SYNTHESIS AND MOLECULAR STRUCTURE OF PROLAME, N-(3-HYDROXY-1,3,5(10)-ESTRATRIEN-17β-YL)-3-HYDROXYPROPYLAMINE; AN AMINO-ESTROGEN WITH PROLONGED ANTICOAGULANT AND BRIEF ESTROGENIC EFFECTS.

Juan M. Fernández-G., Manuel F. Rubio-Arroyo, Manuel Soriano-García, R.A. Toscano and M. del Carmen Pérez-César.

Instituto de Química, Universidad Nacional Autónoma de México. Circuito Exterior, Ciudad Universitaria, Coyoacan 04510, México D.F.

Consuelo Rubio-Póo, Juan J. Mandoki, Aurora de la Peña, Cristina Lemini, Nicandro Mendoza-Patiño and F. Cruz.

Departamento de Farmacología, Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad Universitaria, Coyoacan 04510, México D.F.

Received 6-26-85

ABSTRACT

The synthesis and molecular structure of prolame, N-(3-hydroxy-1,3,5(10)-estratrien-17β-yl)-3-hydroxypropylamine, is described. It was characterized by ir, nmr, mass spectrometry and chemical analysis. The crystal structure of this compound was determined by single-crystal x-ray diffraction. Prolame belongs to space group $P2_12_12_1$. Cell dimensions are: a = 8.356(2), b = 13.343(4) and c = 16.119(4) Å. Z = 4; R = 4.1%.

INTRODUCTION

Recently a biphasic effect of estrone and estradiol-17β on blood clotting has been described (1). The ligand prodiame, N-(3-hydroxy-1,3,5(10)-estratrien-17β-yl)-1,3-propylenediamine (2) a diamino-estrogen synthetized by some of us has shown to have a prolonged anticoagulant effect, however it also increases the motor activity and at larger doses elicits convulsions (3). With the aim of obtaining a compound with prolonged anticoagulant activity similar to prodiame, but without convulsive activity, we have synthetized the monoamino-estrogen prolame, N-(3-hydroxy-1,3,5(10)-estratrien-17β-yl)-3-hydroxypropylamine, and solved its crystal and molecular structure.

EXPERIMENTAL

The melting point obtained on a Fisher-Johns apparatus was uncorrected. The H-nmr spectrum was obtained on a Varian FT-80A spectrometer. The ir spectrum was recorded on a Perkin-Elmer 283-B spectrometer. The mass spectrum was obtained on a Hewlett-Packard Mod. 5985 quadrupole mass spectrometer at 70 eV. The C,H,N, analysis was determined by Dr. E. Pascher (Bonn, W. Germany). The x-ray analysis was performed on a Nicolet R3m diffractometer equipped with a graphite monochromator crystal. Estrone (Syntex), sodium borohydride and 3-amino-1-propanol both from Aldrich were used for the synthesis.

Synthesis

Prolame N-(3-hydroxy-1,3,5(10)-estratrien-17β-yl)-3-hydroxypropylamine was obtained by the usual methods (4,5) reacting estrone and 3-amino-1-propanol followed by reduction with sodium borohydride. A mixture of estrone (1.5 g) and 3-amino-1-propanol (2.5 mL) in 150 mL of toluene was refluxed for 12 h, using a Dean-Stark trap. The reaction mixture was concentrated until it crystallized, and was filtered. The solid product was washed with cold toluene and dried with suction. To this solid, dissolved in methanol (250 mL), sodium borohydride (400 mg) was added portionwise and was refluxed thereafter for 20 min. The solution was poured into ice-water, and the obtained white crystals were filtered and dried. Recrystallization from methanol-water gave 1.2 g of the pure product, m p 152°C. The mass spectrum shows a M^+= 329 m/e and 114 m/e (base peak). The H-nmr (DMSO-d6) spectrum shows signals at 0.65 (s, C-18), 1.15-2.25 (m), 2.62 (t, C-19), 3.15 (s, OH), 3.42 (t, C-21), 6.45 (d, C-4 and C-2), 7.0 (d, C-1). The ir spectrum (KBr wafer) shows bands at 3422 cm^{-1} (OH), 3272 cm^{-1} (NH). Elemental analysis for $C_{21}H_{31}O_2N$.

