REGIO- AND STEREOSELECTIVE TRITIATION OF THE JUVENOID ANALOG W 328

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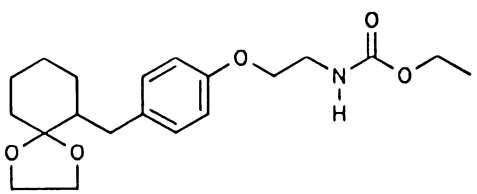
The juvenoid analog 2-(4-(2-(ethoxycarbamato)ethoxy)benzyl)-1-cyclohexanone ethylene acetal (*I*) (W 328) was labelled by ^{3}H in the benzyl position by the CESG method. ^{3}H NMR revealed the stereoselectivity of the labelling. The results are compared with the data published in the literature and discussed in the terms of stereoelectronic requirements.

The labelled juvenoid analog 2-(4-(2-(ethoxycarbamato)ethoxy)benzyl)-1-cyclohexanone ethylene acetal (*I*) (W 328) was required for the metabolic studies of this potential pesticide on the insects. The compound *I* labelled in the urethane carbonyl position by ^{14}C was already prepared[1]. On the basis of the preliminary results it was desirable to label the aromatic portion of the W 328 molecule. According to the literature the labelling of the W 328 by tritium using catalyzed Exchange in Solution with Gas[2] (CESG) should give the product selectively labelled in the benzyl position (with at least 90% selectivity).



I

EXPERIMENTAL

An all glass Toepler pump[3] was used for the tritium gas transfers. The "cool" compound *I* was prepared according to ref.[4]. TLC was performed on the Merck HPTLC Kieselgel 60, UV_{254}, plates, preparative TLC was performed on the 0.2 mm Merck Kieselgel 60, UV_{254}, alufoil supported plate. Radioactivity distribution was analyzed on the Berthold LB 2832 Linear Analyzer combined with multichannel analyzer Berthold-Silena and HP 97-S calculator. HPLC was performed on the Spectra-Physics apparatus equipped with the Separon SGX C18 column (4 × 250 mm, particle size 7 μm, Tessek Ltd.) and coupled with the 171 Radioisotope Detector Beckman (solid scintillator Ultra-Scint™ cell, 125 μl); mobile phase: water with 70% of methanol, flow 1.3 ml/min., retention time 16 min.

Activity of the samples was assayed on liquid scintillation counter Beckman LS 7800 with the correction on quenching (external standard). UV spectra were taken on the Specord UV-VIS apparatus (Karl Zeiss, Jena). ^{3}H NMR spectrum was recorded at 320 MHz on a Bruker AC 300 spectrometer equipped with a ^{1}H/^{3}H dual probe, sample was dissolved in $CDCl_3$. Chemical shifts are given in ppm (δ-scale). Solvent evaporations were done on Büchi rotary evaporator under reduced pressure and bath temperature 30 – 40 °C.

[Benzyl-^{3}H]2-(4-(2-(ethoxycarbamato)ethoxy)benzyl)-1-cyclohexanone Ethylene Acetal (*I*)

In a small reaction vessel (5 ml) equipped with a magnetic stirr bar the compound *I* (5.2 mg, 15 μmol) was dissolved in an ethyl acetate (0.5 ml), the 10% Pd/$BaSO_4$ (10.3 mg) was added and the reaction vessel was connected to the tritiation apparatus. The contents was frozen with liquid nitrogen, reaction vessel and the apparatus were evacuated and tritium gas (60% of tritium, ca 230 GBq) was transferred over the reaction mixture. After thawing the reaction mixture was stirred under tritium gas (78 kPa, room temperature) for 90 min. The tritium gas was trapped on uranium and the mixture was lyophilized in the closed system. The residue was dissolved in methanol (0.5 ml) and the mixture was transferred to centrifugation tube. The reaction vessel was washed with another portion of methanol (0.5 ml) and the catalyst was centrifugated off. The supernatant was separated and the catalyst was washed with methanol (0.3 ml). United methanol solutions were evaporated in the closed system to dryness. The syrupy residue was dissolved in toluene (2 ml). Total activity was 7.77 GBq; according to the radio-TLC (HPTLC Kieselgel 60, hexane–ether 1 : 1) 24% of activity is in the compound *I* (R_F 0.3).

The mixture was purified on the 20 × 20 cm Kieselgel 60 desk (two developments by hexane–ether 1 : 1). The zone containing the product (UV detection) was cut off and eluted by ether. The radio-HPLC revealed another 20% of impurity.

[^{3}H]-*I* was further purified on the Separon RP-18 column (6 × 250 mm), mobile phase water with 80% of methanol, flow 1.2 ml/min, in four portions. The mass of the pure product was assayed by the UV absorption measurement af methanol–water solution (λ_{max} 279 nm, ε 1 502 dm^3 mol^{-1} cm^{-1}) as 1.39 mg. The methanol–water solution was evaporated and the residue was evaporated four times with toluene (5 ml) to remove water completely. The residue was dissolved in toluene (5 ml) and this stock solution was kept at about 5 °C. The yield of [^{3}H]-*I* was 1 236 MBq (33.4 mCi); specific activity (M.w. 363.4) 0.323 TBq/mmol (8.73 Ci/mmol); radiochemical purity (HPLC, TLC) > 96%. ^{3}H NMR (^{1}H broad band decoupling): 1.71 (s, cyclohexane ring, C-2 ^{3}H), 2.15 (s, benzylic ^{3}H), 2.92 (s, benzylic ^{3}H), 6.80 (s, benzene ring ^{3}H).

