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High Resolution ^{1}H NMR Spectroscopic Studies of the Metabolism and Excretion of Ampicillin in Rats and Amoxycillin in Rats and Man

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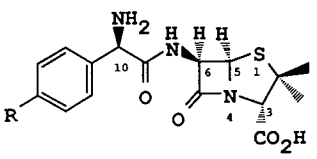
Abstract—High resolution proton nuclear magnetic resonance (^{1}H NMR) spectroscopy has been used to investigate the metabolism and urinary excretion of the aminopenicillins, ampicillin and amoxycillin, in rats and of amoxycillin in man. ^{1}H NMR resonances of the aminopenicillins, together with those for their 5*R*, 6*R* and 5*S*, 6*R* penicilloic acids and diketopiperazine metabolites were detected, assigned and quantified in urine samples with the aid of spin-echo NMR techniques. The dimer of amoxycillin was detected in rat urine for the first time together with novel drug-related resonances assigned to amoxycillin carbamate. Quantitative ^{1}H NMR spectroscopic results were consistent with HPLC and microbiological data considering that only single measurements were recorded. Due to the short analysis time and simple sample preparation, NMR was particularly useful for studying the metabolism of the aminopenicillins for which sample degradation poses analytical problems. The non-invasive character of ^{1}H NMR spectroscopic analysis of urine also provided unique information on a reversible reaction between amoxycillin and bicarbonate, an endogenous urinary metabolite.

Ampicillin (I) and amoxycillin (II) are semisynthetic penicillins, widely used as broad spectrum antibacterial agents (Williams & Kruk 1981). Both drugs have a plasma half-life of approximately 1·5 h in man and undergo little metabolism, being excreted largely unchanged into the urine (Kucers & Bennett 1987). During the first 6 h, ~30% of an oral and ~75% of an intravenous dose of ampicillin are



I R=H
II R=OH

recovered in the urine as unchanged drug, compared with ~60–70% (oral dose) and ~70–80% (i.v.) of amoxycillin (Brogden et al 1979; Simon et al 1985). Both drugs are unstable in aqueous solution and are known to undergo acid and base catalysed degradation to their 5*R*, 6*R* penicilloic acids (III and IV) (Schwartz 1969), which then readily epimerize to the 5*S*, 6*R* penicilloic acids (V and VI, respectively) under acidic and basic conditions (Bird et al 1983). Other degradation products include the diketopiperazines (VII and VIII) (Bundgaard 1977; Bundgaard & Larsen 1979) and the dimers (IX and X) (Bundgaard 1977). Compounds III, IV, V, VI, VII and VIII have been detected in biofluids and reported as metabolites of ampicillin and amoxycillin (Everett et al 1984; Haginaka & Wakai 1987; Haginaka et al 1987).

Excretion products of aminopenicillins have been studied

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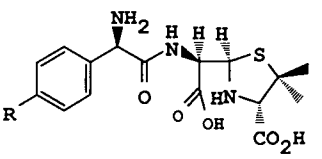
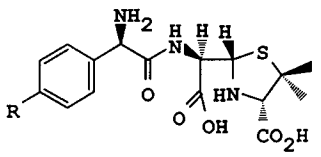
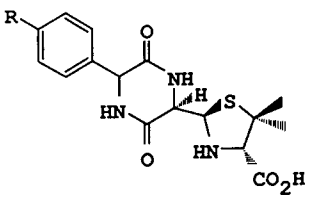


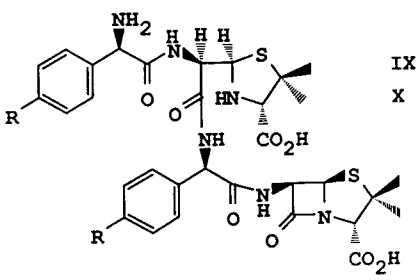
III R=H
IV R=OH



V R=H
VI R=OH



VII R=H
VIII R=OH



IX R=H
X R=OH

in untreated biological fluids by a variety of methods. Microbiological assays have been used extensively, although they are limited to antibacterially active compounds containing an intact β-lactam ring. HPLC has so far been the method of choice for detecting penicillin metabolites in biological samples (Miners 1985). However, extensive pre- or post-column derivatization of drug-related material is usually required to minimize interference by endogenous components before quantitative HPLC data can be obtained (Miners 1985). Ampicillin, III, V and VII have been identified in rat urine, bile and plasma by HPLC (Haginaka et al 1987). Using a similar method, amoxycillin, VI and VIII have been identified in human urine (Haginaka & Wakai 1987).

