A New Synthesis of 3-(3-Carboxy-4-hydroxyphenyl)-L-alanine (3′-Carboxy-L-tyrosine)

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3-(3-Carboxy-4-hydroxyphenyl)-L-alanine (*1*) occurs in various higher plants.[1,2] *1* has been synthesized from L-tyrosine (*2*) *via* 3-(4-hydroxy-3-nitrophenyl)-L-alanine, 3-(3-amino-4-hydroxyphenyl)-L-alanine, and 3-(3-cyano-4-hydroxyphenyl)-L-alanine.[1] However, the yield in the Sandmeyer step has never been satisfactory and the purification from the reaction mixture is difficult. Syntheses of racemic *1* are available.[3,4]

We now report a new, efficient, synthesis of *1* from *2* *via* *N*-acetyl-L-tyrosine (*3*), *N*-acetyl-*O*-methyl-L-tyrosine (*4*), *N*-acetyl-*O*-methyl-L-tyrosine ethyl ester (*5*) and *N*-acetyl-3-(3-formyl-4-methoxyphenyl)-L-alanine ethyl ester (*6*). The step from *5* to *6*, performed with dichloromethyl methyl ether and $TiCl_4$, has recently been described in the literature, although without experimental details.[5] The formylation of *4* with dichloromethyl methyl ether has also recently been described in the literature, although the yield was low.[6,7] Oxidation of *6* to give the desired carboxyl group in 3′-position has after a number of preliminary attempts been accomplished by two different methods. The first, using Ag_2O in NaOH, results in saponification, yielding *N*-acetyl-3-(3-carboxy-4-methoxyphenyl)-L-alanine (*7*) which is esterified to *N*-acetyl-3-(3-carboxy-4-methoxyphenyl)-L-alanine diethyl ester (*8*). Demethylation is performed with BCl_3 to give *N*-acetyl-3-(3-carboxy-4-hydroxyphenyl)-L-alanine diethyl-ester(*9*), and acid hydrolysis finally gives *1*. The second oxidation method, using $KMnO_4$, gives *N*-acetyl-3-(3-carboxy-4-methoxyphenyl)-L-alanine monoethyl ester (*10*) which by demethylation with BCl_3 gives *N*-acetyl-3-(3-carboxy-4-hydroxyphenyl)-L-alanine monoethyl ester (*11*) and by hydrolysis *1*. Both oxidation methods are proceeding with satisfactory yields. All new compounds, including *6*, have been characterized by IR and ^1H NMR spectra, optical rotation and elemental analysis, and mass spectra have been recorded for *7*, *8*, and *10*.

Experimental. M.p.'s and b.p.'s are uncorrected. IR spectra in KBr were recorded on a Perkin-Elmer Infracord 337. ^1H NMR spectra were recorded on a JEOL C-60 HL instrument with TMS as internal standard. MS were recorded on an AEI 3074 instrument at 70 eV with an ion source temperature of 200 °C. Optical rotations were determined on a Perkin-Elmer 141 polarimeter. TLC was performed on Silica Gel F_{254} (Merck) with butanol-acetic acid-water (4:1:1) as the mobile phase. Compounds were visualized with UV light, formyl groups with 2,4-dinitrophenylhydrazine [8] and phenol groups with $FeCl_3$ and $K_3Fe(CN)_6$.[9] Microanalyses were performed by Mr. G. Cornali and his staff.

N-Acetyl-O-methyl-L-tyrosine ethyl ester (5). A solution of *4* (prepared in two steps [10] from *2*) (0.20 mol, 47.4 g) and *p*-toluenesulfonic acid monohydrate (3 g) in absolute ethanol (300 ml) and benzene (500 ml) was refluxed for 15 h. Evaporation and crystallization from light petroleum (b.p. 80–110 °C) gave 0.172 mol (45.5 g, 86 %), m.p. 90–92 °C, $[\alpha]_D^{22}$ +22.2° (*c* 0.6, EtOH). TLC: R_F 0.77. Lit. value: M.p. 85–90 °C,[5] 93–94.5 °C, $[\alpha]_D^{25}$ +23.3° (*c* 1.0, EtOH).[6]

N-Acetyl-3-(3-formyl-4-methoxyphenyl)-L-alanine ethyl ester (6). *Cf.* Ref. 5. To a solution of *5* (0.10 mol, 26.5 g) in dry CH_2Cl_2 (450 ml) at −18 °C was added dropwise $TiCl_4$ (74.5 ml) followed by dichloromethyl methyl ether (30 ml). After 2 h the solution was poured on ice (300 g) and HCl (300 ml 3 N). The organic phase was washed with water, saturated $NaHCO_3$, and water, dried over $CaCl_2$ and evaporated to dryness. Recrystallization from light petroleum (b.p. 60–80 °C) gave 0.717 mol (21.0 g, 72 %), m.p. 86–87 °C, $[\alpha]_D^{22}$ +27.2° (*c* 0.6, EtOH), TLC: R_F 0.76. Anal. $C_{15}H_{19}NO_5$: C, H, N. ^1H NMR ($CDCl_3$): δ 1.25 (3 H, t, $COOCH_2CH_3$), 1.95 (3 H, s, $COCH_3$), 3.06 (2 H, d, CH_2), 3.88 (3 H, s, OCH_3), 4.12 (2 H, q, $COOCH_2CH_3$), 4.76 (1 H, sextet, CH), 6.20 (1 H, d, NH), 6.76–7.50 (3 H, m, aromatic protons), 10.33 (1 H, s, CHO). Lit. value:[5–7] M.p. 85 °C, m.p. 100–101 °C, $[\alpha]_D^{25}$ +24.0° (*c* 1.0, H_2O).,

