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Bio-based crotonic acid from polyhydroxybutyrate: synthesis and photocatalyzed hydroacylation

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A novel thermolytic distillation process was developed to depolymerize polyhydroxybutyrate (PHB) for the selective production of crotonic acid. The conditions adopted (170 °C, 150 mbar) were applied to pure PHB and PHB-enriched bacteria containing 60 and 30% of PHB, giving recovery of crotonic acid of 92, 78 and 58%, respectively. The high efficiency of the developed process poses the basis for a drop-in production of bio-based crotonic acid, whose versatility as a platform chemical has been investigated through photochemical approach. The photocatalytic addition (promoted by tetrabutylammonium decatungstate - TBADT) of aliphatic and aromatic aldehydes to crotonic acid took place under solar-simulated light irradiation. TBADT triggered the *in-situ* formation of valuable acyl radicals from the corresponding aldehydes, thus inducing the desired hydroacylation via radical conjugate addition. Notably, the functionalization took place in a satisfying yield quite independently from the adopted sample of crotonic acid (whether commercial or bio-based).

Introduction

Crotonic acid (**CA**) and its esters find application in coating, paint, textile, binders, adhesives, flocculants, ceramics, and agrochemical industries.[1] The main industrial use of **CA** is as a building block in the synthesis of co-polymers with vinyl acetate (trade name of Mowilith, Vinnapas, and Vinac). **CA** and its *cis*-isomer (isocrotonic acid) are in fact quite prone to polymerization in analogy to other α,β-unsaturated compounds (e.g. acrylic acid). The industrial synthesis of **CA** involves the oxidation of crotonaldehyde in a two-steps process, in which peroxocrotonic acid is initially formed as an intermediate and then reacts with a further molecule of crotonaldehyde to yield **CA**. Crotonaldehyde, the direct precursor of **CA**, is industrially produced through the self-condensation of acetaldehyde followed by dehydration of acetaldol, and acetaldehyde in turns is directly derived from ethylene platform (Wacker oxidation).[2] An interesting alternative pathway for synthesizing bio-based **CA** without the use of fossil resources through a drop-in strategy is the selective depolymerization of specific biopolymers, such as polyhydroxybutyrate (PHB). PHB belongs to the class of polyhydroxyalkanoates (PHAs), bio-based polyesters produced by a variety of aerobic bacteria able to ferment various carbon sources and accumulate PHA-granules inside the cells as energy and carbon storage. Selective depolymerizations of PHB, isolated or as inclusions inside bacterial cells, has been explored applying both thermal (hydrothermal treatment or pyrolysis) and chemical/enzymatic processes demonstrating that, in principle, it is possible to produce different chemicals, including propene,[3-5] **CA** and isocrotonic acid,[4,6,-11] methyl crotonate,[12,13] methyl acrylate,[12] cyclic and linear oligomers,[7,14] 3-hydroxybutyric acid,[4,8] methyl 3-hydroxybutanoate,[15] and hydrocarbon oil.[16] The cleavage and re-arrangement of PHB-bonds is a complementary strategy to the use of PHB as polymer in the bio-based plastic market, and poses the basis for PHB-exploitation as "platform chemical". This strategy can be particularly useful to manage "challenging PHA", such as: i) PHA that does not have suitable chemo-physical properties for the polymer market (e.g. molecular weight below 0.5 MDa);[17] ii) PHA with fluctuating monomer ratio (and properties) due to time-varying carbon source composition (e.g. waste);[13] iii) low amount-PHA inside bacterial cells, thus difficult or non-convenient to be extracted.[17,18] In these contexts, depolymerization approaches dedicated to produce drop-in chemicals from "challenging PHA" appear economically and environmentally preferred to PHA recovery. When intracellular PHA is directly used as feedstock to produce chemicals, no cell release is required, lowering the production costs of such chemicals and the carbon footprint due to lower energy demand.[12] Thermal degradation of PHA and PHB (eventually assisted by $Mg(OH)_2$, MgO or CaO catalysts)[9,19-21] occurs through β-elimination reactions that randomly break the chain and give dehydrated *trans*-alkenoic acids as major products (e.g. **CA** from hydroxybutyrate units).[14] To the best of our

