Reaction of α-hydrazino carboxylic acids with ninhydrin

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Several α-hydrazino carboxylic acids and their *m*-nitrobenzal hydrazones have been prepared and characterized. The latter and two of the former have not been previously reported. The α-hydrazino acids were found to react with ninhydrin to yield Ruhemann's purple, but in much lower yield than the corresponding amino acids. However, hydrazine itself yields a different colored product. Various aspects of the reaction between ninhydrin and the α-hydrazino acids were investigated and a mechanism for the formation of Ruhemann's purple is suggested.

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Introduction

A number of α-hydrazino carboxylic acids have been prepared and characterized. Most of these acids have previously been reported (1–5), with the exception of the α-hydrazino derivatives of palmitic and stearic acids, and the *m*-nitrobenzal hydrazone derivatives (6).

As part of the characterization of the α-hydrazino acids, their reaction with ninhydrin was tested. Some reaction between the carbonyl-rich reagent, and the hydrazino group, to yield a colored complex was anticipated, but it was a complete surprise to find that the reaction gives Ruhemann's purple (7), though in much lower yields compared to the corresponding α-amino acids.

Stimulus for this work derives from the hope of the eventual synthesis of oligo- and poly-hydrazino acids consisting of α-hydrazino carboxylic acid monomers bound to each other in

$$\begin{matrix} O & H & H \\ \| & | & | \end{matrix}$$

"hydrazino peptide" (—C—N—N—) bonds. This would create an entire chemistry analogous to protein chemistry, and this paper is a report of preliminary activities in this area.

As research interests expand in this field, it would be desirable for all to proceed with some common, and hopefully clear, nomenclature and symbolism. In this respect, it is suggested that the monomers, as a group, be called *hydrazino acids* and abbreviated, HA. Where the hydrazino acid has a corresponding characterized amino acid, possessing a common name, the name of the HA should be that of the amino acid, preceded by the word hydrazino. Thus the HA analogous to leucine, α-hydrazinoisocaproic acid, would be called hydrazinoleucine. If no Greek letter precedes the word, hydrazino, the group is to be understood as occupying the α-position. For shorthand notation, hydrazinoleucine would be written, HLeu.

Experimental

Materials

Most of the α-bromo carboxylic acid precursors of the HA were purchased from either Eastman Organic Chemicals or City Chemical Company, New York. The 95% hydrazine used was an Eastman Organic Chemicals product. Ninhydrin and other reagents were available here. Liquid reagents were distilled prior to use and ninhydrin was recrystallized from water.

Preparative Procedure for the Hydrazino Acids

While the exact details may vary slightly from one specific preparation to another, the general method for synthesizing HA is given below (2–5, 8–10).

Ten grams (less if the acid was in short supply or too expensive) of the corresponding α-bromo carboxylic acid were added to 50 ml of absolute ethanol at room temperature. To this mixture, an approximate 5*M* excess of 95% hydrazine was added in small portions. The solution was reduced to one-quarter its original volume over a hot plate and then placed in the deep freeze to initiate crystallization of the product. Usually crystals appeared within 1 h. After 24 h, they were filtered off, washed with absolute ethanol and dried. Recrystallization was performed by solution in ethanol–water mixtures, followed by reduction in volume, addition of absolute ethanol, and subjection to deep freeze temperatures.

In general the HA were soluble in hot and cold water, but essentially insoluble in ethanol as well as in such organic reagents as *p*-dioxane and hexane.

In all cases the racemic α-bromo acid precursors were employed, thus yielding products which were devoid of optical activity.

Preparation of m-Nitrobenzaldehyde Derivatives

Two grams of *m*-nitrobenzaldehyde were dissolved in sufficient ethanol, and 0.5 g of α-hydrazino acid was dissolved in sufficient water, to bring each into solution. The

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TABLE I

α-Hydrazino (carboxylic) acids[a]

Chemical name	Suggested common names	Suggested abbreviation	Melting point (°C)		% yield	Appearance	Nitrogen analysis %[b]	
			Observed	Literature			Calculated	Found
α-Hydrazino-*n*-butyric	Hydrazinobutyric	HAbu	212d.	208[c]	26	White plates	23.7	23.7[d]
α-Hydrazino-3-methyl-*n*-butyric (α-Hydrazinoisovaleric)	Hydrazinovaline	HVal	250d.	230–235[e]	36	White powder	21.2	20.8
α-Hydrazinovaleric	Hydrazinonorvaline	HNval	216d.	215[f]	80	White needles	21.2	21.3[g]
α-Hydrazino-4-methylvaleric (α-Hydrazinoisocaproic)	Hydrazinoleucine	HLeu	227d.	228[h]	32	Silvery powder	19.2	19.1[i]
α-Hydrazinohexanoic (α-Hydrazinocaproic)	Hydrazinonorleucine	HNleu	220d.	221[j] 218[k] 199–201[l]	28	Silvery plates	19.2	19.2[m]
α-Hydrazinophenylacetic	Hydrazinophenylglycine	HPGly	182d.	183–184[n]	55	White powder	16.8	16.6
α-Hydrazinohexadecanoic	Hydrazinopalmitic	HPal	203		51	White powder	9.8	9.9
α-Hydrazinooctadecanoic	Hydrazinostearic	HSte	205d.		66	White powder	8.9	8.7

[a]The general procedure for α-hydrazino acid preparation is described in the Experimental section. All observed melting points are corrected.
[b]Values with footnotes were determined by the Dumas method; the others were obtained by microkjeldahl procedure.
[c]See refs. 1 and 3.
[d]Calcd: C, 40.7; H, 8.5. Found: C, 40.5; H, 8.6.
[e]See refs. 2 and 3.
[f]See refs. 1 and 3.
[g]Calcd: C, 45.4; H, 9.2. Found: C, 45.3; H, 9.1.
[h]See ref. 3.
[i]Calcd: C, 49.3; H, 9.7. Found: C, 48.9; H, 9.6.
[j]Vacirca and Ciocca, Boll. 1st sieroterap. Milanese, 23, 99 (1944).
[k]See ref. 4.
[l]See ref. 3.
[m]Calcd: C, 49.3; H, 9.7. Found: C, 49.5; H, 9.7.
[n]See ref. 5. Results are for the laevorotatory form.

