Anticoagulant and estrogenic effects of two new 17β-aminoestrogens, butolame [17β-(4-hydroxy-1-butylamino)-1,3,5(10)-estratrien-3-ol] and pentolame [17β-(5-hydroxy-1-pentylamino)-1,3,5(10)-estratrien-3-ol]

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The syntheses and characterizations of two new 17β-aminoestrogens, butolame [17β-(4-hydroxy-1-butylamino)-1,3,5(10)-estratrien-3-ol] and pentolame [17β-(5-hydroxy-1-pentylamino)-1,3,5(10)-estratrien-3-ol], are presented. Both compounds, when administered in single subcutaneous injections to male mice and rats, produce dose-dependent increases in blood clotting times that may last several days. The estrogenic effects assessed by the vaginal cornification test are of relatively short duration. (*Steroids* **58**:457–461, 1993)

Keywords: 17β-aminoestrogens; butolame; pentolame; anticoagulant effect; estrogenic effect; steroids

Introduction

The biphasic effects on blood clotting time of estradiol, estrone, diethylstilbestrol, and of mixed conjugated equine estrogens (Premarin) have been described earlier by us; initial anticoagulant effects, lasting few hours, are followed by procoagulant effects lasting several days.[1] These biphasic effects may underlie the discrepant effects of estrogens on thrombogenesis observed in different studies.[2] In contrast, some 17β-aminoestrogens studied by our group produce long-lasting anticoagulant effects,[3–6] and inhibit platelet aggregation.[7] Of these, prolame,[4] and hexolame[5] have an amino-alcohol side chain—NH-(CH_2)n-OH at C-17 with 3 and 6 methylenes, respectively—and lengthen blood clotting times in laboratory animals without increasing motor activity or producing convulsions. In this paper we describe the synthesis, the anticoagulant and estrogenic effects of two new 17β-aminoestrogens, butolame, [17β-(4-hydroxy-1-butylamino)-1,3,5(10)-estratrien-3-ol] and pentolame, [17β-(5-hydroxy-1-pentylamino)-1,3,5(10)-estratrien-3-ol], with side chains of 4 and 5 methylenes, respectively. The information on the pharmacological effects of these compounds may contribute to a better understanding of the structure–activity relationships in this homologous series. Butolame and pentolame in mice and rats produce dose-dependent increases in blood clotting times that last several days, and are apparently not followed by a procoagulant phase. Estradiol, as reported before,[1] has a much shorter anticoagulant effect, that is followed by a sustained procoagulant phase. In contrast, the estrogenic effects of butolame and pentolame are of shorter duration than that of estradiol. The prolonged anticoagulant effects of these 17β-aminoestrogens, and the absence of a procoagulant phase, suggest their possible usefulness as therapeutic agents for prostatic cancer, the menopausal syndrome, and as estrogenic components of contraceptives.

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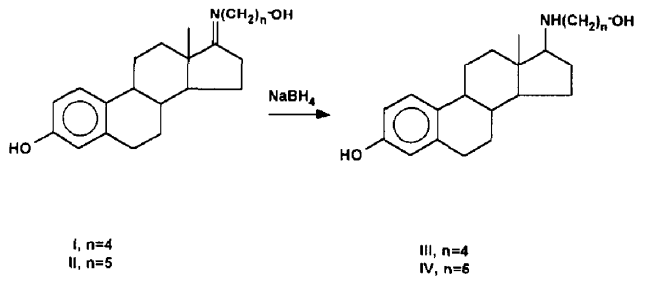

Figure 1 Synthesis of the 17β-aminoestrogens butolame (III) and pentolame (IV).

Experimental

Synthesis

Figure 1 shows the scheme of the butolame and pentolame syntheses. All solvents and reagents were analytical reagent grade, and were used without further purification. Estrone was obtained from Syntex (México), and 4-amino-1-butanol, 5-amino-1-pentanol, and sodium borohydride were purchased from Aldrich (Milwaukee, WI, USA). Proof of chemical purity was established by normal spectral (IR, NMR, MS) and analytical (TLC and chemical analysis) techniques. Melting points were determined on an Electrothermal capillary melting point apparatus, and are uncorrected. IR spectra were obtained in a potassium bromide disc using a Nicolet FT-55X spectrophotometer. ^{1}H NMR spectra were recorded on a Varian-Gemini spectrometer 200 MHz, in $CDCl_3$-$DMSOd_6$, and are reported in ppm downfield from the internal standard tetramethylsilane. MS were obtained on a Hewlett-Packard 5985B quadrupole mass spectrometer at 70 eV.