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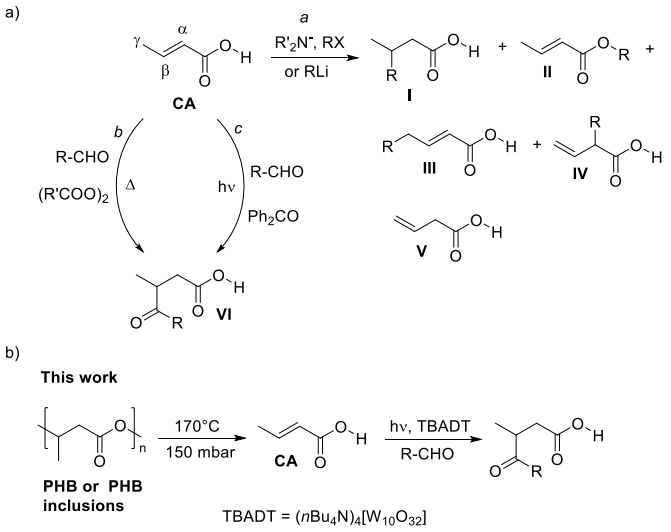
Electronic Supplementary Information (ESI) available: detailed PHB production through mixed microbial culture; description of thermal procedures (TP1-3); NMR spectra of CA_{PHB}, CA_{30} and CA_{60}; characterization and NMR spectra of products **6-12**; yield determination via NMR spectra analysis

knowledge, there are no reports describing the production of alkenoic acids (specifically **CA**) through thermolysis of PHA at temperatures below 200°C, and just three reports on the production of **CA** through thermolysis of PHB inclusions inside bacterial cells.[4,5,11] The chemical reactivity of **CA** explored so far includes polymerizations, esterifications, and additions to the double bond to yield mono and di-substituted butanoic acids.[1] Notably, the selective functionalization of this type of α,β-unsaturated acids is not an easy task because of the presence of acidic hydrogens in the COOH group and the γ-position. The nucleophilic alkylation of **CA** may lead to mixtures of products, as exemplified in Scheme 1 (*path a*). In fact, along with β-alkylation (compound **I**) and *O*-alkylation (**II**), the deprotonation would lead to dienediolates causing a γ- (**III**) and an α-alkylation (**IV**). Moreover, simple protonation of the carbanion may induce a C–C double bond shift to give deconjugated derivative **V**.[22-26] As a further drawback, at least two equivalents of the strong base/nucleophile are required, since one equivalent is simply devoted to the deprotonation of the COOH group. A milder and greener approach for the functionalization of α,β-unsaturated acids could be by conjugate addition of carbon-based radicals generated under photocatalytic conditions via a hydrogen atom transfer (HAT) reaction from suitable hydrogen donors.[27-29] Despite one of the first photocatalytic reactions ever-tested made use of an α,β-unsaturated acid (the addition of isopropanol onto maleic acid to give terebic acid, tetrahydro-2,2-dimethyl-5-oxo-3-furancarboxylic acid),[30] conjugated acids are rarely used as Michael acceptors in photocatalyzed syntheses.[31,32] In particular, the acylation via acyl radicals, a promising route for the preparation of γ-keto acids, is virtually unexplored. As for the case of **CA**, only a couple of examples have been reported for its acylation (to give **VI**), both exploiting the homolytic cleavage of the C–H bond of the formyl group of an aldehyde. The first involves heating in the presence of a peroxide (Scheme 1, *path b*),[33] while the other makes use of a photocatalyzed reaction (*path c*, benzophenone as the photocatalyst).[34]



Scheme 1 a) Functionalization of crotonic acid (**CA**) by nucleophilic alkylation under basic conditions (*path a*) and by addition of an acyl radical generated from the corresponding aldehyde under thermal (*path b*) and photocatalytic (*path c*) conditions. b) Thermochemical-photocatalytic route for the eco-sustainable synthesis of γ-keto acids via bio-based **CA**.

Experimental

Chemicals. All reagents, solvents and chemicals used in this work were of analytical grade and purchased from various commercial suppliers. Commercial polyhydroxybutyrate (PHB) with a mean molecular weight of 0.8 MDa and a polydispersity index of 5.9[18] was bought from Biomer (Germany). Commercial crotonic acid (hereafter named **CAc**) was purchased from Sigma-Aldrich and had a purity of 98%. Aldehydes (**5a-g**) used in the photocatalytic experiments were purified before use. HPLC-grade acetonitrile was employed for the photochemical reactions. Tetrabutylammonium decatungstate (nBu$_4$N)$_4$[W$_{10}$O$_{32}$] (TBADT) was synthesized as previously reported.[47]

PHB production by mixed microbial cultures. PHB-containing mixed microbial cultures (MMC) were cultivated in a 0.75 m^3 prototype consisting of a sequencing batch reactor (500 L) and an accumulation reactor (250 L; Figure S1 and detailed description in ESI). Two batches of freeze-dried MMC samples were prepared and used: one with 30% of PHB (hereafter named PHB-MMC-30) and the other with 60% of PHB (hereafter named PHB-MMC-60).

