B_3(7) yielded a new spot which showed a slightly higher R_f-value than that of detoxinine. The ^{13}C-NMR-spectrum of **6** showed a close similarity to that of **3** except for the new appearance of a methylenic carbon at δ27.4 ppm in the former; this is consistent with the mass spectral data for the N-acetylmethyl ester of 6(**15**), viz., the fragment ions derived from C and D in the mass spectrum of **15** showed a shift by 58 mass units compared with those of **11**. Based on these results, the structure of **6** was established as a new congener, having the deoxydetoxinine nucleus, in the detoxin family.

Next, the fragmentation pattern in the mass spectrum of the N-acetylmethyl ester of detoxin B_3(**16**) showed a close resemblance to that of **15**, the difference being a shift by 28 mass units (ion C) in the latter. This result, along with the detection of isobutyric acid in the acid hydrolysate, gave the evidence that the structure of **7** is as depicted in the figure.

*The structure of detoxin A_1(**8**).* Comparison of the ^{13}C-NMR-spectra of **8** and valyldetoxinine (**9**) showed that they had a close resemblance to each other except for 2 signals assigned to C_5 and C_6 (table 2), respectively. In addition, the peracetate of **8** which is identical to that of **9** indicated that **8** is the C_5 monoacetate of **9** on the basis of the ^{13}C-NMR-data, viz., an acyl shift (about 2.3 ppm) in C_5 is associated with a β-shift (about 1.0 ppm upfield) of C_6 in **8**. Accordingly, the structure of **8** was determined as shown in the figure.

1 Acknowledgment. This work was supported by a Grant-in-Aid for Special Project Research (510208) from the Ministry of Education, Science and Culture, Japan. We are grateful to Kaken Chemical Co. Ltd for the supply of the detoxin complex. This is Part V of studies of Detoxin Complex, the Selective Antagonists of Blasticidin S'. For Part IV, see Ogita et al.[8].

2 H. Yonehara, H. Seto, A. Shimazu, T. Hidaka, K. Kakinuma and N. Ōtake, Agric. Biol. Chem. *37*, 2771 (1973).
3 H. Yonehara, S. Takeuchi, N. Ōtake, T. Endo, Y. Sakagami and Y. Sumiki, J. Antibiot., Tokyo, A *16*, 195 (1963).
4 N. Ōtake, K. Kakinuma and H. Yonehara, Agric. Biol. Chem. *37*, 2777 (1973).
5 K. Kakimuma, N. Ōtake and H. Yonehara, Tetrahedron Lett. *1972*, 2509.
6 K. Kakinuma, N. Ōtake and H. Yonehara, Tetrahedron Lett. *21*, 167 (1980).
7 N. Ōtake, K. Furihata, K. Kakinuma and H. Yonehara, J. Antibiot., Tokyo, *27*, 484 (1974).
8 T. Ogita, N. Ōtake, K. Kakinuma and H. Yonehara, Agric. Biol. Chem. *42*, 2403 (1978).
9 J.B. Stothers, in: Carbon-13 NMR-Spectroscopy, p.479. Ed. Alfred T. Blomquist and Harry Wasserman. Academic Press, New York 1972.

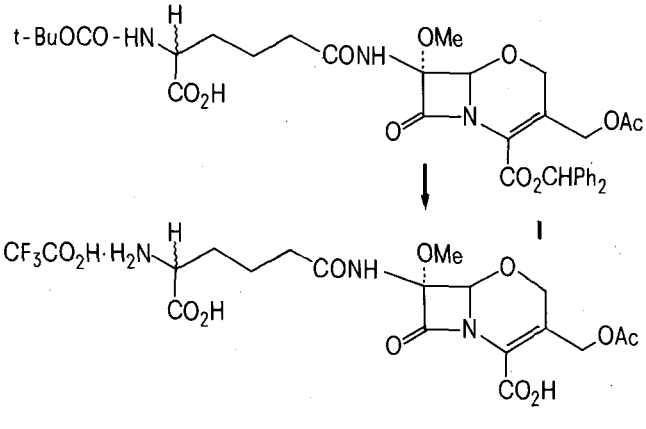
HPLC of cephalosporins and their oxa-derivatives

R.D. Miller, Cynthia Affolder and N. Neuss[1,2]

Lilly Research Laboratories, Eli Lilly and Company, Indianapolis (Indiana 46285, USA), 15 January 1981

Summary. The presence of the oxygen atom in place of the sulfur atom has significant impact on the polarity of the β-lactam derivative. This has been illustrated by direct comparison of HPLC data of 4 different cephalosporin derivatives and their oxa analogues.

