MASS SPECTROMETRIC STUDY OF α-CARBOLINES

P. A. Sharbatyan, A. N. Kost,*
V. V. Men'shikov, L. G. Yudin,
and N. B. Chernyshova

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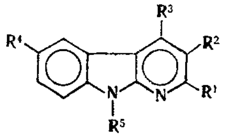
A series of α-carbolines were subjected to mass-spectrometric analysis. It is shown that these compounds are extremely stable with respect to electron impact. From 40 to 60% of the total ion current goes into the production of molecular ions during dissociative ionization. The mass spectra of these compounds are simple: the maximum peak corresponds to the molecular peak in the spectrum. The formation of the $[M-1]^+$ ion and the detachment of a methyl group in monomethylcarbolines ($[M-15]$) are determined by the position of the methyl group. The mass-spectral fragmentation confirms the proposed scheme for the formation of α-carbolines from arylhydrazones and δ-keto nitriles. A method for the synthesis of α-carbolines on the basis of accessible arylsulfamidoindoles is proposed.

New methods for the synthesis of α-carbolines that have made these saltlike compounds more accessible have been found in recent years [1-4]; the N-oxides of these compounds display considerable antivirus activity [5]. Certain difficulties that are a consequence of the insufficient amount of study devoted to structures of this sort have been encountered in the establishment of their structures [1, 6, 7].

To characterize the structures of the synthesized α-carbolines more nearly completely and to study the mechanism of the new method for the synthesis of these compounds that was discovered in our laboratory we subjected a series of α-carbolines to mass-spectrometric analysis. Since the literature contains only fragmentary data on the mass spectra of compounds of this class [3, 4], we studied I-XIV (Table 1).



It seemed of interest to examine the effect of substituents on the W_M value and the pathway of fragmentation of substituted α-carbolines.

α-Carboline is more stable than carbazole. Thus the W_M value for α-carboline is 61.7, as compared with 49.0 for carbazole [8].

The introduction of a methyl group in the pyridine ring reduces the stability of the molecule with respect to electron impact by a factor of almost 1.5 (Table 2) and leads to the formation of a rearranged $[M-1]^+$ ion due to the loss of a hydrogen atom of the methyl group; this is characteristic for the methylpyridine ring [9]. The stability of this ion and its subsequent fragmentation are responsible for the decrease in the W_M value (45.6), since the current of the fragmentary ions increases. The introduction of a second methyl group also lowers the W_M value but to a lesser extent. Another rearrangement process, viz., detachment of a methyl group with migration of a hydrogen atom and with ring expansion (or detachment of a CH_3 group from the rearranged molecular ion), appears simultaneously. Thus yet another fragmentation pathway comes into play, as a result of which a certain decrease in the stability of the molecule ($W_M = 43.2$) is observed.

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*Deceased.

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TABLE 1. Position and Character of the Substituents in Model α-Carbolines (I-XIV)

Compound	R^1	R^2	R^3*
I	H	H	H
II	CH_3	H	H
III	H	CH_3	H
IV	H	H	CH_3
V	H	H	H
VI	CH_3	CH_3	H
VII	CH_3	H	CH_3
VIII	CH_3	H	H
IX	CH_3	CH_3	H
X	CH_3	H	CH_3
XI	C_6H_5	H	CH_3
XII	CH_3	H	C_6H_5
XIII	C_6H_5	H	C_6H_5
XIV	H	H	NO_2

*Note that $R^4 = CH_3$ for VIII and IX, while $R^4 = H$ for the remaining compounds; $R^5 = CH_3$ for V and X, while $R^5 = H$ for the remaining compounds.

