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ENVIRONMENTAL BIOTECHNOLOGY

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White biotechnology for green chemistry: fermentative 2-oxocarboxylic acids as novel building blocks for subsequent chemical syntheses

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Abstract Functionalized compounds, which are difficult to produce by classical chemical synthesis, are of special interest as biotechnologically available targets. They represent useful building blocks for subsequent organic syntheses, wherein they can undergo stereoselective or regioselective reactions. ''White Biotechnology'' (as defined by the European Chemical Industry [http://www.europabio.org/white_biotech.htm], as part of a sustainable ''Green Chemistry,'') supports new applications of chemicals produced via biotechnology. Environmental aspects of this interdisciplinary combination include:

- Use of renewable feedstock
- Optimization of biotechnological processes by means of:
  - New ''high performance'' microorganisms
  - On-line mezasurement of substrates and products in bioreactors
  - Alternative product isolation, resulting in higher yields, and lower energy demand

In this overview we describe biotechnologically produced pyruvic, 2-oxopentaric and 2-oxohexaric acids as promising new building blocks for synthetic chemistry. In the first part, the microbial formation of 2-oxocarboxylic acids (2-OCAs) in general, and optimization of the fermentation steps required to form pyruvic acid, 2-oxoglutaric acid, and 2-oxo-D-gluconic acid are described, highlighting the fundamental advantages in comparison to chemical syntheses. In the second part, a set of chemical formula schemes demonstrate that 2-OCAs are applicable as building blocks in the chemical synthesis of, e.g., hydrophilic triazines, spiro-connected heterocycles, benzotriazines, and pyranoic amino acids. Finally, some perspectives are discussed.

Keywords White biotechnology · Green chemistry · 2-Oxocarboxylic acids · Triazines · Spiro-connected heterocycles

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Introduction

The historical link between industrial microbiology and technical chemistry at the beginning of the twentieth century was fruitful for both technical disciplines. For example, the anaerobic fermentation of acetone, glycerol, and butanol as feed stocks for explosives and synthetic rubber during World War I should be mentioned. However, chemical technologies that were, at that time, both more effective and ''cleaner'' were developed in order to avoid the low productivity and wastewater problems resulting from biotechnological processes.

As result of the oil crisis in the 1970s, various new strategies regarding the use of renewable feedstock for bulk chemical production were discussed. The most frequently used petrochemicals could be derived from a few products obtainable through anaerobic fermentations and dehydrations (Fig. 1).

The term ''biorefinery'' is used in strategic programs in the United States and other countries with an excess of agricultural products and a lack of oil reserves. The biorefinery can be described as ''clusters of bio-based industries producing chemicals, fuels, power, products, and materials'' [47, 50].

The application of biotechnological principles in chemistry has recently been named ''White Biotechnology''

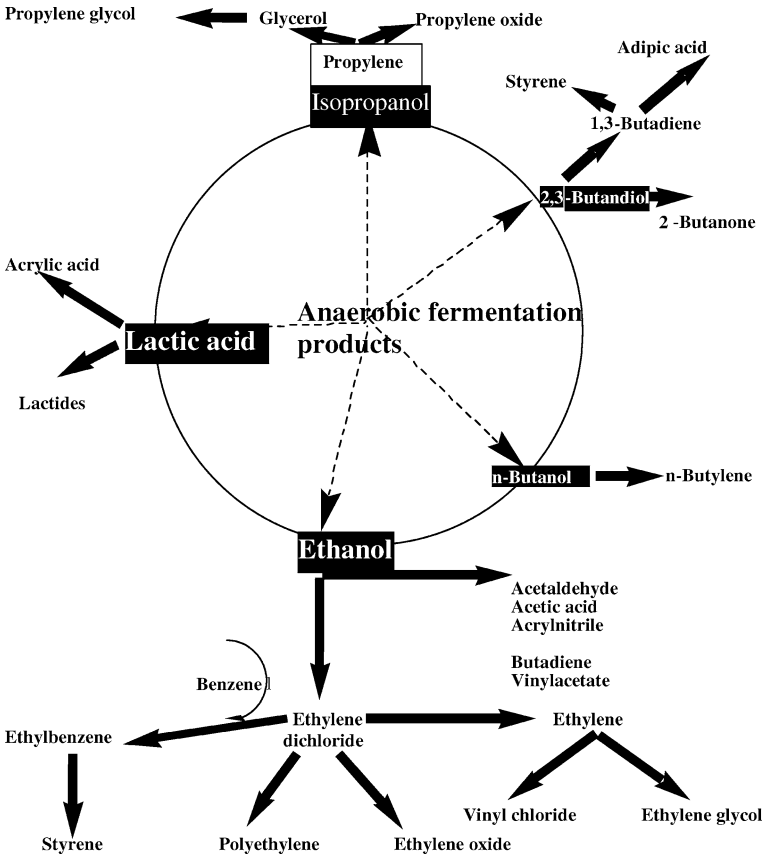


Fig. 1 Fermentation products as building blocks for synthesis of chemicals [17]

by an initiative of the European chemical industry, and is described as follows:

White Biotechnology is an emerging field within modern biotechnology that serves industry. It uses living cells like moulds, yeasts or bacteria, as well as enzymes to produce goods and services [49].

White Biotechnology connects chemistry, biology, and modern technologies and could be considered as a

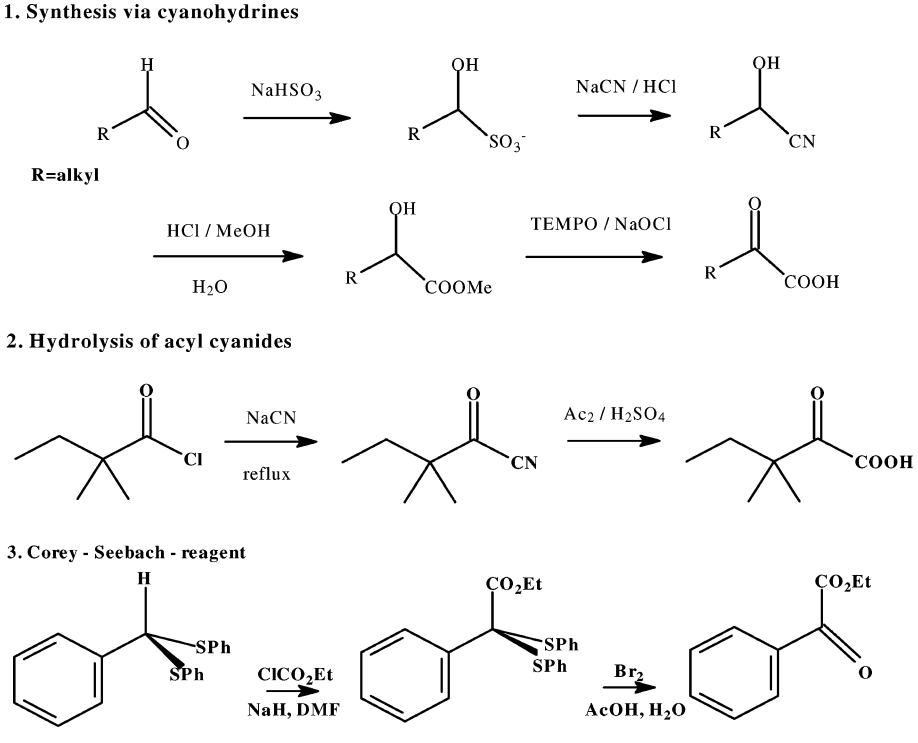
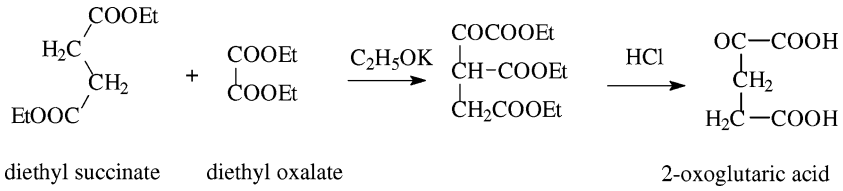


Fig. 2 Some chemical reaction sequences to 2-oxocarboxylic acids (2-OCAs)

Fig. 3 Chemical synthesis of 2-oxoglutaric acid (2-OGA)



promising part of the "Green Chemistry" defined by Anastas and Warner in 1998 [1]:

> *Green chemistry is the utilization of a set of principles that reduces or eliminates the use or generation of hazardous substances in the design, manufacture, and applications of chemical products.*

In general, "White Biotechnology" processes usually include environmental aspects. Although these aspects are often not highlighted in detail, in the ideal case they are always present and active—as should always be the case for modern technologies.

