

SUPPLEMENTARY INFORMATION

AMPK modulatory activity of olive-tree leaves phenolic compounds: bioassay-guided isolation on adipocyte model and in silico approach

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Encinar^{3,§}, Vicente Micol^{3,4§}

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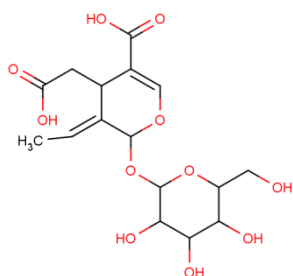
² Research and Development of Functional Food Centre (CIDAF), PTS Granada, Avda. del Conocimiento s/n., Edificio BioRegión, 18016, Granada, Spain.

³ Instituto de Biología Molecular y Celular (IBMC), Avda. de la Universidad s/n, Edificio Torregaitán. Miguel Hernández University (UMH), 03202 Elche, Alicante, Spain

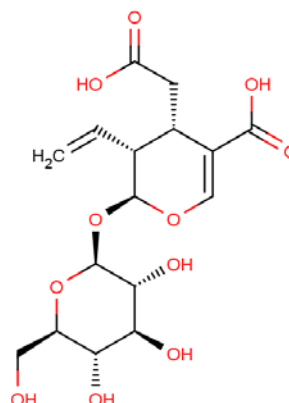
⁴ CIBER: CB12/03/30038, Fisiopatología de la Obesidad y la Nutrición, CIBERobn, Instituto de Salud Carlos III (ISCIII), Cra. de Valldemossa, km 7.5, 07122, Palma de Mallorca, Spain.

Supplementary Figure 1. Proposed molecular structure of the identified compounds showed on Table 1 for selected fractions with biological activity over AMPK kinase.

Peaks 5, 8 and 15.

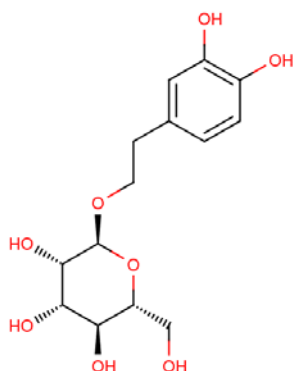


4-(carboxymethyl)-3-ethenyl-2-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]-3,4-dihydro-2H-pyran-5-carboxylic acid
PubChem CID: 101042548, Oleoside.

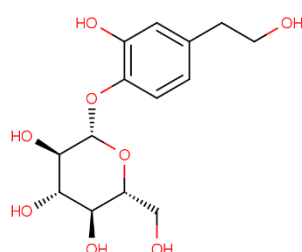


(3E)-4-(carboxymethyl)-3-ethylidene-2-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]-3,4-dihydro-2H-pyran-5-carboxylic acid
PubChem CID: 14136854, Secologanoside.

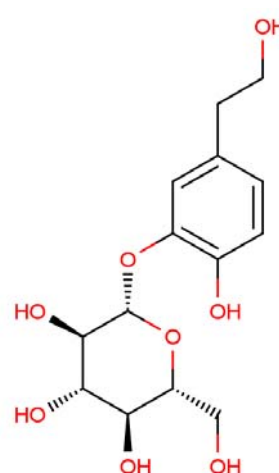
Peaks 6 and 7



2-[2-(3,4-dihydroxyphenyl)ethoxy]-6-(hydroxymethyl)oxane-3,4,5-triol
PubChem CID: 13845930,
Hydroxytyrosol 1-O-glucoside.

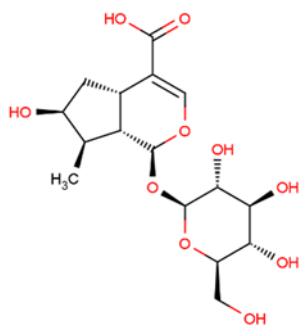


2-[2-hydroxy-4-(2-hydroxyethyl)phenoxy]-6-(hydroxymethyl)oxane-3,4,5-triol
PubChem CID: 6453057,
Hydroxytyrosol 4-beta-D-glucoside.

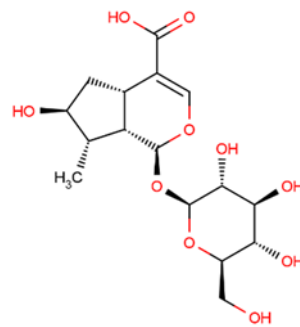


2-[2-hydroxy-5-(2-hydroxyethyl)phenoxy]-6-(hydroxymethyl)oxane-3,4,5-triol
PubChem CID: 5315870,
Cimidahurinine.

Peaks 9.

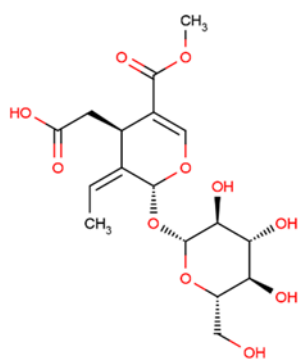


6-hydroxy-7-methyl-1-[[3.4.5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]-1H.4aH.5H.6H.7H.7aH-cyclopenta[c]pyran-4-carboxylic acid
PubChem CID: 158144, 8-Epiloganic acid.

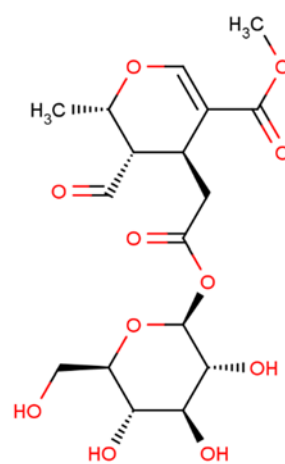


6-hydroxy-7-methyl-1-[[3.4.5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]-1H.4aH.5H.6H.7H.7aH-cyclopenta[c]pyran-4-carboxylic acid
PubChem CID: 89640, Loganic Acid.

Peaks 16 and 22.

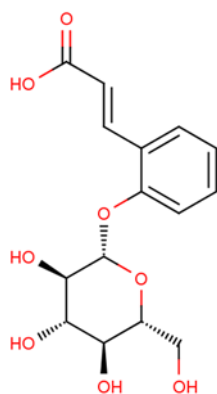


methyl 3-formyl-2-methyl-4-(2-oxo-2-[[3.4.5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]ethyl)-3,4-dihydro-2H-pyran-5-carboxylate
PubChem CID: 24121278, oleoside 11-methyl ester.