Mass spectrometry and nuclear magnetic resonance spectroscopy (NMR) have been traditionally used to identify isolated and purified penicillin compounds. More recently NMR and mass spectrometric methods have been successfully applied to the detection of penicillins and their metabolites in intact biological fluids. For example, the use of LC-MS and MS-MS to investigate ampicillin metabolism has resulted in the identification of the 3*S*, 5*R* and 3*S*, 5*S* epimers of III in urine extracts (Suwanrumpha & Freas 1989). The main advantages of using on-line MS detection with HPLC are the molecular weight and structural information the technique provides, whilst eliminating the need for derivatization procedures.

Over the last ten years NMR spectroscopy has been used increasingly to detect endogenous and exogenous components in biological fluids (Nicholson & Wilson 1989). The analysis of biofluids by NMR has had applications in a number of drug metabolism studies where it has been possible to detect drugs and their metabolites in urine (Nicholson 1989), bile (Gartland et al 1989; Basker et al 1990), tissue extracts, and in-vivo (Prior et al 1990). The metabolism and excretion of the penicillin flucloxacillin, for example, has been successfully investigated using ^{1}H and ^{19}F 1D and 2D NMR (Everett et al 1985, 1989). In the past, because of its relative insensitivity (detection limit of ~ 10 nM mL^{-1} for ^{1}H at 400 MHz), NMR has been most useful for studying drugs which are administered in relatively high doses, excreted mainly via one route, and transformed into a small number of metabolites (Nicholson & Wilson 1989). Investigation of the metabolism of drugs which do not meet these criteria by NMR may still provide useful data especially if the samples are partially purified before analysis (Nicholson & Wilson 1989). The advent of higher field NMR spectrometers, 600 MHz and above, will lead to greater analytical performance in drug metabolism studies.

There are a number of NMR active nuclei which can be used to detect drug metabolites in biofluids including ^{1}H (Nicholson & Wilson 1989), ^{19}F (Everett et al 1985, 1989; Prior et al 1990), ^{13}C (Preece & Timbrell 1990), ^{15}N (Wade et al 1990) and ^{31}P (Malet-Martino & Martino 1989). ^{1}H NMR detection is the most widely applicable, as it can be used to monitor the metabolism of any xenobiotic compound containing a CH_3, CH_2 or CH group with a characteristic NMR resonance in a region of the spectrum, relatively free from interference by endogenous component signals. One of the main advantages of using NMR detection is that it automatically provides structural information, because of the direct and well understood relationships between molecular structure and NMR chemical shifts, coupling constants and relaxation times. In addition, NMR provides an analytical overview of a biofluid sample, and will, in general, detect all compounds present above the detection threshold irrespective of chemical class. NMR may also be used to monitor dynamic processes such as chemical exchange in biofluids as it does not perturb chemical equilibria.

The reactions of aminopenicillin in biofluids are still not fully understood. We have therefore used ^{1}H NMR, HPLC and microbiological assays to investigate the excretion of the metabolites and degradation products of ampicillin and amoxycillin in human and rat urine and their interactions with endogenous components.

Materials and Methods

Dosing and urine collection

Male albino rats (CFY strain, Hacking and Churchill or Sprague-Dawley) were placed in metabolism cages with food and water freely available. On the day of the experiment the animals were given either ampicillin sodium (700 mg kg^{-1}, i.v.; CFY rats) or amoxycillin sodium (700 and 500 mg kg^{-1}, i.v; 200 and 100 mg kg^{-1}, i.p.; Sprague-Dawley) and waters loaded with 2 mL sterile water. Urine was collected directly into individual dry ice-cooled containers for a period of 24 h before dosing and at frequent intervals thereafter. All urine samples were stored at −70°C before assay.

