REGIOSELECTIVE TRITIATION OF CARBAMATE DICYCLIC JUVENOIDS

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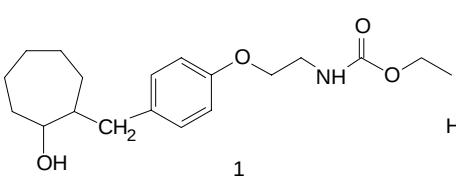
The tritiated juvenoids ethyl *N*-{2-[4-(*cis*-2-hydroxy-1-cycloheptylmethyl)phenoxy]ethyl}carbamate (**1**), 2-[4-(*trans*-2-hydroxy-1-cyclohexylmethyl)phenoxy]ethyl *N*-ethylcarbamate (**2**) and ethyl *N*-{2-[4-(*c*-2-hydroxy-3-methyl-*r*-1-cyclohexylmethyl)phenoxy]ethyl}carbamate (**3**) were prepared by Catalyzed Exchange in Solution with Gas on $PdO/BaSO_4$. The high degree of regio- and stereoselectivity observed in the products by ^3H NMR is discussed in the terms of the stereoelectronic requirements of the exchange reaction.

Key words: CESG; ^3H NMR; Tritiated juvenoids; Stereoselectivity.

The tritiated juvenoids **1–3** were required for metabolic studies of these potential pesticides on selected insects. Recently we have reported the regio- and stereoselective tritiation of the juvenoid ethyl *N*-{2-{4-[2,2-(ethylenedioxy)-1-cyclohexylmethyl]phenoxy}ethyl}carbamate (**4**) in the benzyl position[1] by tritium gas using Catalyzed Exchange in Solution with Gas[2] (CESG). As all here presented juvenoids have the benzyl moiety in the molecule, the CESG was a method of choice.



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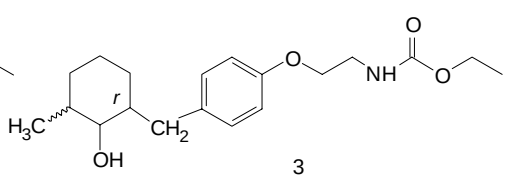

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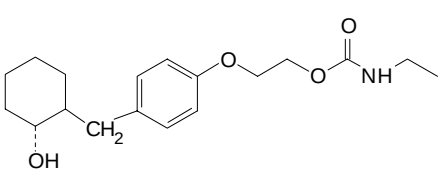

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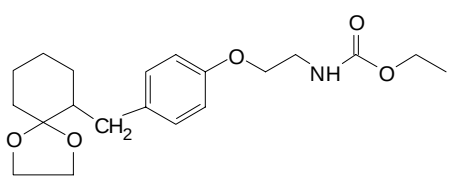

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EXPERIMENTAL

For tritium gas transfers an all stainless-steel pump was used[3]. The "cold" compounds **1–3** were prepared according to ref.[4]. Ethyl acetate was dried over K_2CO_3 and freshly distilled.

Radio-TLC was performed on the Merck silica gel 60 UV_{254} 0.25 mm plates; preparative TLC was performed on the 20 × 20 cm glass supported plates, thickness 2 mm, of the same brand. Radioactivity distribution on the plates was analysed on the Berthold LB 2821 Linear Analyzer combined with PC station equipped with CHROMA software.

Analytical radio-HPLC was performed on Merck–Hitachi apparatus (pump L-6200A, Photo Diode Array Detector L-3000) coupled with the Berthold LB 506 C-1 radioactivity detector (PC station, Berthold HPLC software, liquid scintillation cell 200 μl, scintillation cocktail LUMA Flow II). Preparative HPLC was performed on apparatus formed by two Waters high pressure pumps, Waters Gradient Controller 680, Merck–Hitachi L-400 UV detector, BD 41 Kipp&Zonen flat bed recorder and Pharmacia FRAC-100 fraction collector. Activity of the samples was assayed on liquid scintillation counter Intertechnique SL 3000 in LUMA GEL.

^{3}H NMR spectra were recorded at 266.8 MHz on Bruker WM 250 spectrometer equipped with ^{1}H/^{3}H dual probe, samples were dissolved in $CDCl_3$. The broad band ^{1}H decoupling was used. Chemical shifts are given in ppm (δ-scale), coupling constants (*J*) are given in Hz. The resonance frequency corresponding to 0 ppm was calculated by multiplying the known ^{1}H frequency for the TMS by the γ^3H to γ^1H ratio.

UV spectra were measured in methanol solution, λ_{max} were given in nm and ε in $m^2\ mol^{-1}$.

Mass spectra were recorded on Nermag R3010 triple–quadrupole apparatus coupled with PDP-11. For FAB the glycerol–thioglycerol (20%) matrix was used; DCI (Desorption by Chemical Ionisation) conditions were following: 50–550 mA, 10 mA sec^{-1} in the presence of ammonium gas, source pressure 133 Pa.

Solvent evaporations were done on rotary evaporator under reduced pressure (133–400 Pa) and bath temperature 30–40 °C or Speedvac apparatus (fractions after preparative HPLC).

