Mechanism of the ninhydrin reaction. II. Preparation and spectral properties of reaction products from primary aromatic amines and ninhydrin hydrate[1]

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To elucidate the mechanism of the ninhydrin reaction of aromatic amines, ninhydrin hydrate (I) was treated with equimolar quantities of aniline, *p*-chloroaniline, 2-aminopyridine, *o*-, *m*-, and *p*-aminophenol, and *o*- and *p*-phenylenediamine in aqueous solution. The assignments of structure to the products (see Scheme 1) are based on the elemental analyses and spectroscopic data summarized in Tables I–III.

Ninhydrin hydrate forms a variety of condensation products with nucleophilic reagents including aromatic amines (2–5). The results of this study indicate that the amino group of aromatic amines reacts with ninhydrin hydrate in a nucleophilic displacement to form products having structure III, which were isolated and characterized in the case of aniline, *p*-chloroaniline, 2-aminopyridine, and *m*-aminophenol. The analogous reaction intermediates from *o*- and *p*-aminophenol and *p*-phenylenediamine apparently dehydrate spontaneously to IV, IV*a*, and IV*b*, respectively. The driving force for these dehydrations must come from the stabilization of the products via the indicated resonance interactions, in which unshared electrons on the phenolic and primary amino groups participate (see Scheme 1).

The product from the reaction of ninhydrin with aniline (2-hydroxy-2-phenylamino-1,3-indanedione (III)) and the corresponding analogues from *p*-chloroaniline, 2-aminopyridine, *o*- and *m*-aminophenol, and *o*-phenylenediamine do not absorb in the visible region. However, the products from the reaction of ninhydrin with *p*-aminophenol and with *p*-phenylenediamine (1,3-diketohydrindylidene-*p*-hydroxyphenylamine (IV) and 1,3-diketohydrindylidene-*p*-aminophenylamine (IV*a*), respectively) show strong absorption between 400 and 600 mμ attributable to the extended system of π-electrons.

Although the products from *p*- and *o*-aminophenol (IV and IV*b*) are structural isomers, the former compound absorbs strongly in the visible region whereas the latter does not. Two reasons may account for this. (*i*) The electrons involved in the resonance stabilizations of excited states traverse different pathways in the two compounds, as illustrated. (*ii*) The phenolic group of the ortho isomer could participate in internal hydrogen bonding (IV*b*). If the energetics are favorable, such interactions could distort the coplanarity of the molecule and inhibit resonance stabilization of excited states (6). Internal hydrogen bonding and steric interactions preventing the rings from being fully coplanar are probably the major factors responsible for the lack of visible absorption and for the complex electronic spectrum of IV*b* (Table II).

With *o*-phenylenediamine and ninhydrin, a quinoxaline derivative VI is formed (3*a*) rather than the expected product analogous to IV*a*. This compound shows strong absorption in the ultraviolet region (Table II).

The nuclear magnetic resonance spectra of the compounds listed in Table III reveal pronounced solvent effects on the chemical shifts for the various protons. The striking influence of the solvent on the spectra is illustrated in Fig. 1. The symmetrical ninhydrin hydrate molecule gives rise to two peaks in dimethyl sulfoxide-d_6, with a ratio of intensities of 2:1. The low-field peak is assigned to the four aromatic protons and the high-field peak to the two hydroxyl protons (Fig. 1*a*). The aromatic protons of ninhydrin hydrate give a complex pattern of lines in trifluoroacetic acid. This unsymmetrical multiplet may be caused by protonation of one of the carbonyl groups of ninhydrin hydrate in the acid medium

[1]For part I in this series, see ref. 1.

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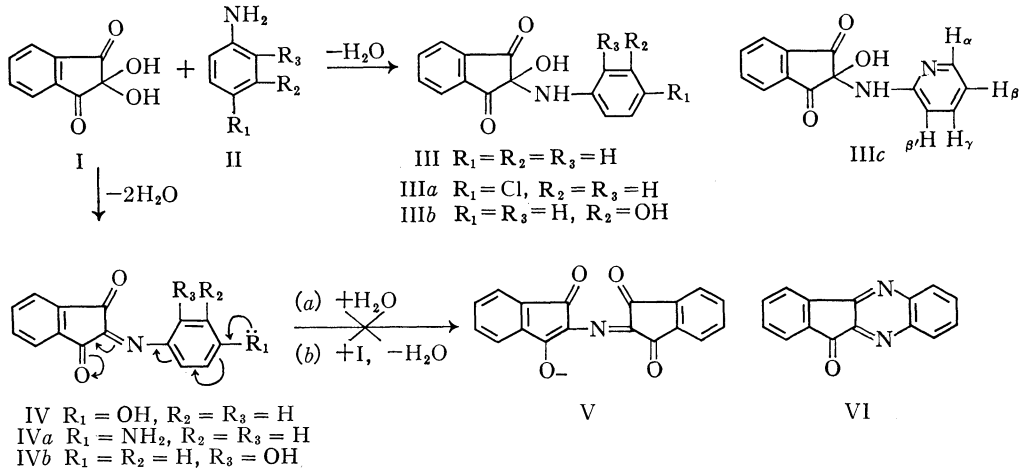
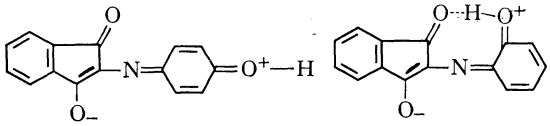


SCHEME 1.

