Formation of oxalo-substituted etheno derivatives in reactions of mucochloric acid with adenosine, guanosine and cytidine

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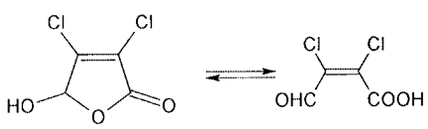
Treatment of the nucleosides adenosine, guanosine and cytidine with mucochloric acid in slightly acid aqueous solutions at 37 °C affords oxalo-substituted etheno derivatives of nucleosides (**2a**, **3a** and **4a**). The derivatives are structurally characterised by UV, ^{1}H and ^{13}C NMR spectroscopy and mass spectrometry. In addition, all the long-range ^{1}H–^{13}C couplings are assigned in the base moieties of the products. A plausible mechanism is presented for the formation of the products.

Introduction

3,4-Dichloro-5-hydroxyfuran-2(5*H*)-one (mucochloric acid, **1**) is easily prepared synthetically either by heating furfural with manganese dioxide and hydrochloric acid [1,2] or by chlorination of butyne-1,4-diol.[3] The compound exists primarily in the ring form at acidic pH conditions and in the open-chain form at neutral conditions (Scheme 1). The compound has been used for the preparation of various pyridazone derivatives.[3,4]



1, ring form **1**, open form

Scheme 1 Tautomeric forms of mucochloric acid.

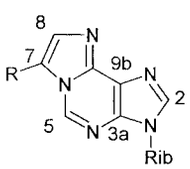
Our interest in **1** originates from a previous work, carried out in our laboratory, where it was reported that **1** is formed by chlorine disinfection of drinking water.[5] The compound has been shown to induce genotoxicity in various biological test systems.[5–9] Generally, it is considered that genotoxicity is attributable to the modification of base units in DNA caused by the genotoxic agent.[10] Therefore, we have studied the reactions of **1** with nucleosides and looked for modified nucleosides. These studies have resulted in the identification of several nucleoside derivatives that are formed at near physiological pH conditions (pH 7–7.5).[11–14]

In this study, we have allowed **1** to react with adenosine, guanosine and cytidine at pH 4.6 and observed the formation of previously unknown nucleoside derivatives in substantial yields. The present paper deals with the structural identification of the derivatives and presents a plausible mechanism for their formation. All the C–H long-range coupling constants have been determined by selective decoupling experiments. The constants have been of critical importance in our previous work on the structural elucidation of nucleoside derivatives.[11–14]

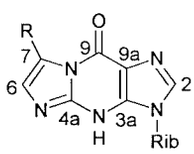
Results and discussion

Chromatographic characteristics

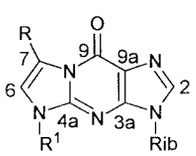
HPLC analyses on a reversed-phase C18 column showed that the unknown derivatives eluted before the corresponding parent nucleosides or any of the previously identified adducts. Secondly, the retention times of the adducts were found to be longer in acidified eluent mixtures than in neutral eluents. These observations implied that the new adducts were polar in nature and contained acid functionalities.



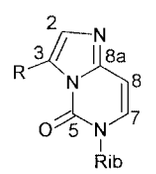
2a: R = -COCOOH
2b: R = -CHO



3a: R = -COCOOH



3b: R = -COCOOCH$_3$, R^1 = -CH$_3$
3c: R = R^1 = -H



4a: R = -COCOOH
4b: R = -CHO

Rib = β-D-ribofuranosyl

Structural characterisation of the adenosine derivative 2a

The UV spectrum of **2a** was very similar to the spectrum of the previously identified formyl etheno derivative of adenosine, **2b**. Compound **2a** exhibited absorption maxima at 226 and 338 nm, a minimum was found at 272 nm, and a shoulder at 294 nm, whereas **2b** gave absorption maxima at 228, 325 and 335 nm, a minimum at 271 nm, and a shoulder at 295 nm.[12] Thus the UV spectra indicated structural similarities between **2a** and **2b**.

In the ^{1}H NMR spectrum of **2a** (Table 1), three singlet resonance signals from the protons in the base unit were observed. The signal at δ_H 8.85 ppm was assigned to H-2 on the basis of the observed long-range H–H correlation with the distinct doublet signal of H-1′ in the ribosyl unit (δ_H 6.16 ppm). The proton signal at δ_H 10.09 ppm was assigned to H-5. The signal showed a long-range H–H correlation with the signal of H-2. The large downfield shift of H-5 can be explained by the electron deficiency at the 5-position. The resonance signal for

Table 1 ^{1}H and ^{13}C chemical shifts (δ) and spin–spin coupling constants ($J_{H,H}$ and $J_{C,H}$) of protons and carbons in **2a**

