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RAPID COMMUNICATION

Ninhydrin reaction with phenylethylamine: unavoidable by-products

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Abstract. Ninhydrin and phenylethylamine reacted to form a complex mixture from which four products were isolated: a benzo-fused spiroheterocyclic and a pyrrole product resulting from tricomponent condensation between ninhydrin, phenylethylamine and phenylacetaldehyde produced *in situ*, a 2-amino-1,3-indandione dimerisation-dehydrogenation product and a Schiff base. This article analyses these compounds' structure and proposes reaction mechanisms contributing to knowledge regarding ninhydrin and phenylethylamine chemical reactivity.

Keywords. Ninhydrin; phenylethylamine; phenylacetaldehyde; Schiff base.

1. Introduction

Ninhydrin **1** is an indane-derived tricarbonyl compound; it is highly electrophilic due to having three consecutive carbonyls. Ninhydrin usually reacts with amines, phenols and enolizable carbonyl compounds yielding differing heterocyclic compounds such as cyclophanes, isoindolinones, hydroindoles, isoquinolines and β-carbolines[1]. Ninhydrin reaction with primary amines yields a dark blue or purple compound, known as Ruhemann's purple. This coloured compound's formation has been useful for developing qualitative and quantitative analytical techniques for amines.[2,3]

The tricomponent reaction between ninhydrin **1**, phenylacetaldehyde **2** and primary amines in equimolar amounts yields fluorescent pyrrole derivatives which are also useful for quantifying primary amines, including peptides. Three structurally related products have been isolated from this reaction: a heterocyclic compound **3**, a spiroheterocyclic compound **4** and a pyrrole **5** (Scheme 1).[1,4]

Recent studies have shown that the reaction of ninhydrin **1** with phenylethylamine **6** has not had the overall behaviour described for amines and produces a benzo-fused spiroheterocyclic product (spiro[furan-2,1′-isoindoline]) **7**. This spiroheterocyclic product's **7** formation can be explained by means of a tricomponent reaction between ninhydrin, phenylethylamine and phenylacetaldehyde, produced *in situ* (Scheme 2).[5]

The spiroheterocyclic product **4** yielded by the reaction between ninhydrin, phenylacetaldehyde and primary amines and the spiroheterocyclic product **7** yielded by the reaction between ninhydrin, phenylethylamine and phenylacetaldehyde produced *in situ* had a great structural similarity.[4,5] They only differed regarding the position of nitrogen and oxygen; **4** had a lactone and an enamine whilst **7** had a lactam and an enol ether. Although having structural similarity, these two spiroheterocycles' had different formation mechanisms. The first step in the formation of **4** implied the reaction of ninhydrin carbon 2 with phenylacetaldehyde carbon α whilst the first step in the formation of **7** occurred through the reaction of ninhydrin carbon 2 with phenylethylamine. This pattern shows that the order of adding the reagents affected the course of the reaction.

Continuing our studies orientated towards understanding phenylethylamines' chemical behaviour regarding carbonyl compounds, this article describes a structural analysis of three unavoidable sub-products resulting from ninhydrin's reaction with phenylethylamine and proposes reaction mechanisms contributing

