Supplementary Glycosides of Digitalis[1]

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Twenty-four derivatives of digitoxigenin, digoxigenin, and gitoxigenin have been studied in cats under ether anesthesia for their cardiotonic action as measured by the mean iv LD. Of 11 glycosides of digitoxigenin, neodigitalinum verum is inactive. Digoxigenin monodigitoxoside is more potent than the bis- and tetradigitoxosides and more than twice as active as digoxin (the tridigitoxoside). The α-oriented aglycone, 16-epigitoxogenin, and has no digitalis-like action. Two glycosides of gitoxigenin and two formyl- and five acetylgitoxins all surpass the activity of the natural aglycone gitoxigenin.

Increasing numbers of cardiac glycosides and aglycones have been isolated, partially synthesized, or otherwise altered, following the development and improvement of chromatography, special spectroscopy, nmr spectroscopy, new synthetic methods, and other techniques. Our interest in the pharmacological action of this class of compounds initiated in 1933. With the constituents of Digitalis species alone, the potency and toxicity of 31 products were presented in 1962,[2] and of 11 in 1965.[3] This communication deals with the results of 24 new compounds. As shown in Table I the substances are derivatives of digitoxigenin, digoxigenin, and gitoxigenin. In order to save space, abbreviations of aglycones, sugars, and acyl groups are used according to Nover, *et al.*[4] Chemical configurations or suggested formulas are to be found in the papers by Haack, *et al.*,[5] Rees, *et al.*,[6] Ragab, *et al.*,[7] Kaiser, *et al.*,[8-10] Nover, *et al.*,[11] and Hupin.[12] Neodigitoxin is an isomer of digitoxin, but its structure has not been elucided.

Methods

The investigations were identical with those previously employed.[3] The most important procedure was to make a 0.1% stock solution with the least amount of EtOH. Twenty compounds were soluble in 47.5–50% EtOH. Monoacetylgitoxin required 60% EtOH, neodigitoxin and digoxoside 70%, and formiloxin 75%. Various dilutions were prepared in order to determine the mean lethal dose (LD) in colonies of 10 cats each within 30–60 min by iv injections—1:100,000, 1:50,000, 1:25,000, and 1:10,000. A few substances were not sufficient in quantity to run 10 animals: thus 5 for monoacetylgitoxin, diacetylgitoxin γ,δ, diacetylgitoxin γ, and triacetylgitoxin, and 7 for pentaacetylgitoxin. Because of spare solubility in saline, digitoxigenin and digoxigenin bisdigitoxoside, formiloxin, and pentaacetylgitoxin had to be administered through a 3-way microburet.[13] No frogs were used in this study.

Results

The mean LD values ± standard errors were converted into the reciprocals as listed in the last column of Table I. This enables us to recognize the structure–activity relationship by direct proportion. Among the 11 glycosides of digitoxigenin the first 5 monosides and biosides are substantially potent. The next 5 biosides and triosides are lower in cardiotoxicity. Digitalinum verum has a low activity (0.7 LD/mg),[14] but its neo form is completely inactive in 4 cats. The nature of carbohydrates conjugated with the secondary hydroxy group at C_3 and the anomeric changes must account for the difference.

Our previous experience[14] indicates that monosides of cardenolides are generally more active than biosides and oligosides. The three glycosides of digoxigenin in Table I bear out the same conclusion. The reverse is true with the two glycosides of gitoxigeningitoroside *vs.* glucogitoroside. When a stereoisomeric change takes place in this aglycone at C_{16} to become 16-epigitoxigenin, the cardioactivity is completely lost (2 cats). Glucolanadoxin, a formic ester of C_{16}, has a respectable activity. Formiloxin is 20% as potent; it has 4 additional formic substituents in the sugar residues as indicated in Table I. Acetylation at various positions does not confer much favorable effect as shown by the last 5 products.

Discussion

Eight glycosides of digitoxigenin have a higher potency in etherized cats than the aglycone (2.18/mg).[2] Neoodorobioside G and neodigitoxin are both less active. Neodigitalinum verum has no evidence of cardiac action in doses exceeding 4 mg/kg. Digoxigenin has a value of 2.26/mg,[2] but its mono- and bisdigitoxosides are far more potent, as shown in Table I. However, its tetradigitoxoside is equally active. Gitoxigenin is the weakest of the 3 aglycones (0.33/mg);[2] its glycosides, formyl and acetyl derivatives all surpass this value. The α-16-OH of gitoxigenin makes it devoid of any cardiac effect in the anesthetized cat although a token response occurs in the papillary muscle of the

