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Cyclopenta[*f*]isoquinoline Derivatives Designed to Bind Specifically to Native Deoxyribonucleic Acid. 2. Synthesis of 6-Carbamylmethyl-8-methyl-7(5)*H*-cyclopenta[*f*]isoquinolin-3(2*H*)-one† and Its Interaction with Deoxyribonucleic Acids and Poly(deoxyribonucleotides)‡

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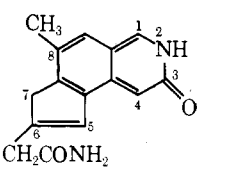
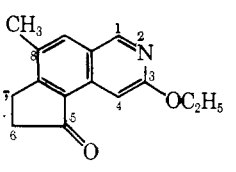
3-Ethoxy-8-methyl-5,6-dihydro-7*H*-cyclopenta[*f*]isoquinolin-5-one (**2**) was converted to 6-carbethoxymethyl-3-ethoxy-8-methyl-5,6-dihydro-7*H*-cyclopenta[*f*]isoquinolin-5-one (**6**) through an oxalyl derivative. Treatment of **6** with ammonia gave the corresponding amide **7** which on sodium borohydride reduction and subsequent dehydration yielded 6-carbamylmethyl-3-ethoxy-8-methyl-7(5)*H*-cyclopenta[*f*]isoquinoline (**9**). The analogous ester **10** was similarly obtained from **6**. Numerous attempts to dealkylate the 3-ethoxy group of **9** or **10** failed. However, **6** could easily be dealkylated on heating with 25% hydrochloric acid in a sealed tube. The ester, 6-carbethoxymethyl-8-methyl-5,6-dihydro-7*H*-cyclopenta[*f*]isoquinoline-3(2*H*),5-dione (**11**), so obtained was converted to the corresponding amide **12** which on reduction with sodium borohydride and subsequent dehydration afforded the desired compound, 6-carbamylmethyl-8-methyl-7(5)*H*-cyclopenta[*f*]isoquinolin-3-(2*H*)-one (**1**). **1** was found to be mildly cytotoxic against L5178Y mouse leukemia cells in culture. **1** was also found to bind to native calf thymus DNA. **1** inhibited RNA synthesis by a DNA-dependent RNA polymerase and a higher inhibition of RNA synthesis was observed when poly(dG-dC) was used as a template than when poly(dA-dT) was used. A significant increase of thermal transition temperature of calf thymus DNA and poly(dG) · poly(dC) was observed in the presence of **1**. The accumulated evidence demonstrates that **1** interacts weakly with calf thymus DNA and interacts preferentially with poly(deoxyribonucleotides)-containing GC pairs.

In the preceding paper,[1] we have described how the compound of structure **1** could bind to guanine–cytosine (GC) pairs of a DNA double helix by hydrogen bond formation. Starting from *m*-methyl-*N*-acetylbenzylamine we have described a multistep synthesis of 3-ethoxy-8-methyl-5,6-dihydro-7*H*-cyclopenta[*f*]isoquinolin-5-one (**2**) and the corresponding unsaturated compound **3** which constitute the basic skeleton of **1**.

In this paper we report the synthesis of compound **1** and its binding to DNA. In order to synthesize **1**, it seemed preferable to put the side chain at the C_6 position on compound **2**, in the first place, followed by dealkylation. Attempts at the direct alkylation of **2** were not very success-



1



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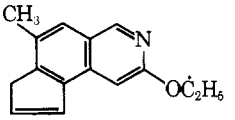

3

† The compound synthesized is a mixture of 7*H* and 5*H* isomers; only the structure corresponding to the 7*H* isomer is shown in the paper.

