



Supplementary Materials

A Gedunin-Type Limonoid, 7-Deacetoxy-7-oxogedunin, from Andiroba (*Carapa guianensis* Aublet) Reduced Intracellular Triglyceride Content and Enhanced Autophagy in HepG2 Cells

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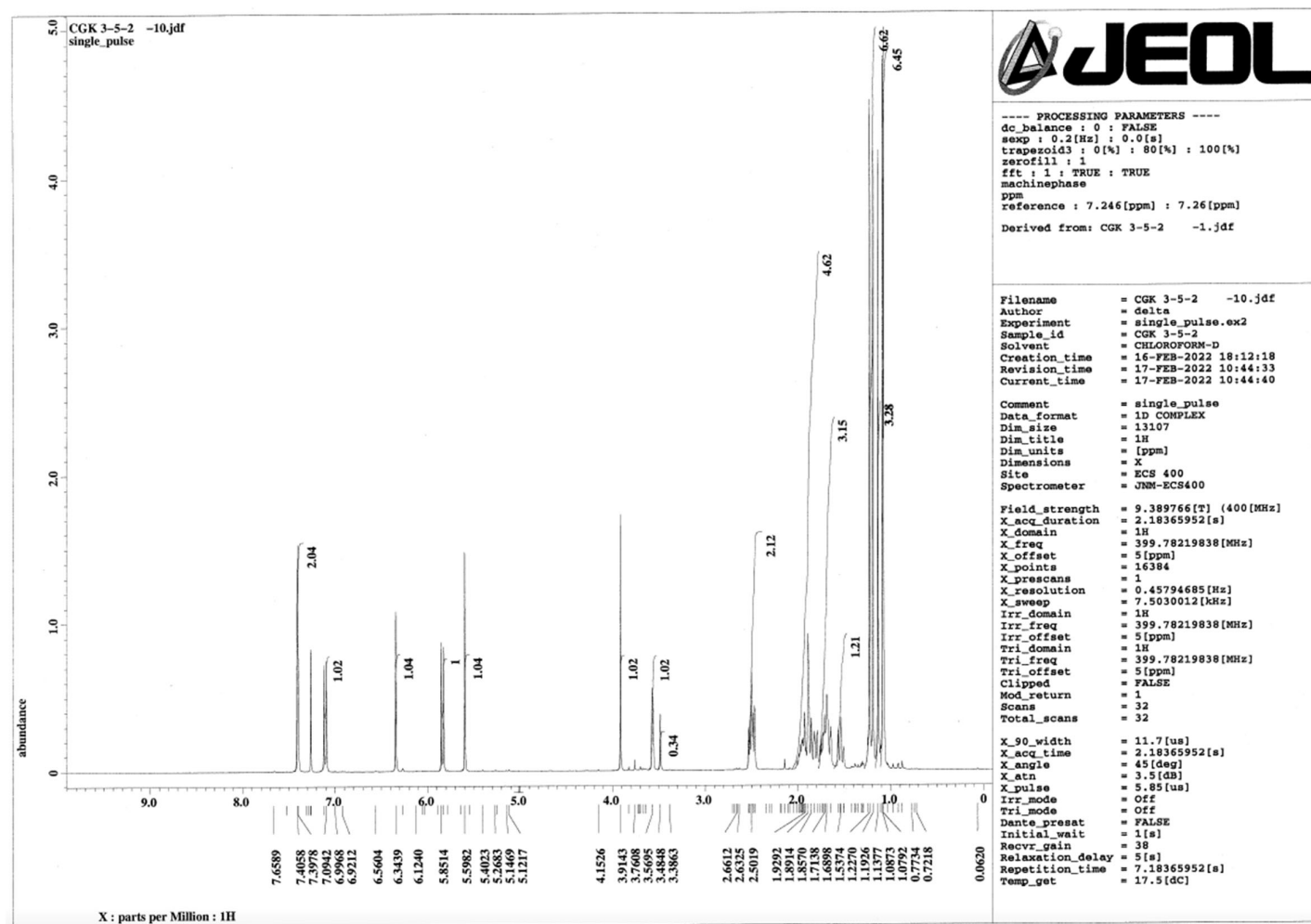
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Figure S1. ^1H -NMR (400 MHz, CDCl_3) spectrum of DAOG (1).

