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Development of a Practical Multikilogram Production of (*R*)-Seudenol by Enzymatic Resolution

R. ter Halle,* Y. Bernet, S. Billard, C. Bufferne, P. Carlier, C. Delaitre, C. Flouzat, G. Humblot, J. C. Laigle, F. Lombard, and S. Wilmouth

Organon S.A., Centre de Développement Préclinique, 20, rue Henri Goudier, B.P. 140, 63203 Riom, Cedex, France

Abstract:

Different enzymatic routes for the production of (*R*)-seudenol were investigated. The Novozym 435-catalyzed reaction of racemic seudenol with vinyl butyrate proved to be the most promising route. By using vinyl laurate as an acyl donor, (*R*)-seudenol laurate could be separated from (*S*)-seudenol by an extractive work-up procedure. A nonaqueous hydrolysis allowed the isolation of pure (*R*)-seudenol. Scaling up to 7 kg resulted in a robust reproducible process.

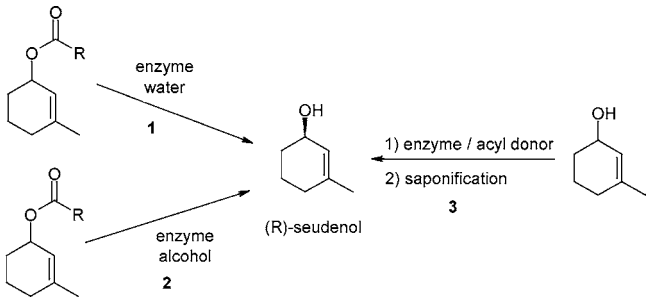
Introduction

In the course of an ongoing project we needed a method for the production of (*R*)-seudenol (3-methyl-2-cyclohexenol) on a multi-kilogram scale. Our goal was to obtain *(R)*-seudenol with at least 95% ee in a practical and economically feasible procedure without the need for chromatographic purification.

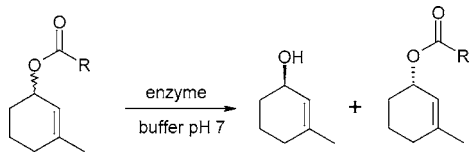
Although different chemical methods are described for the synthesis of (*R*)-seudenol,[1] we estimated that an enzymatic method would be the most attractive. To accomplish this, three different options are suitable for the enzymatic resolution of racemic seudenol (see Scheme 1): (*R*)-selective enzymatic hydrolysis of a seudenol ester, (*R*)-selective enzymatic transesterification of a seudenol ester and (*R*)-selective enzymatic acylation of seudenol followed by hydrolysis of the corresponding ester.

Enzymatic Hydrolysis of Seudenol Esters. Kazlauskas[2] et al. described the hydrolysis of seudenol acetate with bovine pancreatic cholesterol esterase and *Candida Rugosa* lipase. However, both enzymes gave very low selectivities ($E = 1$ and $E = 3.4$ respectively). Therefore, different enzymes[3] were tested for the enzymatic hydrolysis of seudenol acetate,

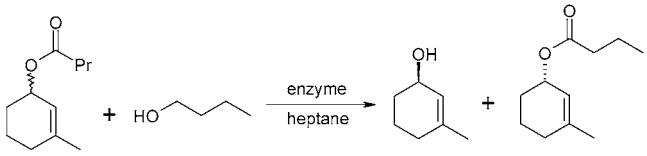
Scheme 1. **Different pathways for enzymatic preparation of *(R)*-seudenol**



Scheme 2. **Enzymatic hydrolysis of seudenol esters**



Scheme 3. **Enzymatic transesterfication of seudenol butyrate by butanol**



seudenol butyrate, and seudenol laurate at pH 7 (Scheme 2).

As was judged from blank experiments, enzymatic reactions with seudenol acetate and seudenol butyrate were plagued by the noncatalysed background reaction. Using buffers with pH 6.0 and 8.0 did not suppress this nonenzymatic hydrolysis. Seudenol laurate was not hydrolyzed without enzyme, but in this case the enzyme-catalyzed reaction is too sluggish to be of any practical interest.

Therefore, it could be concluded that using these enzymes enzymatic hydrolysis of seudenol esters was not a good procedure to obtain (*R*)-seudenol.

