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FAECAL STEROID MARKERS FROM ARCHAEOLOGICAL ANTROPOGENIC SOILS

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Gas chromatography-mass spectrometry (GC-MS) analysis of the lipid extracts from samples collected within soil layers unearthed during archaeological excavations in Alsace (NE France) has been carried out. The soils comprised notably structure (St.) 4564 (Obernai) and St. 3153 (Strasbourg) dated back respectively to the 1st and 15th centuries AD.

High relative amounts of C_{27} and C_{29} steroids were detected in the lipid extracts, comprising mainly 5β-stanols **1** and **2** (Fig. 1a,b), together with minor Δ^5-sterols (**3**) and 5α-stanols (**4**). 5β-stanols are formed by hydrogenation of Δ^5-sterols by enteric microbiota in the digestive tract of animals and humans (Gérard, 2014), and their presence in the environment is considered to be a good indication for faecal inputs (Bull et al., 2002) and/or manuring (Bull et al., 2001). In an archaeological context, **1** and **2** represent usually reliable markers for ancient latrines, as is the case in the present study with the sample collected in structure 4564.

Determination of the ratio (**1a** + **2a**) / (**1b** + **2b**), which can be used to estimate the proportion of animal *vs.* vegetal dietary (Bull et al., 2002), suggests a predominant omnivorous diet according to the faecal sterol distributions in both samples, although the relative abundance of **1b** and **2b** in sample St. 4564 indicates significant plant inputs into the diet. Interestingly, in the same sample, miliacin (**5**; Fig. 1c), a methyl ether triterpenoid biomarker for broomcorn millet (*Panicum miliaceum*; Jacob et al., 2008), is by far the major lipid (Fig. 1c), disclosing consumption of this cereal by the Roman Gaul population settled in Obernai.

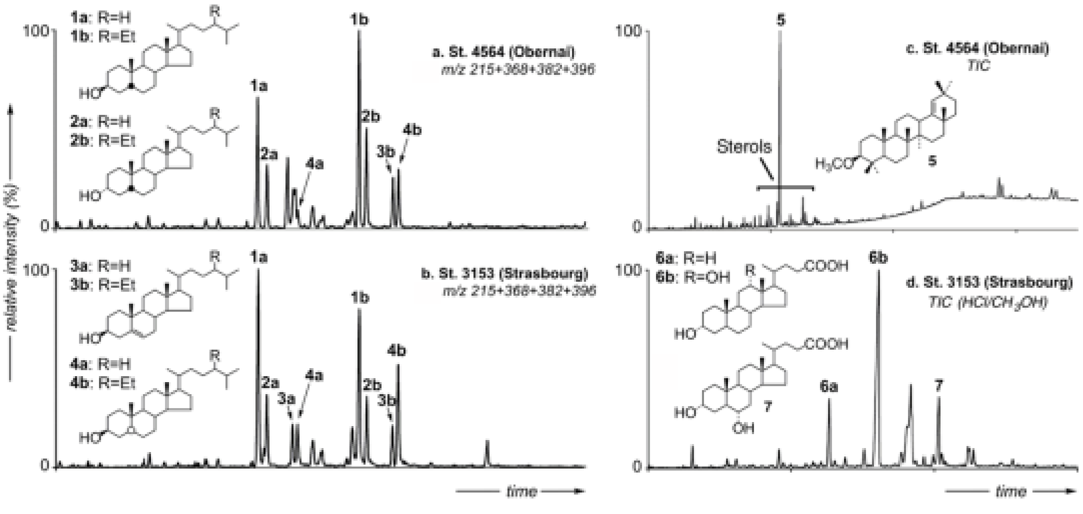

Figure 1 *Mass chromatograms (m/z 215 + 368 + 382 + 396) showing the distribution of stanols and sterols in: a) Sample St. 4564 (Obernai); b) Sample St. 3153 (Strasbourg); c) Gas chromatogram (TIC) of the apolar lipid fraction from sample St. 4564 (Obernai); d) Gas chromatogram (TIC) of the lipids released upon acidic hydrolysis of the polar extract from sample St. 3153 (Strasbourg). Carboxylic acids are analysed as methyl esters and alcohols as acetates.*

Methanolysis (HCl/CH_3OH) of the polar fractions from the lipid extracts has resulted in the release of abundant bile acids, which are degradation products of cholesterol **3a** in the liver (Gérard, 2014). Among them, lithocholic and deoxycholic acids (**6a** and **6b**, Fig. 1d) prevail in both samples, as in the case with human faeces (Batta et al., 1999). However, sample St. 3153 was characterised by the co-occurrence of hyodeoxycholic acid (**7**), a bile acid mainly produced by suids (Tyagi et al., 2008), suggesting that the structure sampled likely corresponded to a place dedicated to pig housing. In this respect, it is noteworthy that the *n*-alcohol distribution from this sample was also characterized by an unusual high contribution of the C_{26}, C_{28} and C_{30} homologues, as well as wax esters dominated by the C_{44} homologue having a C_{18} acyl moiety. The latter, together with high *n*-C_{26} alcohol abundance, occur in rye straw (Ji and Jetter, 2008), whereas predominant *n*-C_{28} alcohol is reported to occur in wheat straw (del Rio et al., 2013).

References

Batta, A.K., Salen, G., Rapole, K.R., Batta, M., Batta, P., Alberts, D., Earnest, D., 1999. Highly simplified method for gas-liquid chromatographic quantitation of bile acids and sterols in human stool. Journal of Lipid Research 40, 1148-1154.

Bull, I.D., Betancourt, P.P., Evershed, R.P., 2001. An organic geochemical investigation of the practice of manuring at a Minoan site on Pseira Island, Crete. Geoarchaeology 16, 223-242.

Bull, I.D., Lockheart, J., Elhmmali, M.M., Roberts, D.J., Evershed, R.P., 2002. The origin of faeces by means of biomarker detection. Environment International 27, 647-654.

Del Rio, J.C., Prinsen, P., Gutiérrez, A., 2013. A comprehensive characterization of lipids in wheat straw. Journal of Agricultural and Food Chemistry 61, 1904-1913.

Gérard, P., 2014. Metabolism of cholesterol and bile acids by the gut microbiota. Pathogens 3, 14-24.

Jacob J., Disnar J.-R., Arnaud F., Chapron E., Debret M., Lallier-Vergès E., Desmet M., Revel-Rolland M., 2008. Millet cultivation history in the French Alps as evidenced by a sedimentary molecule. Journal of Archaeological Science 35, 814-820.

Ji, X., Jetter, R., 2008. Very long chain alkyl resorcinols accumulate in the intracuticular wax of rye (*Secale cereale* L.) leaves near the tissue surface. Phytochemistry 69, 1197-1207.

Tyagi, P., Edwards, D.R., Coyne, M.S., 2008. Use of sterol and bile acid biomarkers to identify domesticated animal sources of faecal pollution. Water Air Soil Pollution 187, 263-274.