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INHIBITION OF HIV-1 INTEGRATION PROTEIN BY AURINTRICARBOXYLIC ACID MONOMERS , MONOMER ANALOGS, AND POLYMER FRACTIONS

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Summary. Several aurintricarboxylic acid (ATA) monomers, monomer analogs, and polymer fractions have been tested as inhibitors of HIV-1 integration protein (IN). Both of the ATA monomers and all of the ATA polymer fractions inhibited a selective DNA cleavage reaction catalyzed by IN. The ATA monomer analogs were inactive or had low activity. The activities of the substances as inhibitors of HIV IN correlated in a positive way with their activities as inhibitors of the cytopathic effect of HIV-1 in CEM and HIV-2 in MT4 cells. These results suggest that inhibition of HIV IN may contribute to the antiviral activity of the ATA monomers and monomer analogs in cell culture.

Aurintricarboxylic acid (ATA, schematic structure **1**(1)), a mixture of polymeric substances that forms when salicylic acid is treated with formaldehyde and sulfuric acid in the presence of sodium nitrite (2), inhibits a variety of enzymes that process nucleic acids, including several RNA polymerases (3, 4), reverse transcriptases (5-7), DNA polymerases (5, 8-11), and nucleases (12-20). ATA inhibits these protein nucleic acid interactions by binding to the protein, not the nucleic acid (8, 13). Recent interest in ATA has resulted from the fact that it prevents the cytopathic effect of HIV-1 and HIV 2 in cell culture (21-23). This antiviral effect may be due to the binding of ATA to gp120 on the surface of the virus and to the CD4 receptor on the cell surface, which prevents the initial binding of the virus to the cell surface (22, 23). ATA is also a potent inhibitor of HIV-1 reverse transcriptase in cell free systems (7, 23). These biological activities of ATA suggest that it might also inhibit the integration protein (IN) of HIV-1. Retroviral integration proteins are responsible for insertion of a double-stranded DNA copy of the viral RNA genome into the host cellular DNA, an essential step in the retroviral life cycle (24). Various ATA molecular weight fractions, as well as ATA monomers **2** and **5** and structurally related compounds **3**, **4**, **6**, and **7** were therefore tested as inhibitors of HIV-1 IN.

The termini of all retroviral DNA's contain a CA dinucleotide positioned, in general, two bases from the 3' end of the minus strand of U3 and the plus strand of U5. Retroviral IN's carry out a selective endonucleolytic processing of the viral DNA, cleaving on the 3' side of the A

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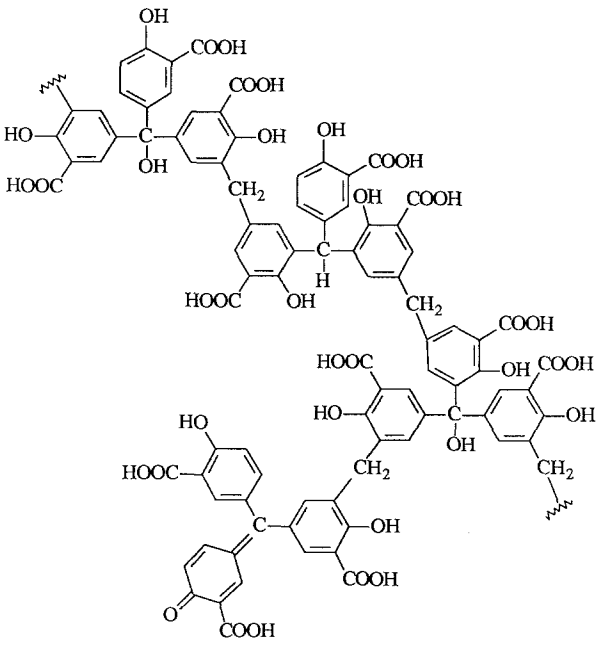
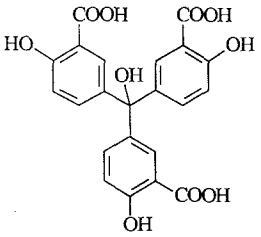


Scheme 1



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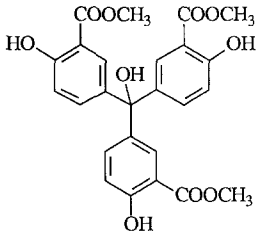

