In a ligroin–propylene glycol descending papergram, overnight development, the R_f for 16α-methyl-17α-hydroxyprogesterone (XVIb) is slightly greater than that of 16β-methyl-17α-hydroxyprogesterone (XVIa).

Anal. Calcd. for $C_{22}H_{32}O_3$: C, 76.70; H, 9.36. Found: C, 76.55; H, 9.32.

Also isolated from this column was a substance identified as 16α-methyl-3β,17α-dihydroxy-5-pregnen-20-one,[21a] which was obtained by incomplete oxidation of XIIIb followed by zinc and ethanol treatment.

16α-Methyl-17α-hydroxy-4-pregnene-3,20-dione 17-Acetate (16α-Methyl-17α-acetoxyprogesterone, XVIIb).—To 0.3 g. of 16α-methyl-17α-hydroxyprogesterone (XVIb) contained in 3 ml. of acetic acid under nitrogen was added 0.6 ml. of trifluoroacetic anhydride. The reation solution was warmed at 90–95° for 1 hr., then diluted with 50 ml. water. The aqueous mixture was extracted with methylene chloride which in turn was washed with 5% aqueous sodium carbonate, then with water. Evaporation of the organic phase to a solid residue afforded, after trituration with hexane, 165 mg. of product, m.p. 213–220°. An analytical sample, crystallized from ether, had m.p. 229–232°, $[\alpha]_D$ + 70.7°; λ_{max} 239 mμ (ϵ 17,050); 5.78, 5.84, 6.04, 6.20, 8.02 μ. Reported by Batres, *et al.*,[21a] m.p. 239–240°, $[\alpha]_D$ + 82° ($CHCl_3$), λ_{max} 240 mμ log ϵ 4.24; and by Bernstein, *et al.*,[21b] m.p. 233–236°; $[\alpha]$ + 80° ($CHCl_3$); λ_{max}^{MeOH} 242 (ϵ 15,200).

Anal. Calcd. for $C_{24}H_{34}O_4$: C, 74.57; H, 8.87. Found: C, 74.76; H, 8.85.

In a ligroin–propylene glycol descending papergram the R_f of the 16α-methyl-17α-acetoxyprogesterone (XVIIb) is slightly less than that of the 16β-methyl-17α-acetoxyprogesterone (XVIIa).

Cardiac Activity of Newer Digitalis Glycosides and Aglycones

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Thirty-one cardiac glycosides or aglycones, isolated from the leaves of *Digitalis lanata* or from the leaves and seeds of *D. purpurea*, or partially synthesized, have been pharmacologically assayed in etherized cats. Certain comparisons were made for structure-activity relationship. Esterification at C_{16} may give rise to aglycones, such as gitaloxigenin and 16-acetylgitoxigenin (oleandrigenin), that have a higher potency than their glycosides. Digipurpurin, a member of the C_{21}-steroids, has no digitalis-like action.

Chemical work during recent years on the leaves and seeds of *Digitalis purpurea* and on the leaves of *D. lanata* has resulted in the isolation of newer glycosides and aglycones and in the partial synthesis of heretofore unknown derivatives. It has been our privilege to evaluate the pharmacological activity of the newer substances

