Cycloaddition Reactions Relevant to the Mechanism of the Ninhydrin Reaction. *X*-Ray Crystal Structure of Protonated Ruhemann's Purple, a Stable 1,3-Dipole

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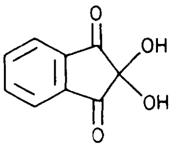
Trapping experiments with dipolarophiles provide evidence for the formation of 1,3-dipoles as intermediates in the ninhydrin reaction and protonated Ruhemann's purple is shown to be a stable N-protonated 1,3-dipole by trapping experiments and by an *X*-ray crystal structure determination.

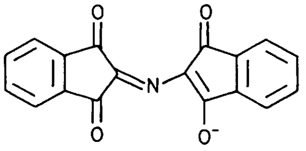
The use of ninhydrin (**1**) for the detection and quantitative estimation of α-amino acids depends on the formation of Ruhemann's purple (**2**), a purple dye. The mechanism of this important reaction has been the subject of numerous investigations,[1,2] and the current mechanistic position has been summarised.[3] Our recent work on decarboxylative transamination[4] suggests a modification of the currently accepted mechanism. Thus we were able to trap intermediate 1,3-dipoles (**3**) as cycloadducts (**5a**) by carrying out the reaction of ninhydrin with α-amino acids in the presence of *N*-phenylmaleimide (**4a**). The observation that the ninhydrin reaction occurs at an optimal pH of 4.5—5.2[5] supports (**3**) as an intermediate rather than the aza-allylic anion (**6**) although this 4 π-anion could also lead to (**5**).

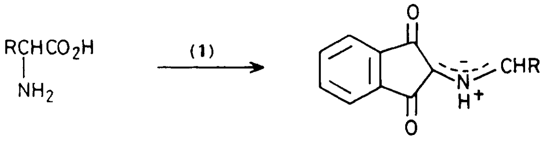
The 1,3-dipole (**3**) in the absence of a dipolarophile is expected to undergo prototropy to (**7**)[4] which leads eventually[3] to Ruhemann's purple. When (**1**) is treated (MeCN, 80 °C, 12 h) with glycine methyl ester hydrochloride and (**4b**), decarboxylation is prevented and the intermediate dipole (**8**) is trapped as the adduct (**5b**), (74%). The generation of the dipole may involve the H-bonded enol (**9**)[6] or a 1,5-H shift (**10**) (arrows).[7] The stereochemistry of (**5b**) was established by nuclear Overhauser difference spectroscopy.

Ruhemann's purple (**2**) is a stabilised aza-allylic anion. Kauffmann's extensive work with aza-allylic anions[8] has shown that they participate in 4π + 2π cycloadditions. However, attempts to prepare a cycloadduct from (**2**) and (**4a**) or (**4b**) were unsuccessful. Protonation of Ruhemann's purple (**2**), an ambident anion, could occur at carbon, oxygen, or nitrogen. A spectroscopic study (i.r., n.m.r., mass) of protonated Ruhemann's purple concluded the protonated form had structure (**11**).[2] However, it seemed to us that the u.v. spectrum of protonated Ruhemann's purple [$\lambda_{max.}$ ($CHCl_3$) 485 (ε 16 000)][2] was more in accord with structure (**12**), a structure considered but discarded by the previous workers.[2] Structure (**12**) is a 1,3-dipole and we therefore attempted cycloaddition reactions between protonated Ruhemann's purple and dipolarophiles. *N*-Phenylmaleimide (**4a**) reacted [dimethylformamide (DMF), 100 °C, 20 min] with protonated Ruhemann's purple to give the cycloadduct (**13a**), 89%. Maleic anhydride (**4c**) reacted [tetrahydrofuran (THF), 60 °C, 24 h] similarly to give (**13b**), 75%, whilst methyl propiolate (THF, 60 °C, 24 h) gave (**14**), 76%.

