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Catalytic approach via retro-aldol condensation of glucose to furanic compounds†

Rui Zhang, a Aleksi Eronen, a Xiangze Du, b Enlu Ma, a Ming Guo, a Karina Moslova a and Timo Repo* a

The synthesis of new type furan-based compounds other than 5-hydroxymethylfurfural from glucose is a very attractive yet underexploited strategy. We report here a catalytic conversion of glucose with acetylacetone (acac) to furan-centered chemicals, 2-methyl-3-acetylfuran (MAF) and 1-(5-(1,2-dihydroxyethyl)-2-methylfuran-3-yl)ethan-1-one (DMAF), which are potential building blocks for the synthesis of fine chemicals. The experimentally supported reaction mechanism is a cascade-type, including glycolaldehyde (GA) formation by H\textsubscript{2}MoO\textsubscript{4} catalysed retro-aldol condensation (C2+C4) of glucose and immediate capture of transient C2 and C4 intermediates by acac to yield MAF and DMAF. To the best of our knowledge, this is the first report on straightforward synthesis of MAF and DMAF from glucose, providing a new, but generic synthesis strategy for GA-based C2 and erythrose-based C4 chemistry in biorefining.

Introduction

The high dependence of modern society on fossil fuel-based resources, together with the associated adverse environmental impacts, motivates research to identify renewable raw materials and to develop new fuel and chemical production methods for these. Glucose has attracted global attention as a representative monosaccharide of non-edible cellulose. Numerous studies were devoted to convert glucose into valuable platform chemicals, such as levulinic acid, 5-hydroxymethylfurfural (5-HMF), lactic acid, sugar alcohols and ethylene glycol (Fig. 1) through isomerization, dehydration, hydrogenation and retro-aldol condensation (RAC). RAC of glucose through a C2+C4 pathway generates glycolaldehyde (GA) and erythrose (Scheme 1, step 1). GA is a remarkable small molecule with both aldehyde and alcohol functionality, which has high potential to be a renewable alternative for petroleum-based ethylene oxide. GA is prone to many side reactions due to its highly reactive nature, thus it is often sequentially stabilized after formation, such as by hydrogenation to ethylene glycol. Other synthetic methods have also been developed for the transformation of GA, mainly including oxidation, aldol reaction, amination etc. for the production of glycolic acid, α-hydroxy acid esters and amines, as summarized recently by William et al. Nevertheless, new transformations that create platform chemicals or building blocks for fine chemicals are greatly needed to boost contemporary biorefinery concepts toward a sustainable world.

In this study, acetylacetone (acac), a typical β-dicarbonyl compound, was employed to capture the in-situ formed, reactive GA. The rapid interconversion between keto and enol tautomers of acac makes it a good nucleophilic reagent to attack aldehyde groups. As shown herein, high temperature...
treatment (220 °C) of aqueous glucose solution in the presence of acac gives a highly intriguing furan-derived product 2-methyl-3-acetylfuran (MAF) (Table 1, entry 1). Addition of H$_2$MoO$_4$ as a catalyst improves the efficiency of the reaction and enables the glucose transformation to MAF under significantly milder conditions. In addition, the catalytic process opens simultaneously a unique possibility to 1-(5-(1,2-di-hydroxyethyl)-2-methylfuran-3-yl)ethan-1-one (DMAF) synthesis, which derived from the reaction between erythrose (C4 fragment) and acac (Fig. 1). It is noteworthy that MAF and DMAF were previously only accessible by multi-step chemical synthesis$^9$ or using isolated and expensive GA and erythrose as starting materials in a ZrCl$_4$ catalysed reaction with acac.$^6$ From the furan-derived products, MAF is considered as a useful intermediate for the synthesis of photochromic molecules,$^9$ pharmaceuticals,$^{10}$ secoprostacyclins and food additives,$^{11}$ while DMAF is seen as underexploited chemical with potential for pharmaceutical and fine chemical industry.$^6,^{12}$

