Nuclear Magnetic Resonance and Circular Dichroism of Penicillins derived from Disubstituted Acetic Acids

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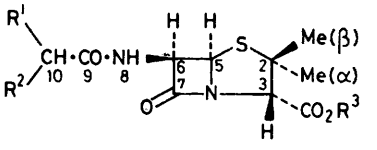
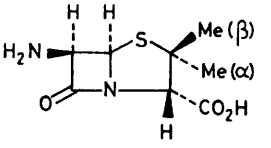
N.m.r. and c.d. spectra of some isomeric pairs of penicillins derived from disubstituted acetic acids are reported. For those penicillins which have an aromatic group attached to C-10 empirical correlations are observed between the configuration at C-10 and, (*a*) the sign of a c.d. band at 200—210 nm, and (*b*) the chemical shift of the 3-H and methyl protons. These correlations are used to assign the *R* configuration to the crystalline methyl (3) and ethyl (4) esters of carbenicillin and to the calcium insoluble isomers of carbenicillin (8) and ticarcillin (9). Many of the penicillins show an upfield shift of the methyl protons relative to their position in 6-aminopenicillanic acid (16). This is ascribed to shielding effects of the side-chain aromatic ring produced by folded conformations of the side-chain. For the penicillins (14) and (15) derived from tyrosine, side-chain rotamer proportions calculated from coupling constants are discussed in relation to chemical shifts. Bands at about 230 and 260—280 nm in the c.d. spectra are discussed in comparison with previous c.d. studies of penicillins and the relevant side-chain acids.

NUCLEAR MAGNETIC RESONANCE [1] and circular dichroism [2] studies have shown significant differences between the spectra of the diastereoisomers of phenethicillin (1). Similar n.m.r. effects have been observed in the Beecham laboratories for other diastereoisomeric pairs of penicillins and this study has been undertaken to investigate differences in n.m.r. and c.d. spectra between penicillin isomers derived from several disubstituted acetic acids. Compounds (1)—(16) have been studied.

The configuration at the C-3, C-5, and C-6 asymmetric centres is *S*, *R*, and *R* respectively, as in all penicillins synthesised from the naturally occurring precursor, 6-aminopenicillanic acid (16). The esters (2), (3), and (4) crystallise from aqueous alcohols as a single diastereoisomer,[3] even though C-10 is not deliberately resolved in the synthesis. An *X*-ray structure determination has shown [4] that the crystalline phenyl ester (2) has the *R* configuration at C-10 and the results reported here show that compounds (3) and (4) also crystallise in the *R* configuration. Compounds (1), (8), and (9) are normally prepared as mixtures of the diastereoisomers. Samples of the separate isomers of (1) prepared from the resolved side-chain acid were used. The isomers of (8) and (9) can be separated by preferential precipitation of one form



(1)–(15)



(16)

Number	R¹	R²	R³	Configuration at C-10	Name
(1)	PhO	Me	K	*R* and *S*	Phenethicillin
(2)	Ph	CO_2Ph	Na	*R*	Carfecillin
(3)	Ph	CO_2Me	Na	See text	
(4)	Ph	CO_2Et	Na	See text	
(5)	Ph	OH	Na	*R*	
(6)	Ph	OH	Na	*S*	
(7)	Ph	SO_3Na	Na	*R* and *S*	Sulbenicillin
(8)	Ph	CO_2Na	Na	*R* and *S*	Carbenicillin
(9)	3-Thienyl	CO_2Na	Na	*R* and *S*	Ticarcillin
(10)	Ph	NH_2	H	*R*	Ampicillin
(11)	Ph	NH_2	H	*S*	
(12)	4-OH.C_6H_4	NH_2	H	*R*	Amoxycillin
(13)	4-OH.C_6H_4	NH_2	H	*S*	
(14)	4-OH.$C_6H_4CH_2$	NH_2	H	*R*	
(15)	4-OH.$C_6H_4CH_2$	NH_2	H	*S*	

as a low solubility calcium salt [5,6] and samples of the separate isomers prepared in this way were used for some of the work reported here.