Thermal treatment of PHB and PHB-MMC samples. Three different thermal procedures (TP1-3, detailed in ESI and summarized in Table 1) with various configurations (Figures S2 and S3) have been applied to PHB (sample of 5 g), PHB-MMC-60 (sample of 8.34 g) and PHB-MMC-30 (sample of 16.67 g), to give a distilled fraction enriched in **CA**.

Table 1 Thermal procedures (TP1-3) applied in this work.

Thermal procedure	Step	T (°C)	Pressure (mbar)	Time (min)
TP1	Thermolysis	290	150	30
	Distillation	170	150	60
TP2	Thermolysis	240 (or 290)	150	30 (or 15)
	Thermolysis	290	150	30
	Distillation	170	150	60
TP3	Thermolytic distillation	170	150	60

The yield of the distilled fractions produced through each TP (on PHB or biomass weight basis in the case of pure PHB or bacterial biomass, respectively) was determined as follows:

$$\text{Yield}_{\text{distillates}}\ (\text{w/w}_{\text{PHB}}\%) = \text{Distillates (g)} / \text{PHB (g)} * 100$$

$$\text{Yield}_{\text{distillates}}\ (\text{w/w}_{\text{biomass}}\%) = \text{Distillates (g)} / \text{Biomass (g)} * 100$$

The yield of **CA** on distilled fraction weight basis ($Yield_{\mathbf{CA}\text{-}distillates}$, $w/w_{distillates}\%$) was calculated by GC-MS analysis as described below.
Consequently, the yield of **CA** on PHB or biomass weight basis was determined as follows:

$$Yield_{\mathbf{CA}}\ (w/w_{PHB}\%) = Yield_{distillates}\ (w/w_{PHB}\%) * Yield_{\mathbf{CA}\text{-}distillates}(w/w_{distillates}\%)$$

$$Yield_{\mathbf{CA}}\ (w/w_{biomass}\%) = Yield_{distillates}\ (w/w_{biomass}\%) * Yield_{\mathbf{CA}\text{-}distillates}(w/w_{distillates}\%)$$

The recovery of **CA** (%) achievable from each sample was calculated as follows, on the assumption that 1 g of PHB will give 1 g of **CA** ($Yield_{\mathbf{CA}\text{-}theoretical}$, $w/w_{PHB}\%$), independently by the fact that pure PHB or PHB inclusions have been treated:

$$Recovery_{\mathbf{CA}}\ (\%) = Yield_{\mathbf{CA}}\ (w/w\%) / Yield_{\mathbf{CA}\text{-}theoretical}\ (w/w_{PHB}\%)$$

General procedure for CA acylation. A degassed solution (by argon bubbling for 10 minutes) of **CA** (0.65 mmol, 0.13 M, 56 mg), the chosen aldehyde **5a-g** (0.75 mmol, 0.15M) and TBADT (2 mol%, 43 mg) in 5 mL of acetonitrile, was exposed for 24 h to simulated sunlight in a closed Pyrex vessel, using a Solarbox (Solarbox 1500e; CO.FO.ME.GRA., Italy, equipped with a 1.5 kW Xenon lamp; light intensity: 500 W m^{-2}). The progress of the reaction was monitored by GC-FID as described below and, upon completion, the crude mixture was poured into a round-bottom flask and the solvent removed via rotary evaporation. Then, the reaction product was isolated by column chromatography using SiO_2 as the stationary phase and mixtures of cyclohexane/ethyl acetate as eluants. Column chromatography was performed on an Isolera Spektra One (Biotage, Sweden), using Sepachrom Purezza Daily - Open Load cartridges (Sepachrom Srl, Italy).

