ISSN 1068-1620, Russian Journal of Bioorganic Chemistry, 2010, Vol. 36, No. 2, pp. 263–268. © Pleiades Publishing, Ltd., 2010.
Original Russian Text © V.P. Shevchenko, I.Yu. Nagaev, I.S. Levina, L.E. Kulikova, N.F. Myasoedov, A.V. Kamernitzky, 2010, published in Bioorganicheskaya Khimiya, 2010, Vol. 36, No. 2, pp. 283–288.

The Synthesis of *O*-Substituted 3-Oximes of 6α-Methyl-16α,17α-cyclohexanopregn-4-ene-3,20-diones Tritium-Labeled in the 1,2-Position

V. P. Shevchenko[a, 1], I. Yu. Nagaev[b], I. S. Levina[b], L. E. Kulikova[b], N. F. Myasoedov[a], and A. V. Kamernitzky[b, †]

[a] *Institute of Molecular Genetics, Russian Academy of Sciences, pl. Kurchatova 2, Moscow, 123182 Russia*
[b] *Zelinskii Institute of Organic Chemistry, Russian Academy of Sciences, Leninskii pr. 47, Moscow, 117913 Russia*

Received April 2, 2009; in final form, October 12, 2009

1,2-Tritium-labeled 3-(*O*-carboxypropyl)- and 3-(*O*-carbomethoxypropyl)-oximes of 6α-methyl-16α,17α-cyclohexanopregn-4-ene-3,20-diones were obtained by the homogeneous catalytic hydrogenation of 1,2-dehydroprecursors with gaseous tritium and the subsequent separation of the resulting mixtures by HPLC. The specific radioactivities of 50–55 Ci/mmol were prepared using tris-(triphenylphosphine)-rhodium chloride.

Key words: pentaranes, synthesis, steroids, oxime, tritium, HPLC

DOI: 10.1134/S1068162010020172

INTRODUCTION

Steroid hormones and their synthetic analogues interact not only with classical nuclear receptors, sometimes they also demonstrate a characteristic binding to other receptor or nonreceptor proteins with a known or potential biological function [1].[2] A specific protein had earlier been shown to occur in the cytosol of the rat uterus. It specifically binds progesterone derivatives containing relatively voluminous 16α,17α-cyclopentane and -cyclohexane substituents (pregna-D'-pentaranes) while demonstrating an exceptionally weak interaction with native progesterone [2, 4]. The isolation and identification of this protein (we named it "uterine pentaranophilin") is of interest to reveal its function in an organism. The use of affinity chromatography on sorbents with immobilized steroid ligands might be an optimal isolation method for the protein. An investigation of the series of our substituted 3- and 19-oximes of 16α,17α-cyclohexanopregnanes with PR and UT showed [5] that pentarane (3*Z*)-(3-methoxycarbonylpropoxyimino) derivatives display a high affinity for UT while demonstrating an extremely weak interaction with PR. The biggest difference between the affinities for the two proteins was observed for (3*Z*)-(3-methoxycarbonylpropoxyimino)-6α-methyl-16α,17α-cyclohexanopregn-4-en-20-one. This contains a sufficiently long chain with a terminal carboxyl group that can form an amide bond with the carrier and, in its acidic form, might turn out to be an efficient ligand for the preparation of an affinity sorbent suitable for UT isolation. The corresponding labeled analogues of pentaranes should be synthesized to carry out such a work.