An overnight-fasted normal volunteer received 3 g of amoxycillin trihydrate orally as a mixture with a sucrose base in water (30 mL). A standard production sachet of Amoxil 3 g was used for this purpose. Pre-dose and frequent post-dose urine samples were collected and aliquots were frozen at −70°C before analysis. This study was part of a larger volunteer study and was performed to a written protocol with the consent of the local ethics committee.

^{1}H NMR analysis

Untreated urine samples were mixed with D_2O (50 or 100 μL) to give a final volume of 500 μL and placed in 5 mm o.d. NMR tubes. Hahn spin-echo and 1-pulse spectra were acquired using saturation of the water resonance by gated decoupling during the relaxation delay. Identification and quantification of metabolites was based on adding accurately weighed quantities of reference standards of known purity into the biofluid and measuring the changes in the integrals (or peak heights where accurate integration was not possible) of the metabolite resonances. The conditions used for NMR analysis are listed in Table 1.

Microbiological analysis

Samples were asssayed microbiologically for ampicillin and amoxycillin using a large-plate agar diffusion technique with *Sarcina lutea* NCTC 8340 as the assay organism.

HPLC analysis

Untreated urine samples collected from rats dosed with ampicillin and amoxycillin and urine diluted with eluent were analysed by HPLC. The conditions used for HPLC analysis are shown in Table 2.

Table 1. Experimental conditions for NMR analysis.

	Ampicillin (rat)	Amoxycillin (rat)	Amoxycillin (man)
Spectrometer	Bruker WH400	Bruker AM400	Bruker WH400
Frequency	400 MHz	400 MHz	400 MHz
Probe	5 mm ^{1}H	5 mm ^{13}C ^{1}H dual	5 mm ^{1}H
Temperature	Ambient	Ambient	Ambient
Spectral width	4808 Hz	4800 Hz	4808 Hz
Transients	144	144	144
Steady-state transients	4	4	4
Data points in FID	16384	16384	16384
Data points in spectrum	16384	32768	16384
Echo time	60 ms	70 ms	60 ms
Relaxation delay	2·4 s	3 s	2·4 s
Linebroadening (Hz)	1	0·3	1
Volume of urine	450 μL	400 μL	450 μL
Volume of D_2O	50 μL	100 μL	50 μL
Reference	HOD (at 4·8 ppm)	TSP[a]	HOD (at 4·8 ppm)

[a] Trimethylsilyl propionic acid (TSP).

Table 2. Experimental conditions for HPLC analysis.

	Ampicillin (rat)	Amoxycillin (rat)
Instrument	Waters	Waters
Pumps	*M6000A*	*M6000A*
Programmer	M660	M660
Injector	7120 (Rheodyne)	7120 (Rheodyne)
Column	C18, 300 cm × 4 mm	C18 300 cm × 4 mm
Detector	Spectromonitor III	Spectromonitor III
Wavelength, λ	240 nm	240 nm
Injection volume	20 μL	10 μL
Flow rate	1 mL min^{-1}	1·2 mL min^{-1}
Eluent A	*0·05 M formate pH 5*	*50% v/v 0·1 M formate pH 4·5* *50% v/v distilled water*
Eluent B	62·5% v/v 0·05 M formate pH 5 37·5% v/v acetonitrile	50% v/v 0·1 M formate pH 4·5 12·5% v/v distilled water 37·5% v/v acetonitrile
Programme	Initial: 90% eluent A 0–5 min: to 70% A	Initial: 90% A 0–15 min: to 65% A 15–20 min: held at 65% A

Results and Discussion

The *gem*-dimethyl group at the C2 position of the thiazolidine ring of compounds I–X gives rise to a pair of sharp three-proton singlets which resonate between δ_H ~0·5–1·7 ppm in the ^{1}H NMR spectrum (Figs 1–3). This region of the control urine spectrum is relatively free from endogenous component resonances allowing penicillin-related signals to be observed, although there is some peak overlap with doublet signals arising from the methyl groups of organic acids and amino acids such as lactate and alanine. Using a Hahn spin-echo pulse sequence, with a refocusing delay of 60–70 ms, NMR spectra were edited according to resonance multiplicities which resulted in drug-related singlet peaks and endogenous component doublets appearing with opposite phase in the spectrum (Fig. 1A). It was then easy to distinguish between exogenous and endogenous NMR resonances. The chemical shifts of the paired methyl singlets for compounds I–X in urine were pH dependent so that to achieve unambiguous assignments it was necessary to add authentic standards directly into the sample and re-acquire the spectrum.