In summary, the environmental aspects of the link between biotechnology and "Green Chemistry" include:

- Use of renewable feedstock.
- Optimization of biotechnological processes, resulting in higher yield, minimized wastes, and lower energy demand, e.g., by means of:
  - new "high performance" microorganisms
  - on-line measurement of substrates and products in bioreactors
  - Integrated consideration of the entire process, including feedstock production, fermentation, and product isolation
- Sustainable socio-economical and regio-structural development.

As a contribution to this current development, we are investigating applications of biotechnologically accessible 2-oxocarboxylic acids (2-OCAs) as novel building blocks for the chemical synthesis of heterocyclic compounds and for regioselective and stereoselective chemical reactions.

Oxocarboxylic acids

General remarks

The chemistry of OCAs depends upon the relationship of carboxylic group(s) and oxo function. The known variants are 2-, 3-, 4-, 5-, and 6-OCAs, often synonymously called α-, β-, γ-, δ-, and ϵ-OCAs. Due to their role in biochemical cycles, 2-OCAs are of special interest. 2-OCAs are usually stable as acids and salts; 3-OCAs decompose by decarboxylation and form the corresponding ketones; 4-OCAs are stable and do not decompose.

Glyoxylic acid (OHC–COOH) [CAS 298-12-4] (synonyms: oxoacetic acid, glyoxalic acid) is the simplest 2-OCA and is important in biochemical metab-

Fig. 4 Chemical oxidation of protected D-fructose by $KMnO_4$ to form 2-oxo-D-gluconic acid (2-OGcA)

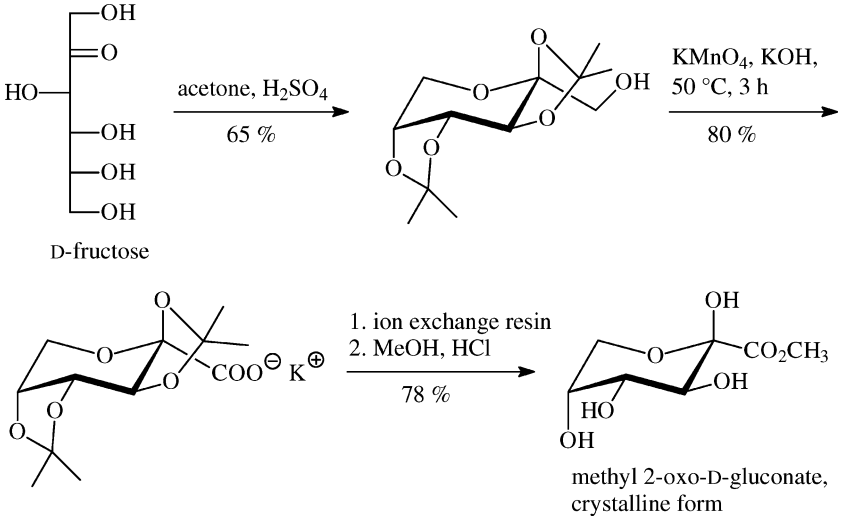


Table 1 2-Oxocarboxylic acids (2-OCAs) in nature and metabolism

OCA	R in R-CO-COOH	and application
Glyoxylic acid	H–	Main metabolite in glyoxylic acid cycle Free in unripe fruits Synthesis of allantoine and other compounds
Pyruvic acid	CH_3–	Central metabolite Synthesis of pharmaceutical compounds
2-Oxobutyric acid	C_2H_5–	Isoleucine-synthesis, threonine metabolite In milk and cheese
2-Oxoisovalerianic acid	$(CH_3)_2CH$–	Intermediate in metabolism of valine In cheese
2-Oxoisocapronic acid	$(CH_3)_2CH–CH_2$–	In amino acid metabolism in equilibrium with valine In cheese
2-Oxophenylpropionic acid	C_6H_5)–CH_2–	Metabolite of phenylalanine In tobacco
2-Oxoglutaric acid	HOOC–$(CH_2)_2$–	Metabolite in TCA-cycle Amino acid metabolism Chemical synthesis of heterocyclic compounds (this review)
2-Oxo-D-gluconic acid	D-*arabino*–CH_2OH–$(CHOH)_3$–	Chemical conversion to isoascorbic acid and synthesis of heterocycles (this review)
2,5-Dioxo-D-gluconic acid	D-*threo*–CH_2OH–CO–$(CHOH)_2$–	Microbial derivitization to 2-oxogulonic acid (vitamin C synthesis)

olism. This acid has two reactive groups and is produced by oxidation of glyoxal by nitric acid, by anodic oxidation, or by catalytic oxidation of ethylene [29]. Glyoxylic acid is used in the synthesis of various heterocyclic compounds, as well as some other technically important syntheses.

The most important OCAs in synthetic chemistry are pyruvic acid (H_3C–CO–COOH) and acetoacetic acid (H_3C–CO–CH_2–COOH) and their derivatives. Pyruvic acid can be synthesized chemically by dehydration and decarboxylation of tartaric acid or by vapor phase catalytic oxidation of lactic acid with molecular oxygen in the presence of a catalyst comprising iron phosphate and palladium [28]; biological methods are described below. Pure acetoacetic acid is a thermally unstable 3-OCA that tends to decarboxylate easily. However, stable esters and amides exist in tautomeric keto-enol forms. Ethyl acetoacetate, the synthesis of which is exclusively chemically, is a very important synthetic precursor for pharmaceuticals, dyes, and pigments. Annual production is reported to be 80,000 t [24]. Acetonedicarboxylic acid (HOOC–CH_2–CO–CH_2–COOH) [CAS 542-05-2] [synonyms: 3-oxoglutaric acid, β-ketoglutaric acid, 3-oxo-pentane dicarboxylic acid], which is stabilized by the second carboxyl group, is also an important starting material for the synthesis of pharmaceutically active alkaloids; it is also used as a stabilizer for natural fats and oils. Production proceeds from citric acid after oleum treatment or by similar paths of citric acid oxidation.

Levulinic acid (H_3C–CO–CH_2–CH_2–COOH) [CAS 123-76-2] (synonyms β-acetylpropionic acid, γ-ketovaleric acid, 4-oxopentanoic acid) is synthesized chemically from fructose via 5-(hydroxyl methyl) furfural. This OCA is used as raw material for the synthesis of pharmaceuticals, and for the production of plasticizers.

General synthetic routes to 2-OCAs

Some recent methods for the synthesis of 2-OCAs and derivatives are summarized in Fig. 2.