17β-(4-Hydroxy-1-butylamino)-1,3,5(10),17(20)-estratetraen-3-ol (I) and 17β-(5-Hydroxy-1-pentylamino)-1,3,5(10),17(20)-estratetraen-3-ol (II)

In separate experiments, mixtures of 1.5 g (5.5 mmol) of estrone and 1 g (11 mmol) of 4-amino-1-butanol or 1 g (9.69 mmol) of 5-amino-1-pentanol in 10 ml of isopropyl ether were heated until dissolution. The solvent was evaporated and the reaction mixture was kept for 4 hours at 80-85 C. After letting the reaction mixture cool to room temperature, the solid formed was triturated with acetone (20 ml). The white solid obtained was filtered and vacuum dried to give 1.870 g (98% yield) of product I, mp 200-201C. IR, ν_{max} 3320-3110 (OH), 1670 (C = N), 1611 (aromatic ring), cm^{-1}. ^{1}H NMR, δ 0.86 (s, 3H, 18-CH_3), 1.35-2.62 (m), 2.82 (m, 2H, 16-CH_2), 3.22 (m, 2H, CH_2-N =), 3.59 (t, J = 5 Hz, 2H, CH_2-OH) 6.55 (d, J 2.4 Hz, 1H, H-4), 6.63 (dd, J = 8.4 Hz and 2.4 Hz, 1H, H-2), 7.10 (d, J = 8.4 Hz, 1H, H-1). Analysis calculated for $C_{22}H_{31}NO_2$: C, 77.37; H, 9.15; N, 4.10. Found: C, 77.26; H, 9.09; N, 4.06. Product II was obtained as described above (1.920 g 98% yield), mp 178-180C. IR, ν_{max} 3330-3105 (OH), 1665 (C = N), 1610 (aromatic ring), cm^{-1}. ^{1}H NMR, δ 0.87 (s, 3H, 18-CH_3), 1.25-2.53 (m), 2.83 (m, 2H, 16-CH_2), 3.27 (m, 2H, CH_2-N =), 3.68 (t, J = 5 Hz, 2H, CH_2-OH) 6.58 (d, J = 2.5 Hz, 1H, H-4), 6.64 (dd, J = 8.5 Hz and J = 2.5 Hz, 1H, H-2), 7.1 (d, J = 8.5 Hz, 1H, H-1). Analysis calculated for $C_{23}H_{33}NO_2$: C, 77.70; H, 9.36; N, 3.94. Found: C, 77.61; H, 9.28; N, 3.91.

17β-(4-Hydroxy-1-butylamino)-1,3,5(10)-estratrien-3-ol (III) and 17β-(5-Hydroxy-1-pentylamino)-1,3,5(10)-estratrien-3-ol (IV).

In separate experiments the products I and II (4.5 mmol) were dissolved in ethanol (20 ml), and sodium borohydride (1.0 g) was added portionwise over 30 minutes. The reaction mixture was heated at reflux for 30 minutes and stirred at room temperature overnight. After diluting with water (100 ml) and cooling on ice bath, the precipitate formed was filtered, washed thoroughly with water, and dried. The corresponding hydrochlorides of products III and IV were obtained by dissolving 2 mmol of the product in 10 ml of ethyl acetate, and adding 0.5 ml of concentrated HCl. The precipitate was filtered and dried to give quantitative yields of the hydrochlorides. These were recrystallized from methanol-ethyl acetate, filtered, and vacuum dried.

