94-5; 4-(trifluoromethyl)phenyl isothiocyanate, 1645-65-4; 4-(phenylsulfonyl)benzoic acid, 5361-54-6; 4-cyano-2-methylaniline, 78881-21-7; *N*-[3-chloro-4-(4′-chlorophenoxy)phenyl]cyanoacetamide, 136186-50-0; *N*-methyl-*N*-[4-(trifluoromethyl)phenyl]-cyanoacetamide, 136186-51-1; benzenesulfinic acid sodium salt, 873-55-2; 4-fluoroacetophenone, 403-42-9; 4-(phenylsulfonyl)-acetophenone, 65085-83-8; 3-methyl-4-nitrobenzonitrile, 96784-54-2; 4-chlorophenol, 106-48-9; 3,4-dichloronitrobenzene, 99-54-7; 4-(4′-chlorophenoxy)-3-chloronitrobenzene, 22544-07-6; *N*-methyl-4-(trifluoromethyl)aniline, 22864-65-9.

Gossypol and Derivatives: A New Class of Aldose Reductase Inhibitors†

Lorraine M. Deck, David L. Vander Jagt, and Robert E. Royer*

Department of Biochemistry, University of New Mexico School of Medicine, Albuquerque, New Mexico 87131.
Received May 24, 1991

Gossypol and 17 derivatives were tested as inhibitors of aldose reductase from human placenta. Gossypol and a number of the derivatives were potent inhibitors. The order of inhibitory activity was interpreted in relation to the Kador–Sharpless pharmacophor model for the aldose reductase inhibitor site. The structural but not the electronic aspects of the model were found to apply to this series of compounds.

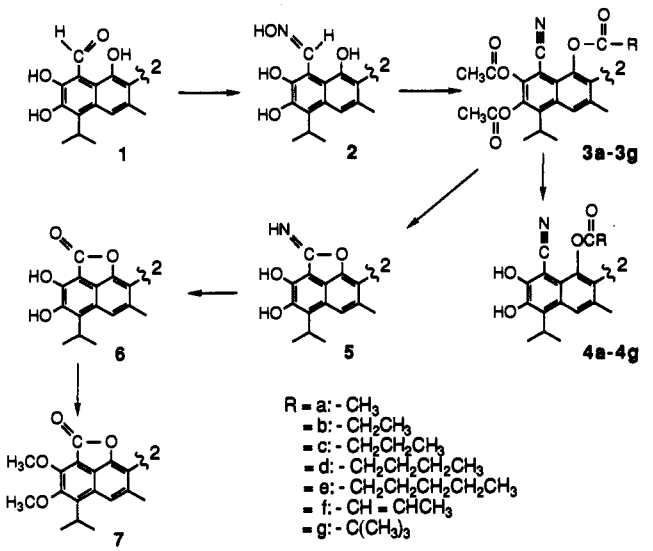
The complications of diabetes mellitus can affect many organs, but especially eye, nerve, and kidney.[1] The mechanisms whereby prolonged hyperglycemia induces these complications are not known, although a number of pathogenic mechanisms have been proposed.[1,2] One contributing mechanism might involve the polyol pathway in which glucose is reduced by NADPH to sorbitol. This reduction is catalyzed by aldose reductase (alditol:NADP$^+$ oxidoreductase, EC 1.1.1.21). Numerous studies with experimental animals support the idea that inhibition of aldose reductase can prevent the development of diabetic complications.[3-6] Therefore, aldose reductase has become a prime target for the development of therapeutic approaches to the treatment of the complications of diabetes.

Aldose reductase inhibitors are structurally diverse compounds. Kador and Sharpless[7] proposed a pharmacophor model for aldose reductase inhibitors based upon examination of the structural and electronic features of some of the known inhibitors of rat lens aldose reductase. Most kinetic studies have used aldose reductase from animal sources.[8,9] It has been shown that the sensitivity of aldose reductase to inhibitors varies with different tissues and species.[10,11] Studies carried out with an enzyme from human tissues might have greater relevance to human disease. The instability of the drug binding site of human aldose reductase has been a problem in the past.[12]

A rapid procedure for the purification of human placental aldose reductase with stabilization of the drug binding site has been described recently.[13,14] The presence of NADP at all stages of purification and during storage of the enzyme stabilizes the inhibitor binding site, making it possible to use human enzyme for kinetic studies of inhibition.

In this paper we examine a set of structurally related compounds as inhibitors in relation to the Kador–Sharpless pharmacophor model. The objective of the work was to test the model with inhibitors of human aldose reductase. The set of compounds chosen was the natural product gossypol (1) and a series of derivatives. Gossypol fits the pharmacophor model reasonably well. The structural features of the model[7,15] and the molecular dimensions of gossypol are indicated in Figure 1. Key structural features of the Kador–Sharpless model include the following: (1) a primary lipophilic (aromatic) region separated (center to center) by approximately 2.8–3.8 Å (d_1) from an electrophilic group; (2) a secondary lipophilic region located about 2.8–6.1 Å (d_2) from the electrophilic group and coplanar with the primary lipophilic region; and

Scheme I



† This paper is dedicated to Professor Herbert C. Brown on the occasion of his 80th birthday.