RESULTS

N.m.r. Spectra.—Preliminary spectra were run at 60 MHz on solutions containing *ca.* 90 mg/ml in D_2O for compounds (1)—(9) and *ca.* 70 mg/ml in D_2O adjusted to pD 8 with NaOD for (10)—(15). These showed differences between the isomers in the methyl, 3-H, 5-H/6-H, and 10-H chemical shifts for most of the isomeric pairs, although in several cases the resolution was not adequate to determine the individual shifts reliably. The major difference reported [1] for the phenethicillin (1) isomers was a singlet for 5-H/6-H in the *R* isomer but a well spaced quartet in the *S* isomer. This behaviour was observed here with the isomeric pairs (10)/(11), and (14)/(15), but all other pairs gave a quartet for these protons in both isomers. The esters (3) and (4) gave spectra in fresh solution in D_2O indicative of a single diastereoisomer, but in D_2O adjusted to pD 9 the spectra showed no 10-H signal and doubling of the methyl, 3-H and 5-H/6-H signals, indicating a mixture of isomers. The spectra in D_2O alone changed with age of solution, indicating gradual conversion into the isomeric mixture. Carfecillin (2) showed similar effects, although the rate of change was much faster than with compounds (3) and (4) and the spectra were abnormally broad-banded so that the only evidence of isomerisation was doubling of the 3-H signal and the development of two small broad lines on either side of a broad singlet for 5-H/6-H.

Spectra were run at 250 MHz on low concentration

TABLE 1

Chemical shifts from 250 MHz spectra, relative to sodium dimethylsilapentane-1-sulphonate