X-Ray analysis

Single crystals of prolame were grown by slow evaporation of a methanol-acetone solution as colorless prisms, which proved suitable for x-ray-analysis. Initially photographic studies showed the m m m Laue symmetry and systematic absences in h00 with h = odd, 0k0 with k = odd and 00l with l = odd, thus uniquely defining the space group as $P2_12_12_1$. Unit cell dimensions were obtained by least-square fit to the angular setting of 25 centered reflections. Crystal data for prolame, $C_{21}H_{31}O_2N$, molecular weight 329.48: a = 8.356(2), b = 13.343(4), c = 16.119(4) Å, V = 1797.1(3) $Å^3$, d_{cal} = 1.222 gcm^{-1}, Z = 4, space group $P2_12_12_1$; (Cu Kα) = 5.65 cm^{-1}.

The crystal chosen for intensity measurement has the dimensions: 0.18 x 0.20 x 0.21 mm, and was mounted approximately along the c axis on a glass fiber. Intensity measurements were made with Cu Kα radiation (λ = 1.5418 Å) utilizing the ω-scan technique; individual scan speeds were determined by a rapid scan at the position of the calculated Bragg peak, and the rate of scanning varied from 4.0 deg-min^{-1} (less than 150 counts during the rapid scan) to 29.3 deg-min^{-1} (more than 2500 counts during the rapid scan). Two reflections were routinely monitored at intervals of 50 reflections. All reflections in the hkl octant to 2θ = 115°were collected ($\sin\theta/\lambda$ = 0.550 $Å^{-1}$). The total number of data collected was

1416, of which 1249 reflections had $I > 2.0\ \sigma(I)$; these formed the basis of the structural solution and refinement, where $\sigma(I)$ was derived from counting statistics. Values of $\sigma(F)$ were determined from the equation $(F) = (F/2)(|\sigma^2(I)|/I^2 + \delta^2)^{1/2}$, where δ is estimated as the instrumental uncertainty ($\delta = 0.020$) obtained from the variation in measured intensities in the periodically scanned standard reflections. The 1249 reflections and their associated standard deviations were corrected for Lorentz and polarization effects: no absorption correction was applied. The data was adjusted to an approximately absolute scale $K = 0.5699$ and an overall U value of 0.050 $Å^2$. The crystal structure was solved by direct methods using the program package SHELTX (6). The program SOLV was employed using 114 phases with $E \geq 1.20$ and 9 reflections in the starting set. The trial structure was refined by blocked cascade least-squares procedure with anisotropic temperature factors for the non-hydrogen atoms and with a fixed isotropic temperature factor $U = 0.06\ Å^2$ for the hydrogen atoms bonded to tertiary CH, secondary CH_2 and primary CH_3 carbon atoms; and the coordinates of the hydrogen, nitrogen and oxygen atoms were refined. The function minimized was $\Sigma w(|F_o| - |F_c|)^2$ with a weighting scheme $w^{-1} = |\sigma^2(F_o) + G(F_o)^2|$, where σ is the standard deviation of the observed amplitudes based on counting statistics and G, a variable to be adjusted after each cycle, final $G = 0.001$; maximum shift of parameters in the last cycle 0.2; no peaks > 0.3 $eÅ^{-3}$; anomalous dispersion corrections were applied to the scattering factors from International Tables for X-ray Crystallography (7); isotropic extinction parameter $x = 0.005$; final $R = 0.041$, $R_w = 0.045$ where $R_w = \Sigma w^{1/2}|F_o| - |F_c|\ \Sigma w^{1/2}\ (|F_o|)$.
All computations were performed in the laboratory on a Nova 4S computer and plots were drawn on a Tektronix plotter .

