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BIOSYNTHESIS OF α-SPINASTEROL IN PUMPKIN*

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Abstract—After administration of [3α-^3H]-5α-stigmast-7-en-3β-ol (I) to the leaves of pumpkin plants (*Cucurbita pepo* L.) radioactive α-spinasterol (II) was isolated and degraded to 3β-acetoxy-5α-bisnorchol-7-enaldehyde (VI) which was shown to be radiochemically pure by TLC and crystallization to constant specific radioactivity.

INTRODUCTION

DEHYDROGENATION of saturated sterol side chains at C-22,23 has been reported by several authors.[1–6] This prompted us to investigate the metabolism of 5α-stigmast-7-en-3β-ol (I) in pumpkin plants (*Cucurbita pepo* L.). I has recently been isolated from other Cucurbitaceae as *Coccinia indica* L.,[7] *Cucumis sativus* L., and *Cucumis melo* L.[8] All attempts to detect I in pumpkin have, however, failed so far. This may be due to a very rapid turn over of this sterol. Pumpkin plants are well suited for the examination of Δ^7-sterol biogenesis since they contain only traces of Δ^5-sterols. In our laboratory four sterols have been isolated from pumpkin[9] (Chart 1): 5α-stigmasta-7,22-dien-3β-ol (α-spinasterol, II), [24(28)Z]-5α-stigmasta-7,24(28)-dien-3β-ol (III), 5α-stigmasta-7,22,25-trien-3β-ol (IV), and 5α-stigmasta-7,25-dien-3β-ol (V). The structures of III, IV, and V have been proved by synthesis.[10] Administration of labelled III to pumpkin plants gave active IV and V, whereas the label of α-spinasterol (II) was rather poor.[11] For that reason we supposed I to be a precursor of II.

RESULTS AND DISCUSSION

5α-Stigmast-7-en-3β-ol was labelled by reduction of 5α-stigmast-7-en-3-one[7] with tritiated sodium borohydride. This afforded a mixture of epimers which were separated

TABLE 1. RECRYSTALLIZATION OF 3β-ACETOXY-5α-BISNORCHOL-7-ENALDEHYDE AND THE CORRESPONDING 2,4-DINITROPHENYL HYDRAZONE

Compound	Solvent for crystallization	Specific activity [dpm/μmole]
3β-Acetoxy-5α-bisnorchol-7-enaldehyde (VI)	petrolether	5890 ± 177
	petrolether	4900 ± 147
	petrolether	4271 ± 128
	petrolether	4263 ± 128
	petrolether	4296 ± 129
	benzene–cyclohexane 1:1	4283 ± 128
2,4-Dinitrophenyl hydrazone of VI	petrolether–10% ether	4140 ± 124
	petrolether–10% ether	4190 ± 126

* Dedicated to Professor F. Bohlmann on the occasion of his 50th birthday.

