Occurrence of tetraester and mixed ether/ester-bound *iso*-diabolic acid membrane-spanning lipids in mineral soils

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Introduction

Branched glycerol dialkyl glycerol tetraethers (brGDGTs) are a suite of membrane lipids that are widely used as empirical proxies for past temperature and pH. Although the stereochemistry of their glycerol moiety suggests they are produced by bacteria, the exact producers and the biosynthetic pathway of brGDGTs (**Fig. 1**) remain unclear.

Here we report the occurrence of tetraester and mixed ester/ether membrane spanning lipids with a backbone consisting of *iso*-diabolic acid (*iso*-DA) containing up to two additional methyl groups in mineral soils. These compounds are presumed intermediate products during brGDGT synthesis[1-3] but had not been detected in the environment before. Notably, they were simultaneously detected in an *Acidobacterium*[4].

Compound identification

UHPLC-MS chromatograms of soils from Nepal and Rwanda revealed an additional peak in the *m/z* 1050 trace eluting at 70.4 mins (**Fig. 2**). These soils stand out for their low pH (<4.5) and high elevation (>1900 masl) compared to other soils analyzed for GDGTs at Utrecht University. Compound identification is based on the accurate mass spectrum generated using UHPLC-HRMS (**Fig. 3**) and comparison with mass spectra of mixed ether/ester lipids with a DA backbone from cell material of *Thermatogales*[6,7]. HRMS spectra further revealed the presence of brGDGTs with one, two, or four ester bounds, as well as up to two additional methylations, as in brGDGT-IIa and IIIa (**Fig. 2, 3**).

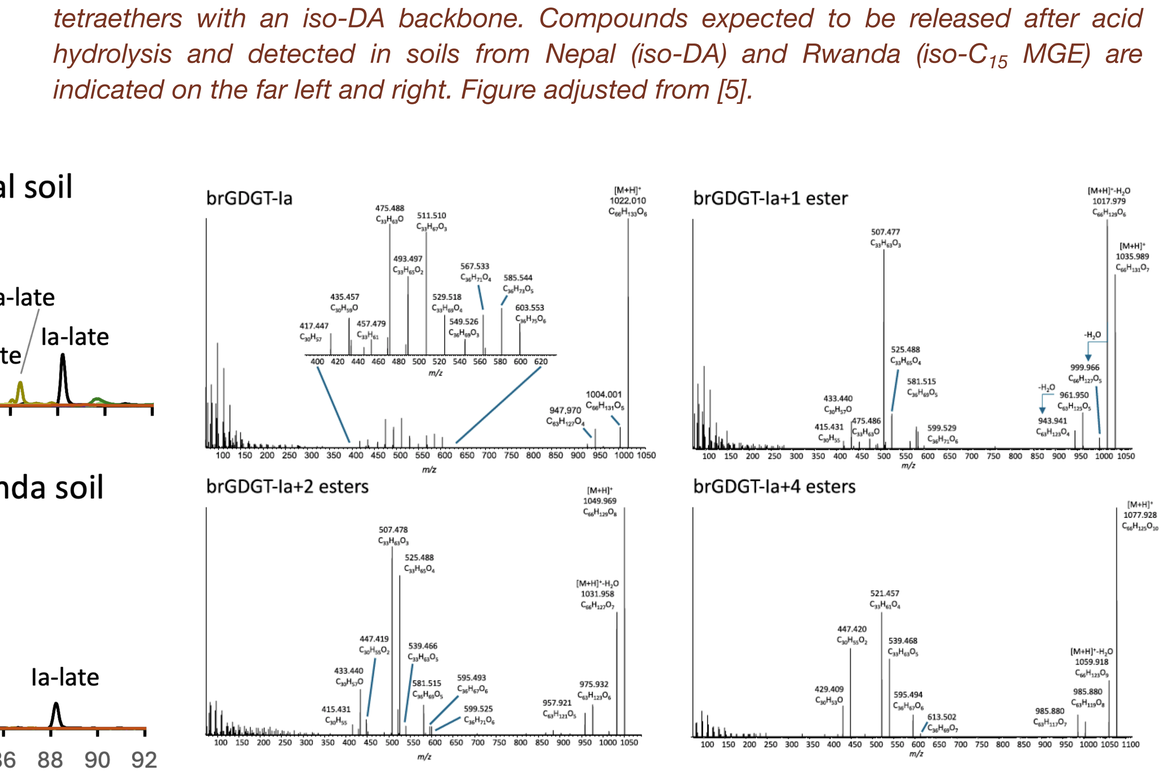

Fig. 1. *Hypothesized biosynthetic pathways for brGDGT production. Left: tail-to-tail condensation of two iso-C_{15} FAs to form tetraesters with an iso-DA backbone followed by ester-bond reduction. Right: ester reduction followed by tail-to-tail condensation to form tetraethers with an iso-DA backbone. Compounds expected to be released after acid hydrolysis and detected in soils from Nepal (iso-DA) and Rwanda (iso-C_{15} MGE) are indicated on the far left and right. Figure adjusted from [5].*