Labelling of Ethyl *N*-{2-[4-(*cis*-2-Hydroxy-1-cycloheptylmethyl)phenoxy]ethyl}carbamate (**1**)

The compound **1** (5.83 mg, 17.4 μmol) was dissolved in anhydrous ethyl acetate (0.5 ml) and 10% $PdO/BaSO_4$ catalyst (16.75 mg) was added. The reaction flask was connected to the tritium gas handling apparatus, the solution was degassed and the tritium gas (approximately 1.11 TBq (30 Ci)) was introduced into the flask under pressure of 0.15 MPa. The reaction mixture was then magnetically stirred for 1.5 h at room temperature. The tritium gas was then pumped off, the catalyst was filtered off using Millipore 0.2 μ PTFE filter and washed three times with methanol (2 ml). The collected filtrates were evaporated to dryness and the residue was evaporated three times with methanol (5 ml) to eliminate the labile activity. The residue after the last evaporation was dissolved in toluene (6 ml). The crude mixture (1 360 MBq (367 mCi)) contained, according to radio-HPLC (column VYDAC 218TP 5 μ, 4.6 × 250 mm, gradient of water (A)–methanol (B) mixture: 1 min 5% B, 5 to 50% (B) in 4 min, 50% (B) for 30 min, flow rate 1 ml min^{-1} H), 37% of **1**. The mixture was purified on the preparative TLC plate, development with ether–hexane (4 : 1) mixture (R_F 0.24), 4.2 GBq (112 mCi) of **1** with radiochemical purity (radio-HPLC) 87% was obtained. The tritium labelled **1** was eventually purified on VYDAC 218TP 5μ, 10 × 250 mm column at flow rate 3.5 ml min^{-1} in three portions. The fractions containing labelled **1** were evaporated to dryness and then with three portions of toluene (10 ml) to remove completely the water. The residue after the last evaporation was dissolved in toluene (10 ml) and stocked. Thus the 2 312 MBq (62.5 mCi) of **1** were obtained, radiochemical purity >99%, molar activity 0.63 TBq $mmol^{-1}$ (17 Ci $mmol^{-1}$). UV spectrum (λ, nm (ε)): 202 (11 100), 223 (11 100), 276 (1 530), 283 (1 270). ^{3}H NMR spectrum: 2.44 d, *J* = 15 and 2.64 d,

J = 15 (benzylic tritons of the ditritiated compound); 2.45 s and 2.67 s (benzylic tritons of the monotritiated compounds). Mass spectrum (FAB), *m/z* (%): 336 (M + H, 53), 338 (M + H, 35), 340 (M + H, 12).

Labelling of 2-[4-(*trans*-2-Hydroxy-1-cyclohexylmethyl)phenoxy]ethyl *N*-Ethylcarbamate (**2**)

Tritiation of **2** (6.4 mg, 19.4 μmol) catalyzed by 10% $PdO/BaSO_4$ (14.6 mg) in anhydrous ethyl acetate (0.5 ml) was performed analogously to **1**. The pressure of tritium gas during exchange was 0.085 MPa. The crude mixture (22.2 GBq (599 mCi)) contained 69% of labelled **2** (column LiChrosphere 100 RP 18 E 5μ, 4 × 250 mm, gradient of water (A)–acetonitrile–water (90%) (B) mixture: 50–70% (B) in 15 min, 70–100% (B) in 5 min, flow rate 1 ml min^{-1}, $k' = 2.5$). About 2 200 MBq were purified in six portions on VYDAC 218TP 5μ, 10 × 250 mm column using isocratic elution with 50% (B) mobile phase at flow rate 3.5 ml min^{-1}. The chromatographic fractions were processed analogously to **1**. The yield was 1.0 GBq (27.3 mCi) of [benzyl-^{3}H] **2** with radiochemical purity >99% and molar activity 0.48 TBq $mmol^{-1}$ (13.2 Ci $mmol^{-1}$). UV spectrum (λ, nm (ε)): 201 (5 660), 222 (5 810), 276 (810), 282 (670). ^{3}H NMR spectrum: 2.25 d, *J* = 14.5 and 2.99 d, *J* = 14.5 (benzylic tritons of ditritiated compound); 2.28 s and 3.02 s (benzylic tritons of monotritiated compounds). Mass spectrum (DCI), *m/z* (%): 322 (M + H, 61), 324 (M + H, 33), 326 (M + H, 6), 339 (M + NH_4, 60), 341 (M + NH_4, 34), 343 (M + NH_4, 6).

