Tetrahedron Letters,Vol.28,No.39,pp 4587-4588,1987 0040-4039/87 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

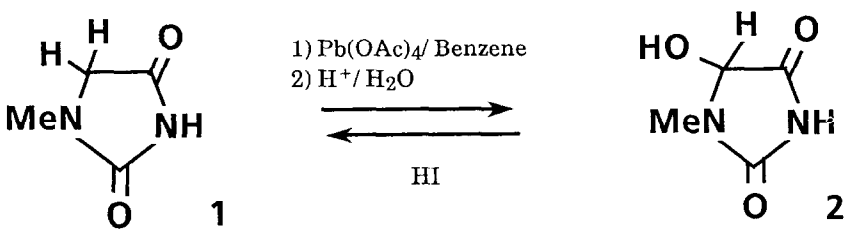
BIOACTIVE COMPOUNDS PRODUCED IN ANIMAL TISSUES (II); TWO HYDANTOIN PLANT GROWTH REGULATORS ISOLATED FROM INFLAMED RABBIT SKIN TISSUE[1]

Kazuharu Ienaga,[a,*] Ko Nakamura,[a] Toshio Goto[b] and Jin-emon Konishi[a]

[a]Institute of Bio-active Science, Nippon Zoki Pharm. Co., Ltd., Yashiro, Hyogo 673-14, Japan, and [b]Laboratory of Organic Chemistry, Faculty of Agriculture, Nagoya University, Nagoya 464, Japan

1-Methylhydantoin and 5-hydroxy-1-methylhydantoin were isolated as plant growth regulators from rabbit skin tissue which had been inflamed by inoculation with vaccinia virus.

During our screening process for unique bio-active metabolites induced in animals by viral infections, two hydantoin plant growth regulators, 1 and 2, were isolated. This kind of approach, in which cells and tissues are used as virtual *in vivo* bioreactors, has not previously been successful in leading to low molecular weight bioactive substances as distinct from high molecular weight products such as the interferons. The first indication that an extract of vaccinia virus-inoculated rabbit skin tissues contained metabolite(s) not present in the extracts of normal skin was obtained by using cut white chrysanthemum flowers; the extract from the inflamed skin prolonged the life of such flowers considerably, whereas the extracts from normal skin did not. Subsequently, the more sensitive rice-germination test was used as the bioassay method during isolation of the active principle(s), since it had been already shown in a previous paper to be useful for this purpose[1] and it could distinguish active fractions, newly obtained from the inflamed skin, from those containing two active diketopiperazines[1] proved to be common in skin.



The skin tissue extract of vaccinia-infected rabbits was prepared by a modified method based on that of Aonuma *et al.*:[2] an aqueous phenol extract from the skin tissue was boiled and filtered. After removal of the solvent, the residue was dissolved in a minimal amount of water and extracted with ethyl acetate. Evaporation of the organic extract *in vacuo*, followed by column

chromatography (silica-gel; ethyl acetate), gave the two active principles as colourless crystalline products, 1 (mp 158 °C) and 2 [mp 138 °C (Found: C,37.1; H,4.7; N,21.5. $C_4H_6N_2O_3$ requires C,36.9; H,4.7; N,21.5 %)]. The first product was clearly 1-methylhydantoin (1) because its NMR and mass spectra were identical with those of an authentic specimen.[3] The even more active rice-germination promoter (2) was assigned the structure, 5-hydroxy-1-methylhydantoin, initially on its elemental analysis, spectral data[4] and its p*K*a value (8.64): the latter value and the chemical shift at 10.64 ppm (in DMSO-d_6) were evidently derived from a free 3-NH rather a free 1-NH grouping because p*K*a values[5] *ca.* 8.5 and >13 and chemical shifts[6] of *ca.* 10.5 and *ca.* 8.5 ppm (in DMSO-d_6) have been reported for the respective groupings in hydantoins. The isolated natural product (2) was almost certainly a one to one racemic mixture because its $[\alpha]_D$ value approximated to zero.

The structure of the new hydantoin 2 was also consistent with the fact that both hydantoins, 1 and 2, could be correlated each other as follows. Treatment of 2 with aqueous HI gave 1 in 92 % yield, whereas 1 was converted into 2 in 64 % yield by oxidation. Thus, 1-methylhydantoin (1: 1.0 mmol) and lead tetraacetate (1.1 mmol) in benzene were refluxed for 6 hours, followed by purification by column chromatography to yield 5-acetoxy-1-methyl-hydantoin,[7] which was hydrolyzed with 0.6N aqH_2SO_4 to 2 (mp 137-138 °C).

A phenol extract from normal skin,[2] was treated by the same procedure. Neither 1 nor 2 could be detected in any fraction. This fact suggested that both hydantoins were not artefacts and that their production has been induced by the inoculation with vaccinia virus.

The major active principle (2) showed its effect on the flowering period of cut white chrysanthemum at a concentration as low as 10^{-6}M; whereas the minor active principle (1) showed no effect on the cut flower at the concentration of 10^{-4}M. It should be noted that 1 has also been isolated and recognized as a germination promoter from microorganism.[8]

REFERENCES

1) Part I: K. Ienaga, K. Nakamura and T. Goto, *Tetrahedron Lett.*, **28**, 1285 (1987).
2) S. Aonuma, Y. Kohama, S. Yashiki, I. J. Chen, H. Egawa and N. Onishi, *Yakugaku Zasshi*, **96**, 1247 (1976).
3) Spectral data for 1: ^{1}H NMR(DMSO-d_6, 100 MHz): 2.69 (3H, s), 3.80 (2H, s), 10.60 (1H, brs) ppm. MS(EI) m/z 114(M^+)(*cf.* authentic sample from Aldrich).
4) Spectral data for 2: ^{1}H NMR(DMSO-d_6, 100 MHz): 2.73 (3H, s), 4.96 (1H, d: J=8 Hz), 6.84 (1H, d: J=8 Hz), 10.64 (1H, brs) ppm. MS(EI) m/z 130 (M^+).
5) J. Gut, M. Prystas, J. Jonas and F. Sorm, *Collection Czechoslov. Chem. Commun.*, **26**, 974 (1961).
6) R.A. Coral and O. Orazi, *Spectrochimica Acta.*, **21**, 2119 (1965).
7) Spectral data for 5-acetoxy-1-methylhydantoin: ^{1}H-NMR(DMSO-d_6, 100 MHz): 2.14 (3H, s), 2.78 (3H, s), 6.04 (1H, s), 11.21 (1H, brs) ppm. MS(EI) m/z 172 (M^+).
8) Y. Koaze, *Bull. Agr. Chem. Soc. Japan*, **22**, 238 (1958).

(Received in Japan 6 June 1987)