



HAL
open science

Sustainable Straightforward Synthesis and Evaluation of the Antioxidant and Antimicrobial activity of Sinapine and Analogs

Louis Mouterde, Aurélien Peru, Matthieu Mention, Fanny Brunissen, Florent Allais

► **To cite this version:**

Louis Mouterde, Aurélien Peru, Matthieu Mention, Fanny Brunissen, Florent Allais. Sustainable Straightforward Synthesis and Evaluation of the Antioxidant and Antimicrobial activity of Sinapine and Analogs. *Journal of Agricultural and Food Chemistry*, 2020, 68 (26), pp.6998-7004. 10.1021/acs.jafc.0c02183 . hal-02876020

HAL Id: hal-02876020

<https://hal.science/hal-02876020v1>

Submitted on 20 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Sustainable Straightforward Synthesis and Evaluation of the Antioxidant and Antimicrobial activity of Sinapine and Analogs

*Louis M. M. Mouterde**, Aurélien A. M. Péru, Matthieu Mention, Fanny Brunissen, Florent

*Allais**

URD Agro-Biotechnologies Industrielles (ABI), CEBB, AgroParisTech, 51110, Pomacle, France

Phone +33 3 52 62 04 65, E-mail louis.mouterde@agroparistech.fr,
florent.allais@agroparistech.fr

*Author to whom correspondence should be addressed

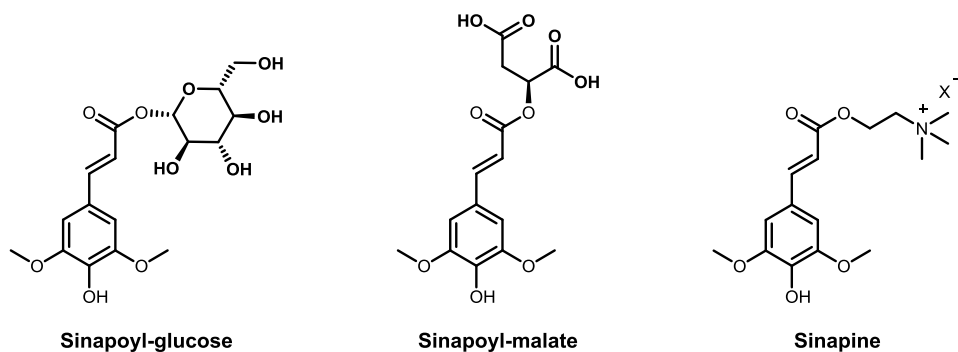
Keywords: Sinapine, Knoevenagel-Doebner, Meldrum's acid, Choline chloride, Choline phenolic esters

Abstract

Naturally occurring sinapine was successfully synthesized through a proline-mediated Knoevenagel-Doebner condensation in ethanol. This synthetic process involving bio-based syringaldehyde, Meldrum's acid and choline chloride, offers a sustainable alternative to the existing low yield pathways. This two-step strategy gives access to sinapine in a 52% overall yield, and has been implemented to the synthesis of sinapine analogs using 4-hydroxybenzaldehyde, 3,4-dihydroxybenzaldehyde and vanillin as precursors giving target molecules with 34-61% overall isolated yields. Purity of synthetic sinapine and that of its analogs (ca. 95%) was assessed by NMR and HPLC-MS analysis. Furthermore, antioxidant and antimicrobial activities were assessed and confirmed the potential of this series of molecules.

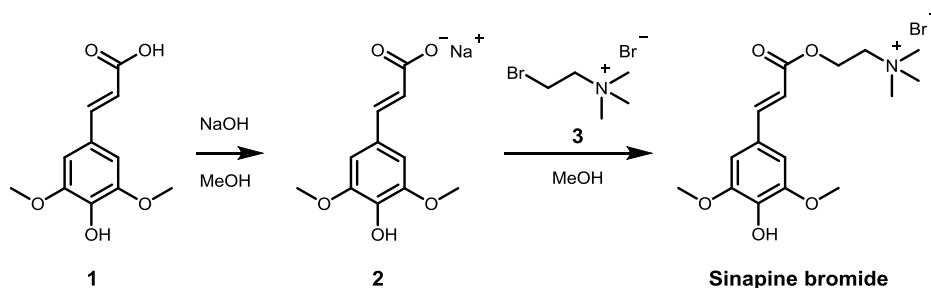
Introduction

Naturally occurring sinapic acid (**1**) and its main esters (*e.g.*, sinapoyl-glucose, sinapoyl-malate and sinapoyl-choline aka sinapine) have shown great potential in a wide range of applications thanks to their potent anti-UV, antioxidant, anti-inflammatory, anti-cancer and/or anti-microbial properties (Scheme 1).¹⁻⁹ These compounds can be found in Nature in a wide variety of products such as fruits, vegetables, cereals or *Brassicaceae* seeds.¹⁰ The latter are particularly of interest due to their relatively high contents in sinapic analogs and their availability, especially rapeseeds that can contain up to 18,000 μg of sinapic derivatives per gram of seed.^{11, 12} After obtaining the oil by cold pressure, the resulting seed cake can contain up to 10,000 μg of sinapic analogs per gram of dry weight.^{12, 13} Moreover, the global production of rapeseed cake was at an average of 69 millions of tons between 2013 and 2016, which represents a capacity of 69 kT sinapic acid per year.¹⁴ This potential source of sinapic derivatives has generated many research works aimed at efficiently extracting sinapic compounds from this agricultural byproduct.¹⁵⁻¹⁸ The same observation can be made for mustard bran that contains up to 125,000 μg of sinapic derivatives per gram of dry weight.^{4, 19, 20} Although great advances have been made in this area, no viable industrial process has been developed yet. Therefore, alternatives must be found to access these valuable molecules.



