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Pyrazinone Conjugates as Potential Cephalosporin Allergens†

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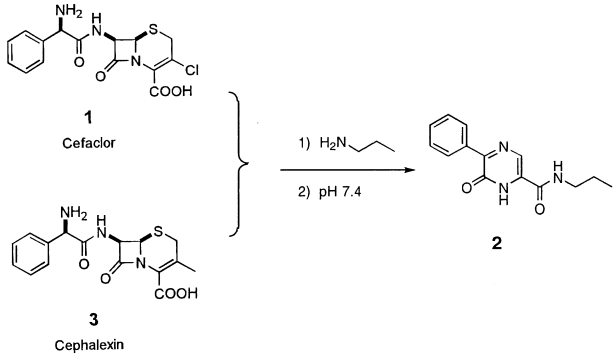
Abstract—Cephalosporins with an α-amino group on the 7-β-acyl substituent have been shown to undergo aminolysis with subsequent formation of a fluorescent pyrazinone degradation product. An analogous reaction seems to take place upon in vitro conjugation of such cephalosporins to albumins and polylysine. A corresponding pyrazinone–polylysine conjugate was able to bind IgE antibodies in sera from patients with suspected allergy to β-lactams. These findings may have applications in the diagnosis of allergy against aminocephalosporins. © 2001 Elsevier Science Ltd. All rights reserved.

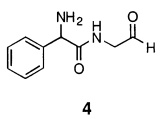
Allergy against β-lactam antibiotics is a widely recognized clinical problem and the proportion of the US population who report a history of penicillin allergy has been estimated to vary from 0.7 to 10% of treated patients.[1] In the cephalosporin subgroup allergic reactions occur in 0.9–3.2% of patients.[2] The major allergenic determinant in penicillin allergy is the amidopenicilloyl unit, which is formed upon aminolytic cleavage of the β-lactam ring. In contrast, the allergenic determinants of cephalosporins are not known, a fact that has delayed the development of test procedures in diagnosis of cephalosporin allergy. The initial intermediates of cephalosporin aminolysis are reported to be unstable and degrade, probably with cleavage of the dihydrothiazine ring.[3,4] However, to the best of our knowledge, no aminolysis products resulting from dihydrothiazine cleavage of cephalosporins have been isolated and characterized. We wish to report studies on the reactions between cefaclor/cephalexin and propylamine in which a fluorescent pyrazinone derivative has been isolated and characterized.

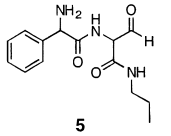
Cefaclor (**1**) was allowed to react with propylamine in aqueous solution (pH > 10) for 30 min and the solution was then lyophilized to give a solid mixture. The solid was then dissolved in phosphate buffer pH 7.4 and left to stand at 37 °C for 2 weeks. Repeated extraction with methylene chloride followed by evaporation gave a crude solid which was purified by flash chromatography to give a 27% yield of 1,6-dihydro-6-oxo-5-phenyl-*N*-propylpyrazinecarboxamide (**2**)[5] (Scheme 1). Aminolysis of cephalexin (**3**), a cephalosporin with an identical C-7 side chain, also gave **2**, albeit in lower (10%) yield.



Scheme 1.

We analyzed the degradation of cefaclor aminolysis mixture using reverse-phase chromatography.[6] The peak corresponding to **2** steadily increased during the course of degradation and appeared to be the final product. The mechanism of degradation of the initial aminolysis intermediates of cefaclor seems to be complex and involve numerous intermediates and byproducts. A degradation route involving the intermediate phenylglycyl-acetaldehyde (**4**) has been proposed for the acidic degradation of cefaclor, which, together with a number of other degradation products, yields different pyrazine derivatives.[7] Analogously, we believe the aldehyde **5** is a key intermediate in the formation of pyrazinone derivative **2**.





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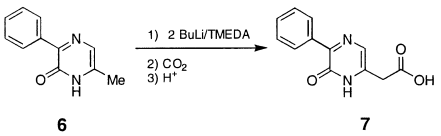
It is well known that cefaclor and other β-lactam antibiotics containing the phenyl glycine side chain degrade under certain conditions to yield 6-methyl-3-phenyl-2(1*H*)-pyrazinone.[8] Furthermore, in a recent study of acidic degradation of cefaclor a number of fluorescent pyrazine derivatives were isolated and characterized.[7] However, in order to become immunogenic a small molecule like cefaclor has to become polymerized or conjugated to a macromolecule and such conjugates are formed by electrophilic attack on lysine ε-amino groups. Thus, in relation to the immunogenicity of cefaclor and other cephalosporins the aminolysis reaction is highly relevant. Therefore, we next applied this reaction to the study of cefaclor conjugation to human serum albumin (HSA) and poly-L-lysine. Thus, cefaclor was conjugated to HSA and poly-L-lysine under slightly alkaline conditions and the conjugates were dialyzed. The conjugates were then stored in phosphate buffer pH 7.4 at 37 °C and the course of degradation was followed by UV-spectroscopy. The UV-spectra were gradually transformed and finally showed the characteristic absorption maximum of 356 nm, which is similar to that of the substituted pyrazine derivative **2**. Furthermore, the fluorescent properties (emission at 444 nm) of the conjugates were identical to that of pyrazine **2**.

