Synthesis of Related Substances of Cefadroxil

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The chemical synthesis of some related substances of the cephalosporin antibiotic cefadroxil is described. These compounds which may be present in commercial samples originate from the semisynthetic preparation of the antibiotic or from degradation. The preparation of these products enables the validation of selective quantitative analytical methods, such as liquid chromatography and tlc.

Synthese von Nebenprodukten bei der Cefadroxil-Herstellung
Die Synthese von einigen möglichen Nebenprodukten des Cephalosporin-Antibiotikums Cefadroxil wird beschrieben. Diese Nebenprodukte, die gelegentlich als Verunreinigung in kommerziellen Proben auftreten, entstehen während der semi-synthetischen Herstellung dieses Antibiotikums oder während dessen Lagerung durch Abbau. Die Darstellung dieser Produkte ermöglicht es, selektive quantitativ-analytische Methoden wie die HPLC und die DC zu validieren.

Cefadroxil (**1**) is a semisynthetic antibiotic which belongs to the cephalosporins. For the quantitative determination of cefadroxil, liquid chromatography (LC) is the method of choice[1]. In order to validate a selective LC method it is necessary to dispose of the potential impurities. These may be formed by side-reactions during the semisynthetic preparation or by decomposition of the relatively unstable molecule.

The structures of **1** and its potential impurities **2** to **10** are shown in Scheme 1. Structures **1** to **9** have D-configuration at C-11 or at the corresponding position, while **10** has L-configuration. Apart from the starting materials for the semi-synthetic preparation, 7-aminodesacetoxy-cephalosporanic acid (7-ADCA) (**7**) and D-4-hydroxyphenylglycine (**D-6**), which are commercially available, these compounds have to be synthesised. This publication deals with the preparation of the potential impurities of cefadroxil (**1**). The methods are mainly derived from those used for the synthesis of the potential impurities of cefalexin and cefradine, which were published[2]. These related substances of cefadroxil (**1**) were not only used in the development of a new LC method[1] but also in a comparative study of LC methods[3].

Results and Discussion

The methods for the preparation of the related substances of cefalexin[2] were applied to cefadroxil. For the synthesis of the Δ^2-isomer of cefalexin, two methods were described[2]. For the Δ^2-isomer of cefadroxil (**8**), however, only the second method was applicable since method 1 caused acetylation of the phenol function of cefadroxil. The isomers **1** and **8** were not separated and the mixture was used as such in LC experiments.

Since 3-hydroxy-4-methyl-2(5*H*)-thiophenone (**4**) originates from the common part of the molecules cefadroxil, cefalexin and cefradine[2], acidic degradation of cefadroxil produces the same product.

The unstable degradation product **5** was prepared *in situ* immediately before use, by dissolving **1** in 0.1 N NaOH (1 mg/ml) and storing the solution at room temp. for 10 min. **5** is a mixture of diastereoisomers, due to isomerisation of C-6 and C-7.

The newly prepared substances were characterised by their melting point, infrared-, ultraviolet-, NMR- and mass spectra.

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Experimental Part

M.p.: Büchi SMP-20 apparatus, corrected.- UV spectra: Philips PU 8740 UV/VIS scanning spectrophotometer.- IR-spectra: Perkin-Elmer 197 spectrophotometer (KBr).- ^{1}H-NMR and ^{13}C-NMR spectra: Jeol FX90Q spectrometer, TMS or sodium 2,2-dimethyl-2-silapentane-5-sulphonate (DSSA) as internal standard. Liquid secondary ion mass spectra (LSIMS): Kratos Concept 1H mass spectrometer. Thioglycerol was used as matrix for the positive-ion mass spectra and sulfolane for the negative-ion mass spectra.

3-Aminomethylene-6-(4-hydroxyphenyl)-piperazine-2,5-dione (**2**)

2 was prepared according to the method described for the preparation of 3-aminomethylene-6-phenyl-piperazine-2,5-dione by *Bundgaard*[4], mol. mass: 233.- Yield 52%. M.p.: the product turns black above 200°C.- UV (H_2O): λmax ($A^{1\%}$) = 229 (382), 283 nm (520).- IR (KBr): 3600-2800 (OH, NH), 1680-1630 (amide, C=C), 1510 cm^{-1} (amide II).- ^{1}H-NMR ([D_6]DMSO, TMS): δ (ppm) = 4.74 (d; J = 2.5 Hz, 6-H), 5.82 (d; J = 11.0 Hz, NH_2), 6.61 (t; J = 11.0 Hz, 7-H), 6.74 (d; J = 8.6 Hz, H_m), 7.08 (d; J = 8.6 Hz, H_0), 7.70 (d; J = 2.5 Hz, 1-NH), 9.35 (br s; 4-NH), OH was not detected, the signals at 5.82, 7.70 and 9.35 disappeared after addition of D_2O, the signals at 4.74 and 6.61 are transformed to singlets.- ^{13}C-NMR ([D_6]DMSO): δ (ppm) = 58.8 (C-6), 101.9 (C-3), 115.2 (C_m), 126.0 (C-7), 127.7 (C_0), 130.7 (C_i), 157.2 (C_p), 161.6 (C-2), 163.8 (C-5).- LSIMS: m/z 232 $(M - H)^-$.