TABLE 2. Mass Spectra of α-Carbolines

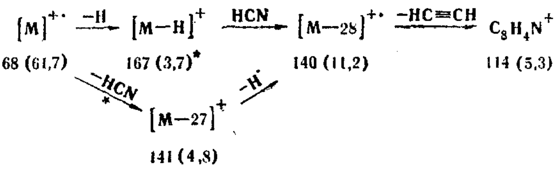
Compound	mp, °C	W_M	m/e (intensity of the peak in percent of the maximum peak)*
I	120	61,7	168 (100), 167 (6), 141 (8), 140 (18), 115 (4), 114 (8), 113 (6), 78 (17), 71 (3), 63 (6)
II	120	44,5	182 (100), 181 (28), 154 (10), 153 (3), 149 (8), 140 (6), 129 (13), 127 (14), 113 (6), 111 (8), 109 (6), 101 (6), 97 (18), 95 (15), 91 (27)
III	90	45,8	182 (100), 181 (52), 180 (11), 155 (3), 154 (8), 130 (5), 129 (9), 127 (4), 91 (5), 81 (3), 77 (6)
IV	90	45,6	182 (100), 181 (28), 153 (3), 154 (7), 129 (3), 128 (5), 127 (8), 91 (9), 81 (6), 77 (7)
VI	120	41,4	196 (100), 195 (48), 181 (31), 168 (6), 167 (4), 155 (7), 154 (10), 139 (3), 127 (7), 125 (8), 97 (5), 71 (5)
VII	120	43,2	196 (100), 195 (32), 194 (3), 182 (3), 181 (20), 168 (4), 167 (3), 155 (3), 154 (5), 97 (3)
XI	140	42,5	258 (100), 257 (8), 243 (3), 242 (3), 236 (3), 129 (4), 111 (7), 109 (4), 97 (17), 96 (10), 95 (8), 67 (9), 60 (8)
XII	150	48,6	258 (100), 257 (20), 241 (4), 128 (8), 127 (7), 120 (4), 114 (3), 77 (3), 68 (3)
XIII	140	57,1	320 (100), 319 (16), 243 (3), 111 (3), 97 (5), 96 (3), 95 (4), 81 (5), 71 (6)
XIV	200	27,6	213 (100), 183 (3), 168 (7), 167 (53), 166 (5), 155 (13), 141 (6), 140 (42)

*Ions that have intensities >3% of the maximum peak are presented.

In the case of 4-methyl-α-carbolines the introduction of a phenyl group in the pyridine ring (4-methyl-2-phenyl-α-carboline) reduces the stability of the molecule with respect to electron impact. On the other hand, the introduction of the same group in position 4 of the pyridine ring (2-methyl-4-phenyl-α-carboline) increases the stability of the molecule with respect to electron impact (W_M = 48.6) somewhat. The introduction of two phenyl groups (2,4-diphenyl-α-carboline) in place of methyl groups in 2,4-dimethyl-α-carboline leads to an increase in W_M to 57.1 due to stabilization of the molecular ion. Similar effects were observed in the case of phenylpyridines [9]. As expected, the introduction of a strong electron-acceptor nitro group lowers the W_M value by a factor of two; however, as before, it remains very high.

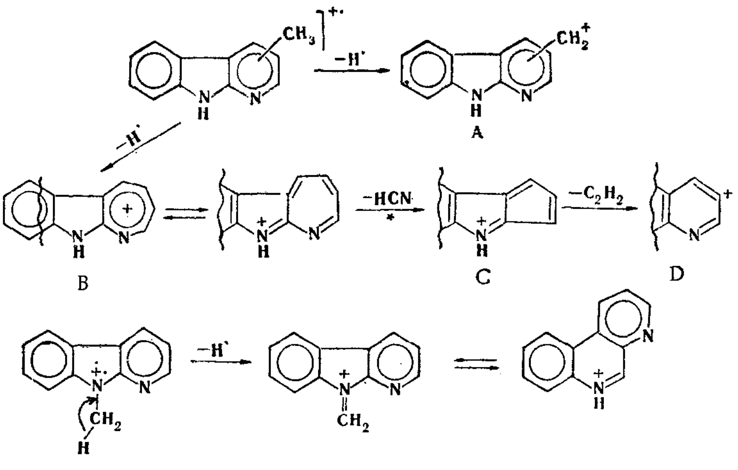
It follows from an analysis of the mass spectra (Table 2) that the molecular ions of α-carbolines have high stabilities; appreciable fragmentation of these molecular ions is observed at ionizing voltages above 40 V. However, if the voltage exceeds 50 V, intense peaks corresponding to doubly charged ions appear in the mass spectra. The presence of an intense M–1 peak in the spectrum is characteristic for these structures.

