Synthesis of Chlorhexidine Digluconate Impurities

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Five new biguanides, found as impurities in chlorhexidine digluconate solutions, were synthesized by addition of amines to aminonitriles. Nonstoichiometric additions required to prepare the unsymmetrical biguanides resulted in low yields of the desired impurities, which were isolated by flash chromatography and characterized by HPLC-UV, HPLC–MS, and ^{1}H NMR.

Keywords: *Chlorhexidine digluconate; impurities; synthesis; antimicrobial*

Chlorhexidine digluconate (CHG) is a very effective antimicrobial agent with many applications and trade names (Budavari et al., 1989). We have previously reported the isolation and identification of 11 impurities in stressed CHG solutions (Revelle et al., 1993). We present here the synthesis of five of these new biguanide impurities (Figure 1).

The impurity syntheses feature several amine addition reactions of the form shown in Figure 2. The hydrochlorides of amines 1,6-hexanediamine, *p*-chloroaniline, aniline, and impurity **F** were added to aminonitriles sodium dicyanamide, cyanamide, and impurity **E** in various ways to generate the five new subject biguanides (Figure 1). The reactions were simple and resulted in good yields except in those cases requiring nonstoichiometric additions.

The synthesis of the **symmetrical** chlorhexidine (Rose and Swain, 1956) was modified by the addition of half of the prescribed stoichiometric equivalent of certain reagents to produce a low yield of desired, otherwise inaccessible, **unsymmetrical** chlorhexidine impurities (Figure 1). Although the yields of some target impurities were low and the purifications by flash chromatography somewhat tedious (Revelle et al., 1993), the starting materials were readily available and inexpensive.

Impurities **F** and **E** were prepared in low overall yield by nonstoichiometric amine addition reactions. In contrast, the stoichiometric amine addition reactions, such as in the preparation of impurities **G** and **H**, resulted in good yields.

The products of impurity **F**/cyanamide reaction (Ainley et al., 1949; Braun et al., 1938; King and Tonkin, 1946) depended upon solvent conditions. Under rigorous anhydrous conditions, the main product was guanidine **G**. When the butanol solvent was not dried properly, urea **G-1** resulted. The hydrolysis of guanidine **G** to urea **G-1** (Figure 1) has literature precedents (Huber, 1969; Revelle et al., 1993; Sayer and Conlon, 1980).

As previously reported for chlorhexidine itself (Revelle et al., 1993), the chlorhexidine impurities also break down in the HPLC–MS thermospray vaporizer probe following known degradation reactions. For example, HPLC–MS of impurity **G** (MH^+ 353) showed two major retro-ene (Hoffman, 1969) degradation products at *m/z* 201 (protonated 1,6-diguanidinohexane) and 170 (protonated *p*-chlorophenylguanidine), and a retro-amine addition degradation product at *m/z* 311 (protonated impurity **F**).

As proof of structure and differentiation from impurity **G**, the HPLC–MS of urea **G-1** (MH^+ 354) showed retro-ene degradation products at *m/z* 202 (protonated urea analog of 1,6-diguanidinohexane) and 170 (protonated *p*-chlorophenylguanidine) and a retro-amine addition degradation product at *m/z* 311 (impurity FH^+).

The HPLC–MS degradation pattern of impurity **H** (MH^+ 471) was particularly interesting in that five retro-ene degradation products as protonated molecular ions were seen: 353 (impurity **G**), 319 (analog of **G** in which chlorine was replaced with hydrogen), 201 (1,6-diguanidinohexane), 170 (*p*-chlorophenylguanidine), and 136 (phenylguanidine). The above HPLC–MS degradation of **H** illustrates the variety of ways chlorhexidine-related molecules can undergo the retro-ene reaction.

EXPERIMENTAL PROCEDURES

For a discussion of materials, instruments, and methods (analytical and preparative), see our previous paper (Revelle et al., 1993). The product impurities were isolated as the dihydrochloride salts and in some cases converted to the diacetate salts for characterization. The impurity dihydrochlorides were dissolved in 0.1 M ammonium acetate and reduced to a residue of constant weight as the diacetate under reduced pressure. The purity level of each synthetic impurity after flash chromatographic isolation was approximately 98% as shown by HPLC-UV analysis and confirmed by ^{1}H NMR and HPLC–MS spectra.