The most versatile acyl anion equivalent (Fig. 2, Eq. 3) is the Corey–Seebach reagent, which may be alkylated and the conjugate anion of the intermediate treated with

Table 2 Selection of pyruvic acid (PA)-producing yeast strains [25]

Microorganism	Carbon source	Nitrogen source	Time (h)	PA (gL^{-1})	Yield (gg^{-1})	Reference
Torulopsis glabrata TR-2026	Glucose	Polypeptone	60	52.1	0.52	[32]
T. glabrata ACII33	Glucose	Peptone	60	56.8	0.58	[33]
T. glabrata IFO 0005	Glucose	Soybean hydrolysate, NH_4^+	67	67.8	0.50	[34]
T. glabrata ACII-3	Glucose	Soybean hydrolysate, NH_4^+	47	60.3	0.68	[35]
T. glabrata WSH-IP 303	Glucose	NH_4^+	56	69.0	0.62	[26]

Table 3 Formation of 2-oxoglutaric acid (2-OGA) by microorganisms

Microorganism	Carbon source	2-OGA (gL^{-1})	Yield (gg^{-1})	References
Arhthrobacter paraffineus	*n*-Paraffins	60	0.74	[43]
Candida lipolytica	*n*-Paraffins	48	0.60	[44]
C. lipolytica (diploid)	*n*-Paraffins	185	0.80	[27]
Yarrowia lipolytica H222-27-11	*n*-Paraffins (fed-batch)	195	0.90	[46]
Y. lipolytica	Ethanol (fed-batch)	49	0.42	[13, 20]
Y. lipolytica H222-27-11	Sunflower oil	35	0.38	[9]
	Rapeseed oil	34	0.37	

ethyl chloroformate at a convenient stage to introduce the carboxyl function. However, low yields and the application of dangerous and toxic chemicals and solvents mean that alternative routes to special 2-OCAs are always welcome.

Deamination of amino acids by trifluoro acetic anhydride results in the corresponding 2-OCAs [24]. Synthetic procedures are known for compounds of special interest, but they are often difficult or costly. Pyruvic acid [CAS 127-17-3] (synonyms: 2-oxopropanoic acid, pyroracemic acid, α-ketopropanoic acid, here abbreviated as PA) is produced on an industrial scale by dehydration and decarboxylation of tartaric acid. The PA is distilled from the mixture of tartaric acid, sodium and potassium sulfate, and purified by vacuum distillation. Other processes, summarized by Klingler and Ebertz [24], are the gas-phase oxidation of lactic acid, recently patented [29], the hydrolysis of 2,2-dihalopropionic acid and the oxidation of methylglyoxal with halogens.

2-Oxoglutaric acid (2-OGA) [CAS 328-42-7], (synonyms: 2-ketoglutaric acid, 2-oxopentanedioic acid, α-ketoglutaric acid, oxoglutaric acid—here taken to mean 2-OGA) can be synthesized from succinic acid and oxalic acid diethyl esters with a yield of 75% (Fig. 3; yields for starting material syntheses not included).

2-Oxo-D-gluconic acid [CAS 669-90-9] (synonyms: D-arabino-2-hexulonic acid, D-arabino-hexaric acid, 2-ketogluconic acid, 2-ketogluconic acid, D-glucosonic acid, mannonic acid, here abbreviated as 2-OGcA) is one of the isomeric oxo-hexaric acids. In principle, 2-OGcA is accessible by chemical oxidation of protected D-fructose with $KMnO_4$ at a 40% yield (Fig. 4); however, this has been performed only on the laboratory scale, and only with difficulty.

A procedure such as this demonstrates some typical disadvantages of chemical synthesis in comparison to biotechnology. An expensive and harmful oxidant ($KMnO_4$) must be used at least in stoichiometric amounts, and a waste product (MnO_2) with oxidizing properties is formed. Furthermore, the reaction requires several steps with individual work-up procedures. Catalytic processes using mixed noble metal catalysts produce desirable mixtures of different acids but show no advantages concerning yield and final concentration in comparison to biological oxidation (see below) [39].

2-OCAs: occurrence in nature and role in metabolism

2-OCAs are important intermediates in metabolism, both in catabolism and anabolism. They form the link between the metabolism of amino acids, carbohydrates, hydrocarbons and fats. Table 1 shows some 2-OCAs detected in foods, excreted from cells, or transformed by direct oxidation from microorganisms in solution.

PA and 2-OGA are formed by microorganisms in concentrations of more than 65 gL^{-1}. 2-OGcA is an intermediate during the technical synthesis of isoascorbic acid. However, this 2-OCA is not commercially available as a free acid or ester. Only the hemicalcium salt is available as an expensive fine chemical [CAS 304655-85-4] on the gram scale (price about €70 g^{-1}).

Microbial synthesis of some 2-OCAs and optimization of fermentation

In general, for application as raw materials for chemical synthesis, an important consideration may be that biologically formed 2-OCAs are not commercially available at acceptable prices. This is mainly the case for the oxohexulosonic acids. However, Li et al. [25] published a cost comparison between chemical and biological con-

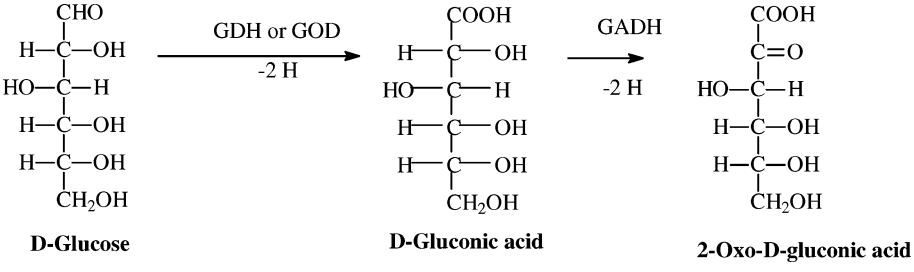


Fig. 5 Biological oxidation of D-glucose to 2-oxo-D-gluconic acid (2-OGcA). *GDH* Glucose dehydrogenase, *GOD* glucose oxidase, *GADH* gluconic acid dehydrogenase

Table 4 Optimization of 2-oxo-D-gluconic acid (2-OGcA) formation [40]

Microorganisms	Substrate	Yield (gg^{-1})	Productivity ($gL^{-1}h^{-1}$)	2-OGcA (gL^{-1})
Gluconobacter oxidans CCM 1804	Glucose	0.85	2.8	100
Serratia marcescens IMET 11312	Gluconic acid	0.95	5.1	185

version methods, calculating, for PA fermentation, costs of only about 15% of the chemical route when compared with the tartaric acid route.

There is general interest in replacing petrochemicals with renewable products. Biotechnological applications are favored as a means to reach this goal. The most promising 2-OCAs are PA, 2-OGA and 2-OGcA, which are all well known as products of biosynthesis and biotransformation. Recently, the preparation of 2-OCAs from carbon dioxide has been patented [19]. Carbon dioxide is made to react with an aldehyde compound (acetaldehyde, propionaldehyde) under mild enzymatic conditions. Decarboxylases, e.g., PA decarboxylase, in the presence of thiamine are suitable for this purpose. Carbon dioxide is applied under supercritical conditions. The yield, relative to the corresponding aldehyde, can be higher than 50%. The following section describes—partly including our own work—the optimization of these "old" reactions by applying some new principles in process optimization.

Pyruvic acid

The microbial oxidation of D-(−)lactic acid offers a high yield [15]. Using *Acinetobacter* strains, conversion was complete after 5 h. L-(+)-Lactic acid was not converted. However, the final concentration of PA was no higher than 19.6 gL^{-1}. Izumi et al. [21] described the microbial oxidation of 1,2-propane diol. However, the highest PA concentration achieved was only 14.6 gL^{-1}.

The recently described screening of PA-producing yeasts was more successful [45]. Using an isolated *Trichosporon cutaneum* strain with glucose as carbon source, the highest reported concentration of PA achieved was 34.6 gL^{-1}, corresponding to a yield of 0.43 gg^{-1}.