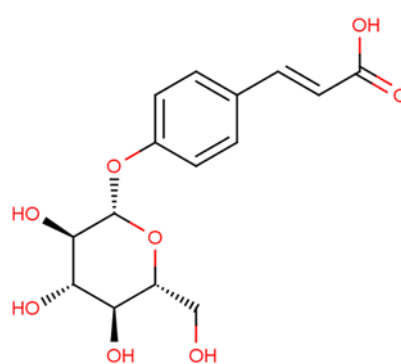


2-[(3Z)-3-ethylidene-5-(methoxycarbonyl)-2-[[3.4.5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]-3,4-dihydro-2H-pyran-4-yl]acetic acid

Peak 17



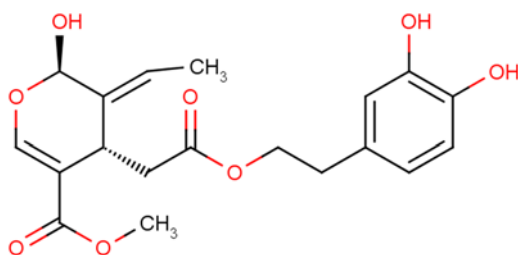
(2E)-3-(2-[[3.4.5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]phenyl)prop-2-enoic acid



(2E)-3-(4-[[3.4.5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]phenyl)prop-2-enoic acid
PubChem CID: 9840292.

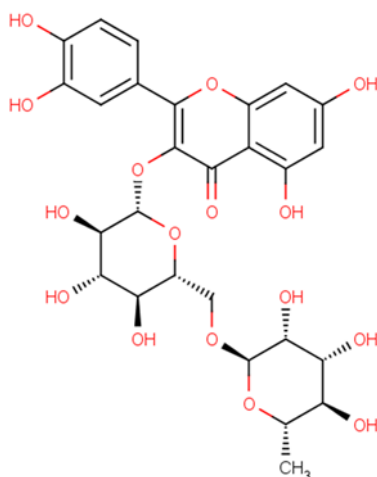
PubChem CID: 6275271.

Peak 24



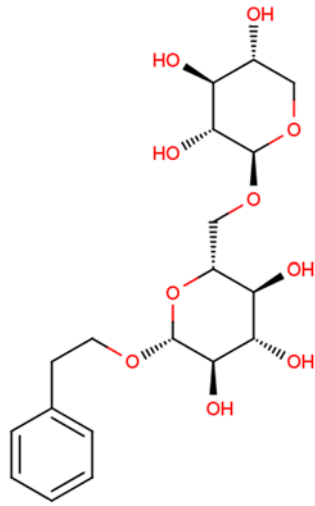
methyl (3E)-4-{2-[2-(3,4-dihydroxyphenyl)ethoxy]-2-oxoethyl}-3-ethylidene-2-hydroxy-3,4-dihydro-2H-pyran-5-carboxylate
PubChem CID: 56842347, Oleuropein Aglycone.

Peak 25 and 33

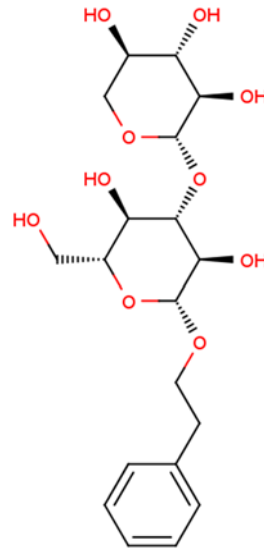


2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(3,4,5-trihydroxy-6-[(3,4,5-trihydroxy-6-methyloxan-2-yl)oxy]methyl)oxan-2-yl]oxy]-4H-chromen-4-one
PubChem CID: 5280805, Rutin.

Peak 27

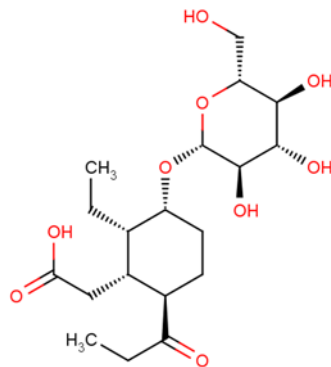


2-(2-phenylethoxy)-6-((3,4,5-trihydroxyoxan-2-yl)oxy)methyl)oxane-3,4,5-triol
 PubChem CID: 131129, 2-Phenylethyl beta-primeveroside.



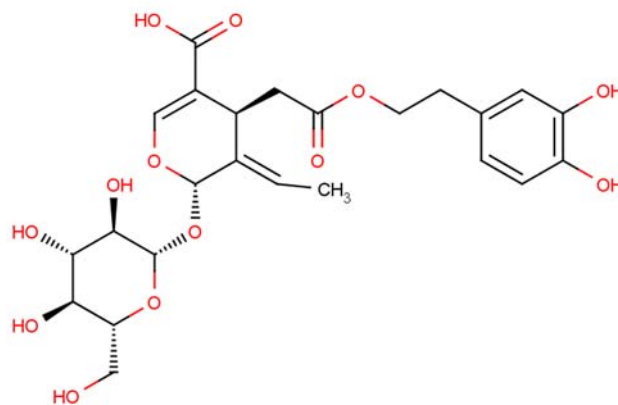
2-((3,5-dihydroxy-2-(hydroxymethyl)oxan-4-yl)oxy)oxane-3,4,5-triol
 PubChem CID: 100930979.

Peak 28



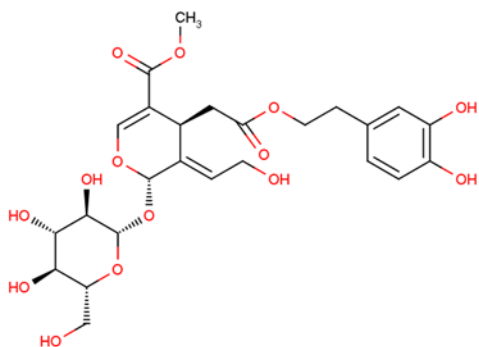
2-(2-ethyl-6-propanoyl-3-((3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl)oxy)cyclohexyl)acetic acid

Peak 30



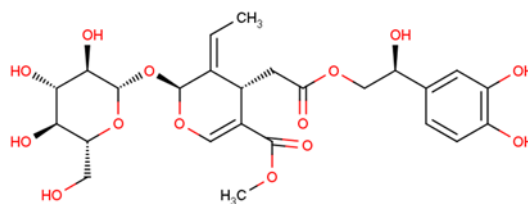
(3E)-4-{2-[2-(3,4-dihydroxyphenyl)ethoxy]-2-oxoethyl}-3-ethylidene-2-((3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl)oxy)-3,4-dihydro-2H-pyran-5-carboxylic acid

Peaks 32 and 39



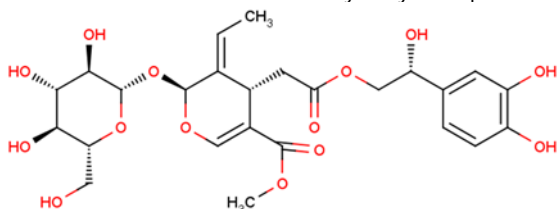
methyl (3E)-4-{2-[2-(3,4-dihydroxyphenyl)ethoxy]-2-oxoethyl}-3-(2-hydroxyethylidene)-2-{{[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}}-3,4-dihydro-2H-pyran-5-carboxylate

