Synthesis and Biological Properties of Some Unique Cytotoxic Steroids

J. E. PIKE, J. E. GRADY, J. S. EVANS, AND C. G. SMITH

Research Laboratories, The Upjohn Company, Kalamazoo, Michigan

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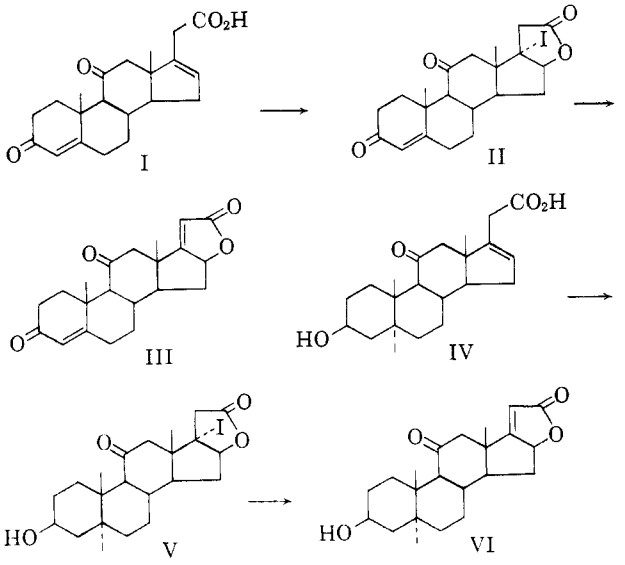
Of more than 150 steroids tested for cytotoxic activity in a growing mammalian cell culture system, approximately 25 were found to have reproducible ID_{50} values below 10 γ/ml. The most active compounds all contain a γ-lactone ring fused to the 16,17-positions. Two chemically related lactones (III and VI), the syntheses of which are described in this paper, had ID_{50} values of 0.2 and 0.18 γ/ml., respectively, acute LD_{50} values in mice of 79 and 115 mg./kg., marginal inhibitory activity against S-180 and T-4 lymphoma implanted in mice with no inhibitory activity against 8 additional tumors tested *in vivo*, relatively low endocrine activity at the concentrations tested, and, in the case of III, blood levels as high as 30 γ/ml. after intravenous administration of a high dose of this compound to dogs. Because of the high and unusual order of cytotoxicity of these steroids, coupled with a relatively low order of whole animal toxicity and endocrine activity as well as good blood levels, these compounds are believed to be interesting candidates for evaluation as antitumor agents in both experimental animals and man.

In the course of examining approximately 150 steroids for cytotoxic activity against mammalian cells *in vitro*, 25 of these agents were found to inhibit reproducibly the growth of KB cells 50% (ID_{50}) at a level of 10 γ/ml. or less. Of these, only 6 showed an ID_{50} of 1 γ/ml. or less under the same test conditions. Two steroids with high cytotoxic activity, 16β-hydroxy-3,11-dioxopregna-4,17(20)-dien-21-oic acid, γ-lactone (III); and 3β,16β-dihydroxy-11-oxo-5α-pregn-17(20)-en-21-oic acid, γ-lactone (VI) were examined for breadth of biological activities in a variety of test systems *in vitro* and for antitumor activity *in vivo*. The synthesis and biological activities of these compounds and the cytotoxic activities of certain other active steroids are described in this paper.

Chemistry.—Alkaline hydrolysis of methyl 3,11-dioxo-*cis*-pregna-4,17(20)-dien-21-oate has been reported to give a mixture of 3,11-dioxo-*cis*-pregna-4,17(20)-dien-21-oic acid and the β,γ-isomer, 3,11-dioxopregna-4,16-dien-21-oic acid (I).[1] Conversion of the β,γ-unsaturated acid (I) to the corresponding iodolactone (II),[2] followed by dehydroiodination[3] gave the unsaturated lactone (III).

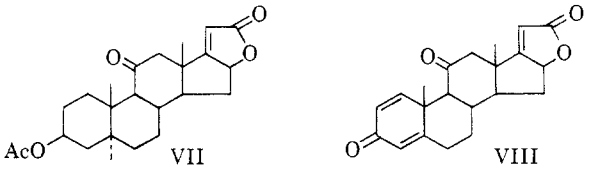


(1) J. A. Hogg, P. F. Beal, A. H. Nathan, F. H. Lincoln, W. P. Schneider, B. J. Magerlein, A. R. Hanze, and R. W. Jackson, *J. Am. Chem. Soc.*, **77**, 4436 (1955).