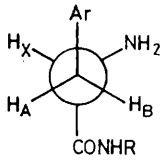
Compound [Solvent]	C-10 Configuration	C-2 methyls	Chemical shift, δ (*J*/Hz) 3-H	5-H and 6-H	10-H	Side-chain
(1) [D_2O]	*R*	1.43s, 1.50s	4.17s	5.44, 5.47q (*J* 3.8)	4.91q (*J* 6.7)	Me 1.59d (*J* 6.7); Ph 6.9—7.5m
	S	1.46s, 1.47s	4.20s	5.37, 5.54q (*J* 3.9)	4.93q (*J* 6.7)	Me 1.59d (*J* 6.7); Ph 7.0—7.5m
(2) [D_2O]	*R* [a]	1.48s, 1.52s	4.23s	5.53, 5.57q (*J* 3.9)	5.3s	Ph 7.2—7.6m
	S [a]	1.52s, 1.59s	4.28s	5.49, 5.64q (*J* 3.9)	*b*	Ph 7.1—7.6m
(3) [D_2O]	Presumed *R* [a]	1.46s, 1.51s	4.20s	5.47, 5.52q (*J* 3.8)	4.98s	Me 3.80s; Ph 7.4—7.5m
	Presumed *S* [a]	1.54s, 1.61s	4.26s	5.47, 5.61q (*J* 4.1)	*b*	Me 3.81s; Ph 7.4—7.5
(4) [D_2O]	Presumed *R* [a]	1.45s, 1.50s	4.20s	5.46, 5.52q (*J* 3.9)	4.95s	Me 1.26t (*J* 7.0); CH_2 4.26q (*J* 7.0); Ph 7.4—7.5m
	Presumed *S* [a]	1.52s, 1.60s	4.25s	5.44, 5.59q (*J* 3.9)	*b*	Me 1.28t (*J* 7.0); CH_2 4.25q (*J* 7.0); Ph 7.4—7.5m
(5) [D_2O]	*R*	1.48s, 1.56s	4.23s	5.48, 5.55q (*J* 3.9)	5.26s	Ph 7.4—7.5m
(6) [D_2O]	*S*	1.50s, 1.61s	4.26s	5.45, 5.56q (*J* 3.9)	5.25s	Ph 7.4—7.5m
(7) [D_2O]	*R* [c]	1.45s, 1.52s	4.22s	5.50, 5.55q (*J* 3.9)	5.08s	Ph 7.4—7.6m
	S [c]	1.52s, 1.60s	4.26s	5.58, 5.61q (*J* 4.4)	5.09s	Ph 7.4—7.6m
(8) [D_2O]	Ca insoluble [d]	1.48s, 1.52s	4.24s	5.49, 5.57q (*J* 3.9)	4.55s	Ph 7.4—7.5m
	Ca soluble [d]	1.52s, 1.61s	4.27s	5.55, 5.59q (*J* 3.9)	4.50s	Ph 7.4—7.5m
(9) [D_2O]	Ca insoluble [e]	1.46s, 1.50s	4.22s	5.46, 5.55q (*J* 4.1)	4.65s	Thiophen 7.1—7.5m
	Ca soluble [e]	1.49s, 1.57s	4.24s	5.52, 5.56q (*J* 4.3)	4.60s	Thiophen 7.1—7.5m
(10) [D_2O]	*R*	1.39s, 1.39s	4.12s	5.47, 5.50q (*J* 3.8)	5.19s	Ph 7.4—7.5m
(11) [D_2O]	*S*	1.37s, 1.45s	4.17s	5.44, 5.57q (*J* 4.0)	5.20s	Ph 7.5brs
(10) [D_2O + NaOD to pD 8]	*R*	1.40s, 1.48s	4.15s	5.44s	4.63s	Ph 7.4brs
(11) [D_2O + NaOD to pD 8]	*S*	1.47s, 1.49s	4.20s	5.39, 5.55q (*J* 4.0)	4.73s	Ph 7.4brs
(12) [D_2O]	*R*	1.40s, 1.41s	4.13s	5.47s [f]	5.08s	C_6H_4 6.9, 7.3 dd
(13) [D_2O]	*S*	1.35s, 1.45s	4.18s	5.42, 5.57q (*J* 4.1)	5.12s	C_6H_4 6.9, 7.3 dd
(12) [D_2O + NaOD to pD 8]	*R*	1.41s, 1.47s	4.15s	5.42, 5.44q (*J* 3.9)	4.56s	C_6H_4 6.8, 7.2 dd
(13) [D_2O + NaOD to pD 8]	*S*	1.45s, 1.46s	4.19s	5.38, 5.55q (*J* 3.9)	4.69s	C_6H_4 6.9, 7.3 dd
(14) [D_2O]	*R*	1.46s, 1.54s	4.15s	5.40, 5.51q (*J* 4.0)	4.23 [g]	CH_2 3.05, 3.15 [g]; C_6H_4 6.9, 7.1 dd
(15) [D_2O]	*S*	1.47s, 1.48s	4.17s	5.44, 5.51q (*J* 4.1)	4.21 [g]	CH_2 3.06, 3.13 [g]; C_6H_4 6.9, 7.1 dd
(14) [D_2O + NaOD to pD 8]	*R*	1.46s, 1.53s	4.15s	5.40, 5.44q (*J* 3.9)	3.80 [g]	CH_2 2.82, 2.97 [g]; C_6H_4 6.7, 7.0 dd
(15) [D_2O + NaOD to pD 8]	*S*	1.46s, 1.49s	4.16s	5.36, 5.48q (*J* 3.9)	3.86 [g]	CH_2 2.89, 2.97 [g] C_6H_4 6.8, 7.1 dd
(16) [D_2O]		1.53s, 1.65s	4.31s	4.98, 5.59q (*J* 4.1)		
(16) [D_2O + NaOD to pD 8]		1.50s, 1.60s	4.16s	4.56, 5.48q (*J* 4.1)		

[a] Shifts for *R* isomers from the spectra of fresh solutions, shifts for *S* isomers from the additional lines in the spectra of isomeric mixtures formed on storing the solutions. [b] Not observed due to deuteriation. [c] Assignments from a spectrum of a mixture of isomers (about 4 : 1, *R* : *S*) are based on those of Morimoto *et al.*[11] [d] Shifts from a spectrum of a mixture of isomer assigned on the basis of 60 MHz spectra of the separate isomers. [e] Shifts from spectra of the calcium salt for the calcium insoluble isomer and of the dibenzylethylenediamine salt for the calcium soluble isomer. [f] Singlet with two very small outer lines *ca.* 4 Hz on either side. [g] Shifts derived from ABX analysis.