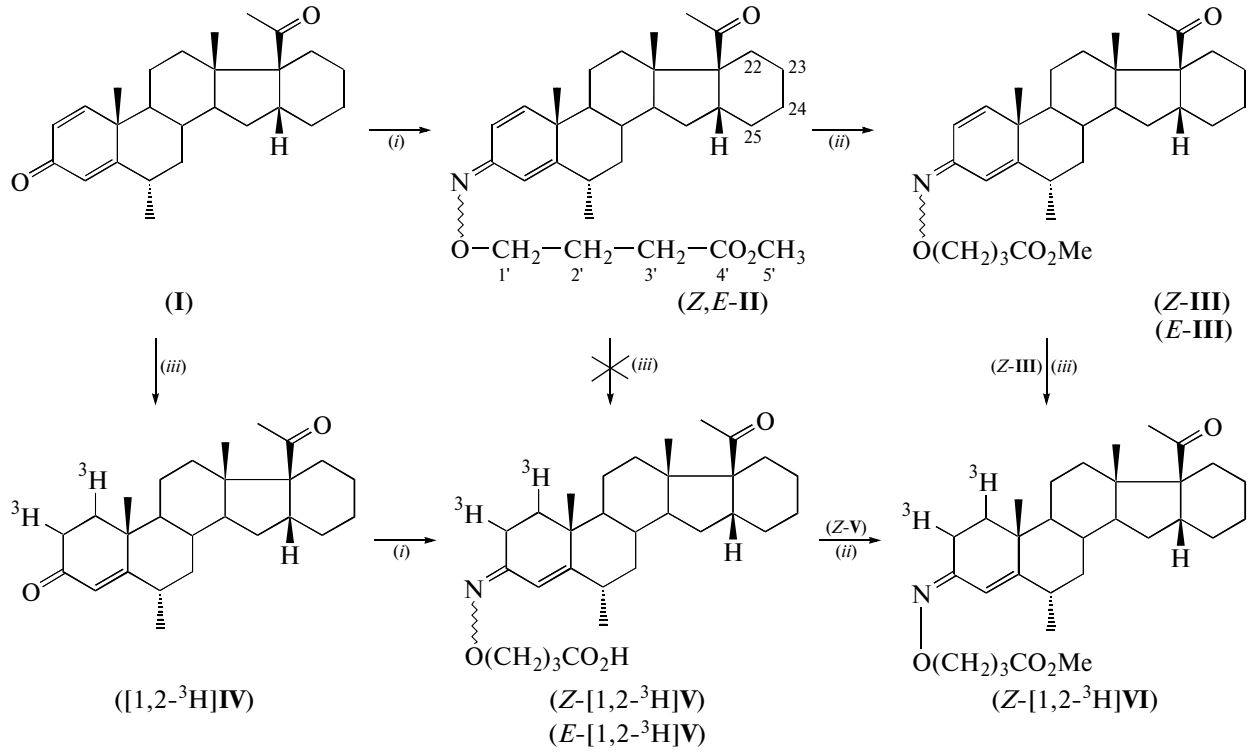
RESULTS AND DISCUSSION

In this work, we describe a method for the preparation of tritium-labeled 3-(*O*-carboxypropyl)- and 3-(*O*-methoxycarbonylpropyl)oximes of 6α-methyl-16α,17α-cyclohexanopregn-4-ene-3,20-dione. The (3*Z*) isomer of the last compound is necessary for studying the degree of completeness of the unlabeled analogue with the corresponding support during the preparation of the affinity sorbent.

The synthesis of these compounds is given in Scheme 1. The interaction of 6α-methyl-16α,17α-cyclohexanopregna-1,4-diene-3,20-dione (**I**) with *O*-(3-carboxypropyl)hydroxylamine hydrochloride led to a mixture of (3*Z*)- and (3*E*)-substituted oximes (**II**) and, further, to individual methyl esters (*Z*-**III**) and (*E*-**III**). The selective reduction of the 1,2 double bond in steroids (**I**), (*Z*,*E*-**II**), and (*Z*-**III**) was effected by gaseous tritium in the presence of tris(triphenylphosphine)rhodium chloride by the procedure in [6]. The rate of the selective hydrogenation of the 1,2 double bond in the imine derivatives of dienes (*Z*,*E*-**II**) and (*Z*-**III**) turned out to be substantially less than that of diene (**I**). The hydrogenation of oxime (*Z*-**III**) for 19 h resulted in a

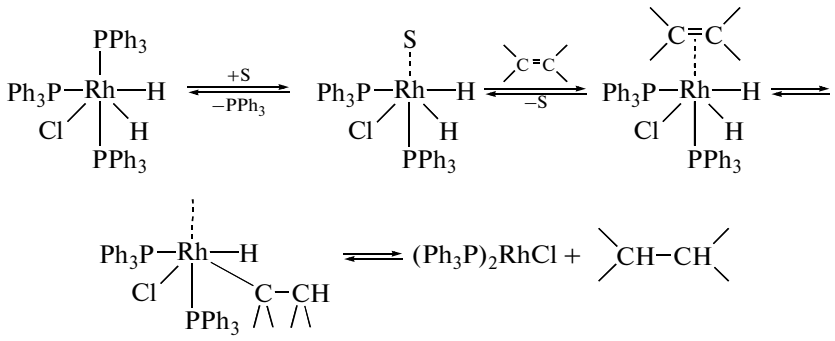
[1] Corresponding author; fax: +7 (499) 196-0221; e-mail: nagaev@img.ras.ru
[†] Deceased.
[2] Abbreviations: UT, uterine pentaranophilin; PR, progesterone receptor.

