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A Novel Glycosidicly 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 alkaloide 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_5H_5N 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 preperative TLC was performed with precoated silica gel F_{254}(20 × 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 Yağlıdere region, in the north of Turkey, in March, 1995. The chopped wet plants (~ 1500 g) were extracted with cold CH_3OH (1.5 lt, 3 times, 24 hours each). The total aqueous CH_3OH 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_3OH (9:1-2:3) and finally with $CHCl_3$-CH_3OH-H_2O (2:2.6:0.4) gave 43 fractions (*ca.* 15-20 ml each). Fractions

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), $CHCl_3$ (30 ml), and then discontinuous gradient elution with $CHCl_3$ CH_3OH (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) ($CHCl_3$-CH_3OH, 0:0.5, R_f = 0.35) IR (KBr) v max 3500-2500, 3500-3200, 3320, 2927, 1630, 1378-1360, 1075, 1048, 1025 cm^{-1}; ^1H NMR (C_5D_5N, 200 MHz) and ^{13}C NMR (C_5D_5N, 50 MHz) (see Table 1); positive FAB-MS (MNBA) m/z 493(5) $[M]^+$, 409(43) $[M-85+H]^+$, 295(100) $[M-175-H_2O+H]^+$, 235(55) $[M-179-2H_2O-CO_2+H]^+$, 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, C_5D_5N).

	1[a]		
No	^{13}C (δ,ppm)[b]	APT	^1H (δ,ppm)[c]
1.....	-	-	8.48 d, J=9 Hz
2.....	70.46	C	
3.....	78.48	CH	5.50
4.....	35.59	CH_2	2.09-1.09
5.....	75.92	CH	4.62
6.....	51.75	CH	5.31
-COOH.....	175.68	C	[d]
1′.....	105.62	CH	5.00 d, J=7.4 Hz
2′.....	75.18	CH	4.02
3′.....	78.48	CH	3.90
4′.....	72.46	CH	4.12
5′.....	78.49	CH	4.30
6′.....	62.64	CH_2	4.50-4.12
1″.....	35.59	CH_2	1.65
2″.....	34.00	CH_2	1.24
3″.....	32.13	CH_2	1.24
4″.....	29.63	CH_2	1.24
5″.....	30.02	CH_2	1.24
6″.....	30.02	CH_2	1.24
7″.....	30.02	CH_2	1.24
8″.....	27.95	CH_2	1.24
9″.....	25.90	CH_2	1.24
10″.....	22.95	CH_2	1.24
11″.....	14.30	CH_3	0.92 t, J=6.15 Hz

[a]Chemical shifts (ppm) are relative to internal TMS in C_5D_5N.

[b]Assignments assisted by HETCOR data.

[c]Assignments assisted by COSY data.

[d]Unobserved, in exchange with solvent

Hydrolysis of Compound 1: Compound **1** (4 mg) was hydrolyzed with 10% H_2SO_4 for 4 hours. The residue obtained showed the presence of D-glucose when compared with an authentic sample of this sugar on TLC (CH_3OH, R_f = 0.74).

Result and Discussion

The new piperidine type of glycosidicly 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 anomeric proton at δ 5.00 ppm. The coupling constant (J = 7.4 Hz) implied a β-configuration of the sugar residue. The ^{13}C NMR (pyridine-d_5, 50 MHz) spectra of **1** showed one signal at δ 105.62 ppm for the anomeric carbon signal, also indicate of a β-configuration[6-7].

The -NH- protons were observed at δ 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 δ 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 δ 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 ($C_6H_9O_4$), a monosaccharide (C_6) sugar and long chain hydrocarbon (C_{11}) moity. 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 δ 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_2SO_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 glucone 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 glucosidicly linked piperidine type alkaloid exhibited prominent ions at m/z 493(5) $[M]^+$, 409(43) $[M\text{-}85+H]^+$, 295(100) $[M\text{-}175\text{-}H_2O+H]^+$, 277(55) $[M\text{-}179\text{-}2H_2O\text{-}CO_2+H]^+$ and 159(10) [M-179-side-chaine(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-β-glycopyranosyl-2-undecil-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.

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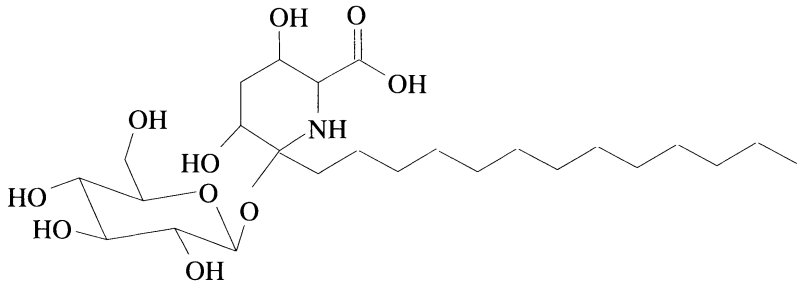

2-β-D-glycopyranosyl-2-undecil-3,5-dihydroxy-6-carboxy piperidine, **1**

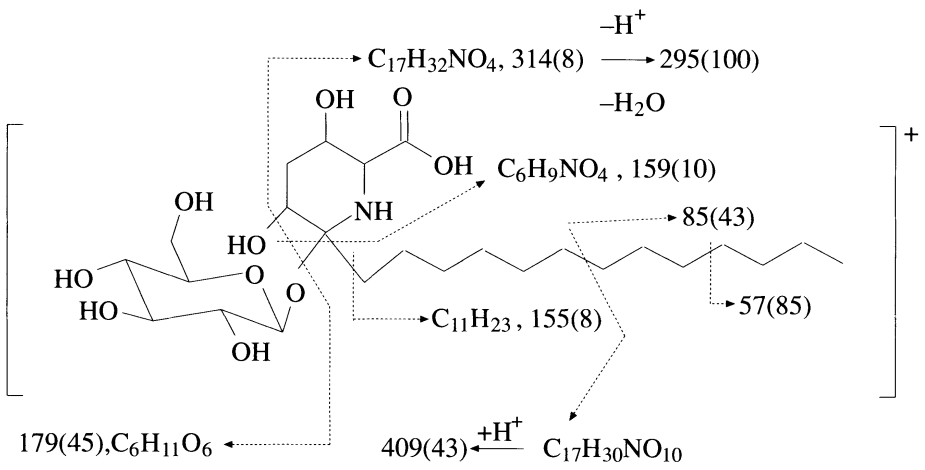

Figure 1. FAB-MS (m/z) spectral analysis of compound **1.**

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