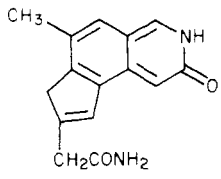
New Cyclopenta[*a*]naphthalene Derivatives. Synthesis of 2-(Carbamylmethyl)-8-hydroxy-3*H*-cyclopenta[*a*]naphthalene as a Possible Deoxyribonucleic Acid Binding Agent

Nitya G. Kundu

School of Pharmacy and LAC-USC Cancer Center Research Laboratory, University of Southern California, Los Angeles, California 90033. *Received September 7, 1979*

8-Methoxy-1-oxo-2,3-dihydro-1*H*-cyclopenta[*a*]naphthalene (**4**) was converted to the oxalyl derivative (**7**) by treatment with diethyl oxalate in the presence of sodium ethoxide. Compound **7** in the form of the sodium salt was alkylated with ethyl bromoacetate in DMF to 2-(carbethoxymethyl)-8-methoxy-1-oxo-2,3-dihydro-1*H*-cyclopenta[*a*]naphthalene (**8**). Treatment of **8** with methanolic ammonia yielded the corresponding amide (**9**). Dealkylation of **8** with 48% HBr and subsequent esterification gave compound **10**. Ammonolysis of **10** led to the amide **11**, which after reduction and subsequent dehydration of the reduced product afforded the desired compound, 2-(carbamylmethyl)-8-hydroxy-3*H*-cyclopenta[*a*]naphthalene (**2**). Compound **2** was found to be mildly growth inhibitory to L1210 and CCRF-CEM leukemic cells in culture. From thermal transition temperature studies, compound **2** was found to bind to calf thymus DNA and the poly(deoxyribonucleotides), e.g., poly(dG)·poly(dC), poly(dG-dC), poly(dA)·poly(dT), and poly(dA-dT).

It is known from studies of antibiotics which have found important clinical applications in cancer chemotherapy that these compounds probably exert their chemotherapeutic activities by inhibition of DNA and/or RNA syntheses.[1-11] These biochemical effects are dependent on the binding of these compounds to double-helical DNA by intercalation, hydrogen bonds, and hydrophobic interactions. We have been interested in the development of molecules on the basis of molecular model studies that will bind to double-helical DNA through simple forces such as hydrogen-bond formation.[12,13] In consequence, we have reported the development of a cyclopenta[*f*]isoquinoline derivative, **1**, which binds weakly to double-helical DNA



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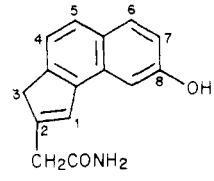

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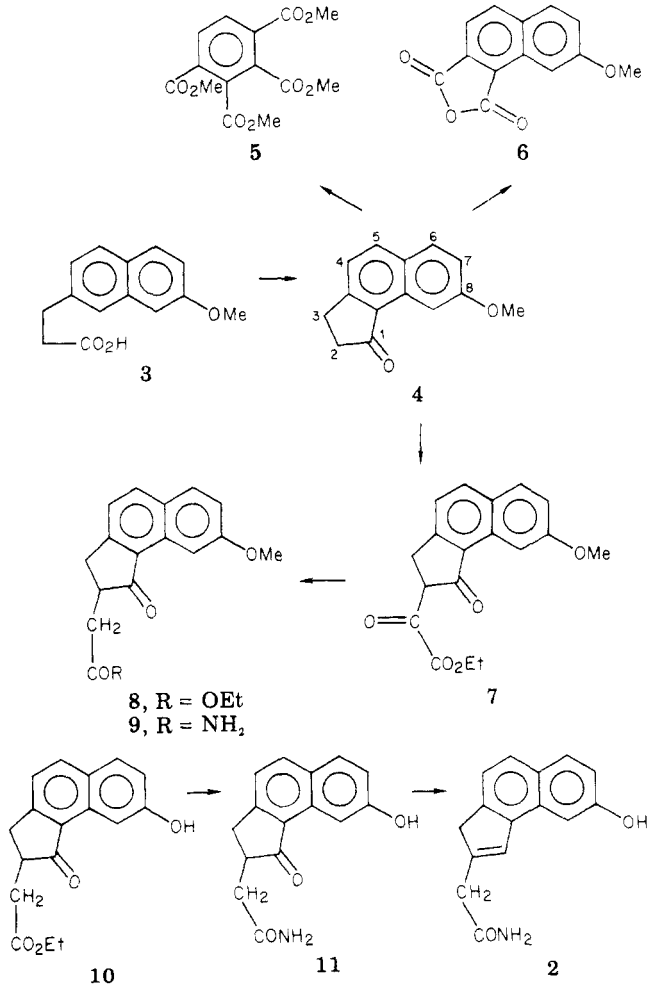
with some GC specificity.[14] The cyclopenta[*a*]naphthalene derivative, **2**, which is the carbocyclic equivalent of **1**, according to molecular models (see Figure 1) should bind to adenine-thymine (AT) pairs of double-helical DNA such that the C-8 hydroxyl group of **2** donates a hydrogen bond to the C-4 carbonyl group of thymine and the amide portion of the side chain donates a hydrogen bond to the N-7 of adenine. Because of the fulfillment of expectations with the cyclopenta[*f*]isoquinoline derivative, **1**, we were encouraged to develop the carbocyclic analogue, **2**, and study its properties. In this paper, the synthesis of compound **2** and related compounds and some of their biological properties are reported.