Urine and plasma contain a wide variety of substances including metal ions, phosphate, OH^-, H^+, alcohols, protein, amino acids and sugars which can enhance the rate of degradation of aminopenicillins (Bundgaard 1977; Bundgaard & Larsen 1979; Tiwari et al 1980; Bundgaard & Hansen 1981, 1982). It is, therefore, unclear whether the penicillin-derived compounds identified in this work are true metabolites or simply degradation products and they will therefore be referred to as metabonates.

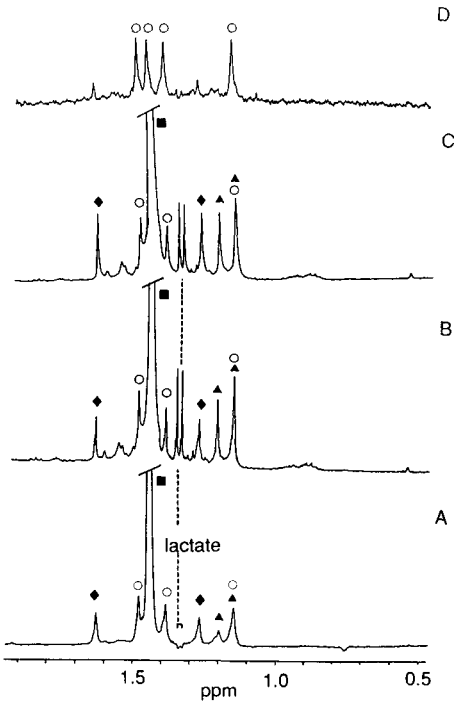


FIG. 1. Low frequency regions of the 400 MHz ^{1}H NMR spectra of rat urine samples collected up to 2 h after dosing with amoxycillin (200 mg kg^{-1}, i.p.). A spin-echo spectrum using a delay, $\tau = 70$ ms; B 1-pulse spectrum of the same sample with 100 μg IV added; C as for B but with added VIII 100 μg; D 1-pulse spectrum of 1 mg mL^{-1} X in D_2O (pD 7·5). ■ Amoxycillin, ◆ VIII, ▲ IV, ○ X.

The metabolism of ampicillin in rats

Quantitative ^{1}H NMR spectroscopy, HPLC and microbiological assays were performed on rat urine samples collected at various time points up to 24 h after dosing with 700 mg kg^{-1} ampicillin (Table 3). In the ^{1}H NMR spectra of post-dose urine samples, the *gem*-dimethyl peaks of ampicillin, III, V and VII were assigned by experiments in which authentic compounds were added to biofluids. Quantitative ^{1}H NMR spectroscopic data for ampicillin and VII in urine samples up to 6 h post-dose agreed well with those obtained from microbiology and HPLC, considering that only single measurements were taken. Agreement between the NMR and microbiological data for the 6–8 and 8–24 h samples was less good, possibly due to the low signal-to-noise ratio of drug-related resonances in the NMR spectra of these urine samples. These data confirm a preliminary ^{1}H NMR study which reported that ampicillin III, V and VII were detected in post-dose urine samples following the administration of ampicillin (Everett et al 1984). This latter study was the first to detect diketopiperazine as a metabonate of an aminopenicillin.

The metabolism of amoxycillin in rats

Quantitative ^{1}H NMR spectroscopy, HPLC and microbiological assays were carried out on urine samples collected from rats dosed with 700, 500, and 100 mg kg^{-1} amoxycillin

Table 3. Quantitative NMR, microbiological and HPLC data for ampicillin and its metabonates (expressed in $\mu g\ mL^{-1}$ urine) following the administration of the sodium salt of ampicillin (700 mg kg^{-1}, i.v.) to rats.

