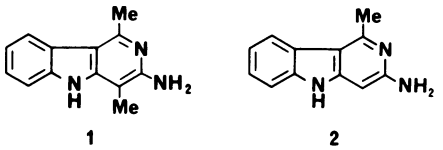
Synthesis of 3-Amino-5*H*-pyrido[4,3-*b*]indoles, Carcinogenic γ-Carbolines

Hiroshi AKIMOTO,* Akiyoshi KAWAI, and Hiroaki NOMURA
Central Research Division, Takeda Chemical Ind., Ltd., Juso, Yodogawa-ku, Osaka 532
(Received May 23, 1984)

The carcinogenic γ-carbolines 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (**1**) and 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (**2**) were synthesized efficiently by the following procedures. The key method involved the acid-catalyzed cyclization of 2-acetamido-3-(2-indolyl)alkanoic acids to 1,2-dihydro-γ-carbolines. This was followed by dehydrogenation to the γ-carbolinecarboxylates and conversion of the ester group to the carboxyl and finally to the amino one by Curtius rearrangement. Alternative methods involved the thermolysis of 4-(1-benzotriazolyl)-3,6-dimethyl-2-pyridinamine to synthesize **1** and the condensation of 3-acetylindole-2- acetonitrile with ammonia to synthesize **2**. The structures of γ-carbolines **1** and **2** were unambiguously established by comparing samples of each synthesized by the two different routes. A selective and one-step synthesis of ethyl 2-acetamido-3-(2-indolyl)alkanoates was newly exploited starting from diethyl acetamidomalonate and quaternary ammonium salts of 2-[1-(dimethylamino)alkyl]indoles.

In a preliminary communication,[1] we reported on the total synthesis of the carcinogenic γ-carboline derivatives, 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (named Trp-P-1, **1**) and 3-amino-1-methyl-5*H*-pyrido-[4,3-*b*]indole (named Trp-P-2, **2**).[2] This study was initiated in order to obtain sufficient amounts of samples to allow extensive biological studies, including carcinogenesis tests of **1** and **2**,[3] and has contributed to the development of new routes for the large-scale preparation of these γ-carbolines.

Massive research on environmental mutagenes and carcinogens over the last decade has led to the identification of many of these substances which occur in food, water and air.[3a] One series of such environmental agents, heterocyclic amines formed by pyrolysis of various amino acids and proteins, was identified as being mutagenic and has been intensively investigated by Sugimura and his co-workers.[3] The first isolated and highly potent mutagens among these compounds are the γ-carboline derivatives Trp-P-1 (**1**) and Trp-P-2 (**2**) isolated from protein pyrolysates[4] and more favorably tryptophan pyrolysates.[5] The structures of these carbolines have been determined from spectral data and, in the case of **1**, also from X-ray analysis.[5] These mutagens were suspected to be carcinogenic, based on their ability to induce morphological transformation of cultured mammalian embryo cells.[6,7] However, sufficient quantities of these compounds were needed for carcinogenicity tests in animals. Their preparation by tryptophan pyrolysis gave extremely low yields.[5] Thus, we developed new procedures for the large-scale synthesis of **1** and **2**.[1]



1 2

Fig. 1. Structures of Trp-P-1 (**1**) and Trp-P-2 (**2**).

Results and Discussion

Only a few reports have appeared regarding the synthesis of γ-carboline and its derivatives. They include 1) cyclization to the γ-carboline pyrrole ring by thermal decomposition of azidobenzenes or benzotriazoles containing a pyridine ring (Graebe-Ullmann type reaction),[8] 2) building the γ-carboline pyridine ring by cyclization of azomethines derived from 3-formylindole and amino acetal,[9] and 3) cyclization to 1,2,3,4-tetrahydro-γ-carbolines by thermal reaction of 4-piperidone phenylhydrazones (Fischer indole synthesis).[10] We tried three approaches to the synthesis of γ-carbolines **1** and **2**: A) Thermolysis of 4-(1-benzotriazolyl)-3,6-dimethyl-2-pyridinamine (**6**) in polyphosphoric acid, B) ring closure from 2-acetamido-3-(2-indolyl)alkanoic acids (**14a,b**) to 1,2-dihydro-γ-carbolines, followed by dehydrogenation, and C) building the γ-carboline pyridine ring by cyclization of the 2,3-disubstituted indole **19** with ammonia.

*Synthesis of 3-Amino-1,4-dimethyl-5*H*-pyrido[4,3-b]indole (1) by Route A.* A key intermediate for the synthesis of **1**, 3,6-dimethyl-4-nitro-2-pyridinecarboxylic acid (**3**), was prepared from 2,5-dimethylpyridine[11] and the nitro group was readily replaced by *o*-phenylenediamine to yield the *N*-phenyl-4-pyridinamine compound (**4**). Upon treatment with nitrous acid, this was converted to the 4-(benzotriazolyl)-pyridine derivative **5** in excellent yield. The carboxyl group of **5** was easily converted to the amino group by Curtius rearrangement using diphenylphosphoryl azide(DPPA)[12] to yield the 2-amino derivative **6**. Heating a sample of **6** in polyphosphoric acid gave the desired product **1**, which was isolated as colorless prisms of the acetic acid salt in moderate yield (46%) (Chart 1). Elemental analysis and mass spectroscopy supported the molecular formula of $C_{13}H_{13}N_3 \cdot CH_3COOH$. The UV spectrum of **1** showed several absorption maxima at 226,

244, 265, 267, 307, and 319 nm in an acidic solution, the pattern being comparable to that of γ-carboline.[13] The IR spectrum showed absorption bands at 1650 (CH_3COOH salt) and 1610 (γ-carboline) cm^{-1}. The ^1H NMR spectrum had signals for three methyl groups, acetic acid and those at the C-1 and C-4 positions at δ 2.08 (3H, s), 2.36 and 2.96 (each 3H, s), respectively, together with the four aromatic protons at δ 7.20—7.42 (3H, m) and 8.03 (1H, m).