Scheme I



Chemistry. The synthesis of compound **2** was accomplished as shown in Scheme I. The basic tricyclic ring system of **2** (compound **4**) has been reported by Moffatt.[15]

(1) D. W. Henry, *ACS Symp. Ser.*, **no. 30**, 15–57 (1976).
(2) E. Calendi, A. DiMarco, M. Reggiani, B. Scarpinato, and L. Valentini, *Biochim. Biophys. Acta*, **103**, 25 (1965).
(3) A. DiMarco, *Cancer Chemother. Rep., Part 3*, **6**, 91 (1975).
(4) F. Arcamone, *Lloydia*, **40**, 45 (1977).
(5) H. M. Sobell, *Prog. Nucleic Acid Res. Mol. Biol.*, **13**, 153 (1973).
(6) P. B. Joel, P. A. Friedman, and I. H. Goldberg, *Biochemistry*, **9**, 4421 (1970).
(7) M. Waring, *J. Mol. Biol.*, **54**, 247 (1970).
(8) R. D. Wells and J. E. Larson, *J. Mol. Biol.*, **49**, 319 (1970).
(9) B. K. Bhuyan and C. G. Smith, *Proc. Natl. Acad. Sci. U.S.A.*, **54**, 566 (1965).
(10) H. Suzuki, K. Nagai, E. Akutsu, H. Yamaki, N. Tanaka, and H. Umezawa, *J. Antibiot.*, **23**, 473 (1970).
(11) P. Chandra and M. Woltersdorf, *Biochem. Pharmacol.*, **25**, 877 (1976).
(12) N. G. Kundu, J. A. Wright, K. L. Perlman, W. Hallett, and C. Heidelberger, *J. Med. Chem.*, **18**, 395 (1975).
(13) N. G. Kundu, W. Hallett, and C. Heidelberger, *J. Med. Chem.*, **18**, 399 (1975).
(14) N. G. Kundu and C. Heidelberger, *Biochem. Biophys. Res. Commun.*, **60**, 561 (1974).

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Table I. UV Spectra of Some Cyclopenta[a]naphthalene Derivatives

no.	λ, nm (ε)				
4	341 (6364)	314 (6182)	262 (11 545)	258 (11 273)	229 (56 091)
7	371 (25 384)			255 (14 835)	222 (54 285)
8	342 (7554)	315 (7173)	263 (11 847)		230 (44 021)
9	343 (7604)	317 (6979)	264 (11 666)		229 (57 291)
10	350 (7252)	318 (5824)	263 (9725)		230 (40 329)
11	349 (7340)	317 (6010)	263 (9574)		223 (37 765)
2	330 (2562)	318 (2397)	294 (5372)	274 (6364)	234 (95 041)

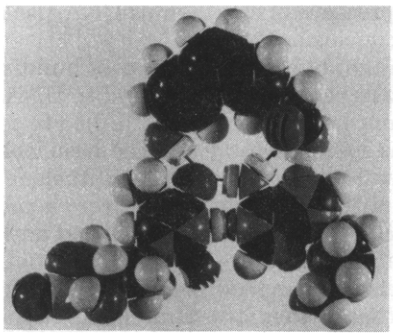


Figure 1. CPK model of the complex between an AT pair of DNA and the cyclopenta[a]naphthalene derivative **2**.

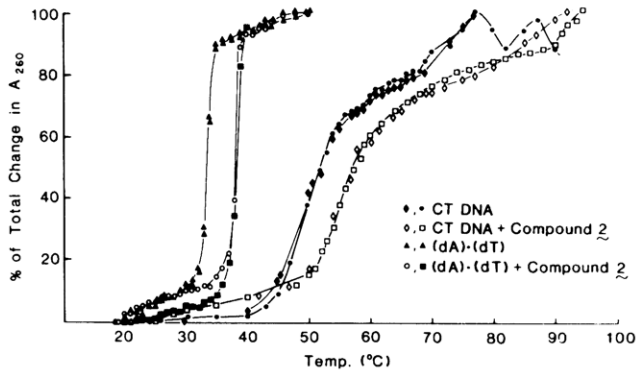


Figure 2. Thermal transition temperature of calf thymus DNA and (dA)·(dT) in the absence and presence of compound **2**. The reaction conditions are described under Experimental Section.