Urine sample	NMR				Microbiology	HPLC	
	Ampicillin	III	V	VII	Ampicillin	Ampicillin	VII
Rat 1[a]							
0–2 h	12040	846	219	406	11685	10906	303
2–4 h	2131	440	120	48	1764	2517	53
4–6 h	1409	245	154	37	1010	1170	43
6–8 h	246	63	51	10	182	270	32
8–24 h	182	—[c]	—[c]	—[c]	175	193	—[c]
Rat 2[b]							
0–3 h	5869[b]	388	101	160	5869[b]	—[d]	—[d]
3–6 h	570	166	52	35	691	—[d]	—[d]
6–8 h	151	19	19	12	261	—[d]	—[d]
8–24 h	130	—[c]	14	11	259	—[d]	—[d]

[a] All quantitative NMR data for rat 1 were based on the change in peak intensity recorded after adding 547 μg ampicillin into the 2–4 h urine sample. [b] All quantitative NMR data for rat 2 was based on the microbiological data for the 0–3 h urine sample. [c] Not detected. [d] Not analysed.

(Tables 4, 5). NMR resonances arising from amoxycillin, IV, VI and VIII were identified in post-dose urine samples by addition of authentic standards. Additional resonances consistent with the dimer of amoxycillin (X) were observed in NMR spectra of 0–2 h urine samples taken from rats dosed with 200 mg kg^{-1} amoxycillin. The molar concentration of X in these urine samples was similar to that of VIII after dosing with 200 mg kg^{-1} amoxycillin (Fig. 1). Variation in the amount of dimer observed is consistent with the known dependence on pH of its formation (Bundgaard 1977).

The NMR spectra of 0–2 and 2–4 h urine samples taken from rats dosed with 100 mg kg^{-1} revealed the presence of an extra pair of methyl peaks, in addition to those corresponding to amoxycillin IV and VIII. These resonances were not assignable to any known metabolite or degradation product of II and were labelled "y". The chemical shifts of resonances

Table 4. Quantitative NMR and HPLC data of amoxycillin, XI, VIII and IV in rat urine expressed as mean (μmol/sample $\pm$ s.e.m.)[a] after dosing with the sodium salt of amoxycillin (100 mg kg^{-1}, i.p.).

Urine sample	NMR				HPLC	
	Amoxycillin	XI	VIII	IV	Amoxycillin	VIII
0–2 h	24·36 ± 10·0	2·50 ± 1·58	0·28 ± 0·12	0·51 ± 0·34	24·81 ± 6·72	0·27 ± 0·09
2–4 h	2·78 ± 1·80	0·31 ± 0·20	0·09 ± 0·05	0·26 ± 0·18	2·98 ± 1·38	0·10 ± 0·06
4–6 h	0·44 ± 0·38	—[b]	0·04 ± 0·04	0·04 ± 0·06	0·56 ± 0·35	0·07 ± 0·06
6–8 h	0·19 ± 0·14	—[b]	0·01 ± 0·01	0·01 ± 0·01	0·24 ± 0·16	0·01 ± 0·01
8–24 h	0·15 ± 0·08	—[b]	0·06 ± 0·05	—[b]	0·85 ± 0·82	0·12 ± 0·11

[a] Standard error of mean, n = 5 rats. [b] Not detected.

Table 5. Quantitative NMR and microbiological data for amoxycillin, IV, VI and VII in rat urine (expressed in $\mu g\ mL^{-1}$) after dosing with the sodium salt of amoxycillin (700 and 500 mg kg^{-1}, i.p.).

Rat (dose)	Urine sample	Microbiology	NMR			
		Amoxycillin	Amoxycillin	IV	VI	VIII
1 (700 mg kg^{-1})	0–2 h	10014[a]	10014[a]	1525	284	1050
	2–4 h	2041	2306	267	69	162
	4–6 h	1721	1868	280	150	192
	6–8 h	656	656	57	—[c]	38
	8–24 h	405	—[d]	—[d]	—[d]	—[d]
2 (500 mg kg^{-1})	0–2 h	5465	5655	171	63	138
	2–4 h	645	1498	95	48	73
	4–6 h	415	398	—[c]	—[c]	—[c]
	6–8 h	565	685	67	21	27
	8–24 h	420	453	—[c]	19	27
3 (500 mg kg^{-1})	0–3 h	6823	5925	—[c]	~35[b]	240
	3–6 h	934	1180	560	29	64
	6–8 h	357	434	300	25	24
	8–24 h	66	43	—[c]	—[c]	—[c]

[a] All quantitative NMR results for rats 1–3 were based on the microbiology data for amoxycillin in the 0–2 h sample of rat 1. [b] Peak obscured. [c] Not detected. [d] Not analysed.

