Crystal Structure of Potassium 4-Androsten-17 α -ol-3-oneglucosiduronate dihydrate (Epitestosterone glucuronide)

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C₂₅H₃₉ KO₁₀ is monoclinic, $P2_1$. The unit-cell dimensions at 293 K are $a = 10.6996(8)$, $b = 7.8839(6)$, $c = 15.8913(11)$ Å, $V = 1313.86(17)$ Å³, $D_x = 1.362$ g/cm³, and $Z = 2$. The R value is $R = 0.037$ for 4604 reflections. The A, B, C, D and E rings occur in a conformation between envelope and half chair, distorted chair, distorted chair, distorted envelope, and distorted chair, respectively. The three-dimensional structure is completed by the formation of hydrogen-bonded chains by the anions.

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The conjugation of steroid hormones preferentially at hydroxyl functional groups includes sulfate and glucuronic acid moieties. Steroid glucuronides are formed mostly in the liver, increasing both the polarity and the hydrosolubility compared with non-conjugated steroids. Particularly, testosterone and its 17 α -epimer epitestosterone are conjugated with the glucuronic moiety at the hydroxyl group at C17. These two glucuronides (17 β (TG) and 17 α (EG), respectively) are excreted in human urine,¹ and can be measured to detect high amounts of testosterone in patients with hyperandrogenism or furtive ingestion in some athletes. Testosterone and epitestosterone, themselves, have some electronic and physicochemical differences,² and epitestosterone may act as an antiandrogen, contrary to testosterone.³ Also, the ratio of production is

different. Interestingly in mass spectrometric experiments, EG readily forms adducts with ammonium than TG.⁴ Likewise, EG

Table 1 Crystal and experimental data

Formula:	C ₂₅ H ₃₉ KO ₁₀
Formula weight	= 538.66
Crystal system:	monoclinic
Space group:	$P2_1$ $Z = 2$
a	= 10.6996(8) Å
b	= 7.8839(6) Å
c	= 15.8913(11) Å
β	= 101.443(1)°
V	= 1313.86(17) Å ³
D_x	= 1.362 g/cm ³
μ (Mo $K\alpha$)	= 0.257 mm ⁻¹
T	= 291 K
Colorless	
$F(0\ 0\ 0)$	= 576
$0.30 \times 0.22 \times 0.11$ mm	
Radiation:	Mo $K\alpha$
ω scans with $\theta_{\max}$	= 25.0°
R	= 0.037 (on F^2)
R_w	= 0.072 (on F^2)
$(\Delta/\sigma)_{\max}$	= 0.000
$(\Delta\rho)_{\max}$	= 0.274 eÅ ⁻³
$(\Delta\rho)_{\min}$	= -0.144 eÅ ⁻³
No. of reflections measured	= 10803
No. of reflections used	= 4604
No. of parameters	= 348
Goodness-of-fit	= 0.921
Measurement:	Bruker Smart Apex CCD diffractometer
Program system:	SMART V 5.625 and SAINT V 6.23C
Structure determination:	direct methods (SHELXS-97)
Refinement:	full matrix least-squares (SHELXL-97)

CCDC 608871 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_required/cif.

Fig. 1 Molecular structure of potassium 4-androsten-17 α -glucosiduronate-3-one dihydrate with atom labeling. Thermal ellipsoids are drawn at the 50% probability level.

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Table 2 Final atomic coordinates and equivalent isotropic temperature factors ($\text{\AA}^2$)