(1) Crabbe, M. J. C. The complications of diabetes. In *Diabetic Complications*; Crabbe, M. J. C., Ed.; Churchill Livingstone: London, 1985; pp 1-24.
(2) Baynes, J. W.; Thorpe, S. R.; Murtiashaw, M. H. Nonenzymatic Glucosylation of Lysine Residues in Albumin. In *Methods in Enzymology: Posttranslational Modifications*; Wold, F., Moldave, K., Eds.; Academic Press: New York, 1984; pp 88-98.
(3) Culebras, A.; Alio, J.; Herrera, J. L.; Lopez-Fraile, M. I. Effect of an Aldose Reductase Inhibitor on Diabetic Peripheral Neuropathy. *Arch. Neurol.* **1981**, *38*, 133-134.
(4) Chylack, L. T.; Henriques, H. F.; Cheng, H. M.; Kinoshita, J. H. Efficacy of Alrestatin, an Aldose Reductase Inhibitor, in Human Diabetic and Nondiabetic Lenses. *Ophthalmology* **1979**, *86*, 1579-1585.
(5) Beyer-Mears, A.; Cruz, E. Reversal of Diabetic Cataract by Sorbinil, an Aldose Reductase Inhibitor. *Diabetes* **1985**, *34*, 15-21.
(6) Christensen, J. E. J.; Varnek, L.; Gregersen, G. The Effect of an Aldose Reductase Inhibitor (Sorbinil) on Diabetic Neuropathy and Neural Function of the Retina: A Double-blind Study. *Acta Neurol. Scand.* **1985**, *71*, 164-167.
(7) Kador, P. F.; Sharpless, N. E. Pharmacophor Requirements of the Aldose Reductase Inhibitor Site. *Mol. Pharmacol.* **1983**, *24*, 521-531.
(8) Doughty, C. C.; Conrad, S. M. A Reaction Mechanism for Aldose Reductase from Lens. *Biochim. Biophys. Acta* **1982**, *708*, 358-364.

0022-2623/91/1834-3301$02.50/0

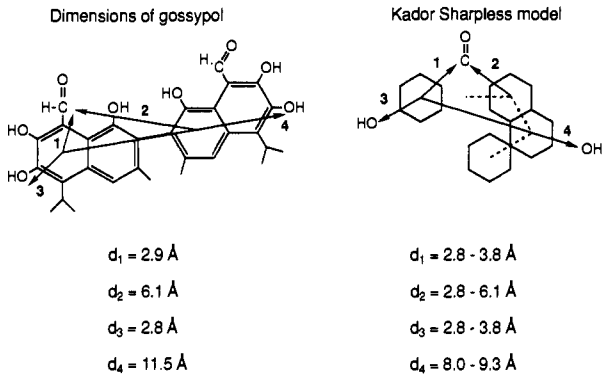


Figure 1. The Kador–Sharpless pharmacophor model for aldose reductase inhibitors and the structure of gossypol.

(3) two hydroxyl groups located 2.8–3.8 Å (d_3) and 8.0–9.3 Å (d_4) from the center of the primary lipophilic region.

In addition to the structural features of the model, Kador and Sharpless noted the following electronic correlations: (1) There is a correlation between the inhibitory activity of a molecule and its LUMO (lowest unoccupied molecular orbital energy). (2) There is a relationship between the ability of compounds to undergo a charge-transfer interaction at a polarized carbonyl group and their ability to inhibit aldose reductase.

Gossypol and the gossypol derivatives used in this study have the same basic structure. They all possess primary and secondary aromatic regions at appropriate distances from an electrophilic carbon. Since all of the compounds are symmetrical dimers, the primary and secondary lipophilic regions are identical. Due to the steric effects of the hydroxyl groups and the methyl groups flanking the binaphthyl bond, the lipophilic regions cannot attain coplanarity as required by the model. The compounds differ from each other in the nature of the electrophilic group, the number and position of free hydroxyl groups, and lipophilicity.

Chemical Synthesis. Compounds were synthesized by altering various sites on the gossypol molecule. The synthesis in this laboratory of gossylic nitrile 1,1′-diacetate (**4a**) and homologues **4b–4g** (Scheme I) from gossypol dioxime (**2**)[16] have been previously reported.[17,18] Hydrolysis of gossylic nitrile 1,1′,6,6′,7,7′-hexaacetate (**3a**) with concentrated sulfuric acid resulted in the formation of gossylic iminolactone (**5**) as indicated in Scheme I. The unusual structure of this compound is indicated by its facile hydrolysis with dilute HCl in ethanol to form gossylic lactone (**6**). Compound **5** is, however, stable in neutral buffered aqueous solution with no detectable hydrolysis