GC-MS analysis. GC-MS analysis of chemicals produced by thermolysis of PHB or PHB-enriched bacteria was performed using an Agilent 7820A gas chromatograph connected to an Agilent 5977E quadrupole mass spectrometer. The injection port temperature was 280°C. Analytes were separated on a DB-FFAP polar column (30 m length, 0.25 mm i.d., 0.25 μm film thickness), with helium flow of 1 mL min^{-1}. Mass spectra were recorded under electron ionization (70 eV) at a frequency of 1 scan s^{-1} within the 29–450 m/z range. The temperature of the column was set to 50°C (5 min) and increased to 250°C (10°C min^{-1}). A calibration curve was performed with **CAc** in a 50-500 ppm range; the yield of **CA** ($Yield_{\mathbf{CA}\text{-}distillates}$) and by-products (isocrotonic acid (**1**), 3-butenoic acid (**2**), crotonamide (**3**), dimer of PHB (**4**)) in each distilled sample was determined by using this calibration curve and confirmed by ^{1}H NMR (see below). The identification of **1-3** was achieved by comparing their mass spectra with the NIST spectra database (https://chemdata.nist.gov/). The identification of dimer of PHB (**4**) was achieved by comparison with mass fragmentation reported in the literature.[50]

GC-FID analysis. GC-FID analysis to monitor the progress of the photocatalytic reactions was performed on an Agilent 7820A instrument. The injection was performed at 250°C in split mode. The initial oven temperature of 80°C was maintained for 2 min, increased by 10°C min^{-1} to 250°C and maintained for 5 min. An Agilent HP5 capillary column (30 m length, 0.32 mm i.d., 0.25 μm film thickness) was used with nitrogen as the carrier gas at a constant flow rate of 6.0 mL min^{-1}.

NMR analysis. ^{1}H and ^{13}C NMR spectra were recorded on a 300 or 200 MHz and a 75 MHz spectrometer, respectively. All NMR spectra were acquired using $CDCl_3$ as the solvent. Attributions were made based on ^{1}H and ^{13}C NMR. Data for ^{1}H NMR are reported as follows: chemical shifts reported in ppm and referred to residual chloroform ($CHCl_3$), multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet), integration and coupling constant (*J*, Hz). Data for ^{13}C NMR are reported as chemical shifts (ppm) and referred to residual chloroform ($CHCl_3$). The reported NMR yields are based on 200 MHz ^{1}H NMR spectra and have been calculated by adding to the crude reaction mixture a known amount of dibromomethane (CH_2Br_2) used as an external standard (see ESI for details).

Results and discussion

Thermal treatment of PHB and PHB-MMC samples. The first thermal treatment here applied to PHB and PHB-containing biomass (TP1, *thermolysis-distillation*) consisted in thermolysis at 290°C (the maximum degradation temperature of PHB),[6,7,9,19] followed by a distillation at 170°C, both under reduced pressure (150 mbar). TP1 was initially tested on pure PHB (Table 2, entries 1 and 2), verifying the effect of each step of the thermal procedure (thermolysis alone, entry 1, or thermolysis followed by distillation, entry 2) on the yield of distilled fraction ($w/w_{PHB}\%$) and **CA** ($w/w_{distillates}\%$). The yields of the distilled fraction were almost quantitative in both cases, in line with what reported by other authors,[9] but the final distillation step increased the yield of **CA** up to 96% (entry 2). Dimer of PHB (**4**) was the by-product whose amount decreased to the largest extent after the distillation, both for its physical separation under reduced pressure and for its further thermolysis that could eventually occur at 170°C under the same condition. **CA** yield slightly increased after the distillation (entries 1 and 2, Table 2), whereas the amount of the other two alkenoic acids (isocrotonic acid **1** and 3-butenoic acid **2**) was substantially the same before and after such step. The yield of **CA** on PHB input basis ($w/w_{PHB}\%$) achieved through TP1 was 88%. TP1 was also tested on bacterial biomass containing 30 and 60% of PHB (entries 3 and 4, Table 2). It appeared evident that the presence of the "non-PHB cell mass" (NPCM) had a detrimental effect on the production of **CA**. The yield of **CA** on distilled fraction basis ($w/w_{distillates}\%$) was significantly lower than that obtained when the same procedure was applied to pure PHB, whereas all GC-MS detectable by-products drastically increased. The amount of

non-GC-MS detectable by-products became relevant, especially in the case of PHB-MMC-30. The yield of **CA** (w/w_{PHB}%) achieved through TP1 applied on PHB-MMC-30 was 8%, corresponding to a recovery of **CA** of 26%, whereas the yield of **CA** (w/w_{PHB}%) applied on PHB-MMC-60 was 31%, corresponding to a recovery of **CA** of 51%.

