^{13}C and ^{15}N Solution and Solid-state Nuclear Magnetic Resonance Study of the Intermolecular Interactions in the 1:1 Trimethoprim‡: Sulphamethoxazole§ Complex

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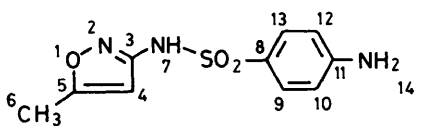
The combined use of natural-abundance solid-state ^{13}C and ^{15}N n.m.r. shows that the structure of the trimethoprim:sulphamethoxazole complex (TMP:SMZ) is best described by transfer of the proton from N(7) of SMZ to N(1) of TMP. Stabilisation of the complex is achieved by the resulting ionic interaction and by the formation of two intermolecular hydrogen bonds. When the complex is dissolved in dimethyl sulphoxide it is largely dissociated into neutral free base TMP and SMZ.

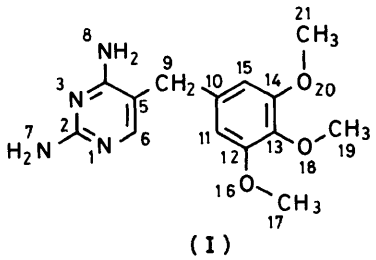
The antibacterial substances trimethoprim (TMP) (**I**) and sulphamethoxazole (SMZ) (**II**) are often used in association, and in such mixtures the components display synergistic effects and sequentially block the biosynthetic route to tetrahydrofolic acid.[1] The most commonly employed pharmaceutical formulation is a 1:5 w/w mixture of TMP:SMZ known as 'Cotrimoxazole' and this is used as an aqueous suspension or in tablet form. Recent studies[2–6] have shown the formation of a 1:1 molecular complex between TMP and SMZ, and this is insoluble in aqueous solution. It is known that the nature of the interaction between the two components is relevant toward the bioavailability of the drug affecting the 'pharmaceutical phase' of the action. Several analytical methods *e.g.* i.r. spectroscopy,[3,4] *X*-ray analysis,[5,6] and thermal analysis (DSC)[2] have been used to study the TMP–SMZ interaction, but some ambiguities exist in the interpretation of the data. In their *X*-ray study Giuseppetti *et al.*[5] proposed that the complex was stabilised by

SMZ



(II)

TMP



(I)

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‡ 2,4-Diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine.
§ 4-Amino-*N*-(5-methylisoxazol-3-yl)benzenesulphonamide.

Table 1. Solution and solid-state ^{13}C chemical shifts for trimethoprim

	Solution ([$^2H_6$]DMSO)			Solid-state		
	TMP	TMP·HCl	TSC	TMP	TMP·HCl	TSC
C(2)	162.2	154.5	161.3	163.3	153.4	155.0
C(4)	162.3	164.0	162.6	163.3	163.3	165.5
C(5)	105.8	108.9	106.3	103.2	112.2	108.8
C(6)	155.8	139.7	153.8	158.5	138.6	140.0
C(9)	33.1	32.1	33.0	32.4	32.0	32.5
C(10)	135.8	136.3	135.5	135.3	136.5	135.9
C(11)	106.0	106.4	106.0	102.6	105.6	107.2
				100.8	104.6	104.7
C(12)	152.8	152.9	152.8	152.7	153.5	153.2
				151.9		152.5
C(13)	135.7	133.1	135.5	135.3	130.0	133.1
C(16)	55.8	56.0	55.9	53.3	54.3	54.0
				52.6		
C(17)	60.0	60.0	60.0	62.6	59.2	58.6

two intermolecular hydrogen bonds *viz.* (TMP)N(7)–H · · · N(2)(SMZ) and (SMZ)N(7)–H · · · N(1)(TMP), while in their *X*-ray study Nakai *et al.*[6] also proposed the former hydrogen bond, but that the second hydrogen bond is of the type (SMZ)$N(7)^-$ · · · H–$N(1)^+$(TMP) where there has been proton-transfer between the two molecules in the complex.

In order to further elucidate the TMP–SMZ interactions we have undertaken a solution-state and solid-state nuclear magnetic resonance study of the complex and its separate components in both their neutral and ionised (TMP hydrochloride and SMZ sodium salt) forms. The spectra obtained are on ^{13}C and ^{15}N at the natural abundance levels as it was anticipated that the isotropic chemical shifts of these nuclei would be sensitive indicators of molecular environment.[7,8]

Discussion

^{13}C *N.m.r. Spectra.*—The solution and solid-state ^{13}C shieldings of TMP as free base, the hydrochloride, and the complex with SMZ are collected in Table 1, whereas the corresponding data for SMZ, its sodium salt, and the complex with TMP are in Table 2.