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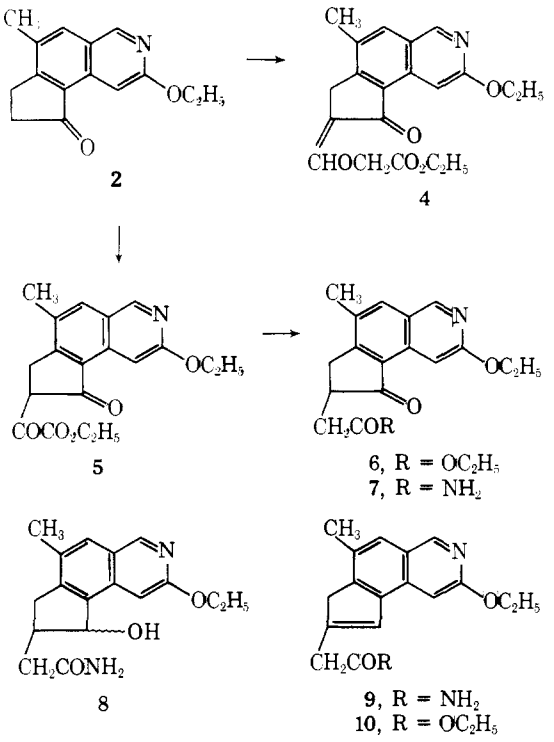
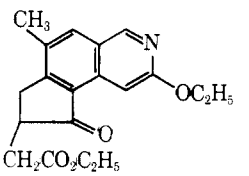
ful. Treatment of 2 with ethyl bromoacetate or iodoacetamide in the presence of potassium *tert*-butoxide[2] in solvents such as DMF, THF, etc., yielded primarily the starting material, with some uncharacterizable water-soluble products. It seems that under the conditions of direct alkylation, due to the low acidity of the methylene group adjacent to the carbonyl function, alkylation takes place predominantly on the nitrogen atom of the isoquinoline moiety, resulting in water-soluble quaternary salts. Similarly, alkylation could also be considered *via* the enamine[3] of 2, but no enamine could be isolated from the reaction of 2 with pyrrolidine in the presence of *p*-toluenesulfonic acid in benzene.

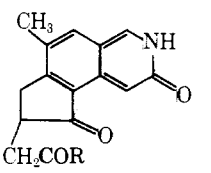
Whereas compound 2 could be easily formylated[4] with ethyl formate in the presence of sodium ethoxide in benzene, the formyl derivative did not give any well-characterizable product from its reaction with iodoacetamide in the presence of sodium ethoxide. However, reaction of the formyl derivative of 2 with ethyl bromoacetate in the presence of potassium *tert*-butoxide in DMF led to compound 4 due to the O-alkylation of the enolic form of the formyl derivative. The structure of 4 was established from its uv and nmr properties. In order to avoid O-alkylation, 2 was converted to the corresponding oxalyl derivative 5, in good yield, by treatment with diethyl oxalate in the presence of sodium ethoxide in benzene. The oxalyl derivative 5 reacted with ethyl bromoacetate in DMF in the presence of potassium *tert*-butoxide at 80°; alkylation and subsequent elimination of the oxalyl group took place. Removal of solvent and work-up yielded an oil which, after chromatography over Florisil [eluent benzene–methanol (10:1)], gave 6 as a crystalline solid. The keto ester 6 was converted to the corresponding amide 7 by heating in an autoclave with liquid ammonia. Alternatively, 6 could be converted by hydrolysis to the corresponding acid which was then converted to the acid chloride with thionyl chloride, and the acid chloride gave the amide by treatment with ammonia. Reduction of the keto amide 7 with sodium borohydride in ethanol furnished the hydroxyamide 8 which was dehydrated with *p*-toluenesulfonic acid in xylene to give the unsaturated amide 9. Similarly, reduction of 6 with sodium borohydride followed by treatment of the product with *p*-toluenesulfonic acid in xylene afforded the unsaturated ester 10.



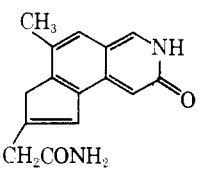
Numerous attempts were made to dealkylate 6-carbamylmethyl-3-ethoxy-8-methyl-7(5)*H*-cyclopenta[*f*]isoquinoline (9), the related ester 10, or the model compound 3. Use of vigorous reaction conditions, *e.g.*, reflux with 48% hydrobromic acid,[5,6] usually led to decomposition of the molecule or to polymeric products. Similarly, use of anhydrous aluminum chloride,[7,8] in benzene, or boron tribromide[9,10] failed to give the desired dealkylated product. A much milder reagent, sodium iodide[6] in acetic acid, is known to dealkylate alkoxypyrimidines but use of this reagent with 9 or 3 likewise failed to yield dealkylated products. An alternative approach[11,12] was to heat the methiodides with lithium bromide in acetonitrile. However, when the methiodides of 3 or 9 were so treated, the corresponding *N*-methylisoquinolones could not be isolated. It is probable that the additional cyclopentane ring destabilizes the molecule to such an extent that the products break down under the reaction conditions. Finally, however, compound 6 was successfully dealkylated by heating with 25% hydrochloric acid in a sealed tube;[13,14] alternatively, 48% hydrobromic acid[5,6] led to the same compound. Esterification of the dealkylated product afforded compound 11 which, on treatment with alcoholic ammonia, was converted to the corresponding amide 12.



