Synthesis and Antifertility Activity of Zoapatanol Analogues

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The synthesis of and guinea pig contragestational screening data for several oxepane and 3,8-dioxabicyclo[3.2.1]octane analogues of zoapatanol (1) are described and their structure–activity relationships discussed. Conversion of the 5-keto group on the nonenyl side chain of 1 into a hydroxyl function enhanced the potency. Further significant enhancement in the potency was realized with the transformation of several oxepanes into the 3,8-dioxabicyclo-[3.2.1]octane-1-acetic acid derivatives. Detailed, comparative contragestational evaluation of the three most potent compounds **9**, **33**, and **37** is presented, which led to the selection of **33** (ORF 13811) for further biological evaluation.

A complete account of the isolation and structural elucidation of the novel oxepane diterpenoids zoapatanol (**1**, structure I, R_4 = H, b = saturated, c = unsaturated) and montanol (**19**, I, R_4 = CH_3, b = unsaturated, c = saturated) from the Mexican plant zoapatle (*Montanoa tomentosa*, Compositae) has recently appeared.[1] The total synthesis of **1** from our laboratories[2,3] and others[4] has also recently been reported.

The use of the aqueous extract or "tea" of the zoapatle leaves for the last several centuries to induce menses and labor and to regulate fertility is well-known.[5] Biological evaluation of the various semipurified extracts as well as purified **1** from the plant showed a contragestational profile in laboratory animals.[6]

In order to delineate the structure–activity relasionships in this series, we have synthesized a number of derivatives[7] from plant-derived **1** and **19** and evaluated their contragestational activity in the guinea pig by the same method.[6] This paper summarizes these studies, which resulted in the identification of several highly potent analogues of **1**. Furthermore, a detailed dose–response evaluation of three of the most interesting compounds, which were selected for possible further development as antifertility agents, is also presented.

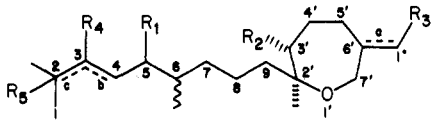
Chemistry. The derivatives prepared from **1** and **19** are broadly grouped into two types: (a) the monocyclic oxepanes (**1–22**, Table I) and (b) the bicyclic oxepanes or 3,8-dioxabicyclo[3.2.1]octane derivatives (**23–47**, Table II). The syntheses of the various monocyclic oxepanes are outlined in Scheme I. A few of these (**2**, **4**, **7–10**, **15**, **16**, **20**, **22**) were characterized earlier in the course of our structure elucidation work.[1] Compounds **17** and **18** (structure II, R_5 = OOH and OH, respectively) were identified as the air-oxidation products of **1**. As is well documented for other β,γ-enones,[8–10] compound **1**, its alcohol derivatives (e.g., **2–5**) and related bicyclic derivatives readily suffered air oxidation during their handling in organic solvents under ambient conditions of air and light to form **18** and its corresponding derivatives.[16] This autoxidation susceptibility of **1** and its β,γ-enone-containing derivatives was overcome by converting them to the corresponding homoallylic alcohols (e.g., IV) which were relatively stable to air and light. The synthetic steps leading to the various bicyclic oxepanes are outlined in Scheme II. The formation of the 3,8-dioxabicyclo-[3.2.1]octane system by transannular ether bridge formation was established during the MnO_2 oxidation of **1** to saturated aldehyde **25** and also during the dehydration of **1** to **23**.[1] The bicyclic acetic acid derivatives VI (**26**, **30**, **33**, **38**, **39**, **43**, **45**) were prepared by the selective oxidation of the primary hydroxyl function of I (**1**, **19**) and IV (**9**, **13**, **14**, **20**), respectively, by use of platinum-catalyzed oxidations.[11] Oxidation of **1** and **19** with chromic acid/H_2SO_4, on the other hand, gave the side chain degraded acids V (**40** and **42**, respectively). The nonenyl side chain degraded bicyclic diacid **46** (IX, R = H, R_1 = O) resulted from treatment of monoacid **26** with OsO_4. Alternatively, its monomethyl ester IX (R = CH_3, R_1 = O) could be obtained by oxidative workup with H_2O_2 following ozonolysis of a mixture of the methyl esters of acids **26**, **30**, and **43**. Selective reduction of the carboxyl function in the presence of the carbomethoxy function in IX was accomplished with catecholborane[12] to afford the primary alcohol **47** (IX, R = CH_3, R_1 = H_2). Preparation of the keto alcohols **24** and **27** (VIII, R_1 = O) entailed the selective reduction of the aldehyde function in the presence of the keto function in VII (**25** and **28**, R_1 = O) with sodium triacetoxyborohydride.[13] The acyclic derivative **48** was obtained by the hydrogenolytic ring opening[1] of **1** upon treatment with H_2/Pd in EtOH (Scheme I).

Structure–Activity Relationship. The contragestational activity of the various zoapatanol-related derivatives is presented in Table I for the monocyclic oxepanes **1–22** and in Table II for the 3,8-dioxabicyclo[3.2.1]octanes **23–47**. The ED_{100} of zoapatanol (**1**, 80–100 mg/kg) has been arbitrarily selected to represent base-line potency and is symbolized as (+). Compounds having activity at a dose greater than 100 mg/kg are considered to be relatively less interesting, and, therefore, their activity is represented by the symbol NS (not significant relative to **1**).[14] The very

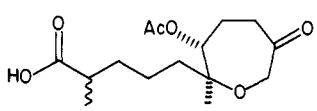
(1) Kanojia, R. M.; Wachter, M. P.; Levine, S. D.; Adams, R. E.; Chen, R.; Chin, E.; Cotter, M. L.; Hirsch, A. F.; Huetteman, R.; Kane, V. V.; Ostrowski, P.; Shaw, C. J.; Mateos, J. L.; Noriega, L.; Guzman, A.; Mijarez, A.; Tovar, L.; Shefter, E. *J. Org. Chem.* **1982**, *47*, 1310–1319.
(2) Chen, R.; Rowand, D. A. *J. Am. Chem. Soc.* **1980**, *102*, 6609–6611.
(3) Kane, V. V.; Doyle, D. L. *Tetrahedron Lett.* **1981**, *22*, 3027–3030.
(4) Nicolaou, K. C.; Claremon, D. A.; Barnett, W. E. *J. Am. Chem. Soc.* **1980**, *102*, 6611–6612.
(5) Levine, S. D.; Hahn, D. W.; Cotter, M. L.; Greenslade, F. C.; Kanojia, R. M.; Pasquale, S. A.; Wachter, M. P.; McGuire, J. L. *J. Reprod. Med.* **1981**, 524–528.
(6) Hahn, D. W.; Ericson, E. W.; Lai, M. T.; Probst, A. *Contraception* **1981**, *23*, 133–140.
(7) Kanojia, R. M; Wachter, M. P.; Chen, R. U.S. Patent 4 130 556, December 19, 1978.
(8) Fieser, L. F.; Greene, T. W.; Bischoff, F.; Lopez, G.; Rupp, J. J. *J. Am. Chem. Soc.* **1955**, *77*, 3928–3929.
(9) Shapiro, E. L.; Legatt, T.; Olivetto, E. P. *Tetrahedron Lett.* **1964**, 663–664.
(10) Furutachi, N.; Nakadaria, Y.; Nakanishi, K. *Chem. Commun.* **1968**, 1625–1626.
(11) Heynes, K.; Paulsen, H. "Newer Methods of Preparative Organic Chemistry"; Forest, W., Kirchner, F. K., Ed.; Academic Press: New York, 1963; Vol. 2, pp 303–335.
(12) Kabalka, G. W.; Baker, J. D., Jr.; Neal, G. W. *J. Org. Chem.* **1977**, *42*, 512–517.
(13) Gribble, G. W.; Ferguson, D. C. *J. Chem. Soc., Chem. Commun.* **1975**, 535.
(14) See footnote *a* under Tables I and II.

0022-2623/85/1828-0796$01.50/0

Table I. Biological Activity of Monocyclic Oxepanes



I-IV
(1-21)

