Sodium 2-Mercaptoethanesulfonate in Reversible Adduct Formation and Water Solubilization

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Sodium 2-mercaptoethanesulfonate (MESNA, coenzyme M) forms 1:1 covalent adducts with highly π-electron deficient heterocycles. The addition is caused by the thiol function, and the adducts become water soluble as sulfonates. ^{1}H NMR spectroscopy has been used to obtain information about electronic and steric effects on the equilibria between 2-pyrimidinones and their 1:1 MESNA adducts. The adducts are potential prodrugs for biologically interesting 2-pyrimidinones.

2(1*H*)-Pyrimidinones are of interest for the control of cell proliferation because of their ability to cause reversible arrest of the cell cycle during metaphase.[1] Attempts have been made to apply this property to the synchronization of rapidly proliferating cells.[2] A synchronizing agent given in a sequential treatment with a phase-specific cytotoxic drug would be of interest in the treatment of diseases caused by uncontrolled, rapidly proliferating cells. Thus the aim is to synchronize the cell-division cycles so that the normal cells are in an insensitive phase and the abnormal cells are in the sensitive phase at the time when a phase-specific cytotoxic agent is given. It is important that the blocking agent has no harmful effects on the normal cell, and that the blocking agent can be rapidly removed in order for the cells to resume the cycle parasynchronously. Cell separation into groups occurs because of kinetic differences. Once it is necessary to remove the supply of the synchronizing agent, there must be no depot in the body from which additional agent can be supplied. As a consequence, the agent must have a reasonably high water solubility.

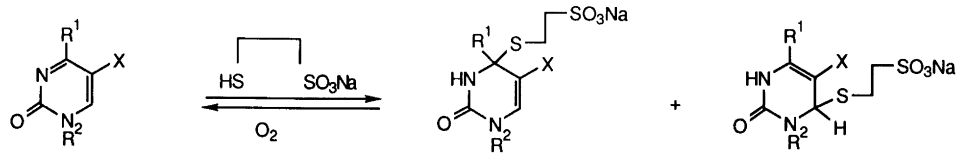
Our most active compounds, however, carry an aralkyl or heteroaralkyl substituent on N-1.[2] The low water solubility of these derivatives cannot be overcome by salt formation as the desired biological activity is reduced by acidic or basic functions in the molecule.[1] We have, in the past, tried to overcome the low water solubility by converting the pyrimidinones into 1:1 bisulfite adducts.[3] The adducts are sulfonic acids which are solubilized as salts, and these readily dissociate to the parent heterocycle which is responsible for the biological activity.[4] The adduct-forming ability of the 2-pyrimidinones is due to the π-electron deficiency of this system. Thus irreversible 1:1 adduct formation takes place with organometallics or metal hydrides.[5,6] With oxygen, sulfur or nitrogen nucleophiles, however, the adduct formation is reversible, and the preferred form is strongly influenced by the nature of the heteroatom.[7] A method for the preparation of the corresponding phosphonates from phosphites has also been worked out.[3] The phosphonates, however, did not possess the metaphase-arresting ability of the parent heterocycle because of their failure to dissociate.

In man the use of bisulfite complexes might be limited because bisulfites have been reported to be mutagenic.[8] We therefore have studied adduct formation with thiols, in particular the use of sodium 2-mercaptoethanesulfonate (coenzyme M or MESNA). MESNA is used in cancer chemotheraphy as an adjuvant to cyclophosphamides in order to reduce the nephrotoxicity caused by cyclophosphamide metabolites, and does not interfere with the action of cytotoxic agents.[9]

The MESNA adducts were formed in aqueous dioxane by the addition of MESNA to a suspension of the pyrimidinone until a clear solution was obtained. The products were isolated by freeze–drying, and were stable when stored in the cold, even with excess of the sulfonate. All the reactions were run in an inert-gas atmosphere in degassed solvents to prevent oxidation of the thiol to its disulfide. In contact with air the slightly soluble pyrimidinone is gradually precipated from a solution of the adduct owing to the removal of the thiol as the disulfide.

Table 1 shows a study of equilibria between 2-pyrimidinones and their MESNA adducts in acetone-d_6 and D_2O (1:1; v:v) using ^{1}H NMR spectroscopic monitoring. The study was aimed at elucidating the influence of steric and electronic effects. Two mole equivalents of MESNA seemed to give the optimal information for the whole series of pyrimidinones. The equilibrations were rapid and the spectra could therefore be recorded after 5 min. The relative concentrations of the components in the mixtures were determined by simple integration of the appropriate resonances.The signals from the methylene protons belonging to the NCH_2 group in most cases appear as an AB system. This patterns is caused by the chiral center created in the adduct at the position where the new carbon–sulfur bond is formed. The chemical-shift difference between the

Table 1. ^{1}H NMR spectroscopic studies of equilibria between 2-pyrimidinones and their adducts with sodium 2-mercaptoethanesulfonate (1:2 w:w) in acetone-d_6–D_2O (1.1 v:v) at 20 °C.