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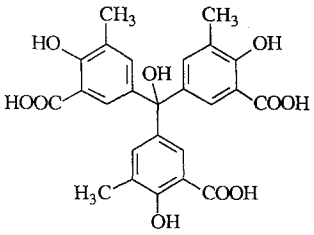

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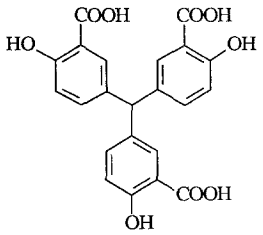

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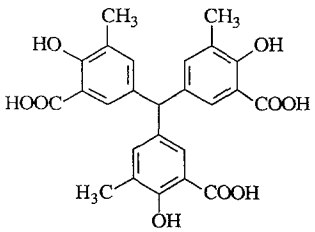
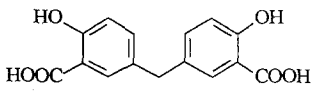


Scheme 6



Scheme 7

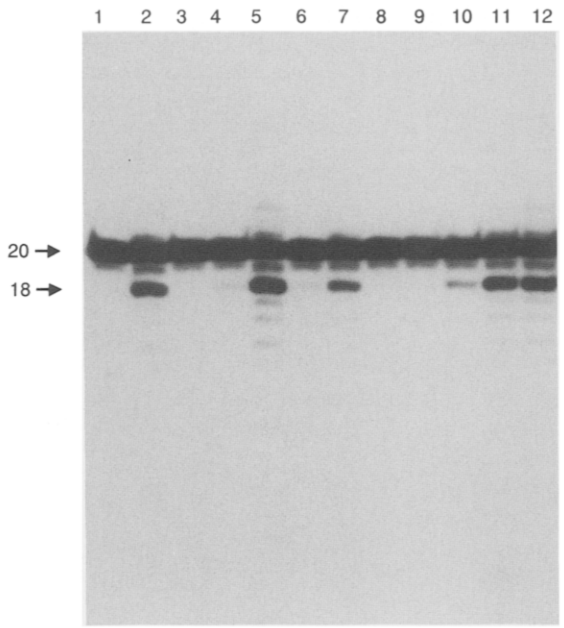


Figure 1. Polyacrylamide gel electrophoresis monitoring the conversion of a 20-mer (plus strand of HIV U5) to an 18-mer by HIV IN in the absence and presence of inhibitors. Lane 1: no protein. Lane 2: no inhibitor. Lane 3: 100 μM **2**. Lane 4: 50 μM **2**. Lane 5: 100 μM **3**. Lane 6: 100 μM 4. Lane 7: 50 μM **4**. Lane 8: 100 μM **5**. Lane 9: 50 μM **5**. Lane 10: 100 μM **6**. Lane 11: 50 μM **6**. Lane 12: 1 mM **7**.

residue in this conserved CA (25, 26). This selective DNA cleavage reaction, which is a prerequisite for insertion of the viral DNA into the host cellular DNA, can be simulated *in vitro* with synthetic double-stranded oligonucleotide mimics of the termini of viral DNA (27, 28). In the present study, inhibition of this selective HIV IN cleavage reaction by various ATA polymer, monomers, and monomer analogs was examined.

Methods. DNA cleavage assays were carried out as previously described (28). Briefly, reaction mixtures (10 μL) contained 1 pmol of an oligonucleotide mimic of HIV U5 DNA (5' end-labeled on the plus strand), 20 mM Tris-HCl (pH 8), 5 mM 2-mercaptoethanol, 1 mM $MgCl_2$ (contributed from the end-labeling reaction), 1 mM $MnCl_2$, 0.5 μg of protein, and the inhibitor. Incubations were for 1 h at 37 °C. The samples were subjected to electrophoresis on a 20 % denaturing polyacrylamide gel. The assay monitors the conversion of a 20-mer (plus strand of HIV U5) to an 18-mer. The preparation of the ATA polymers, monomers, and monomer analogs was described previously (23, 29, 30).

Results and Discussion. The effects of the various low molecular weight ATA components and analogs on the IN cleavage reaction are shown in Figure 1. The IN cleavage reaction was inhibited by compounds **2**, **4**, **5**, and **6**. Compounds **3** and **7** were inactive at the highest concentration tested (100 μM and 1 mM, respectively).