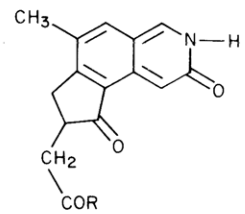
We have successfully followed his procedure of synthesizing **4** from 2-(β-carboxyethyl)-7-methoxynaphthalene (**3**) by hydrofluoric acid cyclization. Compound **3** was synthesized by a slight modification of Moffatt's procedure as described under Experimental Section. Moffatt has deduced the structure of **4** on the basis of ultraviolet spectra. Because of the importance of the structure of **4** to us, we have confirmed his structural assignment by conversion of ketone **4** to tetramethyl benzene-1,2,3,4-tetracarboxylate (**5**)[16] by nitric acid oxidation of **4** and subsequent esterification of the product with methanol and sulfuric acid. Further confirmation of the structure of **4** was obtained by its oxidation with potassium ferricyanide[17] and potassium hydroxide to 7-methoxynaphthalene-1,2-dicarboxylic anhydride (**6**), which was identical with an authentic sample[18] by mixture melting point and NMR comparisons.

Compound **4** was easily converted to the corresponding oxalyl derivative, **7**, by treatment with diethyl oxalate in the presence of sodium ethoxide in benzene. The oxalyl derivative, **7**, in the form of the sodium salt, reacted with ethyl bromoacetate in DMF at 100 °C; alkylation and subsequent elimination of the oxalyl group took place. Removal of DMF and workup yielded an oil that was purified by chromatography over Florisil to give the crystalline keto ester **8**. Treatment of **8** with methanolic ammonia yielded the keto amide **9**. Dealkylation of **8** was effected by refluxing with 48% hydrobromic acid.[19,20] The dealkylated product on esterification led to compound **10**, which on treatment with methanolic ammonia gave compound **11**. Sodium borohydride reduction of **11** and subsequent dehydration of the reduced product with hydrochloric acid in acetic acid led to the target compound, **2**. The 7-methoxycyclopenta[a]naphthalene-1-oxo derivatives, e.g., **4**, **8**, and **9**, are characterized by an ultraviolet absorption band at about 342 nm (Table I). In the phenolic compounds, **10** and **11**, there was a bathochromic shift of this band to 350 nm. However, in contrast to the cyclopenta[f]isoquinoline derivatives, **12** and **13**, which

Table II. Change in Thermal Transition Temperature (ΔT_m) of Calf Thymus DNA and Poly(deoxyribonucleotides) in the Presence of **2**[a]

DNA or poly(deoxyribonucleotides)	T_m, °C
calf thymus DNA	5
(dA)·(dT)	5
(dA-dT)·(dA-dT)	4
(dG)·(dC)	4.5
(dG-dC)·(dG-dC)	6

[a] For conditions, see Experimental Section.



12, R = OEt
13, R = NH_2

show absorption[14] at 420 nm and exist primarily in the lactam form, compounds **10** and **11** did not show any absorption at higher wavelengths, indicating that these compounds exist predominantly in the phenolic forms as shown in structures **10** and **11**. In the target compound, the highest ultraviolet absorbing band was at 330 nm, establishing the predominantly phenolic nature of this compound.

Growth Inhibition Studies. The growth inhibitory effects of these compounds were studied against L1210[21] and CCRF–CEM leukemic cells in culture.[22] Compound

(15) J. S. Moffatt, *J. Chem. Soc. C*, 725 (1966).
(16) W. H. Linnell, D. W. Mathieson, and D. T. Modi, *J. Chem. Soc.*, 3257 (1953).
(17) M. F. Ansell, *J. Chem. Soc.*, 575 (1954).
(18) L. F. Fieser and E. B. Hershberg, *J. Am. Chem. Soc.*, **58**, 2314 (1936).
(19) A. Brossi and S. Teitel, *Helv. Chim. Acta*, **53**, 1779 (1970).
(20) T. L. V. Ulbricht, *J. Chem. Soc.*, 3345 (1961).
(21) G. E. Moore, A. A. Sandberg, and K. Ulrich, *J. Natl. Cancer Inst.*, **36**, 405 (1966).

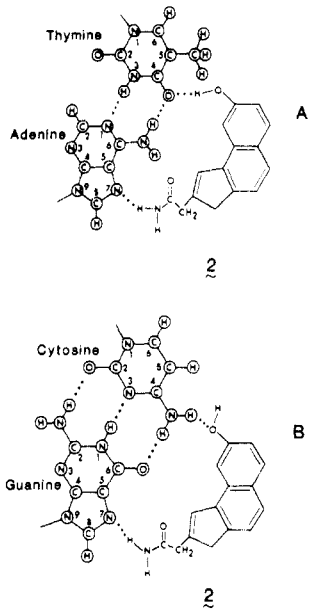


Figure 3. (A) A two-dimensional diagram of the complex between an AT pair of DNA and the cyclopenta[*a*]naphthalene derivative **2**. (B) A two-dimensional diagram of the complex between a GC pair of DNA and the cyclopenta[*a*]naphthalene derivative **2**.

