Food & Function

ROYAL SOCIETY OF CHEMISTRY

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *Food Funct.*, 2022, **13**, 7487

Received 30th May 2022,
Accepted 23rd June 2022

DOI: 10.1039/d2fo01475k

rsc.li/food-function

4-Hydroxydibenzyl: a novel metabolite from the human gut microbiota after consuming resveratrol

C. E. Iglesias-Aguirre, [a] F. Vallejo,[a] D. Beltrán,[a] J. Berná, [b] J. Puigcerver,[b] M. Alajarín, [b] M. V. Selma [a] and J. C. Espín *[a]

Resveratrol (RSV) was known to be metabolised by the gut microbiota to dihydroresveratrol, lunularin (LUNU), and (or) 3,4′-dihydroxy-*trans*-stilbene (DHST). We describe here for the first time that LUNU can be further dehydroxylated, but only at the 3-position, to yield 4-hydroxydibenzyl, a novel metabolite found in human urine after RSV intake in 41 out of 59 healthy participants. In contrast, DHST was not further dehydroxylated, and thus, 4-hydroxy-*trans*-stilbene was not detected as a gut microbial metabolite of RSV. Faecal *in vitro* incubations confirmed the *in vivo* results.

Introduction

The stilbene *trans*-resveratrol (3,5,4′-trihydroxy-*trans*-stilbene, resveratrol, RSV) has been reported to exert health benefits in different clinical studies.[1–5] However, the metabolism of RSV by the human gut microbiota has been scarcely approached so far. An *in vitro* pioneering study by Jung *et al.*[6] screened 43 animal- or human-associated bacterial strains for RSV metabolism. Among the strains metabolizing RSV, *Eggerthella lenta* ATCC 43055 and *Bacteroides uniformis* ATCC 8492 converted RSV efficiently into dihydroresveratrol (DHRSV).

A further *in vitro* and *in vivo* study conducted by Bode *et al.*[7] showed that RSV's main gut microbial metabolites were DHRSV, 3,4′-dihydroxydibenzyl (also known as lunularin, LUNU), and 3,4′-dihydroxy-*trans*-stilbene (DHST). After consuming a single RSV dose, these metabolites were found in hydrolysed urine samples from 12 healthy volunteers. The bacteria *Slackia equolifaciens* and *Adlercreutzia equolifaciens* converted RSV into DHRSV, although the microbial groups involved in the biotransformation of RSV to other metabolites remain mostly unknown.[7] Recently, a bacterial strain isolated from human faeces, capable of reducing RSV to DHRSV has been described and proposed as *Adlercreutzia rubneri* sp. nov. with the type and only strain ResAG-91^T.[8]

Recently, Jarosova *et al.*[9] described the metabolism *in vitro* of RSV, among other stilbenes, using faecal samples from 5 individuals. In contrast to Bode *et al.*,[7] DHRSV was the only metabolite detected, but not LUNU or DHST.

LUNU and DHST are produced by a single dehydroxylation at the 3- or 5-position (it is the same theoretical position as the resorcinol nucleus is symmetrical) in DHRSV and RSV, respectively.[7] Thus, we hypothesized that a similar further dehydroxylation could convert LUNU into 4-hydroxydibenzyl (4HDB) and DHST into 4-hydroxy-*trans*-stilbene (4HST). Therefore, we aimed to identify the presence of both monohydroxylated metabolites, 4HDB and 4HST, in urine and faeces as possible novel RSV-derived metabolites from the human gut microbiota.

Materials and methods

Materials

trans-Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene, resveratrol, RSV, 99% purity), 6,7-dihydroxycoumarin (98%), β-glucuronidase (≥100 000 units per mL), and sulfatase (>10 000 units per g solid) from *Helix pomatia* were purchased from Sigma-Aldrich (St. Louis, MO, USA). 4-Hydroxy-*trans*-stilbene (4HST, 98%) was obtained from ThermoFisher Sci. (Madrid, Spain). Organic solvents were purchased from Merck (Darmstadt, Germany) and Milli-Q ultrapure water from Millipore Corp. (Bedford, MA, USA).

Synthesis and spectroscopy data of resveratrol-derived metabolites

^{1}H NMR spectra were recorded at 25 °C on Avance 300 and 400 MHz instruments (Bruker, Karlsruhe, Germany). ^{1}H NMR chemical shifts are reported relative to tetramethylsilane (Me_4Si) and were referenced *via* residual proton resonances of the corresponding deuterated solvent. Abbreviations of coupling patterns are as follows: br, broad; s, singlet; d, doublet; t,

[a]*Laboratory of Food & Health, Research Group on Quality, Safety and Bioactivity of Plant Foods, CEBAS-CSIC, P. O. Box 164, 30100 Campus de Espinardo, Murcia, Spain. E-mail: jcespin@cebas.csic.es*
[b]*Department of Organic Chemistry, Faculty of Chemistry, University of Murcia, 30100 Murcia, Spain*

triplet; q, quadruplet; m, multiplet. Coupling constants (J) are expressed in Hz.

Dihydroresveratrol (>97%; DHRSV) was synthesized as previously described,[10] and showed identical spectroscopic data as those reported therein: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.11 (s, 1H), 9.02 (s, 2H), 6.99 (d, J = 8.4 Hz, 2H), 6.65 (d, J = 8.5 Hz, 2H), 6.06 (d, J = 2.1 Hz, 2H), 6.02 (t, J = 2.1 Hz, 1H), 2.64 (m, 4H).

