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3-Amino-5-hydroxybenzoic Acid in Antibiotic Biosynthesis. VIII* Synthesis of Chlorinated Analogues

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Abstract

The three isomeric ring monochlorinated derivatives of 3-amino-5-hydroxybenzoic acid (1), required for studies of the biosynthesis and synthesis of several important classes of antibiotics, are prepared from methyl 3-amino-5-hydroxybenzoate (5). *N*-Chlorosuccinimide selectively monochlorinates the 2- and 6-positions of this substrate, whilst perchlorination followed by hydrolysis and regiospecific protodechlorination at the 2- and 6-positions affords the 4-chloro acid.

Introduction

3-Amino-5-hydroxybenzoic acid (1), recently shown to be a naturally occurring amino acid,[1] has been established as the key biogenetic precursor of the aromatic or quinonoid nuclei in antibiotics of the ansamycin,[2–4] mitomycin[5] and maytansinoid[6] groups. The maytansinoids, originally obtained from higher plants[7] and more recently from microorganisms,[8] contain the amino acid in its least modified form. Thus in

* Part VII, *Tetrahedron*, 1983, **39**, 4189. (Part VII was inadvertently referred to as Part V in *J. Antibiot.*, 1983, **36**, 1323.)

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[2] Kibby, J. J., McDonald, I. A., and Rickards, R. W., *J. Chem. Soc., Chem. Commun.*, 1980, 768.
[3] Ghisalba, O., Fuhrer, H., Richter, W. J., and Moss, S., *J. Antibiot.*, 1981, **34**, 58; Ghisalba, O., and Nüesch, J., *J. Antibiot.*, 1981, **34**, 64.
[4] Rinehart, K. L., Potgieter, M., Jin, W.-Z., Pearce, C. J., Wright, D. A., Wright, J. L. C., Walter, J. A., and McInnes, A. G., in 'Trends in Antibiotic Research. Genetics, Biosyntheses, Actions and New Substances' (Eds H. Umezawa, A. L. Demain, T. Hata and C. R. Hutchinson) p. 171 (Japan Antibiotics Research Association: Tokyo 1982).
[5] Anderson, M. G., Kibby, J. J., Rickards, R. W., and Rothschild, J. M., *J. Chem. Soc., Chem. Commun.*, 1980, 1277.
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[7] Komoda, Y., and Kishi, T., in 'Anticancer Agents Based on Natural Product Models' (Eds J. M. Cassady and J. D. Douros) p. 353 (Academic Press: New York 1980); Kupchan, S. M., Komoda, Y., Branfman, A. R., Sneden, A. T., Court, W. A., Thomas, G. J., Hintz, H. P. J., Smith, R. M., Karim, A., Howie, G. A., Verma, A. K., Nagao, Y., Dailey, R. G., Jr, Zimmerly, V. A., and Sumner, W. C., Jr, *J. Org. Chem.*, 1977, **42**, 2349.
[8] Higashide, E., Asai, M., Ootsu, K., Tanida, S., Kozai, Y., Hasegawa, T., Kishi, T., Sugino, Y., and Yoneda, M., *Nature (London)*, 1977, **270**, 721; Asai, M., Mizuta, E., Izawa, M., Haibara, K., and Kishi, T., *Tetrahedron*, 1979, **35**, 1079.

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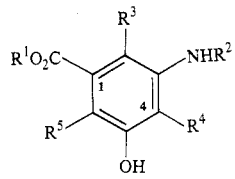
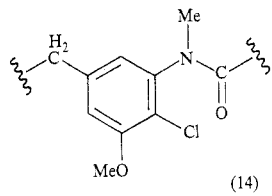
the course of the biosynthesis of maytansine itself, which has the partial structure (14), the amino acid (1) has been modified by ring chlorination, *N*- and *O*-methylation, alkylation and reduction of its carboxy group, and *N*-acylation, but retains its benzenoid nucleus. More extensive modifications occur during its conversion into the complex nuclei of the ansamycin and mitomycin groups. With regard to the present work, however, it is notable that chlorination at what was originally the C4 position of the amino acid (1) also occurs in several ansamycins of the naphthomycin type,[9] which have the partial structure (15) carrying a 3-chloro substituent.