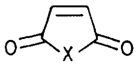
Thus the solution chemistry of protonated Ruhemann's purple accords with its formulation as a stable N-protonated 1,3-dipole (**12**). We have postulated such N-protonated dipoles as intermediates in the thermal cycloaddition reactions of X=Y–ZH systems[6] and in decarboxylative transamination reactions[4] and have sought to prepare stable examples of such species. We therefore undertook an *X*-ray crystal structure


(**1**)

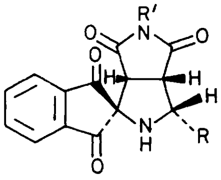

(**2**)



(**3**)

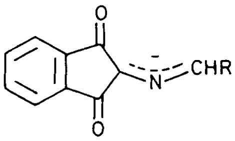

(**4**)

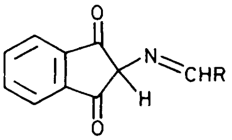
a; X = NPh
b; X = NMe
c; X = O

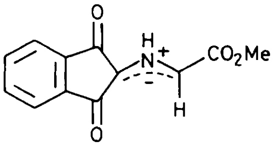


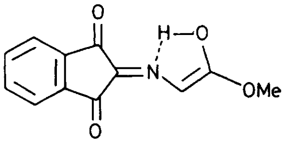
(**5**)

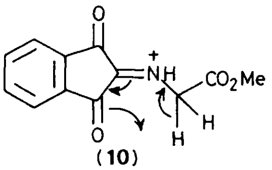
a; R' = Ph
b; R = CO₂Me, R' = Me

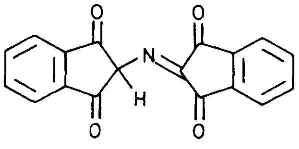

(**6**)

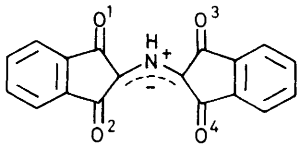

(**7**)

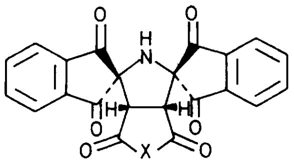

(**8**)


(**9**)


(**10**)


(**11**)

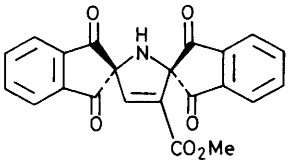

(**12**)



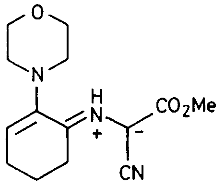
(13)

a; X = NPh

b; X = O



(14)



(15)

determination of protonated Ruhemann's purple and this has unambiguously confirmed the 1,3-dipole structure (**12**) in the solid state.† A projection of the molecule is shown in Figure 1.

The *X*-ray results are completely consistent with structure (**12**), with no significant differences between the two halves of the molecule [*e.g.* N–C bonds are 1.340(5) and 1.346(4) Å]. The CNC bond angle is 127.9(3)° and repulsion between O(2) and O(4) is relieved by rotation of each indan-1,3-dione moiety by about 20° around the C–N bonds. Recently a second example of a stable N-protonated 1,3-dipole (**15**) has come to our attention[9] and this further reinforces our proposals for the intermediacy of such species in the thermal cycloaddition reactions of X=Y–ZH species,[6] and in decarboxylative transamination reactions.[4]

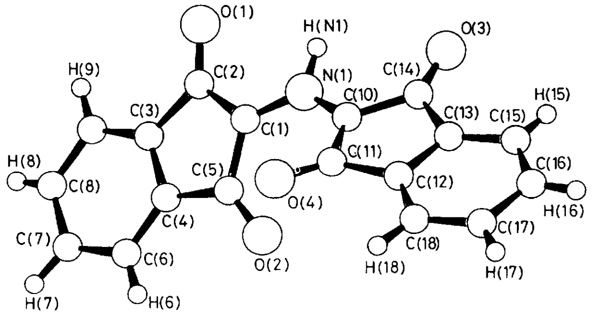


Figure 1

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† *Crystal Data:* $C_{18}H_9NO_4$, M = 303.3, monoclinic, a = 11.659(10), b = 19.398(16), c = 5.931(5) Å, β = 93.15(8)°, U = 1339.3 Å^3, Z = 4, D_c = 1.504 g cm^{-3}, $F(000)$ = 624, space group $P2_1/n$ (No. 14), Mo-K_α radiation, λ = 0.71069 Å, μ(Mo-K_α) = 0.65 cm^{-1}.

Diffraction data were recorded on a Stöe STADI-2 diffractometer. The structure was determined and refined using the program SHELX. All hydrogen atoms were located in a difference Fourier synthesis and were included in the refinement with individual isotropic vibration parameters. Non-hydrogen atoms were allowed to vibrate anisotropically. In the final stages the 998 reflections with $F > 6\sigma(F)$ were used and yielded a final R value of 0.038.

The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW. Any request should be accompanied by the full literature citation for this communication.