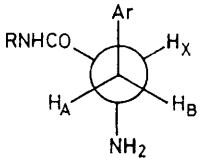
solutions to obtain accurate chemical shifts for all protons under conditions which minimise intermolecular effects of the type reported [7] to affect the spectra of benzyl- and phenoxymethyl-penicillins. The 250 MHz spectra were obtained on solutions containing *ca.* 1 mg/ml in D_2O for all the compounds and also in D_2O adjusted to pD 8 with NaOD for the amino-compounds (10)—(15). Chemical-shift data are given in Table 1. Signals have not been assigned separately to the α- and β-methyl groups or to 5-H and 6-H

TABLE 2

Coupling constants and rotamer populations for the tyrosyl penicillin isomers



(I)



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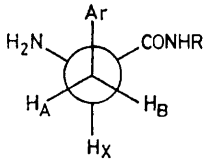

(III)

Compd.	Solvent	J/Hz AB	J/Hz AX	J/Hz BX	Rotamer (%) (I)	Rotamer (%) (II)	Rotamer (%) (III)
(14)	D_2O	13.6	8.9	6.5	57	35	8
(14)	D_2O + NaOD to pD 8	13.5	8.9	5.9	57	30	13
(15)	D_2O	13.9	8.3	6.2	52	33	15
(15)	D_2O + NaOD to pD 8	13.6	7.7	6.0	46	31	23

because such assignments cannot be made reliably on the basis of chemical-shift data alone, except for the 5-H and 6-H of compound (16) where the upfield line is clearly due to 6-H because of the large shift on change of pH. Also the signals at 1.40 and 1.48 p.p.m. in the spectrum of ampicillin (10) in D_2O plus NaOD can be assigned to the β- and α-methyls respectively by comparison with the results of Dobson *et al.*[8] The coupling constants of the 10-H and methylene protons of the tyrosyl penicillins (14) and (15) and the rotamer populations calculated from them by the method of Pachler [9] are given in Table 2.

C.d. Spectra.—The results from c.d. spectra of aqueous solutions of compounds (2)—(7) and (9)—(16) are given in Table 3. Spectra of the esters (2)—(4) were run periodically as the solutions aged. The position and intensity of the band at *ca.* 228 nm remained unchanged but the negative band at 206 nm gradually decreased in intensity until at equilibrium a valley with Δε close to zero was observed. The rate of change was much faster for (2) than for (3) and (4). C.d. spectra of fresh solutions of compounds (2)—(4) at pH 9 also showed Δε close to zero at *ca.* 206 nm. For compounds (6), (9), (11), (14), and (15) the spectrum showed a trough with positive Δε between the strongly positive bands below 200 nm and *ca.* 230 nm. Analysis of these traces by resolution as Gaussian curves showed the presence of hidden positive or negative extrema at 200—210 nm as indicated in Table 3.

DISCUSSION

Comparison of the n.m.r. spectra both from high and low concentration solutions shows a significant effect of concentration on chemical shift for compound (2), smaller effects for (1) and (4) and negligible effect for the other compounds. With compounds (1), (2), and (4) increase of concentration moved some or all of the signals to higher field, the extent of the shift varying for different protons. These effects were not investigated in detail but they are consistent with those reported by Thakkar and Wilham [7] for benzyl- and phenoxymethyl-penicillins and ascribed by them to micelle formation. The apparent absence of micelle formation for most of the compounds studied here may be explained by the presence of an ionised or other hydrophilic group in their side-chains. In addition to the effect on chemical shifts micelle formation probably also explains the broad banded nature of the high concentration spectrum of carfecillin (2). The low concentration spectrum showed normal line widths.