In the mass spectrum of α-carboline itself, in addition to a molecular-ion peak with mass 168* ($[C_{11}H_8N_2]^+$), only the peaks of ions with masses 167 ($[C_{11}H_7N_2]^+$), 141 ($[C_{10}H_7N]^+$), 140 ($[C_{10}H_6N]^+$), and 114 ($[C_8H_4N]^+$) have sufficient intensities. The elementary compositions of these ions were established on the basis of accurate measurements of their masses. The selectivity of the fragmentation is very high, and 82% of the total ion current goes into the production of the ions shown in the scheme. The molecular-ion peak is the maximum peak. The introduction of a methyl group lowers the stabilities of the molecules with respect to electron impact and decreases the probability of detachment of hydrogen cyanide: the intensities of the peaks of the ions with masses 155 for 2-methyl-, 3-methyl-, and 4-methyl-α-carbolines are 1.2-1.4% of the total current. Cleavage at the β bond and the loss of hydrogen by the molecular ions become the predominant processes. The intensity of the $[M{-}1]^+$ ion depends on the position of the methyl group in the pyridine ring. The intensities for 2-methyl- and 4-methyl-α-carbolines are close, whereas in the case of 3-methyl α-carboline the probability of detachment of a hydrogen atom is higher by a factor of two.



In the case of V, which has a CH_3 group bonded to the nitrogen atom, the M–1 peak turns out to be higher than the molecular-ion peak (see the scheme).

The $[M{-}1]^+$ ion may be formed via the following pathways:



The splitting out of HCN from the M–1 ion observed in the mass spectra of 2-, 3-, and 4-methyl-α-carbolines is a confirmation of the possibility of the existence of ion B.† This process is confirmed by metastable ions with m* = 131.0 (calculated value m* = 131.0). The ratios of the intensities of the $[M{-}(H + HCN)]^+$ and $[M{-}H]^+$ ion peaks in the spectra of

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*Here and subsequently, the m/e values (and intensities in percent relative to the total ion current) are presented.

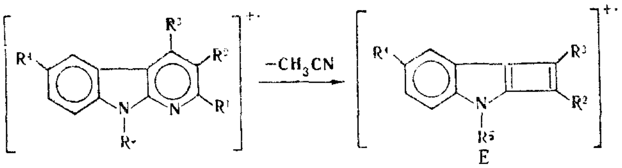
†Detachment of a methyl group from the phenyl ring is also possible in addition to splitting out of a hydrogen atom from the methyl group; this was demonstrated by means of a deuterium label in the case of phthalazones [10]. However, it follows from the mass spectrum of unsubstituted α-carboline that the extent to which this process occurs does not exceed 3.7%.

2-, 3-, and 4-methyl-α-carbolines are inconstant, and this constitutes evidence for the existence of various intermediate states (A and B) for these isomers.

A molecule of acetylene is eliminated from ion C. The resulting D ion probably has a quinoline structure (without a hydrogen atom). This process is confirmed by a metastable peak with m* = 106.4 (calculated value 106.5).

The subsequent fragmentation of ion D leads to an ion with mass 77, which is characteristic for aromatic compounds that contain a benzene ring [9].

