AN UNUSUAL FLUORIDE-MEDIATED OXIDATION OF GITOXIN\(^1\)

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An unusual oxidative transformation of \(\alpha,\beta\)-unsaturated \(\gamma\)-lactone of the cardenolide gitoxin (1a) by tetra-\(n\)-butylammonium fluoride (TBAF) is reported. The structure of the product (2a) was finally confirmed by an X-ray crystal structure analysis of its genin (2b). The oxidation proceeded also in the presence of CsF, and KF + 18-crown-6.

KEY WORDS gitoxin; \(\alpha,\beta\)-unsaturated \(\gamma\)-lactone; oxidation; fluoride ion

Gitoxin (1a) is a cardiac glycoside isolated from Digitaria purpurea as one of its major components along with digitoxin, but is not used clinically because of its low solubility to water and alcohol and its low uptake from the digestive system. Thus, we attempted to create more potent compounds from 1a. In the course of this study, we found that treatment of gitoxin (1a) only with tetra-\(n\)-butylammonium fluoride (TBAF) caused oxidative transformation of the \(\alpha,\beta\)-unsaturated \(\gamma\)-lactone moiety. In this paper, we report identification of this product obtained by the unusual oxidation induced by fluoride anion.

Gitoxin (1a, 50 mg) was treated with TBAF (5 eq)\(^2\) in freshly distilled THF, DMF, or CH\(_2\)Cl\(_2\) (5 ml) at r.t. for an hour to give 2a\(^3\) in 86% yield. FAB-MS spectrum of 2a indicated quasimolecular peaks at 795 (M+H\(^+\)) and 817 (M+Na\(^+\)). In conjunction with elemental analysis data, the molecular formula of 2a was determined as C\(_{41}\)H\(_{62}\)O\(_{15}\). In the \(^1\)H- and \(^13\)C-NMR spectra of 2a, the methylene signals of C-21 disappeared, while an additional quaternary carbon signal appeared and two proton signals of H-16 (\(\delta\) 5.34) and H-22 (\(\delta\) 6.29) were shifted to downfield. The IR spectrum indicated two absorption bands of carbonyl groups at 1660 and 1730 cm\(^{-1}\) in addition to the broad absorption band at 3450 cm\(^{-1}\). These facts suggested that 2a possessed an additional carbonyl group instead of C-21 methylene of 1a. With the UV spectrum taken into consideration, one carbonyl group was supposed to exist as \(\alpha,\beta\)-unsaturated carboxyl group. The other was esterified with 16-OH group. The lactone structure was supported by \(^1\)H-detected heteronuclea multiple bond correlation (HMBC) spectrum (\(J_{\mathrm{C-H}} = 8.3\) Hz), which indicated the correlation from 16-H to C-21 (\(\delta\)C 172.9), from 17-H (\(\delta\) 3.15) to C-20 (\(\delta\)C 140.8), C-21, and C-22 (\(\delta\)C 131.9), and from olefinic H-22 to C-20 and C-21 (Fig. 1A). As the NOE enhancement was observed between 17-H and 22-H and between 18-H\(_3\) (\(\delta\) 0.81) and 22-H, the olefin structure of 20 and 22 position was clarified to be Z-form (Fig. 1B). Thus the structure of 2a was estimated as indicated in Chart 1.

In order to confirm the structure, X-ray crystal structure analysis of the genin of 2a (2b) was carried out. Compound 2b\(^4\) was prepared from gitoxogenin (1b)\(^5\) in a similar manner. The \(^1\)H- and \(^13\)C-NMR spectral data of 2b were almost the same as those of 2a, except for the signals due to the sugar moieties. As shown in Fig. 2, the structure of 2b was unambiguously clarified\(^6\), and at the same time, the structure of 2a was established.

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Table 1. Positional Parameters and Their Estimated Standard Deviations

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<th>Atom</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>Beq(A2)</th>
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a Anisotropically refined atoms are given in the form of the isotropic equivalent displacement parameter defined as: \((4/3) x [a^2 x B(1,1) + b^2 x B(2,2) + c^2 x B(3,3) + ab(cos \gamma) x B(1,2) + ac(cos \beta) x B(1,3) + bc(cos \alpha) x B(2,3)]\). b Atoms of ethanol.

