ISSN 1070-3632, Russian Journal of General Chemistry, 2017, Vol. 87, No. 12, pp. 3052–3058. © Pleiades Publishing, Ltd., 2017.

Synthesis and Elucidation of Structure of Deuterated Androsta-3,5-diene-7,17-dione[1]

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Received December 19, 2017

Abstract—The title compound was synthesized as an internal standard for the quantitative determination of Arimistane in biological samples. [$^2H_7$] Arimistane was obtained from Arimistane-d_0 by alkaline H/^{2}H exchange of the $C^{2,4,6,8,16}$ protons. The location of the labels was confirmed by NMR spectroscopy. The spatial structures were studied by X-ray diffraction.

Keywords: NMR spectroscopy, deuterated steroids, isotopic labelling, deuterium labelling, [2,2,4,6,8,16,16-$^2H_7$] arimistane, X-ray crystallography

DOI: 10.1134/S1070363217120520

INTRODUCTION

DHEA, an important endogenous steroid hormone that has a wide range of biological effects, predominantly adrenal, is one of the most common circulating steroids in adults [1]. Androsta-3,5-diene-7,17-dione (Arimistane), a metabolite of 7-KETO-DHEA, is a potent inhibitor of aromatase. The latter is an adrenal and adipose tissue enzyme catalyzing the conversion of androgens to estrogens, for example, testosterone to beta-estradiol, which increases the content of endogenous anabolic hormones and maintains a high level of endogenous testosterone [2]. Androsta-3,5-diene-7,17-dione is formed in the human body is the product of natural metabolism of DHEA prohormones. Therefore, the quantitative determination of biogenic Arimistane and its pharmacokinetic features are of high interest for biomedical studies. Since estrogen hormones increase the sensitivity of androgen receptors, high dosages of aromatase inhibitors can over-suppress estrogen levels and lead to health problems, including arthralgia development, loss of bone strength, changes in the lipid profile, loss of libido and other diseases [3, 4]. Currently, Arimistane is a part of dietary supplements and its content in such products is often not standardized, that is why it is important to develop reliable methods for its analysis.

To determine reliably the level of biogenic hormones, isotopically labeled standards that demonstrate the mass shift in GC/MS and LC/MS techniques are required [5, 6]. In the current study, we synthesized and elucidated the structure of deuterated Androsta-3,5-diene-7,17-dione as an internal standard for the quantitative determination of Arimistane in biological samples.

EXPERIMENTAL

7-Keto-DHEA acetate was purchased from Chem-Impex Int'l Inc. ^{1}H NMR spectra were measured on a Bruker AVANCE III 400 MHz NMR spectrometer. 2-D COSY, HMQC, and HMBC experiments were carried out to support the assignment. Mass spectra data were measured on a Thermo Scientific TSQ Quantum Access Max Mass spectrometer. Organic solvents used were dried according to the standard methods in cases it was required. Commercially available reagents were used without further purification. All reactions were monitored by TLC with EMD/Merck KGaA silica gel coated plates, the spots were visualized by UV light and by charring with 0.1% ninhydrin in EtOH. Column chromatography was

[1] The text was submitted by the authors in English.

