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On the Identification and Spontaneous Decomposition of Two Galloyl Depsides from *Pistacia atlantica* Desf.

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Introduction

Pistacia atlantica Desf. is a pistachio tree, whose subspecies naturally grow in parts of the Middle-East and in North Africa. In contrast to *Pistacia vera*, which is widely known for its edible fruits (nuts), the importance of *Pistacia atlantica* lies in its resin and essential oil, and in the application of its parts in traditional medicine. Extracts and chemical compounds isolated from different parts of the plant were reported to possess anti-bacterial, anti-fungal, anti-inflammatory, anti-diabetic, wound healing, and other activities.[1,2] The leaves of *Pistacia atlantica* are known have a very high content of tannins and depsides, with several of them being derivatives of gallic acid. In this work the isolation of a mixture of *meta*-digallic acid methyl ester (**1**) and *para*-digallic acid methyl ester (**2**) from *Pistacia atlantica* is briefly described, together with details about the identification process and its spontaneous decomposition.

Materials and Methods

Dried and ground *P. atlantica* leaves were extracted with 100% methanol for 24 hours using a mechanical mixer. This process was repeated five times. The extract filtered through filter paper was concentrated to dryness in a rotary evaporator at a temperature not exceeding 45 °C. The crude extract was suspended in 90% methanol and then fractionated with solvents of increasing polarity with *n*-hexane, chloroform ($CHCl_3$), ethyl acetate (EtOAc), and *n*-butanol/saturated with water (*n*-BuOH). An α-amylase and α-glucosidase inhibitory activity-directed fractionation study was performed on the *P. atlantica* sub-extracts with the EtOAc sub-extract being the most potent amongst them. The EtOAc sub-extract was repeatedly subjected to column chromatography (polyamide, RP-18, Sephadex LH-20) whilst targeting compounds with the inhibitory activity. As a result, a mixture of *meta*-digallic acid methyl ester (**1**) and *para*-digallic acid methyl ester (**2**) was isolated. The complete details about of the purification of **1** and **2** will be additionally published with the isolation data of co-isolated compounds from *P. atlantica*. The data are available from the first author on request.

All presented NMR spectra were recorded on an Avance III HD NMR spectrometer (Bruker) operating at frequencies of 400 MHz for ^{1}H nuclei and 101 MHz for ^{13}C nuclei. The spectrometer was equipped by a nitrogen-cooled Prodigy cryoprobe. The NMR sample of the mixture of **1** and **2** was prepared by dissolving approximately 15 mg of the mixture in 0.6 ml of CD_3OD with 0.03 % TMS (Eurisotop).

Results and Discussion

The ^{1}H NMR spectrum of the sample contained two sets of resolved signals with the integral intensity ratio between the two sets being approximately 5:2; the first set of signals contained two doublets (J_{HH} = 2.0 Hz) with δ_H = 7.389 and 7.264 ppm, one double-intensity singlet with δ_H = 7.222 ppm, and a triple-intensity singlet with δ_H = 3.843 ppm. From the coupling constant value and from the observed HMBC correlations it was determined that the two doublets belong to two protons bonded on a phenyl ring in a mutual *meta*-position. The elevated δ_C values of ^{13}C nuclei interacting with the abovementioned protons suggested the presence of a carbonyl moiety (δ_C = 168.28 ppm) and three oxygen-containing substituents bonded to this aromatic ring. The carbonyl moiety was identified as a methyl ester thanks to the strong $^{3}J_{CH}$ interaction observed between the protons with δ_H = 3.843 ppm and the carbon with δ_C = 168.28 ppm. The identified methyl gallate fragment had to be asymetrically substituted (i. e. not in the *para*-position relative to its carbonyl group) for its protons not to be identical.

From the matching integral intensities it was suggested that the protons with δ_H = 7.222 ppm mentioned at the start of this paragraph belonged to the same compound and possibly belonged to a symmetric aromatic fragment (explaining the double-intensity). This was later proven by the acquisition of an HMBC spectrum with a prolonged time interval for the evolution of small $^{n}J_{CH}$ interactions (the sequence was optimized for J_{CH} = 3 Hz) – a weak heteronuclear interaction could be easily spotted between protons with δ_H = 7.389 and 7.264 ppm, and a carbonyl with δ_C = 166.65 ppm. According to the observed HMBC interaction, the latter atom belonged to an aromatic ring containing the protons with δ_H = 7.222 ppm. The same 2D spectrum, together with the 2D HSQC spectrum clearly proved the symmetry of this aromatic ring as the latter protons showed both a single-bond and a multiple-bond correlation with a carbon with δ_C = 110.84 ppm. The singlet-shaped signal with δ_H = 7.222 ppm was resolved as a doublet of a doublet in the ^{13}C-satellite spectrum ($^{1}J_{CH}$ = 162 Hz, $^{4}J_{HH}$ = 1.8 Hz). It was suggested that the aromatic cycle is another galloyl moiety, thus the compound being identified as *meta*-digallic acid methyl ester (**1**).

