AGROCLAVINE GLYCOSYLATION

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Agroclavine was converted into its 1-hydroxymethyl derivative by condensation with formaldehyde and enzymatically glycosylated using β-galactosidase from *Aspergillus oryzae*.
Key words: Ergot alkaloids; Glycosides; Agroclavine; β-Galactopyranosides; β-Galactosidase; *Aspergillus oryzae*; Enzymatic reactions; Biocatalysis.

Agroclavine (**1**) is a simple ergot alkaloid having an 8-ergolene skeleton that is sometimes trivially classified as clavine. Although this compound itself is not used in medicine, it has interesting biological effects. Agroclavine acts on α-adrenoreceptors or 5-hydroxytryptamine receptors[1] and causes vasoconstriction, exhibits a cytostatic activity comparable with that of clinically used adriamycin[2], possesses a well documented antibiotic activity[3], stimulates the killing capability of NK-leukocytes against the NK-resistant target cells[3,4], and enhances the production of certain interleukins[6] (IFN-γ, IL-2). We have shown in our previous studies that *O*-glycosylation of some ergot alkaloids increases their immunomodulatory effects[7]. Although agroclavine has no functional groups, the corresponding *N*-ribosides[8] and *N*-deoxyribosides[9] were successfully prepared. However, monosaccharide units present on the cell surface are required for proper comparison of these effects. Therefore, we prepared a 1-hydroxymethyl derivative of agroclavine using the known procedure[10] and attempted its *O*-glycosidation by enzymatic methods.

EXPERIMENTAL

NMR spectra were measured on a Varian VXR-400 spectrometer (399.95 MHz for ^{1}H, 100.58 MHz for ^{13}C) at 25 °C. Carbon signal multiplicity was determined by APT (Attached Proton Test). The reported signal assignment is based on COSY, delay-COSY, and HETCOR experiments and is consistent with that of parent compound[11] (**1**).

Positive-ion electrospray ionization mass spectra (ESI-MS) were recorded on a double-focusing instrument Finnigan MAT 95 (Finnigan MAT, Bremen, Germany) of BE geometry. Samples dissolved in $CH_3CN–H_2O$ (1 : 1) were continuously infused through a stainless capillary held at 4.3 kV into the electrospray ion source *via* linear syringe pump at a flow rate of 50 µl/min. For high resolution experiments the instrument was tuned to a resolution of about 6 000 (10% valley definition) and the measurements were carried out by the peak-matching method against a mixture of polypropylene glycols (average M_r = 425) as an internal standard.

1-(Hydroxymethyl)agroclavine (**2**)

Agroclavine (**1**; 234 mg, 1 mmol) was heated under reflux with aqueous formaldehyde (37%, 4 ml). All starting material was converted into the product **2** after 1 h (TLC: $CHCl_3$–MeOH 8 : 2, detection by charring with 5% H_2SO_4). After cooling the reaction mixture was extracted with ethyl acetate (3 × 10 ml), the combined extracts were washed with water and brine. Crude yellowish product (217 mg) was obtained after evaporation. Recrystallization from acetone afforded white crystals of **2** (140 mg, 52%). ESI-MS, *m/z*: 269 $[M + H]^+$ (for $C_{17}H_{21}N_2O$ calculated: 269.1654, found: 269.1655).

1-[(β-D-Galactopyranosyloxy)methyl]agroclavine (**3**)

Compound **2** (80 mg, 0.3 mmol) was dissolved in McIlvain buffer (pH 4.6, 0.5 ml) and the solution was adjusted to pH 4.6 by addition of citric acid (typically 40 mg). 4-Nitrophenyl-β-D-galactopyranoside (60 mg, 0.2 mmol) was added and the reaction was started by addition of β-galactosidase (13 U, 2 mg) from *A. oryzae* (Sigma). The mixture was incubated at 30 °C, after 1 h another portion of 4-nitrophenyl-β-D-galactopyranoside (60 mg, 0.2 mmol) was added and after total 3 h the reaction was stopped by 5 min boiling. The cool mixture was extracted with diethyl ether to remove bulk of the 4-nitrophenol formed, traces of ether were removed by short evaporation *in vacuo*, the mixture was diluted to 10 ml with water and passed slowly through a column (1 × 20 cm) filled with polystyrene resin SM-2 (BioRad, U.S.A.) previously washed with acetone (3 times) and well with water. After passing the mixture, the column was washed with water (50 ml) and then the mixture of alkaloids was eluted with MeOH (20 ml). This treatment removes all salts, free sugars and proteins that would complicate final purification. The methanolic eluate was concentrated *in vacuo*, loaded onto a silica gel G 60 (Merck) flash column and eluted with a mixture $CHCl_3$–MeOH (8 : 2) with slow gradient of MeOH up to (8 : 3.5). The product (42 mg) was recrystallized from MeOH to afford **3** (35 mg, 27%). ESI-MS, *m/z*: 431 $[M + H]^+$ (for $C_{23}H_{31}N_2O_6$ calculated: 431.2182, found: 431.2185). Unreacted **2** can be nearly quantitatively recovered. The purity of the product **3** was higher than 98% according to the HPLC (ref.[12]), no signals of impurity were found in ^1H NMR.

