The Rearrangement of 3-Amino-3,4-dihydro-1-hydroxycarbostyril to 3-Amino-3,4-dihydro-6-hydroxycarbostyril (1)

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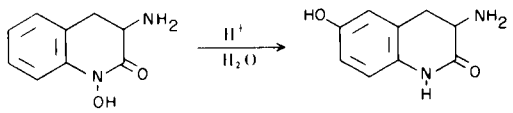
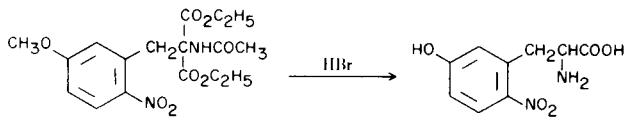
In a previous publication (4) from these laboratories, we described the synthesis of 3-amino-3,4-dihydro-1-hydroxycarbostyril (I) and its potent inhibitory activities against the growth of several microorganisms as part of a program aimed at finding new bioactive compounds. Since this earlier report, we observed that refluxing of I in dilute acidic solution produced a change from its characteristic violet color to green when treated with ferric chloride reagent with a concomitant loss of its microbiological activity. Consequently, we decided to investigate this rather unanticipated result, which has now led us to discover that I undergoes an interesting molecular rearrangement in acidic media. Therefore, it is the purpose of this paper to report the results of this investigation which appears to be the first example of such a rearrangement by a *N*-aryl cyclic hydroxamate system as I.

In the present investigation, we found that treatment of I with dilute aqueous sulfuric acid under reflux conditions results in rearrangement to 3-amino-3,4-dihydro-6-hydroxycarbostyril II.

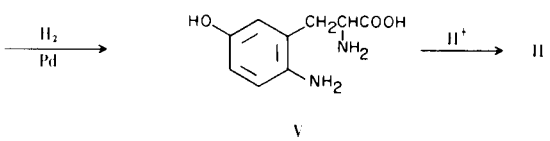


I II

The rearranged product II was isolated as the hydrochloride salt, the structure of which was ultimately established by independent synthesis using an acetamidomalonic ester route similar to that previously described for 3-amino-3,4-dihydrocarbostyril (5).



III IV



V

The condensation of the appropriately substituted 5-methoxy-2-nitrobenzyl bromide (6) with ethyl acetamidomalonate in the presence of sodium ethylate gave 2-acetamido-2-(5-methoxy-2-nitrobenzyl)malonic ester (III). The hydrolysis, decarboxylation and demethylation of III was effected by use of concentrated hydrobromic acid to yield 5-hydroxy-2-nitrophenylalanine (IV) as the hydrobromide. In the subsequent reaction, the latter compound was neutralized by careful addition of concentrated ammonium hydroxide to produce its free base, which was hydrogenated using platinum black catalyst to reduce the nitro to an amino group, forming 2-amino-5-hydroxyphenylalanine (V). In separate experiments, V was converted directly to the desired product, 3-amino-3,4-dihydro-6-hydroxycarbostyril hydrochloride (II), without isolation by acidification of the hydrogenation reaction mixture with concentrated hydrochloric acid, which effects intramolecular cyclization through lactam formation.

Both compounds obtained from the acetamidomalonic ester synthesis (Method A) and the molecular rearrangement of I (Method B) gave similar elemental microanalyses for the hydrochloride monohydrate of II, and the melting point of their admixture was unchanged. Also, samples of II from either one of the two methods were shown to be indistinguishable from each other, and yet distinguishable from the structural isomer I, by a comparison of their melting points, color reactions, and R_f values in two different solvent systems as summarized in Table I. Further, the infrared and the ultraviolet spectra of the rearranged product were identical in every detail with those of the authentic synthesized compound II. The ultraviolet spectra of compounds I and II are recorded in Table II. Therefore, the analytical and the spectral evidence thus obtained establishes unequivocally the assigned structure of II as the rearrangement product of I in acidic solution.

