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Synthesis of Mutagens isolated from Tryptophan Pyrolysate and of Some Analogs, 3-Amino-5*H*-pyrido[4,3-*b*]indoles

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A potent mutagen, 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole, isolated from tryptophan pyrolysate, and some analogs were synthesized. The key reaction was the $AlCl_3$-catalyzed condensation of 2-cyanomethylindole and alkyl (or phenyl) cyanide.

Keywords——tryptophan pyrolysate; mutagen; carcinogen; amino-5*H*-pyrido-[4,3-*b*]indole; 5*H*-pyrido[4,3-*b*]indole; γ-carboline; aminopyridine

Many mutagenic compounds have been found since the development of the rapid detection methods using bacteria. The mutagenic principles in foods have been of particular interest. In connection with the mutagenic principles formed by heating of foods, Sugimura, Kosuge, Iitaka and Okamoto proposed the structures of two potent mutagens, named Trp-P-1 and Trp-P-2, isolated from tryptophan pyrolysate.[1] The structure of Trp-P-1 was determined by X-ray crystallography to be 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (**1**), and that of Trp-P-2 was deduced to be 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (**2**), mainly by spectral comparison with Trp-P-1.[1] We have now synthesized these compounds in order to confirm their structures and to obtain sufficient material for biological tests.[2] Positions 1 and 4 of the compounds seem to be important in relation to the mutagenic activity, since the mutagenic activity of Trp-P-1, in which the 4-hydrogen of Trp-P-2 is replaced by a methyl group, is lower than that of Trp-P-2. In order to examine the substitutent effect at positions 1 and 4 on mutagenic activity, we also synthesized alkyl and aryl derivatives of Trp-P-1 and Trp-P-2 at positions 1 and 4.

The chemistry of γ-carboline (5*H*-pyrido[4,3-*b*]indole), the basic structure of the two mutagenic principles, is little known compared to that of β-carboline, a regioisomer of the nitrogen atom, though synthetic methods for γ-carboline are known, *i.e.*, the Glaebe–Ullmann method[3] starting from *o*-phenylenediamine and chloropyridine, and Dalton's method[4] starting from 3-formylindole. It seems to be difficult to introduce an amino group at position 3 of γ-carboline itself. For example, direct amination at position 3 of γ-carboline under the conditions of the Chichibabine reaction was unsuccessful.

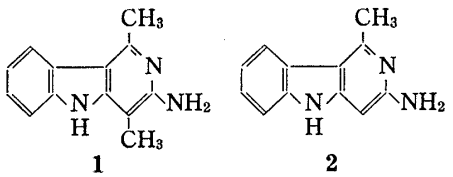


Chart 1

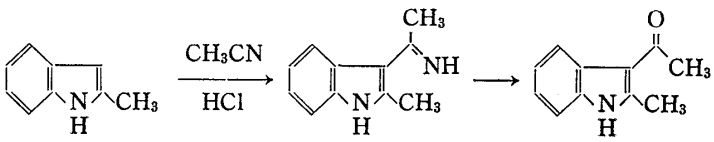


Chart 2

Seka reported that treatment of 2-methylindole (**3**) with acetonitrile in the presence of hydrogen chloride gave 3-acetyl-2-methylindole (**5**).[5] The reaction is believed to proceed *via* an imine intermediate (**4**), which is hydrolyzed to **5**. If the 2-methyl group is replaced by a cyanomethyl group, intramolecular nucleophilic attack of the imine nitrogen on the cyano carbon should give Trp-P-2.

2-Cyanomethylindole (**8**) was photochemically prepared from a quinoline Reissert compound, 2-cyano-N-ethoxycarbonyl-1,2-dihydroquinoline (**6**), according to Ikeda *et al.*[6] Reaction of 2-cyanomethylindole (**8**) with acetonitrile in the presence of hydrogen chloride gave only a trace of the expected compound, but upon condensation with aluminum chloride as a catalyst in acetonitrile, Trp-P-2 was obtained as expected. The compound was identical with Trp-P-2 isolated from the pyrolysate as judged by comparison of the spectral data and determination of the melting point of the acetate salt. The yield was 20—35% after purification.