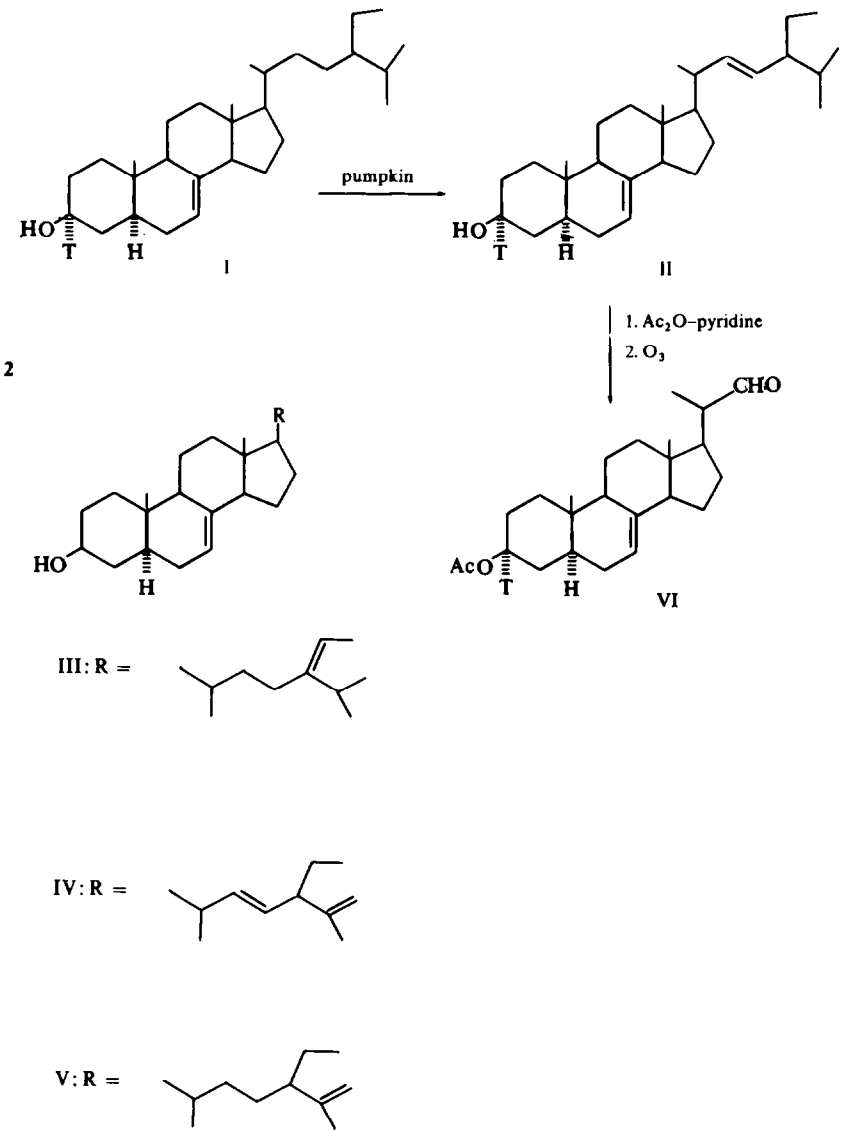

CHART 1

by chromatography. [3α-^{3}H]-5α-Stigmast-7-en-3β-ol (I) was applied to the leaves of pumpkin plants in acetone solution. The sterols were isolated by a common procedure (Experimental) and converted to the acetates which were purified by chromatography on silver nitrate–silica gel. The sterols III–V exhibited low radioactivity, whereas II was highly labelled. Since the precursor I could not be removed completely from II by chromatography and crystallisation we cleaved the side chain of II with ozone and isolated radioactive 3β-acetoxy-5α-pregn-7-en-22-al (VI). VI could easily be purified to constant radioactivity. The preparation of the 2,4-dinitrophenyl hydrazone of VI occurred without loss of specific molar radioactivity (Table 1).

Compound I is *ca* 0·6% incorporated into II. This result supports the assumption that dehydrogeneration of a saturated sterol side chain at C-22,23 is a common process in the biogenesis of sterols.

EXPERIMENTAL

Solvent mixture for column chromatography was light petroleum with increasing amounts of ether. Radioactivity was determined in a Beckman scintillation counter having a general efficiency of 50% for ^3H, and 35% in the case of the 2,4-dinitrophenyl hydrazone.

Preparation of the radioactive precursor. 5α-Stigmast-7-en-3-one (18 mg) dissolved in THF (1 ml) was treated for 30 min with 5 mC of tritiated $NaBH_4$; inactive $NaBH_4$ (15 mg) was added and after 30 min the mixture was poured into water and extracted with ether. After evaporation the residue was purified by column chromatography on silica gel to remove the less polar 3α-hydroxy epimer. Crystallization from MeOH afforded pure I (7 mg), specific activity 4·90. 10^8 dpm/mg. No radioactive impurities were detected by TLC in light petroleum–50% ether (R_f 0·21) or chloroform–10% acetone (R_f 0·35) using a Berthold TLC scanner.

Administration of I to pumpkin plants. [3α-^{3}H]-5α-Stigmast-7-en-3β-ol (1 mg, 4·90 × 10^8 dpm) was administered in doses of 0·98 × 10^8 dpm in acetone soln to the cotyledones of eighty 6-days-old pumpkin plants which were grown from seed in a greenhouse on Knop's soln. A total of 5 such treatments were given to the plants every 2 days. Five days after the last treatment the roots were cut off and the plants (512 g) frozen in liquid N_2 and homogenized in a blender under addition of acetone. The shredded material was extracted with hot acetone in a soxhlet and the evaporated extract saponified with 600 ml of 10% (w/v) KOH in EtOH-water (95–5) for 2 hr on a steam bath. The mixture was evaporated under reduced press, water was added and the non-saponifiable material thoroughly extracted with ether. Evaporation of the extract gave a semi-solid (total activity 3.50. 10^8 dpm). The mixture of Δ^7-sterols was isolated by column chromatography on alumina (50 g, 10% H_2O). Fractions were analyzed by TLC using benzene–4% EtOH in which Δ^7-sterols had R_f 0·33. After acetylation of the Δ^7-sterols (101 mg) with Ac_2O–pyridine they were separated by column chromatography on $AgNO_3$-silica gel. Fractions were analyzed by TLC on $AgNO_3$–silical gel G plates (Merck) using cyclohexane–10% diisopropyl ether. The acetates of III, IV, and V were nearly inactive after several crystallizations from methanol.

Ozonolysis of II-*acetate.* As the α-spinasteryl acetate fraction (2 × 10^8 dpm, 64 mg) still contained unchanged precursor, part of it (25 mg) was cleaved by ozonolysis in methylene chloride (1 ml) with a trace of pyridine. Column chromatography of the reaction mixture on silica gel yielded VI (10 mg) which was diluted with inactive carrier (100 mg). Multiple chromatography and crystallization to constant radioactivity (Table 1) yielded pure aldehyde (18 mg), specific activity 4283 dpm/μmole. Radiochemical purity in TLC was determined by the method of Akthar *et al.*[12] in light petroleum 30% ether (R_f 0·43) or cyclohexane–20% EtOAc (R_f 0·55).

The aldehyde (17 mg) was converted to the 2,4-dinitrophenyl hydrazone. Column chromatography and crystallization from light petroleum–10% ether (Table 1) yielded pure hydrazone (14 mg), m.p. 218–220°, 4190 dpm/μmole. Radiochemical purity was proved by TLC using cyclohexane–30% EtOAc (R_f 0·45) or chloroform–5% acetone (R_f 0·51) as solvents.

Another portion of the α-spinasteryl acetate (39 mg) was saponified and oxidized with Jones reagent in acetone at 10°. As expected the resulting α-spinasten-3-one showed no radioactivity after column chromatography and crystallization from methanol. This result indicates lack of randomization.

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