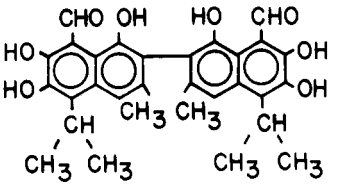
Biologically Active Derivatives of Gossypol: Synthesis and Antimalarial Activities of Peri-Acylated Gossylic Nitriles

Robert E. Royer,† Lorraine M. Deck,‡ Naomi M. Campos,† Lucy A. Hunsaker,† and David L. Vander Jagt*†

Department of Biochemistry, University of New Mexico School of Medicine, Albuquerque, New Mexico 87131, and Department of Chemistry, University of New Mexico, Albuquerque, New Mexico 87131. *Received February 3, 1986*

A series of peri-acylated gossylic nitriles were synthesized from gossypol dioxime by treatment of the dioxime with the appropriate acid anhydride and its salt. The reaction pathway was elucidated by isolation and characterization of intermediates. Peri-acylated gossylic nitriles (acyl = acetyl, propionyl, butyryl, and valeryl) were compared with gossypol for activity against both chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum*. The gossylic nitriles all retain activity, with activity increasing with the length of the *peri*-acyl group. Gossylic nitrile 1,1′-divalerate shows antimalarial activity comparable to gossypol itself. The peri-acylated gossylic nitriles are strong inhibitors of parasite lactate dehydrogenase.

Gossypol (**1**), a toxic disesquiterpene obtained from cotton seeds, has been the subject of research in animal nutrition for many years.[1,2] The toxicity of gossypol specifically has limited the use of cotton seed meal as a source of dietary protein. This toxicity results, at least in part, from reactions involving the two aldehyde functional groups.[3]



1

In recent years there has been an increased interest in gossypol, due mainly to reports from the People's Republic of China that gossypol exhibits antispermatogenic properties. Clinical trials, first reported in 1978,[4] continue in China and elswhere on the usefulness of gossypol as a male antifertility agent.

Gossypol exhibits biological activities in addition to its antispermatogenic activity. These include spermicidal,[5] antiviral,[6,7] and antiparasitic[8,9] activities. In a preliminary report[9] we described the antimalarial activity of gossypol in vitro against the human pathogen *Plasmodium falciparum*, and we reported that a derivative of gossypol retains antimalarial activity.[10] In the present study, we describe the synthesis of a series of peri-acylated gossylic nitriles and report on the antimalarial activities of these derivatives against chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum*. This series of compounds was selected because it involves modification of the aldehyde functional groups of gossypol, groups that are known to contribute to toxicity.

Chemical Synthesis. Gossylic nitrile 1,1′-diacetate (**6a**) was synthesized by the route shown in Scheme I from the previously described[11] gossypol dioxime (**2**). A suspension of **2** was stirred in acetic anhydride to give a yellow intermediate (**3a**), which could be filtered off, but which darkened on standing and decomposed with an attempted recrystallization. The NMR spectrum of **3a** has a single new peak (δ 2.23) corresponding to a pair of chemically equivalent acetyl groups. The phenolic hydroxyls of gossypol are not acetylated under these conditions. During the course of synthesis of **6a**, oxime ester **3a** was not isolated, but sodium acetate was added and the reaction mixture was heated on a boiling water bath to give the bis(anhydro oxime) (**4a**). The NMR spectrum of **4a** indicates the presence of two pairs of equivalent acetyl groups and no free hydroxyls. The aldehyde proton signal of gossypol (δ 11.18) is replaced by one for the benzylimino