6



11, R = OC_2H_5
12, R = NH_2



1

Reduction of 12 with sodium borohydride in ethanol and subsequent dehydration of the reduced product with hydrochloric acid in acetic acid yielded the desired final product, 1. Lactam–lactim tautomerism of the isoquinoline moiety is known to occur in 3-hydroxyisoquinoline[15,16] and its derivatives.[1] In the case of 3-hydroxycyclopenta[*f*]isoquinolines similar tautomerism is also observed. The lactam–lactim conversion was found to be solvent dependent. Uv spectral studies (Table I) indicate that a maximum at 357 nm can be ascribed to the lactim form, whereas the lactam form absorbs at *ca.* 420 nm. It is seen that 6-carbethoxymethyl-8-methyl-5,6-dihydro-7*H*-cyclopenta[*f*]isoquinoline-3(2*H*),5-dione (11) and the corresponding 6-carbamylmethyl derivative 12 exist exclusively in the lactam form in water, whereas in 95% ethanol, 11 and 12 are a mixture of the lactam and the lactim tautomers. 6-Carbamylmethyl-8-methyl-7(5)*H*-cyclopenta[*f*]isoquinolin-3(2*H*)-one (1), however, is predominantly in the lactam form both in water and 95% ethanol, as shown in Table I. The appearance of two aromatic methyl signals in the nmr shows that 1 is a mixture of 7*H* and 5*H* isomers.

Cytotoxicity Studies. Preliminary studies with 1 at 10^{-4} *M*, according to the previously described assay procedure,[17] have shown it to be cytotoxic to L5178Y mouse leukemia cells in culture.

Table I. Uv Spectra of Some Cyclopenta[*f*]isoquinolines

Compound	Solvent	λ, nm (ε)				
6-Carbethoxymethyl-3-ethoxy-8-methyl-5,6-dihydro-7*H*-cyclopenta[*f*]isoquinolin-5-one (6)	95% ethanol		357 (8360)	308 (5380)	296 (5340)	262 (1,150)
6-Carbamylmethyl-3-ethoxy-8-methyl-5,6-dihydro-7*H*-cyclopenta[*f*]isoquinolin-5-one (7)	95% ethanol		353 (7420)	307 (5780)	297 (5720)	
6-Carbethoxymethyl-3-ethoxy-8-methyl-7(5)*H*-cyclopenta[*f*]-isoquinoline (10)	95% ethanol		357 (4580)		285 (1140)	233 (36,390)
6-Carbamylmethyl-3-ethoxy-8-methyl-7(5)*H*-cyclopenta[*f*]-isoquinoline (9)	95% ethanol		357 (5450)	337 (2880)	295 (7060)	238 (4,460)
6-Carbethoxymethyl-8-methyl-5,6-dihydro-7*H*-cyclopenta-[*f*]isoquinoline-3(2*H*),5-dione (11)	95% ethanol Water	420 (3040) 415 (4770)	360 (2940)	325 (4000) 325 (5420)	312 (3830) 275 (4810)	239 (38,080) 238 (49,700)
6-Carbamylmethyl-8-methyl-5,6-dihydro-7*H*-cyclopenta[*f*]-isoquinoline-3(2*H*),5-dione (12)	95% ethanol Water	420 (3900) 405 (5150)	360 (3660)	323 (4800) 317 (4870)	310 (4480) 275 (5170)	
6-Carbamylmethyl-8-methyl-7(5)*H*-cyclopenta[*f*]isoquinolin-3(2*H*)-one (1)	95% ethanol Water	420 (1520) 418 (2500)		347 sh (1810) 348 sh (1600)	332 (2080) 333 (2000)	263 (15,300) 265 (20,000)