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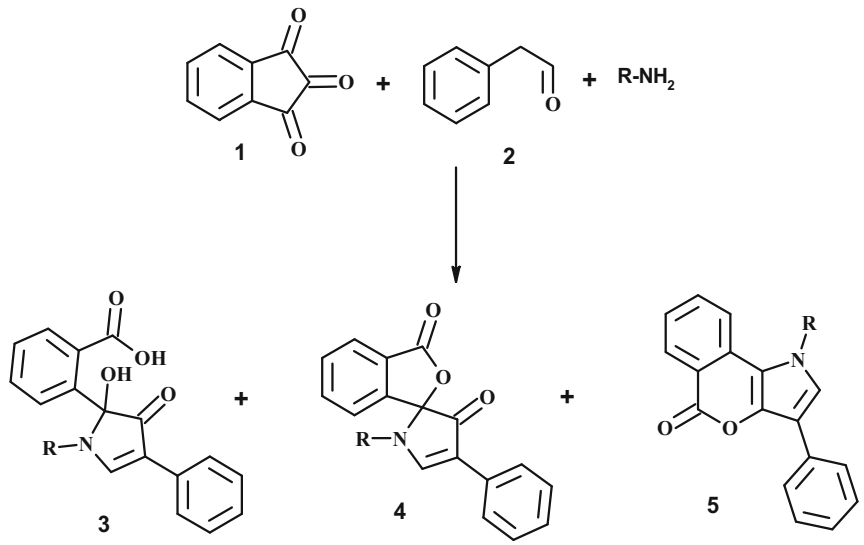
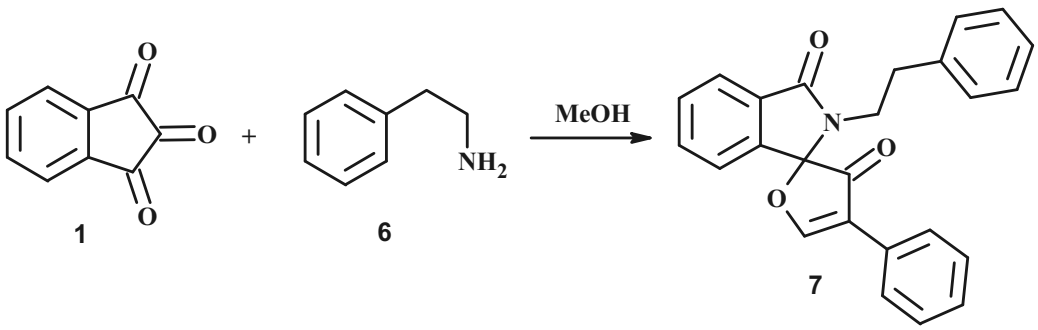


Scheme 1. Tricomponent reaction between ninhydrin **1**, phenylacetaldehyde **2** and primary amines.



Scheme 2. Reaction between ninhydrin **1**, phenylethylamine **6** and phenylacetaldehyde produced *in situ.*

towards knowledge regarding the starting reagents' chemical reactivity.

2. Experimental

Ninhydrin reaction with phenylethylamine: Phenylethylamine (9.0 mmol, 1.092 g) was added to a ninhydrin (9.0 mmol, 1.440 g) in methanol solution. The mixture was stirred at room temperature until the reactants' disappearance was observed by TLC; the solvent was removed under reduced pressure. The residue so obtained was purified by column chromatography on silica gel using toluene:ethyl acetate mixtures as the mobile phase.

2′-phenethyl-4-phenyl-3H-spiro[furan-2,1′-isoindoline]-3,3′-dione **7**, White solid, 785 mg, yield: 46%, M.p. 144-145 °C[5]

2-[(2-phenylethyl)imino]-1H-indene-1,3(2H)-dione **8**, yellow solid, 121 mg, yield: 5%, M.p. 128-130 °C IR (ATR): (cm^{-1}) 3030, 2933, 1714. ^{1}H NMR: (400 MHz, $CDCl_3$) δ (ppm): 3.01 (t, 2H, J = 7.6 Hz), 3.95 (t, 2H, J = 7.6 Hz), 7.24-7.33(m), 7.73 (dd, 2H, J = 5.6; 2.8 Hz), 7.85 (dd, 2H, J = 5.6; 2.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 34.6, 39.3, 123.2, 126.6, 128.6, 128.9, 132.1, 133.9, 138.1, 168.2; EI-MS: (*m/z*) 263.9 (88%), 133.1 (29%), 104.2 (100%)

6,12-dioxo-6,12-dihydro-diindenopyrazine **9**, orange solid, 243 mg, yield: 19%, M.p. did not melt up to 350 °C IR (ATR): (cm^{-1}) 1728. ^{1}H NMR: (400 MHz, $CDCl_3$) δ (ppm): 7.63 (t, 2H, J = 7.6 Hz), 7.76 (t, 2H, J = 7.6 Hz),): 7.87 (d, 2H, J = 7.2 Hz), 8.05 (d, 2H, J = 7.2 Hz), ^{13}C NMR (100 MHz, $CDCl_3$) δ: 122.7, 124.7, 133.0, 134.9, 136.0, 139.7, 147.4, 163.4, 189.2; EI-MS: (*m/z*) 283.8 (78%), 227.9 (100%), 201.0 (20%), 100.1 (18%), 74.0 (14%).

