MHz, $CDCl_3$) exhibited peaks at δ 7·8—6·8 (m, 5H), 4·33 (t, *J* 7·3 Hz, 2H), 1·83 (t of q, $J = J'$ = 7·3 Hz, 2H), 1·22 (s, 9H), and 0·85 (t, J' 7·3 Hz, 3H). Furthermore, the aromatic region displayed a 2:1:2 proton pattern similar to that reported for *N*-methylisoindole.[5]

We conclude that the unisolated, fluorescent *o*-phthalaldehyde reaction products [*e.g.*, (Ia) and (Ib)] possess the 1-alkylthio-2-alkylisoindole structure of (Ic) on the basis of similar modes of preparation and common fluorescent spectra (λ_{excit} 338 nm in 95% EtOH). Compared to (Ia) and (Ib),[6] the emission maximum of (Ic) is shifted to lower wavelengths (Δ *ca.* 40 nm) and is an order of magnitude less intense. Both effects are undoubtedly due to the hydrophobic t-butyl group of (Ic). Finally, the similar i.r. and n.m.r. spectra and mass spectral fragmentation of (Ic) and solutions of (Ia) and (Ib) directly substantiate the isoindole structure for these fluorescent reaction products.

In view of this simple preparation of 1-alkylthio-2-alkylisoindoles, it should now be possible to prepare other derivatives; we have already determined that the use of ethanethiol, in place of β-mercaptoethanol, conveys several advantageous properties to the fluorescent isoindole.[6]

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