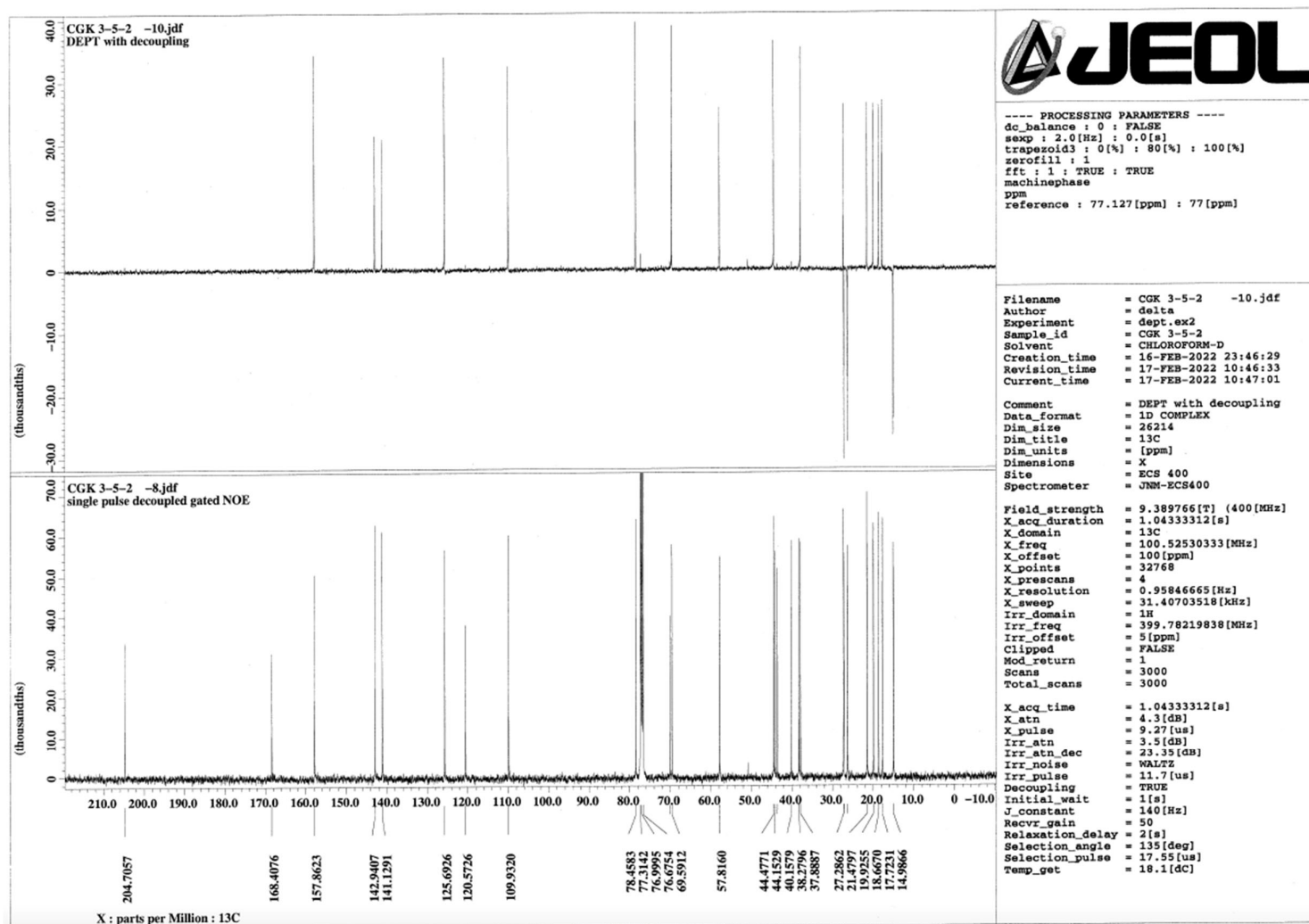


Figure S2. ^{13}C -NMR (100 MHz, CDCl_3) spectrum of DAOG (1).

