CYCLOPENTA[f]ISOQUINOLINE DERIVATIVES DESIGNED TO BIND SPECIFICALLY TO NATIVE DEOXYRIBONUCLEIC ACID. III. INTERACTION OF 6-CARBAMYLMETHYL-8-METHYL-7H-CYCLOPENTA[f]ISOQUINOLIN-3(2H)-ONE WITH DEOXYRIBONUCLEIC ACIDS AND POLYDEOXYRIBONUCLEOTIDES.[1]

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SUMMARY

By the use of space-filling models, a novel compound, 6-carbamylmethyl-8-methyl-7H-cyclopenta[f]isoquinolin-3(2H)-one (1) was devised which would be expected to hydrogen bond specifically to GC pairs in the major groove of the double helix such that (i) the amino group of the cytosine molecule donates a hydrogen bond to the C-3 carbonyl of the isoquinoline moiety and (ii) the amide proton of the side chain donates a hydrogen bond to the N-7 of guanine. From difference spectra studies it was found that 1 binds to native calf thymus DNA better than to denatured DNA; 1 inhibited RNA synthesis by a DNA-dependent RNA polymerase; and equilibrium dialysis experiments revealed that 1 binds to poly(dG).poly(dC), whereas no such binding to poly(dA).poly(dT) was observed.

Many antibiotics bind to cellular DNA in various ways and thereby produce profound biochemical, pharmacological and chemotherapeutic effects (1-9). It was of interest to devise ad hoc, by the use of space-filling models, a synthetic compound that would be expected: 1) to hydrogen-bond specifically to GC pairs in the major groove, across the double helix of DNA; 2) not to be electrostatically attracted to the phosphates of the backbone; and 3) to stack parallel to the base pairs. As shown in Fig. 1, the cyclopenta[f]isoquinoline derivative (1) fulfills

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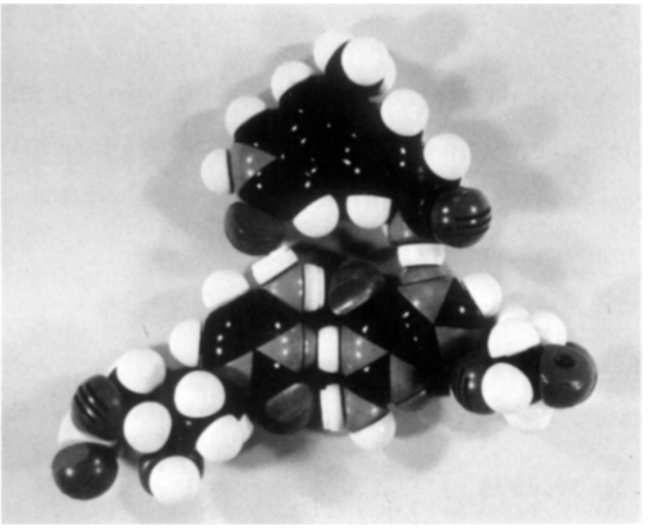

Fig. 1. CPK model of the complex between a GC pair of DNA and the cyclopenta[f]isoquinoline derivative, 1.

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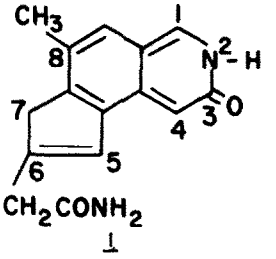
these criteria on the model, such that the amino proton of cytosine donates a hydrogen bond to the C-3 carbonyl group of the isoquinoline and the amide proton of the side-chain donates a hydrogen bond to the N-7 of the guanine. This compound, 1, has a novel ring system and does not resemble in structure any of the known DNA-binding antibiotics. In this communication we report our studies of the interaction of 1 with deoxyribonucleic acids and poly-deoxyribonucleotides.



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EXPERIMENTAL

6-Carbamylmethyl-8-methyl-7H-cyclopenta[f]isoquinolin-3(2H)-one (1) was synthesized by a multistep process starting from m-methyl-N-acetyl-benzylamine. The synthesis of 1 is being reported elsewhere.