Scheme I

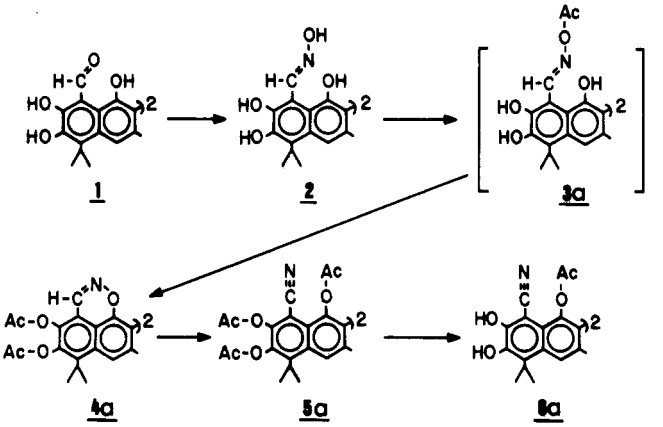


Table I. Antimalarial Activities of Gossypol and *peri*-Acyl Gossylic Nitriles against *P. falciparum*, in Vitro

drug	IC_{50},[a] μM strain FCB/NC-1[b]	strain CDC/I/HB-3
gossypol	13[c]	7
gossylic nitrile 1,1′-diacetate	76	36
gossylic nitrile 1,1′-dipropionate	69	46
gossylic nitrile 1,1′-dibutyrate	26	21
gossylic nitrile 1,1′-divalerate	16	12

[a] IC_{50} represents the drug concentration that produced 50% inhibition of growth of *P. falciparum*. [b] Strains FCB/NC1 and CDC/I/HB-3 are the chloroquine-resistant and chloroquine-sensitive strains of parasite, respectively. [c] IC_{50} values were obtained from plots of the growth inhibition data as shown in Figure 1. All experimental points were obtained in triplicate. These triplicate points showed a range less than ±5%.

† Department of Biochemistry.
‡ Department of Chemistry.

(1) Withers, W. A.; Carruth, F. E. *Science (Washington, D.C.)* **1915**, *41*, 324.
(2) Abou-Donia, M. B. *Residue Rev.* **1976**, *61*, 126.
(3) Clark, E. P. *J. Biol. Chem.* **1928**, *76*, 229.
(4) National Coordinating Group on Male Antifertility Agents *Chin. Med. J.* **1978**, *4*, 417.
(5) Waller, D. P.; Zaneveld, L. J. D.; Fong, H. H. S. *Contraception* **1980**, *22*, 183.
(6) Wichmann, K.; Vaheri, A.; Luukkainen, T. *Am. J. Obstet. Gynecol.* **1982**, *142*, 593.
(7) Dorsett, P. H.; Kerstine, E. E. *J. Pharm. Sci.* **1975**, *64*, 1073.
(8) Montamat, E. E.; Burgos, C.; Gerez de Burgos, N. M.; Rovai, L. E.; Blanco, A. *Science (Washington, D.C.)* **1982**, *218*, 288.
(9) Heidrich, J. E.; Hunsaker, L. A.; Vander Jagt, D. L. *IRCS Med. Sci.* **1983**, *11*, 304.
(10) Vander Jagt, D. L.; Baack, B. R.; Campos, N. M.; Hunsaker, L. A.; Royer, R. E. *IRCS Med. Sci.* **1984**, *12*, 845.
(11) Clark, E. P. *J. Biol. Chem.* **1927**, *75*, 725.

0022-2623/86/1829-1799$01.50/0

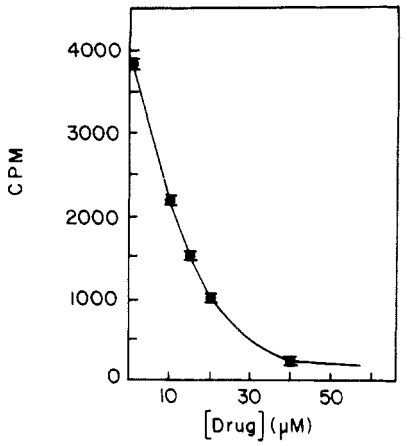


Figure 1. Inhibition of growth of *P. falciparum*, strain CDC/I/HB-3, by gossylic nitrile 1,1′-divalerate, as determined by uptake of [^{3}H]hypoxanthine: LD_{50} = 12 μM.

proton (δ 8.90). The bis(anhydro oxime) was previously known in the form of its tetramethyl ether.[12] Compound **4a** was isolated or was converted directly to gossylic nitrile hexaacetate (**5a**) by bringing the reaction mixture to a boil for 30 min. The formation of the nitrile was clearly indicated by a new band at 2230 cm^{-1} in the IR spectrum. The four acetyl groups protecting the *o*-hydroxyls of **5a** were readily removed by refluxing in aqueous methanol with potassium carbonate to give **6a**. Gossylic nitrile dipropionate (**6b**), dibutyrate (**6c**), and divalerate (**6d**) were synthesized by exactly the same procedure used for **6a**, except that the corresponding acid anhydride and salt were substituted for acetic anhydride and sodium acetate.

