Phytochemistry, 1970, Vol. 9, pp. 2003 to 2005. Pergamon Press. Printed in England.

SHORT COMMUNICATION

THE BIOSYNTHESIS OF STEROLS IN PUMPKIN

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(*Received* 9 *December* 1969)

Abstract—Tritium labeled 3β-hydroxy-5α-stigmasta-7,24(28)Z-diene (I) was administered to pumpkin plants (*Cucurbita pepo* L.) and the sterols were isolated. The specific incorporation rates were: 3β-hydroxy-5α-stigmasta-7,22t,25-triene (II) $6{\cdot}7 \times 10^{-1}$%, 3β-hydroxy-5α-stigmasta-7,25-diene (III) $4{\cdot}1 \times 10^{-2}$%, and α-spinasterol (IV) $3{\cdot}4 \times 10^{-4}$%. From this result, I is tentatively regarded as a precursor for II and III.

INTRODUCTION

PUMPKIN seeds (*Cucurbita pepo* L.) contain several Δ^7-sterols,[1] among them a 24-ethylidene compound 3β-hydroxy-5α-stigmasta-7,24(28)Z-diene,* recently synthesized in this laboratory.[2] 24-Ethylidene sterols can act as key intermediates in phytosterol biogenesis.[3,4]

RESULT AND DISCUSSION

The following radioactive stigmastan derivatives were isolated from pumpkin plants after administration of [23,25-$^3H_3$]-3β-hydroxy-5α-stigmasta-7,24(28)Z-diene (I):

TABLE 1. INCORPORATION OF [23,25-$^3H_3$]-3β-HYDROXY-5α-STIGMASTA-7,24(28)-DIENE INTO THE STEROLS OF PUMPKIN

Compound†	Specific incorporation rate[5] (%)
3β-Hydroxy-5α-stigmasta-7,22t,25-triene (II)	$6{\cdot}7 \times 10^{-1}$
3β-Hydroxy-5α-stigmasta-7,25-diene (III)	$4{\cdot}1 \times 10^{-2}$
3β-Hydroxy-5α-stigmasta-7,22t-diene (IV) (α-spinasterol)	$3{\cdot}4 \times 10^{-4}$

† The configuration at C-24 is assumed to be α_F; see, however, W. SUCROW and B. GIRGENSOHN, *Chem. Ber.* in press.

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* For definition of the symbols Z and E for configuration of double bonds see J. E. BLACKWOOD, C. L. GLADYS, K. L. LOENING, A. E. PETRARCA and J. E. RUSH, *J. Am. Chem. Soc.* **90**, 509 (1968).

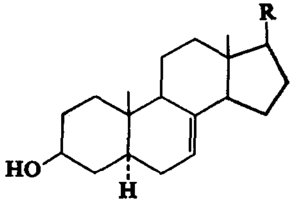
[1] W. SUCROW and A. REIMERDES, *Z. Naturforsch.* **23b**, 42 (1968).

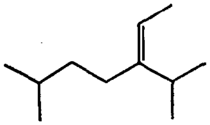
[2] W. SUCROW and B. RADÜCHEL, *Chem. Ber.* **102**, 2629 (1969).

[3] A. R. H. SMITH, L. J. GOAD and T. W. GOODWIN, *Biochem. J.* **104**, 56 c (1967).

[4] D. J. BAISTED, *Phytochem.* **8**, 1697 (1969).

[5] H. R SCHÜTTE, *Radioaktive Isotope in der Organischen Chemie und Biochemie*, p. 86, Verlag Chemie, Weinheim (1966).



(I) R =


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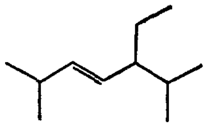

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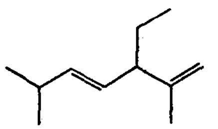

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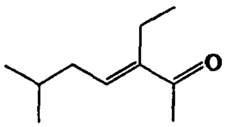

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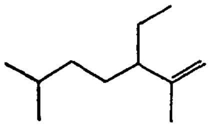

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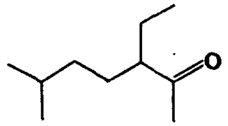