TABLE I

CARDIAC ACTIVITY OF DIGITALIS GLYCOSIDES AND AGLYCONES

Compound	Chemical reference	Concentration	No. of cats	Mean (geo.) LD ± S. E. mg./kg.
Digitoxigenin-β-D-digitoxoside	1	1:100,000	10	0.2168 ± 0.0119
Digitoxigenin-α-D-digitoxoside	2	1:2,000	10	0.5544 ± 0.0475
Desacetyllanatoside A, crystalline	3	1:50,000	10	0.3522 ± 0.0116
Glucodigifucoside	4	1:50,000	10	0.3154 ± 0.0274
17α-Digitoxigenin	5	1:500	2	one survived 5.8; the other, 11.2
3-Epi-17α-digitoxigenin	6	1:1,000	1	animal survived 5
Gitoxigenin	7	1:10,000	10	3.010 ± 0.312
Gitostin	8	1:20,000	10	1.260 ± 0.1372
Desacetyllanatoside B, crystalline	3	1:50,000	10	0.415 ± 0.0299
16-Acetylgitoxigenin	7	1:100,000	10	0.2161 ± 0.0126
16-Acetylstrospeside	9	1:50,000	10	0.2464 ± 0.0117
16-Acetylstrospeside acetate	9	1:50,000	10	0.2908 ± 0.0151
16-Acetyldigitalinum verum	7	1:50,000	10	0.2554 ± 0.0160
16-Propionyldigitalinum verum	7	1:25,000	10	0.8193 ± 0.0666
Diginatigenin	10, 11	1:10,000	5	1.380 ± 0.0978
Diginatin	10	1:50,000	10	0.4734 ± 0.0468
Lanatoside D	12	1:25,000	10	0.5141 ± 0.0272
Gitaloxigenin	13	1:100,000	10	0.0975 ± 0.0037
Verodoxin	14	1:50,000	10	0.2309 ± 0.0154
16-Formyldigitalinum verum	15	1:100,000	10	0.1924 ± 0.0102
Gitaloxin	16	1:1,000	10	0.8999 ± 0.0568
Lanatoside E	13	1:25,000	10	0.6490 ± 0.0241
Neogitostin	17	1:10,000	7	2.818 ± 0.1618
Neogitostin acetate	17	1:10,000	6	2.553 ± 0.3561
Digipurpurin	18	1:1,600	1	cat survived 4.572
Digitoxigenin-α-D-rhamnoside	19, 20	1:25,000	10	0.6149 ± 0.0365
Digitoxigenin-2-deoxy-β-D-glucoside	20	1:100,000	10	0.1895 ± 0.0087
Neodigoxin	21	1:50,000	10	0.2900 ± 0.0160
Lanadoxin	22	1:50,000	10	0.3319 ± 0.0190
Acetylgitaloxin	23	1:25,000	10	0.8420 ± 0.0595
16-Acetylgitoxin	24, 25	1:25,000	10	1.171 ± 0.0521
16-Acetylgitoxin	26	1:25,000	5	1.172 ± 0.0953

supplied by the scholars of different laboratories. The present report deals with thirty-one compounds whose cardiac potencies were measured by their mean lethal doses (LD's) in cats. The results are compared with those of closely allied products for any structure-activity relationship.

Methods.—The names of the 31 compounds are shown in Table I. All of them have a butenolide ring at C_{17}, except digipurpurin, which is one of the C_{21}-steroids.[27] Although 17α-digitoxigenin is a constituent of *Menabea venata*, it is included here because of its close resemblance to digitoxigenin.[5] For animal experiments, stock solutions of 0.1% were prepared with 47.5% ethanol by volume, and dilutions from 1:5,000 to 1:100,000 were made with saline.

The activity of each substance was evaluated in etherized cats by continuous infusion of a suitable dilution into the femoral vein at the rate of 1 ml./min. so that a 2 kg. cat died between 30 and 60 minutes. Slowing of heart rate, arrhythmias, secondary cardiac acceleration, and ventricular fibrillation were determined by palpation and auscultation. These manifestations are simple but reliable evidence of digitalis-like action. The geometrical mean LD of each compound, usually from 10 cats depending on the quantity of mate-

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rial, was computed together with its standard error (S.E.). If the heart rate did not undergo the characteristic changes and the animal survived 4.5 to 11 mg./kg., the substance was considered inactive. Four compounds—gitaloxin, digipurpurin, 3-epi-17α-digitoxigenin and digitoxigenin-α-D-digitoxoside—were so insoluble in saline that various stock solutions were intravenously injected by means of a 3-way microburet at the rate of 0.06 ml./min. and washed in, each time, with 1 ml. of saline.[28] The ethanol content of the solutions of the first three steroids was 47.5%, but that for the last was raised to 59.4%. The purpose was to administer a minimum of ethanol to each animal. The weight range of 277 cats ran between 1.6 and 2.9 kg. Females in advanced stage of pregnancy were eliminated.

Results and Discussion.—According to the data in Table I, last column, the most potent substance is gitaloxigenin and the least potent is gitoxigenin. Digipurpurin, 3-epi-17α- and 17α-digitoxigenin are inactive. Desacetyllanatoside A and B are in crystalline form and prove to be more active than amorphous samples studied previously.[29] Two specimens of 16-acetylgitoxin of independent sources[25,26] yielded practically identical figures in cats, but such closeness is of a rare occurrence.