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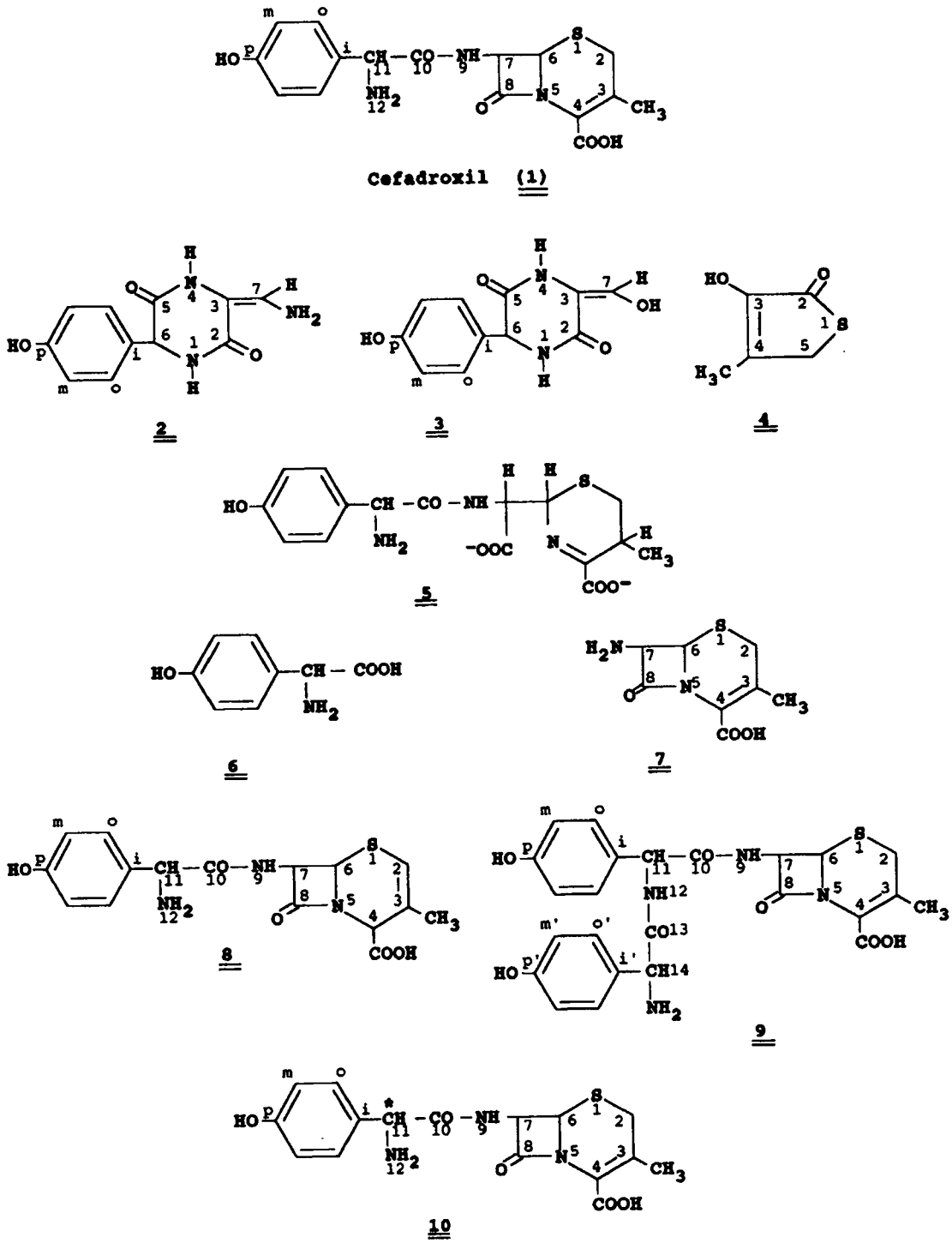

Scheme 1. Structures of cefadroxil (**1**) and its potential impurities.

3-Hydroxymethylene-6-(4-hydroxyphenyl)-piperazine-2,5-dione (**3**)

Compound **2** was transformed into **3**, as described for 3-hydroxymethylene-6-phenylpiperazine-2,5-dione[2]. After 24 h of stirring, the solution was evaporated. The residue was dried under vacuum. Mol. mass = 234.- Yield 100%.- M.p.: the product turns black above 200°C.- UV (H_2O): λmax ($A^{1\%}$) = 228 (354), 261 nm (267).- IR (KBr): 3600-2800 (OH, NH), 1680-1630 cm^{-1} (amide, C=C).- ^{1}H-NMR ([D_6]DMSO, TMS): δ (ppm) = 4.79 (d; J = 2.3 Hz, 6-H), 6.39 (br; OH and phenol), 6.77 (d; J = 8.6 Hz, H_m), 6.93 (s; 7-H), 7.08 (d; J = 8.6, H_o), 8.17 (d; J = 2.3 Hz, 1-NH), 9.41 (br s; 4-NH), the signals at 6.39, 8.17 and 9.41 disappeared after addition of D_2O, the doublet at 4.79 turned into a singlet.- ^{13}C-NMR ([D_6]DMSO):

δ (ppm) = 58.7 (C-6), 109.1 (C-3), 115.3 (C_m), 127.7 (C_0), 130.1 (C_i), 135.8 (C-7), 157.3 (C_p), 161.0 (C-2), 163.8 (C-5).- LSIMS: m/z 233 $(M - H)^-$.

