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TRITERPENOIDS IN EPICUTICULAR WAXES OF *DUDLEYA* SPECIES

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Key Word Index—*Dudleya*; Crassulaceae; epicuticular wax; triterpenoids; β-amyrin acetate; taraxerone.

Abstract—The pentacyclic triterpenoids, β-amyrin acetate and taraxerone, are major components in the epicuticular wax of the genus *Dudleya*.

INTRODUCTION

Heavily glaucous, non-glaucous and intermediately glaucous populations are found in many species of *Dudleya*. Experimental crosses and field observations suggest that glaucescence is a polygenic trait [1]. The constituents of both non-glaucous and glaucous epicuticular waxes are investigated here to determine the chemical compounds possibly responsible for glaucescence in *Dudleya*.

RESULTS AND DISCUSSION

IR and TLC indicated that the epicuticular wax of the two *Dudleya* species examined here consisted of long chain alkanes, wax esters, primary alcohols and carboxylic acids. The glaucous waxes gave a positive reaction to the Lieberman–Burchard test indicating the presence of triterpenoids. By MS, two pentacyclic triterpenoids, β-amyrin acetate and taraxerone, were detected. Both of these compounds have been previously reported in plant waxes. β-amyrin acetate by Horn and Lamberton [2] and taraxerone by Tulloch [3].

The approximate percent composition of the waxes of the green and glaucous forms of two *Dudleya* species is listed in Table 1. These figures were determined by weighing fractions isolated by silicic acid column chromatography. It is of interest to note the dominance of triterpenoids in these conspecific forms. The *D. brittonii* glaucous wax is 40–45% β-amyrin acetate while the non-glaucous form contains only trace amounts of this compound. Similarly, the *D. farinosa* glaucous wax is 39–42% taraxerone while the green form contains trace amounts of this triterpenoid.

Table 1. Approximate percent composition of components in waxes of the green and glaucous forms of *D. farinosa* and *D. brittonii*

Components	*D. farinosa* green	*D. farinosa* glaucous	*D. brittonii* green	*D. brittonii* glaucous
Alkanes	50	28	20	30
Esters	21	11	35	9
β-Amyrin acetate	tr	tr	tr	40–45
Taraxerone	tr	39–42	tr	tr
Primary alcohols	6	4	8	2
Carboxylic acids	10	15	22	3

tr = less than 1%.

Single wax constituents have been shown to cause glaucescence in a variety of plants. Glaucous peas have a C_{31} hydrocarbon as the major constituent [4], while a C_{29} alkane is the predominant component of glaucous cabbage [5]. Major β-diketones cause glaucescence in *Eucalyptus*, [6], *Poa colinsoi* [7], barley [8] and durum wheat [9]. Recrystallization experiments and ultramicroscopical examination of the crystal structure of the epicuticular waxes is currently being performed to determine whether pentacyclic triterpenoids are responsible for glaucescence in *Dudleya*.

Chromatographic analysis of conspecific green and glaucous plants of many *Dudleya* species indicates that the glaucous plants differ from one another in the dominance of one of the two triterpenoids, β-amyrin acetate and taraxerone. The pattern of occurrence of these triterpenoids in glaucous species may be of systematic value in *Dudleya* [10].

EXPERIMENTAL

Plant material. Mature plants grown in a greenhouse in Claremont, California were analysed for their wax constituents. *D. brittonii* green and glaucous form were 6-year-old plants grown from seed collected near the type locality at La Misión, Northwestern Baja California del Norte, Mexico. The green and glaucous forms of *D. farinosa* were mature plants collected from population samples of Point Arena, Mendocino County, California. They were grown for 3 yr under the same conditions as the *D. brittonii* plants. These plants represent the 4 major types of wax constituent patterns in the genus.

Extraction. TLC showed that the qualitative composition of the wax was the same for both young and old leaves of a particular species. Thus, the older leaves closer to the base of the caudex were removed, leaving a rosette of younger leaves for plant survival. The surface wax was extracted by immersion and agitation of the leaves in Et_2O for 5–10 sec. To avoid contamination the cut ends of the leaves were not immersed. The soln was then vacuum filtered to remove dirt and on evaporation the surface lipid was obtained.

Fractionation. The epicuticular wax (*D. brittonii* glau. 229 mg, *D. brittonii* green 148 mg, *D. farinosa* glau. 531 mg, *D. farinosa* green 178 mg) was dissolved in Et_2O and dried into Silic AR-CC-4 100 mesh (Malinckrodt, St. Louis). The silicAR and wax was then placed on a column of silicAR. The column was eluted with *n*-hexane (160 ml), C_6H_6 (160 ml), $CHCl_3$ (160 ml) and MeOH (200 ml). Fractions (20 ml) were collected and evaporated to dryness.

Chromatography. TLC was carried out with commercial 20 × 20 cm plates (Kieselgel 60 F-254) precoated with a 0.25 mm thick layer of Si gel G. C_6H_6 and $CHCl_3$ were used as develop-

ing solvents. The wax constituents were detected by spraying with 50% H_2SO_4 and charring at 100° for 40 min.