1-[N^5-(*p*-Chlorophenyl)biguanido]-6-(N^3-cyanoguanidino)hexane, Impurity E. Sodium dicyanamide (23.5 g, 264 mmol) and 1,6-hexanediamine dihydrochloride (25.0 g, 132 mmol) were added to 200 mL of butanol and refluxed for 16 h. The solvent was removed under reduced pressure to give an opaque oil. Without further purification, the oil was dissolved in 300 mL of 2-ethoxyethanol and *p*-chloroaniline hydrochloride (21.4 g, 132 mmol) added. (The *p*-chloroaniline hydrochloride was prepared by bubbling gaseous hydrogen chloride through a dichloromethane solution of *p*-chloroaniline.) The reaction mixture was refluxed overnight. Under reduced pressure, the filtrate was concentrated to a viscous oil which was purified by four passes through a reversed phase flash chromatography column as previously described. The fractions were analyzed according to the isocratic HPLC method (Revelle et al., 1993) with a mobile phase ratio of 50/50 buffer/methanol. Under the above conditions the retention time of **E** was 3.0 min. The isolated **E** dihydrochloride was collected as a colorless oil (2.0 g, 3.8% overall yield): ^{1}H NMR (CD_3OD) (diacetate of **E**) δ 1.35 (4H, m, $CH_2CH_2CH_2CH_2CH_2CH_2-$), 1.55

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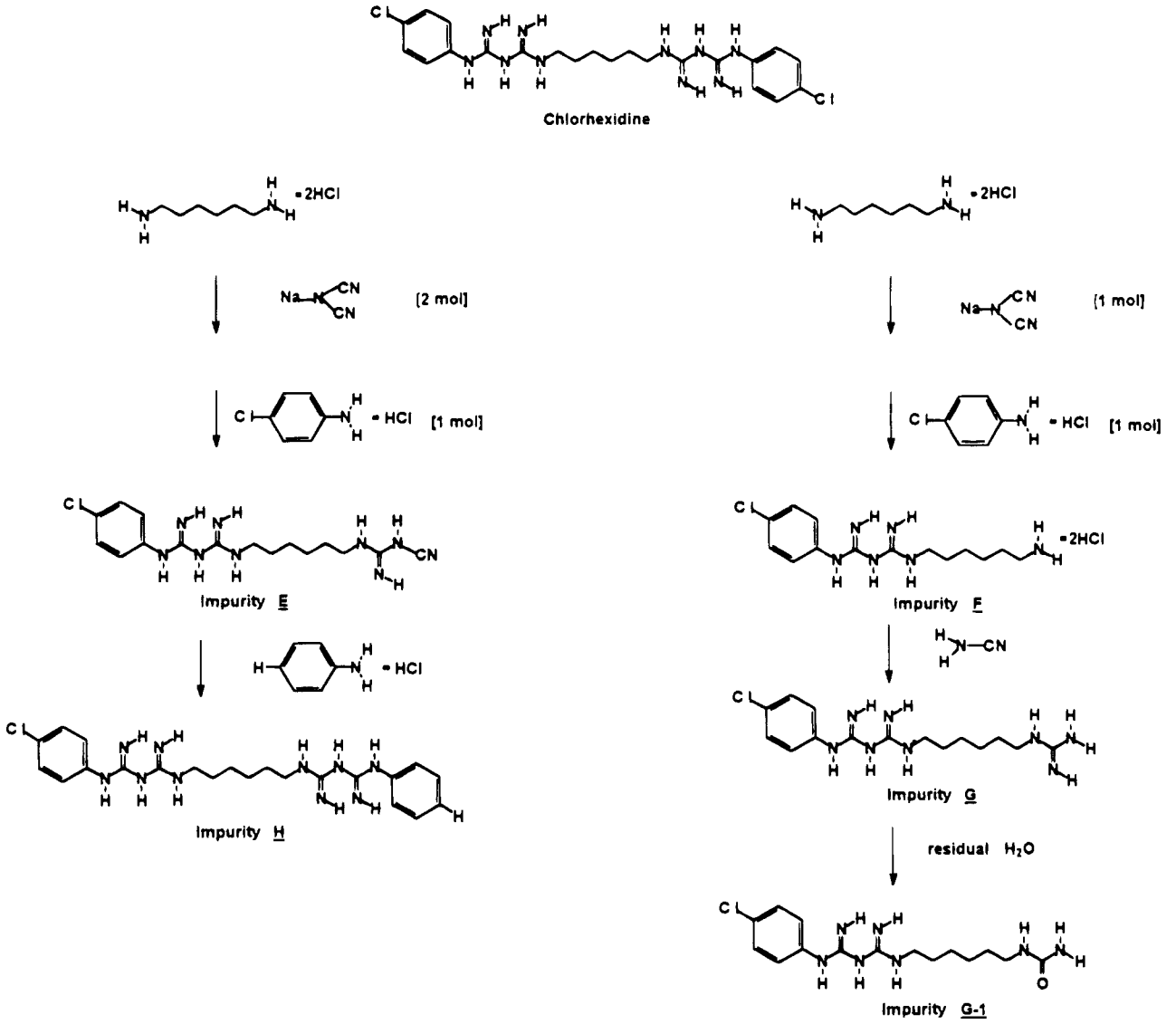

