preparation. In this method, urea in urine did not interfere with derivatization by pentafluoropropionic anhydride, and it was unnecessary to destroy urea by incubation with urease (3). Derivatization of atenolol and metoprolol with pentafluoropropionic anhydride yielded products with good GLC properties, low retention times, and high electron-capture response. Back-extraction procedures ensured removal of extraneous components which normally take a long time to elute, reducing the time between injections to 15 min.

The method was applicable to the measurement of propranolol, which had a retention time of 9 min (Fig. 1*b*).

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New Compounds: Antitumor Activity of 3β-Hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam 4-[*p*-[Bis(2-chloroethyl)amino]phenyl]butyrate

P. CATSOULACOS *x, D. POLITIS *, L. BOUTIS ‡, and A. PAPAGEORGIOU ‡

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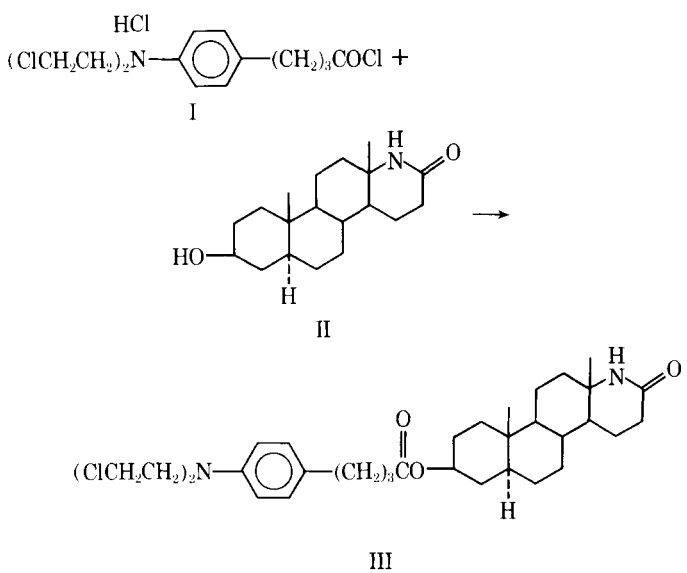
Abstract □ 3β-Hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam 4-[*p*-[bis(2-chloroethyl)amino]phenyl]butyrate was prepared by reacting 4-[*p*-[bis(2-chloroethyl)amino]phenyl]butyryl chloride hydrochloride with 3β-hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam. The cytostatic action of the ester was investigated on two tumor systems (B16 melanoma on C57 bl mice and T8-Guerin on rats).

Keyphrases □ 3β-Hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam ester of chlorambucil—synthesized, antineoplastic activity evaluated, mice and rats □ Chlorambucil, 3β-hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam ester—synthesized, antineoplastic activity evaluated, mice and rats □ Antineoplastic activity—3β-hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam ester of chlorambucil evaluated in mice and rats

3β - Hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam *p*-[bis(2-chloroethyl)amino]phenylacetate exhibited significant antitumor activity against P-388 and L-1210 leukemia in mice (1) and T8-Guerin, B16 melanoma, and Theagenion-Bahner angiosarcoma (2).



Scheme I

Table I—Effect of III on T8-Guerin Tumor and B16 Melanoma

Treatment	Number of Animals	Mean Survival of Noncured Animals, Days	Survivors
T8-Guerin Tumor (on Wistar Rats)			
Controls	10	55	0/10
Chlorambucil, 12.5 mg/kg ip			
Day 2	5	—	5/5
Days 2 and 10	4	65	3/4
Day 10	5	66	1/5
Days 10 and 20	5	60	1/5
III, 70 mg/kg ip			
Day 2	4	54	1/4
Days 1 and 10	5	60	3/5
Day 10	7	58	2/7
Days 10 and 20	4	76	3/4
B16 Melanoma (on C57 Black Mice)			
Controls	10	30	0/10
Chlorambucil, 12.5 mg/kg ip			
Day 2	5	52	0/5
Days 2 and 10	5	49	0/5
Day 10	5	43	0/5
Days 10 and 20	5	56	0/7
III, 70 mg/kg ip			
Day 2	7	48	0/7
Days 2 and 10	9	44	0/9
Day 10	6	47	0/6
Days 10 and 20	5	57	0/5

This fact created an interest in this compound that led to the synthesis of a number of derivatives (3–5).

DISCUSSION

These findings prompted the study of compounds that might have a favorable ratio between lethal and minimum effective doses and at the same time a lower toxicity than the nitrogen mustards. Therefore, 3β-hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam 4-[*p*-[bis(2-chloroethyl)amino]phenyl]butyrate (III, Scheme I) was synthesized by the action of 4-[*p*-[bis(2-chloroethyl)amino]phenyl]butyryl chloride hydrochloride (I) on 3β-hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam (II) (6).

