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Unambiguous structure of atractyloside and carboxyatractyloside

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This Letter is dedicated to the memory of J.-F.S. who passed away after the completion of an earlier version of this manuscript

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ABSTRACT

Atractyloside (ATR) was characterized in 1868 and until now structural studies on diterpenic moiety had been done through the characterization of ATR derivatives; while the glycosidic moiety seemed to be a β-D-glucopyranose a recent crystal structure of the mitochondrial ATP/ADP carrier in complex with CATR showed an α-D-glucopyranose. We decided to re-examine the ATR and CATR structures by crystallographic study of ATR.

Atractyloside (ATR) and carboxyatractyloside (CATR) are diterpenoid glycosides produced by several plants among which *Atractylis gummifera* found in the Mediterranean area. These substances are sometimes responsible for human intoxication (gastrointestinal hemorrhage, hepatic necrosis). Toxicity is explained by their high-affinity binding to and inhibition of the mitochondrial adenine nucleotide carrier (Ancp). The Ancp is a member of the mitochondrial carrier family catalyzing a 1:1 exchange between the uptake of ADP into the mitochondrial matrix and the release toward the cytosol of the ATP produced by ATP synthase. Both ATR and CATR have been extensively used to understand the function of Ancp; they block the carrier in one inhibited conformation by interacting with residues within its cavity facing the inter membrane space thereby preventing ADP/ATP binding.

The first chemical characterization of atractyloside was described in 1868.[1] Later, the structure and stereochemistry of the diterpenic moiety were determined through the characterization of atractyloside derivatives such as (−)-kauren and a β-D-glucoside anomer was indirectly identified by NMR analysis of the methyl ester of atractyloside in DMSO (dimethyl sulfoxide).[2,3] While atractyloside and carboxyatractyloside structures seemed to be established (Fig. 1), a crystal structure of Ancp in complex with CATR was recently solved at 2.2 Å resolution and in this structure CATR showed an α-D-glucoside bound to the (−)-kauren.[4] In a recent report Kedrov et al. also presented CATR and ATR as α-D-glucosides.[5] Some chemical suppliers depict α-glucosides as well and this might cause a complication in the scientific community. The results presented below aimed at elucidating these conflicting assignments of glucoside anomery and determining unambiguously the ATR and CATR structures by X-ray crystallography studies.

In order to understand why the CATR anomer in the Ancp crystal structure was different from that expected, CATR was reconstructed into the electron density map and refined using 1OKC pdb file and electron density data from the EDS server.[6] Ancp and water molecules were kept unmodified. Four enantiomers of CATR were constructed using Pymol software:[7] α-D-glucoside-(−)-kauren, β-D-glucoside-(−)-kauren, α-D-glucoside-(+)-kauren, β-D-glucoside-(+)-kauren and were manually inserted in the density using Coot and refined with Buster-TNT and Phenix.[8–10] All atoms of protein, lipids, detergent and water molecules were kept (2656 atoms), only CATR was replaced (50–51 atoms). As refinement global parameters could not be discriminative between enantiomers, electron density maps needed to be investigated (Fig. 2).

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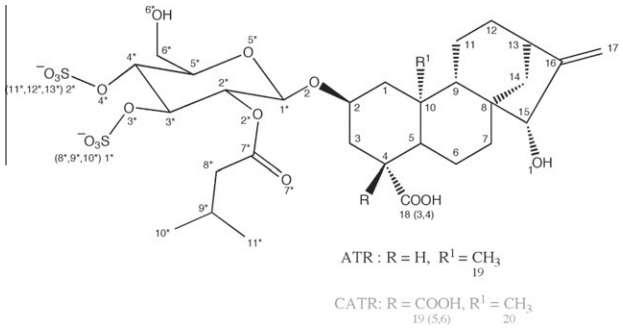

Figure 1. Atomic numbering of ATR and CATR (gray).

Both α-glucoside or β-glucoside (−)-kauren fit well into 2FoFc density map because C-1* and O-2 of glucopyranoside could be translated and still remained into density. Too small atomic position differences and the limited data resolution did not allow discriminating between the two solutions; both had good model statistics.

