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93. Masashi Okada,[*1] Atsushi Yamada,[*1] and Morizo Ishidate[*2]: The Structure of Diginatigenin.

(*Iatrochemical Institute of Pharmacological Research Foundation*[*1] *and Faculty of Pharmaceutical Sciences, University of Tokyo*[*2])

Diginatigenin, a novel cardiac aglycone, was found by Murphy[1] as the aglycone of a new cardiac glycoside, diginatin, which was isolated in a very small amount from *Digitalis lanata*. Angliker, *et al.*[2] later isolated a new cardiac glycoside, lanatoside-D, from *Digitalis lanata* and showed that diginatigenin also constituted the aglycone of this glycoside, which therefore was proved to be the genuine form of diginatin.

Murphy[1] tentatively assigned the structure of 3β,12β,14,16β-tetrahydroxy-5β-card-20(22)-enolide (I) for diginatigenin, which was principally based on the chromogenic and spectroscopic properties of diginatigenin in comparison with those of other cardiac aglycones of digitalis and determination of the number of acylable hydroxyl groups present in diginatigenin, although no acylate was actually prepared. Quite recently it has been found by Tamm, *et al.*[3] that gitoxigenin is converted microbiologically into diginatigenin and the reported yield of this bioconversion by their fungus strain is very low (0.5%). It has also been found independently here that a certain of fungus strain transforms gitoxigenin into diginatigenin in a much better yield (6%).[4] Furthermore, it was demonstrated that incubation of this fungus with oleandrigenin (16-monoacetylgitoxigenin) yields 16-monoacetyldiginatigenin in more than 15%. The strain used by Tamm, *et al.*[5] and this strain[4] are also able to hydroxylate digitoxigenin at 12β-position to give digoxigenin in a good yield. From these facts it seems fairly reasonable, if not conclusive, to adopt the above structure assigned by Murphy to diginatigenin. With sufficient amounts of diginatigenin and 16-monoacetyldiginatigenin prepared by microbiological method in hand, it became possible to establish a definitive structure for diginatigenin.

Specific rotations of diginatigenin (I), 16-monoacetyldiginatigenin (II), and triacetyldiginatigenin (III) were reported for the first time in a previous paper.[4] Molecular rotation of these compounds and that of digoxigenin, 12β-hydroxybufalin[6] and their acetates are compared in Tables I and II. Molecular rotation differences presented in these tables agree fairly well with the contribution of 12β-hydroxyl and 12β-acetoxyl groups.

Table I. Comparison of Molecular Rotation

Compound	$[\alpha]_D$ (MeOH)	$[M]_D$	$\Delta[M]_D$
Digoxigenin	+22.5	+ 87.5	+16
Digitoxigenin	+19	+ 71.5	
12β-Hydroxybufalin	−16	− 65	+12
Bufalin	−20	− 77	
Diginatigenin	+34	+138	+ 9
Gitoxigenin	+33	+129	
16-Monoacetyldiginatigenin	+ 6	+ 27	+70
Oleandrigenin	−10	− 43	

*1 26 Nishigahara 1-chome, Kita-ku, Tokyo (岡田正志, 山田 厚).
*2 Hongo, Tokyo (石館守三).

1) J. E. Murphy: J. Am. Pharm. Assoc., **44**, 719(1955).
2) E. Angliker, F. Barfuss, W. Kussmaul, J. Renz: Ann. Chem. Liebigs, **607**, 131(1957).
3) Ch. Tamm, A. Gubler: Helv. Chim. Acta, **41**, 1762(1958).
4) M. Okada, A. Yamada, M. Ishidate: This Bulletin, 8, 530(1960).
5) Ch. Tamm, A. Gubler: Helv. Chim. Acta, **41**, 297(1958); **42**, 239(1959).
6) *Idem*: *Ibid.*, **42**, 473(1959).