17β-(4-Hydroxy-1-butylamino)-1,3,5(10)-estratrien-3-ol (III)

Obtained in 95% yield. Recrystallized from methanol/isopropyl ether mp 147-148C. IR, ν_{max} 3300 (OH), 3180-3020 (NH), 1610 (aromatic ring) cm^{-1}. ^{1}H NMR, δ 0.75 (s, 3H, 18 CH_3), 1.17-2.31 (m), 2.63 (m, 2H, -OH and NH), 2.77 (m, 3H, -N-CH_2 and 17-CH), 3.57 (t, J = 6.2 Hz, 2H, CH_2-OH), 6.55 (d, J = 2.7 Hz, 1H, H-4), 6.62 (dd, J = 8.4 Hz and 2.7 Hz, 1H, H-2), 7.08 (d, J = 8.4 Hz, 1H, H-1). MS m/z 343 (M$^+$, 9.8%), 284 (12.5), 128 (100%). Analysis calculated for $C_{22}H_{33}NO_2$. C, 76.92; H, 9.68; N, 4.08. Found: C, 76.87; H, 9.57; N, 4.01. Hydrochloride mp 259-260C. IR, ν_{max} 3380-3180 (OH and NH), 2440 (NH$^+$), 1600 (aromatic ring) cm^{-1}.

17β-(5-Hydroxy-1-pentylamino)-1,3,5(10)-estratrien-3-ol (IV)

Obtained in 96% yield. Recrystallized from methanol/isopropyl ether, mp 164-165C. IR, ν_{max} 3400-3240 (OH and NH), 1605 (aromatic ring) cm^{-1}. ^{1}H NMR, δ 0.72 (s, 3H, 18-CH_3), 1.18-2.32 (m), 2.64 (m, 2H, -OH and NH) 2.78 (m, 3H, -N-CH_2 and 17-CH), 3.63 (t, J = 6.40 Hz, 2H, CH_2OH) 6.57 (d, J = 2.6 Hz, 1H, H-4), 6.64 (dd, J = 8.5 Hz and 2.6 Hz, 1H, H-2), 7.11 (d, J = 8.5 Hz, 1H, H-1). MS m/z 357 (M$^+$ 18%), 284 (29%), 142 (100%). Analysis calculated for: $C_{23}H_{35}NO_2$: C, 77.26; H, 9.87; N, 3.92. Found: C, 7.13; H, 9.79; N, 3.86. Hydrochloride mp 257-258C. IR, ν_{max} 3340-3220 (OH and NH), 2420 (NH$^+$), 1615 (aromatic ring) cm^{-1}.

Pharmacological methods

Male rats of Wistar origin, and male mice of CD1 origin from our colony were used. The animals were distributed among the groups according to a balanced, Latin square, block design, based on body weights. The estrogens, dissolved in corn oil, were administered subcutaneously (s.c.); control animals received the solvent only. Blood clotting time measurements also followed a balanced, Latin square, block design, so that there was no difference in the time of testing among the various groups. The experimenter always ignored to which group each animal belonged (blind experiments). For the dose–response curves, the blood clotting times were measured after 11 AM, 24 hours after a single or the last injection. When the time course of the anticoagulant effect was studied, blood clotting times were measured at different hours and days after a single or last administration.

Blood clotting time was measured as reported earlier,[3-6] with slight improvements. The tail of the animal was warmed for one minute in water at 40C to increase blood flow. The tail was dried, and a small cut was made at the tip with a sharp razor blade. A 25 μl sample of capillary blood was collected from the tip of the tail into a microhematocrit glass capillary tube on which two marks, 45 mmol apart, had previously been made. The chronometer was started when the blood first made contact with the glass capillary tube. The blood was made to flow by gravity between the two marks, by tilting the capillary tube alternately to +60°

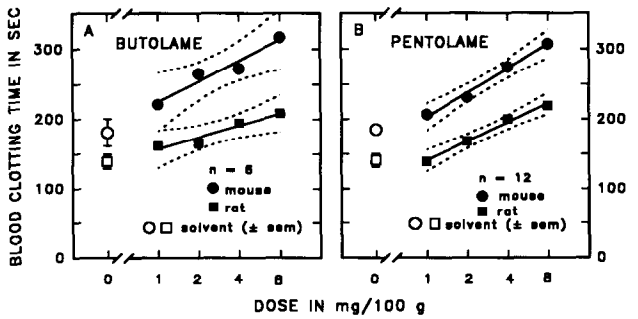

Figure 2 Log dose-response relationships of butolame (A) and pentolame (B) on blood clotting time, in the mouse and the rat, 24 hours after a single s.c. injection.

and −60° angles with respect to the horizontal plane, until the blood ceased to flow (reaction end point). The statistical significance between the mean clotting time of a control and a treated group was estimated, after assessing the homogeneity of the corresponding variances (F test), by the Student-*t* test. Least square linear regressions of blood clotting times on log dose, and their 95% confidence limits were estimated by usual procedures. The significance of the regressions, and the lack of significant deviations from linearity, were estimated by analysis of variance.