Since these results were lower than what achievable from pure PHB (entry 2, Table 2), another thermal procedure named TP2 (*thermolysis-thermolysis-distillation*) was set up and tested. TP2 consisted of a first thermolysis at 240 or 290°C, followed by a second thermolysis at 290°C, and a final distillation at 170°C (each step under reduced pressure, 150 mbar). The main difference between configurations TP1 and TP2 relied on preliminary thermolysis (at 240 or 290°C) dedicated to the pre-formation of PHB-oligomers, to physically separate this fraction from bacterial biomass residue that could have a role in promoting side-reactions and by-products formation. This preliminary thermolysis performed at 290°C, followed by the two steps already adopted in TP1, gave a large improvement in terms of **CA** yield (entries 5 and 6, Table 2). Despite the yield of the distilled fraction was slightly higher than what obtained with TP1 (18 *vs* 12% in the case of PHB-MMC-30, and 50 *vs* 39% in the case of PHB-MMC-60), the yield of **CA** in this fraction increased from 64 to 86% in the case of PHB-MMC-30, and from 79 to 94% in the case of PHB-MMC-60, optimizing the purity of the distillate. The yield of **CA** (w/w_{PHB}%) achieved through TP2 with the first thermolysis at 290°C applied on both PHB-MMC-30 and PHB-MMC-60 was 15 and 47%, respectively (**CA** recovery of 52 and 78%, respectively). Similar results were obtained when the first thermolysis was applied at a lower temperature (240°C, entries 7 and 8, Table 2). Noteworthy, this finding suggested the possibility that a full thermal decomposition of PHB could occur even operating at a lower temperature than that at which PHB usually decomposes. From this evidence, a last thermal procedure (TP3, here named *thermolytic distillation*) has been set up. TP3 consisted of a distillation at 170°C under reduced pressure (150 mbar), largely below the maximum decomposition temperature of PHB (250-310°C).[6,7,9,19] Differently from TP1 and TP2, distillation was the only process applied to PHB or PHB-MMC (entries 9-11, Table 2), thus the only one potentially able to break PHB bonds. The yield of **CA** (92 w/w_{PHB}%, entry 9) here achieved by applying TP3 to PHB was significantly higher than the results obtained through other catalytic/non-catalytic thermal procedures reported in the literature (68-83%).[4,7,17] The yield of **CA** (w/w_{PHB}%) from PHB-MMC-30 with TP3 (entry 10) was analogous to that obtained with TP2 in three steps (compare entries 5, 7 and entry 10), but with a composition of the distilled fraction much more enriched in **CA** than in other by-products. The same held for PHB-MMC-60 treated with TP3 (entry 11), with a final **CA** yield (w/w_{PHB}%) of 47%, and a distilled fraction more selectively enriched in this compound. To the best of our knowledge, the only three thermal procedures already applied in the literature to convert PHB inclusions (10 or 66% PHB content) into **CA**[4,5,11] gave **CA** recoveries of 20-26 and 60%, respectively, mainly due to a large presence of dimers of PHB. By applying TP3 to MMC with 30 or 60% of PHB, the **CA** recovery was 58 and 78%, respectively. The thermolysis conditions adopted in TP3 seem to be the key aspect underpinning the improved performance of the procedure here developed with respect to literature reports, usually performed at the decomposition temperature of PHB (280-290°C). The temperature of 170°C applied under reduced pressure (150 mbar) permits to slowly decompose PHB, and to selectively recover the monomers through distillation. Since oligomers have higher boiling points than monomers, they cannot be distilled under these conditions and remain in the flask, so that their complete monomerization can thus be reached. Moreover, it is reported that heat favours isomerization,[51] thus it is reasonable to suppose that at 170°C (TP3) isomerization side-reactions are inhibited, favouring the formation of the more stable monomer (**CA**, the *trans*-isomer). To the best of our knowledge, the "thermolytic distillation" at 170°C adopted in TP3 is peculiar of the present study and represents a novel one-step procedure for degrading selectively PHB and PHB-inclusions into **CA**, favouring its recovery.

Photocatalyzed hydroacylation of CA. After selecting TP3 as the most promising approach to produce bio-based **CA**, the procedure was applied to PHB, PHB-MMC-60 and PHB-MMC-30 to get three samples of **CA**, hereafter named $\mathbf{CA_{PHB}}$ (almost colourless), $\mathbf{CA_{60}}$ (light brown) and $\mathbf{CA_{30}}$ (dark brown), respectively (Figure 1). These samples were subjected to a photocatalyzed hydroacylation procedure promoted by tetrabutylammonium decatungstate (TBADT). The procedure was initially set up on commercial crotonic acid (**CAc**), focusing on its functionalization with a small library of aldehydes under solar-simulated light irradiation.