Table 2. Solution and solid-state ^{13}C chemical shifts for sulphamethoxazole

	Solution ([$^2H_6$]DMSO)			Solid-state		
	SMZ	$SMZ^- Na^+$	TSC	SMZ	$SMZ^- Na^+$	TSC
C(3)	158.0	165.0	159.0	156.0	165.8	165.0
C(4)	95.4	96.3	95.5	97.4	101.3	95.2
C(5)	169.9	169.4	169.5	171.9	168.2	171.3
C(6)	12.1	11.0	12.1	10.0	10.6	12.7
C(8)	124.4	130.9	125.4	124.1	130.2	129.6
C(9)	128.9	127.4	128.7	128.8	127.2	124.0
C(13)				131.2		126.8
C(10)	112.7	114.5	112.7	114.5	113.3	114.6
C(12)				116.0		
C(11)	153.3	149.6	153.0	154.0	152.5	150.3

One feature of the data in Tables 1 and 2 is that the equivalence in solution of certain ^{13}C resonances [*e.g.* C(11), C(15) of TMP] does not hold in the solid-state due to constraints of the molecular packing. The solution ^{13}C resonances were assigned on the basis of the chemical shifts and the ^1H-coupled multiplicities. For TMP the ^{13}C data on selectively ^{13}C-enriched material reported by Cheung *et al.*[9] provided further information. The pattern of ^{13}C shifts for TMP in solution and solid-state is similar, with the greatest difference occurring for the C(11), C(15) resonances. In parallel with this the solution and solid-state shifts for SMZ are very similar and thus the assignments of the solid-state spectrum follow easily. The solution-state spectrum of TMP·HCl was assigned by comparison with that of TMP and the knowledge[10,11] that the protonation site is N(1). The solution spectrum of SMZ sodium salt was assigned by comparison with that of SMZ, and comparison with ^{13}C data[12] on a range of sulphonamide sodium salts. Again the solid-state chemical shifts for TMP·HCl and SMZ sodium salt quite closely follow the patterns for the corresponding solution spectra thus providing the solid-state assignment. The most pronounced solution-state ^{13}C shielding changes upon protonation of TMP are for C(2) and C(6), whose resonances shift to lower frequency by 7.7 and 16.1 p.p.m. respectively, while upon deprotonation of SMZ, C(3) and C(8) experience high-frequency shifts of 7.0 and 6.5 p.p.m., respectively. Related shielding changes are observed between the solid-state spectra.

Inspection of the data for the complex in the solid-state show that the ^{13}C chemical shifts are consistent with the ionic structure proposed by Nakai *et al.*,[6] (**III**), since the observed shifts are very similar to those measured for TMP·HCl and SMZ sodium salt. Differences between the shifts for the complex and the separate ionic components do exist however and the most significant of these for SMZ sodium salt are (in p.p.m.)

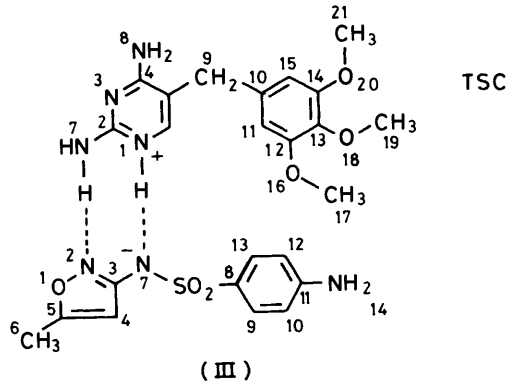

Table 3. Solution and solid-state ^{15}N chemical shifts for trimethoprim and sulphamethoxazole

	Solution ([$^2H_6$]DMSO)			Solid-state		
	TMP	TMP·HCl	TSC	TMP	TMP·HCl	TSC
N(1)	195.6	112.1	179.9	192.5	116.4	117.0
N(3)	184.2	178.4	185.4	177.1	168.9	176.1
N(7)	53.4	62.4	57.9	55.8	56.9	64.0
N(8)	56.3	83.4	64.3	58.2	85.3	74.1
	SMZ	$SMZ^- Na^+$	TSC	SMZ	$SMZ^- Na^+$	TSC
N(2)	324.3	304.9	322.5	324.1	—	309.4
N(7)	95.1	104.9	99.3	93.8	—	113.0
N(14)	51.1	35.9	49.3	48.4	—	42.4

C(4) (−6.1), C(5) (+3.1), and C(9) or C(13) (−3.2), and for TMP·HCl C(5) (−3.4) and C(13) (+3.1) where the + sign indicates the resonance from the complex is at higher frequency. These shift differences will be due to a combination of factors, primarily the presence of the (TMP)N(7)–H · · · N(2)(SMZ) and (SMZ)N(7)$^-$ · · · H–$^+$N(1)(TMP) hydrogen bonds in the complex, and any differences in solid-state conformation induced by the complexation.