A complete survey of microorganisms, as well as carbon and nitrogen sources for PA production by yeast is given by Li et al. [25]. Table 2 shows the highest values reported for concentration and yield.

PA is also formed from glucose by (*Candida*) *Yarrowia lipolytica* under thiamine-limiting conditions, although in lower concentrations than by the described *Torulopsis* strains [25]. Because of the thiamine dependence of the PA dehydrogenase complex, thiamine auxotrophic strains seem more promising for further optimization. Preferred substrates for microbial PA secretion are all C-sources that can be metabolized via glycolysis or related pathways (e.g., carbohydrates and glycerol). This aspect was considered recently in a screening procedure with thiamine

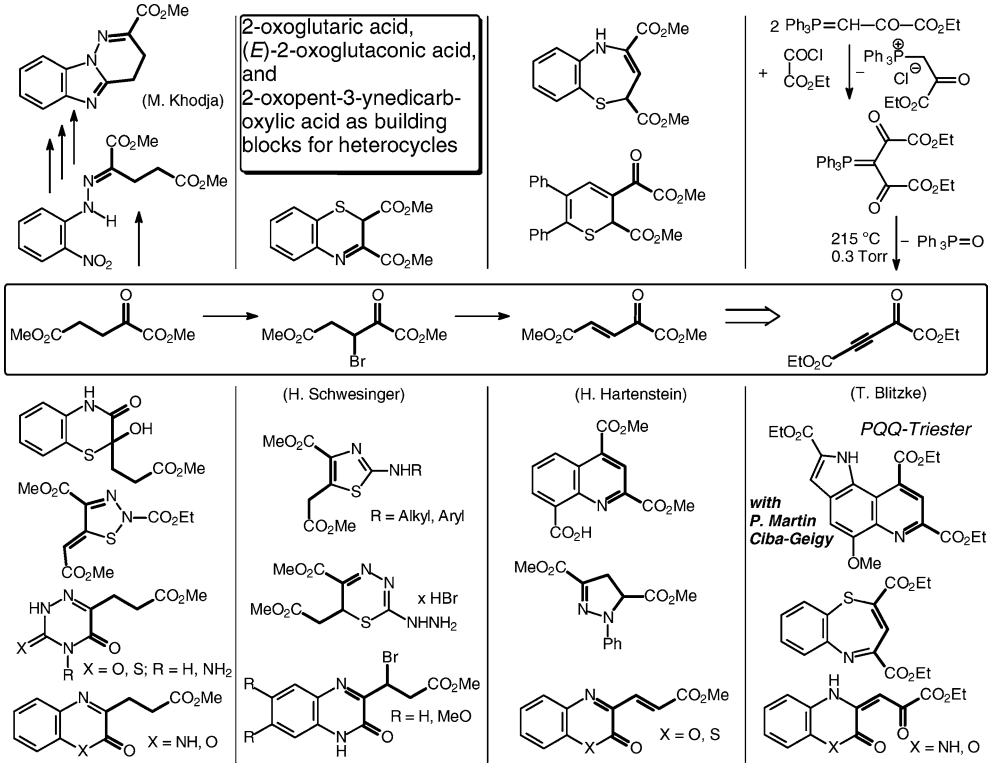


Fig. 6 2-OGA dimethyl ester and analogous diesters as building blocks for heterocycles

Table 5 Types of heterocyclic compounds synthesized from 2-OGA

Type of heterocyclic compound	Features
Five-membered heterocycles	
Monocyclic systems	One hetero atom
Bicyclic systems	One heterocyclic ring with one hetero atom
Polycyclic systems	One or more heterocyclic rings with one hetero atom each
Monocyclic systems	Two hetero atoms
Bicyclic systems	One heterocyclic ring with two hetero atoms
Bicyclic and polycyclic systems	Two und more heterocyclic rings with two hetero atoms
Six-membered heterocycles	
Monocyclic systems	One hetero atom
Polycyclic systems	One heterocyclic ring with one hetero atom
Polycyclic systems	Two hetero cyclic rings with one hetero atom each
Monocyclic systems	Two hetero atoms
Bicyclic systems	One heterocyclic ring with two hetero atoms
Bicyclic systems	Two heterocyclic rings, each with two hetero atoms
Monocyclic systems	Three hetero atoms
Bicyclic systems	Two heterocyclic rings with three hetero atoms
Seven-membered heterocycles	
Polycyclic systems	Two heterocyclic rings

auxotrophic *Y. lipolytica* strains using glucose or glycerol as substrates [36]. Under thiamine limitation (1–3 $\mu g L^{-1}$) of yeast growth, a maximal PA concentration of 61.3 $g L^{-1}$ was reached with glycerol as C-source. The corresponding substrate-related yield was 0.71 $g g^{-1}$. Similarly, Li et al. [25] listed bacteria described as being capable of PA formation. However, the highest PA concentration achieved is only about 30 $g L^{-1}$ (yield 0.60 $g g L^{-1}$)—too low to be of interest for technical application.

The enzymatic oxidation of L-lactic acid by molecular oxygen is possible in the presence of the two enzymes glycolate oxidase [(S)-2-hydroxy-acid oxidase] and catalase. The yield is up to 96%, and concentrations of up to 0.5 M are regarded as commercially acceptable [8].

2-Oxoglutaric acid

As an intermediate of the Krebs cycle, 2-OGA—like citric acid—can be exuded exocellularly by microorganisms (see Table 3). The intracellular energy regulation under conditions of growth limitation and unlimited substrate uptake of the cells grown will now be discussed: a precondition for this process is the limitation of reproductive cell growth by nutrient exhaustion from the culture broth. In the case of 2-OGA, thiamine limitation is necessary because of the thiamine dependence of the 2-OGA-dehydrogenase complex. Inactivity of this enzyme causes a metabolic overflow of 2-OGA. In practice, thiamine limitation can be realized only by thiamine auxotrophic strains, and in the absence of complex suppline sources such as yeast extract. Suitable substrates for 2-OGA production with *Y. lipolytica* are C-sources that are metabolized via the intermediate acetyl-CoA. In this way, accumulation of the undesired by-product PA is minimized.

Y. lipolytica H 222-27-11 is able to synthesize 2-OGA from purified *n*-paraffin with a chain length of C_{12}–C_{18} under aerobic conditions with controlled thiamine limitation (not higher than 1 $\mu g L^{-1}$), and to excrete up to 195 $g L^{-1}$ 2-OGA into the fermentation broth after feeding with *n*-paraffins [46]. The substrate-related yield is 90%, and the productivity is 1.4 $g L^{-1} h^{-1}$. The content of other carboxylic acids after 150 h of fermentation was lower than 5%,

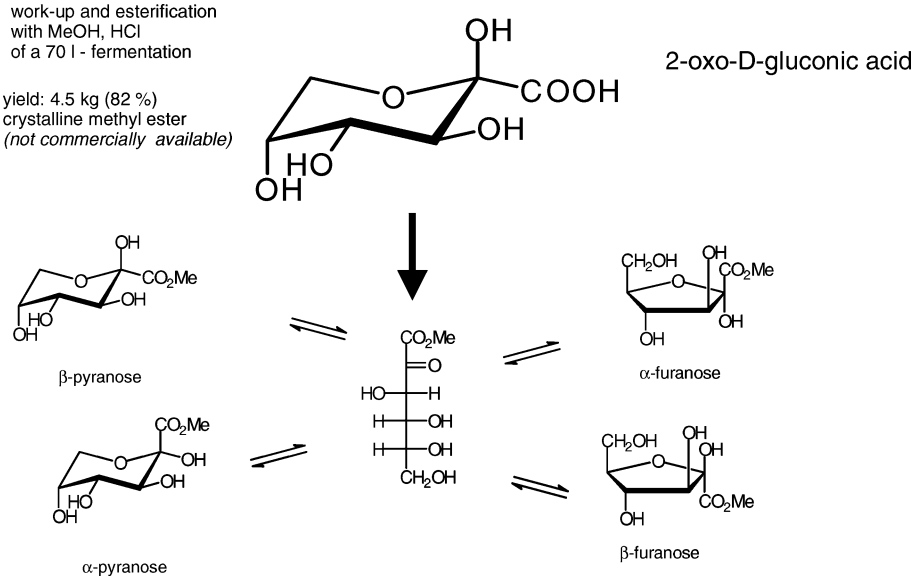


Fig. 7 Isomerism of D-arabino-3,4,5,6-tetrahydroxy-2-oxo-hexaric acid methyl ester