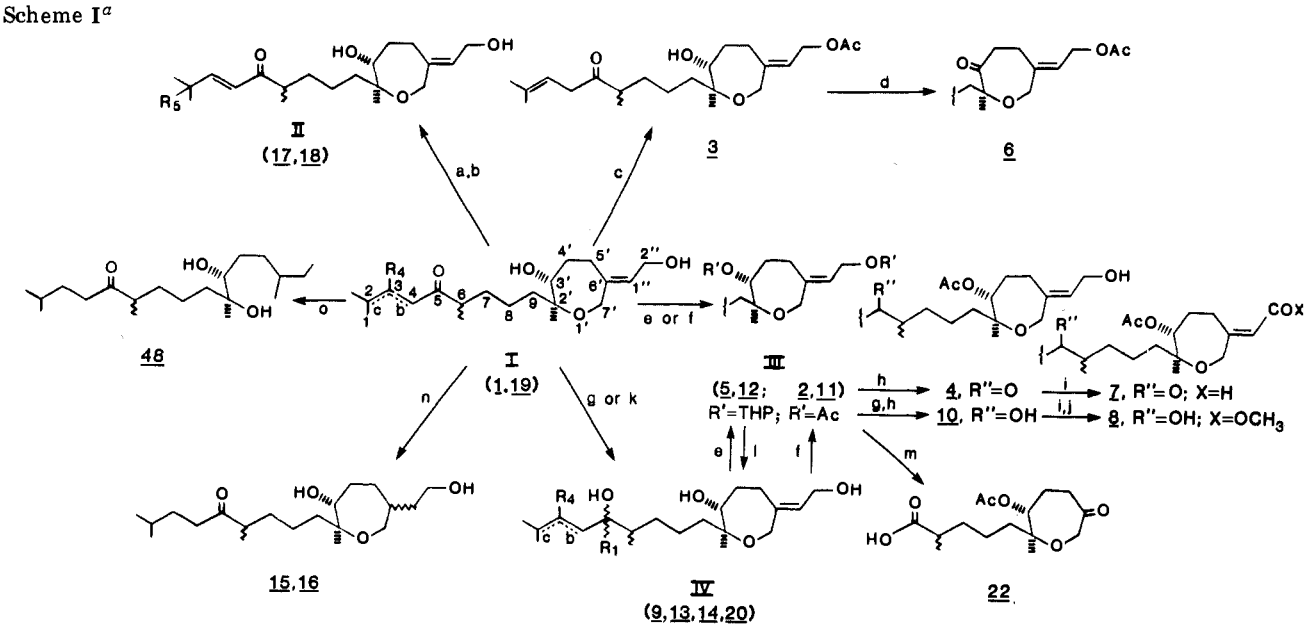


22

no.	R_1	R_2	R_3	R_4	R_5	a	b	c	guinea pig contragestational act.[a]
1	=O	OH	CH_2OH	H		U[b]	S	U	+
2	=O	OAc	CH_2OAc	H		U	S	U	+
3	=O	OH	CH_2OAc	H		U	S	U	+
4	=O	OAc	CH_2OH	H		U	S	U	++
5	=O	OTHP	CH_2OTHP	H		U	S	U	NS
6	=O	=O	CH_2OAc	H		U	S	U	NS
7	=O	OAc	CHO	H		U	S	U	+++
8	~OH	OAc	$COOCH_3$	H		U	S	U	NS
9	~OH	OH	CH_2OH	H		U	S	U	+++
10	~OH	OAc	CH_2OAc	H		U	S	U	+
11	~OAc	OAc	CH_2OAc	H		U	S	U	+
12	~OTHP	OTHP	CH_2OTHP	H		U	S	U	NS
13		OH	CH_2OH	H		U	S	U	+++
14		OH	CH_2OH	H		U	S	U	NS
15	=O	OH	CH_2OH	H		S	S	S	NS
16	=O	OH	CH_2OH	H		S	S	S	NS
17	=O	OH	CH_2OH	H	OOH	U	U	S	NS
18	=O	OH	CH_2OH	H	OH	U	U	S	NS
19	=O	OH	CH_2OH	CH_3	H	U	U	S	NS
20	~OH	OH	CH_2OH	CH_3	H	U	U	S	NS
21		OH	CH_2OH	CH_3	H	U	U	S	NS
22									NS

[a] The contragestational activities of the intraperitoneally (ip) administered compounds on day 22 of gestation according to the published[6] procedure are presented according to the following scale of the dose causing 100% resorption or expulsion of the fetuses: >100 mg/kg = not significant related to 1 (NS); 50–100 mg/kg, +; 25–50 mg/kg, ++; 10–25 mg/kg, +++; and <10 mg/kg, ++++. [b] U = unsaturated, S = saturated.

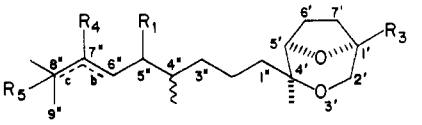
Scheme I[a]



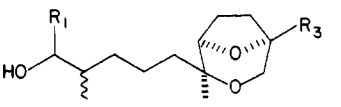
[a] (a) $O_2/h\nu$/Rose Bengal, (b) SiO_2, (c) AcCl/pyr, (d) PCC, (e) DHP/H^+, (f) Ac_2O/pyr, (g) $NaBH_4$, (h) K_2CO_3/MeOH/H_2O, (i) MnO_2, (j) HCN/MeOH/MnO_2, (k) CH_3Li, (l) $LiC{\equiv}CH/H_2N(CH_2)_2NH_2$; H^+, (m) OsO_4, (n) H_2/Pt/$NaNO_2$, (o) H_2Pd/C.

low level of potency of the α,β-enone-containing air-oxidation products **17** and **18** and montanol (**19**) signifies the biological intolerance to a double-bond shift in the β,γ-enone nonenyl side chain of **1**. The contributions of $R_4 = CH_3$ (**19**) and R_5 = oxy (**17** and **18**) to these overall negative effects, however, remain undetermined. Significant enhancement of the potency and activity for the monocyclic oxepanes was realized when the oxo group in the side chain of **1** was converted to a secondary alcohol (**9**) or a tertiary carbinol (**13**). Similar transformation of montanol (**19**) to triols **20** and **21** failed to increase potency. Whereas hydrogenation of both the double bonds of **1** to

Table II. Biological Activity of 3,8-Dioxabicyclo[3.2.1]octanes



V-VIII
(23-45)

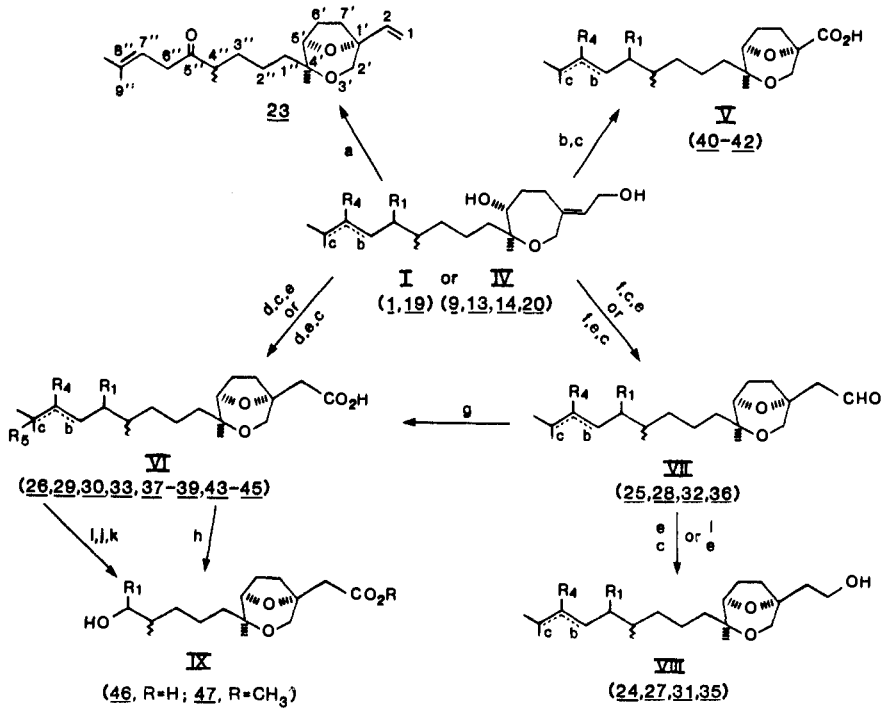


IX
(46-47)

no.	R_1	R_3	R_4	R_5	b	c	guinea pig contragestational act.[a]
23	=O	$CH{=}CH_2$	H		S[(b)]	U	NS
24	=O	CH_2CH_2OH	H		S	U	++++
25	=O	CH_2CHO	H		S	U	++++
26	=O	CH_2CO_2H	H		S	U	++++
27	=O	CH_2OH	H	H	S	S	+++
28	=O	CH_2CHO	H	H	S	S	+++
29	=O	CH_2CO_2H	H	H	S	S	++
30	=O	CH_2CO_2H	H	OH	U	S	NS
31	~OH	CH_2CH_2OH	H		S	U	+
32	~OH	CH_2CHO	H		S	U	++++
33	~OH	CH_2CO_2H	H		S	U	++++
34	~OH	$CH_2CO_2CH_3$	H		S	U	++++
35	~OH	CH_2CH_2OH	H	H	S	S	+++
36	~OH	CH_2CHO	H	H	S	S	++++
37	~OH	CH_2CO_2H	H	H	S	S	++++
38		CH_2CO_2H	H		S	U	++++
39		CH_2CO_2H	H		S	U	+++
40	=O	CO_2H	H		S	U	++
41	~OH	CO_2H	H		S	U	+++
42	=O	CO_2H	CH_3	H	U	S	NS
43	=O	CH_2CO_2H	CH_3	H	U	S	+
44	=O	CH_2CO_2H	CH_3	H	S	S	NS
45	=OH	CH_2CO_2H	CH_3	H	U	S	NS
46	=O	CH_2CO_2H	structure IX				NS
47	H	$CH_2CO_2CH_3$	structure IX				NS

[a] The contragestational activities of the intraperitoneally (ip) administered compounds on day 22 of gestation according to the published[6] procedure are presented according to the following scale of the dose causing 100% resorption or expulsion of the fetuses: >100 mg/kg, not significant relative to 1 (NS); 50–100 mg/kg, +; 25–50 mg/kg, ++; 10–25 mg/kg, +++; <10 mg/kg, ++++. [b] U = unsaturated, S = saturated.

Scheme II[a]



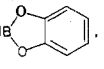
[a] (a) *p*-TsOH/C_6H_6, (b) CrO_3/H_2SO_4/acetone, (c) $NaBH_4$, (d) $Pt/O_2/NaHCO_3$, (e) H_2/Pd, (f) MnO_2, (g) $AgNO_3/NH_4OH$, (h) OsO_4/AcOH, (i) CH_2N_2, (j) O_3/H_2O_2, (k)  , (l) $NaBH(OAc)_3$.