4-Hydroxydibenzyl (or 4-phenethylphenol, 4HDB, >97%) was synthesized according to Camaioni *et al.*,[11] showing the following spectroscopic data: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.12 (s, 1H), 7.30–7.12 (m, 5H), 6.99 (d, J = 8.5 Hz, 2H), 6.64 (d, J = 8.4 Hz, 2H), 2.84–2.71 (m, 4H).

3-Hydroxydibenzyl (or 3-phenethylphenol, 3HDB, >97%) was synthesized as described elsewhere,[12] and showed identical spectroscopic data as those reported therein: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34–7.25 (m, 2H), 7.24–7.12 (m, 4H), 6.77 (dd, J = 7.6, 1.0 Hz, 1H), 6.71–6.65 (m, 2H), 4.71 (s, 1H), 2.96–2.83 (m, 4H).

3,4′-Dihydroxydibenzyl (or 3-(4-hydroxyphenethyl)phenol, lunularin, LUNU, >97%) was synthesized following the procedure described by Ali *et al.*,[13] and showed identical spectroscopic data as those reported therein: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.15 (td, J = 7.6, 0.7 Hz, 1H), 7.07–7.01 (m, 2H), 6.78–6.72 (m, 3H), 6.69–6.63 (m, 2H), 4.66 (s, 1H), 4.61 (s, 1H), 2.83 (br s, 4H).

3,4′-Dihydroxy-*trans*-stilbene (or 3-(4-hydroxystyryl)phenol, DHST, >97%) was synthesized as previously described,[14] showing identical spectroscopic data as those reported therein: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.56 (s, 1H), 9.36 (s, 1H), 7.41 (d, J = 8.5 Hz, 2H), 7.13 (t, J = 7.8 Hz, 1H), 7.03 (d, J = 16.3 Hz, 1H), 6.98–6.88 (m, 3H), 6.76 (d, J = 8.5 Hz, 2H), 6.63 (d, J = 6.8 Hz, 1H) ppm.

Subjects and study design

This dietary intervention followed the ethical guidelines outlined in the Helsinki Declaration of 1975 and its amendments. This study belongs to an ongoing larger trial (MetaboGut), approved (reference PI-042) by IMDEA-Food (Madrid, Spain) and the Spanish National Research Council's Bioethics Committee (Madrid). The present study was not designed to evaluate specific health effects on the volunteers but to increase the current knowledge about the metabolism of RSV by the human gut microbiota.

Volunteers with no diagnosed chronic disease were recruited. Table 1 shows the main characteristics of the participants. Exclusion criteria were as follows: pregnancy/lactation, recent use of antibiotics (within 1-month prior to the study), history of smoking (recent past or present), diagnosed chronic illness, previous gastrointestinal surgery, or taking medication (within 1-month prior to the study). The study was explained to the participants who provided written informed consent.

Participants consumed one daily hard gelatin capsule in the evening for 7 days. The capsules contained 150 mg RSV from *Polygonum cuspidatum* and were manufactured by Laboratorios Admira S.L. (Alcantarilla, Murcia, Spain) following the European Union's Good Manufacturing Practices requirements. The RSV content of the extract was certified by the company (98.7% purity by HPLC) and was free of anthraquinones, metals, solvents, and pathogens.

Table 1 Characteristics of participants (n = 59)

Characteristics	Values[a]
Age (years)	34.2 ± 9.5 (18–52)
Weight (kg)	67.8 ± 10.3 (48.0–95.0)
Normal weight (%)	48 (81.4%)
Overweight (%)	10 (16.9%)
Obese (%)	1 (1.7%)
BMI (kg m^{-2})	23.0 ± 2.7 (18.5–30.7)
Sex (female/male)	28/31

[a] Quantitative values are shown as mean ± SD and (range).

Faecal cultures

Baseline stool samples were provided by 9 volunteers randomly chosen from the group (7 males and 2 females normoweight subjects). Preparation of faecal suspensions and subsequent culturing experiments were conducted with minor modifications as previously described.[15]

Aliquots were prepared with 10 g of stool samples in filter bags and diluted 1 : 10 (w/v) with L-cysteine hydrochloride supplemented (0.05%) Nutrient Broth using a stomacher for homogenization. Filtered faecal suspensions (50 μL) were inoculated into 5 mL of Wilkins-Chalgren anaerobe medium (WAM, Oxoid) added with 0.05% L-cysteine and containing a 30 μM solution of each standard in DMSO (0.6% DMSO in the final culture medium). The compounds (RSV, DHRSV, DHST, LUNU, 4HDB, and 4HST) were individually added to the broth and incubated under anoxic conditions in an anaerobic chamber (Concept 400, Baker Ruskin Technologies Ltd, Bridgend, South Wales, UK) with an atmosphere consisting of $N_2/H_2/CO_2$ (85 : 5 : 10) at 37 °C. Incubations of faecal cultures without added compounds, and incubations of the compounds without fecal inocula, were used as controls. Three replicates were carried out using each faecal suspension and compound. Samples were collected after 7 days of incubation and processed for UPLC-QTOF-MS and(or) GC-MS analyses.

