## Cardiotonic Activities of Four New Compounds Derived from Digitoxigenin and Uzarigenin, and Their Structure-Activity Relationship

It was reported previously that the introduction of a hydroxyl group into the molecule of digitoxigenin (I) at 15α-position resulted, unexpectedly, in a complete loss of the cardiotonic activity[1,2]. This suggested that the essential steric features in the vicinity of the C and D rings, or combination of the molecule with its site of action, may somehow be interfered by a group attached at C-15. In the present study, 3 new compounds derived from digitoxigenin were tested for their cardiotonic activities on the isolated frog's heart, in order to obtain further informations upon the problem. Another new derivative of uzarigenin was also tested, and the result provides an additional evidence for the view that 14β-hydroxyl group is not indispensable for the specific cardiotonic activity[2].

The compounds used were 15β-hydroxydigitoxigenin (II)[3], 15-oxodigitoxigenin (III)[3], 15α-hydroxy-14α-digitoxigenin (V)[3], 14-deoxy-14βH-uzarigenin (VII)[4] as well as digitoxigenin and uzarigenin (VI). The 4 derivatives were newly synthesized by Dr. M. OKADA of Tokyo Biochemical Research Institute, Tokyo, and kindly supplied together with digitoxigenin and uzarigenin (Prof. REICHSTEIN'S specimen).

Stock solutions were prepared, by dissolving each compound in 70% ethanol in a concentration of 1 mg/ml. Immediately before use, these stock solutions were diluted to desired concentrations with Ringer's solution containing 0.6 m*M* of calcium.

STRAUB'S frog heart preparation was used. Impairment of the contractile force was induced by reducing the calcium concentration of the bathing medium to 0.6 m*M*, 1/3 the normal. Then the compound to be tested was applied either by replacing the bathing medium with the test solution, or by adding a small amount of a solution (see below). The bathing medium was aerated via a fine polyethylene tubing inserted into the STRAUB'S cannula. The experiments were performed during the period from September to November, at room temperatures of 20–25°C.

The results are summarized in the Table. Among the 3 compounds (II, III and V) derived from digitoxigenin, II was the most potent. But it is less potent than digitoxigenin, which consistently induced a systolic arrest at a concentration of $10^{-6}$ g/ml. The compound III was weak, but definitely active, inducing a typical systolic arrest when a concentration as high as $3 \times 10^{-5}$ g/ml was applied, while V was inactive. From these results and the previous data on 15α-hydroxydigitoxigenin (IV)[1,2], the order of potency among these allied compounds may arbitrarily be shown as in Figure 1.

The structural formulae of uzarigenin (VI) and VII are shown in Figure 2. Since the water solubility of these com-

Action of 3 derivatives of digitoxigenin, uzarigenin and its derivative on the isolated frog's heart (STRAUB's preparation)

| Concentration (g/ml) | $10^{-7}$ | $3 \times 10^{-7}$ | $10^{-6}$ | $3 \times 10^{-6}$ | $10^{-5}$ | $3 \times 10^{-5}$ |
|---|---|---|---|---|---|---|
| II | | − − + | + + + | + + + | × × × | |
| III | | | − − − | + + + | + + × | × × × |
| V | | | | | − − − | − − − |
| uzarigenin (VI) | + + + | | + + + | × × × | | |
| VII | − − + | | + + + | + + + | ×[a] ×[a] ×[a] | |

− no effect, + improvement of contractility without a tendency to systolic arrest, × systolic arrest. [a] Incompletely dissolved.


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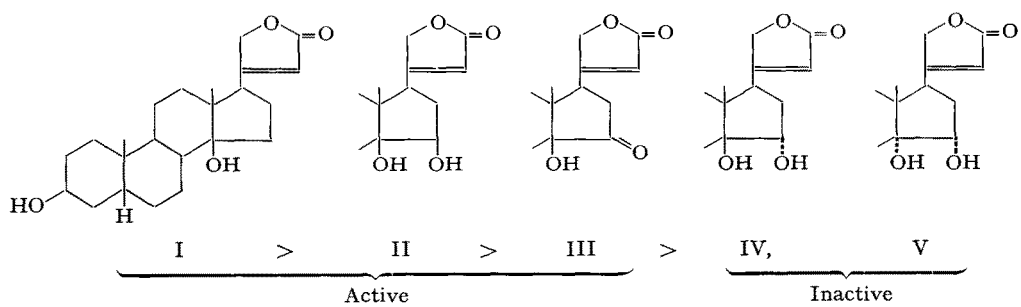


Fig. 1. Schematic presentation of the order of cardiotonic potency among digitoxigenin (I) and allied compounds.

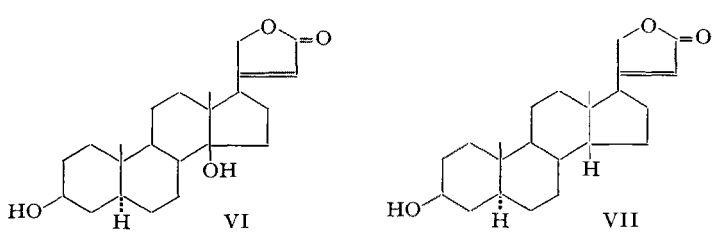


Fig. 2. Structural formulae of uzarigenin (VI) and 14-deoxy-14βH-uzarigenin (VII).

pounds was very low, the stock solutions were diluted with 35% ethanol, and 0.02 ml of the solution was injected with a microsyringe directly into the bathing medium (2.0 ml) to get a final dilution. When the medium contained $10^{-5}$ g/ml of VII, minute crystals were visible. As shown in the Table, this compound seemed to be a little less potent than uzarigenin, but is definitely active, although it lacks 14$\beta$-hydroxyl group.