Table III. Oral Contragestational Activity of Zoapatanol Analogues in Day 22 Pregnant Guinea Pigs

compd	N	mg/kg	implants	% nonviable	ED_{50}, mg/kg (95% FL)[a]
control	9	0	36	5.6	
33	5	10.0	15	46.7	
	5	25.0	20	85.0	10.94 (4.76, 15.22)
	4	50.0	12	100.0	
37	5	25.0	18	11.1	
	5	37.5	18	94.4	29.80 (27.19, 32.86)
	5	50.0	19	100.0	
9	5	25.0	21	9.5	
	5	50.0	18	50.0	46.10 (38.33, 54.21)
	4	75.0	17	88.2	
	5	100.0	15	100.0	

[a] The ED_{50} values and its 95% fiducial limits (FL) were calculated for each compound by using Finney's probit method.[17]

15 and **16** and also the oxidation of the secondary alcohol function of **1** to the oxepanone **6** greatly reduced potency, the oxidation of the primary allylic alcohol function of **4** to the α,β-unsaturated aldehyde **7**, a potential precursor to bicyclic derivatives, greatly increased the potency.

The most dramatic enhancement of potency was realized upon the conversion of monocyclic oxepanes into the 3,8-dioxabicyclo[3.2.1]octane derivatives (Table II). In these bicyclic compounds, the presence of an oxygen-bearing, one- or two-carbon side chain at C-1′ appeared crucial for the biological activity (cf. NS for **22** and **23**). Primary alcohols VIII (**24**, **27**, **31**, and **35**), aldehydes VII (**25**, **28**, **32**, and **36**), and carboxylic acids VI (**26**, **29**, **33**, **37–39**, and even **43**) were all very potent. Even the lower homologous acids V (**40** and **41**) were fairly active although not as potent as the corresponding acetic acids VI (**26** and **37**, respectively). Although, in general, the presence of the 3,8-dioxabicyclo[3.2.1]octane-1-acetic acid system imparted activity to a majority of derivatives, no significant potency was noted for **30**, **43**, and **44–47**, all of which also contain nonenyl side chain modifications that were detrimental to the activity of their monocyclic counterparts. The nonenyl double bond in the bicyclic derivatives contributed little to the potency as evidenced by only slightly diminished potency for several of the saturated compounds (**27**, **29**, **35**, and **37**) as compared to their unsaturated congeners. This observation, when considered together with the fact that the completely hydrogenated monocyclic oxepanes (**15** and **16**) had much lower potency, underlines the biological importance of the allylic alcohol function of **1**. Since this particular unsaturation is also obligatory for the chemical transformation of the monocyclic oxepanes into the more potent bicyclic derivatives, it is tempting to postulate that **1** and related biologically active monocyclic oxepanes undergo similar in vivo transformations to manifest the observed biological activity. The acyclic compound **48** was devoid of activity.

On the basis of these preliminary test results, **9**, **33**, and **37** were selected for further biological evaluation and dose–response studies in the guinea pig by the oral (po) route of administration (Table III). Three principal considerations guided the selection of these derivatives for further studies: (a) their potency in the guinea pig contragestational tests, (b) their chemical stability under ambient conditions, and (c) their varying structural types. In view of the potentially inherent susceptibility of the β,γ-enone-containing compounds to aerobic oxidation, as previously described for **1**, the corresponding homoallylic alcohols **9** and **33** were found fairly stable under ambient conditions and the fully saturated acid **37** even more so. From these studies (Table III) **33** has an oral contragestational ED_{50} of 10.94 mg/kg in the guinea pig, which is approximately 3 times more potent than its hydrogenated derivative **37** and 5 times more potent than **9**, one of the more potent monocyclic oxepanes. On the basis of these results, **33** (ORF 13811) was selected for additional biological evaluation in other animal species[15] for possible further development as an antifertility agent.

Experimental Section

^1H NMR spectra were obtained on either a Varian A-60 or a T-60A spectrometer in $CDCl_3$ with tetramethylsilane as the internal standard unless specified otherwise, and the values were expressed in parts per million (δ). IR spectra were recorded neat on a Beckman IR-8 infrared spectrophotometer unless specified otherwise and the values are expressed in microns (μm). UV spectra were measured on a Cary Model 15 recording spectrophotometer in EtOH and are expressed in nanometers (nm). Optical rotations were determined on a Rudolph Model 70 polarimeter attached to a Model 200 photoelectric unit. Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Symbols of elements refer to microanalyses with results within 0.4% of calculated values. Due to the viscous, oily nature of the products, it was not possible to obtain microanalyses for several of the compounds; however, satisfactory corroborative spectral and GC–MS data were obtained. EI/CI mass spectra were obtained on a Finnigan 1015D quadrupole mass spectrometer coupled to a Finnigan 9500 gas chromatograph via a glass jet separator. Separation was achieved with a 5-ft glass column ($^1/_8$ in. i.d.) packed with 3% OV-1 on Gas Chrom Q (80–100 mesh). The column was temperature programmed from 190 to 250 °C at 15 °C/min, after an initial hold of 30 s. EI spectra were obtained at 70 eV, and CI spectra were recorded at 100 eV with methane and isobutane as reagent gases. All evaporations were carried out in vacuo. TLC analyses were performed on Uniplate Silicar 7GF plates (Analtech) and preparative TLC was carried out on Quantagram PQIF plates (1000 μm). Column chromatographies were performed on Mallinckrodt SilicAR-CC7 unless otherwise specified. Isolation and characterization of natural products **1** and **19** and the syntheses of **2**, **4**, **8–10**, **15**, **16**, **19**, **20**, **22**, **23**, **25**, **32**, and **48** have been described earlier.[1]

9-[(2′*S*,3′*R*)-3′-Hydroxy-6′-[(*E*)-2″-acetoxyethylidene]-2′-methyl-2′-oxepanyl]-2,6-dimethyl-2-nonen-5-one (3). Compound **1** (5.697 g, 16.9 mmol) in benzene (200 mL) and pyridine (20 mL) at 0 °C under N_2 was treated with AcCl (15 mL, excess) during a 15-min period. After 2 h of stirring, the mixture was taken up in ether (300 mL), washed with saturated $CuSO_4$ solution, dried ($MgSO_4$), and evaporated to give a brown oil. This was purified by two successive column chromatographies on silica gel to isolate the diacetate **2** (2.34 g with 30:70 ether/petroleum ether) and the monoacetate **3** (0.48 g, 40:60 ether/petroleum ether). IR (neat) 2.86 (br), 5.76, 5.83, 8.0 μm; NMR δ 3.1 (br d, J = 7 Hz, 2 H, 4-H), 3.5 (m, 1 H, 3′-H), 4.05 (br s, 2 H, 7′-H), 4.6 (d, J = 7 Hz, 2 H, 2″-H), 5.3 (m, 2 H, 3-H, 1″-H).

9-[(2′*S*,3′*R*)-3′-(Tetrahydro-2*H*-pyranyloxy)-6′-[(*E*)-2″-(tetrahydro-2*H*-pyranyloxy)ethylidene]-2′-methyl-2′-oxepanyl]-2,6-dimethyl-2-nonen-5-one (5). A solution of **1** (600 mg, 1.8 mmol) in benzene (5 mL) was treated with dihydropyran (7.8 mL) and *p*-toluenesulfonic acid (3 mg) at room temperature and the mixture was stirred under N_2 for 1.5 h. The reaction mixture was washed several times with 10% aqueous $NaHCO_3$ and the organic phase was dried (Na_2SO_4) and evaporated to dryness to give crude **5** as an oil (871 mg). This was purified once by preparative TLC (30:70 EtOAc/hexane) to obtain semipurified **5** (529 mg), which was further purified on a silica gel column (25 g). Elution with 4–6% EtOAc/hexane gave purified **5** (395 mg, 43%). IR (neat) 5.8 μm; NMR δ 1.05 (d, J = 7 Hz, 3 H, 6-CH_3), 1.10 (s, 3 H, 2′-CH_3), 1.23 (s, 3 H, unassigned), 2.60 (s, 2 H,

(15) Hahn, D. W.; Tobia, A. L.; Rosenthale, M. E.; McGuire, J. L. *Contraception* **1984**, *30*, 39–53.

(16) After the completion of this work, the monoacetate of **18** was also reported from the same plant by Seaman et al.: Seaman, F. C.; Malcolm, A. J.; Fischer, N. H. *Phytochemistry* **1984**, *23*, 464–465.

(17) Finney, D. J. "Statistical Methods in Biological Assay", 2nd ed.; Griffin Press: London, 1971.

unassigned), 3.11 (d, J = 7 Hz, 2 H, 4-H), 4.05 (s, 2 H, CH-O), 4.58 (m, 2 H, 2″-H), 5.10–5.20 (m, 2 H, 3-H, 1″-H); GC–MS, M^+ not observed, 251, 233, 211 (BP), 171, 143, 141, 125, 113.

