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STUDIES OF THE THIADIAZINE, TAUROLIDINE—I. IDENTIFICATION OF THE MOLECULAR SPECIES PRESENT IN AQUEOUS SOLUTIONS BY ^{1}H- AND ^{13}C-NMR SPECTROSCOPY

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Summary—The equilibria in deuterium oxide solutions of the diamine, 4,4′-methylenebis(tetrahydro-1,2,4-thiadiazine-1,1-dioxide), were studied using highfield ^{1}H- and ^{13}C-NMR with the aid of solutions of tetrahydro-2H-1,2,4-thiadiazine-1,1-dioxide (taurultam), its two *N*-methyl derivatives and methylene glycol. Comparison of the ^{1}H-NMR spectrum of taurolidine with the one obtained from a mixture of taurultam and methylene glycol indicated that the same equilibria exists in both these solutions. It was concluded that taurolidine, taurultam and its 4-hydroxymethyl adduct and methylene glycol are the major components present. To facilitate the interpretation of the ^{13}C-spectra, ^{13}C-enriched methylene glycol was added to solutions of taurultam. The ^{13}C-studies confirmed the ^{1}H-NMR study.

Aqueous solutions of either amines or amides with methylene glycol have a complex composition since the reactants are in equilibrium with either *N*-hydroxymethyl(carbinolamine) and *N,N′*-methylenediamine adducts or both.[1,2,3] In some instances these adducts can be isolated. Moreover there has also been a considerable interest in the therapeutic usefulness of insoluble *N*-hydroxymethyl adducts of amines, amides and imides, as potential prodrugs.[4,5,6] Although some of the *N,N′*-methylenediamine adducts are stable in the solid state, once in solution they are hydrolysed and in equilibrium with their respective carbinolamines, amines and methylene glycol.

Taurolidine [4,4′-methylenebis(tetrahydro-1,2,4-thiadiazine-1,1-dioxide)] (IV) is a broad spectrum bactericide[7] and antiendotoxin[8] whose activity is linked to its susceptibility to hydrolysis to carbinolamines. Examination of taurolidine (IV) in solution by ^{1}H-NMR[9,10,11] and ^{13}C-NMR[10] indicated that it is in equilibrium with 4-hydroxymethyltetrahydro-2H-1,2,4-thiadiazine-1,1-dioxide (VII), tetrahydro-2H-1,2,4-thiadiazine-1,1-dioxide (taurultam, I) and methylene glycol (Fig. 1). Although there is conclusive evidence that the N^4-hydroxymethyl compound is formed, no evidence has been presented for the formation of a N^2-hydroxymethyl adduct. With the aid of taurultam and its *N*-methyl derivatives (II, III and V) the equilibria produced in the presence of methylene glycol and ^{13}C-enriched methylene glycol were examined in detail by ^{1}H- and ^{13}C-NMR spectroscopy. The identity of the molecular species present in aqueous solutions of taurolidine was confirmed through complete assignments of ^{1}H- and ^{13}C-spectra complemented by a combination of two-dimensional experiments and long-range correlations.

MATERIAL AND METHODS

Tetrahydro-2H-1,2,4-thiadiazine-1,1-dioxide (I), 2-methyl-(II), 4-methyl-(III) and 2,4-dimethyl-(V) tetrahydro-2H-1,2,4-thiadiazine-1,1-dioxide, 4,4′-methylenebis(tetrahydro-1,2,4-thiadiazine-1,1-dioxide) (IV), and 4,4-methylenebis(2,2′-dimethyltetrahydro-2H-1,2,4-thiadiazine-1,1-dioxide) (VI), (Fig. 2) were kindly donated by Geistlich-Pharma, Wolhusen, Switzerland. A 15% aqueous solution of ^{13}C-enriched methylene glycol was obtained from MSD Isotopes, Cambrian Gases, U.K.