In order to determine immunoglobulin E (IgE) antibodies to the pyrazinone hapten, a polylysine conjugate was prepared. The dianion of 6-methyl-3-phenyl-2(1*H*)-pyrazinone (**6**) was treated with carbon dioxide which upon acidic work up gave the known[7] compound 1,6-dihydro-6-oxo-5-phenylpyrazineacetic acid (**7**) (Scheme 2).

The pyrazinone acid **7** was then conjugated to partly succinylated poly-L-lysine with *O*-(*N*-succinimidyl)-*N,N,N′,N′*-tetramethyluronium tetrafluoroborate (TSTU) as a condensing agent. The conjugate was dialyzed and coupled to the cyanogen bromide-activated cellulose solid phase (ImmunoCAP™). Specific IgE antibodies in 31 sera from patients with suspected β-lactam allergy were measured with UniCAP® specific IgE according to the directions of use (Pharmacia Diagnostics AB, Uppsala, Sweden). The system is calibrated against the World Health Organization (WHO) International Reference Preparation for human IgE 75/502 and permits calculation of IgE antibody levels in allergen specific units (U_A).[9] A positive result was defined as a value greater or equal to 0.35 U_A/mL. A negative pool, consisting sera from 20 different donors, was used as a control. All of the 31 patient sera were positive to one or more of penicilloyl G, penicilloyl V, ampicilloyl, and amoxicilloyl ImmunoCAP. Fifteen sera were also positive to the cefaclor ImmunoCAP. In this cefaclor-positive subgroup specific IgE to the pyrazinone hapten were determined in nine (60%) cases. Interestingly, only two (13%) sera were positive to phenylglycine, which is the C(7) side chain of cefaclor. Among the cefaclor-negative subgroup consisting of 16 patient sera, no pyrazinone positive serum was detected. These results show that the 3-phenyl-2(1*H*)-pyrazinone hapten, linked at the 6-position to a carrier, is recognized by IgE antibodies in sera from certain cefaclor allergic patients. We believe these findings will contribute to the understanding of the epitopes involved in cephalosporin allergy.



6 **7**

Scheme 2.

In summary, cefaclor and cephalexine have been aminolyzed and the initial, unstable intermediates have been shown to degrade and finally yield a substituted pyrazinone derivative (**2**). In vitro conjugation of these cephalosporins to amine-containing macromolecules such as albumin and polylysine seems to give the same pyrazinone derivative as a hapten conjugated to the macromolecules. Pyrazinone-specific IgE antibodies were determined in a number of β-lactam allergic patients.

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References and Notes

1. Idsoe, O.; Guthe, T.; Willcox, R.; deWeck, A. *Bull. WHO* **1968**, *38*, 159.
2. Thompson, J. W.; Jacobs, R. F. *Drug Safety* **1993**, *9*, 132.
3. Hamilton-Miller, J. M. T.; Newton, G. G. F.; Abraham, E. P. *Biochem. J.* **1970**, *116*, 371.
4. Hamilton-Miller, J. M. T.; Richards, E.; Abraham, E. P. *Biochem. J.* **1970**, *116*, 385.
5. Analytical data for compound **2**: mp 202 °C; ^{1}H NMR (400 MHz, 50 °C, $CDCl_3$): δ 8.34 (m, 2H, *ortho*-Ph), 8.22 (s, 1H, H-5), 7.48 (m, 1H, *para*-Ph), 7.46 (m, 2H, *meta*-Ph), 6.79 (br s, 1H, N–H), 3.44 (m, 2H, CH_2-9), 1.65 (m, 2H, CH_2-10), 0.99 (t, J = 7.4 Hz, 3H, CH_3). ^{13}C NMR (100.6 MHz, 50 °C, $CDCl_3$): δ 160.4 (C=O), 154.5 (C-2), 153.7 (C-3), 135.1 *ipso*-Ph), 130.8 (*para*-Ph), 130.6 (C-6), 129.3 (*ortho*-Ph), 128.3 (*meta*-Ph), 125.6 (C-5), 42.0 (C-9), 22.8 (C-10), 11.3 (CH_3). MS (*m*/*z*, %): 257 (M^+, 100%), 242 (1%), 212 (9%), 200 (35%). UV absorption (phosphate buffer pH 7.0): λ_{max} = 356 nm, log ε = 4.11; emission: λ_{max} = 444 nm. Anal. calcd for $C_{14}H_{15}N_3O_2$: C, 65.4; H, 5.88; N, 16.3; O, 12.4. Found: C, 65.2; H, 6.1; N, 16.05.
6. Chromatographic analyses were performed using the SMART® System (Pharmacia Biotech) on a μRPC C2/C18 PC 3.2/3 column eluting with a linear gradient from 0.1% TFA in water to 0.1% TFA in 60% acetonitrile.
7. Baertschi, S. W.; Dorman, D. E.; Occolowitz, J. L.; Collins, M. W.; Spangle, L. A.; Stephenson, G. A.; Lorenz, L. J. *J. Pharm. Sci.* **1997**, *86*, 526.
8. Barbhaiya, R. H.; Brown, R. C.; Payling, D. W.; Turner, P. *J. Pharm. Pharmac.* **1978**, *30*, 224.
9. Yman, L. In *The Immunoassay Handbook,* 2nd ed.; Wild, D., Ed.; Nature Publishing Group: London, 2001; pp 674–675.