Scheme 1. Wathelet et al. synthesis strategy to sinapine

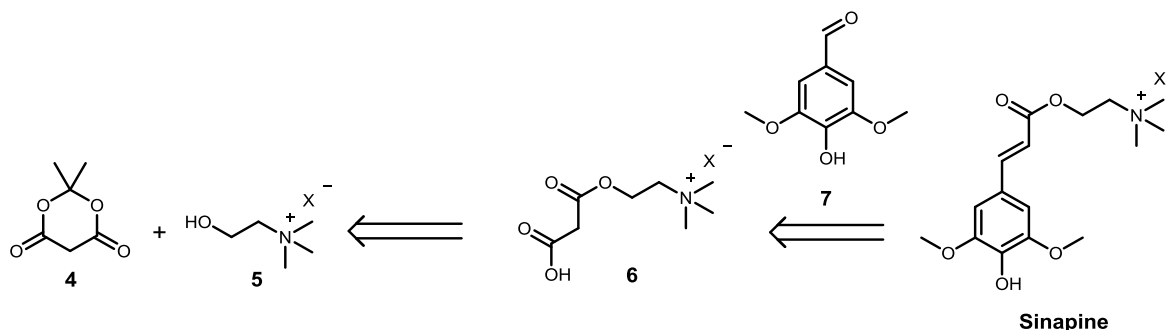
While straightforward synthesis of sinapoyl-malate and sinapoyl-glucose have been described in the literature, it is more ambiguous in the case of sinapine.^{21, 22} A method has been reported by Wathelet et al., based on the work of Clausen et al., in a communication poster involving sinapoate (**2**) and bromocholine bromide (**3**) (Scheme 2).^{23, 24} This method presents several disadvantages because of (1) the relative low availability and high price of sinapic acid (**1**), (2) the significant toxicity as well as the corrosivity of bromocholine bromide (**3**), and (3) the necessity of performing a precise titration for the formation of **2**.²⁵ Moreover, this method has been developed on a ~50 mg scale and the yields are not clearly reported. It is noteworthy to mention that the synthesis of sinapine analogs through this method has been reported in the literature using chlorocholine chloride as precursor.²⁶ Another strategy consisted in an esterification of sinapic acid, *via* an acyl chloride and using dimethyl-ethanolamine, followed by a methylation of the resulting intermediate.^{27, 28}



Scheme 2. Synthetic strategy to sinapine from Wathelet et al. ^[9a]

Under such consideration, a sustainable and straightforward strategy to access sinapine remained to be developed. Herein, we report a convergent retrosynthetic approach starting with the transesterification of Meldrum's acid (**4**) with choline halide (**5**) followed by a Knoevenagel-Doebner condensation of the resulting choline chloride malonate monoester (**6**) with lignin-derived and readily available syringaldehyde (**7**) (Scheme 3). This approach was then

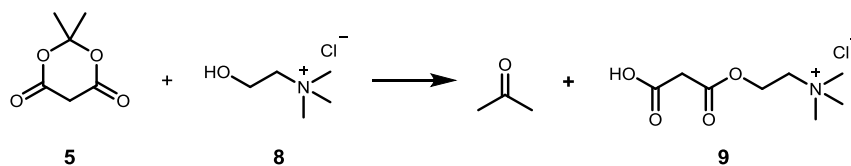
successfully implemented to other *p*-hydroxybenzaldehydes and the antioxidant and antimicrobial properties of the resulting choline esters were assessed.



Scheme 3. Retrosynthetic approach for the synthesis of sinapine

Results and Discussion

Synthesis of sinapine and its analogs. The first step of this study dealt with the formation of the choline chloride malonate monoester (**9**). Unfortunately, the latter cannot be readily obtained through classical Fischer esterification. Indeed, under such conditions, the desymmetrization of malonic acid with choline chloride is difficult due to the even reactivity of the two carboxylic acid functions. Therefore, an alternative strategy consisting in performing a transesterification on Meldrum's acid with choline chloride was investigated (Scheme 4).²⁹



Scheme 4. Formation of the choline chloride malonate monoester (**9**)

Preliminary experiments with an equimolar mixture of choline chloride and Meldrum's acid with or without solvent (acetonitrile 2M) showed the degradation of Meldrum's acid into malonic

acid, and further into acetic acid (Table 1, entries 1 and 4). In order to increase the conversion and to find the optimal conditions, the reaction was carried out with or without solvent at different choline chloride/Meldrum's acid ratio over a 3-hour period. The reactions without solvent were performed at 90 °C, melting point of Meldrum's acid, and the reaction in acetonitrile at 82 °C, boiling temperature of the solvent. A slight excess of Meldrum's acid (1.25 equivalents) resulted in a better conversion with or without solvent (Table 1, entries 2 and 5). Increasing this excess to 1.5 equivalents allowed to reach 100% conversion in presence of solvent (Table 1, entry 3), while 85% conversion was observed without solvent (Table 1, entry 6). It seems that acetic acid formed *in situ* hindered the reaction completion by favorize the degradation of Meldrum's acid if not diluted in a solvent. Therefore, the optimal conditions to obtain the monoester **9** in quantitative conversion appeared to be in acetonitrile at 82 °C with 1.5 equivalents of Meldrum's acid (characteristic peak at 1.70 ppm, ESI-S5) (Table 1, entry 3). However, in such conditions, residual Meldrum's acid was observed. This issue was easily overcome by extending the reaction time to 5 hours (Table 1, entry 7).