(2) J. Bougeault, *Ann. Chim. Phys.*, **14**, 145 (1908); **15**, 296 (1908).

The n.m.r. spectrum[4] of this lactone (III) showed in addition to a peak corresponding to the C-4 vinyl proton, a signal at 352 c.p.s. due to the C-20 hydrogen; this provides additional support for the α,β-unsaturated lactone structure and eliminates for example the possibility that the double bond could be at 16,17. Interestingly, the infrared spectrum of (III) showed two prominent lactone carbonyl peaks [1770 and 1753 cm.$^{-1}$ (Nujol)]; similar examples of this unusual but apparently characteristic result with cyclic α,β-unsaturated 5-ring lactones have been discussed in detail by Jones and co-workers.[5]

Similarly methyl 3β-hydroxy-11-oxo-5α-pregn-17(20)-en-21-oate[6] on basic hydrolysis gave 3β-hydroxy-11-oxo-5α-pregn-16(17)-en-21-oic acid (IV) together with the α,β-unsaturated acid. Iodolactonization of the β,γ-isomer gave V, which followed by dehydroiodination as before produced 3β,16β-dihydroxy-11-oxo-5α-pregn-17(20)-en-21-oic acid, γ-lactone (VI). Acetylation with acetic anhydride in pyridine afforded the corresponding 3-acetate (VII).



A similar hydrolysis, iodolactonization, and dehydrohalogenation sequence was run with methyl 3,11-dioxo-*cis*-pregna-1,4,17(20)-trien-21-oate,[7] without isolating the intermediates, to give the unsaturated lactone (VIII).

The C-16-oxygen function in these lactones has been assigned the 16β-configuration assuming the formation of an intermediate 16α,17α-iodonium species, attack by

(3) J. Klein, *J. Am. Chem. Soc.*, **81**, 3611 (1959).

(4) N.m.r. spectra were obtained and analyzed by Dr. G. Slomp and F. A. MacKellar with a Varian DP-60 or A-60 spectrometer operating at 60 Mc. DP-60 spectra were observed on *ca.* 0.15 *M* solutions (generally unless otherwise indicated in $CDCl_3$) and these spectra were calibrated against internal tetramethylsilane using the audiofrequency side-band technique. Frequencies are reported in cycles/sec. downfield from tetramethylsilane. The A-60 spectra were run on 0.25 *M* solutions.

(5) R. N. Jones and B. S. Gallagher, *J. Am. Chem. Soc.*, **81**, 5242 (1959).

(6) D. E. Ayer and W. P. Schneider, *ibid.*, **82**, 1249 (1960).

(7) J. A. Hogg, F. H. Lincoln, A. H. Nathan, A. R. Hanze, B. J. Magerlein, W. P. Schneider, P. F. Beal, and J. Korman, *ibid.*, **77**, 4438 (1955).

the iodine occurring from the less hindered α-side,[8] and then reaction of this intermediate with the carboxylate anion to give a 17α-iodo-16β-hydroxy lactone.[9]

Experimental[10]

16β-Hydroxy-17α-iodo-3,11-dioxopregn-4-en-21-oic Acid, γ-Lactone (II).—A solution of 45.5 g. of 3,11-dioxopregna-4,16-dien-21-oic acid in aqueous potassium bicarbonate solution, prepared from 30 g. of potassium bicarbonate and 1200 ml. of water, was added with stirring to a solution of iodine (160 g.) and potassium iodide (300 g.) in water (2400 ml.). After 4.5 hr. at room temperature, the organic material was isolated with ethyl acetate. The combined extracts were washed with sodium thiosulfate solution, sodium bicarbonate solution, and water and then dried (sodium sulfate). Removal of the solvent and crystallization of the residue from acetone–Skellysolve B[11] gave II, crop 1, 30.5 g., m.p. 195–205° dec.; crop 2, 3.5 g. Further crystallization of crop 1 from acetone–Skellysolve B raised the m.p. to 218–221° dec.; ν_{max}^{Nujol} 1773, 1708, 1665, 1618, 1200, 1177, 1170, 1045, and 1028 cm.$^{-1}$; λ_{max}^{EtOH} 237.5 mμ (ϵ 16,650).

Anal. Calcd. for $C_{21}H_{23}IO_4$: C, 53.86; H, 5.30; I, 27.1. Found: C, 54.13; H, 5.94; I, 27.4.

