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The use of *Aspergillus terreus* extracts in the preparative synthesis of 2-keto-3-deoxy-ulosonic acids

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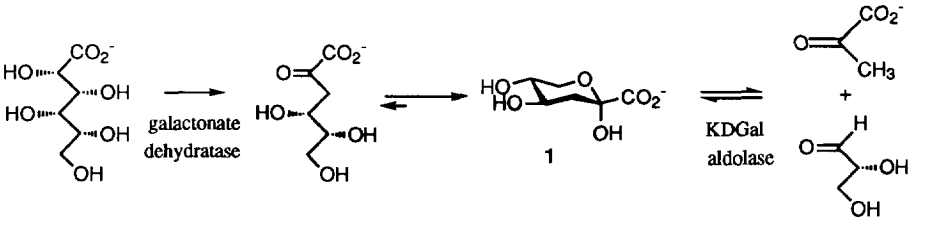
Abstract : Pure 2-keto-3-deoxy-ulosonic acids, KDGal (**1**), DAH (**2**) and its 5-epimer (**3**) were prepared on preparative scales through diastereoselective aldol reactions of pyruvate respectively with D-glyceraldehyde, D-erythrose and D-threose, catalyzed by *Aspergillus terreus* extracts; from 2-deoxy-D-ribose, 5-deoxy-KDO (**4**) could be obtained in a lower yield, with a diastereomeric excess of 72%.

In search of enzymes catalyzing formation of C-C bonds for synthetic purposes, we recently started to investigate aldolases from filamentous fungi, which require pyruvate as the nucleophilic component; in these microorganisms a modified Entner-Doudoroff pathway involving non phosphorylated intermediates has been evidenced.[1] However we observed that KDG aldolase from *Aspergillus niger* did not catalyze a totally diastereoselective aldol reaction between pyruvate and D-glyceraldehyde, suggesting either a lack of selectivity of the enzyme or the occurence of two distinct aldolases with complementary facial selectivity.[2] Therefore we turned to another fungus, *Aspergillus terreus*, that has been reported to be able to metabolize D-galactonate according to Scheme 1.[3] The reversibility of the second reaction prompted us to examine its synthetic utility.



Scheme 1.

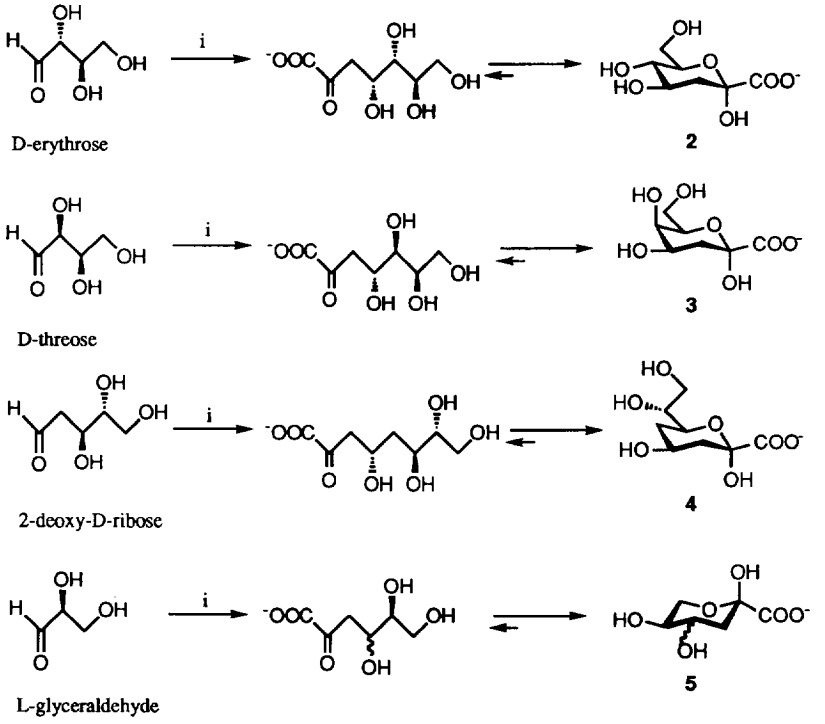
We wish to report here results we obtained in the aldol reaction of pyruvate with D-glyceraldehyde and other acceptor substrates, catalyzed by crude extracts of *Aspergillus terreus* NRRL 265 grown on 2% D-galactonate as the sole source of carbon.[3]

Condensation of D-glyceraldehyde with pyruvate in the presence of the enzymatic extract led to a diastereomerically pure compound identified as 3-deoxy-D-*threo*-2-hexulosonate (KDGal **1**).[4] Several other aldehydes were tested as electrophiles; the rate of formation of the corresponding keto-deoxy-ulosonic acids is reported relative to D-glyceraldehyde in Table 1.[5]

Table 1. Substrate specificity of KDGal Aldolase towards the acceptor.