^{1}H- and ^{13}C-NMR studies were performed on Bruker WM 250 and AME 400 spectrometers. 250 MHz ^{1}H-spectra of I, II, III and IV before and after the addition of known amounts of methylene glycol were determined in deuterium

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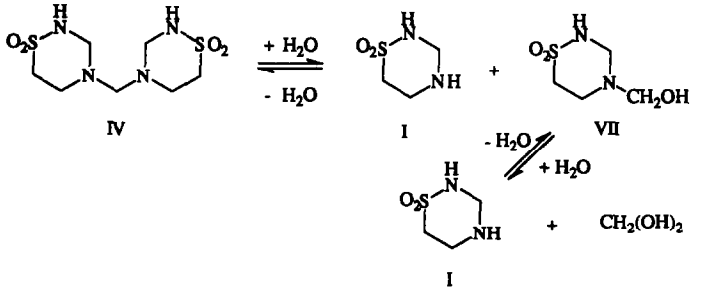


Fig. 1. Chemical equilibria of molecular species present in a solution of taurolidine (IV).

oxide containing acetonitrile as an internal standard. The methylene glycol solution in deuterium oxide was freshly prepared[12] and its concentration determined colorimetrically.[13] 62.9 MHz ^{13}C-spectra of compounds I–III in deuterium oxide (reference standard dioxan) were determined. ^{13}C-spectra of compounds I–III were determined in deuterium oxide containing ^{13}C-enriched methylene glycol. ^{1}H (400 MHz) and ^{13}C (100 MHz) spectra of deuterium oxide solutions of I and IV were recorded. Detailed structural assignments were made from COSY 45 (typical parameters are ^{1}H: 90° pulse = 13 μs and ^{13}C: 90° pulse = 9μs), H–C direct correlation (HCCOBI) and H–C long range (HMBC, inverse detected using pulse program INV4LPLRND) experiments.

RESULTS AND DISCUSSION

Studies with taurolidine (IV) *and analogues*

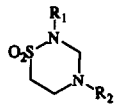
Multiplet signals in the ^{1}H-spectra of I and II in deuterium oxide between δ3.16 and δ3.40 (Table 1) and δ3.15 and δ3.38 (Table 1), respectively with total integral values equivalent to four protons were assigned to the C–5 and C–6 protons. Interestingly the signal assigned to these protons in III appeared as a singlet at

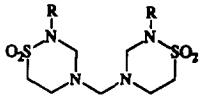
Table 1. ^{1}H assignments of compounds I, II and III in deuterium oxide in the absence and presence of ^{13}C enriched methylene glycol*

Compound	H-position	δ	Compound	H-position	δ†
I	3	4.25(s)	I + $CH_2(OH)_2$	3	4.29(s)
	5, 6	3.29(m) (3.16–3.40)			4.32(s)
				N^4–CH_2OH	4.45(s)
				N^4–CH_2–N	3.66(s)
				5, 6	3.29(m)
			$CH_2(OH)_2$		4.80(s)
II	N^2–CH_3	2.83(s)	II + $CH_2(OH)_2$	N^2–CH_3	2.85(s)
					2.88(s)
	3	4.31(s)		3	4.21(s)
	5, 6	3.27(m) (3.15–3.38)			4.29(s)
				N^4–CH_2OH	4.42(s)
				N^4–CH_2–N	3.61(s)
				5, 6	3.27(m)
			$CH_2(OH)_2$		4.80(s)
III			III + $CH_2(OH)_2$	N^2–CH_2OH	4.86(s)
	3	4.13(s)		3	4.30(s)
					4.38(s)
	N^4–CH_3	2.41(s)		N^4–CH_3	2.39(s)
	5, 6	3.29(s)		5, 6	3.29(s)
			$CH_2(OH)_2$		4.80(s)

*Key: (s) singlet; (m) multiplet.

†The new signals produced when methylene glycol is added.





Compound	R_1	R_2
I	H	H
II	CH_3	H
III	H	CH_3
V	CH_3	CH_3
VII	H	CH_2OH
VIII	CH_3	CH_2OH

Compound	R
IV	H
VI	CH_3

Fig. 2. Thiadiazine-1,1-dioxide derivatives.

δ3.29 (Table 1). The signals due to the C–3 protons in I, II and III appeared downfield at δ4.25, δ4.31 and δ4.13, respectively. Singlets equivalent to three protons appeared upfield in the spectra of II and III, and were assigned to the *N*-methyl protons. Addition of methylene glycol to solutions of I, II and III modified the spectra producing a number of new signals which were not solely due to methylene glycol (Table 1). There were also changes in the integral values of the signals of the parent compounds indicating the formation of new compound(s).