PubChem CID: 6440747, 10-Hydroxyeuropein.



methyl (3E)-4-{2-[2-(3,4-dihydroxyphenyl)-2-hydroxyethoxy]-2-oxoethyl}-3-ethylidene-2-{{[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}}-3,4-dihydro-2H-pyran-5-carboxylate

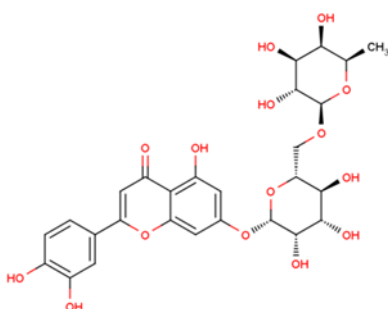
PubChem CID: 102461563, (7''S)-Hydroxyeuropein.



methyl (3E)-4-{2-[2-(3,4-dihydroxyphenyl)-2-hydroxyethoxy]-2-oxoethyl}-3-ethylidene-2-{{[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}}-3,4-dihydro-2H-pyran-5-carboxylate

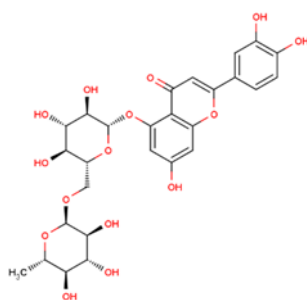
PubChem CID: 102461562, (2''R)-2''-Hydroxyeuropein.

Peak 34



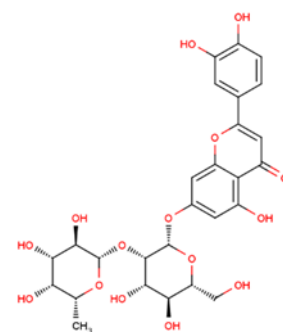
2-(3,4-dihydroxyphenyl)-5-hydroxy-7-{{[3,4,5-trihydroxy-6-{{[3,4,5-trihydroxy-6-methyloxan-2-yl]oxy}methyl]oxan-2-yl]oxy}}-4H-chromen-4-one

PubChem CID: 44258082, Luteolin 7-rutinoside.



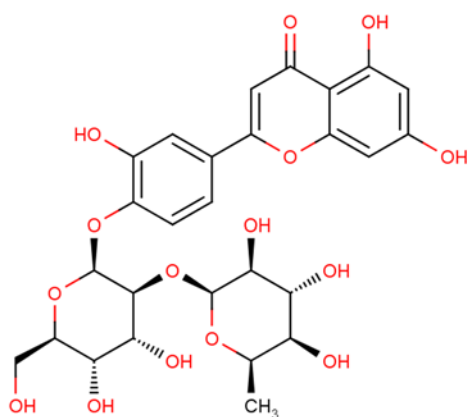
2-(3,4-dihydroxyphenyl)-7-hydroxy-5-{{[3,4,5-trihydroxy-6-{{[3,4,5-trihydroxy-6-methyloxan-2-yl]oxy}methyl]oxan-2-yl]oxy}}-4H-chromen-4-one

PubChem CID: 44258131, Luteolin 5-O-rutinoside.

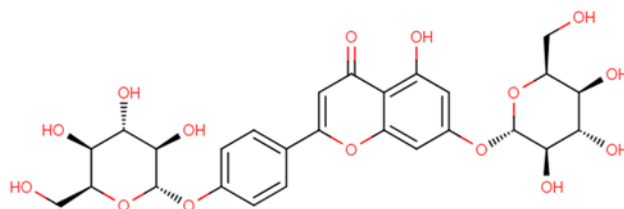


7-{{[4,5-dihydroxy-6-(hydroxymethyl)-3-{{[3,4,5-trihydroxy-6-methyloxan-2-yl]oxy}oxan-2-yl]oxy}}-2-(3,4-dihydroxyphenyl)-5-hydroxy-4H-chromen-4-one

PubChem CID: 44258083, Luteolin 7-neohesperidoside.

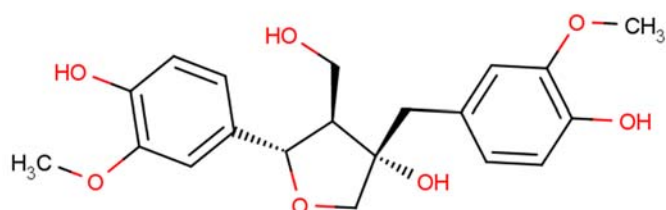


2-(4-([4,5-dihydroxy-6-(hydroxymethyl)-3-[[3,4,5-trihydroxy-6-methyloxan-2-yl]oxy]oxan-2-yl]oxy)-3-hydroxyphenyl)-5,7-dihydroxy-4H-chromen-4-one
 PubChem CID: 44258098, Luteolin 4'-neohesperidoside.



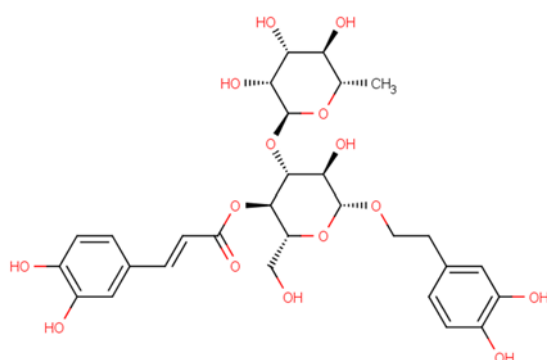
5-hydroxy-7-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]-2-(4-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]phenyl)-4H-chromen-4-one
 PubChem CID: 44257819.

Peak 35



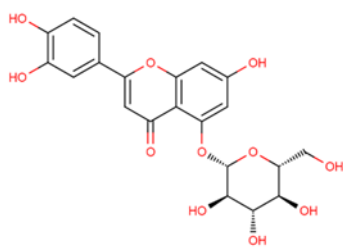
5-(4-hydroxy-3-methoxyphenyl)-3-[(4-hydroxy-3-methoxyphenyl)methyl]-4-(hydroxymethyl)oxolan-3-ol
 PubChem CID: 5273570, Olivil.

Peak 36

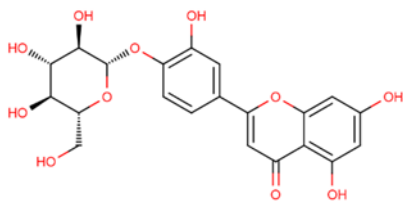


6-[2-(3,4-dihydroxyphenyl)ethoxy]-5-hydroxy-2-(hydroxymethyl)-4-[(3,4,5-trihydroxy-6-methyloxan-2-yl)oxy]oxan-3-yl (2E)-3-(3,4-dihydroxyphenyl)prop-2-enoate
 PubChem CID: 5281800, Acteoside.

