Table II—Determination and Recovery of Promethazine Hydrochloride (Micrograms) in Pharmaceutical Preparations

Preparation	Present	Added	Determined	Recovery, %
Tablet	37.97	41.76	41.30	99.00
Syrup	30.00	23.64	23.70	100.25
Injection	35.00	32.65	32.70	100.15

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NMR Studies and GC Analysis of the Antibacterial Agent Taurolidine

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Received December 9, 1981, from the **Department of Pharmacy, University of Strathclyde, Glasgow G1 1XW, U.K.*; ‡*Monklands District General Hospital, Airdrie ML6 0JS, U.K.*; and §*Geistlich-Pharma, 6110 Wolhusen, Switzerland.* Accepted for publication June 10, 1982.

Abstract □ The NMR spectrum of taurolidine in deuterium oxide was compared with spectra obtained from model experiments with amines and formaldehyde. Head-space analysis combined with capillary GC showed that there was <0.004% free formaldehyde present in 2% solutions of taurolidine. This value is comparable to the concentration of formaldehyde found when the taurolidine solutions were injected directly onto GC columns.

Keyphrases □ Taurolidine—NMR spectral analysis, head-space analysis–GC methods for the determination of formaldehyde □ NMR spectroscopy—analysis of taurolidine □ GC analysis—direct or combined with head-space analysis, quantitation of formaldehyde concentration in taurolidine □ Head-space analysis—with capillary GC, quantitation of formaldehyde concentration in taurolidine

Taurolidine [4,4′-methylenebis(tetrahydro-1,2,4-thiadiazine 1,1-dioxide), I] is a broad spectrum bactericide and antiendotoxin (1–3) which is being used widely in clinical trials to counteract bacterial infections following GI surgery and to treat peritonitis (4–6). It is administered intraperitoneally *via* a catheter inserted when the abdomen is closed, as a 2% aqueous solution[1] containing 5% povidone[2] (added to increase the solubility of taurolidine).

As a result of NMR studies (7) of aqueous solutions of taurolidine, equilibria are considered to exist between taurolidine, 4-hydroxymethyltetrahydro-2*H*-1,2,4-thiadiazine 1,1-dioxide (II), tetrahydro-2*H*-1,2,4-thiadiazine 1,1-dioxide (III)[3], and formaldehyde.

The existence of such equilibria was studied with the aid of solutions of morpholine–formaldehyde and III–formaldehyde as models. Also, because of the concern regarding toxicity, the determination of the formaldehyde concentration in solutions of taurolidine (which have been used in large volumes for the treatment of septicemia) is of considerable interest. Thus, this paper also describes the quantitative determination of formaldehyde using combined head-space analysis–capillary GC and direct GC.

EXPERIMENTAL

NMR—The NMR spectra of taurolidine were determined in deuterium oxide and deuterodimethylsulfoxide. Taurolidine (20 mg/ml) together with 1-vinyl-2-pyrrolidinone polymer (50 mg/ml) in deuterium oxide, and morpholine and III in deuterium oxide (before and after gaseous formaldehyde had been passed through the solution), were also determined[4]. The reference standard was sodium 3-trimethylsilylpropionate-2,2,3,3-$^2H_4$.

GC Analysis of Formaldehyde—*Head-Space Analysis*—Aqueous solutions (10 ml) of formaldehyde (0.005–0.01%) containing sodium chloride (5 g) were added to 100-ml injection vials which were tightly stoppered. The vials were incubated at 20, 40, or 60° for 1 hr to allow the

[1] Taurolin.
[2] Polyvinylpyrrolidone-17, mol. wt. 11,000, BASF (Germany).
[3] Taurultam.
[4] Perkin-Elmer R32 90-MHz NMR spectrometer.

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Table I—Proton NMR Structural Assignments of Taurolidine

Solvent	Chemical Shift (δ)[a]				
	>NH	HN—CH_2—N		>N—CH_2—N<	SO_2CH_2—CH_2—N<
Deuterodimethylsulfoxide	7.25(t)	4.14(d)		3.59(s)	3.17(m)
	CH_2O	>N—CH_2OH	HN—CH_2—N	>N—CH_2—N<	SO_2CH_2—CH_2—N<
Deuterium oxide	4.82(s)	4.47(s)	4.27(s)	3.65(s)	3.30(m)

[a] Key: (s) singlet; (d) doublet; (t) triplet; (m) multiplet.