(Fig. 1*b*), which produces nonequivalent aromatic protons.

An analogous solvent effect is observed with diketohydrindylidenediketohydrindamine (V). In dimethyl sulfoxide-d_6, the spectrum consists of two peaks of equal intensity which originate in the aromatic protons of the two nonequivalent indanedione rings. These two peaks coalesce in trifluoroacetic acid. This changed spectrum could again be caused by protonation on the carbonyl group and hydrogen bonding forming a symmetrical structure, as illustrated in Figs. 1*c* and 1*d*.



IV IVb

The nuclear magnetic resonance spectra of III*b*, IV*b*, and VI in both dimethyl sulfoxide-d_6 and CF_3COOH were too complex to permit unequivocal assignments of chemical shifts and coupling constants to the various protons.

The results of the present investigation are of interest in connection with a study by Suffis and his co-workers (7). They investigated the conditions required for color development during the reaction of ninhydrin with *p*-aminophenol and *p*-phenylenediamine at 100 °C, and concluded that the ninhydrin method can be used for quantitative determination of these two aromatic amines in the presence of their ortho and meta isomers. However, these authors did not isolate any of the postulated products, and their extinction coefficients, determined with the reaction mixtures, appear to be lower by a factor of 3 to 5 than those given in Table II for compounds IV and IV*a*.

The low extinction coefficients may be due to the presence of the citrate buffer in the ninhydrin reagent solution, since the addition of a drop of concentrated HCl to methanolic solutions of IV and IV*a* caused an instantaneous fading of their purple colors. Evidently, protonation of IV and IV*a* in acid media interrupts the resonance stabilization of the quinoid excited states in these compounds.

Treatment of primary aliphatic amines with ninhydrin hydrate at 100 °C in a pH 5.5 buffer medium results in the formation of V, which exhibits a strong absorption maximum in the visible region at 570 mμ (1, 8). However, when the primary aromatic amines of structure II and compounds III–III*b* were subjected to the same reaction conditions, no color was observed. These compounds probably fail to yield V because the electron shifts in IV (see Scheme 1) required for deamination are energetically unfavorable processes as a result of the loss of the aromaticity of the aryl groups

TABLE I

Compound	Melting point (°C)	Formula	Analyses (%) Calculated	Found	Infrared bands (cm^{-1})	Yield (%)
III	92–93	$C_{15}H_{11}NO_3$ (253.27)	C 71.14	71.21	1 710, 1 740 (C=O)	81
			H 4.38	4.24	3 310, 3 325, 3 480 (OH, NH)	
			N 5.53	5.31		
III*a*	112–116	$C_{15}H_{10}NO_3Cl$ (287.70)	C 62.61	62.48	1 705, 1 742 (C=O)	71.5
			H 3.50	3.48	3 290, 3 325 (OH, NH)	
			N 4.87	4.83		
			Cl 12.33	12.54		
III*b*	>300	$C_{15}H_{11}NO_4$ (269.20)	C 66.93	67.02	1 700 (C=O)	97.0
			H 4.12	4.33	3 315, 3 400 (OH, NH)	
			N 5.20	5.22		
III*c*	176–177	$C_{14}H_{10}N_2O_3$ (254.25)	C 66.14	66.16	1 715 (C=O)	96.5
			H 3.96	3.97	3 410 (OH, NH)	
			N 11.02	10.82		
IV	230–232	$C_{15}H_9NO_3$ (251.24)	C 71.71	71.54	1 680, 1 730 (C=O)	75.0
			H 3.61	3.72	3 200 (OH)	
			N 5.60	5.63		
IV*a*	206–208	$C_{15}H_{10}N_2O_2$ (250.26)	C 71.99	72.08	1 633 (C=N)	90.0
			H 3.99	4.12	1 680, 1 718 (C=O)	
			N 11.19	11.20	3 200, 3 300, 3 480 (NH)	
IV*b*	239–240	$C_{15}H_9NO_3$ (251.24)	C 71.70	71.34	1 645 (C=N)	91.5
			H 3.61	3.70	1 742 (C=O)	
			N 5.60	5.65	3 450 (OH, broad)	
VI	225–226	$C_{15}H_8N_2O$ (232.24)	C 77.57	77.23	1 720 (C=O)	98.0
			H 3.47	3.43		
			N 12.06	12.24		