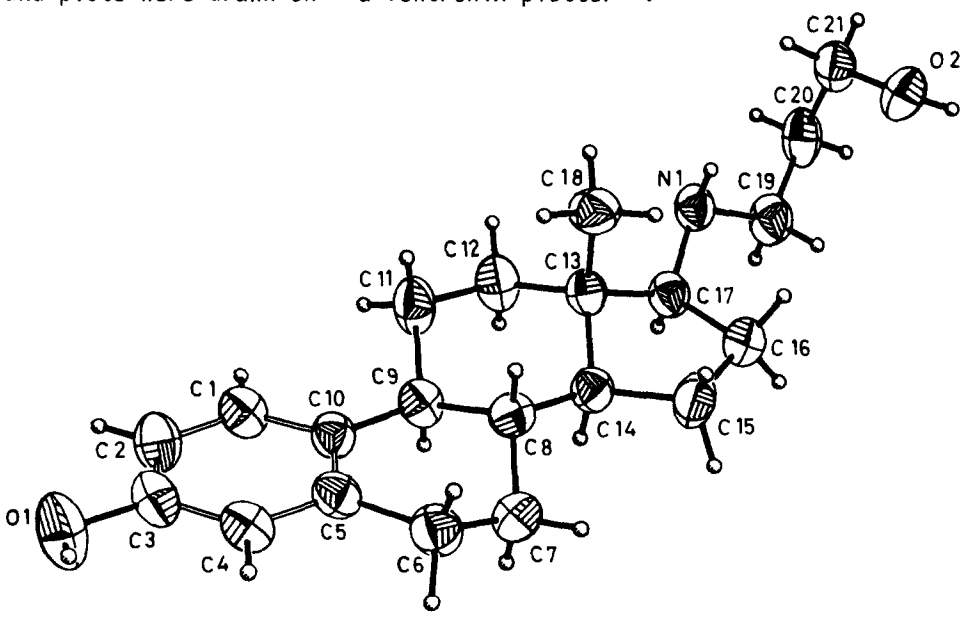

Figure 1. The molecular structure of prolame, N-(3-hydroxy-1,3,5(10)-estratrien-17β-yl)-3-hydroxypropylamine.

DISCUSSION

The observed crystallographic structure of the molecule is shown in Figure 1. Positional parameters and anisotropic thermal parameters for non-H atoms of prolame are listed in Table 1. The hydrogen coordinates and isotropic temperature factors are given in Table 2. Intramolecular bond lengths and angles, together with estimated standard deviations, are given in Table 3. None of the values for bonds and angles in the steroid nucleus are significantly different from those observed in similar molecules (8-10).

The presence of ring A requires formally that atoms C(1), C(2), C(3), C(4), C(5), C(6), C(9), C(10) and O(1) be coplanar; this is found to be so, the maximun deviation from the plane being 0.051 Å at C(6). The B ring can best be described as half chair with a pseudo-diad passing through the C(5)-C(10) and C(7)-C(8) bonds. The C ring conformation has normal chair conformation with substituent at C(13) in axial position, and the D ring conformation is close to a perfect envelope. The D ring/side chain junction geometry is trans to C(13) and gauche to C(16).

The molecules are connected by hydrogen bonds between hydroxyl groups and between a hydroxyl and the N atom of the substituent at C(17), forming a continuous ribbon along the b-axis direction. The O(1) links to O(2)' at (0.5+x, 1.5-y, 2-z)| O(1)...O(2)' = 2.767(4), O(1)-H1A = 0.76(3), O(2)'...H1A = 2.03(3) Å and the angle O(1)-H1A...O(2)' is 165(3)°|. The O(2) links to N' at (0.5+x, 2.5-y, 2-z)| O(2)...N' = 2.861(4), O(2)-H2A = 0.86(3), N'...H2A = 2.04(3) Å and the angle O(2)-H2A...N is 162(3)°|.

