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A New Synthesis of (±)-Trypargine

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A new route for the synthesis of (±)-trypargine (I) is described. The Pictet–Spengler reaction of N_b-benzyltryptamine with α-ketoglutaric acid was carried out in aprotic media to afford the key intermediate, 2-benzyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole-1-propionic acid (IIa), which was converted into (±)-trypargine (I) in five or six steps.

Keywords——trypargine; total synthesis; tetrahydro-β-carboline; N_b-benzyltryptamine; α-ketoglutaric acid; Pictet–Spengler reaction

Trypargine (Ia),[1] isolated from the African rhacophorid frog, *Kassina senegalensis*, is an optically active skin component derived from tryptophan. In our previous paper,[2] the structure of Ia was established as (1*S*)-(—)-1-(3′-guanidinopropyl)-1,2,3,4-tetrahydro-β-carboline by the total synthesis of Ia and by means of optical rotatory dispersion (ORD) and circular dichroism (CD) measurements. Although the previously reported route was convenient for the synthesis of racemic trypargine (I), a complicated optical resolution of the racemate was required to obtain the optically active compound.

We have now developed a new method for the total synthesis of I, which may be applicable to the asymmetric synthesis of Ia and Ib. In our synthetic plan, an N_b-benzyl-tetrahydro-β-carboline derivative (IIa), prepared by the condensation of N_b-benzyltryptamine with α-ketoglutaric acid, was chosen as the synthetic intermediate.

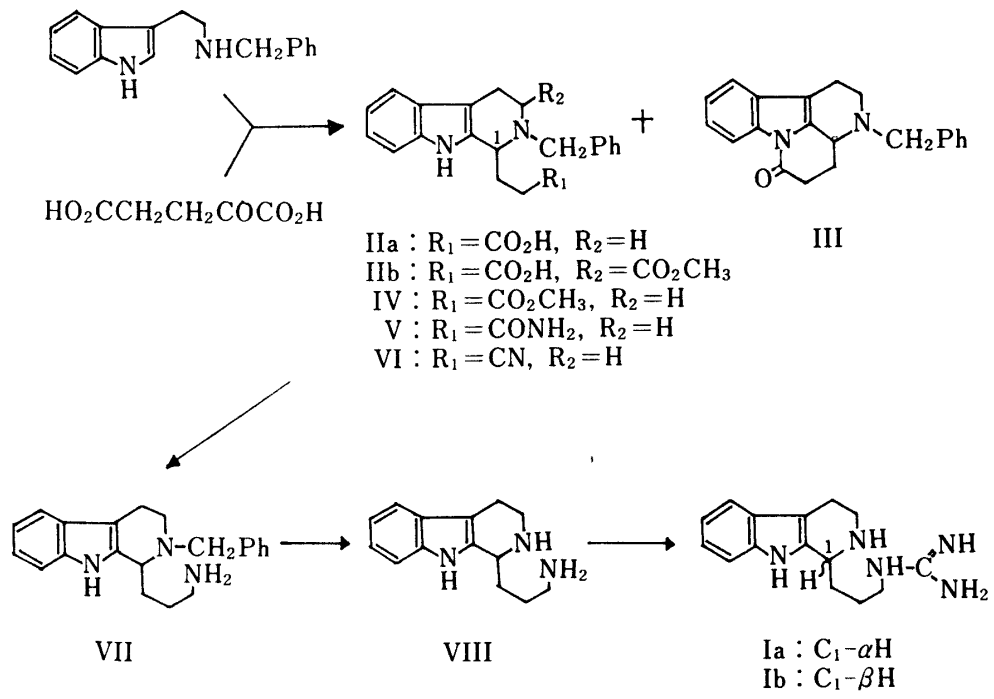


Chart 1

The synthesis of the key intermediate was achieved in an aprotic solvent under non-acidic conditions analogous to those used by Cook *et al.*;[3] the condensation of N_b-benzyltryptamine with α-ketoglutaric acid in dry toluene–dry dioxane for 2—3 h under reflux, with azeotropic removal of water, furnished the desired compound IIa in 86.4% yield. Examination of the crude reaction mixture by high performance liquid chromatography (HPLC) indicated that only a small amount (1—2%) of the oxocanthine derivative (III)[4] was present, and the formation of III was avoided by the use of a shorter reaction time.