1-phenethyl-3-phenylindeno[1,2-b]pyrrol-4(1H)-one **12**, red solid, 440 mg, yield: 28%, M.p. 69-70 °C IR (KBr): (cm^{-1}) 3059, 3028, 1695. ^{1}H NMR: (400 MHz, $CDCl_3$) δ (ppm): 3.13 (t, 2H, J = 7.3 Hz), 4.16 (t, 2H, J = 7.2 Hz), 6.66 (s, 1H), 6.80 (d, 1H, J = 7.3 Hz), 7.05 (t, 1H, J = 7.83), 7.14 (m, 3H), 7.17 (d, 1H, J = 1 Hz), 7.19-7.24 (m, 2H), 7.27 (d, 2H, J = 7.6 Hz), 7.36 (t, 2H, J = 7.7 Hz), 7.40 (d, 1H, J = 7.1 Hz), 7.89 (dd, 2H, J1 = 8.21 Hz,

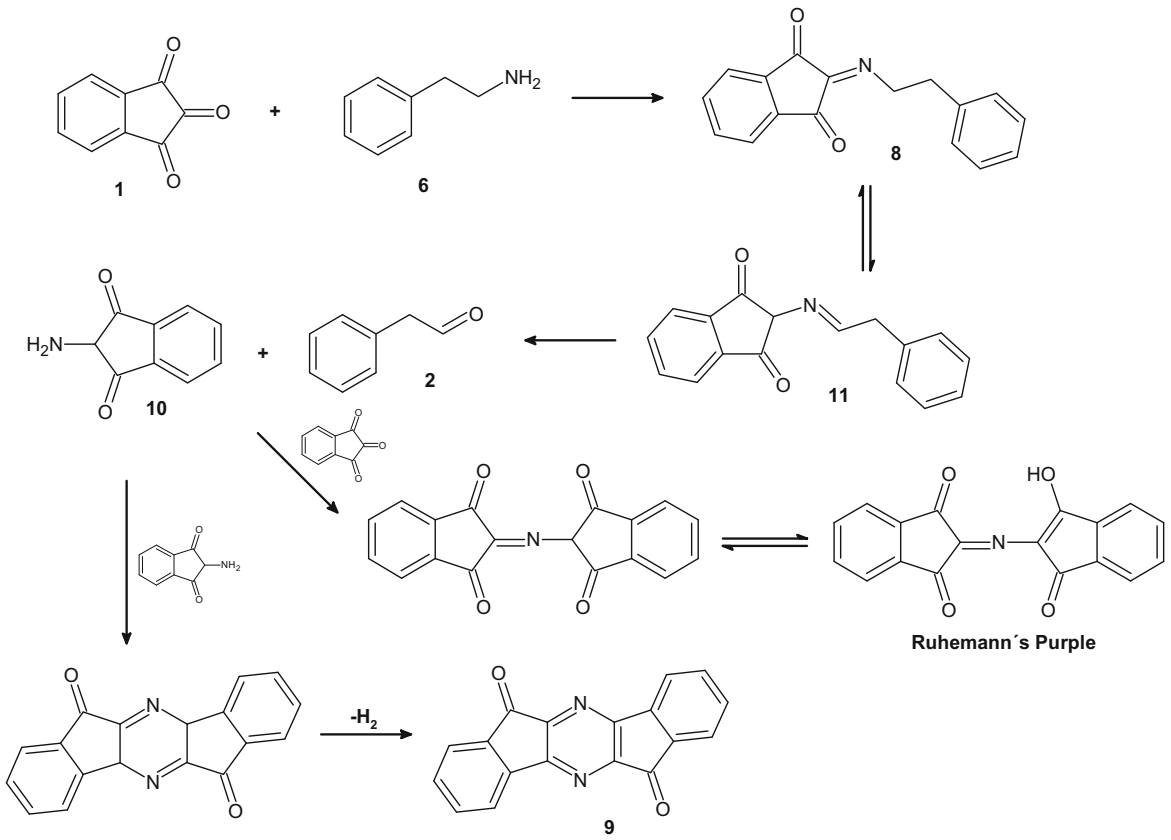
J2 = 1,14 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 37.6, 50.2, 116.5, 120.8, 123.2, 123.4, 129.9, 124.4, 128.5, 128.8, 132.2, 132.9, 133.9, 135, 136.9, 139.7, 150.8, 168.2, 185.8; EI-MS: (*m/z*) 349.1, 258.1, 245.1, 230.2, 217.2, 202.2, 105.2, 91.2, 77.1; ESI-MS: (*m/z*) 350.143, Calc. 350.144

