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4-Hydroxy-17-methylincisterol, an Inhibitor of DNA Polymerase-α Activity and the Growth of Human Cancer Cells *In Vitro*

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ABSTRACT. An ergosterol derivative, 4-hydroxy-17-methylincisterol (HMI), was found to be an inhibitor of mammalian DNA polymerases *in vitro*. HMI inhibited the activity of calf thymus DNA polymerase α (pol. α). Among the polymerases tested, pol. α was the most sensitive to inhibition by HMI, and the inhibition was concentration dependent. The inhibitory effect of HMI on pol. α was almost the same as that shown by aphidicolin, a well-known potent pol. α inhibitor. HMI had relatively less effect on rat DNA pol. β, human immunodeficiency virus type 1 reverse transcriptase (HIV-RT), and calf thymus terminal deoxynucleotidyl transferase (TdT) *in vitro*, and did not influence the activities of prokaryotic DNA polymerases such as Klenow Fragment of DNA polymerase I, or the DNA-metabolic enzyme DNase I. HMI was found to be able to prevent the growth of human cancer cell lines originating from patients with leukemia or various solid tumors; its IC_{50} values ranged from 7.5 to 12 μM. We also synthesized other ergosterol derivatives and tested them, and found that two compounds, 17-methylincisterol and 4-acetyl-17-methylincisterol, have similar inhibitory effects. BIOCHEM PHARMACOL **56**;5:583–590, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. DNA polymerase α; enzyme inhibitor; ergosterol derivative; 4-hydroxy-17-methylincisterol

We have been studying eukaryotic DNA polymerases [1–8]. In the process of our investigations, the need for an inhibitor of each of the DNA polymerases has arisen. Given that the roles of the DNA polymerases *in vivo* are still obscure, the elucidation of the precise role of each DNA polymerase and the use of appropriate inhibitors would be quite useful. We therefore established an assay method to detect DNA polymerase inhibitors, and have used it to screen the extracts of many organisms for the inhibitors. In addition to the use mentioned above, such inhibitors could also be employed as anticancer chemotherapy agents, because they inhibit cell proliferation.

In the extract screening, an important aspect was the type of natural product we examined as a source of inhibitors. Not only were several fungi, mushrooms, and higher plants found to produce such inhibitors [9–12], but some algae were also indicated to produce them (our unpublished data). Some of the inhibitors were a restricted group of highly degraded steroids called incisterols, which are reportedly produced by marine organisms such as sponges and mollusks [13]. A member of the incisterol family has also been synthesized from vitamin D_2 [14]. Another synthesized incisterol, γ-hydroxy-α,β-unsaturated lactone, which is a true natural product [13], was reported to be a potent fish toxic agent. Thus, the principal role of an incisterol in sponges is probably a defensive role against predators [14]. Interestingly, some of the incisterol family members easily synthesized from ergosterol were mammalian DNA polymerase-specific inhibitors. Our primary concern in the present study was to synthesize some members of the incisterol family, and to identify their biochemical properties as DNA polymerase inhibitors. These incisterols were HMI**, MI, and AcMI, which are moderately similar to γ-hydroxy-α,β-unsaturated lactone. They were also found to be able to kill human cancer cells *in vitro*. Our

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** *Abbreviations:* AcMI, 4-acetyl-17-methylincisterol; DNase I, deoxyribonuclease I; HIV-RT, human immunodeficiency virus type 1 reverse transcriptase; HMI, 4-hydroxy-17-methylincisterol; MI, 17-methylincisterol; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; pol., DNA polymerase; TdT, terminal deoxynucleotidyl transferase; and T_m, melting temperature.