N-Acetyl-3-(3-carboxy-4-methoxyphenyl)-L-alanine (7). Moist Ag_2O prepared from $AgNO_3$ (20 mmol, 3.4 g) as described in the literature [11] was covered with water (20 ml) and NaOH pellets (3.4 mmol, 1.37 g) were added under vigorous stirring. The mixture was heated to 55 °C and *6* (6.8 mmol, 2.0 g) was added. After 10 min of stirring, the mixture was filtered, and the precipitated Ag washed with water. The filtrate was poured into ice-cold HCl (7.5 ml, conc.). The precipitated white solid was isolated by filtration and dried. Yield, 6.2 mmol (1.74 g, 91 %), m.p. 188 °C (decomp.), $[\alpha]_D^{22}$ +67.1° (*c* 0.6, phosphate buffer pH 7, 0.2 M). TLC: R_F 0.44. Anal. $C_{13}H_{15}NO_6$: C, H, N. ^1H NMR [$(CD_3)_2SO$]: δ 1.80 (3 H, s, $COCH_3$), 2.90 (2 H, d, CH_2), 3.75 (3 H, s, OCH_3), 4–5 (CH, 2COOH), 6.9–7.4 (3 H, m, aromatic protons), 7.90 (1 H, d, NH). MS: Probe temp. 150 °C; (*m/e* (% rel. int.): 281 (0.4, [M]), 264 (0.7, [M−OH]), 250 0.5, [M−OCH_3]), 236 (5.7, [M−COOH]), 222 (59, [M−CH_3CONH_2]), 165 (100, [M−CH_3-CONHCHCOOH]). Oxidation using standard

conditions[11] resulted in a yield of only about 50 %.

N-Acetyl-3-(3-carboxy-4-methoxyphenyl)-L-alanine diethyl ester (8). To a suspension of *7* (25 mmol, 7.03 g) suspended in absolute ethanol (150 ml) was added $SOCl_2$ (25 mmol, 2.97 g) dropwise under cooling and stirring. After 24 h at room temperature, excess ethanol was removed by evaporation. The crude ester was dissolved in benzene and purified on a column of silica gel (Kiselgel 60, Merck, 70–230 mesh, 83 g, 20 × 360 mm) by elution with benzene (200 ml) and benzene-ethanol (9:1, 200 ml). *8* was obtained as an oil by concentration. $[\alpha]_D^{26}$ +19.7° (*c* 1.0, EtOH), TLC: R_F 0.79. Found: C 61.93; H 6.98; N 3.91. Calc. for $C_{17}H_{23}NO_6$: C 60.52; H 6.87; N 4.15. ^{1}H NMR ($CDCl_3$): δ 1.32 (6 H, q, 2 $COOCH_2CH_3$), 2.02 (3 H, s, $COCH_3$), 3.12 (2 H, d, CH_2), 3.88 (3 H, s, OCH_3), 4.31 (4 H, octet, $2COOCH_2CH_3$), 4.86, (1 H, sextet, CH), 6.10 (1 H, d, NH), 6.8–7.4 (3 H, m, aromatic protons). MS: Probe temp. 70 °C: 337 (0.6, [M]), 292 (22, [M – OC_2H_5]), 278 (100, [M – H_2NCOCH_3]), 264 (2.5, [M – COC_2H_5]), 193 (100, [M – $CH_3CONHCHCOOC_2H_5$]) 141 (264 → 193). The main product of esterification of *7* with *p*-toluenesulfonic acid and ethanol in benzene was *10*.

N-Acetyl-3-(3-carboxy-4-hydroxyphenyl)-L-alanine diethyl ester (9). A solution of *8* (6.0 mmol, 2.20 g) in CH_2Cl_2 (50 ml) was added dropwise at −17 °C into a solution of BCl_3 in CH_2Cl_2 (60 ml containing 60 mmol of BCl_3). After 1 h at −17 °C and 22 h at room temperature, water (100 ml) was added to the solution at −17 °C. After separation of the phases the aqueous phase was extracted with CH_2Cl_2. The combined organic phases were washed with water, $NaHCO_3$-solution, and water, dried over $CaCl_2$ and evaporated to dryness. Crystallization from light petroleum (b.p. 60–80 °C) – benzene (4:1) afforded *9* (3.6 mmol, 1.18 g, 61 %), m.p. 99 °C, $[\alpha]_D^{26}$ +16.8° (*c* 1.0, EtOH), TLC: R_F 0.83. Anal. $C_{16}H_{21}NO_6$: C, H, N, ^{1}H NMR ($CDCl_3$): δ 1.35 (6 H, q, $COOCH_2CH_3$), 2.00 (3 H, s, $COCH_3$), 3.10 (2 H, d, CH_2), 4.28 (4 H, octet, $COOCH_2CH_3$), 4.81 (1 H, q, CH), 5.98 (1 H, d, NH), 6.7–7.4 (3 H, m, aromatic protons).