In connection with our investigations[3] on different metabolites in fermentation broths we were interested in differences in retention times between cephalosporins and their oxa-derivatives[4]. The oxa-β-lactam derivatives were provided as fully blocked compounds[5] and were hydrolyzed to the zwitterionic species prior to the chromatography. This procedure was carried out on small aliquots of the samples and reactions were done in flamed glassware under a nitrogen atmosphere. The following example illustrates the process of deblocking: 16 mg of N-tert-butyloxy-carbonyl-4-benzhydro-derivative **I** (mol. wt 695.7) (0.023 mmole) were added to 0.15 ml anisole in an ice bath.



Trifluoroacetic acid was chilled and 0.45 ml was slowly added to the reaction vessel. The reaction proceeded for 25 min and was confirmed to be complete by TLC on silica developed with 5% acetic acid in ethyl acetate. The reaction mixture was evaporated on a Büchi rotoevaporator, triturated with ether (2-3 ml), filtered and washed with hexane. The product was then dried in high vacuum over P_2O_5/KOH for 1.5 h. The yield of the TFA salt was 10 mg (82% theory). We have chosen 2 well-characterized chromatographic systems to compare these nuclear isomers, and these data are summarized in figures 1 and 2. The 1st system was a reversed phase paired with ion mobile phase on microBondapak C18 (Waters) (fig. 1). The solvent mixture used was prepared by mixing an aqueous solution of 0.005 M tetrabutyl ammonium hydroxide, 1% ammonium phosphate at pH 7 and 1% acetonitrile. The use of the column 4×300 mm with a flow rate of 2 ml/min led to a pressure of 2000 psi. The 2nd system consisted of an ion-exchange stationary phase, microBondapak NH_2 with a mobile phase of acetic acid, methanol, acetonitrile, and water (2/4/7.5/86.5) (fig.2). Using a column 4×300 mm with a flow rate of 3 ml/min created a pressure of 3000 psi. All chromatograms were obtained using Waters M6000A pump, U6K septumless injector (Waters Assoc., Milford, Mass.) with Schoeffel Model 770 UV detector (Schoeffel Inst., Westwood, N.J.) at 254 mm (0.2 Aufs.) and Fisher omniscribe recorder (Fisher Scientific, Cincinnati, Ohio). Solvents used were all of the spectral purity type; solvents