Proton[a]	δ (ppm)	$J_{H,H}$/Hz[b]	Carbon[c]	δ (ppm)	Multiplicity	$^{1}J_{C,H}$/Hz	$^{>1}J_{C,H}$/Hz
H-2	8.85 (1H)		C-2	142.3	dd	215.7	4.1
			C-3a	141.8	dddd		12.8, 5.5, 2.6, 0.5
H-5	10.09 (1H)		C-5	137.0	dd	221.7	0.5
			C-7	121.7	d		16.0
H-8	8.78 (1H)		C-8	147.9	d	195.8	
			C-9a	145.1	ddd		13.2, 4.9, 1.0
			C-9b	122.9	dd		11.9, 1.0
			*C*O	174.6	s		
COO*H*	12.80	br[d]	*C*OOH	163.3	s		
H-1′	6.16 (1H)	5.2 (H1′–H2′)	C-1′	88.0	d	166.6	
H-2′	4.64 (1H)	5.0 (H2′–H3′)	C-2′	74.4	d	147.2	
H-3′	4.27 (1H)	3.9 (H3′–H4′)	C-3′	70.1	d	149.5	
H-4′	4.06 (1H)	3.8 (H4′–H5′)	C-4′	85.6	d	148.7	
H^{a}-5′	3.77 (1H)	3.9 (H4′–H^{b}5′)	C-5′	61.0	td	140.0	3.2
H^{b}-5′	3.66 (1H)	−12.0 (H^{a}5′–H^{b}5′)					
O*H*[e]	5.59 (1H)						
O*H*[e]	5.27 (1H)						
O*H*[e]	5.09 (1H)						

[a] H-1′–H^{b}-5′ Protons in the ribosyl unit; the assignment of signals of the sugar moiety was based on the connectivities in the H–H COSY spectra. [b] Coupling constants were calculated with the PERCH program[18] and using the linewidth of 1.99 Hz. [c] C-1′–C-5′ Carbons in the ribosyl unit. [d] Broad. [e] Signals for the hydroxy protons in the ribosyl unit; no effort was made to assign the signals to specific hydroxy groups.

Table 2 The observed ^{1}H–^{13}C correlations of compounds **2a**, **3a** and **4a**. The recording conditions were optimised for a coupling of 8 Hz

Compound **2a**

	C-2	C-3a	C-5	C-8	C-9a	C-9b
H-2	CH	CNCH	CNCNCH			CNCH
H-5		CNCH	CH		CNCH	
H-8				CH	CNCH	
H-1′	CNCH					

Compound **3a**

	C-2	C-3a	C-4a	C-6	C-7	C-9a
H-2	CH	CNCH				CNCH
H-6			CNCH	CH	CCH	
H-1′	CNCH	CNCH				

Compound **4a**

	C-2	C-3	C-7	C-8	C-8a
H-2	CH	CCH			
H-7			CH		CCCH
H-8				CH	
H-1′			CNCH		

the corresponding proton in unmodified adenosine (labelled as H-2 in adenosine) is observed at δ_H 8.13 ppm, whereas the H-5 in ethenoadenosine is observed at δ_H 9.30 ppm.[11] In compound **2b**, where the electronegative formyl substituent at C-7 makes the 5-position even more electron deficient, the H-5 signal appears at δ_H 9.96 ppm.[12] Similar downfield shifts have also been observed for other ethenoadenosine derivatives that have a formyl or an oxalo group bound at C-7.[13] The electron deficiency of the 5-position is also evident from the observation that ethenoadenosine undergoes ring-opening at C-5 in alkaline solution and produces bi-imidazole due to a loss of C-5 from the original ethenoadenosine moiety.[15] The signal at δ_H 8.78 ppm was assigned to H-8 in the etheno bridge. The signal has a small downfield shift compared with the value observed for **2b** (δ_H 8.62 ppm).[12]

In the ^{13}C spectrum nine signals from the base and five signals from the ribosyl unit were observed (Table 1). The carbons bonded to a hydrogen were assigned from the one-bond C–H correlation spectra and the fully proton-coupled ^{13}C spectrum. The large coupling constants ($^{1}J_{C,H} \approx 200$ Hz) observed for C-2 (δ_C 142.3 ppm), C-5 (δ_C 137.0 ppm), and C-8 (δ_C 147.9 ppm) are in accord with the values usually reported for unsaturated methine carbons.[16] Further, C–H correlations were observed between C-2 and H-1′ of the ribosyl unit and between C-5 and H-2 (Table 2). The two unsplit carbon signals observed at the lowest field, δ_C 174.6 and 163.3 ppm, were assigned to the carbonyl and carboxy carbons of the oxalo group, respectively. The assignment of the other carbon signals was made on the basis of the ^{13}C chemical shift, the observed long-range C–H correlations, and the multiple bond couplings ($^{>1}J_{C,H}$). The signal at δ_C 141.8 ppm showed correlation to H-5 and H-2 and was assigned to C-3a. In the proton-coupled carbon spectrum, this signal was split into four doublets due to coupling to H-5, H-2, H-1′ and to H-8. Selective decoupling of the protons was used in order to specify each C–H coupling. The magnitude of the couplings is given in Fig. 1. The signal at δ_C 145.1 ppm was assigned to C-9a due to a correlation to H-8 and a weaker correlation to H-5. The C-9a signal was split into three doublets due to coupling to all three protons in the base unit (Fig. 1). The signal at δ_C 122.9 ppm showed a long-range correlation to H-2 and was assigned to C-9b. This resonance signal was split into two doublets and the stronger coupling (J 11.9 Hz) was found to be due to coupling to H-2. The weaker coupling (J 1.0 Hz) could not be assigned although all the protons in the base unit and H-1′ in the sugar unit were selectively decoupled. The last non-ribosyl carbon signal was observed at δ_C 121.7 ppm and the signal was assigned to C-7. The signal is shifted upfield by about 3 ppm compared with the value for **2b**.[12] The carbons C-3a, C-9a, and C-9b exhibited $^{3}J_{C,H}$ values of 12.8, 13.2 and 11.9 Hz, respectively. These strong couplings are *trans*-vicinal couplings in unsaturated CNCH fragments (Fig. 1).[16] Also C–H correlations were observed between the carbon and the proton in the same CNCH fragments (Table 2). The splitting of the C-7 signal into a doublet is due to a geminal coupling to H-8. The high value of this coupling (J 16.0 Hz) may be explained by the electronegativity of the nitrogens and the carbonyl group.[16]