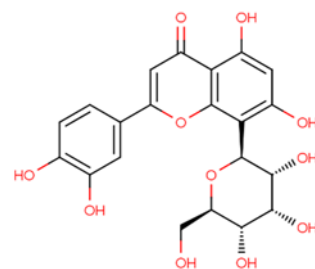
Peaks 37, 48 and 55



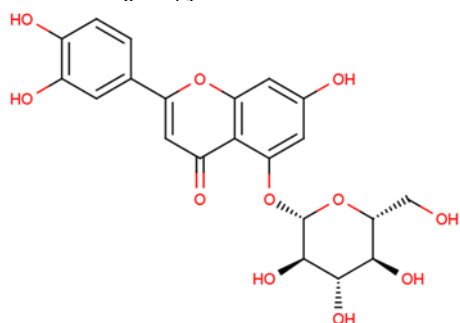
2-(3,4-dihydroxyphenyl)-7-hydroxy-5-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]-4H-chromen-4-one
PubChem CID: 5317471, Luteolin-5-O-beta-D-glucopyranoside.



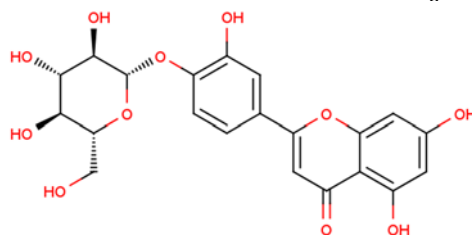
5,7-dihydroxy-2-(3-hydroxy-4-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]phenyl)-4H-chromen-4-one
PubChem CID: 5319116, Luteolin 4'-O-glucoside.



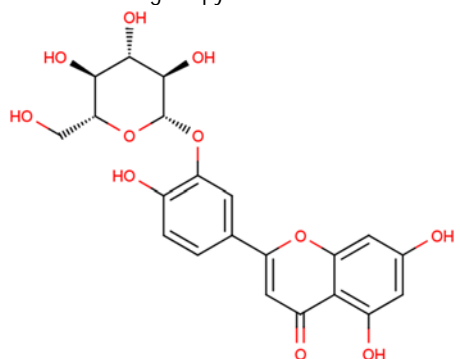
2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-8-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]-4H-chromen-4-one
PubChem CID: 44257907, Luteolin 8-C-beta-D-glucopyranoside.



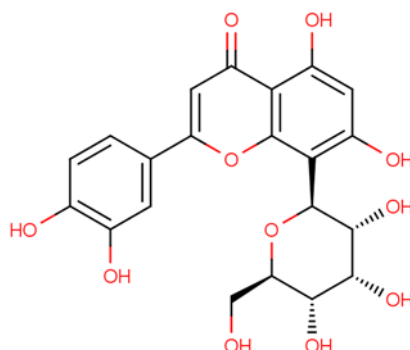
2-(3,4-dihydroxyphenyl)-7-hydroxy-5-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]-4H-chromen-4-one
PubChem CID: 5317471, Luteolin-5-O-beta-D-glucopyranoside.



5,7-dihydroxy-2-(3-hydroxy-4-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]phenyl)-4H-chromen-4-one
PubChem CID: 5319116, Luteolin 4'-O-glucoside.

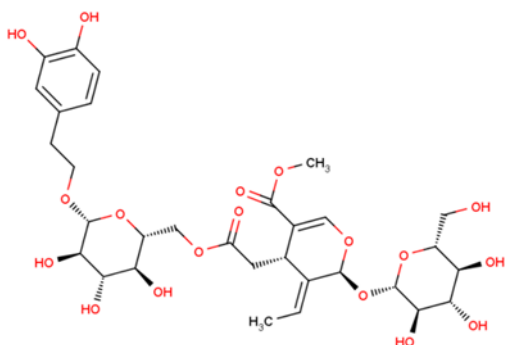


5,7-dihydroxy-2-(4-hydroxy-3-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]phenyl)-4H-chromen-4-one
PubChem CID: 12309350, Luteolin 3'-glucoside.

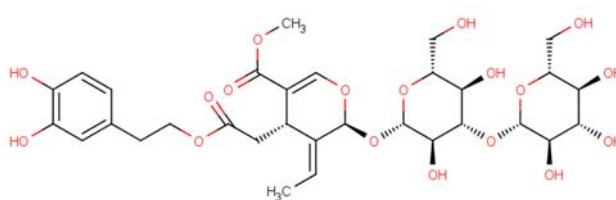


2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-8-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]-4H-chromen-4-one
PubChem CID: 44257907, Luteolin 8-C-beta-D-glucopyranoside.

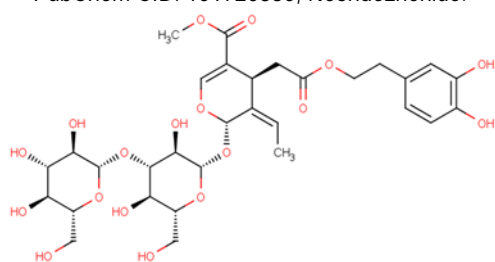
Peaks 40, 42, 43 and 44



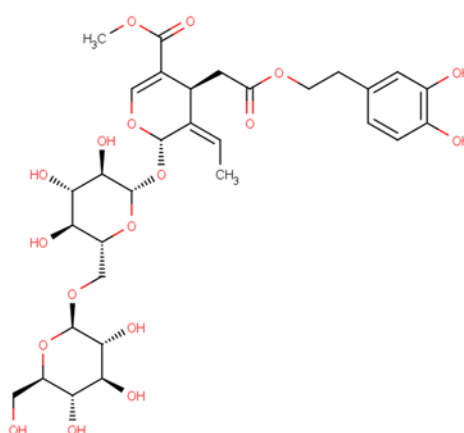
methyl (3E)-4-[2-((6-[2-(3,4-dihydroxyphenyl)ethoxy]-3,4,5-trihydroxyoxan-2-yl)methoxy)-2-oxoethyl]-3-ethylidene-2-((3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl)oxy)-3,4-dihydro-2H-pyran-5-carboxylate
PubChem CID: 101720830, Neonuezhenide.



methyl (3E)-2-((3,5-dihydroxy-6-(hydroxymethyl)-4-((3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl)oxy)oxan-2-yl)oxy)-4-([2-(3,4-dihydroxyphenyl)ethoxy]-2-oxoethyl)-3-ethylidene-3,4-dihydro-2H-pyran-5-carboxylate
PubChem CID: 102031346, Oleuropein 3'-glucoside.