9-[(2′*S*)-6′-[(*E*)-2″-Acetoxyethylidene]-2′-methyl-3-oxooxepanyl]-2,6-dimethyl-2-nonen-5-one (6). A solution of **3** (350 mg, 1 mmol) in CH_2Cl_2 (2 mL) was rapidly added to pyridinium chlorochromate (338 mg, 1.57 mmol) in CH_2Cl_2 (1.5 mL) at room temperature. The solution briefly become homogeneous before depositing a black insoluble layer. After 2 h the reaction mixture was diluted with anhydrous ether (5 mL), the solvent decanted, and the black solid again washed with ether (2 × 50 mL). The organic extracts were filtered through Celite, the solvent was evaporated, and the oily residue (350 mg) was purified by preparative TLC (2:5 EtOAc/$CHCl_3$) to afford **6** (195 mg, 56%) as a colorless oil. IR 5.75, 5.85 μm; NMR δ 1.06 (d, J = 7 Hz, 3 H, 6-CH_3), 1.23 (s, 3 H, 2′-CH_3), 2.03 (s, 3 H, $OCOCH_3$), 3.09 (d, J = 7 Hz, 2 H, 4-H), 4.03 (s, 2 H, 7′-H), 4.56 (d, J = 7 Hz, 2 H, 1″-H), 5.07–5.70 (m, 2 H, 3-H, 2″-H); GC–MS, 378 (M^+), 318 (M − AcOH), 309 (M − 69), 249 (309 − AcOH). Anal. ($C_{22}H_{34}O_5$) C, H.

9-[(2′*S*,3′*R*)-3′-Acetoxy-6′-[(*E*)-2″-oxoethylidene]-2′-methyl-2′-oxepanyl]-2,6-dimethyl-2-nonen-5-one (7). Compound **4** (400 mg, 1.05 mmol) was treated in CH_2Cl_2 (100 mL) with MnO_2 (1.2 g) at room temperature under N_2 for 17 h. The MnO_2 was filtered and washed with CH_2Cl_2 and the solvent evaporated to give an oil (400 mg). This was purified by preparative TLC (EtOAc/hexane, 40:60). The principal and most nonpolar UV-absorbing band was eluted with EtOAc to give **7** as a viscous oil (230 mg, 57%). IR (neat) 5.75, 5.80, 5.97 μm; NMR δ 1.06 (d, J = 7 Hz, 3 H, 6-CH_3), 1.11 (s, 3 H, 2′-CH_3), 1.60 and 1.71 (each s, each 3 H, 1-H), 2.06 (s, 3 H, 3′-$OCOCH_3$), 3.09 (d, J = 7 Hz, 2 H, 4-H), 4.25 (s, 2 H, 7′-H), 4.74 (q, 1 H, 3′-H), 5.11–5.45 (m, 1 H, 3-H); UV (EtOH) 238 nm (ε 10 288); GC–MS, 378 (M^+).

9-[(2′*S*,3′*R*)-3′-Acetoxy-6′-[(*E*)-2″-acetoxyethylidene]-2′-methyl-2′-oxepanyl]-2,6-dimethyl-2-nonen-5-ol Acetate (11). Triol **9** (474 mg, 1.4 mmol) in pyridine (6 mL) was treated with Ac_2O (4.5 mL) at room temperature under N_2 for 18 h. Pyridine and excess Ac_2O were removed in vacuo. The residue was treated with CH_3OH and again taken to dryness to give crude **11** (600 mg). This was purified by preparative TLC (20:80 EtOAc/cyclohexane) to give purified **11** (390 mg, 59%) as a viscous oil. IR (neat) 5.75 μm; NMR δ 0.89 (d, J = 7 Hz, 3 H, 6-CH_3), 1.15 (s, 3 H, 2′-CH_3), 1.60 and 1.66 (each s, each 3 H, 1-H, 2-CH_3), 2.03 (s, 9 H, CH_3COO), 4.08 (s, 2 H, 7′-H), 4.56 (d, J = 7 Hz, 2 H, 2″-H), 4.67–5.0 (m, 2 H, 3′-H), 5.0–5.5 (m, 2 H, 3-H, 1″-H); GC–MS, M^+ not observed, 406 (M − AcOH), 346 (406 − AcOH), 286 (346 − AcOH).

9-[(2′*S*,3′*R*)-3′-(Tetrahydro-2*H*-pyranyloxy)-6′-[(*E*)-2″-(tetrahydro-2*H*-pyranyloxy)ethylidene]-2′-methyl-2′-oxepanyl]-2,6-dimethyl-5-(tetrahydro-2*H*-pyranyloxy)-2-nonene (12). A solution of **9** (450 mg, 1.32 mmol) in dry CH_2Cl_2 (5 mL) was treated with dihydropyran (2.25 mL) and p-toluenesulfonic acid (3.0 mg) at room temperature and stirred under N_2 for 1.5 h. The organic phase was washed several times with 10% $NaHCO_3$ solution, dried (Na_2SO_4), and then evaporated to dryness to give a viscous oil (800 mg). This was purified once on a silica gel column (2.5–4% EtOAc/hexane) followed by a second purification by preparative TLC (20% EtOAc/hexane) to give purified **12** (329 mg, 41.5%) as a viscous oil. IR (neat) 9.3 μm; NMR δ 4.03 (m, 2 H, CH-O), 4.56 (m, 2 H, 2″-H), 5.1–5.5 (m, 2 H, 3-H, 1″-H); GC–MS, M^+ not observed, m/e 85 (diagnostic of THP ethers).

9-[(*E*)-(2′*S*,3′*R*)-3′-Hydroxy-6′-[(*E*)-2″-hydroxyethylidene]-2′-methyl-2′-oxepanyl]-2,5,6-trimethyl-2-nonen-5-ol (13). To a solution of **1** (407 mg, 1.2 mmol) in dry THF (8 mL), cooled to −78 °C, was added under N_2 an excess of CH_3Li (3 mL of 1.6 M in ether). The mixture was stirred at −78 °C for 10 min and then slowly warmed to room temperature. It was diluted with ether (100 mL) and treated with saturated aqueous NH_4Cl solution (50 mL). The organic layer was washed with H_2O (50 mL), dried (Na_2SO_4), and evaporated to give **13** as a viscous oil (410 mg, 96%). IR (neat) 2.87 μm; NMR δ 4.03 (m, 5 H, 3′-H, 7′-H, 2″-H), 5.3 (m, 2 H, 3-H, 1″-H).

9-[(2′*S*,3′*R*)-3′-Hydroxy-6′-[(*E*)-2″-hydroxyethylidene]-2′-methyl-2′-oxepanyl]-2,6-dimethyl-5-ethynyl-2-nonen-5-ol (14). A solution of **5** [obtained from 600 mg of zoapatanol (1)] in anhydrous dioxane (10 mL) was added at room temperature to a stirred mixture of LiC≡CH/$H_2N(CH_2)_2NH_2$ complex (2.6 g) in anhydrous dioxane (30 mL), previously saturated with purified acetylene. A stream of acetylene was passed through this stirred mixture at room temperature for 24 h. The reaction was quenched by the slow addition of a saturated aqueous solution of NH_4Cl (50 mL). The two layers were separated, and the aqueous layer was extracted with ether (4 × 100 mL). The combined organic phases were evaporated to a dark brown oily residue (1.0 g). This was dissolved in 70% AcOH (100 mL), warmed on a steam bath for 2 h, and stirred at room temperature for 19 h. The solution was evaporated to dryness, and the residue was dissolved in ether, washed successively with 5% $NaHCO_3$, H_2O, dried (Na_2SO_4), and concentrated to afford an oily residue (900 mg). This was purified by preparative TLC (48:52 EtOAc/cyclohexane) and the most polar band was eluted (12:88 i-PrOH/$CHCl_3$) to afford **14** as a light yellow oil (0.246 g, 42% based on **1**). IR (neat) 2.93, 3.0 μm; NMR δ 1.0 and 1.03 (a pair of d, J = 7 Hz, 3 H, 6-CH_3, two isomers at C-5), 1.17 (s, 3 H, 2′-CH_3), 1.55, 1.77 [each s, each 3 H, 1-H, 2-CH_3), 2.40 (s, 1 H, C≡CH), 2.63 (s, ~3 H, OH, exchanged with D_2O), 3.53 (t, 1 H, 3′-H), 4.08 (s, 2 H, 7-H), 4.17 (d, J = 6 Hz, 2 H, 2″-H), ~5.4 (m, 2 H, 1″-H, and 3-H); GC–MS, two components, both show identical fragmentation pattern and therefore considered isomeric tertiary ethynyl carbinols. Highest m/e 277 [M^+ − (C_5H_7 and H_2O)]; GC–MS of Me_3Si derivative: one major peak; m/e 511 (M^+ − 69), 421 [511 − 90 (Me_3SiOH)], 331 (421 − 90), 221 (331 − 90).

9-[(2′*S*,3′*R*)-3′-Hydroxy-6′-[(*E*)-2″-hydroxyethylidene]-2′-methyl-2′-oxepanyl]-2,6-dimethyl-2-hydroperoxy-3-nonen-5-one (17). A solution of **1** (0.98 g, 2.9 mmol) in CH_3OH (70 mL, containing Rose Bengal saturated anion-exchange resin AG 1 × 8, 2.5 g) was irradiated with a 300-W tungsten lamp for 92 h while oxygen was bubbled through it and it was cooled with a cold finger and stirred briskly throughout. The resin was removed by filtration and washed with MeOH. The combined filtrate and washings were evaporated to dryness below 40 °C. The residue was taken up in CH_2Cl_2, dried (Na_2SO_4), and purified by preparative TLC (12:88 i-PrOH/$CHCl_3$). The most polar, UV-absorbing band afforded **11** upon elution (0.213 g, 20%), showing a positive starch–iodide test for peroxide. IR (neat) 2.92, 5.94, 6.02, 6.14, 11.8 (OOH) μm; NMR δ 1.08, (d, J = 6 Hz, 3 H, 6-H), 1.15 (s, 3 H, 2′-CH_3), 1.36 (s, 6 H, 1-H, 2-CH_3), 3.50 (m, 1 H, 3′-H), 4.06 (s, 2 H, 7′-H), 4.26 (d, J = 6 Hz, 2 H, 2″-H), 5.4 (m, 1 H, 1″-H), 6.20 (d, J = 16 Hz, 1 H, 4-H), 6.83 (d, J = 16 Hz, 1 H, 3-H); UV (EtOH) 222 nm (ε 5140).

