^{1}H and ^{13}C NMR Spectra of the Rotational Isomers of *N*-Hydroxymethylamides and Derivatives†

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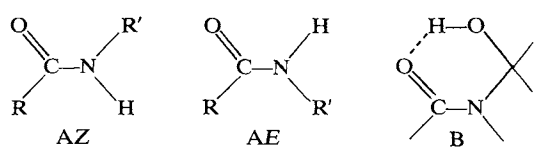
A series of *N*-hydroxymethylamides, $RCONR'CH_2OH$, and their *O*-methyl and *O*-acetyl derivatives, have been studied by ^{13}C and ^{1}H magnetic resonance spectroscopy. Signals have been assigned to the *E*- and *Z*-isomers on the basis of the analysis of the fully coupled spectra, and by comparison of the chemical shifts with those of model compounds. The introduction of the hydroxy, alkoxy or acetoxy groups at the α-position of the *N*-alkyl moiety causes a significant shift in the equilibrium towards the *E*-rotamer compared with the unsubstituted *N*-alkylamide. The predominant effect in determining the *E*:*Z* ratio appears to be the steric interaction between the carbonyl oxygen and the α-oxygen in the alkyl moiety; intramolecular hydrogen bonding does not play a significant role in determining the rotamer populations of these molecules.

INTRODUCTION

Rotational isomerism ($AZ \rightleftharpoons AE$) in *N*-alkylamides has been extensively studied using ^{13}C NMR spectroscopy[1,2] and, recently, with ^{15}N natural abundance spectroscopy.[3] In *N*-methylformamide, the *Z*-isomer is preferred by a factor of 9:1 over the *E*-isomer.[1] This present study of the ^{1}H and ^{13}C NMR spectra of a series of *N*-hydroxymethylamides, and their derivatives, was undertaken to assess the effect of the α-situated oxygen substituent on the chemical shifts in the amide and on the rotational equilibrium $AZ \rightleftharpoons AE$. The presence of a hydroxy group in R' introduces the additional possibility of hydrogen bonding, such as shown in B, which could exert a significant effect on the rotational equilibrium.



N-Hydroxymethylformamide ($HCONHCH_2OH$) has acquired special significance as a metabolite of the anti-tumour agent *N*-methylformamide (NMF).[4] In structure–activity studies of a large number of amides, NMF was found to be the most potent inhibitor of tumour growth; small changes in structure, such as substitution of the methyl with an ethyl group, result in loss of activity.[5] *N*-Hydroxymethylformamide (**1**) has been identified as a urinary metabolite of NMF[6] in mice, and although it does not possess the anti-tumour efficacy of NMF against murine tumours *in vivo*, it is active against the human mammary tumour xenograft (MX-1).[7]

Some *N*-hydroxymethylamides (carbinolamides) can be synthesized readily by reaction of the appropriate amide with formaldehyde,[8] whereas the analogous carbinolamines ($RNHCH_2OH$) usually revert spontaneously to the component alkylamine and formaldehyde.[9] Amongst *N*-hydroxymethylamides, there is often considerable variation in stability. For example, *N*-hydroxymethylbenzamide (**6**) is a stable crystalline substance, readily available from the reaction of benzamide and formaldehyde; however, *N*-methyl-*N*-hydroxymethylbenzamide ($PhCONCH_3CH_2OH$) has not been synthesized chemically, although it has been generated metabolically from *N*,*N*-dimethylbenzamide.[10] The foregoing observations suggest that all carbinolamides of structure $RCONHCH_2OH$, where R = H, alkyl or aryl, are stable; substitution of the amide NH by alkyl groups has a destabilizing effect.[11] It was of interest to examine the NMR spectra of a series of carbinolamides in order to explore whether or not intramolecular hydrogen bonding of type B is a significant factor in the geometry of these molecules and, hence, influences their stability.

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EXPERIMENTAL

Spectroscopy

Nuclear magnetic resonance spectra were obtained at 360 MHz (proton) and 90.8 MHz (^{13}C) with a Nicolet 360 NB spectrometer. The chemical shifts are referenced to TMS.