In the positive-mode ESI mass spectra, the protonated molecular ion was the most abundant ion and it was observed at m/z 364. Also observed were ion peaks at m/z 320 and 232

Table 3 ^{1}H and ^{13}C Chemical shifts (δ) and spin–spin coupling constants ($J_{H,H}$ and $J_{C,H}$) of protons and carbons in **3a**

Proton[a]	δ (ppm)	$J_{H,H}$/Hz[b]	Carbon[c]	δ (ppm)	Multiplicity	$^{1}J_{C,H}$/Hz	$^{>1}J_{C,H}$/Hz
H-2	8.13 (1H)		C-2	137.9	dd	213.2	3.7
			C-3a	149.8	dd		4.8, 3.0
H-4	not obs.[d]						
			C-4a	149.1	d		10.6
H-6	8.04 (1H)		C-6	131.3	d	193.7	
			C-7	121.6	d		10.3
			C-9	153.1	s		
			C-9a	116.8	d		11.4
			*C*O	180.8	s		
COO*H*	12.45	br[e]	*C*OOH	165.7	s		
H-1′	5.83 (1H)	5.8 (H1′–H2′)	C-1′	87.5	d	164.5	
H-2′	4.53 (1H)	5.0 (H2′–H3′)	C-2′	73.8	d	148.2	
H-3′	4.15 (1H)	3.4 (H3′–H4′)	C-3′	70.6	d	149.5	
H-4′	3.94 (1H)	3.7 (H4′–H5′)	C-4′	85.7	d	148.2	
H^{a}-5′	3.66 (1H)	3.7 (H4′–H^{b}5′)	C-5′	61.7	t	140.2	
H^{b}-5′	3.56 (1H)	−12.0 (H^{a}5′–H^{b}5′)					
O*H*[f]	5.40 (1H)						
O*H*[f]	5.09 (2H)						

[a] H-1′–H^{b}-5′ Protons in the ribosyl unit; the assignment of signals of the sugar moiety was based on the connectivities in the H–H COSY spectra. [b] Coupling constants were calculated with the PERCH program[18] and using the linewidth of 1.69 Hz. [c] C-1′–C-5′ Carbons in the ribosyl unit. [d] Not observed. [e] Broad. [f] Signals for the hydroxy protons in the ribosyl unit; no effort was made to assign the signals to specific hydroxy groups.

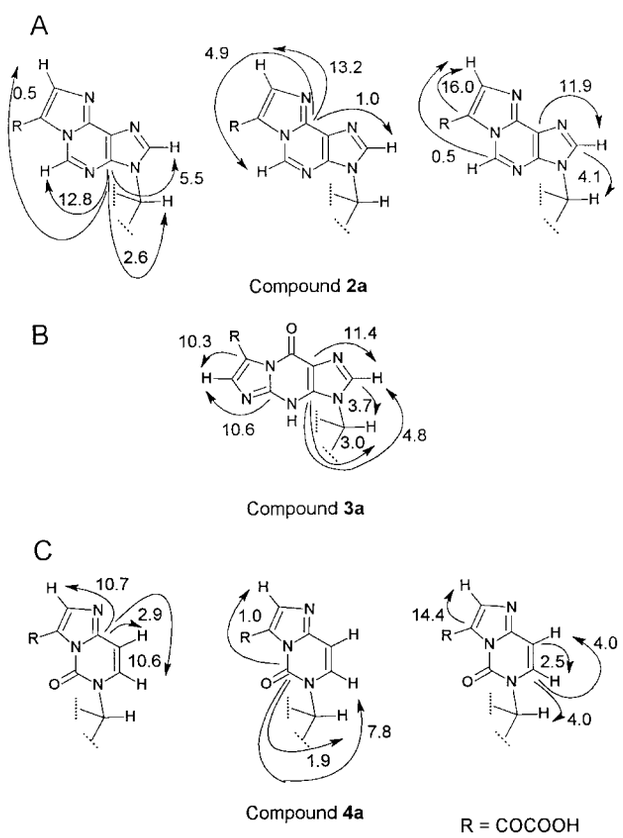


Fig. 1 Long-range ^{1}H–^{13}C couplings in **2a**, **3a** and **4a**. The couplings were assigned on the basis of selective decoupling of the protons.