Scheme II

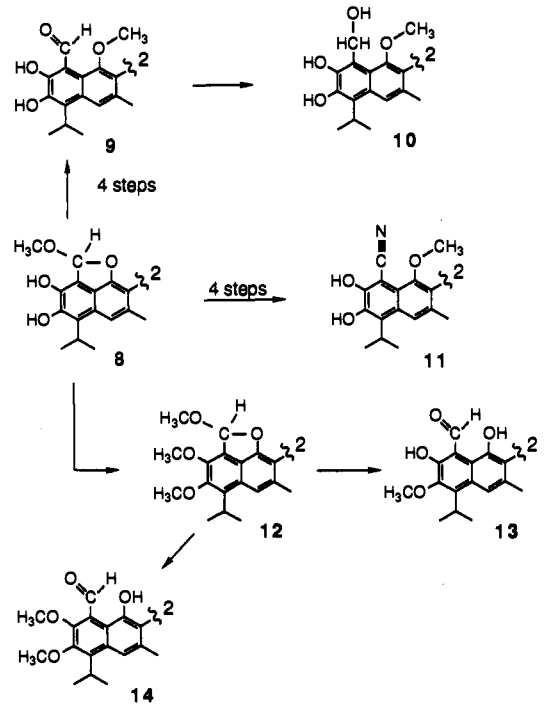


Table I. Gossypol and Derivatives: Constants (K_i) for the Inhibition of Human Placental Aldose Reductase, Binding Energies (G_0) Calculated from K_is, LUMO Values, and Charge Densities at Electrophilic Carbon Atoms

no.	inhibitor	K_i (μM)	G^0	LUMO	charge
1	gossypol	0.50	8.6	−8.94	0.96
2	gossypol dioxime	7.8	6.9	−8.88	0.55
4a	gossylic nitrile 1,1′-diacetate	7.3	7.0	−9.41	0.78
4b	gossylic nitrile 1,1′-dipropionate	6.8	7.0	−9.41	0.78
4c	gossylic nitrile 1,1′-dibutyrate	5.3	7.2	−9.41	0.78
4d	gossylic nitrile 1,1′-dipentanoate	4.7	7.2	−9.41	0.78
4e	gossylic nitrile 1,1′-dihexanoate	3.1	7.5	−9.41	0.78
4f	gossylic nitrile 1,1-dicrotonate	1.3	8.0	−9.68	0.78
4g	gossylic nitrile 1,1′-bis(2,2′-dimethylpropanoate)	2.5	7.6	−9.42	0.77
5	gossylic iminolactone	9.3	6.8	−9.59	0.98
6	gossylic lactone	0.15	9.3	−9.69	1.18
7	gossylic lactone tetramethyl ether	48	5.9	−9.67	1.18
9	gossypol 1,1′-dimethyl ether	0.31	8.8	−8.93	0.94
10	gossylic alcohol 1,1′-dimethyl ether	4.3	7.3	−8.91	0.29
11	gossylic nitrile 1,1′-dimethyl ether	2.8	7.5	−9.42	0.78
13	gossypol-6,6′-dimethyl ether	1.5	7.9	−8.92	0.94
14	gossypol-6,6′,7,7′-tetramethyl ether	9.7	6.8	−8.90	0.92
15	gossypol hexamethyl ether (dialdehyde form)	56	5.8	−8.88	0.90

(9) Conrad, S. M.; Doughty, C. C. Comparative Studies on Aldose Reductase from Bovine, Rat and Human Lens. *Biochim. Biophys. Acta* **1982**, *708*, 348–357.
(10) Hoffman, P. L.; Wermuth, B.; von Wartburg, J. P. Human Brain Aldehyde Reductases: Relationship to Succinic Semialdehyde Reductase and Aldose Reductase. *J. Neurochem.* **1980**, *35*, 354–366.
(11) Kador, P. F.; Kinoshita, J. H.; Tung, W. H.; Chylack, L. T. Differences in the Susceptibility of Aldose Reductase to Inhibition. *Invest. Ophthalmol. Visual Sci.* **1980**, *19*, 980–982.
(12) Kador, P. F.; Kinoshita, J. H.; Sharpless, N. E. The Aldose Reductase Inhibitor Site. *Metabolism* **1986**, *35*, (Suppl. 1), 109–113.
(13) Vander Jagt, D. L.; Stangebye, L. A.; Hunsaker, L. A.; Eaton, R. P.; Sibbitt, W. L. Purification of Aldose Reductase from Human Placenta and Stabilization of the Inhibitor Binding Site. *Biochem. Pharmacol.* **1988**, *37*, 1051–1056.
(14) Vander Jagt, D. L.; Hunsaker, L. A.; Robinson, B. R.; Stangebye, L. A.; Deck, L. M. Aldehyde and Aldose Reductases from Human Placenta. *J. Biol. Chem.* **1990**, *265*, 1–7.
(15) Kador, P. F.; Kinoshita, J. H.; Sharpless, N. E. Aldose Reductase Inhibitors: A Potential New Class of Agents for the Pharmacological Control of Certain Diabetic Complications. *J. Med. Chem.* **1985**, *28*, 841–849.
(16) Clark, E. P. Studies on Gossypol. I. The Preparation, Purification and Some of the Properties of Gossypol, the Toxic Principle of Cottonseed. *J. Biol. Chem.* **1927**, *75*, 725–739.
(17) Royer, R. E.; Deck, L. M.; Campos, N. M.; Hunsaker, L. A.; Vander Jagt, D. L. Biologically Active Derivatives of Gossypol: Synthesis and Antimalarial Activities of Peri-Acylated Gossylic Nitriles. *J. Med. Chem.* **1986**, *29*, 1799–1801.
(18) Deck, L. D. Dissertation, Department of Biochemistry, University of New Mexico School of Medicine.

over a period of 7 days. The major peak in the mass spectrum of **5** corresponds to the mass of one-half of the molecule. The NMR spectrum also supports the structure shown. Compound **6**, prepared from **5**, was methylated with dimethyl sulfate to form gossylic lactone tetramethyl ether (**7**), which was synthesized by Adams and Geissman[19] by a different route.