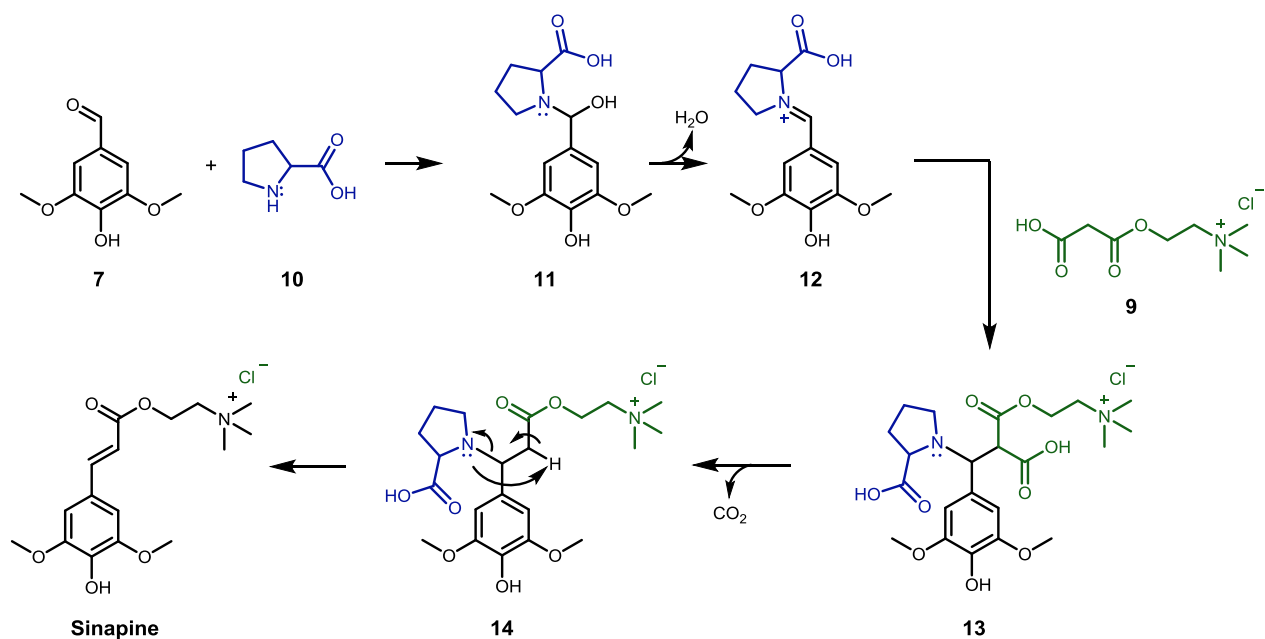
Table 1. Optimization of the choline chloride malonate monoester synthesis.

Entry	Solvent	Temperature (°C)	Duration (h)	Ratio choline chloride/Meldrum's acid	Conversion (%) ^[a]
1	Acetonitrile	82	3	1:1	60
2	Acetonitrile	82	3	1:1.25	76
3	Acetonitrile	82	3	1:1.5	100

4	None	90	3	1:1	65
5	None	90	3	1:1.25	79
6	None	90	3	1:1.5	85
7	Acetonitrile	82	5	1:1.5	100

[a] Conversion were determined by ^1H NMR of the crude reaction mixture.

After concentration in vacuo, the crude reaction mixture (Table 1, entry 7) was directly submitted to a proline-mediated Knoevenagel-Doebner condensation with syringaldehyde in ethanol to afford sinapine.^{30,31} The mechanism of this reaction, previously described by Peyrot et al., involves the activation of the aldehyde moiety through the formation of the iminium **12** which will then react with the monoester **9**. The intermediary **13** thus formed will undergo decarboxylation to yield **14** that rearranges to regenerate proline and provide sinapine (Scheme 5). The latter can be easily purified through C18 reverse phase chromatography giving the pure product in a 48% isolated yield. Such approach avoids the use of the non-sustainable classical pyridine/piperidine system.³² It is worth mentioning that transesterification of sinapine by ethanol occurred (up to 20% yield). Polar aprotic solvents such as THF, AcOEt and Cyrene[®] have been investigated to replace ethanol in order to overcome this issue, unfortunately, the results were not conclusive.



Scheme 5. Proline-mediated Knoevenagel-Doebner condensation's mechanism

This methodology was then implemented to benzaldehyde, 3,4-dihydroxybenzaldehyde and vanillin in order to yield coumaroyl-, caffeoyl- and feruloyl-choline, respectively. In all cases, quantitative consumption of the aldehyde was observed and isolated yields varied from 34 to 61% (Table 2). It is worth mentioning that these procedures have been validated at the gram-scale.

Table 2. Isolated yields of sinapine analogs.

Substrate	Product	Yield (%) ^[a]
4-hydroxybenzaldehyde	Coumaroyl-choline	34
3,4-dihydroxybenzaldehyde	Caffeoyl-choline	61
Vanillin	Feruloyl-choline	50

[a] Yields were calculated from isolated product after purification.

Characterization of sinapine and its analogs. The compounds thus formed and purified were characterized by UHPLC-MS. Mass analysis revealed that in all cases the major peak corresponded to the molecular ion (m/z 250, 266, 280 and 310 for coumaroyl-, caffeoyl-, feruloyl-choline and sinapine, respectively). Additionally, two characteristic fragments corresponding to the loss of trimethylamine and choline were observed for each analog (m/z 59 and 103, respectively). The HPLC UV-detection chromatograms at 320 nm (Figures S-11 and 12) also showed another peak eluting at 0.5 min corresponding to residual choline as confirmed by ^1H NMR analysis (ca. 5% mol). Finally, thanks to very different retention time, it was shown that coumaroyl-, caffeoyl-, feruloyl-choline and sinapine can be efficiently separated using reversed phase C18 chromatography, which is a very interesting feature if one wants to use a crude mixture of *p*-hydroxybenzaldehydes directly obtained after lignin-oxidation (Figure 1).

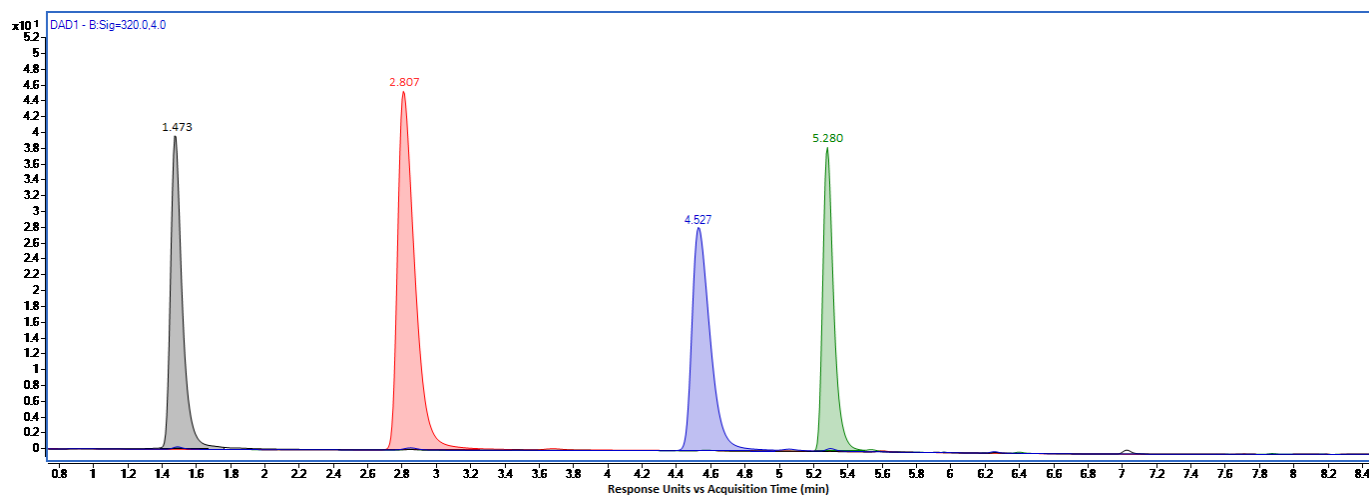


Figure 1. HPLC UV-detection chromatograms superposition of coumaroyl (red peak), caffeoyl (black peak), feruloyl (blue peak) and sinapoyl-choline (green peak)