Enzymatic Transesterification of Seudenol Esters. Different enzymes[3] were tested for transesterification of seudenol butyrate with *n*-butanol (Scheme 3).

The lipases from *Pseudomonas fluorescens* and *Pseudomonas* sp. Type B showed some enantioselectivity (enantiomeric ratio $E = 7-9$[4]), but immobilized *Candida antartica* lipase B (Novozym 435)[5] was by far the most efficient enzyme ($E = 127$). Unfortunately, a rather high catalyst loading of 25 wt % had to be used to reach 38% conversion in 24 h.

* Author to whom correspondence may be addressed. E-mail: rob.halle@organon.fr.

(1) (a) For enantioselective reduction of the corresponding ketone by (−)-*N*-methylephedrine/$LiAlH_4$, see: Kawasaki, M.; Suzuki, Y.; Terashima, S. *Chem. Pharm. Bull*. **1985**, *33*, 52−60 and Wu, K.-M.; Okamura, W. H. *J. Org. Chem*. **1990**, *55*, 4025−4033. (b) For Ru-catalyzed hydrogenation of the corresponding ketone, see: Ohkuma, T.; Ikehira, H.; Ikariya, T.; Noyori, R. *Synlett* **1997**, *5*, 467−468. (c) For hydride transfer hydrogenation of the corresponding ketone, see: Kathó, Á.; Carmona, D.; Viguri, F.; Remacha, C. D.; Kovács, J.; Joó, F.; Oro, L. A. *J. Organomet. Chem*. **2000**, *593*−*594*, 299−306. (d) For kinetic resolutions, see: Kitamura, M.; Kasahara, I.; Manabe, K.; Noyori, R.; Takaya, H. *J. Org. Chem*. **1988**, *53*, 708−710 and Hashiguchi, S.; Fujii, A.; Haack, K.-J.; Matsumura, K.; Ikariya, T.; Noyori, R. *Angew. Chem., Int. Ed. Engl*. **1997**, *36*, 288−290.

(2) Kazlauskas, R. J.; Weissfloch, A. N. E.; Rappaport, A. T.; Cuccia, L. A. *J. Org. Chem.* **1991**, *56*, 2656−2665.

(3) Enzyme screening kits from Fluka (Lipase basic kit (ref 62327), Lipase extension kit (ref 62323) and Esterase basic kit (ref 46041)) were used for the screening of different lipases and esterases.

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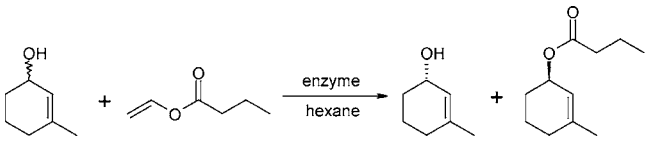
Scheme 4. **Enzyme-catalyzed kinetic resolution of seudenol**



By tuning the reaction temperature, alcohol, and solvent this loading could be reduced to 10 wt %, but still this transformation turned out to be economically less interesting than the enzymatic acylation of seudenol.

Enzymatic Acylation of Seudenol. The enzymatic acylation of seudenol is well-known from the literature. Bergbreiter and Wong[6] reported the acylation of seudenol with different enzymes and various acylating reagents. The best results were obtained with *Pseudomonas* sp. lipase which gave 67% ee for the (*R*)-ester at 32% conversion ($E = 7$). *Mucor miehei*-[7] and Lipase AK-catalyzed[8] acylation with vinyl acetate gave slightly better selectivities ($E = 14$ and 11, respectively). Orrenius et al.[9] observed a better selectivity ($E = 29$) by using a *Candida antartica* lipase B preparation (CAB SP 525) which gave 85.5% ee of (*R*)-seudenol acetate on a preparative scale (1.4 g). The best results (albeit at a small scale) were obtained by using immobilised *Candida antartica* lipase B (Novozym 435) and vinyl butyrate as an acyl donor.[10] An enantiomeric ratio $E = 187$ was obtained for this resolution (see Scheme 4).

We were easily able to perform this reaction on a 100 g-scale in heptane to obtain (*R*)-seudenol butyrate in 48% yield. Hydrolysis of the ester gave (*R*)-seudenol with 95.6% ee. These values fitted perfectly for our requirements which were ee > 95%.