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Table I

Cardioactivity in Cats

Compound	Aglycone abbreviation[a]	Sugar or acyl abbreviation[b]	No. of mean LD's ± std error/mg
Digitoxogenin allomethyloside	Dg	3-O-Alms	5.28 ± 0.33
Digitoxigenin glucomethyloside	Dg	3-O-Glms	4.44 ± 0.37
Digitoxigenin glucosidoglucomethyloside	Dg	3-O-Glms-Glu	5.49 ± 0.19
Glucoevatromonoside	Dg	3-O-Dxs-Glu	6.63 ± 0.23
Glucodigifucoside	Dg	3-O-Fcs-Glu	4.33 ± 0.42
Neoglucodigifucoside	Dg	3-O-Fcs-Glu	2.63 ± 0.24
Digitoxigenin bisdigitoxoside	Dg	3-O-(Dxs)$_2$	2.34 ± 0.08
Neodigitalimum verum	Dg	3-O-Dls-Glu	Inactive
Neoodorobioside G	Dg	3-O-Dls-Glu	1.03 ± 0.13
Glucodigitoxigenin bisdigitoxoside	Dg	3-O-(Dxs)$_2$-Glu	3.02 ± 0.18
Neodigitoxin	Dg	3-O-(Dxs)$_3$	1.50 ± 0.07
Digoxigenin monodigitoxoside	Dxg	3-O-Dxs	9.28 ± 0.01
Digoxigenin bisdigitoxoside	Dxg	3-O-(Dxs)$_2$	5.08 ± 0.23
Digoxoside	Dxg	3-O-(Dxs)$_3$	2.40 ± 0.13
16-Epigitoxigenin	Gg	3β-OH; 16α-OH	Inactive
Gitoroside	Gg	3-O-Dxs	2.38 ± 0.17
Glucogitoroside	Gg	3-O-Dxs-Glu	4.92 ± 0.28
Glucolanadoxin	Gg	3-O-Dxs-Glu; 16β-OFm	5.74 ± 0.27
Formiloxin	Gg	3-O-(Dxs)Fm-(Dxs)Fm-(Dxs) Fm$_3$; 16β-OFm	1.06 ± 0.04
Monoacetylgitoxin	Gg	3-O-(Dxs)Ac-(Dxs)$_2$; 16β-OH	1.01 ± 0.10
Diacetylgitoxin γ,δ	Gg	3-O-(Dxs)Ac-(Dxs)Ac-Dxs; 16β-OH	0.59 ± 0.01
Diacteylgitoxin γ	Gg	3-O-(Dxs)Ac-(Dxs)$_2$; 16β-OAc	0.74 ± 0.12
Triacetylgitoxin	Gg	3-O-(Dxs)Ac-(Dxs)Ac-Dxs; 16β-OAc	0.91 ± 0.11
Pentaacetylgitoxin	Gg	3-O-(Dxs)Ac-(Dxs)Ac-(Dxs) Ac$_3$; 16β-OAc	0.56 ± 0.04

[a] Aglycone abbreviations: Dg = digitoxigenin; Dxg = digoxigenin; Gg = gitoxigenin. [b] Sugar or acyl abbreviations: Dxs = digitoxose; Dls = digitalose; glu = glucose; Glms = glucomethylose; Fcs = fucose; Alms = allomethylose; Ac = acetyl; Fm = formyl.

same animal.[15] Haustein and associates[16] studied 10 acetyl derivatives of gitoxin including the pentaacetyl form in animals and man, and were not impressed by their enteric absorption. Schaumann[17] has reviewed the pharmacology of the entire group of acetylated gitoxins. The structures of these semisynthetic glycosides are not easily identified for Voigtläuder and Balsam[18] could only delineate 15 out of 31 acetyl derivatives of gitoxin and digoxin by nmr spectroscopy.

The cardiac action of formyl derivatives of digitalis glycosides has been reported previously.[19] Formiloxin, or pentaformyl gitoxin, is said to have a favorable intestinal absorption coefficient.[20] A preliminary note on its evaluation in human beings has appeared.[21] It must be realized that it takes extensive studies before a digitalis substitute comes to widespread use. Seventy years passed after digitoxin was isolated when Gold[22] established its full clinical value. This glycoside almost approaches an ideal cardiac therapeutic agent—complete absorption from the intestinal tract, free from impurities, ease of digitalization and maintenance, and economically competitive with the crude drug or other natural digitalis glycosides. Jelliff and coworkers[23] recently showed how digitoxin could be used with mathematical precision. Gold, *et al.*,[24] compared strophanthidinarabinoside with digitoxin in auricular fibrillators, but found it unsuitable for oral administration. Forty-four other cardiotonic substances in our collaborative work similarly failed to achieve a complete absorption from the intestines, the results of which are as yet unpublished. There have been great advances in the chemistry and pharmacology of cardiotonic substances—natural or synthetic. As long as digitoxin is capable of controlling the symptoms of congestive heart failure of large numbers of patients, there is no urgency of developing a substitute unless it offers superiority, such as a shorter latent period.