The ^{13}C shifts for the complex in solution are significantly different to those from the solid-state [particularly so for C(2) and C(6) of TMP and C(3) of SMZ], but are similar to the shifts from the components TSP and SMZ separate in solution. This then indicates that in DMSO solution the complex is largely dissociated into the neutral components.

^{15}N *N.m.r. Spectra.*—Both solution-state and solid-state ^{15}N spectra for TMP, TMP·HCl, SMZ, SMZ sodium salt, and the TMP—SMZ complex are collected in Table 3 and some of the spectra are illustrated in Figure 1.

The solution-state ^{15}N chemical shifts for N(1), N(3), and N(7) for free base TMP and TMP·HCl were assigned by comparison with those reported by Stadeli *et al.*,[11] and by Bevan *et al.*,[13] on the ^{15}N-enriched compounds. Solution-state ^{15}N shifts for N(1), N(7), and N(8) of TMP in the complex were intermediate between those for TMP and TMP·HCl as expected for significant dissociation of the complex (see ^{13}C section) and only partial protonation of the dissociated TMP. The solution ^{15}N assignments for SMZ alone, its sodium salt, and in the complex were made by comparison with the data from Curzon.[12] For the solid-state ^{15}N spectra the assignments for TMP and TMP·HCl follow those for the solution state, and the shifts for TMP in the complex were assigned by comparison with TMP·HCl. We have been unsuccessful in attempts to obtain a solid-state CPMAS (cross-polarisation and magic angle spinning) ^{15}N spectrum of SMZ sodium salt because of its long proton relaxation times (see Experimental section), but the shifts for SMZ alone and complexed were assigned by comparison with the solution spectra. Considering the ^{15}N data in Table 3, in the case of both the solution-state and solid-state results, protonation of TMP at N(1) to form TMP·HCl results in a large low-frequency shift for N(1) (−83.5 and −76.1 p.p.m. respectively for solution and solid state), and additionally gives a high-frequency shift for the remote nitrogen N(8) (+27.1 p.p.m. for both solution and solid state). The structure of TMP·HCl is probably best described as a hybrid of the canonical forms shown in Figure 2, and the ^{15}N shieldings for N(8) indicate an important contribution from canonical form (c) in both solution and solid state.

