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MECHANISM OF METHYLATION OF NUCLEOSIDE SUGAR HYDROXYL GROUPS BY DIAZOMETHANE IN THE PRESENCE OF STANNOUS CHLORIDE

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ABSTRACT

Diazomethane rapidly reacts with $SnCl_2$ in methanol to give MeCl and $Sn(OMe)_2$, both of which were isolated and identified. This reaction is the initial step in the apparent $SnCl_2 \cdot 2H_2O$-catalysed methylation of nucleosides by diazomethane. The actual catalyst, $Sn(OMe)_2$, readily reacts with Brönsted acids, with exchange of ligands. For ribofuranosyl nucleosides, Sn^{2+} binds to site(s) having a labile proton, the effect being particularly predominant with 5- and 6-membered cyclic structures, with the tin ion co-ordinated to the ionised hydroxyl groups of the sugar moiety. These cyclic structures account for the high reactivity of the hydroxyl groups towards Me^+, as in the complexes of ribofuranosyl nucleosides with Bu_2SnO. A similar, if not identical, mechanism operates in the case of glucopyranosides.

INTRODUCTION

Under certain conditions, diazomethane selectively methylates HO-2′ and HO-3′ of adenosine and cytidine[1,2]. Nucleosides containing an acidic aglycon, *e.g.*, uridine or inosine, undergo preferential *N*-methylation. However, in the presence of various salts having the characteristics of Lewis acids, *e.g.*, $SnCl_2 \cdot 2H_2O$, diazomethane selectively and quantitatively methylates[3–5] HO-2′ and HO-3′ of ribonucleosides, including those with an acidic aglycon group. It has been suggested[4,6] that this reaction is due to the formation of an intermediate complex between Sn(II) and HO-2′,3′.

It was subsequently shown[7] that $SnCl_2$ is not directly involved in the methyla-

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tion reaction, but reacts very rapidly with diazomethane to give unidentified tin product(s) that exhibited catalytic properties identical with those ascribed to $SnCl_2$. We now report on the nature of the product(s), and the mechanism leading to selective *O'*-monomethylation.

RESULTS AND DISCUSSION

Addition of ethereal diazomethane to methanolic $SnCl_2 \cdot 2H_2O$ led to rapid formation of a white precipitate, with simultaneous, vigorous evolution of a gas[7]. Following such a reaction under conditions analogous to those employed for the methylation of HO-2',3' of nucleosides, elementary analysis of the solid product confirmed the absence of nitrogen and chlorine, and the presence of tin, carbon, hydrogen, and oxygen. On prolonged storage, the product was slowly converted into hydrated SnO_2.

The i.r. spectrum of the gaseous product, freed from traces of water and methanol, was identical with that of chloromethane. Thus, diazomethane, following protonation, reacts with chloride ions to yield MeCl, so that the reaction mixture contains Sn^{2+} and MeO^- formed from methanol following transfer of a proton to CH_2N_2. This reaction mechanism is consistent with the observed, enhanced electrical conductivity of methanolic solutions of diazomethane, interpreted[8] in terms of the ions $CH_3N_2^+$ and MeO^-.

The foregoing data accord with the analysis of the solid product of the reaction. The Mossbauer spectrum of this product indicated two components, since there were quadrupole-splitting (q.s.) doublets, one with a q.s. of 0.92 $\pm$0.06 and an isomer shift (i.s.) of 0.09 $\pm$0.06, and a second with a q.s. of 1.92 $\pm$0.06 and an i.s. of 2.92 $\pm$0.06. These parameters correspond well with those[9] for SnO_2 and $Sn(OMe)_2$ or MeO-Sn-O-Sn-OMe, respectively. The solid product contained no $SnCl_2$.

Tin methoxylate is readily hydrolysed and oxidised in the presence of moisture and atmospheric oxygen, as follows[10,11]:

$$2\ Sn(OMe)_2 + H_2O \rightarrow MeO\text{-}Sn\text{-}O\text{-}Sn\text{-}OMe \xrightarrow{O_2/H_2O} SnO_2 \cdot nH_2O.$$

