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ISOPRENOID HYDROCARBONS FROM THE FRUIT OF EXTANT PLANTS

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Key Word Index—*Cucumis melo*; Cucurbitaceae; *Lycopersicon esculentum*; Solanaceae; *Diospiros kaki*; Ebenaceae; plant waxes; isoprenoid hydrocarbons; pristane; phytane.

Abstract—The epicuticular waxes from the fruit of melons, tomatoes and paradise apples were found to contain the acyclic isoprenoids, pristane and phytane. The highest content of phytane (10.6%) was identified in the liquid wax of paradise apple. The unusually high pristane–phytane ratio of 2.1 in tomato liquid wax suggests that these isoprenoids could originate in geochemical samples such as coals and sediments not only from bacteria and phytol degradation, as has been previously suggested, but also from plant waxes.

INTRODUCTION

The acyclic isoprenoids, pristane and phytane, have been found as major components in many ancient sediments: coal, crude oils, fossils and meteorites [1–3]. The presence of these isoprenoid alkanes has been established in green algae and in marine organisms, but they were not isolated from extant organisms until recently [4, 5].

Pristane and phytane are used in organic geochemistry, and in the chemistry of extraterrestrial materials as biological markers, because of their stable isoprenoid skeleton and their structural relationship with terpanes and steroids. The nature of sources and the generation of pristane and phytane as biological markers is of considerable scientific interest [1]. It has been assumed that these compounds were derived mainly from the phytyl side chain of chlorophyll-a as well as from chlorophyll-b, bacteriochlorophyll-a, α- and γ-tocopherols and carotenoid pigments [1]. The mechanism by which phytane and pristane are generated from the phytyl moiety and chlorophyll is still unclear [3]. Simulation experiments with phytol and *in situ* incubation with ^{14}C-phytol and thermodegradation experiments by heating of phytol have indicated the possible conversion of phytol to other functionalized isoprenoids [3, 6, 7]. On the basis of these experiments, it has been concluded that phytane is produced in sediments and that extant organisms do not produce phytane, as did some of their progenitors [3].

The presence of minor quantities (0.3–0.8%) of norpristane, pristane and phytane was established recently in the hydrocarbon fraction from the fruit of *Paliurus spina* Christi Mill [8]. Later, more significant quantities of pristane and phytane were identified in the hydrocarbon fractions of watermelon and squash epicuticular waxes [9]. These results led us to examine other extant plants for the content of pristane and phytane, and our results are described in the present paper.

RESULTS AND DISCUSSION

The cuticular waxes of the fruit of melons, tomatoes and paradise apples were isolated by light petroleum extraction. The total wax mixtures were separated into two fractions of liquid (soluble in acetone) and solid (insoluble in acetone) waxes. All precautions were taken during the isolation and separation to exclude contamination and thermodegradation of the samples. The extractions were performed at ambient temperature. The solvents used were checked by GC-mass spectrometry for the presence of organic impurities as well as for isoprenoids. The silica gel TLC plates were prepared from silica gel, previously purified to remove organic impurities [10]. All experimental procedures were run at room temperature.

The hydrocarbon fractions isolated from the liquid wax of melons and tomatoes and from the solid wax of paradise apples were found to contain as the main

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Table 1. Hydrocarbon composition of epicuticular waxes from melons, tomatoes and paradise apples