TABLE 1. Assignments of NMR spectra of HMI

Position	^{13}C	^{1}H	HMBC*
1	171.83		H-2
2	111.97	5.61 (d, J = 2.0 Hz)	H-8
3	171.19		H-2, 8
4	105.39		H-2, 5
5	35.23	2.30 (ddd, J = 2.4, 3.9, 13.7 Hz, α)	H-6
		1.84 (ddd, J = 4.9, 13.0, 13.7 Hz, β)	H-5, 12
6	35.10	1.96 (ddd, J = 2.4, 3.9, 13.7 Hz, β)	H-5, 12
		1.63 (ddd, J = 3.9, 13.0, 13.2 Hz, α)	
7	48.89		H-5, 6, 8, 12
8	50.40	2.66 (ddd, J = 2.0, 5.9, 11.2 Hz)	H-2, 12
9	21.37	1.72 (m, α), 1.48 (m, β)	H-8
10	28.88	1.90 (m, α), 1.47 (m, β)	†
11	55.35	1.50 (m)	H-12, 13, 14, 15
12	11.76	0.60 (s)	H-6, 8
13	40.15	2.05 (m)	H-14, 15, 16
14	21.03	1.04 (d, J = 6.8 Hz)	H-15
15	134.71	5.17 (dd, J = 8.3, 15.2 Hz)	H-13, 14, 16
16	132.84	5.26 (dd, J = 7.3, 15.1 Hz)	H-13, 15, 21
17	42.85	1.86 (m)	H-15, 16, 19, 20, 21
18	33.07	1.49 (m)	H-19, 20
19	19.66	0.83 (d, J = 6.8 Hz)	H-20
20	19.98	0.84 (d, J = 6.8 Hz)	H-19
21	17.62	0.92 (d, J = 6.8 Hz)	H-16

*Heteronuclear multiple bond connectivity.
†No cross-peak was observed in an HMBC experiment.

present study is the first investigation of the mode of the molecular action of the incisterol family agents as eukaryotic DNA polymerase-specific antitumor agents.

MATERIALS AND METHODS

Synthesis of Compounds

The methods used to synthesize HMI, MI, and AcMI were as follows. For HMI, a chloroform solution (300 mL) containing 3 g of ergosterol was irradiated for 9 hr with UV light (wavelength, 312 nm) with constant air-bubbling of the solution. The oily residue after evaporation was dissolved in 5 mL of chloroform, applied to a silica gel column (100–200 mesh, 5.0 × 50.0 cm), and eluted with chloroform. The fractions with the inhibitory activity of DNA polymerase were collected and evaporated *in vacuo*. The residue was dissolved in 1.5 mL of ethyl acetate:hexane (5:4), loaded onto a silica gel column (300 mesh, 2.0 × 15.0 cm), and then eluted with the same solvent. Similarly, the fractions with the inhibitory activity were collected and evaporated *in vacuo*. The residue, after being redissolved in methanol, was further purified by HPLC on a C18 silica gel column (YMC A-323, eluent, 90% acetonitrile; flow rate, 6 mL/min; detection, UV absorbance at 210 nm; retention time, 5.2 min). Finally, 3.6 mg of HMI was obtained. HMI was identified by ^{1}H and ^{13}C NMR data. Analysis of positive FAB mass and FAB high resolution mass were performed on a glycerol matrix, and were recorded on a mass spectrometer (JEOL JMS HX110). $[M + H]^+$:obsd. *m/z* 333.2425; calcd. *m/z* 333.2430 for $C_{21}H_{33}O_3$. NMR measurements were performed on a JEOL JNM Alpha 600 spectrometer. The ^{1}H and ^{13}C spectra were recorded in $CDCl_3$ solution at 600 and 100 MHz, the chemical shifts given relative to tetramethylsilane, and the $CDCl_3$ solvent peaks were δ 0.00 and 77.0 ppm, respectively. The data obtained are summarized in Table 1. MI was obtained from HMI by treating HMI with methanolic HCl [14]. The yield of MI from HMI was 25%. AcMI was also synthesized from HMI. HMI in pyridine and acetic anhydride (1:1) was exposed for 12 hr and then purified using a C18 silica gel column (HPLC, YMC A-323, eluent, 90% acetonitrile; flow rate, 6 mL/min; detection, UV absorbance at 210 nm; retention time, 7.2 min). The yield of AcMI from HMI was 86%. MI and AcMI were confirmed by a comparison of the ^{1}H NMR data of HMI.

Enzymes and DNA Polymerase Assays

The DNA polymerases and the DNA metabolic enzymes used are the same as those described in previous reports [9, 15, 16]. HMI, MI, and AcMI were dissolved in DMSO. Three microliters of the dissolved sample was mixed with 12 μL of each enzyme in 50 mM Tris–HCl (pH 7.5) containing 0.1 mM EDTA, 1 mM dithiothreitol, and 50% glycerol, and then kept at 4° for 10 min. Five microliters of each of the preincubated solutions was added to 20 μL of each of the enzyme standard reaction mixtures, and then each of the enzyme activities was measured under the conditions described in previous reports [9, 15, 16].

