IDENTIFICATION OF THE ACTIVE METABOLITES OF THE ISOXAZOLYL-PENICILLINS BY MEANS OF MASS-SPECTROMETRY

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Electron-impact mass-spectrometry of the methyl esters of the isoxazolylpenicillins and of their active metabolites showed the latter to be formed from their parent compounds by hydroxylation of the 5-methyl group.

The formation of active metabolites from a number of penicillins is well documented[1~4]. For instance, about 10~20% of the penicillin activity in human urine after oral or intravenous administration of the isoxazolylpenicillins is in the form of a metabolite. The antibacterial activities of these metabolites are comparable to the parent compounds[4].

VAN HARKEN *et al.*[5] showed the metabolite of dicloxacillin to be 6-[3-(2,6-dichlorophenyl)-5-hydroxymethyl-4-isoxazolecarboxamido]-penicillanic acid, *i.e.* the hydroxymethyl derivative of the parent compound. As far as the chemical identity of the metabolites of oxacillin, cloxacillin, and flucloxacillin is concerned, in a recent paper it was stated by this author that the metabolites of oxacillin and cloxacillin are 5-hydroxymethyl derivatives too. The metabolite of flucloxacillin, however, should be of a different structure[6]. These propositions were based on differences in chromatographic characteristics observed for the respective metabolites. Mass-spectrometric investigation of the metabolites showed contrary to the afore-said that the metabolites of the various isoxazolylpenicillins all are 5-hydroxymethyl derivatives. The mass-spectrometric investigation will be discussed in this paper.

Experimental

Sodium oxacillin and sodium dicloxacillin were gifts of Bristol Laboratories; sodium cloxacillin and sodium flucloxacillin were gifts of Beecham Research Laboratories.

To obtain alkali-free acids, aqueous solutions of the compounds were extracted with methylene-chloride at pH 2.2. The separated organic layer was dried (anhydrous $MgSO_4$) and evaporated. Methyl esters were obtained by reacting the free acids with ethereal diazomethane.

The active metabolites were isolated from rat urine collected 0~8 hours after i.p. administration of 10 mg of the corresponding penicillin dissolved in saline. The isolation procedure was as described previously[6]. An additional purification was obtained by reverse-phase high performance liquid chromatography. Esterification was achieved as described for the parent compound. Trimethylsilyl (TMS) derivatives of the metabolites were prepared by reacting the methyl esters with BSTFA at room temperature. The low-resolution Electron-Impact(EI) ionization mass spectra were obtained using an LKB 2091 mass spectrometer. The samples to analyse were introduced directly into the source via a heated inlet. The source temperature was maintained at 200°C. Mass spectra were taken at 70 eV with a trap current of 50 μA.