Atom	<i>x</i>	<i>y</i>	<i>z</i>	<i>U</i> _{eq}
K	0.89599(5)	-0.09181(8)	0.62954(3)	0.05212(16)
O1	1.09334(19)	0.1596(3)	1.24093(11)	0.0678(6)
O2	0.71863(14)	0.2259(2)	0.64678(9)	0.0432(4)
O3	0.74331(14)	0.1128(2)	0.52072(9)	0.0392(4)
O4	0.80442(18)	-0.1615(2)	0.45214(11)	0.0540(5)
O5	0.81191(18)	-0.0422(2)	0.32618(11)	0.0581(5)
O6	0.83886(17)	0.2975(2)	0.33110(10)	0.0464(4)
O7	0.92063(16)	0.5452(2)	0.45387(11)	0.0458(4)
O8	0.79612(18)	0.5498(2)	0.59634(12)	0.0529(5)
O9	1.1131(3)	0.1329(3)	0.74022(15)	0.0903(8)
O10	0.5945(2)	-0.2041(4)	0.56159(17)	0.0922(8)
C1	0.9085(3)	-0.0378(4)	1.05353(15)	0.0577(7)
C2	0.9705(3)	-0.0403(4)	1.14834(16)	0.0601(8)
C3	1.0042(3)	0.1327(4)	1.18128(16)	0.0525(7)
C4	0.9204(3)	0.2692(4)	1.14279(16)	0.0607(8)
C5	0.8190(3)	0.2448(4)	1.07886(15)	0.0558(8)
C6	0.7260(3)	0.3864(5)	1.05310(17)	0.0802(10)
C7	0.6994(3)	0.4152(5)	0.95612(16)	0.0726(9)
C8	0.6610(3)	0.2533(4)	0.90652(15)	0.0566(7)
C9	0.7609(2)	0.1140(4)	0.93418(14)	0.0468(6)
C10	0.7914(2)	0.0782(3)	1.03266(15)	0.0508(7)
C11	0.7342(3)	-0.0455(4)	0.87925(15)	0.0592(8)
C12	0.7146(3)	-0.0094(4)	0.78300(15)	0.0551(7)
C13	0.6096(2)	0.1203(4)	0.75583(15)	0.0517(7)
C14	0.6441(2)	0.2805(4)	0.81011(15)	0.0532(7)
C15	0.5491(3)	0.4156(5)	0.76592(16)	0.0762(9)
C16	0.5232(3)	0.3622(4)	0.67077(17)	0.0720(9)
C17	0.5935(2)	0.1955(4)	0.66546(16)	0.0518(7)
C18	0.4786(3)	0.0468(5)	0.7632(2)	0.0892(11)
C19	0.6773(3)	-0.0078(6)	1.06222(18)	0.0913(12)
C1'	0.7175(2)	0.2682(3)	0.56132(14)	0.0368(5)
C2'	0.8203(2)	0.3951(3)	0.55654(13)	0.0386(5)
C3'	0.8228(2)	0.4308(3)	0.46330(14)	0.0356(5)
C4'	0.8447(2)	0.2665(3)	0.42011(14)	0.0357(5)
C5'	0.7496(2)	0.1304(3)	0.43222(13)	0.0353(5)
C6'	0.7917(2)	-0.0405(3)	0.40104(16)	0.0388(6)

$$U_{eq} = (1/3) \sum_i \sum_j U_{ij} (a_i^* a_j^*) (a_i a_j).$$

is better than TG to form adducts with Na⁺. Because of its intriguing biological relevance, and as an important step to know the structural characteristics and the correct orientation of the glucuronide moiety in epitestosterone, as well as its formation of adducts, it was decided to solve by X-ray crystallography the molecule of EG coordinated with a potassium atom.

The X-ray data for the crystals of the title compound were collected by graphite-monochromatized Mo *K*_α radiation at 293 K. No absorption correction was applied. The structure was solved by direct methods and refined by full-matrix least-squares with anisotropic temperature factors for the non-hydrogen atoms. Hydrogen atoms for both water molecules were located from difference Fourier maps and their positions refined. The positions for the other H atoms were calculated geometrically, and a riding model was used in the refinement, with C-H distances in the range of 0.85–0.98 Å and *U*_{iso}(H) = 1.2*U*_{eq}(C). The software used to prepare the material for publication was PARST97.⁵ Table 1 summarizes the crystal and experimental data. The molecular structure is shown in Fig. 1.