The acid (IIa) was further treated with a slight excess of ethereal diazomethane to give the methyl ester (IV) as an amorphous compound in quantitative yield. Without purification, this ester was converted by treatment with methanolic ammonia to the amide (V) in 87.8% yield calculated from the acid (IIa). Dehydration of V with phosphorus oxychloride in a mixture of dry pyridine and *N*,*N*-dimethylformamide (DMF) at 0°C furnished the nitrile (VI) in 89.4% yield. The nitrile (VI) was first reduced with $LiAlH_4$ to afford the amino compound (VII), because the debenzylated derivative of VI was easily cyclized to 1,2,3,5,6,11b-hexahydro-3-imino-11*H*-indolo[3,2-*g*]pyrrocoline, as found in the previous experiment.[2] The unstable product (VII) was directly hydrogenated over 10% palladium on charcoal without purification to give the debenzylated amine (VIII) as the hydrochloride in 89.8% yield from VI. Comparison of this hydrochloride with an authentic sample prepared by the hydrolysis of I with aqueous hydrazine hydrate[2] showed that these substances were indistinguishable.

On the other hand, the reduction of the amide (V) with $LiAlH_4$, followed by catalytic hydrogenation over palladium on charcoal, afforded the above-mentioned hydrochloride of VIII in high yield.

Finally, the free base of VIII was heated with *S*-methylisothiourea sulfate in water or DMF to yield (±)-trypargine (I) as the sulfate in moderate yield . This sulfate was converted to the corresponding hydrochloride according to the procedure described in the previous paper.[2] The hydrochloride of I was identical with an authentic sample on the basis of a comparison of infrared (IR) spectra, and proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra.

The results described above indicate that the optically active Ia or Ib may be synthesized. The Pictet–Spengler condensation of optically active N_b-benzyltryptophan methyl ester of known chirality with α-ketoglutaric acid could occur in a stereospecific fashion to give the *trans*- or *cis*-1,3-disubstituted-tetrahydro-β-carboline (IIb) of known absolute stereochemistry. In addition, functionalization of the 1-substituent of IIb could be carried out in the present synthetic route and the 3-methoxycarbonyl group coud then be removed by the method of Yamada *et al.*[5] to provide optically active Ia or Ib of known absolute configuration. An attempt to prepare IIb and to convert IIb to Ia or Ib is in progress.

Preliminary studies on the biological activity of (±)-trypargine have shown that intravenous administration of (±)-trypargine (10 mg/kg) to mice caused their death within 2 min as a result of respiratory failure and paralysis. Further investigation on the biological activity of trypargine and its derivatives is in progress.

Experimental

All melting points were taken on a Yanagimoto micro melting point apparatus and are uncorrected. Ultraviolet (UV) spectra were determined on a Hitachi 323 spectrophotometer. Infrared (IR) spectra were recorded by using a Hitachi 285 spectrophotometer. Mass spectra (MS) were recorded with a JEOL JMS-D300 mass spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were obtained on a JEOL FX-270 spectrometer. Chemical shifts in the ^{1}H- and ^{13}C-NMR spectra are reported as δ values (parts per million) from tetramethylsilane as an internal standard. Abbreviations used are: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), aromatic (arom). Chromatography was performed on SiO_2 (silica gel 60, 35—70 mesh, Merck) and Al_2O_3 (active basic, Merck). HPLC was carried out in the reverse phase (μ-Bondapak

C_{18}, Waters Associates). The eluting solvent was 60—80% CH_3CN–$AcONH_4$ (0.05 M, pH 4.00).