Table 1. Positional parameters (x 10^4) for non-hydrogen atoms of Prolame [a].

atom	x	y	z	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
N	5210(4)	11356(2)	9443(2)	46(2)	45(2)	48(2)	1(1)	-0(1)	-11(1)
O(1)	814(4)	3762(2)	7967(2)	80(2)	44(1)	90(2)	4(1)	-29(2)	-12(1)
O(2)	7327(3)	12653(2)	11053(2)	40(1)	55(1)	71(2)	7(1)	-3(1)	3(1)
C(1)	1251(4)	6466(3)	7912(2)	54(2)	46(2)	46(2)	0(2)	-10(2)	4(2)
C(2)	60(5)	5525(3)	7830(2)	55(2)	59(2)	51(2)	1(2)	-16(2)	-6(2)
C(3)	1487(4)	4695(3)	8040(2)	63(2)	44(2)	42(2)	0(2)	-8(2)	-9(2)
C(4)	3041(4)	4818(3)	8389(2)	58(2)	41(2)	37(2)	1(2)	-6(2)	4(2)
C(5)	3732(4)	5755(3)	8386(2)	50(2)	46(2)	38(2)	-3(2)	-4(2)	2(2)
C(6)	5455(4)	5829(3)	8669(2)	51(2)	47(2)	63(2)	-8(2)	-9(2)	9(2)
C(7)	6198(4)	6841(3)	8477(2)	42(2)	59(2)	60(2)	-5(2)	-2(2)	4(2)
C(8)	5086(4)	7660(2)	8787(2)	40(2)	47(2)	38(2)	1(2)	-0(2)	4(2)
C(9)	3532(4)	7663(2)	8273(2)	48(2)	41(2)	43(2)	2(2)	-9(2)	3(2)
C(10)	2825(4)	6619(3)	8200(2)	43(2)	45(2)	36(2)	-1(2)	-3(2)	-2(2)
C(11)	2368(5)	8466(2)	8580(3)	45(2)	46(2)	83(3)	-5(2)	-19(2)	1(2)
C(12)	3137(4)	9507(3)	8612(2)	53(2)	42(2)	74(2)	0(2)	-18(2)	3(2)
C(13)	4693(4)	9514(2)	9115(2)	43(2)	42(2)	41(2)	1(2)	-6(2)	-2(2)
C(14)	5795(4)	8711(2)	8771(2)	41(2)	50(2)	38(2)	-0(2)	-2(2)	-1(2)
C(15)	7401(4)	8903(3)	9205(3)	38(2)	58(2)	73(2)	-4(2)	-6(2)	-5(2)
C(16)	7437(5)	10048(3)	9394(2)	41(2)	63(2)	59(2)	-8(2)	3(2)	-9(2)
C(17)	5767(4)	10429(2)	9020(2)	46(2)	46(2)	41(2)	2(2)	1(2)	-10(2)
C(18)	4295(5)	9346(3)	10043(3)	60(2)	52(2)	52(2)	-3(2)	14(2)	-7(2)
C(19)	6354(4)	12188(3)	9311(2)	58(2)	56(2)	58(2)	4(2)	3(2)	-19(2)
C(20)	5926(5)	13146(2)	9779(2)	56(2)	40(2)	79(3)	11(2)	-17(2)	-13(2)
C(21)	5896(4)	13048(3)	10708(2)	46(2)	42(2)	70(2)	-9(2)	-9(2)	3(2)

[a] Positional parameters are listed as fractions of cell edges. Anisotropic temperature factor exponent takes the form: $-2\pi^2(h^2a^{*2}U_{11} + k^2b^{*2}U_{22} + \ell^2c^{*2}U_{33} + 2k\ell b^*c^*U_{23} + 2h\ell a^*c^*U_{13} + 2hka^*b^*U_{12})$. Estimated standard deviations with respect to the last digit listed are given in parenthesis.