Materials

***N*-Methylformamide (9).** This was purchased from Aldrich Chemical Co. and redistilled (b.p. 65 °C at 2 Torr) before use.

***N*-Hydroxymethylformamide (1).** Obtained by reaction of formamide with paraformaldehyde according to the method of Grady and Stott.[12] Analysis: found, C 31.73, H 6.84, N 18.22; calculated for $C_2H_5NO_2$, C 32.00, H 6.71, N 18.66%.

***N*-Methoxymethylformamide (2).** Aqueous potassium hydroxide (40%) (2 ml) was added to a stirred mixture of formamide (45 g) and paraformaldehyde (33 g). After 0.5 h, methanol (200 ml) and concentrated sulphuric acid (3 ml) were added to the clear mixture, which was stirred for a further 6 h. After filtration to remove a white precipitate, dry diethyl ether (20 ml) was added to the filtrate, which was allowed to stand over sodium hydrogen carbonate overnight. After filtration, the mixture was concentrated under reduced pressure and the residue dissolved in water (50 ml) and extracted with dichloromethane (5 × 100 ml). The combined extracts were washed with water, dried and evaporated under reduced pressure to give an oil (12 g), which was distilled under reduced pressure. Three fractions were collected: (i) 1.9 g, b.p. 80–90 °C at 3 Torr; (ii) 1.7 g, b.p. 90–98 °C at 3 Torr; and (iii) 2.4 g, b.p. 98–110 °C at 3 Torr (lit.,[13] b.p. 55–58 °C at 0.1 Torr).

Fractions (i) and (ii) were combined and purified by chromatography on a silica gel (Merck, 70–230 mesh) column (60 × 1.5 cm i.d.) with chloroform as the eluent. Chloroform fractions were concentrated at room temperature under reduced pressure (2 Torr) to afford *N*-methoxymethylformamide as a colourless liquid (0.7 g). The product gave a single spot with R_F 0.5 on TLC using 5% methanol in chloroform on silica gel, developed with iron(III) chloride after conversion to the hydroxamic acid.[14] Gas chromatographic analysis of the product (1% w/v solution in acetone), injected on to a glass column (1.5 m × 4 mm i.d.) packed with 10% w/w PEGA on Chromosorb W AW DMCS (100–120 mesh) maintained at 180 °C in a Pye Unicam 204 gas chromatograph with the injector and detector maintained at 200 and 250 °C, respectively, gave a single peak with retention time 4.3 min (carrier gas, N_2 at a flow-rate of 25 ml min^{-1}) using flame-ionization detection (H_2, 45 ml min^{-1}; air, 325 ml min^{-1}).

***N*-Hydroxymethyl-*N*-methylformamide (3).** This was synthesized by the method of Grady and Stott[12] from **9** and paraformaldehyde.

***N*-Acetoxymethyl-*N*-methylformamide (4).** Crude *N*-acetoxymethyl-*N*-methylformamide was synthesized by the method of Ross *et al.*[15] The crude product (12 g) was dissolved in formamide (50 ml) and extracted with dichloromethane (3 × 150 ml). The lower layer was separated and washed with water (75 ml), dried and evaporated under reduced pressure to yield the acetate **4** as a pale yellow oil (4.7 g), free from *N*-methylformamide.

***N*-Hydroxymethylacetamide (5).** This was synthesized by the method of Milkowski *et al.*[16]

***N*-Hydroxymethylbenzamide (6).** A mixture of benzamide (20 g), formalin (50 ml) and potassium carbonate (20 g) in tetrahydrofuran (500 ml) was refluxed for 5 h, then left overnight at room temperature. The organic layer was separated from a gummy residue and the solvent evaporated under reduced pressure. The residual oil solidified slowly and was recrystallized from ethyl acetate to give hydroxymethylbenzamide. A further batch was obtained by dissolving the gummy residue in water and extracting the resulting dark red solution with ethyl acetate. The ethyl acetate extracts were washed, dried and evaporated under reduced pressure to afford the second batch of the hydroxymethylbenzamide (total yield 8.6 g, 34%), m.p. 115–117 °C (lit.,[17] m.p. 104–106 °C).