Labelling of Ethyl *N*-{2-[4-(*c*-2-Hydroxy-3-methyl-*r*-1-cyclohexylmethyl)phenoxy]ethyl}carbamate (**3**)

Tritiation of **3** (4.9 mg, 13.5 μmol) catalyzed by 10% $PdO/BaSO_4$ (15.5 mg) in anhydrous ethyl acetate (0.5 ml) was performed analogously to **1**. The pressure of tritium gas during exchange was 0.165 MPa. The crude mixture (14.2 GBq (383 mCi)) contained 45% of labelled **3** (column LiChrosphere 100 RP 18 E 5μ, 4 × 250 mm, gradient of water (A)–acetonitrile–water (90%) (B) mixture: 50–70% (B) in 15 min, 70–100% (B) in 5 min, flow rate 0.7 ml min^{-1}, $k' = 4.7$). The crude mixture was purified in five portions on LiChrosphere 100 RP18 E 10μ, 10 × 250 mm column using gradient elution 50–60% (B) in 20 min, flow rate 4 ml min^{-1}. 1.9 GBq (51.5 mCi) of [benzyl-^{3}H] **3** with radiochemical purity >98% and molar activity 0.58 TBq $mmol^{-1}$ (15.7 Ci $mmol^{-1}$) were obtained. UV spectrum (λ, nm (ε)): 201 (10 700), 222 (10 600), 276 (1 480), 282 (1 220). ^{3}H NMR spectrum: 2.24 d, *J* = 15 and 3.00 d, *J* = 15 (benzylic tritons of ditritiated compound); 2.27 s and 3.03 s (benzylic tritons of monotritiated compounds). Mass spectrum (FAB), *m/z* (%): 336 (M + H, 50), 338 (M + H, 46), 340 (M + H, 4).

RESULTS AND DISCUSSION

In the previously published paper[1] we have formulated the stereoelectronic requirements for the exchange of benzylic hydrogen for tritium on palladium catalyst – the benzene ring coplanar with the surface of the catalyst and the C–H bond lying in the plane bisecting the benzene ring and perpendicular to the catalyst surface. Due to the presence of the spiro-1,3-dioxolane ring vicinal to the benzylic methylene in **4** there is a high – 92% – stereoselectivity of the exchange of the less hindered hydrogen and no double labelling of this methylene was observed. For the compounds described in this paper the common feature is the free hydroxyl group in the vicinity of the benzylic methylene, which is obviously less space demanding than the spiro-1,3-dioxolane ring. This fact is reflected by a decreased selectivity (64 to 77%) and an increased double labelling (15 to 26%), see Table I.

To support by us formulated stereoelectronic requirements we calculated the distribution of tritium in concerned molecules and compared the results with the experiment. To distinguish between two benzylic hydrogens we call *pro-R*H the hydrogen the exchange of which for tritium gives rise to *R* configuration of benzylic carbon and *pro-S*H the hydrogen the exchange of which gives rise to *S* configuration. The respective tritium atoms are designed as *R*T and *S*T. The stereochemical formula for **2** (Fig. 1) illustrates the situation.

The calculation of the distribution of tritium was based on the presumption, that the energy difference between two transition states, giving rise to *R* or *S* configuration, respectively, is equal to energy difference between the two different conformations having *pro-R*H or *pro-S*H in the favourable position for exchange. Then the ratio of *R*T/*S*T will be

$$\frac{R\mathrm{T}}{S\mathrm{T}} = \mathrm{e}^{-(F_R - F_S)} \ ,$$

where F_R and F_S are energies of respective conformations. From the ratio obtained in this way the percentages of *R*T and *S*T were calculated. For the calculation of F_R and

TABLE I
Distribution of ^{3}H in the benzyl group of labelled juvenoids

Compound	Distribution of ^{3}H, %				Double labelling %	Molar activity TBq mmol^{-1}
	experimental		calculated			
	low field	upper field	*R*T	*S*T		
1	75	25	73	27	31	0.63
2	36	64	25	75	14	0.48
3	23	77	23	77	15	0.58
4	8	92	14	86	0	0.33

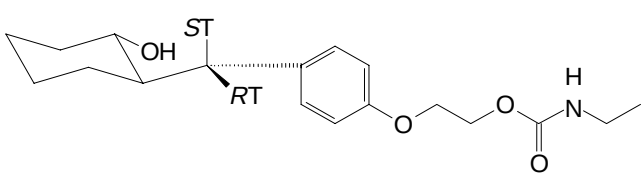

FIG. 1
Stereochemical formula of **2**

F_S we used MACROMODEL software using the MM2 force field method for minimization of energy. The respective hydrogen to be exchanged was kept in the plane perpendicular to the benzene ring plane with accuracy ±1°. The cyclohexane (or cycloheptane) ring was oriented in such a way as to keep the least distance between the exchanged hydrogen and the ring hydroxyl oxygen, that is exchanged hydrogen and the ring hydroxyl should be both very close to the surface of catalyst. The results are summarized in the Table I. In the columns "experimental low field" and "experimental upper field" there are the percentages of the corresponding tritium content as found in the ^{3}H NMR spectrum. In the columns "calculated *R*T" and "calculated *S*T" there are the percentages calculated from the MACROMODEL software data by the above mentioned procedure. In the column "Double labelling %" there is percentage of tritiated molecules containing two tritiums (a mean of MS and ^{3}H NMR data). The molar activities in the last column are derived from MS data.

It can be seen, that the calculated distributions correlate very well with those found. The above mentioned calculation also enabled us to assign the less shielded benzylic hydrogen as *pro-R* and the more shielded one as *pro-S*.

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