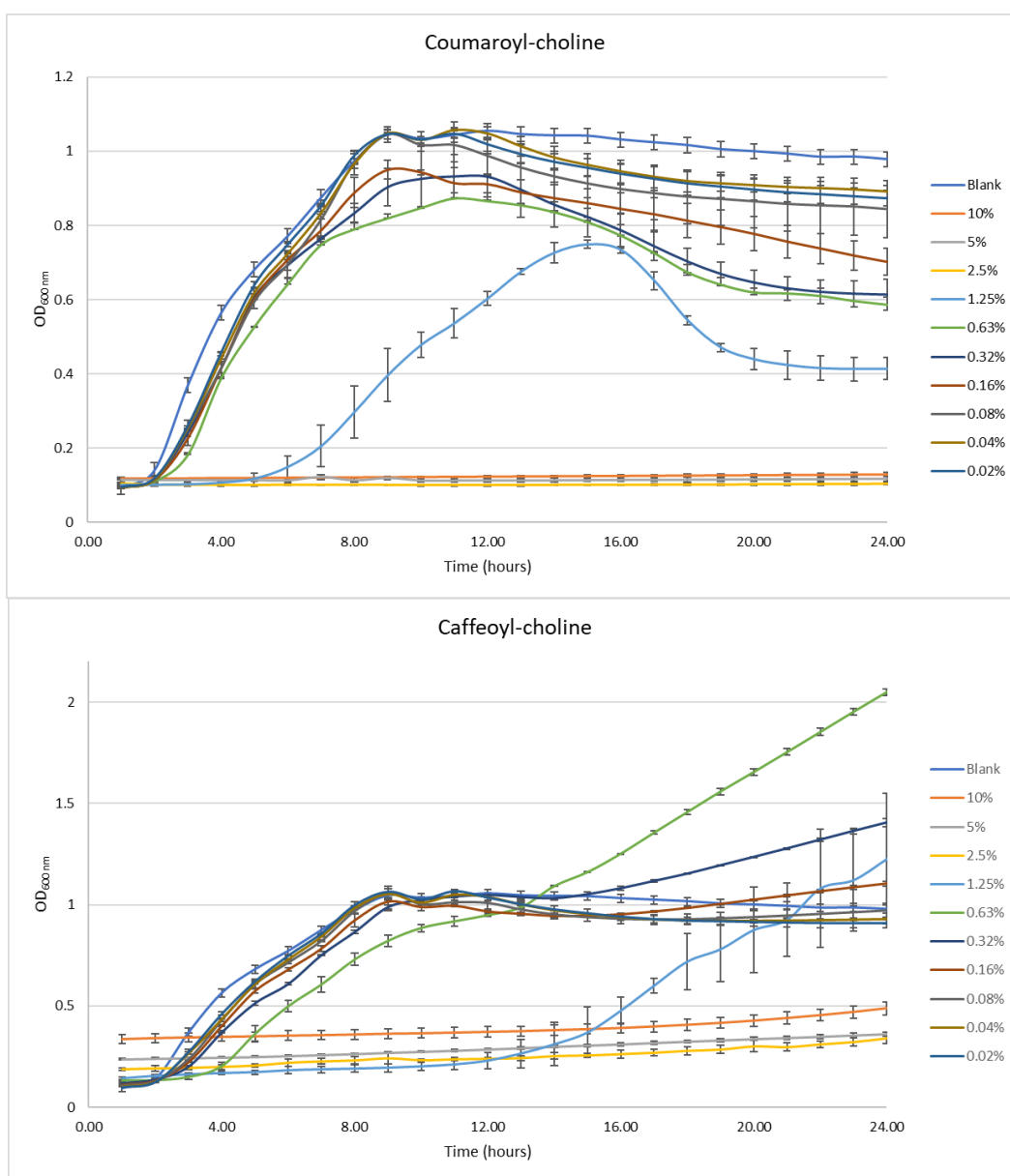
Evaluation of the antioxidant activity of sinapine and analogs. Antioxidant activities of *p*-hydroxycinnamic acids are well described in the literature.¹⁻⁹ An efficient method to assess this property is through DPPH assay which determines the capacity of a given molecule to scavenge stable DPPH free radicals, usually expressed as the quantity of molecule needed to reduce half of the initial population of DPPH radicals (aka EC₅₀, the lower the EC₅₀, the higher the antioxidant activity is). In this study, the EC₅₀ value for sinapine was determined as being 18.1 nmol, which is of the same order of magnitude than that of sinapine thiocyanate measured by Wei et al. using the same assay (17.6 nmol).³³ This study was extended to coumaroyl-, caffeoyl- and feruloyl-choline, giving EC₅₀ values of >150, 6.26 and 36.73 nmol, respectively. The impact on the antioxidant activity of the substituents nature, number, and position on the aromatic cycle is well known phenomenon³⁴ As already reported, the extra methoxy group of sinapine increases the antiradical activity compared to that of feruloyl-choline (18.1 nmol vs. 36.73 nmol); so does the extra hydroxy group of the caffeoyl-choline (6.26 nmol vs. 36.73 nmol). It is noteworthy to mention that the impact of the hydroxy group on the antioxidant properties is higher than that of the methoxy group (6.26 nmol vs. 18.1 nmol), as it provides an extra phenol moiety able to quench a second radical. Compared to commercially available fossil-based antioxidant such as Irganox 1010 (plastic industry) and Trolox (pharmaceutical industry), sinapine and its analogs have slightly lower free radicals scavenging abilities (Table 3). Nevertheless, these molecules are biobased and show high-water solubility which can be real benefits in the cosmetics and agri-food industry.