Table 6. ^{1}H NMR chemical shift (δ in ppm) and coupling constant data (J in Hz) for amoxycillin and y in rat urine at pH 8·9.

	δ_H ppm CH_3's	δ_H ppm H3	δ_H ppm H5 and H6	δ_H ppm H10	δ_H ppm Phenyl
Amoxycillin	1·43	4·17	5·47; 5·44	—[a]	6·90; 7·29
	1·48		($^3J_{H5,H6}$ = 3·9)		(^3J = 8·9)
y	1·47	4·23	5·46; 5·50	—[a]	6·89; 7·28
	1·53		($^3J_{H5,H6}$ = 3·9)		(^3J = 8·9)

[a] The H10 proton resonances of amoxycillin and y were obscured by the water peak.

arising from amoxycillin and y were similar (Table 6), with the aromatic signals almost coincident but with small high frequency shifts for protons H3, H5, H6 and the *gem*-dimethyl groups of y relative to amoxycillin. There was no change in the $^3J_{H5,H6}$ coupling constant (3·9 Hz) indicating that the relative configuration at C5 and C6 was unchanged. It was inferred that the β-lactam ring of y was still intact, as its hydrolysis generally results in a substantial increase in $\Delta\delta$ (H5–H6) (Bird et al 1983; Haginaka & Wakai 1986).

Dilution and variable temperature experiments suggested that y was the product of a reversible reaction involving amoxycillin rather than being a true metabolite. Dilution of a rat urine sample containing a 2:1 ratio of amoxycillin:y resulted in a conversion of y into amoxycillin to give a new ratio of approximately 4:1 (Fig. 2). Increasing the temperature of a urine sample containing y and amoxycillin to 349 K resulted in a more substantial change in the ratio which was reversed on cooling the sample back to room temperature (Fig. 3, Table 7). At high temperatures (349 K) there was also

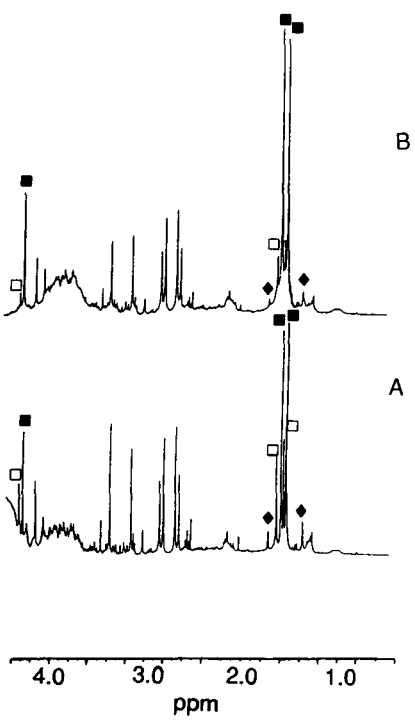


FIG. 2. Low frequency regions of 400 MHz ^{1}H NMR spectra showing the effect of dilution on the relative intensities of II and y in rat urine collected 2 h after dosing with amoxycillin (100 mg kg^{-1}, i.p.). A 1-Pulse spectrum, B 1-pulse spectrum of the same sample after a 1 in 10 dilution. ■ Amoxycillin, ◆ VIII, □ y.

Table 7. Changes in the ratio of the intensities of the *gem*-dimethyl peaks of amoxycillin and y (II:y) and their observed spin–spin relaxation times (T_{2obs} in ms) with temperature (K).