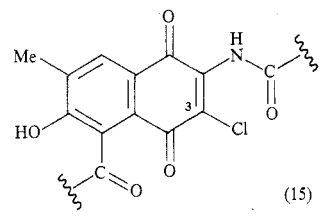


	R^1	R^2	R^3	R^4	R^5
(1)	H	H	H	H	H
(2)	H	H	Cl	H	H
(3)	H	H	H	Cl	H
(4)	H	H	H	H	Cl
(5)	Me	H	H	H	H
(6)	H	Ac	H	H	H
(7)	H	Ac	Cl	Cl	Cl

	R^1	R^2	R^3	R^4	R^5
(8)	Me	H	Cl	H	H
(9)	Me	H	H	H	Cl
(10)	Me	H	Cl	H	Cl
(11)	Me	H	Cl	Cl	Cl
(12)	Me	Cl	Cl	Cl	Cl
(13)	Me	H	H	Cl	H



(14)



(15)

Little is known as to when such modifications occur to the amino acid skeleton during its conversion into these antibiotics. Directed biosynthesis experiments in these laboratories[10] with 4-chloro and *N*-methyl substituted 3-amino-5-hydroxybenzoic acids, and mutasynthesis experiments by Traxler and Ghisalba[11] with the 4-hydroxy and 4-methyl derivatives of the same amino acid (1), failed in each case to yield the appropriately substituted actamycin or rifamycin. These results indicated that the corresonding chloro [as in (14) and (15)], hydroxy, methyl and *N*-methyl substituents when present in the nuclei of ansamycins and maytansinoids are introduced at biosynthetic stages beyond the level of 3-amino-5-hydroxybenzoic acid (1).

Amongst the derivatives of the amino acid (1) of particular interest for directed biosynthesis studies with ansamycin, maytansinoid and mitomycin antibiotics were

[9] Keller-Schierlein, W., Meyer, M., Zeeck, A., Damberg, M., Machinek, R., Zähner, H., and Lazar, G., *J. Antibiot.*, 1983, **36**, 484, and references therein.

[10] Becker, A. M., Herlt, A. J., Hilton, G. L., Kibby, J. J., and Rickards, R. W., *J. Antibiot.*, 1983, **36**, 1323.

[11] Traxler, P., and Ghisalba, O., *J. Antibiot.*, 1982, **35**, 1361.

the 2-, 4-, and 6-chloro isomers (2), (3) and (4).* Efficient access to the 4-chloro isomer (3) would also be of value in connection with the total synthesis of maytansinoids.[12] Tetrasubstituted aromatics of the type (3), carrying the unusual array of three different contiguous hetero-substituents, constitute important synthons for the so-called 'western zone' (14) of maytansine. Four previous routes to such substituted aromatics have been published. Those of Kane and Meyers[13] and Götschi *et al.*[14] adjust the functionality on the aromatic ring of methyl and ethyl vanillate, respectively, whilst those of Corey *et al.*[15] and Foy and Ganem[16] proceed via dihydroaromatic systems and require subsequent aromatization steps. Only the first three of these routes yield products in which the benzylic carbon is at the oxidation level of carboxyl, and of these only the aromatic routes of Kane and Meyers[13] and Götschi *et al.*[14] are readily adaptable to preparation of the unmethylated 3-amino-4-chloro-5-hydroxybenzoic acid (3) required in the present work.

In view of the ready availability of 3-amino-5-hydroxybenzoic acid (1) and its methyl ester (5) from amination of 3,5-dihydroxybenzoic acid,[17] we have examined the chlorination of these substrates. We describe here the synthesis of the three required monochloro isomers (2), (3) and (4) of the amino acid (1). Further biosynthetic studies[18] with these compounds will be reported elsewhere.

Synthesis of the Chlorinated Amino Acids

Since the various monochloro isomers were required on preparative scale, we sought selective chlorination processes which would involve the minimum of protection–deprotection steps. Direct chlorination of the free amino acid (1) with chlorine in acetic acid led to complex mixtures containing chlorinated and unchanged materials and decomposition products. As expected, the hydrochloride salt of the amino acid (1), which would form in the course of the reaction, was inert under these chlorination conditions. The addition of sodium acetate as a buffer to prevent such protection of the starting material promoted decomposition but not chlorination. Decomposition was reduced by prior conversion of the amino acid into its *N*-acetyl derivative (6), but attempted monochlorination with chlorine gave mixtures of mono-, di- and tri-chlorinated products together with starting material. The methyl ester (5) behaved similarly with chlorine, sulfuryl chloride or t-butyl hypochlorite. The *N*-acetyl derivative (6) with excess of chlorine afforded the trichlorinated product (7).