In the case of di- and trimethyl-α-carbolines, which contain a CH_3 group attached to the C_2 atom (as well as in the case of 2-methyl-α-carboline), in addition to detachment of a molecule of HCN, one observes elimination of a CH_2CN group; this process, as demonstrated by the metastable peaks, may take place both from the molecular ion and from the M–1 ion:



E

The E ion has masses of 141 (II), 155 (VI), and 169 (IX or X). The possibility that ring expansion due to the inclusion of a methyl group to give a five-membered ring occurs in addition to the formation of ion E is not excluded. The subsequent fragmentation of this ion will be similar to the fragmentation of ion C.

In addition to the pathway of fragmentation of the molecular ions of methylcarbolines examined above, detachment of a methyl group is also observed. Like the formation of an M–1 ion, the detachment of a methyl group in monomethylcarbolines ([M–15]) is determined by the position of the methyl group. In fact, in a comparison of the mass spectra (Table 2) it is apparent that an M–15 peak is absent in the case of 3-methylcarboline, while the intensities of the M–15 peaks in the spectra of 4-methyl- and 2-methyl-α-carbolines are, respectively, 1.1 (±0.2) and 0.6% (±0.1) of the intensity of the molecular peak.

An important feature of the spectrum of 2-methyl-α-carboline is the presence of an ion with mass 165, which is formed from the M–15 ion by elimination of a molecule of hydrogen. This process is confirmed by a metastable peak with m* = 162.9 (calculated value 163). Ejection of a molecule of hydrogen from the M–15 ion is not observed in the case of 4-methyl-α-carboline. The decrease in the intensity of the peak at m/e 167 in the mass spectrum of 2-methyl-α-carboline as compared with 4-methyl-α-carboline is evidently associated with dehydrogenation of the $[M{-}15]^+$ ion. It should be noted that the sum of the intensities of the peaks with m/e 165 and 167 in the mass spectrum of 2-methyl-α-carboline is equal to the intensity of the M–15 peak in the case of 4-methyl-α-carboline:

$$0.6(\pm 0.1) + 0.4(\pm 0.1) = 1(\pm 0.2).$$

Thus 2-methyl-α-carboline can be distinguished from 4-methyl-α-carboline from the intensity of the $[M{-}CH_3, {-}H_2]^+$ peak.

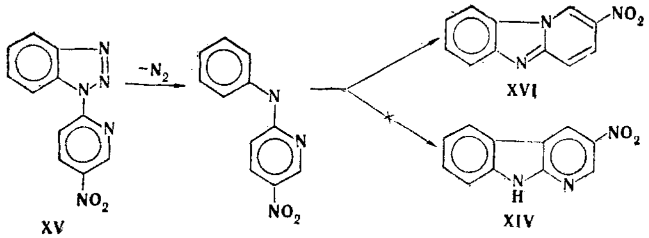
The elimination of a molecule of hydrogen from the M–15 fragment is observed in all cases in which the CH_3 group is in the α position with respect to the pyridine nitrogen atom. For example, a $[M{-}CH_3, {-}H_2]^+$ peak is present in the mass spectra of 2,4,9-trimethyl-α-carboline, 2,3-dimethyl-α-carboline, and 2,3,6-trimethyl-α-carboline. The presence of a metastable peak with m* = 117.3 (calculated value 117.3) in the mass spectra of 2- and 4-monomethylcarbolines constitutes evidence that the M–15 ion subsequently eliminates a molecule of hydrogen cyanide with the formation of an ion with mass 140. In addition, an ion with mass 141 may be formed from the M–15 ion as a result of elimination of a molecule of acetylene. An ion with mass 115, which is present in the spectra of all indole derivatives [11], is formed in the subsequent fragmentation of the ions with masses 141 and 140.

It is apparent from the mass spectra of di- and trimethylcarbolines that an increase in the number of methyl groups in the pyridine ring as compared with monomethylcarbolines gives rise to an increase in the intensity of the M–15 peak. However, an analysis shows that the relative intensity of the M–15 peak increases sharply in the case of a vicinal orientation of the methyl groups in the pyridine ring. A similar increase in the relative intensity of the M–15 peak was observed in the case of dimethylindoles [12]. We assume that the explanation of this fact must be sought in the fact that expansion of the pyridine ring in the case of 2,3-dimethyl-α-carboline occurs primarily through the methyl group attached to the C_3 atom. The $[M-CH_3]^+$ ion is formed as a result of detachment of the methyl group attached to the C_2 atom.

