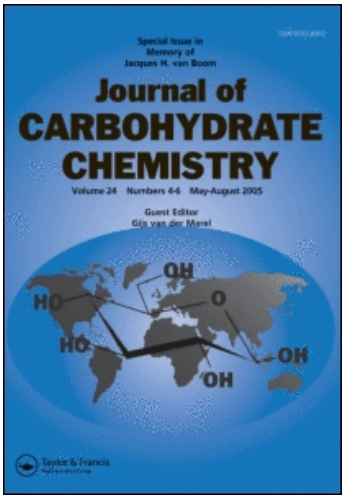


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*COMMUNICATION*

# GLYCOSIDASE-CATALYSED SYNTHESIS OF ERGOT ALKALOID α-GLYCOSIDES

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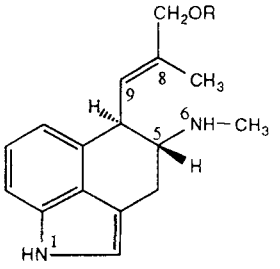
Glycosides are widely occurring natural compounds. Among them, ergot alkaloids, therapeutically potent substances,[1] have been isolated only as mono-[2-4] and oligofructosides.[4,5] The formation of these metabolic by-products in saprophytic cultures of some *Claviceps* strains grown in sucrose media is catalysed by invertase present in fungal mycelia.[3] The other *O*-glycosides of ergot alkaloids, obtained either synthetically[6] using peracetylated glycosyl bromides and $Ag_2CO_3$ as catalyst, or enzymatically,[7-12] have a β-configuration at the sugar anomeric centre. Preparation of some ergot alkaloid α-glycosides needed to complete our studies regarding their potential immunomodulatory and antiviral activities is the subject of this paper.

Enzymatic synthesis was chosen since it avoids multiple protection/deprotection procedures, corresponding enzymes are readily available and their rather low specificity for acceptors allows a reaction with a variety of substrates.[13,14] Parent compounds - chanoclavine (**1a**), elymoclavine (**2a**), lysergol (**3a**), and 9,10-dihydrolysergol (**4a**), were kindly donated by Galena Pharmaceuticals (Opava, Czech Republic). Enzyme preparations of α-galactosidase (EC 3.2.1.22; from green coffee beans), α-glucosidases (EC 3.2.1.20; maltase, type IV from brewers yeast, type V from rice, from *Bacillus*

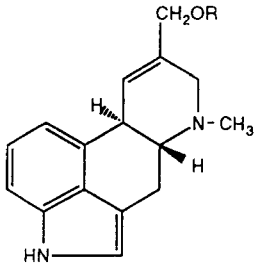
*stearothermophilus*), β-galactosidase (from *Aspergillus oryzae*, grade XI, source of α-galactosidase), β-glucuronidase (from limpets, source of α-L-fucosidase), and defatted almond meal (source of α-mannosidase) were obtained from Sigma. The purification of α-galactosidase from β-galactosidase (*A. oryzae*) and α-mannosidase from almond meal was carried out as described elsewhere.[15] Other chemicals were of analytical grade.