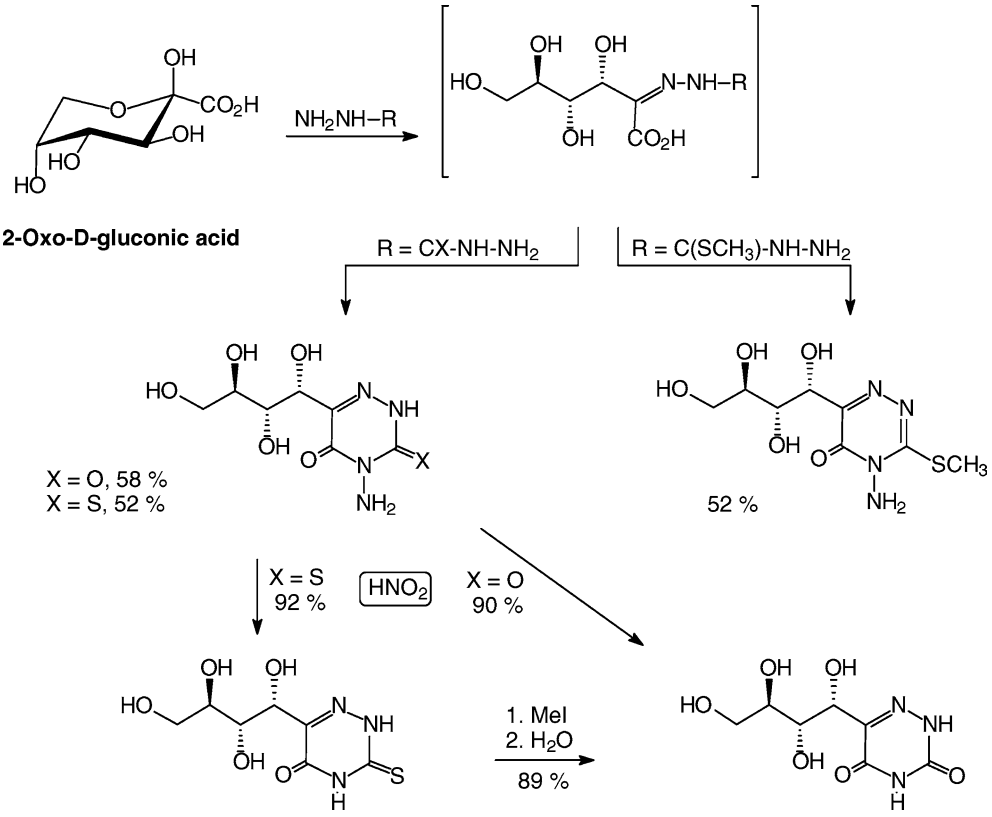


Fig. 8 Direct synthesis of 6-(D-arabino-tetritol-1-yl)-1,2,4-triazines from 2-OGcA

indicating the specificity of the process. This fermentation process results in final concentrations that are higher than in the commercial citric acid process. Experiments using substrates from renewable feedstock in shake-flasks indicate that triglycerides (sunflower and rapeseed oil) or fatty acids are potentially efficient C-sources for 2-OGA production with *Y. lipolytica* H 222-27-11 [9]. Preconditions for process optimization include a suitable mutant of *Y. lipolytica*, and thiamine limitation between 0.1 and 1 μgL^{-1}. Balancing the thiamine requirement of the growing biomass and the limitation for optimum 2-OGA excretion is the current challenge facing process optimization and modeling in this case. Ethanol can also serve as a carbon source (Table 3).

2-Oxo-D-gluconic acid

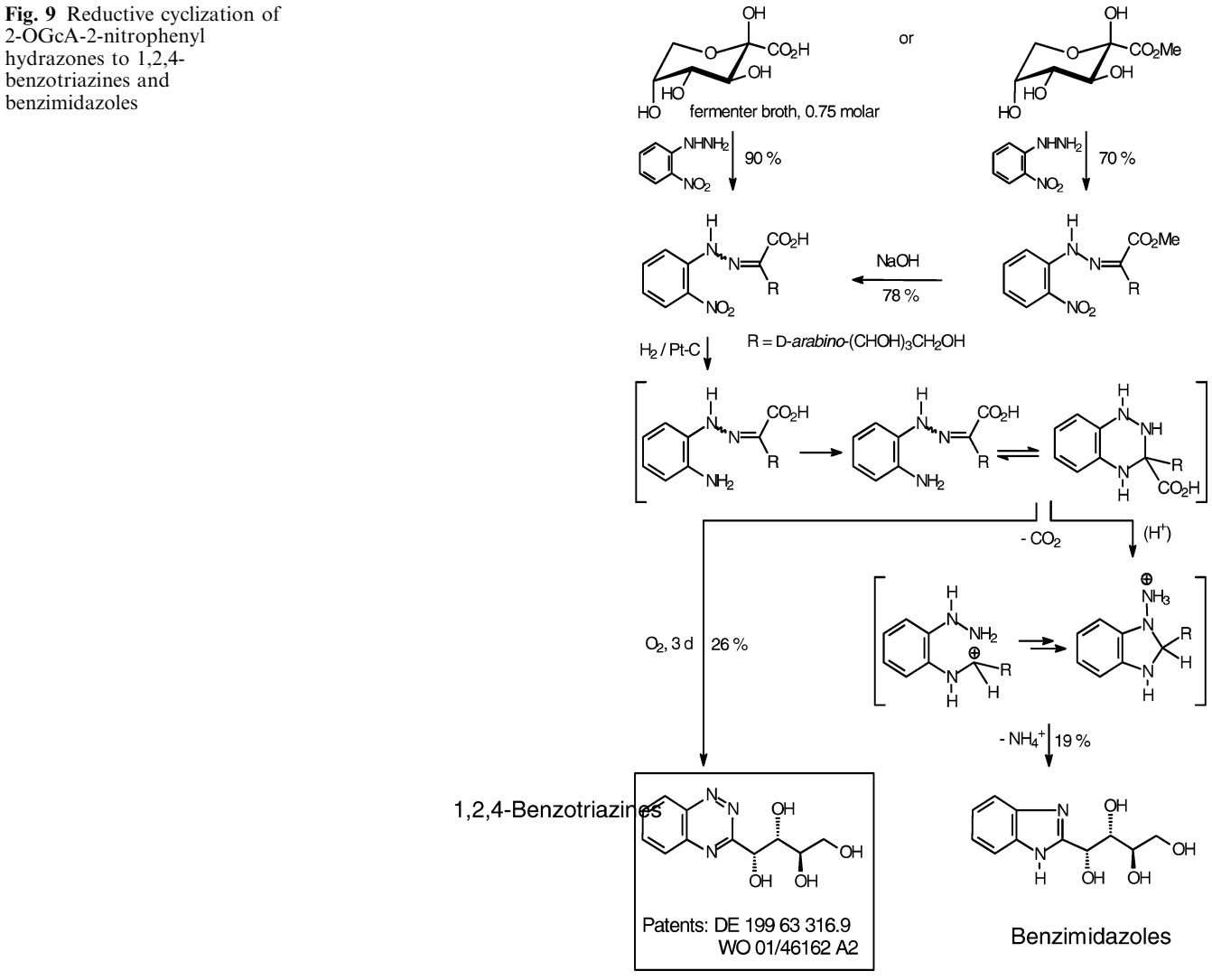
Many bacteria and fungi show the ability to oxidize exocellular glucose and gluconic acid to 2-OGcA. The reaction steps and participating enzymes are shown in Fig. 5. However, older papers, especially those published before the 1960s, should be considered critically, because reliable analytical speciation of the various oxidation products of glucose was possible only after the availability of HPLC or IC.

