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PRESQUALENE ALCOHOL FROM *GIBBERELLA FUJIKUROI*

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Key Word Index—*Gibberella fujikuroi*; Ascomycetes; presqualene alcohol; sterol biosynthesis.

Abstract—The sequencing of steps between farnesyl pyrophosphate (FPP) and squalene, in *Gibberella fujikuroi* was investigated.

INTRODUCTION

Gibberella fujikuroi is a plant pathogen of cereal crops which demonstrably produces farnesyl pyrophosphate (FPP), squalene, squalene-oxide, lanosterol and multiple sterol end products [1–4]. However, sequencing of steps between FPP and squalene is undefined in *G. fujikuroi*. It is assumed [5–7] that the immediate precursor of squalene is a phosphorylated version of the cyclopropyl-containing polyolefin presqualene alcohol (PSA), which is formed from the direct head-to-head union of two molecules of FPP. Except for yeast [5], PSA has never been detected in any fungi. The results described herein show that the occurrence of PSA is not confined to yeast and support the implied intermediacy of phosphorylated PSA in squalene biosynthesis in *G. fujikuroi*.

RESULTS AND DISCUSSION

When *G. fujikuroi* mycelia were cultured as previously described [1] to monitor for sterol levels in the vegetative mycelia, no PSA was detected at $\leqslant 0.0001\%$ dry wt mycelia. However, in the presence of an ethanolic solution of 10 ppm bifarnesol, a potential intermediate of PSA [8], PSA accumulated at a concentration sufficient for its isolation and characterization. The bifarnesol treatment did not affect the growth-response. The wet mycelia from 10 flasks (3 g dry wt) were ground with sea sand and acetone at room temperature and extracted (Soxhlet) overnight with acetone. The acetone (total lipid) extract was subjected to TLC analysis (benzene–ether, 9:1) to separate hydrocarbons (e.g. squalene and squalene oxide, R_f 0.96) esterified materials (0.96), polar compounds (e.g. phospholipids, $R_f = 0$), 4,4-desmethyl (e.g. cholesterol, R_f 0.33), 4-monomethyl (e.g. lophenol, R_f 0.46) and 4,4-dimethyl (e.g. lanosterol, R_f 0.50) sterols from bifarnesol (R_f 0.33) and PSA (R_f 0.76; authentic sample provided by Prof. Coates [9]). The TLC zone matching PSA was scraped and eluted from the adsorbant with diethyl ether. The material thus obtained was analyzed by GC (3% SE-30 packed column operated at 245°). In the chromatogram a single dominant peak with a R_t similar to that of authentic PSA (RR_t cholesterol 0.71) was evident. The total amount of PSA in the combined material was determined by GC and then corroborated by HPLC [reversed phase C_{18} column: flow rate 1.6 ml/min; solvent, 4% aq. MeOH; UV detector at 205 nm; of PSA acetate (free PSA unstable on column) relative to free cholesterol 0.91] to be 50 μg. The EI mass spectrum of the sample possessed the characteristic fragments of PSA, possessing a $[M]^+$ at m/z 426 and other important ions at m/z 395, 357, 339, 289, 273, and 271. Efforts are currently in progress to determine the biosynthetic mechanism for the bifarnesol-induced elevation in PSA content and to prepare bioregulators of sterol biosynthesis [1, 3], which have been designed to interfere with early and late stages of the pathway, including PSA formation.

EXPERIMENTAL

Full details of the experimental procedures used in the studies with *G. fujikuroi* are presented elsewhere [1–3].

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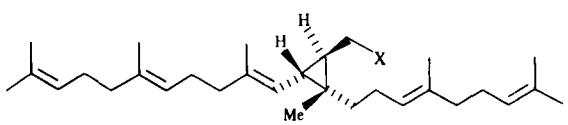

Presqualene alcohol X = OH
Presqualene alcohol pyrophosphate X = OPP

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24-METHYLENE-25-METHYLCHOLESTEROL IN *PHASEOLUS VULGARIS* SEED: STRUCTURAL RELATION TO BRASSINOSTEROIDS*

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Key Word Index—*Phaseolus vulgaris*; Leguminosae; seed; sterol; brassinosteroid; 24-methylene-25-methylcholesterol; 24-ethyldesmosterol; clerosterol.