Table 1. Crystal data and structure refinement for Arimistane and Arimistane-d_7

Parameter	Value	
	Arimistane	Arimistane-d_7
Empirical formula	$C_{19}H_{24}O_2$	$C_{19}H_{17}D_7O_2$
Molecular weight	284.38	291.44
Temperature, K	100(2)	100(2)
Crystal system	Orthorhombic	Orthorhombic
Space group	$P2_12_12_1$	$P2_12_12_1$
a, Å	8.3252(2)	8.3456(5)
b, Å	17.0398(4)	17.0404(10)
c , Å	32.4428(7)	32.546(3)
α = β = γ, deg	90	90
Volume, Å^3	4602.4(2)	4628.5(5)
Z	12	12
ρ_{calc}, g/cm^3	1.231	1.224
μ, mm^{-1}	0.608	0.077
F(000)	1848.0	1848.0
Crystal size, mm^3	0.25 × 0.14 × 0.12	0.21 × 0.15 × 0.12
Radiation	CuK_α (λ = 1.54184)	MoK_α (λ = 0.71073)
2θ range for data collection, deg	5.858 to 139.99	5.396 to 52.992
Index ranges	$-9 \le h \le 10$, $-12 \le k \le 20$, $-39 \le l \le 27$	$-9 \le h \le 10$, $-21 \le k \le 10$, $-40 \le l \le 31$
Reflections collected	13210	14316
Independent reflections	8237 [R_{int} = 0.0306, R_{sigma} = 0.0322]	8882 [R_{int} = 0.0471, R_{sigma} = 0.1065]
Data/restraints/parameters	8237/0/574	8882/0/574
Goodness-of-fit on F^2	1.051	1.093
Final *R* indexes [$I \ge 2\sigma(I)$]	R_1 = 0.0413, wR_2 = 0.1075	R_1 = 0.0769, wR_2 = 0.1283
Final *R* indexes [all data]	R_1 = 0.0440, wR_2 = 0.1101	R_1 = 0.1095, wR_2 = 0.1486
Largest difference peak/hole, e/Å^3	0.18/–0.29	0.27/–0.29
Flack parameter	0.05(13)	–

performed using Merck 60 Å 70–230 mesh silica gel. Colourless single crystals of Arimistane and Arimistane-d_7 were obtained for X-ray analysis by recrystallization from heptane.

Crystal structure determination and refinement. For the single crystal X-ray diffraction, two prismatic translucent crystals of Arimistane and its deuterated form were scanned on an Agilent Technologies SuperNova and Xcalibur EOS diffractometers at the temperature of 100K. The structures have been solved by the direct methods by means of the SHELX program [7] incorporated in the OLEX2 program package [8]. The carbon-bound H atoms were placed in calculated positions (including H^8, H^9, and H^{14}, localized from the difference Fourier maps) and were included in the refinement in the "riding" model approximation. Empirical absorption correction was