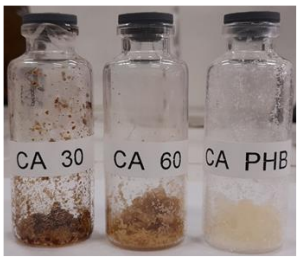
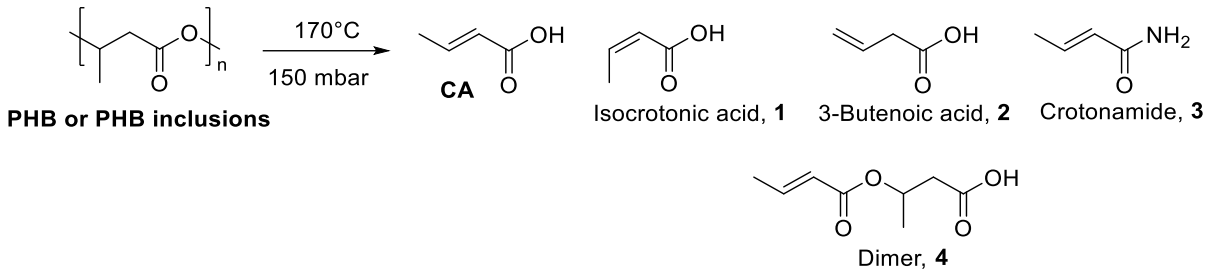


Fig. 1 CA samples obtained by applying TP3 procedure to PHB ($\mathbf{CA_{PHB}}$), PHB-MMC-60 ($\mathbf{CA_{60}}$) and PHB-MMC-30 ($\mathbf{CA_{30}}$).

Table 2 Yield of distilled fraction (on PHB or PHB-MMC input basis) and its composition in GC-MS detectable compounds, yield of **CA** (on PHB or PHB-MMC input basis) and recovery of **CA** through thermal procedures TP1-3 applied to PHB and PHB-MMC samples.



Entry	TP	TP Steps	Substrate	$Yield_{distillates}$ (w/w_{PHB}% or $w/w_{biomass}$%)	Product Yield ($w/w_{distillates}$%)					$Yield_{CA}$ (w/w_{PHB}% or $w/w_{biomass}$%)[a]	$Recovery_{CA}$ (%)[b]
					CA	1	2	3	4		
1	TP1	thermolysis	PHB	99	85	3.5	0.6	-	9.3	84	84
2	TP1	thermolysis-distillation	PHB	92	96	2.6	0.2	-	-	88	88
3	TP1	thermolysis-distillation	PHB-MMC-30	12	64	10.6	3.8	0.7	5.2	8	26
4	TP1	thermolysis-distillation	PHB-MMC-60	39	79	8.5	3.5	0.4	1.0	31	51
5	TP2	thermolysis[c]-thermolysis-distillation	PHB-MMC-30	18	86	8.4	1.8	0.3	-	15	52
6	TP2	thermolysis[c]-thermolysis-distillation	PHB-MMC-60	50	94	1.4	0.6	-	-	47	78
7	TP2	thermolysis[d]-thermolysis-distillation	PHB-MMC-30	21	87	8.7	1.8	0.6	-	18	61
8	TP2	thermolysis[d]-thermolysis-distillation	PHB-MMC-60	49	94	4.1	0.9	-	-	46	77
9	TP3	thermolytic distillation	PHB	94	98	0.1	-	-	0.2	92	92
10	TP3	thermolytic distillation	PHB-MMC-30	19	92	0.1	-	0.5	-	17	58
11	TP3	thermolytic distillation	PHB-MMC-60	49	96	0.5	-	-	-	47	78

[a] calculated as: $Yield_{CA} = Yield_{distillates} * Yield_{CA\text{-}distillates}$

[b] calculated on the assumption that 1 g of PHB would give 1 g of **CA**, independently by the fact that pure PHB or PHB inclusions have been treated, as: $Recovery_{CA} = Yield_{CA} / Yield_{CA\text{-}theoretical}$