RESULTS AND DISCUSSION

Agroclavine (**1**) has no hydroxyl available for glycosidation. Thus, a different strategy was adopted, attaching a suitable spacer with a free OH group to the indole N–H and glycosylation. Hydroxymethylation of agroclavine was attained by condensation with formaldehyde[10]. The reaction product **2**

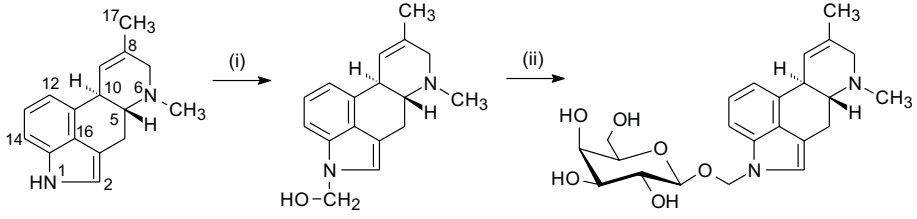
TABLE I
^{1}H and ^{13}C NMR spectra of compounds **2** and **3** (for detailed NMR spectra of **1** see ref.[11]). Chemical shifts in ppm (δ-scale), coupling constants (*J*) in Hz

Atom	**2**[a]			**3**[b]		
	δ_C	δ_H	*J*	δ_C	δ_H	*J*
			Alkaloid unit			
2	119.86	6.881 dd	1.8, 0.6	118.29	7.091 dd	1.8, 0.4
3	112.35	–		113.36	–	
4	26.64	2.716 ddd	14.3, 11.7, 1.8	27.31	2.817 ddd	13.7, 11.8, 1.8
		3.259 ddd	14.3, 4.0, 0.6		3.415 ddd	13.7, 3.2, 0.4
5	64.05	2.528 ddd	11.7, 9.5, 4.0	65.53	2.721 ddd	11.8, 9.4, 3.2
7	60.64	2.946 dddq	16.3, 4.0, 2.3, 1.2	61.25	3.412 dm	16.0
		3.193 dddq	16.3, 2.4, 1.2, 0.6		3.914 dm	16.0
8	132.12	–		132.82	–	
9	121.73	6.151 m		121.04	6.283 m	
10	40.57	3.680 dm	9.5	41.02	3.762 m	
11	132.53	–		132.88	–	
12	113.47	6.970 ddd	7.1, 1.4, 0.7	114.79	7.037 ddd	7.1, 1.4, 0.8
13	123.34	7.158 dd	8.3, 7.1	124.67	7.192 dd	8.2, 7.1
14	107.73	7.232 ddd	8.3, 1.0, 0.7	109.17	7.389 ddd	8.2, 0.8, 0.6
15	134.21	–		136.71	–	
16	127.64	–		128.49	–	
17	21.06	1.735 dddd	2.3, 1.2, 1.0, 0.6	21.07	1.841 m	
NCH_3	40.71	2.450 s		41.65	2.589 s	
NCH_2O	69.60	5.487 s		73.80	5.673 d	11.1
					5.900 d	11.1
			Carbohydrate unit			
1′				101.05	4.128 d	7.8
2′				72.47	3.529 dd	9.7, 7.8
3′				75.11	3.420 dd	9.7, 3.5
4′				70.60	3.793 dd	3.5, 1.1
5′				77.40	3.423 ddd	7.2, 5.0, 1.1
6′				61.25	3.732 dd	11.5, 5.0
					3.818 dd	11.5, 7.2

[a] Measured in $CDCl_3$–CD_3OD (4 : 1) mixture. [b] Measured in CD_3OD.

gave a $[M + H]^+$ ion at *m/z* 269 ($C_{17}H_{21}N_2O$) in ESI-MS. Its NMR spectra (Table I) lacked the signal of indole N–H and its couplings but it contained signals due to an additional OCH_2 group. Long-range couplings of its protons to H-2 and H-14 confirmed the localization at N-1. This reaction also afforded an analogous product with 17-acetoxyagroclavine (17-*O*-acetylelymoclavine) in yield of *ca* 85% (data not given).

Because of many drawbacks of chemical glycosylation of ergot alkaloids[10] (decomposition of the starting material, orthoester formation), we used a proven enzymatic method[13,14]. Successful β-galactosylation of **2** was achieved by β-galactosidase from *Aspergillus oryzae* (Scheme 1). The resulting glycoside **3** had a $[M + H]^+$ ion at *m/z* 431 ($C_{23}H_{31}N_2O_6$) in its ESI-MS and NMR spectra were consistent with this structure. The D-*galacto*-configu-



(i) aqueous CH_2O, 1 h, 100°C;
(ii) 4-nitrophenyl β-D-galactopyranoside, β-galactosidase from *Aspergillus oryzae*

SCHEME 1

ration was inferred from the characteristic vicinal couplings (Table I), anomeric configuration from $J(1',2') = 7.8$ Hz. In ^{13}C NMR the NCH_2O carbon is shifted 4.2 ppm downfield with respect to the parent compound; its protons exhibit a magnetic nonequivalence (0.227 ppm) in ^1H NMR and one of them (5.673 ppm) a NOE to H-1′.

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