TABLE 3

Circular dichroism results *

Compound	C-10 Configuration	λ/nm(Δε	λ/nm (Δε)	λ/nm (Δε)	λ/nm (Δε)	λ/nm (Δε)	λ/nm (Δε)
(2) (fresh soln.)	*R*				228 (+12.4),	206 (−4.8),	193 (+16.1 !)
(2) (after 4 h)	*R* + *S*				229 (+11.4),		196 (+11.0 !)
(3) (fresh soln.)	Presumed *R*				227 (+13.2),	206 (−4.2),	195 (+18.4 !)
(3) (after 24 h)	*R* + *S*				228 (+13.7),		195 (+15.5 !)
(4) (fresh soln.)	Presumed *R*				227 (+12.8),	206 (−4.3),	195 (+13.7 !)
(4) (after 48 h)	*R* + *S*				228 (+12.2),		195 (+11.8 !)
(5)	*R*	280 (+0.5),	265s (+1.7),		229 (+9.3),	204 (−4.6),	187 (+23.1 !)
(6)	*S*	287 (−0.02),	268 (−0.02),		224 (+13.0),	205 (+2.0), [a]	187 (+26.7 !)
(7)	80%*R* + 20%*S*	280 (−0.1),	267s (+0.7),		225 (+9.4),	202 (−2.0),	186 (+24.6 !)
(9)	Ca insoluble [b]				223 (+12.8),	203 (−3.5), [a]	180 (+27.5 !)
(9)	Ca soluble [c]				230 (+6.0),	210 (+1.7) [a],	182 (+25.6 !)
(10)	*R*		267 (+0.3),	259s (+0.7),	232 (+12.7),	206 (−11.4),	192 (+29.8 !)
(11)	*S*		266 (−0.3),	261 (−0.1),	224 (+10.3),	210 (+3.1) [a],	198 (+11.3 !)
(12)	*R*	278 (−0.5),	270 (−0.7),		238 (+10.8),	207 (−6.4),	190 (+46.3 !)
(13)	*S*	275 (+0.9),		243s (+0.8),	226 (+7.1),	208 (+9.1),	198 (−7.1 !)
(14)	*R*	275 (+0.3),		235 (+8.2),	221s (+6.7),		195 (+6.9 !)
(15)	*S*	275 (+0.2),			228 (+7.8),	203 (+3.5), [a]	190 (+10.6 !)
(16)					229 (+15.8),	201 (−14.2),	184 (+26.2 !)

* All Δε values are for c.d. extrema except those indicated by s (= shoulder) or ! (= Δε at lowest wavelength measured). [a] Extrema located by application of Gaussian curve resolver. Δε values very approximate. [b] From the spectrum of the calcium salt. [c] From the spectrum of the dibenzylethylenediamine salt.

All of the pairs of isomers listed in Table 1 show chemical-shift differences for the methyl, 3-H, 5-H/6-H, and 10-H signals which must arise from the change of configuration at C-10 and the consequent effects on the conformation of the molecule. There are seven pairs of isomers [(1), (2), (5)/(6), (7), (10)/(11), (12)/(13), and (14)/(15)] where the C-10 configuration is definitely known. In each case the 3-H signal of the *R* isomer is upfield of that in the corresponding *S* isomer, although in some cases the difference is only 0.01 or 0.02 p.p.m. The difference in the 10-H shifts between the isomers ranges from 0.01 to 0.13 p.p.m. with the signal at higher field in the *R* isomers except for the hydroxy-pair (5) and (6), where a difference of 0.01 p.p.m. in the opposite direction occurs. There is also a no entirely consistent difference between the isomers in the methyl and 5-H/6-H shifts. The absence of specific assignment of these signals to the α- and β-methyls and to 5-H and 6-H means that only empirical comparisons can be made. Nevertheless, some trends can be discerned. The methyl-group shifts in the isomeric pairs (2), (5)/(6), (7), (10)/(11), and (12)/(13) tend to be at lower field in the *S* than in the *R* isomers whereas the reverse occurs with the pairs (1) and (14)/(15). The separation between the 5-H and 6-H chemical shifts is greater in the *S* than in the *R* isomers with the exception of sulbenicillin (7) and the tyrosyl penicillins (14)/(15) when dissolved in D_2O. In those cases where the *S* isomer has this greater separation the chemical shift of both protons in the *R* isomer lies between the shifts in the *S* isomer, so there is a downfield shift of one proton and an upfield shift of the other on inversion of the C-10 configuration from *R* to *S*.