At concentrations of less than 0.5% w/v in aqueous solution NMR studies have indicated that methylene glycol exists solely as a monomer in its hydrated form.[14] With increasing concentration, however, the composition of the solution changes with the production of oligomers. Whilst in this present study the signal due to HOD varied in chemical shift, the one attributed to the monomer was at δ4.80, which is consistent with previous reports.[10,14]

In the presence of acetonitrile as internal standard, the sum of the integral ratio values of the signals at δ4.25, δ4.29 and δ4.32 (relative to the signal due to the methyl group of acetonitrile) in the spectrum of I after the addition of methylene glycol, had the same integral ratio value as the C–3 protons at δ4.25 in the spectrum of I (Table 2). This suggests that the signals at δ4.32 and δ4.29 were due to the C–3 methylene group protons of newly formed species. The integral ratio of the new signal at δ3.66 was half that observed for the signal at δ4.29; the signals at δ3.66 and at δ4.29 were therefore, respectively assigned to the bridging methylene protons and the C–3 protons of IV. The downfield resonance at δ4.45 was assigned to the methylene protons of a *N*-hydroxymethyl group, and the signal observed at δ4.32 which had the same integral ratio as the signal at δ4.45 to the C–3 protons of the *N*-hydroxymethyl compound (VII, Fig. 1). Thus the NMR data provides conclusive evidence that when methylene glycol is added to a solution of I, *N*,*N*′-methylenediamine (IV) and *N*-hydroxymethyl adducts of I are formed, with the hydroxymethyl group presumably at the 4-position (VII) as has previously been proposed in studies on the hydrolysis of IV[9,10,11] (Fig. 1). The studies with methylene glycol were repeated with the two *N*-methyl analogues of I, compounds II and III (Table 1). There were similar changes in the spectrum of II on the addition of methylene glycol as had been observed with I, which aided the assignment of the new signals. Apart from the signal at δ2.83, assigned to the methyl group of II, two new signals at δ2.85 and δ2.88 were also present. Similarly the new signals at δ4.21 and δ4.29 were assigned to C–3 methylene protons of new molecular species. With the aid of integral ratios (Table 2) the signals of δ4.42 and δ4.29 were assigned to a hydroxymethyl

Table 2. Proportions of thiadiazine species present in solutions of I and II containing varying concentrations of methylene glycol

	Proportions (%) Compound (C–3 protons δ values)		
Compound I (60 m*M*) Methylene glycol (m*M*)	I (4.25)	IV (4.29)	VII (4.32)
0	100	0	0
13.5	87.9	6.05	6.05
27.0	64.1	24.3	11.7
40.5	54.5	33.4	12.1
Compound II (60 m*M*) Methylene glycol (m*M*)	II (4.31)	VI (4.21)	VIII (4.30)
0	100	0	0
12.0	81.1*	6.0*	12.9*
30.0	67.6	8.7	23.6
60.0	45.8	8.4	45.8

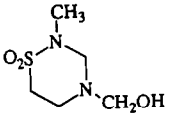
*Calculated from the N–CH_3 signal.

Table 3. ^{1}H-NMR structural assignments of the molecular species present in a deuterium oxide solution of IV (35 m*M*)*

Chemical shift (δ)				
$CH_2(OH)_2$	N^4–CH_2OH	HNC^3H_2N–	N–CH_2–N–	$O_2SCH_2CH_2N$–
4.81(s)	4.45(s)	I 4.25(s)	IV 3.66(s)	3.15–3.48(m)
		IV 4.29(s)		
		VII	4.32(s)	

*Key: (s) singlet: (m) multiplet.

group and C–3 protons, respectively of the 4-hydroxymethyl adduct II, and the signals at δ3.61 and δ4.21 were therefore assigned, respectively to the bridging methylene protons and to the C-3 methylene protons of VI. Thus the signals at δ2.85 and δ2.88 are assigned to the *N*-methyl protons of VI and the 4-hydroxymethyl adduct (VIII).



VIII

Interestingly the NMR spectrum of III is different from those obtained with I and II, in that the signals for the C–5 and C–6 methylene protons appear not as multiplets but as a singlet (Table 1). Addition of methylene glycol to a solution of III, although modifying the spectrum (Table 1), produced a spectrum which was simpler than that for II after the addition of methylene glycol (Table 1). Integral ratio values obtained with acetonitrile as internal standard indicated that the signals at δ2.39, δ4.30 and δ4.86 were due to the *N*-methyl, C–3 and hydroxy-methyl protons, respectively; and the molecular species was identified as the 2-hydoxymethyl adduct of III.

The difference between the signals of the hydroxymethyl group, at the 4- and 2-positions is 0.44 ppm, which is similar to the difference between the δ value of the two *N*-methyl group signals for II and III, *i.e.* 0.42 ppm.