The molar activity calculated from the signal attenuation of the exchanged protons in the ^{1}H NMR of the [^{3}H]-W 328 is 8.95 Ci/mmol.

RESULTS AND DISCUSSION

The ^3H NMR analysis of the obtained [^3H]-*I* showed that 95.1% of incorporated tritium is in the benzylic position. The rest was found in the position 2 of the cyclohexane ring (3.2%, β-position with respect to the benzene ring) and on the benzene ring (1.7%). The signals of the two benzylic protons are well resolved due to the neighboring chiral carbon atom C-2 and thus in the ^3H NMR spectrum (Fig. 1) it is clearly seen, that the distribution of the tritium within the benzyl group is uneven – 91.9% are found in the tritium at δ = 2.15. The two minor signals at δ = 1.31 and 1.76 do not correlate with the ^1H NMR signals[4] of compound *I* and were thus assigned to radiochemical impurities. From the ^1H coupling constants[4] of the predominantly exchanged benzylic proton it follows, that this proton is oriented antiperiplanary to the C-2 proton of the adjacent cyclohexane ring.

Similar stereospecifity of the benzylic exchange was observed[5,6] at the estrogen steroids of the general formula *II*. The facile exchange of the conformationally fixed 9α proton in estrogens suggests, that its position matches with the stereoelectronic requirements of the exchange. Hence, these stereoelectronic requirements can be formulated as follows – 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 (Fig. 2). This arrangement makes possible the overlap of the benzene ring π-orbital with the σ-orbital of the C–H bond which in turn facilitates rupture of this

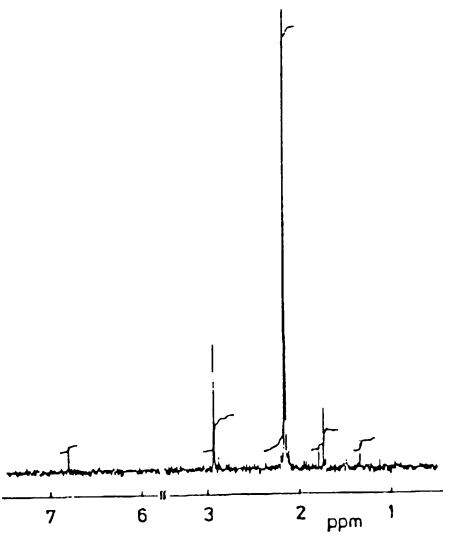


FIG. 1
^3H NMR Spectrum of compound [^3H] - *I*

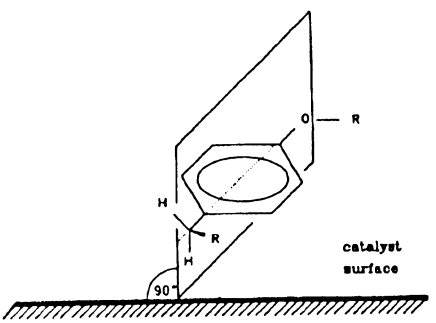
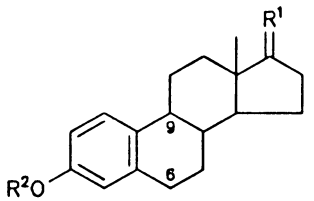


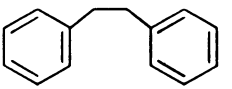
FIG. 2
The arrangement of benzylic moiety on the surface of the catalyst

bond (in comparison to other alkyl C–H bonds) and replacement of the hydrogen by the tritium from the catalyst surface.

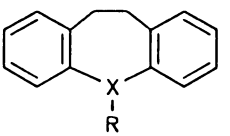
Inspection of the Dreiding model of the compound *I* molecule confirms the above mentioned results. The conformation of the molecule exposing the benzylic proton with the high observed exchange rate is relatively free of unfavorable interactions. On the other hand, exposing the second, less readily exchanged proton requires such an arrangement, in which the five membered ketal ring and benzene ring are brought to great proximity with the resulting H–H interactions.



II



III



IV, X = CH; R = H

V, X = N; R = $CH_2CH_2CH_2N(CH_3)_2$

The results of Buchman et al.[7], who observed the significant drop of obtained molar activities in the series bibenzyl (*III*) – 10,11-dihydrobenzocycloheptane (*IV*) – imipramine (*V*) (under otherwise identical exchange conditions), also support the here presented stereoelectronical restraints for the catalytical tritium exchange over Pd. While in the bibenzyl the completely staggered conformation of the central ethane moiety exposes one proton of each side to the exchange, the introduction of the central cycloheptandiene ring requires eclipsed conformation of the ethane bridge for the exchange ot these bridge protons. It would be interesting to analyze the ^3H spectrum of the product, since from the examination of the model it follows, that the majority of the tritium should be in the methylene bridge. The roles of the ring nitrogen and the side chain nitrogen in further lowering the exchange ratio in imipramine and its analogs are most likely due to the lower rate of desorption of the product from the catalyst surface – desorption, conformation change and adsorption sequence is required to expose another proton to exchange reaction.

The controversy exists in the literature on the stereoselectivity of the exchange in estrogens in the position 6. In the ref.[5] the exclusive 6α labelling is claimed whereas the authors of the paper[6] assess the 6β position for the triton on the basis of its vicinal coupling constant. Whereas the axial 6β proton is in favorable position for the exchange (as is 9α proton), the exchange of the equatorial 6α proton would require the change of the conformation of the cycle B from the half-chair to the boat. This fact should be borne in the mind in the case of the reexamination of the published results.

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