[c] first thermolysis step performed at 290°C

[d] first thermolysis step performed at 240°C

As shown in Figure 2 and according to literature precedents,[45,46] the process is triggered by excited TBADT that abstracts a hydrogen atom from the formyl C(sp^2)-H bond in aldehydes **5a-g** to afford the corresponding acyl radicals. This in turn adds onto the β-position of **CAc** to give a radical adduct that leads to the formation of the final product via a back hydrogen-atom transfer (b-HAT) step. The optimized conditions to reach complete conversion of the starting materials are as follows: an acetonitrile solution of **CAc** (0.13 M) and a slight excess of the chosen aldehyde (**5a-g**, 0.15 M; 1.15 equiv.) was irradiated in the presence of a catalytic amount of TBADT (2 mol%) for 24 h. To our delight, the process worked well both with aliphatic and aromatic aldehydes to give the corresponding adducts in yields ranging from good to excellent. Thus, hydrocinnamaldehyde **5a** and heptaldehyde **5b** gave adducts **6** and **7** in 71 and 58% isolated yield, respectively. Control experiments related to the preparation of **6** demonstrated that the presence of both light and TBADT are mandatory for the occurrence of the process. A decrease of the yield to 36% (according to NMR analysis) was observed in the synthesis of **6** by adding TEMPO (2,2,6,6-tetramethylpiperidine *N*-oxyl, 1.15 equiv.) to the reaction mixture, thus confirming the radical nature of this photocatalytic hydroacylation (see Table S1).

Moving to aromatic aldehydes, the reaction worked well on parent benzaldehyde **5c** to give **8** (70% yield), while the presence of a substituent in the *para*-position was tolerated, independently from its electronic character, as demonstrated by *para*-anisaldehyde **5d** and *para*-chlorobenzaldehyde **5e** (products **9**, **10**). Further modification of the substitution pattern on the aromatic ring, as in the case of *meta*-chlorobenzaldehyde **5f** and 3,4,5-trimethoxybenzaldehyde **5g**, smoothly gave the expected adducts **11** and **12** in up to 89% isolated yield.

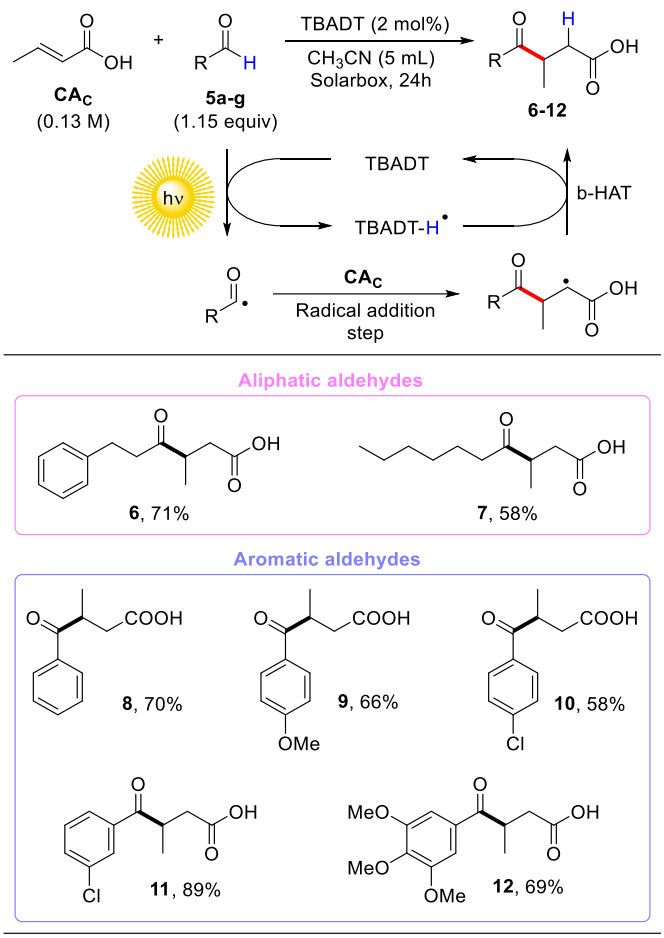


Fig. 2 Photocatalytic hydroacylation of **CA**$_C$ with aldehydes **5a-g**. Reaction conditions: an Ar-bubbled acetonitrile solution (5 mL) containing **CA**c (0.13 M, 0.65 mmol), aldehydes **5a-g** (0.15 M, 0.75 mmol, 1.15 equiv.) and TBADT (2 mol%) was irradiated with a Solarbox (1.5 kW Xe lamp; light intensity: 500 W m^{-2}) for 24 h. The reported yields are referred to the isolated products after column chromatography (for further details see Experimental section and ESI).