*Zusammenfassung.* Die kardiotonischen Wirkungen der vier neuen Cardenolid-Derivate auf das isolierte Froschherz wurden untersucht und die Beziehungen zwischen ihren chemischen Strukturen und Wirkungen diskutiert.

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## On the Metabolism of Prenylamine (Segontin®)

Prenylamine, *N*-[3'-phenylpropyl-(2')]-1,1-diphenylpropylamine-(3), (see Figure 3) introduced in 1960 for treatment of angina pectoris[1] is able to decrease the catecholamine content of various organs[2–4]. The mechanism underlying its amine releasing action was interpreted as reserpine – like inter alia because prenylamine was also found to release serotonin from brain[2,4,5]; this finding was not confirmed by other authors[6].

Investigations on the pharmacological properties of the drug showed that it has sympathomimetic actions like an indirectly acting amine, e.g. phenylethylamine or tyramine. In addition it has imipramine-like qualities, i.e. it inhibits the uptake of $^3$H-noradrenaline at the level of the cellular membrane[6].

After i.v. injection of $^{14}$C-prenylamine in rats, despite its high lipid solubility, radioactivity declined rapidly in the organs with a half life of about 15 min in heart and brain non-exponentially and in a multiphasic manner[6]. This may be due to a rapid metabolism of prenylamine, which is supported by the fact that after oral administration of the $^{14}$C-labelled drug in rats $^{14}CO_2$ appeared very soon in the expiration air[7].

These results prompted us to investigate the metabolism of prenylamine.

*Methods and materials.* Rats (150 g) were treated orally with 100 mg/kg D,L-$^{14}$C-prenylamine-lactate[8] (specific activity 30 mC/g; purification of the labelled drug was done by preparative thicklayer chromatography).

Urine was collected in 5 h intervals up to 72 h. During this time only 17% of the radioactivity ingested was excreted into the urine. The urinary radioactive metabolites were characterized by means of highvoltage electrophoresis: paper Schleicher & Schüll 2043 b; pyridine/acetic acid/$H_2O$ = 100/10/890; pH 6.0; 2000 V, 40 mA; 2.5 h; further identification by column chromatography: hydrolyzed urine specimens (2*N* HCl, 2 h, 100°C) after neutralization and addition of amphetamine and derivatives mentioned below were absorbed on Dowex 50 (200–400 mesh), washed with 0.01*N* HCl, and the basic metabolites eluted with 2*N* HCl. The eluted metabolites were separated and identified by thinlayer chromatography (solvent: benzene/pyridine/acetic acid = 62/19/19; Eastman chromagram sheet K 301 R 2, silica gel).

Radioactivity was localized on the chromatograms autoradiographically (Doneo Clear base film Fa. Adox).

*Results.* Figure 1 shows an autoradiogram of urinary metabolites excreted 5, 10 and 24 h after application of the drug. Qualitatively, the same pattern of metabolites was observed up to 72 h. The main metabolites (I), about 60% of the urinary radioactivity, moved to the anode.

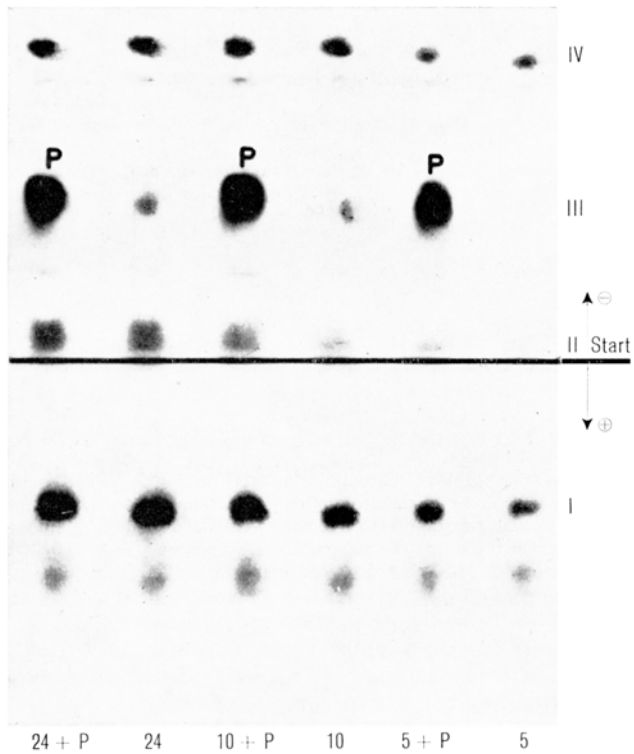


Fig. 1. Autoradiographic demonstration of urinary metabolites of prenylamine excreted 5, 10, and 24 h after administration of 100 mg/kg D,L-$^{14}$C-prenylamine-lactate orally, separated by highvoltage electrophoresis. + P = $^{14}$C-prenylamine added to the respective fraction before separation (for details see text).

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[8] We are indebted to the Farbwerke Hoechst AG for a generous gift of $^{14}$C-prenylamine.