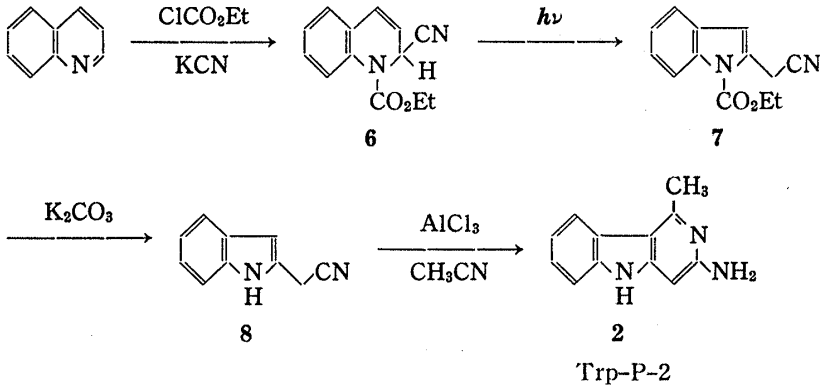


Chart 3

Trp-P-1 was similarly prepared from 2-(1-cyanoethyl)indole (**9**), which was obtained by treating 2-cyanomethyl-N-ethoxycarbonylindole (**7**) with lithium diisopropylamide (LDA)–methyl iodide followed by hydrolysis. The compound was identical with Trp-P-1 as judged by comparison of the spectral data and determination of the melting point of the acetate (Chart 4).

Derivatives having an alkyl (or phenyl) group at position 1 were similarly prepared by using alkylcyanides and 2-(1-cyanoalkyl)indoles (Chart 5).

Studies on the effect of a methyl group on the biological activity required the synthesis of 1-unsubstituted compounds. Since the above reaction for this purpose requires hydrogen cyanide, a stepwise pathway was adopted. Application of the Vilsmeier reaction[7] to 2-cyanoethylindole (**9**) gave 3-formylindole in 80% yield. Treatment of the compound with NH_3 in methanol gave 3-amino-4-methylpyrido[4,3-*b*]indole (**18**) in 30% yield through an imine intermediate[8] (Chart 6).

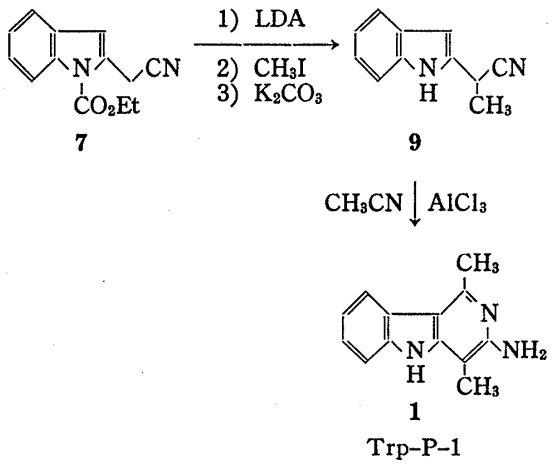


Chart 4

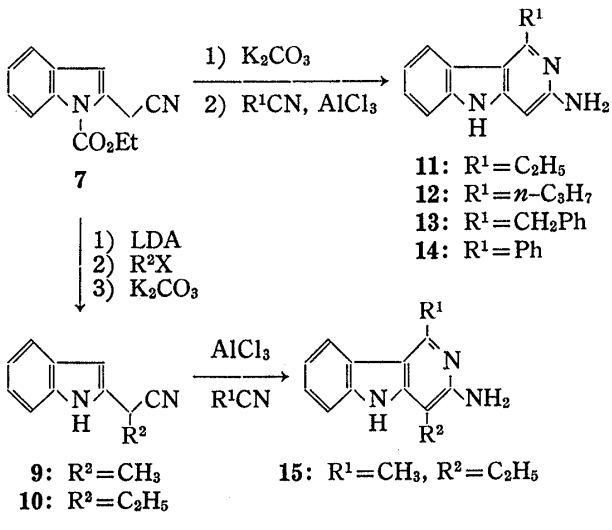
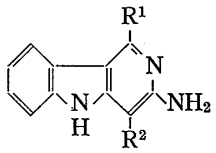


Chart 5

TABLE I.