16β-Hydroxy-3,11-dioxopregna-4,17(20)-dien-21-oic Acid, γ-Lactone (III).—A solution of 16β-hydroxy-17α-iodo-3,11-dioxopregn-4-en-21-oic acid, γ-lactone (34.0 g., m.p. 205° dec.) in pyridine (200 ml.) was stirred at room temperature for 60 hr. At the end of this time methylene chloride was added and the organic layer washed successively with water, dilute hydrochloric acid, sodium bicarbonate solution, and water. The organic layer was dried over sodium sulfate and the solvent removed to give a crystalline solid, which was dissolved in methylene chloride and chromatographed on Florisil® (1500 g.). Elution was effected with increasing percentages of acetone in Skellysolve B; crystalline material was obtained from the 50% acetone–Skellysolve B eluates. These fractions were combined and recrystallized from acetone–Skellysolve B to give crop 1, 14.7 g., m.p. 235–238°; crop 2, 3.95 g., m.p. 228–233°. Further crystallization of crop 1 from the same solvent gave III, m.p. 229–236°; λ_{max}^{EtOH} 230 mμ (ϵ 22,500); ν_{max}^{Nujol} 3080, 1770, 1753, 1743, 1697, 1663, 1635, 1605, 1247, 1233, 1175, 1163, 1133, and 1085 cm.$^{-1}$.

Anal. Calcd. for $C_{21}H_{24}O_4$: C, 74.09; H, 7.11. Found: C, 73.96; H, 7.30.

The n.m.r. spectrum showed a singlet at 58 c.p.s. (C-18 methyl), a singlet at 90 c.p.s. (C-19 methyl), a doublet at 352 c.p.s. (20-H), and a complex multiplet centered at 301 c.p.s. (16-H).

3β,11β-Dihydroxy-17α-iodo-11-oxo-5α-pregnan-21-oic Acid, γ-Lactone. (V).—To a solution of methyl 3β-hydroxy-11-oxo-5α-pregn-17(20)-en-21-oate (29.1 g.) in methanol (350 ml.) was added potassium hydroxide (29.1 g.) in water (100 ml.), and the mixture heated under reflux for 3 hr. Isolation was effected, after cooling, by pouring the solution into water and the organic material was extracted with methylene chloride. The alkaline aqueous solution was acidified with dilute hydrochloric acid and the acid extracted with ethyl acetate. The ethyl acetate extracts were washed with water until neutral, dried (sodium sulfate), and the solvent removed *in vacuo*. Direct crystallization gave crop 1 (from acetone) and crop 2 (from ether) which were combined to give 3β-hydroxy-11-oxo-5α-pregn-17(20)-en-21-oic acid (6.95 g.), m.p. 270–276°. Further crystallization from methanol gave m.p. 273–276°.

The mother liquors from this crystallization gave a third crop, 8.16 g. (from ether), m.p. 176–184°. Further crystallization from acetone–Skellysolve B (twice) gave 3β-hydroxy-11-oxo-5α-pregn-16(17)-en-21-oic acid (IV); m.p. 188–190°; ν_{max}^{Nujol} 3450, 3370, 2720, 2600, 1725, 1700, 1210, 1170, and 1030 cm.$^{-1}$.

Anal. Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.64; H, 8.87.

IV (1.0 g.) dissolved in a solution of potassium bicarbonate (1.6 g.) in 25 ml. of water was added to a solution of iodine (2.5 g.) in potassium iodide (6.0 g.) and 50 ml. of water. This mixture was stirred for 30 min. and then allowed to stand overnight at room temperature. Isolation was effected by the addition of methylene chloride; then the organic solution was washed with sodium thiosulfate solution, sodium bicarbonate solution, and water and then dried (sodium sulfate). Removal of the solvent gave crystalline material which was triturated with methanol to give the γ-lactone V, m.p. 220° dec. Further crystallization from acetone–Skellysolve B gave material, m.p. 223–229° dec.; ν_{max}^{Nujol} 3540, 1770, 1705, 1165, 1135, and 1035 cm.$^{-1}$.

Anal. Calcd. for $C_{21}H_{29}IO_4$: C, 53.34; H, 6.14; I, 26.9. Found: C, 53.16; H, 6.40; I, 26.26.