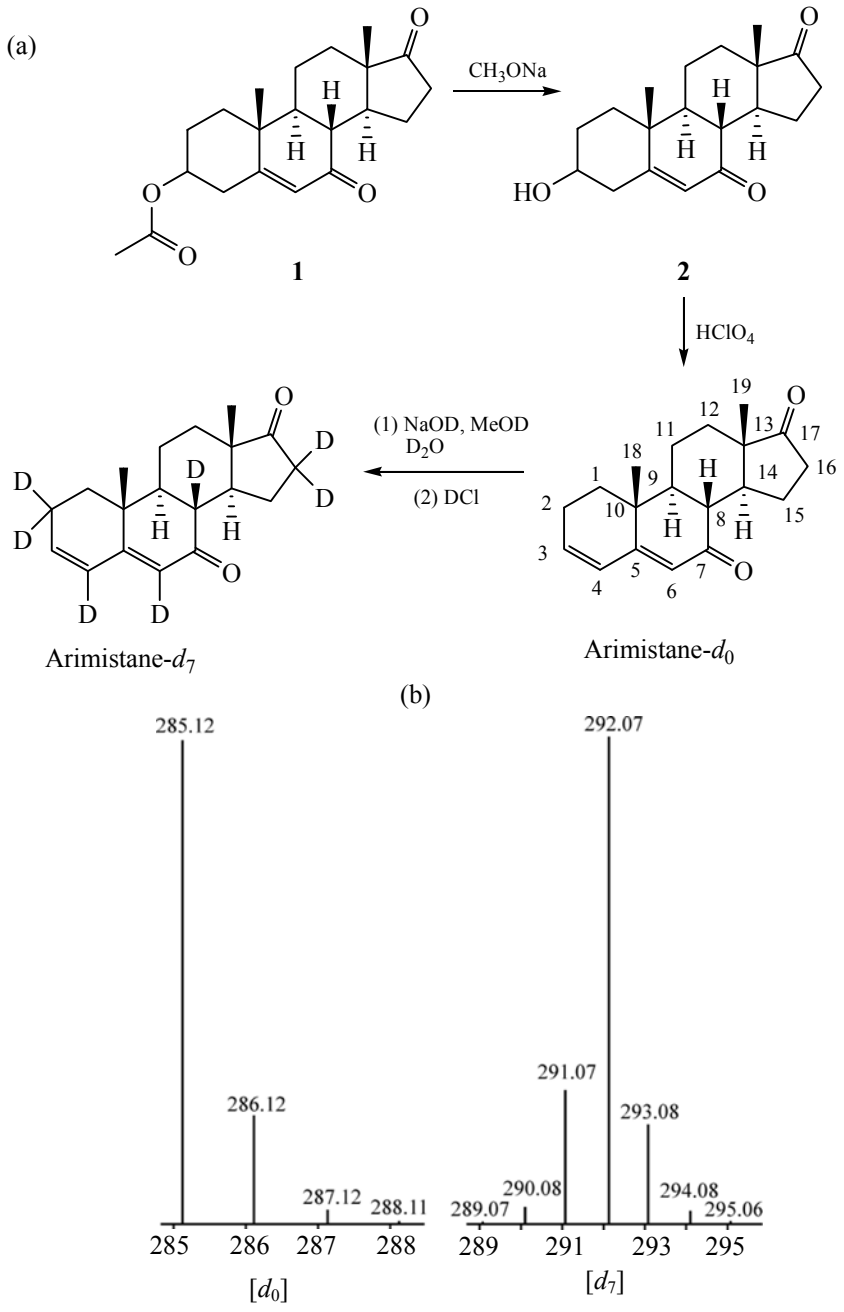

Fig. 1. (a) Synthesis of Arimistane-d_7 and (b) MS molecular ion cluster of Arimistane-d_0 and Arimistane-d_7.

applied in CrysAlisPro [9] program complex using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm. Experimental details and crystallographic parameters are listed in Table 1. The Bijvoet pair analysis from PLATON [10] was performed to determine the absolute structure.

Supplementary crystallographic data for this paper have been deposited at Cambridge Crystallographic Data Centre (CCDC 1536546 and 1547721) and can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif.

Synthetic procedures. 7-Keto-DHEA **2** was synthesized (Fig. 1) by hydrolysis of 7-keto-DHEA acetate **1** in aqueous methanol with quantitative yield and used without further purification [1]. ^{1}H NMR spectrum of the compound was identical to the earlier

Table 2. ^{1}H chemical shifts, δ, ppm (TMS)

Atom	δ, ppm	*J*, Hz	Protons	Atom	δ, ppm	*J*, Hz	Protons
1	1.97–1.85 m	–	CH_2	12	1.97–1.85 m	–	CH_2
	1.44–1.34 m	–					
2	2.43–2.27 m	–	CH_2	14	1.75–1.64 m	–	CH
3	6.29–6.20 m	–	CH	15	2.88–2.77 m	–	CH_2
4	6.14 d	9.9	CH		1.97–1.85 m	–	CH_2
6	5.67 s	-	CH	16	2.49 d.d	20.7, 7.6	CH_2
8	2.54 t	12.1	CH		2.20–2.09 d.t	19.5, 9.3	CH_2
9	1.75–1.64 m	-	CH	18	1.17 s	–	CH_3
11	1.84–1.77 m	-	CH_2	19	0.94 s	–	CH_3
	1.62–1.50 q.d	12.9, 4.1					

Table 3. ^{13}C chemical shifts, δ, ppm (TMS)

Atom	δ, ppm	Atom	δ, ppm	Atom	δ, ppm	Atom	δ, ppm	Atom	δ, ppm
1	32.78	5	161.71	9	49.78	13	48.28	17	>210
2	23.37	6	123.94	10	36.43	14	46.46	18	16.69
3	137.26	7	201.24	11	20.54	15	24.31	19	13.87
4	127.61	8	44.95	12	30.84	16	35.71		

data [11]. Carbon atoms in Arimistane are numbered (Fig. 1) according to the numbering common for aromatase steroids [12].

Arimistane was synthesized by treating compound **2** with perchloric acid in methanol [13] and isolated as colourless solid, yield 86%, mp 167°C (lit. 167–168°C).