No.	R^1	R^2	Yield (%)	Method	mp (°C)	Formula	Analysis (%) Calcd (Found) C	H	N	IR ν_{max}^{KBr} cm^{-1}	MS *m/e*, (M^+)	NMR (solvent) δ, ppm	UV λ_{max}^{MeOH} nm (log ε)
23[b]	H	H	15	C	266—267[b]	$C_{11}H_{10}N_3Cl$[b]	60.14 (59.83	4.54 4.49	19.13 18.77)	3360, 3280, 3180, 1665, 1625, 1458, 1247, 748	183	(D_2O–d_6-DMSO); 6.10 (1H, s), 7.00—7.40 (3H, m), 7.50 (1H, dd, J=8 Hz), 7.77 (1H, s)	(acidic); 238 (3.82), 262 (4.12), 290—340 (3.22), (alkaline); 262 (4.17), 268 (4.28), 290—350 (3.08)
2[a]	CH_3	H	24	A	250—260[a]	$C_{14}H_{15}N_3O_2$[a]	=Trp-P-2			3050, 3020, 1670, 1655, 1615, 1540, 1462, 1403, 1340, 1268, 1205, 842, 734	197	(d_6-DMSO); 1.88 (3H, s), 2.68 (3H, s) 6.23 (1H, s), 7.00—7.35 (3H, m), 7.80 (1H, d, J=8 Hz)	(acidic); 224 (4.03), 245 (sh), 266 (4.64), 269 (4.68), 300—330 (broad, 3.4—3.6), (alkaline); 243 (4.46), 263 (4.51), 300—330 (broad, 3.6—3.7)
11[a]	C_2H_5	H	27	A	236—237[a]	$C_{15}H_{17}N_3O_2$[a]	66.42 (66.18	6.27 6.24	15.50 15.75)	3320, 3070, 2600, 1650, 1570, 750	211	(CD_3OD); 1.42 (3H, t, J=8 Hz), 1.92 (3H, s), 3.23 (2H, q, J=8 Hz), 6.45 (1H, s), 7.1—7.4 (3H, m), 7.85 (1H, d, J=8 Hz)	(acidic); 210 (4.63), 245 (sh), 268 (5.14), 300—325 (broad, 4.05), (alkaline); 2.04 (4.95), 242 (4.08), 262 (4.95), 295—335 (4.15)
12[a]	n-C_3H_7	H	29	A	217—218[a]	$C_{16}H_{19}N_3O_2$[a]	67.37 (67.61	6.67 6.73	14.74 14.58)	3060, 2950, 1672, 1650, 1620, 1545, 1405, 745, 732	225	(C_5D_5N); 1.12 (3H, t, J=8 Hz), 2.00—2.30 (2H, m), 2.2 (3H, s), 3.45 (2H, t, J=Hz), 6.70 (1H, s), 12.20 (1H, br. s)	(acidic); 210 (5.50), 265 (sh), 270 (5.9), 295—325 (3.92), (alkaline); 242 (4.68), 262 (4.82), 290—335 (4.63)