The minor signal set contained two singlet-shaped with δ_H = 7.104 and 7.238 ppm, and a non-integer intensity singlet with δ_H = 3.865 ppm. Thanks to the knowledge of the structure of the major compound it was suggested that the two singlet-shaped proton signals belong to two pairs of protons from two symmetric galloyl moieties, thus the signal δ_H = 3.865 ppm actually being a standard triple-intensity -O-CH_3 group. With the same procedure the compound was identified as *para*-digallic acid methyl ester (**2**).

A set of signals that did not correspond with the ^{1}H and ^{13}C spectra was observed in the 2D NMR spectra measured afterwards (especially in the HMBC spectrum optimized for very small couplings that was measured a week after the sample preparation). A careful examination of a re-measured ^{1}H spectrum revealed the presence of an aromatic singlet shaped proton with δ_H = 7.048 ppm and a methoxy-group with δ_H = 3.815 ppm with non-corresponding integral intensities. Expectedly, the HMBC spectrum revealed that these signals belong to a symmetric galloyl moiety with δ_C = 52.32, 110.12 (2C), 121.51, 139.82, 146.55 (2C), and 169.09 ppm. No other correlation signals were obtained that would suggest a substitution of the aromatic ring; therefore, the compound was identified as methyl gallate (**3a**). The only discrepancy was found in the non-corresponding integral intensities of the two proton signals of the compound – the integral intensity ratio 4:3 was observed instead of 2:3 as would be expected for methyl gallate. It was revealed that the *meta*- and *para*-digallic acid methyl esters gradually decompose and form the additionally observed methyl gallate (see Figure 1).

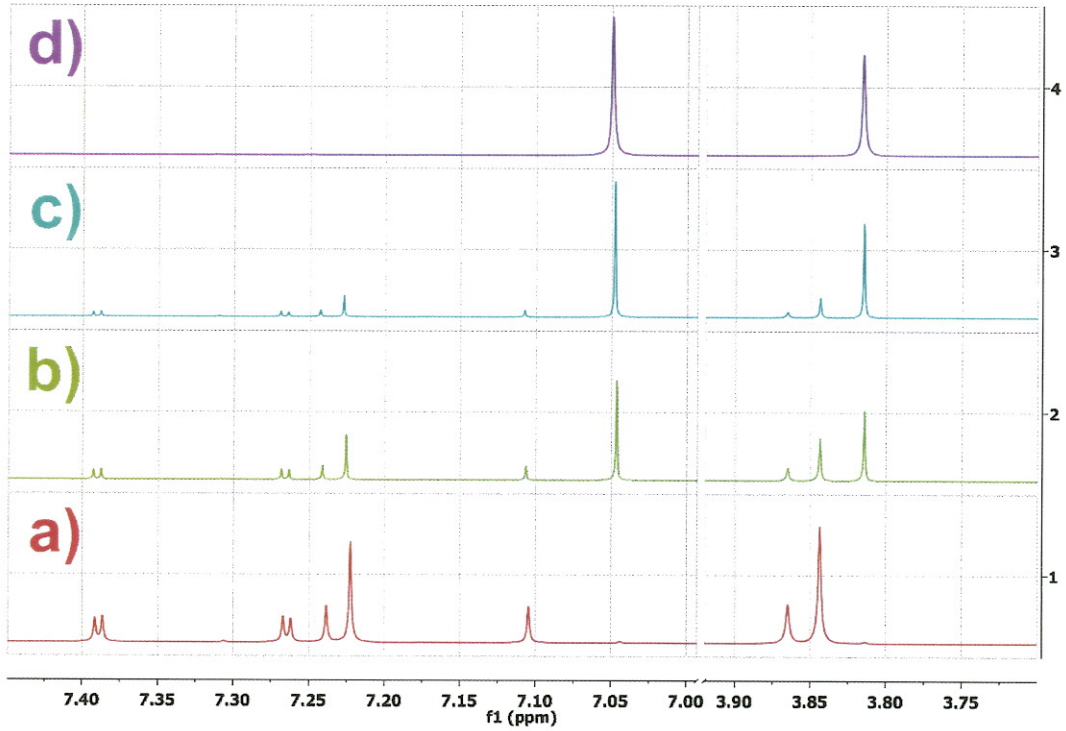

Fig. 1: The gradual decomposition of *o*- and *p*-digallic acid methyl ester as observed by ^{1}H NMR spectroscopy. The individual spectra have been measured a) immediately after sample preparation, b) after 4 days, c) after 7 days, and d) after 35 days of sample storage in varied temperatures (4 °C - 25 °C).