### Results and discussion

#### Investigation on MAF formation

To open RAC pathway for glucose conversion in the presence of acac, we chose the high temperature reaction conditions (220 °C). It was confirmed to be essential reaction parameter for high yield of MAF (Table S1 and Fig. S1-4). The indispensable role of acac as a nucleophile in this transformation was further confirmed with a series of experiments. H$_2$O/acac and H$_2$O/ethyl acetate reaction media were unsuccessful in this transformation and gave only insoluble humins, while all reactions with acac involved gave MAF. From the studied combinations, H$_2$O with acac offered a satisfactory yield of 46% for this cascade-type reaction (Table 1, entries 1-5).

| Entry | Solvent     | Substrate | MAF Yield$^{+}$ (mol %) |
|-------|-------------|-----------|-------------------------|
| 1     | H$_2$O/acac | Glucose   | 46                      |
| 2     | H$_2$O/acetone | Glucose  | -                       |
| 3     | H$_2$O/ethyl acetate | Glucose | -                       |
| 4     | acac        | Glucose   | 18                      |
| 5     | EtOH/acac   | Glucose   | 16                      |
| 6     | H$_2$O/acac | GA        | 83                      |
| 7     | H$_2$O/acac | GA        | 82                      |
| 8     | H$_2$O/acac | Mannose   | 46                      |
| 9     | H$_2$O/acac | Xylose    | 12                      |
| 10    | H$_2$O/acac | Fructose  | 9                       |
| 11    | acac        | GA        | 66                      |

Reaction conditions: 300 mg substrate, total solvent volume 13 mL, H$_2$O:solvent = 1/1, 220 °C, 30 min, 2.5 MPa N$_2$, 600 r/min. $^a$ MAF yield = mol of MAF/mol of glucose $\times$ 100%, the amount of MAF is determined by GC using acetophenone as internal standard; $^b$ 80 °C.

We also studied the role of GA as the key intermediate. Indeed, use of pure GA as a starting material instead of glucose raises the MAF yield greatly to 83% under the same reaction conditions (Table 1, entry 6). The central role of GA in the MAF forming reaction is also consistent with the results obtained from a series of carbohydrate substrates. Mannose, as a C-2 epimer of glucose, gave a comparable yield of MAF as glucose, while xylose and fructose gave much lower yields (Table 1, entries 8-10). Xylose as an aldopentose only gave around 1/3 of MAF compared with the amount derived from glucose, in accord with C2+C3 RAC of xylose. Fructose is prone to undergo C3+C3 RAC (which produces glyceraldehyde and dihydroxyacetone) rather than the desired C2+C4 pathway and thus gave only a small amount of MAF.$^{13}$ We confirmed this observation by a direct reaction between acac and glyceraldehyde at 220 °C, yielding only 4% of MAF and other unidentifiable products. Further studies revealed that acetic acid as a general carbohydrate decomposition product along with typical dehydration products with furan structures (such as furfural, 2-methylfuran and 5-HMF) are not involved in the MAF forming reaction (Table S2). These results confirm the pivotal role of GA in the condensation reaction with acac and further the formation of the furan structure of MAF under the applied conditions.

The reaction pathway involves the cleavage of glucose through C2+C4 RAC, followed by the aldol condensation of GA with acac (Scheme 1, steps 1 and 2). In this reaction, the presence of water is necessary for high yields in both steps (Table 1, entries 1 vs 4, 6 vs 11). It is known that water, besides of being an efficient proton carrier, undergoes autoprotolysis. Therefore, the concentration of hydronium (H$_3$O$^+$) and hydroxide (OH$^-$) ions increases with increasing temperature and

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Table 1: Effect of different reaction media and substrates on the yield of MAF without catalyst
Table 2 MAF and DMAF yield under various reaction conditions with H$_2$MoO$_4$ catalyst and proposed reaction pathway