N-Hydroxymethyl-*p*-*tert*-butylbenzamide (**8**) was obtained in an analogous manner from *p*-*tert*-butylbenzamide (yield 86%), m.p. 133–134 °C (lit.,[18] m.p. 134–135 °C); $\nu_{max.}$ 3230 and 1645 cm^{-1}.

***N*-Acetoxymethylbenzamide (7).** *N*-Hydroxymethylbenzamide (5 g) and acetic anhydride (2.4 ml) were dissolved in KOH-dried, redistilled pyridine (13.3 ml) and stirred for 48 h at room temperature. The clear solution was poured over crushed ice (28 g) and left in the cold overnight. The mixture was extracted with chloroform and the extracts were washed, dried and evaporated. The residual liquid was distilled under reduced pressure to afford *N*-acetoxy-methylbenzamide (0.53 g), b.p. 150 °C at 4 Torr. (This compound has been reported[19] as the product of the lead tetraacetate oxidation of hippuric acid and, although described as a 'syrup,' no physical data have been given.)

DISCUSSION

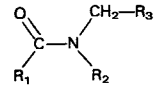
The preparation of *N*-hydroxymethylformamide (**1**) has been described.[12] The reaction of formamide with an anhydrous polymer of formaldehyde, preferably paraformaldehyde, in the presence of an alkali metal hydroxide as catalyst, is reported[12] to give 'an almost water-white pure product in 98% yield.' We have repeated this procedure, and also obtained a high yield of a colourless liquid that gave a C,H,N analysis in

agreement with the formula of hydroxymethylformamide, $C_2H_5NO_2$. However, such an analysis could also arise from a variety of mixtures, e.g. (a) a 1:1 mixture of formamide and formaldehyde, (b) a 1:1 mixture of methylenebisformamide [$(HCONH)_2CH_2$] and bishydroxymethylformamide [$HCON(CH_2OH)_2$] or (c) a mixture containing all of these species in addition to **1**. Indeed, the ^1H NMR spectrum of a sample of **1** prepared by Grady and Stott's method indicates that it is not pure *N*-hydroxymethylformamide. Further, the ^{13}C spectrum of this material is exceedingly complex and shows eight signals in the carbonyl-carbon region of the spectrum, *ca* 166–172 ppm. Attempts to purify the hydroxymethylformamide by chromatography or fractional distillation were unsuccessful. Clearly, spectral analysis of this complex mixture is not possible without reference to model compounds.

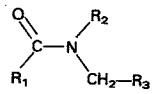
N-Methoxymethylformamide (**2**), which is a suitable model compound, was obtained in a pure form by distillation and chromatography, and a complete ^1H and ^{13}C NMR spectral analysis is possible. The ^1H NMR spectrum of **2** in D_2O clearly shows two rotational isomers. The major rotamer (*ca* 60%) is the *Z*-isomer (**2***Z*), which gives rise to signals at 8.32 (1 H, s, formyl CH), 4.72 (2 H, d, *J* 7.2 Hz, CH_2) and 3.36 (3 H, s, OMe) ppm. The minor rotamer (**2***E*) gives proton signals at 8.20 (1 H, d, *J* 11.5 Hz, formyl CH), 4.61 (2 H, d, *J* 7.2 Hz, CH_2) and 3.32 (3 H, s, OMe) ppm. The NH signals are broadened at *ca* 6.0 and 5.7 ppm. The major difference between the spectra of the rotamers is in the coupling of the formyl CH and the NH protons. The *trans* coupling constant of 11.5 Hz in **2***E* is close to the expected value for monosubstituted formamides.[1] The *cis* coupling constant is invariably lower in the *Z*-isomer (*ca* 2.0 Hz in formamides), and the apparent absence of *cis* coupling in **2***Z* is probably attributable to the electron-withdrawing effect of the $—CH_2OCH_3$ fragment (Taft σ^* +0.64), which is *trans*-coplanar to the formyl proton in **2***Z*.