This result indicates that compounds II and III are synthesized in the plant from I as a precursor. Goodwin *et al.*[3] have proposed a C-24 carbonium ion as a common intermediate in the biosynthesis of both Δ^{25} and $\Delta^{24(28)}$-compounds. Our result shows that this mechanism should be modified for pumpkin plants since the Δ^{25} compound (II) is generated from the $\Delta^{24(28)}$ compound (I). It is, however, possible that I is converted into a C-24 carbonium ion by addition of a proton. Hydride transfer from C-25 to C-24 would yield a C-25 carbonium ion which, by proton loss from C-26, would lead to a Δ^{25} double bond. Whether II and III are formed successively from the precursor I (I $\rightarrow$ II $\rightarrow$ III) or independently (III $\leftarrow$ I $\rightarrow$ II) will be the subject of further studies by administration of labeled II and III. In our opinion, α-spinasterol (IV) is not generated directly from I because of its very small incorporation rate. Ellouz and Lenfant[6] have administered labeled stigmastanol to *Dictyostelium discoideum* and shown that it is a precursor of 3β-hydroxy-5α-stigmast-22-ene. According to this scheme, α-spinasterol could be biosynthesized from 3β-hydroxy-5α-stigmast-7-ene which, however, has not yet been isolated from pumpkin.

EXPERIMENTAL

Plant Material

Pumpkin seeds were germinated for 4 days and then cultivated on Knop's solution for 12 days in a greenhouse at 22°.

Preparation of the Radioactive Precursor

3β-Acetoxy-5α-cholest-7-ene-24-one[2] was treated with T_2O in basic medium.[7] Wittig reaction with triphenyl ethylidene phosphorane and saponification yielded [23,25-$^3H_3$]-3β-hydroxy-5α-stigmasta-7,24(28)Z-diene (I), specific activity 3·50 × 10^9 counts/min/mmole.

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[6] R. Ellouz and M. Lenfant, *Tetrahedron Letters* 2655 (1969).

[7] M. Lindberg, F. Gautschi and K. Bloch, *J. Biol. Chem.* **238**, 1661 (1963).

Administration of the Labeled Compound

Twenty mg labeled I (total activity 1·70 × 10^8 counts/min) were emulsified in the system cotton seed oil–sucrose monostearate–water. The roots were separated from the plants and the stalks dipped into the emulsion which was consumed within 24 hr. After an additional 3 days on Knop's solution the sterol mixture was extracted by the usual procedure. Chromatography on alumina and silver nitrate impregnated silica gel[8] yielded the sterols II–IV which were recrystallized from methanol to constant radioactivity. Because of the relatively high specific radioactivities of II and III* (3·610, 3·211, 3·100, 3·082, 3·08·, 3·081 × 10^4 counts/min/mg for II and 7·850, 7·820, 7·819 × 10^3 counts/min/mg for III) it was supposed that they still contained some radioactive precursor (I), which cannot be removed by crystallization. Therefore, II and III were converted to the ketones V and VI,[8] which were diluted with inactive carriers (V, 1:2; VI, 1:5·5), equilibrated in basic medium and purified by preparative TLC with petrol ether–ether (6:4) on silica gel. As expected, this resulted in a loss of radioactivity. Several crystallizations from methanol yielded pure V (821, 935, 935, 928 counts/min/mg) and VI (680, 625, 630, 632 counts/min/mg). No radioactive impurities were detected by TLC in $CHCl_3$–EtOAc (9:1). The specific incorporation rates were calculated to 6·1 × 10^{-1}% for II and 4·1 × 10^{-2}% for III.

Ozonization of α-spinasterol (IV) which showed only small radioactivity afforded 2-ethyl-3-methyl-butyraldehyde isolated as 2,4-dinitrophenylhydrazone[9] and crystallized twice from methanol (42 and 40 counts/min/mg). The steroidal aldehyde 3β-hydroxy-5α-pregn-7-ene-21-al was isolated likewise and showed no radioactivity. Specific incorporation rate for IV is 3·4 × 10^{-4}%.

Radioactivity measurements were carried out in a Beckman scintillation counter, using toluene as solvent with the usual scintillators.

Acknowledgements—Financial support by Deutsche Forschungsgemeinschaft and Stiftung Stipendien-Fonds des Verbandes der Chemischen Industrie is gratefully acknowledged.

* II has been diluted 1:28 with inactive carrier.

[8] W. Sucrow, *Chem. Ber.* **99**, 3559 (1966).

[9] M. Devys, A. Alcaide, M. Barbier and E. Lederer, *Phytochem.* **7**, 613 (1968).