Synthesis of 3-Amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (1) by Route B. 2-Acetamido-3-(2-indolyl)-butyric acid (**14a**) was synthesized by subjecting the intermediary 2-[1-(dimethylamino)ethyl]indole (**11**), which was prepared from 2-indolecarboxylic acid (**7**) *via* 2-acetylindole (**8**), the dialkylamine **9** and *N,N*-dialkylformamide **10**, to malonic ester synthesis.[14] Quaternarization of **11** and subsequent reaction with diethyl acetamidomalonate gave 2-acetamido-3-(2-indolyl)butyrate (**13a**) in 81% yield from **11**. This ester was subjected to alkaline hydrolysis to yield 2-acetamido-3-(2-indolyl)butyric acid (**14a**) in quantitative yield. Treatment of **14a** with thionyl chloride in ethanol resulted in intramolecular cyclization and a simultaneous esterification to yield the dihydro-γ-carboline ester **15a**.[15] Dehydrogenation with sulfur quantitatively gave γ-carboline-3-carboxylate **16a**, and subsequent alkaline hydrolysis provided the γ-carbolinecarboxylic acid **17a** in high yield (68% yield from **13a**). The final step was Curtius rearrangement of **17a** using DPPA which gave **1** in 41% yield (Chart 2). Elemental analysis, UV, IR, and mass spectra gave results identical to those of the product obtained by route A.

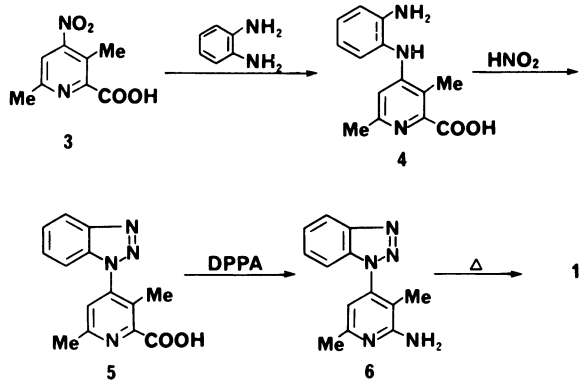


Chart 1. Synthesis of Trp-P-1 by Route A.

Synthesis of 3-Amino-1-methyl-5H-pyrido[4,3-b]indole (2) by Route B. Since route B was very efficient for the synthesis of **1**, we applied it to the synthesis of **2**. As described above, the trimethylammonium salt derived from 2-(dimethylaminomethyl)indole (**12**) was subjected to a malonic ester synthesis using diethyl acetamidomalonate, which yielded 2-acetamido-3-(2-indolyl)propionate (**13b**) in high yield (82%). This compound was cyclized to the dihydro-γ-carboline ester **15b** by treatment with thionyl chloride in ethanol. Dehydrogenation and alkaline hydrolysis, as in the synthesis of **17a** from **11**, gave γ-carbolinecarboxylic acid **17b** in good yield (77.8% from **13b**). Conversion of the carboxyl group to the amino function using DPPA yielded the desired γ-carboline **2** (Chart 2). The physical properties were determined with the crystalline acetic acid salt. The molecular for-

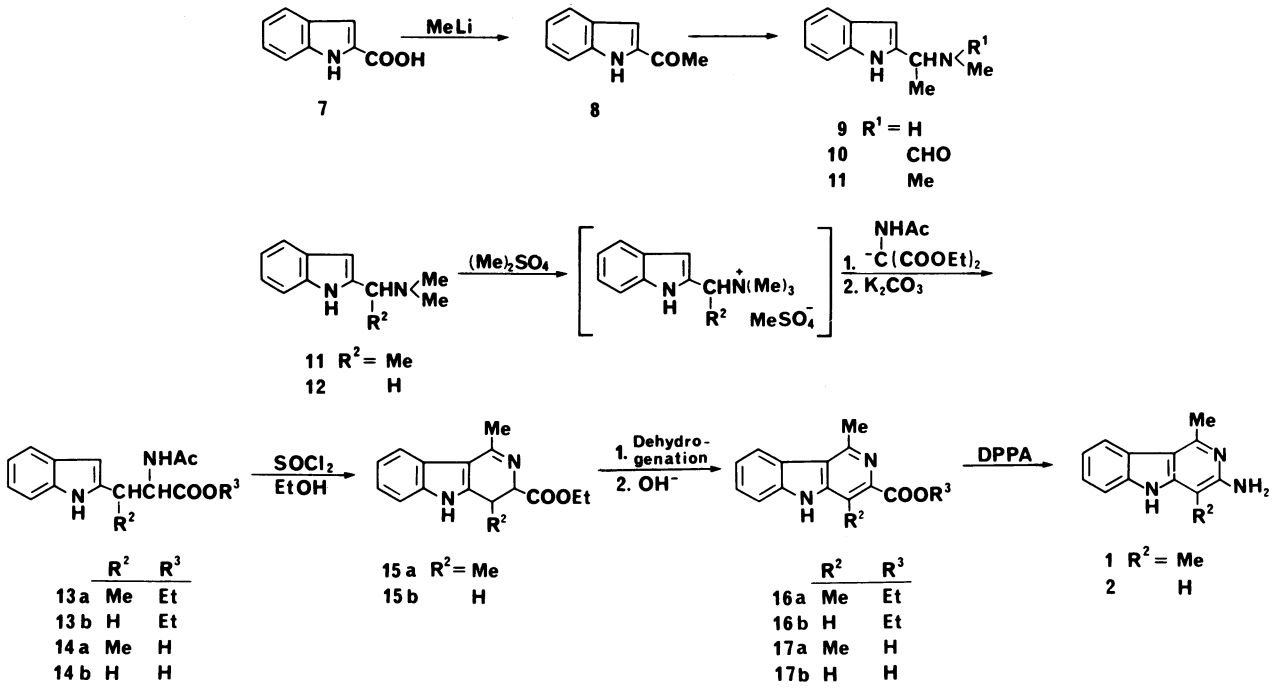


Chart 2. Synthesis of Trp-P-1 and Trp-P-2 by Route B.

mula agreed with $C_{12}H_{11}N_3 \cdot CH_3COOH$ on elemental analysis and mass spectroscopy. Its IR and UV spectra showed the absorption bands characteristic to γ-carboline and were very similar to those of **1** (the acetic acid salt). The structure was further supported by the ^1H NMR spectrum, which showed the signals at δ 1.93 (3H, s) due to acetic acid methyl protons, at δ 2.74 (3H, s) due to C-1 methyl protons, at δ 6.37 (1H, s) due to C-4 proton and at δ 7.07—7.53 (3H, m) and 7.97 (1H, d) due to four aromatic protons.

