Tetrahedron: Asymmetry Vol. 4, No. 6, pp. 1165-1168, 1993
Printed in Great Britain

0957-4166/93 $6.00+.00
Pergamon Press Ltd

Microbiological Aldolisations. Synthesis of 2-Keto-3-Deoxy-D-Gluconate

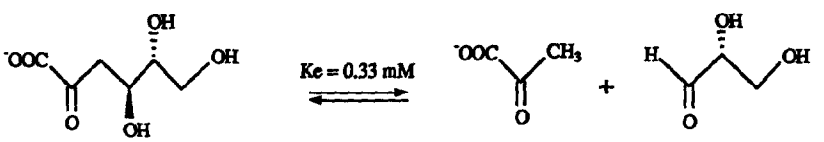
Claudine Augé* and Véronique Delest

Laboratoire de Chimie Organique Multifonctionnelle, Institut de Chimie Moléculaire d'Orsay, URA CNRS 462, bât 420, 91405 Orsay cédex, France

(*Received* 15 *February* 1993)

Abstract : An efficient synthesis of 2-keto-3-deoxy-D-gluconate (KDG) was achieved *via* stereospecific aldolisation using the fungus *Aspergillus niger*. Whereas incubation of D-glyceraldehyde and pyruvate with enzymatic extract led to a diastereomeric mixture of threo/erythro compounds in the 13 : 87 ratio, incubation with resting cells turned out to enhance this ratio up to 4 : 96 in favor of KDG.

For some years we have been extensively using, for synthetic purposes, *N*-acetylneuraminic acid aldolase, one of the aldolases which utilizes pyruvate as the nucleophile.[1] No very much attention has been paid to other pyruvate aldolases, except recently HKG and KDPG aldolases.[2,3] KDPG aldolase from *Pseudomonas putida* seems to exhibit a specificity not broad enough towards the acceptor for synthetic applications. This observation prompted us to turn to another aldolase, KDG aldolase, involving non phosphorylated intermediates. This enzyme has been found in some filamentous fungi and specially in *Aspergillus niger* grown on 2% D-gluconate as the sole source of carbon.[4] The metabolic function of this enzyme, which is induced by D-gluconate, is the degradation of gluconate *via* 2-keto-3-deoxy-D-gluconate **1** into D-glyceraldehyde **2** and pyruvate **3** :



1 **3** **2**

We wish to report here, initial studies in the investigation of this reaction of aldolisation for organic synthesis, carried out with enzymatic extracts and resting cells as well. *Aspergillus niger* was grown on D-

gluconate, the mycelium was recovered after 3 days, then either used like that or ground with the French Press. From the crude extract, the enzyme was partially purified by heat treatment and precipitation with acetone according to the published procedure (specific activity : 0.32 U/mg).[4] Alternatively *Aspergillus niger* was grown on D-galactonate.

Results are summarized in the table.

Table. Diastereomeric compositions of the products resulting from aldol condensation of **2** and **3**.[a]

Source of Carbon	glyceraldehyde	Enzymatic extracts	Mycelium	Isolated yield %	Threo/Erythro ratio
D-gluconate	D	+		73	13 : 87
D-gluconate	L	+		74	40 : 60
D-gluconate	D,L		+	36	46 : 54
D-gluconate	D		+	65	4 : 96
D-galactonate	D		+	88	9 : 91

[a] depending upon three factors : the source of Carbon used in the culture, the enantiomeric form of glyceraldehyde, the incubation procedure.