In many patents and papers, the following microorganisms are described as having the ability to oxidize glucose or gluconic acid : *Gluconobacter cerinus*, *Pseudomonas aeroginosa*, *Pseudomonas fluorescens*, *Gluconobacter oxydans*, *Acetobacter methanolicus*, *Serratia marcescens*, *Klebsiella aerogenes*, *Cyanococcus chromospirans*, and *Erwinia* sp.

The yield is described as being between 0.85 and 0.90 gg^{-1}, with final concentrations of no more than 100 gL^{-1}. Stubbs et al. [42] calculated a productivity of 3.2 gL^{-1} h^{-1}. Starting with 120 gL^{-1} glucose, concentration-dependent inhibition was observed in batch fermentations. Therefore, a glucose feeding process was developed by Misenheimer et al. [31]. The highest productivity calculated by these latter authors was 7.50 gL^{-1} h^{-1}, with a yield of 0.95–1.0 gg^{-1}.

Controlled substrate feeding is very important. If the concentration of glucose is too low or is limiting, the oxidation process is decreased, probably due to biomass growth or shock effects. If the concentration is too high (fed-batch), the overall yield is decreased or the oxygen dissolution is lower than the supply by the stirrer. The application of glucose-specific biosensors will certainly offer new possibilities for feeding strategies.

Stottmeister et al. [40] have developed a process with high productivity and final concentrations of up to 185 gL^{-1} 2-OGcA (Table 4). This process was based on the oxidation of gluconic acid with *S. marcescens*. It was favorable to combine this procedure with the Vogelbusch process [51] of gluconic acid formation by rapid glucose oxidation with *A. methanolicus*. The formation of 5-OGcA or 2,5-OGcA as unwanted by-products was not observed.



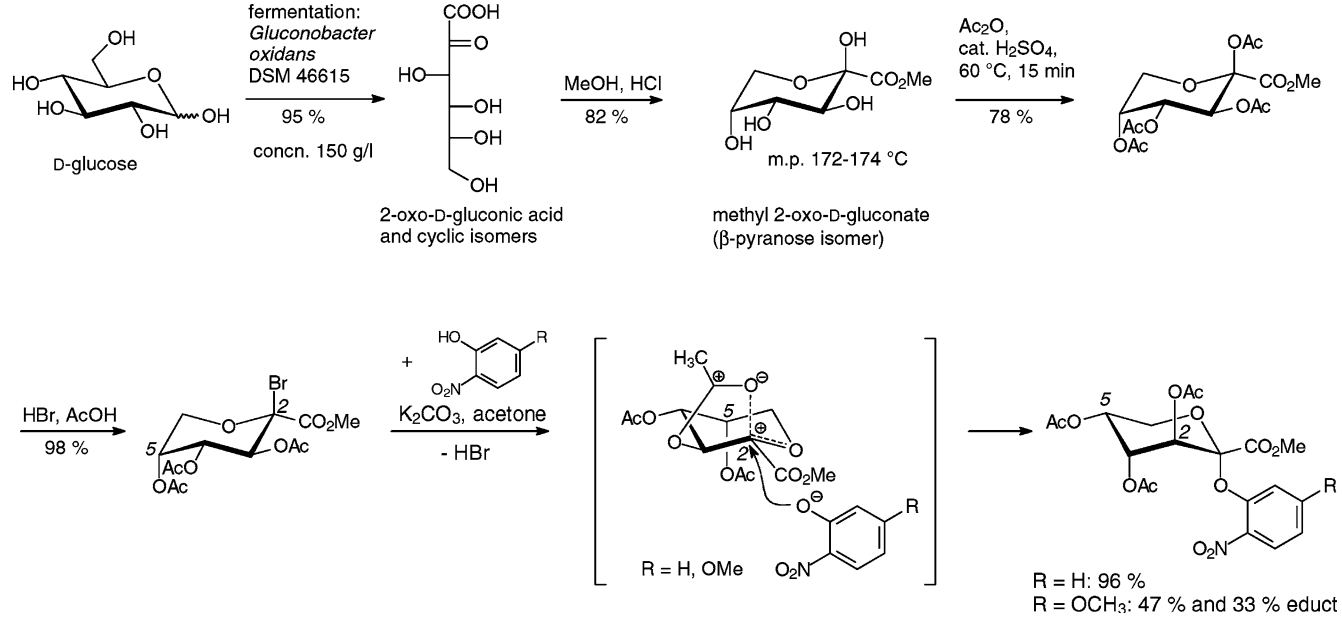


Fig. 9 Reductive cyclization of 2-OGcA-2-nitrophenyl hydrazones to 1,2,4-benzotriazines and benzimidazoles

Fig. 10 Diastereoselective 2,3-trans-glycosidation to form precursors for reductive cyclizations

Selected OCAs as building blocks

Pyruvic acid

PA is a highly reactive 2-oxo acid and is therefore often used in synthetic organic chemistry [14]. The molecule can react as both an acid and a ketone. The corresponding α-amino acid is alanine. Reaction products via various routes include tetrahydroisoquinolines, quinoxalinols, hydroxypteridines, diarylpropionic acids, among others (for an overview, see [24]). The formation of heterocyclic compounds, especially of substituted asymmetric triazines, is of interest as a source of intermediates for pharmaceutical and agrochemical products. The formation of 3-amino-as-triazines from PA (and related compounds) is described in a patent [30]. PA is used in a cyclocondensation reaction to form condensed 1,2,4-triazines with antibacterial activity [38].

2-Oxoglutaric acid

Despite its high reactivity, the application of 2-OGA is limited chiefly to condensation and cyclocondensation reactions, which include the carbon atoms C-1 and C-2.

As an advantage in the isolation of the 2-OGA from fermentation broth, it is possible to directly precipitate the corresponding hydrazones after biomass filtration. The formation of these hydrazones is the first step in the cyclization reaction sequences. In this way, the expensive isolation of 2-OGA (as in the case of other fermentatively formed 2-OGAs) is not necessary. In principle, this is an example of an elegant "direct product