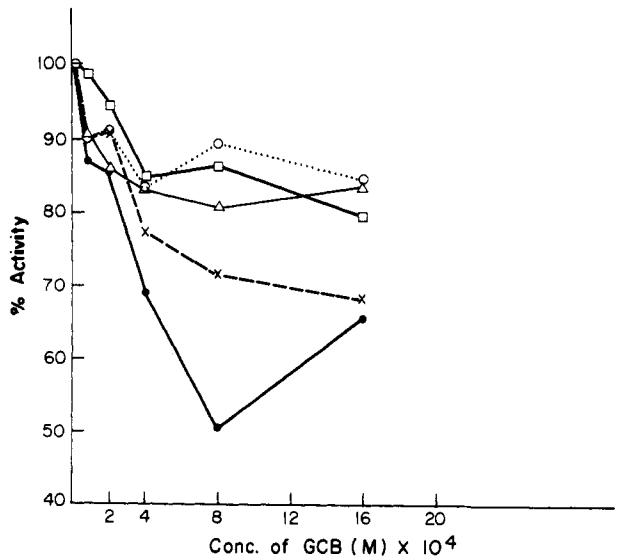


Figure 1. Inhibition of DNA-dependent RNA polymerase reaction (*E. coli*) in the presence of 1. The templates used: calf thymus DNA ●, poly(dA–dT) · poly(dA–dT) ○, poly(dA) · poly(dT) △, poly(dG) · poly(dC) □, poly(dG–dC) · poly(dG–dC) X. The reaction conditions are described in the Experimental Section. GCB (abbreviation for GC binder) refers to compound 1.

Interaction with DNA's and Poly(deoxyribonucleotides). The cyclopenta[*f*]isoquinoline derivative 1 interacts with DNA's and poly(deoxyribonucleotides). This is evident from the effect of 1 on the DNA-dependent RNA polymerase (*Escherichia coli*) reaction as is shown in Figure 1. A higher inhibition of RNA synthesis was observed when poly(deoxyguanine–deoxycytosine) [poly(dG–dC)] was used as a template than when poly(deoxyadenine–deoxythymine) [poly(dA–dT)] was used. The data from the inhibition of the RNA-polymerase reaction are in good agreement with those we obtained from difference spectra and equilibrium dialysis experiments.[18] Additional difference spectra studies have revealed the importance of the tricyclic moiety and the carbamylmethyl side chain for the binding of 1 with calf thymus DNA. As is seen in Table II, no interaction between 6-carbethoxymethyl-7-methylisoquinolin-3(2*H*)-one and calf thymus DNA could be observed.

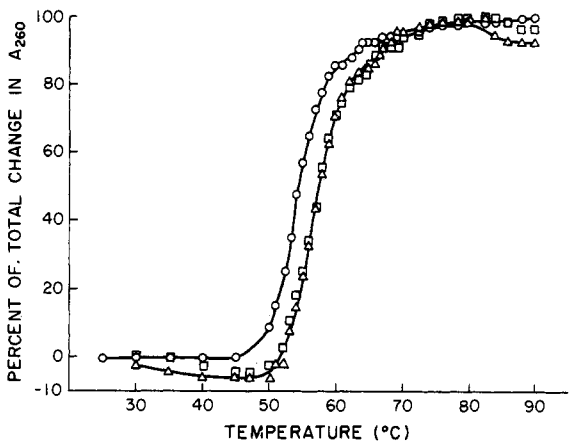
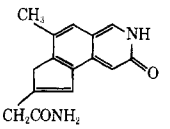
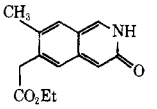


Figure 2. Thermal transition temperature of calf thymus DNA (○) and calf thymus DNA in the presence of 1 (△, □). The reaction conditions are described in the Experimental Section.

Table II. Binding Studies with Calf Thymus DNA as Determined by Difference Spectroscopy

Compound	ΔOD_{400}
	−0.031
	0.00

The interaction between 1 and calf thymus DNA is also demonstrated by the effect of 1 on the thermal transition temperature (T_m) of native calf thymus DNA (Figure 2). A small but significant increase in T_m of 3.5° was observed.

More significantly, in the presence of 1 although a small increase in T_m of 3° was observed for poly(dG) · poly(dC), no such change of T_m was seen in the case of poly(dG–dC), poly(dA–dT), and poly(dA) · poly(dT).

From the above studies, it can be concluded that our predictions based on molecular models are fulfilled to a considerable extent. It is evident that 1 definitely interacts with DNA's and polydeoxyribonucleotides although the magnitude of the interaction is small. This agrees well with our hydrogen bonded model since only two hydrogen bonds are formed per molecule of 1 in its interaction with DNA's and poly(deoxyribonucleotides). Also, in certain cases, 1 binds preferentially to poly(deoxyribonucleotides) containing GC pairs. In conclusion, it can be said that although alternative mechanisms of interaction are possible for the interaction of 1 with DNA's and poly(deoxyribonucleotides), the experiments we have reported so far favor our hydrogen bonded model. The effect of salt concentration on the stability of the complex between 1 and calf thymus DNA as evidenced by difference spectra results,[18] the failure to isolate a complex by gel filtration, and the absence of any effect on the circular dichroism spectra of calf thymus DNA in the presence of 1 also support the hydrogen bonded model and disfavor any intercalation model for the binding of 1 with DNA's and poly(deoxyribonucleotides).