Deuterated Arimistane was synthesized according to the earlier developed procedure providing deuterontestosterone [14]. Neat pieces of Na (0.13 g) were added to a 10-mL solution of deuterated methanol in small portions. When all of sodium had reacted, 10 mL of D_2O were added. The solution was allowed to cool down to room temperature, and 200 mg of unlabeled Arimistane (d_0) were added. The solution was stirred upon refluxing for 24 h. The solvent was removed *in vacuum*, and D_2O (10 mL) was added. The suspension was cooled down on ice and acidified by concentrated DCl. The suspension was extracted with EtOAc (3×15 mL), the organic phase was dried over $MgSO_4$ and concentrated to dryness (40°C). The crude solid was purified by column chromatography (EtOAc : Hex = 1 : 2, v/v).

Arimistane-d_7. Yield 50%, colourless solid, mp 167°C. LC–MS, ESI, *m/z* (%): 292.07 $[M + H]^+$ (100). Deuterium distribution (%): d_7 (78.50), d_6 (17.87), d_5 (3.22), and d_4 (0.41).

RESULTS AND DISCUSSION

NMR spectrometry. For determining locations of labels in the structure of deuterated Arimistane, it was necessary to solve the ^{1}H and ^{13}C NMR spectra of Androsta-3,5-diene-7,17-dione, which had not been done earlier.

All assignments of the ^{1}H and ^{13}C chemical shifts and coupling constants are presented in Tables 2 and 3. Signals at 6.29–6.20, 6.14, and 5.67 ppm were assigned to olefinic protons. The signal at 5.67 ppm (H^6) was not correlated with other protons in the COSY spectrum. The doublet at 6.14 ppm (H^4) was coupled to the only signal at 6.29–6.20 ppm. The lower field olefinic proton at 6.29–6.20 ppm (H^3) was assigned to H^4.

Carbon atoms of the types (C, CH, CH_2, CH_3) were determined using the DEPT experiments. Attached protons for each carbon were established by ^{1}H–^{13}C HMQC. H^3 Demonstrated COSY correlation to a proton at 2.43–2.27 ppm and $^{1}H^{13}C$ HMBC were

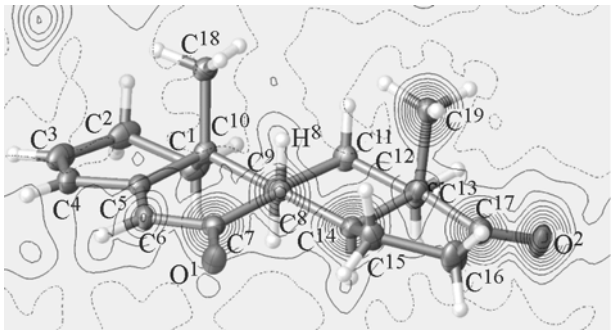

Fig. 2. Molecular structure of Arimistane combined with graph of observed electron density map around C^6, C^7, C^8, C^{14}, C^{19}, C^{13}, C^{17}, O^2 sites, contour intervals are 0.1 e/Å^3. Displacement ellipsoids are drawn at the 50% probability level.

attributed to carbon signals at 161.71, 32.78, and 23.37 ppm. The protons signals recorded at 1.97–1.85 and 1.44–1.34 ppm were attached to carbon atoms measured by signal at 32.78 ppm. The protons recorded at 2.43–2.27 ppm were attached to the carbon recorded at 23.37 ppm. Owing to these correlations, the signal at 2.43–2.27 ppm was assigned to the methylene protons H^2 and the proton signals at 1.97–1.85 and 1.44–1.34 ppm corresponded presumably to the methylene protons H^1. This assumption was confirmed by the correlation of the carbon at 32.78 ppm with H^3, with the methyl protons at 1.17 ppm and with the methine proton at 1.75–1.64 ppm, which in turn was possible only if the signals at 1.97–1.85 and 1.44–1.34 ppm were assigned to H^1, the signal at 1.17 ppm was assigned to H^{18} and the signal at 1.75–1.64 ppm was assigned to H^9. The signal at 0.94 ppm was assigned to H^{19}. The methine proton at 2.54 ppm was recorded as a triplet and assigned to H^8 owing to its coupling with H^9 and H^{14}, which was confirmed by a correlation on a COSY spectrum. The proton at 1.75–1.64 ppm was assigned to H^{14}, as well as earlier to H^9.