TABLE II

Electronic absorption maxima and extinction coefficients in methanol for ninhydrin and its derivatives

Compound	λ_{max} (mμ)	ε (l/mole cm)
I	250	12 500
	228	45 000
III	255	14 700
	226	42 500
III*a*	245	21 500
	228	33 400
III*b*	360	2 500
	300	4 500
	243	16 940
III*c*	250	14 500
	228	44 500
IV	545	5 800
	420	11 200
	310	3 500
	250	22 000
	228	19 800
IV*a*	535	26 500
	465	25 000
	330	4 200
	275	13 000
	255	22 600
IV*b*	330	10 560
	294	12 750
	280	12 110
	237	23 860
	220	23 400
VI	289	41 300
	223	25 600

during the transformation. Aromatic amines therefore resist deamination under the conditions of the ninhydrin reaction used for α-amino acids.

EXPERIMENTAL

Melting points were taken on a Fisher–Johns apparatus[2] and are not corrected. Nuclear magnetic resonance spectra were determined on a Varian model A-60 spectrometer, with the integrator at 60 Mc.p.s. and with tetramethylsilane as an internal reference; electronic spectra on a Cary model 14 spectrophotometer; and infrared spectra on a Perkin–Elmer model 421 spectrophotometer in KBr pellets.

2-Hydroxy-2-N-phenylamino-1,3-indanedione (III)

To a solution of 1.83 ml (0.02 mole) of aniline in 75 ml of water was added dropwise, with stirring, a solution of 3.56 g (0.02 mole) of ninhydrin hydrate in 50 ml of water during about 10 min. A fluffy, bright-yellow precipitate began settling almost immediately. Stirring was continued for 12 h, and the precipitate was filtered off and dried in a desiccator. Attempted recrystallizations of the product from ethanol or aqueous ethanol solutions yielded a dark polymeric material.

[2]The mention of trade names or products does not constitute endorsement by the U.S. Department of Agriculture over those not named.

TABLE III

Summary of the nuclear magnetic resonance data for ninhydrin and its derivatives

Compound	Chemical shifts (τ scale)	Remarks
I in CF_3COOH	1.75–2.08	Complicated multiplet (Fig. 1*b*)
I in dimethyl sulfoxide-d_6	1.87 (four benzene protons)	See Fig. 1*a*
	2.37 (two hydroxyl protons)	
III in CF_3COOH	1.82 (indanedione protons)	Broad peak
	2.45 (benzene protons)	Slightly split peak
III in $CDCl_3$	2.12 (indanedione protons)	Symmetrical multiplet
	2.83 (benzene protons)	Broad absorption
III*a* in CF_3COOH	1.97 (indanedione protons)	Two symmetrical multiplets
	2.63 (benzene protons)	
III*b* in CF_3COOH	1.92 (benzene protons)	ABCD system; unsymmetrical multiplet
	1.27 (α proton)	
	2.62 (β proton)	ABXY system
	2.70 (β' proton)	$J_{\alpha,\beta}$ 6 c.p.s.; $J_{\beta',\gamma}$ 9 c.p.s.
	2.00 (γ proton)	$J_{\beta,\gamma}$ 6.5 c.p.s.
IV in CF_3COOH	1.78 (indanedione protons)	Sharp peak
	2.87 (A protons)	A_2B_2 quartet with $J_{A,B}$ 9 c.p.s.
	2.53 (B protons)	
IV in dimethyl sulfoxide-d_6	1.95 (indanedione protons)	Sharp peak
	3.13 (A protons)	A_2B_2 quartet with $J_{A,B}$ 9 c.p.s.
	2.42 (B protons)	
IV*a* in CF_3COOH	1.72 (indanedione protons)	Broad peak
	2.12 (benzene protons)	Sharp peak
IV*a* in D_2O	1.90 (indanedione protons)	Sharp peak
	2.30 (benzene protons)	Sharp peak
V in CF_3COOH	2.37 (aromatic protons)	Nearly symmetrical (Fig. 1*d*)
V in dimethyl sulfoxide-d_6	1.97 and 2.32 (aromatic protons)	Two peaks of equal intensity (Fig. 1*c*)

2-Hydroxy-2-N-(p-chlorophenylamino)-1,3-indanedione (IIIa)

The reaction was similar to that described for III, except that the *p*-chloroaniline was dissolved in 750 ml of hot water. The bright-yellow product was recrystallized from aqueous ethanol as a fluffy brownish material that had the same melting point and infrared spectrum as the yellow compound. The color change appears to be due to a surface phenomenon.