TABLE II

m-Nitrobenzal hydrazones of α-hydrazino acids*

Hydrazone of	Melting point (°C)	% yield	Appearance	Nitrogen analysis %†	
				Calculated	Found
Hydrazino-*n*-butyric	112	71	Yellow powder	16.6	16.4
Hydrazinonorvaline	191–192	41	Light-yellow needles	15.8	15.6
Hydrazinovaline	162–163	27	Yellow powder	15.8	15.5
Hydrazinoleucine	82	44	Yellow needles	15.0	15.0
Hydrazinonorleucine	97–98	54	Yellow needles	15.0	14.9
Hydrazinophenylglycine	195–197	35	Yellow leaflets	14.0	13.9

*The general preparative procedure is described in the Experimental section. All melting points are corrected.
†Determined by microkjeldahl analysis in which sodium thiosulfate was added to the digestion mixture to facilitate the reduction of the nitro group.

two solutions were slowly mixed. During this process product precipitation was observed in some cases. After complete mixing, the resulting solution was reduced on a hot plate to approximately three-quarters of its original volume and cooled. The *m*-nitrobenzaldehyde derivatives were filtered off and recrystallized from 50% ethanol.

Ninhydrin Reaction Procedure for the Hydrazino Acids

Essentially, the ninhydrin reaction with HA's was carried out in a manner similar to that described for amino acids by Moore and Stein (11). In the first set of conditions, various aliquots of 0.002 *M* solutions of either α-hydrazino or α-amino acids were brought to 1 ml volumes with water in suitable test tubes. They were then treated with 1 ml of 0.2 *M* citrate buffer, pH 5, which contained 0.8 g $SnCl_2 \cdot 2H_2O$/l as reducing agent, and 20 g of ninhydrin/l. Methyl cellosolve was absent from the reagent. After mixing the HA and ninhydrin solutions, the tubes were capped and immersed in a water bath maintained at 100° for various periods of time. This set of conditions will be called the "aqueous system" in this report. The second set of conditions varied only in that methyl cellosolve was present in a 1:1 ratio with the aqueous citrate buffer, but concentrations of citrate, stannous chloride, and ninhydrin were identical to those in the aqueous system described above. However, tubes containing the reaction mixture were immersed in an oil bath maintained at 124° (the b.p. of methyl cellosolve) and kept in the bath for various times. In the 1h treatment the water would evaporate, leaving essentially a methyl cellosolve solution of reactants and products. Except for the bath temperature and reaction volumes, this system is closest to that given by Moore and Stein (11) and is referred to here as the "cellosolve–water system." A third ninhydrin reagent, which was used only in the reaction with hydrazine hydrate, consisted of a solution of ninhydrin and $SnCl_2 \cdot 2H_2O$ in methyl cellosolve (no citrate) in identical concentrations as in the above reagent solutions.

After appropriate bath immersion times, the tube contents were diluted with 5 ml (or more if required) of 1:1; *n*-propanol:H_2O, mixed well, and read in either a Bausch & Lomb Spectronic 20 or a Beckman DU recording spectrophotometer, accompanied by suitable blanks. Due to reduced atmospheric pressure indigenous to this region, salt was added to our water baths to achieve and maintain 100°.

Results and Discussion

The α-Hydrazino Acids and their m-Nitrobenzal Hydrazones

Table I presents pertinent data on the α-hydrazino acids prepared in this work including the two new ones, hydrazinopalmitic and hydrazinostearic acids. Appropriate data for the *m*-nitrobenzaldehyde derivatives are given in Table II. The *m*-nitrobenzal hydrazones of hydrazinopalmitic and hydrazinostearic acids could not be obtained due to solubility problems.

Paper Chromatography

Comparative runs were performed in duplicate for norleucine and HNleu using Whatman No. 1 paper and an eluant consisting of 60:30:10; *n*-butanol:glacial acetic acid:water, in a closed system. Approximately 26 μg of DL-norleucine were spotted and approximately 142 μg of DL-HNleu were used. The chromatograms were run for 6 h, using the ascending method. They were then dried and the color was developed with ninhydrin spray in *n*-butanol (1 g ninhydrin in 25 ml *n*-butanol). This gave the following R_f values: DL-norleucine: 0.67; DL-hydrazinonorleucine: 0.41.

Infrared Spectral Comparison of α-Hydrazino- and α-Amino-n-butyric Acids

The infrared (i.r.) spectra of α-hydrazino-*n*-butyric acid and α-amino-*n*-butyric acid were compared (Fig. 1). Four bands are germane (12). The first two are the N—H bending peaks known as amino acid I and II, which are usually seen at 6.03–6.22 μ and 6.46–6.74 μ, respectively. For aminobutyric, these are observed at 6.03–6.10 μ and 6.63 μ, respectively. Although these bands are purported to disappear on *N*-substitution in the amino acid (12), both bands are