3β,16β-Dihydroxy-11-oxo-5α-pregn-17(20)-en-21-oic Acid, γ-Lactone. (VI).—A solution of 3β,16β-dihydroxy-17α-iodo-11-oxo-5α-pregnan-21-oic acid, γ-lactone (14.85 g.) in pyridine (50 ml.) was stirred at room temperature for 3 days. The pyridine solution was then diluted with methylene chloride and washed successively with dilute hydrochloric acid, sodium bicarbonate solution, and water and then dried (sodium sulfate). Removal of the solvent gave crystalline material; this was crystallized from acetone–Skellysolve B to give VI, crop 1, 8.5 g., m.p. 259–264°; crop 2, 0.90 g., m.p. 260–265°. Further crystallization from acetone–Skellysolve B gave m.p. 259–264°; ν_{max}^{Nujol} 3460, 1780, 1733, 1700, 1640, 1165, 1125, 1080, 1040, 1015 cm.$^{-1}$; λ_{max}^{EtOH} 216 mμ (ϵ 13,300).

Anal. Calcd. for $C_{21}H_{28}O_4$: C, 73.22; H, 8.19. Found: C, 73.02; H, 8.20.

The n.m.r. spectrum showed a singlet at 65 c.p.s. (C-18 methyl); a singlet at 88 c.p.s. (C-19 methyl); a doublet at 352 c.p.s. (20-H), and a complex multiplet centered at 304 c.p.s. (C-16 H).

16β-Hydroxy-3,11-dioxopregna-1,4,17(20)-trien-21-oic Acid, γ-Lactone (VIII).—A suspension of methyl 3,11-dioxopregna-1,4,17(20)-trien-21-oate (29.6 g.) in 500 ml. of methanol was heated to reflux under nitrogen for 3 hr. with potassium hydroxide (30.0 g.) dissolved in water (100 ml.). At the end of this time most of the methanol was removed *in vacuo*. The residual brown solution was boiled with Darco G (*ca.* 2.0 g.), filtered through Celite, and the solution extracted with methylene chloride. The aqueous layer was then acidified and extracted with ethyl acetate. The ethyl acetate extracts were washed with water, then saturated sodium bicarbonate solution (two 500-ml. portions). This sodium bicarbonate solution was then added to a solution of iodine (29.6 g.) in potassium iodide (40.0 g.) in water (500 ml.). After standing 18 hr. at room temperature the solution was extracted with ethyl acetate–methylene chloride (1:1) and washed with sodium thiosulfate solution, saturated aqueous sodium bicarbonate solution, and water and then dried (sodium sulfate). Removal of the solvent and trituration of the residue with ether–methanol gave the iodolactone, 2.4 g., m.p. 205° dec.

A suspension of this lactone (2.0 g.) was stirred for 3 days at room temperature with pyridine (20 ml.). The mixture was then diluted with water and extracted with methylene chloride. The organic extracts were washed with ice-cold, dilute hydrochloric acid, sodium bicarbonate solution, and water and then dried (sodium sulfate). Removal of the solvent gave an oil. This was dissolved in methylene chloride (20 ml.) and chromatographed on Florisil (100 g.) made up in Skellysolve B. Elution with increasing proportions of acetone in Skellysolve B gave crystalline material from the 25–40% acetone–Skellysolve B eluates. These crystalline fractions were combined and crystallized from methanol to give crop 1, 0.47 g., m.p. 216–219°; crop 2, 0.34 g., m.p. 216–219°. Further crystallization of crop 1 from acetone–Skellysolve B gave VIII, m.p. 218–220°; ν_{max}^{Nujol} 1770, 1745, 1710, 1670, 1630, 1610, 1235, 1155, 1120, 1070 cm.$^{-1}$; λ_{max}^{EtOH} 228 mμ (ϵ 22,300).

Anal. Calcd. for $C_{21}H_{22}O_4$: C, 74.53; H, 6.55. Found: C, 74.53; H, 6.81.

The n.m.r. spectrum showed a singlet at 42 c.p.s. (C-18 methyl), a singlet at 40.5 c.p.s. (C-19 methyl), a doublet at 349.5 c.p.s. (20-H), and a complex multiplet centered at 298 c.p.s. (16-H).