2-Hydroxy-2-N-(m-hydroxyphenylamino)-1,3-indanedione (IIIb)

The reaction mixture was similar to that described for III, except that solid ninhydrin was added to the *m*-aminophenol dissolved in 250 ml of water. The precipitate was filtered off and recrystallized from ethanol as yellowish-white needles.

2-Hydroxy-2-N-(2-aminopyridyl)-1,3-indanedione (IIIc)

The reaction was similar to that described for III, except that the 2-aminopyridine was dissolved in 40 ml of water. The whitish precipitate was recrystallized from ethanol.

1,3-Diketohydrindylidene-2-p-hydroxyphenylamine (IV)

To a solution of 2.18 g (0.02 mole) of *p*-aminophenol in 250 ml of water was added 3.56 g (0.02 mole) of solid ninhydrin hydrate. The reaction mixture was stirred for 12 h, and the red precipitate was filtered off and washed with cold methanol. The product was recrystallized from hot methanol.

1,3-Diketohydrindylidene-2-p-aminophenylamine (IVa)

To a solution of 5.34 g (0.03 mole) of ninhydrin hydrate in 250 ml of water was added, during 60 min, a solution of 5.13 g of phenylenediamine dihydrochloride in 80 ml of water which had been adjusted to pH 7 with LiOH. The fluffy red precipitate was recrystallized from absolute ethanol as glistening, purple-red crystals.

1,3-Diketohydrindylindene-2-o-hydroxyphenylamine (IVb)

The reaction was similar to that described for III, except that the *o*-aminophenol was dissolved in 500 ml of water. The compound was recrystallized from ethanol as fluffy brownish crystals.

11-Oxo-indeno(1,2-b)quinoxaline (VI)

The reaction was similar to that described for III, except that solid ninhydrin hydrate was added to the *o*-phenylenediamine dissolved in 200 ml of water. The yellow precipitate was recrystallized from ethanol as fluffy needles (lit. m.p. 219 °C (3*a*)).

Reaction with Ninhydrin

To 1 mg samples of compounds III–III*b*, as well as starting aromatic amines in duplicate matched tubes, were added 4 ml of ninhydrin reagent solution, prepared according to Stein and Moore (8), and 2 ml of water. All of the compounds dissolved. The tubes were covered with aluminium caps and placed in a 100 °C water bath for 20 min. To each of the cooled solutions was added 10 ml of 50% ethanol–water. The tubes were mixed and read against a blank solution in a Beckman model B spectrophotometer.

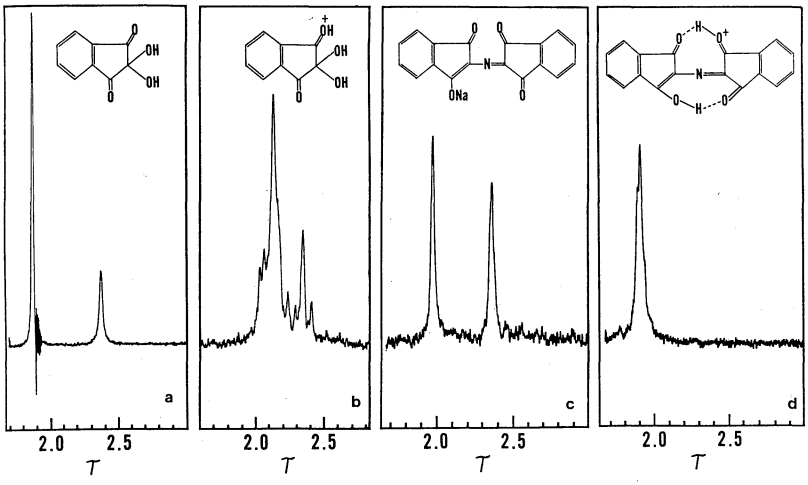


FIG. 1. Nuclear magnetic resonance spectra of ninhydrin hydrate in (*a*) dimethyl sulfoxide-d_6 and in (*b*) trifluoroacetic acid, and of diketohydrindylidenediketohydrindamine (sodium salt) in (*c*) dimethyl sulfoxide-d_6 and in (*d*) trifluoroacetic acid.

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Optical properties of the dimedonyl derivative of α-phenylethylamine

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The search for new chromophoric groupings for the assignment of configuration in optically active amino acids and amines has led to the investigation of numerous derivatives (1, 2). Among the chromophores which have been suggested recently, the dimedone condensation compound of optically active amino acids (3) and amines (4) has been shown to exhibit interesting properties. This derivative, which is easily prepared, has a chromophore in the 280 mμ region, with which an intense Cotton effect is associated. Hence, in dimedonyl derivatives of optically active amines, the absolute configuration at the asymmetric center can be deduced from the sign of the Cotton effect experimentally observed (4).

It has been shown that the dimedone condensation compounds of primary and secondary amines with the (*R*) configuration (5) exhibit a positive Cotton effect, whereas the same derivative of amines