Table 3. EC₅₀ values for commercially available antioxidants, sinapine and its analogs.

Compound	EC ₅₀ (nmol)
Irganox 1010	6.89 ± 0.28
Trolox	4.02 ± 0.34
BHT	7.11 ± 0.26
BHA	3.67 ± 0.41
Ethyl-Sinapate	13.7 ± 0.66
Coumaroyl-choline	>150
Caffeoyl-choline	6.26 ± 0.11
Feruloyl-choline	36.73 ± 1.52
Sinapine	18.1 ± 0.57

Antimicrobial activity of sinapine and analogs. The antimicrobial potential of sinapine and its analogs has also been assessed using *Escherichia coli* K12 strain. The microorganism was put in presence of different concentrations of the studied compounds and the growth was measured by optical density at 600 nm (OD₆₀₀). While sinapine showed minimal inhibitory concentration (MIC) at 10%w, feruloyl-, caffeoyl- and coumaroyl-choline gave better results at 5, 1.25 and 1.25%w, respectively. The minimal bactericidal concentration (MBC) was also observed at 10, 5, 2.5 and 2.5%w for sinapine, feruloyl-, caffeoyl- and coumaroyl-choline, respectively (Figure

2). It has to be noted that, as previously observed for phenolic acids.³⁵ the more methoxy groups on the aromatic ring, the lower the antimicrobial activity. **As a comparison, natural antibacterial thymol has a MBC of 0.2% w.³⁶** MCI and MCB were confirmed by streaking the culture media on agar plates followed by an incubation at 37 °C for 24 hours. MCI showed colonies in the agar plates, where MCBs showed no colonies. It is important to note that the augmentation of the absorbance at the MCBs of caffeoyl-choline and sinapine are due to strong coloration of the culture media (most likely due to degradation products).



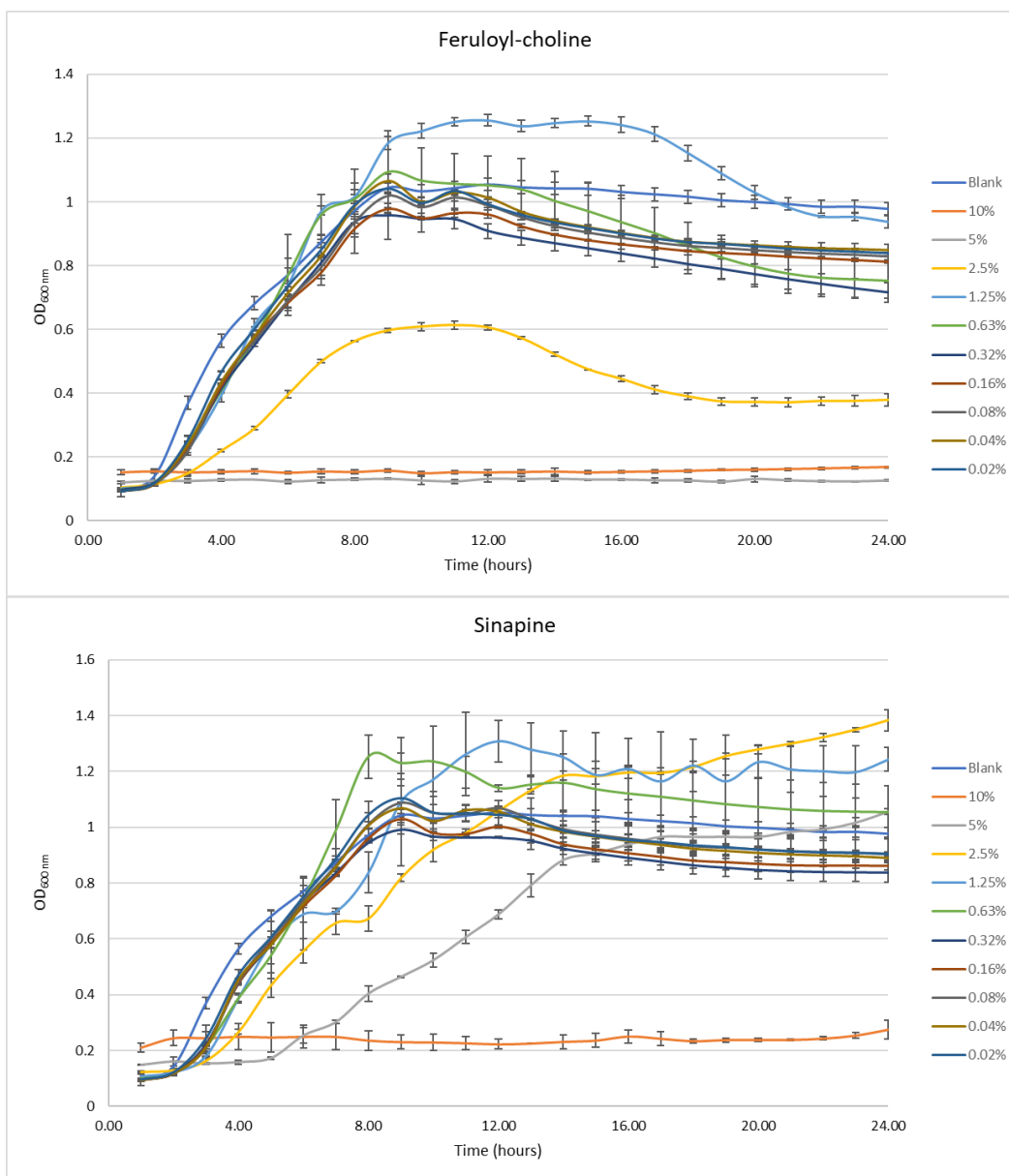


Figure 2. Growth pattern *Escherichia coli* K12 strain in the presence of coumaroyl-, caffeoyl-, feruloyl-choline and sinapine. *E. coli* was grown in LB without (0) or with addition of the indicated concentrations of studied compounds (in % w), and the OD₆₀₀ was measured at the indicated time points. Results are average of two independent assays performed in duplicate, and error bars correspond to standard deviations

Conclusion

Herein, we have developed and optimized a sustainable synthetic approach to sinapine, feruloyl-, caffeoyl- and coumaroyl-choline through a Knoevenagel-Doebner condensation of the corresponding *p*-hydroxybenzaldehydes and the asymmetric choline chloride malonate monoester obtained from Meldrum's acid. This two-step strategy provides the desired products in isolated yields from 34 to 61%. The compounds thus formed were characterized through UHPLC-MS and NMR analysis evaluating their purities being ~95%. Finally, their antioxidant and antimicrobial activities were assessed, and data confirmed their potential as (1) alternatives to current fossil-based antioxidant, and (2) antimicrobial.