Effects of HMI on Various Human Cancer Cells In Vitro

For the investigation of the *in vitro* effects of HMI, K562 (leukemia), MKN28 (stomach cancer), PC6 (lung cancer), MCF7 (breast cancer), HT29 (colon cancer), and HT1080 (fibrosarcoma) cells were used. The cells were cultured using RPMI 1640 medium without phenol red, supplemented with 10% fetal bovine serum as a standard medium. The cells were cultured at 37° in the standard medium in humidified 5% CO_2–95% air. The cytotoxicity of HMI was investigated as follows. A high concentration of HMI dissolved in DMSO was stocked. Approximately 2×10^3 cells/well were inoculated in a 96-well micro-plate, and the HMI stock solution, diluted with standard medium to various concentrations, was applied to each well. After incubation for 72 h, the survival rate was measured by an MTT assay [17].

Thermal Transition of DNA

Thermal transition profiles of double-stranded DNA to single-stranded DNA with or without HMI were obtained with a spectrophotometer (U3210) equipped with a thermoelectric cell holder. Calf thymus DNA (6 mg/mL) was dissolved in 0.1 M sodium phosphate buffer (pH 7.0) containing 1% DMSO. The solution temperature was equilibrated at 78° for 10 min, and then increased by 1° at 2-min intervals for each measurement point. Any change in the absorbance of HMI itself at each temperature point was automatically subtracted from that of DNA plus HMI in the spectrophotometer.

RESULTS

Chemical Structures of the Incisterols as DNA Polymerase Inhibitors

As noted earlier in the paper, some incisterols are produced by marine organisms such as sponges and mollusks [13], and others are easily synthesized from ergosterol. One of the synthesized incisterols, γ-hydroxy-α,β-unsaturated lactone, has been found to be a potent fish toxic agent [14]. We found here that some of the incisterols are mammalian DNA polymerase-specific inhibitors: HMI, MI, and AcMI.

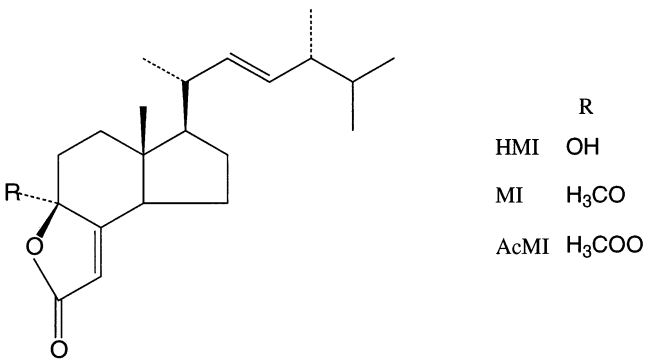


FIG. 1. Chemical structures of HMI, MI, and AcMI.

TABLE 2. Abilities of three ergosterol derivatives to inhibit cell proliferation of various cancer cells (IC_{50})

	IC_{50} (μg/mL)					
	K562	MKN28	PC6	MCF7	HT29	HT1080
HMI	2.5	2.7	2.9	4	2.9	2.5
MI	4.3	12.5	16.5	12.5	13.5	7
AcMI	12	5.6	17	13.5	6.6	5.4

Concentration–response studies were performed twice, the results were plotted on graphs, and the effective concentration of each analog that inhibited 50% growth was calculated. Cell lines: K562 (leukemia), MKN28 (stomach cancer), PC6 (lung cancer), MCF7 (breast cancer), HT29 (colon cancer), and HT1080 (fibrosarcoma).

Their chemical structures, as shown in Fig. 1, are moderately similar to that of γ-hydroxy-α,β-unsaturated lactone. As described below, they were found not only to be a DNA polymerase inhibitor, but were also shown to efficiently prevent the growth of human cancer cells *in vitro* as follows.

Effects of the Incisterols on Various Human Cancer Cells In Vitro

At first, we investigated *in vitro* the cytotoxic effect of HMI on K562, MKN28, PC6, MCF7, HT29, and HT1080 cells originating from patients with cancer. The incisterols prevented the proliferation of each of these cell lines, as shown in Table 2. All of the cell lines were influenced by the addition of HMI at similar concentrations and in a concentration-response fashion (10^{-9} to 10^{-4} mol/mL). The concentrations of HMI required for 50% growth inhibition (IC_{50}) were 2.5 to 4 μg/mL (7.5 to 12 μM). MI and AcMI also inhibited the proliferation of all of the cell