After refinement of (+)-kauren enantiomers, FoFc map clearly showed a bad position of C-12 and C-20 from diterpene indicating that the correct enantiomer was (−)-kauren. In order to determinate anomeric configuration of glucosidic part of CATR its crystallization was investigated.

The ATR crystals were grown from ammonium sulfate solutions but CATR could not crystallized under these conditions.[11] The crystal data, intensity measurement, and refinement details are summarized in Table 1. Crystallographic data for the structure of ATR have been deposited with the Cambridge Crystallographic Data Centre (deposition number: 840440). The asymmetric unit contains three ATR molecules, each bound to two ammonium ions. Hydrogen atoms were placed in geometrically idealized positions and constrained to ride on their parent atoms. Hydrogen atoms from ammonium and water molecules were not built. Most of non-hydrogen atoms were refined anisotropically and hydrogen atoms were refined isotropically.

In this structure ATR is unambiguously shown to be a β-D-glucoside-(−)-kauren. The three ATR structures (molecules 1, 2, 3 respectively) display a few differences: at the torsion angle between glucoside and diterpene (C1–C2–O2–C1*: 154.36°, 137.36°, 162.33°), at the torsion angle within the isovaleric acid moiety (C1*–C2*–O2*–C7*: 134.39°, 140.34°, 128.91°) and at glucose O-6* atomic positions. As illustrated in Figure 3, anisotropic factors are significantly higher for isovaleric acid atoms, and glucose C-6* and O-6*. CATR was then reconstructed from ATR structure using the Builder module of Pymol.[7]

Table 1
Crystallographic data of atractyloside

Crystal data	
Formula = $3(C_{30}H_{43}O_{16}S_2)$, 7(O), 6(N)	
Space group = C2221	$Z = 8$
$a = 22.592\ (5)$ Å, $b = 42.609(9)$ Å, $c = 24.735(5)$ Å	$D = 1.321$ g cm^{-3}
$\alpha = 90°$, $\beta = 90°$, $\gamma = 90°$	$F(000) = 9976$
$V = 23810\ (8)$ Å^3	$\mu = 1.87\ 3$ mm^{-1}
$T = 213$ K	CuK/α radiation = 1.54178 Å
Data collection	
Total reflections = 50296	$-27 \leqslant h \leqslant 23$
Unique reflections = 20985	$-52 \leqslant k \leqslant 42$
Reflections Fo > 4sig(Fo) = 9666	$-27 \leqslant l \leqslant 13$
$R_{int} = 0.1246$	$2.84° \leqslant \theta \leqslant 72.51°$
$R_{sigma} = 0.11137$	
Refinement	
Refinement on F^2	
$wR_2 = 0.3509$	$R1$ (all data) = 0.1407
Flack parameter = 0.13(0.0388)	Good of fitness = 1.1870
$w = 1/[\sigma^2(Fo^2)+(0.2000P)^2 + 0.0000P]$ where $P = (Fo^2+2Fc^2)/3$	

In conclusion based on these results a new refinement of crystallographic data of the Ancp–CATR complex was performed that gives a better structure model (Table 2). In contrast to previous reports,[4,5] the CATR is present as a β-glucoside and in addition the carboxylate group common to CATR and ATR is now interacting with R279 (in α helix 6) via a water molecule while the extra carboxylate specific of CATR forms a salt bridge with R79 (in α helix 2) better explaining the increase of CATR affinity as compared to ATR. This also easily explains the results of Kedrov et al. using the atomic force microscopy in single-molecule force spectroscopy mode which showed that α helix H2 is better stabilized when CATR rather than ATR is bound to Ancp.[5]