Experimental Section

General: Evaporations were conducted under reduced pressure at temperatures below 40 °C unless otherwise noted. ¹H NMR spectra were recorded at 300 MHz at 25 °C in the indicated solvent and referenced to residual protons (CD₃OD, 4.87 ppm). ¹³C NMR spectra were recorded at 75 MHz at 25 °C in the indicated solvent and referenced to solvent (CD₃OD, 49.2 ppm).

HPLC/MS method: LC-MS analyzes were performed on an Agilent 1290 system, equipped with a PDA UV detector, and a 6545 Q-ToF mass spectrometer (Wilmington, DE, USA). The source is equipped with a JetStream ESI probe operating at atmospheric pressure. The spectrometer was configured according to the following settings: mass range *m/z* 50–1000, gas temperature 325 °C, gas flow 8 L/min, nebulizer 35 psi, sheath gas temperature 350 °C, sheath gas flow 11 L/min. Results were recorded and processed with Mass Hunter B.08.000 software. Elution was performed using a Zorbax Eclipse plus C18 (1.8 μm, 50 x 2.1 mm; Agilent) with the column heated at 40 °C. The mobile phases were 0.1% formic acid in water (solvent A) and acetonitrile (solvent B), the flow rate was set at 0.4 mL/min and followed the gradient: 0-3 min

at 5% of B, 3-4 min from 5% to 10% B, 4-13 min from 10% to 99% B, 13-16 min at 99% B, 16-18 min from 99% to 5% B. The sample injection volume was 1 μ L and the autosampler was tempered at 10 °C. The UV acquisition was carried out at 250 nm and 320 nm with a reference set at 360 nm.

Method for the synthesis of Sinapine and its analogs: Meldrum's acid (1.51 g, 10.5 mmol) and choline chloride (980 mg, 7 mmol) were mixed together in acetonitrile (3.5 mL, 2M) and heated at reflux for 5 hours. The resulting mixture was dried in vacuo and dissolved in ethanol (630 mM, 10 mL). The corresponding aldehyde (6.9 mmol) and proline (725 mg, 6.9 mmol) were then added and the solution was refluxed overnight. The crude mixture was concentrated *in vacuo*, dissolved in deionized water (10 mL) and directly applied to a C18 reversed phase flash (95/5, water/methanol). The fraction containing the desired product were combined and dried *in vacuo* to yield the pure product.

Coumaroyl-Choline: 34% isolated yield. M.p. 240-242 °C. ^1H NMR (300 MHz, CD_3OD): δ = 7.70 (d, J = 15.9 Hz, 1H, H-3), 7.50 (d, J = 8.4 Hz, 1H, H-5 and H-9), 6.83 (d, J = 8.4 Hz, 2H, H-6 and H-8), 6.39 (d, J = 15.9 Hz, 1H, H-2), 4.67 (m, 2H, H-10), 3.81 (m, 2H, H-11), 3.30 (s, 9H, H-12). ^{13}C NMR (75 MHz, CD_3OD): δ = 167.0 (s, C-1), 160.7 (s, C-7), 146.8 (d, C-3), 130.5 (d, C-5 and C-9), 125.9 (s, C-4), 116.0 (d, C-6 and C-8), 113.1 (d, C-2), 65.3 (t, C-11), 57.9 (t, C-10), 53.6 (q, C-12). TOF MS ES+: $[\text{M}]^+$ for $\text{C}_{14}\text{H}_{20}\text{NO}_3^+$: m/z 250.1438; found: m/z 250.1437.

Caffeoyl-Choline: 61% isolated yield. M.p. 183-185 °C. ^1H NMR (300 MHz, CD_3OD): δ = 7.61 (d, J = 15.9 Hz, 1H, H-3), 7.09 (d, J = 1.8 Hz, 1H, H-9), 6.98 (dd, J = 1.8 and 8.4 Hz, 1H, H-8), 6.80 (d, J = 8.4 Hz, 1H, H-5), 6.31 (d, J = 15.9 Hz, 1H, H-2), 4.65 (m, J = Hz, 1H, H-10), 3.80 (m, 5H, H-11 and H-13), 3.27 (s, 9H, H-12). ^{13}C NMR (75 MHz, CD_3OD): δ = 166.5 (s, C-1),

148.5 (s, C-7), 146.7 (s, C-6), 145.5 (d, C-3), 126.1 (s,C-4), 121.9 (d, C-9), 115.2 (d, C-8), 114.0 (d, C-2), 112.6 (d, C-5), 64.8 (t, C-11), 57.4 (t, C-10), 53.1 (q, C-12). TOF MS ES+: $[M]^+$ for $C_{14}H_{20}NO_4^+$: m/z 266.1387; found: m/z 266.1385.

Feruloyl-Choline: 50% isolated yield. M.p. 109-111 °C. 1H NMR (300 MHz, CD_3OD): δ = 7.50 (d, J = 15.9 Hz, 1H, H-3), 7.06 (d, J = 1.8 Hz, 1H, H-9), 6.96 (dd, J = 1.8 and 8.1 Hz, 1H, H-6), 6.76 (d, J = 8.1 Hz, 1H, H-5), 6.27 (d, J = 15.9 Hz, 1H, H-2), 4.57 (m, J = Hz, 1H, H-10), 3.76 (m, 5H, H-11 and H-13), 3.23 (s, 9H, H-12). ^{13}C NMR (75 MHz, CD_3OD): δ = 167.9 (s, C-1), 150.6 (s, C-8), 149.2 (s, C-7), 147.7 (d, C-3), 127.3 (s,C-4), 124.4 (d, C-5), 116.5 (d, C-6), 114.3 (d, C-2), 111.8 (d, C-9), 66.0 (t, C-11), 58.9 (t, C-10), 56.5 (q, C-13), 54.6 (q, C-12). TOF MS ES+: $[M]^+$ for $C_{15}H_{22}NO_4^+$: m/z 280.1543; found: m/z 280.1545.