Figure 1. Synthesis of five chlorhexidine digluconate impurities.

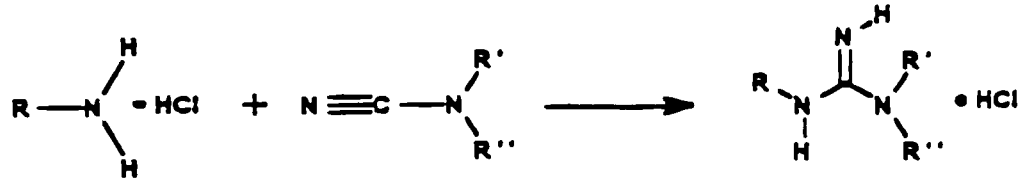

Figure 2. Amine addition reactions.

(4H, m, $-CH_2CH_2CH_2CH_2CH_2CH_2-$), 1.95 (6H, s, $-CH_3$), 3.15 (4H, m, $CH_2CH_2CH_2CH_2CH_2CH_2-$), 7.30 (2H, d, J = 8 Hz, Ar-H), 7.42 (2H, d, J = 8 Hz, Ar-H); HPLC–MS thermospray, *m/e* 378 (MH^+, 10%), 226 (protonated cyano derivative of 1,6-diguanidinohexane, 100%), 184 (60%), 201 (protonated 1,6-diguanidinohexane, 15%).

1-[N^5-(*p*-Chlorophenyl)biguanido]-6-aminohexane, Impurity F. Sodium dicyanamide (14 g, 158 mmol) and 1,6-hexanediamine dihydrochloride (30 g, 158 mmol) were added to 200 mL of butanol and refluxed for 16 h. The solvent was removed under reduced pressure to give an oil. Without further purification, the oil was dissolved in 300 mL of 2-ethoxyethanol and *p*-chloroaniline hydrochloride (25.6 g, 158 mmol) added. The mixture was refluxed for 16 h and filtered. The filtrate was concentrated to a dark oil under reduced pressure and purified by four passes through a flash chromatography column. Fractions were analyzed by the isocratic HPLC method with a mobile phase ratio of 50/50 buffer/methanol. The retention time of **F** under the above conditions was 3.0 min. The isolated product, **F** dihydrochloride, was collected a colorless oil (2.85 g, 5.8% overall yield): ^{1}H NMR ($CDCl_3$) δ 1.30 (4H, m, $-CH_2CH_2CH_2CH_2CH_2CH_2-$), 1.50 (2H, m, $-CH_2CH_2NH_2$), 1.70 (2H, m, $-NHCH_2CH_2-$), 2.90 (2H, m, CH_2NH_2-), 3.20 (2H, m, $-NHCH_2-$), 7.25 (2H, d, J = 10 Hz, Ar-H), 7.45 (2H, d, J = 10 Hz, Ar-H); HPLC–MS thermospray, *m/e* 311 (MH^+, 100%).