9-[(2′*S*,3′*R*)-3′-Hydroxy-6′-[(*E*)-2″-hydroxyethylidene]-2′-methyl-2′-oxepanyl]-2,6-dimethyl-2-hydroxy-3-nonen-5-one (18). Compound **18** was isolated by column chromatography of a semipurified preparation of **1**, which was exposed to air and light for 1 week. The product **18** was eluted, following **1** (8:92 i-PrOH/$CHCl_3$). IR (neat) 2.95, 3.25, 5.95, 6.03, 6.15 μm; NMR δ 1.07 (d, J = 7 Hz, 3 H, 6-H), 1.08 (s, 3 H, 2′-CH_3), 1.35 (s, 6 H, 1-H, 2-CH_3), 2.50 (br s, OH), 3.63 (m, 1 H, 3′-H), 4.05 (s, 2 H, 7′-H), 4.1 (d, J = 7 Hz, 2 H, 2″-H), 5.36 (m, 1 H, 1″-H), 6.26 (d, J = 16 Hz, 1 H, 4-H), 6.85 (d, J = 16 Hz, 1 H, 3-H), UV (EtOH) 224 nm (ε 9420).

(*E*)-9-[(2′*S*,3′*R*)-3′-Hydroxy-6-[(*E*)-2″-hydroxyethylidene]-2′-methyl-2′-oxepanyl]-2,3,5,6-tetramethyl-3-nonen-5-ol (21). To a solution of **19** (430 mg, 1.22 mmol) in dry THF (10 mL) was slowly added at −78 °C under N_2 an excess of CH_3Li in ether (1.6M, 3 mL). After 30 min at −78 °C, the reaction mixture was slowly allowed to attain room temperature and then treated with wet THF (10 mL), CH_2Cl_2 (100 mL), and saturated NH_4Cl solution (50 mL). The organic layer was washed with H_2O (100 mL), dried (Na_2SO_4), and evaporated to give **21** as a colorless oil (450 mg, 98%). IR (CCl_4) 2.78, 3.92 μm; NMR δ 1.8 (2 d, J = 1 Hz, 3 H, 3-CH_3), 3.8 (m, 3 H, 3′-H, 2″-H), 5.2 (br s, 1 H, 4′-H), 5.4 (m, 1 H, 1″-H).

(1′*R*,4′*S*,5′*R*)-4′-(4″,8″-Dimethyl-5″-oxo-7″-nonenyl)-4′-methyl-3′,8′-dioxabicyclo[3.2.1]octane-1′-ethan-1-ol (24). A slurry of $NaBH_4$ (70 mg) in benzene (155 mL) was treated with AcOH (92 mg) and refluxed under N_2 for 1 h to obtain a clear solution of $NaBH(OAc)_3$. To this was added **25** (158 mg, 0.47 mmol in benzene, 5 mL) and the mixture refluxed for 5 h. The solvent was evaporated and the residue was diluted with H_2O (10 mL) and extracted with CH_2Cl_2 (2 × 75 mL). The organic layer

was dried (Na_2SO_4) and evaporated to give a yellow oil (158 mg). This was purified by preparative TLC (4:1 EtOAc/$CHCl_3$) to afford **24** as a colorless oil (121 mg, 76%). IR (neat) 2.85, 5.82 μm; NMR δ 1.06 (d, J = 6 Hz, 3 H, 4″-CH_3), 1.27 (s, 3 H, 4′-CH_3), 1.60 and 1.73 (each s, each 3 H, 9″-H, 8″-CH_3), 3.09 (d, J = 7 Hz, 2 H, 6″-H), 3.17 and 3.75 (each d, J = 11 Hz, each 1 H, 2′-H) 3.75 (t, 3 H, 1-H, 5′-H), 5.06–5.43 (br m, 1 H, 7″-H); GC–MS, M^+ not observed, 269 (M − 69), 251 (269 − H_2O), 239, 212/211.

(1′*R*,4′*S*,5′*R*)-4′-(4″,8″-Dimethyl-5″-oxo-7″-nonenyl)-4′-methyl-3′,8′-dioxabicyclo[3.2.1]octane-1′-acetic Acid (26). To a stirred suspension of prereduced PtO_2 (500 mg) in water (75 mL) were added $NaHCO_3$ (1.58 g) and 1 [(600 mg) in acetone (48 mL) and water (117 mL)]. The mixture was stirred briskly at room temperature and protected from light under an oxygen atmosphere for 24 h. Platinum metal was removed by filtration on a pad of Celite and washed with 10% acetone in water. The combined filtrate and washings were evaporated to dryness in vacuo, and the residue was dissolved in water (125 mL) and extracted with EtOAc (2 × 100 mL). The aqueous layer was acidified to pH 3 with dilute HCl and quickly extracted with EtOAc (2 × 100 mL). This was separated by preparative TLC (*i*-PrOH/$CHCl_3$/AcOH, 9:90.75:0.25) into two UV-absorbing, colorless bands. The more polar and more intensely UV-absorbing band was **30** (0.238 g, 37%). The less polar and lightly UV-absorbing band was **26** (0.170 g, 26%). IR (neat) 2.8–3.3, 5.80, 5.85 μm; NMR: 1.03 (d, J = 7 Hz, 3 H, 4″-CH_3), 1.27 (s, 3 H, 4′-CH_3), 1.60 and 1.70 (each s, each 3 H, 8″-CH_3 and 9″-H), 2.57 (s, 2 H, 1′-H), 3.10 (d, J = 7 Hz, 2 H, 6″-CH_2), 3.40 and 3.73 (each d, J = 11 Hz, 2 H, 2′-H), 3.85 (br s, 1 H, 5′-H), 5.23 (m, 1 H, 7″-H), 6.83 (br s, ∼1 H, CO_2H). Anal. ($C_{20}H_{32}O_5$) C, H.

(1′*R*,4′*S*,5′*R*)-4′-(4″,8″-Dimethyl-5″-oxononanyl)-4′-methyl-3′,8′-dioxabicyclo[3.2.1]octane-1′-ethan-1-ol (27). Following the procedure described for the preparation of **24**, **28** (286 mg, 1.2 mmol) in benzene (10 mL) was reduced with $NaBH(OAc)_3$, prepared from $NaBH_4$ (125 mg) and AcOH (165 mg) to give **27** (197 mg, 68%) as a colorless viscous oil, which solidified to a low-melting waxy solid on prolonged standing. IR (neat) 2.86, 5.85 μm; NMR δ 0.88 (d, J = 6 Hz, 6 H, 8″-CH_3, 9″-H), 1.06 (d, J = 7 Hz, 3 H, 4″-CH_3), 1.30 (s, 3 H, 4′-CH_3), 3.19 and 3.78 (each d, J = 11 Hz, each 1 H, 2′-H), 3.78 (m, 3 H, 1-H, 5′-H); GC–MS, 340 (M^+), 322 (M − H_2O), 304 (322 − H_2O), 292, 212. Anal. ($C_{20}H_{36}O_4$) C, H.

(1′*R*,4′*S*,5′*R*)-4′-(4″,8″-Dimethyl-5″-oxononanyl)-4′-methyl-3′,8′-dioxabicyclo[3.2.1]octane-1′-acetaldehyde (28). Compound **25** (611 mg, 1.82 mmol) in EtOAc (300 mL) was hydrogenated in the presence of 10% Pd/C (400 mg) at atmospheric pressure for 1 h. The reaction mixture was filtered through a pad of Celite and the solvent evaporated to give an oily residue (608 mg), which was purified by preparative TLC (30:70 EtOAc/hexane) to afford **28** as a viscous oil (272 mg, 88%). IR (neat) 3.63, 5.80, 5.82 μm; NMR 0.8 (d, J = 6 Hz, 6 H, 8″-CH_3, 9″-H), 1.06 (d, J = 7 Hz, 3 H, 4″-CH_3), 1.30 (s, 3 H, 4′-CH_3), 2.58 (d, J = 2 Hz, 2 H, 2-H), 3.29 and 3.73 (each d, J = 11 Hz, 2 H, 2′-H), 3.82 (m, 1 H, 5′-H), 9.80 (t, 1 H, 1-H); GC–MS, 338 (M^+), 320 (M − H_2O), 294, 267.

(1′*R*,4′*S*,5′*R*)-4′-(4″,8″-Dimethyl-5″-oxononanyl)-4′-methyl-3′,8′-dioxabicyclo[3.2.1]octane-1′-acetic Acid (29). A solution of $AgNO_3$ (1.328 g) in H_2O (18.9 mL) was treated with 10% NaOH (11.7 mL) while being stirred under N_2 to form a black precipitate of silver oxide. A 15% solution of NH_4OH (12.9 mL) was added dropwise with stirring until the precipitate first disappeared, followed by the dropwise addition of **28** (300 mg, 0.89 mmol) in MeOH (10.28 mL). The reaction mixture was refluxed at 85–90 °C for 5 h and then poured in ice–H_2O (160 mL). EtOAc was added and the solution acidified with 5% HCl to pH 3. The organic layer was washed with brine and dried (Na_2SO_4) and the solvent removed to give an oily residue (258 mg), which was purified by preparative TLC (8:92 *i*-PrOH/$CHCl_3$ + 2 drops of AcOH) to give **29** as a viscous oil (167 mg, 53%). IR (neat) 5.75, 5.83 μm; NMR δ 0.88 (d, J = 6 Hz, 6 H, 8″-CH_3, 9″-H), 1.06 (d, J = 7 Hz, 3 H, 4″-CH_3), 1.30 (s, 3 H, 4′-CH_3), 2.62 (s, 2 H, 2-H), 3.36 and 3.78 (each d, J = 11 Hz, 2 H, 2′-H), 3.85 (m, 1 H, 5′-H), 4.67–5.05 (br s, 1 H, CO_2H, D_2O exchanged); GC–MS, M^+ not observed, 237 (M − 99), 226.