* IUPAC nomenclature rules require the acid (4) and its ester (9) to be named as 5-amino-2-chloro-3-hydroxybenzoic acid and benzoate, and these designations will be used in the Experimental. The '6-chloro isomer' terminology will be used in the Discussion to avoid confusion which would otherwise arise from reversing the basic 3-amino-5-hydroxybenzoic acid numbering for these two compounds.

[12] Meyers, A. I., Babiak, K. A., Campbell, A. L., Comins, D. L., Fleming, M. P., Henning, R., Henschmann, M., Hudspeth, J. P., Kane, J. M., Reider, P. J., Roland, D. M., Shimizu, K., Tomioka, K., and Walkup, R. D., *J. Am. Chem. Soc.*, 1983, **105**, 5015, and references therein.

[13] Kane, J. M., and Meyers, A. I., *Tetrahedron Lett.*, 1977, 771.

[14] Götschi, E., Schneider, F., Wagner, H., and Bernauer, K., *Helv. Chim. Acta*, 1977, **60**, 1416; Götschi, E., Schneider, F., Wagner, H., and Bernauer, K., *Org. Prep. Proced. Int.*, 1981, **13**, 23.

[15] Corey, E. J., Wetter, H. F., Kozikowski, A. P., and Rama Rao, A. V., *Tetrahedron Lett.*, 1977, 777.

[16] Foy, J. E., and Ganem, B., *Tetrahedron Lett.*, 1977, 775.

[17] Becker, A. M., Rickards, R. W., and Brown, R. F. C., *Tetrahedron*, 1983, **39**, 4189.

[18] Becker, A. M., and Rickards, R. W., unpublished data.

Reaction of the methyl ester (5) with 1·1 equivalents of *N*-chlorosuccinimide at −40°, however, afforded selectively the 2- and 6-chloro isomers (8) and (9) in yields of 35% and 45% after separation by medium pressure liquid chromatography, together with a small amount of methyl 3-amino-2,6-dichloro-5-hydroxybenzoate (10). Hydrolysis to the required monochloro amino acids (2) and (4) proceeded smoothly in refluxing hydrochloric acid.

If the methyl ester (5) was treated with an excess of *N*-chlorosuccinimide at room temperature, only two products, the 2,4,6-trichloro- and *N*,2,4,6-tetrachloro-benzoates (11) and (12), were formed in 65% and 12% yield respectively. Treatment of these perchlorinated products, either alone or as the mixture, with refluxing aqueous hydrobromic acid effected ester hydrolysis and selective dechlorination to afford the third required isomer, 3-amino-4-chloro-5-hydroxybenzoic acid (3), in 80% yield. Other strong acids at elevated temperatures, such as trifluoroacetic acid at 60°, aqueous hydrochloric acid at reflux, trifluoromethanesulfonic acid at 130°, or concentrated sulfuric acid at 160°, did not effect dechlorination. The observed protodechlorination[19] involves electrophilic aromatic substitution of chlorine by protons, and reduction by nucleophilic bromide ions is required to assist removal of the two chlorine substituents. The remaining 4-chloro substituent is probably the most thermodynamically stable of the ring chlorine atoms, relative to the 2- and 6-chloro substituents which were both introduced and removed preferentially.

Structures of the Chlorinated Amino Acids

Differences in both ^{1}H and ^{13}C n.m.r. chemical shifts between the three isomeric monochloro amino acids were small, and did not permit assignment of the structures (2), (3) and (4) to the specific products obtained as above. The structure of the 4-chloro acid (3) could be confirmed by demethylation of methyl 3-amino-4-chloro-5-methoxybenzoate, prepared from methyl vanillate by the method of Kane and Meyers.[13] The position of the chlorine substituents in all three isomeric acids could, however, be assigned unambiguously by the nuclear Overhauser enhancement difference technique.[20] The ^{1}H n.m.r. spectrum of the methyl ester of each isomer was recorded in (D_6)acetone at −15° to prevent exchange of its OH and NH_2 protons, which were then irradiated in turn whilst observing the intensity of the aromatic protons. The substitution pattern of compound (9), for example, follows from irradiation of its OH proton signal at δ 8·45 when only one of the two aromatic proton signals is enhanced, whereas both are enhanced upon irradiation of the NH_2 proton signal at 4·99. The position of the chlorine atoms in the other two isomeric monochloro methyl esters (8) and (13) were assigned similarly with equal certainty. The technique was also used to establish the structure of the 2,6-dichloro methyl ester (10), and to assign specific chemical shifts to the three aromatic protons in methyl 3-amino-5-hydroxybenzoate itself (5). Where intensity enhancements occurred, they were in all cases substantial and in the range 11–27%.