K	Ratio	T_{2obs} amoxycillin	T_{2obs} y	T_{2obs} VIII
294	2·3:1·0	~130	~130	~110
304	3·2:1·0	~130	~130	~110
319	6·2:1·0	—	—	—
329	47:1·0	~100	~60	~130
339	166:1·0	~70	~60	~130
349	∞	~70	~60	~100
329	70:1·0	~70	~60	~100
294	2·9:1·0	~100	~110	~100

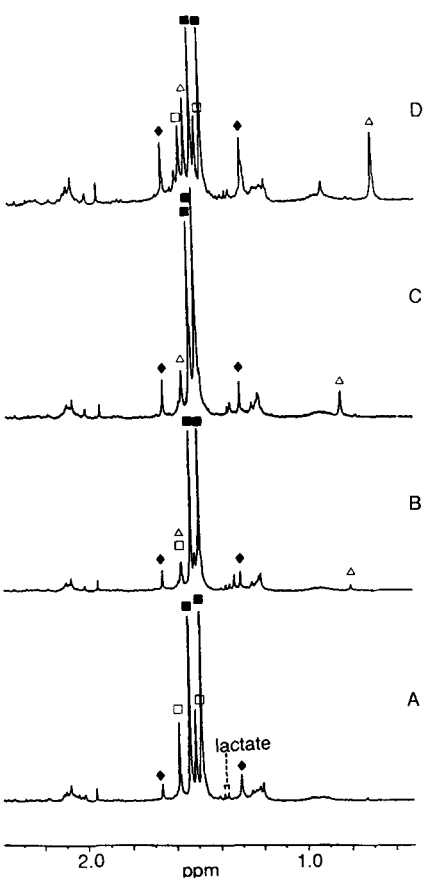


FIG. 3. Low frequency regions of 400 MHz ^{1}H NMR spectra showing the effect of temperature on the relative intensities of amoxycillin and y in a 0–2 h urine sample. A 294 K; B 309 K (the relative intensities of resonances from y and amoxycillin have decreased and increased, respectively); C 339 K (some y may be present under the VI resonance at 1·53 ppm); D after recooling to 294 K (y has reappeared together with resonances at 0·6 and 1·5 ppm corresponding to VI). The chemical shifts of amoxycillin, IV, VI and VIII are temperature dependent. In C the peak at 1·53 ppm was assumed to be mainly due to VI as the resonance for y at 4·14 ppm has disappeared. ■ Amoxycillin, △ VI, ◆ VIII, □ y.

a slight decrease in the observed spin–spin relaxation time (T_{2obs}) of the *gem*-dimethyl resonances of amoxycillin and y (from ~130 to ~70 and ~60 ms, respectively) whilst there was no change in the corresponding T_{2obs} for other metabolites. All these results indicated that there was an interconversion which was slow on the NMR timescale and that the position of equilibrium was altered in favour of amoxycillin at high temperatures. These observations suggested that y may result from the reversible reaction of amoxycillin with a "^{1}H NMR-invisible" endogenous compound (such as an inorganic ion or urinary protein), a self-associated form of amoxycillin (previously reported for a number of penicillins (Thakkar & Wilham 1971; Bird et al 1982)) or a rotamer of amoxycillin. The latter two suggestions were thought less likely as it was not possible to form y in model solutions, by varying pH or concentration, or by adding amoxycillin into control urine. Similar experiments with the 10*S* isomer of amoxycillin confirmed that y was not the product of racemization at C10.

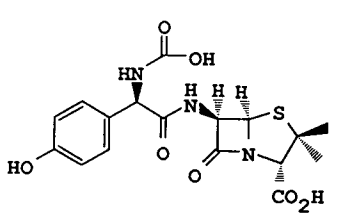
An NMR study involving model solutions of II and various inorganic urinary metabolites revealed that a chemical species XI with identical NMR properties to y (Tables 6, 8) was produced in the presence of 1 M HCO_3^- ions at pH 7·5.

Table 8. NMR chemical shift (δ in ppm) and coupling constant (J in Hz) data for amoxycillin and XI in 1 M carbonate solutions (pD 8·5 in D_2O).

	CH_3's	H3	H5 and H6	H10	Phenyl
Amoxycillin	1·45	4·19	5·46; 5·50	—[a]	6·92
	1·48		($^3J_{H5,H6}$ = 3·8)		7·32
XI	1·47	4·23	5·48; 5·53	—[a]	6·94
	1·54		($^3J_{H5,H6}$ = 3·8)		7·30

[a] The H10 proton resonances of amoxycillin and XI were obscured by the water peak.