13[a]	CH_2Ph	H	9	A	249[a]	$C_{20}H_{19}N_3O_2$[a]	72.07 (71.68	5.70 5.67	12.61 12.46)	3400, 3060, 1673, 1620, 1545, 740, 700	273	(CD_3OD); 1.92 (3H, s), 4.55 (2H, s), 6.52 (1H, s), 7.00—7.40 (8H, m), 7.80 (1H, d, J=8 Hz)	(acidic); 242 (sh), 247 (sh), 252 (sh), 262 (sh), 268 (4.66), 290—325 (3.91), (alkaline); 242 (sh), 247 (sh), 252 (sh), 262 (4.60), 290—330 (4.04)
14[a]	Ph	H	10	A	246—247[a]	$C_{19}H_{17}N_3O_2$[a]	71.47 (71.09	5.33 5.48	13.16 12.97)	3356, 3056, 1655, 1512, 1750, 715	259	(CD_3OD); 1.92 (3H, s), 6.55 (1H, s), 6.80—7.70 (9H, m)	(acidic); 220 (4.29), 242 (sh), 252 (sh), 270 (4.47), 295 (3.68), (alkaline), 264 (4.45), 298 (3.99), 305—335 (4.03)
18[a]	H	CH_3	30	B	295	$C_{14}H_{15}N_3O_2$[a]	73.07 (72.68	5.58 5.56	21.32 21.13)	3360, 3120, 1665, 1635, 1245, 750	197	(CD_3OD); 2.00 (3H, s), 2.25 (3H, s), 7.00—7.40 ((3H, m), 7.8 (1H, d, J=8 Hz), 8.28 (1H, s)	(acidic); 262 (4.60), 267 (4.69), 290—320 (4.75), (alkaline); 242 (sh) 258 (4.40) 290—330 (3.88)
1[a]	CH_3	CH_3	23	A	250	$C_{15}H_{17}N_3O_2$	=Trp-P-1			3350, 3100, 1650, 1560, 1400, 750, 740	211	(C_5D_5N); 2.05 (3H, s), 2.42 (3H, s), 2.90 (3H, s)	(acidic); 262 (4.73), 290—330 (4.19), (alkaline); 242 (4.59), 258 (4.62), 212 (4.09), 225 (4.06)
19[a]	H	C_2H_5	88	B	243[a]	$C_{13}H_{13}N_3$[c]	73.93 (73.73	6.16 6.25	19.90 19.84)	3320, 3080, 1665, 1560, 1400, 750, 740	211	(CD_3OD); 1.20 (3H, t, J=8 Hz), 1.90 (3H, s), 2.75 (2H, q, J=8 Hz), 7.00—7.30 (3H, m), 7.80 (1H, d, J=8 Hz), 8.26 (1H, s)	(acidic); 262 (4.61), 267 (4.67), 290—320 (3.87), (alkaline); 242 (4.41), 261 (4.57), 290—335 (3.87)
15[a]	CH_3	C_2H_5	12	A	257	$C_{16}H_{19}N_3O_2$[a]	67.37 (67.63	6.68 6.78	14.74 14.84)	3340, 3080, 1652, 1550, 750	225	(CD_3OD); 1.24 (3H, t, J=8 Hz), 1.98 (3H, s), 2.82 (2H, q, J=8 Hz), 2.88 (3H, s), 7.10—7.40 (3H, m), 7.85 (1H, d, J=8 Hz)	(acidic); 222 (sh), 262 (4.83), 265 (4.83), 295—325 (4.09), (alkaline); 243 (sh), 257 (4.49), 290—330 (3.82)