(1′*R*,4′*S*,5′*R*)-4′-(4″,8″-Dimethyl-8″-hydroxy-6″(*E*)-nonenyl)-4′-methyl-3′,8′-dioxabicyclo[3.2.1]octane-1′-acetic Acid (30). In the Pt-catalyzed oxidation of 1 to obtain **26**, **30** was isolated as the more polar colorless oil (0.238 g, 37%). IR (CCl_4) 2.8–3.3, 5.65 (sh), 5.82 μm; NMR δ 1.08 (d, J = 7 Hz, 3 H, 4″-CH_3), 1.30 (s, 3 H, 4′-CH_3), 1.36 (s, 6 H, 8″-CH_3, 9″-H), 2.58 (s, 2 H, 2-CH_2), 3.40 and 3.73 (each d, J = 11 Hz, each 1 H, 2′-H), 3.85 (br s, 1 H, 5′-H), 6.25 (d, J = 16 Hz, 1 H, 6″-H), 6.83 (d, J = 16 Hz, 1 H, 7″-H), 5.10 (br s, OH, CO_2H).

(1′*R*,4′*S*,5′*R*)-4′-(4″,8″-Dimethyl-5″-hydroxy-7″-nonenyl)-4′-methyl-3′,8′-dioxabicyclo[3.2.1]octane-1′-ethan-1-ol (31). A solution of **25** (200 mg, 0.6 mmol) in EtOH (10 mL) was treated with $NaBH_4$ (55 mg) at room temperature under N_2 for 18 h. The solvent was evaporated and the residue diluted with H_2O and neutralized with 5% HCl. The solution was extracted with CH_2Cl_2 (2 × 50 mL), and the organic layer was dried (Na_2SO_4) and evaporated to give a yellow oil (208 mg), which was purified by preparative TLC (1:9 *i*-PrOH/$CHCl_3$) to afford purified **31** as a viscous oil (152 mg, 75.3%). IR (neat) 2.90 μm; NMR δ 0.88 (d, J = 6 Hz, 3 H, 4″-CH_3), 1.32 (s, 3 H, 4′-CH_3), 1.63 and 1.72 (each s, each 3 H, 8″-CH_3, 9″-H), 3.08 (m, 6 H, CH–O), 3.18 and 3.60 (each d, J = 12 Hz, 1 H, 2′-H), 3.60 (t, J = 6 Hz, 3 H, 5′-H, 1-H), 3.20–3.40 (br m, ∼1 H, 5″-H), 5.12 (m, 1 H, 7″-H), GC–MS, M^+ not observed, 271 (M − 69), 253.

(1′*R*,4′*S*,5′*R*)-4′-(4″,8″-Dimethyl-5″-hydroxy-7″-nonenyl)-4′-methyl-3′,8′-dioxabicyclo[3.2.1]octane-1′-acetic Acid (33). Pt-catalyzed oxidation of **9** (260 mg, 0.764 mmol) afforded after preparative TLC (12:87:1 *i*-PrOH/$CHCl_3$/AcOH) **33** (192 mg, 70%) as a colorless viscous oil. IR 2.89, 5.80 μm; NMR δ 0.88 (d, J = 7 Hz, 3 H, 4″-CH_3), 1.31 (s, 3 H, 4′-CH_3) 1.63 and 1.71 (each s, each 3 H, 8″-CH_3, 9″-H), 2.60 (s, 2 H, 2-CH_2), 3.39 and 3.76 (each d, J = 11 Hz, each 1 H, 2′-H), 3.45 (m, 1 H, 5″-H), 3.85 (m, 1 H, 5′-H), 4.91–5.16 (m, 1 H, 7″-H), 6.03 (br s, COOH, OH); GC–MS, (M^+ not observed), 285 (M − 69), 267, 199. Anal. ($C_{20}H_{34}O_5$) C, H.

Methyl (1′*R*,4′*S*,5′*R*)-4′-(4″,8″-Dimethyl-5″-hydroxy-7″-nonenyl)-4′-methyl-3′,8′-dioxabicyclo[3.2.1]octane-1′-acetate (34). Acid **33** (77 mg, 0.22 mmol) was esterified with diazomethane in ether and the crude ester purified by preparative TLC (10:89:1 *i*-PrOH/$CHCl_3$/AcOH) to give **34** (74 mg, 91%) as a colorless, viscous oil. IR 2.85, 5.75 μm; NMR δ 0.85 (d, J = 7 Hz, 3 H, 4″-CH_3), 1.30 (s, 3 H, 4′-CH_3), 1.63 and 1.73 (each s, each 3 H, 8″-CH_3, 9″-H), 2.58 (s, 2 H, 2-H), 3.65 (s, 3 H, CH_3OCO), 3.40 and 3.77 (each d, J = 11 Hz, each 1 H, 2′-H), 3.23 (m, 2 H, 5′-H, 5″-H), 5.15 (m, 1 H, 7″-H); GC–MS, M^+ not observed, 299 (M − 69), 281, 267.

(1′*R*,4′*S*,5′*R*)-4′-(4″,8″-Dimethyl-5″-hydroxynonanyl)-4′-methyl-3′,8′-dioxabicyclo[3.2.1]octane-1′-ethan-1-ol (35). Compound **31** (234 mg, 0.69 mmol) in EtOAc (150 mL) was hydrogenated in the presence of 5% Pd/C (200 mg) at atmospheric pressure for 2 h and then purified by preparative TLC (1:9 *i*-PrOH/$CHCl_3$) to give purified **35** (60 mg, 25%) as a waxy solid, mp 88–89 °C. IR (neat) 2.90 μm; NMR δ 0.88 (d, J = 6 Hz, 9 H, 8″-CH_3, 9″-H, 4″-CH_3), 1.30 (s, 3 H, 4′-CH_3), 3.19 and 3.78 (each d, J = 11 Hz, each 1 H, 2′-H), 3.37 (m, 1 H, 5″-H), 3.87 (m, 3 H, 2-H, 5′-H); GC–MS, 342 (M^+), 324, (M − H_2O), 271, 253. Anal. ($C_{20}H_{38}O_4$) C, H.

(1′*R*,4′*S*,5′*R*)-4′-(4″,8″-Dimethyl-5″-hydroxynonanyl)-4′-methyl-3′,8′-dioxabicyclo[3.2.1]octane-1′-acetaldehyde (36). A solution of **32** (320 mg, 0.95 mmol) in EtOAc (175 mL) was hydrogenated in the presence of 10% Pd/C (250 mg) at atmospheric pressure for 1 h. The crude product was purified by preparative TLC (2:5 EtOAc/$CHCl_3$) to give purified **36** as a colorless viscous oil (173 mg, 54%). IR (neat) 2.86, 3.64, 5.80 μm; NMR δ 0.90 (d, J = 6 Hz, 9 H, 8″-CH_3, 9″-H, 4″-CH_3), 1.33 (s, 3 H, 4′-CH_3), 2.60 (d, J = 2 Hz, 2 H, 2-H), 3.29, 3.74 (each d, J = 11 Hz, each 1 H, 2′-H), 3.38 (m, 1 H, 5″-H), 3.83 (m, 1 H, 5′-H), 9.78 (t, J = 2 Hz, 1-H); GC–MS, M^+ not observed, 322 (M − H_2O), 307, 296, 269, 197. Anal. ($C_{20}H_{36}O_4$) C, H.

(1′*R*,4′*S*,5′*R*)-4′-(4″,8″-Dimethyl-5″-hydroxynonanyl)-4′-methyl-3′,8′-dioxabicyclo[3.2.1]octane-1′-acetic Acid (37). A solution of **33** (442 mg, 1.25 mmol) in EtOAc (200 mL) was hydrogenated in the presence of 10% Pd/C (350 mg) at atmospheric pressure for 2 h. The reaction mixture was filtered (Celite) and the solvent evaporated to give an oily residue (411 mg), which was purified by preparative TLC (1:9 *i*-PrOH/$CHCl_3$ with three drops of AcOH) to give **37** (306 mg, 69%) as a waxy solid. Recrystallization from isopropyl ether gave an analytical sample (142

mg, mp 93–94 °C). IR (neat) 2.90, 5.80 μm; NMR δ 0.88 (d, J = 6 Hz, 9 H, 4″-CH_3, 8″-CH_3, 9″-H), 1.33 (s, 3 H, 4′-CH_3), 2.60 (s, 2 H, 2-H), 3.30 (m, 1 H, 5″-H), 3.39 and 3.78 (both d, J = 11 Hz, each 1 H, 2′-H), 3.86 (m, 1 H, 5′-H), 5.58 (m, 1 H, CO_2H); GC–MS (Me_3Si derivative) 500 (M^+), 485 (M – CH_3), 429 (M – C_5H_{11}), 400, 370, 339, 311, 309, 257, 240, 215. Anal. ($C_{20}H_{36}O_5$) C, H.