Compound III was isolated in pure form after silica gel column chromatography. It gave a 50% increased lifespan over controls in the treatment of L-1210 leukemia in mice by the intraperitoneal, subcutaneous, and oral routes. In contrast, unmodified steroidal esters were inactive

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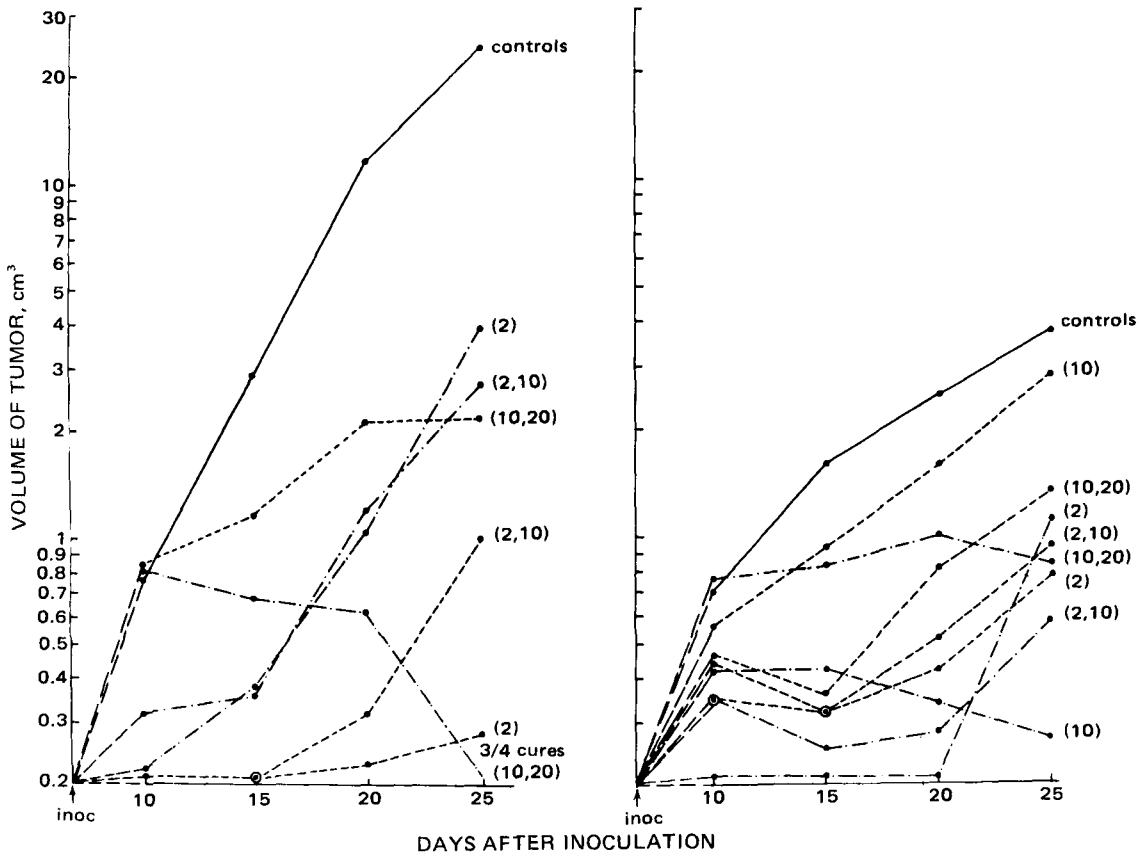

Figure 1—*Tumor growth curves of T8-Guerin tumor (left) and B16 melanoma (right) after treatment with chlorambucil and III. Key: —, controls; - - -, chlorambucil, 12.5 mg/kg ip; and –·–, ester, 70 mg/kg ip. Numbers in parentheses are the days after tumor inoculation.*

in L-1210 leukemia (7). The cytostatic action of III was investigated on two tumor systems (B16 melanoma on C57 bl mice and T8-Guerin tumor on rats).

Compounds II, III, and chlorambucil [4-[*p*-[bis(2-chloroethyl)amino]phenyl]butyric acid] were used as 1–5-mg/ml suspensions in corn oil in various dose schedules. For rats and mice, the LD_{50} (30 days) of III was 120 mg/kg after intraperitoneal injection. Compound II had no direct cytostatic effect on the tumors. The results of treatment of T8-Guerin tumor and B16 melanoma with III and chlorambucil are shown in Table I and Fig. 1.

Both III and chlorambucil, when given to animals with growing tumors, gave an increased lifespan. Clinical observations on the response of the animals and of the tumors and pathological studies revealed about the same antineoplastic activity of both chlorambucil and III but more potent early toxic effects of chlorambucil; the action of III was more prolonged.

EXPERIMENTAL[1]

To a solution of 7 g of II in 250 ml of dry benzene was added 9 g of I. The mixture was heated under reflux under nitrogen for 24 hr. Then the reaction mixture was concentrated under reduced pressure. The remaining residue was dissolved in chloroform, chromatographed on a silica gel column, and eluted with chloroform.

After solvent evaporation, the residue was crystallized from ethyl acetate–*n*-hexane to give 8.5 g (62%) of III, mp 118–121°; IR: ν_{max} 3145, 3030 (NH), 1715 ($COOCH_2$), 1670 (NHCO), 800, and 700 (aromatic ring) cm^{-1}; NMR: τ 9.2 (18-CH_3), 8.85 (19-CH_3), 5.25 (C_3-H), 3.45 (NH), 6.3 [$N(CH_2CH_2)_2$], 7.6 [$(CH_2)_3$] and the four aromatic protons at ~3; mass spectrum: molecular ion M^+ 590.

Anal.—Calc. for $C_{33}H_{48}Cl_2N_2O_3$: C, 67.01; H, 8.12; N, 4.73. Found: C, 67.15; H, 7.71; N, 4.47.

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[1] Melting points were determined on a Gallenkamp melting-point apparatus and are uncorrected. IR spectra were recorded with a Perkin-Elmer 521 in solid phase potassium bromide. NMR spectra were determined with a Varian Associates A-60 instrument, using deuterochloroform as a solvent and tetramethylsilane as the internal standard. Elemental analyses were performed by the Analytical Laboratory of the Chemistry Department, Nuclear Research Center "Demokritos."