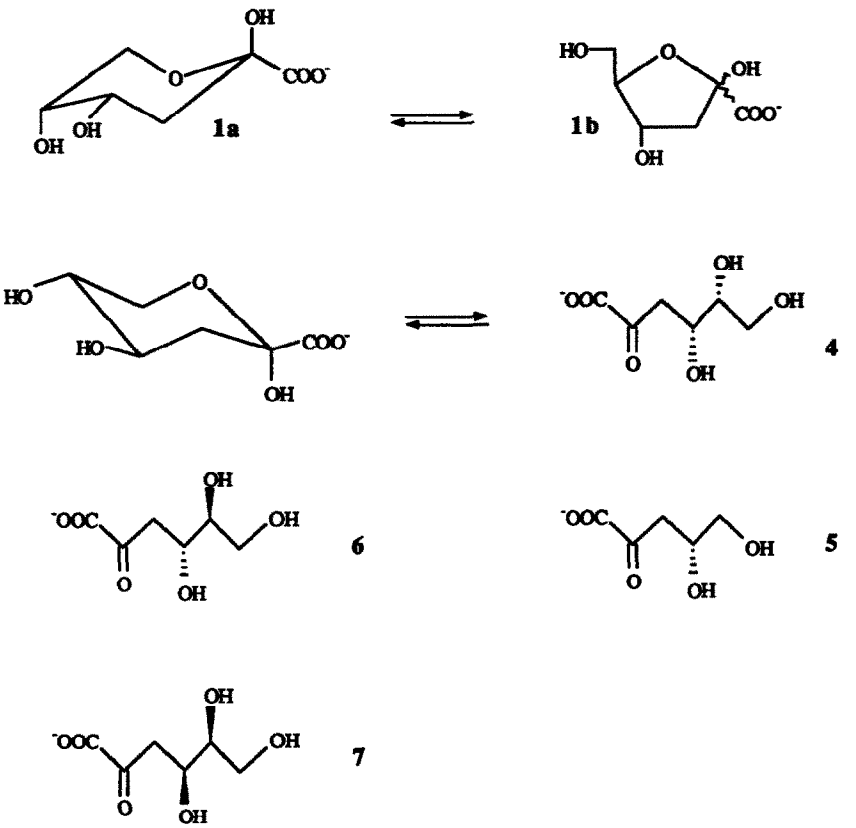
Surprisingly condensation of D-glyceraldehyde **2** with pyruvate **3** in the presence of the enzymatic extract did not lead to a single compound but to a mixture of two diastereomers in the 13 : 87 ratio.[5] From NMR spectroscopy, the major compound, 3-deoxy-D-*erythro*-2-hexulosonate (KDG) was identified as a mixture of pyranose **1a** and furanose **1b**, and the minor one, 3-deoxy-D-*threo*-2-hexulosonate **4**, as the pyranose form.[6] The enantiomeric purity of the starting material, D-glyceraldehyde, was ascertained by derivatization with (+) α-methyl-benzylamine into the Schiff base, followed by reduction and peracetylation.[7] Therefore, we must admit that the enzymatic preparation contained two distinct aldolases with complementary facial selectivity. Indeed, two different degrading systems co-exist in D-gluconate-grown *Aspergillus niger*, the one degrading D-gluconate, the other degrading L-arabonate and involving KDA aldolase.[8] Compound **4** exhibited the same stereochemistry on C-4 than 2-keto-3-deoxy-L-arabonate (KDA **5**), the intermediate degradation product of L-arabonate into pyruvate and glycolaldehyde.[9]

Enzymatic condensation of L-glyceraldehyde with **3** led to a mixture of threo/erythro compounds in the ratio 40 : 60 in 73% yield. The condensation products **6** and **7** are the enantiomers of **1** and **4**, and in that case this is the L-threo compound **7** that results from the action of KDG aldolase. Thus KDA aldolase was supposed to have higher affinity for L-glyceraldehyde than KDG aldolase.

Moreover we observed that the use of resting cells instead of enzymatic extract was of major interest. Indeed in that case, the diastereomeric ratio was enhanced up to 96% in favor of the erythro.[10] The reason for this increase is still not clear; the threo compound might be metabolized more quickly than the erythro. Furthermore KDG synthesis was much more efficient by incubation with the mycelium than with the enzymatic extract: ten millimoles of KDG could be easily prepared with the amount of mycelium obtained from 1 L of culture. KDG has just been discovered as a component of bacterial polysaccharide;[10] it is also supposed to play a role as an inducer in the synthesis of the pectate lyase, an enzyme involved in the cleavage of

polygalacturonate.[6] Chemical syntheses of this compound have been recently reported.[6,12]

Condensation of D,L-glyceraldehyde under the same conditions led to an equimolar mixture of threo and erythro.



Growing *Aspergillus niger* on D-galactonate instead of D-gluconate, did not make great difference in the diastereomeric mixture obtained by incubation with mycelium; the erythro compound was predominantly formed.

Work is now in progress to better elucidate these microbiological aldolisations and to explore the possibilities for the synthesis of analogs. Preliminary experiments let us suppose that these aldolases exhibit broad specificity towards the acceptor.

Typical experiment with enzymatic extract : 0.2 M solution of D or L or D,L-glyceraldehyde[13] in 0.02 M potassium phosphate buffer pH 8.0 (5 mL) was incubated with 1.1 equiv. of pyruvate in the presence of 2 U KDG aldolase at 30° for 8 h.