(1) (2) (3)

	X	R^1	R^2	(1)	(2)	(3)
a	H	H	H	100.0	0.0	0.0
b	H	H	CH_2Ph	62.6	17.7	17.7
c	H	H	Ph	37.3	33.6	29.1
d	Cl	H	H	6.5	93.5	–
e	Br	H	H	8.3	91.7	–
f	I	H	H	7.8	92.2	–
g	Cl	CO_2Me	H	0.0	–	100.0
h	F	H	CH_2Ph	<1.0	41.4	58.6
i	Cl	H	CH_2Ph	<1.0	39.4	60.6
j	Br	H	CH_2Ph	<1.0	42.4	57.6
k	I	H	CH_2Ph	<1.0	44.3	55.7
l	Cl	H	CH_2OCH_2Ph	<1.0	48.5	51.5
m	Br	H	CH_2OCH_2Ph	<1.0	46.2	53.8
n	Cl	H	CH_2COPh	<1.0	40.7	59.3
o	Br	H	CH_2COPh	7.3	36.4	56.3
p	Cl	H	$CH_2OC_6H_4CHO$-*p*	<1.0	52.5	47.5
q	Cl	H	$CH_2SC_6H_4Cl$-*p*	12.9	28.8	58.3

methylene protons is larger in the 3,6- than in the 3,4-regioisomer. The chiral center is closer to the methylene protons in the 3,6-isomer. The signals from the methylene protons in 3,4-isomer often appear as a broad singlet in the spectra. The H-4 signal from the 3,6-adduct appears at a lower field than does the H-6 signal from its 3,4-dihydro isomer; both signals are found in the region 6–7 ppm. When the solvent is changed to DMSO-d_6, however, this pattern is reversed.[10] Allylic couplings in the adducts were not observed, and coupling with the NH proton was excluded because of deuterium exchange.

From Table 1 it is seen that under the conditions of the measurements, no adduct formation was detected for 2(1*H*)-pyrimidinone (**1a**) itself. With the *N*-benzyl derivative (**1b**), the main component is the conjugated heterocycle. With the more electronegative *N*-phenyl substituent (**1c**) adduct formation is promoted. Presumably the compounds **1b**, **1c** and **1a** differ in their tendency to adduct formation because the polarization of the N–H (O–H bond in **1c**), as seen by hydrogen bonding or acid dissociation, results in a higher electron density in the heterocyclic ring in compounds **1a** than in **1b** or **1c**. An electronegative substituent at C-5 favors adduct formation; the 5-halogeno derivatives (**1d**, **1e**, **1f**) of the parent compound (**1a**) are present in the mixture as > 90 % adducts. With an additional electron-withdrawing 4-carboxylic ester group (**1g**) adduct formation goes to completion.

The *N*-alkylated 5-halogenopyrimidinones (**1h–1m**) are almost entirely present as adducts. The nature of the 5-halogen atom has little influence on the adduct formation, although this may not be true in other series as seen by comparison of the phenacyl derivaties (**1n** and **1o**). The 3,6-/3,4-adduct isomer ratio is in most cases close to 3:2. In bisulfite- and phosphite-adduct formation, however, the 3,4-dihydro isomer is preferred, and this was also the case in the irreversible reactions with most of the organometallics, where in some cases, only the 3,4-isomer was obtained.[5] On the basis of steric arguments, formation of the 3,4-adduct in preference to the 3,6-adduct would be expected because the 6-position has two *ortho* substituents, and N-1 and at C-5, whereas the 4-position has one *ortho* substituent, at C-5, and a lone pair of electrons on the vicinal nitrogen atom.

Thiol addition to the formyl group in the benzaldehyde derivative (**1p**) was not seen under the experimental conditions used. The same selectivity for the pyrimidine ring has also been found in the adduct formation between bisulfites and pyrimidinones containing acyl functions.[3]

Experimental

Preparation of the adducts **2** *and* **3**. The 2(1*H*)-pyrimidinone (2 mmol) was suspended in degassed water–dioxane 1:1 under nitrogen, and sodium 2-mercaptoethanesulfonate was added gradually with stirring until a clear solution resulted. The product was isolated by freeze–drying. The

ble 2. ^{1}H NMR spectra (acetone-d_6–D_2O 1:1) for sodium 2-(2-oxo-1,2,3,4-tetrahydropyrimidin-4-yl)thioethanesulfonates (**2**) and sodium 2-(2-oxo-?,3,6-tetrahydropyrimidin-6-yl)thioethanesulfonates (**3**).