30–35% yield of (*Z*-[1,2-^{3}H]**VI**) with a molar radioactivity of 25 Ci/mmol, whereas the 2-h hydrogenation of pentarane (**I**) led to a 65% yield of ([1,2-^{3}H]**IV**) with a 50- to 55-Ci/mmol molar radioactivity. An extremely low yield was observed during the hydrogenation of the 1,2 double bond in the steroid oxime (**II**) with the terminal carboxyl group. This seems to be connected with the fact that in contrast to methyl ester of oxime (*Z*-**III**), the free acid (*Z*-(**II**) forms a stable complex with the homogeneous catalyst that inhibits the transition into the activated state (Scheme 2) [7].



Reaction conditions: *i* – $HO_2C(CH_2)_3ONH_2 \cdot HCl$ – Py, 60°C, 5 h; *ii* – CH_2N_2, MeOH, Et_2O; *iii* – $^3H_{(gaseous)}$, $(Ph_3P)_3RhCl$, dioxane (or benzene).

Scheme 1.



where S is a solvent molecule.

Scheme 2.

Therefore, the use of a homogeneous catalyst for the obtainment of the target steroid ([1,2-^{3}H]**V**) from (**II**) was unpractical. All of the efforts to carry out the selective hydrogenation using heterogeneous catalysts (5% Pd/C, dioxane, 30 min; 5% Pd/$BaSO_4$ dioxane, 30 min; Lindlar catalyst, ethyl acetate, 30 min; Lindlar catalyst, dioxane, 30 min; and Lindlar catalyst, dioxane, 90 min) were also unsuccessful because a lot

of side labeled compounds were formed. Hence, the synthesis of isomers of the labeled steroid (**V**) was achieved by the selective hydrogenation of 1,4-bisdehydrosteroid (**I**) to ([1,2-^{3}H]**IV**) by a modified procedure [6] with the subsequent interaction of the resulting labeled steroid with *O*-(3-carboxypropyl)hydroxylamine. After chromatographic purification and separation, the individual isomers (*Z*-[1,2-^{3}H]**V**) and (*E*-[1,2-^{3}H]**V**) were finally isolated in 43 and 36% yields, respectively. The molar radioactivities of both labeled preparations were equal (50 Ci/mmol). The treatment of acid (*Z*-[1,2-^{3}H]**V**) with diazomethane yielded the individually labeled 3-*O*-[3-(methoxycarbonyl)propyl]oxime (*Z*-**VI**).

EXPERIMENTAL

One and two-dimensional NMR spectra (δ, ppm) were registered in $CDCl_3$ at 30°C on a Bruker DRX-500 spectrometer (Germany) with working frequencies of 500.13 and 125.76 MHz for protons and ^{13}C nuclei, respectively. The signals of residual $CHCl_3$ (7.27 ppm) for the ^{1}H NMR spectra and $CDCl_3$ (77 ppm) for the ^{13}C NMR spectra were used as references. When measuring two-dimensional spectra, the standard Bruker programs were applied. The spectra were decoded using the COSY, HSQC, and HMBC procedures. The configuration of oximes was determined by the use of 2D-NOESY spectroscopy.

Tris(triphenylphosphine)rhodium chloride (Fluka) was used as a catalyst. Radioactivity was measured on an LKB1215 scintillation counter with the efficiency of tritium registration near 30% in a dioxane scintillator. A MultiChrom 1.5 system (ZAO Ampersend, Russia) was used for the collection and processing of chromatographic data. Plates coated with Sorbfil-UV silica gel (Russia) were used for TLC. The optimal conditions for carrying out the reactions were found by the use of a 1% tritium–protium mixture according to the procedures in [8–12]. The stability and distribution of the label were tested by the method in [8].