Similarly, tin methoxylate, resulting from the reaction of $SnCl_2 \cdot 2H_2O$ with diazomethane, may be partially hydrolysed in a reaction

$$2\ MeOH + SnCl_2 + 2\ CH_2N_2 \rightarrow \underset{\substack{H_2O\downarrow \\ SnO_2 \cdot nH_2O}}{Sn(OMe)_2} + 2\ MeCl + 2\ N_2,$$

which resembles that[10] utilised for the synthesis of $Sn(OMe)_2$, *viz.*,

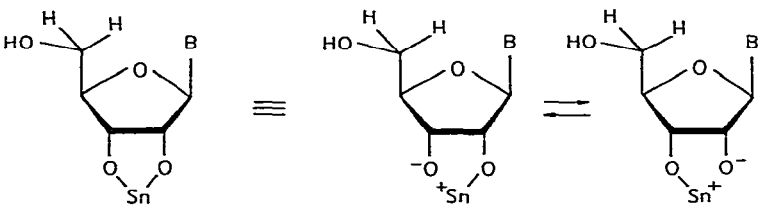
$$SnCl_2 + 2\ MeOH + 2\ NEt_3 \rightarrow Sn(OMe)_2 + 2\ Et_3NH^+\ Cl^-.$$

The difference between these two reactions is simply the proton acceptor.

The products of the reaction between $SnCl_2$ and diazomethane in non-polar solvents are different from those reported here[12].

Using pure SnO_2 and $Sn(OMe)_2$, it could be shown that hydrated SnO_2 had no influence on the methylation reaction, whereas $Sn(OMe)_2$ reacted in a manner identical with that of $SnCl_2 \cdot 2H_2O$, with the exception that the former did not lead to liberation of gaseous products on addition of diazomethane. It follows that diazomethane is initially utilised, in the presence of $SnCl_2 \cdot 2H_2O$, for the formation of $Sn(OMe)_2$.

Stannous methoxide reacts with Brönsted acids to liberate methanol[13,14]. This reaction may lead to bonding of Sn to oxygen, nitrogen, or sulphur by ligand exchange. It is reasonable to expect $Sn(OMe)_2$ to form a cyclic complex with the *cis*-2′,3′-diol group of ribonucleosides (**1–3**); such a cyclic structure is more stable thermodynamically than acyclic, alkoxytin derivatives. This, in turn, leads to the ionisation of HO-2′ and HO-3′, with consequent high reactivity towards the methyl carbocation.



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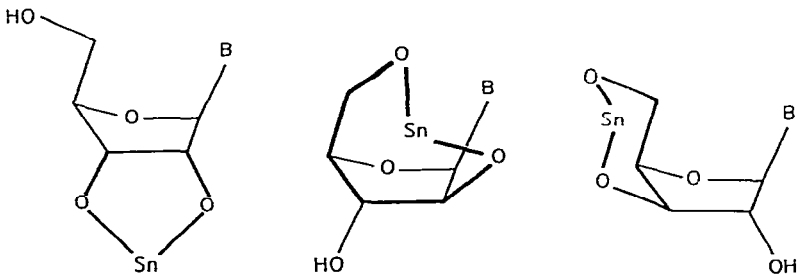
The foregoing interpretation is analogous to that involved in the synthesis and reactivity of 2′,3′-*O*-(dibutylstannylene)cytidine, prepared from Bu_2SnO and cytidine in methanol[15]. It has been shown[16] that Bu_2SnO is converted in methanolic medium into $Bu_2Sn(OMe)_2$, which probably reacts with cytidine. On treatment of the complex of $Bu_2Sn(OMe_2)$ and cytidine with ethereal diazomethane in methanol, the reaction products were, as expected, quantitatively 2′- and 3′-*O*-methylcytidines.

The similarity in catalytic properties[5] of $Sn(OMe)_2$, $Bu_2Sn(OMe)_2$, and $SnCl_4$, suggests a common pathway for the activation of HO-2′,3′ of ribofuranose *via* a 2-stanna-1,3-dioxolane structure (**4**), independently of the valency state (II or IV) of the Sn atom. Formation of such a structure, which is known to be thermodynami-



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cally more stable than acyclic, alkoxytin derivatives[17], adequately explains the Sn-catalysed selectivity and quantitative *O'*-methylation by diazomethane.

Under similar conditions, 1-β-D-arabinofuranosylcytosine and 9-β-D-arabinofuranosyladenine were resistant to methylation, thus excluding the formation of an alternative, cyclic structure (**5**) with tin, involving HO-2',5'. However, this nucleoside can adopt a conformation in which HO-2',5' are hydrogen-bonded to form a seven-membered ring[18]. The 2',5'-cyclic phosphate of this nucleoside is known[19].