2-Benzyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole-1-propionic Acid (IIa)——N_b-Benzyltryptamine (5.73 g, 22.9 mM) was dissolved in dry toluene–dry dioxane (1: 1, v/v; 20 ml), and α-ketoglutaric acid (4.01 g, 1.2 eq) was added in one portion to the stirred solution at room temperature. The mixture was heated to reflux (120—125°C) for 2—3 h with water separation *via* a Dean–Stark trap. After removal of the solvent, the residual oil (8.62 g) was chromatographed on silica gel (150 g) packed with the aid of *n*-hexane and eluted with 5% MeOH in AcOEt to provide IIa (7.24 g, 86.4% yield) as colorless crystals. Recrystallization from MeOH afforded colorless prisms. mp 125—130°C. *Anal.* Calcd for $C_{21}H_{22}N_2O_2 \cdot CH_3OH$: C, 72.10; H, 7.15; N, 7.65. Found: C, 71.80; H, 7.11; N, 7.39. MS *m/z* (%): 334 (M^+, 3.9), 261 (100), 197 (27.5), 169 (13.7), 156 (7.8), 115 (3.9), 91 (52.9). UV λ_{max}^{MeOH} nm (log ε): 221 (4.59), 273 (3.87), 279 (3.86), 289.5 (3.76). ^{1}H-NMR ($CDCl_3$) δ: 3.46 (s, CH_3OH), 3.85 and 4.03 (2H, AB-q, J=13.0 Hz, C$\underline{H}_2$Ph), 4.20—4.28 (1H, m, C_1–H), 7.06—7.49 (9H, m, arom H).

Methyl 2-Benzyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole-1-propionate (IV) and 2-Benzyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole-1-propionamide (V)——A slight excess of ethereal diazomethane was added in small portions to a stirred solution of IIa (2.00 g, 5.5 mM) in MeOH–CH_2Cl_2 (1: 1; v/v; 40 ml) over a period of 20 min at room temperature, and then the mixture was stirred for an additional 0.5—1 h. Evaporation of the solvent under reduced pressure provided a pale orange viscous oil (IV, 1.99 g), which was shown by HPLC analysis to be of over 98% purity. This methyl ester (IV) was used in the following reaction without purification. An analytical sample of IV was obtained by chromatography on Al_2O_3 with *n*-hexane–benzene (1: 1; v/v) and benzene as eluents. MS *m/z* (%): 348 (M^+, 3.7), 261 (100), 197 (7.3), 169 (12.2), 156 (8.5), 115 (4.9), 91 (53.7). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3470 (NH), 1730 (ester). UV λ_{max}^{MeOH} nm: 226, 276 (shoulder), 282, 290. ^{1}H-NMR ($CDCl_3$) δ: 3.54 (3H, s, CO_2CH_3), 3.52—3.60 (1H, m, C_1–H), 3.63 and 3.70 (2H, AB-q, J=13.2 Hz, C$\underline{H}_2$Ph), 7.02—7.36 (8H, m, arom H), 7.48 (1H, dd, arom H), 8.09 (1H, s, indole NH).

An ice-cooled solution of the foregoing methyl ester IV (1.99 g) in MeOH (50 ml) was saturated with ammonia, and the mixture was allowed to stand in a closed bottle at room temperature for 10—14 d. The solvent was concentrated *in vacuo*, and the residue (1.92 g) was chromatographed on Al_2O_3 (77 g) with a mixture of benzene–$CHCl_3$ (1: 1; v/v) to provide the amide (V, 1.97 g, 87.8% yield from IIa) as a colorless solid, which was recrystallized from benzene to afford colorless needles. mp 129—130°C. *Anal.* Calcd for $C_{21}H_{23}N_3O \cdot C_6H_6$: C, 78.80; H, 7.10; N, 10.21. Found: C, 78.52; H, 7.08; N, 9.91. MS *m/z* (%): 333 (M^+, 3.0), 315 (2.3), 261 (100), 242 (22.0), 234 (4.5), 197 (7.6), 169 (15.2), 156 (6.8), 115 (4.5), 91 (53.0). IR ν_{max}^{KBr} cm^{-1}: 3460 (NH), 1675 (amide). UV λ_{max}^{MeOH} nm (log ε): 226 (4.51), 276 (shoulder, 3.80), 283 (3.82), 290 (3.74). ^{1}H-NMR ($CDCl_3$) δ: 3.58—3.66 (1H, m, C_1–H), 3.67 and 3.72 (2H, AB-q, J=13.9 Hz, C$\underline{H}_2$Ph), 7.04—7.52 (9H, m, arom H), 7.34 (s, C_6H_6), 8.55 (1H, s, indole NH).