mp. X	R^1	R^2	Comp.	δ	J/Hz	H-4	H-6	H-5	R
			2						
H	H	H	**3**						
			2	brs	4.54	d, 5.37, *J* 4.02	d, 6.24, *J* 7.37	brt, 4.79*	s, 7.22
H	CH_2Ph	H	**3**	AB	4.23/5.04, *J* 15.33	d, 6.37, *J* 7.81	brs, 5.11	brt, 4.98*	s, 7.22
			2			d, 5.67, *J* 4.69	d, 6.53, *J* 7.81	dd, 5.14	7.3–7.6
H	Ph	H	**3**			d, 6.53, *J* 7.81	d, 5.54, *J* 4.63	dd, 5.22	7.3–7.6
			2			s, 5.44	s, 6.64		
Cl	H	H	**3**						
			2			s, 5.41	s, 6.66		
Br	H	H	**3**						
			2			s,5.44	s, 6.64		
I	H	H	**3**						
			2						
Cl	H	CO_2Me	**3**				s, 5.44		3.88 (OMe)
			2	AB	4.40/5.19, *J* 13.96	d, 5.64, J_{HF} 6.34	d, 6.50, J_{HF} 6.34		7.3–7.4
F	CH_2Ph	H	**3**	AB	3.50/4.61, *J* 15.41	d, 6.62, J_{HF} 5.61	d, 5.21, J_{HF} 4.40		7.3–7.4
			2	s	4.68	s, 5.43	s, 6.58		7.3–7.4
Cl	CH_2Ph	H	**3**	AB	4.33/5.26, *J* 15.14	s, 6.71	s, 5.01		7.3–7.4
			2	s	4.68	s, 5.43	s, 6.63		7.3–7.5
Br	CH_2Ph	H	**3**	AB	4.30/5.25, *J* 15.20	s, 6.77	s, 5.01		7.3–7.5
			2	d‡	4.66	s, 5.35	s, 6.65		7.3–7.4/7.5–7.6
I	CH_2Ph	H	**3**	AB	4.28/5.24, *J* 15.26	s, 6.80	s, 4.95		7.3–7.4/7.5–7.6
			2	AB	5.00/5.02, *J* 10.62	s, 5.32	s, 6.55		s, 7.36, 4.56 (s, OCH_2Ph)
Cl	CH_2OCH_2Ph	H	**3**	AB	4.90/5.46, *J* 10.56	s, 6.72	s, 5.28		s, 7.37, 4.58 (s, OCH_2Ph)
			2	AB	4.94/4.98, *J* 10.84	s, 5.35	s, 6.56		s, 7.33, 4.52 (s, OCH_2Ph)
Br	CH_2OCH_2Ph	H	**3**	AB	4.86/5.42, *J* 10.74	s, 6.73	s, 5.25		s, 7.33, 4.58 (s, OCH_2Ph)
			2	AB	4.91/5.20, *J* 18.44	s, 5.46	s, 6.65		7.6–7.7/8.0–8.1
Cl	CH_2COPh	H	**3**	AB	4.91/5.26, *J* 18.31	s, 6.73	s, 5.36		7.6–7.7/8.0–8.1
			2	AB	4.87/5.15, *J* 18.77	s, 5.44	s, 6.66		7.5–7.7/7.9–8.0
Br	CH_2COPh	H	**3**	AB	4.86/5.22, *J* 18.44	s, 6.75	s, 5.33		7.5–7.7/7.9–8.0
			2	AB	5.55/5.67, *J* 9.95	s, 5.51	s, 6.68		7.25/7.95, 9.87 (*CHO*)
Cl	$CH_2OC_6H_4CHO$-*p*	H	**3**	AB	5.54/6.05, *J* 10.38	s, 6.90	s, 5.41		7.25/7.95, 9.87 (*CHO*)
			2	AB	4.91/4.97, *J* 14.16	s, 5.32	s, 6.40		d, 7.36/7.52
Cl	$CH_2SC_6H_4Cl$-*p*	H	**3**	AB	4.58/5.61, *J* 14.14	s, 6.59	s, 5.42		d, 7.33/7.45

he assignments of these resonances are tentative and may be interchanged. However, they are in accord with the corresponding values for (**2c**) d (**3c**). ‡Inner resonances of AB spin system, outer resonances not seen.

off-white product, which consisted of the adduct isomers **2** and **3** could be stored even in the presence of excess sodium 2-mercaptoethanesulfonate, for months in the refrigerator. It dissolved to a clear solution in oxygen-free water, and was used in biological studies without any further purification.[4]

^{1}H NMR studies: The ^{1}H NMR data were recorded on a Varian XL 300 spectrometer tuned to 299.92 MHz. The 2(1*H*)-pyrimidinone (0.1 mmol) was added to degassed (ultrasound) acetone-d_6–D_2O (1:1 v:v; 1.5 ml) followed by the addition of sodium 2-mercaptoethanesulfonate (0.2 mmol). The solid dissolved after the mixture had been shaken for *ca.* 2 min, and the solution was then transferred to an NMR tube under nitrogen by means of a gas-tight syringe. The ^{1}H NMR signals were recorded after 3 min at 20 °C, total time after mixing: 5 min. After this period there was no change in the spectra. The reference was the water signal at 4.61 ppm. The signals from the methylene protons of the mercaptoethyl groups appear as multiplets (2.7–3.2 ppm).

The pyrimidonones were available from other work and their ^{1}H NMR spectra have been recorded: **1b**, **1j** and **1k**,[5a] **1c**,[11] **1n** and **1o**,[12] **1d**, **1e** and **1i**,[13] **1f**,[14] **1g**[7a] **1h**,[6b] **1l** and **1m**,[5b] and **1p** and **1q**.[3]

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