[19] De la Mare, P. B. D., and Swedlund, B. E., in 'The Chemistry of the Carbon–Halogen Bond' (Ed. S. Patai) Part 1, p. 407 (John Wiley: London 1973).
[20] Richarz, R., and Wüthrich, K., *J. Magn. Reson.*, 1978, **30**, 147; Kotovych, G., and Aarts, G. H. M., *Can. J. Chem.*, 1980, **58**, 2649.

Experimental

Melting points were determined on a Kofler stage and are uncorrected. For flash chromatography silica gel (Merck, 230–400 mesh) was used. N.m.r. spectra were recorded on Varian HA-100 (^{1}H) and Bruker CXP-200 (^{1}H and ^{13}C) spectrometers, with tetramethylsilane as internal reference. Nuclear Overhauser enhancement experiments were carried out at −15° on the latter instrument. Chemical shifts at this temperature differ slightly from the reported room temperature spectra. Mass spectra were run on GEC–AEI MS 902 or VG-Micromass 7070F spectrometers operating at 70 eV. Elemental analyses were carried out by the Analytical Services Unit of the Australian National University.

3-Acetylamino-5-hydroxybenzoic Acid (*6*)

To 3-amino-5-hydroxybenzoic acid hydrochloride (948 mg, 5 mmol) in water (10 ml) was added acetic anhydride (4 ml) under ice-cooling. After 1 h at room temperature the white precipitate was filtered, washed and dried to give the amorphous N-*acetyl derivative* (6) (927 mg, 95%), m.p. 245–253° from methanol/water (Found: $M^{+\cdot}$, 195·0527. $C_9H_9NO_4$ requires $M^{+\cdot}$, 195·0532). ^{1}H n.m.r. (CD_3OD) δ 7·59, m, H2; 7·45, m, H6; 7·39, m, H4; 2·11, s, $COCH_3$. Mass spectrum: *m/z* 195 (M, 35%), 153 (M−CH_2CO, 100), 136 (M−CH_2CO−OH, 10).

3-Acetylamino-2,4,6-trichloro-5-hydroxybenzoic Acid (*7*)

To 3-acetylamino-5-hydroxybenzoic acid (6) (5 mg, 0·026 mmol) in acetic acid (1 ml) was added chlorine in acetic acid (1 M, 0·2 ml) with stirring at room temperature. After 50 min the excess of chlorine was removed and the solvent evaporated to give *3-acetylamino-2,4,6-trichloro-5-hydroxybenzoic acid* (7) (6 mg, 77%) (Found: $M^{+\cdot}$, 296·9362. $C_9H_6Cl_3NO_4$ requires $M^{+\cdot}$, 296·9362). ^{1}H n.m.r. (CD_3OD) δ 2·16, s, $COCH_3$. Mass spectrum: *m/z* 301/299/297 (M, 1, 2, 2%), 265/263/261 (M−HCl, 7, 36, 55), 259/257/255 (M−CH_2CO, 33, 96, 100), 215/213/211 (M−CH_2CO−CO_2, 6, 20, 21).

Methyl 3-Amino-5-hydroxybenzoate (*5*)

The ester (5) was prepared by the method of Becker *et al.*[17] ^{1}H n.m.r. (CD_3COCD_3) δ 8·16, bs, OH; 6·86, dd, *J* 1·5 and 2·2 Hz, H2; 6·77, dd, *J* 1·5 and 2·2 Hz, H6; 6·42, t, *J* 2·2 Hz; 4·80, bs, NH_2; 3·80, s, $COOCH_3$; OH irradiated, H4 +11%, H6 +15%; NH_2 irradiated, H4 +19%, H2 +24%.