Table 2 Hydrogen coordinates ($x10^4$) and isotropic temperature factors ($x10^3$) of Prolame.

atom	x	y	z	U ($Å^2$)
H	5217(45)	11192(23)	10004(22)	60
H(1a)	1352(40)	3396(23)	8170(20)	60
H(2a)	8153(39)	13025(23)	11004(19)	60
H(1)	611	7038	7770	60
H(2)	-472	5448	7625	60
H(4)	3661	4236	8448	60
H(6a)	6066	5319	8393	60
H(6b)	5494	5722	9257	60
H(7a)	7215	6996	8758	60
H(7b)	6343	6908	7889	60
H(8)	4939	7501	9358	60
H(9)	3788	7854	7713	60
H(11a)	1463	8488	9214	60
H(11b)	2018	8290	9128	60
H(12a)	3369	9715	8056	60
H(12b)	2395	9967	8361	60
H(14)	5960	8750	8182	60
H(15a)	8279	8671	8872	60
H(15b)	7434	8582	9737	60
H(16a)	7637	10231	9861	60
H(16b)	8255	10328	8946	60
H(17)	5766	10667	8458	60
H(18a)	5265	9361	10762	60
H(18b)	3785	8787	10110	60
H(18c)	3590	9865	10232	60
H(19a)	7392	11972	9492	60
H(19b)	6386	12338	8729	60
H(20a)	4885	12759	9535	60
H(20b)	6701	13649	9636	60
H(21a)	5028	12612	10856	60
H(21b)	5716	13700	10941	60

Table 3 Bond lengths (Å) and angles (°) of Prolame. The estimated standard deviations are given in parenthesis.

N-C(17)	1.496(4)	N-C(19)	1.480(4)
O(1)-C(3)	1.371(4)	O(2)-C(21)	1.421(4)
C(1)-C(2)	1.375(5)	C(1)-C(10)	1.410(5)
C(2)-C(3)	1.376(5)	C(3)-C(4)	1.378(5)
C(4)-C(5)	1.383(5)	C(5)-C(6)	1.513(5)
C(5)-C(10)	1.412(5)	C(6)-C(7)	1.518(5)
C(7)-C(8)	1.519(5)	C(8)-C(9)	1.540(5)
C(8)-C(14)	1.522(4)	C(9)-C(10)	1.518(5)
C(9)-C(11)	1.529(5)	C(11)-C(12)	1.532(5)
C(12)-C(13)	1.531(5)	C(13)-C(14)	1.518(5)
C(13)-C(17)	1.523(5)	C(13)-C(18)	1.549(4)
C(14)-C(15)	1.535(5)	C(15)-C(16)	1.535(5)
C(16)-C(17)	1.550(5)	C(19)-C(20)	1.526(5)
C(20)-C(21)	1.504(5)		