a) Acetate. *b*) Hydrochloride. *c*) Free.

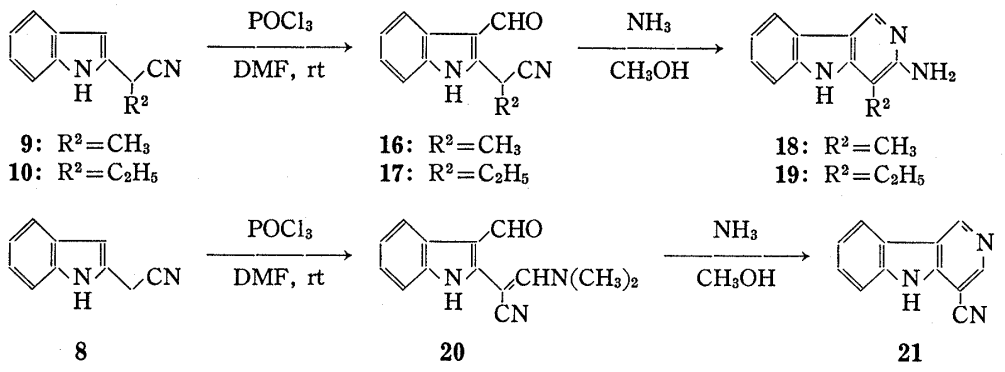


Chart 6

When the Vilsmeier reaction of 2-cyanomethylindole (**8**) was performed under similar conditions at room temperatures, the product obtained was a dimethylamino derivative (**20**) whose structure was deduced from the elemental analysis ($C_{14}H_{13}N_3O$) and mass (MS) and nuclear magnetic resonance (NMR) spectra. Treatment of the dimethylamino derivative (**20**) with NH_3 in methanol gave 4-cyano-γ-carboline (**21**). When the Vilsmeier reaction was performed at low temperature and stopped before the complete disappearance of the starting material, the desired product (**22**) was obtained as the major product. The subsequent ring closure step also encountered a difficulty. When 2-cyano-3-formylindole (**22**) was refluxed in methanol saturated with NH_3, the yield of the unsubstituted 3-amino-γ-carboline (**23**) was negligible. However, the presence of a drop of trifluoroacetic acid and the divided addition of NH_3 were helpful, though the yield was improved only to 15%. The physical data of the derivatives thus obtained are shown in Table I.

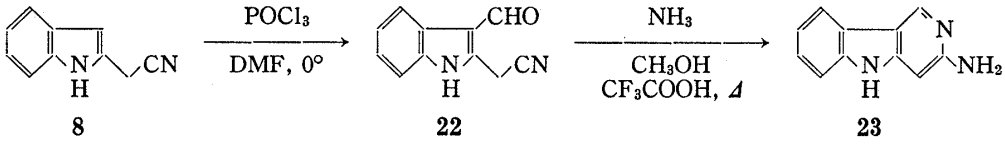


Chart 7

The isolated yields are not very good, but the small number of steps required for the synthesis of 3-amino-γ-carbolines is very advantageous. The mutagenic activity of these compounds will be described elsewhere.[9] The presence of a methyl group at position 1 enhances the activity, while an alkyl group at position 3 decreases it. The activity of a compound substituted by one or two bulky groups is much lower.

Experimental[10]

2-Cyanomethylindole (8)——The compound was prepared from 2-cyano-N-ethoxycarbonyl-1,2-dihydroquinoline (Reissert compound) (**6**) according to Ikeda *et al.*, mp 99° (lit. 101°).[6] As reported, the reaction temperature should be kept at near 0°.

2-(1-Cyanoethyl)-N-ethoxycarbonylindole——2-(Cyanomethyl)-N-ethoxycarbonylindole (**7**) (800 mg, 3.5 mmol) was added to a tetrahydrofuran (THF) solution (30 ml) of LDA (5.26 mmol) in THF (40 ml) at −78°. After 5 min, an excess of methyl iodide was added, and the mixture was stirred for 5 min at the same temperature, then allowed to come to room temperature. Dilute hydrochloric acid was added to the solution, then the mixture was extracted with methylene chloride. The organic layer was separated, washed with water, and dried over sodium sulfate, and the solvent was removed. The resulting solid was recrystallized from benzene–*n*-hexane to give 760 mg of the title compound, 90%, mp 78°. IR ν_{max}^{KBr} cm^{-1}: 3400, 2980, 2240, 1740, 1380, 1335, 767, 750. MS *m/e*: 242 (M^+). NMR (in $CDCl_3$): 1.52 (3H, t, J=7 Hz), 1.75 (3H, d, J=7 Hz), 4.77 (1H, q, J=7 Hz), 6.75 (1H, s), 7.10—7.50 (3H, m), 8.00 (1H, dd, J=7 Hz). *Anal.* Calcd for $C_{14}H_{14}N_2O_2$: C, 69.13; H, 6.17; N, 11.52. Found: C, 69.19; H, 5.82; N, 11.29.