Sequential addition of methylene glycol to solutions of I and II changed the equilibria, in that as the concentration of methylene glycol was increased, the concentrations of I and II decreased with concomitant increases in their respective hydroxymethyl adducts (VII and VIII), whilst the concentrations of the *bis*-compounds IV and VI increased to 12 and 9%, respectively of the components present in the solution, whereafter they remain constant (Table 2). Increasing the concentration of methylene glycol in solutions of III also produced both a decrease in the concentration of III and an increase in the concentration of the hydroxymethyl compound. There was, however, no evidence for the presence of the *bis*-compound.

Comparison of the ^{1}H-NMR spectrum of IV (Table 3) in deuterium oxide with the one obtained when methylene glycol is added to a solution of I (Table 1) indicates that the same equilibria exist between the molecular species present in the respective solutions. Thus, apart from IV, I and its 4-hydroxymethyl adduct (VII) and methylene glycol are also present. A minor signal at δ4.51 may be due to the hydroxymethyl group protons of the 2-hydroxymethyl derivative of I. Moreover the intensity of this signal increased when methylene glycol is added to the solution of IV. Unfortunately it was not possible to identify signals due to the C–3, C–5 and C–6 methylene protons of I because of overlapping signals, from the methylene protons of the other molecular species, in these regions.

As was observed with I, the equilibria changed in a solution of IV when incremental amounts of methylene glycol were added, resulting in a decrease in the concentration of I and an increase in the concentration of the 4-hydroxymethyl derivative of I, (VII), while the concentration of IV remained constant.

Interestingly the NMR spectrum of VI in deuterium oxide was identical to that for II plus methylene glycol, suggesting that VI is not stable in solution, being hydrolysed completely to II and VIII. Unfortunately taurolidine (IV) and some of the analogues (V and VI) were not sufficiently soluble in deuterium oxide to obtain ^{13}C-spectra at 62.9 MHz. However it was possible to obtain 62.9 MHz ^{13}C-spectra of compounds I, II and III in deuterium oxide in the absence and presence of ^{13}C-enriched methylene glycol (Table 4).

At the concentrations of methylene glycol used in this study there was only one significant signal for methylene glycol and ^{13}C-enriched

Table 4. ^{13}C-NMR structural assignment of compounds I, II and III in the absence and presence of ^{13}C-methylene glycol

Compound	Chemical shift (δ)*						
	$CH_2(OH)_2$		C-3	C-5	C-6	C-2'‡	C-4'‡
I			60.00	42.98	50.17		
†I + $CH_2(OH)_2$	81.70	76.98 (N^4CH_2OH)	62.98	46.43	48.49		
		65.09 (–NCH_2N–)	62.85	46.21	46.71		
II			66.82	43.18	45.54	33.64	
†II + $CH_2(OH)_2$	81.70	77.28 (N^4CH_2OH)	69.79	45.11	46.45	34.64	
		69.19 (–NCH_2N–)	70.21				
III			65.97	45.34	50.28		37.56
†III + $CH_2(OH)_2$	81.70	72.12 (N^2CH_2OH)	70.83	46.68	51.21		38.64

*Internal standard dioxan.
†The new signals produced when methylene glycol is added.
‡Methyl group.

methylene glycol in deuterium oxide at δ81.70, with minor ones at δ85.65 and δ89.45 in the enriched methylene glycol spectrum.

Intensification of the signals at δ60.00, δ66.82 and δ65.97 (Table 4), in the presence ^{13}C-enriched methylene glycol, facilitated their assignment to the C-3 atoms of I, II and III, respectively. This phenomenon occurs because ^{13}C-methylene glycol exchanges with the C-3 atoms in these compounds due to ring opening.[15] Assignment of δ values for the C-5 and C-6 atoms of I, II and III (Table 4) was done