The protons at 1.97–1.85 and 1.29 ppm were attached to the carbon recorded at 30.84 ppm. This carbon demonstrated ^1H–^{13}C HMBC correlations to methyl protons at 0.94 ppm (H^{19}), methylene protons at 1.84–1.77 ppm and 1.62–1.50 ppm and the methine proton at 1.75–1.64 ppm. Owing to these correlations, the signals at 1.97–1.85 and 1.29 ppm were assigned to H^{12} and the signals at 1.84–1.77 and 1.62–1.50 ppm were assigned to H^{11}. The protons at 2.88–2.77 and 1.97–1.85 ppm were attached to the carbon recorded at 24.31 ppm. This methylene carbon was correlated with the methylene protons measured at 2.49 and 2.20–2.09 ppm and the methine proton at 1.75–1.64 ppm. The protons at 2.49 ppm and 2.20–2.09 ppm were attached to the carbon recorded at 35.71 ppm. This methylene carbon was correlated with the methylene protons at 2.88–2.77 and 1.97–1.85 ppm. Owing to these correlations, the signals at 2.88–2.77 and 1.97–1.85 ppm were assigned to H^{15} and the signals at 2.49 and 2.20–2.09 ppm were assigned to H^{16}.

There were no signals corresponding to 2, 4, 6, 8, 16 atoms in the ^1H NMR spectrum of deuterated Arimistane. Thus, we obtained 2,2,4,6,8,16,16-d_7-Arimistane. It was impossible to determine the orientation of the deuterium atom in the 8th position by NMR experiments. To solve this problem, we performed X-ray diffraction of Androsta-3,5-diene-7,17-dione and 2,2,4,6,8,16,16-d_7-Arimistane.

Crystal structure. Crystal structures were solved in the non-centrosymmetryc $P2_12_12_1$ space group with R_1 = 0.041 and 0.077, respectively. Determination of the absolute structure of Arimistane was confirmed by Bijvoet pair analysis, which gave 87% coverage for

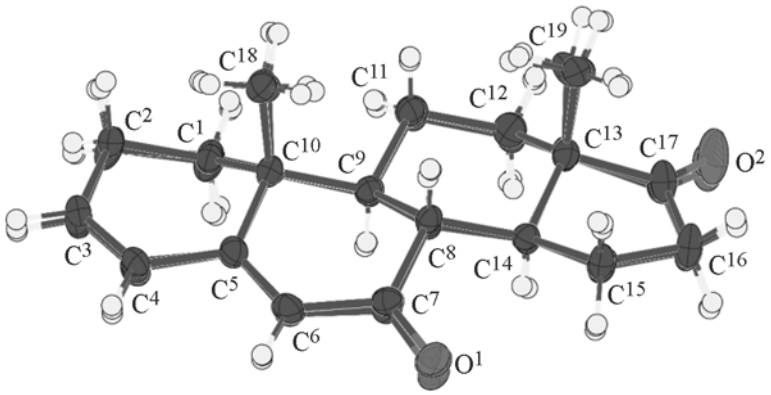

Fig. 3. Superposition of three independent Arimistane molecules. Displacement ellipsoids are drawn at the 50% probability level.