corresponding to the loss of carbon dioxide and the ribosyl unit (followed by protonation at N-3) from the protonated molecule, respectively. The accurate mass measurement for the protonated product (*m*/*z* 364.0899) agreed with the calculated value (*m*/*z* 364.0893). The NMR and UV spectroscopic and the mass spectrometric data are consistent with the structure of **2a**.

Structural characterisation of the guanosine derivative 3a

The UV spectrum of **3a** exhibited absorption maxima at 224 (strong), 290 and 328 nm. Absorption minima were observed at 274 and 306 nm.

In the ^{1}H NMR spectrum of **3a** (Table 3), two low-field, unsplit proton signals attributable to the base unit were observed at δ_H 8.13 ppm and δ_H 8.04 ppm. The former signal was assigned to H-2 on the basis of the observed H–H correlation to the H-1′ signal of the ribose unit at δ_H 5.83 ppm. Consequently, the latter signal was assigned to H-6.

The assignment of the signals in the ^{13}C spectrum was based on the chemical shifts, C–H correlations and one and multiple-bond couplings. Nine signals were found to arise from the modified base unit (Table 3). The methine carbons at δ_C 137.9 ppm and δ_C 131.3 ppm were assigned to C-2 and C-6, respectively. Further, the signal of C-2 displayed a weak coupling (*J* 3.7 Hz) to H-1′ (Fig. 1). The carbonyl and carboxy carbons of the oxalo group were observed at δ_C 180.8 and 165.7 ppm, respectively. The signal at δ_C 149.8 ppm was split into two small doublets (*J* 4.8 and 3.0 Hz) due to coupling with H-2 and H-1′ and further displayed a correlation to H-2 (Table 2). Thus, the signal was assigned to C-3a. The signal at δ 116.8 ppm was assigned to C-9a on the basis of the chemical shift, the three-bond correlation to H-2 and the large *trans*-vicinal coupling (*J* 11.4 Hz) to H-2 transmitted through the unsaturated CNCH fragment.[16] The two carbon signals at δ_C 121.6 ppm and δ_C 149.1 ppm displayed correlation with H-6 and these signals were attributed to C-7 and C-4a, respectively. Both signals were split into doublets (*J* 10.3 Hz for C-7 and *J* 10.6 Hz for C-4a) due to the coupling to H-6. The large coupling constant observed for C-4a implies that the exchangeable N–H proton must be bound to N-4 and not to N-5. Finally, the resonance signal at δ_C 153.1 ppm was assigned to C-9 based on the chemical shift.

In the ESI mass spectrum of **3a**, the protonated molecular ion was observed at *m*/*z* 380. Fragment ions at *m*/*z* 308 and 248 corresponded to a loss of C_2O_3-fragment and the ribosyl unit (followed by protonation at N-3) from the protonated molecular ion, respectively. The elemental composition was verified by accurate mass measurement. The observed protonated molecular ion (*m*/*z* 380.0844) is in agreement with the calculated value (*m*/*z* 380.0842).

Although the NMR and mass spectral data are fully consistent with the structure **3a**, the position of the oxalo group is still arguable. In order to unequivocally determine the position, compound **3b** was prepared by methylation of **3a** with methyl iodide. In **3b**, the correlation found between H-6 and the N-5 methyl protons in the 2D NOESY spectrum showed that the oxalo group must be bound at C-7 and not at C-6. The

Table 4 ^{1}H and ^{13}C Chemical shifts (δ) and spin–spin coupling constants ($J_{H,H}$ and $J_{C,H}$) of protons and carbons in **4a**

Proton[a]	δ (ppm)	$J_{H,H}$/Hz[b]	Carbon[c]	δ (ppm)	Multiplicity	$^{1}J_{C,H}$/Hz	$^{>1}J_{C,H}$/Hz
H-2	8.10 (1H)		C-2	141.7	d	192.4	
			C-3	125.5	d		14.4
			C-5	145.9	ddd		7.8, 1.9, 1.0
H-7	8.07 (1H)	7.8 (H7–H8)	C-7	131.9	ddd	187.1	4.0, 4.0
H-8	6.91 (1H)	7.8 (H8–H7)	C-8	98.3	dd	178.0	2.5
			C-8a	148.9	ddd		10.7, 10.6, 2.9
			*C*O	178.7	s		
COO*H*	not obs.[d]		*C*OOH	163.8	s		
H-1′	5.97 (1H)	4.4 (H1′–H2′)	C-1′	89.5	d	170.5	
H-2′	4.14 (1H)	5.4 (H2′–H3′)	C-2′	74.4	d	149.6	
H-3′	4.06 (1H)	4.3 (H3′–H4′)	C-3′	69.4	td	148.8	4.8
H-4′	3.97 (1H)	2.3 (H4′–H^{a}5′)	C-4′	85.1	d	148.0	
H^{a}-5′	3.73 (1H)	3.1 (H4′–H^{b}5′)	C-5′	60.3	t	141.0	
H^{b}-5′[a]	3.64 (1H)	−12.2 (H^{a}5′–H^{b}5′)					
O*H*[e]	5.69 (1H)						
O*H*[e]	5.31 (2H)						