The ^{13}C NMR spectrum of **2** confirms the presence of the rotational isomers **2***Z* and **2***E* in both D_2O and $CDCl_3$ solutions. In the fully decoupled spectrum in deuteriochloroform (see Table 1), the major isomer **2***Z* gives rise to signals at 161.74 (C=O), 69.72 (CH_2) and 56.02 (CH_3) ppm. Corresponding signals from the minor *E*-isomer **2***E* are seen at 165.28, 74.55 and 54.78 ppm. The conformations were assigned by comparison of ^{13}C–^1H coupling constants, obtained from the coupled spectrum of **2** in $CDCl_3$ (see Table 2), with those reported for the corresponding conformations of *N*-methylformamide.[2] The carbonyl carbon of the *Z*-rotamer (**2***Z*) is identified by its larger ^1J(CH) value (195.2 Hz compared with 191.5 Hz in **2***E*) and by the equality of ^2J(CNH) and ^3J(CNCH) (4.3 Hz), resulting in the doublet of quartets pattern in the fully ^1H coupled spectrum. The corresponding absorption of the *E*-rotamer is a doublet of triplets with the ^2J(CNH) not resolved. The value of 6.65 Hz for ^3J(CNCH) in **2***E*, compared with 3.1 Hz in NMF, is an effect[20] of the electronegative methoxy substituent on the coupled system. The CH_2 absorptions of the two rotamers differ mainly in the observation of an additional coupling, probably to the NH proton, in the

Table 1. ^{13}C chemical shifts of a series of *N*-hydroxymethyl-, *N*-methoxymethyl- and *N*-acetoxymethylamides



Z-Isomer



E-Isomer

No.	R_1	R_2	R_3	C=O	CH_2	R_1	R_2	R_3	C=O	CH_2	R_1	R_2	R_3	Solvent[a]
1	H	H	OH	167.28	67.25	—	—	—	168.2	73.1	—	—	—	A
2	H	H	OMe	161.74	69.72	—	—	56.02	165.28	74.55	—	—	54.8	B
				167.92	72.22	—	—	57.99	171.29	77.15	—	—	57.2	A
3	H	Me	OH	167.26	69.45	—	35.9	—	168.0	76.0	—	31.1	—	A
4	H	Me	OAc	167.9	70.7	—	36.4	22.5 174.6	168.3	76.6	—	31.6	22.6 174.7	A
5	Me	H	OH	177.2	65.23	24.7	—	—	179.6	71.1	23.9	—	—	A
6	Ph	H	OH	168.4	65.04	127.2 128.7 132.1 133.6	—	—	—	—	—	—	—	B
7	Ph	H	OAc	167.65	64.54	127.1 128.7 131.8 132.8	—	20.54 171.35	—	—	—	—	—	B
8	p-*t*-Bu-C_6H_4	H	OH	168.7	65.32	31.1 35.0 125.6 126.9 130.5 155.7	—	—	—	—	—	—	—	B
9	H	H	H	166.5	26.8	—	—	—	169.75	30.3	—	—	—	A

[a] Solvent: A, deuterium oxide; B, deuteriochloroform.

Table 2. ^{13}C–^{1}H coupling constants (Hz) in *N*-methoxymethylformamide (2) in $CDCl_3$

	2Z	2E
^1J(CH)(C=O)	195.2	191.5
^2J(CNH)(C=O)	4.3	[a]
^3J(CNCH)(C=O)	4.3	6.65
^1J(CH)(CH_2)	157.3	155.7
^3J(COCH)(CH_2)	5.4	5.4
^3J(CNCH)(CH_2)	5.4	[a]
^1J(CH)(CH_3)	142.1	141.9
^3J(COCH)(CH_3)	5.6	5.4

[a] Not resolved.

spectrum of the *Z*-isomer. The methylene carbon of the *E*-isomer is a triplet of quartets, arising from the directly bonded C–H coupling and the three-bond coupling to the *O*-methyl protons, whereas in the spectrum of **2***Z* the methylene carbon is observed as a triplet of quintets. The additional multiplicity presumably arises from coupling to the formyl proton, which is suitably situated in a *trans*-periplanar position in **2***Z*, unlike the situation in **2***E*. The signals from the *O*-methyl carbons are quartets of triplets in both isomers.

