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15-Oxygenated 14-Chloro-5β,14β-cardenolides

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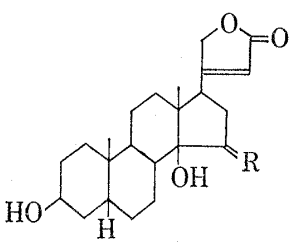
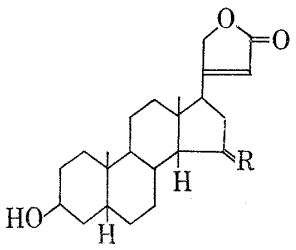
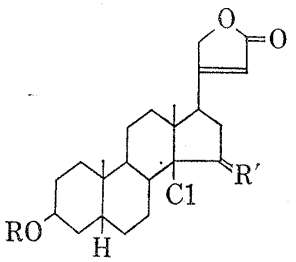
The introduction of an oxygen function into C-15 of digitoxigenin (I) profoundly affected the cardiotonic (inotropic) activity determined by using the isolated frog's heart (Straub's preparation) in the following order: digitoxigenin (I)>15β-hydroxydigitoxigenin (IV)[2]>15-oxodigitoxigenin (II)[2]>15α-hydroxydigitoxigenin (III),[3,4] the last being practically inactive.[5] In the 14-deoxy-14β-cardenolide series, a similar relationship has been observed, as a whole, to that in the 14β-hydroxy series.[6] Thus, among four cardenolides[7] (V, VI, VII, VIII) indicated in Chart 1 only 15α-hydroxy one was found to be inactive again. Extending these observations three 15-oxygenated 14-chloro-5β,14β-cardenolides, 3β-hydroxy-14-chloro-15-oxo-5β,14β-card-20(22)-enolide (IXa), 3β,15α-dihydroxy-14-chloro-5β,14β-card-20(22)-enolide (Xa), 3β,15β-dihydroxy-14-chloro-5β,14β-card-20(22)-enolide (XIa), were prepared for pharmacological examinations.



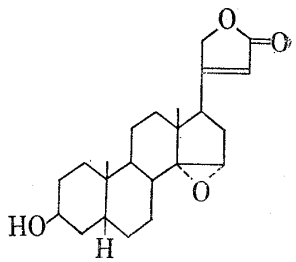
I: R=H_2
II: R=O
III: R=α-OH, β-H
IV: R=α-H, β-OH



V: R=H_2
VI: R=O
VII: R=α-OH, β-H
VIII: R=α-H, β-OH



IXa : R=H, R′=O
IXb : R=Ac, R′=O
Xa : R=H, R′=α-OH, β-H
Xb : R=Ac, R′=α-OH, β-H
XIa : R=H, R′=α-H, β-OH
XIb : R=Ac, R′=α-H, β-OH



XII

Ac=CH_3CO

Chart 1

The first cardenolide IXa was prepared by hydrolysis of its acetate (IXb) reported earlier[4] in methanol with hydrochloric acid. On acetylation with acetic anhydride and pyridine it reverted to IXb. Reduction of IXa with sodium borohydride yielded a mixture, whose thin-layer chromatography (TLC) revealed the formation of two chlorohydrins, Xa and XIa, as shown in Fig. 1a, the latter being predominant. Only a small amount of XIa was obtainable in pure state after repeated fractional crystallizations, since both chlorohydrins were fairly labile to be separated by various chromatographic procedures. It was different from Xa (15α-hydroxy epimer) described below.

1) Location: *Takada 3-Chome, Toshima-ku, Tokyo.*
2) M. Okada and Y. Saito, *Chem. Pharm. Bull.* (Tokyo), **15**, 352 (1967); *idem, ibid.*, **17**, 515 (1969).
3) M. Okada and M. Hasunuma, *Yakugaku Zasshi*, **85**, 822 (1965).
4) H. Ishii, T. Tozyo, and D. Satoh, *Chem. Pharm. Bull.* (Tokyo), **11**, 576 (1963).
5) T. Shigei and S. Mineshita, *Experientia*, **24**, 466 (1968).
6) T. Shigei, H. Tsuru, Y. Saito, and M. Okada, *Experientia*, submitted.
7) Preparation of these cardenolides will be reported elsewhere.