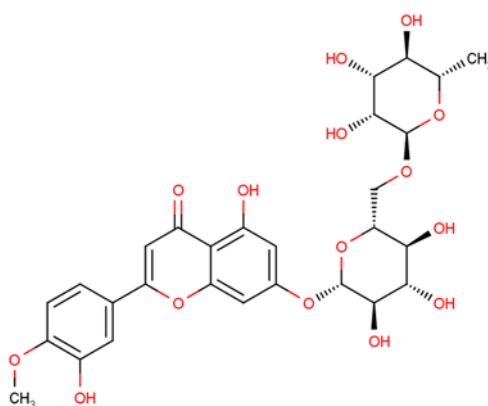


methyl (3Z)-2-((3,5-dihydroxy-6-(hydroxymethyl)-4-((3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl)oxy)oxan-2-yl)oxy)-4-([2-(3,4-dihydroxyphenyl)ethoxy]-2-oxoethyl)-3-ethylidene-3,4-dihydro-2H-pyran-5-carboxylate
PubChem CID: 101447987.



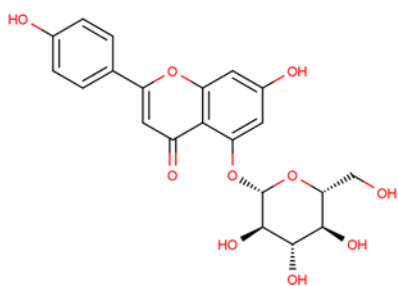
methyl (3Z)-4-([2-(3,4-dihydroxyphenyl)ethoxy]-2-oxoethyl)-3-ethylidene-2-((3,4,5-trihydroxy-6-((3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl)oxy)methyl)oxan-2-yl)oxy)-3,4-dihydro-2H-pyran-5-carboxylate
PubChem CID: 102078602, 6'-O-beta-D-Glucopyranosyloleuropein.

Peaks 45 and 46

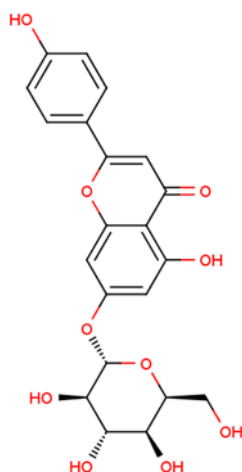


5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-((3,4,5-trihydroxy-6-((3,4,5-trihydroxy-6-methyloxan-2-yl)oxy)methyl)oxan-2-yl)oxy]-4H-chromen-4-one
PubChem CID: 5281613, Diosmin.

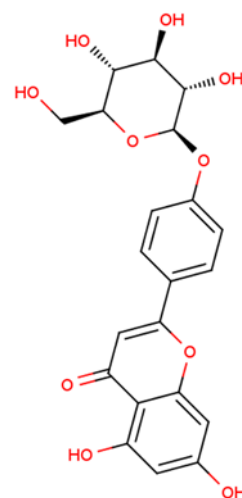
Peak 47



7-hydroxy-2-(4-hydroxyphenyl)-5-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]-4H-chromen-4-one
PubChem CID: 14730806.

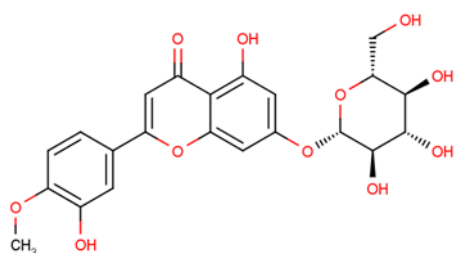


5-hydroxy-2-(4-hydroxyphenyl)-7-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]-4H-chromen-4-one
PubChem CID: 44257792.

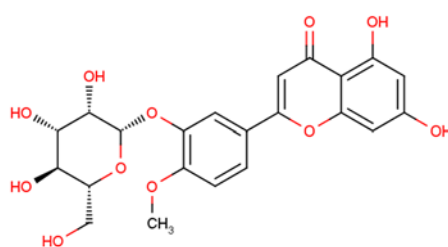


5,7-dihydroxy-2-(4-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]phenyl)-4H-chromen-4-one
PubChem CID: 101135001.

Peak 49

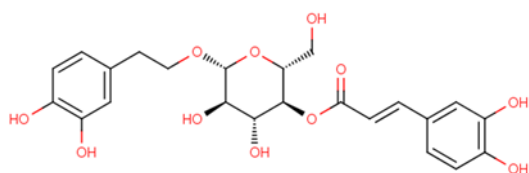


5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]-4H-chromen-4-one
PubChem CID: 11016019, Diosmetol 7-glucoside.

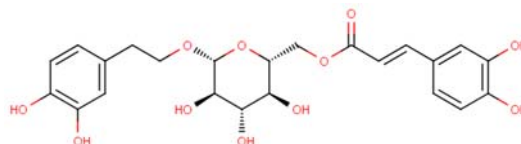


5,7-dihydroxy-2-(4-methoxy-3-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]phenyl)-4H-chromen-4-one
PubChem CID: 44258229, Diosmetin 3'-glucoside.

Peak 50

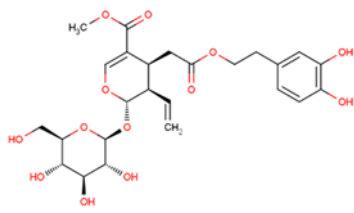


6-[2-(3,4-dihydroxyphenyl)ethoxy]-4,5-dihydroxy-2-(hydroxymethyl)oxan-3-yl (2E)-3-(3,4-dihydroxyphenyl)prop-2-enoate
PubChem CID: 5273566, Calceolarioside A.

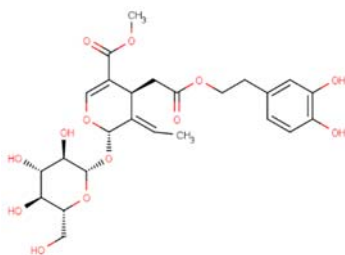


(6-[2-(3,4-dihydroxyphenyl)ethoxy]-3,4,5-trihydroxyoxan-2-yl)methyl (2E)-3-(3,4-dihydroxyphenyl)prop-2-enoate
PubChem CID: 5273567, Calceolarioside B.

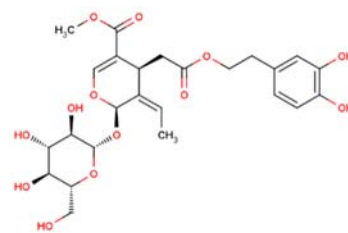
Peaks 59 and 68



methyl 4-{2-[2-(3,4-dihydroxyphenyl)ethoxy]-2-oxoethyl}-3-ethenyl-2-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]-3,4-dihydro-2H-pyran-5-carboxylate
PubChem CID: 102016333,
Oleuroside.

