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A Dinucleoside Phosphorothioate

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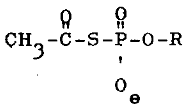
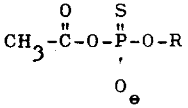
We are interested in dinucleoside phosphorothioates as substrates for phosphodiesterases and nucleases. As a representative of this class of compounds we synthesized dithymidine-3',5'-phosphorothioate in the following way:

Thymidine-5'-phosphorothioate (Ia)¹⁾, containing about 5 % of thymidine-5'-phosphate (Ib), was acetylated in the usual manner with Ac_2O/pyridine²⁾. Analysis by paper chromatography revealed about 90 % of acetylated mononucleotide and about 10 % of dinucleoside pyrophosphate. To determine the ratio of S-nucleotide (IIa) to O-nucleotide (IIb) the acetylated mononucleotide was digested with E. coli alkaline phosphatase³⁾. It has been shown previously that nucleoside phosphorothioates are resistant to this enzyme¹⁾. Paper chromatography of the digest showed 20 % of 3'-O-acetylthymidine, 14 % of an unidentified product, presumably dinucleoside pyrophosphate, and 66 % of 3'-O-acetylthymidine-5'-phosphorothioate (IIa), thus indicating a replacement of sulfur by oxygen to about 15 % during acetylation.

This loss of sulfur presumably arises from hydrolysis of the mixed anhydride IIIa during work up with aqueous pyridine. In which ratio the mixed anhydrides IIIa and IIIb are formed[4)] and by which mechanism they are hydrolized cannot be decided by these experiments.

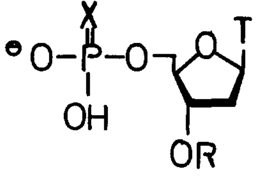

$CH_3-\overset{O}{\overset{\|}{C}}-S-\underset{O^{\ominus}}{\underset{|}{\overset{O}{\overset{\|}{P}}}}-O-R$

R = 5'-thymidyl


$CH_3-\overset{O}{\overset{\|}{C}}-O-\underset{O^{\ominus}}{\underset{|}{\overset{S}{\overset{\|}{P}}}}-O-R$

IIIa IIIb

The acetylated mixture of O- and S-nucleotides IIa and IIb was condensed with 5'-tritylthymidine in pyridine using 2 eq. of triisopropylbenzenesulfonyl chloride[5)] as condensing reagent. After removal of protective groups and separation on a DEAE-cellulose column (HCO_3^- -form) we obtained the dinucleoside phosphorothioate IVa in 18 % (Anal. (as sodium salt) Calcd.: N, 9.60; S, 5.49. Found: N, 9.30; S, 5.08; P/thymidine, 1:1.07. $\lambda_{max}^{H_2O}$: 267 mμ (ε = 18 500)] and the dinucleoside phosphate IVb in 33 % yield. IVa and IVb can be distinguished by paper chromatography but not by electrophoresis at pH 3.5 (formate buffer) or pH 7.5 (0.1 M triethylammoniumbicarbonate).



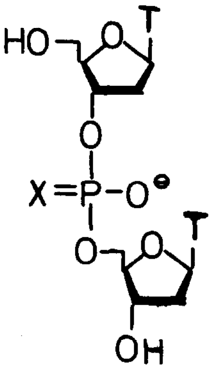
T = thymyl

Ia, X = S
R = H

Ib, X = O
R = H

IIa, X = S
R = Acetyl

IIb, X = O
R = Acetyl



IVa, X = S

IVb, X = O

It could be shown that IVa is stable to triisopropylbenzenesulfonyl chloride under the reaction conditions. The disproportionally high ratio of IVb to IVa, therefore, is a reflection of the ambident character of Ia towards this reagent.

Using dicyclohexylcarbodiimide (DCC) as condensing reagent the sole product was IVb. From the work of Mikolajczyk[6)] it is known that in phosphonothioates and phosphinothioates it is exclusively the sulfur which attacks DCC. This apparently is also true for phosphorothioates like Ia. Since a cyclic trimetaphosphate is believed to be the phosphorylating species in phosphorylations with DCC[7)] the activation of the thiopyrophosphate to the trimetaphosphate presumably takes place with complete loss of sulfur.

In an attempted oxidation of IVa with H_2O_2, 70 % was recovered unchanged, 28 % was desulfurized to IVb. IVa also was desulfurized to about 10 % by the action of 1,1'-carbonyldiimidazole in DMF, a reaction which is reminiscent of the action of $COCl_2$ on 0,0-diethyl phosphorothioate[8)]. Contrary to our expectations IVa could not be transformed into IVb by DMSO[9)].

IVa was completely resistant to snake venom phosphodiesterase[3)] and spleen phosphodiesterase[10)].

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