| Entry | T (°C) | Time (min) | Conversion$^a$ (mol %) | Mannose Yield$^a$ (mol %) | MAF Yield (mol %) / (C%)$^b$ | DMAF yield$^d$ (mol %) / (C%)$^d$ |
|-------|--------|------------|------------------------|---------------------------|-----------------------------|-------------------------------|
| 1     | 60     | 30         | 54                     | 8                         | 16 / 5                      | 19 / 13                       |
| 2     | 80     | 30         | 84                     | 6                         | 45 / 15                     | 36 / 23                       |
| 3     | 80     | 120        | 93                     | 4                         | 56 / 19                     | 36 / 24                       |
| 4     | 80     | 180        | 98                     | 2                         | 59 / 20                     | 41 / 28                       |
| 5     | 80     | 240        | 99                     | 1                         | 59 / 20                     | 42 / 28                       |
| 6     | 100    | 15         | 98                     | 2                         | 55 / 18                     | 40 / 27                       |
| 7     | 100    | 30         | 100                    | 1                         | 59 / 20                     | 39 / 26                       |
| 8     | 120    | 30         | 100                    | -                         | 59 / 20                     | 37 / 25                       |
| 9     | 220    | 30         | 100                    | -                         | 66 / 22                     | -                             |
| 10$^d$| 100    | 30         | 64                     | 44                        | -                           | -                             |
| 11$^e$| 80     | 30         | -                      | -                         | 87                          | -                             |
| 12$^f$| 100    | 120        | 100                    | -                         | 52 / 17                     | 37 / 25                       |

Reaction conditions: 300 mg (46 g L$^{-1}$) glucose, 100 mg H$_2$MoO$_4$, H$_2$O/acac = 1/1 (6.5 mL/6.5 mL), 2.5 MPa N$_2$, 600 r/min. $^a$ Measured by HPLC using authentic glucose and mannose as standards; $^b$ DMAF yield = mol of DMAF/mol of glucose × 100%, the amount of DMAF is determined by $^1$H NMR in MeOD using 2-methylfuran as internal standard; $^c$ Carbon yield is calculated based on carbon atoms in glucose. Carbon yield of MAF = 2 × mol of MAF/6 × mol of glucose × 100%; Carbon yield of DMAF = 4 × mol of DMAF/6 × mol of glucose × 100%; $^d$ In the absence of acac; $^e$ GA as starting material; $^f$ 975 mg (150 g L$^{-1}$) glucose, 321 mg H$_2$MoO$_4$. 

Water is a prominent proton or hydroxide ion source at high temperatures. However, in these MAF forming reactions the measured pH of aqueous phase ranges from 2.7 to 3.0 pH units depending on the applied reaction conditions (Table S3). This phenomenon is likely due to the dissociation of acac in water, as the pH value for H$_2$O/acac reaction medium at room temperature is measured as 3.1. In addition to above, water is beneficial as it dissolves glucose well and generates a homogeneous reaction medium for the reaction.

As we searched for ways to improve efficiency of reaction, it soon became clear that the RAC, which forms GA, is the high temperature step in the concerted reaction. The high yield reaction between pure GA and acac can occur even at 80 °C (Table 1, entry 7). An additional $^1$H NMR study revealed that GA reacts fast with acac and the GA signal disappears in 1 min at 100 °C (Fig. 5S-6). Previous publications support the reasoning; high GA yields are normally obtained under supercritical water (>373 °C, >22 MPa) and in a flow reactor where the formed GA can be rapidly separated. In general, the elevated reaction temperature is related to high activation energy of RAC of glucose. Following this idea and raising the reaction temperature, we achieved a yield of 49% for MAF at 240 °C, but at 260 °C the yield already decreased slightly (Table S1). Therefore, raising the temperature quickly hit the limit and we had to look for alternative solutions.

H$_2$MoO$_4$ catalysed reaction

The ideal catalyst to enhance the RAC towards GA should be able to reduce the required activation energy while minimizing the isomerization of glucose to fructose. In this respect Mo(VI) compounds (i.e., molybdic acid, molybdenum oxide, polyoxometalates) are attractive, as they are known to catalyse epimerization of glucose to mannose through 1,2-intramolecular carbon shift (1,2-CS), known as Bilik reaction.
production of formic acid and glycolic acid from cellulose via RAC pathway combined with oxidation. Therefore, we introduced commercially available H$_2$MoO$_4$ to our studies. When we performed the H$_2$MoO$_4$ catalysed reaction at 220 °C, a significant increase in MAF yield to 66% was observed (Table 2, entry 9). To our surprise, reducing the reaction temperature as low as 100 °C gave a MAF yield 59% (Table 2, entry 7), while in contrast no transformation occurs without H$_2$MoO$_4$ catalyst. This is marked enhancement when compared to uncatalysed reaction with the record yield of 46% at 220 °C (Table 1, entry 1).