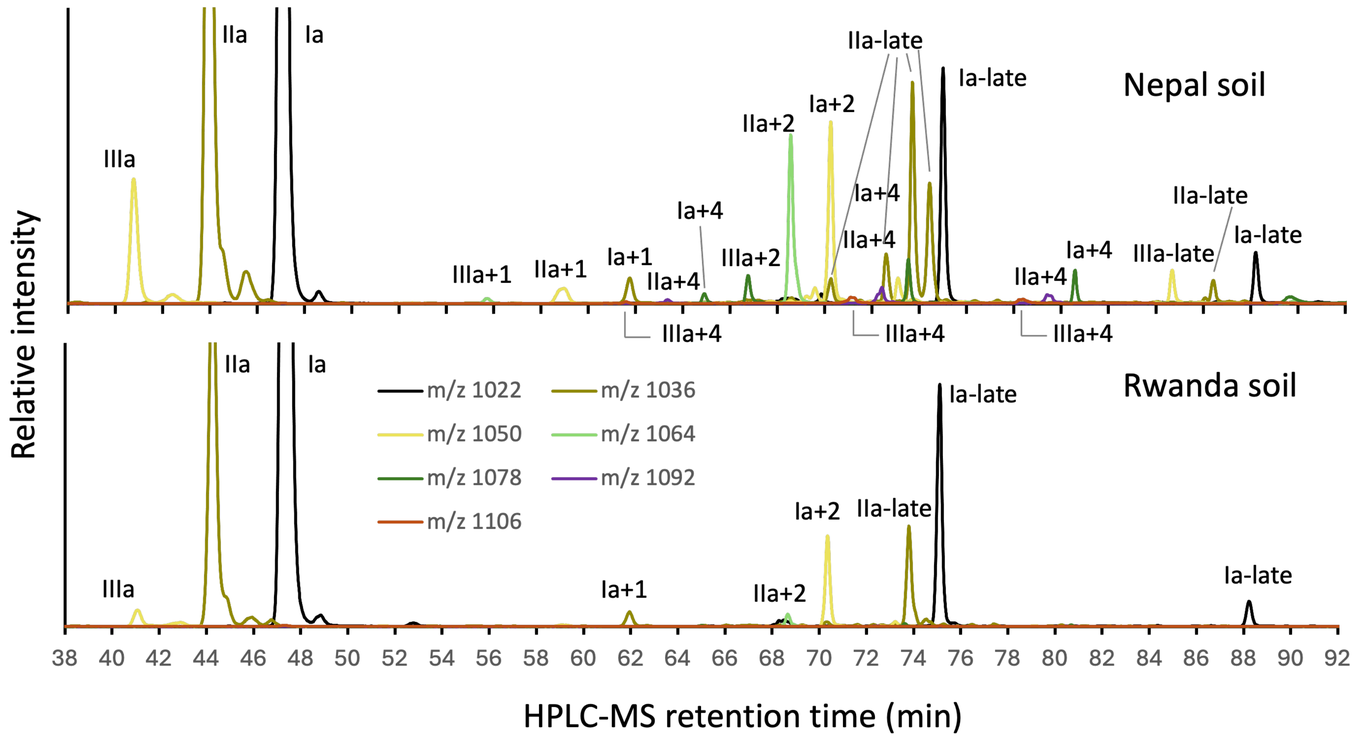

Fig. 2. *UHPLC-MS chromatograms of brGDGTs in which up to four ethers (+1, 2, or 4) are replaced by esters and up to two additional methylations (I, II, or III) in mineral soils from Nepal and Rwanda. Late-eluting isomers of brGDGT-Ia, IIa, and IIIa are also found.*

Fig. 3. *UHPLC-HRMS mass spectra of brGDGT-Ia, mixed ether/ester, and tetraester-bound iso-DA membrane-spanning lipids in the Nepal soil based on accurate mass.*

Potential clues on biosynthesis pathways

Acid hydrolysis resulted in disappearance of all suspected mixed ether/ester and tetraester lipids in the UHPLC-MS chromatogram, confirming the presence of ester bonds.

GC-MS analysis of the hydrolysed fractions revealed that *iso*-DA was released from the Nepal soil, whereas the Rwanda soil released *iso*-C_{15} and *iso*-C_{17} monoalkyl glycerol ethers (MGE). We thus speculate that mixed ether/ester lipids in these two soils are produced following distinct biosynthesis pathways: the formation of *iso*-DA tetraesters and subsequent ester bond reduction (Nepal; **Fig. 1, left**), or through the reduction of diesters into diethers followed by carbon-carbon linking (Rwanda; **Fig. 1, right**).

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Conclusions

- The occurrence of tetraester and mixed ether/ester-bound *iso*-DA membrane-spanning lipids in the environment confirms their existence as hypothesized intermediates during brGDGT synthesis.
- Differences in compounds released after acid hydrolysis of the GDGT fractions support both current hypothesized biosynthetic pathways.
- Identification of all responsible enzymes or gene clusters in brGDGT biosynthesis complicates identification of brGDGT producers through bioinformatics.

References: *[1]Sinninghe Damsté et al. 2011 AEM; [2]Sinninghe Damsté et al. 2014 AEM; [3]Sinninghe Damsté et al. 2018 Org Geochem; [4]Chen et al. 2025 GCA; [5]Halamka et al. 2023 Geobiol.; [6]Sinninghe Damsté et al. 2007 Archiv. Microbiol.; [7]Sahonero-Canavesi et al. 2022 Sci Adv.*