These differences can be used to consider the probable C-10 configuration for compounds (3) and (4) and the calcium soluble and insoluble isomers of compounds (8) and (9); this has not previously been established. In the esters (3) and (4) the methyl and 3-H shifts for the isomer present in fresh solution and for that formed as the solution ages are very close to those for the *R* and *S* isomers respectively of carfecillin (2). Similarly the separation between the 5-H and 6-H signals for the isomers of (3) and (4) parallels that for (2). These results, together with those from c.d. spectra (see below), strongly support assignment of the *R* configuration to compounds (3) and (4) in fresh aqueous solution and so to their crystalline forms. The methyl and 3-H shifts of the calcium insoluble and soluble isomers of (8) and (9) are close to those of the *R* and *S* isomers respectively of (2) and of sulbenicillin (7). Also the 5-H/6-H separation parallels that of the *R* and *S* isomers of (7), which is the only penicillin of known C-10 configuration studied which has an anionic group in the side-chain, rather than that of (2) and other compounds with neutral side-chain groups. These results imply assignment of the *R* configuration to the calcium insoluble isomers of compounds (8) and (9). This assignment is supported for compound (9) by c.d. results (see below) and is consistent with the observed [5,10] greater antibacterial activity of the calcium insoluble isomers on plate bioassay because several α-substituted phenylacetamidopenicillins show [11,12,13] greater antibacterial activity for the *R* than for the *S* isomer.

The above assignment for compounds (8) and (9) means that 10-H is at higher field in the *S* isomers by 0.05 p.p.m. This is the reverse of the difference in the 10-H shifts for most of the other isomeric pairs. Several factors can be envisaged as contributing to the shift difference of 10-H between isomers. These include the effect of the change of configuration on the position of the proton relative to the R^2 group and the effect of conformational differences on the position of the proton relative to the shielding and deshielding regions exerted by the C-9 carbonyl and the aromatic ring. In view of the variety of these possible effects it would perhaps be surprising if an entirely consistent trend were observed in the isomeric difference for this proton. A similar multiplicity of effects could contribute to the 5-H/6-H shift differences. Because of this, and the lack of assignment to 5-H and 6-H separately, the 5-H/6-H and 10-H shifts will not be considered further. The methyl and 3-H shifts are, however, amenable to analysis by comparison with their values in the parent molecule, 6-aminopenicillanic acid (16), and these are now discussed in more detail.

Blanpain *et al.* [14] have studied the n.m.r. spectra of some penicillins with aromatic side-chains whose crystal structures have been determined by *X*-ray methods. They found that those with a flexible side-chain which is folded back over the penam nucleus in the crystal gave methyl and 3-H signals shifted upfield of those in (16) and in some other penicillins where the side-chain cannot fold in this way. These shifts, which were attributed to the shielding effect of the aromatic ring, imply that these penicillins exist in a side-chain folded conformation in aqueous solution as well as in the crystal. The crystal structure of three of the penicillins studied here has been determined. Ampicillin (10) [15] and amoxycillin (12) [16] have very similar structures with the benzene ring folded partly towards the penam nucleus, but the distance of the benzene ring from the methyls is so great that their upfield shift of 0.1 to 0.2 p.p.m. relative to (16) could not possibly be produced in this conformation. Carfecillin (2) [4] adopts a conformation in the crystal in which the ester phenyl would have negligible shielding or deshielding effect on the methyls and the C-10 phenyl would exert a slight deshielding effect on them. Thus the observed shifts for compounds (2), (10), and (12) are not consistent with their having the same conformation in aqueous solution as in the crystal. However, examination of molecular models shows that the penicillins studied here can adopt folded conformations in which the aromatic ring would affect the methyl and 3-H shifts.

Comparison of chemical shifts of compounds (2)—(9) with those of (16) at pD 8 shows small and negligible shielding respectively for the methyls of the *R* and *S* isomers, and slight deshielding of 3-H in both isomers. By contrast, compounds (10)—(13) show significant shielding of the methyls and a negligible effect on 3-H in both isomers. The different shielding effects in these

groups could arise from conformational differences in the side-chain or the thiazolidine ring.

Apart from compounds (5) and (6), all the compounds which show small shielding effects have a bulky or negatively charged R^2 group. The side-chain conformation of these compounds will be affected by repulsion between R^2 and the C-9 carbonyl and by steric compression of R^2 with the *ortho*-hydrogens of R^1. Inspection of molecular models shows that these effects inhibit folding of the side-chain into conformations in which R^1 can produce significant shielding of the methyls. Similar repulsion and steric effects are not produced by the amino-group so the side-chains of compounds (10)—(13) can fold to bring the methyls well into the shielding region of the benzene ring. The observed shifts imply that this folding occurs to a greater extent when the amino-group is in the ionised than in the un-ionised form (spectra in D_2O and at pD 8 respectively). There is no obvious reason why folding of the hydroxy side-chain of compounds (5) and (6) should be inhibited and the reason for the observed small shielding effect in these compounds is obscure.