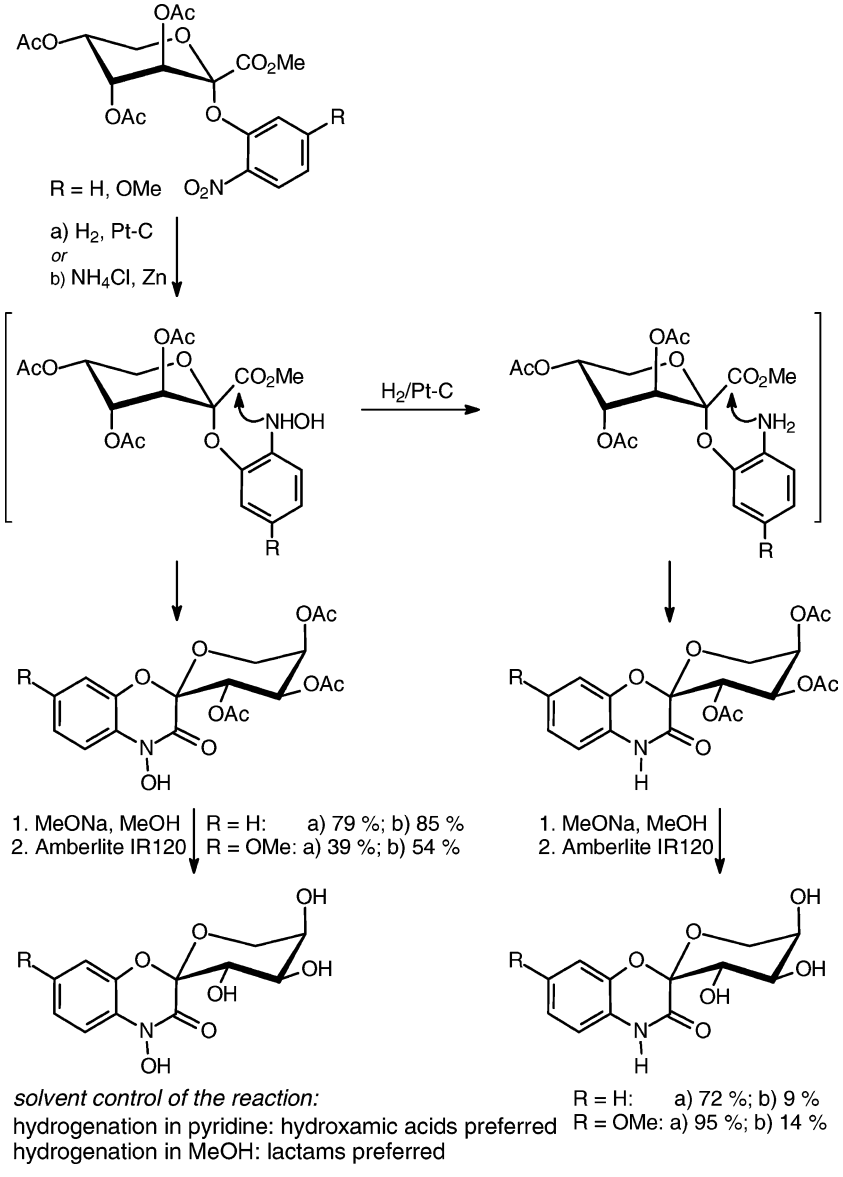


Fig. 11 Reductive cyclization to form spiro-connected saccharidic heterocycles

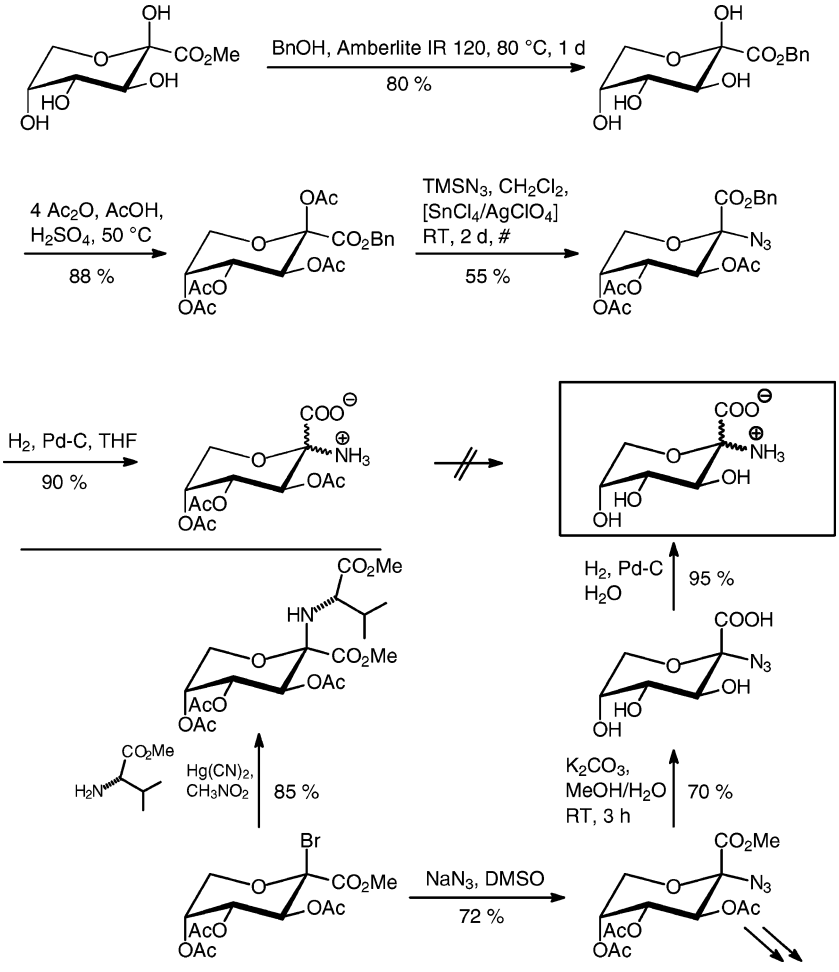


Fig. 12 Possible pathway from methyl 2-oxo-D-gluconate (OGcMe) to a novel type of sugar amino acid

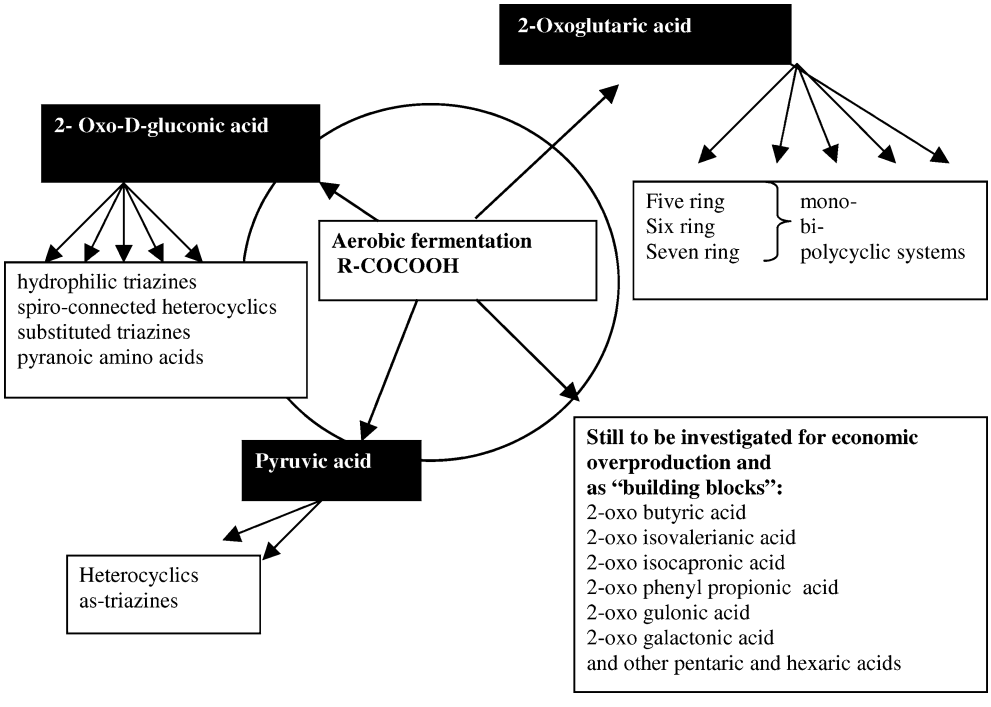


Fig. 13 Fermentative 2-OCAs—existing building blocks and future candidates

recovery'' or ''direct capture of products'' from fermentation broth (see [48]).