Scheme II outlines the synthesis of gossypol derivatives with selectively methylated phenolic groups. Gossypol was treated with methanol and acid to form dimethyl dilactol **8** as a mixture of diastereomers. Compound **8** was converted to gossypol 1,1′-dimethyl ether (**9**) in four steps. Although the aldehyde groups of gossypol are not reduced with sodium borohydride, aldehyde **9** was readily reduced to alcohol **10** with this reagent. Compound **8** was also converted in four steps to gossylic nitrile 1,1′-dimethyl ether (**11**). Compound **8** was methylated with dimethyl sulfate to form the known[20] gossypol hexamethyl ether, **12**. This procedure resulted in the exclusive formation of the dilactol form of the hexamethyl ether with no free aldehyde form. The acetal methyl groups and the methyl groups at positions 7 and 7′ in **12** were removed by treatment with sulfuric acid in acetic acid according to a published procedure[21] to form gossypol 6,6′-dimethyl ether (**13**). The acetal methyl groups of **12** were selectively hydrolyzed under milder conditions to form gossypol 6,6′,7,7′-tetramethyl ether (**14**). The dialdehyde form of gossypol hexamethyl ether (**15**) was prepared by the method of Datta and co-workers.[22] This method involves methylating gossypol to form three gossypol hexamethyl ethers (dialdehyde, dilactol, and monoaldehyde–monolactol) and isolating the dialdehyde form by chromatography.

Inhibition of Aldose Reductase. Table I lists the inhibition constants (K_i) for the inhibition of human placenta aldose reductase by gossypol and gossypol derivatives, with glyceraldehyde as substrate. For convenience in the following discussion, inhibition constants were converted to binding energies ($G^0 = -RT \ln K_i$), which are also listed in Table I.

Of the gossypol derivatives tested, the best inhibitor was gossylic lactone, $K_i = 0.15$ μM. This compares favorably with sorbinil ($K_i = 0.28$ μM), which has been used as a benchmark compound for comparing aldose reductase inhibitors. While gossypol and gossylic lactone are good inhibitors of human aldose reductase, gossypol hexamethyl ether (dialdehyde form, **15**) and gossylic lactone tetramethyl ether are much poorer inhibitors. The binding energy of gossypol hexamethyl ether is 3.0 kcal lower than that of gossypol 1,1′-dimethyl ether. The binding energy of gossylic lactone tetramethyl ether is 3.4 kcal lower than that of gossylic lactone. This suggests that one or more of the three pairs of free hydroxyl groups of gossypol are required for reasonably tight binding to the enzyme. The difference in binding energy between gossypol and gossypol 6,6′-dimethyl ether supplies an estimate of 0.7 kcal for the contribution to binding made by the 6- and 6′-hydroxyl groups in the presence of other free hydroxyl groups. The 0.2 kcal greater binding energy of 1,1′-dimethylgossypol over gossypol suggests that the 1- and 1′-hydroxyl groups are not important for binding to the enzyme when other hydroxyl groups are present. The binding energy of gossylic nitrile 1,1′-dimethyl ether is slightly greater (0.5 kcal) than that of gossylic nitrile 1,1′-diacetate, and the binding energies of the gossylic nitrile 1,1′-diacylates increase slightly (0.5 kcal) as the acyl groups are increased in chain length from acetyl to hexanoyl. This suggests that lipophilicity rather than hydrogen-bonding ability is important at these positions. The binding energy of gossylic nitrile 1,1′-bis(2,2-dimethylpropanoate) is 0.4 kcal greater than that of its straight chain analogue, gossylic nitrile 1,1′-dipentanoate, and the binding energy of gossylic nitrile 1,1′-dicrotonate is 0.6 kcal greater than that of its saturated analogue, gossylic nitrile 1,1′-dibutyrate. Binding is apparently affected by steric properties of the acyl carbon chains. The binding energy of gossypol 6,6′,7,7′-tetramethyl ether is 1.0 kcal greater than that of gossypol hexamethyl ether. It appears that the 1,1′-hydroxyl groups can function, to some extent, in binding to the enzyme when other free hydroxyl groups are not present.

The binding energy of gossypol is 1.7 kcal greater than that of gossypol dioxime. The binding energy of gossypol 1,1′-dimethyl ether is 1.5 kcal greater than that of gossylic alcohol 1,1′-dimethyl ether and 1.3 kcal greater than that of gossylic nitrile 1,1′-dimethyl ether. These data suggest that the aldehyde groups contribute substantially to binding. The binding energy of gossylic lactone is 0.7 kcal greater than that of gossypol, so the lactone appears to contribute more to binding than does the aldehyde. The lactone differs from the aldehyde in that the carbonyl group is locked into a specific orientation with respect to the naphthalene ring system, the 1,1′-hydroxyl groups are esterified, and the electron density at the carbon atom is about 0.22 less. The effect of esterifying the 1,1′-hydroxyl groups can be estimated from the slightly greater binding energy (0.2 kcal) of gossypol 1,1′-dimethyl ether over that gossypol. The rest of the binding energy difference (0.5 kcal) is assumed to be due to steric or electronic differences between the aldehyde and lactone functional groups. An estimate of 4.3 kcal for the contribution of the lipophilic regions to the binding energy of gossypol can be made by subtracting 1.5 kcal (contribution of the carbonyl) from the binding energy of gossypol hexamethyl ether (5.8 kcal).