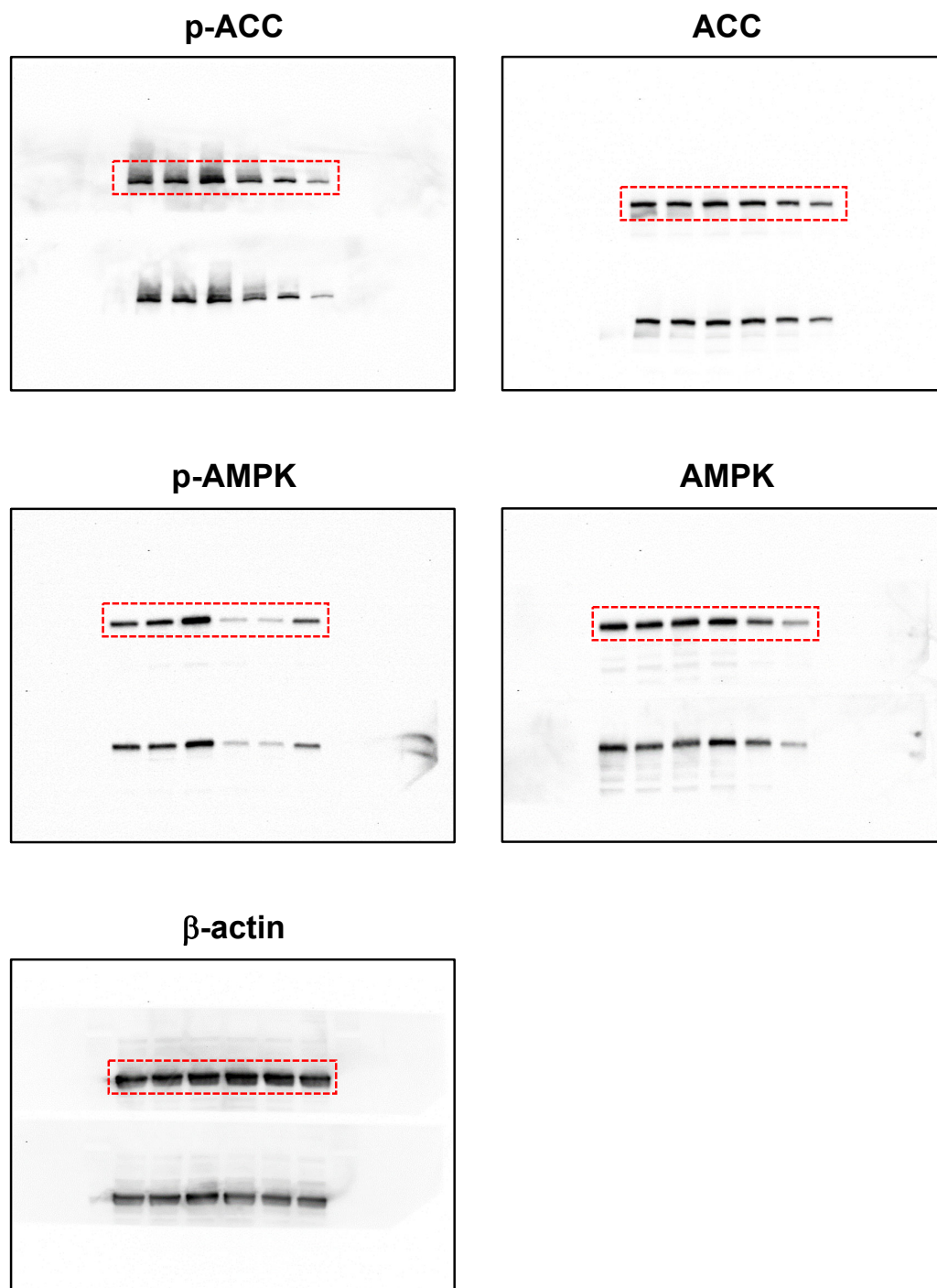


Figure S3. Unedited blots for Figure 4B.