Table II. Comparison of Molecular Rotation

Compound	$[\alpha]_D$ (MeOH)	$[M]_D$	$\Delta[M]_D$
Diacetyldigoxigenin	+50	+239	+151
Acetyldigitoxigenin	+21	+ 88	
Diacetyl-12β-hydroxybufalin	+34	+151	+177
Acetylbufalin	− 6	− 26	
Triacetyldiginatigenin	+32	+170	+209
Diacetylgitoxigenin	− 8	− 39	

A solution of 16-monoacetyldiginatigenin (II) in 80% ethanol containing 3.5% of hydrochloric acid was refluxed for 4 hours and the reaction product was acetylated in the usual way with acetic anhydride and pyridine. Chromatographic purification of the acetylated product gave a crystalline substance of m.p. 234~241°, $[\alpha]_D$ +492° ($CHCl_3$), UV λ_{max}^{EtOH} mμ (log ε): 330(4.36), 222(4.05) (Fig. 1). It is well-known that gitoxigenin, oleandrigenin, and their glycosides are converted into $\Delta^{14,16}$-dianhydrogitoxigenin[7,8] on heating in approximately 1*N* hydrochloric acid, which possesses very high dextrorotation, $[\alpha]_D$ +579° (MeOH), and exhibits two bands at 337.5 mμ (log ε 4.31) and 222.5 mμ (log ε 4.06) in its ultraviolet absorption spectrum.[9]

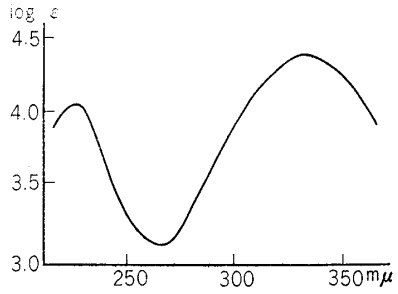


Fig. 1.

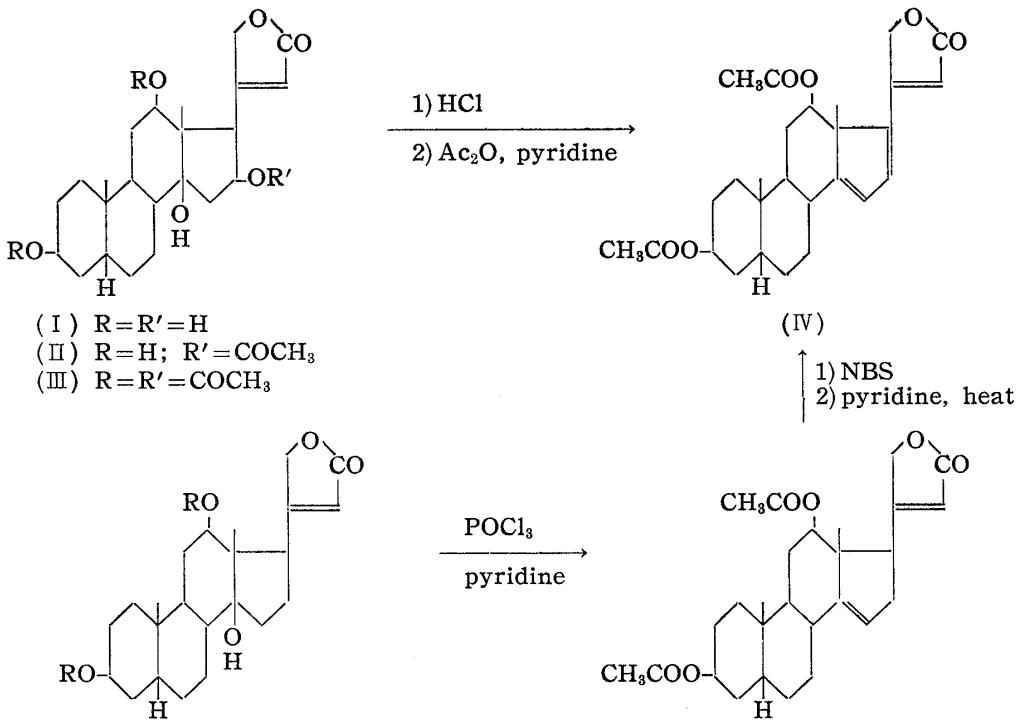
Ultraviolet Absorption Spectrum of Diacetyl-$\Delta^{14,16}$-dianhydrodiginatigenin

