A Novel Glycosidically Linked Piperidine Alkaloid
From Cyclamen Coum

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The structure of a novel piperidine type alkaloid from Cyclamen coum was established as 2-β-D-glycopyranosyl-2-undecil-3,5-dihydroxy-6-carboxypiperidine, 1, whose structure has been deduced from spectral data.

Introduction

The occurrence of piperidine alkaloid in marine organisms is mentioned in the literature\(^1-6\). In the course of our studies on bioactive substances from cyclamen organisms, we isolated glucose and undecil substituted piperidine-type alkaioide derivative from the Cyclamen coum and its structure was deduced as 1 from its NMR and FAB-MS spectral data.

Experimental

**Instrumentation:** NMR spectra were recorded on a Bruker AC 200L NMR at 200 MHz instrument in C\(_6\)H\(_5\)N using TMS as internal standard. IR spectra were taken on a Perkin Elmer 1600 spectrophotometer. (+) FAB-MS spectra were recorded on a Zabspec MS instrument, Flash column chromatography was performed on a silica gel 60 (230-400 mesh) and preparative TLC was performed with precoated silica gel F\(_{254}\) (20 x 20 cm 0.2 mm) plates. A voucher specimen has been deposited in deepfreeze at the Department of Chemistry, Karadeniz Technical University.

**Isolation of compound 1:** Specimens of the Cyclamen coum were collected in the Giresun Yaghdere region, in the north of Turkey, in March, 1995. The chopped wet plants (~1500 g) were extracted with cold CH\(_3\)OH (1.5 lt, 3 times, 24 hours each). The total aqueous CH\(_3\)OH extract was filtered, and the filtrate was concentrated on a rotary evaporator at 30°C. The aqueous extract thus obtained (0.4 liter) was extracted with CHCl\(_3\) (150 ml, 3 times). After collecting CHCl\(_3\) extract (450 ml), it was evaporated in vacuo at 30-35 °C. The crude mixture obtained (0.9 g) was chromatographed on a Kieselgel 60 (40 g, 230-400 mesh) flash column chromatograph. Elution with n-hexane, followed by discontinuous gradient elution with n-hexane-CHCl\(_3\) (3:1-1:4) and CHCl\(_3\) and then discontinuous gradient elution with CHCl\(_3\)-CH\(_3\)OH (9:1-2:3) and finally with CHCl\(_3\)-CH\(_3\)OH-H\(_2\)O (2:2.6:0.4) gave 43 fractions (ca. 15-20 ml each). Fractions
A Novel Glycosidicly Linked Piperidine Alkaloid From Cyclamen Coum, N. YAYLI & C. BALTACI

39-40 were combined after the analyses of TLC to give the ninth fraction (24.2 mg). The ninth fraction was chromatographed on a Kieselgel 60 (6 g, 230-400 mesh) flash column chromatograph. Elution with, respectively, n-hexane (30 ml), CHCl₃ (30 ml), and then discontinuous gradient elution with CHCl₃CH₃OH (50 ml) (10:1-10:2) gave 34 fractions (ca. 3-4 ml each). Fractions 25-28 were combined after TLC analysis to give compound 1 (17.2 mg) (CHCl₃CH₃OH, 0:0.5, Rf = 0.35) IR (KBr) ν max 3500-2500, 3500-3200, 3320, 2927, 1630, 1378-1360, 1075, 1048, 1025 cm⁻¹; ¹H NMR (C₅D₅N, 200 MHz) and ¹³C NMR (C₅D₅N, 50 MHz) (see Table 1); positive FAB-MS (MNBA) m/z 493(5) [M]+, 409(43) [M-85+H]+, 295(100) [M-175-H₂O+H]+, 235(55) [M-179-2H₂O-CO₂ + H]+, 159(10) [M-179-side-chaine(155)]+; and 155, 141, 127, 113, 99, 85, 71, 57.

Table 1. NMR Data for Compound 1 (200 MHz, C₅D₅N).

<table>
<thead>
<tr>
<th>No</th>
<th>¹³C (δ,ppm)b</th>
<th>APT</th>
<th>¹H (δ,ppm)c</th>
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<tr>
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<td>-</td>
<td>-</td>
<td>8.48 d, J=9 Hz</td>
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<tr>
<td>2</td>
<td>70.46</td>
<td>C</td>
<td></td>
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<tr>
<td>3</td>
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<td>CH</td>
<td>5.50</td>
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<td>18'</td>
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<td>CH₃</td>
<td>0.92 t, J=6.15 Hz</td>
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aChemical shifts (ppm) are relative to internal TMS in C₅D₅N.
bAssignments assisted by HETCOR data.
cAssignments assisted by COSY data.
dUnobserved, in exchange with solvent

Hydrolysis of Compound 1: Compound 1 (4 mg) was hydrolyzed with 10% H₂SO₄ for 4 hours. The residue obtained showed the presence of D-glucose when compared with an authentic sample of this sugar on TLC (CH₃OH, Rf = 0.74).
Result and Discussion