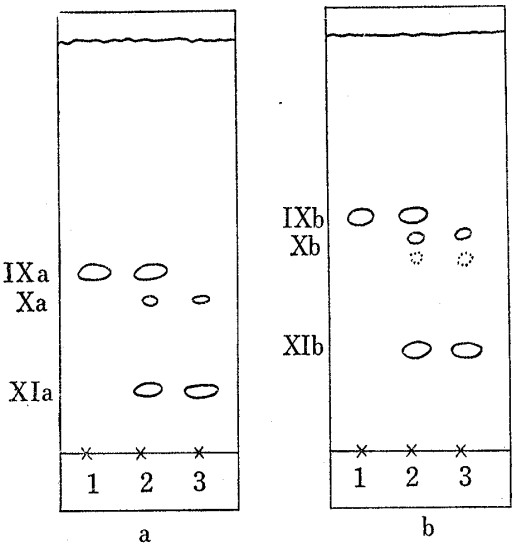


Fig. 1. Thin-Layer Chromatography

a: reduction product of IXa with $NaBH_4$: 1) IXa, 2) IXa+reduction product, 3) reduction product
b: reduction product of IXb with $NaBH_4$: 1) IXb, 2) IXb+reduction product, 3) reduction product

In contrast to the above chlorohydrins (Xa, XIa), their acetates, Xb and XIb, were stable and obtained in fairly good yield by reducing IXb with sodium borohydride, XIb (15β-hydroxy epimer) being the principal product again (Fig. 1b). Attempts to prepare Xa and XIa from Xb and XIb respectively by acid hydrolysis described above were unsuccessful owing to their instability.

Preparation of Xa was finally achieved by treating 3β-hydroxy-14α,15α-epoxy-5β-card-20(22)-enolide (XII)[3,4,8] in chloroform by anhydrous hydrogen chloride. Acetylation of Xa with acetic anhydride and pyridine gave a monoacetate which was identical with the 3-monoacetate of 3β,15α-dihydroxy-14-chloro-5β,14β-card-20(22)-enolide[4] (Xb), thus being established the structure and configuration of Xa. Preferentical acetylation of the 3-hydroxy group in 15α-hydroxydigitoxygenin had been observed.[3]

The results of pharmacological examinations by Straub's method were quite unexpected.[6] IXa and XIa were both inactive, while Xa possessed a definite cardiotonic activity.

Experimental[9]

3β-Hydroxy-14-chloro-15-oxo-5β,14β-card-20(22)-enolide (IXa)——A solution of IXb (80 mg) in a mixture of MeOH (20 ml) and 10% HCl (20 ml) was allowed to stand for 43 hr at room temperature. The solution was neutralized with 10% Na_2CO_3 and MeOH was removed *in vacuo* to yield a crystalline precipitate which was recrystallized from MeOH to give IXa (45 mg). mp 174—177°, UV $\lambda_{max}^{MeOH}m\mu(\log\varepsilon)$: 215 (4.16), IR $\nu_{max}cm^{-1}$: 3480 (OH), 1791, 1762 (sh), 1747, 1637 (butenolide and 15 C=O). *Anal.* Calcd. for $C_{23}H_{31}O_4Cl\cdot H_2O$: C, 65.00; H, 7.83. Found: C, 65.29; H, 7.56.

Acetylation of the above IXa (4.2 mg) in the usual way with acetic anhydride and pyridine gave IXb, mp 200—204° (decomp.), which was identical with an authentic specimen of IXb in the mixed melting point, TLC, and comparison of the IR spectrum.

3β,15α-Dihydroxy-14-chloro-5β,14β-card-20(22)-enolide (Xa)——Into a solution of XII (50 mg) in $CHCl_3$ (3 ml) was introduced anhydrous hydrogen chloride at −10° for 10 min and the solution was allowed to stand at 0° for 15 min. After adding $CHCl_3$ (20 ml) to the solution, it was washed consecutively with H_2O, 5% Na_2CO_3 and H_2O, and dried over anhyd. Na_2SO_4. Evaporation of the solvent *in vacuo* gave a crystalline residue which was recrystallized from MeOH-ether to afford Xa (19 mg). mp 185—190°, UV $\lambda_{max}^{MeOH}m\mu(\log\varepsilon)$: 217 (4.19), IR $\nu_{max}cm^{-1}$: 3410, 3270 (OH), 1806, 1748, 1624 (butenolide). *Anal.* Calcd. for $C_{23}H_{33}O_4Cl$: C, 67.55; H, 8.13. Found: C, 67.66; H, 8.15.

Acetylation of Xa (38 mg) with acetic anhydride (1 ml) and pyridine (0.5 ml) at 20° for 15 hr afforded an acetate (20 mg), mp 210—215°, after recrystallization from acetone–petroleum ether, which was identical with an authentic sample of Xb in the usual criteria (TLC, mixed melting point, IR).