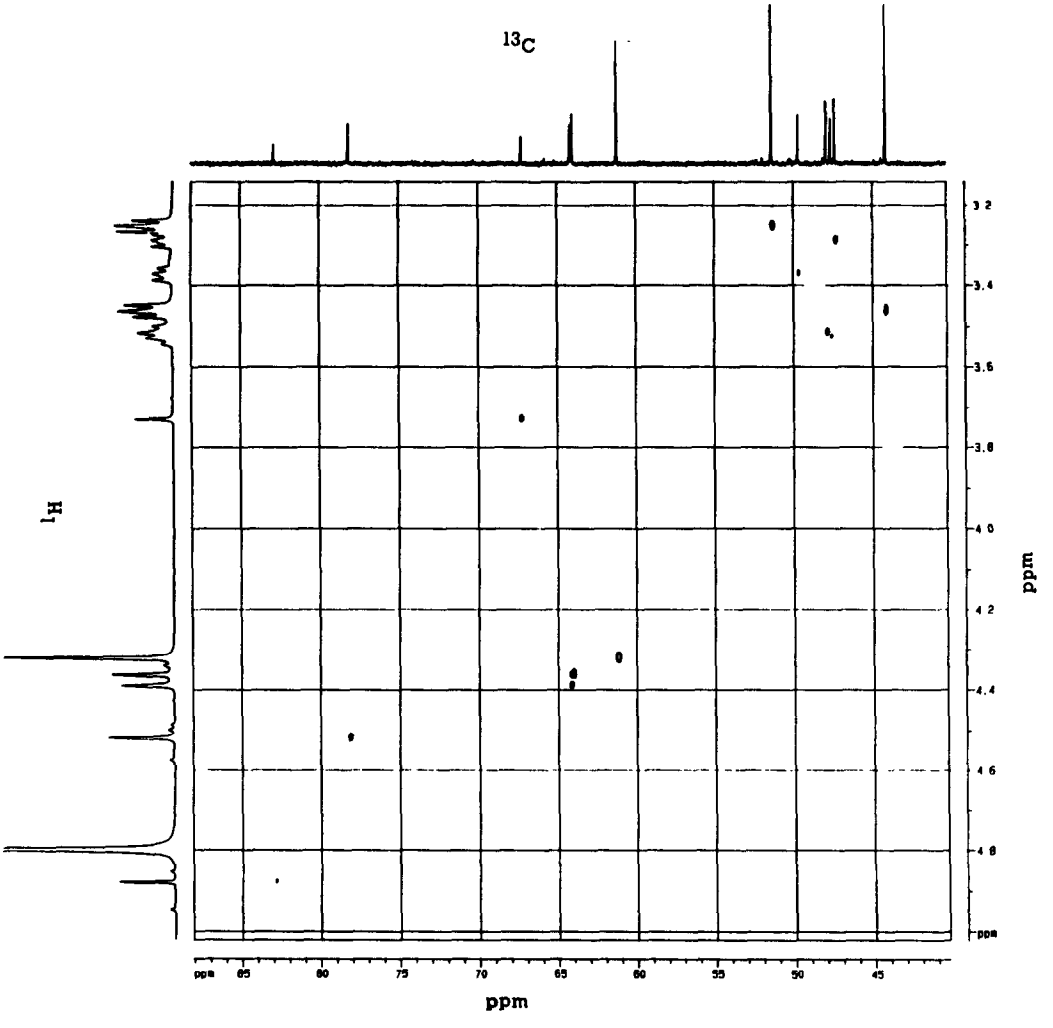


Fig. 3. ^{1}H–^{13}C direct correlation (HCCOBI) spectrum of taurolidine (IV) in solution.

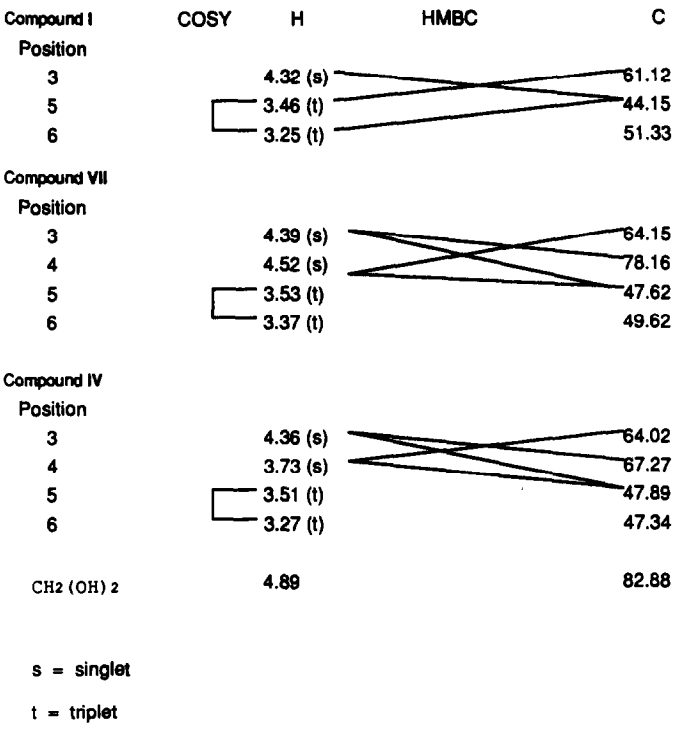


Fig. 4. 400 MHz spectral data of solutions of compound I and taurolidine (IV).

on the basis of the effect the introduction of a methyl group, at either position 2 or 4, had on the δ values.

