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AN EXPEDIENT SYNTHESIS OF 3-AMINO-5-HYDROXY-BENZOIC ACID AND ITS N-ALKYL ANALOGUES

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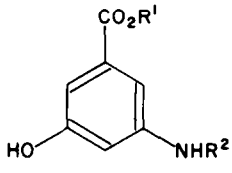
Abstract—The natural aromatic amino acid **1**, an intermediate in the biosynthesis of several important groups of antibiotics, and its N-alkyl derivatives are efficiently prepared by direct amination of 3,5-dihydroxybenzoic acid.

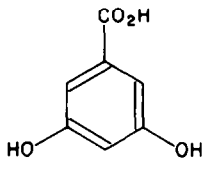
3-Amino-5-hydroxybenzoic acid **1** has recently been shown to be a naturally occurring amino acid[1] which functions as a key intermediate in the microbial biosynthesis of antibiotics of the ansamycin,[2-4] maytansinoid,[5] and mitomycin[6] groups. These groups of antibiotics provide several clinically important pharmaceuticals, such as the antitumor agent mitomycin C[7] and the antibacterial agent rifampicin, a synthetic modification of the ansamycin antibiotic rifamycin S.[8] The same amino acid **1** may also be involved in the biosynthesis of other types of antibiotic,[2] including ferrimycin A_1,[9] manumycin[10] and the related asukamycin[11] and U-62162.[12]

The molecular complexity of all these antibiotics is such that the quantities required for laboratory studies and clinical use can be produced only by fermentation, and not by total synthesis. In some fermentations, the production of the key precursor 3 - amino - 5 - hydroxybenzoic acid **1** may be limited, either nutritionally or genetically, to the extent that the full potential of the microorganism for antibiotic production may not be realized. In these circumstances, the addition of exogenous amino acid to the fermentation may increase the antibiotic yield. For example, such supplementation increased the production of the ansamycin actamycin[13] by a *Streptomyces* species several-fold even in a complex medium,[14] and restored the production of rifamycin B by a genetically impaired mutant of *Nocardia mediterranei* to the level of the parent strain.[4]

The use of 3 - amino - 5 - hydroxybenzoic acid **1** as a fermentation additive necessitates ready access to significant quantities of the material, particularly if applied on commercial scale. Published syntheses of the amino acid **1** all start from 3,5-dinitrobenzoic acid. The first synthesis by Bray *et al.* used partial reduction to differentiate the nitro groups, and proceeded in 1% yield over five steps.[15] This route was modified and improved by Bickel *et al.*[9] and by Ghisalba and Nüesch.[4] Herlt *et al.*[16] displaced one of the two nitro groups by methoxide ion, and achieved a 77% overall yield of the amino acid **1** in three steps. We now report the synthesis of the amino



	R^1	R^2
1:	H	H
2:	Me	H
3:	H	Me
4:	H	Et
5:	Me	Me



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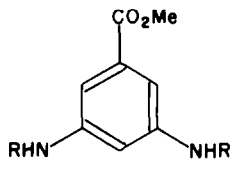
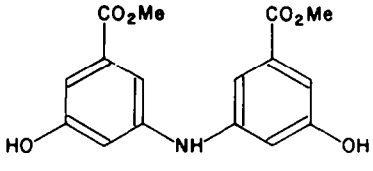


	R
7:	H
8:	Me



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acid **1** by direct amination of 3,5-dihydroxybenzoic acid **6**. The process uses cheap materials, proceeds in high yield without complex purification steps, and can be readily adapted to large scale production.

Resorcinol and its alkyl analogues yielded aminophenols when heated under pressure with a mixture of the amine (or ammonium hydroxide) and the amine hydrochloride, either alone[17] or under Bucherer conditions with added sodium bisulfite.[18] Furthermore, 3,5-dihydroxybenzoic acid **6** yielded diphenylamines when heated with an arylamine in the presence of zinc chloride or hydrochloric acid.[19] We have extended these findings, which arise from the potential ketonic character of resorcinols, to the reaction of the dihydroxy acid **6** with aqueous ammonium hydroxide and ammonium chloride. Heating under pressure at 180° for 40 hr gave a mixture consisting mainly of the amide and the ammonium salt of 3 - amino - 5 - hydroxybenzoic acid. Methanolysis of the mixture allowed the separation of methyl 3 - amino - 5 - hydroxybenzoate **2** in 75% yield, from the ester of starting material (10%), methyl 3, 5-diaminobenzoate **7** (7%) and dimethyl 5,5'-dihydroxy-3,3'-iminodibenzoate **9** (2%). Acid hydrolysis of the ester **2** gave 3 - amino - 5 - hydroxybenzoic acid as its hydrochloride salt in 95% yield, from which the free amino acid **1** can be recovered in 96% yield by precipitation at its isoelectric point. Alternatively, the original product mixture could be hydrolyzed directly to the required amino acid **1**, which was isolated without chromatography as its hydrochloride in 70% overall yield, together with starting material (14%).