Experimental

Materials.—Trimethoprim (m.p. 199—200 °C) and sulpha-

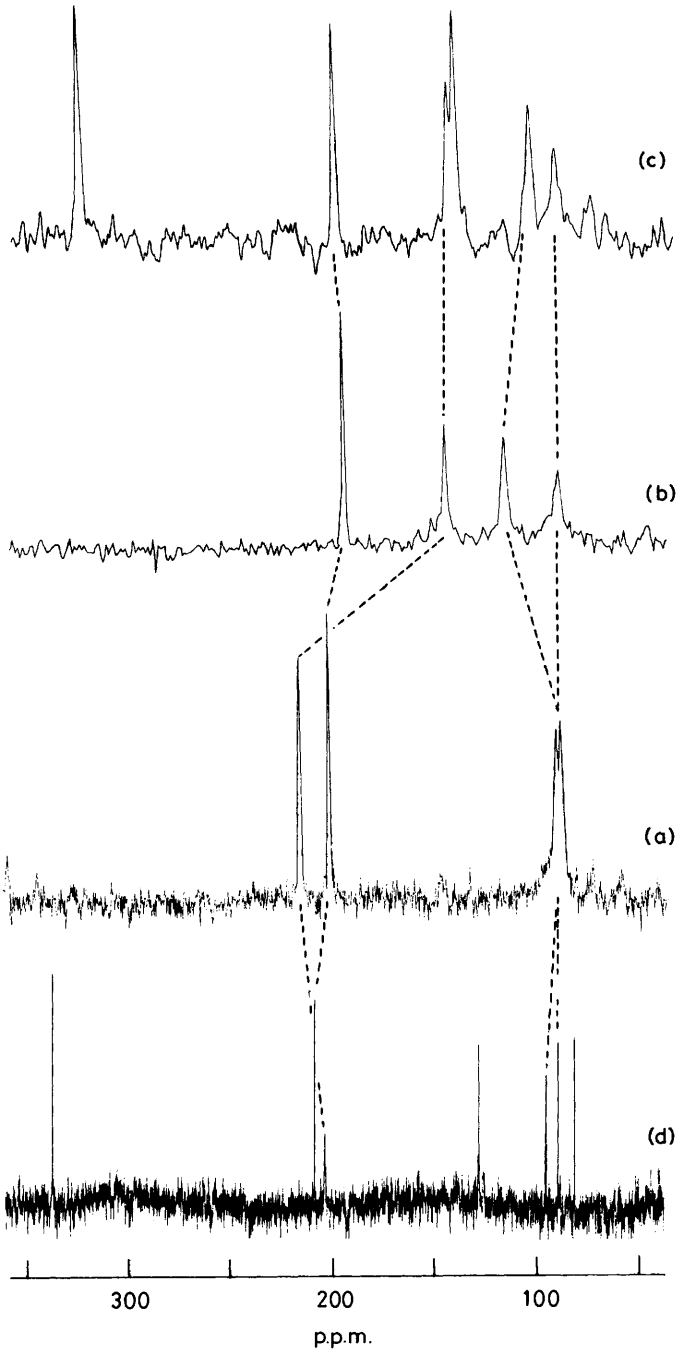

Figure 1. Natural-abundance solid-state ^{15}N spectra of (a) TMP, (b) TMP·HCl, and (c) the complex TSC, and (d) solution-state ^{15}N spectrum of TSC

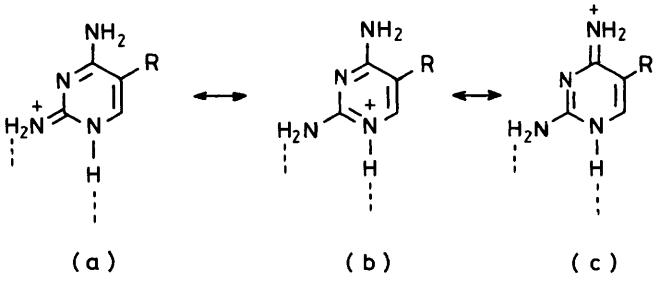

Figure 2. Possible canonical forms contributing to the hybrid structure of TMP·HCl

Table 4. Solid-state ^1H relaxation data for trimethoprim hydrochloride and sulphamethoxazole and its sodium salt

	^1H T_1/s	^1H $T_{1\rho}$/ms
TMP·HCl	1.9	348
SMZ	74	6.9
SMZ·Na	31	—

methoxazole (m.p. 169—170 °C) were kindly provided by La Roche S.p.a. and not further purified.

1:1 Trimethoprim:sulphamethoxazole complex was prepared according to ref. 2 (m.p. 176—177 °C) (lit., 178—179 °C) (Found: C, 53.12; H, 5.45; N, 17.93. $C_{24}H_{29}N_7O_6S$ requires C, 53.03; H, 5.38; N, 17.66%).

Trimethoprim hydrochloride was prepared by treating a suspension of TMP (1.45 g, 0.005 mol) in anhydrous ether with HCl gas. After filtration, the crude hydrochloride was recrystallised from ethanol, m.p. 252—254 °C (decomp.) (Found: C, 51.56; H, 5.90; N, 17.12. $C_{14}H_{19}ClN_4O_3$ requires C, 51.54; H, 5.86; N, 17.15%).

Sulphamethoxazole sodium salt was obtained by treating an ethanolic solution of SMZ (1.27 g, 0.005 mol) with an equimolar amount of ethanolic NaOH. Partial removal of the solvent produced a crystalline precipitate which was purified by recrystallisation from ethanol, m.p. 280—282 °C (decomp.) (Found: C, 43.64; H, 3.67; N, 15.17. $C_{10}H_{10}N_3NaO_3S$ requires C, 43.63; H, 3.66; N, 15.26%).

