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METABOLISM OF THE SESQUITERPENOID PHYTOALEXINS CAPSIDIOL AND RISHITIN TO THEIR 13-HYDROXY DERIVATIVES BY PLANT CELLS

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Key Word Index—*Capsicum frutescens*; *Solanum tuberosum*; Solanaceae; tissue cultures: phytoalexins; plant metabolism sesquiterpenes; capsidiol; rishitin; hydroxycapsidiol; hydroxyrishitin.

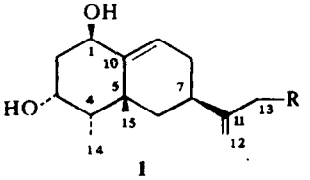
We recently reported that fruits of sweet pepper (*Ca3sicum frutescens*) metabolize exogenously supplied solutions of capsidiol (**1**, R = H), the pepper phytoalexin [1]. Since this raised the possibility that capsidiol might be a normal, if transitory constituent of peppers [1], further work was undertaken. This has led to the isolation of one of the metabolites and its identification as the 13-hydroxy-derivative (**1**, R = OH). Further, the use of tissue cultures confirmed that the metabolism of capsidiol is indeed effected by the plant and not by contaminating micro-organisms possibly present in the fruit. Tissue cultures were used similarly to determine the fate of potato phytoalexins. One of these, rishitin (**2**, R = H) was oxidized by potato-cell suspensions to 13-hydroxyrishitin (**2**, R = OH). Lubimin (**3**), the other potato phytoalexin tested, was metabolized very rapidly to unidentified products.

Loss of capsidiol or rishitin and lubimin during incubation with pepper- or potato-cell suspensions was readily demonstrated by GLC of ether extracts. Thus, for example, in a 24 hr incubation period capsidiol decreased from an initial concentration of 1×10^{-4} M to 5×10^{-5} M, rishitin decreased from 5×10^{-5} M to 2.9×10^{-5} M and lubimin decreased from 5×10^{-5} M to undetectable levels. Using ^{14}C-labelled preparations of the compounds, TLC of the ether extracts from such incubation mixtures revealed two major radioactive components, derived from capsidiol and rishitin respectively, but no detectable amounts of any metabolite derived from lubimin. The products from capsidiol and rishitin had lower R_f values than their parent compounds and were isolated from repetitive cell suspension culture experiments in mg amounts. The same metabolite as that from pepper cell suspensions was also isolated from pepper fruits incubated with 1×10^{-4} M capsidiol. Its structure (**1**, R = OH) followed readily from precise mass measurements and from a comparison of its ^{1}H- and ^{13}C-NMR spectra with those of (**1**, R = H) [2, 3]. The key feature in both spectra was the absence of absorption ascribable to a methyl group attached to a double bond while both spectra contained absorption characteristic of a new hydroxymethyl group. The presence of the additional hydroxyl group was confirmed by the ready formation of a triacetate. The structure of the rishitin metabolite as (**2**, R = OH) followed from entirely analogous results.

While this manuscript was in preparation, the isolation of the glucosides of several 13-hydroxylated derivatives of solavetivone (**4**) was reported from a flue-cured tobacco [4]. It thus appears that 13-hydroxylation may be a general metabolic process in the Solanaceae and, particularly after a further step such as glucosidation, might function as a detoxification mechanism.

EXPERIMENTAL

Calluses were initiated from shoot segments of peppers (*Capsicum frutescens* L., cultivar 'Keystone Resistant Giant') and potatoes (*Solanum tuberosum* L., cultivar 'Kennebec') by standard procedures on Murashige and Skoog agar medium [5].



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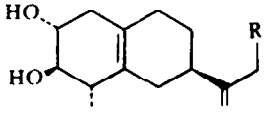

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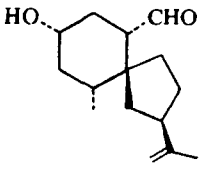

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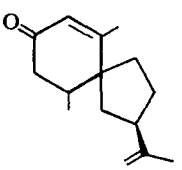