The new piperidine type of glycosidically linked alkaloid was isolated from a methanolic extract of *Cyclamen coum*. We assigned the alkaloid structure based on the following evidence. The $^1$H NMR (pyridine-d$_5$, 200 MHz) spectrum of the compound exhibited a characteristic signal for an anomic proton at $\delta$ 5.00 ppm. The coupling constant ($J = 7.4$ Hz) implied a $\beta$-configuration of the sugar residue. The $^{13}$C NMR (pyridine-d$_5$, 50 MHz) spectra of 1 showed one signal at $\delta$ 105.62 ppm for the anomic carbon signal, also indicate of a $\beta$-configuration$^6$-$^7$.

The -NH- protons were observed at $\delta$ 8.48 (1H, d, J=9 Hz) ppm in the $^1$H NMR spectrum. The carboxylic proton was unobserved in the $^1$H NMR spectrum because of exchange with the solvent$^8$. The $^1$H NMR spectrum further showed piperidine ring signals at $\delta$ 5.50 (1H), 5.31(1H), 4.62 (1H) and 2.09-1.09 (2H) ppm. The side chain protons were also observed in the $^1$H NMR spectrum at $\delta$ 1.65 (2H), 1.24 (18H) and 0.92 (3H, t, J = 6.15 Hz) ppm.

Standard 1D and 2D NMR procedures were employed to elucidate the structure of compound 1. Conventional $^1$H (200 MHz) and $^{13}$C (50 MHz) NMR spectra combined with multiplicity-selected (APT) $^{13}$C data yielded the gross structure of the molecule and showed it to consist of a hydroxy and carboxy substituted piperidine ring (C$_8$H$_9$O$_4$), a monosaccharide (C$_6$) sugar and long chain hydrocarbon (C$_{11}$) moiety. The COSY map afforded a comprehensive description of through-bond proton-proton connectivities. Corroborative evidence for the molecular structure thus derived was gleaned from the $^{13}$C-$^1$H chemical shift correlation (HETCOR).

The broad-bond $^{13}$C NMR spectrum (pyridine-d$_5$, 50 MHz) of compound 1 showed a carboxylic carbonyl signal at $\delta$ 175.68 ppm. The IR spectrum also showed bands for carboxyl (C=O; 1630, COO-H; 2500-3500 cm$^{-1}$) and 2° amine (-NH-; 3320 cm$^{-1}$) functionalities.

In order to identify the sugar moiety, compound 1 was hydrolyzed with 10% H$_2$SO$_4$. The residue obtained showed the presence of D-glucose when compared with the authentic sample of this sugar on TLC.

The assigned $^1$H and $^{13}$C resonance for compound 1 is shown in Table 1. Comparisons of the spectral data in Table 1 with the published spectra of related alkaloids$^1$-$^5$,$^9$-$^{13}$ showed glucose to be a glucopyranosyl, and alkaloid a substituted piperidine type ring having hydroxy, carboxy and long chain hydrocarbon. The positive ion FAB mass spectrum (MNBA) of glycosidically linked piperidine type alkaloid exhibited prominent ions at m/z 493(5) [M]$^+$, 409(43) [M-85+H]$^+$, 295(100) [M-175-H$_2$O+H]$^+$, 2$^+$,55) [M-179-2H$_2$O-CO$_2$+H]$^+$ and 159(10) [M-179-side-chain(155)]$^+$ corresponding to C$_{23}$H$_{43}$NO$_{10}$ (Figure 1). The length of the side-chain was determined with the aid of $^{13}$C, APT NMR and FAB-MS spectra$^{14}$.

Thus, we conclude that compound 1 has the structure 2-$\beta$-glycopyranosyl-2-undecyl-3,5-dihydroxy-6-carboxy piperidine, which is a novel natural product elucidate from the *Cyclamen coum*. The stereochemistry and synthesis of this compound is currently under investigation.

Acknowledgements

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A Novel Glycosidically Linked Piperidine Alkaloid From Cyclamen Coum, N. YAYLI & C. BALTACI

\[
\begin{align*}
&\text{OH} & \text{O} \\
&\text{HO} & \text{HO} \\
&\text{HO} & \text{O} \\
&\text{OH} & \\
&\text{OH} & \\
\end{align*}
\]

2-\(\beta\)-D-glucopyranosyl-2-undecyl-3,5-dihydroxy-6-carboxy piperidine, \(1\)

\[
\begin{align*}
&\text{OH} & \text{O} \\
&\text{HO} & \text{HO} \\
&\text{HO} & \text{O} \\
&\text{OH} & \\
&\text{OH} & \\
\end{align*}
\]

\(\text{C}_{23}\text{H}_{43}\text{NO}_{10}, \{\text{M}\}^+, 493(5)\)

**Figure 1.** FAB-MS (m/z) spectral analysis of compound 1.

**References**