Liebermann–Burchard test. Triterpenoids turn red on the addition of concd H_2SO_4 followed by acetic anhydride.

MS were measured at 70 eV through a direct inlet system. At low resolution the source temp. was 140°; for high resolution the source temp. was 160°, with the trap at 300°.

IR were measured in KBr pellets (0.5 mg sample per 100 mg KBr).

Alkanes. The IR spectrum showed the absence of all oxygenated absorption bands. TLC gave $R_f = 0.83$ (C_6H_6), $R_f = 0.74$ ($CHCl_3$). These values correspond to those reported for hydrocarbons in *Triticum aestivum* [4].

Esters. The IR spectrum gave a strong band at 1150 (C—O) and at 1725 (C=O) cm^{-1}. TLC indicated $R_f = 0.66$ (C_6H_6), $R_f = 0.75$ ($CHCl_3$). These figures correspond to those reported for such compounds in many plants [4, 11–13].

Primary alcohols. IR revealed one strong band at 1100 (C—O) cm^{-1}. TLC gave $R_f = 0.07$ (C_6H_6), $R_f = 0.15$ ($CHCl_3$). These values are also very similar to those reported for such components in many plant waxes [4, 11–13].

Carboxylic acids. The IR spectrum showed a strong band at 1720 (C=O) cm^{-1} and TLC gave $R_f = 0$ (C_6H_6 and $CHCl_3$). These figures have been previously reported for fatty acids isolated from plant waxes [4, 11–13].

β-amyrin acetate. The IR spectrum gave very strong bands at 1720 (C=O) and 1235 (acetate) cm^{-1}. UV: $\lambda_{max}^{cyclopentane}$ nm: 2357. MS: *m/e* 468, 257, 249, 218 (base peak), 204, 203, 189, 175, 159, 147, 133, 119, 109, 94, 93, 81, 79, 69, 67, 65, 55, 43. (most intense peaks listed). This compound undergoes a retro Diels–Alder fragmentation which gives a characteristic ion *m/e* 218 [14]. This leaves a neutral fragment of 250 which is equal to 191 (unsubstituted fragment) + 59 (acetate). The MS figures correspond to those reported for this compound previously [13]. High resolution MS gave the empirical formula of $C_{32}H_{52}O_2$. PMR (99.8% $CDCl_3$) δ 0.76, 0.85, 0.9, 1.1, 1.2, *s* at 2.05 ($OCOCH_3$), *m* at 4.3–4.65 (CHOAc), multiplets at 5.1–5.25 (C=CH—). These values are in accordance with those reported earlier [15]. TLC gave $R_f = 0.32$ (C_6H_6) and $R_f = 0.48$ ($CHCl_3$). The mp was 231–234°. LB test: maroon red. An authentic sample of this compound gave the same IR spectrum, R_f values in both C_6H_6 and $CHCl_3$, and λ_{max}. The mmp was not depressed.

Taraxerone. The IR spectrum showed a strong wide carbonyl band at 1690–1700 cm^{-1}. UV [illegible] 2357. MS: *m/e* 424, 409, 300 (base peak), 285, [illegible] 205, 204, 189, 149, 133, 121, 119, 109, 107, 95, 93, 91, 67, 57, 43. (most intense peaks listed). This compound undergoes a retro Diels–Alder fragmentation to furnish *m/e* 300. High resolution MS indicated an empirical formula of $C_{30}H_{48}O$ and revealed that the *m/e* 300 fragment was $C_{21}H_{32}O_2$. This corresponds to the postulated structure for this fragment. The MS was identical to that published in ref. [16]. PMR (99.8% $CDCl_3$): δ 0.4, 0.75, 0.76, 0.85, 0.92, 0.96, 1.06, 1.1, 1.2, 1.4, 1.6, *m* at 1.8–1.95, 2.40–2.55, *s* at 3.5, 4.9, *m* at 5.5–5.7. TLC gave $R_f = 0.25$ (C_6H_6) and $R_f = 0.36$ ($CHCl_3$). The mp was 240–244°. LB test: maroon red. An authentic sample of taraxerone gave the same IR spectrum, R_f values in both solvents and λ_{max}. The mmp was not depressed.

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BIOSYNTHETIC INTERMEDIATES IN THE CONVERSION OF FUCOSTEROL TO OOGONIOL

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Key Word Index—*Achlya heterosexualis*; [CD_3]-methionine; fucosterol; antheridiol; oogoniol; steroid biosynthesis.

Abstract—When grown in the presence of [CD_3]-methionine *Achlya heterosexualis* produces oogoniols containing two deuterium atoms which are located at C-28 and C-29. This is consistent with conversion of fucosterol to a C-29 aldehyde followed by reduction to the C-29 hydroxyl present in oogoniol.

The oogoniols (**1**) are a group of closely related steroids which induce the formation of oogonia or female sex organs in the water mould *Achlya* [1]. A study of the biosynthesis of these steroids has revealed that fucosterol, the major sterol present in *Achlya*, is an intermediate in their biosynthesis [2]. When added to a culture of the