The principal possibility of inclusion of the methyl group in the C_3 position in a ring is in agreement with the fact that the intensities of the peaks of $[M-H]^+$ ions in the mass spectra of 3-methyl-2,3-dimethyl- and 2,3,6-trimethyl-α-carbolines have close values (52, 48, and 50%, respectively).

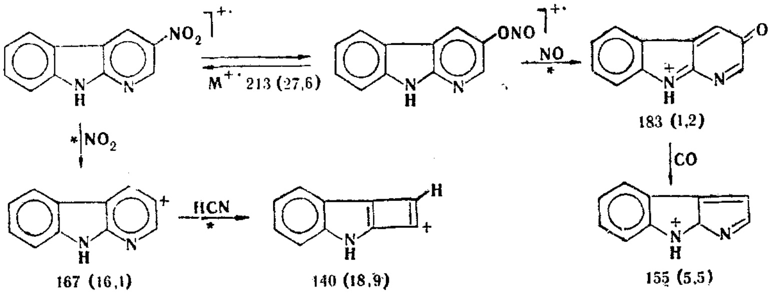
Different intensities of the peaks of the $[M-H]^+$ ions are characteristic for the mass spectra of isomeric methylphenyl-α-carbolines. Thus the $I_{(M-H)}/I_M$ value for 2-methyl-4-phenyl-α-carboline is higher by a factor of 2.5 than the value for 2-phenyl-4-methyl-α-carboline, while just the opposite effect is observed for isomeric phenylpyridines [9]. This fact indicates that the cyclization with the participation of the nitrogen atom of the pyridine ring and the carbon atom of the phenyl ring that is characteristic for 2-phenylpyridines under the influence of electron impact in hindered in the case of 2-phenyl-α-carboline. The intensity of the peak of $[M-H]^+$ ions in the mass spectrum of 2,4-diphenyl-α-carboline is low (1.3%), and the remaining peaks have intensities lower than 1%.

The effect of an electron-acceptor substituent in the pyridine ring on the fragmentation pathways during electron impact has been examined in the case of 3-nitro-α-carboline (XIV). Previous attempts to obtain carbolines with a nitro group in the pyridine ring have been unsuccessful. It has been reported [13] that 3-nitro-α-carboline (XIV) is formed in 29% yield when 1-(5-nitro-2-pyridyl)benzotriazole (XV) is heated in pyrophosphoric acid. In fact, however [1, 7], the cyclization proceeds with the participation of the pyridine nitrogen atom and the formation of 2-nitropyrimido[1,2-a]benzimidazole (XVI).



We obtained a mixture in which the major component was a compound with the empirical formula $C_{11}H_6N_3O_2$ in the condensation of 2-arylsulfamidoindole with the sodium salt of nitromalondialdehyde. The IR spectrum of the major component does not contain stretching vibrations of a carbonyl group at 1680-1720 cm^{-1}. The absorption bands at 1338 and 1540 cm^{-1} are related to the stretching vibrations of the NO_2 group, while the absorption bands at 1598 and 1610 cm^{-1} pertain to C=N or C=C stretching vibrations. The UV spectrum (in $CHCl_3$) has absorption maxima at 282, 298, and 351 nm; this is typical for α-carbolines [4]. The PMR spectrum (in CF_3COOH) characterizes this substance as an aromatic system with a multiplet at 7.40-8.38 ppm. Two singlets (at 7.1 and 7.23 ppm) are related to the α and γ protons of the pyridine ring.