2-(1-Cyanoethyl)indole (9)——2-(1-Cyanoethyl)-N-ethoxycarbonylindole (600 mg, 2.48 mmol) and potassium carbonate (600 mg) were suspended in methanol, and the mixture was stirred for 55 min at room

temperature. After being acidified with dilute hydrochloric acid, the mixture was extracted with methylene chloride. The extract was washed with water and dried over sodium sulfate, and the solvent was removed. The resulting solid was recrystallized from benzene–*n*-hexane to give 353 mg of the title compound, 84%, mp 88°. IR ν_{max}^{KBr} cm^{-1}: 3150, 2240, 1635, 1585, 1465, 1340, 765, 750. MS *m/e*: 170 (M$^+$). NMR (in $CDCl_3$): 1.74 (3H, d, J=8 Hz), 5.08 (1H, q, J=8 Hz), 7.00—7.60 (3H, m), 7.78 (1H, dd, J=7 Hz), 8.51 (1H, br s). *Anal.* Calcd for $C_{11}H_{10}N_2$: C, 77.64; H, 5.88; N, 16.47. Found: C, 77.47; H, 5.97; N, 16.35.

2-(1-Cyanopropyl)-N-ethoxycarbonylindole——This compound was prepared by a procedure similar to that described for the above methylation, 55%, mp 78—79°. IR ν_{max}^{KBr} cm^{-1}: 3400, 2950, 2240, 1740, 1595, 1565, 1464, 1387, 1330, 766, 756. MS *m/e*: 256 (M$^+$). NMR (in $CDCl_3$): 1.20 (3H, t, J=8 Hz), 1.52 (3H, t, J=6 Hz), 2.02 (2H, m), 4.55 (1H, q, J=6 Hz), 4.77 (1H, q, J=5 Hz), 7.26—7.60 (3H, m), 8.08 (1H, dd, J=8 Hz). *Anal.* Calcd for $C_{15}H_{16}N_2O_2$: C, 70.31; H, 6.25; N, 10.94. Found: C, 70.24; H, 6.26; N, 10.85.

2-(1-Cyanopropyl)indole (10)——The compound was prepared by a procedure similar to that described for 2-(1-cyanoethyl)indole (**9**), 90%, mp 62°. IR ν_{max}^{KBr} cm^{-1}: 3320, 2960, 2250, 1460, 1430, 800, 750. MS *m/e*: 184 (M$^+$). NMR (in $CDCl_3$): 1.11 (3H, t, J=8 Hz), 2.03 (2H, quintet), 3.95 (1H, t, J=6 Hz), 6.46 (1H, s), 7.00—7.40 (3H, m), 7.55 (1H, dd, J=5 Hz), 8.35 (1H, b, s). *Anal.* Calcd for $C_{12}H_{12}N_2$: C, 78.26; H, 6.25; N, 15.22. Found: C, 78.09; H, 6.59; N, 15.05.

2-Cyanomethyl-3-formylindole (22)——Phosphorus oxychloride (300 mg, 1.95 mmol) was added to dimethylformamide (DMF) (0.5 ml) in an ice bath. After 5 min, a DMF solution of 2-cyanomethylindole (**8**) (300 mg, 1.92 mmol) was added to the solution, and the reaction mixture was stirred for 1 hr at room temperature. The mixture was then poured into ice-water, and basified with aqueous sodium hydroxide solution. The resulting precipitates were collected, and recrystallized from methanol to give 105 mg of 2-(1-cyano-2-N,N-dimethylamino)vinyl-3-formylindole (**20**), 23%, mp 248—250°. IR ν_{max}^{KBr} cm^{-1}: 3400, 3160, 2200, 1630, 1572, 1460, 1400, 1370, 1172, 750. MS *m/e*: 239 (M$^+$). NMR (in DMSO-d_6): 7.20—7.40 (3H, m), 7.62 (1H, s), 8.05 (1H, m), 7.60 (1H, s), 11.80 (1H, br s), (in DMSO-d_6–D_2O): 3.30 (6H, s), 7.20—7.40 (3H, m), 7.60 (1H, m), 8.10 (1H, m), 10.06 (1H, s). *Anal.* Calcd for $C_{14}H_{13}N_3O$: C, 70.29; H, 5.44; N, 17.57. Found: C, 70.44; H, 5.47; N, 17.50.