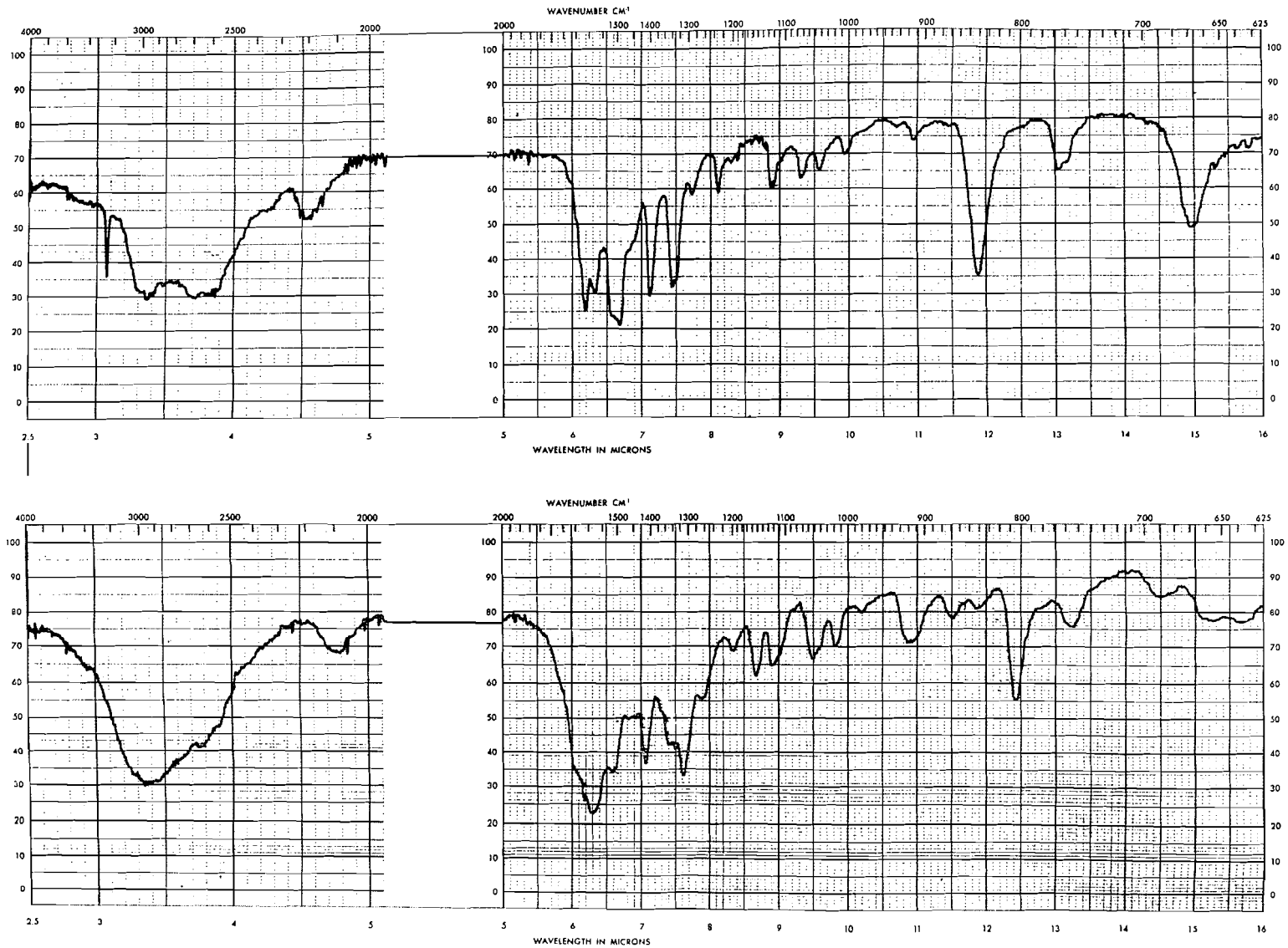

FIG. 1. Comparison of the infrared spectra of α-amino-*n*-butyric acid and α-hydrazino-*n*-butyric acid which were obtained on KBr pellets in a Beckman IR-8 recording spectrophotometer. Upper section, α-hydrazino-*n*-butyric acid; lower section, α-amino-*n*-butyric acid.

observed in similar intensity as sharper peaks at 6.19 μ and 6.68 μ for the hydrazino acid. While additional evidence will be recited below supporting a zwitterion structure for the hydrazino acid, the above observation suggests that the proton dissociated from the carboxyl group resides on the β-nitrogen atom. The amino acid I and II bands arise from N—H bending involving a nitrogen atom situate as, $(R—NH_3)^+$. Further substitution of the nitrogen atom to create, $(R—NH_2R')^+$, causes band disappearance. If the proton in the HA zwitterion resided on the α-nitrogen, this nitrogen would be situate as $(R—NH_2R')^+$ and the bands should not be observed; however, if the β-nitrogen carried the proton its structure would be, $(R—NH_3)^+$ and the bands should be seen, as is the case. (The fact that the amino acid nitrogen atom is attached to a carbon in the R group and the HA's β-nitrogen atom would be attached to a nitrogen in the R group, should influence only band position and intensity.)

The second two bands of interest are those shown by free amino acids in the zwitterion form for ionized carboxyl absorption at 6.25–6.42 μ and at approximately 7.15 μ. For aminobutyric, a strong band is seen at 6.25–6.38 μ and a somewhat weaker one is in evidence at 7.08–7.10 μ. For the HA, both peaks are distinct and sharp at 6.32 μ and 7.12 μ. These observations, considered with the complete absence of unionized carboxyl group absorption in the 5–6 μ region, and the generally similar infrared (i.r.) spectral graphs for both acids, reasonably suggest that the free α-hydrazino aliphatic acids exist as zwitterions.