(1′*R*,4′*S*,5′*R*)-4′-(4″,5″,8″-Trimethyl-5″-hydroxy-7″-nonenyl)-4′-methyl-3′,8′-dioxabicyclo[3.2.1]octane-1′-acetic Acid (38). Pt-catalyzed oxidation of **13** (400 mg, 1.13 mmol) afforded after preparative TLC purification (10:89:1 i-PrOH/$CHCl_3$/AcOH) **38** as a colorless viscous oil (0.236 g, 57%). IR 2.90, 5.80 μm; NMR δ 0.86 and 0.89 (each d, J = 7 Hz, 3 H, 4″-CH_3), 1.05 (s, 3 H, 5″-CH_3), 1.31 (s, 3 H, 4′-CH_3) 1.63 and 1.73 (each s, each 3 H, 8′-CH_3, 9′-H), 2.60 (s, 2 H, 2-H), 3.36 and 3.73 (each d, J = 11 Hz, each 1 H, 2′-H), 3.82 (t, 1 H, 5′-H), 5.43 (m, 1 H, 7″-H); GC–MS, M^+ not observed, m/e 199, 151, 149. Me_3Si derivative: m/e 497 (M^+ – CH_3), 443 (M – 69, C_5H_9), 353 (443 – Me_3SiOH), 325, 272, 259, 215.

(1′*R*,4′*S*,5′*R*)-4′-(4″,8″-Dimethyl-5″-ethynyl-5″-hydroxy-7″-nonenyl)-4′-methyl-3′,8′-dioxabicyclo[3.2.1]octane-1′-acetic Acid (39). Pt-catalyzed oxidation of **14** (130 mg, 0.36 mmol) afforded after preparative TLC purification (10:89:1 i-PrOH/$CHCl_3$/ACOH) **39** (90 mg, 66%) as a colorless viscous oil. IR 2.89, 5.80 μm; NMR δ 0.98 and 1.03 (a pair of d, J = 6 Hz, 3 H, 4″-CH_3 isomer mix at 4″ and 5″), 1.65 and 1.77 (each d, each 3 H, 8″-CH_3, 9″-H), 2.38 (s, 1 H, C≡CH), 2.60 (s, 2 H, 2-H), 3.37 and 3.75 (each d, J = 11 Hz, 2 H, 2′-H), 3.88 (m, 1 H, 5′-H), 5.12–5.48 (m, 1 H, 7″-H); GC–MS (Me_3Si derivative); M^+ not observed, m/e 453 (M – 69, C_5H_9), 363 (453 – Me_3SiOH), 327 (417 – Me_3SiOH).

(1′*R*,4′*S*,5′*R*)-4′-(4″,8″-Dimethyl-5″-oxo-7″-nonenyl)-4′-methyl-3′,8′-dioxabicyclo[3.2.1]octane-1′-carboxylic Acid (40). A solution of **1** (250 mg, 0.74 mmol) in acetone (15 mL) at 0 °C was treated slowly with an excess of Jones reagent (6 mL, 6 mmol) under N_2. The reaction mixture was kept at 0 °C for 10 min and then treated with ether (100 mL) and H_2O (20 mL). The organic layer was washed with 10% $NaHCO_3$ (3 × 100 mL). The aqueous phase was combined and acidified at 0 °C to pH 2–3 with 10% HCl. The mixture was extracted with ether (2 × 150 mL), and the organic extracts were dried ($MgSO_4$) and evaporated to give **40** (121 mg, 50%). IR (neat) 2.78–4.0, 5.78, 5.87 μm; NMR δ 1.62 (br s, 3 H, 8″-CH_3), 1.75 (br s, 3 H, 9″-H), 3.1 (br d, J = 7 Hz, 2 H, 6″-H), 3.80 (m, 3 H, 2′, 5-H), 5.3 (br, 1 H, 7″-H), 8.80 (br s, 1′-CO_2H, exchanged with D_2O).

(1′*R*,4′*S*,5′*R*)-4′-(4″,8″-Dimethyl-5″-hydroxy-7″-nonenyl)-4′-methyl-3′,8′-dioxabicyclo[3.2.1]octane-1′-carboxylic Acid (41). A solution of **40** (500 mg, 1.47 mmol) in EtOH (25 mL) at 0 °C was treated under N_2 with excess $NaBH_4$ (200 mg, 5.2 mmol). After stirring of the reaction mixture for 20 min, it was treated with saturated NH_4Cl solution (25 mL) and ether (150 mL). The organic layer was extracted with 10% $NaHCO_3$ (3 × 50 mL). The combined aqueous phase was acidified at 0 °C to pH 2–3 with 10% HCl. The mixture was extracted with ether (3 × 100 mL), and the organic extracts were dried ($MgSO_4$) and evaporated to give **41** as a viscous oil (370 mg, 74%). IR (neat) 2.78–4.00, 5.78 cm^{-1}; NMR δ 1.62 (br s, 3 H, 8″-CH_3), 1.75 (br s, 3 H, 9″-H), 3.8 (m, 4 H, 2′,5′-H, 5″-H), 5.3 (br, 1 H, 7″-H).

(1′*R*,4′*S*,5′*R*)-4′-(4″,7″,8″-Trimethyl-5″-oxo-6″-nonenyl)-4′-methyl-3′,8′-dioxabicyclo[3.2.1]octane-1′-carboxylic Acid (42). Oxidation of **19** (235 mg, 0.66 mmol) in acetone (20 mL) with Jones reagent (3 mL, 3 mmol) at 0 °C as described for the preparation of **40** afforded **42** as a viscous oil (150 mg, 68%). IR (neat) 2.78–4.0, 5.78, 5.93, 6.17 μm; NMR δ 2.06 (d, J = 1 Hz, 3 H, 7″-CH_3), 3.8 (m, 3 H, 2′-H), 6.02 (br, 1 H, 6″-H), 8.60 (br s, 1 H, CO_2H, exchanged with D_2O).

(1′*R*,4′*S*,5′*R*)-4′-(4″,7″,8″-Trimethyl-5″-oxo-6″-nonenyl)-4′-methyl-3′,8′-dioxabicyclo[3.2.1]octane-1′-acetic Acid (43). The Pt-catalyzed oxidation of **19** (1.3 g, 3.67 mmol) gave after preparative TLC purification (10:89:1 i-PrOH/$CHCl_3$/AcOH) **43** as a colorless oil (0.40 g, 64%). IR (neat) 5.81, 5.90, 6.15 μm; NMR δ 1.06 (d, J = 7 Hz, 9 H, 4″, 8″-CH_3, 9″-H), 1.30 (s, 3 H, 4′-CH_3), 2.07 (s, 3 H, 7″-CH_3), 2.60 (s, 2 H, 2-H), 3.35 and 3.75 (each d, J = 12 Hz, each 1 H, 2′-H), 3.85 (t, 1 H, 5′-H), 6.03 (br s, 1 H, 6″-H); GC–MS: M^+ not observed, m/e 111; UV (EtOH) 240 nm (ε 8230).

(1′*R*,4′*S*,5′*R*)-4′-(4″,7″,8″-Trimethyl-5″-oxononanyl)-4′-methyl-3′,8′-dioxabicyclo[3.2.1]octane-1′-acetic Acid (44). A solution of **43** (200 mg, 0.546 mmol) in EtOAc (150 mL) was hydrogenated in the presence of 10% Pd/C (150 mg) at atmospheric pressure for 1 h. The usual workup afforded **44** as a viscous oil (152 mg, 75%). IR (neat) 5.75, 5.82 μm; NMR δ 0.83 (m, 9 H, 7″, 8″-CH_3, 9″-H), 1.04 (d, J = 7 Hz, 3 H, 4″-CH_3), 1.30 (s, 3 H, 4′-CH_3), 2.62 (s, 2 H, 2-H), 3.38 and 3.77 (each d, J = 12 Hz, each 1 H, 2′-H), 3.87 (t, 1 H, 5′-H); GC–MS, M^+ not observed, 226, 215, 185.

(1′*R*,4′*S*,5′*R*)-4′-(4″,7″,8″-Trimethyl-5″-hydroxy-6″-nonenyl)-4′-methyl-3′,8′-dioxabicyclo[3.2.1]octane-1′-acetic Acid (45). Pt-catalyzed oxidation of **20** (120 mg, 0.34 mmol) afforded after preparative TLC purification (90:9:1 EtOAc/hexane/AcOH) **45** (65%) as a colorless viscous oil. IR 2.89, 5.82 μm; NMR δ 0.82 (d, J = 7 Hz, 3 H, 4″-CH_3), 0.98 (d, J = 7 Hz, 6 H, 8″-CH_3, 9″-H), 1.32 (s, 3 H, 4′-CH_3), 1.62 (d, J = 2 Hz, 3 H, 7″-CH_3), 2.60 (s, 2 H, 2-H), 3.37 and 3.75 (each d, J = 11 Hz, each 1 H, 2′-H), 3.90 (m, 1 H, 5″-H), 4.10 (m, 1 H, 5′-H), 5.08 (br s, 3 H, 6″-H, OH); GC/MS (Me_3Si derivative) M^+ not observed, m/e 497 (M – CH_3), 469 (M – C_3H_7), 408 (497 – OMe_3Si), 257 (M – side chain). Anal. ($C_{21}H_{36}O_5$) C, H.