Sampling procedure and processing

Volunteers provided urine and faeces at baseline and after 7 days. Samples were frozen at −80 °C until further analysis. The urine samples were centrifuged, filtered through a 0.22 μm PVDF filter, and diluted with acidified water (0.1% formic acid) before analysis by UPLC-QTOF-MS. The urinary excretion of creatinine was measured to allow standardisation of diuresis, as reported elsewhere.[16]

For the complete deconjugation of phase-II conjugated metabolites, urine samples were treated overnight with glucuronidase and sulfatase from *Helix pomatia*, as previously described.[16] Hydrolysed urine samples were analysed by UPLC-QTOF-MS and GC-MS.

Stool samples (1 g) were processed as previously described.[17] Faecal samples (5 mL) from fermentation experiments were extracted with 5 mL of ethyl acetate plus 1.5% formic acid and processed, as described elsewhere.[15] Both faecal and *in vitro* fermented samples were analysed by LC-MS and GC-MS.

In the case of GC-MS analyses, urine, faeces, and faecal fermentation samples were extracted as described above and analyzed with and without derivatisation. The evaporated residues were dissolved in 30 μL pyridine and converted to trimethylsilyl derivatives by adding 30 μL of *N*,*O*-bis-(trimethylsilyl) trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) and incubated at 100 °C for 15 min. The sample residues after speed vacuum evaporation were dissolved in acetone and injected into the GC-MS equipment for the analyses without derivatization.

Analysis of resveratrol and derived metabolites

UPLC-ESI-QTOF-MS. Analyses were performed on an Agilent 1290 Infinity UPLC system coupled to a 6550 Accurate-Mass quadrupole-time-of-flight (QTOF) mass spectrometer (Agilent Technologies, Waldbronn, Germany) using an electrospray interface (Jet Stream Technology) as previously described, using 6,7-dihydroxycoumarin as an internal control of the ionisation signal. A previously validated method (linearity, precision, accuracy, limits of detection, and quantification) was used to analyze RSV and derived metabolites.[16]

GC-MS. Silylated samples were analysed using an HP 8890 gas chromatograph with an HP 5977B mass selective detector (MSD) (Agilent). An HP5-MS (30 m × 0.25 mm ID and film thickness of 0.25 μm) phase capillary column was used with helium as a carrier gas at a constant rate of 1 mL min^{-1}. The temperature of the injector and MS source was maintained at 200 °C. The column temperature program consisted of injection at 60 °C (hold time 1 min), which was raised at 7 °C min^{-1} to 180 °C, at 3 °C min^{-1} to 200 °C (hold time 1 min), and finally at 10 °C min^{-1} to 230 °C (hold time 10 min). A solvent delay of 3.5 min was selected. The samples were analysed in splitless mode with a purge flow to a split vent of 50 mL min^{-1} at 0.8 min. An Agilent 5190-2292-900 μL (Splitlless, single taper, ultra inert) liner was used. The MS was operated in the electron impact mode with an ionisation energy of 70 eV. The mass spectrum was acquired in positive electron impact (70 eV). Two methods with different MS parameters were performed depending on the metabolites to be analysed.

The first methodology was optimised for non-silylated metabolites, 3HDB and 4HDB, and the MS parameters were the following: SIM acquisition type with both 198 and 107 *m*/*z* ions selected, segment retention times fixed between 21 and 24 min with high-resolution mode, dwell time of 25 ms and cycle time of 13.96 Hz, and finally, a calculated EMV of 1700.

The second methodology was used for silylated metabolites, *i.e.*, LUNU, DHP, RSV, DHRSV, DHST, and 4HST, and the MS parameters were the following: SCAN acquisition type with a threshold of 1000, scan mass range from 50 to 800 Da at 2.0 scan per s, a scan speed of 1.562 u/s, a cycle time of 502.60 ms and finally, a calculated EMV of 1360.

Compounds were identified by direct comparison with the available standards and confirmed by their spectral properties, molecular mass, and fragmentation pattern. The quantification of RSV and derived metabolites and their conjugates were determined by interpolation in the calibration curves obtained with their corresponding available standards in the urine and faecal matrices.

Results

Resveratrol metabolites in urine and faeces

The UPLC-ESI-QTOF-MS analysis of urine samples allowed the tentative identification of 25 RSV-derived metabolites, mostly phase-II derived from both human and microbial origin (results not shown). Thus, the hydrolysis of urine samples was carried out to identify the parent (unconjugated) RSV and its derived gut microbial metabolites using the available standards. As a result, RSV, *cis*-RSV, DHRSV, LUNU, and DHST were identified in urine. Fig. 1 shows representative extracted ion chromatograms of these metabolites, although high inter-individual variability was observed since RSV, *cis*-RSV, and DHRSV were observed in all the samples, while LUNU was detected in 41 and DHST only in 14 of the 59 volunteers. The same metabolites were also detected in faeces (results not shown). However, no monohydroxylated metabolites were found in urine or faeces despite different modifications of the UPLC-ESI-QTOF protocol, or UPLC-QQQ analyses were tested (results not shown).