Synthesis of 3-Amino-1-methyl-5H-pyrido[4,3-b]indole (2) by Route C. In order to confirm the structure of **1**, the alternative, more short-cut method was exploited. Chart 3 shows the synthetic route of **2**, *via* indole-2-acetonitrile (**18**)[16] which was readily available from 2-(dimethylaminomethyl)indole (**12**). Compound **18** was converted to 2-acetylindole-2-acetonitrile (**19**) by the Vilsmeier-Haack reaction[17] with *N,N*-dimethylacetamide (DMA) and phosphoryl chloride. This key intermediate **19**, when treated with methanolic ammonia, underwent spontaneous condensation and aromatization to give the desired product **2** in 58% yield (Chart 3). The physical data regarding the product agreed with those of the sample obtained by route B.

Malonic Ester Synthesis Starting from 1-(2-Indolyl)alkylamines, 11 and 12. When quaternary ammonium salt derived from **11** and **12** were allowed to react with excess sodioacetamidomalonate in ethanol at room temperature, the corresponding monocarboxylates **13a,b** were obtained selectively and quantitatively without hydrolytic decarboxylation procedure (Table 1, runs 3 and 6). In each case, an equimolar quantity of diethyl carbonate was formed in the reaction mixture, offering some suggestion for the reaction mechanism.

In the present reaction, the composition and yields of the products were markedly influenced by changes in the reaction conditions, particularly, the molar ratio of 1-(2-indolyl)alkylamines **11** and **12** and sodioacetamidomalonate. In fact, an entirely different mode of reaction was encountered when the reaction was carried out using sodioacetamidomalonate in amounts less than equimolar to that of the quaternary salts derived from **11** and **12**. The reaction products were the indolylmethyl derivatives of 2-acetamidomalonates **20a,b** (Table 1, runs 1 and 4). When the sodiomalonate was used in slight excess of the equimolar quantity of **11**, the dihydropyrrolo[1,2-*a*]indole **21a** was produced selectively (Table 1, run 2).

These substituted carboxylates, **20a,b**, **21a** and **13a,b**, when treated with alkali, gave the identical 2-acetamidoalkanoic acids **14a,b** in quantitative yields. Thus, this tricyclic hetero system **21** is assumed to be a precursor which can be readily converted to **13a,b**. Several attempts to isolate the tricyclic compound **21b** were unsuccessful under a range of reaction conditions, presumably due to the higher lability of **21b**, because the less hindered ring-carbonyl is considered to be more susceptible to nucleophile attack than that of the sterically more crowed pyrrolidone **21a** during the reaction. These results suggest that, under conditions using excess sodioacetamidomalonate, the reaction proceeds from 1-(2-indolyl)alkylamines **11** and **12** to the monocarboxylates **13a,b** *via* **20** and **21** (Chart 4). An intramolecular attack by the indole anionic nitrogen of **20** upon one of the malonic ester carbonyls results in the formation of the tricyclic pyrrolidone **21**, which, on attack by ethoxide, affords the corresponding monocarboxylates **13a,b**, through C–C bond cleavage of the pyrrolidone ring and another attack by ethoxide ion on the resulting ethoxycarbonyl group of the presumed intermediate (Chart 4). Thus, the present malonic ester-type reaction opens an efficient one-step synthesis of the 3-(2-indolyl)alkanoates, extending a two-carbon chain at the methyl position of the 2-indolylmethyl group

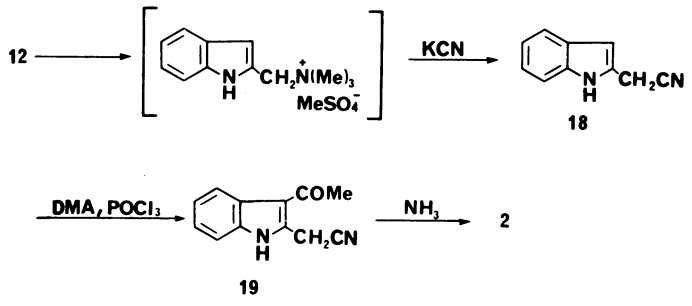


Chart 3. Synthesis of Trp-P-2 by Route C.

Table 1. Malonic ester synthesis starting from quaternary salts of **11** and **12**[a]

Run	Molar ratio: Sodiomalonate[b] / Quaternary salt of **11**[c]	Product ratio/%[d]: **20a**	**21a**	**13a**
1	0.98	82 (67)	16	2[e]
2	1.10	1	88 (71)	11
3	1.25	—	6	94 (81)[f]
	Sodiomalonate[b] / Quaternary salt of **12**[c]	**20b**	**21b**	**13b**
4	0.98	94 (76)	—	6[e]
5	1.10	42	—	58
6	1.25	3	—	97 (82)[f]

a) See text for reaction conditions. b) Calculated as sodium ethoxide which was employed in the reaction with the equimolar quantity of diethyl acetamidomalonate. c) Prepared from equimolar amounts of the corresponding amine **11** or **12** and dimethyl sulfate. d) For evaluation of the product ratios (%), each sample was analyzed by HPLC (Waters Associates; μ-Porasil, 3.9 mm × 30 cm, $CHCl_3$: AcOEt = 4 : 1; detection at 254 nm). Figures in parentheses express the isolated yields. e) Diethyl carbonate was not detected. f) A nearly quantitative yield of diethyl carbonate was detected by HPLC analysis.

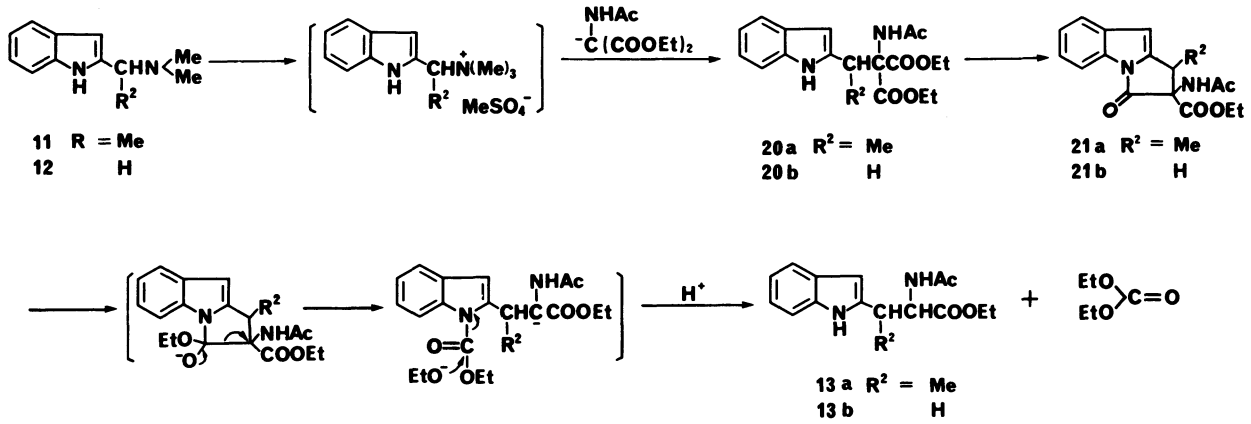


Chart 4. Reaction Mechanism of Monocarboxylic Ester Formation.

under mild reaction conditions.

