Reduction of steroidal ketones by *C. acidophila*

Substrates[a]	Products (yields %)[b]
5α-Pregnan-3,6,20-trione (**2**)	3β-Hydroxy-5α-pregnan-6,20-dione (**3**; 45%)
5α-Androstan-3,17-dione (**4**)	3β-Hydroxy-5α-androstan-17-one (**5**; 10%)
	3β,17β-Dihydroxy-5α-androstane (**6**; 2%)
5α-Pregnan-3,11,20-trione (**7**)	3β-Hydroxy-5α-pregnan-11,20-dione (**8**; 3%)
5β-Pregnan-3,12,20-trione (**9**)	None
Androst-4-en-3,11,17-trione (**10**)	None
Androst-1,4-dien-3,11,17-trione (**11**)	None
6β-Hydroxy-pregn-4-en-3,20-dione (**12**)	Pregn-4-en-3,6,20-trione (**13**; 4%)
	5α-Pregnan-3,6,20-trione (**2**; 25%)
6β-Hydroxy-androst-4-en-3,17-dione (**14**)	5α-Androstan-3,6,17-trione (**15**; 35%)
Pregn-4-en-3,60,20-trione (**13**)	5α-Pregnan-3,6,20-trione (**2**; 4%)
	3β-Hydroxy-5α-pregnan-6,20-dione (**3**; 25%)
	3β-Hydroxy-pregn-4-en-6,20-dione (**16**; 13%)
Androst-4-en-3,6,17-trione (**17**)	5α-Androstan-3,6,17-trione (**15**; 10%)
5α-Cholestan-3,6-dione (**18**)	None
5β-Cholanic acid-3,7-dione-methyl ester (**19**)	None

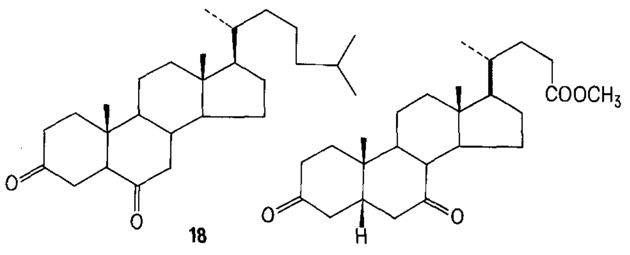
[a] Purchased from Steraloids, Wilton, USA. [b] Isolated yields.

(substrates **7** and **9**) are poorly or not reduced; the presence of carbonyl groups at C-11 also inhibits the hydroxylation-oxidation at C-6 (substrates **10** and **11**) expected because of similarity with the progesterone framework[1]. Δ^4, 6β-hydroxy compounds (substrates **12** and **14**) are oxidized to the corresponding 6-oxo derivatives and, subsequently, reduction of the Δ^4-double bond takes place.

The reduction of the Δ^4-double bond also occurs in Δ^4, 6-oxo derivatives (substrates **13** and **17**); in 1 case (substrate **13**), the product of reduction at C-3 occurring before Δ^4 reduction was also isolated.

Compounds containing a voluminous hydrophobic side chain (substrates **18** and **19**) are not reduced; this result agrees with a previous report[5] of inhibition of the bacterial reductase activity by steroids carrying an aliphatic side chain.

No attempts were made to optimize the single processes; however, acetonized[1] resting cells failed to transform the given substrates, indicating that, unlike other enzymatic activities[6], the reductase activity is lost when *C. acidophila* cells are treated with organic solvents.



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Helminthosporoside, a host-specific toxin from *Helminthosporium sacchari*: a structure revision and a new partial structure[1]

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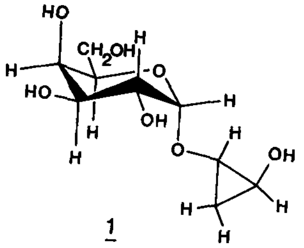
Summary. Reevaluation of the previously proposed structure of helminthosporoside, a host-specific toxin from *Helminthosporium sacchari*, reveals a sesquiterpenoid bis-digalactoside. The carbohydrate portion of the toxin was characterized by ^{13}C-NMR spectroscopy, methylation analyses, FD and FAB mass spectroscopy. The ring size and anomeric configuration of the galactose moieties were determined by utilizing a ^{13}C-NMR structural analysis method. A new partial structure is proposed.