Next, we searched for the possible monohydroxylated metabolites using GC-MS. Table 2 shows the compounds detected using GC-MS in silylated and non-silylated samples. Silylation allowed for determining the same metabolites detected by UPLC-QTOF-MS (Table 2 and Fig. 2). The available standard of the monohydroxylated stilbene 4HST was detected by GC-MS, but its ion mass was not found in any urine or

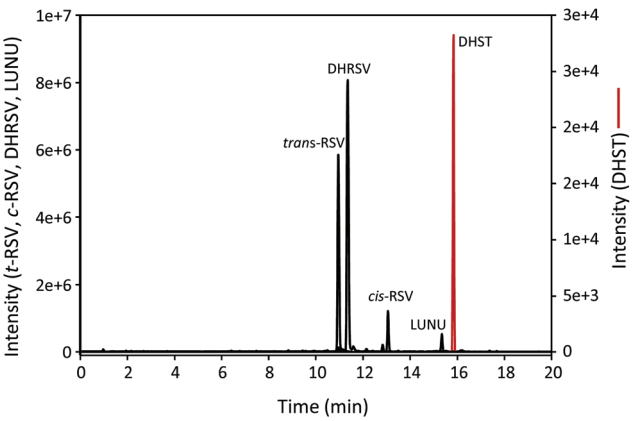

Fig. 1 UPLC-ESI-QTOF-MS extracted ion chromatograms of hydrolysed urine samples after RSV intake for 7 days.

Table 2 RSV and its gut microbial-derived metabolites determined by GC-MS in urine[a] and faeces after RSV intake (n = 59) and (or) faecal cultures after individual incubation of RSV, DHRSV, LUNU, DHST, and 4HST (n = 9)

No.	Compound	RT (min)	Mass	Target ion	LOD; LOQ (nM)	Occurrence	Urine (μg mg^{-1} creatinine)[b]	Faeces (μg g^{-1})[b]
Silylated								
1	4HST	28.1	268	268	200; 500	ND	—	—
2	LUNU	29.3	358	179	50; 100	U, F, FC	85.7 ± 129.5	30.1 ± 29.3
3	*cis*-RSV	32.0	444	444	—	U	268.2 ± 150.3[c]	—
4	DHRSV	32.4	446	179	100; 250	U, F, FC	909.2 ± 649.2	33.6 ± 50.2
5	DHST	32.9	356	356	200; 500	U, F	10.0 ± 4.4	D
6	RSV	38.2	444	444	100; 250	U, F, FC	464.8 ± 266.3	1.5 ± 3.0
Non-silylated								
1′	3HDB	21.8	198[d]	107	200; 500	ND	—	—
2′	4HDB	22.1	198[d]	107	100; 250	U, FC	12.5 ± 36.6	—

U, urine; F, faeces; FC, faecal culture; ND, not detected; D, detected but not quantified; 4HST, 4-hydroxy-*trans*-stilbene; LUNU, lunularin; DHRSV, dihydroresveratrol; DHST, 3,4′-dihydroxy-*trans*-stilbene; RSV (*trans*-resveratrol); 3HDB, 3-hydroxydibenzyl; 4HDB, 4-hydroxydibenzyl. [a] Hydrolysed samples. [b] Mean ± SD. [c] Tentatively quantified as RSV. [d] Mass without silylation.

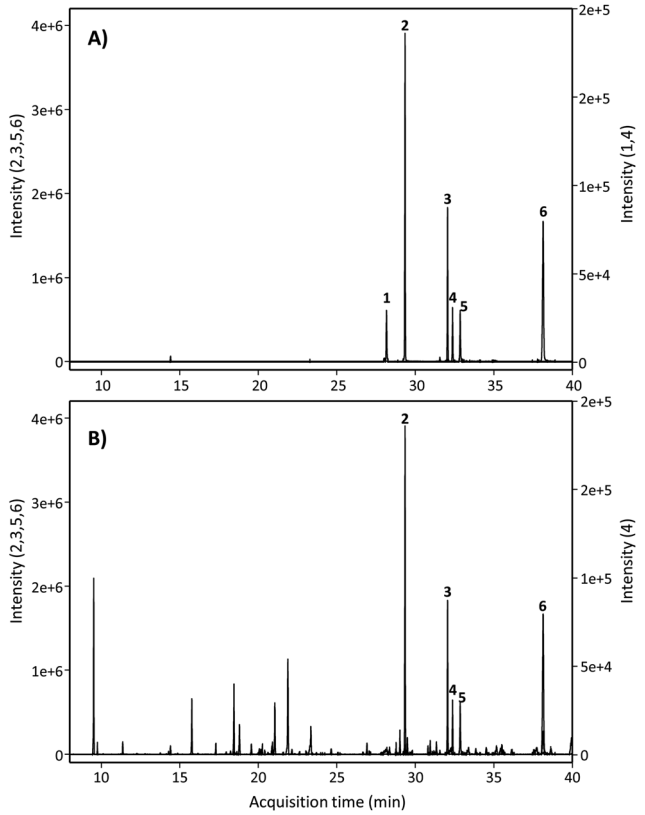

Fig. 2 GC-MS extracted ion chromatograms after silylation of (A) standards and (B) hydrolysed urine samples. Peak numbers are listed in Table 2 (silylated samples).