[a] H-1′–H^{b}5′ Protons in the ribosyl unit; the assignment of signals of the sugar moiety was based on the connectivities in the H–H COSY spectra. [b] Coupling constants were calculated with the PERCH program[18] and using the linewidth of 1.47 Hz. [c] C-1′–C-5′ Carbons in the ribosyl unit. [d] Not observed. [e] Signals for the hydroxy protons in the ribosyl unit; no effort was made to assign the signals to specific hydroxy groups.

assignment of the structure to **3b** was further supported by the observed long-range correlations.

The NMR and UV spectroscopic and mass spectrometric data are consistent with the structure of **3a**.

Structural characterisation of the cytidine derivative 4a

The cytidine derivative **4a** exhibited absorption maxima at 222, 270 and 328 (strong) nm, a shoulder at 280 nm and minima at 255 and 290 nm. The spectrum was very similar to that of the formyl etheno derivative of cytidine **4b**, which exhibited absorption maxima at 220, 273, 284 and 326 nm and minima at 256, 279 and 289 nm[12] and thus indicated similarities in the structures of **4a** and **4b**.

In the ^{1}H NMR spectrum of the product, two doublet and one singlet signals from the base moiety were observed (Table 4). The doublet at δ_H 8.07 ppm (J 7.8 Hz) was assigned to H-7 on the basis of the long-range correlation to H-1′ in the ribosyl unit, which was observed at δ_H 5.97 ppm as a distinct doublet. The H–H COSY spectrum displayed a strong correlation between H-7 and the doublet at δ_H 6.91 ppm (J 7.8 Hz), which was consequently assigned to H-8. The third signal originating from the base moiety was observed at δ_H 8.10 ppm as a singlet and was assigned to H-2 in the etheno bridge. The chemical shift is close to that observed for the corresponding proton in **4b** (δ_H 8.18 ppm).[12]

The ^{13}C spectrum displayed eight signals that originated from the modified base unit (Table 4). The singlet signals representing the carbonyl and carboxy carbons in the oxalo group were observed at the lowest field, δ_C 178.7 and 163.8 ppm, respectively. The methine carbons were easily assigned from the fully proton coupled ^{13}C spectrum and the observed one-bond C–H correlations. Thus the signals at δ_C 141.7, 131.9 and 98.3 ppm could be assigned to C-2, C-7 and C-8, respectively. The assignments of the other signals were based on the chemical shifts and on the long-range C–H correlations and couplings. The signal at δ_C 125.5 ppm displayed a two-bond correlation and a large coupling constant (J 14.4 Hz) to H-2 (Fig. 1). Consequently, the signal was assigned to C-3. The signal at δ_C 145.9 ppm was assigned to C-5 due to the couplings with H-7 (J 7.8 Hz), H-1′ (J 1.9 Hz) and H-2 (J 1.0 Hz). The last signal at δ_C 148.9 ppm was assigned to C-8a. The signal displayed a correlation to H-7 and it was also split into three doublets. The large couplings (J 10.7 and 10.6 Hz) represent *trans*-vicinal couplings to H-2 and H-7, respectively, and the small coupling (J 2.9 Hz) that to H-8.

The ESI mass spectra displayed the protonated molecular ion at m/z 340 and a fragment ion at m/z 208 corresponding to the loss of the ribosyl unit followed by protonation at N-6. The accurate mass measurement for the protonated product (m/z 340.0791) agreed with the calculated value (m/z 340.0781). The NMR and the UV spectroscopic and the mass spectrometric data are consistent with the structure of **4a**.

Product yields

At pH 4.6 and a reaction time of eight days, the yields of **2a**, **3a** and **4a** were 58%, 41% and 31%, respectively. At pH 3.0, the yields were lowered to approximately half of the yields obtained at pH 4.6. At pH 7.0 and 8.5, the yields for **2a** and **3a** were, at maximum, only 3 and 6%, respectively and the product **4a** could not be observed at all under these conditions.

Upon prolonged storage of the products at pH 4.6 and 37 °C, compounds **2a** and **4a** underwent decarboxylation and the formyl etheno derivatives (**2b** and **4b**) were formed, while in the case of compound **3a** the whole oxalo group was lost and ethenoguanosine **3c** was obtained.