Labeled preparations were analyzed and purified by HPLC on a Reprosil pur C18aq, 5 μm, 4 × 150-mm column at a flow rate of 1 ml/min in a system of methanol–5 mM H_3PO_4 9 : 1. Retention times (min): 6.08 for (**IV**), 6.75 for (*Z*-**II**), 7.53 for (*Z*-**V**), 9.83 for (*Z*-**III**), 10.86 for (*Z*-**VI**), and 12.21 min for (*E*-**VI**). The preparative purification was carried out on a Kromasil 100C18, 7 μm, 4.6 × 150-mm column, flow rate 1 ml/min; systems: (A) 1 : 1 methanol–10 mM H_3PO_4, (B) methanol, 50% B for 5 min, then a linear gradient from 50 to 80% B for 10 min; retention time (min): 8.41 (*Z*-**III**), 9.52 (*Z*-**VI**), 5.49 (*Z*-**II**), and 6.19 (*Z*-**V**).

3*E*- and 3*Z*-(3-Methoxycarbonylpropoxyimino)-6α-methyl-16α,17α-cyclohexanopregna-1,4-diene-20-ones (*E*-III) and *Z*-III). A mixture of *O*-(3-carboxypropyl)hydroxylamine hydrochloride [13] (304 mg, 1.95 mmol) and 1,4-didehydrosteroid (**I**) [12] (415 mg, 1.09 mmol) in pyridine (5 ml) was stirred at 60°C for 6 h. The solvent was removed in a vacuum, and the residue was coevaporated with toluene (3 × 30 ml). Ethyl acetate and water were added to the resulting residue, the water phase was extracted with ethyl acetate, and the combined organic layers were washed with 5% HCl and water and dried over Na_2SO_4. A mixture of isomeric 3-*O*-(3-carboxypropyl)oximes (**II**) was dissolved in methanol (5 ml) and kept for 0.5 h at 20°C with 10 ml of an ethereal solution of diazomethane (from 460 mg of nitrosomethylurea and 0.92 ml of 45% KOH). The solvents were evaporated in a vacuum, and the residue was chromatographed on a column filled with Kieselgel 60 (0.063–0.100 mm) (Merck). Elution with a petroleum ether–acetone mixture of 99 : 1 to 96 : 4 gave individual 3-*O*-[3-(methoxycarbonyl)propyl]oximes: (*E*-**III**), mp 73–75°C (hexane) (229 mg, 42%) and (*Z*-**III**), mp 101–103°C (215 mg, 40%). The ^{1}H- and ^{13}C NMR spectra of these compounds are given in the table.

3*Z*-(3-Carboxypropoxyimino)-6α-methyl-16α,17α-cyclohexanopregna-1,4-dien-20-one (*Z*-II). Ester (*Z*-**III**) (70 mg, 0.14 mmol) and NaOH (110 mg, 2.75 mmol) in methanol (6 ml) were kept for 20 h at room temperature, and ether (20 ml) and 10% HCl were added. The organic layer was washed with water, dried over Na_2SO_4, and the solvent was removed in a vacuum. The residue was chromatographed on a column filled with Kieselgel 60. Elution with a 78 : 20 : 2 petroleum ether–acetone–methanol system led to the isolation of (*Z*-**II**), yield 56 mg, mp 223–225°C (ether–hexane).

3*Z*-(3-Methoxycarbonylpropoxyimino)-6α-methyl-16α,17α-cyclohexano-[1,2-^{3}H]pregn-4-en-20-one (*Z*-[1,2-^{3}H]VI). (*a*) A solution of 1,4-bisdehydroprecursor (*Z*-**III**) (2.2 mg, 0.0044 mmol) and tris(triphenylphosphine)rhodium chloride (2.2 mg, 0.0024 mmol) in benzene (0.3 ml) was placed in an ampoule, frozen in liquid nitrogen, evacuated, filled with a protium–tritium (1 : 1 ratio) mixture up to a pressure of 400 hPa, and kept under stirring for 19 h at room temperature. The ampoule content was frozen again in liquid nitrogen and evacuated. The labile tritium was removed by the three-time evaporation of the reaction mixture with methanol (5 : 1). Analysis was carried out by HPLC (Fig. 1).

To remove the soluble catalyst, the reaction mixture was applied to a Sorbfil-UV plate, which was developed with a 7 : 3 hexane–acetone system. The zone containing the target labeled substance was cut out, and the reaction products were eluted with methanol (5 × 10 ml). The eluates were filtered and evaporated. The analysis and the preparative purification of the preparation were carried out by HPLC. The radiochemical purity of (*Z*-[1,2-^{3}H]**VI**) was 95–97% after the chromatography; yield 30–35%; molar radioactivity, 25 Ci/mmol. The labeled steroid (**VI**) was stored as a solution in methanol at 10–15°C.