C(17)-N-C(19)	110.9(3)	C(2)-C(1)-C(10)	122.3(3)
C(1)-C(2)-C(3)	119.9(3)	O(1)-C(3)-C(2)	119.3(3)
O(1)-C(3)-C(4)	121.5(3)	C(2)-C(3)-C(4)	119.3(3)
C(3)-C(4)-C(5)	121.9(3)	C(4)-C(5)-C(6)	118.9(3)
C(4)-C(5)-C(10)	119.7(3)	C(6)-C(5)-C(10)	121.4(3)
C(5)-C(6)-C(7)	112.7(3)	C(6)-C(7)-C(8)	108.8(3)
C(7)-C(8)-C(9)	109.9(3)	C(7)-C(8)-C(14)	114.2(3)
C(9)-C(8)-C(14)	108.4(3)	C(8)-C(9)-C(10)	111.5(3)
C(8)-C(9)-C(11)	111.3(3)	C(10)-C(9)-C(11)	114.9(3)
C(1)-C(10)-C(5)	116.8(3)	C(1)-C(10)-C(9)	121.5(3)
C(5)-C(10)-C(9)	121.6(3)	C(9)-C(11)-C(12)	112.3(3)
C(11)-C(12)-C(13)	112.3(3)	C(12)-C(13)-C(14)	108.5(3)
C(12)-C(13)-C(17)	116.9(3)	C(14)-C(13)-C(17)	99.9(3)
C(12)-C(13)-C(18)	109.2(3)	C(14)-C(13)-C(18)	112.4(3)
C(17)-C(13)-C(18)	109.8(3)	C(8)-C(14)-C(13)	114.1(3)
C(8)-C(14)-C(15)	119.1(3)	C(13)-C(14)-C(15)	104.2(3)
C(14)-C(15)-C(16)	103.1(3)	C(15)-C(16)-C(17)	105.4(3)
N-C(17)-C(13)	115.9(3)	N-C(17)-C(16)	115.1(3)
C(13)-C(17)-C(16)	103.8(3)	N-C(19)-C(20)	114.0(3)
C(19)-C(20)-C(21)	115.0(3)	O(2)-C(21)-C(20)	114.1(3)

The biological studies of prolame are reported in the following paper of this issue.

ACKNOWLEDGEMENTS

The authors from Instituto de Química are indebted to Consejo Nacional de Ciencia y Tecnología (CONACYT), for funds which enabled us to obtain a single phase uninterruptible power system to protect our diffractometer computers. We wish to express our gratitude to Dr. A. Guzmán of Syntex, S.A., for the generous supply of estrone; and Mr. A. Cuellar from Instituto de Química.

REFERENCES

1. Mandoki, J.J., Zavala, E., Silva, G., Mendoza-Patiño, N., Rubio-Póo, C., Medina-Martínez, S. and Domínguez-Escoto, P., PROC. WEST. PHARMACOL. SOC. 26, 45 (1983).
2. Fernández-G., J.M., Rubio-Arroyo, M.F., Rubio-Póo, C. and de la Peña, A., MONATSH. CHEM. 114, 535 (1983).
3. Rubio-Póo, C., Mandoki, J.J., Jayme, V., Mendoza-Patiño, N., Alvarado, C., Silva, G., Zavala, E., Fernández-G., J.M. and Rubio-Arroyo, M.F., PROC. WEST. PHARMACOL. SOC. 26, 111 (1983).
4. Counsell, R.E. and Klimstra, P.D., J. MED. CHEM. 7,119 (1964).
5. Lu, M.C., Delaney, N.G. and Counsell, R.E., J. MED. CHEM. 24, 1038 (1981).
6. Sheldrick, G.M., SHELTX. Revision 3; An integrated system for solving, refining and displaying crystal structures from diffraction data, Univ. of Gottingen (1981) Federal Republic of Germany.
7. International Tables for X-ray Crystallography. Vol. IV. Kynoch Press (1974). Birmingham.
8. Norton, D.A., Kartha, G. and Lu, C.T., ACTA CRYSTALLOGR. 16, 89 (1963).
9. Norton, D.A., Kartha, G. and Lu, C.T., ACTA CRYSTALLOGR. 17, 77 (1964).
10. Cooper, A., Norton, D.A. and Hauptman, H., ACTA CRYSTALLOGR. SECT. B. B25, 814 (1969).

APPENDIX

The following trivial names have been used:
Estradiol-17β: 1,3,5(10)-estratriene-3,17β-diol
Estrone: 3-hydroxy-1,3,5(10)-estratrien-17-one
Prodiame: N-(3-hydroxy-1,3,5(10)-estratrien-17β-yl)-1,3-propylenediamine
Prolame: N-(3-hydroxy-1,3,5(10)-estratrien-17β-yl)-3-hydroxypropylamine