Antiviral data are presented together with data on inhibition of IN in Table 1. The most active of the structurally defined, low molecular weight compounds, both as inhibitors of the IN cleavage reaction and as antiviral agents, were the triphenylcarbinol **2** and the triphenylmethane **5**. The introduction of three methyl groups ortho to the phenolic hydroxyl groups in **2**, resulting in

Table 1. Prevention of HIV-1 and HIV-2 cytopathogenicity in cell culture and inhibition of HIV-1 IN by ATA monomers, monomer analogs, and polymer fractions

Substance	IC_{50} HIV-1/CEM[a]	IC_{50} HIV-2/MT4[a]	IC_{50} (HIV-1 IN)[b]
2	110 μM (50 μg/mL)	34 μM (15 μg/mL)	10-50 μM (4-22 μg/mL)
3	>160 μM (>78 μg/mL)	>200 μM (>100 μg/mL)	>100 μM (>48 μg/mL)
4	>200 μM (>205 μg/mL)	>200 μM (>100 μg/mL)	50-100 μM (24-48 μg/mL)
5	77 μM (32 μg/mL)	31 μM (13 μg/mL)	10-50 μM (4-21 μg/mL)
6	>200 μM (>186 μg/mL)	140 μM (67 μg/mL)	50-100 μM (23-46 μg/mL)
7	>200 μM (276 μg/mL)	>200 μM (100 μg/mL)	>1 mM
ATA (M_W 3336)[c]	0.8 μg/mL	0.7 μg/mL	3 μg/mL
ATA (M_W 2326)	2.0 μg/mL	1.1 μg/mL	3 μg/mL
ATA (M_W 1609)	2.9 μg/mL	2.4 μg/mL	1 μg/mL
ATA (M_W 1437)	6.4 μg/mL	3.1 μg/mL	1 μg/mL
ATA (M_W 1149)	12 μg/mL	3.3 μg/mL	1 μg/mL
ATA (M_W 475)	18 μg/mL	11 μg/mL	10 μg/mL

[a]Data for the prevention of HIV-1 cytopathogenicity in CEM cells and HIV-2 in MT4 cells are from references 23 and 25. [b]Data for inhibition of HIV IN were obtained from DNA cleavage assays. [c]M_W is the weight average molecular weight determined by the universal calibration method (23).

compound **4**, reduced the activity against IN. Compound **4** was also inactive in the antiviral assays at the highest concentration tested. A similar modification of the triphenylmethane **5**, affording compound **6**, also decreased inhibitory activity in both the IN and the antiviral assays. Methylation of the three carboxylic acid groups of **2**, affording the trimethyl ester **3**, abolished the activity against IN and the antiviral activity. This indicates that the free carboxylic acid groups are essential for activity. The diphenylmethane derivative **7** was inactive as an inhibitor of IN at a concentration of 1 mM, and was also inactive in antiviral assays.

Various ATA molecular weight fractions have been obtained by equilibrium dialysis, ultrafiltration, and gel permeation chromatography (23). Previous studies revealed a direct relationship between the molecular weights of these fractions and their antiviral activities in a

number of assays. These included prevention of the binding of the OKT4A monoclonal antibody to the CD4 receptor, inhibition of the binding of anti-gp 120 monoclonal antibody to gp 120, inhibition of the attachment of HIV-1 virions to cells, and inhibition of HIV-1 reverse transcriptase (23). In all of these assays, potency increased with molecular weight. A similar relationship was established between molecular weight of the various ATA fractions and their potencies as inhibitors of the cytopathic effects of HIV-1 and HIV-2 in cell culture. The ATA fractions were tested as inhibitors of HIV IN. As shown in Table I, all of the fractions inhibited the IN-catalyzed selective cleavage reaction. Although the highest molecular weight fraction was a more potent inhibitor of HIV IN than the lowest molecular weight fraction, the correlation between molecular weight and potency was not as strong for IN inhibition as for antiviral activity.

We pointed out previously that whereas the polymeric substance ATA prevents the binding of the OKT4A monoclonal antibody to the CD4 receptor and inhibits HIV-1 reverse transcriptase, the triphenylcarbinol **2** and the triphenylmethane **4** do not (30). The mechanism of action of these low molecular weight substances has been unknown. The present results, showing a general correlation between the activity of the compounds **2-7** as IN inhibitors and as antiviral agents, suggest that inhibition of HIV IN may contribute to their antiviral activity.

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