Sinapine: 48% isolated yield. M.p. 82-84 °C. 1H NMR (300 MHz, CD_3OD): δ = 7.44 (d, J = 15.9 Hz, 1H, H-3), 6.74 (s, 2H, H-5 and 9), 6.24 (d, J = 15.9 Hz, 1H, H-2), 4.56 (m, 2H, H-10), 3.78 (m, 2H, H-11), 3.74 (s, 6H, H-13), 3.24 (s, 9H, H-12). ^{13}C NMR (75 MHz, CD_3OD): δ = 167.7 (s, C-1), 149.2 (s, C-6 and 8), 147.8 (d, C-3), 139.5 (s, C-7), 126.2 (s, C-4), 114.7 (d, C-2), 106.9 (d, C-5 and 9), 66.1 (t, C-11), 59.9 (t, C-10), 56.8 (q, C-12), 54.6 (q, C-13). TOF MS ES+: $[M]^+$ for $C_{16}H_{24}NO_5^+$: m/z 310.1649; found: m/z 310.1648.

Antioxidant assay: 190 μ L of homogeneous DPPH solution (200 μ M) in ethanol was added to a well containing 10 μ L of coumaroyl-, caffeoyl-, feruloyl-choline or sinapine in ethanol at different concentrations (from 400 μ M to 12.5 μ M). The reaction was followed by a microplate Multiskan FC, performing 1 scan every 5 min for 7.5 h at 515 nm. The use of different amounts of substrate give the EC_{50} value, which is describe as the efficient concentration needed to reduce the initial population of DPPH by half.

Antimicrobial assay: Overnight cultures of *Escherichia coli* K12 strain were diluted to an $OD_{600} = 0.1$ in a sterile 96-well microplate with fresh LB medium with or without the addition of coumaroyl-, caffeoyl-, feruloyl-choline or sinapine at a final concentration of 10, 5, 2.5, 1.25, 0.630, 0.320, 0.160, 0.080, 0.040 or 0.020 %w, and the optical density at 600 nm was measured every 15 min. Proper blank controls were used in each microplate. Wells containing the MCBs and MCIs were streaked onto Agar plates and incubated for 24 hours.

Acknowledgements

This work was supported by the Région Grand Est, Conseil Départemental de la Marne and Grand Reims.

References

- (1) Nowak, H.; Kujawa, K.; Zadernowski, R.; Rocznik, B.; Kozłowska, H. Antioxidative and bactericidal properties of phenolic compounds in rapeseeds. *Fett Wiss. Technol.* **1992**, *94*, 149-52.
- (2) Tesaki, S.; Tanabe, S.; Ono, H.; Fukushi, E.; Kawabata, J.; Watanabe, M. 4-Hydroxy-3-nitrophenylacetic and sinapic acids as antibacterial compounds from mustard seeds. *Biosci., Biotechnol., Biochem.* **1998**, *62*, 998-1000.
- (3) Maddox, C. E.; Laur, L. M.; Tian, L. Antibacterial activity of phenolic compounds against the phytopathogen *Xylella fastidiosa*. *Curr. Microbiol.* **2010**, *60*, 53-58.
- (4) Engels, C.; Schieber, A.; Gaenzle, M. G. Sinapic acid derivatives in defatted Oriental mustard (*Brassica juncea* L.) seed meal extracts using UHPLC-DAD-ESI-MS n and

identification of compounds with antibacterial activity. *Eur. Food Res. Technol.* **2012**, *234*, 535-542.

(5) Yun, K.-J.; Koh, D.-J.; Kim, S.-H.; Park, S. J.; Ryu, J. H.; Kim, D.-G.; Lee, J.-Y.; Lee, K.-T. Anti-inflammatory effects of sinapic acid through the suppression of inducible nitric oxide synthase, cyclooxygenase-2, and proinflammatory cytokines expressions via nuclear factor- κ B inactivation. *J. Agric. Food Chem.* **2008**, *56*, 10265-10272.

(6) Hudson, E. A.; Dinh, P. A.; Kokubun, T.; Simmonds, M. S. J.; Gescher, A. Characterization of potentially chemopreventive phenols in extracts of brown rice that inhibit the growth of human breast and colon cancer cells. *Cancer Epidemiol., Biomarkers Prev.* **2000**, *9*, 1163-1170.

(7) Dean, J.; Kusaka, R.; Walsh, P.; Allais, F.; Zwier, T. Plant Sunscreens in the UV-B: Ultraviolet Spectroscopy of Jet-cooled Sinapoyl Malate, Sinapic Acid, and Sinapate Ester Derivatives. *J. Am. Chem. Soc.* **2014**, *136*, 14780.

(8) He, L.; Li, H.; Guo, S.; Liu, L.; Qiu, J.; Li, F.; Cai, B. Inhibitory effects of sinapine on activity of acetylcholinesterase in cerebral homogenate and blood serum of rats. *Zhongguo Zhongyao Zazhi* **2008**, *33*, 813-815.

(9) Ferreres, F.; Fernandes, F.; Sousa, C.; Valentao, P.; Pereira, J. A.; Andrade, P. B. Metabolic and Bioactivity Insights into *Brassica oleracea* var. *acephala*. *J. Agric. Food Chem.* **2009**, *57*, 8884-8892.

(10) Niciforovic, N.; Abramovic, H. Sinapic Acid and Its Derivatives: Natural Sources and Bioactivity. *Compr. Rev. Food Sci. Food Saf.* **2014**, *13*, 34-51.