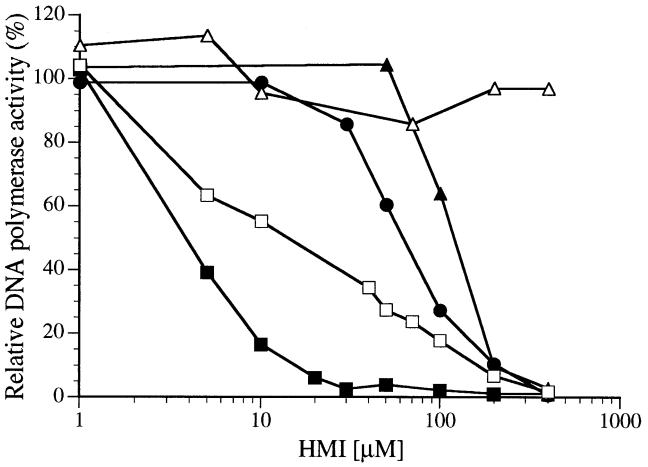
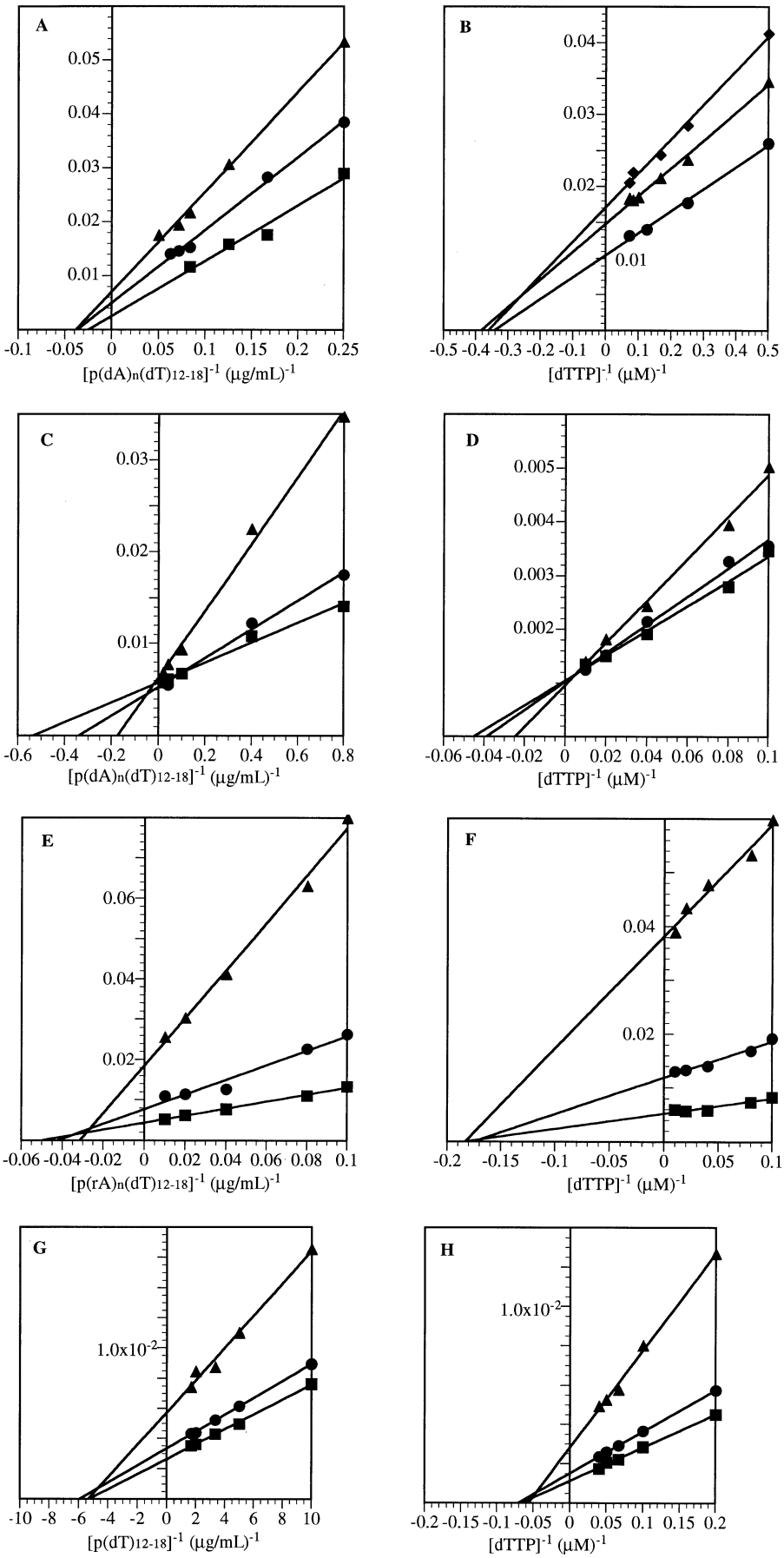