Further support for this thesis is found in the relatively good water solubility and poor organic

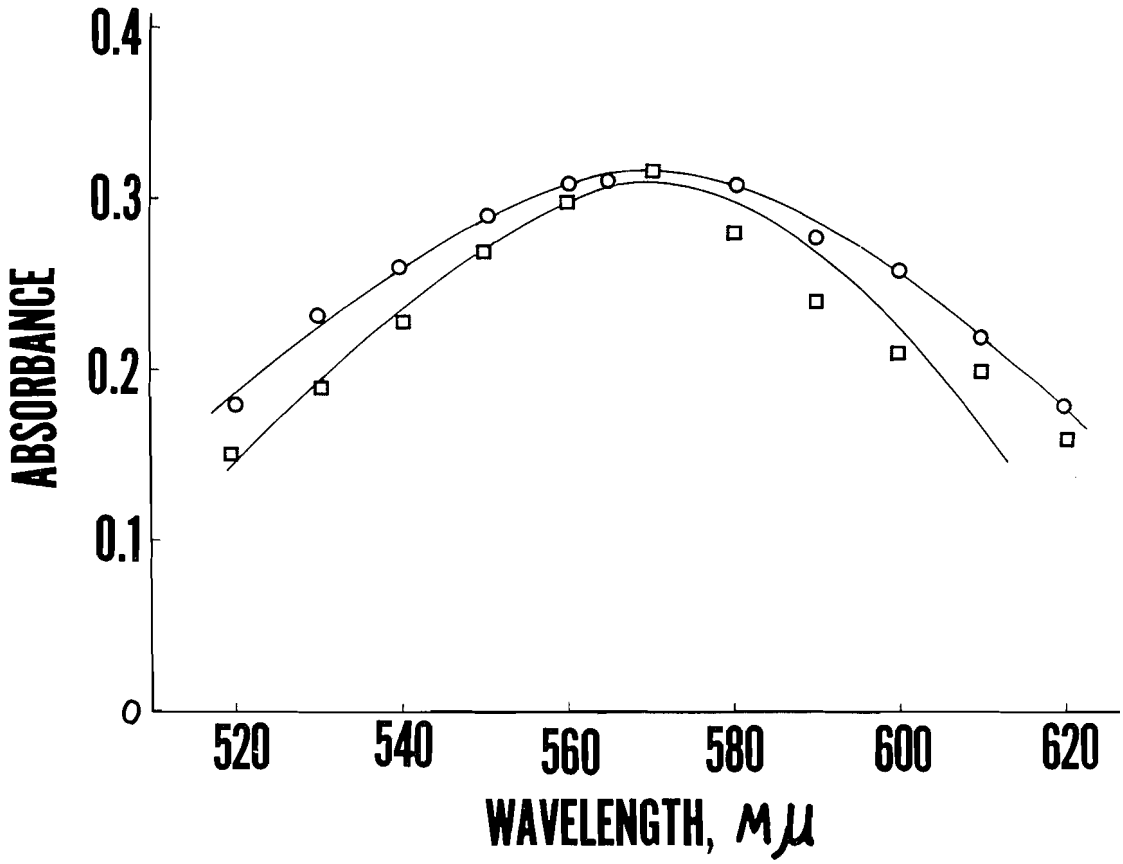

FIG. 2. Absorption spectra for hydrazinonorleucine and norleucine. Aqueous system conditions; bath time: 20 min. Acid concentration in reaction: 80γ of 0.002 *M* norleucine; 600γ of 0.002 *M* hydrazinonorleucine. ○, DL-Hydrazinonorleucine; □, DL-Norleucine.

solvent solubility displayed by the hydrazino acids; this is analogous to amino acid solubility behavior.

The infrared spectra of α-hydrazinonorleucine and norleucine were almost identical to the corresponding *n*-butyric acid analogues.

The Ninhydrin Reaction in the Aqueous System at 100°

Absorption Spectra

The complex formed in the reaction between either HA or amino acids and ninhydrin generally has a maximum absorption at 570–572 mμ; although, it is quite broad and tends to range from 560–580 mμ. This is illustrated in Fig. 2 for the ninhydrin reaction product for norleucine and the corresponding HA. Note that extraction of comparable color yields for these related acids required a 7.5 times greater molar concentration of the HA. Even higher ratios were required for other pairs.

Beer–Lambert Relationship

Figure 3 shows a graph of absorbance vs. concentration for hydrazinonorleucine, hydrazinonorvaline, hydrazinoleucine, and hydrazino-*n*-butyric acids. All were reacted with ninhydrin under aqueous system conditions. While color yields vary despite identical initial HA concentrations, it is clear that the Beer–Lambert relationship is followed. The curves give evidence that color yields among the straight chain acids increase with increasing hydrocarbon chain lengths. Further, as judged by comparison between HNleu and HLeu, branching markedly reduces color yields. (In separate data, HNval yielded roughly three times more color than HVal compared at a single concentration.)

While the results with the corresponding amino acids at 100 °C reaction temperature (11) contain no hint of either of the above phenomena, support for steric and polar effects was demonstrated by Friedman and Sigel (13), who observed a decided relationship between color yield and structure at 30°, as well as at 100°. Unfortunately, comparisons are precluded, as none of the amino acids examined in their work at 100 °C correspond to the HA in this work, and they do not correspond to those in Moore