Chemical shifts (δ, ppm) in the ^{1}H- and ^{13}C NMR spectra of (3*Z*)- and (3*E*)-(3-methoxycarbonylpropoxyimino)-6α-methyl-16α,17α-cyclohexanopregna-1,4-dien-20-ones (*Z*-**III**) and (*E*-**III**)

(*Z*-**III**)						(*E*-**III**)					
Atom number	^{1}H	^{13}C	Atom number	^{1}H	^{13}C	Atom number	^{1}H	^{13}C	Atom number	^{1}H	^{13}C
1	6.20	142.10	16	2.95	33.87	1	6.34	146.48	16	2.96	33.87
2	6.13	120.83	17	–	64.10	2	6.77	113.77	17	–	64.11
3	–	149.40	18	0.73	15.94	3	–	149.81	18	0.73	15.93
4	6.57	106.59	19	1.15	20.27	4	5.93	113.25	19	1.15	19.94
5	–	161.66	20	–	212.40	5	–	156.92	20	–	212.36
6	2.52 1.13 (CH_3)	33.68 18.23	21	2.12	27.90	6	2.45 1.09 (CH_3)	33.04 18.16	21	2.12	27.89
7	1.85 0.76	42.41	22	1.96 1.50	26.98	7	1.81 0.71	42.32	22	1.95 1.48	26.97
8	1.67	35.53	23	1.57 0.86	22.23	8	1.65	35.54	23	1.58 0.86	22.22
9	1.04	53.12	24	1.42 1.13	20.97	9	1.04	52.85	24	1.43 1.13	20.95
10	–	43.13	25	1.54 1.46	27.47	10	–	43.08	25	1.54 1.45	27.46
11	1.75 1.58	21.73	1'	4.12	72.76	11	1.73 1.57	21.76	1'	4.11	72.70
12	1.66 1.58	31.87	2'	2.03	24.65	12	1.66 1.57	31.86	2'	2.02	24.62
13	–	46.94	3'	2.45	30.76	13	–	46.91	3'	2.43	30.77
14	1.63	49.47	4'		173.82	14	1.62	49.50	4'	–	173.79
15	1.53 1.32	29.97	5'	3.67	51.53	15	1.52 1.33	30.00	5'	3.67	51.50

(*b*) The *Z*-isomer of ([1,2-^{3}H]**V**) was dissolved in methanol (0.5 ml) and kept for 0.5 h at 20°C with 1 ml of an ethereal solution of diazomethane. The methyl ester (*Z*-[1,2-^{3}H]**VI**) was isolated by HPLC; its molar radioactivity was 50 Ci/mmol.

6α-Methyl-16α,17α-cyclohexano-[1,2-^{3}H]pregn-4-ene-3,20-dione ([1,2-^{3}H]IV). A solution of 1,4-bisdehydrosteroid (**I**) [12] (4 mg, 0.008 mmol) and tris(triphenylphosphine)rhodium chloride in dioxane (0.2 ml) was placed in an ampoule, frozen in liquid nitrogen, evacuated, filled with 100% gaseous tritium up to a pressure of 400 hPa, and kept under stirring for 120 min at room temperature. The ampoule content was again frozen in liquid nitrogen and evacuated. The labile tritium was removed by the three-time coevaporation of the reaction mixture with a 5 : 1 ethyl acetate–methanol mixture. The soluble catalyst was removed by application on a Sorbfil-UV plate and three-times eluted with a 1 : 1 hexane–ether system. The zone containing the target labeled substance ([1,2-^{3}H]**IV**) was cut out, and the reaction products were eluted with ethyl acetate (5 × 10 ml), filtered, and evaporated. The analysis and preparative purification of the preparation were achieved by HPLC. The radiochemical purity achieved 95–97% after the chromatography; the yield was 65%, and molar radioactivity, 50–55 Ci/mmol. The labeled steroid ([1,2-^{3}H]**IV**) was stored as a solution in a 2 : 1 ethyl acetate–methanol mixture at 10–15°C.