X-Ray studies have shown [14] that the thiazolidine ring of penicillins can exist in two different conformations in the solid state with the C-3 carboxy-group either axial or equatorial. When the side-chain is folded towards the penam nucleus the methyls are closer to the aromatic ring in the axial than in the equatorial conformation, so differences in extent of shielding could occur between penicillins with similar side-chain conformations. However, this is an unlikely explanation for the results discussed here because it would require compounds (10)—(13), which show the largest shielding, to have the carboxy-group in the axial conformation. This is contrary to the finding [8] that ampicillin (10) exists predominantly in the equatorial form in aqueous solution. Thus differences in extent of side-chain folding seem the most likely cause of the observed differences in extent of methyl and 3-H shielding, both between isomers and between compounds.

Compounds (1), (14), and (15) have greater conformational flexibility of the side-chain than those discussed so far because they have an additional atom between the benzene ring and C-10. Their methyl shifts compared with compound (16) show only small shielding effects. For compounds (14) and (15) this can be considered in relation to the rotamer proportions about the C(10)–CH_2 bond given in Table 2. Examination of molecular models shows that rotamer (I) produces open conformations whatever the extent of rotation about other bonds in the side-chain, so the hydroxyphenyl group cannot approach the thiazolidine ring and exert a shielding effect. Although in both rotamers (II) and (III) rotation about the C(9)–C(10) bond can bring the methyl groups into the shielding region from the benzene ring, this rotation is sterically hindered so that the preferred conformations would be expected to maintain a fairly large separation consistent with the observed small shielding effects. The slightly greater shielding in the *S* than the *R* isomer is consistent with the lower proportion of rotamer (I) in the *S* isomer, but the similarly decreased proportion of (I) in the *S* isomer at pD 8 compared with the D_2O solution is not accompanied by any change in the extent of shielding. This presumably indicates a difference in the preferred conformations of the (II) and (III) rotamers at the two pD values.

Comparison of the rotamer populations in Table 2 with those of tyrosine [17] shows a similar proportion of rotamer (I) but a lower proportion of (III) and a higher proportion of (II) in the penicillins. This preponderance of rotamer (II) over (III) in the penicillins does not seem readily explicable in steric terms and may arise from solvation effects. Models show that if rotation about the C(9)—C(10) bond is restricted to a region around that in which the C-9 carbonyl eclipses the C(10)–N bond, then rotamer (II) is more folded than (III) and so will be preferred because it presents a smaller hydrophobic surface to the aqueous solvent. This rotational restriction could arise by attraction between the C-9 carbonyl and the ionised amino-group in the solutions in D_2O. If this is so, neutralisation of the amino-group at pD 8 would remove the restriction and favour rotamer (II) relative to the other rotamers, as is in fact observed.

The c.d. results given in Table 3 show a strong positive Cotton effect near 230 nm which is a characteristic of c.d. spectra of penams.[18–23] The intensity of this band is reasonably constant for all the penicillins studied here and, in common with a previously reported [20] finding, is lower than in the parent compound, (16). In the hydroxy- and amino-compounds (5), (6), and (10)—(15) this extremum occurs at higher wavelength in the *R* than the *S* isomers, but in the acidic compounds (7) and (9) the reverse seems to occur. Samples of both isomers of (7) were not available, but the observed wavelength of 225 nm from the predominantly *R* isomer sample is at the low end of the range found for the other compounds, implying that the *S* isomer will give an extremum at higher wavelength.

The origin of this characteristic band in penams has been the subject of detailed theoretical study which concludes [23] that in (16) it arises from overlapping transitions associated with the β-lactam amide and the thiazolidine sulphur. In the penicillins themselves the side-chain amide and aromatic chromophores could also contribute Cotton effects in this region, so the observed band can be a combination of several different transitions. The presence of shoulders at 243 and 221 nm in the spectra of compounds (13) and (14), respectively, presumably arises from partial resolution of some of these transitions. Similarly the differences in position of the band between isomers can be ascribed to differences in the relative intensities, and perhaps the sign, of the various transitions.