Application of the Bucherer conditions[18] to this system was not advantageous, since not only mono- but also di-amination was promoted. Thus the addition of sodium bisulfite (1.2 eq; 0.11 eq had little effect) to the original reaction mixture resulted in complete consumption of starting material after only 22 hr at 180°. However, a complex mixture of amides and organic salts was formed, which upon methanolysis yielded a 4 : 3 mixture of methyl 3-amino-5-hydroxybenzoate **2** and methyl 3,5-diaminobenzoate **7** in 73% yield. Reduction of the reaction temperature to 140° for the same time merely reduced both amination rates, leaving starting material (36%) in addition to mono- and di-aminated products (6 : 1 *mixture*, 51%).

For studies of directed biosynthesis[20] of modified antibiotics, N-alkyl derivatives of the amino acid **1**, such as the N-Me and N-Et derivatives **3** and **4**, are required. These analogues can also be prepared by direct amination of 3,5-dihydroxybenzoic acid **6**, but the higher nucleophilicity of the reagent alkylamines necessitates modification of the conditions to avoid over-reaction. Reaction of the acid **6** with aqueous methylamine and methylamine hydrochloride under the conditions used with ammonium salts yielded equal amounts of 3-hydroxy-5-methylamino- and 3,5 - bis(methylamino) - substituted products, mainly as their methylamides. Further study established that the reaction proceeded slowly at 140° or below, and that, particularly at higher temperatures, methylamine salts (the hydrochloride or the benzoate) promoted over-amination which could be suppressed by omitting the methylamine hydrochloride and adding sodium carbonate or hydroxide. Ionization of the carboxyl group as its sodium salt also reduced amide formation dramatically. Thus reaction with aqueous methylamine in the presence of sodium carbonate at 180° for 16 hr gave after hydrolysis and methylation† the ester **5** of 3-hydroxy - 5 - methylaminobenzoic acid in 56% yield (77% allowing for recovered starting material). Under these amination conditions, starting material (29%) and 3,5-bis(methylamino)benzoic acid (10%) can be recovered as their methyl esters, but the use of a longer amination time in an attempt to achieve higher conversion to the desired product only increased the yield of the bis(methylamino)benzoate **8**. The free methylamino acid **3**, which in contrast to the amino acid **1** is extractable from water at pH 4, can be obtained in similar yield to the ester **5** directly from acid hydrolysis of the original amination mixture, or by acid or alkaline hydrolysis of the ester **5**.

The methyl ester of 3-ethylamino-5-hydroxybenzoic acid **4** can be prepared in an analogous manner in 66% yield (allowing for recovered starting material) from 3,5-dihydroxybenzoic acid **6** and ethylamine. Directed biosynthesis studies with these compounds are under way.

†Direct methanolysis failed to convert the methylamides to the corresponding methyl esters.

EXPERIMENTAL

^{1}H NMR spectra were determined on Varian HA-100 or Jeol FX-200 spectrometers, ^{13}C NMR spectra on a Bruker CXP-200 spectrometer. Mass spectra were run on GEC-AEI MS 902 or VG-Micromass 7070F instruments operating at 70 eV. M.ps were taken on a Kofler hot-stage apparatus. Microanalyses were carried out by the Analytical Service of the Australian National University.