Substrates	Relative rate	Substrates	Relative rate
D-glyceraldehyde	100	D,L-lactaldehyde	50
glycolaldehyde	115	D-arabinose	0
D-erythrose	85	acetaldehyde	0
D-threose	54	propionaldehyde	0

The high relative rates we measured with hydroxylated aldehydes allowed us to anticipate that these aldehydes could be good substrates. This was confirmed by large scale incubation of fungi extracts in the presence of pyruvate with D-erythrose and D-threose,[6] affording respectively pure compounds **2** and **3** (Scheme 2).[7]



Scheme 2. i) *Aspergillus terreus* extracts, 1 eq. pyruvate, 2 days, 27 °C

Table 2. Aldol reaction of pyruvate and various acceptors catalyzed by extracts of *Aspergillus terreus*

Acceptors	Products	Scale of synthesis (mmol)	Enzyme U/mmol [a]	Isolated yield %	de (%)
D-glyceraldehyde	**1**	1	0.5	55	100
D-erythrose	**2**	4	1.3	58	100
D-threose	**3**	4	2.4	85	100
2-deoxy-D-ribose	**4**	0.1	2.8	33	72
L-glyceraldehyde	**5**	1	0.5	50	48

[a] One enzyme unit (U) is the amount of enzyme that catalyzes the formation of 1 μmole of KDGal per minute.

On the other hand incubation with 2-deoxy-D-ribose achieved on a smaller scale was not completely diastereoselective (de=72%, Table 2).[8] Moreover contrary to D-glyceraldehyde, L-glyceraldehyde led to a diastereomeric mixture of threo and erythro compounds in the 26:74 ratio. Results are summarized in Table 2. It is worth mentioning that compounds **2** and **4** occur exclusively in the pyranose form, whereas compound **3** occurs in both pyranose and furanose forms.

In conclusion we have shown that *Aspergillus terreus* extracts are able to catalyze a stereoselective aldol reaction, creating a new asymmetric center of *R* configuration, resulting from the *re* face attack of the aldehyde. To our knowledge this is the first example of the use of aldolases from filamentous fungi. Thus biologically significant compounds have been prepared: compound **2** (DAH) is the dephosphorylated form of the biosynthetic precursor of aromatic amino acids, compound **3** oxidized on carbon 7 is a lipopolysaccharide constituent of bacteria of the genus Rhizobium fixing nitrogen and living in symbiosis with legume plants[9], whereas compound **4** is the 5-deoxy derivative of KDO, the major sugar of the lipopolysaccharide of Gram-negative bacteria. This enzymatic route, providing an easy access to this class of compounds, is a good alternative to the previously reported ones.[10] Purification of the KDGal aldolase is now on the way in order to further explore the potential of the enzyme.

Experimental

The mycelium was recovered after 3 days, filtered, then ground with the French Press in 0.02 M potassium phosphate buffer pH 8, affording after centrifugation at 5000 rpm for 30 mn a crude extract (specific activity: 0.04 U/mg) further used in synthesis. In a typical experiment a 0.1 M solution of acceptor was incubated with crude extracts in 0.02 M potassium phosphate buffer pH 8 with 1 equivalent of pyruvate at 27°C. When all pyruvate has been consumed, incubation was stopped and the products were purified by anion exchange chromatography on AG1-X8 resin (HCO_3^-, 100-200 mesh) and elution with a linear gradient of 0 to 0.4 M ammonium bicarbonate. After deionization with AG50-X8 (H^+), neutralisation with dilute ammonia and freeze-drying, the compounds were isolated as their ammonium salts.

