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Regioselective Schiff's Base Mediated Deglycosidation of Digitalis Glycosides. New Efficient Synthesis of Digoxigenin Bis-Digitoxoside and Digoxigenin Mono-Digitoxoside

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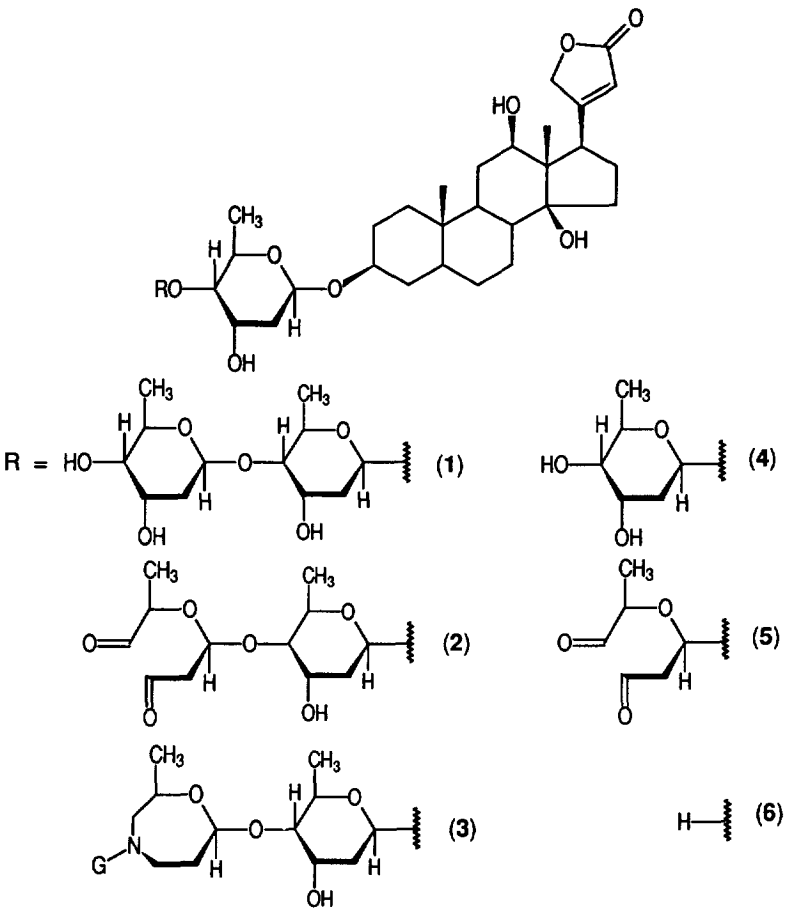
Abstract: Dialdehydes derived from digoxin glycosides via sodium periodate oxidation were regioselectively deglycosylated by various amino acids in *anhydrous* methanolic solution to afford excellent yields of digoxigenin bis-digitoxoside and digoxigenin mono-digitoxoside in a single step.

Plants have been a rich source of bioactive glycosides. The *Digitalis* family, in particular, has produced a number of steroidal glycosides which have been utilized medicinally in the treatment of cardiac diseases for centuries.[1] These glycosides are extensively metabolized in man, producing partially and fully deglycosylated species: for example, the aglycone digoxigenin and its mono- and bis-digitoxosides are the three products resulting from full and partial cleavage of digoxin.[2]

Despite their unique pharmacological properties, monitoring of the serum levels of *Digitalis* glycosides is required for maintenance of clinical efficacy, since the compounds are toxic at higher concentrations.[3] Monitoring by specific immunoassay is further complicated by the cross-reactivities of structurally similar metabolites.[3] There is thus a significant need for quantities of the metabolites of these glycosides for use as cross reactants and for construction of immunological reagents. Although the aglycones can be easily produced by complete hydrolysis of the glycoside moiety, the procedures used to prepare mono- and bis-glycosides are cumbersome[4-7] and result in mixtures of products.[4,6,7]

Here we describe a regioselective method for deglycosidation, using digoxin, the second most commonly prescribed drug in the US,[8] as an example. The method provides the bis- and mono-glycosides in excellent, reproducible yield. The reactions proceed cleanly to completion, and product isolation can be achieved by simple column chromatography.

The method is initiated by oxidative opening of the terminal digitoxose of digoxin with sodium periodate to produce dialdehyde **2**, a transformation well precedented in the carbohydrate literature.[9] Dialdehyde **2** has been reported to undergo reductive aminations with amines to form 3'-oxaperhydro-azepine derivatives (**3**).[10] However, treatment of the dialdehyde with an amino acid in *anhydrous* methanol results in the regioselective cleavage of the oxidized sugar residue to exclusively produce digoxigenin bis-digitoxoside (**4**) *in a single step*. The digoxigenin bis-digitoxoside can be treated similarly to produce the corresponding dialdehyde (**5**) and digoxigenin mono-digitoxoside (**6**).