There is a consistent correlation between the sign of the 200 and 210 nm band and the C-10 configuration for those penicillins in which the aromatic ring is directly attached to C-10. For compounds (5), (6), and (10)—

(13) the *R* and *S* isomers have this band negative and positive respectively. This also applies to the esters (2)—(4) because the changes observed in their 206 nm band with increase in age and change in pH of solution must be due to isomerisation at C-10 since this is the only difference seen in the n.m.r. spectra run under similar conditions. Consequently the results indicate that the *R* and *S* isomers of these compounds have Cotton effects of opposite sign and about equal intensity at this wavelength, which cancel to near zero in the equilibrium mixtures. The similarity of behaviour of the three esters supports the deduction from the n.m.r. results that crystalline compounds (3) and (4) have the *R* configuration at C-10. The assignment from n.m.r. results of the *R* configuration to the calcium insoluble salt of (9) is also supported by the sign of the 200—210 nm band in the c.d. spectra of its isomers.

The origin of the band at 200—210 nm in the c.d. spectra of penicillins is uncertain. 6-Aminopenicillanic acid (16) shows a Cotton effect in this region which is strongly negative when the amino-group is ionised and weakly positive when it is neutral.[24] This band has been tentatively assigned [23] to a conformationally modified π-π^* β-lactam excited state. However, in the penicillins the additional side-chain amide and aromatic chromophores may also contribute to this band. In this context it is noteworthy that the *R* negative *S* positive correlation is the same as that found for an unassigned band at 208—210 nm in the c.d. spectra of phenylglycineamide,[25] and *p*-hydroxyphenylglycineamide,[26] which are the side-chains of (10)/(11) and (12)/(13) respectively. In view of this correspondence of sign and position of the band in compounds with and without the aminopenicillanate nucleus it is tempting to assign the 200—210 nm band in the penicillins predominantly to the side-chain amide or aromatic chromophores. However, in the absence of a clear understanding of its origin the empirical correlation between the sign and the C-10 configuration clearly needs to be used with caution.

The aromatic Cotton effects above *ca.* 260 nm are of opposite sign for the *R* and *S* isomers in compounds (5), (6), and (10)—(13), as was found [2] for the isomers of phenethicillin. The sign of these bands is the same as that for the aromatic bands in the c.d. spectra of the corresponding isomers of their side-chain acids; mandelic acid,[27] phenylglycine,[25] and *p*-hydroxyphenylglycine.[26] This suggests that the conformation of these penicillins is such that the penam nucleus does not exert a significant sign-determining effect on the aromatic bands. By contrast the tyrosylpenicillins (14) and (15) both give a positive aromatic band, which is the sign observed [28] for this band in *S*-tyrosine. This behaviour is presumably related to the greater conformational mobility of the aromatic ring in these compounds. In the *R* isomer, (14), this flexibility must bring the penam nucleus into a positive sign-determining region for the aromatic band to produce the reversal of sign from that in *R*-tyrosine.

EXPERIMENTAL

N.m.r.—60 MHz Spectra were run on a Perkin-Elmer R12A at a probe temperature of *ca.* 35 °C.

250 MHz Spectra were run on a Brüker WM250 at a probe temperature of *ca.* 21 °C. Spectra were acquired into 16K data points with a spectral width of 2 000 Hz except for the spectra of compounds (14) and (15) from which the coupling constants in Table 2 were measured where a spectral width of 1 000 Hz was used. The coupling constants were obtained from an ABX analysis of the observed shifts and the rotamer populations in Table 2 were calculated by Pachler's [9] method using his values of 2.6 and 13.6 Hz for the gauche and *trans* vicinal coupling constants respectively. This method does not differentiate between rotamers (I) and (II) and the ambiguity was resolved on the basis of results for tyrosine obtained by selective deuteriation.[17]

C.d.—Spectra were run on a Cary-61 spectropolarimeter at room temperature at concentrations between 0.1 and 6.8 mg/ml in cells of path-length 0.5 to 5.0 mm. Gaussian curve resolution was carried out on a du Pont curve resolver.

Materials.—Sulbenicillin was a commercial sample from the Takeda company. The other penicillins were either Beecham commercial materials or were prepared in the Beecham laboratories by standard synthetic methods from 6-aminopenicillanic acid and the appropriate side-chain acid.

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