Tetrahedron Letters,Vol.23,No.39,pp 4055-4056,1982 0040-4039/82/394055-02$03.00/0
Printed in Great Britain

REACTION OF 4-ACETOXY-1,4-BENZOXAZIN-3-ONE WITH DNA. A POSSIBLE CHEMICAL MECHANISM FOR THE ANTIFUNGAL AND MUTAGENIC ACTIVITIES

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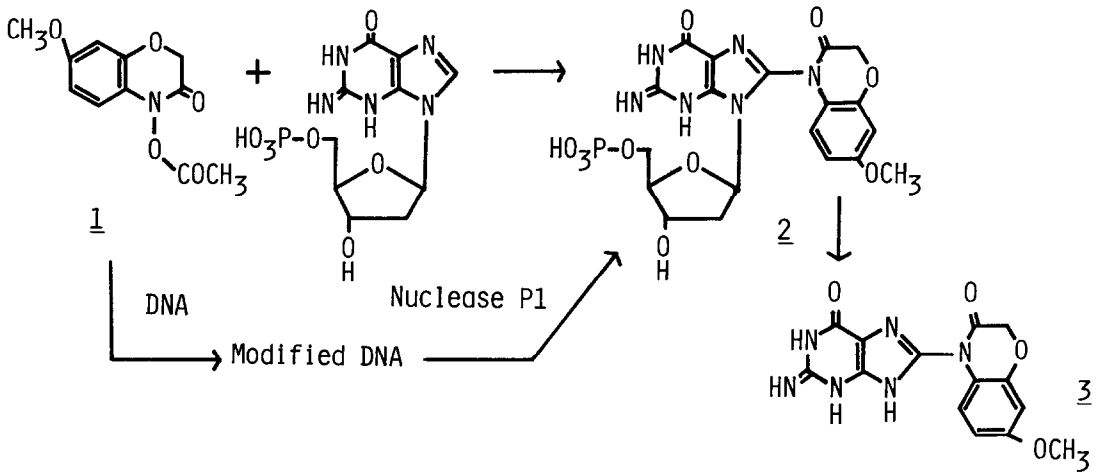
Abstract A reaction of 4-acetoxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one with DNA and nucleotides was described. The N^4 position of the benzoxazinone reacted with the C^8 position of the guanine residue of DNA and guanylic acid.

A group of 1,4-benzoxazin-3-ones was isolated from Job's tears, rye, wheat, and corn.[1] Derivatives of 2,4-dihydroxy-1,4-benzoxazin-3-one occur as glycosides from which the aglycones are rapidly released by enzymatic hydolysis after physical and biological injury of the plants. They exibit antifungal and insectistat properties. We found that some of them are moderately strong mutagens to bacteria.[2] Nothing is known about the mechanism of biological actions of these unique cyclic hydroxamic acids. On the other hand, aromatic hydroxylamines and hydroxamic acids are quite obligatory to the initiation of mutation and cancer and they react with nucleic acids and proteins.[3] Therefore, we anticipated that 4-hydroxy-1,4-benzoxazinones react with biological molecules, and already reported[4] that the acetate (1) of 4-hydroxy-7-methoxy-benzoxazinone reacted with nucleophiles such as phenols and indoles which are models of nucleophilic fragments of proteins and nucleic acids. In this paper, we describe a reaction of 1 with DNA and nucleotides.

A freshly prepared 1 (0.11 mmole) was added to a solution of 5'-deoxyguanylic acid (2 equiv.) in water-DMF (5 1, 5 ml) and the mixture was stirred at room temperature for 30 min On concentrating the reaction mixture and chromatography on Sephadex LH 20 (elution with water), a major product (2) was isolated in 58% yield after reprecipitation from ethanol-methanol. The nmr spectrum of 2 suggested the presence of the benzoxazinone ring and a guanylic acid moiety, though the spectrum is understood as a mixture of two conformational isomers. Hydrolysis of 2 with trifluoroacetic acid gave a guanine-benzoxazinone adduct (3) in a good yield, mp >300°. Anal. Calcd. for $C_{14}H_{12}N_6O_4$, C 51.22, H: 3.69, N 25.60. Found C 51.35, H 3.81, N 25.38. The nmr of 3 was well analyzed, NMR (DMSO, ppm from TMS) 3.71 (s, 3H), 4.81 (s, 2H), 6.54 (m, 2H), and 6.69 (d, 2H, J=2) (those are assigned to the hydrogens of the benzoxazinone ring), 6.42 (s, br, 2H), 10.63 (s, br, 1H), and 12.72 (s, br, 1H) (those are assigned to the guanine moiety). The nmr shows that the hydrogen atom at guanine C-8 was absent, and three hydrogens of the benzoxazinone ring were assigned. Consequently, the binding sites were the nitrogen atom of the benzoxazinone and the position 8 of the guanine. The carbon-nmr also supported this structure. The same compound could be prepared from guanine and 1, though the yield was low (6%). A very similar result was obtained in the reaction of 1 with 5'-guanylic acid. The product (4, 47% yield) was hydrolyzed to give 3 in a



quantitative yield. 1 did not react with deoxyadenylic acid, deoxycytidylic acid, and thymidylic acid.

The reaction of 1 with calf thymus DNA was studied. DNA (0.5 g, Na salt, Sigma Chemical Co.) in water-DMF (5:1, 240 ml) was treated with 1 (370 mg) at room temperature for 30 min. DNA modified with 1 was recovered by addition of cold ethanol and centrifugation. The modified DNA was dissolved in water (pH 5, adjusted by acetic acid) and hydrolyzed by Nuclease P1 at 50° to a nucleotide mixture. The hydrolysate was subjected to a Sephadex LH 20 column, eluted with water. The modified nucleotide was eluted later than normal nucleotides. The isolated compound was identified with 2 prepared from deoxyguanylic acid and 1 by comparison of IR, UV, and NMR spectra. The amount of the nucleotide modified by 1 was as much as 4% of the total guanine in the calf thymus DNA.

It is concluded that the acetate 1 reacted with DNA and bound covalently to the guanine residue of the DNA. The mode of binding is similar to the binding to DNA of carcinogenic 2-acetylaminofluorene[3] and of some heteroaromatic amines isolated from food pyrolysates.[5] The present results suggest that the mutagenicity and possibly other biological activities of 4-hydroxybenzoxazinones might be caused by a similar chemical reaction.

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(Received in Japan 25 June 1982)