(1′*R*,4′*S*,5′*R*)-4′-(4″-Carboxypentyl)-4′-methyl-3′,8′-dioxabicyclo[3.2.1]octane-1′-acetic Acid (46). An excess of $NaIO_4$ (1.26 g) was added to **43** (538 mg, 1.47 mmol), AcOH (20 mL), H_2O (5 mL), and OsO_4 (catalytic amount, a few crystals) and the mixture stirred at room temperature for 19 h. The solvent was removed in vacuo to give a white solid, which was treated with H_2O (50 mL) and EtOAc (100 mL). The organic layer was separated and dried (Na_2SO_4) and the solvent evaporated to give an oil. This was dissolved in ether (100 mL) and extracted with 5% K_2CO_3 (2 × 75 mL). The aqueous layer was combined and acidified at 0 °C to pH 2–3 with concentrated HCl. The mixture was extracted with ether (200 mL), the organic layer dried (Na_2SO_4), and the solvent evaporated to give an oil (249 mg), which was purified by preparative TLC (1:9 i-PrOH/$CHCl_3$ + three drops of AcOH) to give **46** as a colorless viscous oil (194 mg, 42.3%). IR 5.82 μm; NMR δ 1.17 and 1.20 (each d, J = 6 Hz, 3 H, 4″-CH_3), 1.30 (s, 3 H, 4′-CH_3), 2.60 (s, 2 H, 2-H), 3.39 and 3.78 (each d, J = 11 Hz, each 1 H, 2′-H), 3.87 (t, 1 H, 5′-H); GC–MS, M^+ not observed, 226, 185. Anal. ($C_{15}H_{24}O_6$) C, H.

Methyl (1′*R*,4′*S*)-4′-[4″-(Hydroxymethyl)pentyl]-4′-methyl-3′,8′-dioxabicyclo[3.2.1]octane-1′-acetate (47). (a) Pt-Catalyzed Oxidation of Triplet Mixture (1 and 19) To Obtain a Mixture of Acids 26, 30, and 43. Triplet mixture[1] (1 + 19, 6.88 g) was oxidized in an oxygen atmosphere in the presence of PtO_2 (0.5 g), H_2O (500 mL), $NaHCO_3$ (3.3 g), and acetone (138 mL) by briskly stirring for 5 days at room temperature and working up the reaction as described for the preparation of **26** to obtain a mixture of acids **26**, **30**, and **43** (4.7 g). This was then treated at 10 °C with excess diazomethane in ether to afford a mixture of the corresponding methyl esters (5.0 g).

(b) Ozonolysis To Obtain the Methyl Ester of 46. The methyl ester mixture (2.5 g) was ozonolyzed in EtOAc (40 mL) at –10 to –15 °C for 2 h and then treated with 30% H_2O_2 (7 mL) and stirred at room temperature for 24 h. Following the usual acid–base workup, the acidic components were isolated and purified on a silica gel column (25–30% EtOAc/cyclohexane) to afford the methyl ester of **46** (0.453 g, ~20% from **1** and **19** mixture). IR (neat) 3.0–3.2, 5.76, 5.87 μm; NMR δ 1.18 (d, J = 7 Hz, 3 H, 5″-H), 1.31 (s, 3 H, 4′-CH_3), 2.61 (s, 2 H, 2-H), 3.68 (s, 3 H, CO_2CH_3), 3.47 and 3.80 (each d, J = 11 Hz, 2 H, 2′-H), 3.79 (m, 1 H, 5′-H), 10.3 (br s, 1 H, CO_2H); MS (probe), 314 (M^+); GC–MS (Me_3Si derivative) 386 (M^+). Anal. ($C_{16}H_{26}O_6$) C, H.

(c) Borane Reduction. Catecholborane (0.51 mL, 4.6 mmol) was slowly added to a stirred solution of the methyl ester of **46** (0.44 g, 1.42 mmol) in dry $CHCl_3$ (3 mL) at room temperature under N_2 and the mixture stirred for 42 h. Water and $CHCl_3$ were added, the organic layer was successively extracted with aqueous 10% $NaHCO_3$, 1 N NaOH, and H_2O and dried (Na_2SO_4), and the solvent was evaporated to give an oily residue (380 mg). This was purified by two successive preparative TLC purifications [(i) 7:93 i-PrOH/$CHCl_3$, (ii) 1:1 EtOAc/cyclohexane)] to afford **47** (277 mg, 66%). $[\alpha]^{23}_D$ +8° (0.5, $CHCl_3$); IR (neat) 2.88, 5.78 μm; NMR δ 0.92 (d, J = 7 Hz, 3 H, 4″-CH_3), 1.32 (s, 3 H, 4′-CH_3), 2.60 (s, 2 H, 2-H), 3.43 (d, J = 6 Hz, 2 H, 5″-H), 3.43 and 3.78 (each d,

J = 11 Hz, 2 H, 2′-H), 3.67 (s, 3 H, CO_2CH_3), 3.80 (br s, 1 H, 5′-H); GC–MS (Me_3Si derivative) 372 (M^+). Anal. ($C_{16}H_{28}O_5$) C, H.

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7α-Substituted Derivatives of Androstenedione as Inhibitors of Estrogen Biosynthesis

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In an effort to obtain more information on the structure–activity relationship among the 7α-(phenylthio)androstenedione inhibitors of the enzyme aromatase, a series of compounds containing both electron-donating and electron-withdrawing ring substituents was synthesized and tested for aromatase inhibitory activity. No linear correlation between substituent electronic effects and inhibitory activity was observed. The halogen-containing compounds, particularly 8, appeared to be quite potent inhibitors. The ^{125}I analogue of 8 was synthesized in order to evaluate the possibility of side-chain elimination under the assay conditions. Approximately 90% of [^{125}I]-8 remained intact for up to 1 h under assay conditions.

Agents that control estrogen biosynthesis may be of therapeutic usefulness. Estrogens have been implicated in the development or maintenance of endometrial and mammary carcinoma.[2] Moreover, correlations have been made between estrogen receptor levels and the responsiveness of breast tumors to hormone therapy.[3] Particularly important may be the effect of peripheral extraglandular estrogen production on metastatic carcinoma in post menopausal women.[4]

Because of this association of estrogens with various disease states, interest has focused on the synthesis of agents that will competitively inhibit the enzyme aromatase. This enzyme is responsible for the conversion of androstenedione into estrone, the last step in estrogen biosynthesis. In particular, Brodie's group[5] has demonstrated the effectiveness of a number of analogues of androstenedione as inhibitors of aromatase. For example, 4-hydroxyandrostene-3,17-dione and its acetylated derivative 4-acetoxyandrostene-3,17-dione were shown to be competitive inhibitors of placental aromatase and also caused the regression of 7,12-dimethylbenzanthracene-induced mammary tumors in rats.[6,7]

Complementing these investigations has been the search for irreversible inhibitors of aromatase. Covey et al.[8] have developed compounds that may act as mechanism-based irreversible inhibitors of aromatase. Other potential mechanism-based irreversible inhibitors have been synthesized by Marcotte and Robinson[9] and by Metcalf et al.[10] Moreover, it has recently been found that some of the competitive inhibitors such as 4-hydroxyandrostenedione have an irreversible component associated with their inhibition of aromatase.[7,11,12]

Previous work from our laboratory has demonstrated the effectiveness of various 7α-phenylthio derivatives of androstenedione as inhibitors of aromatase. In particular 7α-[(4-aminophenyl)thio]androst-4-ene-3,17-dione (1) was found to be one of the most potent in vitro inhibitors of aromatase reported to date with a K_i of 18 nM.[13,14] Replacing the p-amino substituent with p-methoxy or hy-

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(2) (a) K. J. Ryan, *Cancer Res.*, **42**, 3342s (1982); (b) M. A. Kirschner, G. Schneider, N. H. Ertel, and E. Worten, *Cancer Res.*, **42**, 3281s (1982).
(3) W. R. Miller, R. A. Hawkins, and A. P. M. Forrest, *Cancer Res.*, **42**, 3365s (1982).
(4) Y. L. Abul-Hajj, *J. Steroid Biochem.*, **13**, 1395 (1980); (b) H. L. Judd, R. M. Borone, L. R. Laufer, J. C. Gambone, S. L. Monfort, and B. L. Lasley, *Cancer Res.*, **42**, 3345s (1982).
(5) A. M. H. Brodie, *Cancer Res.*, **42**, 3312s (1982).
(6) A. M. H. Brodie, D. Marsh, and H. J. Brodie, *J. Steroid Biochem.*, **10**, 432 (1979).
(7) A. M. H. Brodie, H. J. Brodie, W. M. Garrett, J. R. Hendrickson, D. A. Marsh, and C. H. Tsai, *Biochem. Pharmacol.*, **31**, 2017 (1982).
(8) D. F. Covey, W. F. Hood, and V. D. Parikh, *J. Biol. Chem.*, **256**, 1076 (1981).
(9) P. A. Marcotte and C. H. Robinson, *Biochemistry*, **21**, 2773 (1982).
(10) B. W. Metcalf, C. L. Wright, J. P. Burkhart, and J. O. Johnston, *J. Am. Chem. Soc.*, **103**, 3221 (1981).
(11) A. M. H. Brodie, W. M. Garrett, J. R. Hendrickson, C. H. Tsai-Morris, P. A. Marcotte, and C. H. Robinson, *Steroids*, **38**, 693 (1981).
(12) D. F. Covey and W. F. Hood, *Endocrinology*, **108**, 1597 (1981).
(13) R. W. Brueggemeier, E. E. Floyd, and R. E. Counsell, *J. Med. Chem.*, **21**, 1007 (1978).
(14) R. W. Brueggemeier, C. A. Snider, and R. E. Counsell, *Cancer Res.*, **42**, 3334s (1982).

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