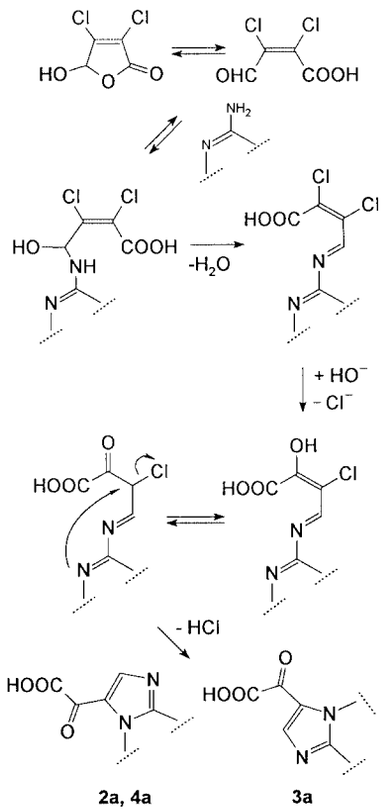
A plausible reaction mechanism

A plausible mechanism for the formation of the oxalo-substituted etheno derivatives is outlined in Scheme 2. Initially, the carbonyl carbon in **1** is attacked by the exocyclic amino group of the nucleosides, yielding a carbinolamine. Loss of water produces an intermediate where the carbon next to the carboxy group is susceptible to attack by a hydroxy group, resulting in displacement of the chlorine. Following tautomerisation to the keto form the remaining chlorine is displaced by an attack from the endocyclic nitrogen in the pyrimidine rings. Finally, loss of a proton will yield the products.

Wasserman and Precopio[17] have shown that in mucochloric acid, the chlorine at the β-carbon is replaced by a hydroxy group and mucoxychloric acid is formed. We have treated mucoxychloric acid with adenosine and found that the yield of **2a** is lower than that obtained from the reaction with **1**. Therefore, we suggest that the chlorine replacement mainly takes place after coupling of **1** to adenosine and the formation of the imine, although some of the product may be produced through the initial formation of mucoxychloric acid.

Experimental

CAUTION. Mucochloric acid **1** has been tested positive in the Ames mutagenicity assay with *Salmonella typhimurium*



Scheme 2 Proposed mechanism for the formation of products **2a**, **3a** and **4a**.

(TA100) without metabolic activation. Therefore, caution should be exercised in the handling and disposal of the compounds described herein.

Chromatographic methods and isolation of products

Analytical HPLC was performed on a Kontron Instruments liquid chromatographic system consisting of a model 322 pump, a 440 diode-array detector (UV), and a KromaSystem 2000 data-handling program (Kontron Instruments S.P.A., Milan, Italy). The reaction mixtures were chromatographed on a 5 μm, 4 mm × 125 mm reversed-phase C18 analytical column (Spherisorb ODS2, Hewlett Packard, Espoo/Esbo, Finland). The column was eluted isocratically for 5 min with water or potassium dihydrogen phosphate (0.01 M or 0.02 M, pH 4.6) and then with gradient of acetonitrile from 0% to 30% over the course of 25 min at a flow rate of 1 ml min^{-1}. The compounds were isolated from the reaction mixtures by column chromatography on a 4 cm × 4 cm column of preparative C18 bonded silica grade (40 μm, Bondesil, Analytichem International, Harbor City, CA, USA). As the mobile phases, water and mixtures of water–acetonitrile were used. Semi-preparative-scale separations were carried out with an HPLC system consisting of Shimadzu LC-9A pumps, a variable-wavelength Shimadzu SPD-6A UV detector and semi-preparative 8 μm, 10 mm × 250 mm reversed-phase C18 column (Hyperprep ODS, Hypersil, Krotek, Tampere/Tammerfors, Finland). As eluent, 2% acetic acid in water was used.

Compounds **2a**, **3a** and **4a** were obtained as powders. The powders were re-dissolved in water–ethanol–diethyl ether (1 : 3 : 3) and upon storage in the refrigerator the compounds precipitated as powders. The powders started to decompose at about 150 °C.

Spectroscopic and spectrometric methods

^1H and ^{13}C NMR spectra were recorded at 30 °C on a JEOL JNM-A500 Fourier transform NMR spectrometer at 500 and 125 MHz, respectively. The samples were dissolved in DMSO-d_6, and tetramethylsilane (TMS) was used as internal standard. The ^1H NMR signal assignments were based on proton chemical shifts and 2D homonuclear H–H correlation (COSY) experiments. For the ribosyl units in **2a**, **3a** and **4a**, the chemical shifts and the coupling constants of the multiplets of the proton signals were calculated using the PERCH program.[18] The linewidths used in the simulation are given in the footnotes of the corresponding Tables. The assignment of the carbon signals was based on carbon chemical shifts, 2D heteronuclear ^1H–^{13}C correlation experiments (HETCOR and COLOC) and fully proton-coupled carbon spectra. The multiple-bond proton–carbon coupling constants were assigned by selective decoupling of the protons in the base moieties and the H-1′ in the ribosyl units. The small proton–carbon couplings observed for **2a** ($^{>1}J_{C,H} < 1.0$ Hz) were extracted from the resolution-enhanced ^{13}C spectra. All the coupling constants are given in Hz. The positive-mode electrospray ionisation mass spectra were recorded on a Fisions ZABSpec-oaTOF spectrometer (Manchester, UK). Ionisation was carried out using nitrogen as both nebulising and bath gas. A potential of 8.0 kV was applied to the ESI needle. The temperature of the pepperpot counter electrode was 90 °C. The samples were introduced by loop injection at a flow rate of 20 μl min^{-1} (H_2O–CH_3CN–AcOH 80 : 20 : 1). PEG 200 was used as standard for the exact mass determinations. The mass spectrometer had a resolution of 7000. UV spectra were recorded with a Shimadzu UV-160 A spectrophotometer (Shimadzu Europe, Germany).