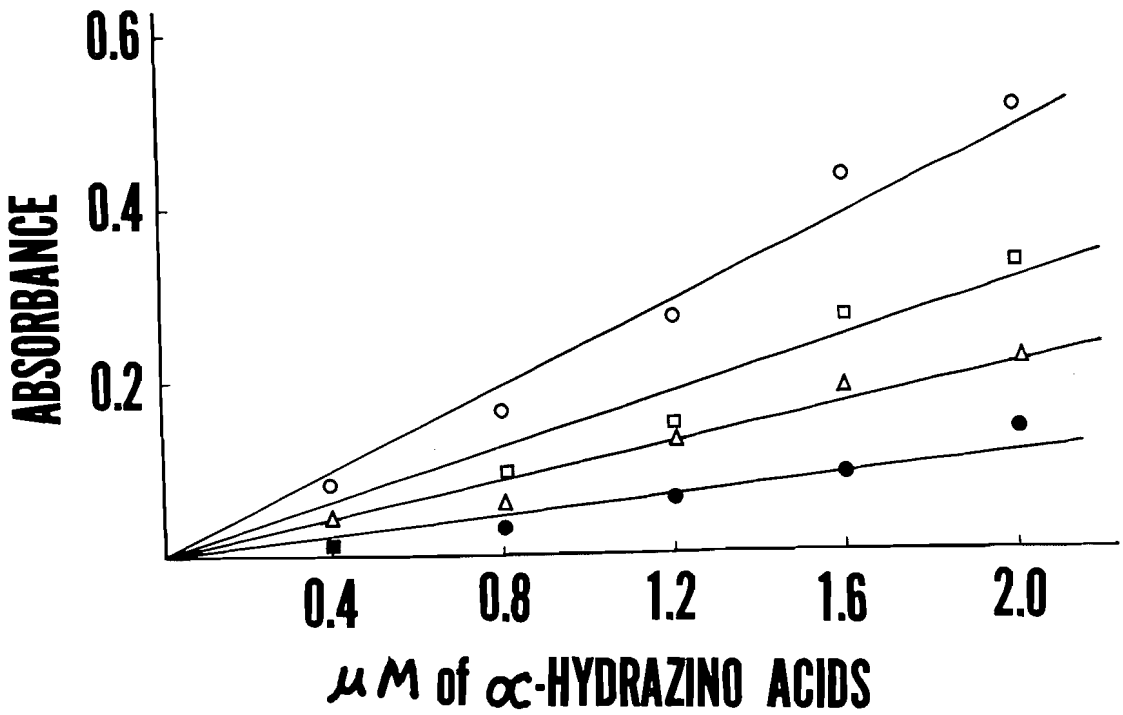

FIG. 3. Absorbance at 570 mμ vs. concentration for the hydrazino acids. Aqueous system conditions; bath time: 20 min. ○, DL-Hydrazinonorleucine; □, DL-Hydrazinonorvaline; △, DL-Hydrazino-*n*-butyric acid; ●, DL-Hydrazinoleucine.

TABLE III

Color stability of 20 min ninhydrin reaction product*

Compound	Aliquot of standard solution† (μl)	Wavelength (mμ)	Absorbance at various times after removal from bath				
			15 min	6 h	11 h	20 h	24 h
Valine	80	565	0.30	0.28	0.25	0.23	0.20
		570	0.31	0.27	0.24	0.23	0.20
Norleucine	80	565	0.30	0.28	0.27	0.23	0.20
		570	0.32	0.28	0.26	0.22	0.20
Hydrazinonorvaline	600	565	0.22	0.19	0.17	0.15	0.12
		570	0.23	0.20	0.17	0.14	0.12
Hydrazinonorleucine	600	565	0.31	0.29	0.26	0.23	0.20
		570	0.32	0.28	0.25	0.23	0.20
Hydrazinobutyric	1200	565	0.29	0.26	0.24	0.21	0.19
		570	0.30	0.26	0.23	0.21	0.20
Hydrazinovaline	1200	565	0.13	0.11	0.10	0.08	0.07
		570	0.14	0.13	0.13	0.11	0.09
Hydrazinoleucine	1200	565	0.15	0.14	0.13	0.10	0.08
		570	0.16	0.15	0.13	0.11	0.09

*Aqueous system conditions; bath temperature 100 °C.
†0.002 *M*.

and Stein's list (11), (except leucine which Friedman and Sigel used as a standard).

Color Stability as a Function of Time

Color stability as a function of time is given in Tables III and IV for the 20 min and 60 min ninhydrin reaction product, respectively. In all cases, gradual color loss is experienced with time. The exact decay rate varies with the specific acid (whether amino acid or HA) but on the whole, ranges from 30–50% over a 24 h period or approximately 1–2% per h, which corresponds with the observations of Moore and Stein for amino acids (11).

Effect of Reaction Time on Initial Color Yield

Some comparison of this relationship is possible in the data of Tables III and IV where HAbu and HVal give a slight color increase after 1 h reaction time, whereas HNleu shows a decrease, compared to the results obtained from 20 min of reaction (adjusted for concentration differentials).

In a further study, a comparison of color yield vs. reaction time was made for the ninhydrin–norleucine and ninhydrin–hydrazinonorleucine reactions (Fig. 4). Both curves have similar form, rising steeply early in the reaction and approaching maxima of color yield in the

TABLE IV

Color stability of 1 h ninhydrin reaction product*

Compound	Aliquot of standard solution† (μl)	Wavelength (mμ)	Absorbance at various times after removal from bath		
			15 min	1 h	24 h
Hydrazinobutyric	600	565	0.19	0.14	0.11
		567	0.18	0.14	0.12
		570	0.18	0.13	0.11
Hydrazinonorleucine	600	565	0.19	0.14	0.12
		567	0.17	0.15	0.11
		570	0.17	0.13	0.10
Hydrazinovaline	600	565	0.10	0.07	0.05
		567	0.09	0.08	0.04
		570	0.09	0.07	0.05

*Aqueous system conditions; bath temperature 100 °C.
†0.002 *M*.