Although these results were easily obtained on a small scale, the separation of (*R*)-seudenol butyrate and (*R*)-seudenol after enzymatic resolution by column chromatography was costly and troublesome on a large scale. Purification by distillation could not be considered as an option since our kilogram laboratory is not adapted for distillation on a large scale.

Extractive Work-up with Seudenol Butyrate. We decided to investigate the extractive work-up for the separation of seudenol and seudenol butyrate. After filtering off the immobilized enzyme, the reaction mixture consisted of seudenol, seudenol butyrate, and excess vinyl butyrate in heptane. The idea was to remove seudenol by washing this heptane phase with aqueous methanol. Being apolar, seudenol butyrate and vinyl butyrate should remain in the heptane layer, while seudenol would be extracted into the methanol layer.

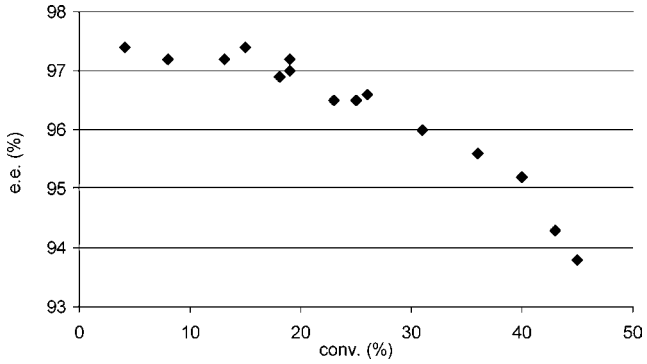
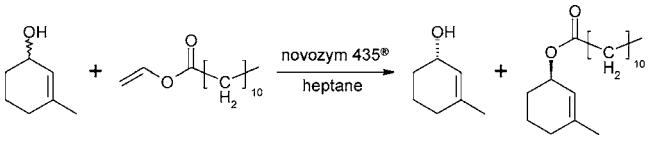


Figure 1. **Enantiomeric excess (ee) vs conversion for resolution with vinyl laurate.**

Scheme 5. **Enzymatic resolution with vinyl laurate at acylating reagent**



When 5 g of seudenol and 5 g of seudenol butyrate were dissolved in 90 mL of heptane and washed with 10 mL of 10% aqueous methanol, 1.3% of seudenol was left in the heptane phase after nine extractions, and 88.2% of seudenol butyrate was recovered from this phase.

Although these results showed that the principle of the extractive work-up was valid, the losses of seudenol butyrate were too large. Moreover, an extractive work-up that uses more than five extractions could not be considered as a practical method.

To obtain (*R*)-seudenol with a good ee it was necessary to remove as much seudenol as possible. We chose to set the maximum allowed level of seudenol after extraction at 0.1%. Obviously, separating a mixture of seudenol butyrate and seudenol could never be done with such efficiency. Therefore, we decided to increase the difference in polarity between the two compounds by chosing a long-chain fatty acid as acyl donor

Extractive Work-up with Seudenol Laurate. A look in catalogues of different suppliers showed that most vinyl alkanoate esters are sold as a mixture of isomers. We assumed that an isomerically pure vinyl alkanoate ester was necessary for efficient kinetic resolution, and therefore vinyl laurate was chosen as long-chain acyl donor.

The kinetic resolution (Scheme 5) was less efficient with vinyl laurate as compared to that with vinyl butyrate.

Instead of obtaining high ee's at 50% conversion, enantioselectivities dropped below the specification limit of 95% around 38% conversion (see Figure 1). The calculated enantiomeric ratio *E* of 76 was significantly lower compared to the reaction with vinyl butyrate (enantiomeric ratio $E = 187$).[5c]

Therefore, it was decided to stop the reaction at 35% conversion to obtain (*R*)-seudenol with a sufficiently high ee. The amount of catalyst could be further reduced from

(4) Enantiomeric ratios *E*'s were calculated according to the following formulas: $E = \ln[(1 - c)(1 - \mathrm{ee(S)})]/\ln[(1 - c)(1 + \mathrm{ee(S)})]$ for the substrate and $E = \ln[1 - c(1 + \mathrm{ee(P)})]/\ln[1 - c(1 - \mathrm{ee(P)})]$ as was described by Sih (Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294−7299).