There is no correlation between the logs of the inhibition constants for the compounds listed in Table I and their LUMO values. There is likewise little or no correlation (coefficient = 0.15) between the logs of the inhibition constants and electron density at the electropositive carbon atoms (corresponding to the aldehyde carbons of gossypol). Apparently hydrogen bonding and steric interactions are more important than electronic effects in this series of compounds.

Discussion

The results of this study show that the Kador–Sharpless pharmacophor model for aldose reductase inhibitors is partially applicable to the inhibition of human aldose reductase by gossypol and derivatives. The model should be useful in the design of better inhibitors in this series. The results correspond with those which would be expected from the structural aspects of the model. No support was found, however, for the electronic aspects of the model.[7]

The lipophilic regions of gossypol and derivatives contribute most (about 4.3 kcal) to binding energy. With the gossypol derivatives at hand, it was not possible to separate the contributions of the primary and secondary lipophilic

(19) Adams, R.; Geissman, T. A. Structure of Gossypol. VIII. Derivatives of Ethers of Gossypol. *J. Am. Chem. Soc.* **1938**, *60*, 2166–2170.
(20) Seshadri, T. R.; Sharma, N. N. Isolation of the Hexamethyl Ether of the Dilactol Form of (±) Gossypol. *Indian J. Chem.* **1975**, *13*, 865–866.
(21) Adams, R.; Geissman, T. A. Structure of Gossypol. VII. Gossypol Dimethyl Ether. *J. Am. Chem. Soc.* **1938**, *60*, 2163–2166.
(22) Datta, S. C.; Murti, V. V. S.; Seshadri, T. R. A Study of the Derivatives of (±) Gossypol. *Indian J. Chem.* **1972**, *10*, 691–693.

regions. Since the pharmacophor model calls for the primary and secondary lipophilic regions to be coplanar, it seems likely that compounds based on the gossypol structure in which coplanarity could be attained would be superior inhibitors. The aldehyde groups contribute about 1.5 kcal to binding energy. According to the pharmacophor model, one aldehyde group would be expected to contribute the bulk of this binding energy. Unsymmetrical molecules with only one aldehyde might be better inhibitors. The phenolic hydroxyl groups in the 6,6',7,7'-positions contribute about 3.0 kcal to binding energy. They might be serving in the role of the hydroxyl groups at d_3 or d_4 in the model or both. Gossypol exhibits atropisomerism due to hindered rotation about the binaphthyl bond. Since the gossypol used in this work was racemic and none of the derivatives were resolved, the differences in inhibitory properties of their (+) and (-) isomers were not determined.

Experimental Section

Chemical Synthesis. The gossypol used in this research was provided as the acetic acid complex by the Southern Regional Research Center of the USDA. Solvents and other chemicals were reagent grade. IR spectra were recorded on a Beckman Model IR-33 spectrometer in KBr pellets. NMR spectra were recorded on a Varian FT 80-A (80 MHz) spectrometer in $CDCl_3$ unless otherwise indicated. Chemical shifts are in δ units relative to TMS. The mass spectral data was obtained with a Finnegan Model 4500 GC/MS/DS (EI, 70 eV) using the solids probe. Melting points were determined with a VWR Scientific Electrothermal capillary melting point apparatus and are uncorrected. Gossypol dioxime (**2**) was prepared as described by Clark.[16] Compounds **4a–g** were made by a general method developed in this laboratory as previously described.[17] Gossypol 6,6'-dimethyl ether (**13**) was prepared by the method of Adams and Geissman.[21] The dialdehyde form of gossypol hexamethyl ether (**15**) was prepared by the method of Datta and co-workers.[22]

Gossylic Iminolactone (5; 9,9',10,10'-Tetrahydroxy-6,6'-dimethyl-8,8'-bis(1-methylethyl)[5,5'-bi-2*H*-naphtho[1,8-*bc*]furan]-2,2'-diimine). One gram (1.3 mmol) of gossylic nitrile 1,1',2,2',3,3'-hexaacetate (**3a**)[17] was stirred in 10 mL of concentrated sulfuric acid at 25 °C for 30 min. Ice was added slowly, and the yellow solid was filtered off and recrystallized twice from acetone to yield 0.58 g (1.1 mmol, 85%) of yellow **4** which retained 1 equiv of water: mp 260 °C dec; IR 1665 (imino) cm^{-1}; MS m/z 256 (0.5 M^+, 100); NMR (perdeuteriodioxane) δ 1.52 (d, 12 H, J = 7 Hz), 2.3 (s, 6 H), 3.65 (sept, 2 H, J = 7 Hz), 6.99 (br s, 6 H), 7.60 (s, 2 H). Anal. ($C_{30}H_{28}N_2O_6 \cdot H_2O$) C, H, N.