The afore-mentioned compound obtained from 16-monoacetyldiginatigenin (II), therefore, should be diacetyl-$\Delta^{14,16}$-dianhydrodiginatigenin of the presented structure (IV), supposing that the third acylable hydroxyl group of diginatigenin is really located at 12β-position. The compound correspoding to this structure (IV), diacetyl-Δ^{14}-anhydro-16,17-dehydrodigoxigenin, on the other hand, had already been synthesized by Plattner, *et al.*[10] from digoxigenin (3β,12β,14-trihydroxy-5β-card-20(22)-enolide[11]) (Va), according to the scheme in Chart 1. The reported properties of diacetyl-Δ^{14}-anhydro-16,17-dehydrodigoxigenin, m.p. 235~237°, $[\alpha]_D$ +393° ($CHCl_3$), UV λ_{max}^{EtOH} 332 mμ (log ε 4.25), are in agreement with those of the diacetyl-$\Delta^{14,16}$-dianhydrodiginatigenin obtained in the present series of work, except for its rotation.*3

*3 The molecular rotation (+2238°) of the present diacetyl-$\Delta^{14,16}$-dianhydrodiginatigenin is in much better agreement with that calculated (+2225°) as indicated below, than that (+1788°) of diacetyl-Δ^{14}-anhydro-16,17-dehydrodigoxigenin reported by Plattner, *et al.*[10]

$[M]_D$ of $\Delta^{14,16}$-dianhydrogitoxigenin	+ 2055°
Increment of 12β-$OCOCH_3$ (Diacetyldigoxigenin — acetyldigitoxigenin)	+ 151°
Increment of acetyl group at 3β-OH (Acetyldigitoxigenin — digitoxigenin)	+ 17°
Calculated $[M]_D$ for (IV)	+ 2225

7) L. F. Fieser, M. Fieser: "Steroids," 758(1959), Reinhold Publishing Co., New York.
8) F. Thudium, O. Schindler, T. Reichstein: Helv. Chim. Acta, **42**, 2(1959).
9) O. Schindler, T. Reichstein: *Ibid.*, **35**, 442(1952).
10) Pl. A. Plattner, H. Heusser: *Ibid.*, **29**, 727(1946).
11) S. Pataki, K. Meyer, T. Reichstein: *Ibid.*, **36**, 1295(1953).



(I) R=R'=H
(II) R=H; R'=$COCH_3$
(III) R=R'=$COCH_3$

(IV)

(Va) R=H
(Vb) R=$COCH_3$

Chart 1.

Diacetyl-Δ^{14}-anhydro-16,17-dehydrodigoxigenin (IV) was prepared after the procedure described by Plattner, *et al.*, and it was found that diacetyl-$\Delta^{14,16}$-dianhydrodiginatigenin is completely identical with diacetyl-Δ^{14}-anhydro-16,17-dehydrodigoxigenin by mixed melting point and infrared comparison (Fig. 2). The results presented in this paper, therefore, coupled with the bioconversion of gitoxigenin to diginatigenin have established definitely that the structure of diginatigenin is 3β,12β,14,16β-tetrahydroxy-5β-card-20(22)-enolide (I).