A host-specific toxin of sugarcane produced by *Helminthosporium sacchari* was isolated by Steiner and Byther[3]. An isolated fraction was characterized by NMR, IR, and mass spectroscopy. A linkage assignment was made after enzymatic analyses, and a structure was proposed (**1**). The trivial name of helminthosporoside was suggested for this toxin[4].

In an attempt to verify the validity of the proposed structure for helminthosporoside, we had to repeat the early work. Isolation techniques and bioassays were similar to the original methods of Steiner and Strobel[4]. The yellow-colored toxin preparation obtained by their procedure was decolorized by treatment with activated charcoal in boiling

95% ethanol. The mixture was filtered through Whatman No. 2 filter paper and the filtrate was concentrated by rotary evaporation (40 °C) with no apparent loss of activity. The preparation was separated on a μBondaPAK Carbohydrate Column (Waters Associates, Inc.) with a solvent system of acetonitrile/water 78:22 v/v[5]. The peak containing toxic activity was further purified on a 295 × 0.8 cm Bio-gel P-2 (−400 mesh) chromatography column[6]. A single peak was obtained as determined by monitoring at 196 nm, total carbon analysis, and by toxicity studies.



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The single peak from the Bio-gel column was analyzed by PMR, CMR, field desorption (FD) and fast atom bombardment (FAB) mass spectroscopy. FAB mass spectroscopy revealed a parent ion of 884 m/e. FD mass spectroscopy revealed a $C_{15}H_{22}$ residue, presumably a sesquiterpene[13]. Integration of the proton NMR-spectrum reveals approximately 4 hexose residues to 1 sesquiterpene moiety.

Methylation analyses independently conducted by 2 research groups revealed that galactose was the only carbohydrate present. The analyses also confirmed the presence of a terminal galactofuranosyl residue and an internal 4-linked or 5-linked galactosyl residue. The data from a carbon-13 NMR spectrum of the carbohydrate portion of the toxin reveal resonances corresponding to carbons of the galactose moieties (table).

However, galactose in 2 different chemical arrangements can be observed by ^{13}C-NMR, one as a terminal β-galactofuranosyl and the other as an internal β-galactofuranoside. Since there are 4 galactosyl residues per molecule, this suggests that 2 groups of the digalactofuranosyl moiety must be present. The ring size and stereochemistry at C-1 of the 2 different types of residues can be found by using a new CMR-method for structural analyses of galactosides[7]. The figure shows the CMR-resonances of the carbohydrate moieties and their assigned carbons using the fingerprint method. The CMR-resonances of the carbohydrate moieties compare only with the β-D-galactofuranosides, 1 terminal and 1 internal. Carbon-13 NMR in D_3COD-D_2O, (60:40), revealed 2 resonances at 77.1 ppm and 3 resonances at 81.9 ppm, thereby confirming the carbon assignments made by the fingerprint method.

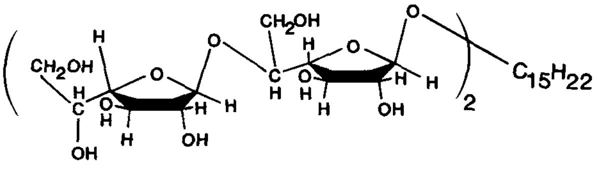
Carbon-13 NMR resonances of the carbohydrate portion of helminthosporoside (90 MHz, D_2O, referenced to external TMS)

ppm	Intensity	Carbon assignments*
107.8	8480	C1′‡
107.1	4135	C1″‡
83.3	9143	C4″
81.9	16166	C2′, C4′, C2″
77.1	11022	C3′, C3″
76.8	7910	C5′
71.2	9520	C5″
63.5	9292	C6″
61.9	8108	C6′

* The single prime refers to the internal galactose and the double prime to the terminal residue. ‡ We recognize that these resonances are very similar, and that the assignments may be reversed.