In order to facilitate comparisons by direct proportion, the mean LD's of active compounds are converted to their reciprocals with the S.E.'s, as listed in the last column of Table II. Previously published data of digitoxigenin, evomonoside, digitoxin, lanatoside A and B, digoxigenin, digoxin and lanatoside C[29,30] are included. The glycosides of digitalis fall into the formulas of 5 cardenolides (I to V) as reviewed by Stoll.[31] It is obvious that the addition of an OH group to digitoxigenin at C_{16} greatly diminishes the cardiac activity. Digoxigenin with an OH group at C_{12} has a potency comparable to that of digitoxigenin. The presence of two OH groups at C_{12} and C_{16} in the molecule of digitoxigenin diminishes the activity by two-thirds. Surprisingly, esterification of gitoxigenin with formic acid results in the most potent aglycone of the entire series. A similar favorable influence is exerted upon 16-acetyl- and 16-propionylgitoxigenin (VI and VII), the former also being known as oleandrigenin. The order of activity of three esters is formyl > acetyl > propionyl. The enhancement of action is more pronounced than that due to acetyla-

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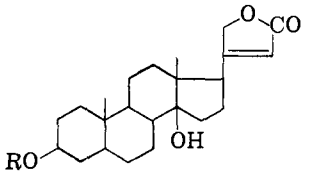
TABLE II

STRUCTURE-ACTIVITY RELATIONSHIP

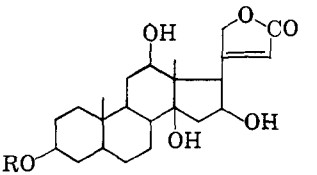
Formula	R	Compound name	Reciprocal of mean LD ± S. E.
I	H	Digitoxigenin	2.18 ± 0.17
	H	17α-Digitoxigenin	inactive
	H	3-Epi-17α-digitoxigenin	inactive
	β-D-Digitoxose	Digitoxigenin-β-D-digitoxoside	4.61 ± 0.25
	α-D-Digitoxose	Digitoxigenin-α-D-digitoxoside	1.80 ± 0.15
	α-D-Rhamnose	Digitoxigenin-α-D-rhamnose	1.63 ± 0.10
	α-L-Rhamnose	Evomonoside	3.59 ± 0.19
	2-Deoxy-β-D-glucose	Digitoxigenin 2-deoxy-glucoside	5.28 ± 0.24
	Fucose-glucose	Glucodigifucoside	3.17 ± 0.27
	3(Digitoxose)	Digitoxin	3.07 ± 0.11
	3(Digitoxose)-glucose	Desacetyllanatoside A	2.84 ± 0.09
	2(Digitoxose)-digitoxose-(Ac)-glucose	Lanatoside A	2.77 ± 0.13
II	H	Gitoxigenin	0.33 ± 0.03
	β-D-Digitalose-β-cellobiose	Gitostin	0.79 ± 0.09
	β-D-Digitalose-β-gentiobiose	Neogitostin	0.35 ± 0.02
	β-D-Digitalose(Ac)-β-gentiobiose	Neogitostin acetate	0.39 ± 0.05
	3(Digitoxose)-glucose	Desacetyllanatoside B	2.41 ± 0.17
	2(Digitoxose)-digitoxose-(Ac)-glucose	Lanatoside B	2.58 ± 0.18
III	H	Digoxigenin	2.26 ± 0.21
	3(Digitoxose)	Digoxin	4.33 ± 0.17
	2(Digitoxose)-digitoxose-(Ac)-glucose	Lanatoside C	4.30 ± 0.33
IV	H	Diginatigenin	0.72 ± 0.05
	3(Digitoxose)	Diginatin	2.11 ± 0.21
	2(Digitoxose)-digitoxose-(Ac)-glucose	Lanatoside D	1.94 ± 0.10
V	H	Gitalotoxigenin	10.26 ± 0.39
	Digitoxose	Lanadoxin	3.01 ± 0.17
	Digitalose	Verodoxin	4.33 ± 0.29
	Digitalose-glucose	16-Formyldigitalinum verum	5.20 ± 0.28
	3(Digitoxose)	Gitaloxin	1.11 ± 0.07
	2(Digitoxose)-digitoxose-(Ac)	Acetylgitaloxin	1.19 ± 0.08
	2(Digitoxose)-digitoxose-(Ac)-glucose	Lanatoside E	1.54 ± 0.06
VI	H	16-Acetylgitoxigenin (Oleandrogenin)	4.63 ± 0.27
	Digitalose	16-Acetylstrospeside	4.06 ± 0.19