Methyl 3-Amino-2-chloro-5-hydroxybenzoate (*8*) *and Methyl 5-Amino-2-chloro-3-hydroxybenzoate* (*9*)

To methyl 3-amino-5-hydroxybenzoate (5) (6·0 g, 36 mmol) in acetonitrile at −40° (100 ml) was added *N*-chlorosuccinimide (5·29 g, 39·6 mmol) in acetonitrile (120 ml) over 30 min. After an additional 30 min at −40°, the solution was evaporated and the residue suspended in dichloromethane/ethyl acetate (6 : 1). Insoluble succinimide was removed and the filtrate subjected to flash chromatography in the same solvent system to give as the first eluted component *methyl 3-amino-2,6-dichloro-5-hydroxybenzoate* (*10*) (1·00 g, 12%), m.p. 146–148° from ether/light petroleum (b.p. 60–80°) (Found: C, 40·6; H, 2·9; Cl, 30·0; N, 5·7; $M^{+\cdot}$, 234·9801. $C_8H_7Cl_2NO_3$ requires C, 40·7; H, 3·0; Cl, 30·0; N, 5·9%; $M^{+\cdot}$, 234·9803). ^{1}H n.m.r. (CD_3COCD_3) δ 9·26, bs, OH; 6·63, s, H4; 5·40, bs, NH_2; 3·92, s, $COOCH_3$; OH irradiated, H4 +24%; NH_2 irradiated, H4 +14%. ^{13}C n.m.r. (CD_3COCD_3) δ 166·1, q, *J* 4 Hz, $COOCH_3$; 153·4, d, *J* 3·5 Hz, C5; 145·5, d, *J* 1·5 Hz, C3; 135·3, s, C1; 106·5, m, C2 or C6; 105·8, d, *J* 9 Hz, C2 or C6; 103·6, dt, J_d 161, J_t 5·6 Hz, C4; 52·9, q, *J* 148 Hz, $COOCH_3$. Mass spectrum: *m/z* 239/237/235 (M, 10, 62, 100%), 208/206/204 (M−OMe, 10, 59, 88), 180/178/176 (M−COOMe, 2, 14, 21), 170 (11), 112 (11).

The mixture of monochloro compounds which eluted after the dichloro ester (10) was subjected to medium pressure liquid chromatography on silica (Merck LiChroprep Si 60) in ethyl acetate/light petroleum (b.p. 40–60°, 6 : 1) to give first *methyl 3-amino-2-chloro-5-hydroxybenzoate* (8) (2·52 g, 35%), m.p. 143–146° from acetone/ether/light petroleum (b.p. 60–80°) (Found: C, 47·4; H, 4·0; Cl, 17·6; N, 6·9; $M^{+\cdot}$, 201·0195. $C_8H_8ClNO_3$ requires C, 47·7; H, 4·0; Cl, 17·6; N, 6·9%;

$M^{\cdot+}$, 201·0193). ^1H n.m.r. (CD_3COCD_3) δ 8·40, bs, OH; 6·54, narrow AB system, lower-field doublet H4, higher-field doublet H6; 5·06, bs, NH_2; 3·84, s, $COOCH_3$; OH irradiated, H4 +18%, H6 +18%; NH_2 irradiated, H4 +20%. ^{13}C n.m.r. (CD_3COCD_3) δ 167·2, m, $COOCH_3$; 157·1, t, J 3 Hz, C5; 147·2, s, C3; 132·9, s, C1; 108·0, m, C2; 107·1, dd, J 165 and 6 Hz, C4 or C6; 105·2, dq, J_d 159, J_q 6 Hz, collapsed to dd on addition of D_2O, J_d 159 and 6 Hz, C4 or C6; 52·4, q, J 148 Hz, $COOCH_3$. Mass spectrum: m/z 203/201 (M, 33, 100%), 172/170 (M−OMe, 27, 76), 145/143 (M−$C_2H_2O_2$, 6, 19), 144/142 (M−COOMe, 12, 31), 116/114 (M−COOMe−CO, 4, 10).

