Substitution of hydrogen by deuterium hindering the biochemical dehydrogenation of alditols in the sense of Bertrand—Hudson rule

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It has been found that the biochemical dehydrogenation by the microorganism *Acetobacter pasteurianus BS 1775* of D-arabinitols labelled with deuterium in the C-4 position does not proceed. Similarly, the reaction was totally blocked with D-[4-^{2}H]ribitol. The D-[2-^{2}H]mannitol which has theoretically two reaction centres was dehydrogenated only at C-5 position.

Было найдено, что биохимическое дегидрирование с микроорганизмом *Acetobacter pasteurianus BS 1775* не происходит в случае D-арабинитолов меченых дейтерием на атоме С-4. Аналогично не происходит биохимическое дегидрирование D-[4-^{2}H]рибитола, а D-[2-^{2}H]маннитол дегидрируется только на атоме С-5.

Nonsubstituted alditols which possess two vicinal hydroxyl groups in D-*erythro* configuration adjacent to a terminal primary hydroxyl group are dehydrogenated under the action of *Acetobacter sp.* to corresponding 2-ketoses [1]. This biochemical dehydrogenation is with advantage used for the preparation of D-*threo*--pentulose from D-arabinitol and L-*erythro*-pentulose from ribitol [2—4]. For the purpose of the study of the mechanism of epimerization of aldoses and ketoses catalyzed by molybdate ions [5—7] we focused our attention to preparation of ^{2}H-labelled 2-ketoses in a biochemical way.

The biochemical dehydrogenation of ^{2}H-labelled D-arabinitols *I—IV* and ^{2}H-labelled ribitols *VIII, IX* was performed under the same conditions as biochemical dehydrogenation of D-arabinitol *VII* and D-ribitol *X* (Table 1). These pentitols, as well as D-[2-^{2}H]mannitol and D-mannitol were dehydrogenated by the microorganism *Acetobacter pasteurianus BS 1775* which is commonly used for the preparation of D-*threo*-pentulose and L-*erythro*-pentulose. D-Arabinitols labelled with ^{2}H on carbon atoms C-1 (*I*), C-2 (*II*), C-3 (*III*), and C-5 (*V*) are biochemically dehydrogenated approximately with the same parameters (reaction time, degree of conversion) as is the unlabelled D-arabinitol (*VII*). Similarly, the reaction of [3-^{2}H]ribitol (*VIII*) can be compared with that of unlabelled ribitol (*X*). However, in case of D-arabinitol labelled with ^{2}H on C-4 (*IV, VI*), as well as in the case of

Table 1

Dehydrogenation of alditols to corresponding pentuloses (%) by *Acetobacter pasteurianus BS 1775*

No.	Pentitol	Reaction time, h			
		24	48	72	96
I	D-[1-^{2}H]Arabinitol	64	75	59	53
II	D-[2-^{2}H]Arabinitol	61	69	53	50
III	D-[3-^{2}H]Arabinitol	43	40	30	28
IV	D-[4-^{2}H]Arabinitol	<5	<5	<5	<5
V	D-[5-^{2}H]Arabinitol	66	75	75	57
VI	D-[2,4-^{2}H]Arabinitol	<5	<5	<5	<5
VII	D-Arabinitol	63	73	55	49
VIII	[3-^{2}H]Ribitol	46	61	54	50
IX	D-[4-^{2}H]Ribitol	<5	<5	<5	<5
X	Ribitol	51	66	52	48

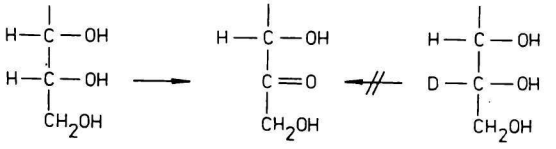
D-[4-^{2}H]ribitol (*IX*) the degree of conversion to corresponding pentuloses did not reach 5% after 96 h (determined by polarographic method). After 10 days of incubation of compounds *IV*, *VI*, and *IX* no pentuloses or aldopentoses were found besides the starting alditol in the reaction mixture by means of paper chromatography.

The effect of the presence of deuterium in the reaction centre of the molecule of alditol was investigated using D-[2-^{2}H]mannitol as a model compound. This alditol possesses, according to Bertrand—Hudson rule two reaction centres. The D-[2-^{2}H]mannitol used in the reaction was deuterized to 88% in the indicated position. After 72 h D-[5-^{2}H]*arabino*-hexulose was isolated having the degree of deuterization of 70—72%. The degree of deuterization was determined by means of mass spectrometry after transforming D-mannitol and D-*arabino*-hexulose to their corresponding per-*O*-acetyl derivatives (the intensities of characteristic ion pairs of known structures had the *m/z* values: 361, 362, 289, 290, 217, 218 for D-mannitol and 347, 348, 331, 332, 317, 318, 289, 290, 275, 276 for D-*arabino*--hexulose, respectively. The D-*arabino*-hexulose was transformed also to per-*O*--acetyl-*N*-(*p*-tolyl)glycoside exhibiting the *m/z* values 437, 438, 363, 364. Using the described microbial strain only one third of D-mannitol was converted to D-*arabino*-hexulose under the given reaction conditions (two thirds of the starting D-mannitol were recovered from the reaction mixture).

The decrease of the degree of deuterization by 16—18% found during the biochemical dehydrogenation of D-[2-^{2}H]mannitol to D-[5-^{2}H]*arabino*-hexulose can be explained by preferential dehydrogenation of unlabelled D-mannitol which was present in the proportion of 12% in the starting material. This assumption is

supported well by the fact that remaining two thirds of unreacted D-mannitol isolated from the reaction mixture had the degree of deuterization 94%. From these data it also follows that the biochemical dehydrogenation of D-[2-^{2}H]mannitol proceeds only on one reaction centre, *i.e.* on the carbon atom C-5. Slow, but measurable biochemical dehydrogenation (<5%) detected with D-pentitols labelled with deuterium on C-4 (*IV, VI, IX*) can be ascribed to dehydrogenation of unlabelled pentitol present in the investigated sample.