3*Z*- and 3*E*-(3-Carboxypropoxyimino)-6α-methyl-16α,17α-cyclohexano-[1,2-^{3}H]pregn-4-en-20-ones (*Z*-[1,2-^{3}H]V) and (*E*-[1,2-^{3}H]V). A methanolic solution of ([1,2-^{3}H]**IV**) (50 μg, 0.132 μmol, 7 μCi) was placed in an ampoule, evaporated, and *O*-(3-carboxypropyl)hydroxylamine hydrochloride (0.4 mg, 2.57 μmol) and dry pyridine (0.1 ml) were added. The ampoule was filled with argon, sealed, and stirred on a rotary evaporator for 3 h at a bath temperature of 60°C. After the end of the reaction, the ampoule was opened and the pyridine was evaporated. The residue was purified by preparative HPLC and (*Z*-[1,2-^{3}H]**V**) (43%)

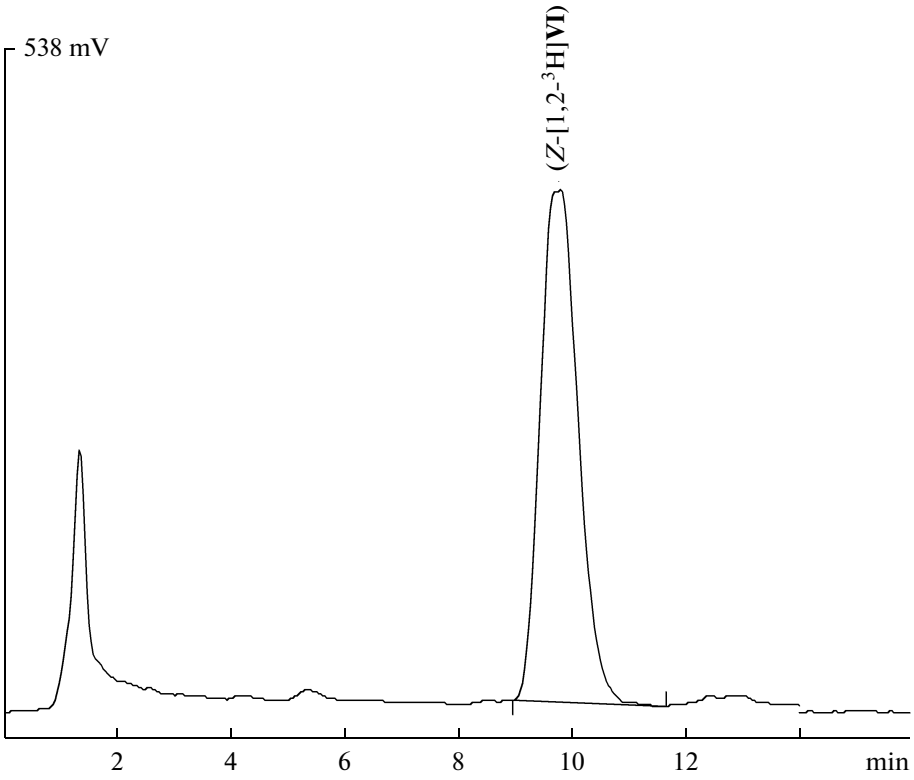


Fig. 1. Radioactivity profile in the chromatogram of the reaction mixture obtained upon the steroid (**III**) hydrogenation by gaseous tritium.

