Faculty of Pharmaceutical Sciences,
University of Tokyo
Hongo-7-3-1, Bunkyo-ku, Tokyo,
Japan, 113

HIROSHI NAKAMURA
KEIJIRO SAMEJIMA
YUKIKO SAITO
ZENZO TAMURA

Received November 11, 1970

[Chem. Pharm. Bull. 19(2) 438—439 (1971)]

UDC 581.192.04 : 582.663

Occurrence of Insect-Moulting Substances Ecdysterone and Inokosterone in Callus Tissues of *Achyranthes*

Achyranthes fauriei LÉVEILLÉ et VANIOT (Amaranthaceae) is one of the plant sources which were first demonstrated to contain ecdysterols.[1] Later the ecdysterols ecdysterone and inokosterone were shown to occur also in the other *Achyranthes* spp.[2] While ecdysterols are quite interesting substances in the respects that they disclose not only the dramatic effects in the moulting and metamorphosis of arthropods but also the unique physiological activities in higher animals.

In the hope that plant tissue cultures may provide an effective tool in studying the production and the metabolic pathway of ecdysterols, we have started the present work. Thus, induction and growth of seedling callus tissues from the *Achyranthes* plants were examined on the surface of basal media containg some of coconut milk, yeast extract, casein hydrolysate, and plant growth regulators (2,4-dichlorophenoxyacetic acid (2,4-D), α-naphthaleneacetic acid, 3-indolacetic acid, kinetin, and gibberellin). As the results, it was shown that callus tissues are best induced when the White's basal medium was supplemented with 10% of coconut milk and 1 ppm of 2,4-D for *A. fauriei*, *A. japonica*, *A. japonica* var. *hachijoensis*, *A. obtusifolia*, and *A. rubrofusca*, and with 10% of coconut milk and 4 ppm 2,4-D for *A. longifolia*. On the other hand, it was found that the growth of the callus tissues is best effected when the Murashige-Skoog's basal medium was supplemented with 10% of coconut milk, 1 ppm of 2,4-D, and 1 ppm of kinetin for *A. fauriei*, *A. japonica*, *A. japonica* var. *hachijoensis*, *A. obtusifolia*, and *A. rubrofusca*, and with 10% of coconut milk, 4 ppm of 2,4-D, and 1 ppm of kinetin for *A. longifolia*. Extracts of the callus tissues grown under various conditions were shown to exhibit intense insect moulting hormone activity in the *Sarcophaga* test, indicating the presence of ecdysterols. Identification of the ecdysterols as ecdysterone and inokosterone in the extracts was carried out by thin-layer chromatography of the extracts and their acetylation products. Certain extracts were further subjected to the liquid chromatography using an Amberlite XAD-2 column[3] to corroborate the identity. However, the contents of the ecdysterols in the callus tissues were very small ($<0.002\%$) as compared with those in the normal plants. Increase of the ecdysterols contents in callus tissues by modification of the medium components is the future problem.

Acknowledgement We thank Dr. T. Kameya, Institute for Agricultural Research, this University, for helpful advice.

1) T. Takemoto, S. Ogawa, and N. Nishimoto, *Yakugaku Zasshi*, **87**, 325, 1469, 1474 (1967).
2) T. Takemoto, S. Ogawa, N. Nishimoto, and S. Taniguchi, *Yakugaku Zasshi*, **87**, 1478 (1967); T. Takemoto, S. Ogawa, N. Nishimoto, K.-Y. Yen, K. Abe, T. Sato, K. Osawa, and M. Takahashi, *ibid.*, **87**, 1521 (1967); T. Takemoto, S. Ogawa, N. Nishimoto, H. Hirayama, and S. Taniguchi, *ibid.*, **88**, 1293 (1968).
3) M. Hori, *Steroids*, **14**, 33 (1969).

