The hydroxy-lactone (I) which we[3] have previously postulated as a missing link in the biosynthetic pathway of the ecdysterols in this plant, has here been revealed to occur in reality.

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Biotransformation of Digitoxin by Suspension Callus Culture of *Digitalis purpurea*

Microbial transformation[1] has extensively been studied and is great significance for the synthesis of steroids and other organic compounds, but very few[2] have hitherto been reported on the biotransformation of organic compounds by plant tissue (callus) culture.

Now we wish to report the biotransformation of digitoxin to gitoxin, purpurea glycoside A and purpurea glycoside B by *Digitalis purpurea* callus tissue.

The callus tissue derived from aseptically seedling of *Digitalis purpurea* was grown on Murashige's and Skoog's agar medium containing 1 mg/liter of 2,4-D (2,4-dichlorophenoxyacetic acid) and 0.1 mg/liter of kinetin. The callus tissue was subcultured at three weeks intervals for about three years. No cardenolides are found in the callus tissue until now.

Digitoxin (total 190 mg) was administered to the suspension callus cultures of *Digitalis purpurea*. After shaking culture for 26 days, the callus (1030.3 g) were harvested and homogenized with 3 liter cold methanol in a Waring blender. The callus tissue (28.9 g dry weight) was filtered and the filtrate was evaporated under reduced pressure. The concentrated aqueous solution was extracted with *n*-hexane, chloroform and chloroform–methanol mixed solvent, respectively.

The chloroform solution was concentrated and column-chromatographyed using silica gel as adsorbent and chloroform–ethanol as developing solvent. The non-converted digitoxin was first separated and the following white substance was identified by thin-layer chromatography with authenitc gitoxin (*Rf* 0.48; chloroform: methanol=4:1 as developing solvent). Furthermore, the next following white substance (about 78 mg) was eluted out. This third substance was several times recrystallized from ethanol–ether mixed solvent. The white powder obtained (11.5 mg) showed mp 218—223° and no depression with authentic sample of purpurea glycoside A. The ultraviolet (UV) and infrared (IR) spectrum were identical with those of purpurea glycoside A. The powder was further hydrolyzed with 0.05N H_2SO_4 in 50% methanol and the hydrolysate obtained gave the same thin-layer chromatogram as that of authentic purpurea glycoside A.

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2) S.J. Stohs and E.J. Staba, *J. Pharm. Sci.*, **54**, 56 (1965); J.M.H. Graves and W.K. Smith, *Nature*, **214**, 1248 (1967).

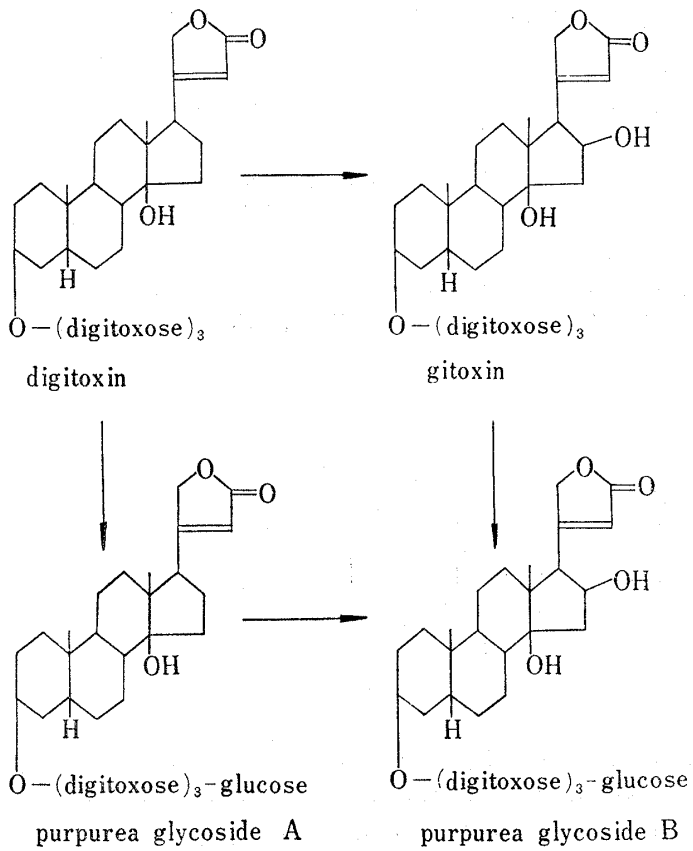

Chart 1

The presence of purpurea glycoside B was also detected in chloroform–methanol fraction by thin-layer chromatography (*Rf* 0.40; methylene chloride : methanol : formamide=80:19:1 as developing solvent).

It is notable that gitoxin, purpurea glycoside A and purpurea glycoside B were found in the metabolites by the callus tissue. These compounds would be formed by the pathway shown in Chart 1.

The separation of a considerable amount of purpurea glycoside A might indicate that exogeneous D-glucose is partly transformed to the bound D-glucose of purpurea glycoside A as well as *Digitalis purpurea* leaves[3a] and its enzyme solution.[3b] The formation of purpurea glycoside B could be also explained by assuming that digitoxin is glucosylated *via* gitoxin formed by 16β-hydroxylation, or 16β-hydroxylated *via* purpurea glycoside A formed by glucosylation. The 16β-hydroxylation of digitoxin to gitoxin is the same reaction as that of digitoxigenin to gitoxigenin by a fungi such as *Helicostylum piriforme*.[4a] Tschesche, *et al.*[4b] suggested that the 16β-hydroxylation in cardenolides of *Digitalis lanata* occurs only before the formation of γ-lactone ring (digitoxigenin). On the contrary, our results showed that 16β-hydroxylation occurs after the formation of γ-lactone ring.

It is of much interest that the glucosylation and 16β-hydroxylation by callus tissue are easily proceeded, and the metabolic pathway in callus tissue suggests the biosynthetic pathway of purpurea glycosides in plants.

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