A 2-stanna-1,3-dioxolane is not necessarily the only structure that may account for selective methylation with diazomethane. Another possibility is a 2-stanna-1,3-dioxane (**6**), which may be formed theoretically with xylofuranosides, lyxofuranosides, and also 1,2-*O*-isopropylidene-α-D-glucofuranose[20].

Treatment of 9-β-D-xylofuranosyladenine (XylA) with diazomethane in methanol in the absence of $SnCl_2$ gave, surprisingly, appreciable proportions of 5'-*O*-methylXylA and traces of 3'-*O*-methylXylA, but the reaction was sluggish and proceeded to only 40%. On addition of $SnCl_2$, the reaction proceeded more rapidly and was quantitative, the products being 5'-*O*-methylXylA (80%), 3'-*O*-methylXylA (15%), and traces of 2',3'(5')-di-*O*-methylXylA.

With 1-β-D-lyxofuranosylcytosine, the products of alkylation with diazomethane in the presence of $SnCl_2$ were, as anticipated, principally the 2'-*O*-methyl (36%) and 3'-*O*-methyl (36%) derivatives, accompanied by the 2',5'- (22%) and 3',5'-di-*O*-methyl (6%) derivatives.

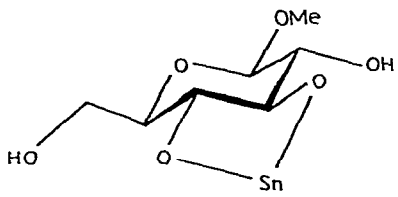
Methylation of 1,2-*O*-isopropylidene-α-D-glucofuranose with diazomethane in the presence of $SnCl_2 \cdot 2H_2O$ gave[20] the 5-*O*-methyl and 3,5-di-*O*-methyl derivatives. As for XylA, a 2-stanna-1,3-dioxane structure involving HO-3,5 is formed, but, in contrast to XylA, the 3-*O*-methyl derivative may further react with diazomethane, *via* a complex involving HO-5,6, to yield the 3,5-di-*O*-methyl derivative. Thus, it is HO-5, and not the primary hydroxyl group, which is preferentially substituted. This result is most likely due to a preference for the 2-stanna-1,3-dioxolane structure, because of entropy considerations and, perhaps, the greater acidity of HO-5 as compared to that of HO-6. By contrast, in the 2-stanna-1,3-dioxane structure, steric factors may be more important and favour attack of HO-5 (or HO-5' in XylA) by the methylating agent. Such steric effects are particularly pronounced in the methylation of α- and β-D-glucopyranosides[21] and β-D-galactopyranosides[22].

The cyclic complexes between sugar hydroxyl groups and Sn are more stable than acyclic complexes, and this explains the initial, exclusive methylation of the sugar hydroxyl groups in such nucleosides as uridine or formycin, the aglycons of which possess acidic protons. Tin can form complexes with phenolic groups and with such bases as imidazole and pyrazole, as well as with hydroxyl groups[21]. However, the ability to activate exclusively the hydroxyl groups involved in the complex during the methylation reaction points to the predominant role of the cyclic structure.

The above conclusions are probably applicable to glucopyranosides[22]. Methylation of methyl 4,6-*O*-benzylidene-β-D-glucopyranoside in the presence of $SnCl_2$

yields comparable proportions of monoalkyl derivatives, but the α anomer yields different proportions and methylation is quantitative.

Methylation of methyl β- and α-D-glucopyranosides in the presence of $SnCl_2$ yields the 3-*O*-methyl and 2,3-di-*O*-methyl derivatives, pointing to formation of a complex with Sn of at least HO-2,3 and HO-3,4. Initial methylation of HO-2 or HO-3 could involve complexes of tin with HO-2,3 and HO-3,4; subsequent reaction of the 2-*O*-methyl derivative *via* the complex (7) of tin with HO-3,4 would yield the 2,3-di-*O*-methyl derivative. For the 3-*O*-methyl derivative, no cyclic complex with tin is possible.



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The distribution of mono- and di-alkylated products, which are formed in comparable proportions with the β, but not the α, anomer, is analogous to the distribution of the monoalkylated products formed with methyl 4,6-*O*-benzylidene-β- and -α-D-glucopyranosides. These results indicate that the complex of tin with HO-2,3, which is sensitive to the orientation of the substituent at C-1, is the same in the glucopyranosides and their 4,6-*O*-benzylidene derivatives. It would be of interest to compare the relative proportions of the monoalkylated products of the α- and β-D-ribofuranosyl nucleosides.