(5) Produced by Novozymes A/S.

(6) Wang, Y.-F.; Lalonde, J. J.; Momongan, M.; Bergbreiter, D. E.; Wong, C.-H. *J. Am. Chem. Soc.* **1988**, *110*, 7200−7205.

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Scheme 6. **Hydrolysis of (*R*)-seudenol laurate**

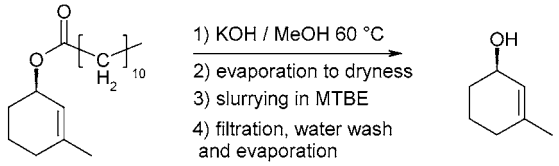

Table 1. **Multikilogram enzymatic resolution**

seudenol (kg)	reaction time (h)	conversion (%)	seudenol laurate (g)	yield mol/mol (%)	ee (%)	GC purity (a/a) (%)
6.7	15	33.8	6695	38	96.2	79.0
6.7	15	34.3	7146	41	95.6	79.7
6.6	15	33.6	6952	40	95.6	79.8

Table 2. **Multikilogram hydrolysis**

seudenol laurate (kg)	seudenol (g)	yield (mol/mol %)	ee (%)	GC purity (a/a) (%)
10	2487	65	96.0	98.4
10.775	2969	72	95.8	98.4

1.78 to 0.67 wt % to obtain the desired conversion around 16 h.

Application of the extractive work-up procedure after the enzymatic resolution proved that the purification proceeded smoothly. Extracting the heptane layer (200 mL) four times with 100 mL of 10% aqueous methanol left less than 0.1% of seudenol in the heptane layer, and 98% of seudenol laurate was recovered after evaporation of heptane.

Hydrolysis of Seudenol Laurate. After the enzymatic resolution with vinyl butyrate, the product, seudenol butyrate, was smoothly hydrolyzed by reaction with NaOH in a mixture of methanol and water. After extraction with dichloromethane, seudenol was obtained in quantitative yield. When the same procedure was tried with seudenol laurate, a sticky solid was formed that could be redissolved by adding more water. However, extraction with dichloromethane was not successful due to excessive emulsion formation. Clearly, sodium laurate was much harder to remove from the organic layer than sodium butyrate.

Attempts were made to remove the salts by precipitation of the calcium salts by adding $CaCl_2$ after hydrolysis. Although this method worked on small-scale experiments, difficult filtrations were observed on a larger scale.

However, when the hydrolysis was performed with a nonaqueous 4 M solution of KOH in methanol, good results were obtained (Scheme 6). Concentrating the reaction mixture to dryness and slurrying the resulting solid with MTBE resulted in the precipitation of potassium laurate salts. These salts were removed by filtration, whereas (*R*)-seudenol remained in the MTBE. After washing the MTBE filtrate with water and evaporation of the solvent, pure (*R*)-seudenol was obtained in 83% yield and 95.8% ee.

Kilolab Production. This process was finally performed on a multikilogram scale to give the results shown inTables 1 and 2.

As can be seen in Table 1, the enzymatic resolution was remarkably reproducible: all three batches were stopped after 15 h at the same conversion, yielding (*R*)-seudenol laurate in nearly identical yield and purity. These batches were combined and subsequently allowed to hydrolyze in two batches. Although the yields are somewhat lower, (*R*)-seudenol is produced with a purity and enantioselectivity that meet our specifications (ee > 95%; purity > 95%). The lower yields can be explained by losses due to azeotropic coevaporation of seudenol during methanol and MTBE evaporations.

Conclusions

A practical method was developed for the production of (*R*)-seudenol based on an enzymatic resolution with vinyl laurate and Novozym 435 as a catalyst. By using an extractive work-up, seudenol and (*R*)-seudenol laurate could be separated without the need for chromatography. By hydrolyzing (*R*)-seudenol laurate with methanolic KOH, potassium laurate precipitated, and emulsions were avoided during work-up. With these modifications, the synthesis was performed on multikilogram scale in a reproducible manner to yield (*R*)-seudenol with 95.9% ee and >98% purity.