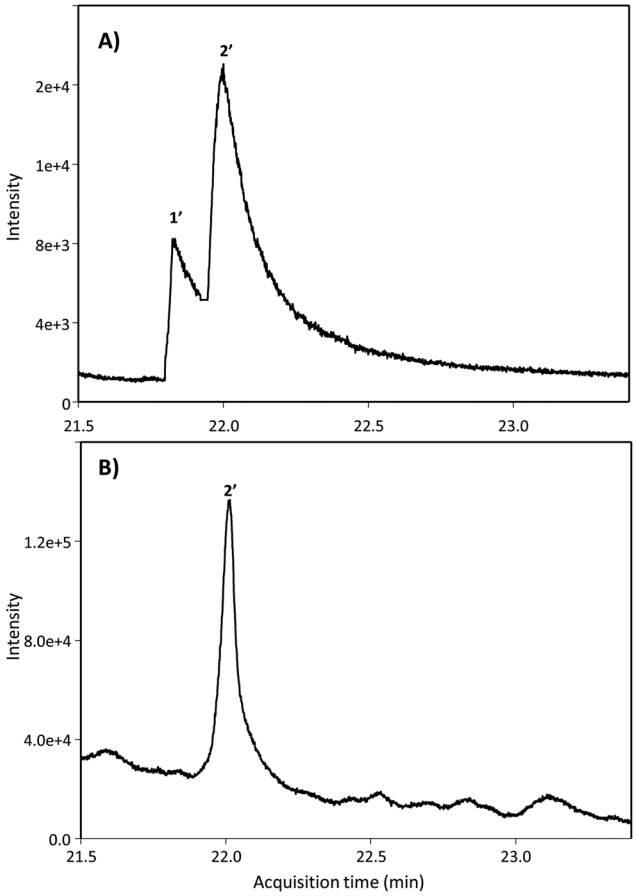

Fig. 3 GC-MS extracted ion chromatograms of non-silylated samples of (A) dibenzyl standards and (B) hydrolysed urine. Peak numbers are listed in Table 2 (non-silylated samples).

faeces sample, which also excluded the presence of the isomer 3-hydroxy-*trans*-stilbene (3HST). Since detecting the monohydroxylated dibenzyls 3HDB and 4HDB remained elusive, we next analyzed the hydrolysed urine samples by GC-MS with no silylation. Finally, both available 3HDB and 4HDB standards were detected in non-silylated samples (Table 2 and Fig. 3A). The metabolite 4HDB, with an ion mass of 198 and the main fragment of 107, was found in the urine of 23 individuals after consuming RSV but not in their control urine samples (Fig. 3B). The comparison of the peak detected in the participants' urine with the available standard allowed us the identification of 4HDB as a novel metabolite produced by the human gut microbiota after consuming RSV (Table 2 and Fig. 3). In contrast, the isomer 3HDB was not detected in any sample.

Faecal cultures to confirm the production of 4HDB and (or) 4HST

Next, we separately incubated RSV, DHRSV, LUNU, DHST, and 4HST in faecal cultures from 9 participants randomly chosen to detect the corresponding derived metabolites. After testing different incubation times, we chose 7 days since longer incubation times did not modify the results (results not shown). Again, interindividual variability was observed, as in the case of the urine and faeces analysed. After 7 days of incubation, RSV yielded DHRSV in all the samples (something readily observed at shorter incubation times). LUNU and 4HDB were also detected, but only in faecal cultures from 6 of 9 individuals (steps a–d, Fig. 4), thus confirming the *in vivo* results. 4HST was not detected in any sample, also confirming the lack of 4HST derivatives *in vivo* after RSV intake.

The incubation of DHRSV produced the metabolites LUNU and 4HDB (steps b and c, Fig. 4), but only in some volunteers. However, 3HDB was not detected in any sample. Next, the incubation of LUNU confirmed the lack of 3HDB production, and only 4HDB was detected in some samples.

Finally, the incubation of DHST did not produce 4HST but LUNU (step f, Fig. 4), something observed in all the samples analysed, and 4HDB in 7 samples (step c, Fig. 4). No further degradation of 4HDB was observed in any sample (results not shown). Similarly, the incubation of 4HST only produced 4HDB in all the individuals (step g, Fig. 4). Overall, and considering a high interindividual variability in RSV metabolism, faecal incubations confirmed the results obtained *in vivo* after RSV intake.

Discussion

Since Jang *et al.*[18] reported the RSV's anticancer activity, RSV has been described as a compound that could increase lifespan and prevent or reduce many pathological processes or diseases in preclinical and clinical studies.[19–22]

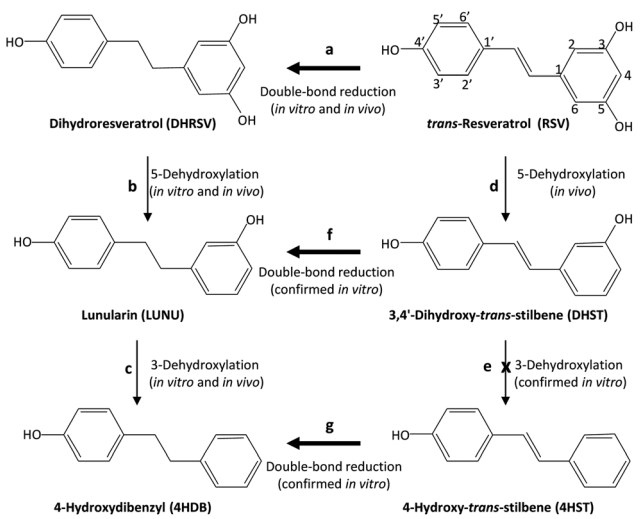

Fig. 4 *In vivo* and *in vitro* production of 4HDB, but not 4HST, by the gut microbiota from RSV. Thicker arrows indicate favoured reactions.