2 inhibited by 25% the growth of L1210 and CCRF-CEM cells at concentrations of 3.2×10^{-5} and 1.0×10^{-4} M, respectively (see Experimental Section).

Interaction with DNAs and Poly(deoxyribonucleotides). The cyclopenta[*a*]naphthalene derivative, **2**, was found to interact with DNAs and poly(deoxyribonucleotides) very weakly. From different spectra studies,[14] a ΔOD of +0.007 was found for the interaction between **2** and native calf thymus DNA. The fluorescence spectra of **2** did not show any change in the presence of calf thymus DNA. The weak nature of the binding between **2** and calf thymus DNA is also apparent from the effect of **2** on the thermal transition temperature (T_m) of native calf thymus DNA (Figure 2). A small but significant increase in T_m of 5 °C was observed. In the case of other poly(deoxyribonucleotides), similar small increases in T_m (Table II) were observed in the presence of **2**. However, no difference of ΔT_m was observed in the interaction of **2** with G-C and A-T containing poly(deoxyribonucleotides).

Discussion

This study has shown that the cyclopenta[*a*]naphthalene derivative **2**, which from molecular models is expected to bind to DNA, does bind to native calf thymus DNA, although very weakly. However, in contrast to the cyclopenta[*f*]isoquinoline derivative, **1**, which binds with DNA with some GC specificity,[13,14] the binding of the cyclopenta[*a*]naphthalene derivative **2** was nonspecific. This probably can be explained by the possibility that the phenolic 8-hydroxyl group in **2** can bind with the C_4 carbonyl group of thymine through a H bond (Figure 3A) but also can hydrogen bond with the amino group of cytosine (Figure 3B). Ts'o and co-workers[24] have observed a lowering of T_m of poly(A) and calf thymus DNA in the presence of phenol, pyrimidine, uridine, thymidine, purine, caffeine, and coumarin. They attributed this to hydrophobic interactions between the small molecules and DNA. Kersten and co-workers[25] have observed that the aromatic hydrocarbon 7,12-dimethylbenz[*a*]anthracene did not influence the melting behavior of DNA. The increase in T_m of DNA and poly(deoxyribonucleotides) in the presence of **2** can better be explained by hydrogen bonded rather than hydrophobic interactions. Since we observe only a negligible change in the difference spectra of **2** at 300 nm, the intercalative interaction between **2** and DNA and poly(deoxyribonucleotides) does not seem of much significance. The lack of change in the fluorescence spectra of **2** in the presence of calf thymus DNA also agrees with the above conclusion.[26]

It is seen that our basic objective of building up molecules that will be cytotoxic and bind to DNAs and poly(deoxyribonucleotides) has been fulfilled to some extent, although the specificity which we had been looking for was not found with the cyclopenta[*a*]naphthalene derivative, **2**. In view of the importance in cancer chemotherapy of small molecules, such as tilorone[27-31] and anthramycin,[32] and polynuclear structures, such as ellipticine,[33] which exert their chemotherapeutic activities by binding to DNA, we believe that further structural manipulations of molecules such as compounds **1** and **2**, that were devised on the basis of molecular model studies, might lead to useful cancer chemotherapeutic agents.

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 taken in 95% ethanol unless otherwise mentioned. The IR spectra were done on a Beckman IR-10 as KBr plates. NMR spectra (reported in δ) were recorded on a Varian EM-390 90-MHz NMR spectrometer in solvents indicated, 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 unless otherwise noted. Mass spectra were taken on a Varian MS-9 spectrophotometer. TLC was performed on an Eastman Chromagram sheet (6060 silica gel with fluorescent indicator) in the indicated solvents: solvent A, benzene; solvent B, chloroform; solvent C, chloroform/methanol (10:1).

Calf thymus deoxyribonucleic acid was purchased from Worthington Biochemical Corp., Freehold, N.J., and was their highest grade of purity. It was further purified[23] by treatment with pronase and ribonuclease, 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. 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.

2-(β-Carboxyethyl)-7-methoxynaphthalene (3). A mixture of 2-(hydroxymethyl)-7-methoxynaphthalene[15] (1.8 g, 9.57 mmol) and freshly distilled PBr_3 (10 mL) was shaken for 4 days at room temperature. The mixture was cautiously dropped onto ice and