Experimental Section

General. All reagents and solvents were used without further purification. All NMR spectra were recorded on a Bruker 300 MHz spectrometer with TMS as internal standard. GC was conducted with an Agilent Technologies HP 6890 instrument with FID equipped with a HP 5 column (30 m × 320 μm × 0.25 μm). All reported values are based on the corrected integration values by using response factors. Pressure 10.7 psi; injector temperature 250 °C; detector temperature 300 °C. Temperature program: initial temperature 60 °C; initial time 10 min; rate 5 °C/min; final temperature 300 °C. Typical retention times (t_R's) were 7.8 min seudenol; 16.8 min seudenol acetate; 23.1 min seudenol butyrate; 42.1 min seudenol laurate.

HPLC was conducted with an Agilent Technologies HP 1090 instrument with DAD detection equipped with a Chiracel OD column (250 mm × 4.6 mm × 10 μm). Eluent heptane/2-propanol (99/1); flow rate 1 mL/min; temperature 40 °C. Typical retention times (t_R's) were 12.6 min (*S*)-seudenol; 13.5 min (*R*)-seudenol.

Standard Procedure for the Enzymatic Hydrolysis of Seudenol Esters. To 5 mL of a phosphate buffer solution of pH = 7.0 (50mM) were added 1 mmol of seudenol ester and 10 mg of enzyme. After stirring for 4 h at 37 °C, a 0.1 mL aliquot was taken and filtered over Celite. The Celite was washed with 0.5 mL of water and 1.5 mL of heptane. The organic layer was analysed by HPLC to determine both conversion and enantioselectivity.

Standard Procedure for the Enzymatic Transesterification of Seudenol Esters. In 2.5 mL of heptane were dissolved 1.1 mmol of seudenol ester and 4.4 mmol (4 equiv) of butanol. Ten milligrams of enzyme was added, and the reaction mixture was stirred at 50 °C. When according to TLC analysis a reasonable conversion was obtained, a 0.1 mL aliquot was taken and filtered over Celite. The Celite was washed with 1 mL of heptane, and the combined filtrate was analysed by HPLC to determine both the conversion and enantioselectivity.

Enzymatic Resolution of 100 g of Seudenol with Vinyl Butyrate. Seudenol (100 g, 0.892 mol), vinyl butyrate (204 g, 1.78 mmol), and Novozym 435 (2.22 g, 2.2 wt %) were added to 900 mL of heptane and stirred for 2.5 h at room temperature at 200 rpm. The suspension was filtered, and the conversion was determined by GC analysis.

After evaporation of the solvent on the rotary evaporator 143.2 g of a colorless oil was obtained, that was split in two portions and purified by column chromatography (silica gel; CH_2Cl_2; 10% ethanolic H_2SO_4 solution as revealer) to yield 77.1 g of seudenol butyrate (0.42 mol; 48% yield).

The product was dissolved in 625 mL of methanol, and 225 mL of a 4 M NaOH solution was added. An exothermic reaction took place, and the temperature rose to 35 °C. After stirring for 1 h the mixture was diluted with 500 mL of water and extracted with 400, 300, and 200 mL of CH_2Cl_2. The combined organic phases were washed with 400 mL of brine and dried over Na_2SO_4. After filtration and evaporation of the solvent 41.9 g (0.37 mol; 90% yield) of a yellow oil was obtained.

HPLC: 95.6% ee; GC: 98.1% purity; NMR (DMSO, 300 MHz) δ 1.33 (1H, m, C$\underline{H}_2$CHOH), 1.44 (1H, m, C$\underline{H}_2$CH$_2$CHOH), 1.61 (3H, s, C$\underline{H}_3$), 1.66 (1H, m, C$\underline{H}_2$CHOH), 1.72 (1H, m, C$\underline{H}_2$CH$_2$CHOH), 1.81 (2H, m, C$\underline{H}_2$CCH$_3$CHCHOH), 3.95 (1H, s(br), C$\underline{H}$OH), 4.44 (2H, d, CHO$\underline{H}$), 5.36 (1H, s, C$\underline{H}$CHOH).