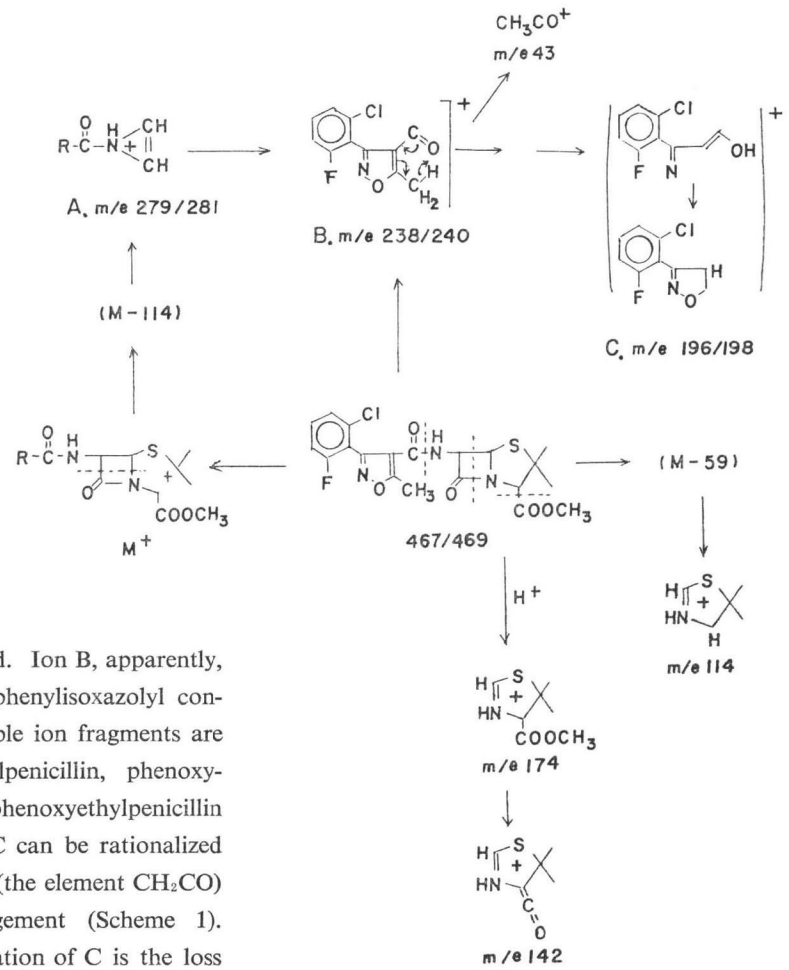
Results and Discussion

In as much as the partial mass spectra of the methyl esters of the various isoxazolylpenicillins and metabolites show the same characteristics, the fragmentation pattern of flucloxacillin and its metabolite will be illustrative for this group of compounds (Scheme 1; Fig. 1a, 1b).

The fragmentation pattern of the methyl esters of benzylpenicillin and phenoxymethylpenicillin following EI ionization is described in detail by RICHTER and BIEMANN[7]. Analogous pathways can be formulated for the methyl esters of the isoxazolylpenicillins. The base peak (*m/e* 174) corresponding to the protonated thiazolidine ion results from fragmentation of the β-lactam ring. This fragmentation probably follows on the protonation of the parent molecule[8]. Other fragments containing the thiazolidine structure are the ions *m/e* 142 and *m/e* 114, respectively. Ion fragments A, B, and C (Scheme 1) representing characteristic fragments for the isoxazolyl moiety are moderately abundant ions (Fig. 1a, 1b; Table 1).

Ion A can be rationalized from M^+ by successive loss of the elements $OCNCHCO_2CH_3$ (M−114) and $(CH_3)_2CS$. The fragment M−114 which was shown to be present in the spectra of the methyl esters of benzylpenicillin and phenoxymethylpenicillin[7] and also was seen for the methyl ester of phenoxyethylpenicillin (own observation) was not found for the isoxazolylpenicillins. Fragment B may result from A by elimination of the element NH C_2H_2 or it may result from the parent molecule by cleavage of the carbonyl-amide bonding. The counterpart of the cleaved parent molecule (*i.e.* the radical ion of the methyl ester of 6-aminopenicillanic acid), however, has not been observed. Ion B, apparently, is stabilized through the phenylisoxazolyl conjugated system. Comparable ion fragments are not observed for benzylpenicillin, phenoxymethylpenicillin[7], and phenoxyethylpenicillin (own observation). Ion C can be rationalized from B by loss of mass 42 (the element CH_2CO) secondary to a rearrangement (Scheme 1). Competitive with the formation of C is the loss

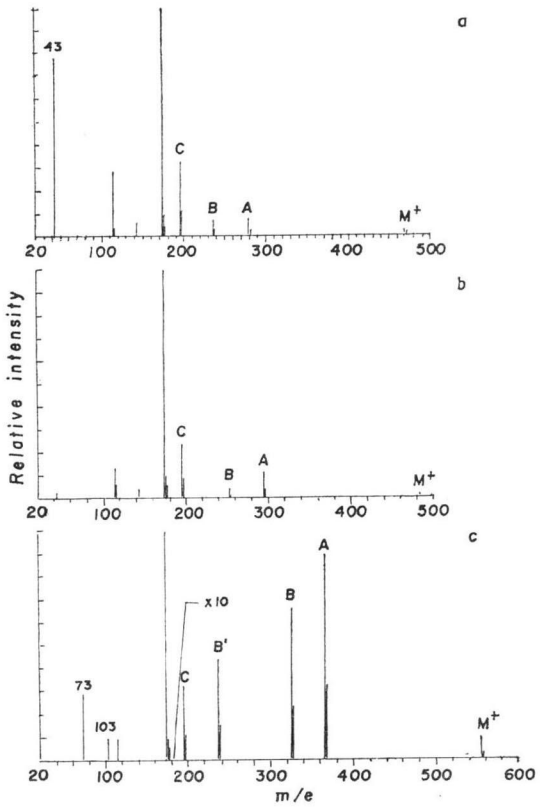
Scheme 1.



of the ion CH_3CO^+, *m/e* 43 (Fig. 1a).