Our new routes for synthesizing mutagenic γ-carbolines **1** and **2** are efficient and useful for preparing large quantities of these compounds. These methods enabled the synthesis of sufficient amounts of **1** and **2** for carginogenesis testing, including an almost one-year administration of **1** and **2** to mice, by Sugimura and his groups.[3,7] The results have proven that these γ-carbolines are carcinogenic.[3a,18]

Experimental

Melting points were measured on a Yanagimoto MP-S3 hot-plate apparatus and are uncorrected. Infrared spectra were recorded on a Hitachi 215 spectrometer. UV spectra were taken on a Hitachi EPS-3T spectrometer. Mass spectra were determined with a Hitachi RMU-6D spectrometer equipped with a direct-inlet system. ^{1}H NMR spectra were obtained using a Varian XL-100-12 instrument: Chemical shifts (δ) are expressed in ppm downfield from internal TMS. Preparative column chromatography was carried out on a Kieselgel 60 (E. Merck, Art. 7743).

3,6-Dimethyl-4-nitro-2-pyridinecarboxylic Acid (3). This compound was prepared from 2,5-dimethylpyridine according to a method[12] described in the synthesis of 6-ethyl-3-methyl-4-nitro-2-pyridinecarboxylic acid. Recrystallization from acetonitrile gave **3** as colorless needles: Mp 145—146 °C; IR (KBr) 3420, 3100, 1720, 1610, 1540, 1520, 1440, 1390, 1295, 1220, 1190, 1120, 960, 890, 800, and 780 cm^{-1}; ^{1}H NMR (CD_3OD) δ=2.46 (3H, s), 2.60 (3H, s), 7.67 (1H, s). Found: C, 48.99; H, 4.03; N, 14.38%. Calcd for $C_8H_8N_2O_4$: C, 48.98; H, 4.11; N, 14.28%.

3,6-Dimethyl-4-(2-aminophenylamino)-2-pyridinecarboxylic Acid (4). A solution of **3** (3.6 g) and *o*-phenylenediamine (5.4 g) in water (100 cm^3) was heated under reflux for 1 h, and evaporated *in vacuo*. The residue was charged on Amberlite XAD-II (500 cm^3) column which was then washed with water (1000 cm^3) and eluted with 20% aqueous MeOH. The combined fractions containing the desired product were evaporated *in vacuo* and gave a pale-yellow solid. Recrystallization from water provided colorless needles (4.4 g, 93%): Mp 241—242 °C; IR (KBr) 2700—2400, and 1600 cm^{-1}; ^{1}H NMR (CD_3OD) δ=2.03 (3H, s), 2.16 (3H, s), 5.80 (1H, s), 6.33—6.68 (3H, m). Found: C, 65.30; H, 5.79; N, 16.28%. Calcd for $C_{14}H_{15}N_3O_2$: C, 65.36; H, 5.88; N, 16.33%.

4-(1-Benzotriazolyl)-3,6-dimethyl-2-pyridinecarboxylic Acid (5). A 10% hydrochloric acid solution (30 cm^3) of **4** (2.0 g) was cooled in an ice bath and then sodium nitrite (1.1 g) in water (30 cm^3) was added. This mixture was left stainding at 0 °C for 2 h. The precipitated solid was collected, washed with 10% hydrochloric acid and dissolved in 2 mol dm^{-3} NaOH. The solution was filtered to remove any insoluble material and then hydrochloric acid was slowly added. Filtration and drying of the resulting crystalline precipitate gave the hydrochloride of **5** (2.25 g, 93%) as colorless needles: Mp 187—188 °C; IR (KBr) 2850—2300, and 1700 cm^{-1}; ^{1}H NMR (CD_3OD) δ=2.60 (3H, s), 2.93 (3H, s), 7.73 (3H, m), 8.13 (1H, m), 8.26 (1H, s). Found: C, 54.89; H, 4.36; N, 18.33; Cl, 11.69%. Calcd for $C_{14}H_{12}N_4O_2 \cdot HCl$: C, 55.18; H, 4.38; N, 18.39; Cl, 11.63%.

4-(1-Benzotriazolyl)-3,6-dimethyl-2-pyridinamine (6). A mixture of **5** (10 g), diphenylphosphoryl azide (DPPA; 10.8 g), and triethylamine (8.3 g) in *t*-BuOH (200 cm^3) was heated under reflux for 1.5 h and gave a residue when evaporated *in vacuo*. This was dissolved in ethyl acetate (200 cm^3), washed successively with saturated aqueous NaCl and saturated aqueous $NaHCO_3$, dried over $MgSO_4$ and evaporated. The resulting solid was dissolved in 25% HBr-acetic acid (140 cm^3) and the solution was kept standing at 0 °C for 30 min, then diethyl ether (140 cm^3) was added. When the mixed solution was left standing for another 1 h, a white precipitate separated. This was collected by filtration, washed with diethyl ether, and dried to give an amorphous powder (the hydrobromide of **6**). This material was suspended in 15% aqueous ammonia (140 cm^3) and extracted with ethyl acetate. The extract was washed with saturated aqueous NaCl, dried over $MgSO_4$, and evaporated *in vacuo*. Recrystallization of the residue from diethyl ether gave the free amino compound **6** (6.1 g, 78%) as pale yellow needles: Mp 139—140 °C; IR (KBr) 3320, 3200, 1620, and 1590 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ=1.83 (3H, s), 2.34 (3H, s), 4.80 (2H, bs), 6.54 (1H, s), 7.40 (3H, m), 8.06 (1H, m). Found: C, 65.28; H, 5.34; N, 29.34%. Calcd for $C_{13}H_{13}N_5$: C, 65.26; H, 5.48; N, 29.27%.