We studied the reaction parameters to optimize the yield and gain further insight to the H$_2$MoO$_4$ catalysed transformations at low temperatures. At fixed reaction time (30 min), the yield of MAF improved markedly from 16% to 59% as temperature raised from 60 °C to 100 °C (Table 2, runs 1, 2 and 7). Similarly, there was a positive correlation on MAF yield when catalyst loading amount range from 10-33 wt%, above this the generation of MAF remained consistent at 59% even when nearly stoichiometric amount of H$_2$MoO$_4$ was used (Table S5). The extension of reaction time from 30 to 180 min at 80 °C resulted in the same MAF yield as for 100 °C, 30 min (Table 2, entries 2-4 vs. 7).

In the catalysed, low temperature reactions, our attention was drawn to the formation of a new product. The detailed $^{1}$$H$, $^{13}$$C$, 2D NMR and HRESI-MS (High-resolution electrospray-ionization mass spectra) analysis confirmed that the isolated product is 1-(5-[1,2-dihydroxyethyl]-2-methylfuran-3-yl)ethan-1-one (DMAF), an aldol condensation product of acac and the C4 fragment, erythrose (Table 2; Fig. S7-10). DMAF can be biorefining. When we performed the H$_2$MoO$_4$ catalysed reaction at 220 °C, it demonstrated a perfect reagent. It is here a component of very high atom economy and can be prepared directly from the glucose via biosynthetic pathway or via bio-based triacetic acid lactone pathway in almost quantitative yield (Fig. S12). Carbon yield towards glucose, as a sum of MAF and DMAF formations in the catalysed reaction under optimized conditions is 48% (Table 2, entry 4). Although this is a rather good number for a RAC derived cascade-type reaction, there is scope for further catalysis or synthesis strategy development to improve the carbon efficiency.

In summary, we have developed a new strategy to convert glucose directly to MAF and DMAF via RAC with subsequent aldol condensation. The reaction benefits from several elementary steps. Non-catalytic approach requires high temperature for C2+C4 RAC and it was shown that GA is a key intermediate in the MAF forming reaction. Acac, which is a good nucleophile and weak acid in water, is essential to capture GA in-situ through aldol condensation. Catalytic approach for MAF was opened with the use of H$_2$MoO$_4$. Its capability for 1,2-CS transformation of glucose is known, but here it demonstrated a pivotal role in C2+C4 type RAC and enables GA and erythrose formation under significantly mild reaction conditions (80 °C). As a result, a novel route to MAF and DMAF synthesis is established. They both can be seen as chemicals with potential for pharmaceutical and fine chemical industry. Notably, natural carbohydrates including cellulose and raw wood materials can be converted to MAF using the presented approaches.
studies are focused on catalyst design to improve the carbon efficiency and the synthesis of other value-added chemicals with this strategy.

Author Contributions

Rui Zhang: investigation, methodology, data curation, formal analysis, software, writing - original draft, writing - review & editing;Aleksi Eronen: data curation, formal analysis, software; Xiangze Du: methodology, data curation; Enlu Ma: methodology, formal analysis; Ming Guo: methodology; Karina Moslova: resources; Prof. Timo Repo*: conceptualization, fund acquisition, supervision, resources, project administration, writing - review & editing.