Estrogenic action was assessed by the vaginal cornification test. Female mice were ovariectomized three weeks before the test. Six groups of seven ovariectomized mice received s.c. each day, for three days, butolame or pentolame (1.3 μg/mouse/day), estradiol-17β at an equivalent dose, or the solvent (corn oil). Vaginal smears obtained with cotton swabs on days 4, 7, 9, and 11 were stained with Giemsa and examined under the microscope. In the presence of cornified cells, the absence of leukocytes in the vaginal smear was considered a positive response.

Results and discussion

The two new 17β-aminoestrogens (Figure 1) butolame (III) and pentolame (IV), were prepared from estrone according to methods described in the literature,[8,9] with minor changes described above. The compounds were characterized by usual spectroscopic methods and by satisfactory elemental microanalysis. Effects of these compounds on blood clotting time were measured, and estrogenic actions were assessed by the vaginal cornification test.

The effects of butolame and pentolame on blood clotting time

Log dose-response relationships with single administrations. Figures 2A and 2B show the log dose-response relationships of butolame and pentolame, respectively, on blood clotting times. The results were obtained in adult mice and rats 24 hours after single s.c. injections. The corresponding linear regressions and 95% confidence limits are also shown. In both species, butolame and pentolame produced linear increases in blood clotting times with respect to log doses. The regression coefficients for butolame were b = 49, P = 0.003, and b = 28, and P = 0.002, for mice and rats, respectively. For pentolame, the respective values were b = 57, P = 0.0001, and b = 45, P = 0.0004. In no case was deviation from linearity statistically significant. With the largest dose (8 mg/100 g), butolame increased blood clotting times by +75% and +50% in the mouse and rat, respectively, and pentolame by +65% and +55%.

Log dose-response relationship of pentolame with repeated administrations. The daily s.c. administration of pentolame for three days, produced in the mouse a linear increase in blood clotting time with respect to log dose (b = 89, P = 0.0005), as shown in Figure 3.

Time course of the anticoagulant effects. Figure 4 shows the time course of the anticoagulant effects in the mouse of a single dose (8 mg/100 g) of butolame and pentolame. A significant increase in blood clotting time was observed 12 hours after the injection (+33%, P = 0.049 with butolame, and +42%, P = 0.043 with pentolame). After 24 hours, the maximal anticoagulant effect was observed with both 17β-amino estrogens; butolame produced a mean increase in blood clotting time of +89% (P = 0.0002) and pentolame of +81% (P = 0.0006), with respect to the control group. Ninety-six hours after the single injection, the blood clotting times were still increased by +28% (P = 0.006) and +11% (P = NS) with butolame and pentolame, respectively.

The rate of decrease in the anticoagulant effects in

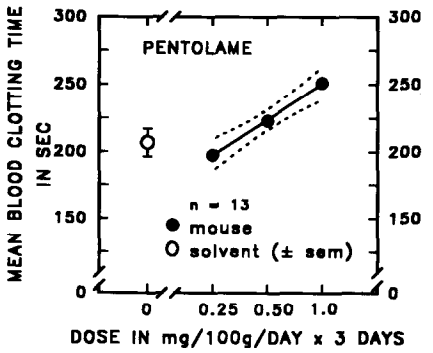

Figure 3 Log dose-response relationship of pentolame, after three administrations at daily intervals, on blood clotting time in the mouse.

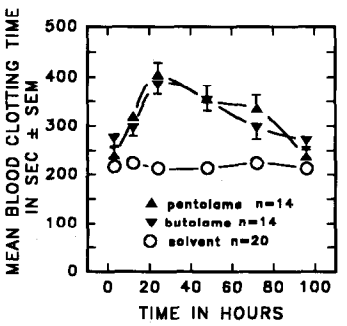

Figure 4 Time course of the anticoagulant effects in the mouse of butolame and pentolame (single s.c. injection of 8 mg/100 g).