It was concluded, on examination of the spectrum of I after the addition of ^{13}C-methylene glycol, that four compounds were present in the solution; methylene glycol and I were in equilibrium with IV and the 4-hydroxymethyl adduct of I (VII). This is in agreement with the present ^{1}H-study and a previous report.[10]

Similar arguments were used for assigning the signals in the ^{13}C-spectra of II and III in the presence of ^{13}C-enriched methylene glycol. For II it would appear again that four compounds are present in equilibrium with each other; methylene glycol, II, and its *N,N'*-methylenediamine (VI) and 4-hydroxymethyl adducts.

High field NMR studies

With the aid of COSY 45, ^{1}H–^{13}C direct (Fig. 3) and H–C long-range (HMBC) correlations over three bonds (Fig. 4) it was possible to assign fully all the signals in the ^{1}H- and ^{13}C-spectra of a solution of taurolidine (IV). Interestingly with the 400 MHz ^{1}H-proton spectrum there was in general a downfield shift of approximately 0.07 ppm compared with the 250 MHz spectrum. This apparent downfield shift and that observed when different instruments were used for the ^{13}C experiments is probably due to instrumental variation related to calibration. Again four molecular species including methylene glycol could be clearly identified. Because the signals due to the formation of I in a solution of IV could be identified by references to its spectrum, the assignment of the signals due to IV, VII and methylene glycol was simplified. This not only confirmed the 250 MHz assignments but made possible, because of improved resolution, assignment of the C–5 and C–6 methylene protons for I, IV and VII. Again there was a downfield shift in the 100 MHz ^{13}C-signals, of between 0.9 and 1.19 compared with the 62.9 MHz study. The H–C long-range correlations (Fig. 4) confirm the assignments of the 250 MHz study for the four molecular species. Thus it can be concluded from the ^{1}H- and ^{13}C-studies that the main hydrolytic components in solutions of taurolidine (IV) apart from the parent compound are the thiadiazine I, and its 4-hydroxymethyl derivative (VIII) and methylene glycol which confirms previous observations.[9,10,11] However, it could be tentatively concluded that a small amount of the 2-hydroxymethyl derivative (VIII) of I may

also be produced *in situ* due to the methylene glycol reacting with the less basic N–2 ring nitrogen.

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REFERENCES

1. W. R. Abrams and R. G. Kallen, *J. Amer. Chem. Soc.*, 1976, **98**, 7777.
2. R. G. Kallen and W. P. Jencks, *J. Biol. Chem.*, 1966, **241**, 5864.
3. R. G. Kallen, R. O. Viale and L. K. Smith, *J. Amer. Chem. Soc.*, 1972, **94**, 576.
4. P. C. Bansal, I. H. Pitman, J. N. S. Tam, M. Mertes and J. J. Kaminski, *J. Pharm. Sci.*, 1981, **70**, 850.
5. P. C. Bansal, I. H. Pitman and T. Higuchi, *J. Pharm. Sci.*, 1981a, **70**, 855.
6. I. H. Pitman, *Med. Res. Rev.*, 1981, **1**, 189.
7. M. K. Browne, G. B. Leslie and R. W. Pfirrmann, *J. Appl. Bacteriol.*, 1976, **41**, 363.
8. I. Bedrosian, R. D. Sofia, S. M. Wolff and C. A. Dinarello, *Cytokine*, 1991, **3**, 568.
9. E. Myers, M. C. Allwood, M. J. Gidley and J. K. M. Saunders, *J. Appl. Bacteriol*, 1980, **48**, 89.
10. F. Erb, N. Febvay and M. Imbenotte, *Talanta*, 1982, **29**, 953.
11. B. I. Knight, G. G. Skellern, G. A. Smail, M. K. Browne and R. W. Pfirrmann, *J. Pharm. Sci.*, 1983, **72**, 705.
12. A. I. B. Vogel, *Textbook of Quantitative Inorganic Analysis*, 4th ed. Longman, London, 1978.
13. T. Nash, *Biochem. J.*, 1953, **55**, 416.
14. D. J. Le Botlan, B. G. Mechin and G. J. Martin, *Anal. Chem.*, 1983, **55**, 587.
15. M. J. Gidley, J. K. M. Sanders, E. R. Myers and M. C. Allwood, *FEBS Letters*, 1981, **127**, 225.