FIG. 2. Effect of HMI on various DNA polymerases. Reactions were carried out for 1 hr under the conditions described in Materials and Methods with the indicated concentrations of HMI. This experiment was performed three times. The enzymes tested and the symbols used are as follows: calf thymus pol. α (■); rat pol. β (●); HIV-RT (▲); calf thymus TdT (□); and the Klenow Fragment of pol. I (△). The specific radioactivity of [^{3}H]dTTP was 1660 cpm/pmol. The 100% values were 57 pmol (■); 138 pmol (●); 72 pmol (▲); 38 pmol (□); and 80 pmol (△).


A
0.05
0.04
0.03
0.02
0.01
-0.1 -0.05 0 0.05 0.1 0.15 0.2 0.25
[p(dA)n(dT)12-18]-1 (μg/mL)-1
B
0.04
0.03
0.02
0.01
-0.5 -0.4 -0.3 -0.2 -0.1 0 0.1 0.2 0.3 0.4 0.5
[dTTP]-1 (μM)-1
C
0.03
0.02
0.01
-0.6 -0.4 -0.2 0 0.2 0.4 0.6 0.8
[p(dA)n(dT)12-18]-1 (μg/mL)-1
D
0.005
0.004
0.003
0.002
-0.06 -0.04 -0.02 0 0.02 0.04 0.06 0.08 0.1
[dTTP]-1 (μM)-1
E
0.06
0.04
0.02
-0.06 -0.04 -0.02 0 0.02 0.04 0.06 0.08 0.1
[p(rA)n(dT)12-18]-1 (μg/mL)-1
F
0.04
0.02
-0.2 -0.15 -0.1 -0.05 0 0.05 0.1
[dTTP]-1 (μM)-1
G
1.0x10-2
-10 -8 -6 -4 -2 0 2 4 6 8 10
[p(dT)12-18]-1 (μg/mL)-1
H
1.0x10-2
-0.2 -0.15 -0.1 -0.05 0 0.05 0.1 0.15 0.2
[dTTP]-1 (μM)-1

lines at concentrations similar to those of HMI (Table 2). The incisterols as DNA polymerase inhibitors were cytotoxic to the human cancer cells *in vitro*.

Inhibition by HMI of DNA Polymerases and Other DNA Metabolic Enzymes

Next we investigated the effect of one of the cytotoxic incisterols, HMI, on DNA polymerases and other DNA metabolic enzymes. As shown in Fig. 2, HMI at 10 μM was found to strongly inhibit the activities of calf thymus pol. α and to moderately inhibit the activities of calf thymus TdT, but this concentration did not influence the activities of rat pol. β, HIV-RT, or the Klenow Fragment of pol. I. The inhibition of HMI was concentration dependent, with 50% inhibition for pol. α and TdT observed at 4 and 10 μM, respectively, and almost complete inhibition (more than 80%) at 10 and 100 μM, respectively. A difference of inhibition was not observed with the use of calf thymus activated DNA as the template-primer instead of poly(dA)/oligo(dT)$_{12–18}$. Actually, neither pol. I, the other prokaryotic DNA polymerase, *Taq* polymerase, nor the DNA metabolic enzyme bovine DNase I was inhibited by 10 μM HMI (data not shown). HMI at less than 10 μM hardly inhibited the activities of pol. β and HIV-RT (Fig. 2). HMI appeared to be an inhibitor specifically for the mammalian DNA polymerases, especially the α-type, *in vitro*.

The effect of HMI on pol. α was almost the same as that of aphidicolin, the well-known pol. α inhibitor [18]. The finding that a derivative of ergosterol or vitamin D_2 is a potent pol. α inhibitor has never been described, to our knowledge.

Mode of Inhibition of Pol. α, Pol. β, HIV-RT, and TdT by HMI

To elucidate the mechanism of inhibition by HMI of pol. α, pol. β, HIV-RT, and TdT, the extent of inhibition as a function of the DNA template-primer or nucleotide substrate concentration was studied (Fig. 3). In the kinetic analysis, poly(dA)/oligo(dT)$_{12–18}$ and dTTP were used as the DNA template-primer and substrate, respectively.