3. Results and Discussion

As mentioned beforehand, the ninhydrin **1** reaction with phenylethylamine **6**, which involved using methanol as solvent, led to producing 2′-phenethyl-1,4-phenyl-3*H*-spiro[furan-2,1′-isoindoline]-3,3′-dione **7**. In addition to spiro **7**, three minority compounds having different structural characteristics were isolated in his work.

The ^{1}H-NMR spectra for the first of them revealed two signals in the aliphatic region which were characteristic for the ethylene from phenylethylamine and a multiplet in the aromatic region between 7.2 and 7.4 ppm, suggestive of the hydrogens from a phenylethylamine ring, and two doublets of doublets at 7.7 and 7.8 ppm, suggestive of the hydrogenous from a ninhydrin ring. The ^{13}C-NMR spectra gave the expected signals for both these systems. The EI-MS spectra revealed a molecular ion at m/z 263.9 and a base peak at *m/z* = 104.2. This information, together with the simplicity of the NMR spectra, led to conclude that the compound was a Schiff base **8**, being a product of the reaction between phenylethylamine and carbon 2 from ninhydrin (Scheme 3). Obtaining this compound confirmed that carbon 2 was ninhydrin's most electrophilic site due to the ring's electro-attractor effect and that of the two adjacent carbonyls. It also showed that the reaction in the conditions used in this work did not completely favour products having greater complexity.

^{1}H- and ^{13}C NMR spectra for the second compound **9** gave signals only coming from ninhydrin **1**. It was thus deduced that neither phenylethylamine nor phenylacetaldehyde produced *in situ* were involved in the formation of this product. Elemental analysis and the molecular ion at m/z 283.8 in EI-MS spectra enabled the $C_{18}H_8N_2O_2$ molecular formula to be calculated. The information so obtained led to establishing that compound **9** was a 6,12-dioxo-6,12, dihydrodiindenopyrazine (Scheme 3). This compound has been previously synthesised by photolytic dimerisation of 3-azido-1-indenone and by dimerisation of 1-nitro-1,3-indandione in ethanol; such dimerisation occurred through intermediary 2-amino-



Scheme 3. The mechanism proposed for the formation of the new products (**9**, **10**) from the ninhydrin reaction with phenylethylamine.

1*H*-indene-1,3(2*H*)-dione **10**, a compound which has been proposed as an intermediary in ninhydrin reaction with amines.[6,7]

The two compounds so isolated **8**, **9** confirmed the general mechanism for the ninhydrin reaction with amines. The first step involved the formation of ketimine **8** through the reaction between carbon 2 from ninhydrin **1** and phenylethylamine **6**. Ketimine **8** - aldimine **11** equilibrium, followed by hydrolysis, led to the formation of phenylacetaldehyde **2** and 2-amino-1,3-indandione **10**, a compound which was isolated by Ruhemann during one of his first studies (Scheme 3).[8] 2-amino-1,3-indandione **10** can be condensed with a ninhydrin molecule to form Ruhemann's purple or dimerised, followed by dehydrogenation, forming 6,12-dioxo-6,12,dihydrodiindenopyrazine **9**. The Schiff base **8** and dimer **9** can be considered general products arising from ninhydrin's reaction with primary amines.