Table II—Proton NMR Structural Assignments of Morpholine and III Solutions in the Absence or Presence of Formaldehyde

	Chemical Shift (δ)[a]				
Morpholine			—CH_2—O—CH_2— 3.5(t)		—CH_2NHCH_2— 2.58(t)
Morpholine + formaldehyde	CH_2O 4.88(s)	>N—CH_2OH 4.44(s)	—CH_2—O—CH_2- 3.93(t)	>N—CH_2—N< 3.25(s)	—CH_2NHCH_2- 2.77(t)
Compound III			HN—CH_2—N— 4.25(s)		SO_2CH_2—CH_2—N< 3.30(m)
Compound III + formaldehyde	CH_2O 4.82(s)	>N—CH_2OH 4.47(s)	HN—CH_2—N— 4.27(s)	>N—CH_2—N< 3.65(s)	SO_2CH_2—CH_2—N< 3.30(m)

[a] Key: (s) singlet; (d) doublet; (t) triplet; (m) multiplet.

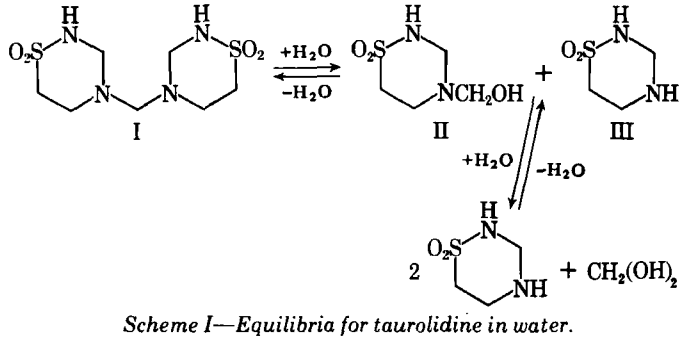
system to equilibrate. A 200-μl sample of the gaseous phase was removed from the vial with a microliter syringe and injected into a gas chromatograph[5] fitted with a 15 m × 0.47-mm capillary column[6] (column A). The temperatures of the injection port, column, and detector were 175, 65, and 150°, respectively. The carrier gas was helium at a pressure of 0.2 bar. Aqueous solutions of taurolidine and taurolidine with 5% 1-vinyl-2-pyrrolidinone polymer were analyzed in a similar manner.

Direct Analysis—Formaldehyde concentrations were also determined by injecting aliquots (1 μl) of taurolidine solution into the gas chromatograph fitted with a glass column (2.1 m × 4 mm) packed with 5% polyoxyethylene glycol stearate[7] on 80–100 mesh chromosorb W-AW (column B). The temperatures of the injection port, column, and detector were 125, 95, and 200°, respectively. The carrier gas was helium at a flow rate of 40 ml/min.

Aqueous solutions of formaldehyde (0.0025–0.01%) were prepared as calibration standards. Formaldehyde (0.0025–0.01%) was added to the taurolidine and taurolidine plus 1-vinyl-2-pyrrolidinone polymer solutions. Formaldehyde concentrations were also determined by injecting aliquots (0.5 μl) of taurolidine solution onto the capillary column (column A) used for head-space analysis.

RESULTS AND DISCUSSION

The singlet at δ3.59 in the NMR spectrum of taurolidine in deuterodimethylsulfoxide (Table I) was assigned to the protons of the methylene group joining the two molecules of II (Scheme I). However in deuterium oxide the signal (δ3.65) due to these protons is reduced to <10% of the intensity observed in deuterodimethylsulfoxide. In deuterium oxide the signals at δ4.47 and δ4.82 have been assigned to the hydroxymethyl group of II and formaldehyde, respectively. That the signal at δ4.47 arises from the hydroxymethyl group has been shown in model experiments with morpholine and formaldehyde, which behave in a manner analogous to solutions of III and formaldehyde (Table II). Thus, the NMR spectrum of a solution of morpholine in deuterium oxide is modified by treatment with formaldehyde gas, the additional peaks observed at δ4.44, δ3.25, and δ4.88 being ascribed to a hydroxymethyl group on the morpholine nitrogen, the methylene bridge of the *N,N'*-methylenediamine, and formaldehyde, respectively. The addition of sodium carbonate eliminated the signal ascribed to the hydroxymethyl group (δ4.44); these derivatives are known to undergo hydrolysis at alkaline pH (8). Solutions of III and



Scheme I—Equilibria for taurolidine in water.

formaldehyde, in turn, mimic the behavior of taurolidine in deuterium oxide: III plus formaldehyde gives an NMR spectrum identical to that of taurolidine in deuterium oxide (Table II).

The equilibria existing between amines and aldehydes in aqueous solutions are not simple (8, 9). There is also evidence[8] to suggest that the equilibria shown in Scheme I only partially represent those that exist in solutions of taurolidine. With saturated solutions of taurolidine in deuterium oxide, the intensity of the signal due to the bridging methylene group (δ3.65) increased and the peak height ratio of the signals ascribed to formaldehyde and the hydroxymethyl group of II decreased in comparison with the ratio of these peaks in the spectrum of the 1% taurolidine solution. Moreover the NMR spectrum of taurolidine (2%) in deuterium oxide containing 5% 1-vinyl-2-pyrrolidinone polymer was comparable to the spectrum of a saturated solution of taurolidine, with respect to the signal intensities due to the bridging methylene protons and formaldehyde, suggesting this substance may influence the equilibria.