Enzymatic Resolution of Seudenol with Vinyl Laurate (Determination of Conversion vs ee Curve). seudenol (20 g, 17.8 mmol), vinyl laurate (16.7 g, 73.6 mmol), and Novozym 435 (33 mg, 0.18 wt %) were allowed to stir for 2 days. After 3.5, 6.5, 22.5, 32, and 48 h a 5 mL sample was taken, filtered, and analyzed by GC to determine the conversion.

The heptane solution was washed four times with 5 mL of 10% aqueous methanol after which no more seudenol was present in the heptane layer, according to TLC analysis. The heptane was evaporated, and the resulting oil was dissolved in 1 mL of ethanol and 0.75 mL of 4 M KOH solution. After stirring for 1 h 0.23 g of $CaCl_2$ and some Celite were added, and the suspension was stirred for 15 min. After filtration, the filtercake was washed three times with EtOAc, and the combined organic layers were washed with brine and dried over Na_2SO_4 to yield a red oil that was analyzed by HPLC to determine the ee.

(*R*)-Seudenol Laurate by Extractive Work-up. Seudenol (20 g, 17.8 mmol), vinyl laurate (16.7 g, 73.7 mmol), and Novozym 435 (0.133 g, 0.67 wt %) were allowed to stir for 24 h. After filtering off the catalyst, the heptane phase was washed four times with 100 mL of 10% aqueous methanol and once with 100 mL of brine. Drying over Na_2SO_4, followed by filtration and evaporation, yielded 23.26 g of a colorless oil. GC analysis showed the presence of less than 0.1% of seudenol.

(*R*)-Seudenol by Nonaqueous Hydrolysis. Seudenol laurate (20.0 g, 67.9 mmol) was dissolved in a solution of 5.7 g (102 mmol) of KOH in 100 mL of methanol. After stirring for 1 h the methanol was evaporated, and the solid was suspended in 50 mL of MTBE. The suspension was filtered and washed with 60 mL of MTBE.

The combined filtrates were washed with 75 mL of water, and the aqueous phase was extracted with 50 mL of MTBE. The combined organic layers were washed with 50 mL of brine and dried over Na_2SO_4. Filtration and evaporation of the solvent yielded 5.33 g (70% yield) of a red oil.

Kilogram-Scale Production of (*R*)-Seudenol Laurate. To a stirred (±400 rpm) solution of 6.588 kg (58.73 mol) of seudenol and 5.98 kg of vinyl laurate in 53 L (8 vol) of heptane was added 44 g of Novozym 435 (0.67 wt %). After stirring for 15 h under a nitrogen atmosphere, a sample was taken, and GC analysis indicated a conversion of 34%. After filtering off the enzyme, the heptane phase was washed four times with 30 L of 10% aqueous methanol. A sample was taken, and GC analysis showed the presence of less than 0.1% of seudenol. The heptane layer was dried over Na_2SO_4, and the solvent was evaporated after filtration to yield 6.952 kg (40% yield) of colorless oil. GC shows a purity of 79.8%. The ee was shown to be 95.6% by HPLC analysis by hydrolyzing a sample of the obtained oil.

Kilogram-Scale Hydrolysis of (*R*)-Seudenol Laurate. KOH (4.2 kg) was dissolved at 0 °C in 35 L of methanol. To this solution was added a solution of 10 kg of (*R*)-seudenol laurate in 20 L of methanol. Five liters of methanol was used to rinse dropping funnel and lines, and the reaction mixture was heated at 60 °C for 30 min. After controlling the disappearance of seudenol laurate by TLC analysis, the reaction mixture was cooled to 40 °C, and the solution was concentrated to 15 L by applying a vacuum. This solution was allowed to evaporate further at the rotary evaporator at 40 °C and 200 mbar until no more distillate was collected.

Fifteen liters of MTBE was added, and the suspension was evaporated until no more distillate was collected. Fifty liters of MTBE was added, and the suspension was stirred for 5 min, after which it was filtered. The residue was washed with 15 L of MTBE, and the combined organic phases were washed with 25 and 15 L of water. The organic phase was dried over Na_2SO_4. After filtration the MTBE was evaporated at 40 °C at 300 mbar to yield 2.49 kg (65% yield) of (*R*)-seudenol. GC: 98.4%; HPLC: 96.0% ee.

Acknowledgment

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