Pharmaceutical Institute,
Tohoku University
Aoba-yama, Sendai

HIROSHI HIKINO
HISANORI JIN
TSUNEMATSU TAKEMOTO

Received November 17, 1970

[Chem. Pharm. Bull. 19(2) 439—440 (1971)]

UDC 547.586.2.09 : 615.31.076.9

The Plasma Levels of Dopa and Catecholamines after Oral Administration of L-Dopa

L-3,4-Dihydroxyphenylalanine (L-dopa) has been used recently as an effective drug for Parkinson's disease. The administered L-dopa was said to be converted to dopamine in brain by L-aromatic amino acids decarboxylase [EC 4.1.1.26].[1] The urinary metabolites of L-dopa have been analysed by many investigators after oral administration to the patients.[2] On the other hand, only the plasma levels of dopa have been measured by isotopic[3] and fluorometric[4] methods and those of dopa and its metabolites have not been estimated simultaneously.

The patients who had been administered with 3 g of L-dopa per day were stopped with the treatment and, after a period of twelve hours, were orally administered with 1 g of the drug. The procedure of the preparation of the samples from plasma was the same as in the previous

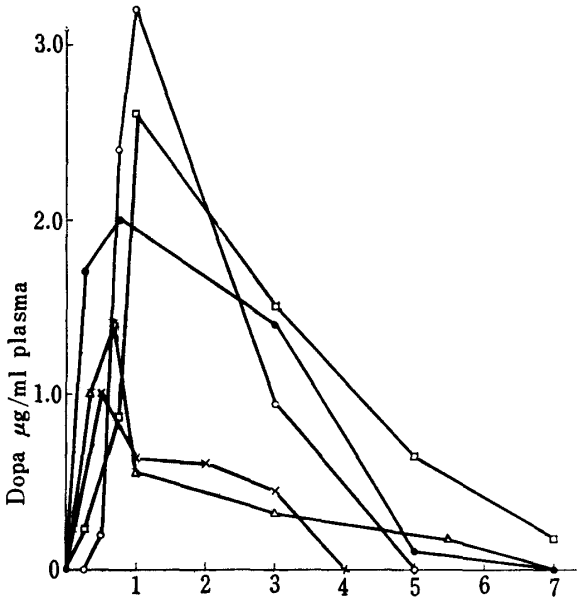

Fig. 1. Plasma Level of Dopa

—○—: patient S.K. —●—: patient H.G.
—×—: patient K.H. —△—: patient S.T.
—□—: patient S.O.

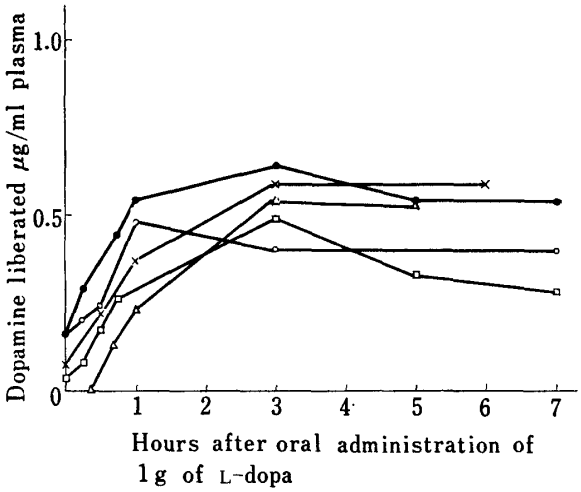

Fig. 2. Plasma Level of Dopamine liberated after Acid Hydrolysis

—○—: patient S.K. —●—: patient H.G.
—×—: patient K.H. —△—: patient S.T.
—□—: patient S.O.

1) K.F. Gey and A. Pletscher, *Biochem. J.*, **92**, 300 (1964).
2) D.B. Calne, F. Karoum, C.R.J. Ruthven and M. Sandler, *Brit. J. Pharm.*, **37**, 57 (1969).
3) M.J. Peaston and J.R. Bianchie, *Brit. J. Med.*, **1**, 400 (1970).
4) G.H. Tyce, M.D. Muenter and C.A. Owen, Jr., *Mayo Clin. Proc.*, **15**, 438 (1970).