In addition to **8** and **9**, a red solid **12** was isolated, having a more complex structure as established by spectroscopic IR, NMR (^{1}H and ^{13}C, HMBC and HMQC) and mass spectrometry techniques (EI-MS and ESI-MS). The signals having the greatest relevance in ^{1}H NMR spectra revealed two triplets at 3.15 and 4.20 ppm, suggestive of methylenes from a unit of phenylethylamine, various signals between 6.8 and 8 ppm, suggestive of three aromatic rings, one disubstituted and two monosubstituted rings and a singlet at 6.69 ppm. The HMBC spectra correlation with the triplet at 4.20 ppm and the singlet at 6.69 ppm provided the necessary information for the structural elucidation of an indene-fused pyrrole (Figure 1). Figure 1 shows the HMBC spectra, highlighting some correlations with four bonds which were extremely useful for structural elucidation; this type of spectroscopic pattern has been observed previously for similar unsaturated compounds.[8–10]

Structural analysis of the three compounds previously isolated by tricomponent reaction between ninhydrin, phenylacetaldehyde and primary amines led to proposing that the initial nucleophilic attack would occur on carbon 2 from ninhydrin and that carbon α from phenylacetaldehyde (in its enol form) would act as a nucleophile (Scheme 4). The subsequent amino group's nucleophilic attack on carbon 1 from ninhydrin and the carbonyl carbon from phenylacetaldehyde would lead to the formation of dihydroxypyrrole **13**. Compound **3** would thus have been formed by a water molecule's nucleophilic attack on carbon 3, followed by oxidative rupture of the cycle. This compound was the precursor of another two products isolated in this work **4**, **5** (Scheme 4).

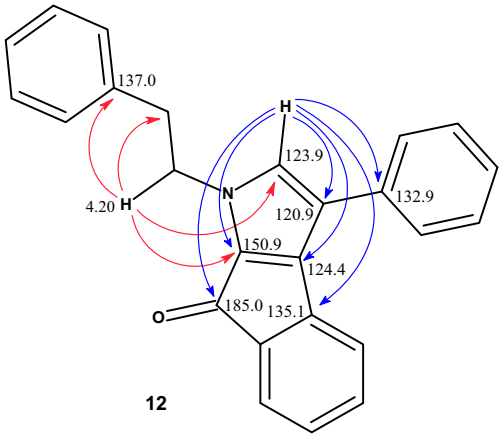


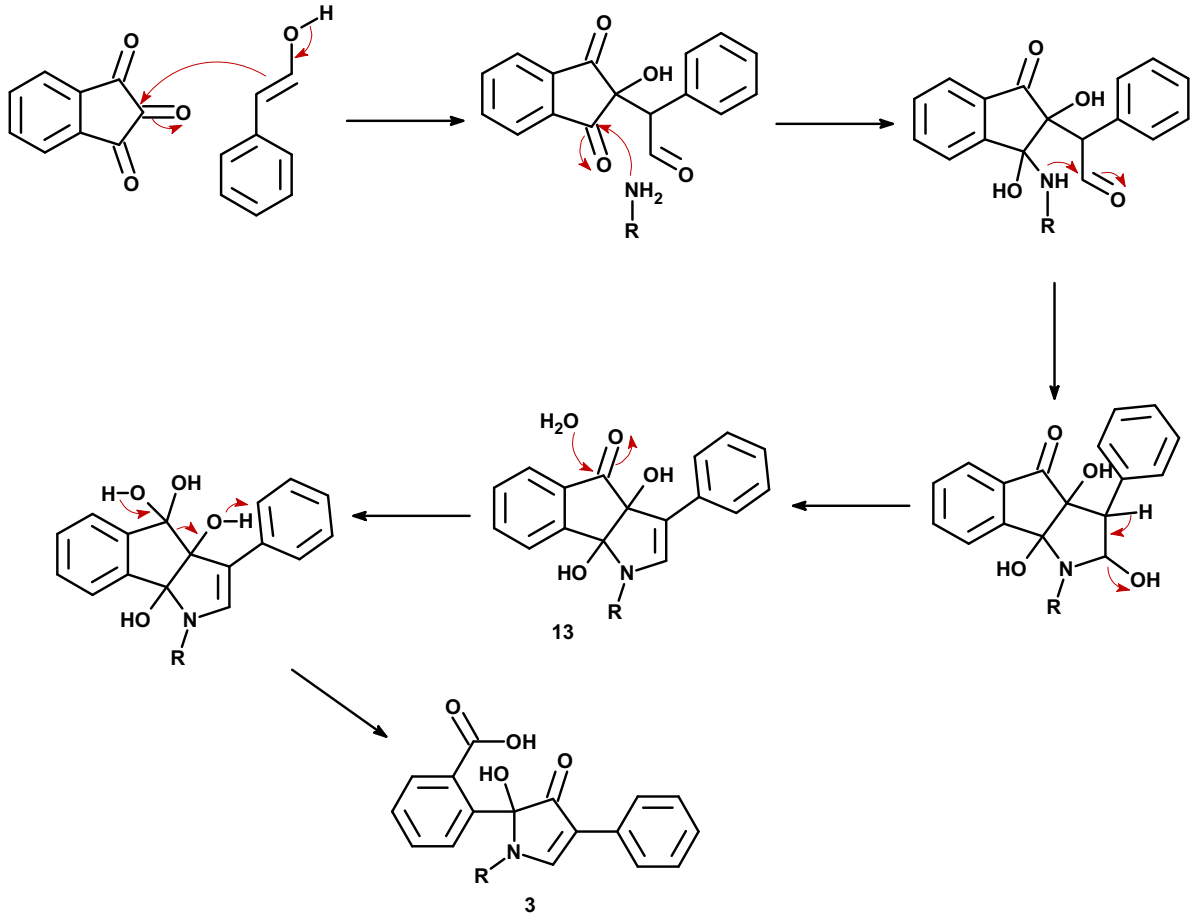
Figure 1. Selected HMBC correlations of **12**.