The ^{13}C spectrum of **2** in D_2O is similar to that in $CDCl_3$, showing two rotamers but with slightly modified chemical shifts for each carbon. The trend of the chemical shifts is similar in the two solvents; the carbonyl and methylene carbons of the *Z*-isomer absorb at higher field than those of the *E*-isomer. These assignments were used to assign the ^{13}C signals of the rotamers of hydroxymethylformamide (**1**) and other derivatives (Table 1). Hydroxymethylformamide is a mixture of *E*- and *Z*-isomers with a slight preference for the *E*-isomer, which has ^{13}C signals at 168.2 (C=O) and 73.1 (CH_2) ppm compared with 167.3 and 67.25 for the *Z*-isomer.

These chemical shifts are remarkably close to those observed in the rotamers of *N*-methyl-*N*-hydroxymethylformamide (**3***Z* and **3***E*), which shows a further pronounced shift in equilibrium towards the *E*-isomer (approximately 87% based on the relative intensity of the carbon signals at 168.0/76.0 and 167.3/69.5 ppm). The assignment of the signals at 35.9 and 31.1 ppm to the *N*-methyl carbons of **3***Z* and **3***E*, respectively, is consistent with the general observation that *N*-methyl carbons which are *syn* to the carbonyl oxygen are invariably shielded relative to the *anti* case.[2] The assignment of the signals in the spectrum of **3** was confirmed by comparison with the spectrum of the *O*-acetate derivative (**4**), which also shows a greater than 80% preference for the *E*-isomer.

The preference for the *E*-isomer of the hydroxymethylformamides in D_2O is not shared by *N*-hydroxymethylacetamide (**5**). The major form (*ca* 90%) is the *Z*-isomer with carbon signals at 177.2 (C=O), 65.23 (CH_2) and 24.7 (CH_3) ppm; the signals of the *E*-isomer appear at 179.6, 71.1 and 23.9 ppm. The carbonyl carbon atoms are deshielded relative to the formamide analogues, which is consistent with reported observations with NMF and acetamide.[2] The proportion of *E*- and *Z*-isomers in **5** is similar to that in NMF itself; in the present work, the major (90%) *Z*-isomer of NMF (**9***Z*) had carbon signals in D_2O at 166.5 (C=O) and 26.8 (CH_3) ppm, compared with the published values of 164.8 and 24.7 ppm.[2] Compound **9***E* has carbon signals at 169.75 and 30.3 ppm (published values 168.0 and 28.3 ppm). Significantly, both **5** and **9** follow the general trend that the *N*-alkyl carbon which is *syn* to the carbonyl oxygen is shielded relative to the *anti* case.

The shift to the *Z*-isomer by the introduction of bulkier groups at the R position is completed in the case of *N*-hydroxymethylbenzamide (**6**), which exists exclusively as the *Z*-isomer in $CDCl_3$. The single species (**6***Z*) has a carbonyl carbon which absorbs at 168.4 ppm and a methylene carbon at 65.04 ppm, in addition to the four different aromatic signals (Table 1). The introduction of an electron-donating *tert*-butyl group in the *para*-position of **6** has no effect on the equilibrium; **8***Z* is a single species (Table 1) with chemical shifts almost identical with those of **6***Z*. The complete preference of the benzamides (**6** and **8**) for the *Z*-isomer could be an indication that intramolecular hydrogen bonding, of the type shown in B, is exerting an effect. However, the acetate derivative **7** of *N*-hydroxymethylbenzamide also exists as a single species in $CDCl_3$; the chemical shifts of the carbonyl (167.65 ppm) and methylene (64.54 ppm) carbons show that the single species is also the *Z*-isomer. This observation suggests that intramolecular hydrogen bonding is not a significant factor in determining the equilibrium between *E*- and *Z*-isomers of *N*-hydroxymethylbenzamide, and that steric factors are more important. The preference of compounds **6**, **7** and **8** for the *Z*-isomer in $CDCl_3$ would thus be a consequence of steric interaction between the aryl and hydroxymethyl groups in the *E*-isomer.