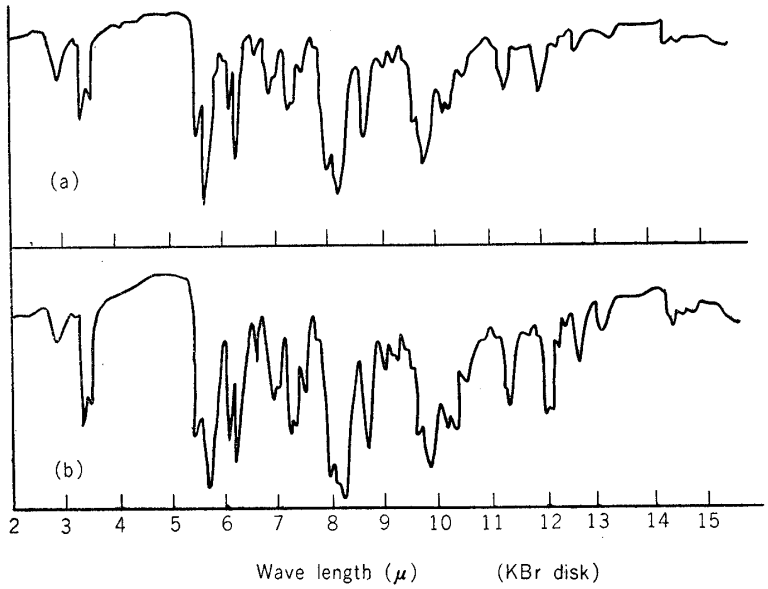


Fig. 2. Infrared Absorption Spectra

(a) Diacetyl-Δ^{14}-anhydro-16,17-dehydrodigoxigenin
(b) Diacetyl-$\Delta^{14,16}$-dianhydrodiginatigenin

In Table III the toxicity of diginatigenin and 16-monoacetyldiginatigenin determined by pigeon method is compared with that of other related cardiac aglycones.[12] A remarkable enhancement of toxicity was observed by acetylation of 16β-hydroxyl group of diginatigenin as in the case of gitoxigenin.

Table III. Toxicity by Pigeon Method[12] *4

Compound	No. of pigeons	LD_{50} (mg./kg.)	Compound	No. of pigeons	LD_{50} (mg./kg.)
Digitoxigenin	6	0.60	Diginatigenin	4	5.75
Digoxigenin	5	1.62	Oleandrigenin	6	0.95
Gitoxigenin	6	5.40	16-Monoacetyldiginatigenin	4	1.84

Experimental*5

Diacetyl-$\Delta^{14,16}$-dianhydrodiginatigenin (=Diacetyl-Δ^{14}-anhydro-16,17-dehydrodigoxigenin) (IV) from 16-Monoacetyldiginatigenin (II)—To a solution of 30 mg. of (II) in 4.5 cc. of 80% EtOH, 0.5 cc. of conc. HCl was added and the solution was refluxed for 4 hr. H_2O was added to the solution and EtOH was evaporated under a reduced pressure. After dilution with AcOEt and washing with 5% Na_2CO_3 and H_2O, the organic phase was dried and the solvent was evaporated. The yellowish residue (26 mg.) was acetylated in the usual way with Ac_2O and pyridine, and the acetylated product (29 mg.) was chromatographed on 1 g. of acid-washed alumina by successive elution with petr. ether, benzene, and $CHCl_3$. The fraction (21 mg.) eluted with petr. ether-benzene mixture (1:3) and benzene gave 14 mg. of (IV), m.p. 232~238°, after crystallization from Me_2CO-petr. ether. Recrystallization from EtOH afforded 11 mg. of (IV), m.p. 234~241°, $[\alpha]_D^{22}$ +492° ($CHCl_3$), UV (Fig. 1.). Admixture with a sample (m.p. 233~239°, UV λ_{max}^{EtOH} mμ (log ε): 332 (4.20), 222 (4.04)) prepared from (V b) according to the procedure described by Plattner, *et al.*[10] melted at 233~240°, and the IR spectra of the two samples were identical in all respects (Fig. 2).

The authors are very grateful to the Research Laboratories of Takeda Pharmaceutical Industries, Ltd. for the infrared spectrographic data and rotation.

Summary

16-Monoacetyldiginatigenin was converted into diacetyl-$\Delta^{14,16}$-dianhydrodiginatigenin, which was identical with diacetyl-Δ^{14}-anhydro-16,17-dehydrodigoxigenin prepared from digoxigenin. Thus, the structure of diginatigenin has been definitely established as 3β,12β,14,16β-tetrahydroxy-5β-card-20(22)-enolide (I).

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〔Added in proof〕 After submission of this paper, H. Linde, J.E. Murphy, and K. Meyer (Helv. Chim. Acta, **42**, 2040, 2753(1959)) also proved the structure of diginatigenin as 3β,12β,14,16β-tetrahydroxy-5β-card-20(22)-enolide using diginatin and essentially the same experimental procedures as reported in this paper.

*4 Toxicity determination was performed by Mr. C. Isono of this Institute under the direction of Prof. K. Tokita, to whom the authors' thanks are due.

*5 All m.p.s are not corrected.

12) A. Yamada: This Bulletin, 8, 18(1960).