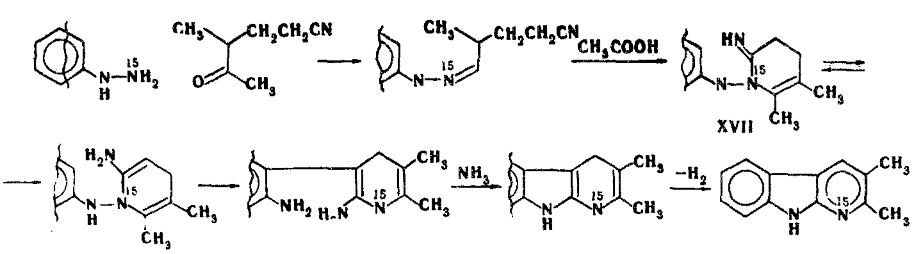
In the mass spectrum of 3-nitro-α-carboline, fragmentation is determined by the presence of a nitro group, and virtually no splitting out of a hydrogen atom from the molecular ion is realized.



The probability of nitro–nitrite rearrangement, which is determined by the ratio of the intensities of the ions viz., $[M-NO]^+/[M-NO_2]^+$, is 0.06, i.e., the nonisomerized form of the molecular ion predominates. The twofold decrease in the contribution of the nitro-nitrite form of M^+ as compared with nitrocarbazoles is evidently associated with the acceptor properties of the pyridine ring.

The data obtained make it possible to verify the proposed mechanism of the formation of α-carbolines from arylhydrazones of δ-keto nitriles [2]. With this end in mind, we synthesized 4-methyl-5-ketocapronitrile and subjected it to reaction with β-N^{15}-phenylhydrazine. To obtain a high-purity substance and to simultaneously verify the effect of possible impurities on the course of the reaction we did not subject methyl ethyl ketone to direct cyanoethylation (which is accompanied by the formation of a number of side products) but rather obtained initially α-methyl-acetoacetic ester, which via cyanoethylation and ketone cleavage led to 4-methyl-5-ketocapronitrile, the percentage of the principal substance in which [according to the results of gas—liquid chromatography (GLCY] was 99.5%. This keto nitrile gave 2,3-dimethyl-α-carboline (VI) in 32% yield upon reaction with phenylhydrazine free of aniline (from special monitoring) and subsequent refluxing with pure acetic acid. Thus the impurities contained in the starting substance do not determine the course of the cyclization.

If α-carbolines are formed via the scheme presented below, elimination of a nitrogen atom included in the composition of the nitrile group should occur after cleavage of the N—N bond in intermediate structure XVII, while the β-nitrogen atom of phenylhydrazine is lost in the usual processes involved in the Fischer synthesis.



We started from sodium nitrite containing 10.75% N^{15}, and the relative intensity of the isotope peak with mass 197 (with respect to the molecular peak with mass 196) underwent a 10.5% increase in the mass spectrum after its conversion to phenylhydrazine and subsequently to carboline VI. Thus the labeled nitrogen atom was not lost during the synthesis, and this constitutes evidence for inclusion of the β-nitrogen atom of phenylhydrazine in the carboline molecule and is a confirmation of the proposed scheme.

EXPERIMENTAL

The mass spectra of the compounds were obtained with an MKh-1303 mass spectrometer with a system for direct introduction of the samples into the ion source at an ionizing-electron energy of 50 eV. The elementary compositions of the characteristic ions in the mass spectra of I, VII, and XIV were determined with a high-resolution Jeol IMS-01-SG-2 spectrometer (Japan).

The synthesis of α-carbolines was described in [2-4]. The substances were purified by numerous recrystallizations from benzene with subsequent sublimation. The purity of the samples was monitored by chromatography (TLC) in a thin layer of aluminum oxide in a benzene—ethyl acetate system (2:1) with development in UV light and with iodine vapors.