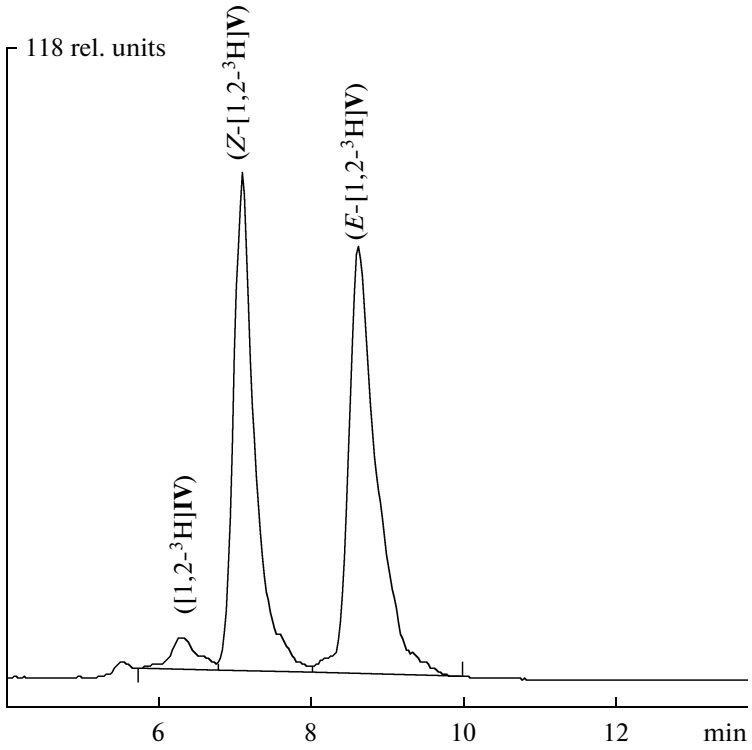


Fig. 2. Radioactivity profile in the chromatogram of the reaction mixture obtained upon the condensation of ([1,2-^{3}H]**IV**) with *O*-(3-carboxypropyl)hydroxylamine hydrochloride.

and (*E*-[1,2-^{3}H]**V**) (36%) were obtained (Fig. 2); the molar radioactivities of each isomer were 50 Ci/mmol.

ACKNOWLEDGMENTS

This work was partially supported by the Program of Basic Studies of the Presidium of the Russian Academy of Sciences of Molecular and Cellular Biology and the Scientific Schools grant SC-2150.2003.4.

REFERENCES

1. Smirnov, A.N., (Element of Endocrine Regulation), Moscow: GEODAR-Media, 2006 [in Russian].
2. Smirnov, A.N., Pokrovskaya, E.V., Shevchenko, V.P., Levina, I.S., and Kamernitskii, A.V., *Bioorg. Khim.*, 1999, vol. 25, pp. 774–781.
3. Smirnov, A.N., Pokrovskaya, E.V., Kogteva, G.S., Shevchenko, V.P., Levina, I.S., Kulikova, L.E., and Kamernitzky, A.V., *Steroids*, 2000, vol. 65, pp. 163–170.
4. Smirnov, A.N., Pokrovskaya, E.V., Shevshenko, V.P., Nagaev, I.Yu., Myasoedov, N.F., Levina, I.S., Kulikova, L.E., and Kamernitzky, A.V., *Bioorg. Khim.*, 2002, vol. 28, pp. 251-257 [*Russ. J. Bioorg. Chem.* (Engl. Transl.), 2002, vol. 28, pp. 224–230].
5. Levina, I.S., Pokrovskaya, E.V., Kulikova, L.E., Kamernitzky, A.V., Kachala, V.V., and Smirnov, A.N., *Steroids*, 2008, vol. 73, pp. 815–827.
6. Shevchenko, V.P., Nagaev, I.Yu., Potapova, A.V., Myasoedov, N.F., Kamernitsky, A.V., Levina, I.S., Kulikova, L.E., and Smirnov, A.N., *J. Label. Comp. Radiopharm*, 1998, vol. 41, pp. 919–925.
7. Makkvillin, F. J., *Gomogennoe gidrirovanie v organicheskoi khimii* (Homogeneous Hydration in Organic Chemistry), Moscow: Khimiya, 1980.
8. Shevchenko, V.P., Nagaev, I.Yu., and Myasoedov, N.F., *Mechennye tritiem lipofil'nye soedineniya* (Tritium-Labeled Lipophylic Compounds), Moscow: Nauka, 2003.
9. Shevchenko, V.P., Nagaev, I.Yu., Bezuglov, V.V., and Myasoedov, N.F., *Radiokhimiya*, 1989, vol. 31, pp. 121–125.
10. Shevchenko, V.P., Nagaev, I.Yu., Myasoedov, N.F., Kamernitskii, A.V., Levina, I.S., and Kulikova, L.E., *Radiokhimiya*, 2000, vol. 42, pp. 176–179.
11. Shevchenko, V.P., Nagaev, I.Yu., and Myasoedov, N.F., *Usp. Khim.*, 1999, vol. 68, pp. 944–966.
12. Kamernitskii, A.V., Levina, I.S., Kulikova, L.E., Galakhova, T.N., Shevchenko, V.P., Nagaev, I.Yu., Myasoedov, N.F., Smirnov, A.N., Pokrovskaya, E.V., and Shchelkunova, T.A., *Izv. Ros. Akad. Nauk, Ser. Khim.*, 1997, no. 8, pp. 1532–1535.
13. Khomutov, R.M., Karpeiskii, M.Ya., and Severin, E.S., *Izv. AN SSSR. Ser. Khim.*, 1962, no. 5, pp. 1074–1076.