Calf thymus deoxyribonucleic acid was purchased from Worthington Biochemical Corp., Freehold, N. J. and was their highest grade of purity. It was

further purified (10) by treatment with pronase, ribonuclease, and chloroform-isoamyl alcohol extraction, and subsequent precipitation with alcohol. No difference, however, was seen between the results obtained with commercial and purified calf thymus DNA. Denatured calf thymus DNA (20 O.D. units) was obtained by heating native DNA in a boiling water bath for 10 min and cooling rapidly in an ice-bath. DNA's from Micrococcus lysodeikticus, Escherichia coli and Clostridium perfringens were purchased from Sigma Chemical Co., St. Louis, Mo., and were their highest grade of purity. Poly(dG-dC), poly(dA-dT), poly(dG).poly(dC) and poly(dA).poly(dT) in their duplex forms were purchased from P-L Biochemicals, Inc., Milwaukee, Wis.

Difference Spectra Studies

Difference spectra were taken on a Cary 15 spectrophotometer with 0 to 0.1 O.D. scale expansion, between 500 and 380 nm. Spectra were obtained using split-compartment mixing cells (Pyrocell Co., Westwood, N. J.) in which equal volumes of solutions of 1 and the DNA or polydeoxyribonucleotide were placed in separate compartments of both cells. After the baseline was obtained, the solutions in the sample cell were mixed and the difference spectrum was recorded. All solutions were made in phosphate buffer, pH 7.21 ± 0.01 (PO_4''' = 0.001 M, NaCl = 0.01 M). The concentration of 1 after mixing was 2 x 10^{-4} M, which gave a total O.D. of 0.5 at 418 nm; DNA or polydeoxyribonucleotide concentrations, after mixing, are given in O.D. units. All reactions were carried out at 24°.

Equilibrium Dialysis Experiments

Equilibrium dialysis experiments were carried out in an apparatus

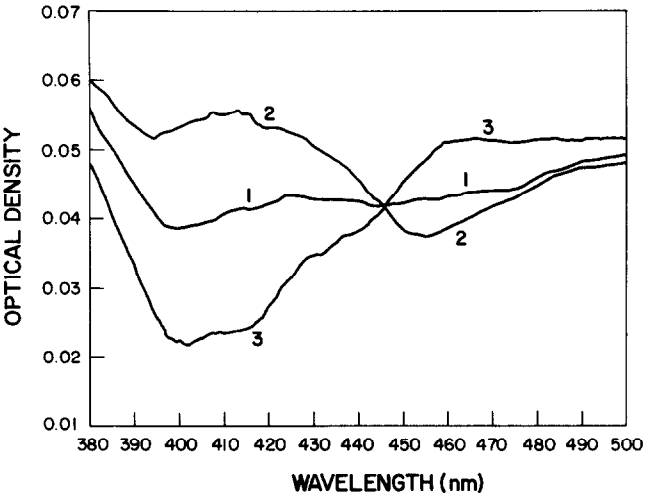

Fig. 2. Difference spectra obtained on a Cary 15 spectrophotometer on mixing a solution of calf thymus DNA with 6-carbamylmethyl-8-methyl-7H-cyclopenta[f]isoquinolin-3(2H)-one, 1. Curve 1 is the baseline, curve 2 is the mixture of compound 1 with buffer only, and curve 3 is the mixture of 1 with a solution of calf thymus DNA (concentration after mixing 1 = 2 x 10^{-4} M, DNA = 10 O.D. units; pH 7.21 ± 0.01, PO_4''' = 0.001 M, NaCl = 0.01 M; T = 24°).