Semiquantification of the amount of free formaldehyde present in aqueous solutions of taurolidine has been attempted using NMR spectroscopy (7). Measurement of the area of the signal due to the bridging methylene protons presents problems, since there is not a complete conversion of taurolidine to its hydrolysis products. Theoretically, this means comparing the taurolidine spectrum in deuterodimethylsulfoxide (where no such equilibria exist) with that in deuterium oxide with the assumption that the methylene bridge proton signals are comparable in both solvents. Although NMR spectroscopy would be the method of choice since it is a noninvasive technique and does not disturb the proposed equilibria, integration of this minor peak is unsatisfactory, espe-

[5] Pye Unicam Model 204.
[6] Silar-5CP (Silicone).
[7] Ethofat 60/25.

[8] Unpublished results.

Table III—Percentage of Free Formaldehyde Present in Aqueous Solutions of Taurolidine and Taurolidine Plus 1-Vinyl-2-pyrrolidinone Polymer [a]

Method	Taurolidine 1%	Taurolidine 2%	Taurolidine (2%) plus 1-vinyl-2-pyrrolidinone polymer (5%)
Head-space with capillary column A	0.0036 ± 0.0004	0.0037 ± 0.0004	Not measurable due to the presence of isopropyl alcohol
Direct analysis			
Column A	0.0022 ± 0.0006	0.0038 ± 0.0003	Not measurable due to the presence of isopropyl alcohol
Column B	0.0013 ± 0.0001	0.0027 ± 0.0001	0.0032 ± 0.0006

[a] Mean ± *SD*.

cially in the presence of 1-vinyl-2-pyrrolidinone polymer, where signals in this molecule interfere with the signal assigned to the bridging methylene protons.

GC combined with head-space analysis can also be regarded as a noninvasive technique, the amount of gaseous formaldehyde in the head-space being proportional to the concentration of formaldehyde present in the aqueous phase. The assay was carried out at different temperatures to determine whether the equilibria changed. At all temperatures the formaldehyde calibration curves were linear. The equations for the plots at 20, 40, and 60° were $y = 0.032 + 40.93x$, $y = 120x$, and $y = 0.036 + 266.2x$, respectively. The correlation coefficients (r) were 0.99, 1, and 0.99 respectively. The precision of the method was ±3% for a 0.01% formaldehyde solution at 40°. At the three assay temperatures, there was <0.004% free formaldehyde present in the taurolidine solutions (Table III).

Rectilinear relationships between detector response and formaldehyde concentrations for the direct GC method were also observed when known amounts of formaldehyde were added to water or taurolidine solutions, or taurolidine solutions containing 1-vinyl-2-pyrrolidinone polymer ($y = 0.165 + 1407x$, $r = 0.99$). That the slope and intercept were the same in all cases, would indicate that the equilibria did not change, especially in the presence of the polymer. Unfortunately, 1-vinyl-2-pyrrolidinone polymer contains a small amount (60–100 ppm) of isopropyl alcohol (1-vinyl-2-pyrrolidinone is polymerized in this solvent). Since isopropyl alcohol has the same retention time as formaldehyde (1.25 min) on column B, it was necessary to subtract the height of the peak observed when an aliquot of a 5% 1-vinyl-2-pyrrolidinone polymer solution was injected on this column from the height of the peak from the formaldehyde–isopropyl alcohol in the taurolidine–1-vinyl-2-pyrrolidinone polymer solution. Comparable amounts of free formaldehyde were found when solutions of taurolidine were injected directly onto either column A or B, indicating that the direct method could be regarded as a noninvasive technique and was suitable for the determination of formaldehyde.

That the equilibria may be sensitive to the concentrations of the molecular species present but not to the presence of 1-vinyl-2-pyrrolidinone polymer is shown by direct GC analysis (Table III) but head-space analysis GC does not support this contention. The concentration of free formaldehyde is not clinically important since no formaldehyde toxicity has been observed with solutions of taurolidine containing 1-vinyl-2-pyrrolidinone polymer either in humans or experimental animals. Moreover, the formaldehyde concentration is considerably lower than the amount of formaldehyde produced in the body after administration of certain antibiotic prodrugs (10).

Compound III has also been shown to possess antibacterial and antiendotoxin activity similar to taurolidine, although higher concentrations are required[8]. This activity also is not related to the presence of free formaldehyde, since solutions of III have been shown to be stable by NMR and GC, liberating no measurable amounts of formaldehyde.

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