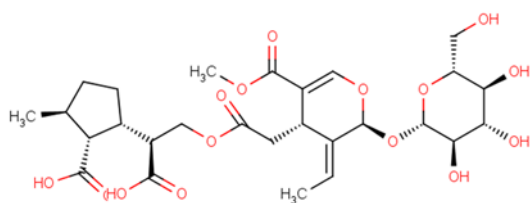


methyl (3E)-4-{2-[2-(3,4-dihydroxyphenyl)ethoxy]-2-oxoethyl}-3-ethylidene-2-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]-3,4-dihydro-2H-pyran-5-carboxylate
PubChem CID: 5281544, Oleuropein.

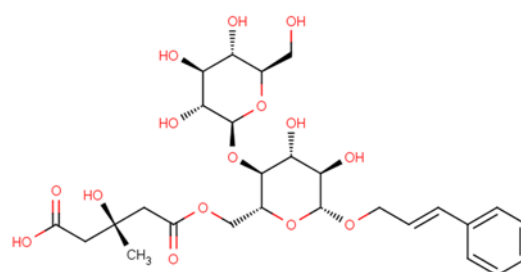


methyl (3Z)-4-{2-[2-(3,4-dihydroxyphenyl)ethoxy]-2-oxoethyl}-3-ethylidene-2-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]-3,4-dihydro-2H-pyran-5-carboxylate
PubChem CID: 53297357,
Oleuropein.

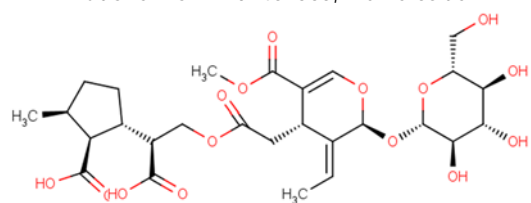
Peak 62



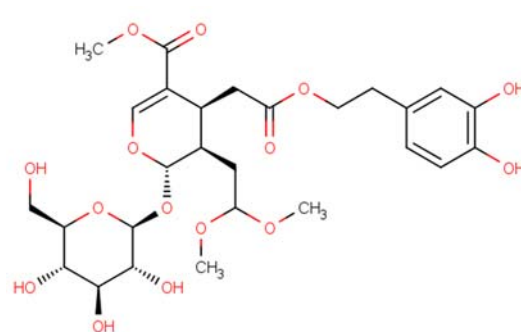
2-[1-carboxy-2-((2-((3E)-3-ethylidene-5-(methoxycarbonyl)-2-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]-3,4-dihydro-2H-pyran-4-yl]acetyl)oxy)ethyl]-5-methylcyclopentane-1-carboxylic acid
PubChem CID: 102461565, Frameroside.



5-[(4,5-dihydroxy-6-((2E)-3-phenylprop-2-en-1-yl]oxy)-3-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]oxan-2-yl)methoxy]-3-hydroxy-3-methyl-5-oxopentanoic acid
PubChem CID: 44521607, Piperchabaoside B.

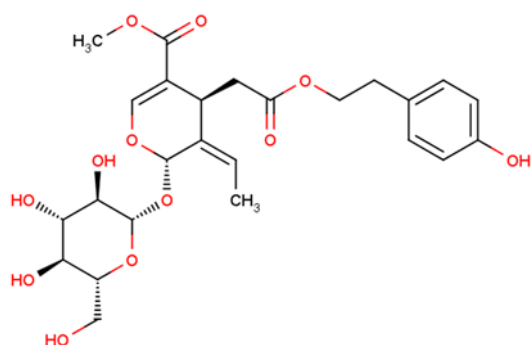


2-[1-carboxy-2-((2-((3E)-3-ethylidene-5-(methoxycarbonyl)-2-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]-3,4-dihydro-2H-pyran-4-yl]acetyl)oxy)ethyl]-5-methylcyclopentane-1-carboxylic acid
PubChem CID: 11968448, 2''-epi-Frameroside.

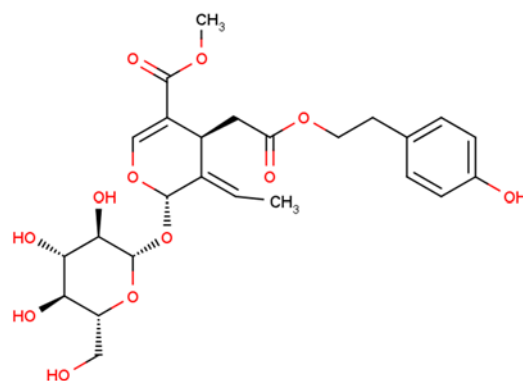


methyl 4-{2-[2-(3,4-dihydroxyphenyl)ethoxy]-2-oxoethyl}-3-(2,2-dimethoxyethyl)-2-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]-3,4-dihydro-2H-pyran-5-carboxylate
PubChem CID: 44521607, Piperchabaoside B.

Peaks 63 and 66

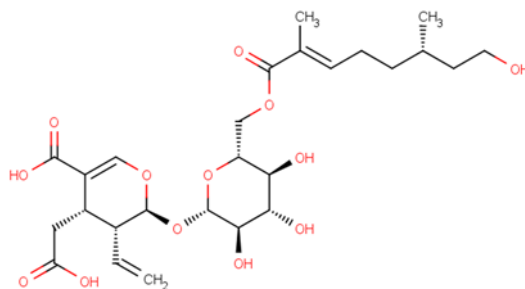


methyl (3Z)-3-ethylidene-4-{2-[2-(4-hydroxyphenyl)ethoxy]-2-oxoethyl}-2-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]-3,4-dihydro-2H-pyran-5-carboxylate
PubChem CID: 10392063, (8Z)-Ligstroside.



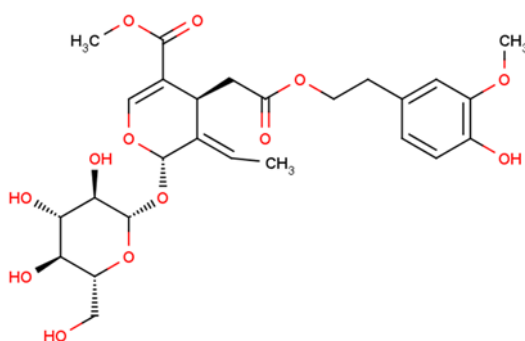
methyl (3E)-3-ethylidene-4-{2-[2-(4-hydroxyphenyl)ethoxy]-2-oxoethyl}-2-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]-3,4-dihydro-2H-pyran-5-carboxylate
PubChem CID: 14136859, Ligstroside.

Peak 65



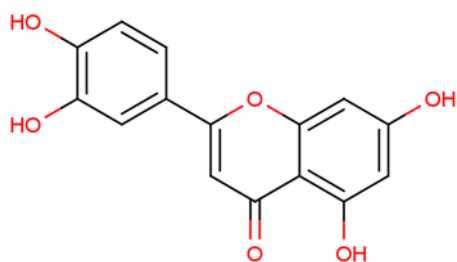
4-(carboxymethyl)-3-ethenyl-2-[[3,4,5-trihydroxy-6-(((2E)-8-hydroxy-2,6-dimethyloct-2-enyl)oxy)methyl]oxan-2-yl]oxy]-3,4-dihydro-2H-pyran-5-carboxylic acid
PubChem CID: 101406919.