Peak no.	Component	Liquid wax			Solid wax		
		Melon	Tomato	Paradise apple	Melon	Tomato	Paradise apple
1	*n*-Heptadecane	3.4	2.3	12.3	—	—	—
2	2,6,10,14-Tetramethylpentadecane (pristane)	1.9	3.8	4.9	—	—	—
3	*n*-Octadecane	5.1	3.1	16.1	—	—	—
4	2,6,10,14-Tetramethylhexadecane (phytane)	3.9	1.8	10.6	—	—	—
5	*n*-Nonadecane	6.8	5.5	19.5	—	—	3.5
6	*n*-Eicosane	2.9	3.1	10.1	—	—	4.4
7	*n*-Heneicosane	9.7	2.3	6.3	—	—	6.3
8	*n*-Docosane	4.4	7.0	16.1	—	18.8	5.1
9	*n*-Tricosane	16.4	4.5	4.1	—	81.2	31.8
10	*n*-Tetracosane	6.9	3.1	—	—	—	6.5
11	*n*-Pentacosane	34.9	17.2	—	—	—	42.1
12	*n*-Hexacosane	3.8	8.1	—	—	—	—
13	*n*-Heptacosane	—	6.5	—	0.6	—	—
14	*n*-Octacosane	—	11.1	—	1.3	—	—
15	*n*-Nonacosane	—	12.0	—	4.2	—	—
16	*n*-Triacontane	—	8.6	—	1.7	—	—
17	*n*-Hentriacontane	—	—	—	8.3	—	—
18	*n*-Dotriacontane	—	—	—	3.2	—	—
19	*n*-Tritriacontane	—	—	—	30.5	—	—
20	*n*-Tetratriacontane	—	—	—	3.7	—	—
21	*n*-Pentatriacontane	—	—	—	40.6	—	—
22	*n*-Hexatriacontane	—	—	—	5.9	—	—

component a straight chain alkane pentacosane, while nonadecane was found as the predominant component in the paradise apple liquid wax (Table 1). The highest content of phytane (10.6%) was found in the liquid wax of paradise apples. A prevalence of pristane (3.0%) over phytane (1.1%) was determined so far in the epicuticular wax of squash (*Cucurbita maxina* Duch.; pristane/phytane (Pr/Ph) ratio equal to 2.7) [9]. A similar prevalence of pristane (3.8%) over phytane (1.8%) is now apparent in the liquid wax of tomatoes (Pr/Ph ratio to 2.1), (Table 1).

The Pr–Ph ratio is used as an indicator of the paleoenvironment and thermal maturity of coals and sediments. The main reason for this use is due to the fact that these isoprenoids have not been found in extant plants [2]. The presence of pristane and phytane in the investigated waxes is not a result of degradation of phytol or phytanic and phytenic acids, because they were not identified among the alchols and the fatty acids of these fruit [9, 11]. The components of the hydrocarbon fractions of melons, tomatoes and paradise apples were identified by comparison of their GC retention times with those of reference compounds. Mass spectra were compared with those of reference compounds and with published data [4].

The experimental results now obtained contradict the previously expressed opinion that "phytane is not produced by extant organisms" [2]. The paucity of data for the presence of pristane and phytane in hydrocarbon fractions from higher plant waxes in previous reports may be the result of insufficient resolution of gas chromatography columns employed for analyses. As a consequence, some of the isoprenoid alkanes, such as pristane and phytane, found in geological samples could have as precursors not only bacterial and phytol degradation, but they may well also originate from plant waxes.

EXPERIMENTAL

General. Carlo Erba HR6G with FID instrument; DB-1 fused silica capillary column (15 m × 0.25 mm) (J and W Scientific U.S.A.); programmed temperature 120–240° (10° min^{-1}); carrier gas N_2, 30 ml min^{-1}; GC-MS: Finnigan MAT SSQ 700.

Plant material. Ripe fruit of *Cucumis melo* L., var. Queen of Colorado (melon), (Cucurbitaceae), *Lycopersicon esculentum* Mill. (tomato), (Solanaceae) and *Diospiros kaki* L., (paradise apple), (Ebenaceae) were collected from the plants growing in Dobrich and Varna regions in 1993.

Extraction and isolation. The epicuticular waxes were extracted with light petroleum from the cuticle of the fruits and sepd on liquid and solid fr. according the known procedures [9, 11]. Anal. and prep. TLC were performed on the prepared silica gel (pure from

organic impurity) plates in developing system petrol–Et_2O–HOAc (90:10:0.5). The hydrocarbons were purified by prep. TLC developed in petrol.

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