3β,16β-Dihydroxy-11-oxo-5α-pregn-17(20)-en-21-oic Acid, γ-Lactone, 3-Acetate. (VII).—3β,16β-Dihydroxy-11-oxo-5α-pregn-17(20)-en-21-oic acid, γ-lactone (1.0 g.) was allowed to stand 18 hr. at room temperature with acetic anhydride (2 ml.) in pyridine (100 ml.). Isolation was effected by pouring the reaction mixture into ice–water and collecting the crystalline material formed by filtration, washing thoroughly with water, and drying *in vacuo*. This material (810 mg.) was crystallized from acetone–Skellysolve B to give the acetate, 0.63 g., m.p. 235–240°. Further crystallization from acetone–Skellysolve B

(8) L. F. Fieser and M. Fieser, *Experientia*, **4**, 285 (1948).

(9) E. E. van Tamelen and M. Shamma, *J. Am. Chem. Soc.*, **76**, 2315 (1954).

(10) Melting points were taken on a Unimelt apparatus (Arthur H. Thomas Co., Philadelphia, Pa.) and are corrected. Infrared spectra were recorded on a Perkin-Elmer Model 221 spectrophotometer from Nujol mulls. Ultraviolet spectra were taken on 95% ethanol solutions using a Cary Model 14 spectrophotometer.

(11) A saturated hydrocarbon fraction, b.p. 64–70°.

Table I

Cytotoxicity of Various Steroids

Compound	Median ID_{50}, γ/ml.[a]
16β-Hydroxy-3,11-dioxo-pregna-1,4,17(20)-trien-21-oic acid, γ-lactone	0.1
3β,16β-Dihydroxy-11-oxo-5α-pregn-17(20)-en-21-oic acid, γ-lactone, 3-acetate	0.15
3β,16β-Dihydroxy-11-oxo-5α-pregn-17(20)-en-21-oic acid, γ-lactone	0.18 ± 0.04[b]
16β-Hydroxy-3,11-dioxo-pregna-4,17(20)-dien-21-oic acid, γ-lactone	0.20 ± 0.04[b]
3β,14β,16-Trihydroxy-20(22)-5β-cardenolide or gitoxigenin	1
Progesterone	3
Androst-4-en-3,17-dione	4
3β-(2-Diethylaminoethoxy)-5α-androstan-17-one	4
9α-Fluoro-11β,17α-dihydroxy-6α-methyl-1,4-pregnadiene-3,20-dione, 17-acetate	4
3β-(2-Diethylaminoethoxy)androst-5-en-17-one, HCl	5
17α,21-Dihydroxypregn-4-ene-3,20,-dione, dioxime[c]	5
Testosterone, 1-aziridinecarboxylate[d]	5
21-Fluoro-11β,17α-dihydroxy-4-pregnene-3,20-dione	8

[a] Dose for 50% inhibition of protein synthesis by KB cells *in vitro;* see ref. 14. [b] 95% confidence limits for mean ID_{50}. [c] We are indebted to Dr. W. J. Wechter, Chemistry Dept., Upjohn Co. for supplying this compound. [d] We are indebted to M. E. Herr, Dept. of Microbiology, Upjohn Co. for supplying this material.

(twice) gave VII, m.p. 255–258°. The infrared spectrum shows ν_{max}^{Nujol} 1780, 1735, 1710, 1645, 1240, 1160, 1210, and 1010 cm.$^{-1}$; λ_{max}^{EtOH} 217 mμ (ε 14,350).

Anal. Calcd. for $C_{23}H_{30}O_5$: C, 71.48; H, 7.82. Found: C, 71.43; H, 7.94.

The n.m.r. spectrum shows a singlet at 58 c.p.s. (C-18 methyl), a singlet at 62.5 c.p.s. (C-19 methyl), a doublet at 351 c.p.s. (20-H), and a complex multiplet centered at 304.5 c.p.s. (16-H).

Biological Testing.—For biological testing, the steroids were dissolved in dimethylformamide and the solutions were diluted to a nontoxic concentration of DMF in all cases. *In vitro* testing for inhibition of bacteria, yeasts, and viruses were performed according to methods described by Hanka and Smith[12] and Siminoff.[13] Certain of the *in vivo* antitumor tests were performed by the contracting laboratories of the Cancer Chemotherapy National Service Center. All *in vitro* assays were performed at multiple dose levels and repeated *in toto* at least twice before averaging the data. The standard error of the tissue culture assay in these studies has been estimated at 15–20%.[14]