7-Oxalo-3-(β-D-ribofuranosyl)-3*H*-imidazo[2,1-*i*]purine 2a

Adenosine (0.2104 g, 0.787 mmol) was treated with mucochloric acid **1** (0.5529 g, 3.31 mmol) in 100 ml of 0.5 M phosphate buffer (pH 4.6) at 37 °C for eight days. After the reaction was complete, the mixture was extracted with diethyl ether (3 × 50 ml) to remove the unchanged **1**. The volume of the aqueous phase was reduced to ≈50 ml by rotatory evaporation. The mixture was filtered, and fractionated on a preparative C18 column. The column was first eluted with 100 ml of water and then with 100 ml batches of 2, 5 and 15% acetonitrile in water. Fractions of 30 ml were collected. The fractions containing the title compound **2a** (0–5% acetonitrile) were combined and the volume was reduced by rotatory evaporation to about 30 ml. To remove the phosphate buffer and the remaining impurities, the concentrated mixture was purified twice more as described above. The desalted solution was evaporated to dryness and dried in a vacuum desiccator over diphosphorus pentoxide to give **2a** (0.1780 g, 62%) as a yellow powder, $\lambda_{max}(H_2O)$/nm 226 (ε/dm^3 mol^{-1} cm^{-1} 2.5×10^4) and 338 (1.8×10^4), λ_{min}/nm 272, 294sh; δ_H(500 MHz; DMSO-d_6; TMS) and δ_C(125 MHz; DMSO-d_6; TMS), see Table 1; *m*/*z* (ESI) 364 (100%, MH^+), 320 (36, $MH^+ - CO_2$), 232 (77, MH^+ − ribosyl + H), 133 (45, ribosyl) [HRMS: Calc. for ($C_{14}H_{13}N_5O_7$ + H): *m*/*z*, 364.0893. Found: *m*/*z*, 364.0899].

4,9-Dihydro-7-oxalo-9-oxo-3-(β-D-ribofuranosyl)-3*H*-imidazo-[1,2-*a*]purine 3a

Guanosine (0.2004 g, 0.708 mmol) was treated with **1** (0.4727 g, 2.80 mmol) as described above for **2a**. The mixture was extracted, concentrated and passed through a preparative column. Fractions containing the title compound **3a** (0–2% acetonitrile) were combined and concentrated to a volume of 20 ml. The product was desalted by passing it twice through the preparative column, eluting first with 50 ml of water (containing 2% acetic acid) and then with 50 ml of 10% acetonitrile in water. The solution containing the product was evaporated to dryness and dried in a vacuum desiccator over diphosphorus pentoxide to give **3a** (0.1212 g, 45%) as a yellow powder, $\lambda_{max}(H_2O)$/nm 224 (ε/dm^3 mol^{-1} cm^{-1} 1.6×10^4), 290 and 328; λ_{min}/nm 274 and 306 with 246sh; δ_H(500 MHz; DMSO-d_6;

TMS) and δ_C(125 MHz; DMSO-d_6; TMS), see Table 3; *m*/*z* (ESI) 380 (57%, MH^+), 308 (39, $MH^+ - C_2O_3$), 248 (100, MH^+ − ribosyl + H) [HRMS: Calc. for ($C_{14}H_{13}N_5O_8$ + H): *m*/*z*, 380.0842. Found: *m*/*z*, 380.0844].