The formation of XI was pH-dependent, with its concentration increasing relative to amoxycillin as the pH of the solution was increased and disappearing below pH 7. It was concluded that amoxycillin had reacted with CO_2 to form a carbamate (XI) in the HCO_3^- solution and also that XI corresponded to y. The reversible formation of amino acid carbamates in the presence of CO_2 is a well documented reaction (Lemieux & Barton 1971), with the rate of reaction and amount of carbamate formed depending on CO_2 concentration and the presence of an uncharged amino group. The C10 amino group of amoxycillin has a pK_a of 7·55 (Bird et al 1983) so that, at the pH of the urine samples in which y was observed (pH $\geqslant$ 7·9 (Connor 1989)), the NH_2 group of amoxycillin was mainly uncharged. The extent of carbamate formation in each sample would then be dependent on the extent of bicarbonate excretion (pK_a = 6·37). The conclusion that y was the carbamate of amoxycillin was supported by the fact that the changes in the ratio of amoxycillin:y produced by dilution and by variations in temperature were reproduced for amoxycillin:XI in model solutions. Additional evidence that y was the product of a reversible reaction of amoxycillin with bicarbonate (rather than being a metabolite of amoxycillin) was the observation that 10*S* amoxycillin carbamate was formed when the 10*S* isomer was added into a "problem" urine sample (Connor 1989). The presence of XI in some samples provided indirect evidence of high amounts of carbonate excretion by some animals (normal range = 0·5–12·0 mg kg^{-1} day^{-1} (Altman & Dittmer 1974)). The formation of this novel metabonate of amoxycillin may have biological significance as it would be favoured in conditions of metabolic alkalosis and therefore could influence the pharmacokinetics or degradation rate of this drug in the body. This could then give rise to some variation between patients treated with this compound.



XI

Quantitative NMR results for amoxycillin in urine were compared with those obtained from HPLC and microbiological assays (Tables 4, 5). For urine samples which contained XI, NMR values for amoxycillin and XI were compared with HPLC values for amoxycillin, as dilution of the urines for HPLC assay would destroy XI. The values in Table 4 are expressed as mean results averaged for five rats. Of particular significance were the results for the 0–2 h urine from rat 1 (NMR 8·1 and 4·5 μM respectively for amoxycillin and XI; HPLC 13·4 μM for amoxycillin). Considering that each figure represented single measurements, data from all three assays compared favourably. For urine samples collected after dosing with 100 mg kg^{-1} amoxycillin, the NMR data for VIII compared less well with HPLC data. This was due partly to the less favourable signal-to-noise ratio in the NMR spectra and partly due to increased interference in HPLC traces from endogenous components. It was not possible to obtain HPLC data for IV and VI in untreated urine due to peak overlap with endogenous components.

The metabolism of amoxycillin in man

Quantitative NMR and microbiological assays were performed on urine samples collected from a subject dosed orally with 3 g amoxycillin (Table 9). NMR resonances for amoxycillin, IV, VI and VIII were unambiguously identified in all urine samples collected up to 24 h post-dose. These

Table 9. Quantitative NMR and microbiological data for amoxycillin, IV, VI and VIII in human urine (expressed as μg mL^{-1}) after oral dosing with 3 g amoxycillin trihydrate.

Urine sample	Microbiology	NMR[a]			
	Amoxycillin	Amoxycillin	IV	VI	VIII
0–2 h	2123	1755	125	60	90
2–4 h	5959	3332	1249	724	143
4–6 h	1074	1202	381	306	36
6–8 h	233	269	92	155	21
8–10 h	55	76	(a)	66	30
10–12 h	29	41	(a)	41	(a)

[a] Not detected. All quantitative NMR data was based on the change in peak intensity produced by adding 562 μg IV into the 2–4 h urine sample.

observations are consistent with literature data on the metabolism of amoxycillin in man using HPLC detection which identified amoxycillin, IV, VI and VIII in urine (Haginaka & Wakai 1987).

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