The most outstanding structural feature of I is the cyclic hydroxamate linkage as part of the heterocycle fused to the benzene ring. This system constitutes a structural counterpart of *N*-phenylhydroxylamine, whose marked lability to rearrangement under acidic conditions has been well documented over the years (7). Apparently, the rearrangement of I to II in acidic media occurs in a manner analogous to the acid-catalyzed rearrangement of *N*-phenylhydroxylamine to *p*-aminophenol (8).

From this preliminary study, it seems reasonable to

TABLE I

Melting Points, Color Reactions, and R_f Values
of Some Hydroxy Substituted 3-Amino-3,4-dihydrocarbostyrils

Compound	M.p. (a) °C	Ninhydrin Reaction	$FeCl_3$ Reaction	Millon Reaction	R_f (b)
I. 3-Amino-3,4-dihydro-1-hydroxycarbostyril·HCl	258-263	+ (yellow)	+ (violet)	- (c)	0.48 (A), 0.75 (B)
II. 3-Amino-3,4-dihydro-6-hydroxycarbostyril·HCl (Method A or Method B)	285-286	+ (yellow)	+ (green)	+ (red)	0.28 (A), 0.79)B)

(a) Melting points are for analytical samples. (b) The R_f values given for the appropriate solvents are indicated by letter: A, 1-BuOH-AcOH-H_2O (4:1:1); B, 65% pyridine. (c) Characteristic red color develops after heating due to rearrangement in acidic media.

TABLE II

Ultraviolet Spectra of Some Hydroxy
Substituted 3-Amino-3,4-dihydrocarbostyrils

Compound	*p*H	λ max, mμ (log ε)	λ min, mμ (log ε)	λ shoulder, mμ (log ε)
I. 3-Amino-3,4-dihydro-1-hydroxycarbostyril·HCl	2	257 (4.111)	226 (3.449)	
	7	258 (4.149)	228 (3.949)	
	10	290 (3.859)	240 (3.590)	267 (3.773)
II. 3-Amino-3,4-dihydro-6-hydroxycarbostyril·HCl (Method A or Method B)	2	258 (4.664)	226 (4.034)	290 (4.123)
	7	258 (4.676)	226 (4.124)	291 (4.149)
	10	273 (4.682)	231 (4.184)	

conclude that *N*-aryl cyclic hydroxamate systems as exemplified by I are susceptible to rearrangement under acidic conditions with formation of the related substituted *N*-aryl lactams. Studies are now in progress to further extend the acid-catalyzed rearrangement of I to yield still other 6-substituted derivatives of II.

EXPERIMENTAL

All melting points are corrected. The R_f data were determined using the ascending technique in the solvents indicated, and ninhydrin reagent was used for development of the spots. The infrared analyses were performed on a Beckman IR-8 spectrophotometer using the potassium bromide pellet technique. The ultraviolet absorption spectra were obtained on a Bausch and Lomb 505 recording spectrophotometer at concentrations of 10 μg./ml. in aqueous solutions at *p*H 2, 7, and 10 in the 200-350 mμ region. The *p*H of each sample was adjusted by the dropwise addition of either concentrated hydrochloric acid or 2*N* sodium hydroxide solutions. The microanalyses were performed by M-H-W Laboratories, Garden City, Michigan.

Ethyl 2-Acetamido-2-(5-methoxy-2-nitrobenzyl)malonate.

To a solution of 11.4 g. of ethyl acetamidomalonate in 140 ml. of magnesium-dried ethanol containing 1.2 g. of sodium was added 12.5 g. of 5-methoxy-2-nitrobenzyl bromide (6). The reaction mixture was stirred at room temperature for 9 hours after which time 550 ml. of water was added to cause precipitation. The solid was removed by filtration, washed with cold ethyl ether, and dried over phosphorus pentoxide *in vacuo* to yield 12 g. (62%) of product, m.p. 133°.

Anal. Calcd. for $C_{17}H_{22}N_2O_8$: C, 53.39; H, 5.79; N, 7.32. Found: C, 53.27; H, 5.80; N, 7.30.

5-Hydroxy-2-nitrophenylalanine.