Conclusion can be made from the presented results that the alditols which can undergo biochemical dehydrogenation in the sense of Bertrand—Hudson rule do not react in the presence of *Acetobacter pasteurianus BS 1775* when the hydrogen atom in the reaction centre is replaced by deuterium (Scheme 1). According to our knowledge, this is the first case described when deuterium present in the reaction centre totally blocks the biochemical reaction.



Scheme 1

Experimental

The individual alditols labelled with ^{2}H were prepared by reduction of corresponding ketose, D-lyxose or D-arabinose, respectively (250 mg) in water (2.5 ml) by sodium borodeuteride (30—40 mg), Czechoslovak Academy of Science, Prague, isotopical purity 98%. After 3 h standing at room temperature the reaction mixture was deionized (katex), concentrated *in vacuo* and after triple evaporation of the residue from methanol (3 ml each time) the epimeric alditols were isolated by chromatography on the column of Dowex 50 W in La cycle by elution with water. The elution volumes for the individual sugars relative to pentaerythritol were 0.89 for ribitol, 1.39 for arabinitol, 2.52 for xylitol, 1.20 for mannitol, and 2.50 for glucitol [8]. The pentitols were isolated also by paper chromatography on Whatman No. 1 paper using the elution system cyclohexanol—pyridine—water saturated by boric acid 6 : 5 : 2 (the mobilities relative to that of xylitol were 1.22 for arabinitol and 1.55 for ribitol [9]). In case of reduction of D-*arabino*-hexulose the chromatographically pure D-[2-^{2}H]mannitol was obtained by direct crystallization from methanol in 2.5% yield. By a similar procedure D-[1-^{2}H]arabinitol was obtained from D-arabinose, D-[2-^{2}H]arabinitol from D-*erythro*-pentulose [10], D-[3-^{2}H]arabinitol from D-*threo*--3-pentulose [11], D-[4-^{2}H]arabinitol from D-*threo*-pentulose [4], D-[5-^{2}H]arabinitol from D-lyxose, D[2,4-^{2}H]arabinitol from D-[4-^{2}H]*threo*-pentulose, [3-^{2}H]ribitol from *erythro*-3-pentulose [11], D-[4-^{2}H]ribitol from L-*erythro*-pentulose [4], and D-[2-^{2}H]mannitol from D-*arabino*-hexulose.

Biochemical dehydrogenation of alditols

Pentitols

The individual alditols were dehydrogenated by the strain *Acetobacter pasteurianus BS 1775* (Czechoslovak Collection of Microorganisms, Prague). The microorganism was cultivated in a medium containing 1.2 g of D-arabinitol and 1.2 g of yeast autolyzate in water (60 ml) at 28°C on a reciprocal shaker (108 cycles per min) for 48 h. The grown microbial cells were then transferred to 3 ml of medium containing 100 mg of the corresponding alditol, 15 mg of yeast autolyzate, 6 mg of $(NH_4)_2HPO_4$, 3 mg of KH_2PO_4, and 0.8 mg of $MgSO_4 \cdot 7H_2O$. The concentration of pentulose formed by biochemical dehydrogenation was estimated polarographically (Polarograph PO 4, Radiometer, Copenhagen). The formed pentulose was separated from the starting pentitol by paper chromatography in the system 2-butanone—butanol—water 16 : 2 : 1, whereby the pentuloses migrated three times faster than the pentitols.

D-[3-^{2}H]*threo*-Pentulose was prepared in greater amount as follows: D-[3-^{2}H]arabinitol (2 g) was biochemically dehydrogenated by the already described procedure for 24 h, the reaction mixture was then filtered and deionized on a mixed-bed resin column. After evaporation to dryness the sugars were separated by chromatography on the column of cellulose (80 × 2 cm) eluted by the above described chromatographic solvent system. By this procedure D-[3-^{2}H]*threo*-pentulose (0.8 g) was obtained; $[\alpha]_D^{23} = -32 \pm 1°$ (*c* 2, water).

D-*[2-^{2}H]Mannitol*

The biochemical dehydrogenation of D-[2-^{2}H]mannitol (200 mg) was carried out for 72 h. The reaction mixture was then chromatographed as described above whereby D-*arabino*-hexulose (45 mg) and the starting D-[2-^{2}H]mannitol (110 mg) were isolated. The deuterium content in the obtained D-*arabino*-hexulose as well as in the starting D-[2-^{2}H]mannitol was determined by mass spectrometry after their conversion to corresponding per-*O*-acetyl derivatives or, in case of D-*arabino*-hexulose, also to per-*O*-acetyl-*N*-(*p*-tolyl)glycoside. The mass spectra were measured on a JMS-D 100, Jeol mass spectrometer at 12 eV electron energy and the emission of 300 μA. The temperature in the evaporation chamber was 70—80°C and 120°C in the ionization chamber. The samples for mass spectrometry were prepared as follows: D-mannitol, or D-*arabino*--hexulose (40 mg) were dissolved in dry pyridine (1 ml) and after adding acetic anhydride (0.5 ml) the mixture was allowed to stand for 24 h at room temperature. A portion (1 ml) of the reaction mixture from the preparation of per-*O*-acetylated D-*arabino*-hexulose was concentrated in a rotary vacuum evaporator to dryness (temperature of water bath 60°C). To the residue absolute ethanol (1 ml), *p*-toluidine (100 mg), and acetic acid (0.1 ml) were added and after 30 min heating at 80°C the sample was used for mass spectrometry.

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