Some difference in behaviour is also observed between β-D-glycopyranosides and *C*-β-D-glycopyranosyl derivatives; the latter preferentially yield the 3-*O*-methyl isomer, whereas the former yield comparable proportions of the 3-*O*-methyl and 2,3-di-*O*-methyl derivatives. This difference is presumably due to the influence of the electronegative substituent, which makes HO-2 more acidic.

EXPERIMENTAL

AraA was a gift from Dr. P. Stoss (Heinrich Mack Nachf., Illetissen/Bayern, G.F.R.), and XylA was kindly provided by Dr. Harry B. Wood (N.C.I., Bethesda, Md., U.S.A.). Methylated derivatives of adenosine, cytidine, and XylA, used as reference compounds in chromatography, were prepared as described elsewhere[24-26] and identified by their ^{1}H-n.m.r. spectra.

Hydrated SnO_2 and Bu_2SnO were kindly provided by Dr. P. J. Smith (International Tin Research Institute). The procedure of Wagner *et al.*[14] was used for the preparation of 2',3'-di-*O*-(butylstannylene)cytidine. Anhydrous $SnCl_2$ and tin methoxylate were prepared according to standard procedures[9]. The $SnCl_2 \cdot 2H_2O$ used in the foregoing preparations was obtained from POCH (Gliwice, Poland).

Solutions of diazomethane were prepared essentially as described by Robins and Naik[4], but using ether in place of 1,2-dimethoxyethane. Methanol was distilled before use as a solvent.

Samples for Mossbauer spectroscopy were pressed from polycrystalline powders containing 15 mg/cm^2 of Sn. The spectra were obtained with a constant-acceleration spectrometer fitted with an 800-channel, Nokia Electronics Co. (Tokyo, Japan) analyser. The source consisted of 5 mCi of ^{119}Sn in $BaSnO_3$. The spectral parameters were computed by fitting Lorentzian line-shapes to the experimental data, using the least-squares method.

Reaction products of diazomethane with $SnCl_2 \cdot 2H_2O$. — To $SnCl_2 \cdot 2H_2O$ (1 g) in methanol (20 ml) was added, portionwise, ethereal diazomethane until the yellow colour persisted. This led to evolution of a gas and formation of a white, amorphous precipitate. Solvent was removed under diminished pressure and the residue was stored *in vacuo* over P_2O_5.

Analysis of this product revealed N (micro-Kjeidahl), $<0.06 \pm 0.02\%$; Cl, $<0.001\%$ (conversion into chloride) and $<0.01\%$ (X-ray fluorescence); Sn, 69.3% [microcombustion, assuming ash to be SnO_2 (as shown by X-ray diffraction)]. However, a subsequent analysis on the same sample gave Sn, 73%, pointing to the instability of the product, which made accurate C and H analyses unreliable.

In separate experiments, gaseous diazomethane was led into a 20% solution of $SnCl_2 \cdot 2H_2O$ in methanol (5–10 ml). The liberated gas was freed from methanol and moisture by passage through a trap containing conc. H_2SO_4 and collected in a cuvette (10-cm pathlength) with KBr windows. The i.r. spectrum was recorded with a Perkin–Elmer Model 580 B spectrophotometer.

Methylation with ethereal diazomethane. — The reaction mixture included ~50 mg of nucleoside in 10 ml of methanol. When catalyst was employed, this consisted of a 10-fold lower (molar) concentration of $SnCl_2 \cdot 2H_2O$, or the product of reaction of this with diazomethane, or a saturated solution of $Sn(OMe)_2$ (which is sparingly soluble in methanol). Ethereal diazomethane was added portionwise, awaiting disappearance of the yellow colour before addition of the next portion. The reaction was monitored by t.l.c. on silica gel F_{254} (Merck) with methanol–chloroform (1:4), and cellulose F_{254} with 1-butanol–ethanol–water (16:2:5)[24–26]. The composition of methylated products was determined by elution with aqueous methanol from small columns of Dowex 1-X4 (HO^-) resin previously calibrated with a mixture of known, methylated nucleosides[24–26], the effluents being monitored and recorded on an LKB 8300A-2 UV instrument.

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