Typical experiment with mycelium : 0.05 M solution of D-glyceraldehyde in 0.02 M potassium phosphate buffer pH 8.0 (20 mL) was gently stirred at 27° for 24 h, with 1.1 equiv. of pyruvate and mycelium obtained from 40 mL of culture.

After incubation the products were purified by anion exchange chromatography on AG 1-X8 resin (HCO_3^-, 100-200 mesh) and isolated as their ammonium salts according to the following procedure : elution with a 0-0.4 M ammonium bicarbonate linear gradient, freeze-drying, deionization with Dowex $50H^+$, neutralization with dilute ammonia and again freeze-drying.

We thank Professor A. Lubineau for his encouragements.

References and Notes

1. C. Augé, C. Gautheron, S. David, A. Malleron, B. Cavayé and B. Bouxom, *Tetrahedron*, 1990, **46**, 201.
2. N.C. Floyd, M.H. Liebster and N.J. Turner, *J. Chem. Soc. Perkin Trans., 1*, 1992, 1085.
3. S.T. Allen, G.R. Heintzelman and E.J. Toone, *J. Org. Chem.*, 1992, **57**, 426.
4. A.M. Allam, M.M. Hassan, T.A. Elzainy, *J. Bacteriol.*, 1975, 1128 ; the enzymatic assay for KDG aldolase was carried out by determining pyruvate liberated from KDG. KDG has been obtained by enzymatic synthesis with KDPG aldolase from *Pseudomonas putida* , see ref. 3.
5. The amount of threo compound was estimated from ^1H NMR spectrum by the intensity of the peak at 1.77 ppm attributed to H-3ax and from ^{13}C NMR by comparing the intensity of the peak at 39.00 and 33. 94, corresponding respectively to the C-3 signal of threo and erythro in the β pyranose form. Correction was made since threo compounds occur mainly in this conformation, whereas the percentage of erythro in this conformation is only 49%. Similar results were found from both estimations.
6. Compound **1** : ^1H and ^{13}C NMR identical to the literature : R. Plantier-Royon, F. Cardona, D. Anker, G. Condemine, W. Nasser and J. Robert-Baudouy, *J. Carbohydr. Chem.*, 1991, **10**, 787 ; compound **4**: ^1H NMR (250 MHz, D_2O, HOD= 4.80 ppm) δ : 1.77 (dd, $J_{3ax,3eq}$=13Hz, $J_{3ax,4}$=11.5Hz, H-3ax), 2.22 (dd, $J_{3eq,4}$=5Hz, H-3eq), ^{13}C NMR (62.9MHz, external 1,4-dioxane reference, δ 66.64) δ : 39.00 C-3, 62.94 C-6, 68.93 C-4, 70.00 C-5, 96.60 C-2, 176.29 C-1. These values are very close to the ones observed for H-3ax and H-3eq in 3-deoxy-D-*glycero*-D-*galacto*-nonulosonic acid (KDN) which exhibits the same stereochemistry on C-4 and C-5 as compound **4**: C. Augé and C. Gautheron, *J. Chem. Soc., Chem. Commun.*, 1987, 859.
7. R. Oshima and J. Kumanotani, *Chem. Lett.*, 1981, 943.
8. M.M. Hassan, A.M. Allam, A.M. Elshafei and T.A. Elzainy, *Egypt. J. Bot.*, 1985, **28**, 165.
9. T.A. Elzainy, M.M. Hassan and A.M. Elshafei, *Ann. Microbiol.*, 1981, **31**, 9.
10. $[\alpha]^D_{20°C}$ -26 (*c* , 2, water at pH 7) ; literature $[\alpha]^D_{22°C}$ -31.6 (*c* , 1.67, water at pH 6) : D. Portsmouth, *Carbohydr. Res.*, 1968, **8**, 193.
11. F. Ferreira, L. Kenne, B. Lindberg and W. Nimmich, *Carbohydr. Res.*, 1991, **210**, 255.
12. R. Ramage, A.M. MacLeod and G.W. Rose, *Tetrahedron*, 1991, **47**, 5625.
13. D-glyceraldehyde was either purchased from Fluka or prepared from D-fructose by oxidation with lead tetraacetate : A.S. Perlin, *Methods in Carbohydr. Chem.* , 1962, **1**, 61.