The molecule consists of four six-membered rings (A, B, C and E) and one five-membered ring (D). Rings A, B, C and D are all *trans* fused. For the puckering-parameters values (ϕ_2 , θ_2

Table 3 Bond distances (Å)

O1 - C3	1.222(3)	O3 - C5'	1.428(2)
O3 - C1'	1.437(3)	O2 - C1'	1.396(3)
O2 - C17	1.448(3)	O8 - C2'	1.421(3)
O7 - C3'	1.412(3)	O6 - C4'	1.425(3)
O4 - C6'	1.242(3)	O5 - C6'	1.251(3)
C1 - C2	1.521(3)	C1 - C10	1.533(4)
C2 - C3	1.480(4)	C3 - C4	1.456(4)
C4 - C5	1.345(4)	C5 - C6	1.498(4)
C5 - C10	1.505(4)	C6 - C7	1.528(4)
C7 - C8	1.513(5)	C8 - C14	1.522(3)
C8 - C9	1.535(4)	C9 - C11	1.525(4)
C9 - C10	1.560(3)	C10 - C19	1.549(4)
C11 - C12	1.529(3)	C12 - C13	1.518(4)
C13 - C17	1.531(3)	C13 - C14	1.532(4)
C13 - C18	1.542(4)	C14 - C15	1.542(4)
C15 - C16	1.541(4)	C16 - C17	1.525(4)
C1' - C2'	1.499(3)	C2' - C3'	1.514(3)
C3' - C4'	1.506(3)	C4' - C5'	1.517(3)
C5' - C6'	1.533(3)		

and Q),⁶ rings A, B, C, D and E occur in a conformation between envelope (¹*E*) and half chair (¹*H*₂), distorted chair (¹*C*₄), distorted chair (¹*C*₄), distorted envelope and distorted chair (¹*C*₄) conformations, respectively. The bond lengths and angles are normal. All O atoms, except O5, are involved in the K coordination. The K...O distances range from 2.6723(16) to 3.313(3) Å. The stereochemistry of the title compound is as follows: C8-βH is *trans* to C9-αH; C9-αH is *trans* to C10-βCH₃; C13-βCH₃ is *trans* to C14-αH; and C13-βCH₃ is *trans* to C17-α-glucoronide. The angle between the mean plane of the D ring of the steroid (C13–C17) and the mean plane of the glucoronide (C1'–C5', O3) is 55.08(8)°, with a torsion angle of C17–O2–C1'–C2' of 144.5(2)°. All of the H-atom substituents of the carbohydrate moiety occupy axial sites, which result in the adoption of *trans* positions for the H atoms attached to adjacent C atoms with respect to each other. There is one intramolecular O6–H6...O5 and five intermolecular O8–H8A...O1, O7–H7A...O4, O9–H9C...O5, O9–H9D...O6 and O10–H10A...O8 hydrogen bonds. The D...A, H...A and D–H...A values are, respectively [2.693(2), 2.761(2), 2.623(2), 2.942(3), 2.962(3), 2.872(3) Å], [1.93 (3), 2.01(3), 1.86(3), 2.15(5), 2.14(5), 1.59(4) Å] and [146(3), 144(3), 167(3), 172(5), 151(4), 162(3)°]. In addition, there are two intermolecular C–H...O interactions <3.5 Å, C4'...O7 (–*x* + 2, +*y* – 1/2, –*z* + 1); C2...O9 (–*x* + 2, +*y* – 1/2, –*z* + 2), 3.371(3) and 3.347(4) Å, respectively.

References

1. A.-F. De Nicola, R. I. Dorfman, and E. Forchielli, *Steroids*, **1966**, 7, 351.
2. C. Kubli-Garfias, *J. Mol. Struct. (Theochem)*, **1998**, 422, 167.
3. L. Stárka, *J. Steroid Biochem. Mol. Biol.*, **2003**, 87, 27.
4. T. Kuuranne, M. Vahermo, A. Leinonen, and R. Kostianen, *J. Am. Soc. Mass Spectrom.*, **2000**, 11, 722.
5. M. Nardelli, *J. Appl. Cryst.*, **1995**, 28, 659.
6. D. Cremer and J. A. Pople, *J. Am. Chem. Soc.*, **1975**, 97, 1354.