Δ^3- and Δ^2-Cefadroxil (**1** *and* **8**)

The isomerisation of 7-ADCA (**7**) to the mixture of Δ^3- and Δ^2-7-ADCA was described[2]. The mixture of **1** and **8** was prepared according to method 2 as described for cefalexin[2]. Mol. mass = 363.- Yield 52%. The two isomers were identified in the reaction mixture by comparison of their relative positions after LC analysis with those of cefalexin and Δ^2-cefalexin and by comparative examination of the UV-spectra by means of photodiode array detection after LC analysis.

4-Hydroxyphenylglycylcefadroxil (**9**)

The intermediate product *N-tert*-butyloxycarbonyl-D-4-hydroxyphenylglycine was prepared as described for the analogous *N-tert*-butyloxycarbonyl-D-phenylglycine[2]. The product was crystallized from ethyl acetate - CCl_4. Yield 80%. Compound **9** was synthesized by condensation of *N-tert*-butyloxycarbonyl-D-4-hydroxyphenylglycine with cefadroxil (**1**), according to the method described for phenylglycylcefalexin[2]. Mol. mass = 512.- Yield 29%.- M.p.: dec. at 170.5°C.- UV (H_2O): λmax ($A^{1\%}$) = 228 (224), 264 nm (92).- IR (KBr): 3600-2800 (OH and amide), 1760 (β-lactam), 1680 (amide I, COOH), 1510 (amide II), 830 cm^{-1} (parasubstituted phenyl).- ^{1}H-NMR (D_2O/CF_3COOD, DSSA): δ (ppm) = 2.11 (s; CH_3), 3.28 (AB, J = 18 Hz; CH_2), 4.91 (d; J = 4.0, 6-H), 5.20 (s; 14-H), 5.51 (s; 11-H overlapping with d of 7-H), 6.89 (d; J = 8.6 Hz, H_m), 6.98 (d; J = 8.6 Hz, $H_{m'}$), 7.28 (d; J = 8.6 Hz, H_0), 7.45 (d; J = 8.6 Hz, H_d).- ^{13}C-NMR (D_2O/CF_3COOD): δ (ppm) = 20.2 (CH_3), 30.9 (CH_2), 57.5, 58.5, 58.7, 59.9 (C-6, C-7, C-11, C-14), 117.1 ($C_{m'}$), 117.7 (C_m), 123.5 ($C_{i'}$), 124.3 (C-4), 127.9 (C_i), 130.4 ($C_{0'}$), 131.2 (C_0), 139.2 (C-3), 157.6 ($C_{p'}$), 158.8 (C_p), 166.2 (β-lactam C=O and COOH), 169.3 (C-13), 172.9 (C-10).- LSIMS: m/z 513 $(M + H)^+$.

L-Cefadroxil (**10**)

The intermediate *N-tert*-butyloxycarbonyl-L-4-hydroxyphenylglycine was prepared as described above for the D-isomer. **10** was synthesized by condensation of *N-tert*-butyloxycarbonyl-L-4-hydroxyphenylglycine with 7-ADCA (**7**) according to the method described for phenylglycylcefalexin[2]. Mol. mass = 363.- Yield 55% .- M.p.: the product swells and becomes glassy above 150°C.- UV (H_2O): λmax ($A^{1\%}$) = 227 (275), 262 nm (153).- IR (KBr): 3600-3200 (OH and amide), 1760 (β-lactam), 1680 (amide I and COOH), 1520 (amide II), 830 cm^{-1} (*p*-substituted phenyl).- ^{1}H-NMR (D_2O/CF_3COOD, DSSA): δ (ppm) = 2.18 (s; CH_3), 3.42 (AB, J = 18 Hz; CH_2), 5.09 (d; J = 4.6 Hz, 6-H), 5.22 (s; 11-H), 5.45 (d; J = 4.6 Hz, 7-H), 6.98 (d; J = 8.6 Hz, H_m), 7.42 (d; J = 8.6 Hz, H_0).- ^{13}C-NMR (D_2O/CF_3COOD): δ (ppm) = 20.2 (CH_3), 31.1 (CH_2), 57.5, 58.8, 60.5 (C-6, C-7 and C-11), 117.6 (C_m), 123.5 (C_i), 124.0 (C-4), 131.2 (C_0), 140.9 (C-3), 159.3 (C_p), 165.9, 166.2 (β-lactam C=O and COOH), 170.3 (C-10).- LSIMS: m/z 364 $(M + H)^+$.

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