Methyl 3-amino-5-hydroxybenzoate **2**

A mixture of **6** (2.0 g, 13 mmol), NH_4Cl (1.7 g, 32 mmol) and 28% aq NH_3 (6 ml) was heated in a steel bomb at 180° for 40 hr. After cooling the soln was evaporated to dryness and the residue taken up in MeOH (100 ml). Concd H_2SO_4 (3 ml) was added dropwise and the soln kept at reflux for 36 hr. The solvent was evaporated under reduced pressure, the residue taken up in ice-cold H_2O, and the aqueous soln extracted with Et_2O. The combined extracts were washed with cold 1N H_2SO_4, then with brine. Drying ($MgSO_4$) and evaporation of the Et_2O gave the methyl ester of starting material (220 mg, 10%) together with a small amount of **9** (70 mg, 2%) (Found: M^+ 317.0891, $C_{16}H_{15}NO_6$ requires: M^+ 317.0839); ^{1}H NMR (CD_3COCD_3) δ 8.5 (2H, bs, OH), 7.6 (1H, bs NH), 7.27 (2H, m, ArH), 7.03 (2H, m, ArH), 6.88 (2H, t, J = 2.2 Hz, H-4), 3.81 ($COOCH_3$).

The aqueous soln and H_2SO_4 washings were combined, adjusted to pH 7 with solid $NaHCO_3$ and extracted with EtOAc. The extracts were washed with brine, dried ($MgSO_4$) and evaporated. Flash chromatography on SiO_2 with EtOAc–CH_2Cl_2 (2 : 3) as eluant or recrystallization from MeOAc–$CHCl_3$ to remove **7** gave **2** (1.63 g, 75%), m.p. 125–127° (Found: C, 57.5; H, 5.42; N, 8.18; $C_8H_9NO_3$ requires: C, 57.5; H, 5.43; N, 8.38%); ^{1}H NMR (CD_3COCD_3) δ 8.16 (1H, bs, OH), 6.86 (1H, dd, J = 1.5 Hz, 2.0 Hz, H-2), 6.77 (1H, dd, J = 1.5 Hz, 2.2 Hz, 2.2 Hz, H-6), 6.42 (1H, t, J = 2.2 Hz, H-4), 4.80 (2H, bs, NH_2), 3.80 (3H, s, $COOCH_3$), assignment of ring protons based on NOE experiments; ^{13}C NMR (CD_3COCD_3) δ 167.7 (s, $COOCH_3$), 159.0 (s, C-5), 150.7 (s, C-3), 132.7 (s, C-1), 108.1 (d, J = 161 Hz, C-2 or C-6), 105.8 (d, J = 164 Hz, C-2 or C-6), 106.4 (d, J = 156 Hz, C-4, assignment based on selective decoupling of H-4), 52.1 (q, J = 147 Hz, $COOCH_3$); MS *m/z* 167 (M^+, 100%), 136 (M^+–OMe, 55), 109 (M^+–$C_2H_2O_2$, 35), 108 (M^+–COOMe, 35). From flash chromatography was also isolated **7** (162 mg, 7%), m.p. 123–126° after sublimation. ^{1}H NMR (CD_3COCD_3) δ 6.62 (2H, d, J = 2.5 Hz, H-2 and H-6), 6.19 (1H, t, J = 2.5 Hz, H-4), 4.42 (4H, bs, NH_2), 3.75 (3H, s, $COOCH_3$); MS *m/z* 166 (M^+, 100%), 135 (M^+–OMe, 25), 108 (M^+–$C_2H_2O_2$, 50), 107 (M^+–COOMe, 42).

3-Amino-5-hydroxybenzoic acid hydrochloride

(a) *From acid* **6**. The acid **6** was reacted with NH_4Cl and NH_4OH as described. The mixture was evaporated to dryness and the residue taken up in 6N HCl (100 ml). The soln was kept at reflux for 16 hr, filtered and concentrated (ca. 25 ml). On cooling 3 - amino - 5 - hydroxybenzoic acid hydrochloride (1.72 g, 70%), pure by ^1H NMR, was collected as greyish crystals. Treatment with charcoal and recrystallization from 6N HCl gave the hydrochloride of **1** as white crystals, m.p. 200–230° (dec), identical by spectroscopic and mixed m.p. comparison with an authentic sample.[16] Extraction with EtOAc of the mother liquor of the original crystallization led to the recovery of starting material (288 mg, 14%).

(b) *From ester* **2**. The ester **2** (200 mg, 1.2 mmol) in 6N HCl (5 ml) was heated at reflux for 2 hr. The soln on cooling deposited crystalline, analytically pure 3 - amino - 5 - hydroxybenzoic acid hydrochloride (215 mg, 95%), identical with the material prepared above.