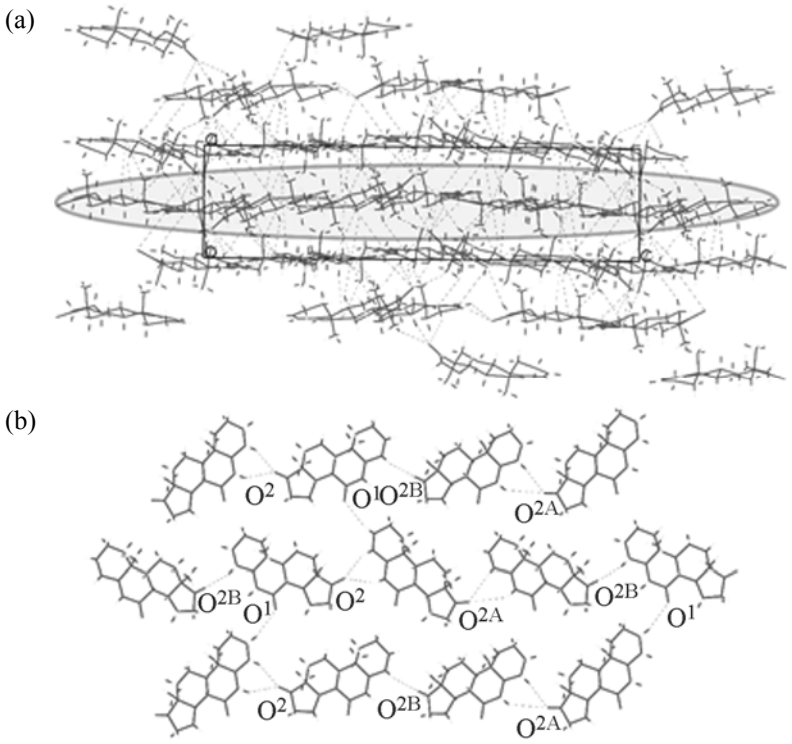

Fig. 4. Weak hydrogen bonding system between molecular sheets (marked by ellipse) in the crystal structure of (a) Arimistane projected on (010) plane and (b) hydrogen bonding system in the molecular sheet projected on (100) plane.

2987 Friedel pairs and Hooft parameter of 0.04(9) [15]. The asymmetric unit of both forms contained three independent molecules. Each molecule consisted of common for steroid-like compounds ring system (ABCD), shown (Fig. 2) with IUPAC-approved ring lettering and atoms numbering [16].

Mean bond lengths in ABCD cycles and adjacent methyl groups (C^{18} and C^{19}) were consistent with similar compounds [17]. Distances between O^1–C^7 and O^2–C^{17} ranged from 2.207 to 2.235 Å and corresponded to the double character of the carbon-oxygen bond. Absolute stereochemistry of the steroid was defined by the parent name for some chiral centers (hydrogen atoms at the bridgehead C^8, C^9, and C^{14}); according to the IUPAC recommendations, they could be defined as oriented 8β, 9α, 14α.

All independent molecules had the same configuration and slightly differed by location of C^{18}, C^{19} methyl groups and O^1, O^2 oxygen atoms due to their complexity in the hydrogen bonding system (Fig. 3),

Table 4. Selected interatomic distances, angles and distances between nearest anions for hydrogen bonds in the structure of Arimistane

Bond	D–H, Å	H–A, Å	D–A, Å	∠DHA, deg	Bond	D–H, Å	H–A, Å	D–A, Å	∠DHA, deg
C^{6A}–H^{6A}…$O^{2\,a}$	0.93	2.77	3.518(3)	138.4	C^{6B}–H^{6B}…$O^{2A\,e}$	0.93	3.41	4.118(3)	134.6
C^{6A}–H^{6A}…$O^{2A\,b}$	0.93	3.49	4.123(4)	127.6	C^{6}–H^{6}…$O^{2B\,d}$	0.93	3.45	4.030(3)	122.5
C^{8A}–H^{8A}…O^{1Ab}	0.98	2.90	3.879(3)	174.7	C^{16B}–H^{16E}…$O^{2B\,f}$	0.97	2.77	3.620(3)	147.0
C^{8B}–H^{8B}…$O^{1\,c}$	0.98	3.03	3.933(3)	153.6	C^{16A}–H^{16A}…$O^{2\,g}$	0.97	3.41	4.068(4)	126.8
C^{8}–H^{8}…$O^{1B\,d}$	0.98	3.18	4.155(3)	174.7	C^{16}–H^{16C}…$O^{2A\,h}$	0.97	3.60	4.204(4)	123.0

[a] 3/2 – *x*, 1 – *y*, 1/2 + *z*. [b] 1/2 + *x*, 1/2 – *y*, 1 – *z*. [c] –3/2 + *x*, 3/2 – *y*, 1 – *z*. [d] 1/2 + *x*, 3/2 – *y*, 1 – *z*. [e] 1/2 – *x*, 1 – *y*, 1/2 + *z*. [f] –1/2 + *x*, 3/2 – *y*, 1 – *z*. [g] 2 – *x*, –1/2 + *y*, 1/2 – *z*. [h] 1 – *x*, 1/2 + *y*, 1/2 – *z*.

which, according to Jeffrey's [18] classification, could be characterized as "weak, electrostatic."