Gossylic Lactone (6; 9,9',10,10'-Tetrahydroxy-6,6'-dimethyl-8,8'-bis(1-methylethyl)[5,5'-bi-2*H*-naphtho[1,8-*bc*]furan]-2,2'-dione). One gram (1.9 mmol) of crude **5** was refluxed with 20 mL of ethanol and 5 mL of 6 M HCl for 1 h. Toluene (30 mL) was added, and the reaction mixture was concentrated to 20 mL by distillation. The hot solution was filtered under pressure through a scintered glass funnel and the product allowed to crystallize at room temperature and then at 0 °C to yield 0.69 g (1.3 mmol, 68%) of brown **5**: mp 230–235 °C dec; IR 1745 (carbonyl) cm^{-1}; NMR δ 1.55 (d, 14 H, J = 7 Hz), 2.34 (s, 6 H), 3.88 (sept, 2 H, J = 7 Hz), 6.3 (br s, 2 H), 7.7 (s, 2 H). Anal. ($C_{30}H_{26}O_8$) C, H.

Gossylic Lactone Tetramethyl Ether (7; 9,9',10,10'-Tetramethoxy-6,6'-dimethyl-8,8'-bis(1-methylethyl)[5,5'-bi-2*H*-naphtho[1,8-*bc*]furan]-2,2'-dione). Compound **6** (0.2 g, 0.39 mmol) was dissolved in 40 mL of acetone. Two grams of potassium carbonate and 1 mL (10.5 mmol) of dimethyl sulfate were added. The suspension was heated under reflux for 20 h. The mixture was poured over ice, and the precipitate was filtered off and recrystallized from acetone/acetic acid to yield 0.18 g (0.32 mmol, 82%) of pale yellow **7**: mp 315–317 °C (lit.[19] mp 315–317 °C).

Gossypol Dimethyl Dilactol (8; 9,9',10,10'-Tetrahydroxy-2,2'-dimethoxy-6,6'-dimethyl-8,8'-bis(1-methylethyl)[5,5'-bi-2*H*-naphtho[1,8-*bc*]furan]). Gossypol acetic acid (0.35 g, 0.6 mmol) was dissolved in 30 mL of methanol, 0.1 g of *p*-toluenesulfonic acid and 1 mL of dimethoxymethane were added, and the mixture was refluxed for 1 h. After cooling, the solution was poured over ice and the precipitate was filtered off to yield 0.28 g (0.51 mmol, 83%) of gold **8**: dec at 180 °C without melting; IR 1100 (methyl acetal) cm^{-1}; NMR δ 1.52 (d, 12 H, J = 7 Hz), 2.07 (d, 6 H), 2.3 (s, 6 H), 3.88 (sept, 2 H, J = 7 Hz), 5.7, 5.8 (2 br s, 2 H total), 6.4 (br s, 2 H), 7.7 (s, 2 H), 8.4 (s, 2 H). Anal. ($C_{32}H_{34}O_8$) C, H.

Gossypol 1,1'-Dimethyl Ether (9; 6,6',7,7'-Tetrahydroxy-1,1'-dimethoxy-3,3'-dimethyl-5,5'-bis(1-methylethyl)[2,2'-binaphthalene]-8,8'-dicarboxaldehyde). Compound **8** (0.7 g, 1.28 mmol) was dissolved in 25 mL of acetone, potassium carbonate (0.85 g, 6.16 mmol) was added, and the mixture was stirred at room temperature for 0.5 h. Chloromethyl methyl ether (0.54 g, 6.71 mmol) was added dropwise, and the mixture was stirred for 3 h. The mixture was filtered onto ice containing a few drops of acetic acid. The lactol hydrolyzed on contact with the water and the yellow tetramethoxymethyl gossypol (0.7 g) was filtered off. This crude material was dissolved in 20 mL of methanol and a neutral solution of hydroxylamine hydrochloride (2 g, 61 mmol) in methanol was added. The mixture was heated under reflux for 1 h, cooled, and poured over ice. The buff oxime (0.7 g) was filtered off. This crude material was dissolved in 15 mL of methanol with KOH (0.6 g, 10.7 mmol). Dimethyl sulfate (0.8 g, 6.4 mmol) was added dropwise with stirring, which was continued for 4 h. The mixture was poured over ice and acidified with hydrochloric acid. The solid was filtered off and dissolved in 20 mL of methanol containing 5 mL of 1 N HCl, and the mixture was refluxed for 0.5 h to hydrolyze the methoxymethyl protecting groups. Ice was added to the cooled solution, and the precipitate was filtered off. It was crystallized from ligroin to yield 0.35 g (0.64 mmol, 50%) of **9** as orange crystals: dec 180 °C without melting; IR 1620 (aldehyde) cm^{-1}; NMR δ 1.51 (d, 14 H, J = 7 Hz), 2.25 (s, 6 H), 3.43 (br m, 4 H, J = 7 Hz), 4.00 (s, 6 H), 7.89 (br s, 2 H), 11.2 (br s, 2 H). Anal. ($C_{32}H_{34}O_8$) C, H.