Peak 67



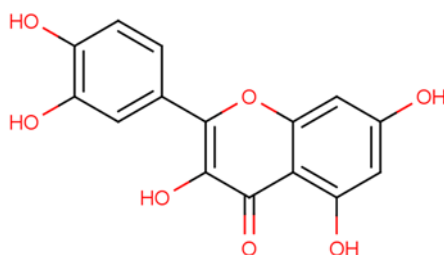
methyl (3E)-3-ethylidene-4-{2-[2-(4-hydroxy-3-methoxyphenyl)ethoxy]-2-oxoethyl}-2-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]-3,4-dihydro-2H-pyran-5-carboxylate
PubChem CID: 102047330.

Peak 69



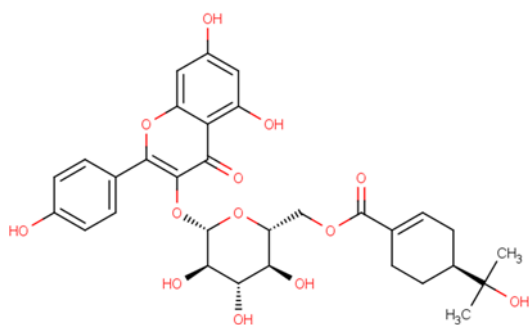
2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4H-chromen-4-one
PubChem CID: 5280445, Luteolin.

Peak 70

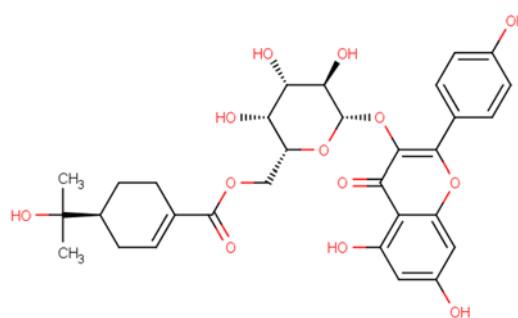


2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one
PubChem CID: 5280343, Quercetin.

Peak 71

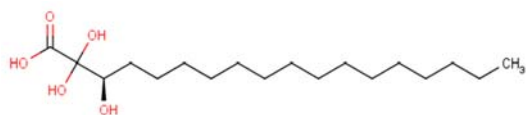


(6-[[5,7-dihydroxy-2-(4-hydroxyphenyl)-4-oxo-4H-chromen-3-yl]oxy]-3,4,5-trihydroxyoxan-2-yl)methyl 4-(2-hydroxypropan-2-yl)cyclohex-1-ene-1-carboxylate
PubChem CID: 15172373, Resinoside A.

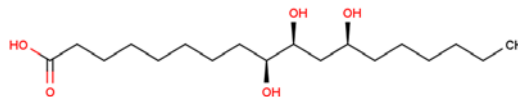


(6-[[5,7-dihydroxy-2-(4-hydroxyphenyl)-4-oxo-4H-chromen-3-yl]oxy]-3,4,5-trihydroxyoxan-2-yl)methyl 4-(2-hydroxypropan-2-yl)cyclohex-1-ene-1-carboxylate
PubChem CID: 101618995, Resinoside B.

Peak 74

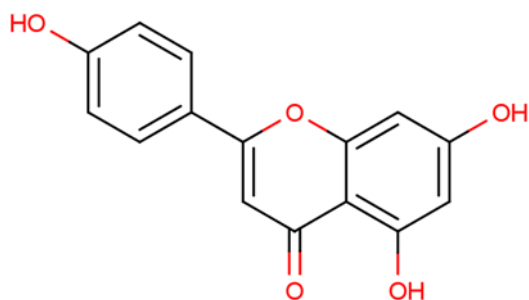


2,2,3-trihydroxyoctadecanoic acid
PubChem CID: 147011, 2,2,3-trihydroxyoctadecanoic acid.



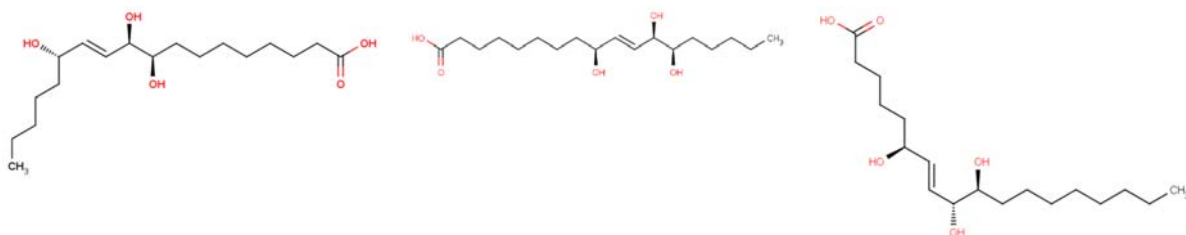
9,10,12-trihydroxyoctadecanoic acid
PubChem CID: 4252742, 9,10,12-trihydroxyoctadecanoic Acid.

Peak 75



5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one
PubChem CID: 5280443.

Peak 76

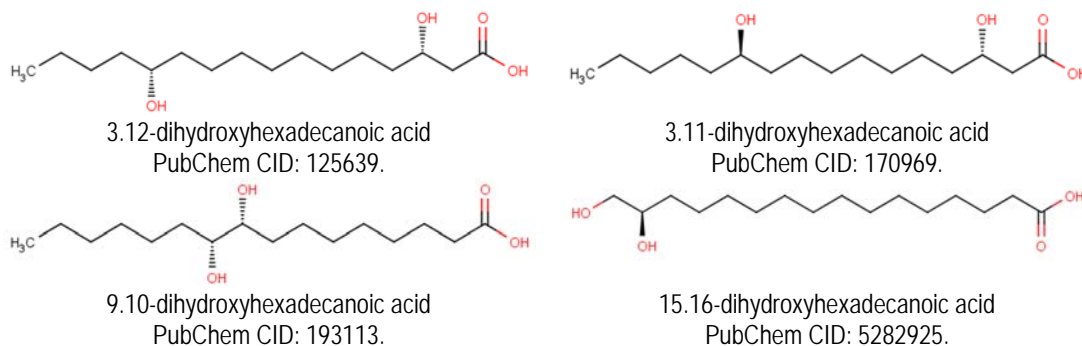


(11E)-9,10,13-trihydroxyoctadec-11-enoic acid
PubChem CID: 5282965.

(10E)-9,12,13-trihydroxyoctadec-10-enoic acid
PubChem CID: 5282966.