Biological Data. Antimalarial Activities, in Vitro, of Peri-Acylated Gossylic Nitriles. The antimalarial activities of a series of peri-acylated gossylic nitriles against the human malarial parasite *Plasmodium falciparum* were determined in an in vitro assay system. Two cloned strains of *P. falciparum* were used. The activities, expressed as IC_{50} values, for the peri-acylated gossylic nitriles and for gossypol against these two strains are listed in Table I. All of the compounds are active against both strains of parasite. The activity of the peri-acylated gossylic nitriles increases with increased length of the *peri*-acyl substituent. Gossylic nitrile 1,1′-divalerate is almost as active as gossypol in both systems. A representative plot is shown in Figure 1 for the inhibition of growth of the chloroquine-sensitive strain CDC/I/HB-3 by gossylic nitrile 1,1′-divalerate.

Inhibition of Parasite Lactate Dehydrogenase by Peri-Acylated Gossylic Nitriles. The putative target for the antimalarial activity of gossypol is parasite lactate dehydrogenase, which is irreversibly inactivated by gossypol.[9,10] The inhibitory properties of the peri-acylated gossylic nitriles against lactate dehydrogenase isolated from strain FCB/NC1[9,10] were determined. The results are listed in Table II. In general, the inhibition of lactate dehydrogenase by the peri-acylated gossylic nitriles increases with increased length of the *peri*-acyl group, except for gossylic nitrile 1,1′-diacetate, which is a stronger inhibitor of lactate dehydrogenase than would be predicted from its antimalarial activity. The peri-acylated gossylic nitriles are competitive inhibitors of the binding of NADH. A representative plot is shown in Figure 2 for inhibition by gossylic nitrile 1,1′-dibutyrate. All of the data are consistent with the idea that parasite lactate dehydrogenase is the target site for these compounds.

(12) Adams, R.; Geissman, T. A. *J. Am. Chem. Soc.* **1938**, *60*, 2184.

Table II. Inhibition of Lactate Dehydrogenase from a Chloroquine-Resistant Strain (FCB/NC-1) of *P. falciparum* by a Series of Peri-Acylated Gossylic Nitriles

drug	K_i,[a] M
gossylic nitrile 1,1′-diacetate	8.3×10^{-7}
gossylic nitrile 1,1′-dipropionate	1.1×10^{-6}
gossylic nitrile 1,1′-dibutyrate	6.2×10^{-7}
gossylic nitrile 1,1′-divalerate	3.3×10^{-7}

[a] K_i values were obtained from double-reciprocal plots of the inhibition kinetics determined in 0.025 M Tris, pH 7.5, using 10 mM pyruvate as the fixed substrate and NADH as the varied substrate.

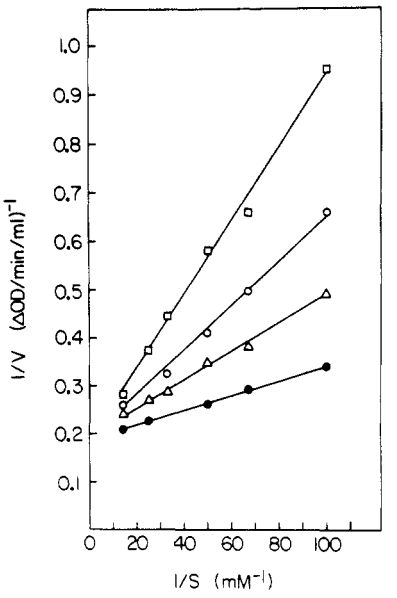


Figure 2. Inhibition of parasite lactate dehydrogenase by gossylic nitrile 1,1′-dibutyrate. Initial rates were determined in the absence of drug (●) or in the presence of 0.625 μM (△), 1.25 μM (○), or 2.50 μM (□) drug. NADH is the varied substrate, S. Inhibition is competitive, $K_i = (6.2 \pm 0.2) \times 10^{-7}$ M.