N-Acetyl-3-(3-carboxy-4-methoxyphenyl)-L-alanine monoethyl ester (10). To *6* (50 mmol, 14.7 g) in water (150 ml, 70–80 °C) was added a solution of $KMnO_4$ (75 mmol, 11.9 g) in water (200 ml) in a few portions. After 5 min, the solution was filtered and the MnO_2 washed with water. The filtrate was concentrated to 50 ml, cooled and conc. HCl added in excess. Extraction with CH_2Cl_2 with subsequent drying of the organic phase and concentration yielded a yellow oil which afforded *10* by crystallization from ethanol – light petroleum (b.p. 60–80 °C) (1:3). Yield 33.4 mmol (10.3 g, 67 %), m.p. 117–119 °C, $[\alpha]_D^{22}$ +27.0° (*c* 0.6, phosphate buffer pH 7, 0.2 M). TLC: R_F 0.69. Anal. $C_{15}H_{19}NO_6$: C, H, N. ^{1}H NMR ($CDCl_3$): δ 1.29 (3 H, t, $COOCH_2CH_3$), 2.03 (3 H, s, $COCH_3$), 3.13 (2 H, d, CH_2), 4.05 (3 H, s, OCH_3), 4.23 (2 H, q, $COOCH_2CH_3$), 4.85 (1 H, q, CH), 6.30 (1 H, d, NH), 6.9–7.9 (3 H, m, aromatic protons). MS: Probe temp. < 50 °C: 309 (0.3, [M]), 292 (22, [M – OH]), 276 (0.3, [M – OOH]), 264 (1.1, [M – COOH]), 250 (100, [M – CH_3CONH_2]), 236 (3.2, [M – $COOC_2H_5$]), 165 (77, [M – CH_3-$CONHCHCOOC_2H_5$]).

N-Acetyl-3-(3-carboxy-4-hydroxyphenyl)-L-alanine monoethyl ester (11). *1* (20 mmol, 6.19 g) in CH_2Cl_2 (100 ml) was added dropwise under stirring to a solution of BCl_3 in CH_2Cl_2 (200 ml containing 0.2 mol of BCl_3) at −17 °C. After 30 min water (100 ml) was added. The phases were separated, and the aqueous phase extracted with CH_2Cl_2. The combined organic phases were washed with water, dried over $CaCl_2$ and concentrated to dryness. Yield 5.2 g (88 %), m.p. 68–70 °C, $[\alpha]_D^{26}$ +24.2° (*c* 0.6, phosphate buffer pH 7, 0.2 M), TLC: R_F 0.71. Purification was accomplished by use of a silica gel column as described for *8*. Anal. $C_{14}H_{17}NO_6$: C, H, N. ^{1}H NMR ($CDCl_3$): δ 1.31 (3 H, t, $COOCH_2CH_3$), 2.09 (3 H, s, $COCH_3$), 3.14 (2 H, d, CH_2), 4.25 (2 H, q, $COOCH_2CH_3$), 4.92 (1 H, q, CH), 6.50 (1 H, d, NH), 6.8–7.7 (3 H, m, aromatic protons).

3-(3-Carboxy-4-hydroxyphenyl)-L-alanine (1) from 9. *9* (0.62 mmol, 200 mg) was refluxed in 4 N HCl (5 ml) for 1 h. After cooling of the solution pH was adjusted to 2.5 with ammonia. After a night in the refrigerator white crystals of *1* were collected. Yield 0.47 mmol (106 mg, 78 %). After recrystallization from water pure *1* was obtained with IR identical with that of authentic material.[1] $[\alpha]_D^{24}$ −30.5° (*c* 0.6, phosphate buffer pH 7, 0.2 M), m.p. 273 °C (decomp.). Lit. values:[1] $[\alpha]_D^{24}$ −29.9° (*c* 0.6, phosphate buffer pH 7, 0.2 M), m.p. 263–264 °C.

1 from *11*. *11* (500 mg) was refluxed in 4N HCl for 1 h. *1* was isolated as described above. Yield, 0.858 mmol (193 mg, 51 %). Recrystallization from water afforded pure *1*, $[\alpha]_D^{26}$ −29.8° (*c* 0.6, phosphate buffer), m.p. 272 °C. ^{1}H NMR [$(CD_3)_2SO$]: δ 3.04 (2 H, d, CH_2), 3.93 (1 H, q, CH), 6.6–7.7 (3 H, m, aromatic protons), 9.1 (5 H, broad s, OH, NH_3^+, COOH).

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