The ^{13}C-NMR result also explains why Steiner and Strobel only observed less that 1% release of galactose with α- or β-galactosidase[4]. Most likely, the enzyme preparation that they used was slightly contaminated by a β-galactofuranosidase. A β-galactofuranosidase has been isolated that very specifically hydrolyzes β-galactofuranosides, but not α-galactofuranosides or α- and β-galactopyranosides[8].

Partial structure (2), can be written for helminthosporoside. Further work is required to complete the structural determination of the aglycone[10].



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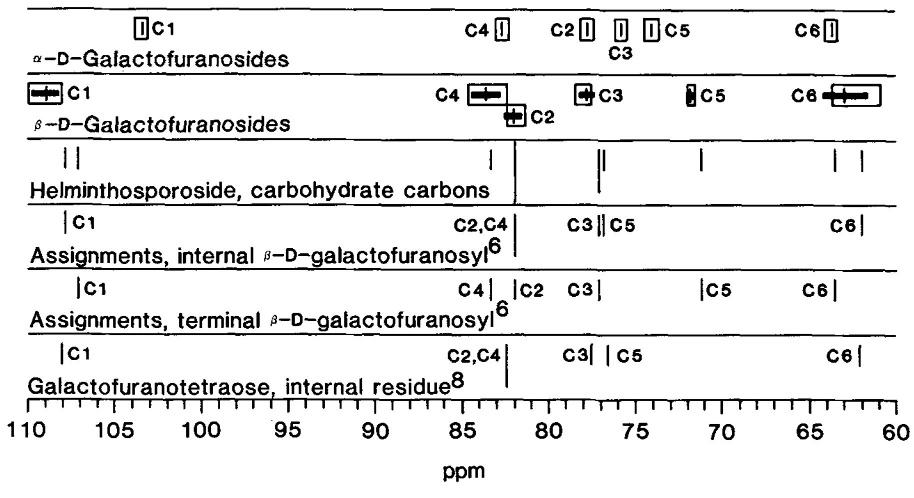

Carbon-13 NMR assignments of the carbons in the carbohydrate portion of helminthosporoside. A rectangular box shows the extreme resonance values for each carbon, a vertical line within the box represents the mean, and a horizontal bar establishes the standard deviation.

1 Acknowledgment. We extend special thanks to C.E. Costello, Department of Chemistry, M.I.T., Cambridge, MA, for FD mass spectral analyses, and to C.E. Ballou, Department of Biochemistry, University of California, Berkeley, CA, for FAB mass spectral analyses. We submit sincere appreciation to both V.N. Reihold, Harvard Medical School, Boston, MA, and P. Albersheim, Department of Chemistry, University of Colorado, Boulder, CO, for methylation analyses. We thank J.S. Frye at the Colorado State University Regional NMR-Center, funded by National Science Foundation grant No.DHE78-18581, Department of Chemistry, Colorado State University, Fort Collins, Co, for high resolution CMR and NMR, and we are grateful to P.W. Jennings, Department of Chemistry, Montana State University, Bozeman, Mt, for total carbon analyses. This work was supported in part by a Herman Frasch Foundation grant to G.A. Strobel, by NSF grant PCM-78-22517, and funds from the Montana Agricultural Experiment Station. B.P. Mundy acknowledges the partial support of NSF (ISP-801149).

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10 A previous structure revision has been published[11]. Although our data do not result in the same conclusions reached by Livingston et al., the importance of understanding the correct structure of this toxin prompts us to report these results. A 2nd effort towards structure determination has been reported[12], and again some differences in proposed structures are found.