Next, selected hydroacylations of different **CA** samples (**CA$_{PHB}$**, **CA$_{60}$** and **CA$_{30}$**) obtained via the thermolytic distillation approach optimized in the present work (TP3) were tested, also to check whether the coloured impurities present in the samples may affect the reaction course (see Figure 1). Thus, the hydroacylations of **CA$_{PHB}$**, **CA$_{60}$** and **CA$_{30}$** to give **6** (from hydrocinnamaldehyde) and **8** (from benzaldehyde) were tested as model reactions. As shown in Table 3, the substitution of **CA$_C$** with the hereby prepared **CA$_{PHB}$**, **CA$_{60}$** and **CA$_{30}$** did not hamper the observed reactivity, with a trend following the order: **CA$_{PHB}$** ~ **CA$_{60}$** > **CA$_{30}$**. Indeed, a limited yield drop (around 10%) has been observed in the formation of **6**, with the samples prepared via TP3 showing a very similar performance.

A difference of only 4% in terms of yield from the best to the worst sample was observed, albeit the colour of the irradiated mixture was markedly different (see Figure 3a and Figures S4-S6). However, in each case, the final irradiated solutions showed the blue colour characteristic of the TBADT-H• species,[39,40] although less apparent in the case of **CA$_{30}$** (Figure 3b). On the other hand, the colour of the starting solution seems to have a role in the preparation of **8**, wherein, contrary to the case of **CA$_{PHB}$** and **CA$_{60}$**, the functionalization of **CA$_{30}$** afforded the desired product in a markedly lower yield (50% by NMR).

Table 3. Synthesis of products **6** and **8** by adopting different **CA** samples as starting materials.[a]

Product	Yield (%)			
	CA$_C$	**CA$_{PHB}$**	**CA$_{60}$**	**CA$_{30}$**
6	74	66	65	62
8	79	75	73	50

[a] NMR yields calculated by adding to the crude reaction mixture a known amount of dibromomethane (CH_2Br_2) used as the external standard (for further details see Experimental section and ESI).

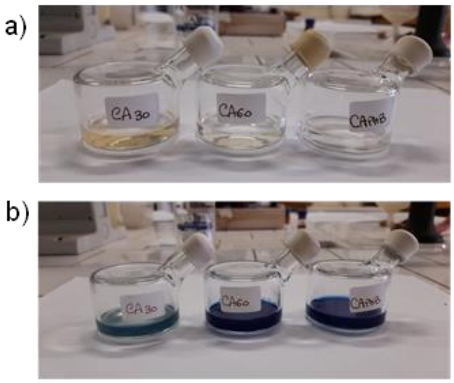
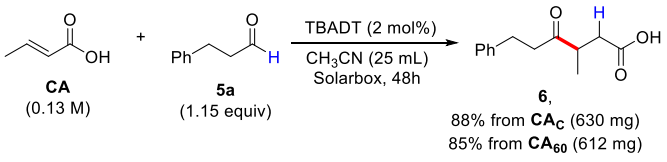


Fig. 3 Glass vessels used for the preparation of γ-keto acid **6** before (a) and after (b) the irradiation in the SolarBox.

Finally, we repeated the preparation of **6** on a larger scale (3.25 mmol, 25 mL solution) by reacting **CA$_C$** and **CA$_{60}$** with aldehyde **5a**. To our delight, a very high isolated yield of **6** has been obtained in both cases (88 and 85% from **CA$_C$** and **CA$_{60}$**, respectively), delivering the desired product in > 600 mg upon irradiation for 48 h (Scheme 2).



Scheme 2 Large scale preparation (3.25 mmol) of **6** starting from **CA$_C$** or **CA$_{60}$**.

Conclusions

This work illustrates an improved green route for the efficient synthesis of bio-based crotonic acid from PHB and extends the knowledge on its potential use as a platform chemical through photocatalyzed transformations. The novel thermolytic distillation process here developed was characterized by milder conditions (170°C without catalysts) than what has been reported so far in the field of PHB-depolymerization processes, but enough to give better results in terms of PHB depolymerization and **CA** purity. The recovery of **CA** from pure PHB and bacteria containing 30 and 60% of PHB were 92, 58, and 78%, respectively, testifying as this procedure can be exploited in a chemical recycling perspective independently from the actual PHB "form" (extracted polymer or bacterial inclusions).

The bio-based crotonic acid so obtained was easily derivatized under mild conditions (room temperature with the help of a small amount of an inorganic photocatalyst), avoiding the use of aggressive bases and nucleophiles, to give valuable γ-keto acids. Despite its high purity, the **CA** derived from PHB-enriched bacteria containing 30 and 60% of PHB, shows a brown coloration. This, however, did not hamper the use of solar light for the hydroacylation step.

Conflicts of interest

There are no conflicts to declare.

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