3-Nitro-α-carboline (XIV). A mixture of 0.5 g (1.7 mmole) of 2-tosylaminoindole, and 0.4 g (3 mmole) of the sodium salt of nitromalondialdehyde in 15 ml of absolute ethanol and 3 ml of 48% HBr was heated at 80-100°C for 3 h, after which it was cooled, and 5-7 ml of water was added. The solution was treated with 0.2 N sodium hydroxide solution up to pH 8-9 and extracted with chloroform, and the extract was dried over magnesium sulfate. The solvent was evaporated in vacuo, and the residue was purified preparatively on plates with aluminum oxide to give 0.21 g (56%) of 3-nitro-α-carboline (XIV) with mp 321-323°C (80% methanol). UV spectrum ($CHCl_3$), λ_{max} (log ε): 282 (4.04), 298 (3.79), and 351 nm (3.56). PMR spectrum (in CF_3COOH): 7.1 (s, H), 7.2 (s, H), and 7.3-7.9 ppm (m, four aromatic protons). IR spectrum: 3400 (N—H); 1540, 1338 cm^{-1} (NO_2). Found: C 61.9; H 3.1; N 19.7%. $C_{11}H_6N_3O_2$. Calculated: C 62.0; H 3.3; N 19.7%.

α-Carboline (I). A mixture of 0.5 g (1.7 mmole) of 2-tosylaminoindole, 0.4 g (2 mmole) of freshly distilled malonaldehyde bis(diethylacetal), 10 ml of absolute ethanol, and 2 ml of 48% hydrobromic acid was heated at 80-100°C, another 0.4 g of malonaldehyde bis(diethylacetal) in 2 ml of absolute alcohol was added dropwise after 30 min, and the mixture was heated for another hour. The isolation of the product was carried out by the method indicated above to give 0.25 g (76%) of α-carboline I with mp 211-212°C (from benzene).

N-Methyl-α-carboline (V). This compound was similarly obtained from 0.2 g (0.6 mmole) of 1-methyl-2-tosylaminoindole and 0.4 g (2 mmole) of malonaldehyde bis(diethylacetal). Workup gave 0.06 g (51%) of a product with mp 52-53°C (from benzene).

β-N^{15}-Phenylhydrazine. A 1.48-g sample of freshly distilled aniline was dissolved in 14.8 ml of concentrated HCl, and the solution was cooled to —4°C and treated with a solution of 1.1 g of $NaNO_2$ (the N^{15} content was 10.75%) in 4.85 ml of H_2O at such a rate that the temperature did not rise above 0°C. The resulting solution of the diazonium salt was filtered thoroughly. An 8.6-g sample of $SnCl_2 \cdot 2H_2O$ was then dissolved in 10 ml of concentrated HCl, the resulting solution was filtered thoroughly, and the filtrate was added dropwise to the solution of the diazonium salt at such a rate that the temperature did not rise above 0°C. The mixture was then stirred with cooling for another 2 h, and the resulting precipitate was removed by filtration, washed on the filter with cooled (to —10°C) concentrated HCl, and added in portions with stirring to 30 ml of 2 N NaOH solution. The mixture was extracted with ether, the ether extract was dried with calcined K_2CO_3, and the ether was evaporated to give 0.9 g (53.5%) of β-N^{15}-phenylhydrazine. According to the results of TLC, the product contained only traces of aniline.

1-N^{15}-2,3-Dimethyl-α-carboline (VI). A mixture of 0.85 g of labeled phenylhydrazine and 0.98 g of 4-methyl-5-ketocapronitrile (obtained from acetoacetic ester) was heated on a water bath for 3 h, after which the water was removed by distillation with benzene, 8 ml of glacial acetic acid was added to the residue, and the mixture was refluxed for 12 h. It was then cooled, diluted with water, and neutralized with 2 N NaOH, and the precipitate was removed by filtration, washed on the filter with water, and crystallized from benzene to give 0.32 g (21%) of labeled carboline VI with mp 262-263°C and R_f 0.4 [benzene—ethyl acetate (2:1), Al_2O_3].

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