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13 *Note added in proof:* This residue correlates to an aglycone of formula $C_{15}H_{24}O_2$. In a recent abstract (Macko, V., Acklin, W., and Agrigoni, D., 183rd American Chemical Society National Meeting, Las Vegas, Nevada, 1982, abstract 252) the structures of 3 isomeric aglycones were reported. We thank Prof. Arigoni for bringing this work to our attention. Prof. Arigoni has also suggested that our toxin, although we thought it to be homogenous, may have also contained the 3 isomers.

Response of serum calcium to administration of an extract from Stannius corpuscles in the anurans

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Summary. The effect of i.p. injection of an aqueous extract of Stannius corpuscles (10 mg/ml/100 g b.wt) on serum calcium levels was investigated in *Rana cyanophlyctis* and *Bufo andersonii.* This treatment evokes in both species hypocalcemia over a period of 1-4 h, followed by hypercalcemia at 6 h, with a return to control at 8 h.

Amphibia occupy an important position in vertebrate phylogeny as they are the 1st class of vertebrates to occupy a terrestrial, as opposed to an aquatic environment. In amphibia, the parathyroid glands make their 1st phylogenic appearance and the corpuscles of Stannius (CS) (present in their piscine ancestors) disappear. However, they possess ultimobranchial bodies similar to those of their piscine ancestors.

It is now generally accepted that the CS produce the hypocalcemic hormones, hypocalcin[1] and teleocalcin[2]. The removal of this gland in teleosts leads to hypercalcemia[3-5] which can be corrected either by homotransplantation[6,7] or by the injections of corpuscular extract[3,7].

So far, there is no report concerning the possible effects of fish Stannius corpuscle extract on amphibian calcium regulation. The present report is, perhaps, the 1st attempt to describe such an effect in anurans, *Rana cyanophlyctis* and *Bufo andersonii.*

Materials and methods. The CS used in this study were obtained from both sexes of adult freshwater catfish, *Heteropneustes fossilis.* The glands were stored in ice and then used almost immediately. The glands were weighed wet and homogenized in ice-cold 0.6% sodium chloride solution (saline). The homogenate was centrifuged (5000 rev/min for 10 min) and the supernatant was collected. The final volume of the supernatant was made up so that 1 ml of the solution contained the extract from 10 mg of wet CS. 60 male *Rana cyanophlyctis* (b.wt 15-20 g) and 60 male *Bufo andersonii* (b.wt 30-45 g) were acclimatized under laboratory conditions for 1 week prior to use. They were then divided into 2 numerically equal groups - a) saline-injected (control); and b) CS extract-injected (experimental).

The experimental specimens were injected i.p. with CS extract at a dosage of 10 mg/ml/100 g b.wt. The control specimens were injected i.p. with 1 ml/100 g b.wt of saline. Blood samples from both the groups were collected by cardiac puncture under ether anesthesia at 0.5, 1, 2, 4, 6, and 8 h following the onset of the treatment. The analysis of serum calcium was made by Trinder's[8] method.

Effect of Stannius corpuscle extract on serum calcium level (mg/100 ml) of *Rana cyanophlyctis* and *Bufo andersonii*

h	*Rana cyanophlyctis*		*Bufo andersonii*	
	Saline	CS extract	Saline	CS extract
0.5	10.61±0.19	10.00±0.33[a]	17.23±0.15	15.45±0.21[c]
1	10.78±0.22	9.28±0.33[b]	16.64±0.16	15.05±0.12[c]
2	10.86±0.17	9.13±0.34[c]	16.85±0.32	14.44±0.19[c]
4	10.74±0.19	9.42±0.41[b]	17.07±0.28	12.17±0.49[c]
6	10.68±0.23	11.26±0.28[a]	17.67±0.09	18.58±0.23[b]
8	10.59±0.15	10.40±0.28	17.80±0.05	18.01±0.34

The values are mean±SD of 5 determinations. [a,b,c] Indicate significant responses. $p < 0.025$, $p < 0.005$ and $p < 0.001$, respectively.