(22) G. E. Foley, H. Lazarus, S. Farber, B. G. Uzman, B. A. Boone, and R. E. McCarthy, *Cancer*, **18**, 522 (1965).
(23) J. Murmur, *J. Mol. Biol.*, **3**, 208 (1961).
(24) P. O. P. Ts'o, G. K. Helmkamp, and C. Sander, *Proc. Natl. Acad. Sci. U.S.A.*, **48**, 686 (1962).
(25) W. Kersten, H. Kersten, and W. Szybalski, *Biochemistry*, **5**, 236 (1966).
(26) E. Boyland and B. Green, *Br. J. Cancer*, **16**, 347 (1962).
(27) R. H. Adamson, *J. Natl. Cancer Inst.*, **46**, 431 (1971).
(28) G. L. Wampler, M. Kuperminc, and W. Regelson, *Cancer Chemother. Rep.*, **57**, 209 (1973).
(29) M. P. Schafer, M. A. Chirigos, and T. S. Papas, *Cancer Chemother. Rep., Part 1*, **58**, 821 (1974).
(30) P. Chandra, F. Zunino, V. P. Gaur, A. Zaccara, M. Woltersdorf, G. Luoni, and A. Gotz, *FEBS Lett.*, **28**, 5 (1972).
(31) P. Chandra and M. Woltersdorf, *FEBS Lett.*, **41**, 169 (1974).
(32) S. B. Horwitz, in "Antineoplastic and Immunosuppressive Agents", Part II, A. C. Sartorelli and D. G. Johns, Eds., Springer-Verlag, New York, 1975, Chapter 64, p 642.
(33) J.-B. Le Pecq, N. D. Xuong, C. Gosse, and C. Paoletti, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 5078 (1974).

extracted with ether. The ether extract was washed with brine and dried, and the solvent was removed; a white residue (2 g) was obtained.

Sodium (1 g, 43.37 mmol) was dissolved in absolute EtOH (20 mL). To the cold solution, distilled diethyl malonate (7 g, 43.75 mmol) in EtOH (10 mL) was added dropwise, and the mixture was stirred overnight. EtOH was removed and the dry residue was dissolved in anhydrous DMF (25 mL). To the cooled (ice bath) solution, the above bromo compound dissolved in DMF (10 mL) was added. The mixture was stirred in the cold for 2 h and then heated at 100 °C for 16 h. DMF was removed under vacuum and the residue was treated with water (25 mL), acidified to pH 1, and extracted with ether (3 × 100 mL). The ethereal layer on removal of solvent gave a residue which was hydrolyzed by refluxing with NaOH (3 g) and H_2O (30 mL) for 3 h. After removal of the neutral material by extraction with ether, the alkaline layer on acidification yielded an acid (2.1 g), mp 148–150 °C, which was filtered and dried. The acid was decarboxylated by heating at 200–210 °C for 45 min. The residue was taken up in ether and extracted with $NaHCO_3$ (5%) solution. On acidification of the bicarbonate extract, a white solid (1.65 g, 7.17 mmol, 75%) was obtained which was filtered, dried, and crystallized from 95% EtOH into glistening white plates of **3**: mp 165–166 °C (lit.[15] mp 164–166 °C); NMR (Me_2SO-d_6) δ 2.7 (t, 2 H, J = 6 Hz, $-CH_2-$), 3.03 (t, 2 H, J = 6 Hz, $-CH_2-$), 3.88 (s, 3 H, $-OCH_3$), between 7.0 and 8.0 (m, 6 H, aro-H).

8-Methoxy-1-oxo-2,3-dihydro-1*H*-cyclopenta[*a*]naphthalene (4). A mixture of **3** (1.5 g, 6.52 mmol) and anhydrous HF (15 mL) was allowed to stand at room temperature for 90 h. The residue (1.3 g, 6.13 mmol, 94%) obtained after removal of HF was crystallized from 95% EtOH into cream-colored needles of **4**: mp 117–118 °C (lit.[15] mp 119 °C); TLC R_f 0.15, 0.38, and 0.66 in solvents A, B, and C, respectively; IR ν_{max} 1680, 1620 cm^{-1}; UV λ_{max} 341 nm (ε 6364), 314 (6182), 262 (11 545), 258 (11 273), 229 (56 091); MS m/e 212 (M^+).

Conversion of 8-Methoxy-1-oxo-2,3-dihydro-1*H*-cyclopenta[*a*]naphthalene (4) to Tetramethyl Benzene-1,2,3,4-tetracarboxylate (5). A mixture of **4** (500 mg), fuming HNO_3 (1.5 mL), and H_2O (0.75 mL) was heated in a sealed tube at 160–170 °C for 24 h. The reaction mixture was evaporated to dryness and the residue esterified with MeOH–H_2SO_4. After the usual workup and crystallization from MeOH, tetramethyl benzene-1,2,3,4-tetracarboxylate (**5**) was obtained as colorless needles, mp 131–132 °C (lit.[16] 132–133 °C).

Conversion of 8-Methoxy-1-oxo-2,3-dihydro-1*H*-cyclopenta[*a*]naphthalene (4) to 7-Methoxynaphthalene-1,2-dicarboxylic Anhydride (6). To a solution of potassium ferricyanide (50 g), KOH (9.0 g), and H_2O (180 mL), the ketone **4** (800 mg) was added. The mixture was heated with stirring at 60–70 °C for 24 h, cooled to room temperature, and filtered. The filtrate was acidified to pH 1 with dilute H_2SO_4, and the separated solids were filtered and air-dried. The solids were then sublimed and the sublimate crystallized from benzene in bright yellow crystals of **6**: mp 193–194 °C (lit.[18] mp 194–195 °C); IR ν_{max} 1840, 1760 cm^{-1}; mixture melting point with an authentic sample remained undepressed and the NMR spectra were identical.