Double-reciprocal plots of the results show that the inhibition of pol. α, HIV-RT, and TdT by HMI was non-competitive with the DNA template (Fig. 3, A, E, and G), since there was no change in the apparent K_m value (21 μg/mL for pol. α, 20 μg/mL for HIV-RT, and 0.19 μg/mL for TdT), while the V_{max} value decreased to 35% in the presence of 10 μM HMI for pol. α (Fig. 3A), to 56% in the presence of 70 μM HMI for HIV-RT (Fig. 3E), and to 46% in the presence of 10 μM HMI for TdT (Fig. 3G). Similarly, the apparent K_m for the substrate dTTP was unchanged, and the V_{max} was decreased to 61% in the presence of 15 μM HMI for pol. α (Fig. 3B), to 43% in the presence of 70 μM HMI for HIV-RT (Fig. 3F), and to 40% in the presence of 5 μM HMI for TdT (Fig. 3H). The inhibition, therefore, was also clearly noncompetitive with respect to the substrate dTTP (Fig. 3). In contrast, HMI inhibition of pol. β activity was competitive with the DNA template and the substrate (Fig. 3, C and D). In the case of the DNA template, the apparent V_{max} was unchanged at 17 pmol/hr, whereas a three-fold increase in K_m was observed in the presence of 50 μM HMI (Fig. 3C). The V_{max} for the substrate (dTTP) was 1 nmol/hr, and the K_m for the substrate increased from 22 to 26 and 40 pmol/mL in the presence of 0, 30, and 50 μM HMI, respectively (Fig. 3D). HMI may interact with or affect both of the binding sites on pol. β, thereby decreasing its affinity with the DNA template and substrate, whereas HMI may bind or interact with a domain distinct from the template or substrate binding sites on pol. α, HIV-RT, and TdT. The results indicate that the HMI-binding sites on the enzymes are structurally different from each other, especially between pol. β and the others, and that HMI binds directly to the DNA template-binding or the substrate-binding site on pol. β, since the incisterols bear no structural resemblance to either the DNA template or the substrate. Competitive and non-competitive inhibitors bind within and outside the active sites of the enzymes, respectively.

Interaction of HMI and Double-Stranded DNA

To determine whether the effect of HMI resulted in binding to DNA or enzyme, the interaction of HMI with double-stranded DNA (dsDNA) was investigated by means

FIG. 3. Kinetic analysis of the inhibition of DNA polymerases by HMI. The effects of HMI on the K_m and V_{max} values of the DNA template-primer and dTTP substrate were determined, and the results are displayed as Lineweaver–Burk plots. These experiments were performed twice. (A) Pol. α activity was assayed with the concentrations of template-primer (poly(dA)$_n$/oligo(dT)$_{12–18}$) indicated, after preincubation of the enzyme with 5 μM (■), 10 μM (●), or 15 μM (▲) HMI. (B) Pol. α activity was assayed with the concentrations of substrate (dTTP) indicated, after preincubation of the enzyme with 1 μM (●), 5 μM (▲) or 10 μM (◆) HMI. (C) DNA polymerase β activity was assayed with the concentrations of template-primer (poly(dA)$_n$/oligo(dT)$_{12–18}$) indicated, after preincubation of the enzyme without (■) or with 30 μM (●) or 50 μM (▲) HMI. (D) Pol. β activity was assayed with the concentrations of substrate (dTTP) indicated, after preincubation of the enzyme without (■) or with 30 μM (●) or 50 μM (▲) HMI. (E) HIV-RT activity was assayed with the concentrations of template-primer (poly(rA)$_n$/oligo(dT)$_{12–18}$) indicated, after preincubation of the enzyme without (■) or with 70 μM (●) or 120 μM (▲) HMI. (F) HIV-RT activity was assayed with the concentrations of substrate (dTTP) indicated, after preincubation of the enzyme without (■) or with 70 μM (●) or 120 μM (▲) HMI. (G) TdT activity was assayed with the concentrations of substrate (oligo(dT)$_{12–18}$) indicated, after preincubation of the enzyme without (■) or with 5 μM (●) or 10 μM (▲) HMI. (H) TdT activity was assayed with the concentrations of substrate (dTTP) indicated, after preincubation of the enzyme without (■) or with 5 μM (●) or 10 μM (▲) HMI.