5,9-Dihydro-7-methoxalyl-5-methyl-9-oxo-3-(β-D-ribofuranosyl)-3*H*-imidazo[1,2-*a*]purine 3b

Compound **3a** (0.1118 g, 0.295 mmol) was dissolved in 80 ml of dimethylformamide. Triethylamine (0.0935 g, 0.926 mmol) and methyl iodide (0.1630 g, 1.15 mmol) were added to the solution and the reaction was allowed to proceed for two days at 37 °C. The course of the reaction was followed by HPLC. After the peak for the starting material **3a** had disappeared, the reaction was stopped. The solvent was removed by rotatory evaporation and the residual yellow oil was diluted to 20 ml with water. The mixture was fractionated by column chromatography. The column was eluted with 0, 4, 8 and 15% acetonitrile in water; volume of each batch was 50 ml. The fractions containing the adduct (8% acetonitrile) were evaporated to dryness to give title compound **3b** (0.0710 g, 59%) as a yellow powder, $\lambda_{max}(H_2O)$/nm 226, 269 and 356; λ_{min}/nm 246 and 316, with 282sh; δ_H(500 MHz; DMSO-d_6; TMS) 8.57 (1H, s, H-6), 8.35 (1H, s, H-2), 5.96 (1H, d, *J* 6.1, H-1′), 5.56 (1H, d, *J* 6.7, HO-2′), 5.32 (1H, d, *J* 5.0, HO-3′), 5.07 (1H, t, *J* 6.0, HO-5′), 4.65 (1H, dd, *J* 5.5 and 5.0, H-2′), 4.24 (1H, m, H-3′), 4.02 (1H, dd, *J* 4.0 and 3.5, H-4′), 3.87 (3H, s, $COOCH_3$), 3.81 (3H, s, N5-CH_3), 3.73 (1H, d, *J* 11.6, H^a-5′), 3.65 (1H, d, *J* 11.6, H^b-5′); δ_C(125 MHz; DMSO-d_6; TMS), 175.9 (d, *J* 1.3, *C*O), 161.9 (q, *J* 4.0, *C*OOCH$_3$), 152.5 (dd, *J* 1.2 and 0.6, C-9), 149.6 (dd, *J* 4.9 and 3.1, C-3a), 145.2 (dq, *J* 7.8 and 3.1, C-4a), 138.8 (dd, *J* 214.8 and 4.3, C-2), 130.8 (dq, *J* 201.8 and 3.3, C-6), 118.4 (d, *J* 9.1, C-7), 117.0 (d, *J* 11.1, C-9a), 87.1 (d, *J* 164.7, C-1′), 85.7 (d, *J* 149.2, C-4′), 73.6 (d, *J* 147.4, C-2′), 70.6 (d, *J* 149.5, C-3′), 61.5 (t, *J* 140.6, C-5′), 52.7 (q, *J* 148.7, COO*C*H$_3$), 32.4 (qd, *J* 143.0 and 1.7, N5-*C*H$_3$); *m*/*z* (ESI) 408 (51%, MH^+), 276 (100, MH^+ − ribosyl + H) [HRMS: Calc. for ($C_{16}H_{17}N_5O_8$ + H): *m*/*z*, 408.1155. Found: *m*/*z*, 408.1158].

5,6-Dihydro-3-oxalo-5-oxo-6-(β-D-ribofuranosyl)imidazo[1,2-*c*]-pyrimidine 4a

Cytidine (0.2483 g, 1.02 mmol) was treated with **1** (0.7010 g, 4.15 mmol) for seven days as described for **2a**. After the reaction had gone to completion, the mixture was extracted, concentrated and passed through a preparative column. Fractions containing compound **4a** (0% acetonitrile) were combined and concentrated to a volume of 20 ml. The procedure was repeated twice more to remove most of the impurities. Due to the close retention times of **4a** and cytidine, the product could not be separated in pure form by column chromatography. Therefore, final purification was done by semi-preparative-scale HPLC using 2% acetic acid as eluent. The fraction containing the product was evaporated to dryness and dried in a vacuum desiccator over diphosphorus pentoxide to give title compound **4a** (0.0872 g, 25%) as a pale yellow powder, $\lambda_{max}(H_2O)$/nm 222 (ε/dm^3 mol^{-1} cm^{-1} 3.8×10^3), 270 and 328 (4.9×10^3); λ_{min}/nm 212, 255 and 290, 280sh; δ_H(500 MHz; DMSO-d_6; TMS) and δ_C(125 MHz; DMSO-d_6; TMS), see Table 4; *m*/*z* (ESI) 340 (80%, MH^+), 208 (100, MH^+ − ribosyl + H) [HRMS: Calc. for ($C_{13}H_{13}N_3O_8$ + H): *m*/*z*, 340.0781. Found: *m*/*z*, 340.0791].

Determination of product yields

Standard solutions of the products were prepared for determination of the yields. Exact amounts of nucleosides (≈10 mg) were allowed to react with four equivalents of mucochloric acid at 37 °C in 4 ml of various buffer solutions (pH 3.0, 4.6, 7.0 and 8.5). Aliquots were taken at suitable intervals and analysed by HPLC. The amount of the adducts in the reaction mixtures was determined by comparing the peak areas of the mixtures with those observed for standard solutions. The molar yields were calculated from the original amount of nucleosides in the reaction mixtures.

Decomposition of products

Approximately 1 mg of the product (**2a**, **3a** or **4a**) was dissolved in 2 ml of 0.5 M phosphate buffer solution (pH 4.6). The mixture was stored at 37 °C for several days and the reaction was followed by HPLC. Identification of the decomposition products was done by comparison of retention times and UV spectra with the standard solutions of **2b**, **3c** and **4b**.

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