TABLE II (*Continued*)

Formula	R	Compound name	Reciprocal of mean LD ± S. E.
	Digitalose(Ac)	16-Acetylstrospeside acetate	3.44 ± 0.18
	Digitalose-glucose	16-Acetyldigitalinum verum	3.92 ± 0.25
	3(Digitoxose)	16-Acetylgitoxin	0.85 ± 0.03 (combined)
VII	Digitalose-glucose	Propionyldigitalinum verum	1.22 ± 0.10



I



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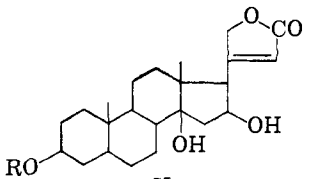

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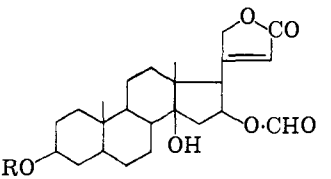

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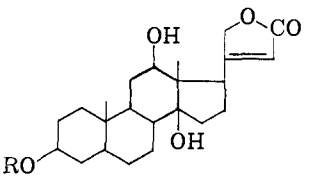
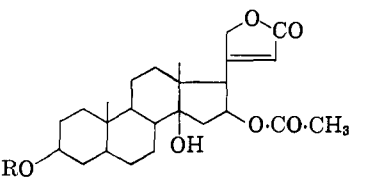
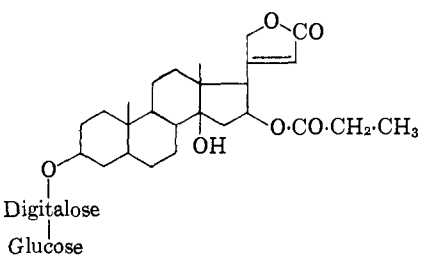


III



VI



VII

tion of the OH group at C_3 of strophanthidin, hellebrigenin, marinobufagin, and cinobufagin.[32-34]

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A change of spatial isomerism from the usual configuration frequently results in loss or decrease of activity. Thus, 17α- and 3-epi-17α-digitoxigenin are inactive in contrast to the β-form (Table II). Digitoxigenin-β-D-digitoxoside, synonymous with evatromonoside, occurs in nature, but the synthetic α-D-digitoxoside is one-third as potent. Similarly, digitoxigenin-α-D-rhamnoside is barely one-half as active as the α-L-rhamnoside (evomonoside).

Most of the glycosides of the first four cardenolides (I to IV, Table II) are more potent than their aglycones. The exceptions are digitoxigenin-α-D-digitoxoside and digitoxigenin-α-rhamnoside. Two single sugar conjugates (monosides), digitoxigenin-2-deoxyglucoside and digitoxigenin-β-D-digitoxoside, are outstanding in their potency as compared with the glycosides of the first group. Monoacetylation with the sugar molecule does not change the activity because the difference in potency is insignificant between lanatoside A and desacetyllanatoside A, between neogitostin and its acetate, between lanatoside B and desacetyllanatoside B, and between gitaloxin and acetylgitaloxin. There is a borderline difference of significance between 16-acetylstrospeside and its acetyldigitalosido derivative at $P = 0.05$ by the t test.

The most remarkable feature is the superior potency of gitaloxigenin and 16-acetylgitoxigenin to their respective glycosides, which is due to the esterification at C_{16}. These are the first instances in which a cardenolide exceeds its glycosides in activity.

Kroneberg[35] studied digitoxigenin-β-D-digitoxoside in seven cats and found it to have an LD of 0.27 to 0.3 mg./kg. Because of the insolubility of gitoxin in both ethanol and water, its potency in cats had not been determined by intravenous infusion. It is interesting to note that Achelis and Kroneberg[36] recorded an LD of 0.465 mg./kg. when they employed a mixed solvent. The same investigators[37] reported a mean LD of 1.01 for gitaloxin in cats. Favorable clinical results in 20 patients with gitaloxin have been published by Storz.[38] Pharmacological studies have been made by Kroneberg and Achelis[39] on verodoxin, by Kovarikovà and associates[40,41] on lanatoside D, and by Sekiya and co-workers[42] on 16-acetyldigitalinum verum.

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