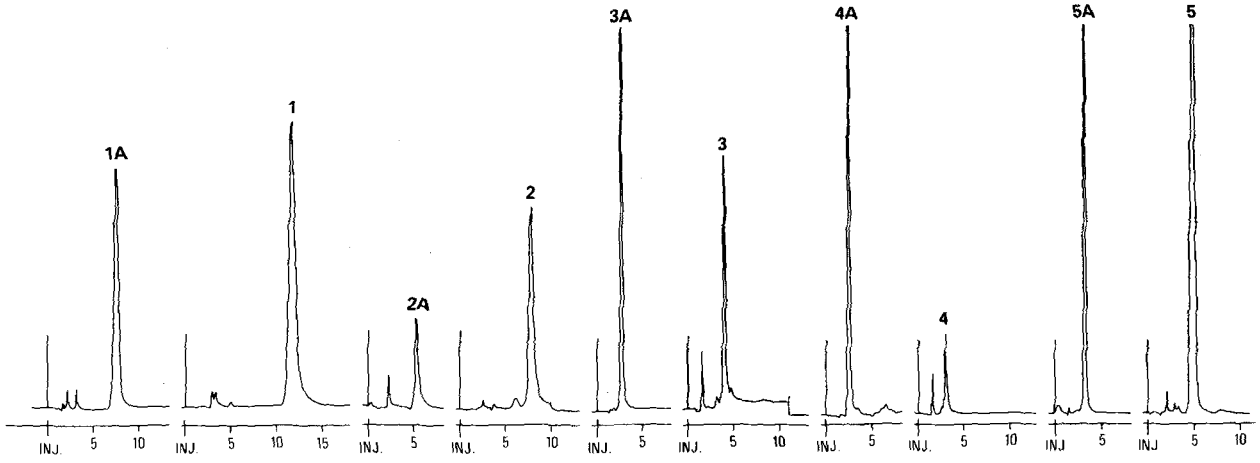


Figure 1. UV-range: 254 mm (0.2 Aufs.); chart speed: 5 mm/min; flow rate: 2 ml/min; column size: 4×300 mm; packing: microBondapak C18 (Waters); psi: 2000; solvent: 0.005 M TBA OH 1% $NH_4H_2PO_4$, pH 7.0, 1% CH_3CN.

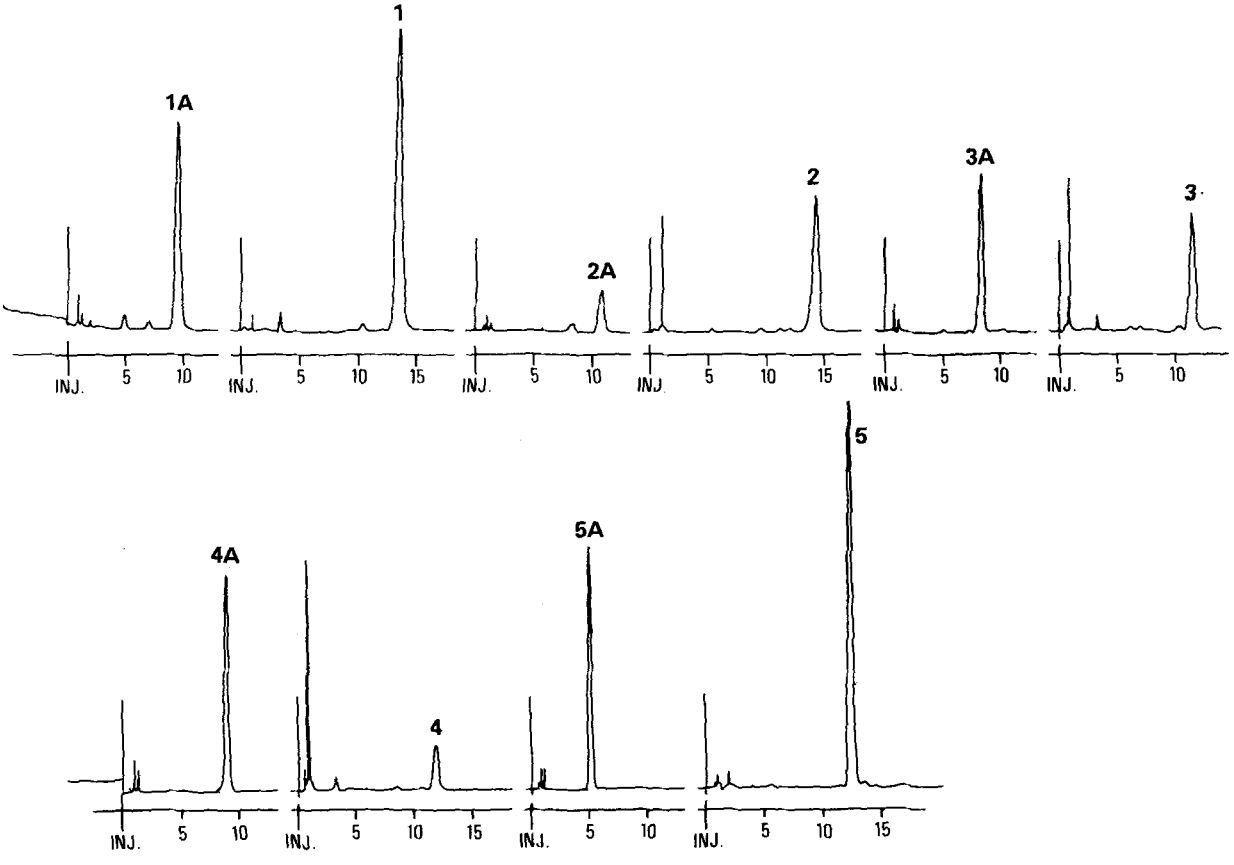
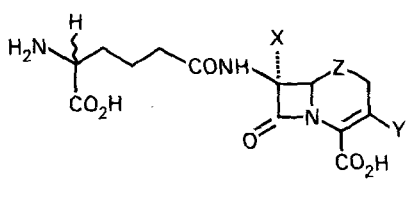