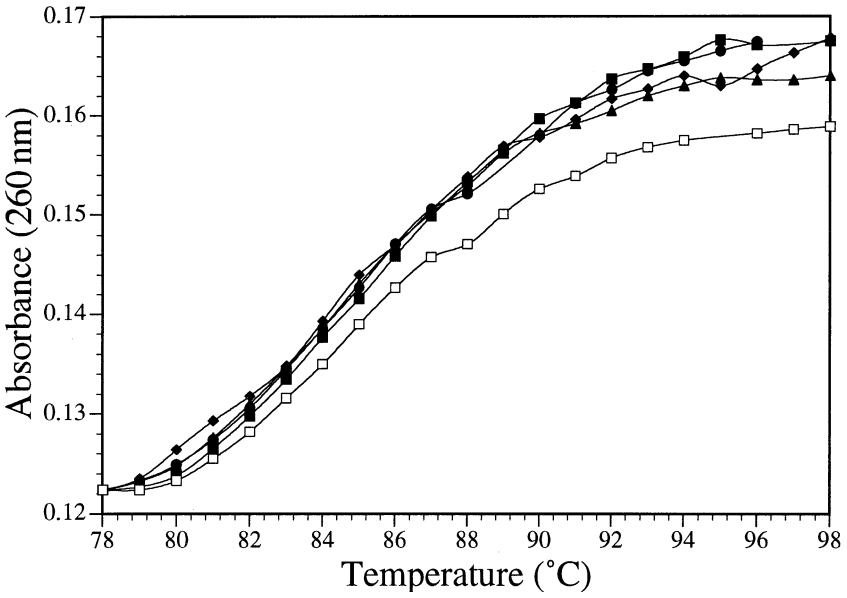


FIG. 4. Effect of HMI on the thermal transition of calf thymus double-stranded DNA in 0.1 M sodium-phosphate buffer (pH 7.0). This experiment was performed three times. The concentrations (μM) of HMI used were 0 (■); 12.5 (●); 25 (▲); and 50 μM (◆), and that of ethidium bromide was 5 μg/mL (□).

of thermal transition of dsDNA with or without HMI. The T_m of dsDNA, with increasing concentrations of HMI (from 0 to 50 μM), was measured precisely by a spectrophotometer equipped with a thermoelectric cell holder. As described in Materials and Methods, calf thymus dsDNA at 6 mg/mL was dissolved in 0.1 M sodium phosphate buffer (pH 7.0) containing 1% DMSO. HMI at more than 50 μM could not be dissolved under these conditions. In the concentration range used, the thermal transition of T_m was not observed (Fig. 4), whereas the ethidium bromide used as the positive control, a typical intercalating compound, produced a clear thermal transition (Fig. 4). These results indicated that HMI did not bind to DNA.

Effects of MI and AcMI on DNA Polymerases

We studied the changes in polymerase inhibition when the hydroxyl group at position 4 in HMI was replaced with a methyl or acetyl group. These alterations produced MI and AcMI, respectively (Fig. 1). As shown in Fig. 5, substitution of the hydroxyl group with the methyl group or the acetyl group in HMI decreased the inhibitory effects on pol. β, HIV-RT, and TdT. Interestingly, the inhibition by MI or AcMI of pol. α remained strong (Fig. 5). These data suggest that the hydroxyl group at position 4 of an incisterol may be required for inhibition.

DISCUSSION

Our original interest was to study the *in vivo* roles of eukaryotic DNA polymerases. Another interest was to find a cytotoxic agent useful as an anticancer chemotherapy agent. As described earlier, we have established an assay method to detect DNA polymerase inhibitors, and have used it to screen extracts of many organisms to find DNA polymerase inhibitors [9, 15]. Some of the inhibitors found were a restricted group of highly degraded steroids called incisterols. The incisterols have been synthesized from vitamin D_2. They were HMI, MI, and AcMI, which are moderately similar to γ-hydroxy-α,β-unsaturated lactone, and which are easily synthesized from ergosterol.