Gossylic Alcohol 1,1'-Dimethyl Ether (10; 6,6',7,7'-Tetrahydroxy-8,8'-bis(hydroxymethyl)-1,1'-dimethoxy-3,3'-dimethyl-5,5'-bis(1-methylethyl)[2,2'-binaphthalene]). A solution containing 0.5 g (0.91 mmol) of **9**, 0.1 g (2.6 mmol) of sodium borohydride, and 30 mL of absolute ethanol was heated under reflux for 0.5 h. The solution was cooled and acidified with dilute HCl. Ice was added, and the precipitate was filtered off and crystallized from toluene/petroleum ether to yield 0.4 g (0.73 mmol, 80%) of gold **10**: dec at 210 °C without melting; IR 3430 (alcohol) cm^{-1}. Anal. ($C_{32}H_{38}O_8$) C, H.

Gossylic Nitrile 1,1'-Dimethyl Ether (11; 6,6',7,7'-Tetrahydroxy-1,1'-dimethoxy-3,3'-dimethyl-5,5'-bis(1-methylethyl)[2,2'-binaphthalene]-8,8'-dinitrile). This compound was synthesized by the same procedure as **9** except that instead of treating the oxime with KOH and dimethyl sulfate at room temperature, the reaction mixture was refluxed for 3 h. After cooling, the solution was acidified with dilute HCl and poured over ice. The precipitate (0.5 g) was filtered off and dissolved in 20 mL of methanol. Five milliliters of 1 M HCl was added, and the solution was refluxed for 30 min, cooled, and poured over ice. The gold solid was filtered off and crystallized from toluene/ligroin to yield 0.35 g (0.65 mmol, 51%) of **11**: dec at 180 °C without melting; IR 2230 (nitrile) cm^{-1}; NMR δ 1.57 (d, 12 H, J = 7 Hz), 2.16 (s, 6 H), 3.51 (sept, 2 H, J = 7 Hz), 3.90 (br s, 6 H, overlaps sept), 4.81 (br s, 4 H), 7.85 (s, 2 H). Anal. ($C_{32}H_{32}N_2O_6$) C, H, N.

Gossypol Hexamethyl Ether (Dilactol Form) (12; 2,2',9,9',10,10'-Hexamethoxy-6,6'-dimethyl-8,8'-bis(1-methylethyl)[5,5'-bi-2*H*-naphtho[1,8-*bc*]furan]). Compound **8** (1 g, 1.83 mmol) was dissolved in 5 mL of methanol, and dimethyl sulfate (5 mL, 53 mmol) was added. The solution was cooled in an ice bath and 7.5 mL of 10% potassium hydroxide in methanol was added dropwise. The mixture was allowed to stand for 4 days. A solution of 10% aqueous potassium hydroxide was added dropwise with cooling. The resulting precipitate was filtered off, washed with water, and recrystallized from ethanol to yield 0.9 g (1.49 mmol, 80%) of **11**: mp 270–271 °C (lit.[20] mp 270–271 °C).

Gossypol 6,6',7,7'-Tetramethyl Ether (14; 1,1'-Dihydroxy-6,6',7,7'-tetramethoxy-3,3'-dimethyl-5,5'-bis(1-methylethyl)[2,2'-binaphthalene]-8,8'-dicarboxaldehyde). Compound **12** (1 g, 1.7 mmol) was dissolved in 10 mL of acetone, and 2 mL of 1 N HCl was added. The mixture was refluxed for 30 min and

poured onto ice. The solid was filtered off and recrystallized once from ethanol and twice from ether/ligroin to yield 0.75 g (1.3 mmol, 76%) of 14 as microcrystalline needles: mp 162-163 °C); IR 3550 (alcohol) cm^{-1}. This compound which exists in several forms and retains solvent tenaciously was analyzed as its (2,4-dinitrophenyl)hydrazine derivative. Anal. ($C_{46}H_{46}N_8O_{14}$) C, H, N.

Molecular Orbital Calculations. The molecular orbital calculations for gossypol and derivatives were carried out using a computer program provided by Professor V. Ortiz, Department of Chemistry, University of New Mexico. The program incorporates features described in the literature.[23-25] Along with other parameters, it calculates the energy of the lowest unoccupied molecular orbital (LUMO) and the charge density at each atom in the molecule. The LUMO units are electron volts, and the charge density units are electrons. The LUMO values calculated by this program are different from the values calculated by Kador and Sharpless[7] for the same compounds. Therefore, LUMO calculations on several of the compounds used in their study were carried out with this program, and the values were found to be proportional to those reported by Kador and Sharpless.

Enzyme Purification and Kinetic Studies. Aldose reductase from human placenta was purified by the method of Vander Jagt and co-workers.[13,14] The purified enzyme was shown to retain its native properties. The sensitivity of the purified enzyme to inhibition by sorbinil did not change during storage at -20 °C for up to 6 months. Assays were conducted in 1 cm path length quartz cuvettes containing 10 mM D,L-glyceraldehyde and 0.1 mM NADPH in 1 mL of buffer (0.1 M sodium phosphate, pH 7). The temperature was maintained at 25 ± 1 °C with a thermostated circulating water bath. The reaction was followed by monitoring the absorbance decrease at 340 nm (the oxidation of NADPH to $NADP^+$) with a Perkin-Elmer Lambda 6 UV/vis spectrophotometer. Kinetic studies of aldose reductase inhibition were carried out under the same conditions. It has been shown for aldose reductases from some sources that inhibition constants vary greatly with substrate.[26] The human placental aldose reductase used in this study was therefore assayed with sorbinil as inhibitor and with the following substrates: glyceraldehyde (K_i = 0.28 μM), *p*-nitrobenzaldehyde (K_i = 0.29 μM), glucose K_i = 0.096 μM), and glucuronic acid (K_i = 0.17 μM). Since these values varied by only about a factor of 3, glyceraldehyde was used as substrate in the kinetic studies for the sake of convenience. Initial rates were corrected for the rate of absorbance decrease detected in the absence of the aldehyde substrate. The inhibitory properties of compounds were analyzed using Dixon plots. Two plots were made using different concentrations of NADPH. The experimental data were entered into the Enzfit program (Elsevier-Biosoft) for calculation of K_i.