Solution-state N.m.r. spectra.—Most spectra were obtained with a JEOL GX 270/89 spectrometer operating at 67.8 and 27.25 MHz respectively for ^{13}C and ^{15}N. Some ^{15}N spectra were obtained with a Bruker WH-400 instrument operating at 40.56 MHz for ^{15}N observation. For ^{13}C spectra the samples were dissolved in [$^2H_6$]dimethyl sulphoxide ([$^2H_6$]DMSO), and were contained in 5 mm o.d. tubes. The ^{15}N spectra were from 0.8M solutions in [$^2H_6$]DMSO contained in 10 mm o.d. tubes. ^{13}C Chemical shifts are reported relative to tetramethylsilane (TMS; high frequency positive convention), and ^{15}N chemical shifts are reported relative to the $^{15}NH_4$ resonance from external 6M $^{15}NH_4{}^{15}NO_3$ in 2M HNO_3. Typically for ^{15}N spectral accumulation the inverse gated decoupling mode was used to suppress often unfavourable ^{15}N-{^1H} nuclear Overhauser enhancements, and pulse-flip angles of *ca.* 30° with relaxation delays of up to 25 s.

Solid-state N.m.r. spectra.—Spectra were obtained with a Bruker MSL-300 instrument operating at 75.47 and 30.42 MHz, respectively, for ^{13}C and ^{15}N. Cross polarisation and magic angle spinning were used, and the samples were contained in 7 mm o.d. rotors in double air-bearing probes. The efficiency of the cross polarisation process depends in part on there being a relatively short proton spin-lattice relaxation time (T_1) to allow optimum cycling through the accumulation routine, and a rotating frame proton relaxation time ($T_{1\rho}$) that is long in comparison to the heteronuclear cross relaxation time (T_{CH} or T_{NH}). Typically T_{CH} values are in the range 0.5—5 ms and are expected to be longer for T_{NH}. In some cases the proton T_1 and $T_{1\rho}$ values were measured (see Table 4) using a 5 mm wideline probe in order to optimise the ^{15}N experimental cross polarisation conditions, and we believe that it is likely that our difficulty in obtaining the ^{15}N spectrum of SMZ sodium salt originates in an adverse combination of proton relaxation parameters. The magic angle spinning rates were in the region 4.3—4.8 kHz, and the TOSS technique[14] was used to simplify some ^{13}C spectra by elimination of the spinning side bands. Single contact cross-polarisation with flip-back was used in all

cases, and ^{1}H decoupling fields were about 70 kHz and 30 kHz, respectively for ^{13}C and ^{15}N observation. The acquisition parameters included a 30 kHz sweep width (35 or 70 ms acquisition time), 1 to 10 ms contact, and 3—90 s recycle delay. ^{13}C Assignment was assisted by measurement of 'non-quaternary suppressed' spectra,[15] incorporating 40 μs decoupler interruption. ^{13}C Chemical shifts were referenced to external TMS *via* the secondary standard adamantane, and the ^{15}N shifts were referenced to the ammonium resonance from doubly-^{15}N labelled ammonium nitrate.

Conclusion

Both ^{13}C and ^{15}N solid-state chemical shifts confirm that the structure of the 1:1 TMP–SMZ complex is best described by structure (**III**) wherein a proton has been transferred from N(7) of SMZ to N(1) of TMP. This specific proton transfer is quite reasonable in view of the acidic nature of sulphonamido proton ($pK_a = 5.6$)[16] and the observation that the preferred protonation site for TMP is at N(1) ($pK_a = 7.2$).[10,11]

The complex is then stabilised by the resulting ionic interaction and by the formation of the two intermolecular hydrogen bonds. The ^{15}N chemical shift changes are greater than for ^{13}C, in particular there is a 75.5 p.p.m. shift difference for N(1) in solid TMP and TMP in the TMP–SMZ complex. It is clear that upon dissolution in DMSO, the TMP–SMZ complex largely reverts to a mixture of free TMP and SMZ as indicated by the observation that the shifts for N(1) and N(8) of TMP in the complex in solution are closer to those for free base TMP then TMP·HCl.

As discussed above the high-frequency solution and solid-state shifts for N(8) in TMP·HCl indicate a significant contribution of canonical form (c) (Figure 2) to the hybrid structure. However, in the complex N(8) experiences a low-frequency shift of 11.2 p.p.m. from that in TMP·HCl, and since N(8) is not directly involved in the complexation to SMZ, then this low-frequency shift must be due to a decrease in the importance of form (c) in the complex when compared to TMP·HCl. The solid-state resonance position for N(1) from TMP·HCl and the complex are almost identical ($\Delta\delta = 0.6$ p.p.m.) and this must be due to a competition of the opposing effects of decreasing the importance of the form (c) (Figure 2) and forming the hydrogen bond (*via* its proton) to N(7) of SMZ in the complex.

Acknowledgements

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