The reason of the abovementioned integral ratio could be explained by the analysis of the mechanism of the formation of the new compound. It was suggested that the digallic acid methyl esters **1** and **2** undergo an alcoholysis (transesterification) by the NMR solvent (MeOH-d_4), see Figure 2. If a standard transesterification mechanism is assumed, statistically, half of the methyl groups of the formed methyl gallate molecules must be fully deuterated – this is in complete accordance with the integral intensity ratio mentioned in the previous paragraph. Also, the ^{1}H NMR spectra suggest that the originally present methyl ester groups do not undergo transesterification by MeOH-d_4 as the methyl group's ^{1}H signal would have statistically had to disappear, and a large quantity of CH_3OD would be detected – both phenomena were not observed. This implies that the initial ionization of **1** and **2** at the depside bond is extremely favored over the ionization of the methyl ester bond of **1**, **2** or **3a**. The reason of such behavior remains unclear; such data suggest that the deuteron could be obtained by an intramolecular transfer from the hydroxy-group bonded to the *ortho*-position relative to the depside linking group *via* a 7-membered cycle. The presence of **3b** was later confirmed by the observation of several ^{13}C signals shifted by isotope effects and the $^1J_{CD}$ interaction within its trideuteromethyl group.

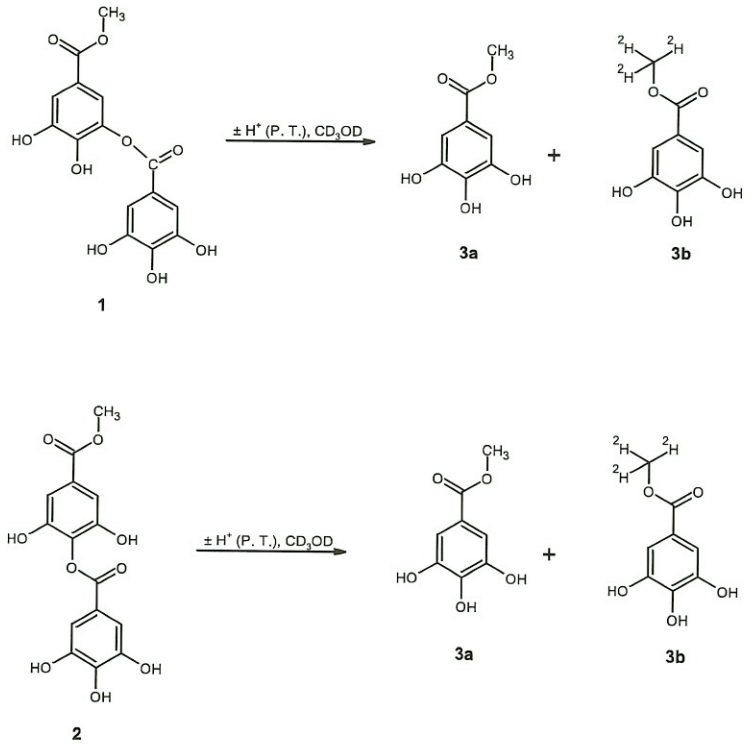

Fig. 2: The decomposition of **1** and **2** in CD_3OD with the formation of methyl gallate (**3a**) and trideuteromethyl gallate (**3b**). The source of the proton (or deuteron) needed in the initial ionization step was omitted on purpose, because of the existence of ambiguous explanations of this reaction step. P. T. = Proton Transfer.

To our knowledge, the compounds **1** and **2** were only characterized by NMR in a 7:3 mixture isolated from Panamian plants by Guldbrandsen et al.[3] Pierson et al. claims to have detected the both compounds in mango fruit using HPLC-MS.[4] The depside bond cleavage is commonly used to obtain gallic acid from gallotannins, but depsidases (tannases) are employed for the catalysis of the ester hydrolysis. The possibility of a spontaneous hydrolysis/alcoholysis can play a negative role in any application of compounds **1** and **2** as drugs. Despite this finding, the mixture of compounds was found to have inhibitory activity against α-amylase and α-glucosidase during the extract fractionation, and the mixture is undergoing pharmaceutical profiling.

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Literature

[1] H. Benamar, A. Marouf, M. Bennaceur, Phytochemical composition, antioxidant and acetylcholinesterase inhibitory activities of aqueous extract and fractions of *Pistacia atlantica* subsp. *atlantica* from Algeria. *J. Herbs, Spices, Med. Plants* 2018, vol. 24, iss. 3, pp. 229-244.

[2] Z. Ben Ahmed, M. Yousfi, J. Viaene, B. Dejaegher, K. Demeyer, Y. V. Heyden, Four *Pistacia atlantica* subspecies (*atlantica*, *cabulica*, *kurdica* and *mutica*): A review of their botany, ethnobotany, phytochemistry and pharmacology. *J. Ethnopharmacol.* 2021, vol. 265, article number 113329.

[3] N. Guldbrandsen, M. De Mieri, M. Gupta, E. Liakou, H. Pratsinis, D. Kletsas, E. Chaita, N. Aligiannis, A.-L. Skaltsounis, M. Hamburger, Screening of Panamanian plants for cosmetic properties, and HPLC-based identification of constituents with antioxidant and UV-B protecting activities. *Scientia Pharmaceutica* 2015, vol. 83, iss. 1, pp. 177-190.

[4] J. T. Pierson, G. R. Monteith, S. J. Roberts-Thomson, R. G. Dietzgen, M. J. Gidley, P. N. Shaw, Phytochemical extraction, characterisation and comparative distribution across four mango (*Mangifera indica* L.) fruit varieties. *Food Chem.* 2014, vol. 149, pp. 253-263.