Impurities G, 1-[N^5-(*p*-Chlorophenyl)biguanido]-6-guanidinohexane, and G-1, 1-[N^5-(*p*-Chlorophenyl)biguanido]-6-ureidohexane. The dihydrochloride of impurity **F** (420 mg, 1.35 mmol) was dissolved in 25 mL of butanol which was freshly distilled from calcium oxide. Cyanamide (100 mg, 2.4 mmol) was added, and the solution was refluxed for 25 h. The progress of the reaction was followed by the HPLC isocratic method with a mobile phase ratio of 70/30 buffer/methanol. The retention time for **F** was 4.2 min, for **G** was 6.2 min, and for **G-1** was 7.0 min. The resulting oil was purified by the flash chromatography method to give 80 mg of highly purified **G** dihydrochloride (17% yield): ^{1}H NMR (CD_3OD) δ 1.40 (4H, m, $-CH_2CH_2CH_2CH_2CH_2CH_2$), 1.60 (4H, m, $-CH_2CH_2CH_2CH_2CH_2CH_2-$), 3.20 (4H, m, $-CH_2CH_2CH_2CH_2-$

CH_2CH_2), 7.3 (2H, d, J = 8 Hz, Ar-H), 7.42 (2H, d, J = 8 Hz, Ar-H); HPLC–MS thermospray, *m/e* 353 (MH^+, 50%), 311 (FH^+, 40%), 201 (protonated 1,6-diguanidinohexane, 100%), 170 (protonated *p*-chlorophenylguanidine, 20%). The other product isolated was 300 mg of **G-1** dihydrochloride (71% yield): ^{1}H NMR (CD_3OD) (**G-1** diacetate) δ 1.40 (4H, m, CH_2-$CH_2CH_2CH_2CH_2CH_2$), 1.60 (4H, m, $CH_2CH_2CH_2CH_2CH_2CH_2$–), 1.95 (6H, s, $-CH_3$), 3.20 (4H, m, $CH_2CH_2CH_2CH_2CH_2CH_2$), 7.3 (2H, d, J = 8 Hz, Ar-H), 7.42 (2H, d, J = 8 Hz, Ar-H); HPLC–MS thermospray, *m/e* 354 (M + H, 100%) 311 (F + H, 50%) 202 (protonated urea analog of 1,6-diguanidinohexane, 90%), 170 (protonated *p*-chlorophenylguanidine, 35%).

The above reaction was repeated under more rigorous anhydrous conditions in which the butanol solvent was refluxed over calcium oxide for 24 h prior to distillation; all glassware was dried overnight at 50 °C, reagents were dried at reduced pressure, and the reaction vessel was kept under a constant positive pressure of nitrogen. Impurity **F** diacetate (30 mg) and cyanamide (20 mg) dissolved in 10 mL of butanol were reacted at 90 °C under the anhydrous conditions outlined above. After 5 h, the reaction mixture was analyzed by HPLC to show impurity **G** (44%, 6.11 min), unreacted impurity **F** (44%, 4.31 min), and *p*-chlorophenylguanidine (11%, 2.70 min). Indicated yields were based on area percent calculations. Under the above anhydrous reaction conditions impurity **G-1** was not detected.

1-[N^5-(*p*-Chlorophenyl)biguanido]-6-(N^5-phenylbiguanido)hexane, Impurity H. Aniline hydrochloride (1.70 g, 13.2 mmol) and 5.0 g of impurity **E** dihydrochloride (13.2 mmol) were dissolved in 100 mL of 2-ethoxyethanol and refluxed for 3 h. After cooling, the precipitate was collected and purified according to the flash chromatography method to give 2.5 g of colorless solid **H** dihydrochloride (4.2 mmol, 32% yield). The retention time under the isocratic method conditions with a mobile phase ratio of 50/50 buffer/methanol was 4.0 min. ^{1}H NMR (CD_3OD) (diacetate of **H**) δ 1.35 (4H, m, $-CH_2CH_2CH_2CH_2CH_2CH_2-$), 1.55 (4H, m, $-CH_2CH_2CH_2$-$CH_2CH_2CH_2-$), 1.95 (6H, s, $-CH_3$), 3.16 (4H, m, $CH_2CH_2CH_2$-$CH_2CH_2CH_2-$), 7.10 (1H, m, Ar-H), 7.30 (8H, m, Ar-H); HPLC–MS thermospray, *m/e* 471 (M + H, 35%), 353 (GH^+, 20%) 319 [(**G** – Cl + H)H^+, 21%], 201 (protonated 1,6-diguanidinohexane, 68%), 170 (protonated *p*-chlorophenylguanidine, 28%) 136 (protonated phenylguanidine, 100%).

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