The mass spectrum of the methyl ester of the flucloxacillin metabolite is shown in Fig. 1b. The molecular ion and the ions A and B are shifted towards higher masses by 16 units indicating the introduction of an oxygen atom in the isoxazolyl system (ions at *m/e*: 483/485, 295/297, and 254/256, respectively). Ion C is identical to C from the parent compound. The acetyl ion (*m/e* 43) is only weakly present (Fig. 1b) and probably results from impurities (Table 1). The obtained results are indicative for a hydroxymethyl structure of the metabolite. Ultimate proof of its structure is obtained from the mass spectrum of the TMS derivative of the methyl ester of the metabolite (Fig. 1c). In addition to the presence of the TMS derivatives of the ions A and B (ions at *m/e*: 367/369, and 326/328, respectively), and to the presence of ion C, two particular ions are found in the spectrum: (1) ion B′ (*m/e* 237/239) which can be rationalized from the loss of the element O-TMS from B; (2) ion *m/e* 103 which can be ascribed to the fragment CH_2O-TMS$^+$. Scheme 2 summarizes the latter fragmentations. The occurrence of ion B′ irrefutably identifies the metabolite to be the hydroxymethyl derivative. The main characteristics of the mass spectra of the methyl esters of the various isoxazolylpenicillins are given in Table 1.

Fig. 1. EI mass spectrum of the methyl ester of (a) flucloxacillin, (b) flucloxacillin metabolite, and (c) flucloxacillin metabolite-TMS derivative.



Scheme 2.

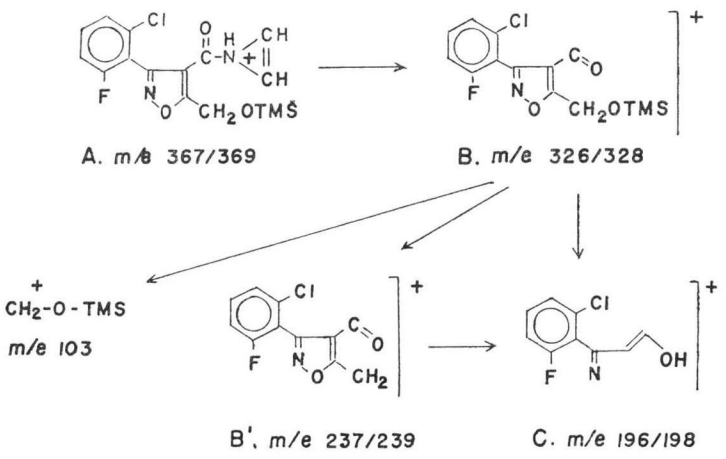


Table 1. Partial mass spectra of the methyl esters of the various isoxazolylpenicillins.

Ion[a]	Oxacillin						Cloxacillin						Flucloxacillin						Dicloxacillin					
	P[c]		M[d]		M-TMS[e]		P		M		M-TMS		P		M		M-TMS		P		M		M-TMS	
	m/e	RI[b]	*m/e*	RI	*m/e*	RI	*m/e*	RI	*m/e*	RI	*m/e*	RI	*m/e*	RI	*m/e*	RI	*m/e*	RI	*m/e*	RI	*m/e*	RI	*m/e*	RI
M+	415	4	431	2	503	<1	449	2	465	2	536	<1	467	1	483	1	555	<1	483	3	499		570	
							451	<1	467	<1	538		469	<1	485	<1	557		485	2	501	<1	572	<1
																			487	<1	503		574	
A	227	21	243	14	315	10	261	13	277	11	348	10	279	8	295	11	367	9	295	10	311	7	383	7
							263	5	279	4	350	3	281	3	297	4	369	3	297	7	313	5	385	5
																			299	1	315	<1	387	<1
B	186	15	202	9	274	10	220	10	236	7	307	8	238	6	254	4	326	7	254	6	270	4	342	5
							222	3	238	2	309	3	240	2	256	2	328	3	256	4	272	3	344	3
																			258	<1	274	<1	346	<1
B′					185	4					219	4					237	5					253	3
											221	1					239	2					255	2
																							257	<1
C	144	56	144	37	144	14	178	43	178	28	178	3	196	33	196	24	196	3	212	24	212	17	212	2
							180	16	180	10	180	1	198	11	198	8	198	1	214	15	214	11	214	1
																			216	2	216	2	216	<1
43		40						73						80		3				37		4		
103						8						10						9						8
174		100		100		100		100		100		100		100		100		100		100		100		100
114		17		14		10		46		18		10		27		14		9		14		18		9

a) Type of ion; see scheme 1 and 2. b) Relative intensity of the ion. c) Parent compound. d) Metabolite.
e) O-TMS derivative of the metabolite.

Conclusion

The active metabolites of the isoxazolylpenicillins are formed by hydroxylation of the 5-methyl group of the isoxazole ring system. This reaction probably is catalyzed by the monooxygenases present mainly in the liver.

The observed differences in the chromatographic behaviour between the metabolite of flucloxacillin on the one side and the metabolites of oxacillin, cloxacillin, and dicloxacillin on the other, therefore, are not the result of differences in chemical structure as has been suggested[6]. The reason for the difference in chromatography is not clear. The results, however, show that ΔRm-, $\Delta \log k'$-, and $\Delta \log P$ values not always are as predictable for a chemical structure as is suggested[9~11].

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