Materials and Methods.—The cytotoxic steroids were tested *in vivo* against four mouse tumors, the T-4 lymphoma, lymphoblast L-5178Y, Ehrlich's carcinoma, and Sarcoma 180. A suspension of T-4 lymphoma cells from donor mice was implanted subcutaneously in the inguinal region of female A/Heston mice supplied by Cumberland Farms. Treatment was started when the tumors reached a measurable size and was continued for 7 consecutive days. The L-5178Y cells from donor mice were injected intraperitoneally into female BDF_1 mice received from the Jackson Memorial Laboratories. The drugs were given intraperitoneally for 7 days starting 20 hr. after implanting the tumor cells. The Ehrlich carcinoma and Sarcoma 180 tests were conducted by implanting the respective tumor cell suspension subcutaneously in the inguinal region. The drugs were given intraperitoneally for 7 consecutive days starting 20 hr. after implanting the tumor cells.

The criteria for evaluating the effectiveness of therapy was either tumor size or survival in the case of L-5178Y. Body weights taken before and after treatment also served as an index of drug toxicity.

Cytotoxicity of Steroids.—Cytotoxicities of a variety of steroids with confirmed ID_{50} values below 10 γ/ml. are shown in Table I. Marked cytotoxic activity in cell culture was observed only with steroids containing the unsaturated γ-lactone ring. These steroids were also cytotoxic to KB cells in a disk-plate assay where inhibition of dye reduction is the end point, as shown in Table II. Because of availability of these compounds, III and VI were chosen for further study.

Antimicrobial Activity *in vitro*.—When tested in conventional agar-plate systems[12] for antibacterial, antifungal, and antiprotozoal activities, III, VI, and VIII showed the activities presented in Table II. The lack of correlation with some of the organisms in Table II was confirmed in subsequent experiments and is unexplained. It should be noted that VIII and III both contain a keto group in the 3-position whereas VI has a hydroxyl group instead. The compounds were inactive against the following spectrum of microorganisms at concentrations of 100 γ per 6 mm. paper disk: *Streptococcus faecalis, Salmonella gallinarum, Lactobacillus casei, Azotobacter vinelandii, Escherichia coli, Proteus vulgaris, Proteus rettgeri, Salmonella schottmeulleri, Candida albicans, Prototheca zopfii, Pichia chodati, Saccharomyces pastorianus, Penicillium oxalicum, Ochromonas danica, Chlorella vulgaris,* and *Crithidia fasciculata.*

Table II

In Vitro Spectrum of Activity

Organism	Zone of inhibition, mm.[a] VIII	III	VI
Bacillus pumilis	21 h[b]	11 h	0
Bacillus subtilis	20 h	30 h	9 h
Klebsiella pneumoniae	8	0	0
Micrococcus aureus	19	11	0
Mycobacterium avium	13 h	10 h	9
Sarcina lutea	17	9	0
KB cells[c]	19	19	17

[a] Compounds dissolved in MeOH at 2 mg./ml. and spotted on a 6 mm. paper disk. [b] h = hazy zone of inhibition. [c] Suspended in agar. Zone of inhibition relates to inhibition of reduction of oxidation–reduction dye. Details of method in preparation.

Acute Animal Toxicity.—The toxicity of III and VI was estimated in Rockland strain albino mice weighing 18–22 g. Compounds were administered intraperitoneally as a suspension in 0.25% aqueous methylcellulose. Groups of 10 mice were dosed with compound at 400 mg./kg. and at doses decreasing in 0.2 log intervals to 40 mg./kg. With both compounds delayed deaths were noted (deaths beyond the first 24 hr.). The LD_{50} values were calculated by the method of Spearman and Karber.[15] The LD_{50} for III was 79 mg./kg. with 95% confidence limits of 65–96 mg./kg. For VI the calculated LD_{50} was 115 mg./kg. with 95% confidence limits of 87–151 mg./kg.

The maximum tolerated dose at which no deaths occurred, estimated from *in vivo* antitumor studies performed at Sloan-Kettering Institute, Southern Research Institute, and Upjohn Laboratories, was approximately 20 mg./kg. when the agent was administered intraperitoneally for 7 days.

In Vivo **Antitumor Studies.**—Both III and VI showed marginal inhibitory activity *vs.* newly transplanted S-180 and estalished T_4 lymphoma in mice at toxic doses, as shown in Table III. Neither steroid showed activity against newly transplanted L-5178Y, the solid form of Ehrlich's carcinoma, or Walker 256 tumor; III showed no activity against solid form of H leukemia in rats, Murphy–Sturm tumor or W-256; VI showed no activity against P-1798 lymphosarcoma, T_4 lymphoma, or fibroadenoma. VIII was marginally active *vs.* S-180 (Table III) and inactive *vs.* T_4 lymphoma, L-5178Y, and Ehrlich's carcinoma at the dose levels tested.