Conflicts of interest

There are no conflicts to declare

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References

1 (a) Y. Liao, S.-F. Koelewijn, G. Van den Bossche, J. Van Aelst, S. Van den Bosch, T. Renders, K. Navare, T. Nicolai, K. Van Aelst, M. Maesen, H. Mitsushima, J. M. Thevelein, K. Van Acker, B. Lagrain, D. Verboekend and B. F. Sels, Science, 2020, 367, 1385-1390; (b) Y. Liu, Y. Nie, X. Lu, X. Zhang, H. He, F. Pan, L. Zhou, X. Liu, X. Ji and S. Zhang, Green Chem., 2019, 21, 3499-3535; (c) P. Sudarsanam, E. Peeters, E. V. Makshina, V. I. Parvulescu and B. F. Sels, Chem. Soc. Rev., 2019, 48, 2366-2421; (d) Z. Sun, G. Bottari, A. Afanasenko, M. C. A. Stuart, P. J. Deuss, B. Fridrich and K. Barta, Nat. Catal., 2018, 1, 82-92; (e) P. Ferrini and R. Rinaldi, Angew. Chem. Int. Ed., 2014, 53, 8634-8639; (f) R. Gérardy, D. P. Debecker, J. Estager, P. Luis and J.-C. M. Monbaliu, Chem. Rev., 2020, 120, 7193-7247; (g) X. Luo, Y. Li, N. K. Gupta, B. Sels, J. Ralph and L. Shuai, Angew. Chem. Int. Ed., 2020, 59, 11704-11716; (h) Y. Liu, C. Luo and H. Liu, Angew. Chem. Int. Ed., 2012, 51, 3249-3253; (i) P. N. R. Vennestrøm, C. M. Osmundsen, C. H. Christensen and E. Taarning, Angew. Chem. Int. Ed., 2011, 50, 10502-10509.

2 (a) J. Song, H. Fan, J. Ma and B. Han, Green Chem., 2013, 15, 2619-2635; (b) S. Kang, J. Fu and G. Zhang, Renew. Sustain. Energy Rev., 2018, 94, 340-362; (c) Y. Yang, C.-w. Hu and M. M. Abu-Omar, Green Chem., 2012, 14, 509-513; (d) X. Zhang, P. Murria, Y. Jiang, W. Xiao, H. I. Kettättäma, M. M. Abu-Omar and N. S. Mosier, Green Chem., 2016, 18, 5219-5229; (e) M. E. Zakrzewska, E. Bogel-Lukasik and R. Bogel-Lukasik, Chem. Rev., 2011, 111, 397-417; (f) L. T. Mika, E. Csfalvay and Á. Németh, Chem. Rev., 2018, 118, 505-613.

3 W. Faveere, S. Van Praet, B. Vermeer, K. N. R. Dumoleijn, K. Moonen, E. Taarning and B. F. Sels, Angew. Chem. Int. Ed., 2021, 60, 12204-12223.

4 (a) M. Zheng, J. Pang, R. Sun, A. Wang and T. Zhang, ACS Catal., 2017, 7, 1939-1954; (b) J. Pang, M. Zheng, R. Sun, A. Wang, X. Wang and T. Zhang, Green Chem., 2016, 18, 342-359.

5 (a) W. Faveere, T. Mihaylov, M. Pelckmans, K. Moonen, F. Gillis-D’Hammers, R. Bosschaert, K. Pierloot and B. F. Sels, ACS Catal., 2020, 10, 391-404; (b) G. Liang, A. Wang, L. Li, G. Xu, N. Yan and T. Zhang, Angew. Chem. Int. Ed., 2017, 56, 3050-3054.

6 (a) W. Caminati and J.-U. Grabow, J. Am. Chem. Soc., 2006, 128, 854-857; (b) Y. Jing, Y. Zhang, Q. Lv, Y. Guo, X. Liu and Y. Wang, Green Chem., 2019, 21, 6236-6240.

7 (a) E. Bacicocchi and R. Zuzcioni, Synth. Commun., 1988, 18, 1841-1846; (b) Y. Ji, J. Pan, P. Dauenhauer and R. J. Gorte, Appl. Catal. A-Gen., 2019, 577, 107-112.

8 (a) Y. Remizov, L. M. Pevzner and M. L. Petrov, Russ. J. Gen. Chem., 2018, 88, 1402-1410; (b) L. M. Pevzner, Y. O. Remizov and M. L. Petrov, Russ. J. Gen. Chem., 2015, 85, 61-70.

9 (a) A. Kruse and E. Dinjus, J. Supercrit. Fluids, 2012, 23, 4147-4150.

10 The possible reaction routes for DMAF like Polyhydroxyalkyl furans as platform molecules for other final products has been reported previously, see reference: V. Escande, T. K. Olszewski, E. Petit and C. Grison, ChemSusChem, 2014, 7, 1915-1923.