(7E)-6,9,10-trihydroxyoctadec-7-enoic acid
PubChem CID: 53248432.

Peak 78

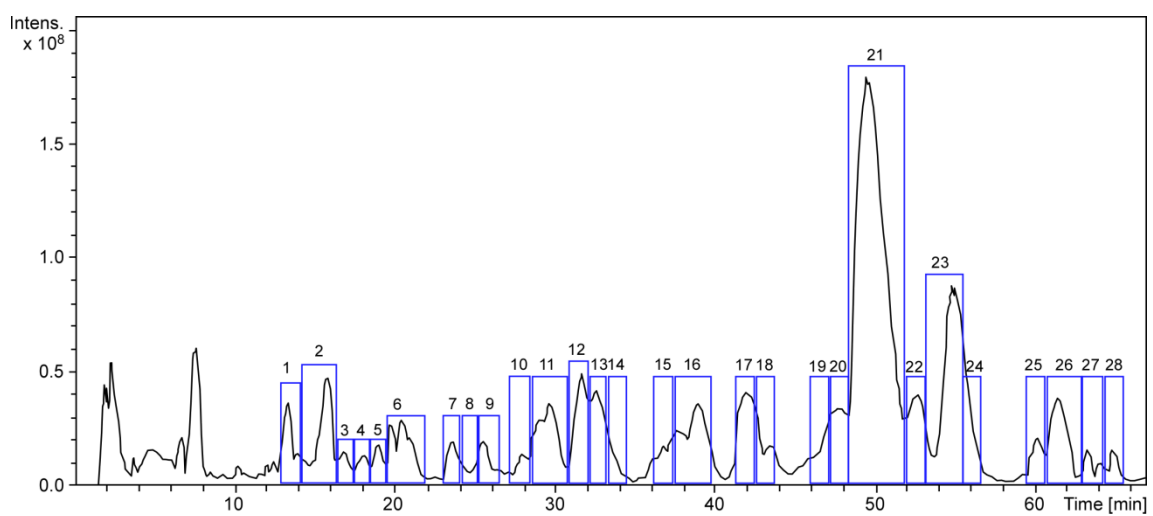


3,12-dihydroxyhexadecanoic acid
PubChem CID: 125639.

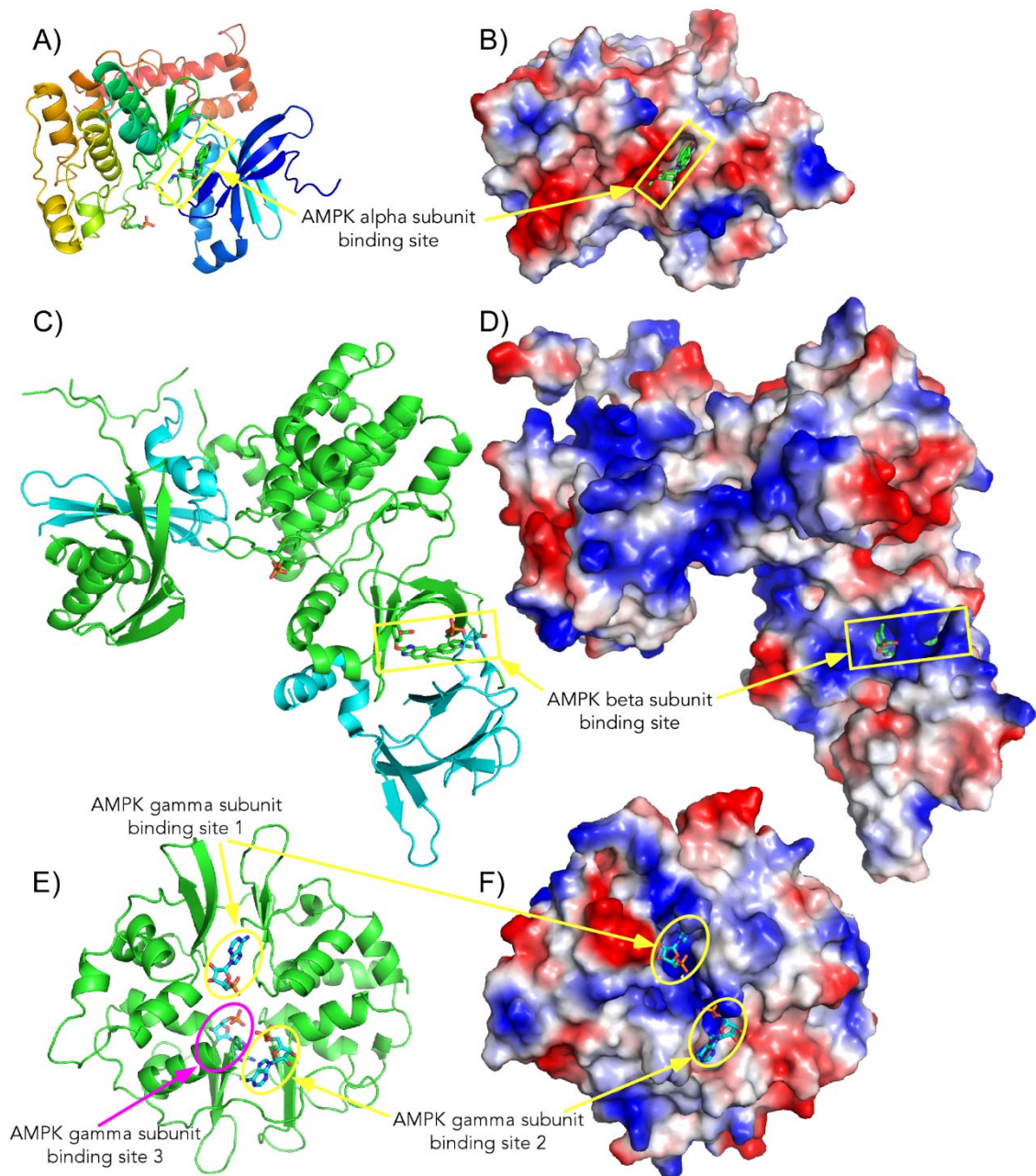
3,11-dihydroxyhexadecanoic acid
PubChem CID: 170969.

9,10-dihydroxyhexadecanoic acid
PubChem CID: 193113.

15,16-dihydroxyhexadecanoic acid
PubChem CID: 5282925.



Supplementary Figure 2. Base peak chromatogram (BPC) made by HPLC-ESI-TOF in negative ion mode of the olive leaf extract, obtained with the optimized semi-preparative conditions, and the collected fractions highlighted in blue.



Supplementary Figure 3. AMPK binding sites. Panels A, C and E show the secondary structure of alpha, beta and gamma subunits of AMPK kinase (4CFE.pdb), respectively. Panels B, D and F represent the electrostatic potential surface with a co-crystallized ligand indicating the position of each binding sites.