Experimental Section

Melting points were determined on a VWR Scientific Electrothermal capillary melting point apparatus and are uncorrected. Infrared spectra were obtained on a Beckman IR-33 spectrophotometer in KBr pellets. Those IR bands are given that clearly indicate the nature of a structural change accomplished in a synthetic step or series of steps. The ^{1}H NMR spectra were taken with a Varian FT 80-A (80-MHz) spectrometer, and chemical shifts are reported in δ units downfield from Me_4Si. The samples were dissolved in $CDCl_3$, and the residual $CHCl_3$ signal was used as an internal standard. The gossypol acetic acid used in this research was provided by the Southern Regional Research Center of the USDA. Solvents and other chemicals were reagent grade. Gossypol dioxime (**2**) was prepared by heating gossypol with neutralized hydroxylamine hydrochloride in ethanol according to the method of Clark.[11]

Gossypol Bis(anhydro oxime) Tetraacetate (4a). One gram (1.8 mmol) of **2** was stirred in 5 mL of acetic anhydride for 5 h at room temperature. Freshly fused and powdered sodium acetate (1 g) was added to the light-yellow suspension, and stirring was continued for 2 h. The reaction flask was then placed in a water bath, which was slowly heated to boiling over a period of 30 min and held at boiling for an additional 30 min. The reaction mixture was allowed to cool, poured onto 50 g of ice, and stirred until the acetic anhydride was hydrolyzed. The light-yellow crude product was filtered off, washed with water, and recrystallized from methanol/acetone to give 800 mg (1.18 mmol, 65%) of **4a** as white microcrystalline plates: mp 210–215 °C; IR 1775 (acetyl) cm^{-1}; NMR 1.61 (d, 12 H, J = 7 Hz), 2.05 (s, 6 H), 2.36 (s, 6 H), 2.59 (s, 6 H), 4.02 (m, 2 H, J = 7 Hz), 8.21 (s, 2 H), 8.90 (s, 2 H). Anal. ($C_{38}H_{36}N_2O_{10}$) C, H, N.

Gossylic Nitrile Hexaacetate (5a). Compound **4a** was prepared as described above, but was not isolated. Instead the

reaction flask was taken off the boiling water bath, placed on a hot plate, and brought to a slow boil for 30 min. The reaction mixture was then cooled and hydrolyzed on 50 g of ice. The product was filtered, washed with water, and recrystallized from methanol/acetone to give 980 mg (1.28 mmol, 71%) of white, microcrystalline **5a**: mp 281–284 °C; IR 2230 (nitrile) cm^{-1}; NMR 1.52 (d, 12 H, J = 7 Hz), 2.08 (s, 6 H), 2.28 (s, 6 H), 2.48 (s, 12 H), 3.85 (sept, 2 H), 8.13 (s, 2 H). Anal. ($C_{42}H_{40}N_2O_{12}$) C, H, N.

Gossylic Nitrile 1,1′-Diacetate (6a; 6,6′,7,7′-Tetrahydroxy-3,3′-dimethyl-5,5′-bis(1-methylethyl)-1,1′-bis(ethanoyloxy)[2,2′-binaphthalene]-8,8′-dinitrile). Compound **5a** (500 mg, 0.65 mmol) was added to 5 mL of methanol. One milliliter of water and 500 mg of potassium carbonate were added, and the mixture was refluxed for 30 min. The mixture was allowed to cool and was acidified by dropwise addition of acetic acid. Ten milliliters of water was added, and the reaction mixture was chilled. The off-white product was filtered, washed with water, and dried. It was recrystallized once from methanol/water and once from toluene/acetone to give 275 mg (0.46 mmol, 71%) of microcrystalline needles: mp 300–302 °C dec; IR 2220 (nitrile), 1765 (acetyl) cm^{-1}; NMR 1.58 (d, 12 H, J = 7 Hz), 1.98 (s, 6 H), 2.24 (s, 6 H), 3.96 (sept, 2 H, J = 7 Hz), 6.4 (br s, 4 H), 7.98 (s, 2 H). Anal. ($C_{34}H_{32}N_2O_8$) C, H, N.