3-Amino-5-hydroxybenzoic acid **1**

To 3 - amino - 5 - hydroxybenzoic acid hydrochloride (47 mg, 0.25 mmol) in H_2O (0.8 ml) was added 2N NaOH dropwise with stirring until the soln reached pH 4. On cooling crystalline **1** (37 mg, 96%) was obtained, identical by spectroscopic and mixed m.p. comparison with an authentic sample.[16]

Methyl 3-hydroxy-5-methylaminobenzoate **5**

The acid **6** (2.0 g, 13 mmol) was added under vigorous stirring to a soln of Na_2CO_3 (1.033 g, 9.75 mmol) in H_2O (5 ml). Stirring was continued until no further CO_2 evolved (4 hr). After addition of 40% $MeNH_2$ (5 ml) under ice-cooling, the soln was heated in a steel bomb at 180° for 16 hr. The cooled soln was evaporated to dryness, the residue taken up in 6N HCl (100 ml), and the soln heated at reflux for 36 hr. After removal of the solvent under vacuum, the residue was taken up in MeOH (100 ml). Concd H_2SO_4 (3 ml) was added dropwise and the soln kept at reflux for 16 hr. The residue remaining on evaporation was taken up in ice-cold H_2O and extracted with Et_2O. The combined extracts were washed with cold 1N H_2SO_4, then with brine. Drying ($MgSO_4$) and evaporation of the Et_2O gave the methyl ester of starting material (633 mg, 29%).

The aq soln and H_2SO_4 washings were combined, adjusted to pH 7 with solid $NaHCO_3$ and extracted with Et_2O. The dried extracts were evaporated and subjected to flash chromatography on SiO_2 in MeOH–CH_2Cl_2 (5 : 95) to give **5** (1.32 g, 56%), m.p. 51–53° from $CHCl_3$ (Found: C, 59.5; H, 6.27; N, 7.49; $C_9H_{11}NO_3$ requires: C, 59.7; H, 6.12; N, 7.73%); ^1H NMR (CD_3COCD_3) δ 8.18 (1H, bs, OH), 6.78 (2H, bd, H-2 and H-6), 6.31 (1H, bt, H-4), 5.06 (1H, bs, NH), 3.80 (3H, s, $COOCH_3$), 2.78 (3H, d, J = 5 Hz, $NHCH_3$); MS *m/z* 181 (M^+, 100%), 180 (M^+–H, 19), 150 (M^+–OMe, 28), 123 (M^+–$C_2H_2O_2$, 41), 122 (M^+–COOMe, 43).

3-Hydroxy-5-methylaminobenzoic acid **3**

(a) *From acid* **6**. Reaction of **6** with $MeNH_2$ was carried out as described using a temp of 170°. Following hydrolysis in 6N HCl the acidic soln was extracted with EtOAc. The combined organic phases were washed with cold 1N HCl, then with brine. Drying ($MgSO_4$) and evaporation of the organic solvent gave starting material (962 mg, 48%).

The combined aq solns were adjusted to pH 4–5 with solid $NaHCO_3$, saturated with NaCl and extracted with EtOAc. Evaporation of the dried extracts gave **3** (1.052 g, 48%), m.p. 205–208° (dec) after recrystallization from EtOAc–hexane (Found: C, 57.6; H, 5.48; N, 8.13; $C_8H_9NO_3$ requires: C, 57.5; H, 5.43; N, 8.38%); ^1H NMR (CD_3OD) δ 6.82 (2H, m, H-2 and H-6), 6.29 (1H, bt, H-4), 2.75 (3H, s, $NHCH_3$); MS *m/z* 167 (M^+, 100%), 166 (M^+–H, 85), 122 (M^+–COOH, 12).