The reaction between ninhydrin **1**, phenylethylamine **6** and phenylacetaldehyde **2** produced *in situ* would have to involve a different mechanism to that described above. It is thus proposed that pyrrole **12** formation would involve phenylethylamine's nucleophilic attack on carbon 2 from ninhydrin to form hemiaminal **14**, followed by a nucleophilic attack by carbon α from phenylacetaldehyde on carbon 1 from ninhydrin. The intramolecular condensation between the carbonyl from phenylacetaldehyde and the amino group would enable dihydroxypyrrole **15** formation which would form pyrrole **12** by deoxygenation (Scheme 5).

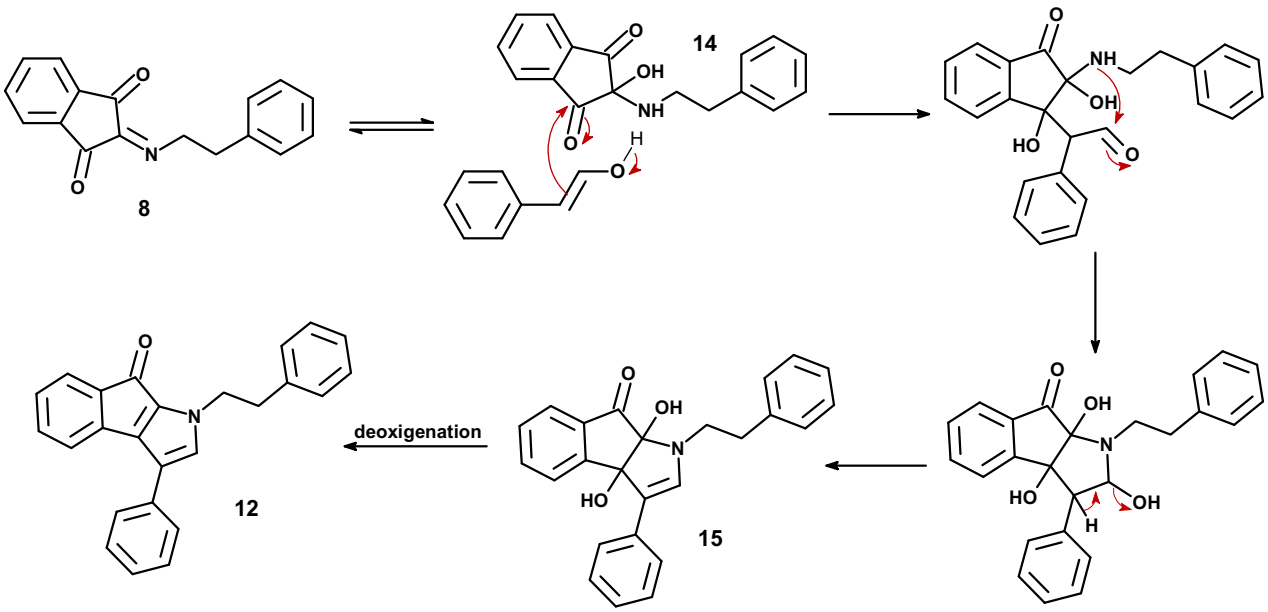
Overall, the difference regarding the proposed mechanisms lies in the initial nucleophilic attack on carbon 2 from ninhydrin. The formation of the isolated products resulting from the tricomponent reaction between ninhydrin **1**, phenylethylamine **6** and phenylacetaldehyde **2** can be explained by the initial attack by phenylacetaldehyde, whose enol form must predominate due to the presence of electro-attractor groups stabilising it through the effect of resonance. However, when the reaction involves ninhydrin and phenylethylamine, the initial nucleophilic attack comes from phenylethylamine and, when phenylacetaldehyde is produced *in situ*, it makes new nucleophilic attacks leading to the formation of pyrrole **12** and spiro **7**.