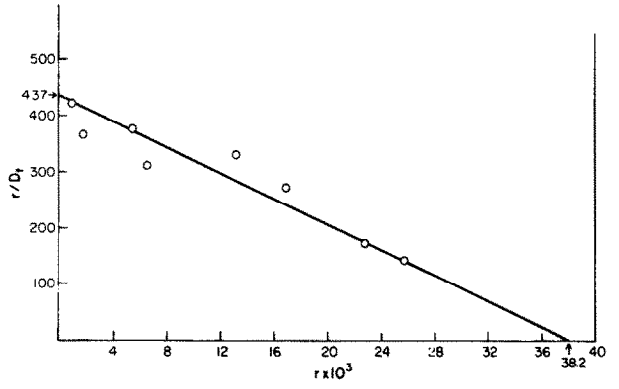

Fig. 3. Scatchard plot of the binding of 1 to calf thymus DNA. r, Moles of bound 1/total moles DNA nucleotide; D_f, moles of free 1.

designed by Wells and Larson (5). A dialysis tubing (La Pine Scientific Co., Chicago, Ill.) separates two holes which are based in two plexiglass sheets and held together with screws. The dialysis tubing was boiled in 0.01 M NaCl-0.001 M EDTA (pH 7.5) for 30 min and then stored at 5° in deionized water. Solutions of 1 (0.2 ml; 4×10^{-4} to 6.125×10^{-6} M) were added to one compartment and a DNA solution (0.2 ml; c = 10 O.D. units) was added to the other compartment. All solutions were made in a phosphate buffer (pH = 7.21 ± 0.01, NaCl = 0.01 M, PO_4''' = 0.001 M). Equilibration was done in the dark in a cold room (T = 5°C) for 3 days with gentle shaking, by which time complete equilibrium was established. Concentration of 1, after equilibrium was established, was measured on a spectrofluorometer (Model 430, G.K. Turner Associates, Palo Alto, Calif.) which was standardized with a quinine sulfate solution (10 ppm). Data were treated in the classical manner (11) (Fig. 3). The slope of the curve gives the binding constant, Kapp (M^{-1}), whereas the reciprocal of the intercept on the abscissa yields the number of nucleotides per binding site.

RESULTS AND DISCUSSION

The uv-visible spectrum of 6-carbamylmethyl-8-methyl-7H-cyclopenta[f]-isoquinolin-3(2H)-one, 1, shows a maximum at 418 nm (ε = 2,500), pH 7.2. On mixing a solution of 1 with calf thymus deoxyribonucleic acid, a hypochromic effect was seen between 380 and 430 nm in the difference spectrum (Curve 3, Fig. 2), which was most pronounced at 400 nm. When a solution of 1 was mixed with buffer in the absence of DNA, a hyperchromic shift at 400 nm was observed (Curve 2, Fig. 2), presumably due to a decrease of self-association which is usually seen among purines, pyrimidines

and related bases (12). All changes in O.D. recorded in the tables have been corrected for this effect. In Table I the effect of DNA concentration on the hypochromicity at 400 nm is shown, and is pronounced only at high concentrations of DNA. The magnitude of the hypochromicity is considerably less than that seen with actinomycin D (1), but is comparable with that obtained with tilorone hydrochloride (13), both of which bind to DNA. This relatively low hypochromic effect is consistent with the model we propose for the binding of 1 with DNA, since only two hydrogen bonds are

Table I

The Binding of 1 to DNA's and Polydeoxyribonucleotides as Determined by Difference Spectroscopy

DNA or Polynucleotide	GC Content %	State	DNA Concentration (O.D. units/ml)	NaCl Concentration (M)	ΔO.D.$^{a}_{400}$
Calf thymus	39	Native	10.0	0.01	-0.031
"	"	"	5.0	"	-0.019
"	"	"	2.5	"	-0.004
"	"	"	10.0	0.001	-0.018
"	"	"	"	0.1	-0.018
"	"	Denatured	"	0.01	-0.019
Micrococcus Luteus	71	Native	5.0	"	-0.016
"	"	"	2.5	"	-0.007
Escherichia coli	50	"	5.0	"	-0.015
"	"	"	2.5	"	-0.004
Clostridium perfringens	31	"	5.0	"	-0.008
"	"	"	2.5	"	-0.005
Poly(dG-dC).Poly(dG-dC)	100	"	2.5	"	-0.016
" "	"	"	1.25	"	-0.011
Poly(dA-dT).Poly(dA-dT)	0	Native	2.50	0.01	-0.012
" "	"	"	1.25	"	-0.007
Poly(dG).Poly(dC)	100	"	2.5	"	-0.014
" "	"	"	1.25	"	-0.004
Poly(dA).Poly(dT)	0	"	2.5	"	-0.007
" "	0	"	1.25	"	+0.002

[a]The complete difference spectrum (cf. Fig. 2) was taken in all cases. Only the corrected ΔO.D. at 400 nm is recorded in Table 1.

formed per molecule of 1. The effect of salt concentration on the difference spectrum (Table 1) is also consistent with this hydrogen-bonded model. The interaction between 1 and calf thymus DNA is easily reversible; hence, attempts to isolate a bound complex by gel filtration on Sephadex G-25 were unsuccessful.