11 (a) S. Yamaguchi and T. Baba, Molecules, 2016, 21; (b) M. Orazov and M. E. Davis, Proc. Natl. Acad. Sci. U.S.A. 2015, 112, 11777; (c) Y. Yan, L. Feng, G. Li, S. Lin, Z. Sun, Y. Zhang and Y. Tang, ACS Catal., 2017, 7, 4473-4478; (d) A. Bayu, A. Abudula and G. Guan, Fuel Process. Technol., 2019, 196, 106162; (e) C. B. Schandel, M. Hej, C. M. Osmundsen, A. D. Jensen and E. Taarning, ChemSusChem, 2020, 13, 688-692.

12 (a) C. Luo, S. Wang and H. Liu, Angew. Chem. Int. Ed., 2007, 46, 7636-7639; (b) A. Kruse and E. Dinjus, J. Supercrit. Fluids, 2007, 41, 361-379.13.

13 J. Stary and J. O. Liljenzin, Pure Appl. Chem., 1982, 54, 2557-2592.

14 M. Sasaki, K. Goto, K. Tajima, T. Adschiri and K. Arai, Green Chem., 2002, 4, 285-287.

15 J. Zhang, B. Hou, A. Wang, Z. Li, H. Wang and T. Zhang, AIChE J., 2014, 60, 3804-3813.

16 (a) V. Bílik, L. Petruš, and V. Farkaš, Chem. zvesti, 1975, 5, 690-693; (b) L. Petruš, M. Petrušová and Z. Hricovičová, in Glycoscience: Epimerisation, Isomerisation and Rearrangement Reactions of Carbohydrates, ed. A. E. Stütz, Springer Berlin Heidelberg, Berlin, Heidelberg, 2001, DOI: 10.1007/3-540-44422-x_2, pp. 15-41; (a) C. Takagaki, S. Furusato, R. Kikuchi and S. T. Oyama, ChemSusChem, 2015, 8, 3769-3772; (d) F. Ju, D. VanderVelde and E. Nikolla, ACS Catal., 2014, 4, 1358-1364; (e) I. Delidovich and R. Palkovits, ChemSusChem, 2016, 9, 547-561; (f) M. Rellán-Piñeiro, M. Garcia-Ratés and N. López, Green Chem., 2017, 19, 5932-5939.

17 (a) J. Albert, R. Wölfel, A. Bösmann and P. Wasserscheid, Energy Environ. Sci., 2012, 5, 7956-7962; (b) J. Zhang, X. Liu, M. Sun, X. Ma and Y. Han, ACS Catal., 2012, 2, 1698-1702; (c) T. Lu, M. Niu, Y. Hou, W. Wu, S. Ren and F. Yang, Green Chem., 2016, 18, 4725-4732.

18 B. K. Chethana, D. Lee and S. H. Mushrif, J. Mol. Catal. A: Chem., 2015, 410, 66-73.
21 M. L. Hayes, N. J. Pennings, A. S. Serianni and R. Barker, 
*J. Am. Chem. Soc.*, 1982, **104**, 6764-6769.

22 J. Iglesias, I. Martinez-Salazar, P. Maireles-Torres, D. Martin Alonso, R. Mariscal and M. Lopez Granados, *Chem. Soc. Rev.*, 2020, **49**, 5704-5771.

23 Y. Zhou, Y. Ding, W. Gao, J. Wang, X. Liu, M. Xian, X. Feng and G. Zhao, *Biotechnol. Biofuels*, 2020, **13**, 88.

24 (a) B. H. Shanks and P. L. Keeling, *Green Chem.*, 2017, **19**, 3177-3185; (b) M. Chia, T. J. Schwartz, B. H. Shanks and J. A. Dumesic, *Green Chem.*, 2012, **14**, 1850-1853; (c) L. P. Saunders, M. J. Bowman, J. A. Mertens, N. A. Da Silva and R. E. Hector, *J. Ind. Microbiol. Biotechnol.*, 2015, **42**, 711-721; (d) D. Xie, Z. Shao, J. Achkar, W. Zha, J. W. Frost and H. Zhao, *Biotechnol. Bioeng.*, 2006, **93**, 727-736.