Experimental Section

Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. The uv spectra were recorded on a Beckman DB-G and quantitative measurements done on a Gilford 2400-S. Spectra were usually taken in 95% ethanol unless otherwise mentioned. The ir spectra were taken on a Beckman IR-10 as KBr plates. Nmr spectra (reported in δ) were recorded on a Perkin-Elmer R-12 in deuteriochloroform, using tetramethylsilane as internal reference. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn., or Spang Microanalytical Laboratory, Ann Arbor, Mich. All analytical results were within 0.4% of the theoretical values.

6-(Carbethoxymethylyloxy)methylene-3-ethoxy-8-methyl-5,6-dihydro-7*H*-cyclopenta[*f*]isoquinolin-5-one (4). To a well-stirred suspension of NaOMe (prepared from Na, 120 mg, 5.22 mmol) in dry benzene (2 ml), ethyl formate (400 mg, 5.40 mmol) in 2 ml of benzene was added, and the mixture was stirred for 0.5 hr. The reaction was cooled in an ice bath and compound **2** (105 mg, 0.44 mmol) was added in 15 ml of benzene. The whole mixture was stirred in an ice bath for 15 min and at room temperature for 45 min and was then decomposed with a phosphate buffer (0.05 *M*, pH 8) and extracted with $CHCl_3$. After removal of solvent, 90 mg (0.25 mmol, 57%) of the formyl derivative, mp 150–152°, was obtained. It gives a bluish violet color with a solution of alcoholic $FeCl_3$; 220 mg (0.62 mmol) of the crude formyl derivative in 10 ml of DMF was added to KO-*t*-Bu (100 mg, 0.90 mmol) in 5 ml of DMF. The solution was stirred at room temperature for 1 hr followed by addition of ethyl bromoacetate (140 mg, 0.84 mmol) in 10 ml of DMF. The mixture was stirred at room temperature for 3 hr and at 100–110° for 17 hr. After work-up and crystallization from alcohol, **4** (60 mg, 0.17 mmol, 20%), mp 150–152°, was obtained. The analytical sample melts at 157–158°: ν max 1740, 1685, 1625 cm^{-1}; λ_{max} 364 (12,500), 313 (12,200), 277 (12,800), 230 (58,700); nmr 1.28 (t, 3 H, J = 7 Hz), 1.48 (t, 3 H, J = 7 Hz), 2.38 (s, 3 H), 3.6 (s, 1 H), 3.64 (s, 1 H), 4.35 (m, 4 H), 4.63 (s, 2 H), 7.35 (s, 1 H), 7.75 (s, 1 H), 8.25 (s, 1 H), 8.85 (s, 1 H). *Anal.* ($C_{20}H_{21}NO_5$) C, H, N.

6-Ethoxalyl-3-ethoxy-8-methyl-5,6-dihydro-7*H*-cyclopenta[*f*]isoquinolin-5-one (5). To a suspension of NaOEt (from 230 mg of Na, 10 mmol) in 10 ml of benzene, diethyl oxalate (1.46 g, 10 mmol) in 3 ml of benzene was added and stirred for 15 min, followed by the addition of **2** (240 mg, 1.0 mmol) in 15 ml of benzene. The mixture was stirred under N_2 for 20 hr, decomposed with 0.05 *M* phosphate buffer (pH 8), and extracted with $CHCl_3$. The $CHCl_3$ extract after removal of solvent yielded a yellow solid (285 mg, 0.82 mmol, 82%) which was crystallized from $CHCl_3$–EtOH as yellow needles: mp 195–196°. It gives a bluish violet color in alcoholic $FeCl_3$ solution: ν max 1730, 1650, 1625 cm^{-1}; λ_{max} 387 (15,200), 315 (6280), 287 (8640), 230 (44,100). *Anal.* ($C_{19}H_{19}NO_5$) C, H, N.