Abstract—24-Methylene-25-methylcholesterol, 24-ethyldesmosterol and clerosterol in addition to several common phytosterols were identified in immature seed of *Phaseolus vulgaris*. 24-Methylene-25-methylcholesterol is considered closely correlated biogenetically with 25-methyldolichosterone, a brassinosteroid recently isolated from the same plant material, because both of them have the same basic side-chain structure.

INTRODUCTION

Immature seed of *Phaseolus vulgaris* cv. Kentucky Wonder has recently been demonstrated to contain 25-methyldolichosterone (**1**) as one of the major brassinosteroids [1]. The structure of this brassinosteroid is unusual because it contains a tertiary butyl moiety in the side chain. We have also isolated 24-methylene-25-methylcholest-5-en-3β-ol (**3c**, 24-methylene-25-methylcholesterol) [2] and 24-methylene-25-methyl-5α-cholest-7-en-3β-ol (**4c**, 24-methylene-25-methyllathosterol) [3] from other higher plant sources, both of which carry a tertiary butyl moiety in the side chain. This prompted us to investigate the sterol constituents of the immature seed of *P. vulgaris* in order to examine the presence of sterols possessing a tertiary butyl group in the side chain. This paper describes the isolation and identification of **3c** and two other uncommon sterols, 24-ethylcholesta-5,24(25)-dien-3β-ol (**3g**, 24-ethyldesmosterol) and 24β(*S*)-ethylcholesta-5,25-dien-3β-ol (**3h**, clerosterol), besides several common phytosterols, in the immature seed of *P. vulgaris*. In relation to the sterol constituents, some aspects of biogenesis of brassinosteroids are discussed.

*Brassinosteroids in *Phaseolus vulgaris* Part IV. For Part III, see ref. [1].

RESULTS AND DISCUSSION

The sterol fraction obtained from the extract of the *P. vulgaris* seed was acetylated, and a portion (600 mg) of the steryl acetate was fractionated by argentation TLC into five fractions (referred to as fractions 1–5 in the order of mobility). Fraction 1 (R_f 0.64–0.77, 333 mg) was a mixture of two components which was then subjected to reverse-phase HPLC to give fractions 1A and 1B. Fraction 1A (mp 143–144°) was a 57:43 mixture of 24α-methylcholesterol (24α-**3a**, campesterol) acetate and 24β-methylcholesterol (24β-**3a**, 22-dihydrobrassicasterol) acetate. Fraction 1B was pure 24α-ethylcholesterol (**3d**, sitosterol) acetate (mp 122–123°). Fraction 2 (R_f 0.37–0.64, 119 mg) was 24α-ethyl-22*E*-dehydrocholesterol (**3e**, stigmasterol) acetate (mp 140–142°). Fraction 3 (R_f 0.25–0.37, 13 mg), on further purification by HPLC, afforded **3g**-acetate (mp 137–139°). Fraction 4 (R_f 0.10–0.25, 34 mg) was further fractionated by HPLC, giving fractions 4A and 4B Fraction 4A was **3h**-acetate (mp 128–129°) and fraction 4B (mp 137–139°) was 24*Z*-ethylidenecholesterol (24*Z*-**3f**, isofucosterol) acetate accompanied by small amounts of the acetates of 24*E*-ethylidenecholesterol (24*E*-**3f**, fucosterol) and 24*Z*-ethylidenelathosterol (24*Z*-**4f**, avenasterol). Fraction 5 (R_f 0.02–0.10, 11 mg) was subjected to HPLC which gave fractions 5A and 5B. Fraction 5A (mp 131–134°) was a mixture (*ca* 8:2) of the acetates of 24-