Phosphorus oxychloride (321 mg, 2.09 mmol) was added to DMF (0.5 ml) in an ice bath. After 5 min, the solution was added dropwise to a DMF solution (1 ml) of 2-cyanomethylindole (**8**) (300 mg, 1.92 mmol) under stirring in an ice bath, and the mixture was poured into ice-water after 15 min. The mixture was basified with aqueous sodium hydroxide solution, warmed up on a steam bath for a few minutes, and extracted with ethyl acetate. The organic layer was washed with water, then dried over sodium sulfate and the solvent was evaporated off. The resulting solid was chromatographed over silica gel to give crude crystals, which were recrystallized from methanol–ethyl acetate to give 171 mg of the title compound, 48%, mp 185—187°. IR ν_{max}^{KBr} cm^{-1}: 3400, 3100, 2230, 1630, 1582, 1466, 1390, 1238, 752. MS *m/e*: 184 (M$^+$). NMR (in CD_3OD): 7.18—7.50 (6H, m), 8.10 (1H, m), 10.13 (1H, s). *Anal.* Calcd for $C_{11}H_8N_2O$: C, 71.74; H, 4.35; N, 15.22. Found: C, 71.73; H, 4.35; N, 15.02.

2-(1-Cyanoethyl)-3-formylindole (16)——Phosphorous oxychloride (270 mg, 1.76 mmol) was added to 0.5 ml of DMF in an ice bath. After 5 min, a DMF solution of 2-(1-cyanoethyl)indole (**9**) (250 mg, 1.47 mmol) was added to the solution, and the reaction mixture was stirred for 1 hr at room temperature. The mixture was poured into ice-water, then the whole was basified with aqueous sodium hydroxide solution, and extracted with ethyl acetate. The organic layer was washed with water, then dried over sodium sulfate, and the solvent was evaporated off. The resulting solid was recrystallized from chloroform–acetone to give 231 mg of 2-(1-cyanoethyl)-3-formylindole (**16**), 80%, mp 205°. IR ν_{max}^{KBr} cm^{-1}: 3400, 3150, 2240, 1635, 1585, 1465, 1390, 765, 750. MS *m/e*: 198 (M$^+$). NMR (in CD_3OD): 1.80 (3H, t, J=8 Hz), 5.08 (2H, q, J=8 Hz), 7.20—7.60 (3H, m), 8.20 (1H, dd, J=8 Hz), 10.12 (1H, s). *Anal.* Calcd for $C_{12}H_{10}N_2O$: C, 72.72; H, 5.05; N, 14.14. Found: C, 72.43; H, 5.17; N, 13.99.

2-(1-Cyanopropyl)-3-formylindole (17)——The compound was prepared according to the procedure described for the formylation of 2-(1-cyanoethyl)indole (**9**). 47%, mp 182°. IR ν_{max}^{KBr} cm^{-1}: 3400, 3160, 2240, 1632, 1590, 1465, 1390, 765. MS *m/e*: 212 (M$^+$). NMR (in CD_3OD): 1.15 (3H, t, J=8 Hz), 2.13 (2H, quintet), 4.86 (1H, t, J=6 Hz), 7.20—7.50 (4H, m), 8.08 (1H, dd, J=6 Hz), 10.30 (1H, s). *Anal.* Calcd for $C_{13}H_{12}N_2O$: C, 73.58; H, 5.66; N, 13.21. Found: C, 73.49; H, 5.70; N, 12.96.

4-Cyano-γ-carboline (21)——2-(1-Cyano-2-N,N-dimethylamino)vinyl-3-formylindole (**20**) (100 mg, 0.518 mmol) was dissolved in 20 ml of methanol saturated with NH_3, and the mixture was allowed to stand at room temperature overnight. The solvent was evaporated off, and the residue was recrystallized from methyl acetate containing a drop of acetic acid to give 47 mg of 4-cyano-γ-carboline (**21**), 44%, mp 290—300°. IR ν_{max}^{KBr} cm^{-1}: 3400, 3050—2680, 2240, 1612, 1593, 1457, 1330, 1280, 1255, 1143, 1118, 1085, 903, 777, 755. MS *m/e*: 193 (M$^+$). NMR (in DMSO-d_6): 7.30—7.70 (3H, m), 8.35 (1H, d, J=8 Hz), 8.87 (1H, s), 9.56 (1H, s), 12.76 (1H, br s). *Anal.* Calcd for $C_{12}H_7N_3$: C, 74.61; H, 3.63; N, 21.76. Found: C, 74.26; H, 3.65; N, 21.79.