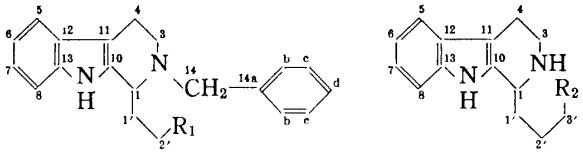
2-Benzyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole-1-propionitrile (VI)——Phosphorus oxychloride (184 mg, 1.5 eq) was added to a stirred mixture of V (333 mg, 0.81 mM) in dry pyridine (791 mg) and dry DMF (5.0 ml) below 0°C. The mixture was stirred at 0°C for 1 h, diluted with $CHCl_3$ (100 ml) and ice-water (50 ml), and then basified with 10% aqueous sodium carbonate. The $CHCl_3$ layer was separated, and the aqueous solution was extracted with $CHCl_3$ (2×100 ml). The combined $CHCl_3$ layer was washed with brine (2×30 ml), dried over anhydrous potassium carbonate, and concentrated *in vacuo*. The residue (426 mg) was purified by silica gel (10 g) column chromatography using a mixture of *n*-hexane–AcOEt (8: 1; v/v) to give the nitrile (VI, 228 mg, 89.4% yield) as a pale yellow solid. Recrystallization from Et_2O–petroleum ether afforded colorless prisms. mp 155—156°C. *Anal.* Calcd for $C_{21}H_{21}N_3$: C, 79.96; H, 6.71; N, 13.32. Found: C, 79.85; H, 6.69; N, 13.23. MS *m/z* (%): 315 (M^+, 41.2), 261 (100), 234 (5.9), 169 (10.0), 156 (13.7), 115 (2.0), 91 (47.1). IR ν_{max}^{KBr} cm^{-1}: 3350 (NH), 2250 (C≡N). UV λ_{max}^{MeOH} nm (log ε): 225 (4.61), 275 (shoulder, 3.89), 282 (3.91), 290 (3.83). ^{1}H-NMR ($CDCl_3$) δ: 3.68—3.76 (1H, m, C_1–H), 3.75 (2H, s, C$\underline{H}_2$Ph), 7.08—7.36 (8H, m, arom H), 7.51 (1H, d, J=7.6 Hz, arom H), 7.79 (1H, s, indole NH).

1,2,3,4-Tetrahydro-9*H*-pyrido[3,4-*b*]indole-1-propylamine (VIII)——1) A solution of VI (94 mg, 0.3 mM) in dry Et_2O (10 ml) was added dropwise to a stirred suspension of $LiAlH_4$ (23 mg, 2 eq) in dry Et_2O (25 ml) over a period of 20—30 min at room temperature. The mixture was refluxed for 9 h under nitrogen, and was then worked up in the usual manner to afford a pale yellow viscous oil (VII, 94 mg).

The above-mentioned oil (VII) (94 mg) was dissolved in EtOH (6 ml) and concd. HCl (0.1 ml). The mixture was hydrogenated at 30°C under atmospheric pressure over 10% palladium on charcoal (19 mg) until the hydrogen uptake ceased (5—6 h). The catalyst was separated by filtration through Celite and washed thoroughly with EtOH. The combined filtrate and washings were concentrated *in vacuo* to provide VIII as a pale orange crystalline hydrochloride (81 mg, 89.8% yield from VI), which was recrystallized from MeOH–EtOH–Et_2O to afford the pure hydrochloride as pale yellow prisms. mp 243—245°C (dec.) [lit.[2] 245—247°C (dec.)]. MS *m/z* (%): 229 (M^+, 36.7), 211 (11.9), 198 (12.8), 182 (18.3), 171 (100), 156 (11.0), 154 (10.6), 144 (13.8), 130 (17.4), 115 (8.7). UV λ_{max}^{MeOH} nm (log ε): 221.5 (4.54), 272 (3.87), 279 (3.85), 289 (3.74). The IR (KBr), mass and ^{13}C-NMR spectra of the hydrochloride of VIII were identical with those of an authentic sample.

2) The amide V (333 mg, 0.81 mM) in dry Et_2O (40 ml) was added to a stirred suspension of $LiAlH_4$ (5 eq) in dry Et_2O (25 ml). The mixture was heated to reflux for 8 h. On work-up as usual, the desired product (VII, 257 mg) was obtained.