Enzymes listed in Table 1 were employed for the reactions on a semipreparative scale. In a typical experiment, the alkaloid (150 mg, 0.5 mmol), activated donor (*p*NP-α-Gal or *p*NP-α-Man, 150 mg, 0.5 mmol; *p*NP-α-Fuc, 30 mg, 0.1 mmol; maltose, 1.04 g, 3 mmol, respectively) were dissolved in McIlvain buffer (pH 5, 2 mL, 50 mM), and citric acid (approx. 60 mg) was added to adjust pH to 5. The enzyme (α-galactosidase 2.3 U, α-mannosidase 1.3 U, α-L-fucosidase 0.35 U, α-glucosidase 6 U, respectively) was added to this solution at 30 °C, the mixture was stirred until reaching a maximum conversion (less that 24 h). Product formation was monitored by TLC (Kieselgel, Merck, Germany; dichloromethane-methanol-ammonia 80:20:0.1, detection with Ehrlich reagent) or HPLC (column 150 x 3.3 mm, Separon SGX $C_{18}$ 7 μm, Tessek, Czech Republic, methanol-water-ammonia 60:40:0.04, flow rate 0.6 mL/min). Upon enzyme deactivation by heating (5 min, 100 °C) the liberated *p*-nitrophenol was extracted with diethyl ether. The products were isolated by solid phase extraction (XAD-2 resin, BDH) followed by flash chromatography (silica gel column, Merck, 0.04 - 0.063 mm; dichloromethane-ammonia (0.1%)-methanol (5 - 25% according to aglycone polarity). Some products were further purified by preparative HPLC (Spectra Physics, injected volume 100 μL containing 3 mg of alkaloid, detection at 280 nm, flow rate 3.0 mL/min, column 250 x 8 mm, Separon SGX $C_{18}$ 7 μm, Tessek, Czech Republic, methanol-water-ammonia 50:50:0.04 (**2b**, **2c**), 55:45:0.04 (**2d**), 60:40:0.04 (**3c**), 70:30:0.04 (**4b**, **4d**), 80:20:0.04 (**1d**)).

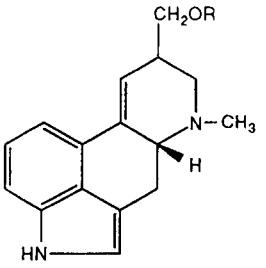
All new compounds gave the expected $[M+H]^+$ and $[M+Na]^+$ peaks in their mass spectra (Table 1) as recorded on a double-sector instrument Finnigan MAT 95 (positive-ion electrospray ionisation mass spectra), and on a double-focusing Finnigan MAT 90 instrument (positive-ion fast atom bombardment mass spectra). $^1H$ and $^{13}C$ NMR parameters, obtained with Varian VXR-400 spectrometer, were similar to those of the parent compounds, with the exception of glycosidation shifts at C-17 varying according to aglycon (chanoclavine 8.6 - 9.0 ppm, elymoclavine 0.8 - 0.9 ppm, lysergol 5.1 - 5.2 ppm, dihydrolysergol 1.8 - 2.3 ppm). The nature of attached sugar follows from characteristic vicinal H,H couplings (Table 2). The α-linkage was inferred from the typical $J_{1,2}$ couplings[16] (ibid.). With compound **3c**, this deduction was confirmed by direct coupling[17,18] of C-1 (168.3 Hz). The structure of αGal*p*(1→3)αGal*p*(1→17-*O*)-elymoclavine was assigned to **2c** using the data in Tables 1 and 2, and the $^{13}C$ chemical



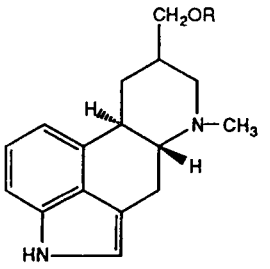

| | R |
|---|---|
| **1a** | H |
| **1b** | α-D-Gal |
| **1c** | α-D-Glc |
| **1d** | α-D-Man |
| **1e** | α-L-Fuc |




| | R |
|---|---|
| **2a** | H |
| **2b** | α-D-Gal |
| **2c** | α-D-Gal(1→3) α-D-Gal |
| **2d** | α-D-Man |




| | R |
|---|---|
| **3a** | H |
| **3b** | α-D-Gal |
| **3c** | α-D-Man |




| | R |
|---|---|
| **4a** | H |
| **4b** | α-D-Gal |
| **4c** | α-D-Glc |
| **4d** | α-D-Man |

