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5β-HYDROXYGITOXIGENIN, A PRODUCT OF GITOXIGENIN PRODUCED BY *DAUCUS CAROTA* CULTURE*

IVAN A. VELIKY, ALAN JONES,† RICHARD S. OZUBKO,‡ MARIA PRZYBYLSKA and FARID P. AHMED

Division of Biological Sciences, National Research Council of Canada, Ottawa, Canada, K1A 0R6

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Abstract—The product of gitoxigenin transformation by *Daucus carota* Ca68 cell suspension culture has been isolated from culture filtrates and identified as 5β-hydroxygitoxigenin. A summary of the physico-chemical data for this novel compound is reported for the first time.

INTRODUCTION

Biological transformations serve as important tools in the structural modification of compounds possessing useful therapeutic activity. Digitalis glycosides and aglycones are widely prescribed drugs for patients with some cardiac disorders. Problems encountered in the administration of digitalis drugs (dosage, toxicity, etc.) have induced the search for new cardiotonic agents with lower toxicity and with a greater specificity for the target site. An alternative approach to the chemical modification of known cardenolides has been microbial transformations. The results have proved limited in the production of useful agents and the yields of products have been low [1]. A more recent and promising advance has utilized plant cell suspension cultures in the biotransformation of cardenolides as well as other steroid skeletons [2]. One of the latest contributions has been the thoroughly studied 12β-hydroxylation of the semisynthetic β-methyldigitoxin by a culture of *Digitalis lanata* cell line 291 [3]. Formation of β-methyldigoxin can be achieved with an overall yield of 66% using an airlift bioreactor system. Quantitative conversion of the aglycone digitoxigenin to 5β-hydroxydigitoxigenin (periplogenin) using suspension cultures of *Daucus carota* Ca68 has previously been reported from these laboratories [4].

In this paper we report the isolation and identification of 3β,5β,14β,16β-tetrahydroxy-card-20(22)-enolide formed as the sole conversion product on incubation of 3β,14β,16β-trihydroxy-5β-card-20(22)-enolide (gitoxigenin) with cultures of *Daucus carota* Ca68. This compound has not been reported previously as either a naturally occurring or chemically synthesized substance.

RESULTS AND DISCUSSION

In preliminary experiments TLC analysis of extracted culture filtrates had shown that a growing suspension of *Daucus carota* cells quantitatively converted gitoxigenin to a more polar product during the 7-day incubation period. A sufficient amount (150 mg) of the purified product was isolated from 5 l. of this culture supplied with gitoxigenin (250 mg) to allow a complete, physico-chemical identification to be made.

The crystals melted at 234–236° (dec.) and elementary analysis showed 67.86% C and 8.48% H, consistent with a molecular formula of $C_{23}H_{34}O_6$. This indicated introduction of a hydroxyl group into the molecule of gitoxigenin. The IR spectrum showed bands at 3535 and 3480 (—OH); 1795 and 1740 (—C=O in enolide ring) and 1625, 1472, 1445 and 1432 cm^{-1} (—C=C— in unsaturated enolide ring). The compound was more dextrorotatory ($[\alpha]_D^{20}$ + 48.6°, c = 0.62, MeOH) than the parent gitoxigenin ($[\alpha]_D^{20}$ + 34.2°, c = 0.64, MeOH) and the shift compares with that observed between digitoxigenin ($[\alpha]_D^{20}$ + 20.9°, c = 1.045, MeOH) and its 5β-hydroxy derivative, periplogenin ($[\alpha]_D^{20}$ + 31.7°, c = 1.08, EtOH).

High resolution MS (200–250°) gave a base peak at m/e 334.1787 ($C_{19}H_{26}O_5$) formed by dehydration at C-3 followed by retro-Diels–Alder elimination of ring A of the steroid skeleton. Other major peaks were present at m/e 370.2135 ($C_{23}H_{30}O_4$, 21.4%), 352.2047 ($C_{23}H_{28}O_3$, 22.6%), and 201.1643 ($C_{15}H_{21}$, 71.7%). No parent ion was observed.