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Small Ring Analogs of Acetylcholine. Synthesis and Absolute Configurations of Cyclopropane Derivatives[1a]

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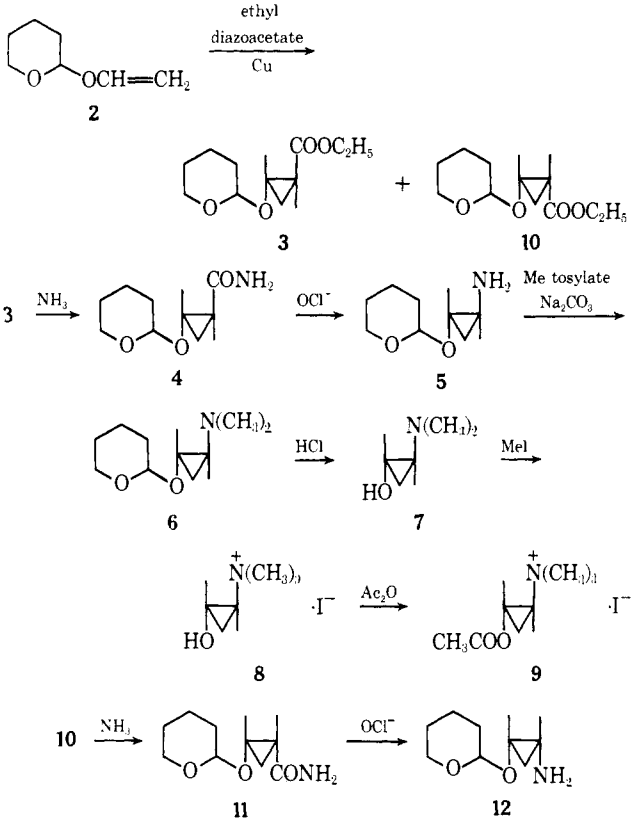
Conformational rigidity has been conferred upon the OCCN portion of acetylcholine by incorporation of the C atoms into a cyclopropane ring. *trans*-2-Acetoxycyclopropyltrimethylammonium iodide has been prepared from an olefinic starting material, 2-vinyloxytetrahydropyran; assignment of the trans configuration to the 1,2-disubstituted cyclopropane systems was based upon literature precedent and upon nmr data, and was confirmed by X-ray crystallographic analysis. Resolution of one of the racemic intermediates in the reaction sequence was achieved, which permitted preparation of both enantiomers of the final product. X-Ray crystallographic analysis has demonstrated that the muscarinically active (+)-*trans*-2-acetoxycyclopropyltrimethylammonium iodide possesses the same absolute configuration as the muscarinically active enantiomers of acetyl-β-methylcholine and muscarine.

Earlier communications have presented preliminary reports of the synthesis[2] of (+)- and (−)-*trans*-2-acetoxycyclopropyltrimethylammonium iodide **9** and the details of the cholinergic effects and enzymatic hydrolysis rates[3] of the two isomers. Herein are presented the details of synthesis of (+)- and (−)-**9** (outlined in Scheme I), and assignments of absolute configurations to the enantiomers.

The 2-tetrahydropyranyl moiety was utilized to mask the cyclopropanol group which is highly susceptible to ring-opening and other undesirable reactions.[4] This protecting group is stable in neutral and basic media, and is easily cleaved under very mild acidic conditions.[5] 2-Vinyloxytetrahydropyran (**2**) reacted with ethyl diazoacetate to give a mixture of the *cis*- and *trans*-cyclopropane isomers (**10** and **3**) which were separable by distillation. The configurational assignments at this stage were tentative and were based upon the expected predominance of the sterically favored trans isomer in reactions of this type.[6] Vpc analysis of the crude reaction mixture indicated a ratio of *trans*-**3** to *cis*-**10** of 45:1. Attempts to modify this ring-closure reaction to attain larger proportions of the cis isomer failed, as did efforts to photoisomerize the trans isomer to cis.[7]

The trans isomer **3** could be converted into the amide **4** either with anhyd NH_3 in ethylene glycol or by use of *n*-BuLi and liq NH_3;[8] while the *n*-BuLi method afforded lower yields than the ethylene glycol method, it permitted a less tedious, less time-consuming work-up. Ammonolysis of the cis ester **10** by the ethylene glycol method (Chart I) gave rise to an amide which could not be obtained analytically pure. Both the trans amide **4** and its cis isomer **11** underwent the Hofmann hypohalite reaction to form the trans and cis primary amines **5** and **12**. The cis amine **12** did not yield a satisfactory analysis for all elements, although spectral data supported its structure. The use of the reaction sequence in Scheme I as a route to the cis isomer of structure **9** was concluded to be unpromising and was abandoned.

SCHEME I



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