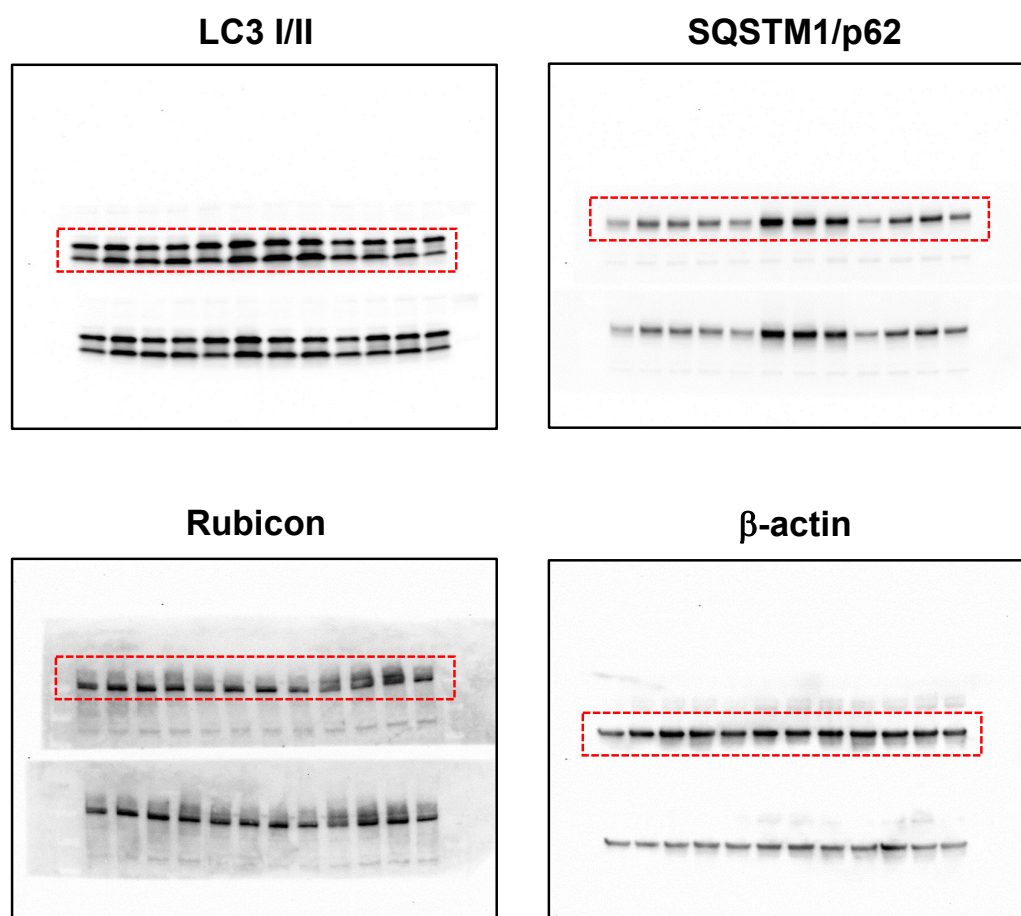


Figure S4. Unedited blots for Figure 5A.

Table S1. Numerical data of MTT assay for Figure 3.

| Treatment | Cell viability (%) | | | | | |
|-----------|--------------------|-----------------|-----------------|------------------|------------------|------------------|
| | 0 μ M | 2.5 μ M | 5 μ M | 10 μ M | 20 μ M | 40 μ M |
| DAOG (1) | 100.0 \pm 1.4 | 97.8 \pm 0.6 | 98.0 \pm 0.1 | 96.2 \pm 0.5** | 95.6 \pm 0.2** | 94.5 \pm 0.7** |
| BBR | 0 μ M | 1 μ M | 3 μ M | 10 μ M | 30 μ M | 100 μ M |
| | 100.0 \pm 0.5 | 100.0 \pm 0.5 | 100.0 \pm 0.6 | 98.0 \pm 0.2 | 96.2 \pm 0.4** | 1.6 \pm 0.7** |

Each value represents the mean \pm S.E.M. ($n = 4$). Significantly different from the control, ** $p < 0.01$ (Dunnett). DAOG; 7-deacetoxy-7-oxogedunin, BBR; berberine chloride.

Table S2. Numerical data of intracellular TG reduction assay for Figure 4A.

| Treatment | Compound C 20 μ M | TG/protein in the homogenate (% of control) | | | |
|-----------|--------------------------|---|--------------------|--------------------|---------------------|
| | | 0 μ M | 5 μ M | 10 μ M | 20 μ M |
| DAOG (1) | – | 100.0 \pm 1.7 | 91.2 \pm 1.7* | 80.1 \pm 0.9** | 71.3 \pm 1.6** |
| | + | 100.0 \pm 2.0 | 90.5 \pm 1.3** | 86.3 \pm 1.2**## | 97.9 \pm 0.9## |
| | | 0 μ M | 7.5 μ M | 15 μ M | 30 μ M |
| BBR | – | 100.0 \pm 3.8 | 89.3 \pm 3.4 | 91.2 \pm 2.8 | 82.6 \pm 2.4** |
| | + | 100.0 \pm 1.1 | 109.6 \pm 1.8*## | 107.3 \pm 3.1## | 149.0 \pm 2.6**## |

Each value represents the mean \pm S.E.M. ($n = 4$). * $p < 0.05$, ** $p < 0.01$ vs. control cells treated with vehicle (Dunnett); ## $p < 0.01$ vs. compound C-nontreated cells (Student's t). DAOG; 7-deacetoxy-7-oxogedunin, BBR; berberine chloride.

