Communication to the Editor

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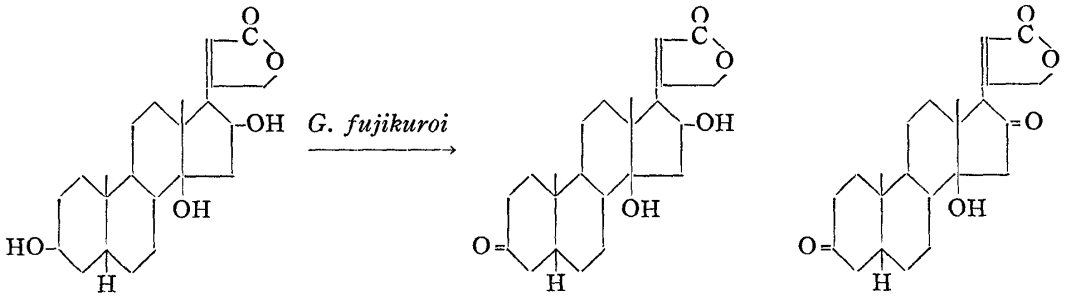
Microbiological Transformation of a Cardiac Aglycone, Oxidation of Hydroxyl Group of Gitoxigenin

Microbiological transformation of steroids, which proved most fruitful in the preparation of hormones, has recently been applied to cardiac aglycones, and there have so far been reported hydroxylation at 6β-, 11α-, 12β-, and 16β-positions, dehydrogenation at 16-position, reduction of 3-carbonyl group, oxidation of 3-hydroxyl group,*1 and hydrolysis of 3- and 16-acyloxyl groups.[1]

It has been reported from these Laboratories[2] that three strains of microörganisms convert digitoxigenin into gitoxigenin, digoxigenin, and an unidentified product. The present communication deals with the oxidation of gitoxigenin (I) to 3-dehydrogitoxigenin (II) and an unknown substance (III) by *Gibbellera fujikuroi* (Saw.) and by an unidentified *Fusarium* species isolated from soil.*2



(I) Gitoxigenin (II) 3-Dehydrogitoxigenin (III) (α-Gitoxigenone ?)

The procedures used for the present experiment were essentially the same as those described in a previous paper.[2] Gitoxigenin (I) was incubated with *G. fujikuroi* and a crude extract obtained by extraction of the culture broth with ethyl acetate was examined by paper partition chromatography with two solvent systems.[2] The results indicated that this microörganism gave two transformation products. On the paper chromatogram developed by solvent system 1, there was observed, besides a spot for the substrate (Rf 0.67), one long-tailing spot (Rf ca. 0.78) which was separated into two (Rf 0.70, 0.35) when chromatographed with solvent system 2. From the intensity of fluorescence produced by Chloramine-T and trichloroacetic acid, the spot with Rf value of 0.35 was considered to represent the major procuct and the other spot (Rf 0.70), the minor one. Since the Rf values of these two products are larger than that of the substrate, hydroxylation at any position of gitoxigenin (I) is not conceivable.

For isolation and structural determination, the mixture of products was subjected to absorption chromatography on alumina and the eluates were examined by paper partition chromatography. Elution with chloroform containing 0.5% of methanol afforded the minor

*1 Oxidation of 3-hydroxyl group of digitoxigenin by *Nigrospora sphaerica* was recently described by Nozaki, *et al.*[1]

*2 The soil was collected in the garden where digitalis plants had been growing.

1) A. Gubler, Ch. Tamm : Helv. Chim. Acta, **41**, 297, 1762 (1958); **42**, 239, 473 (1959); M. Okada, A. Yamada, M. Ishidate : This Bulletin, **8**, 530 (1960); E. Titus, A.W. Murray, H.E. Spiegel: J. Biol. Chem., **235**, 3399 (1960); Y. Nozaki, E. Masuo, H. Ishii, T. Okumura, D. Satoh : Abstracts of Papers, 80th Annual Meeting of the Pharmaceutical Society of Japan (Tokyo, 1960) 114.

2) H. Nawa, M. Uchibayashi, T. Kamiya, T. Yamano, H. Arai, M. Abe : Nature, **184**, 469 (1959).

product which was most likely to be α-gitoxigenone (3,16-bisdehydrogitoxigenin) (Ⅲ) judging from its Rf value and fluorescence by trichloroacetic acid. Detailed study on this compound, however, was not carried out due to its low yield.

Elution with chloroform containing 1% of methanol yielded the major product as colorless plates, m.p. 199~202°/215~220°*3 (from methanol and methanol-ether) in the yield of one-tenth the weight of gitoxigenin used. The melting point, analytical data (Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found : C, 70.86; H, 8.20), Rf value, infrared spectrum, fluorescence by trichloroacetic acid, and Kedde's color reaction were all in good agreement with those of 3-dehydrogitoxigenin (Ⅱ), m.p. 198~200°/226°.[3] The identity was further ascertained by a mixed melting point determination with the authentic 3-dehydrogitoxigenin*4 (Ⅱ). From the above results it is concluded that gitoxigenin (I) was converted by *G. fujikuroi*, chiefly into 3-dehydrogitoxigenin (Ⅱ) and, to some extent, to a less polar substance (Ⅲ) (probably α-gitoxigenone).

Similar pattern of transformation was observed with a species of *Fusarium* separated from the soil.*2 Transformation products of gitoxigenin (I) by this organism gave four spots of products on the paper chromatogram (Rf (solvent system 1) 0.78, 0.52, 0.38, 0.10). Two (0.78 and 0.10) of them were identified as 3-dehydrogitoxigenin (Ⅱ) and diginatigenin, respectively, by comparison with the authentic samples. Though identification of the fungi has not been made so far, it is clear that this microörganism, like *G. fujikuroi*, has an ability of transforming gitoxigenin (I) to 3-dehydrogitoxigenin (Ⅱ).

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*3 All m.p.s were measured on a Kofler block and are uncorrected.
*4 The writers express their thanks to Dr. Satoh for his supply of a sample of 3-dehydrogitoxigenin, m.p. 189~191°/202~211°. The mixed m.p. was 189~199°/202~218°.
3) R. Tschesche, G. Grimmer : Ber., **93**, 1477 (1960).