6-Carbethoxymethyl-3-ethoxy-8-methyl-5,6-dihydro-7*H*-cyclopenta[*f*]isoquinolin-5-one (6). Sodium ethoxide (made from 230 mg, 10 mmol of sodium) was suspended in 100 ml of benzene, the oxalyl derivative **5** (2.3 g, 6.74 mmol) was added, and the mixture was stirred under N_2 for 2 hr. Benzene was removed *in vacuo*. The sodium salt was suspended in 50 ml of DMF, and ethyl bromoacetate (1.4 g, 8.38 mmol) in 20 ml of DMF was added dropwise with stirring at room temperature. The mixture was then heated at 80° with stirring for 12 hr, DMF was removed *in vacuo*, and the residue was treated with H_2O and extracted with $CHCl_3$. The $CHCl_3$ extract was dried and evaporated to yield an oil, which was chromatographed over Florisil (60–100 mesh) using benzene and benzene–MeOH (10:1) as eluent. The benzene–MeOH fractions were combined and evaporated to yield an oil (2 g, 6.11 mmol, 90%, which was crystallized from ethanol as a light yellow solid: mp 102–103°; ν max 1735, 1720, 1690 cm^{-1}; λ_{max} 357 (8360), 308 (5380), 296 (5340), 262 (1150); nmr ($CDCl_3$) 1.24 (t, 3 *H*, J = 7 Hz), 1.46 (t, 3 H, J = 7 Hz), 2.42 (s, 3 H), 4.15 and 4.45 (two overlapping quartets, 4 H), 7.72 (s, 1 H), 8.1 (s, 1 H), 8.81 (s, 1 H). *Anal.* ($C_{19}H_{21}NO_4$) C, H, N.

6-Carbamylmethyl-3-ethoxy-8-methyl-5,6-dihydro-7*H*-cyclopenta[*f*]isoquinolin-5-one (7). Method A. The keto ester **6** (500 mg, 1.53 mmol) was treated with liquid NH_3 at 132° (pressure 1500 lb) for 24 hr; NH_3 was then evaporated and the residue was chromatographed on Florisil. The first eluents (acetone–cyclohexane, 1:1) yielded the unreacted ester. Further elution with benzene–MeOH (3:1) afforded the amide **7** (100 mg, 0.34 mmol, 22%), which crystallized from EtOH as small white needles: mp 239–240°; ν max 1685, 1630 cm^{-1}; λ_{max} 353 (7420), 307 (5780), 297 (5720). *Anal.* ($C_{17}H_{18}N_2O_3$) C, H, N.

Method B. The keto ester **6** (100 mg, 0.3 mmol) was refluxed with 2 ml of concentrated HCl for 1 hr. After cooling overnight in the refrigerator, 80 mg (0.24 mmol) of yellow crystals of the keto acid was obtained: mp 205–206°. The keto acid (40 mg, 0.13 mmol) was allowed to stand at room temperature with $SOCl_2$ (2 ml) for 10 hr, and $SOCl_2$ was then removed *in vacuo*. The residue was added in benzene suspension to ether–benzene (1:1) saturated with NH_3. The reaction was stirred at room temperature for 16 hr and then refluxed for 2 hr with occasional saturation with NH_3. Solvent was removed to yield a solid (14 mg, 0.05 mmol, 38%), which crystallized from EtOH as white needles: mp and mmp (with a sample prepared by method A) 239–240°.

6-Carbamylmethyl-3-ethoxy-5-hydroxy-8-methyl-5,6-dihydro-7*H*-cyclopenta[*f*]isoquinoline (8). A suspension of **7** (75 mg, 0.25 mmol) and $NaBH_4$ (100 mg, 2.64 mmol) in EtOH (25 ml) was stirred at room temperature for 4 hr. EtOH was removed *in vacuo* and the residue treated with H_2O and filtered to yield a white solid (70 mg, 0.25 mmol, 92%) which was crystallized from EtOH: mp 188–189°; ν max 3360, 1670 cm^{-1}; λ_{max} 340 (3810), 282 (4110), 273 (4600). *Anal.* ($C_{17}H_{20}N_2O_3$) C, H, N.