2-Ethoxalyl-8-methoxy-1-oxo-2,3-dihydro-1*H*-cyclopenta[*a*]naphthalene (7). To a suspension of sodium ethoxide (from 0.93 g of sodium, 40.4 mmol) in 40 mL of benzene, diethyl oxalate (4.06 mL) in 15 mL of benzene was added and stirred for 1 h, followed by the addition of **4** (1.0 g, 4.7 mmol) in 60 mL of benzene. The mixture was stirred under N_2 for 24 h, decomposed with 6 N hydrochloric acid, and extracted with chloroform. The combined benzene–chloroform layer was dried (Na_2SO_4) and the solvent was removed. The residue was washed with ether, filtered to yield a yellow solid (1.2 g, 3.84 mmol, 81.7%), and crystallized from $CHCl_3$ into yellow silky needles of **7**: mp 156–157 °C; TLC R_f 0.09, 0.21, and 0.82 in solvents A, B, and C, respectively; IR ν_{max} 1730, 1670, 1620 cm^{-1}; UV λ_{max} 371 nm (ε 25 384), 255 (14 835), 222 (54 285). Anal. ($C_{18}H_{16}O_5$) C, H.

2-(Carbethoxymethyl)-8-methoxy-1-oxo-2,3-dihydro-1*H*-cyclopenta[*a*]naphthalene (8). The oxalyl derivative **7** (1 g, 3.2 mmol) was added to a suspension of sodium ethoxide (made from 100 mg of sodium, 4.35 mmol) in benzene (50 mL). The mixture was stirred under a nitrogen atmosphere for 2 h and then benzene was removed in vacuo. The dry sodium salt was suspended in DMF (25 mL), and ethyl bromoacetate (830 mg, 4.97 mmol) in 10 mL of DMF was added dropwise with stirring at room temperature. The mixture was then heated at 100 °C with stirring for 20 h, DMF was removed in vacuo, and the residue was treated with H_2O and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with water and dried (Na_2SO_4), and the solvent was removed to yield an oil (800 mg, 2.69 mmol, 84%). This was purified by chromatography on a column of Florisil (60–100 mesh) using $CHCl_3$ as eluent. The $CHCl_3$ eluents were combined and evaporated to dryness. The residue was crystallized from 95% EtOH as light yellow colored needles of **8**: mp 110–111 °C; TLC R_f 0.07, 0.14, and 0.84 in solvents A, B, and C, respectively; IR ν_{max} 1730, 1680, 1620 cm^{-1}; UV λ_{max} 342 nm (ε 7554), 315 (7173), 263 (11 847), 230 (44 021); NMR ($CDCl_3$) δ 1.13 (t, 3 H, J = 7 Hz, CH_3- of ester), 3.9 (s, 3 H, $-OCH_3$), 4.08 (q, 2 H, $-CH_2-$ of ester), between 2.3 and 3.4 (m, 5 H), between 7 and 8.4 (m, 5 H, aro-H's); MS m/e 298 (M^+), 253 (M^+ – OEt), 225 (M^+ – $CO_2C_2H_5$). Anal. ($C_{18}H_{18}O_4$) C, H.

2-(Carbamylmethyl)-8-methoxy-1-oxo-2,3-dihydro-1*H*-cyclopenta[*a*]naphthalene (9). A solution of the keto ester **8** (600 mg, 2.0 mmol) in methanolic NH_3 (50 mL) was stirred at room temperature for 2 weeks. The reaction mixture was evaporated to dryness and the residue crystallized from 95% EtOH to white fine needles of **9** (500 mg, 1.86 mmol, 93%): mp 186–187 °C; TLC R_f 0.60 in solvent C; UV λ_{max} 343 nm (ε 7604), 317 (6979), 264 (11 666), 229 (57 291). Anal. ($C_{16}H_{15}NO_3$) C, H, N.

2-(Carbethoxymethyl)-8-hydroxy-1-oxo-2,3-dihydro-1*H*-cyclopenta[*a*]naphthalene (10). The keto ester **8** (1.15 g, 3.85 mmol) was refluxed with 48% hydrobromic acid (20 mL) and glacial acetic acid (10 mL) for 5 days. The mixture was evaporated to dryness in vacuo and the dry residue was esterified with EtOH and sulfuric acid. After usual workup, the residue was chromatographed on Florisil. The first eluents ($CHCl_3$) yielded the unreacted ester. Further elution with $CHCl_3$–MeOH (10:1) afforded the dealkylated ester **10** (900 mg, 3.17 mmol, 82.3%), which was crystallized from 95% EtOH into light yellow needles: mp 182–183 °C; TLC R_f 0.77 in solvent C; IR ν_{max} 3200, 1720, 1670, 1620 cm^{-1}; UV λ_{max} 350 nm (ε 7252), 318 (5824), 263 (9725), 230 (40 329); NMR (Me_2SO-d_6) 1.18 (t, 3 H, J = 7 Hz, CH_3- of ester), 4.05 (q, 2 H, J = 7 Hz, $-CH_2-$ of ester), between 2.5 and 3.5 (m, 5 H), between 7 and 8.4 (m, 5 H, aro-H); MS m/e 284 (M^+), 239 (M^+ – OC_2H_5), 211 (M^+ – $CO_2C_2H_5$). Anal. ($C_{17}H_{16}O_4$) C, H.