Figure 2. UV-range: 254 mm (0.2 Aufs.); chart speed: 5 mm/min; flow rate: 3 ml/min; column size: 4×300 mm; packing: microBondapak NH_2 (Waters); psi: 3000; solvent: 2/4/7.5/86.5 HOAc/MeOH/MeCN/H_2O.



1. X = H; Y = CH_2OAc; Z = S
1A. X = H; Y = CH_2OAc; Z = O
2. X = OCH_3; Y = CH_2OAc; Z = S
2A. X = OCH_3; Y = CH_2OAc; Z = O
3. X = H; Y = $CH_2O\overset{O}{\overset{\|}{C}}NH_2$; Z = S
3A. X = H; Y = $CH_2O\overset{O}{\overset{\|}{C}}NH_2$; Z = O
4. X = OCH_3; Y = $CH_2O\overset{O}{\overset{\|}{C}}NH_2$; Z = S
4A. X = OCH_3; Y = $CH_2O\overset{O}{\overset{\|}{C}}NH_2$; Z = O
5. X = H; Y = CH_3; Z = S
5A. X = H; Y = CH_3; Z = O

Table 1. Comparison of cephalosporins and corresponding oxa-β-lactam derivatives (paired ion chromatography (PIC) tetrabutyl ammonium hydroxide (0.005 M) 1% CH_3CN)

Compounds 1A–5A k'O	Compounds 1–5 k'S	k'S/k'O
1A 4.85	1 8.15	1.68
2A 3.23	2 5.15	1.59
3A 1.08	3 2.08	1.93
4A 0.92	4 1.31	1.42
5A 1.54	5 2.85	1.85

Table 2. Comparison of cephalosporins and corresponding oxa-β-lactam derivatives μ NH_2 bonded phase propylamine on 10 μm Silica (Waters) 2/4/7.5/86.5 $HOAc/CH_3OH/CH_3CN/H_2O$

Compounds 1A–5A k'O	Compounds 1–5 k'S	k'S/k'O
1A 14.2	1 20.3	1.43
2A 16.2	2 21.3	1.31
3A 12.0	3 17.1	1.43
4A 13.2	4 17.6	1.33
5A 7.6	5 18.8	2.47

which were not available commercially were distilled in glass before usage.

The data clearly indicate that a consistent trend in k'-values should be expected with nuclear variation in these compounds. In the case of the reversed phase system, changing from sulfur [S] to oxygen [O] derivative gave rise to a nearly linear decrease in k'-values. Table 1 illustrates this relationship and indicates that the ratio k'S/k'O is uniform. The microBondapak NH_2 system also yielded a decrease in k'-values in going from [S] to [O], but the data were not linear (table 2). However, it indicates that the ratio k'S/k'O is uniform in 4 out of 5 cases under these conditions. It is obvious that the presence of the oxygen atom in place of the sulfur atom has significant impact on the polarity of the entire β-lactam compound, and if this property alone is used to consider the chromatography of the derivative, a linear response could be expected. Although, when the additional ion-exchange property is exploited with the microBondapak NH_2 column, non-linearity and change of eluting order indicate that the effect of hetero-atom replacement can have varied effects on the pKa and therefore the retention properties in this system.