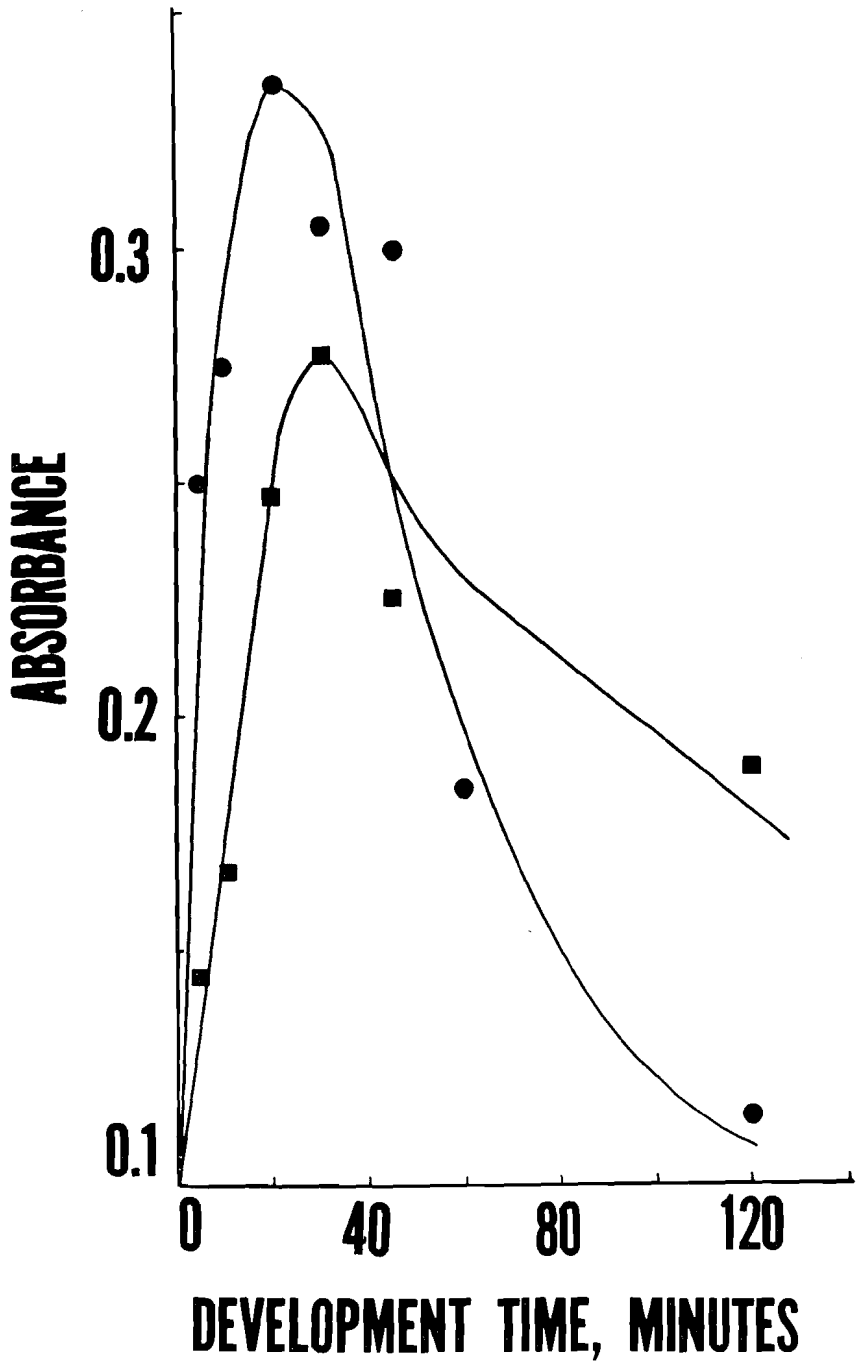

FIG. 4. Absorbance at 570 mμ vs. ninhydrin reaction time (color development time). Aqueous system conditions. Acid concentration in reaction: 80 γ of 0.002 *M* norleucine; 600 γ of 0.002 *M* hydrazinonorleucine. ●, DL-Norleucine; ■, DL-Hydrazinonorleucine.