The success of this transformation requires both Schiff's base formation and mild acid catalysis. In the presence of mild acids alone or alkylamines alone, selective cleavage does not occur. Mild acids cause no reaction; stronger acids unselectively cleave glycosidic linkages, as noted in the previously described method of mono- and bis-digitoxoside preparation.[6,7] In the case of alkylamines alone, Schiff's base formation occurs, but no cleavage takes place, allowing for subsequent reduction as described above[10] to take place.

We have studied several alkylamino acids. As Table 1 illustrates, varying the distance between the amine and acid groups has no effect on the course or yield of the reaction for either the mono- or the bis-digitoxoside formation. Although alkylamine salts of weak acids may produce the desired

product in comparable yield and purity, crystalline amino acids are more easily measured and handled. However, we have observed that salts of alkylamines with strong acids such as hydrochlorides do not react selectively, and produce other products as well as the desired product.

Table 1. Formation of digoxigenin bis-digitoxoside (**4**)[11] and mono-digitoxoside (**6**).[12,13]

Amino Acid	Starting Material	Product	Yield	Purity[a]
glycine	**2**	**4**	84	~100
β-alanine	**2**	**4**	89	99.3
GABA	**2**	**4**	87	98.9
δ-aminovaleric acid	**2**	**4**	91	99.6
ε-aminocaproic acid	**2**	**4**	93	99.3
glycine	**5**	**6**	85	~100
β-alanine	**5**	**6**	87	99.9
GABA	**5**	**6**	81	99.3
δ-aminovaleric acid	**5**	**6**	88	99.7
ε-aminocaproic acid	**5**	**6**	83	99.3

[a] As determined by HPLC.[14]

In summary, we have described a simple method for regioselective deglycosylation, as exemplified by the synthesis of digoxigenin mono- and bis-digitoxosides. Currently, we are exploring the applicability of this method for the synthesis of other natural products. These results will be reported in due course.

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11. Digoxigenin bis-digitoxoside (**4**) showed a mp 217-219°C (lit[6] mp 219-222°C). ^{1}H NMR, ^{13}C NMR, and MS were identical to an authentic sample of digoxigenin bis-digitoxoside (Serva Chemical).
12. Digoxigenin mono-digitoxoside (**6**) showed a mp 212-214°C (lit[6] mp 212-215°C); ^{1}H NMR: (CD_3OD) δ 5.90 (s,1H), 4.93 (m, 4H), 4.00 (m, 2H), 3.73 (m, 1H), 3.32 (m, 2H), 3.14 (dd, 1H, J=9.5 and 5.2 Hz), 2.20-1.25 (m, 19H), 1.22 (d, 3H, J=6.2 Hz), 0.95 (s, 3H), 0.78 (s, 3H); ^{13}C NMR (CD_3OD) δ 178.5, 177.3, 117.7, 96.9, 86.8, 83.8, 75.7, 75.5, 74.4, 70.8, 69.3, 57.3, 47.0, 42.2, 39.9, 38.0, 36.2, 33.6, 33.5, 31.4, 31.0, 30.9, 28.4, 27.8, 27.5, 24.3, 22.8, 18.6, 9.9. HRMS Calc'd for $C_{29}H_{45}O_8$ 521.3114. Found 521.3114.
13. General procedure for the preparation of digoxigenin bis- (**4**) and mono-digitoxoside (**6**): Dialdehyde **2** and dialdehyde **5** were prepared from digoxin and digoxigenin bis-digitoxoside according to a previously described method.[4] Dialdehyde **2** (100 mg, 0.13 mmol) or dialdehyde **5** (100 mg, 0.15 mmol) was dissolved in anhydrous methanol (1.0 ml). Aminocaproic acid (for **2**: 19 mg, 0.12 mmol; for **5**: 18 mg, 0.14 mmol) was added, and the reaction stirred for 12 hrs. at ambient temperature. The orange residue was purified by silica gel chromatography (7% MeOH/ $CHCl_3$), to provide 78 mg (93%) of the bis-digitoxoside or 68 mg (83%) of the mono-digitoxoside as white solids.
14. HPLC Conditions: 8 mm x 10 cm C18 μBondapack column; digoxigenin bis-digitoxoside, 50% acetonitrile/50% water; digoxigenin mono-digitoxoside, 45% acetonitrile/55% water.

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