Compounds **6b–d** were synthesized by using the same procedures and molar ratios of the corresponding acid anhydrides and acid salts that were used for **6a**. Intermediates were not isolated, and yields are based on **2** as starting material.

Gossylic Nitrile 1,1′-Dipropionate (6b; 6,6′,7,7′-Tetrahydroxy-3,3′-dimethyl-5,5′-bis(1-methylethyl)-1,1′-bis(propanoyloxy)[2,2′-binaphthalene]-8,8′-dinitrile). Compound **6b** was recrystallized from toluene to give a tan powder in 62% yield: darkened at 145 °C, dec without melting; IR 2220 (nitrile), 1770 (propionyl) cm^{-1}; NMR 0.69 (t, 6 H, J = 7 Hz), 1.52 (d, 12 H, J = 7 Hz) 2.21 (s, 6 H), 2.24 (q, 4 H, overlaps 2.21s), 3.96 (sept, 2 H, J = 7 Hz), 6.05 (br s, 4 H), 7.93 (s, 2 H). Anal. ($C_{36}H_{36}N_2O_8$) C, H, N.

Gossylic Nitrile 1,1′-Dibutyrate (6c; 6,6′,7,7′-Tetrahydroxy-3,3′-dimethyl-5,5′-bis(1-methylethyl)-1,1′-bis(butanoyloxy)[2,2′-binaphthalene]-8,8′-dinitrile). Compound **6c** was recrystallized from toluene to give tan microcrystalline plates in 58% yield: darkened at 150 °C, dec without melting; IR 2240 (nitrile), 1775 (butyryl) cm^{-1}; NMR 0.43 (t, 6 H, J = 7 Hz), 1.10 (m, 4 H), 1.51 (d, 12 H, J = 7 Hz), 2.20 (s, 6 H), 2.27 (t, 4 H, overlaps 2.20s), 3.96 (sept, 2 H, J = 7 Hz), 6.05 (br s, 4 H), 7.91 (s, 2 H). Anal. ($C_{38}H_{40}N_2O_8$) C, H, N.

Gossylic Nitrile 1,1′-Divalerate (6d; 6,6′,7,7′-Tetrahydroxy-3,3′-dimethyl-5,5′-bis(1-methylethyl)-1,1′-bis(pentanoyloxy)[2,2′-binaphthalene]-8,8′-dinitrile). Compound **6d** was recrystallized from toluene to give a tan powder in 58% yield: softened at 135 °C, dec without melting; IR 2230 (nitrile), 1765 (valeryl) cm^{-1}; NMR 0.47 (t, 6 H, J = 7 Hz), 0.92 (m, 8 H), 1.53 (d, 12 H, J = 7 Hz), 2.22 (s, 6 H), 2.29 (t, 4 H, overlaps 2.22s), 3.96 (sept, 2 H, J = 7 Hz), 6.05 (br s, 4 H), 7.93 (s, 2 H). Anal. ($C_{40}H_{44}N_2O_8$) C, H, N.

Strains of *P. falciparum*. The chloroquine-resistant strain FCB, a Colombian strain of *P. falciparum*, was cloned by limiting dilution. The clone used in the present study is denoted as clone NC-1. The chloroquine-sensitive strain of *P. falciparum*, strain CDC/I/HB-3, is a Honduras strain that was cloned by Prof. W. Trager, Rockefeller University. This clone was obtained from the Malaria Branch, Center for Disease Control, Altanta, GA, with permission from Prof. Trager.