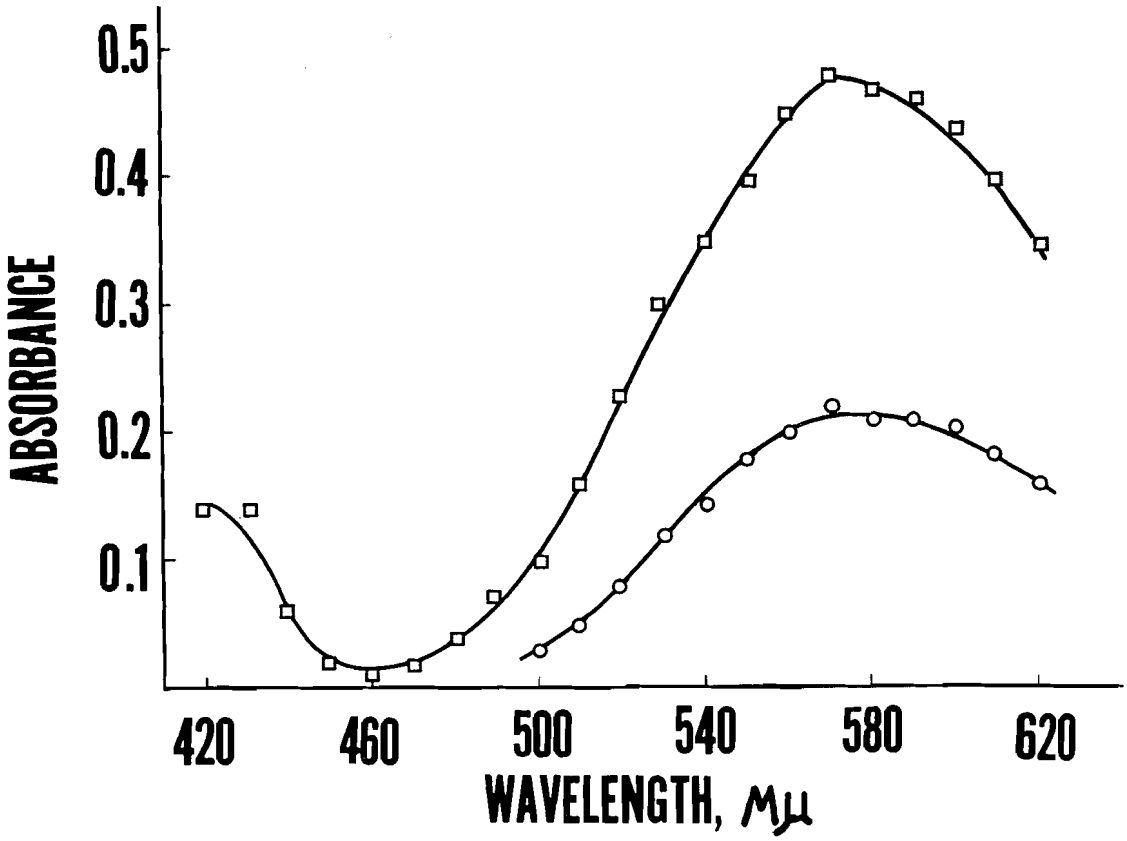

FIG. 5. Absorption spectrum of the hydrazinonorleucine and norleucine ninhydrin reaction products developed in the cellosolve–water system. Acid concentration in reaction: 80γ of 0.002 *M* norleucine and 600γ of 0.002 *M* hydrazinonorleucine. The norleucine reaction required 10 ml of 1:1; *n*-propanol:H_2O diluent rather than 5 ml as in the case of hydrazinonorleucine. Bath time: 1 h; oil bath temperature: 124 °C. ○, DL-Hydrazinonorleucine; □, DL-Norleucine.

vicinity of 22 to 30 min. Thereafter, the color yield in the HNleu case falls off rapidly, while Nleu shows a more gradual decline. The disparity in molar concentrations (7.5 times more HA) must be kept in mind for curve comparison. Also, the ordinate values tend to magnify the variances, which are minor over the first 60 min of development times.

The Ninhydrin Reaction in the Cellosolve–Water System at 124°

Absorption Spectra

The spectra of the products of the ninhydrin–hydrazinonorleucine and ninhydrin–norleucine reactions, run under cellosolve–water conditions, were compared (Fig. 5). The 570 mμ maxima in both cases is characteristic of Ruhemann's purple.

Initially, the reaction conditions are mainly aqueous, but the 1 h reaction time at 124° results in complete evaporation of the water in the reaction mixture.

Although the ratio of the initial color yield in the completely aqueous system of Nleu/HNleu was approximately 7.5, the ratio in the cellosolve–water system is more like 30. Nleu gave more than twice the color yield under these conditions compared to the aqueous system, while HNleu gave approximately one-third less color. It would appear that these conditions offer a more sensitive analytical procedure for amino acid determination than 100° bath temperatures.

Color Stability as a Function of Time

The data in Table V give the color stability for each acid as a function of time for the color developed in the methyl cellosolve–water system. These rates of color decay are comparable to