The human gut microbiota catabolises RSV to produce DHRSV and, to a lower extent, LUNU, and DHST, showing interindividual variability.[7] In this regard, as recently suggested, different human gut microbiota compositions might affect the outcome of trials with RSV since the biological activity of the microbial metabolites might differ from that of RSV,[23,24] which might explain the lack of consensus on RSV as a health-promoting polyphenol.[25]

In the present study and, in agreement with Bode *et al.*,[7] we detected the gut microbial metabolites DHRSV, LUNU, and DHST after RSV intake and confirmed the interindividual variability of RSV metabolism in a group of 59 volunteers.

However, confirming this study's hypothesis, we describe here for the first time that LUNU can be further dehydroxylated at the 3-position to yield 4HDB as a novel metabolite produced by the human gut microbiota from RSV. We have adopted the IUPAC nomenclature to name this metabolite, *i.e.*, 4-hydroxydibenzyl instead of 4-hydroxybibenzyl since the dibenzyl core is 1,2-diphenylethane, being correct names 4-(2-phenylethyl)phenol, 4-hydroxydibenzyl, 4-phenethylphenol, *p*-phenylethylphenol. In this regard, we have used 4-hydroxydibenzyl to highlight the position of the –OH group. Notably, 4HDB was a minor metabolite found only in those participants that produced LUNU. In contrast, LUNU was not dehydroxylated at the 4-position to yield 3-hydroxydibenzyl (3HDB), which was not detected *in vivo* or *in vitro*. Therefore, these results suggest that the human gut microbiota lacks the capacity to dehydroxylate stilbenes and dibenzyls at the 4-position. The specificity of dehydroxylases is also relevant in the metabolism of bile acids[26] and the sequential dehydroxylation pathway of urolithins and different phenolics.[27,28] Although this requires further research, we hypothesize the different capability to dehydroxylate DHRSV and LUNU is a hallmark of inter-individual variability of RSV metabolism. Whether this metabolism, including the production of 4HDB, impacts health upon RSV intake deserves further investigation.

The double bond enzymatic reduction of the 4-styrylphenol core seemed to be the most favoured step since DHRSV was detected in the urine and faeces of all individuals after RSV intake, which was confirmed *in vitro* after incubation of the stilbenes RSV, DHST, and 4HST to produce DHRSV, LUNU, and 4HDB, respectively. 4HST reduction only occurred in the presence of faecal inocula in the *in vitro* studies. Therefore, the most abundant substrates for dehydroxylases were dibenzyls (DHRSV and LUNU), which might explain the sporadic detection of DHST (14 individuals out of 59). Similarly, 4HST was not detected in any *in vivo* or *in vitro* samples because DHST was reduced to LUNU before producing 4HST, which, if any were produced, would be immediately reduced to 4HDB, preventing 4HST accumulation. Therefore, assuming 4HST could be potentially an RSV-metabolite produced by the human gut microbiota, other more favoured reactions prevented its detection, *i.e.*, double bond reduction of the 4-styrylphenol core, even after 7 days of static *in vitro* incubation. This finding agrees with the metabolic profile of RSV-derived metabolites in non-hydrolysed samples previously reported, including the

tentative identification of different LUNU and DHST glucuronide and sulfate conjugates, but not 4HST conjugates.[16,29]

4HDB was not found in faeces, probably due to (i) its rapid absorption and phase-II conjugation to be excreted in the urine, (ii) its low content (below LOD), and (or) (iii) possible interferences between stool matrix and GC-MS analysis without silylation. In contrast, 4HDB was detected in hydrolysed urine samples. However, we could not detect the corresponding 4HDB conjugates because of the lack of signal in UPLC-QTOF-MS and UPLC-QQQ. Besides, as expected, 4HDB conjugates were not detected by GC-MS either because they lack the volatility required for analysis by GC-MS.[30] Although challenging, we acknowledge that 4HDB detection should be improved in further studies.

Conclusions

The compound 4-hydroxydibenzyl (4HDB) is described here for the first time as a novel metabolite produced by the human gut microbiota after consuming RSV and specifically produced after dehydroxylation of lunularin (LUNU) at the 3-position. GC-MS was crucial for detecting this metabolite, which had remained undetected despite many investigations on RSV metabolism.

Our results suggest that the human gut microbiota could produce, at least theoretically in some volunteers, the compound 4-hydroxy-*trans*-stilbene (4HST). However, this metabolite was not detected in any sample because reducing the double bond of the 4-styrylphenol core of its potential precursors DHST and RSV was preferential, thus favouring the 3-dehydroxylation of the dibenzyls DHRSV and LUNU resulting from that reduction.

Further research on the biological activity and safety issues of 4HDB is warranted. In addition, our ongoing research addresses the observed high variability in RSV metabolism by the human gut microbiota, emphasizing why not all individuals can dehydroxylate stilbenes or dibenzyls.