2-Acetylindole (8). A solution of 2-indolecarboxylic acid (**7**; 14.2 g, Fluka AG, Switzerland) and methyl lithium, prepared from lithium (7.2 g) and methyl iodide (29 g), in

diethyl ether (300 cm^3) was refluxed for 1 h. The reaction mixture was poured into ice water and extracted with diethyl ether. The extract was washed with saturated aqueous NaCl, dried over $MgSO_4$ and evaporated to dryness *in vacuo*. Recrystallization of the residue from ethyl acetate gave 11.7 g (84%) of **8** as colorless needles: Mp 154—155 °C; IR (KBr) 3300, 1640, and 1610 cm^{-1}; ^1H NMR ($CDCl_3$) δ=2.57 (3H, s), 7.12 (2H, m), 7.40 (2H, m), 7.67 (1H, d). Found: C, 75.31; H, 5.62; N, 8.78%. Calcd for $C_{10}H_9NO$: C, 75.45; H, 5.70; N, 8.80%.

2-[1-(Methylamino)ethyl]indole (9). A solution of **8** (11.8 g) in 40% methanolic methylamine (350 cm^3) was heated under reflux for 5 h and left overnight at room temperature. After evaporation, the residue was dissolved in MeOH (200 cm^3) and treated with $NaBH_4$ (4.5 g) under stirring at room temperature for 1 h. Evaporation of the reaction mixture *in vacuo* gave a residue which was suspended in 4% aqueous NaOH (100 cm^3) and extracted with ethyl acetate. The extract was washed with saturated aqueous NaCl, dried over $MgSO_4$ and evaporated *in vacuo* to yield the desired product as colorless needles (11.0 g, 89%): Mp 95—95.5 °C; IR (KBr) 3170, 1450, 1430, and 1290 cm^{-1}; ^1H NMR ($CDCl_3$) δ=1.46 (3H, s), 2.33 (3H, s), 3.93 (1H, q), 6.30 (1H, s), 6.97-7.33 (3H, m), 7.50 (1H, m). Found: C, 75.88; H, 8.16; N, 16.01%. Calcd for $C_{11}H_{14}N_2$: C, 75.82; H, 8.10; N, 16.08%.

2-[1-(N-Formylmethylamino)ethyl]indole (10). A solution of **9** (25 g) and 4-nitrophenyl formate (28.8 g) in ethyl acetate (500 cm^3) was stirred at room temperature for 1 h. The reaction mixture was washed successively with 1 mol dm^{-3} NaOH and saturated aqueous NaCl, dried and evaporated *in vacuo*. Recrystallization of the residue from diethyl ether yielded the desired product as colorless needles (23.6 g, 91%): Mp 129—130 °C; IR (KBr) 3230, 3200, 1660, and 1300 cm^{-1}; ^1H NMR ($CDCl_3$) δ=1.67, 1.70 (3H, d, ratio 45:55), 2.67, 2.70 (3H, s, ratio 55:45), 4.96, 5.83 (1H, q, ratio 55:45), 6.46 (1H, s), 7.00—7.70 (4H, m), 8.13, 8.33 (1H, s, ratio 45:55). Found: C, 71.39; H, 6.98; N, 13.68%. Calcd for $C_{10}H_{14}N_2O$: C, 71.26; H, 6.98; N, 13.85%.

2-[1-(Dimethylamino)ethyl]indole (11). A mixture of **10** (45 g) and $LiAlH_4$ (10.6 g) in dry tetrahydrofuran (1000 cm^3) was placed in a flask equipped with a calcium chloride drying tube and stirred at room temperature for 1 h. After evaporation *in vacuo*, the residue was decomposed by the addition of water (100 cm^3) and extracted with diethyl ether. The extract was dried over $MgSO_4$ and evaporated *in vacuo*. The residual oil was distilled under reduced pressure to yield the desired product as a colorless oil (36 g, 86%): Bp 111—112 °C/1 mmHg (1 mmHg≈133.322 Pa), mp 48—49 °C (solidified on standing); IR (KBr) 3400, 2970, 1460, and 1300 cm^{-1}; ^1H NMR ($CDCl_3$) δ=1.35 (3H, d), 2.22 (6H, s), 3.75 (1H, q), 6.22 (1H, s), 6.95—7.30 (3H, m), 7.47 (1H, m), 8.53 (1H, bs). Found: C, 76.55; H, 8.69; N, 14.90%. Calcd for $C_{12}H_{16}N_2$: C, 76.56; H, 8.57; N, 14.88%.

Diethyl 2-Acetamido-2-[1-(2-indolyl)ethyl]malonate (20a). To a solution of **11** (500 mg, 2.65 mmol) in abs EtOH(5 cm^3), dimethyl sulfate (332 mg, 2.65 mmol) was added dropwise at a temperature below 35 °C. The solution was kept standing at room temperature for 1 h and was then added to an ice-cooled, stirred solution prepared from sodium (59.7 mg, 2.60 mmol) and diethyl acetamidomalonate (564 mg, 2.60 mmol) in abs EtOH (10 cm^3). The mixture was allowed to react at room temperature for 2 d and evaporated *in vacuo*. The resulting residue was dissolved in $CHCl_3$ and washed successively with water, aqueous Na_2CO_3, and dil HCl. Evaporation followed by recrystallization from benzene gave the desired product as an amorphous powder (641 mg, 67%): IR (KBr) 3380, 1735, 1720, 1690, 1670, and 1500 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.90 (3H, t), 1.26 (3H, t), 1.47 (3H, d), 1.96 (3H, s), 3.96 (1H, m), 4.30 (2H×2, q), 6.23 (1H, s), 7.00—7.67 (4H, m). Found: C, 63.17; H, 6.68; N, 7.81%. Calcd for $C_{19}H_{24}N_2O_5$: C, 63.32; H, 6.71; N, 7.77%.