Table S3. Numerical data of western blot analysis for Figure 4C.

| Protein | Compound C 20 μ M | Relative expression (fold increase) | | |
|-------------|--------------------------|-------------------------------------|----------------------|-------------------|
| | | Control | DAOG (1, 20 μ M) | BBR (30 μ M) |
| p-AMPK/AMPK | – | 1.00 \pm 0.00 | 1.43 \pm 0.07 | 3.21 \pm 0.66** |
| | + | 0.26 \pm 0.04## | 0.41 \pm 0.06## | 1.68 \pm 0.47** |
| p-ACC/ACC | – | 1.00 \pm 0.00 | 0.86 \pm 0.09 | 0.96 \pm 0.14 |
| | + | 0.57 \pm 0.09## | 0.57 \pm 0.04# | 0.37 \pm 0.06## |

Each value represents the mean \pm S.E.M. ($n = 4$). ** $p < 0.01$ vs. control cells treated with vehicle (Dunnett); # $p < 0.05$, ## $p < 0.01$ vs. compound C-nontreated cells (Student's t). DAOG; 7-deacetoxy-7-oxogedunin, BBR; berberine chloride.

Table S4. Numerical data of western blot analysis for Figure 5B.

| Protein | Treatment | Relative expression (fold increase) | | | |
|-----------------------------|----------------------|-------------------------------------|---------------------|---------------------|---------------------|
| | | 0 h | 6 h | 12 h | 24 h |
| LC3-II / β -actin | Control | 1.00 \pm 0.00 | 1.25 \pm 0.10 | 0.93 \pm 0.08 | 1.13 \pm 0.08 |
| | DAOG (1, 20 μ M) | 1.00 \pm 0.00 | 1.43 \pm 0.12 | 1.48 \pm 0.18# | 1.55 \pm 0.17* |
| | BBR (30 μ M) | 1.00 \pm 0.00 | 0.90 \pm 0.15 | 1.13 \pm 0.15 | 0.66 \pm 0.18 |
| SQSTM1/p62 / β -actin | Control | 1.00 \pm 0.00 | 1.60 \pm 0.18* | 1.24 \pm 0.15 | 1.30 \pm 0.12 |
| | DAOG (1, 20 μ M) | 1.00 \pm 0.00 | 2.74 \pm 0.15**## | 2.70 \pm 0.22**## | 2.18 \pm 0.17**## |
| | BBR (30 μ M) | 1.00 \pm 0.00 | 1.67 \pm 0.19 | 2.06 \pm 0.31** | 1.28 \pm 0.20 |
| Rubicon / β -actin | Control | 1.00 \pm 0.00 | 1.08 \pm 0.11 | 1.08 \pm 0.12 | 1.12 \pm 0.10 |
| | DAOG (1, 20 μ M) | 1.00 \pm 0.00 | 0.75 \pm 0.03** | 0.76 \pm 0.03** | 0.62 \pm 0.08**# |
| | BBR (30 μ M) | 1.00 \pm 0.00 | 1.03 \pm 0.22 | 1.18 \pm 0.30 | 0.80 \pm 0.19 |

Each value represents the mean \pm S.E.M. ($n = 4$); * $p < 0.05$, ** $p < 0.01$ vs. initial (0 h) expression (Dunnett); # $p < 0.05$, ## $p < 0.01$ vs. control group at each sampling point (Dunnett). DAOG; 7-deacetoxy-7-oxogedunin, BBR; berberine chloride.

Table S5. Numerical data of autophagy flux assay for Figure 5C.

| Protein | CQ | Relative expression (fold increase) | | |
|-------------------------|-------------|-------------------------------------|-------------------------------|------------------------------|
| | 100 μ M | Control | DAOG (1, 20 μ M) | BBR (30 μ M) |
| LC3-II / β -actin | – | 1.01 \pm 0.06 ^{ab} | 1.34 \pm 0.06 ^{ac} | 0.72 \pm 0.04 ^b |
| | + | 1.84 \pm 0.05 ^c | 2.58 \pm 0.21 ^d | 1.77 \pm 0.21 ^c |

Each value represents the mean \pm S.E.M. ($n = 4$). Different letters indicate significant differences, $p < 0.05$ (Tukey-Kramer's HSD). CQ; chloroquine, DAOG; 7-deacetoxy-7-oxogedunin, BBR; berberine chloride.

Table S6. Numerical data of mRNA expressions for Figure 5D.

| Treatment | mRNA level | |
|----------------------|-------------------|-------------------|
| | SQSTM1 / RPLP0 | RUBCN / RPLP0 |
| Control | 1.02 \pm 0.13 | 1.01 \pm 0.08 |
| DAOG (1, 20 μ M) | 2.93 \pm 0.07** | 0.63 \pm 0.02** |

Each value represents the mean \pm S.E.M. ($n = 4$). Significantly different from the control, ** $p < 0.01$ (Student's t). DAOG; 7-deacetoxy-7-oxogedunin, BBR; berberine chloride.