In order to determine if a strongly hydrogen-bonding solvent could cause a population change in the hydroxymethylbenzamides, spectra of **6** and **8** were recorded in DMSO-d_6. However, these spectra were consistent with the presence of a single species, **6***Z* or **8***Z*, although small differences in chemical shift were observed. For example, hydroxymethylbenzamide (**6**) gave the following chemical shifts in DMSO-d_6: 166.2 (C=O), 134.2, 131.3, 128.2, 127.2 (aromatic) and 62.9 (CH_2) ppm.

Table 3 summarizes the results of analysis of the

Table 3. Percentage distribution of *E*- and *Z*-isomers of *N*-hydroxymethylamides and derivatives from NMR measurements

Compound	Solvent	*Z*(%)	*E*(%)	Nucleus
1	D_2O	43±5	57±5	^{13}C
2	$CDCl_3$	56±4	44±4	^{13}C
	$CDCl_3$	58±5	42±5	^{1}H
	D_2O	62	38	^{1}H
3	D_2O	13±6	87±6	^{13}C
4	D_2O	17.6±0.5	82.4±0.5	^{13}C
5	D_2O	90.7●3.3	9.3±3.3	^{13}C
6	$CDCl_3$	100	—	^{13}C
7	$CDCl_3$	100	—	^{13}C
8	$CDCl_3$	100	—	^{13}C
9	D_2O	90.5±0.5	9.5±0.5	^{13}C

NMR spectra to determine the percentage distribution of *E*- and *Z*-isomers in compounds **1–9**. In order to test the reliability of such data obtained from the relative intensities of signals in the ^{13}C spectra, the methoxymethylformamide **2** was analysed by both ^{1}H and ^{13}C NMR. The results are identical, within the limits of experimental error; much of the data in Table 3 can only be obtained from ^{13}C data, as the proton spectra are less well resolved in most cases. The most significant observation is the greater preference for the *E*-isomer shown by the hydroxy-, alkoxy- and acetoxy-formamides **1–4** compared with the simple *N*-methylformamide (**9**). This trend is counter to the predicted effect of hydrogen bonding (structure B), which should favour the *Z*-isomer. The percentage of the *Z*-rotamer of *N*-methylformamide observed in this study (90.5%) is close to the reported value (92%).[21] Introduction of the OH group in **1** or the OMe group in **2** shifts the equilibrium towards the *E*-rotamer, so that an almost 1:1 mixture is observed in both cases; this observation must reflect an increase in steric interaction between the carbonyl oxygen atom and the oxygen atom in the *N*-hydroxymethyl or *N*-methoxymethyl group. Introduction of a methyl group in place of the formyl proton, as in **5**, pushes the equilibrium back towards the *Z*-rotamer (>90%); in **5** the overriding factor appears to be the steric interaction between CMe and NCH_2OH groups, which destabilizes the *E*-rotamer. This trend is completed in the hydroxymethylbenzamides (**6**, **7**, and **8**), where the steric interaction with the aryl group is expected to be more pronounced and only the *Z*-rotamer is observed. In *N*-hydroxymethyl-*N*-methylformamide (**3**), the steric factor again overrides the effect of hydrogen bonding; the preferred rotamer is the one with the smaller group *syn* to the carbonyl oxygen.

The effect of introducing the α-oxygen-containing substituent into the *N*-alkyl group of *N*-methylformamide on the rotamer distribution is significantly greater than the change brought about by simply increasing the size of the alkyl group.[22] The replacement of the methyl group in NMF by the *n*-propyl group results in a slight shift towards the *E*-rotamer (8–14%), whereas the substitution of a methyl group by a methoxymethyl group results in a more significant shift to *ca* 40% of the *E*-rotamer. Evidently, the interaction between the methoxymethyl group and the carbonyl oxygen in **2** cannot simply be explained by the size of the methoxymethyl group, and may involve an electronic repulsion not present in simple *N*-alkylformamides.

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