2-(Carbamylmethyl)-8-hydroxy-1-oxo-2,3-dihydro-1*H*-cyclopenta[*a*]naphthalene (11). The keto ester **10** (400 mg, 1.41 mmol) was stirred with MeOH (50 mL) saturated with ammonia gas for 7 days at room temperature. The mixture was evaporated to dryness and the residue chromatographed over Florisil. After initial washing with $CHCl_3$, the product was eluted with $CHCl_3$–MeOH (10:1), yielding a solid (100 mg, 0.39 mmol, 27.6%), which was crystallized from 95% EtOH into light yellow fine crystals of **11**: mp 252–253 °C dec; TLC R_f 0.48 in solvent C; IR ν_{max} 3400, 3200, 1680, 1650, 1620 cm^{-1}; UV λ_{max} 349 nm (ε 7340), 317 (6010), 263 (9574), 223 (37 765); MS m/e 255 (M^+), 238 (M^+ – OH), 211 (M^+ – $CONH_2$). Anal. ($C_{15}H_{13}NO_3$) C, H, N.

2-(Carbamylmethyl)-8-hydroxy-3*H*-cyclopenta[*a*]naphthalene (2). A mixture of **11** (500 mg, 1.96 mmol) and $NaBH_4$ (400 mg, 10.5 mmol) in EtOH (200 mL) was stirred at room temperature for 20 h. Excess borohydride was decomposed with glacial acetic acid and the mixture was evaporated to dryness. The residue was refluxed with concentrated hydrochloric acid (1 mL) in glacial acetic acid (20 mL) for 2 h under nitrogen. The reaction mixture was evaporated to dryness and crystallized from ethanol into small very light pinkish needles of **2** (240 mg, 1.0 mmol, 51%): mp 284 °C (presoftening takes place at 249–250 °C); TLC R_f 0.60 in solvent C; IR ν_{max} 3200, 1670, 1630 cm^{-1}; UV λ_{max} 330 nm (ε 2562), 318 (2397), 294 (5372), 274 (6364), 234 (95 041); MS m/e 239 (M^+), 195 (M^+ – $CONH_2$). Anal. ($C_{15}H_{13}NO_2$) C, H, N.

Difference Spectra. Difference spectra were determined[14] on a Cary 15 spectrophotometer with 0–0.1 OD scale expansion between 400 and 250 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 7.00 ● 0.01 (PO_4^{3-} = 0.001 M; NaCl = 0.002 M; EDTA = 10^{-4} M). The concentration of the

compound after mixing was 5×10^{-5} M and the concentration of DNA-P for calf thymus DNA was 1.7×10^{-3} M. All reactions were carried out at 24 °C.

Thermal Transition Temperature. Thermal transition temperature was determined on a Gilford 2400-S, equipped with a variable-temperature bath. Compound **2** was dissolved in 2 drops of 0.1 M NaOH solution and then diluted with H_2O to appropriate concentration, pH being adjusted to 7.8. Studies on calf thymus DNA and the poly(deoxyribonucleotides) were made in a phosphate–EDTA buffer (pH 7.8): final concentrations were PO_4^{3-} = 0.001 M; Na^+ = 0.002 M; EDTA = 10^{-4} M; **2** = 2.5×10^{-5} M. Concentrations of DNA-P for calf thymus DNA, poly(dA)·poly(dT), poly(dA-dT), poly(dG)·poly(dC), and poly(dG-dC) were 4.0×10^{-5}, 5.4×10^{-5}, 5.1×10^{-5}, 5.6×10^{-5}, and 5.7×10^{-5} M, respectively.

Fluorescence Spectra. Fluorescence spectra were determined on an Aminco Bowman spectrofluorometer, American Instruments Co., Silver Springs, Md. Spectra of **2** were taken at a concentration of 1×10^{-6} M in phosphate buffer, pH 7.80 ± 0.01 (PO_4^{3} = 0.001 M; NaCl = 0.002 M). Compound **2** showed an emission band at 408 nm corresponding to excitation at 300 nm. No change in the fluorescence spectra of **2** was seen in the presence of calf thymus DNA; concentration of DNA-P was 1.6×10^{-3} M.