Author contributions

Conceptualization: J. C. E.; investigation: C. E. I. A., F. V., D. B.; methodology: F. V., J. B., J. P., M. A.; visualization: C. E. I. A., F. V., M. V. S.; validation: F. V., C. E. I. A., J. C. E.; resources: J. C. E, M. V. S.; supervision: J. C. E.; writing original draft: J. C. E.; writing – review & editing: all authors.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This research has been supported by Project PID2019-103914RB-I00 from the Ministry of Science and Innovation (MICINN, Spain). C. E. I. A. and J. P. are the holders of predoctoral grants FPU18/03961 and FPU19/05419, respectively, from MICINN.

References

1 J. Tomé-Carneiro, M. Gonzálvez, M. Larrosa, M. J. Yáñez-Gascón, F. J. García-Almagro, J. A. Ruiz-Ros, F. A. Tomás-Barberán, M. T. García-Conesa and J. C. Espín, Grape resveratrol increases serum adiponectin and downregulates inflammatory genes in peripheral blood mononuclear cells: a triple-blind, placebo-controlled, one-year clinical trial in patients with stable coronary artery disease, *Cardiovasc. Drugs Ther.*, 2013, **27**, 37–48.

2 C. Moussa, M. Hebron, X. Huang, J. Ahn, R. A. Rissman, P. S. Aisen and R. S. Turner, Resveratrol regulates neuroinflammation and induces adaptive immunity in Alzheimer's disease, *J. Neuroinflammation*, 2017, **14**, 1.

3 A. Hoseini, G. Namazi, A. Farrokhian, Ž. Reiner, E. Aghadavod, F. Bahmani and Z. Asemi, The effects of resveratrol on metabolic status in patients with type 2 diabetes mellitus and coronary heart disease, *Food Funct.*, 2019, **10**, 6042–6051.

4 R. H. Wong, J. J. Thaung Zaw, C. J. Xian and P. R. Howe, Regular Supplementation With Resveratrol Improves Bone Mineral Density in Postmenopausal Women: A Randomized, Placebo-Controlled Trial, *J. Bone Miner. Res.*, 2020, **35**, 2121–2131.

5 J. J. Thaung Zaw, P. R. Howe and R. H. Wong, Long-term effects of resveratrol on cognition, cerebrovascular function and cardio-metabolic markers in postmenopausal women: A 24-month randomised, double-blind, placebo-controlled, crossover study, *Clin. Nutr.*, 2021, **40**, 820–829.

6 C. M. Jung, T. M. Heinze, L. K. Schnackenberg, L. B. Mullis, S. A. Elkins, C. A. Elkins, R. S. Steele and J. B. Sutherland, Interaction of dietary resveratrol with animal-associated bacteria, *FEMS Microbiol. Lett.*, 2009, **297**, 266–273.

7 L. M. Bode, D. Bunzel, M. Huch, G.-S. Cho, D. Ruhland, M. Bunzel, A. Bub, C. M. Franz and S. E. Kulling, *In vivo* and *in vitro* metabolism of trans-resveratrol by human gut microbiota, *Am. J. Clin. Nutr.*, 2013, **97**, 295–309.

8 D. A. Stoll, N. Danylec, S. T. Soukup, B. Hetzer, S. E. Kulling and M. Huch, Adlercreutzia rubneri sp. nov., a resveratrol-metabolizing bacterium isolated from human faeces and emended description of the genus Adlercreutzia, *Int. J. Syst. Evol. Microbiol.*, 2021, **71**, 004987.

9 V. Jarosova, O. Vesely, P. Marsik, J. Jaimes, K. Smejkal, P. Kloucek and J. Havlik, Metabolism of Stilbenoids by Human Faecal Microbiota, *Molecules*, 2019, **24**, 1155.

10 V. Cardile, L. Lombardo, C. Spatafora and C. Tringali, Chemo-enzymatic synthesis and cell-growth inhibition activity of resveratrol analogues, *Bioorg. Chem.*, 2005, **33**, 22–33.

11 D. M. Camaioni and J. A. Franz, Carbon-hydrogen vs. carbon-carbon bond cleavage of 1,2-diarylethane radical

cations in acetonitrile-water, *J. Org. Chem.*, 1984, **49**, 1607–1613.

12 A. Wilhelm, P. Kendrekar, A. E. M. Noreljaleel, E. T. Abay, S. L. Bonnet, L. Wiesner, C. de Kock, K. J. Swart and J. H. van der Westhuizen, Synthesis and in vitro antiplasmodial activity of aminoalkylated chalcones and analogues, *J. Nat. Prod.*, 2015, **78**, 1848–1858.

13 M. A. Ali, K. Kondo and Y. Tsuda, Synthesis and Nematocidal Activity of Hydroxystilbenes, *Chem. Pharm. Bull.*, 1992, **40**, 1130–1136.

14 R. Martí-Centelles, E. Falomir, J. Murga, M. Carda and J. A. Marco, Inhibitory effect of cytotoxic stilbenes related to resveratrol on the expression of the VEGF, hTERT and c-Myc genes, *Eur. J. Med. Chem.*, 2015, **103**, 488–496.

15 D. Beltrán, M. D. Frutos-Lisón, J. C. Espín and R. García-Villalba, Re-examining the role of the gut microbiota in the conversion of the lipid-lowering statin monacolin K (lovastatin) into its active β-hydroxy acid metabolite, *Food Funct.*, 2019, **10**, 1787–1791.