6-Carbamylmethyl-3-ethoxy-8-methyl-7(5)*H*-cyclopenta-[*f*]isoquinoline (9). A mixture of **8** (25 mg, 0.08 mmol) and *p*-toluenesulfonic acid (5 mg, 29 μmol) was refluxed in 15 ml of xylene under N_2. Xylene was removed *in vacuo* and the residue was treated with 5 ml of 2 *N* NaOH solution and extracted with $CHCl_3$. On drying (Na_2SO_4) and removal of solvent, the $CHCl_3$ extract afforded **9** (23 mg, 0.08 mmol, 98%), which crystallized from benzene as a pinkish solid: mp 208°; ν max 3400, 1645 cm^{-1}; λ_{max} 357 (5450), 337 (2880), 295 (7060), 238 (4460). *Anal.* ($C_{17}H_{18}N_2O_2$) C, H, N.

6-Carbethoxymethyl-3-ethoxy-8-methyl-7(5)*H*-cyclopenta[*f*]isoquinoline (10). The keto ester **6** (1 g, 3.05 mmol) and sodium borohydride (200 mg, 5.28 mmol) were stirred in 100 ml of EtOH at room temperature for 8 hr. Alcohol was removed *in vacuo* and the residue treated with 50 ml of H_2O and extracted with $CHCl_3$. On removal of solvent from the $CHCl_3$ layer, 980 mg of crude reduced product was obtained which was heated with *p*-toluenesulfonic acid (200 mg, 1.18 mmol) in xylene (100 ml) under N_2 for 5 hr. The xylene was removed and the residue treated with 20 ml of cold 1 *N* NaOH solution and extracted with $CHCl_3$. The material obtained after the removal of $CHCl_3$ was chromatographed over Florisil with $CHCl_3$ as eluent. The oily substance (450 mg, 1.4 mmol, 46%) from the column was crystallized from benzene–Skellysolve B to yield small white needles: mp 90–91°; ν max 1725, 1625 cm^{-1}; λ_{max} 357 (4580), 285 (1140), 233 (36,390); nmr 1.28 (t, 3 H, J = 7 Hz), 1.45 (t, 3 H, J = Hz) 2.45 (s, 3 H), 3.5 (s, 2 H), 3.62 (s, 2 H), 4.2 and 4.42 (two overlapping quartets), 7.15 (m, 2 H), 7.45 (s, 1 H), and 8.9 (s, 1 H). *Anal.* ($C_{19}H_{21}NO_3$) C, H, N.

6-Carbethoxymethyl-8-methyl-5,6-dihydro-7*H*-cyclopenta[*f*]isoquinoline-3(2*H*),5-dione (11). The keto ester **6** (50 mg, 0.15 mmol) was dissolved in 10 ml of HCl (25%) and heated in a sealed tube at 140° for 4 hr. The solution was evaporated and the

residue esterified with EtOH–H_2SO_4. After the usual work-up and crystallization from benzene, 11 was obtained as bright yellow solid (25 mg, 0.08 mmol, 53%): mp 210–211°; ν max 3430, 1725, 1700, 1640 cm^{-1}; λ_{max} (95% EtOH) 420 (3040), 360 (2940), 325 (4000), 312 (3830), 239 (38,080); λ_{max} (H_2O) 415 (4770), 325 (5420), 275 (4810), 238 (49,700); nmr ($CDCl_3$–CD_3OD) 1.25 (t, 3 H, J = 7 Hz), 2.4 (s, 3 H), 2.92 (m, 2 H), 3.35 (m, 3 H), 4.18 (q, 2 H, J = 7 Hz), 7.7 (s, 1 H), 7.95 (s, 1 H), 8.45 (s, 1 H). *Anal.* ($C_{17}H_{17}NO_4$) C, H, N.

6-Carbamylmethyl-8-methyl-5,6-dihydro-7*H*-cyclopenta-[*f*]isoquinoline-3(2*H*),5-dione (12). A suspension of 11 (150 mg, 0.50 mmol) in 40 ml of EtOH was treated with 60 ml of alcoholic NH_3. A clear yellow solution was formed, which was allowed to stand at room temperature for 20 hr and then refluxed for 4 hr. The solution was evaporated and crystallized from EtOH to afford 12 as a yellow solid (15 mg, 0.06 mmol, 11%). The compound does not melt, but gradually decomposes above 300°: ν max 3450, 1700, 1680 cm^{-1}; λ_{max} (95% EtOH) 420 (3900), 360 (3660), 323 (4800), 310 (4480); λ_{max} (H_2O) 405 (5150), 317 (4870), 275 (5170). *Anal.* ($C_{15}H_{14}N_2O_3$) C, H, N.