Ethyl 2-Acetamido-1-methyl-3-oxo-1H-2,3-dihydropyrolo[1,2-a]indole-2-carboxylate (21a). This compound (2.35 g, 71%) was synthesized from the dimethylamino compound **11** (2.0 g) by a procedure similar to that described in the preceding section except that 1.1 equivalents of sodioacetamidomalonate were used. IR (KBr) 3360, 1750, 1740, 1680, and 1610 cm^{-1}; MS *m/z* 314 (M^+); ^1H NMR ($CDCl_3$) δ=1.07, 1.20 (3H, t, ratio 6:4) 1.30, 1.34 (3H, d, ratio 4:6), 2.06, 2.08 (3H, s, ratio 4:6), 3.87, 4.03 (1H, m, ratio 4:6), 4.14, 4.21 (2H, q, ratio 6:4), 6.25, 6.26 (1H, m, ratio 6:4), 7.20—7.50 (3H, m), 8.00 (1H, m). Found: C, 65.11; H, 5.80; N, 8.90%. Calcd for $C_{17}H_{18}N_2O_4$: C, 64.96; H, 5.77; N, 8.91%.

Ethyl 2-Acetamido-3-(2-indolyl)butyrate (13a). To a stirred solution of **11** (9.17 g, 48.8 mmol) in abs EtOH (50 cm^3), dimethyl sulfate (6.3 g, 48.8 mmol) was added dropwise at a temperature below 35 °C. The mixture, after being left standing at room temperature for 1 h, was added to an ice-cooled, stirred solution prepared from sodium (1.4 g, 61 mmol) and diethyl acetamidomalonate (10.6 g, 61 mmol) in abs EtOH (100 cm^3). The combined mixture was allowed to stand at room temperature for 3 d and then evaporated *in vacuo*. After work-up, the desired product was obtained as an amorphous powder (11.3 g, 81%): IR (KBr) 3370, 3360, 3300, 3260, 1730, 1650, 1550, and 1530 cm^{-1}; ^1H NMR (CD_3OD) δ=0.93, 1.16 (3H, t, ratio 1:9), 1.43 (3H, d), 1.90, 1.96 (3H, s, ratio 9:1), 3.56 (1H, q), 4.00, 4.16 (2H, q, ratio 1:9), 4.87, 4.89 (1H, d, ratio 1:9), 6.16, 6.26 (1H, s, ratio 1:9), 6.83—7.60 (4H, m). Found: C, 66.79; H, 6.98; N, 9.16%. Calcd for $C_{16}H_{20}N_2O_3$: C, 66.54; H, 6.99; N, 9.71%.

Ethyl 2-Acetamido-3-(2-indolyl)propionate (13b). From the dimethylamino compound[14] **12** (8.5 g), the desired product **13b** (11.0 g, 82%) was obtained as colorless needles by a procedure similar to that described in the preceding section: Mp 134—135 °C (from benzene); IR (KBr) 3420, 3300, 1740, 1620, 1220, 1200, 1175, and 1020 cm^{-1}; ^1H NMR ($CDCl_3$) δ=1.18 (3H, t), 1.93 (3H, s), 3.23 (2H, d), 4.17 (2H, q), 4.90 (1H, m), 6.23 (1H, s), 6.98—7.67 (4H, m). Found: C, 65.59; H, 6.73; N, 10.01%. Calcd for $C_{15}H_{18}N_2O_3$: C, 65.68; H, 6.61; N, 10.21%.

Malonic Ester Synthesis starting from 2-[1-(Dimethylamino)-alkyl]indoles (11 and 12). A solution of **11** or **12** (0.5 mmol) in abs EtOH (0.5 cm^3) was treated with dimethyl sulfate (0.5 mmol) at a temperature below 35 °C. After the mixture had stood at 20 °C for 1 h, it was added to an ethanolic solution (1.0 cm^3) prepared from equimolar quantities of 0.49, 0.55, or 0.63 mmol of sodium and diethyl acetamidomalonate (Table 1). The mixture was allowed to react at 20 °C for 3 d while being isolated from moisture. The product composition was analyzed by HPLC (μ-Porasil, 3.9 mm×30, $CHCl_3$:AcOEt=4:1). The results are summarized in Table 1.

2-Acetamido-3-(2-indolyl)butyric Acid (14a). *a)* A mixture of **20a** (185 mg) and Na_2CO_3 (180 mg) in water (2.0 cm^3) was refluxed for 15 h and then cooled. This was acidified with concd HCl (0.3 cm^3) and extracted with ethyl acetate. The extract was washed with saturated aqueous

NaCl, dried over $MgSO_4$ and evaporated to dryness *in vacuo*. Precipitation from aqueous EtOH gave the desired product as an amorphous powder (122 mg, 91%): IR (KBr) 3450, 3340, 1740, 1720, and 1600 cm^{-1}; ^1H NMR ($CDCl_3$) δ=1.41, 1.56 (3H, d, ratio 3:7), 2.03, 2.06 (3H, s, ratio 3:7), 3.56 (1H, m), 4.98 (1H, m), 6.36 (1H, m), 6.92—7.66 (4H, m). Found: C, 64.55; H, 6.36; N, 10.73%. Calcd for $C_{14}H_{16}N_2O_3$: C, 64.60; H, 6.20; N, 10.76%.

b) In a similar manner, the tricyclic carboxylate **21a** (288 mg) was subjected to alkaline hydrolysis and gave **14a** (239 mg, 92%).

c) The 2-acetamidobutyrate **13a** (2.15 g) similarly gave **14a** (1.71 g, 97%).

2-Acetamido-3-(2-indolyl)propionic Acid (14a). *a)* In a manner similar to that described in the preceding section, the 2-acetamidomalonate **20b** (3.46 g), on being refluxed in alkaline solution for 15 h, gave **14b** (2.41 g, 98%) as pale yellow needles: Mp 187—188 °C (lit,[14] 190—191 °C); IR (KBr) 3390, 3340, 1695, 1605, and 1535 cm^{-1}; ^1H NMR ($CDCl_3$) δ=1.92 (3H, s), 3.27 (2H, d), 4.80 (1H, m), 6.03 (1H, s), 6.77—7.57 (4H, m). Found: C, 63.57; H, 5.82; N, 11.46%. Calcd for $C_{13}H_{14}N_2O_3$: C, 63.40; H, 5.73; N, 11.38%.

b) The 2-acetamidopropionate **13b** (2.74 g) similarly gave **14b** (2.34 g, 95%).