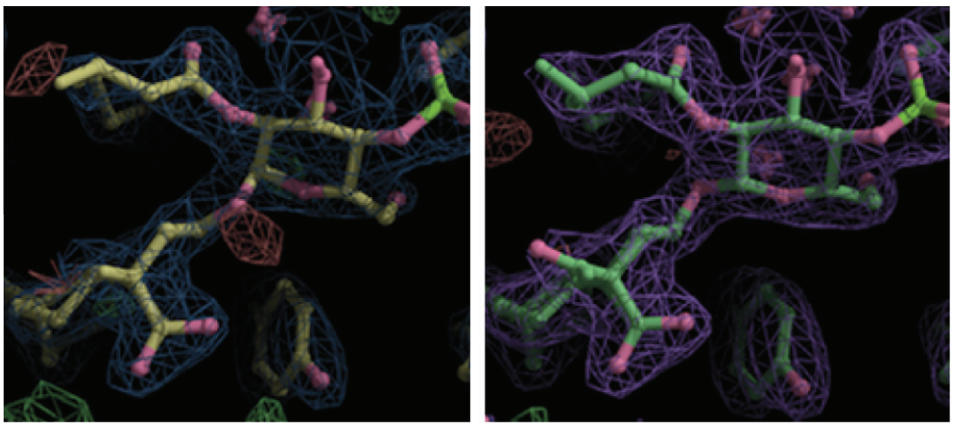

Figure 2. Electron density maps superimposed to CATR enantiomers. Left: map calculated on 1OKC structure with α-D-glucoside-(−)-kauren. Right: map calculated on refined 1OKC structure with β-D-glucoside-(−)-kauren. 2FoFc maps (blue and violet) are contoured at 1 σ, and FoFc at −3 σ (red) and 3 σ (green).

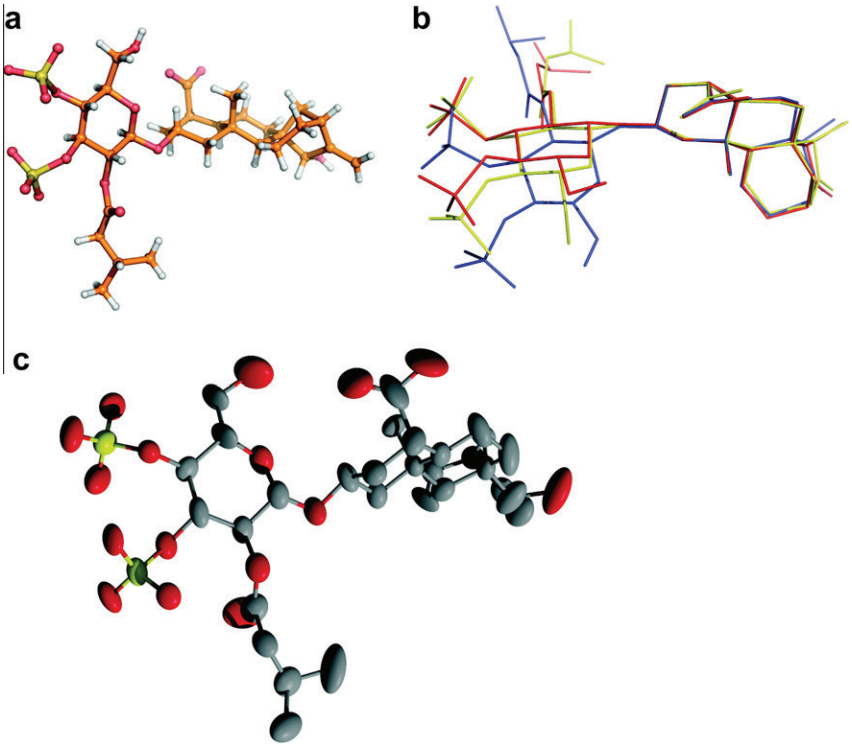

Figure 3. ATR crystal structure. (a) one of the 3 ATR molecules of the asymmetric unit is represented (drawn with the program PyMol), (b), superimposition of the 3 ATR molecules from the asymmetric unit, (c) thermal ellipsoid representation of one of the 3 ATR molecules with thermal motion probability of 30% (drawn with the program Mercury).[14]

Table 2
Crystallographic data of new refined structure of the CATR–protein complex

Unit cell	85.437 83.463 49.922 90.00 90.00 90.00
Space group	*P* 21 21 2
R_{work}	0.1883
R_{free}	0.2510
Rmsd on bonds	0.007 Å
Rmsd on angles	1.118°
97 water molecules: 15 more than in 1okc structure	

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2012.02.040.

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