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containing 5 mg napthaleneacetic acid and 0.1 mg kinetin per l. Cell suspensions were obtained from calluses in the same medium lacking agar (50 ml medium in 200 ml Erlenmeyer flasks, incubated at 25° on a rotary shaker) and routinely transferred to fresh medium every 7 days. ^{14}C-Labelled capsidiol, rishitin and lubimin, available from previous studies [1, 6], were used initially to facilitate recognition of the products on chromatograms. Non-radioactive starting materials were used subsequently, and all available materials were bulked for isolation. Procedures for GLC have been described [7]. Si gel was used for both TLC and CC. 13-Hydroxycapsidiol (1, R = OH) was isolated by PLC (*t*-BuOH–EtOAc–HOAc, (5:95:0.5); $R_{capsidiol}$ *ca* 0.6) and purified by CC in *iso*-PrOH–EtOAc. (1:9). The product (5.5 mg from 5 l. diffusates) crystallized from $CHCl_3$ on cooling: colourless crystals containing solvent (typical $CHCl_3$ peaks in MS), mp indistinct at 85–95°. MS *m/e* (rel. int.): 252 (< 1, M^+) 237 (18, M—Me), 234 (8, M—H_2O), 219 (10, M—Me—H_2O), 216 (32, M—$2H_2O$), 201 (24, M—Me—$2H_2O$), 198 (17, M—$3H_2O$), 183 (28, M—Me—$3H_2O$), 105 (100). Precise mass measurements: calculated for $C_{15}H_{24}O_3$—Me, 237.1491: found, 237.1498; calculated for $C_{15}H_{24}O_3$—H_2O, 234.1620; found 234.1622. PMR (CD_3CN) δ: 5.84 (*q*, 1H, 9-H), 4.82 (*m*, 1H), 4.99 (*m*, 1H) (12-H's), 4.01 (*bs*, 2H, 13-H's), 4.21 (*dd*, 1H, 1-H), 4.41 (*d* of *t*, 1H, 3-H), 1.31 (*s*, 3H, 14-Me), 0.82 (*d*, 3H, 15-Me); (CD_3OD) δ: 5.85 (*q*, 1H, 9-H), 4.99 (*m*, 1H), 4.79 (*m*, 1H) (12-H's), 4.40 (*d* of *t*, 1H, 3-H), 4.22 (*dd*, 1H, 1-H), 4.01 (*bs*, 2H, 13-H's), 1.32 (*s*, 3H, 14-Me), 0.85 (*d*, 3H, 15-Me); ^{13}C-NMR (CD_3OD) δ: 154.7 (C-11), 108.5 (C-12), 129.3 (C-9), 141.4 (C-10), 75.6 (C-1), 66.0 (C-3), 65.4 (C-13), 49.0 (C-4), 47.1 (C-6), 40.3 (C-5), 37.1 (C-7), 37.1 (C-2), 31.9 (C-8), 32.5 (C-14), 9.6 (C-15). Material (6.1 mg) similarly isolated and crystallized from pepper tissue cultures (4 l.) was identical with the compound from fruit diffusates by TLC, mp, PMR, and MS. Acetylation (Ac_2O–Py at room temp.) gave the triacetate, whose PMR contained the requisite three acetate methyl signals at 1.98, 2.00 and 2.03 ppm. MS *m/e* (rel. int.): 336 (2, M—C_2H_2O), 318 (4, M—$C_2H_4O_2$), 276 (22, M—$C_2H_4O_2$—C_2H_2O), 258 (100, M—$2C_2H_4O_2$), 216 (42, M—$2C_2H_4O_2$—C_2H_2O), 198 (81, M—$3C_2H_4O_2$), and 183 (27, M—$3C_2H_4O_2$—CH_3); precise mass, calculated for $C_{21}H_{30}O_6$—$2C_2H_4O_2$, 258.1620; found, 258.1621.

13-Hydroxyrishitin. The ether-extracted material from potato tissue cultures (total, 3.5 l.) was chromatographed (5 ml fractions) first over Si gel (65 g) in MeOH–$CHCl_3$ (5:95), and again over Si gel (40 g) in *iso*-PrOH–EtOAc (1:9). Evapn of the only major radioactive band (fractions 19–23, 2nd system) furnished chromatographically almost homogeneous 13-hydroxyrishitin (2, R = OH) as a syrup (3.8 mg); PMR (CD_3OD) δ: 4.97, 4.74 (*m*, 1H each, 12-H's), 3.96 (*bs*, 2H, 13-H's) 3.38 (*m*, 1H, 2-H), 3.30 (*dd*, 1H, 3-H), 1.05 (*d*, 3H, 15-H's); ^{13}C-NMR (CD_3OD) δ: 153.5 (C-11), 108.8 (C-12), 130.2 (C-5), 125.9 (C-10), 79.8 (C-3), 72.0 (C-2), 65.2 (C-13), 43.1 (C-4), 39.2 (C-1), 37.0 (C-7), 32.7 (C-6), 30.2 (C-9), 27.7 (C-8), 17.0 (C-15); MS *m/e* (rel. int.): 238 (7, M^+), 220 (60, M—H_2O), 205 (14, M—H_2O—Me), 202 (24, M—$2H_2O$), 187 (34, M—$2H_2O$—Me), 184 (11, M—$3H_2O$), and 91 (100); precise mass, calculated for $C_{14}H_{22}O_3$, 238.1569; found, 238.1568. Additional peaks in the MS at *m/e* 278 (7), 236 (3, 278—C_2H_2O), and 218 (7, 278—$C_2H_4O_2$) suggested that the sample contained a little monoacetate as an impurity but in too low a concentration to be detectable in the NMR spectrum. Acetylation of the substance (2 mg) furnished the triacetate, which exhibited three 3-proton singlets at 2.01, 2.05 and 2.08 ppm as clear proof of triacetylation. MS *m/e* (rel. int.): (1.2, M—$C_2H_4O_2$), 262 (1.8, M—$C_2H_4O_2$—C_2H_2O), 244 (4.0, M—$2C_2H_4O_2$), 202 (7.0, M—$2C_2H_4O_2$—C_2H_2O), 184 (86, M—$3C_2H_4O_2$), 169 (31, M—$3C_2H_4O_2$—Me), and 143 (100); precise mass, calculated for $C_{20}H_{28}O_6$—$C_2H_4O_2$, 304.1674; found 304.1679.

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THE CARBON-13 NUCLEAR MAGNETIC RESONANCE SPECTRA OF FOUR EUDESMANE SESQUITERPENOLS

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Abstract—The ^{13}C NMR spectra of geosmin, selina-4(14),7(11)-diene-99-ol and two dihydroeudesmol isomers have been obtained and the individual resonances assigned. Several different empirical correlations developed by others have been combined in simple calculations to predict chemical shift values for sesquiterpenols of the eudesmane group.

INTRODUCTION

With the availability of pulsed-Fourier transform ^{13}C NMR spectroscopy it became of interest to see how useful this physical method might be in the structure