Tetrahedron Letters No.16, pp. 1131-1132, 1971. Pergamon Press. Printed in Great Britain.

BIOSYNTHESIS OF GERMACRENE-C

Kumiko Morikawa and Yoshio Hirose
The Institute of Food Chemistry
2-chome 43, Dojima-naka, Kita-ku, Osaka, Japan
and
Shigeo Nozoe
Institute of Applied Microbiology, University of Tokyo
Bunkyo-ku, Tokyo, Japan

(Received in Japan 22 January 1971; received in UK for publication 19 March 1971)

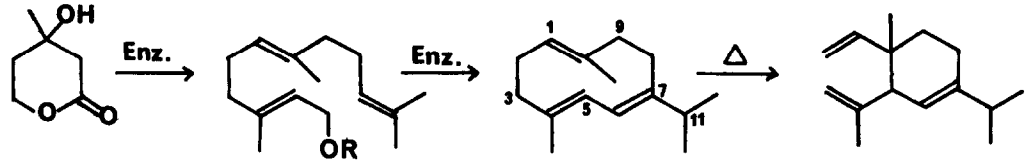
The isolation and the structural elucidation of sesquiterpene hydrocarbon, germacrene-C (I), which is a main constituent in the essential oil of seed of Kadsura japonica Dunal have been reported recently[1].

As a preliminary experiment concerning cyclization mechanism of an acyclic precursor, farnesyl pyrophosphate, into sesquiterpenoids, we attempted to obtain a cell free preparation having an enzymic activity to convert mevalonic acid lactone into germacrene-C. It has been found that supernatent of the homogenate of immature seed of Kadsura japonica Dunel catalyzed the formation of the hydrocarbon (I), trans, trans-farnesol (II) and all trans-geranylgeraniol (III) from 2-^{14}C-mevalonic acid lactone (IV). The supernatant also converted trans, trans-farnesyl pyrophosphate (V)[2] into I and II. However, any significant radioactivity corresponding to III was not recognized in the products from the latter substrate.

On referring to the reported procedure[3] the cell free extracts were prepared with 0.1 M phosphate buffer of PH 7.3 containing glutathione (reduced form). A mixture of the supernatant and IV or V was incubated in the presence of ATP and $MgCl_2$ at 30° for 3hr and then hydrolyzed with alkali. The n-pentane extracts were chromatographed on neutral alumina and divided into hydrocarbons and oxygenated compounds by eluting with n-pentane and ethyl ether respectively.

Radioactive product in the hydrocarbon fraction was identified as germacrene-C by autoradiography on TLC plate impregnated with $AgNO_3$, in which a radioactive spot behaved identically with authentic germacrene-C. The product shows a peak of radioactivity on RI-GLC (column: carbowax 20M, 20% on chromosorb W, 3mm x 3m) at a same retention time with that of δ-elemene (VI) which was produced from I under these circumstances by a Cope rearrangement as reported previously[1]. Further support of identity was obtained by the

fact that germacrene-C in the fraction showed almost constant specific radioactivity during purification steps. Purification was carried out by repeated recrystallization with 95% ethanol in the state of silver nitrate adduct of the germacrene-C with carrier substance.



(IV) (II) : R = H (V) : R = PP (I) (VI)

Radioactive compounds in the ether fraction were shown to be trans, trans-farnesol (II) and all trans-geranylgeraniol (III) by RI-GLC analysis (column: OV-17, 1% on Shimalito W or OV-1,1.5% on chromosorb B, 4mm x 2m) and by autoradiography on TLC developed with different solvent systems on silica gel, on silica gel impregnated with silver nitrate and on paraffin coated silica gel plate.

Enzymic cyclization of farnesyl pyrophosphate specifically labelled with tritium is in progress to ascertain the origin of C-11 hydrogen atom of germacrene-C.

Acknowledgement: We are deeply thankful to Prof. S. Hatsushima and Assistant Prof. S. Sako, Kagoshima University, to Mr. Y. Sakamoto, Hidaka High School, and to Prof. M. Yoneyama, Hiroshima University, for helping collection of the fruits of Kadsura japonica Dunal and to Dr. A. Kawaguchi, Institute of Applied Microbiology, University of Tokyo, for supplying radioisotopic farnesyl pyrophosphate. We are also grateful to Department of Biochemistry, Osaka University Dental School and to Prof. Y. Sato, Kyoritsu Pharm. Colledge, for the provision of facilities for radioactivity measurement.

REFERENCES

(1) K. Morikawa and Y. Hirose, Tetrahedron Letters, 22,1799 (1969).
(2) G. Popják, J. Edmond, K. Clefford and V. Williams, J. Biol. Chem., 244, 1897 (1969).
(3) J.E. Grave, C.T. Dennis, C.D. Uppe and C.A. West, J. Biol. Chem., 240,1847 (1965).