Reduction of 3β-Hydroxy-14-chloro-15-oxo-5β,14β-card-20(22)-enolide (IXa) with $NaBH_4$——To a solution of IXa (50 mg) in MeOH (40 ml) was added $NaBH_4$ (40 mg) at 0° while stirring, and the solution was allowed to stand at 0° for 1.5 hr. After addition of AcOH (1 ml) and H_2O (25 ml) to the solution, it was concentrated *in vacuo* to a small volume and the product was extracted with $CHCl_3$. The organic layer

8) P. Hofer, H. Linde, and K. Meyer, *Helv. Chim. Acta*, **45**, 1041 (1962).
9) Melting points were determined on a Kofler block and are uncorrected. Ultraviolet (UV) spectra were measured in 99% EtOH solution. Infrared (IR) spectra were determined in KBr disks on Hitachi EPI-S2 spectrophotometer; sh=shoulder. TLC plates were prepared according to the Stahl's procedure using silica gel H (E. Merck AG) as adsorbent. The solvent system used was methyl ethyl ketone–heptane (1: 1), and the cardenolide spots were revealed by heating plates at 110° for 10 min after spraying 95% H_2SO_4 or by Kedde reagent.

was washed with H_2O and dried over anhyd. Na_2SO_4. Evaporation of the solvent gave a crystalline residue, whose TLC is shown in Fig. 1a. It was repeatedly recrystallized from MeOH to afford XIa (7 mg). mp 257—264° (decomp.), UV $\lambda_{max}^{max}m\mu(\log\varepsilon)$: 217 (4.18), IR ν_{max} cm^{-1}: 3410 (OH), 1796 (sh), 1771 (sh), 1731, 1626 (butenolide). *Anal.* Calcd. for $C_{23}H_{33}O_4Cl$: C, 67.55; H, 8.13. Found: C, 67.33; H, 8.05.

Reduction of 3β-Acetoxy-14-chloro-15-oxo-5β,14β-card-20(22)-enolide (IXb) with $NaBH_4$——To a solution of IXb (300 mg) in MeOH (150 ml) was added $NaBH_4$ (300 mg) at 0° for 10 min, and the reaction mixture was allowed to stand at 0° for 1.5 hr. After addition of AcOH (1.5 ml) and H_2O (50 ml) it was concentrated *in vacuo* and the product was extracted with $CHCl_3$. The organic layer was washed with H_2O and dried over anhyd. Na_2SO_4. Evaporation of the solvent gave a crystalline residue, whose TLC is shown in Fig. 1b. Repeated fractional crystallizations of the residue from aectone–ether afforded Xb (63 mg), which was identical with an authentic specimen in the usual criteria (TLC, mixed melting point, IR), and XIb (180 mg). mp 160—166° (decomp.), $[\alpha]_D^{25}$ −14.9° (c=1.35, $CHCl_3$), UV $\lambda_{max}^{max}m\mu(\log\varepsilon)$: 217 (4.20), IR ν_{max} cm^{-1}: 3480 (OH), 1803 (sh), 1778 (sh), 1741, 1730 (sh), 1628 (butenolide and acetyl C=O). *Anal.* Calcd. for $C_{25}H_{35}O_5Cl$: C, 66.57; H, 7.82. Found: C, 66.86; H, 8.09.

A solution of XIb (5 mg) and CrO_3 (1.5 mg) in AcOH (0.3 ml) was allowed to stand at 20° for 18 hr. To the reaction mixture was added MeOH (0.5 ml) and then H_2O (1 ml). The product was extracted with $CHCl_3$, and the organic layer was washed with H_2O and dried over anhyd. Na_2SO_4. Evaporation of the solvent *in vacuo* gave a crystalline residue which was recrystallized from MeOH to afford IXb (2 mg), mp 200—203° (decomp.), identical with an authentic sample in the usual criteria (TLC, mixed melting point, IR).

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Drug Absorption and Metabolism Studies by Use of Portal Vein Infusion in the Rat. I. Pyloric Vein Cannulation and Its Application to Study of First-Pass Effect on Bioavailability of Propranolol

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The rate and extent of drug absorption into the systemic circulation have been estimated by pharmacokinetic analysis of plasma concentration-time data or urinary excretion data.[2,3] The percent of absorption can be assessed by comparison of the relative areas under the plasma concentration-time curves after oral and intravenous administration. This method is based on the presumptions that the distribution and elimination of a drug may be expressed in terms of first-order kinetics within the dose ranges studied and that the parameters of these processes remain constant after administering the same quantity of drug by different routes. Thus, the resultant areas are independent of the route of administration and proportional to the dose even when given by different routes. However, it has recently been shown that the areas under the blood level-time curves for aspirin[4] and lidocaine[5] after infusion into a peripheral vein were considerably great as compared with results observed after infusion of an equal dose

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2) J.G. Wagner and E. Nelson, *J. Pharm. Sci.*, **52**, 610 (1963).
3) J.C.K. Loo and S. Riegelman, *J. Pharm. Sci.*, **57**, 918 (1968).
4) P.A. Harris and S. Riegelman, *J. Pharm. Sci*, **58**, 71 (1969).
5) R.N. Boyes, H.J. Adams, and B.R. Duce, *J. Pharmacol. Exptl. Therap.*, **174**, 1 (1970).