16 M. Á. Ávila-Gálvez, R. García-Villalba, F. Martínez-Díaz, B. Ocaña-Castillo, T. Monedero-Saiz, A. Torrecillas-Sánchez, B. Abellán, A. González-Sarrías and J. C. Espín, Metabolic Profiling of Dietary Polyphenols and Methylxanthines in Normal and Malignant Mammary Tissues from Breast Cancer Patients, *Mol. Nutr. Food Res.*, 2019, **63**, 1801239.

17 R. García-Villalba, J. C. Espín and F. A. Tomás-Barberán, Chromatographic and spectroscopic characterization of urolithins for their determination in biological samples after the intake of foods containing ellagitannins and ellagic acid, *J. Chromatogr. A*, 2016, **1428**, 162–175.

18 M. Jang, L. Cai, G. O. Udeani, K. V. Slowing, C. F. Thomas, C. W. W. Beecher, H. H. S. Fong, N. R. Farnsworth, A. D. Kinghorn, R. G. Mehta, R. C. Moon and J. M. Pezzuto, Cancer chemopreventive activity of resveratrol, a natural product derived from grapes, *Science*, 1997, **275**, 218–220.

19 J. Tomé-Carneiro, M. Larrosa, A. González-Sarrías, F. Tomás-Barberán, M. García-Conesa and J. C. Espín, Resveratrol and clinical trials: the crossroad from *in vitro*, studies to human evidence, *Curr. Pharm. Des.*, 2013, **19**, 6064–6093.

20 F. Fogacci, G. Tocci, V. Presta, A. Fratter, C. Borghi and A. F. G. Cicero, Effect of resveratrol on blood pressure: A systematic review and meta-analysis of randomized, controlled, clinical trials, *Crit. Rev. Food Sci. Nutr.*, 2019, **59**, 1605–1618.

21 F. M. Delpino and L. M. Figueiredo, Resveratrol supplementation and type 2 diabetes: a systematic review and meta-analysis, *Crit. Rev. Food Sci. Nutr.*, 2021, 1–16.

22 D. Delmas, C. Cornebise, F. Courtaut, J. Xiao and V. Aires, New Highlights of Resveratrol: A Review of Properties against Ocular Diseases, *Int. J. Mol. Sci.*, 2021, **22**, 1295.

23 K. Pallauf, D. Chin, I. Günther, M. Birringer, K. Lüersen, G. Schultheiß, S. Vieten, J. Krauß, F. Bracher, N. Danylec, S. T. Soukup, S. E. Kulling and G. Rimbach, Resveratrol, lunularin and dihydroresveratrol do not act as caloric restriction mimetics when administered intraperitoneally in mice, *Sci. Rep.*, 2019, **9**, 4445.

24 I. Günther, G. Rimbach, C. I. Mack, C. H. Weinert, N. Danylec, K. Lüersen, M. Birringer, F. Bracher, S. T. Soukup, S. E. Kulling and K. Pallauf, The Putative Caloric Restriction Mimetic Resveratrol has Moderate Impact on Insulin Sensitivity, Body Composition, and the Metabolome in Mice, *Mol. Nutr. Food Res.*, 2020, **64**, 1901116.

25 P. C.-T. Tang, Y.-F. Ng, S. Ho, M. Gyda and S.-W. Chan, Resveratrol and cardiovascular health–promising therapeutic or hopeless illusion?, *Pharmacol. Res.*, 2014, **90**, 88–115.

26 M. Funabashi, T. L. Grove, M. Wang, Y. Varma, M. E. McFadden, L. C. Brown, C. Guo, S. Higginbottom, S. C. Almo and M. A. Fischbach, A metabolic pathway for bile acid dehydroxylation by the gut microbiome, *Nature*, 2020, **582**, 566–570.

27 M. V. Selma, J. C. Espín and F. A. Tomás-Barberán, Interaction between phenolics and gut microbiota: role in human health, *J. Agric. Food Chem.*, 2009, **57**, 6485–6501.

28 R. García-Villalba, D. Beltrán, M. D. Frutos, M. V. Selma, J. C. Espín and F. A. Tomás-Barberán, Metabolism of different dietary phenolic compounds by the urolithin-producing human-gut bacteria *Gordonibacter urolithinfaciens* and *Ellagibacter isourolithinifaciens*, *Food Funct.*, 2020, **11**, 7012–7022.

29 M. Á. Ávila-Gálvez, A. González-Sarrías, F. Martínez-Díaz, B. Abellán, A. J. Martínez-Torrano, A. J. Fernández-López, J. A. Giménez-Bastida and J. C. Espín, Disposition of Dietary Polyphenols in Breast Cancer Patients' Tumors, and Their Associated Anticancer Activity: The Particular Case of Curcumin, *Mol. Nutr. Food Res.*, 2021, **65**, 2100163.

30 J. L. Ordóñez, G. Pereira-Caro, I. Ludwig, J. M. Muñoz-Redondo, M. J. Ruiz-Moreno, A. Crozier and J. M. Moreno-Rojas, A critical evaluation of the use of gas chromatography- and high performance liquid chromatography-mass spectrometry techniques for the analysis of microbial metabolites in human urine after consumption of orange juice, *J. Chromatogr. A*, 2018, **1575**, 100–112.