Table 1 Comparison of the duration of the estrogenic effect (vaginal cornification) of estradiol, butolame, and pentolame

Treatment	Dose/mouse/day for 3 days	N	Body Weight in g ± SEM	Vaginal cornification* on day				
				0	4	7	9	11
Solvent	0.1 mL	14	32.0 ± 0.8	0/14	0/14	0/14	0/14	0/14
Estradiol	1.0 μg	14	32.0 ± 0.9	0/14	14/14	12/14	6/14	0/14
Butolame	1.3 μg	7	30.0 ± 0.6	0/7	7/7	0/7	0/7	0/7
Pentolame	1.3 μg	7	31.0 ± 0.7	0/7	7/7	2/7	0/7	0/7

* Number of mice with vaginal cornification/number of mice tested

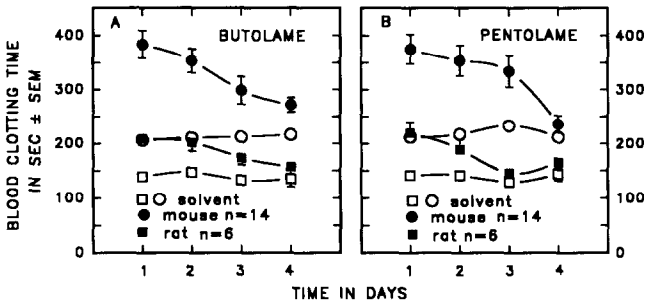

Figure 5 The rates of disappearance of the anticoagulant effects of butolame (A) and pentolame (B) in the mouse and the rat, after a single subcutaneous injection (8 mg/100 g).

mice and rats after 24 hours of a single s.c. injection of butolame or pentolame (8 mg/100g), is shown in Figure 5. The blood clotting times were measured at daily intervals for four days in the aminoestrogen-treated and solvent-treated groups. In both species, the increased blood clotting times observed after 24 hours, diminished gradually on the following three days; with butolame, the maximal values of +86% and +50%, decreased to +25% and 16% in mice and rats, respectively. The increased blood clotting times produced with pentolame (Figure 5B) of +77%, and +55%, decreased to +11% (NS) on the fourth day in the mouse, and to +13% (NS) on the third day in the rat.

Estrogenic Effects

After three days of equimolar s.c. administrations of butolame (1.3 μg), pentolame (1.3 μg), estradiol (1.0 μg), or solvent (corn oil 0.1 ml) to ovariectomized mice, the estrogenic effect was assessed by the vaginal cornification test on days 4, 7, 9, and 11. Table 1 shows the number of animals with vaginal cornification per number of animals assayed. Both butolame and pentolame produced vaginal cornification in all animals tested. The duration of this estrogenic effect was shorter with the aminoestrogens than with estradiol. Our results suggest that butolame may have an even shorter estrogenic effect than pentolame.

Conclusions

Butolame and pentolame have prolonged anticoagulant effects, which may last several days even after a single s.c. injection. These effects are similar to those described by us earlier for prolame and hexolame, and differ from the brief anticoagulant effects of estradiol, diethylstilbestrol and conjugated equine estrogens, which last only few hours, and are followed by sustained procoagulant effects. In contrast, the estrogenic effects (vaginal cornification) of both butolame and pentolame are of shorter duration than those produced with estradiol.

The administration of estrogens without a 17β-amino sidechain to humans, has been reported in different studies,[2] to increase the risk of thromboembolic phenomena, to decrease such risks, and to increase the risk of cerebral hemorrhage. The biphasic effects on blood clotting time in laboratory animals of the estrogens used clinically may explain the discrepant results observed in different studies. The prolonged anticoagulant effects, and the apparent absence of a procoagulant phase of butolame and pentolame, reported in this paper, and of prolame and hexolame published earlier,[4,5] suggest that these 17β-aminoestrogens may be useful in the treatment of prostatic cancer, the menopausal syndrome, or as the estrogenic components of contraceptives; they would probably reduce, instead of increase, the risk of thromboembolic accidents.

Acknowledgments

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