Table 1 also shows the interaction of 1 with DNA's from different natural sources. From our model we expect that 1 would bind more to a DNA of higher GC content than to one of higher AT content. However, only in the case of DNA from Clostridium Perfringens, which has a GC content of 31%, is the binding appreciably lower than to the other DNA's. Presumably because of the high concentration of DNA used, there is an excess of GC pairs available for binding.

It is seen, however (Table 1), that 1 interacts with synthetic polydeoxyribonucleotides containing only GC pairs more effectively than with AT polymers, in agreement with our predictions. Furthermore (Table 1), 1 binds better to double stranded DNA (native), than to single stranded DNA (denatured). Additional studies with related compounds (unpublished) show that the tricylic moiety is essential for binding, and that the side-chain greatly enhances the interaction, as anticipated from the models.

The interaction of 1 with DNA was further substantiated by the finding that 1 caused inhibition of RNA synthesis by a DNA-dependent RNA polymerase from E. coli (14). More than 35% inhibition of RNA synthesis was obtained in the presence of 1 (4×10^{-4} M) with calf thymus DNA as template (3.3×10^{-5} M). Also, 1 caused no inhibition of DNA synthesis by a DNA dependent DNA polymerase obtained from HeLa cells (15).

Further information on the binding of 1 with DNA's and polydeoxyribonucleotides was obtained from equilibrium dialysis experiments. A Scatchard plot (16) of the data obtained from the interaction of native calf thymus DNA with 1 is shown in Fig. 3. From the slope of the curve

Table II

Equilibrium Constants and Number of Binding Sites

for Interactions of 1 with DNA and Polydeoxyribonucleotides

DNA or POLYDEOXYRIBO-NUCLEOTIDES	GC Content %	Kapp (M^{-1})	Nucleotides per site
Calf thymus DNA	39	1.1×10^4	26
Poly(dG-dC).poly(dG-dC)	100	1.6×10^4	36
Poly(dA-dT).poly(dA-dT)	0	1.0×10^4	23
Poly(dG).poly(dC)	100	2.2×10^4	49
Poly(dA).poly(dT)	0	---	--

a Kapp (M^{-1}) value of 1.14×10^4 (Table II)[3] was calculated and the number of nucleotides per binding site was 26. The linear plot indicates that the binding takes place by a single mechanism. It can also be seen from Table II that 1 binds to poly(dG-dC) somewhat better than to poly(dA-dT). However, whereas binding to poly(dG).poly(dC) was observed, no binding to poly(dA).poly(dT) could be detected.

It is evident from these experimental results that our predictions based on molecular models are fulfilled to a considerable extent. The hypochromic effect in the difference spectrum on mixing a solution of 1 with DNA's (in contrast to the hyperchromic effect observed due to dilution of 1), the small magnitude of the effect, its dependence on concentration of DNA, and the effect of salt concentration all agree well with a hydrogen bonded model. Similarly, failure to isolate a complex by gel filtration indicates that the forces responsible for the interaction of 1 with DNA's are very weak and presumably of the hydrogen bonded type. The magnitude of the Kapp values from dialysis experiments

[3] Kapp (M-1) value was incorrectly reported as 1.14×10^5 in N. G. Kundu and C. Heidelberger, Fed. Proc., 33, 556 (1974).

is in agreement with such a model, and the equilibrium dialysis experiments show that 1 binds preferentially to polydeoxyribonucleotides containing GC pairs. Although we cannot completely exclude other mechanisms for the interaction of 1 and DNA's, the experiments we have done so far support our hydrogen-bonded model.

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