TABLE I. ^{13}C Chemical Shifts



	IIa[a] $R_1=CO_2H$	IV[a] $R_1=CO_2CH_3$	V[a] $R_1=CONH_2$	VI[a] $R_1=CN$	VIII[b] $R_2=NH_2$	I[b] $R_2=NH\text{-}C(NH_2)=NH$
C (1)	59.5	55.5	55.9	55.0	54.0	54.4
C (1′)	28.8	29.2[d]	29.8	30.1	30.1	30.5
C (2′)	35.9	30.4[d]	32.2	13.8	24.2	25.7
C (3)	42.4	44.6	45.1	44.4	42.8	42.9
C (3′)					40.1	42.0
C (4)	16.4	17.9	18.0	17.6	19.3	19.4
C (5)	118.1	118.0	118.0	118.2	119.0	119.1
C (6)	119.5	119.2	119.1	119.6	120.5	120.6
C (7)	122.2	121.4	121.4	121.9	123.5	123.5
C (8)	111.7	110.8	111.0	110.9	112.3	112.4
C (10)	132.9	134.4	134.4	132.9	129.2	129.6
C (11)	105.3	107.9	107.8	108.8	107.5	107.4
C (12)	126.1	127.2	127.1	127.1	127.2	127.3
C (13)	136.8	135.9	136.0	136.1	138.1	138.2
C (14)	56.4	57.4	57.4	57.5		
C (14a)	130.4	139.6	139.8	139.0		
C (14b)	129.1[c]	128.1[c]	128.3[c]	128.4[c]		
C (14c)	130.1[c]	129.0[c]	129.2[c]	129.1[c]		
C (14d)	128.9	126.9	127.1	127.4		
	177.5 (CO_2H)	174.6 ($\underline{C}O_2CH_3$)	176.1 ($CONH_2$)	120.0 (CN)		158.6 ($NH\text{-}\underline{C}(NH_2)=NH$)
	50.6 (CH_3OH)	51.5 ($CO_2\underline{C}H_3$)	128.3 (C_6H_6)			

a) In $CDCl_3$ solution. *b*) In CD_3OD solution.
c, *d*) The indicated assignments in each column may be reversed.

Catalytic hydrogenation of VII (257 mg) in EtOH (12.5 ml) and concd. HCl (0.25 ml) over 10% palladium on charcoal (52 mg) proceeded smoothly to give the hydrochloride of VIII (223 mg, 91.2% yield from V).

(±)-Trypargine (I)——A mixture of the free base VIII (229 mg, 1 mM) and *S*-methylisothiourea sulfate (1.2 eq) in water (10 ml) was heated at 50°C for 12 h then stirred at room temperature for 12 h. The solution was concentrated to a small volume *in vacuo*, and the resulting crystals were filtered off and washed with EtOH to give the sulfate of I (240 mg).

This sulfate (240 mg) was converted to the corresponding diformate by the method described in the previous paper.[2] The hydrochloride of I (203 mg, 59% yield) was obtained by treatment of the diformate with 10% w/v EtOH–HCl, and was recrystallized from MeOH–Et_2O to afford pale yellow prisms. mp 210—214°C (lit.[2] 202—206°C). *Anal.* Calcd for $C_{15}H_{21}N_5 \cdot 2HCl$: C, 52.33; H, 6.73; N, 20.34. Found: C, 52.12; H, 6.70; N, 20.41. IR ν_{max}^{KBr} cm^{-1}: 1675, 1640, 1620. UV λ_{max}^{MeOH} nm (log ε): 222 (4.55), 272 (3.87), 279 (3.86), 289 (3.74). The UV, IR (KBr), ^1H- and ^{13}C-NMR spectra of the hydrochloride of I were identical with those of an authentic sample.

References and Notes

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4) Compound III: mp 174—175°C (colorless plates from MeOH–AcOEt). MS *m/z* (%): 316 (M^+, 26), 261 (16), 225 (11), 197 (100), 168 (23), 91 (54). UV λ_{max}^{MeOH} nm (log ε): 241 (4.30), 265 (4.04), 271 (shoulder, 4.00), 293.5 (3.63), 301.5 (3.625). ^{1}H-NMR ($CDCl_3$) δ: 1.80—2.00 (1H, m), 2.44—2.94 (6H, m), 3.15—3.27 (1H, m), 3.39 and 4.21 (2H, AB-q, J=13.5 Hz, $C\underline{H}_2Ph$), 3.50—3.60 (1H, m, C_1-H), 7.22—7.45 (8H, m, arom H), 8.37—8.40 (1H, m, arom H).
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