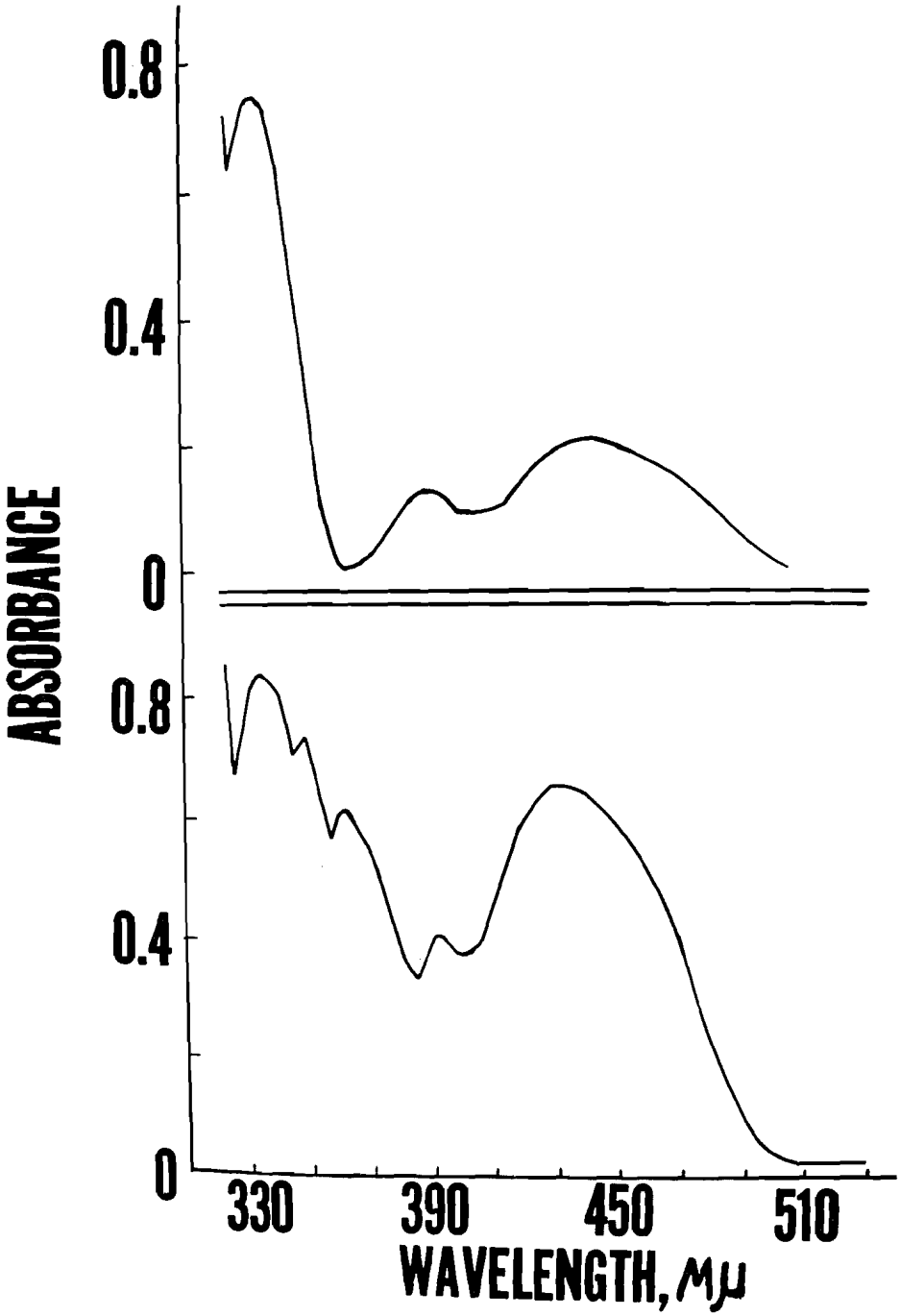

FIG. 6. Absorption spectra of the hydrazine–ninhydrin reaction product developed in two different media. Upper section: aqueous system conditions; bath time: 20 min. Lower section: anhydrous methyl cellosolve containing ninhydrin and stannous chloride, but no citrate. Product developed immediately upon mixing of the reactants at room temperature and was read as such. Concentration of hydrazine: 80 γ of the commercial preparation. The reaction mixture was diluted 1 to 25 with 1:1; *n*-propanol:H_2O, in the case of the anhydrous cellosolve system and 1:50 in the case of the aqueous system prior to reading.

TABLE V

Color stability of ninhydrin reaction product in methyl cellosolve medium*

Compound	Aliquot of standard solution† (μl)	Absorbance at various times after removal from bath				
		Initial	4 h	6 h	11 h	24 h
Norleucine	80	0.48‡	0.44	0.40	0.33	0.27
Hydrazinonorleucine	600	0.22	0.20	0.17	0.15	0.13

*1h color development time. Bath temperature, 124 °C.
†0.002 *M*.
‡10 ml of 1:1; *n*-propanol:H_2O diluent used in this case.

those observed for the reaction run in totally aqueous media.

Hydrazine Reaction with Ninhydrin

Reaction in the Aqueous System

Figure 6, upper section, presents a plot of the data for the color complex formed in the reaction between hydrazine and ninhydrin. No absorption was observed beyond 500 mμ. The first peak was seen at 440 mμ and a weaker one is observed at 385–390 mμ. In the upper ultraviolet region a pronounced peak appears at 332 mμ. Further wavelength reduction was accompanied by absorption that was too intense to be recorded. The reaction mixture was a reddish–brown in appearance. Very little fine structure was seen in the curve and then only from 335 mμ to 324 mμ.

Reaction in Anhydrous Methyl Cellosolve System

Treatment of concentrations of hydrazine and ninhydrin that were identical to those used in the aqueous system, in an anhydrous methyl cellosolve medium containing only ninhydrin and stannous chloride resulted in immediate color development at room temperature and gave the absorption spectrum shown in Fig. 6, lower section. This spectrum is essentially identical to that in Fig. 6, upper section, with major peaks appearing at 430, 390, and 334 mμ. The sharp absorptions as the wavelengths are further reduced are probably due to the keto–phenyl groupings on the ninhydrin or its reduced product or its reaction product with hydrazine. Much more fine structure was observed in this case, than in the product formed in the aqueous medium; however, no fine structure was seen for wavelengths higher than 385 mμ. In the region of the fine structure, two other broad peaks could be observed, one at 348 mμ and the other at 359 mμ and two narrow peaks appeared at 366 and 373 mμ. These additional peaks may reflect the variance in ionization states of the product in the two different solvents. The color yield in anhydrous methyl cellosolve was approximately one-half that in the water system.

The identity of the curves over much of their course point to the formation of the same color complex in both cases, which is obviously different from Ruhemann's purple.

The correspondence between the peak seen with hydrazine at 430–440 mμ and that given at the same wavelength by proline and hydroxyproline may signify some similarity of structure for the ninhydrin reaction product in each case.

Conclusions on the Ninhydrin Studies

In general, the similar absorption maxima and curve shapes of the amino acid and hydrazino acid reaction products with ninhydrin, as well as the color stability results in both the aqueous and cellosolve–water systems, reinforce with considerable confidence the notion that Ruhemann's purple (7) is formed in the reaction between hydrazino acids and ninhydrin, as is known between amino acids and the same reagent.

To account for the appearance of Ruhemann's purple in the hydrazino acid case, one could write a mechanism similar to that suggested for amino acids by Friedman and Sigel (13), which calls for an initial nucleophilic attack by the α-amino nitrogen atom of an amino acid on the C-2 of the ninhydrin molecule (with HA, the attack would be by the β-nitrogen atom of the α-hydrazino group). With the HA hydrazone, tautomerization to an azo compound followed by reduction in the stannous chloride containing medium would yield 2-amino-1,3-diketoindan.

This compound and a molecule of ninhydrin can react to give Ruhemann's purple.

One of the two anonymous referees suggested an interesting alternative mechanism, which presumably includes an initial nucleophilic attack to yield the corresponding hydrazone. A rearrangement of bonds and a splitting off of a fragment from the hydrazone would yield 2-imino-1,3-diketoindan which could then be reduced and reacted with another molecule of ninhydrin to give Ruhemann's purple.

Whatever the exact mechanism for the ninhydrin reaction with either amino acids or HA to yield Ruhemann's purple, they all appear to have two common requirements (*i*) with HA, an initial nucleophilic attack yielding a hydrazone (with amino acids, a Schiff base), and (*ii*) the formation of 2-amino-1,3-diketoindan or its corresponding anion at the 2-position carbon atom.

With hydrazine itself, a hydrazone could form which might not permit reduction to 2-amino-1,3-diketoindan or which could react with a second molecule of ninhydrin to yield the corresponding azo compound also incapable of yielding 2-amino-1,3-diketoindan.

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