Acknowledgment. We thank the Southern Regional Research Center of the USDA for providing the gossypol. We thank Professor Vincent Ortiz for assistance with the molecular orbital calculations. This work was supported in part by USPHS/NIH Grant AI25869.

Registry No. **2**, 17337-96-1; **3a**, 103068-47-9; **4a**, 94242-60-1; **4b**, 103094-22-0; **4c**, 103068-51-5; **4d**, 103068-52-6; **4e**, 136545-55-6; **4f**, 136545-56-7; **4g**, 136545-57-8; **5**, 136105-62-9; **6**, 135692-96-5; **7**, 136545-58-9; **8**, 124616-37-1; **9**, 136545-59-0; **10**, 136545-60-3; **11**, 136545-61-4; **12**, 31591-07-8; **13**, 1110-58-3; **14**, 136545-62-5; **15**, 27864-29-5; gossypol acetic acid, 12542-36-8; aldose reductase, 9028-31-3.

(23) Hoffmann, R.; Lipscomb, W. N. Boron Hydrides: LCAO-MO and Resonance Studies. *J. Chem. Phys.* **1962**, *37*, 2872-2883.
(24) Hoffmann, R. An Extended Huckel Theory. I. Hydrocarbons. *J. Chem. Phys.* **1963**, *39*, 1397-1412.
(25) Ammeter, J. H.; Burgi, H. B.; Thibeault, J. C.; Hoffmann, R. Counterintuitive Orbital Mixing in Semiempirical and Ab Initio Molecular Orbital Calculations. *J. Am. Chem. Soc.* **1978**, *100*, 3686-3692.
(26) Poulsom, R. Comparison of Aldose Reductase Inhibitors in vitro. Effects of Enzyme Purification and Substrate Type. *Biochem. Pharmacol.* **1987**, *36*, 1577-1581.

Activity of Acyclic 6-(Phenylselenenyl)pyrimidine Nucleosides against Human Immunodeficiency Viruses in Primary Lymphocytes

Naganna M. Goudgaon and Raymond F. Schinazi*

Veterans Affairs Medical Center, Atlanta, Georgia 30033, and Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, 1670 Clairmont Road, Decatur, Georgia 30322. *Received June 7, 1991*

Several 6-phenylselenenyl-substituted acyclouridine derivatives were prepared for evaluation as antiviral agents. Lithiation of the *tert*-butyldimethylsilyl-protected acyclonucleosides **4a-f** with lithium diisopropylamide at -78 °C, followed by reaction with diphenyl diselenide as an electrophile, and subsequent removal of the protecting group with tetra *n*-butylammonium fluoride gave 1-[(2-hydroxyethoxy)methyl]-6-(phenylselenenyl)uracils **6a-f** in 50-70% overall yield. The potency and spectrum of activity of compounds **6a-f** against HIV-1 in vitro was similar to HEPT (1), a related 6-phenylthio acyclonucleoside. However, whereas HEPT inhibited HIV-1 reverse transcriptase, the selenium-containing derivatives were ineffective, suggesting a different mechanism of action. Of significance was the finding that the 6-phenylselenenyl acyclonucleosides inhibited also HIV-2 in primary human lymphocytes.

Introduction

In the search for more selective and effective agents against human immunodeficiency virus type 1 (HIV-1), which is the causative agent for the acquired immunodeficiency syndrome (AIDS), a large number of nucleoside analogues with modifications being made in the heterocyclic base and/or the sugar moiety have been extensively investigated for their antiviral activities.[1,2] Several 2',3'-dideoxy nucleosides are approved or are currently undergoing clinical trials in patients with AIDS and AIDS-related complex. These include 3'-azido-3'-deoxythymidine (AZT), 2',3'-didehydro-3'-deoxythymidine (D4T), 3'-azido-2',3'-dideoxyuridine (AzddU), 2',3'-dideoxycytidine (DDC), 2',3'-dideoxyinosine (DDI), and 2',3'-dideoxy-3'-thiacytidine (BCH-189).[3a-d] Recently, it

* To whom correspondence should be addressed.

(1) Mitsuya, H.; Yarchoan, R.; Broder, S. Molecular Targets for AIDS Therapy. *Science (Washington, D.C.)* **1990**, *249*, 1533-1544.
(2) Schinazi, R. F. Strategies and Targets of Anti-Human Immunodeficiency Virus Type 1 Chemotherapy. In *AIDS in Children, Adolescents and Heterosexual Adults; An Interdisciplinary Approach to prevention*; Schinazi, R. F., Nahmias, A. J., Eds.; Elsevier: New York, 1988; pp 126-143.

0022-2623/91/1834-3305$02.50/0