General Proccdure for the Preparation of 3-Amino-γ-carboline Derivatives——Method A: 2-Cyanoalkylindole was heated with aluminum chloride (10—15 eq.) in alkyl (phenyl) cyanide (50—100 eq.) at 100—120° for 12 hr. Alkyl (phenyl) cyanide was removed *in vacuo*, water was added, neutral and acidic products were removed by ether extraction, and the basic fraction was extracted with ethyl acetate after basification by the addition of solid K_2CO_3. The organic layer was dried over K_2CO_3, and the solvent was

removed. The resulting crude product was dissolved in a small amount of methanol and a few milliliters of ethyl acetate, and a drop of acetic acid was added. The resulting crystalline precipitates were collected and recrystallized from methanol–ethyl acetate to give the acetate.

Method B: Ammonia gas was passed through a solution of 2-(1-cyanoalkyl)-3-formylindole (200 mg) in 50 ml of methanol for about 10 min, then the solution was allowed to stand at room temperature overnight. The solvent was evaporated off, ethylacetate and a drop of acetic acid were added, and the resulting crystalline precipitates were recrystallized from methanol–ethyl acetate to give the acetate salt.

Method C: 2-Cyanomethyl-3-formylindole (1800 mg) was dissolved in 20 ml of methanol saturated with NH_3, and a drop of trifluoroacetic acid was added. The solution was refluxed with addition of methanol saturated with NH_3 about every 30 min for 8 hr. The total amount of NH_3-saturated methanol added was about 10 ml. The solvent was evaporated off, and the residue was chromatographed over silica gel (ethyl acetate–ethanol = 4 : 1) to give 15 mg of 2-amino-γ-carboline, which was purified as the hydrochloride by recrystallization from methanol–ethyl acetate containing a drop of hydrochloric acid.

References and Notes

1) T. Sugimura, T. Kawachi, M. Nagao, T. Yahagi, Y. Seino, T. Okamoto, K. Shudo, T. Kosuge, K. Tsuji, K. Wakabayashi, Y. Iitaka, and A. Itai, *Proc. Jpn. Acad.*, **53**, 58 (1977).
2) K. Takeda, T. Ohta, K. Shudo, T. Okamoto, K. Tsuji, and T. Kosuge, *Chem. Pharm. Bull.*, **25**, 2145 (1977).
3) W. Lawson, W.H. Perkin, and R. Robinson, *J. Chem. Soc.*, **1924**, 626.
4) L.K. Dalton, S. Demerac, and T. Teitei, *Aust. J. Chem.*, **22**, 185 (1969).
5) R. Seka, *Chem. Ber.*, **56**, 2058 (1925).
6) M. Ikeda, S. Matsugashita, and T. Tamura, *J. Chem. Soc. Perkin. I*, **1976**, 2587. We acknowledge to Dr. Ikeda for teaching the details of the reaction.
7) L.F. Fieser and M. Fieser, "Reagents for Organic Synthesis," Vol. I, John Wiley and Sons, Inc., New York, 1967, p. 284.
8) A similar method has been reported by H. Akimoto, A. Kawai, H. Nomura, M. Nagao, T. Kawachi, and T. Sugimura, *Chem. Lett.*, **1977**, 1061.
9) M. Nagao, Y. Takahashi, T. Yahagi, T. Sugimura, K. Takeda, K. Shudo, and T. Okamoto, *Carcinogenesis*, **1**, 451 (1980).
10) All melting points were determined in a Yanagimoto melting point apparatus and are uncorrected; the UV spectra were taken on a Shimadzu UV-200S machine; IR spectra were recorded with a JASCO DS-402G or a JASCO IRA-I grating infrared spectrometer; the mass spectra were measured with a JEOL JMS SG-2 machine. The NMR spectra were measured with a JEOL JMM-PS-100 spectrometer, and the chemical shifts are quoted as ppm downfield from tetramethylsilane used as an internal standard. Abbreviations: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m) and broad (br).