The second component to elute was *methyl 5-amino-2-chloro-3-hydroxybenzoate* (9) (3·26 g, 45%), m.p. 85–88° from ether/light petroleum (b.p. 60–80°) (Found: C, 47·8; H, 4·0; Cl, 17·5; N, 6·8; $M^{+\cdot}$, 201·0199. $C_8H_8ClNO_3$ requires C, 47·7; H, 4·0; Cl, 17·6; N, 6·9%; $M^{+\cdot}$, 201·0193). ^1H n.m.r. (CD_3COCD_3) δ 8·32, bs, OH; 6·63, d, J 2·6 Hz, H6; 6·52, d, J 2·6 Hz, H4; 4·84, bs, NH_2; 3·83, s, $COOCH_3$; OH irradiated, H4 +17%; NH_2 irradiated, H4 +20%, H6 +27%. ^{13}C n.m.r. (CD_3COCD_3) δ 167·3, q, J 4 Hz, $COOCH_3$; 154·9, d, J 3 Hz, C3; 148·8, s, C5; 132·9, s, C1; 109·0, dd, J 163 and 6 Hz, C4 or C6; 107·0, s, C2; 105·4, dd, J 158 and 6 Hz, C4 or C6; 52·3, q, J 147 Hz, $COOCH_3$. Mass spectrum: m/z 203/201 (M, 32, 100%), 172/170 (M−OMe, 27, 78), 145/143 (M−$C_2H_2O_2$, 5, 16), 144/142 (M−COOMe, 13, 36), 116/114 (M−COOMe−CO, 4, 10).

3-Amino-2-chloro-5-hydroxybenzoic Acid (*2*)

The ester (8) (1·30 g, 6·4 mmol) in aqueous hydrochloric acid (6 N, 40 ml) was kept at reflux for 16 h. On cooling the *hydrochloride of the acid* (2) (1·43 g, 99%) crystallized, m.p. 228–232° (dec.) from aqueous hydrochloric acid (6 N) (Found: $M^{+\cdot}$ of acid, 187·0036. $C_7H_6ClNO_3$ requires $M^{+\cdot}$, 187·0036). ^1H n.m.r. (CD_3OD) δ 7·30, d, J 2·9 Hz, H6; 7·11, d, J 2·9 Hz, H4. Mass spectrum: m/z 189/187 (M of acid, 32, 100%), 172/170 (M−OH, 6, 17), 144/142 (M−COOH, 4, 11).

5-Amino-2-chloro-3-hydroxybenzoic Acid (*4*)

Hydrolysis of the ester (9) as described for the ester (8) afforded the *hydrochloride of the acid* (4) (98%), m.p. 267–269° (dec.) from aqueous hydrochloric acid (6 N) (Found: $M^{+\cdot}$ of acid, 187·0036. $C_7H_6ClNO_3$ requires $M^{+\cdot}$, 187·0036). ^1H n.m.r. (CD_3OD) δ 7·29, d, J 2·6 Hz, H6; 7·15, d, J 2·6 Hz, H4. Mass spectrum: m/z 189/187 (M of acid, 33, 100%), 172/170 (M−OH, 5, 15), 144/142 (M−COOH, 4, 13).

Methyl 3-Amino-2,4,6-trichloro-5-hydroxybenzoate (*11*)

To methyl 3-amino-5-hydroxybenzoate (5) (3·00 g, 18 mmol) in acetonitrile (50 ml) was added *N*-chlorosuccinimide (9·61 g, 72 mmol) in acetonitrile (50 ml) at 15–20°. After 2 h further *N*-chlorosuccinimide (1·20 g, 9 mmol) was added and stirring continued for 2 h. The solvent was evaporated, the residue extracted with dichloromethane/ethyl acetate (12 : 1) and the extract subjected to flash chromatography in the same solvent system to yield *methyl 3-amino-2,4,6-trichloro-5-hydroxybenzoate* (11) (3·15 g, 65%), m.p. 100–102° from dichloromethane/carbon tetrachloride (Found: C, 35·4; H, 2·1; Cl, 39·1; N, 5·0. $C_8H_6Cl_3NO_3$ requires C, 35·5; H, 2·2; Cl, 39·3; N, 5·2%). ^1H n.m.r. (CD_3COCD_3) δ 9·14, s, OH; 5·48, bs, NH_2; 3·94, s, $COOCH_3$. Mass spectrum: m/z 273/271/269 (M, 31, 96, 99%), 242/240/238 (M−OMe, 35, 98, 100), 214/212/210 (M−COOMe, 8, 25, 25).