Ethyl 1,4-Dimethyl-5H-3,4-dihydropyrido[4,3-b]indole-3-carboxylate (15a). To an ice-cooled, stirred suspension of **14a** (1.57 g) in EtOH (30 cm^3) was added dropwise thionyl chloride (1.5 cm^3). The mixture was refluxed for 30 min and evaporated *in vacuo*, giving a dark-red residue. This was dissolved in water (30 cm^3), washed with ethyl acetate and neutralized with 1 mol dm^{-3} NaOH to give the desired product (1.40 g, 86%) as an amorphous powder: IR (KBr) 1740, 1600, 1500, and 1470 cm^{-1}; ^1H NMR (CD_3OD) δ=0.77, (3H, t, ratio 3:7), 2.53, 2.58 (3H, s, ratio 3:7), 2.85, 2.92 (3H, s, ratio 3:7), 3.20, 3.55 (1H, d, ratio 3:7), 3.87, 3.93 (2H, q, ratio 3:7), 6.80—7.53 (4H, m). Found: C, 69.70; H, 6.65; N, 10.32%. Calcd for $C_{16}H_{18}N_2O_2 \cdot 1/4H_2O$: C, 69.92; H, 6.60; N, 10.19%.

Ethyl 1-Methyl-5H-3,4-dihydropyrido[4,3-b]indo-3-carboxylate (15b). In a manner similar to that described the 3-indolylpropionic acid **14b** (49.2 g) was treated with thionyl chloride and afforded **15b** (44.5 g, 87%) as pale yellow needles: Mp 181—183 °C (from EtOH); IR (KBr) 1735, 1605, 1545, and 1465 cm^{-1}; ^1H NMR (DMSO-d_6) δ=1.22 (3H, t), 2.43 (3H, s), 3.00 (2H, m), 4.15 (2H, q), 4.43 (1H, m), 7.00—7.47 (4H, m).

Ethyl 1,4-Dimethyl-5H-pyrido[4,3-b]indole-3-carboxylate (16a). A stirred suspension of **15a** (1.2 g) and sulfur (150 mg) in dry xylene (25 cm^3) was refluxed for 5 h. The reaction mixture was evaporated *in vacuo* to yield a yellow residue. Recrystallization from EtOH gave **16a** (1.11 g, 93%) as pale yellow needles: Mp 257—258 °C; IR (KBr) 1700, 1620, and 1200 cm^{-1}; ^1H NMR (DMSO-d_6) δ=1.30 (3H, t), 2.53 (3H, s), 2.80 (3H, s), 4.26 (2H, q), 7.00—7.66 (3H, m), 8.10 (1H, m). Found: C, 71.60; H, 5.92; N, 10.30%. Calcd for $C_{16}H_{16}N_2O_2$: C, 71.62; H, 6.01; N, 10.44%.

Ethyl 1-Methyl-5H-pyrido[4,3-b]indole-3-carboxylate (16b).

a) By Dehydrogenation of 15b with Lead Tetraacetate: Lead tetraacetate (117 g) was added in small portions to an ice-cooled, stirred solution of **15b** (51.2 g) in dry pyridine (600 cm^3). The suspension was allowed to react at room temperature for 20 min and then poured into a mixture of ethyl acetate (2000 cm^3) and water (1000 cm^3) with stirring. The organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined extract was washed successively with saturated aqueous NaCl and saturated aqueous $NaHCO_3$, dried over $MgSO_4$ and evaporated to dryness *in vacuo*. Recrystallization of the resulting reddish solid from EtOH gave pale yellow needles (39.9 g, 78%): Mp 252—254 °C; IR (KBr) 1725, 1620, 1595, 1560, and 1345 cm^{-1}; ^1H NMR ($CDCl_3$) δ=1.45 (3H, t), 3.10 (3H, s), 4.47 (2H, q), 7.10—7.67 (4H, m), 8.13 (1H, s). Found: C, 70.65; H, 5.36; N, 10.84%. Calcd for $C_{15}H_{14}N_2O_5$: C, 70.85; H, 5.55; N, 11.02%.

b) By Dehydrogenation of 15b with Sulfur: In a manner similar to that described in the preceding section, the dihydro-γ-carbolinecarboxylate **15b** (25.6 g) was dehydrogenated with sulfur (3.3 g) to give the desired γ-carbolinecarboxylate **16b** (25.1 g, 98%) as pale yellow needles. All spectral and analytical data were identical with those of the sample described above.

1,4-Dimethyl-5H-pyrido[4,3-b]indole-3-carboxylic Acid (17a). A suspension of **16a** (268 mg) in EtOH–1 mol dm^{-3} NaOH (1:1, 5.0 cm^3) was refluxed for 5 h and then concentrated *in vacuo* to half of its original volume. The concentrate, after being neutralized with 1 mol dm^{-3} HCl (2.5 cm^3), gave a pale-yellow precipitate, which was collected by filtration, washed successively with water, ethanol and diethyl ether, and dried. Recrystallization from a large amount of boiling water gave **17a** (212 mg, 88%) as pale yellow prisms: Mp 283.5—284.5 °C; IR (KBr) 1640, 1620, and 1590 cm^{-1}; ^1H NMR (CD_3OD/NaOH) δ=2.67 (3H, s), 2.95 (3H, s), 7.13—7.73 (3H, m), 8.07 (1H, d). Found: C, 69.72; H, 4.87; N, 11.43%. Calcd for $C_{14}H_{12}N_2O_2$: C, 69.99; H, 5.03; N, 11.66%.

1-Methyl-5H-pyrido[4,3-b]indole-3-carboxylic Acid (17b). In a manner similar to that described in the preceding section, the γ-carboline-3-carboxylic acid ester **16b** (63.5 g) gave the desired 3-carboxylic acid **17b** (54.5 g, 96%) as pale yellow prisms: Mp 275—277 °C; IR (KBr) 1650, 1620, 1590, 1400, and 1220 cm^{-1}; ^1H NMR (CF_3COOH) δ=3.43 (3H, s), 7.73—7.93 (3H, m), 8.30 (1H, m), 8.62 (1H, s). Found: C, 63.68; H, 4.72; N, 11.46%. Calcd for $C_{13}H_{10}N_2O_2 \cdot H_2O$: C, 63.91; H, 4.90; N, 11.46%.