- (11) Cai, R.; Arntfield, S. D. A rapid high-performance liquid chromatographic method for the determination of sinapine and sinapic acid in canola seed and meal. *J. Am. Oil Chem. Soc.* **2001**, *78*, 903-910.
- (12) Khattab, R.; Eskin, M.; Aliani, M.; Thiyam, U. Determination of sinapic acid derivatives in canola extracts using high-performance liquid chromatography. *J. Am. Oil Chem. Soc.* **2010**, *87*, 147-155.
- (13) Vuorela, S.; Meyer, A. S.; Heinonen, M. Quantitative analysis of the main phenolics in rapeseed meal and oils processed differently using enzymatic hydrolysis and HPLC. *Eur. Food Res. Technol.* **2003**, *217*, 517-523.
- (14) Oilseeds: World Markets and Trade. USDA, Ed.2016.
- (15) Thiyam, U.; Stoeckmann, H.; Schwarz, K. Antioxidant activity of rapeseed phenolics and their interactions with tocopherols during lipid oxidation. *J. Am. Oil Chem. Soc.* **2006**, *83*, 523-528.
- (16) Yang, M.; Zheng, C.; Zhou, Q.; Liu, C.; Li, W.; Huang, F. Influence of Microwaves Treatment of Rapeseed on Phenolic Compounds and Canolol Content. *J. Agric. Food Chem.* **2014**, *62*, 1956-1963.
- (17) Szydłowska-Czerniak, A.; Amarowicz, R.; Szlyk, E. Antioxidant capacity of rapeseed meal and rapeseed oils enriched with meal extract. *Eur. J. Lipid Sci. Technol.* **2010**, *112*, 750-760.
- (18) Hruschka, S.; Ullmann, D.; Boszulak, W.; Thiel, A. WO2015181203A1, 2015.
- (19) Matthaeus, B. Antioxidant Activity of Extracts Obtained from Residues of Different Oilseeds. *J. Agric. Food Chem.* **2002**, *50*, 3444-3452.

- (20) Flourat, A. L.; Willig, G.; Teixeira, A. R. S.; Allais, F. Eco-Friendly Extraction of Sinapine From Residues of Mustard Production. *Front. Sustain. Food Syst.* **2019**, 12/1-12/10.
- (21) Allais, F.; Martinet, S.; Ducrot, P. H. Straightforward total synthesis of 2-O-feruloyl-L-malate, 2-O-sinapoyl-L-malate and 2-O-5-hydroxyferuloyl-L-malate. *Synthesis* **2009**, 21, 3571-3578.
- (22) Zhu, Y.; Ralph, J. Stereoselective synthesis of 1-O- β -feruloyl and 1-O- β -sinapoyl glucopyranoses. *Tetrahedron Lett.* **2011**, 52, 3729-3731.
- (23) Clausen, S.; Olsen, O.; Soerensen, H. Separation of aromatic choline esters by high-performance liquid chromatography. *J. Chromatogr.* **1983**, 260, 193-9.
- (24) Whatelet, J. P.; Mabon, N.; Marlier, M., Synthesis and Purification of 36 Aromatic Choline Esters as Standards. In *GCIRC Internatiional Congress*: Canberra, Australie, 1988.
- (25) A precise titration for the formation of sinpoate would be difficult to reproduce on an industrial scale.
- (26) Sebestik, J.; Marques, S. M.; Fale, P. L.; Santos, S.; Arduino, D. M.; Cardoso, S. M.; Oliveira, C. R.; Serralheiro, M. L. M.; Santos, M. A. Bifunctional phenolic-choline conjugates as anti-oxidants and acetylcholinesterase inhibitors. *J. Enzyme Inhib. Med. Chem.* **2011**, 26, 485-497.
- (27) An, Y.; Zhang, Y.; Wang, H.; Feng, B. Synthesis of seven sinapine analogs and their effects on the tension of smooth muscle of intestines isolated from rabbit. *Yaoxue Xuebao* **2011**, 46, 1466-1470.
- (28) Spath, E. Synthesis of sinapine. *Monatsh. Chem.* **1920**, 41, 271-85.
- (29) Brooks, D. W.; De Lee, N. C.; Peevey, R. Remote substituent effects in microbial reductions of 3-ketoglutarate and 3-ketoadipate esters. *Tetrahedron Lett.* **1984**, 25, 4623-6.

- (30) de Winter, T. M.; Balland, Y.; Neski, A. E.; Petitjean, L.; Erythropel, H. C.; Moreau, M.; Hitce, J.; Coish, P.; Zimmerman, J. B.; Anastas, P. T. Exploration of a Novel, Enamine-Solid-Base Catalyzed Aldol Condensation with C-Glycosidic Pyranoses and Furanoses. *ACS Sustainable Chem. Eng.* **2018**, *6*, 11196-11199.
- (31) Peyrot, C.; Peru, A. A. M.; Mouterde, L. M. M.; Allais, F. Proline-Mediated Knoevenagel-Doebner Condensation in Ethanol: A Sustainable Access to *p*-Hydroxycinnamic Acids. *ACS Sustainable Chem. Eng.* **2019**, *7*, 9422-9427.
- (32) Knoevenagel, E. E. Knoevenagel: Ueber eine Darstellungsweise der Glutarsaure. *Ber. Dtsch. Chem. Ges.* **1894**, *27*, 2345-2346.
- (33) Li, X.; Han, L.; Li, Y.; Zhang, J.; Chen, J.; Lu, W.; Zhao, X.; Lai, Y.; Chen, D.; Wei, G. Protective effect of sinapine against hydroxyl radical-induced damage to mesenchymal stem cells and possible mechanisms. *Chem. Pharm. Bull.* **2016**, *64*, 319-325.
- (34) Reano, A.; Cherubin, J.; Peru, A. M.; Wang, Q.; Clement, T.; Domenek, S.; Allais, F. Structure-activity Relationships and Structural design Optimization of a Series of *p*-hydroxycinnamic Acids-based bis- and trisphenols as novel sustainable Antiradical/antioxidant additives. *ACS sustainable Chem. Eng.* **2015**, *3*, 3486-3496.
- (35) Sanchez-Maldonado, A. F.; Schieber, A.; Ganzle, M. G. Structure-function relationships of the antibacterial activity of phenolic acids and their metabolism by lactic acid bacteria. *J. Appl. Microbiol.* **2011**, *111*, 1176-1184.
- (36) Gavarić, N.; Kovac, J.; Kretschmer, N.; Kladar, N.; Mozina, S. S.; Bucar, F.; Bauer, R.; Bozin, B. In *Natural products as antibacterial agents - antibacterial potential and safety of post-distillation and waste material from thymus vulgaris l., lamiaceae* 2015 InTech; pp 123-151.