The ^{13}C NMR spectrum of the compound was obtained and compared with the spectrum obtained for gitoxigenin. The most noticeable difference was the presence of a peak at δ 75.3 (relative to internal TMS) in the spectrum of the new compound. This chemical shift indicated the introduction of a hydroxyl group into the gitoxigenin skeleton. The techniques used in the identification of periplogenin [4] showed that the product was 5β-hydroxygitoxigenin and hydroxylation had taken place without inversion of configuration at this carbon.

The stereochemistry of 5β-hydroxygitoxigenin was confirmed by an X-ray analysis based on 1847 observed reflections [5]. The crystals are orthorhombic, space group $P2_12_12_1$, with unit-cell dimensions a = 11.290, b = 24.638, c = 7.122 Å. The structure was determined by

* NRCC No. 18080.

† Research associate, NRCC.

‡ Present address: Imperial Oil Limited, Research Department, P.O. Box 3022, Sarnia, Canada, N7T 7M1.

direct phasing methods and refined using least-squares procedure. The stereochemistry is similar to that of digitoxigenin [6] with both A/B and C/D ring junctions being *cis* and B/C *trans*. Ring D assumes the α-envelope conformation at C-15.

The physico-chemical data for 5β-hydroxygitoxigenin are reported for the first time in this paper. The new cardenolide is being evaluated for cardiotonic activity both *in vitro* and *in vivo*. Formation of this novel cardenolide stresses the importance of plant cell suspension cultures in conversion of existing medicinal agents to new and possibly more active and/or less toxic pharmaceuticals which may be difficult to prepare by chemical synthesis.

EXPERIMENTAL

The origin and maintenance of the suspension culture of *Daucus carota* Ca68 have been described previously [4]. Bioconversion of gitoxigenin was achieved in a 7.5 l. Microferm fermentor (New Brunswick Fermentors Ltd., New Jersey U.S.A.) containing 5 l. 71V medium [7] supplemented with 250 mg gitoxigenin inoculated with a 5-day-old culture of *Daucus carota*. Fermentation was performed at 26° with agitation and aeration rates of 150 rpm and 1500 ml air/min, respectively. Conversion of the cardenolide was monitored daily. On completion of the reaction the medium was separated from the cells by filtration and both fractions were freeze-dried.

The dry residue of the medium filtrate was taken up in MeOH and treated in the same manner described in the isolation of periplogenin [4]. Partial purification of the conversion product from the crude extract was achieved by CC on Florisil eluted in a stepwise manner with 0–20% MeOH in C_6H_6 mixtures. Further chromatography of the 10–15% MeOH fractions on Sephadex LH-20 eluted with $CHCl_3$–heptane–EtOH (50:50:10) yielded a pure compound which on recrystallization from EtOH–H_2O gave colourless, orthorhombic crystals of 5β-hydroxygitoxigenin.

Elemental analysis, IR, MS and ^{13}C NMR spectroscopy were performed as described previously [4]. Optical rotation data were obtained with a 1 dm cell of 1 ml volume. X-ray data were collected using a Picker four-circle automatic diffractometer with Ni-filtered Cu radiation.

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REFERENCES

1. Titus, E. (1961) *Adv. Appl. Microbiol.* **3**, 279.
2. Alferman, A. W., Boy, H. M., Doller, P. C., Hagedorn, W., Heins, M., Wahl, J. and Reinhard, E. (1977) in *Plant Tissue Culture and its Bio-technological Application* (Barz, W., Reinhard, E. and Zenk, M. H., eds.) pp. 125–141. Springer, Berlin.
3. Heins, M., Wahl, J., Lerch, H., Kaiser, F. and Reinhard, E. (1978) *Planta Med.* **33**, 57.
4. Jones, A., Veliky, I. A. and Ozubko, R. S. (1978) *Lloydia* **41**, 476.
5. Przybylska, M. and Ahmed, F. R. (1979) *Acta Crystallogr. Sect. B* **35**, 2436.
6. Karle, I. L. and Karle, J. (1969) *Acta Crystallogr. Sect. B* **25**, 434.
7. Veliky I. A. and Rose, D. (1973) *Can. J. Botany* **51**, 1837.