**Table 1.** α-Glycosides of ergot alkaloids - yields and MS spectra.

| Enzyme (source) | Substrate | Donor | Product | Yield [%] | $[M+H]^+$ | $[M+Na]^+$ | Method |
|---|---|---|---|---|---|---|---|
| α-galactosidase (*A. oryzae*) | **1a** | *p*NP | **1b** | 24 | 419 | 441 | ESI |
| | **2a** | *p*NP | **2b** | 23 | 417 | 439 | ESI |
| | | | **2c** | 3 | 579 | 601 | FAB |
| | **3a** | *p*NP | **3b** | 6 | 417 | 439 | ESI |
| | **4a** | *p*NP | **4b** | 11 | 419 | 441 | FAB |
| α-glucosidase (rice, type V) | **1a** | maltose | **1c** | 8 | 419 | 441 | FAB |
| | **4a** | maltose | **4c** | 3 | 419 | 441 | FAB |
| α-mannosidase (almond) | **1a** | *p*NP | **1d** | 18 | 419 | 441 | ESI |
| | **2a** | *p*NP | **2d** | 3 | 417 | 439 | ESI |
| | **3a** | *p*NP | **3c** | 3 | 417 | 439 | ESI |
| | **4a** | *p*NP | **4d** | 2 | 419 | 441 | FAB |
| α-L-fucosidase (limpet) | **1a** | *p*NP | **1e** | 2 | 403 | 425 | ESI |

**Table 2.** Diagnostic proton-proton couplings $J_{i,j}$ [Hz] of sugar moieties (400 MHz, $CD_3OD$, 25 °C)

| Compound | Configuration | $J_{1,2}$ | $J_{2,3}$ | $J_{3,4}$ | $J_{4,5}$ | $J_{5,6u}$ | $J_{5,6d}$ | $J_{6u,6d}$ |
|---|---|---|---|---|---|---|---|---|
| **1b** | *galacto-* | 3.6 | 10.1 | 3.1 | 1.3 | 5.3 | 6.7 | 11.4 |
| **2b** | *galacto-* | 3.5 | 10.1 | 3.1 | 1.4 | 5.2 | 6.8 | 11.3 |
| **2c** | *galacto-* | 3.1 | 10.0 | 2.6 | 1.4 | 6.0 | 6.8 | 11.3 |
| | *galacto-* | 3.2 | 9.9 | 2.1 | 1.2 | 5.9 | 7.2 | 11.3 |
| **3b** | *galacto-* | 3.5 | 10.1 | 3.1 | 1.3 | 6.1 | 6.0 | 11.9 |
| **4b** | *galacto-* | 3.5 | 10.1 | 3.1 | 1.3 | 5.7 | 6.5 | 11.3 |
| **1c** | *gluco-* | 3.8 | 9.7 | n.d. | 10.9 | 5.8 | 2.0 | 10.9 |
| **4c** | *gluco-* | 3.8 | 9.7 | 9.0 | 10.8 | 5.8 | 2.0 | 11.7 |
| **1d** | *manno-* | 1.7 | 3.4 | 9.5 | 9.5 | 6.0 | 2.0 | 11.8 |
| **2d** | *manno-* | 1.7 | 3.4 | 8.9 | 9.7 | 5.8 | 2.0 | 11.8 |
| **3c** | *manno-* | 1.7 | 3.2 | 8.8 | 9.6 | 5.6 | 2.1 | 12.0 |
| **4d** | *manno-* | 1.7 | 3.4 | 9.3 | 9.6 | 6.0 | 2.3 | 11.7 |
| **1e** | *fuco-* | 3.0 | 11.1 | 2.4 | 1.2 | 6.6 | - | - |

n.d. not determined

shift examination (downfield shift of C-3', upfield shifts of C-2' and C-4' in comparison with **2b**).

Digalactoside **2c** contains the same type 1→3 bond as found with this enzyme acting on lactose thioglycosides[19,20] or in the side reaction of β-*N*-acetylhexosaminidase with elymoclavine.[8] The formation of **1c** was also detected with α-glucosidase from *Bacillus stearothermophilus*; no products were found using maltase or α-glucosidase from brewers yeast. The α-glucosidase from rice has also been reported to glucosylate ascorbic acid.[21] Our yields of mannosylated ergot alkaloids were similar to those observed with α-mannosidase from jack beans.[10]

The application of crude enzymatic preparations for a one-step synthesis of ergot alkaloid glycosides makes this approach an interesting alternative to conventional chemical synthesis. Despite low yields obtained with some derivatives, the separation of final products was fairly straightforward and the method is worth consideration. Biological activities of new compounds will be reported elsewhere.



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