3-Acetylindole-2-acetonitrile (19). Indole-2-acetonitrile[16] (**18**; 1.56 g), prepared from indole-2-carboxylic acid (**7**; Fluka Chem. Corp., Swiss), was dissolved in a mixture of phosphoryl chloride (1.16 cm^3) and *N,N*-dimethylacetamide (3.0 cm^3), and this solution was heated at 85 °C for 2 h. The reaction mixture was poured onto crushed ice (20 g) and washed with ethyl acetate. Unreacted indole-2-acetonitrile (**18**; 840 mg) was recovered from the ethyl acetate layer. The aqueous layer was neutralized with 5 mol dm^{-3} NaOH and extracted with ethyl acetate–tetrahydrofuran (1:1). The extract was washed with saturated aqueous $NaHCO_3$ and dried over $MgSO_4$. After evaporation *in vacuo*, the residue was chromatographed on a column of silica gel using EtOH–$CHCl_3$ (3:7) as an eluent. Work-up, as usual, gave **19** (479 mg, 24%) as fine yellow needles: Mp 223—225 °C; IR (KBr) 3250, 2245, 1620, 1605, 1525, 1490, 1450, and 1180 cm^{-1}; ^1H NMR (DMSO-d_6) δ=2.63 (3H, s), 4.47 (2H, s), 7.07—7.67 (4H, m), 7.83—8.13 (1H, m). Found: C, 72.58; H, 4.98; N, 13.97%. Calcd for $C_{12}H_{10}N_2O$: C, 72.71; H, 5.08; N, 14.13%.

3-Amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1; 1). *a) Thermal Decomposition of 4-(1-Benzotriazolyl)-3,6-*

dimethyl-2-pyridinamine (6): A mixture of **6** (2.0 g) and polyphosphoric acid (16 g) was allowed to decompose by rapid heating over a free flame until evolution of nitrogen gas ceased. After the reaction mixture had cooled to room temperature, ice-water was added and the mixture was made strongly basic with 6 mol dm^{-3} NaOH and extracted with ethyl acetate. The extract was washed with saturated aqueous NaCl, dried over $MgSO_4$ and evaporated to dryness *in vacuo*. The residue was chromatographed on silica gel using 30% MeOH in ethyl acetate as an eluent. The fractions containing **1** were combined on the basis of TLC analysis and evaporated *in vacuo*, yielding a reddish yellow solid, which upon recrystallization from ethyl acetate (20 cm^3) containing a small amount of acetic acid (0.3 cm^3), gave the desired product, the acetic acid salt of **1** (1.04 g, 46%) as colorless prisms: Mp 248—256 °C; IR (KBr) 3350, 3050, 1650, 1550, and 1400 cm^{-1}; UV (0.1 mol dm^{-3} HCl/MeOH) 227 (ε 16000), 242 (sh, 24000), 265 (67000), 267 (sh, 66000), 307 (7900),and 318 (9800); MS *m/z* 211 (M^+); ^1H NMR (pyridine-d_5) δ=2.08 (3H, s), 2.36 (3H, s), 2.96 (3H, s), 7.20—7.42 (3H, m) 8.03 (1H, m). Found: C, 66.27; H, 6.42; N, 15.20%. Calcd for $C_{13}H_{13}N_3 \cdot CH_3COOH$: C, 66.40; H, 6.32; N, 15.49%.

b) By Curtius Rearrangement of 1,4-Dimethyl-5H-pyrido[4,3-b]indole-3-carboxylic Acid (17a): A mixture of **17a** (110 mg) and triethylamine (0.15 g) in DMA (5.0 cm^3) was kept at 60 °C for 1 h with stirring and cooled to 20 °C. DPPA (0.16 g) was added and the resulting mixture was allowed to react at room temperature for 2 h, during which time the suspension became almost clear. Aqueous acetic acid (50%, 2.0 cm^3) was added dropwise and the mixed solution was heated at 90 °C for 1 h, and then poured into ice-cooled aqueous NaOH (0.75 g in 20 cm^3). The combined mixture was extracted with ethyl acetate, and the extract, after being washed with saturated aqueous NaCl and dried over $MgSO_4$, was concentrated *in vacuo* to one-fifth of its original volume. The residual ethyl acetate solution, upon the treatment with acetic acid (0.3 cm^3), afforded the desired compound (51 mg, 41%) as colorless prisms. All spectral and analytical data were identical with those of the sample obtained by the method described above. The starting material (28 mg) was recovered from the aqueous layer of the ethyl acetate extraction when the pH of the solution was adjusted to 4.5 with concd HCl.

3-Amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2; 2).
a) By Curtius Rearrangement of 1-Methyl-5H-pyrido[4,3-b]-indole-3-carboxylic Acid (17b): Treatment of γ-carboline-3-carboxylic acid **17b** (1.13 g) by a procedure similar to that of section **1***b*) gave the desired product (594 mg, 46%) as colorless prisms: Mp 242—247 °C; IR (KBr) 1670, 1655, 1615, 1460, and 1400 cm^{-1}; UV (0.1 mol dm^{-3} HCl/MeOH) 224 (ε 13000), 243 (sh, 24000), 265 (64000), 269 (70000), 306 (5700), and 318 nm (6400); MS *m/z* 197 (M^+); ^1H NMR (DMSO-d_6) δ= 1.93 (3H, s), 2.74 (3H, s), 6.37 (1H, s), 7.07—7.53 (3H, m), 7.97 (1H, d). Found: C, 65.13; H, 5.71; N, 16.25%. Calcd for $C_{12}H_{11}N_3 \cdot CH_3COOH$: C, 65.36; H, 5.88; N, 16.33%.

b) By Cyclization Reaction of 3-Acetylindole-2-acetonitrile (19) with Ammonia: A solution of **19** (39.6 mg) in 20% methanolic ammonia was heated in a sealed tube at 100 °C for 5 h and evaporated *in vacuo*. The drak brown residue was purified through both silica-gel and alumina (Woelm B, Akt. I) columns, using MeOH in ethyl acetate as an eluent, to give **2** (23 mg, 58%). This product was dissolved in ethyl acetate. Then, a small amount of acetic acid was added and the acetic acid salt of **2** was obtained as colorless prisms. The physicochemical data were identical with those of the sample obtained by the method described above.

We are grateful to Dr. Katsura Morita, General Manager of this Division, and to Drs. Masahiko Fujino and Masao Nishikawa for their encouragement throughout this work. We also thank Dr. Morimasa Arakawa (Nard Institute, Ltd., Osaka) for his kind cooperation.

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