(b) *Via ester* **5**. Reaction of **6** with $MeNH_2$, followed by hydrolysis and esterification was carried out as described for the preparation of **5**. The dried Et_2O extracts from the initial fraction, however, were not evaporated but extracted with cold 2N NaOH, washed with brine, dried (Na_2SO_4) and evaporated to give **8** (254 mg, 10%), m.p. 79–82° after sublimation (Found: C, 61.7; H, 7.32; N, 14.2; $C_{10}H_{14}N_2O_2$ requires: C, 61.8; H, 7.27; N, 14.4%); ^1H NMR (CD_3COCD_3) δ 6.58 (2H, d, J = 2 Hz, H-2 and H-6), 6.05 (1H, t, J = 2 Hz, H-4), 4.82 (2H, bs, NH), 3.77 (3H, s, $COOCH_3$), 2.75 (6H, d, J = 5 Hz, $NHCH_3$); MS *m/z* 194 (M^+, 100%), 193 (M^+–H, 10), 163 (M^+–OMe, 8), 136 (M^+–$C_2H_2O_2$, 33), 135 (M^+–COOMe, 25).

The NaOH aq extracts were then acidified to pH 5 under ice-cooling with concd H_3PO_4, saturated with NaCl, and extracted with EtOAc. Drying (Na_2SO_4) and evaporation of the solvent gave **3** (1.17 g, 54%), identical with the material prepared above.

(c) *From ester* **5**. The ester **5** (500 mg, 2.76 mmol) in 6N HCl (4 ml) was heated at reflux for 14 hr. The crystals obtained on cooling were filtered and dried to give 3-hydroxy-5-methyl-aminobenzoic acid hydrochloride (512 mg, 91%), m.p. 190–193° (dec); ^1H NMR (CD_3OD) δ 7.58 (2H, m, H-2 and H-6), 7.19 (1H, m, H-4), 3.07 (3H, s, $NHCH_3$); MS *m/z* 167 (M^+, 100%), 166 (M^+–H, 93), 122 (M^+–COOH, 12).

To the hydrochloride (500 mg, 2.5 mmol) in H_2O (7 ml) was added 2N NaOH until the soln reached pH 4. Saturation with NaCl and extraction with EtOAc gave **3** (363 mg, 88%) identical with the material described above.

Methyl 3-ethylamino-5-hydroxybenzoate

Acid **6** (2.0 g, 13 mmol) and Na_2CO_3 (1.033 g, 9.75 mmol) in H_2O (6 ml) were reacted with $EtNH_2$ (5.1 ml) as described for the preparation of **5**. Hydrolysis with HCl, esterification with MeOH and H_2SO_4 followed by work-up gave methyl 3,5-dihydroxybenzoate (1.05 g, 48%) and, after flash chromatography on SiO_2 in EtOAc–CH_2Cl_2 (1:6), methyl 3,5-bis(ethylamino)benzoate (57 mg, 2%), m.p. 79–80° from $CHCl_3$-hexane (Found: C, 64.7; H, 8.30; N, 12.5; $C_{12}H_{18}N_2O_2$ requires: C, 64.8; H, 8.16; N, 12.6%); ^1H NMR (CD_3COCD_3) δ 6.59 (2H, d, J = 2.5 Hz, H-2 and H-6), 6.11 (1H, t, J = 2.5 Hz, H-4), 4.70 (2H, bs, NH), 3.79 (3H, s, $COOCH_3$), 3.15 (4H, bq, J = 7 Hz, $NHCH_2CH_3$), 1.22 (6H, t, J = 7 Hz, CH_2CH_3); MS *m/z* 222 (M^+, 67%), 207 (M^+–Me, 100), 191 (M^+–OMe, 10), 164 (M^+–$C_2H_2O_2$, 6), 163 (M^+–COOMe, 6). The main component eluted from flash chromatography was methyl 3 - ethylamino - 5 - hydroxybenzoate (940 mg, 37%), m.p. 107–108° from $CHCl_3$ (Found: C, 61.2; H, 6.82; N, 7.00; $C_{10}H_{13}NO_3$ requires: C, 61.5; H, 6.71; N, 7.18%); ^1H NMR (CD_3COCD_3) δ 8.14 (1H, s, OH), 6.80 (2H, m, H-2 and H-6), 6.34 (1H, m, H-4), 4.95 (1H, bs, NH), 3.81 (3H, s, $COOCH_3$), 3.15 (2H, dq, J = 5 Hz and J = 7 Hz, $NHCH_2CH_3$), 1.24 (3H, t, J = 7 Hz, CH_2CH_3); MS *m/z* 195 (M^+, 75), 180 (M^+–Me, 100), 164 (M^+–OMe, 12), 136 (M^+–COOMe, 10).

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