6-Carbamylmethyl-8-methyl-7(5)*H*-cyclopenta[*f*]isoquinolin-3(2*H*)-one (1). A mixture of 12 (65 mg, 0.24 mmol) and $NaBH_4$ (90 mg, 2.38 mmol) in 100 ml of EtOH was stirred at room temperature for 24 hr. The residue obtained after the usual work-up was refluxed with HCl in glacial HOAc (10 ml) for 1 hr. It was then evaporated to dryness and the residue was crystallized from EtOH to yield a yellow solid (10 mg, 0.04 mmol, 16%), which gradually decomposed above 250°: ν max 3440, 1645 cm^{-1}; λ_{max} (95% EtOH) 420 (1520), 347 sh (1810), 332 (2080), 263 (15,300); λ_{max} [H_2O (3% DMSO)] 418 (2500), 348 sh (1600), 333 (2000), 265 (20,000); nmr (pyridine-d_5) 2.28, 2.38 (s, 3 H, 2:1, $ArCH_3$); mass spectrum 254 (M^+). *Anal.* ($C_{15}H_{14}N_2O_2$) C, H, N.

RNA Polymerase Reaction. RNA polymerase was isolated from *E. coli* and inhibition of the RNA polymerase reaction was carried out as described by Burgess.[19] The reaction mixtures contained 10 μg of template per milliliter and 5% of dimethyl sulfoxide, the enzyme concentration being 29 μg/ml.

Thermal Transition Temperature. Thermal transition temperature was determined on a Gilford 2400-S, equipped with a variable temperature bath. Studies on calf thymus DNA and the poly(deoxyribonucleotides) were made in a phosphate–EDTA buffer (pH 7.8): PO_4^{3-} = 0.001 M, Na^+ = 0.002 M, EDTA = 10^{-4} M, DMSO = 0.6% by volume; concentration of DNA-P = 4×10^{-5} M and concentration of 1 = 2.5×10^{-5} M.

Difference Spectra. Difference spectra were determined[18] on a Cary 15 spectrophotometer with 0–0.1 OD 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 the compound and calf thymus DNA were placed in separate compartments of both cells. All solutions were made in phosphate buffer, pH 7.21 ± 0.01 (PO_4^{3-} = 0.001 M, NaCl = 0.01 M). The concentration of the compound after mixing was 2×10^{-4} M and calf thymus DNA concentration was 10 OD. All reactions were carried out at 24°.

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Chemistry of Cephalosporin Antibiotics. 30.[1] 3-Methoxy- and 3-Halo-3-cephems

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The *exo*-methylene group in esters of 7-acylamido- and 7-amino-3-methylenecephams was ozonized to give 3-hydroxy-3-cephems. Conditions are described to effect a selective N-acylation of a 3-hydroxy-3-cephem nucleus ester. Vilsmeier reagents converted 7-acylamido-3-hydroxy compounds to 3-halo-3-cephem derivatives. Diazomethane converted the 3-hydroxy compounds to 3-methoxy-3-cephem derivatives. Removal of the ester-protecting group at the C_4-carboxyl afforded a select group of cephalosporins with direct halo and methoxy substitution at C_3. A number of these compounds are potent antibiotics.

We have recently reported on the preparation of 3-methylenecephams from reductions of cephalosporanic acids in which the acetoxy group is displaced by sulfur nucleophiles[2a] (Scheme I). A reductive cleavage of the acetoxy group in cephalosporanic acids leading to 3-methylenecephams using chromium(II) salts has also been reported.[2b] As esters of the 3-methylenecephams can be readily isomerized to deacetoxycephalosporins, they were shown to be useful precursors to intermediates in published syntheses[3] of cephalexin (1).[4] This paper focuses on the oxidation of the 3-*exo*-methylene grouping in 3-methylenecephams as a step in the preparation of a new class of cephalosporins with the unique structural feature of direct heteroatom substitution at C_3.[5] These compounds possess useful antimicrobial activity.

Our initial oxidation studies were performed on 7-acylamido-3-methylenecepham esters 2 (Scheme II). Low-temperature ozonolysis of methyl 7-phenoxyacetamido-3-methylenecepham-4-carboxylate (**2a**) gave the 3-hydroxy derivative **3a**, some 1-sulfoxide of the starting cephalosporin, and decomposition products. Compound **3a** was isolated pure by preparative tlc silica gel and was shown by uv analysis (pH 7 buffer) to exist largely in the enol (3-hydroxy-3-cephem) form. Absorption in the region of 268 mμ for the