Figure 6 gives a schematic overview of various different types of heterocycles synthesized in our group from dimethyl 2-OGA and dimethyl (*E*)-2-glutaconate (obtained from 2-OGA), together with products obtained from the homologous diethyl 2-oxo-pent-3-ynedicarboxylate (obtained independently). This shows the huge potential of 2-OGA as a building block or building block precursor. Based on this principal scheme, we were able to synthesize more than 70 novel compounds on the basis of 2-OGA and its analogs [10–12, 18, 22, 37]. Various parts of the C_5-chain of the educts have been incorporated into rings. The multiple applications of 2-OGA as building blocks in the synthesis of heterocyclic compounds is summarized in Table 5.

2-Oxo-D-gluconic acid

The chemical structure of 2-OGcA was also considered very promising for the synthesis of heterocyclic compounds, based on practical experience with 2-OGA as a building block. Due to the polyol functionalization and chirality of this saccharidic acid, any heterocycles obtained from it are also equipped with hydrophilicity and chirality. Heterocycles based on 2-OGcA have not previously been reported. Obviously, a principal reason for this is that the free acid is not commercially available and the hemicalcium salt is sold only in analytical amounts at a high price (see above). In contrast to its relatively simple biotechnological formation, isolation of 2-OGcA from the fermentation broth is quite demanding. Attempts to isolate 2-OGcA from biomass-free fermentation solution to a solid state by removal of water failed. Like other carbohydrates, 2-OGcA proved to exist as a syrup in pure form. It turned out, however, that refluxing 2-OGcA syrup in methanol led to the methyl ester, which crystallized with good yields into the β-pyranose form, similar to solid D-fructose. This methyl 2-oxo-D-gluconate (2-OGcMe) is now available from us on the kilogram scale and has proved to be a versatile starting material for syntheses; it is stable and stores excellently under normal conditions [23]. The preparative method has been optimized several times.

Alternatively, a chemical synthesis can in some cases be started directly with the 2-OGcA solution. For instance, substituted hydrazines can be added to cause the well-known formation of insoluble hydrazones, which precipitate and can be filtered off. The biggest advantage of this procedure is that the valuable product is removed from the fermentation broth in the form of an insoluble derivative without the need to remove the water by distillation, which is an expensive task.

To illustrate various synthetic possibilities, the following section contains an overview of the synthesis of several types of saccharide-heterocycle combinations, illustrating the formation of hydrophilic triazines, benzotriazines and benzimidazoles, which contain the saccharidic moiety in the form of an open chain tetritolyl unit. Additionally, annelated heterocycles to which a cyclic sugar part is attached in spiro-bonded form are described. In some circumstances, the possibility of a heteroanalogous modification of the cyclic oxo acid into a special sugar amino acid is reported. In such a hybrid structure, the anomeric C-atom of the sugar moiety resembles the α-C-atom of an amino acid.

Figure 7 shows the equilibrium between the pyranose and furanose isomers of 2-OGcMe, which has already been elucidated in detail by Crawford et al. [16]. The β-pyranose isomer obtained by crystallization was used for all described reactions.

2-OGcA, e.g., in the form of the original fermentation solution, can be reacted with carbohydrazide, thiocarbohydrazide or S-methylthiocarbohydrazide to give the corresponding carbohydrazones and thiocarbohydrazones, respectively. These can all be cyclocondensed to form novel 1,2,4-triazinones with a hydrophilic chiral side chain (Fig. 8).

A fermentation broth of either 2-OGcA or crystalline 2-OGcMe can be used as a precursor for the preparation of functionalized 1,2,4-benzotriazines and benzimidazoles according to Andersch and Sicker [2] (Fig. 9). 2-Nitrophenylhydrazones precipitated in the first step are reacted in a sequence of reductive cyclization and eliminations to form the above heterocycles. As with 1,2,4-triazinones, protecting groups are not necessary here. Such benzotriazines have been patented as potential anti-cancer compounds [41].

The formation of spiro-connected saccharidic heterocycles requires the use of protecting groups. In pure acetic anhydride, a rapid reaction led exclusively to the β-pyranose tetraacetate of 2-OGcMe with good yield. Nucleophilic substitution of the acetate at the anomeric centre with a solution of 33% hydrogen bromide in acetic acid yields the glycosyl bromide, which can then react diastereoselectively with various nucleophiles, such as 2-nitrophenols (shown in Fig. 10), 2-nitrohydroxypyridine, 2-nitrothiophenol and 2-aminothiophenol, producing *ortho*-nitro-aryl-*O*-glycosides and *S*-glycosides as glycosidic precursors for heterocyclizations.

In the next step, glycosides of this type can be reductively cyclized to obtain a spiro junction of the sugar unit with a heterocycle. For example (Fig. 11), 2-nitrophenyl-*O*-glycosides can also be cyclized under different reducing conditions to yield hydrophilic cyclic hydroxamic acids or lactams, which belong to the spiro[pyrido[3,2-*b*][oxazin-2,2′-pyran] skeleton [6]. Similarly, suitable precursors have been cyclized to spiro[1,4-benzothiazin-2,2′-pyrans] [3], spiro[1,4-benzoxazine-2,2′pyrans] [5] and spiro[pyrido[3,2-*b*][1, 4]oxazin-2,2′-pyrans] [4]. These substances represent a novel spiro compound type.

Finally, Fig. 12 provides an overview of first results obtained from a heteroanalogous modification of the cyclic oxo acid into a special sugar amino acid [7]. In such a hybrid structure, the anomeric C-atom of the sugar moiety is also the α-C-atom of an amino acid.

Properties of such hybrid natural products are under investigation.

Perspectives

Well-known metabolites such as 2-OCAs show a high potential for novel applications in organic synthesis. In the present review, we have summarized the application of some well-known microbial metabolites and oxidation products as building blocks in organic synthesis, with special emphasis on *N*-heterocyclic compounds.

From the structural point of view, many metabolites show promising features for the chemist, e.g., reactive groups and even chirality, if they are produced by fermentation from chiral pool carbohydrates. However, not all of these compounds are, at present, easily accessible. Many highly functionalized compounds are not yet of interest for the preparative chemist because they are only available, if at all, as very expensive fine chemicals. Thus, accessibility and availability are the most important prerequisites in order to awaken the interest of chemists, with the price of the biotechnologically generated product being an important precondition for technical interest. The price of the carbon source and the optimization of the standard fermentation are therefore the most important factors determining the applicability of a given product as a "building block" for chemistry.

In general, renewable carbon sources, and inclusion of environmental aspects in the process, are of great relevance for the future. We have demonstrated that the three selected examples of 2-OCAs show the potential for cheaper production via the biotechnological method than via the traditional chemical method. In summary, these compounds should no longer be regarded only as exotic fine chemicals or biochemicals to be used exclusively for research.

For the chemical production of PA, Li et al. [25] calculated costs of US \$8,650 t^{-1}, in contrast to fermentation costs of US \$1,255 t^{-1}. According to our own work, 2-OGA is produced by a new strain of *Y. lipolytica* from *n*-paraffins in concentrations comparable to, or higher than, those achieved by commercial citric acid fermentation. In addition, in the case of synthesis of *N*-heterocycles via the reaction route of reductive cyclization of hydrazones, a direct product recovery isolation/reaction step could be performed via hydrazone precipitation (Fig. 9). 2-OGcA can be produced from D-glucose as well as from D-gluconic acid. The latter is a mass product (about €1,000 t^{-1}) and could be utilized in direct microbial oxidation. 2-OGcA and 2-OGcMe are promising building blocks for cyclization reactions leading to novel types of heterocycle–saccharide combinations.

Finally, we would like to reiterate that many compounds arising from metabolism, or as natural products, with the promising 2-OCA group in their structure are suitable for use as potential building blocks in "green" chemistry. Figure 13 summarizes some of the existing applications of 2-OCAs in organic chemistry, and outlines some promising candidates for future biotechnological production.

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