Miscellaneous *In Vivo* **Tests.**—Both steroids were inactive or showed low orders of activity at the doses tested in the anti-

(12) L. J. Hanka and C. G. Smith, "Antimicrobial Agents and Chemotherapy," Braun–Brumfield, Inc., Ann Arbor, Mich., 1962, p. 677.

(13) P. Siminoff, *Appl. Microbiol.*, **9**, 66 (1961).

(14) C. G. Smith, W. L. Lummis, and J. E. Grady, *Cancer Res.*, **19**, 843 (1959). After the completion of this work a paper appeared describing the cell culture L5187Y inhibition as a method for evaluation of antileukemic corticoids prior to trial in animal systems: J. J. Jaffe, G. A. Fisher, and A. D. Welch, *Biochemical Pharmacology*, **12**, 1081 (1963).

(15) D. J. Finney, "Statistical Methods in Biological Assay," Hafner Publ. Co., New York, N. Y., 1952, p. 524.

inflammatory, diuretic (orally at 5, 10, and 20 mg./kg.), and mineralcorticoid (III only) assays performed in the Upjohn Laboratories.[16]

Blood and Urine Levels.—The applicability of the tissue cell culture test method[17] to the determination of blood and urine levels of III was demonstrated in dogs. Blood levels of 29, 18, 17, and 17 γ/ml. were observed at 15, 30, 60, and 120 min., respectively, after administration of 113 mg./kg., intravenously. These observations were confirmed and extended with this steroid as described in another publication.[18]

Discussion

The marked cytotoxic activity of the steroid lactones described above, combined with their relatively low order of whole animal toxicity, low order of endocrine bioactivity as indicated by the assaysre ported here, and high blood level makes them unique and interesting compounds for further antitumor evaluation.

Cytotoxicity studies reported by other investigators[19,20] also have shown marked activities for certain of these compounds. The steroid lactones reported here were the most active class of compounds of those evaluated simultaneously in the same bioassay system in our laboratories.

Of ten tumors tested *in vivo* in our laboratories or by the CCNSC, only S-180 was inhibited (marginally) by these steroids at toxic doses. All of the tumors tested thus far were transplanted and no studies have been reported with spontaneous tumors in animals or human tumors in heterologous hosts.

The early observations[21–23] that treatment of rats with cortisone resulted in atrophy of the thymus, adrenals, and lymph nodes was suggestive that certain tumor types might respond to steroid therapy. Certain transplantable tumors, such as the lymphocytic neoplasm P1798, have been very sensitive to certain steroids.[24] Other transplantable tumors,[25,26] such as S-180, are relatively resistant to steroid therapy except at toxic levels. The clinical use of potent steroids has been limited to their use in the treatment of either neoplasias of the lymphoid system or hormonally dependent neoplasias. Intensive therapeutic treatment of neoplasms with steroids may be limited because of the hormonal side effects which may interfere either because of the metabolic activity, or because of the host's psychological response (treatment of males with estrogens or females with androgens). In view of these factors, III, VI and other unique cytotoxic steroid lactones are considered interesting candidates for broad biological and pharmacological evaluation in animals and man where freedom from hormonal effects is to be desired.

TABLE III

In Vivo ANTITUMOR ACTIVITIES OF CYTOTOXIC STEROIDS

Compound	Dosage, mg./kg./day	Tumor	Degree of activity	Remarks
III	20	S-180	± (59% I)[a]	
III	32	T-4 lymphoma	−	Tested against established tumor
III	50	T-4 lymphoma	±	Tested against established tumor
VI	40	S-180	± (75% I)	
VIII	6.3	S-180 (solid)	−	
VIII	8.0	S-180 (solid)	±	

[a] 59% inhibition of tumor diameter.

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(16) Unpublished data furnished by Dr. E. M. Glenn, B. Graham, and S. Lyster. Duiresis determined by a modification of the method of Lipschitz, Hadidian, and Kerpesar, *J. Pharmacol. Exptl. Therap.*, **79**, 97 (1943).

(17) C. G. Smith, J. E. Grady, and F. P. Kupiecki, *Proc. Am. Assoc. Cancer Res.*, **4**, 63 (1963).

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