Growth Inhibition Assay. L1210 cells and CCRF–CEM cells were grown in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% dialyzed fetal calf serum (DFCS) with a doubling time of 10–12 and 18–24 h, respectively. Compound **2** was dissolved in 2 drops of 0.1 M NaOH solution, and compound **1** was dissolved in a few drops of Me_2SO. The solutions were diluted to a stock solution of 10^{-3} M with phosphate-buffered saline, sterilely filtered, and aseptically diluted by half log increments. Each concentration (0.7 mL) was added to duplicate 13 × 75 mm test tubes. Cells from logarithmically growing stock culture were suspended in prewarmed RPMI 1640 medium supplemented with 10% dialyzed fetal calf serum, 10 mM Mops (morpholinepropanesulfonic acid), and 20 mM Hepes [*N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid]. Cell suspension (1.8 mL) was added to each tube. The tubes were incubated upright at 37 °C in a warm room or dry incubator. Under these conditions, L1210 cells grew exponentially 15- to 25-fold from an initial density of $2\text{–}2.5 \times 10^4/cm^3$; CCRF–CEM cells grew exponentially 8- to 10-fold. After 48 h (for L1210 cells), or 72 h (for CCRF–CEM cells), the incubation was terminated and the cell densities were determined using a Coulter Counter. The degree of proliferation of each 2-mL culture was expressed as the ratio of the final cell density to the initial cell density; this index was plotted against the drug concentration employed. The concentration of drug which depresses the ratio to 50% of control (the IC_{50}) is graphically determined. Compound **1** inhibited by 25% the growth of L1210 and CCRF–CEM cells at concentrations of 1.0×10^{-4} and 8.0×10^{-5} M, respectively. For the clinically effective drug, adriamycin, $IC_{50} = 1.9 \times 10^{-9}$ and 2.0×10^{-8} M were found for L1210 and CCRF–CEM cells in culture, respectively. IC_{50} for compounds **2** and **1** could not be determined due to solubility problem; at higher concentrations, the compounds crystallized out from the medium.

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4-[(Aminoalkyl)amino]-1,2-dimethoxynaphthalenes as Antimalarial Agents

Sydney Archer,* Peter Osei-Gyimah, and Stephen Silbering

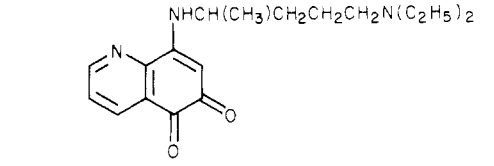
Chemistry Department, Rensselaer Polytechnic Institute, Troy, New York 12181. *Received September 5, 1979*

A series of 4-[*N*-(aminoalkyl)amino]-1,2-dimethoxynaphthalenes was prepared and tested for radical curative activity in rhesus monkeys infected with *P. cynomolgi*. Some radical curative activity was observed.

Antimalarial agents possessing suppressive activity are represented by many different structural types.[1] On the other hand, "the only drugs that accomplish radical cure in *vivax malaria* are the 8-aminoquinolines of which only *primaquine* is used now".[2] Primaquine and its congeners are toxic to some groups of individuals;[3] thus, it would be desirable to have available a less toxic radical curative agent, preferably one of a different structural type.

It is generally believed that the 8-amino-6-methoxyquinolines are biotransformed to active metabolites in the host and that the active form of pamaquine is the quinone **1**.[1] The hydroxynaphthoquinone, menoctone, was shown to have causal prophylactic activity in mice infected with *P. berghei*.[4]

Beaudoin and his colleagues devised a tissue culture system wherein they were able to study the action of drugs on the exoerythrocytic forms of the avian malaria parasite *P. fallax*.[5] Morphologically they were able to distinguish between the effects of drugs which were suppressive from the effects of drugs which were radically curative. In this system, menoctone resembled primaquine more than it did pyrimethamine, a causal prophylactic drug.



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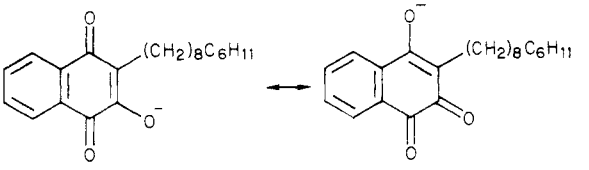

2 3

In aqueous solution menoctone exists as the ion **2**, a resonance form of which is **3**. The *o*-quinonoid form **3** resembles **1** in the sense that both are *o*-quinones which have electron-donating groups in the "para" position directly opposite one of the quinone carbonyl groups. Since

(1) R. N. Pinder, "Medicinal Chemistry", 2nd ed, A. E. Burger, Ed., Wiley-Interscience, New York, 1970, p 492.
(2) I. M. Rollo, "The Pharmacological Basis of Therapeutics", 5th ed, L. S. Goodman and A. Gilman, Eds., MacMillan, London and Toronto, 1975, p 1047.
(3) I. M. Rollo, ref 2, pp 1060–1061.
(4) D. A. Berberian, R. G. Slighter, and H. W. Freele, *J. Parasitol.*, **54**, 1181 (1968).
(5) R. L. Beaudoin, C. P. A. Strome, and W. E. Clutter, *Mil. Med.*, **134**, 979 (1969).

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