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References and Notes

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2. C. Augé and V. Delest, *Tetrahedron: Asymm.*, 1993, **4**, 1165.
3. A. M. Elshafei and O. M. Abdel-Fatah, *Enzyme Microb. Technol.*, 1991, **13**, 930.
4. Compound **1** : $[\alpha]_D^{28}$ + 9 (*c* 1, H_2O); literature $[\alpha]_D^{19}$ + 7.9 (*c* 1.65, H_2O): R. Kuhn, D. Weiser and H. Fischer, *Liebigs Ann. Chem.*, 1959, **628**, 207; ^1H NMR (250 MHz, D_2O, HOD at 4.8 ppm) (α pyranose as the predominent form): δ 1,72 (dd, 1H, $J_{3ax,3eq}$ 13, $J_{3ax,4}$ 11.5 Hz, H-3ax), 2.16 (dd, 1H, $J_{3eq,4}$ 5 Hz, H-3eq); ^{13}C NMR (62.9 MHz, internal 1,4-dioxane reference at δ 66.64): δ 39.02 (C3), 62.93 (C6), 68.93 (C4), 70.72 (C5), 96.64(C2), 176.38 (C1).
5. The rate of formation of keto-deoxy-ulosonic acids was estimated either by HPLC (on GlycoPak DEAE column, Waters), or by colorimetric test with thiobarbituric acid, depending upon the substrates.
6. D-erythrose, 2-deoxy-D-ribose were purchased from Sigma, D-threose was synthesized from 4,6-O-benzylidene-D-galactopyranose according to P. Zimmermann and R.R. Schmidt, *Liebigs Ann. Chem.*, 1988, 663.
7. Compound **2** : $[\alpha]_D^{30}$ + 33.5 (*c* 2, H_2O); literature $[\alpha]_D^{20}$ + 33 (*c* 1, H_2O) : R. Ramage, A.M. MacLeod and G. W. Rose, *Tetrahedron*, 1991, **47**, 5625; ^1H NMR (250 MHz, D_2O, HOD at 4.8 ppm) : δ 1.70 (dd, 1H, $J_{3ax,3eq}$ 13, $J_{3ax,4}$ 12 Hz, H-3ax), 2.13 (dd, 1H, $J_{3eq,4}$ 5 Hz, H-3eq), 3.38 (t, 1 H, $J_{4,5} = J_{5,6}$ 9.5 Hz, H-5), 3.88 (ddd, 1H, H-4), 3.50-3.80 (m, 3H, H-6, H-7, H-7') ; ^{13}C NMR (62.9 MHz, internal 1,4-dioxane reference at δ 66.64) : δ 39.27 (C3), 60.76 (C7), 68.92 (C4), 70.76 (C5), 73.70 (C6), 96.28 (C2), 176.65 (C1). Compound **3** : $[\alpha]_D^{28}$ + 37 (*c* 2.25, H_2O); α pyranose : ^1H NMR (250 MHz, D_2O, HOD at 4.8 ppm): δ 1.8-2.0 (m, 1.4 H, H-3ax, H-3eq), ^{13}C NMR (62.9 MHz, internal 1,4-dioxane reference at δ 66.64): δ 33.82 (C2), 61.68 (C7), 66.02 (C4), 67.70 (C5) 72.61 (C6), 96.39 (C2) 176.82 (C1); furanose : ^1H NMR (250 MHz, D_2O, HOD at 4.8 ppm): δ 2.02 (dd, 0.15 H, $J_{3a,3b}$ 14.5, $J_{3,4}$ 3 Hz, H-3a α), 2.24 (dd, 0.15H, $J_{3a,3b}$ 13, $J_{3a,4}$ 6.5 Hz, H-3a β) 2.34 (dd, 0.15 H, $J_{3b,4}$ 7 Hz, H3b β), 2.52 (dd, 0.15H, $J_{3b,4}$ 7 Hz, H-3b α); ^{13}C NMR (62.9 MHz, internal 1,4 dioxane reference at δ 66.64): 44,48 (C3), 63.11 (C7), 71.28, 71.76, 72.33 (C4, C5, C6), 86,30 (C2). Compound **4** : ^1H NMR (250 MHz, D_2O, HOD at 4.8 ppm) : δ 1.4 (q, 1H, $J_{5ax,5eq} = J_{5ax,4} = J_{5ax,6}$ 12 Hz, H-5ax), 1.55 (t, 1H, $J_{3ax,3eq} = J_{3ax,4}$ 12 Hz, H-3ax), 1.95 (m, 1H, H-5eq), 2.65(ddd, $J_{3eq,4}$ 5, $J_{3eq,5eq}$ 2 Hz, H-3 eq), 3.55 (dd, 1H, $J_{8,8'}$ 12, $J_{8,7}$ 7 Hz, H-8), 3.68 (dd,1H, $J_{8',7}$ 4 Hz, H-8'), 3.76 (dt, 1H, $J_{7,6}$ 4 Hz, H-7), 3.92 (ddd, 1H, $J_{6,5ax}$ 12.5, $J_{6,5eq}$ 2.5 Hz, H-6), 4.07 (m, 1H, H-4); ^{13}C NMR (62.9 MHz, internal 1,4-dioxane reference at δ 66.64) : δ 40.99 (C3), 33.74 (C5), 62.27 (C8), 64.16 (C4), 68.55 (C6), 73.50 (C7), 97.01 (C2), in good agreement with R. Cherniak, R. G. Jones and D. Sen Gupta, *Carbohydr. Res.* , 1979, **75**, 39.
8. The diastereomeric excess was estimated from the intensity of the C-3 signals of both isomers in their pyranose forms; the C-3 signal of the compound exhibiting *R* configuration on C-4 is shifted downfield compared with the one of the compound exhibiting *S* configuration: U. Kragl, A. Gödde, C. Wandrey, N. Lubin and C. Augé, *J. Chem. Soc. Perkin Trans. 1*, 1994, 119.
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