To elucidate the inhibition mechanism of HMI, the extent of inhibition as a function of DNA template-primer or dTTP substrate concentrations was studied (Fig. 3). The addition of an excess of protein (100 μg/mL of BSA) did not affect the binding in either case (data not shown), suggesting that the effect of HMI did not result from the nonspecific adhesion of HMI to the enzymes, but rather that it bound selectively to special sites. The K_i values for pol. α, pol. β, HIV-RT, and TdT, obtained from Dixon plots calculated from the kinetic values in Fig. 3, were found to be 4.8, 16.0, 14.0, and 7.2 μM for template (-primer), and 13.6, 46.5, 6.0, and 4.4 μM for substrate dTTP, respectively. The data suggest that the lower the K_i value, the stronger the inhibition. The K_i value of pol. α for the DNA template was higher than those of the other enzymes, suggesting that the affinity of HMI to pol. α is higher at the DNA template-binding site than to the others. HMI was thought to have little or no effect on the T_m of dsDNA (Fig. 4), and could be expected to interact with each of the enzymes themselves directly.

Because the incisterols found here are broadly effective (although relatively specific for pol. α) on mammalian polymerases, the compounds examined are not inhibitors applicable to our original interest. The incisterols, therefore, should be tested to determine whether they are capable of preventing mammalian cell proliferation, since they can inhibit the activity of the replicative polymerase. They were found to be able to kill human cancer cells *in vitro* (Table 2). The concentration range of IC_{50} values for

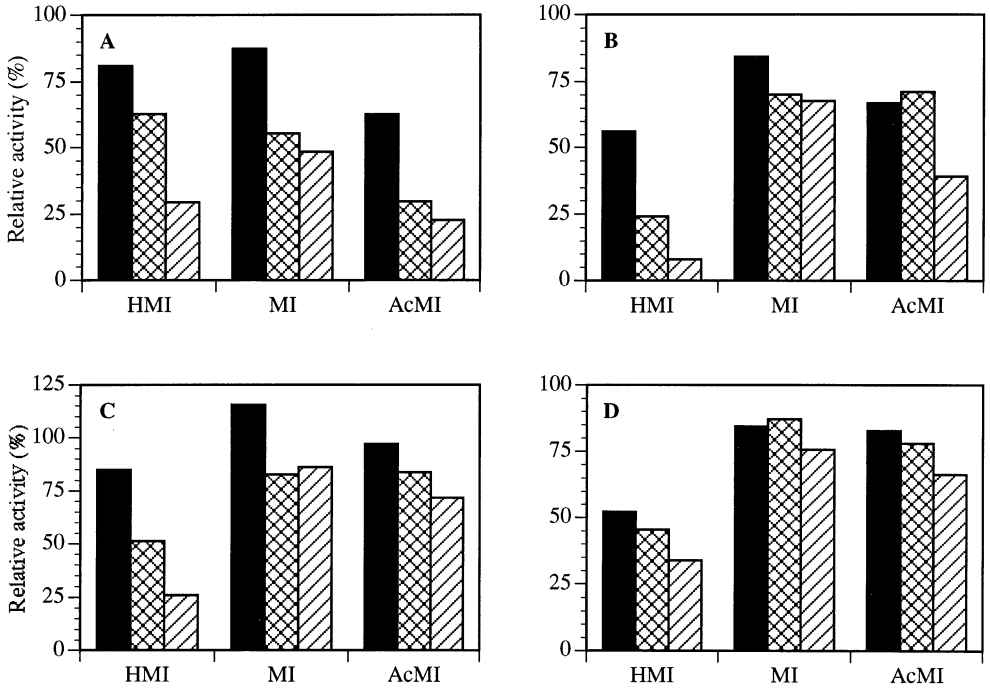


FIG. 5. Effects of derivatives on various DNA polymerases. Three derivatives dissolved in DMSO were preincubated with pol. α (A), pol. β (B), TdT (C), and HIV-RT (D) on ice for 20 min. After preincubation, the DNA polymerase activity was measured as described in Materials and Methods. These experiments were performed twice. [^{3}H]-dTMP incorporation in the absence of compounds was taken as 100%, corresponding to 4.2 pmol/hr (A); 2.8 pmol/hr (B); 13 pmol/hr (C); and 10 pmol/hr (D). The solid bars, cross-hatched bars, and lined bars indicate 3.75, 7.5, and 15 μM of derivatives (A); 80, 100, and 120 μM (B); 7.5, 15, and 30 μM (C); and 30, 60, and 120 μM (D), respectively.

these cells was quite similar to that of the 50% inhibition for pol. α *in vitro*, suggesting that the prevention of the cell proliferation resulted from the inhibition of the replicative polymerase; these compounds may be clinically useful as cytotoxic agents.

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