1 Acknowledgment. We gratefully acknowledge the cooperation of Drs Yuji Sendo, Toshiro Konoike, Masayuki Murakami and Mitsuru Yoshioka of Shionogi Research Laboratories, Shionogi and Co., Ltd, Fukushima-ku, Osaka, 553, Japan, who synthesized the oxa-β-lactams and made them available for this study.

2 Author for reprint requests: Norbert Neuss, Lilly Research Laboratories, Indianapolis/USA.

3 N. Neuss, R.D. Miller, C.A. Affolder, W. Nakatsukasa, J. Mabe, L.L. Huckstep, N. De La Higuera, A.H. Hunt, J.L. Occolowitz and J.H. Gilliam, Helv. chim. Acta *63*, 1119 (1980).

4 W. Nagata, in: Penicillin fifty years after Fleming, a Royal Society Discussion Organized by Sir James Baddiley FRS and E.P. Abraham, FRS, May 1979, p.225. The Royal Society, London.

5 Y. Sendo, T. Konoike, M. Murakami and M. Yoshioka, Heterocycles (1981).

The occurrence of 5-hydroxytryptamine in the holothurian, *Pentacter crassa*

R.P. Gregson, J.F. Marwood and R.J. Quinn

Roche Research Institute of Marine Pharmacology, P.O. Box 255, Dee Why, N.S.W. 2099 (Australia), 12 December 1980

Summary. 5-Hydroxytryptamine (5-HT) and hypoxanthine were isolated chromatographically from the holothurian *Pentacter crassa.* This study was initiated as a result of the observed hypotensive activity of a *P. crassa* extract. This activity was also encountered in extracts of the holothurians *Thelenota ananus* and *Stichopus chloronatus* and can be attributed to 5-HT.

In the marine environment 5-HT has been identified as a constituent of some species of molluscs[1,2], sea anemones[3], annelids[3], tunicates[4,5] and gorgonians[4]. The function of 5-HT in marine invertebrates, particularly molluscs, has been the subject of much physiological and pharmacological research[2,6] and it is now accepted that 5-HT does act as a neurotransmitter and in some cases, e.g. anemones, as a toxin. Few studies on the occurrence of 5-HT in echinoderms have been undertaken. It has been reported[7] that the level of 5-HT in echinoderm nervous tissue is very low and in the case of the sunflower starfish, 5-HT was not detected[8]. The presence of 5-HT in a sea urchin has been indicated by fluorescence[9] and cytochemical analysis indicated its presence in a starfish and a holothurian *Pentacter peterseni*[10]. This report describes the isolation of 5-HT, and hypoxanthine, from the holothurian *Pentacter crassa* (Ekman 1918) (Echinodermata: Holothuroidea)[11]. Hypoxanthine is present in the marine environment in clams, fish and soft corals[1].

This study was undertaken as a result of the biological activity displayed by an aqueous extract of *P. crassa,* which was trawled from N.E. of Cape Peron, Shark Bay, Western Australia and frozen upon collection. The frozen material (1.39 kg) was ground cryogenically, extracted with water and the aqueous extract **A** was subjected to a variety of pharmacological and microbiological bioassays. Potent hypotensive activity was observed when **A** was administered i.v. to pentobarbitone-sodium anaesthetized, deoxycorticosterone acetate-salt, hypertensive rats. Fractionation of **A** was guided by monitoring each separation for hypotensive activity. A solution of **A** (350 g) in water (3 l) was pumped through a column (52 × 45 cm) of Amberlite XAD-2 resin which was then washed with water (5 l) and methanol (9 l). Evaporation of the methanolic fraction gave **B** (16 g) which was partitioned between ethyl acetate and water to give an active, aqueous fraction **C** (9 g). An aqueous solution of **C** (9 g) was chromatographed on a column (7 × 10 cm) of octadecyl silica which was eluted with a water then water-methanol gradient. The material eluted in water **D** (1 g) was inactive, however, it was chromatogaphed on Sephadex G-25, fine, and a colourless solid (22 mg) obtained at V_R/V_M 2.57–2.94 which was