Increased solvent polarity eluted *methyl 3-amino-N,2,4,6-tetrachloro-5-hydroxybenzoate* (12) (680 mg, 12%), m.p. 148–153° (dec.) from dichloromethane/carbon tetrachloride (Found: C, 31·8; H, 1·6; Cl, 46·5; N, 4·6. $C_8H_5Cl_4NO_3$ requires C, 31·5; H, 1·7; Cl, 46·5; N, 4·6%). ^1H n.m.r. (CD_2Cl_2) δ 6·01, bs, NH and OH; 3·96, s, $COOCH_3$. Mass spectrum: m/z 307/305/303 (M, 2, 5, 4%), 275/273/271/269 (M+H−Cl, 4, 29, 88, 91), 244/242/240/238 (M+H−Cl−OMe, 10, 38, 100, 96), 216/214/212/210 (M+H−Cl−COOMe, 3, 10, 28, 27).

3-Amino-4-chloro-5-hydroxybenzoic Acid (*3*)

(*a*) *From methyl 3-amino-2,4,6-trichloro-5-hydroxybenzoate or methyl 3-amino-N,2,4,6-tetrachloro-5-hydroxybenzoate.*—The ester (11) (1·35 g, 4·95 mmol) in aqueous hydrobromic acid (48%, 180 ml) was kept at reflux for 16 h under a stream of nitrogen. Hydrobromic acid was then distilled off at normal pressure, a constant reaction volume being maintained by continuous addition of

fresh aqueous hydrobromic acid (48%). After 12 h the solvent was removed under vacuum and the residue taken up in water containing sodium bicarbonate (*c.* 1 equiv.). The solution (pH 5) was saturated with sodium chloride and extracted with ethyl acetate; the combined dried extracts were evaporated to yield *3-amino-4-chloro-5-hydroxybenzoic acid* (3) (746 mg, 80%), m.p. 248–252° (dec.) from ethyl acetate/light petroleum, identical in m.p. and spectra with the reference sample prepared below. An analytical sample was obtained by sublimation (Found: C, 45·2; H, 3·2; N, 7·6. $C_7H_6ClNO_3$ requires C, 44·8; H, 3·2; N, 7·5%).

(*b*) *From methyl 3-amino-4-chloro-5-methoxybenzoate.*—Methyl 3-amino-4-chloro-5-methoxybenzoate (180 mg, 0·84 mmol), prepared by the method of Kane and Meyers,[13] in a mixture of acetic acid (3 ml) and aqueous hydrobromic acid (48%, 3 ml) was kept at reflux for 15 h. The hot solution after filtration deposited on cooling greyish crystals of the hydrobromide salt of 3-amino-4-chloro-5-hydroxybenzoic acid (187 mg, 83%), m.p. 233–236° (dec.). ^{1}H n.m.r. (CD_3OD) δ 7·54, s, H2 and H6. Mass spectrum: *m/z* 189/187 (M of acid, 32, 100%), 172/170 (M−OH, 8, 27), 144/142 (M−COOH, 4, 15), 82/80 (HBr).

The hydrobromide of the acid (3) (165 mg, 0·61 mmol) was dissolved in water containing sodium bicarbonate (*c.* 1 equiv.) and the resulting free acid was extracted as described in (*a*) to give crystalline *3-amino-4-chloro-5-hydroxybenzoic acid* (3) (113 mg, 98%), m.p. 248–252° (dec.) (Found: $M^{+\cdot}$, 187·0036. $C_7H_6ClNO_3$ requires $M^{+\cdot}$, 187·0036). ^{1}H n.m.r. (CD_3OD) δ 7·02, d, *J* 2·0 Hz, H2; 6·89, d, *J* 2·0 Hz, H6. Mass spectrum: *m/z* 189/187 (M, 33, 100%), 172/170 (M−OH, 8, 25), 144/142 (M−COOH, 5, 15).

Methyl 3-Amino-4-chloro-5-hydroxybenzoate (13)

Esterification of the acid (3) with methanol and conc. sulfuric acid gave *methyl 3-amino-4-chloro-5-hydroxybenzoate* (13) (93%), m.p. 185–187° after sublimation. ^{1}H n.m.r. (CD_3COCD_3) δ 9·17, s, OH; 7·09, d, *J* 2·8 Hz, H2; 6·94, d, *J* 2·8 Hz, H6; 5·37, bs, NH_2; 3·82, s, $COOCH_3$; OH irradiated, H6 +27%; NH_2 irradiated, H2 +25%. Mass spectrum: *m/z* 203/201 (M, 32, 100%), 172/170 (M−OMe, 27, 79), 145/143 (M−$C_2H_2O_2$, 12, 36), 144/142 (M−COOMe, 15, 37).

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