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Appendix 2: Natural Product Literature Searches

Discovering unique, well-preserved biomarkers of natural products are essential to any successful ancient organic residue investigation. Biomarkers should also be specific to a given geographic area-west-central Mexico in this instance--and the possibility of importation of another natural product containing the same biomarker needs to be addressed and ruled out if possible. Many compounds are not unique to a given natural product of biomolecular archaeological interest, principally because metabolic pathways and biological processes are too closely shared by related organisms. In the biological sciences, bioinformatics as a separate discipline has arisen to meet the need of searching through massive databases (whether DNA sequences or the compositions of modern natural products) for relevant chemical data on biomarkers. Search engines, based on statistical algorithms, are employed, which can be as readily available and easy to use as Google or Yahoo. For more intensive literature searches, Sci Finder Scholar, PubMed, the Institute for Scientific Information (ISI), the Kirk-Othmer Encyclopedia of Chemical Technology (2004), Dr. Duke's Phytochemical and Ethnobotanical Databases, and the Amber Research Laboratory's chemical database are essential resources. For testing Needham's pre-Hispanic distillation hypothesis, the chemical and archaeological literature was extensively searched for the relevant compounds for possible plants processed in the pottery from the west-central region of Mexico, including *Agave* spp., hog plum (*Spondias purpurea L*), mesquite fruits (*Prosopis* spp.), prickly pear and other cacti (*Opuntia* spp., *Stenocereus* sp.), guava (*Psidium guajava L.*), maize (*Zea* spp.), sotol (*Dasylirion wheeleri*), *Yucca* spp., chile (*Capsicum* spp.), etc. Most of these plants have a long history of use in this region, dating back to ca. 10,000 B.P. Quids (chewed-up remains) and plant parts have been found together in numerous caves, which attest to their probable importance in beverage and food production, as well as their early domestication (Flannery 1986; Byers1967).We focused our searches on Agave spp., because this plant was deemed most likely for fermentation and distillation and has been the principal natural product for making alcoholic beverages—traditional mescal and tequila--since the Spanish arrived in the sixteenth century (Colunga-García Marín and Zizumbo-Villarreal 2007; Zizumbo-Villarreal et al. 2009b; and Zizumbo-Villarreal and Colunga-GarcíaMarín 2008). A table of over 150 compounds in Agave spp, was compiled based on the recent scientific literature using the most sensitive and precise chemical techniques (especially GC-MS and SPME-GC-MS). It included research by Arellano et al. 2008; De León-Rodríguez et al. 2006, 2008; Nevárez et al. 2011; Peña-Alvarez et al. 2004; Prado-Jaramillo et al. 2015; Riffell et al. 2008; Vera-Guzmán, Guzmán-Gerónimo and López 2010. As already pointed out, lower molecular weight compounds with up to six carbons are likely to be contaminants or microbial products. Differences in which chemical compounds were identified from one report to another can be explained by which Agave spp. were analyzed. For example, De León-Rodríguez, et al. 2008 studied a mixture of *A. angustifolia*, *A. durangensis*, *A. potatorum*, and *A. salmiana*, whereas Vera-Guzmán, Guzmán-Gerónimo and López 2010 focused on *A.* angustifolia and *A. potatorum*, and Prado-Jaramillo et al. 2015 dealt solely with *A. tequilana* (*azul*). Species information is wholly lacking for Molina-Guerrero 2007. Extraction procedures, beverage processing, and plant part (e.g., flower aromas are examined in Riffell et al. 2008) also differed from study to study and contribute to divergent reporting. Such variations were less important in our study, since chemical comparisons of the ancient vessels from ancient El Diezmo-Adonaí vessels were made to undefined *Agave* spp. that were distilled in the modern replica vessels. The composite table, however, provided an all-important starting-point to assess possible chemical compounds derived from agave and other natural products of the region. Contaminants and degradation products could more readily be screened out, and biomarkers established. Except for maize, other possible natural products of the region that might have been associated with or processed in the ancient double-chambered jars have been chemically analyzed to a far lesser degree than agave. Comparable analyses of prickly pear, guava, and hog plum have been published, and were sufficient to establish that the compounds that were identified by SPME-GC-MS in the modern replica and ancient El Diezmo-Adonaí vessels could not serve as biomarkers. But detailed chemical investigation of other plants, such as sotol, *Yucca*, mesquite, and chiles have not been done yet. Possibly, some of the VOCs seen in our analyses by SPME-GC-MS are due to these plants. Much remains, however, to learn of the chemical composition of these plants and the microorganisms of west-central Mexico.