However, the presence of weak hydrogen bonds (Table 4) resulted in the presence of three independent molecules in the crystal structures of Arimistane and Arimistane-d_7. In the crystal of Arimistane, the complex hydrogen bond system (Fig. 4a) was based on nearly planar sheets, parallel to the (100) plane (Fig. 4b). The shortest D–A distances of 3.518(3) and 3.620(3) Å corresponded to the weak hydrogen bonds C^{6A}–$H^{6A}\cdots O^{2}$ inside sheets and C^{16B}–$H^{16E}\cdots O^{2B}$ between sheets, accordingly. The crystal structure of Arimistane-d_7 had the same hydrogen bonding system, which gave grounds to assume the same absolute configuration.

CONCLUSIONS

We have synthesized deuterated Arimistane and completely proved its structure by NMR spectroscopy and X-ray diffraction.

REFERENCES

1. Kolatorova Sosvorova, L., Sarek, J., Vitku, J., and Kvasnica, M., *Steroids*, 2016, vol. 112, p. 88. doi 10.1016/j.steroids.2016.05.001
2. Stocco, C., *Steroids*, 2013, vol. 77, p. 27. doi 10.1016/j.steroids.2011.10.013
3. Goodman, A., *Oncology Times*, 2008, vol. 30, p. 401. doi 10.1097/01.COT.0000313055.56216.26
4. Garreau, J.R., Delamelena, T., Walts, D., KaramLou, K., and Johnson, N., *Am. J. Surg*., 2006, vol. 192, 2006, p. 496. doi https://doi.org/10.1016/j.amjsurg.2006.06.018
5. Atzrodt, J., Blankenstein, J., Brasseur, D., Calvo-Vicente, S., Denoux, M., Derdau, V., Lavisse, M., Perard, S., Roy, S., Sandvoss, M., Schofield, J., and Zimmermann, J., *Bioorg. Med. Chem*., 2012, vol. 20, p. 5658. doi https://doi.org/10.1016/j.bmc.2012.06.052
6. Massey, I.J. and Tökés, L., *J. Labelled Comp. Radiopharm*., 1981, vol. 18, p. 1535. doi 10.1002/jlcr.2580181018
7. Sheldrick, G.M., *Acta Crystallogr., Sect. A*, 2008, vol. 64, p. 112. doi 10.1107/S0108767307043930
8. Dolomanov, O.V., Bourhis, L.J., Gildea, R.J., Howard, J.A.K., and Puschmann, H., *J. Appl. Crystallogr*., 2009, vol. 42, p. 339. doi 10.1107/S0021889808042726
9. *CrysAlisPro*, Agilent Technologies, Version 1.171.36.20, Release 27.06.2012.
10. Spek, A.L., *Acta Crystallogr., Sect. D*, 2009, vol. 65, p. 148. doi 10.1107/S090744490804362X
11. Silvestre, S.M. and Salvador, J.A.R., *Tetrahedron*, 2007, vol. 63, p. 2439. doi 10.1016/j.tet.2007.01.012
12. Beger, R.D., Harris, S., and Xie, Q., *J. Chem. Inf. Comput. Sci*., 2004, vol. 44, p. 1489. doi 10.1021/ci049925e
13. Li, C., Qiu, W., Yang, Z., Luo, J., Yang, F., Liu, M., Xie, J., and Tang, J., *Steroids*, 2010, vol. 75, p. 859. doi 10.1016/j.steroids.2010.05.008
14. Krauser, J.A. and Guengerich, F.P., *J. Biol. Chem*., 2005, vol. 280, p. 19496. doi 10.1074/jbc.M501854200
15. Hooft, R.W.W., Straver, L.H., and Spek, A.L., *J. Appl. Crystallogr*., 2008, vol. 41, p. 963. doi 10.1107/S0021889807059870
16. Moss, G.P., *Pure Appl. Chem*., 1989, vol. 61, p. 1783. doi 10.1351/pac198961101783
17. Cichowicz, N.R., Kaplan, W., Khomutnyk, Y., Bhattarai, B., Sun, Z., and Nagorny, P., *J. Am. Chem. Soc*., 2015, vol. 137, p. 14341. doi 10.1021/jacs.5b08528
18. Jeffrey, G.A., *An Introduction to Hydrogen Bonding*, Oxford: Oxford University Press, 1997.