Parasite Growth. *P. falciparum* was grown in vitro in human erythrocytes. Culture dishes contained 2% erythrocytes in RPMI 1640 medium supplemented with 5 mM glutamine, 35 mM Hepes, 24 mM sodium bicarbonate, and gentamicin, 33 mg/L. The medium contained 10% (v/v) horse serum, pH 7.2. Parasite growth was monitored by measuring the uptake of [^{3}H]hypoxanthine and its incorporation into nucleic acid. Aliquots of media, 250 μL, containing parasitized erythrocytes, 0.5% parasetemia, were added to microtiter wells. Drug at the appropriate concentration was added to the wells in 1 μL of Me_2SO. Control wells received 1 μL of Me_2SO. Samples were run in triplicate at each concentration of drug. After addition of the drug, [^{3}H]hypoxanthine (New England Nuclear), 0.5 μCi, was added to each well. Microtiter plates were incubated for 3 days, 37 °C, in 5:95 CO_2/air, after which the contents were collected on glass fiber filters, using a cell harvester. The filters were washed with distilled water and then were counted by liquid scintillation. In separate experiments, the [^{3}H]hypoxanthine was omitted, and parasite growth was monitored by differential cell counting of Giemsa-stained slides. This confirmed that the uptake of [^{3}H]hypoxanthine provided a good indication of parasite growth.

Acknowledgment. This work was supported by USPHS/NIH Grants GM 25295 and AI 21214. Gossypol was provided by the Southern Regional Research Center for the USDA through S. P. Koltun, Actg. Research Leader, Food and Feed Engineering.

Registry No. **1**, 303-45-7; **2**, 17337-96-1; **4a**, 103068-43-5; **4b**, 103068-44-6; **4c**, 103068-45-7; **4d**, 103068-46-8; **5a**, 103068-47-9; **5b**, 103068-48-0; **5c**, 103068-49-1; **5d**, 103068-50-4; **6a**, 94242-60-1; **6b**, 103094-22-0; **6c**, 103068-51-5; **6d**, 103068-52-6.

Studies in Antifertility Agents. 50. Stereoselective Binding of *d*- and *l*-Centchromans to Estrogen Receptors and Their Antifertility Activity[1]

M. Salman,[†] Suprabhat Ray,*[†] Nitya Anand,[†] A. K. Agarwal,[‡] M. M. Singh,[‡] B. S. Setty,[‡] and V. P. Kamboj[‡]

Medicinal Chemistry and Endocrinology Divisions, Central Drug Research Institute, Lucknow 226001, India.
Received December 3, 1985

Centchroman [*dl*-3,4-*trans*-2,2-dimethyl-3-phenyl-4-[*p*-(β-pyrrolidinoethoxy)phenyl]-7-methoxychroman hydrochloride], an antifertility agent under clinical evaluation, has been resolved into its optical enantiomers. The cytosol estrogen receptor binding affinity and estrogenic, antiestrogenic and antiimplantation activities of the two enantiomers have been determined. The enantiomers display a 7-fold difference in receptor affinity, and a corresponding difference in stimulation of the uterine growth and antiimplantation activity was observed in rats.

As a result of studies carried out in this laboratory on estrogen receptor (ER) binding to various types of di- and triarylethylenes, -ethanes[4] and -alkanones,[5] some generalizations have emerged for the contribution of different substituents of the prototypes of ligand–receptor binding

[†] Medicinal Chemistry Division.
[‡] Endocrinology Division.

(1) CDRI Communication No. 3735.
(2) Ray, S.; Grover, P. K.; Kamboj, V. P.; Setty, B. S.; Kar, A. B.; Anand, N. *J. Med. Chem.* **1976**, *19*, 276.
(3) Anand, N.; Ray, S. *Indian J. Exp. Biol.* **1977**, *15*, 1142.
(4) Durani, S.; Agarwal, A. K.; Saxena, R.; Setty, B. S.; Gupta, R. C.; Kole, P. L.; Ray, S.; Anand, N. *J. Steroid Biochem.* **1979**, *11*, 67.
(5) Gopalachari, R.; Iyer, R. N.; Kamboj, V. P.; Kar, A. B. *Contraception* **1970**, *22*, 962.

0022-2623/86/1829-1801$01.50/0