4. Conclusions

It can thus be concluded that the reaction between ninhydrin and phenylethylamine produced a complex mixture from which it has been possible to isolate four products to date. The previously reported benzo-fused spiroheterocyclic product **7** and pyrrole **12** were

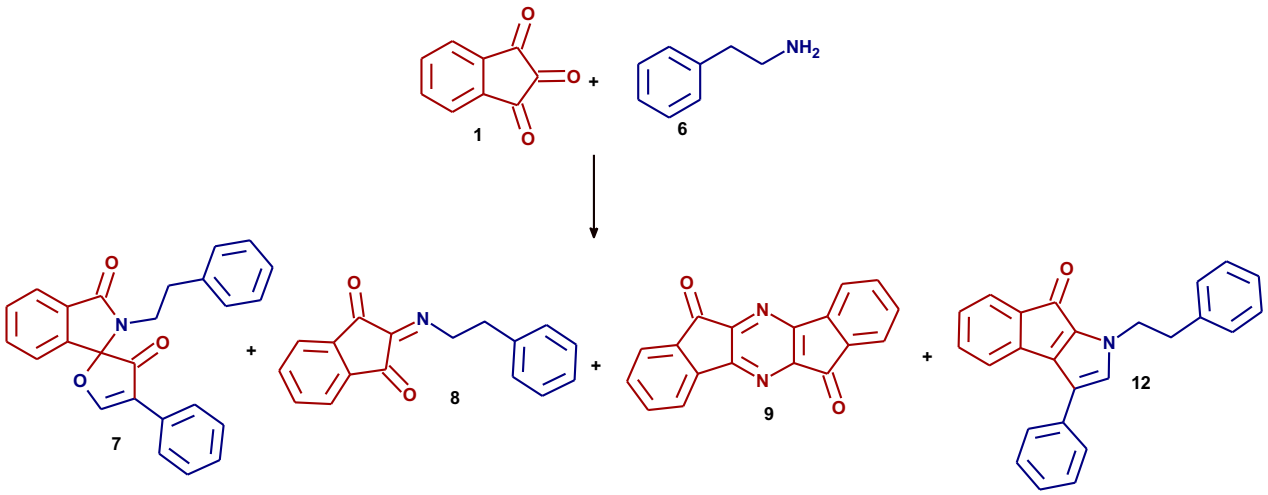


Scheme 4. The mechanism proposed for explaining the tricomponent reaction between ninhydrin **1**, phenylacetaldehyde **2** and primary amines.



Scheme 5. The reaction mechanism proposed for pyrrole **12** formation.

formed by a tricomponent reaction between ninhydrin, phenylethylamine and phenylacetaldehyde produced *in situ*. The following were also isolated: the dimerisation product and subsequent dehydrogenation of 2-amino-1,3-indandione **10** and the Schiff base **8** produced by ninhydrin condensation with



Scheme 6. Reaction of ninhydrin **1** and phenylethylamine **6**.

phenylethylamine (Scheme 6). This work involved the structural analysis of the isolated compounds (**8**, **9** and **12**) and a reaction mechanism has been proposed enabling the formation of each one to be rationalised.

Supplementary Information (SI)

General reaction procedure and NMR data of the compounds are available at www.ias.ac.in/chemsci.

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