Since compounds \(2a\-b\) must be produced by the oxidation of \(1a\-b\) in the presence of either tetrabutylammonium ion or fluoride ion, \(1b\) was treated with tetra-\(n\)-butylammonium bromide (TBAB), tetra-\(n\)-butylammonium
chloride (TBAC), CsF, and KF. The reaction never proceeded in the presence of TBAB and TBAC. The reaction with CsF gave 2b in high yield. Although the reaction with KF proceeded very slowly, the addition of 18-crown-6 to the mixture enhanced the reaction speed. These facts indicated that fluoride ion must be deeply involved in the oxidation. In order to confirm what is the oxidant, 1b was treated with TBAF under argon or oxygen atmosphere. The reaction under oxygen was completed within 30 min to give 2b, while that under argon for 24 h afforded a trace amount of 2b, which was probably yielded in the reaction of 1b with trace oxygen remaining in the reaction system, and another major product as yet unidentified. Although oxidations using fluoride ion and oxygen were reported, the reactions were essentially the photooxidation which was performed by use of strong visible-light and dye-sensitizer in the oxygen-purged well. Compounds 1a-b were converted to 2a-b in air and in the presence of only TBAF, CsF, or KF+18-crown-6, and the reaction proceeded also in the dark. An investigation on the mechanism of this unusual oxidative transformation is now in progress.

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REFERENCES AND NOTES
2) TBAF used for the reaction was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan) as trihydrate, Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan) as 1 M THF solution, and Aldrich Co. Inc. (Milwaukee, U.S.A.) as 1 M THF solution.
3) Data for 2a: colourless crystalline powder. m.p. 169-171°C (EtOH-diisopropyl ether). [α]D25 -21.4° (c=0.5, MeOH). IR (KBr, cm⁻¹): 3450 (OH), 1730 (CO), 1660 (CO). UV (λmax, MeOH) nm (ε): 232.7 (7.0×10⁴). ¹H-NMR (CDCl3, 500 MHz, δ): 3.15 (1H, dd, J=2.4, 6.7 Hz, 17-H), 4.02 (1H, br s, 3-H), 4.13 (1H, dd, J=3.1, 6.1 Hz, 3''-H), 4.24 (2H, dd, J=3.1, 8.0 Hz, 3',3''-H), 4.89 (3H, m, 1', 1'', 1'''-H), 5.34 (1H, dd, J=6.7, 6.7 Hz, 16-H), 6.29 (1H, d, J=2.4 Hz, 22-H). ¹³C-NMR (CDCl3, 125 MHz, δ): 56.7 (C-17), 84.3 (C-16), 131.9 (C-22), 140.8 (C-20), 163.6 (C-23), 172.9 (C-21). FAB-MS (m/z) 795 (M+H)⁺, 817 (M+Na)⁺. Anal. Calcd for C41H62O15: C, 61.93; H, 7.87. Found: C, 62.08; H, 7.94.
4) Data for 2b: colourless plates. m.p. 252-253°C (EtOH-diisopropyl ether). [α]D25 -38.8° (c=0.5, MeOH). IR (KBr, cm⁻¹): 3450 (OH), 1720 (CO), 1660 (CO). ¹H-NMR (CDCl3, 270 MHz, δ): 0.97 (3H, s, 19-CH3), 1.07 (3H, s, 18-CH3), 3.14 (1H, dd, J=2.2, 6.9 Hz, 17-H), 4.01 (1H, br s, 3-H), 5.32 (1H, dd, J=6.6, 6.9 Hz, 16-H), 6.27 (1H, d, J=2.2 Hz, 22-H). FAB-MS (m/z) 405 (M+H)⁺, 427 (M+Na)⁺. Anal. Calcd for C23H32O6: C, 68.28; H, 7.98. Found: C, 68.08; H, 7.94.
6) Crystal data for 2b: C23H32O6•C2H6O, monoclinic, a = 7.049(1), b = 16.861(2), c = 10.088(1) Å, β = 103.81(1)°, V = 1164(3) Å³, Z = 2, space group P21, Dc = 1.285 gcm⁻³, μ (Mo-Kα) = 0.9 cm⁻¹. Data were collected on an Enraf-Nonius CAD4 diffractometer with monochromated Mo-Kα radiation, and corrected for Lorentz-polarization. No absorption correction was made. Full-matrix least-squares refinement of 288 parameters gave R = 0.045 for 2249 observed reflections. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre.
7) In each case, 100 mg of 1b was treated with 5 eq of TBAF in 8 ml of freshly distilled THF at r.t.

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