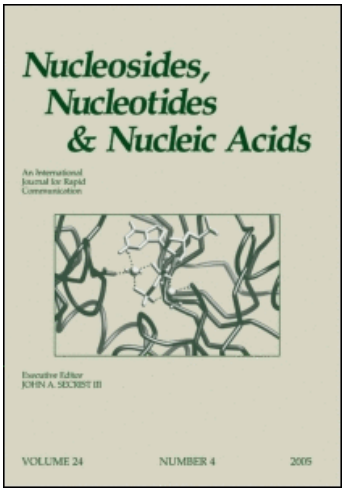

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Evidence for Cyclophosphate Formation During Hydrolysis of 3-Methyl-*cyclo*Sal-PCVMP

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EVIDENCE FOR CYCLOPHOSPHATE FORMATION DURING HYDROLYSIS OF 3-METHYL-*CYCLO*SAL-PCVMP

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Abstract: A hydrolysis study of 3-methyl-*cyclo*Sal-PCVMP **2** is described. Surprisingly, phosphotriester **5** released in this study not the expected PCVMP, but cycloPCVMP.

Here we describe the properties of 3-methyl-***cyclo*sal**igenyl pencicloviR**mono**-**p**hosphate **2**. Penciclovir **1** and the title compound **2** were prepared according to syntheses that have been described previously[1,2].

Compound **2** has been studied for its lipophilic property (partition coefficient in *n*-octanol/water (log*P* value)) as well as for its hydrolytic stability in 25 mM isotonic PBS, pH 7.3. For this hydrolysis experiment we proceeded as described before[3]. The log*P* value, the half-life ($t_{1/2}$) and the rate constant k have already been published[2].

Surprisingly, 3-methyl-*cyclo*Sal-PCVMP **2** showed a pronounced decrease in half-life as compared to other *cyclo*Sal-NMP triesters[2]. We assumed that the reason for this marked increase in hydrolytic lability could be either an assisting effect of the second, unprotected primary hydroxy group in the vicinity of the phosphate triester moiety due to a preorientation or an activation of the nucleophile water or a direct nucleophilic attack of this hydroxyl group at the phosphorus center. The consequences are i) the formation of PCVMP in the former case (except the rate acceleration) and ii) the formation of cyclic PCVMP **3** (cPCVMP). In order to identify the hydrolysis product of the chemical hydrolysis of 3-methyl-*cyclo*Sal-PCVMP **2** two experiments were conducted: First, a ^{31}P-NMR experiment in a DMSO-d_6/water mixture containing small amounts of TRIS-buffer,

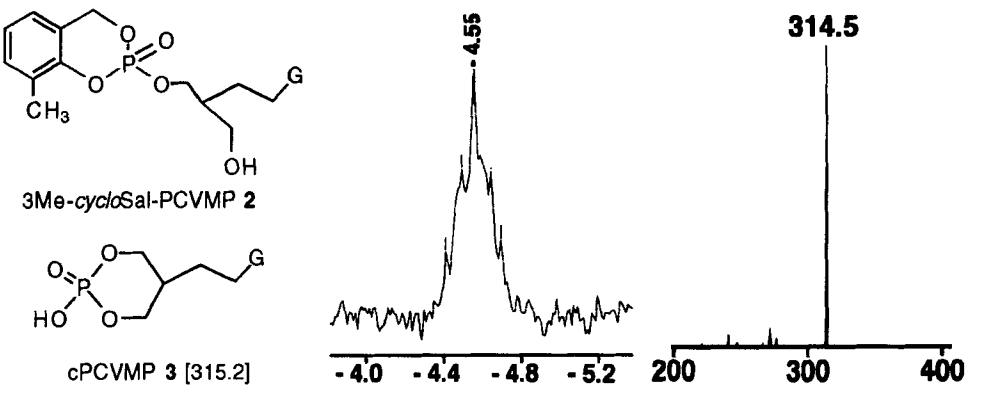

Figure 1: Structures of **2** and **3**, H-coupled ^{31}P-NMR and ESI-mass spectrum of hydrolysis product **3**

pH 8.8 was carried out. After complete hydrolysis, a proton-coupled ^{31}P-NMR spectrum was recorded. From the chemical shift and the coupling pattern of the phosphorus signal (a singulet at -4.5 ppm in the decoupled mode) it became apparent that the hydrolysis of **2** obviously did not lead to the expected PCVMP but to a product which showed a quintet in the proton-coupled mode (Figure 1). In contrast, PCVMP should split into a triplet in the proton-coupled mode of the ^{31}P-NMR spectrum[4]. Consequently, the multiplet observed strongly indicates the formation of the cPCVMP **3** (Figure 1). A further indication for the formation of cPCVMP **3** was taken from electrospray mass spectrometry. PCVMP has a mass of 333.2 u and should yield molecular ions $[M-H]^-$ and $[M-2H]^-$ at m/z 332 and m/z 165.5, respectively. However, the product observed in the NMR experiment was characterized by a molecular ion $[M-H]^-$ at m/z 314 (Figure 1). Further product ion experiments unambiguously demonstrated the presence of a phosphate moiety (m/z 97 and m/z 79). Hence, both experiments prove the formation of cPCVMP **3** (MW 315.2 g/mol) from the *cyclo*Sal-PCVMP derivatives **2** instead of PCVMP.

Compound **2** has been evaluated for its inhibition of HSV-1, HSV-1/TK$^-$ and EBV replication in Vero and P3HR-1 cells. As expected, **2** devoid of any antiviral activity[2].

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