A 5.0 g. sample of ethyl 2-acetamido-2-(5-methoxy-2-nitrobenzyl)malonate was suspended in 50 ml. of 48% hydrobromic acid and heated under reflux for 12 hours. The reaction mixture was taken to dryness under reduced pressure with heating. The solid residue was dissolved in a minimum amount of water, and ammonium hydroxide was added dropwise to *p*H 7.0 to yield 2.6 g. (76%) of product, m.p. 220° dec.

Anal. Calcd. for $C_9H_{10}N_2O_5$: C, 47.79; H, 4.46; N, 12.38. Found: C, 48.04; H, 4.46; N, 12.12.

2-Amino-5-hydroxyphenylalanine.

One gram of 5-hydroxy-2-nitrophenylalanine in 30 ml. of 50% aqueous methanol was hydrogenated under 3.67 kg./cm.^{2} of hydrogen pressure in the presence of 50 mg. of platinum black for 3 hours. The catalyst was removed by filtration, and the resulting solution was concentrated in volume by removal of the solvent under reduced pressure. The residue was recrystallized from ethanol-ether to yield 0.69 g. of crude material. Recrystallization of the latter from 95% ethanol gave 0.51 g. (44%) of product, m.p. 263-264°.

Anal. Calcd. for $C_9H_{12}N_2O_3 \cdot H_2O$: C, 50.46; H, 6.59; N, 13.08. Found: C, 50.56; H, 6.73; N, 13.17.

3-Amino-3,4-dihydro-6-hydroxycarbostyril Hydrochloride.

Method A.

A solution of 1.0 g. of 5-hydroxy-2-nitrophenylalanine in 30 ml. of 50% methanol was hydrogenated under 3.67 kg./cm.^{2} of hydrogen pressure in the presence of 50 mg. of platinum black for 3 hours. After removal of the catalyst by filtration there was added 30 ml. of concentrated hydrochloric acid and 5 ml. of acetone to the filtrate, which was placed in the freezer overnight. The precipitate was collected by filtration and recrystallized from aqueous methanol and hydrochloric acid to yield 0.71 g. (79.5%) of product. The melting point, color reactions and R_f values are given in Table I, and the ultraviolet absorption spectra are recorded in Table II. The infrared spectrum showed major absorption bands at 3.2, 5.9, 6.7, 7.1, 7.5, 7.9, 8.1, 8.7, 11.3, and 12.5μ.

Anal. Calcd. for $C_9H_{10}N_2O_2 \cdot HCl \cdot H_2O$: C, 46.46; H, 5.63; N, 12.00. Found: C, 46.79; H, 5.64; N, 11.97.

Method B.

A solution of 250 mg. of 3-amino-3,4-dihydro-1-hydroxycarbostyril (4) in 25 ml. of 1*N* sulfuric acid was heated under reflux for 24 hours. The course of reaction was followed by removing samples periodically and testing for a change in the characteristic violet color with 10% ferric chloride reagent (in 0.1*N* hydrochloric acid). No color change occurred until after 15 hours at which time a distinctive green was observed for the positive ferric chloride test. After 24 hours the reaction mixture was cooled to room temperature, and then carefully neutralized by the addition of solid sodium hydroxide. At this stage, a small amount of insoluble material was removed by filtration, and the filtrate was taken to dryness by removal of the liquid phase under reduced pressure with warming. The solid residue was leached four times with small amounts of hot methanol. The alcoholic extracts were combined and then reduced to dryness by evaporation of the methanol *in vacuo* to give 230 mg. of solid, which was subsequently dissolved in a small amount of hot methanol and acidified to *p*H 1 by the dropwise addition of concentrated hydrochloric acid. After the resulting solution was placed in a deep freeze overnight, there was recovered a total of 107 mg. (36%) of product. This product was shown to be identical with the authentic sample obtained from Method A by a comparison of their infrared red spectra in conjunction with the physical and chemical data given in Table I and Table II.

Anal. Calcd. for $C_9H_{10}N_2O_2 \cdot HCl \cdot H_2O$: C, 46.46; H, 5.63; N, 12.00. Found: C, 46.84; H, 5.92; N, 11.97.

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