Pergamon

PII: S0031-9422(97)00978-3

Phytochemistry, Vol. 48, No. 5, pp. 881–884, 1998

Printed in Great Britain
0031-9422/98 $19.00+0.00

A TRITERPENOID SAPONIN FROM *CYCLAMEN COUM*

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(*Received in revised form* 15 *October* 1997)

Key Word Index—*Cyclamen coum*; triterpene saponins; olean-12-ene; 30,28β-lactone.

Abstract—A new triterpenoid saponin, coumoside A, has been isolated from the whole plant of *Cyclamen coum* and the structure of this novel saponin ($C_{58}H_{92}O_{27}$) has been deduced by NMR methods based on ^{1}H, ^{13}C, DEPT, ^{1}H-^{1}H COSY, HETCOR, NOESY-NMR experiments and the FAB-mass spectrum. It has the structure 3β-*O*-{β-D-glucopyranosyl-(1-6)-[α-L-arabinopyranosyl-(1-2)]-β-D-glucopyranosyl-(1-4)-[β-D-glucopyranosyl-(1-2)]-α-L-arabinopyranosyl}-16α-hydroxy-30,28β-lactone-olean-12-ene and is called coumoside A.

INTRODUCTION

The genus *Cyclamen* contains 19 species [1]. Previous studies on the genus of *Cyclamen* reported only saponins and sapogenins. The saponin mixture of these species consists mainly of cyclamin, desglucocyclamin I, a small amount of desglucocyclamin II and isocyclamin [2]. Most of the *Cyclamen* species are grown in different regions of Turkey.

One of the *Cyclamen* species is *Cyclamen coum*, widely distributed in northern Turkey. Recently we reported the sterols [3] and a piperidine type alkaloid [4] from *Cyclamen coum*. In our ongoing studies, we have elucidated the structure of a novel triterpene saponin isolated from the methanolic extract of this plant.

RESULTS AND DISCUSSION

The wet bulb of *Cyclamen coum* was extracted with methanol and the saponon fraction was isolated by the classical procedure [2, 5–9]. Flash column chromatography on silica gel gave crude **1**. This was further purified by reverse-phase RP-18 flash column chromatography.

Standard 1D and 2D NMR procedures were employed to elucidate the structure of coumoside A (**1**). Conventional ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra combined with DEPT data gave the gross structure of the molecule and showed that it consisted of a pentacyclic triterpenoid aglycone ($C_{30}H_{45}O_4$) and a pentasaccharide (C_{28}) sugar moiety. The COSY and NOESY maps afforded a comprehensive description of through-bond and through-space proton-proton connectivities which were then interpreted in terms of constitution and relative stereochemistry of the molecule. Corroborative evidence for the molecular structure obtained was gleaned from ^{13}C–^{1}H chemical shift correlation (HETCOR) experiments and FAB-mass spectra.

The ^{13}C NMR spectrum showed 58 carbon resonances (Table 1), supporting the molecular formula **1**. The presence of five sugars in **1** was apparent from the five anomeric carbon signals [δ 106.70, 104.33 (two peaks observed due to intensity and HETCOR), 104.06 and 103.43] [2, 5]. Two of these sugars were arabinose (δ 106.70 and 104.06); the distinct anomeric proton doublets at δ 4.29 (*d*, J = 5.3 Hz) and δ 4.43 (*d*, J = 5.2 Hz) were assigned to two L-arabinose moieties [5, 6]. Three anomeric protons at δ 4.40, 4.41, and 4.49 (*d*, J = 7.9, 7.9, 8.0 Hz), correlating with carbon signals at δ 104.33, 104.33 and 103.43 respectively were assigned to three D-glucose units [2, 5–6].

The ^{13}C NMR downfield signals for the aglycone showed that there were three oxygenated sp^3 carbons (C-3: δ 88.99; C-16: δ 75.33; C-28: δ 81.43 and the remaining oxygenated carbon resonances were accounted for by the five sugars. The thirty carbon aglycone was shown by the DEPT spectrum to have six quaternary carbons [δ 37.12, 39.67, 40.80 (two peaks), 42.06, 43.08]; five methines (δ 41.70, 47.16, 56.04, 75.33, 88.99), ten methylenes (δ 18.59, 22.06, 23.82, 26.55, 28.41, 32.68, 37.12, 38.76, 41.96, 81.43), six methyl groups (δ 16.11, 16.41, 16,99, 27.53, 28.39, 28.79), and three sp^2 carbons [δ 124.25 (CH), 140.90 (C), 177.61 (C=O)]. The aglycone, as shown by the ^{1}H NMR spectrum, had six methyl proton singlets (δ 0.78, 0.79, 0.87, 0.99, 1.07, 1.20) and also one broad vinyl proton singlet (δ 5.25) attached to the carbon at

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Table 1. ^{13}C and ^{1}H NMR spectral data for coumoside A in DMSO-d_6

Aglycone of **1***				Sugar moiety**1***†			
C/H	^{13}C (δ, ppm)	DEPT	^{1}H (δ, ppm)	C/H (δ, ppm)	^{13}C	DEPT (δ, ppm)	^{1}H
1	38.76	CH_2	0.90, 1.32	Ara I 1	104.06	CH	4.29 *d*, J = 5.3 Hz
2	26.55	CH_2	1.84, 1.90	2	78.70	CH	3.62
3	88.99	CH	3.00	3	74.25	CH	3.44
4	39.67	C	—	4	79.27	CH	3.80
5	56.04	CH	0.80	5	66.72	CH_2	3.78, 3.14
6	18.59	CH_2	1.40				
7	32.68	CH_2	1.34	Ara II 1	106.70	CH	4.43 *d*, J = 5.2 Hz
8	40.80	C	—	2	75.33	CH	3.23
9	47.16	CH	1.52	3	77.31	CH	3.38
10	37.12	C	—	4	70.96	CH	3.52
11	23.82	CH_2	1.82, 2.32	5	64.21	CH_2	3.74, 3.40
12	124.25	CH	5.25				
13	140.90	C	—	Glc I 1	104.33	CH	4.40 *d*, J = 7.9 Hz
14	42.06	C	—	2	84.59	CH	3.39
15	37.12	CH_2	1.16, 1.80	3	77.29	CH	3.47
16	75.33	CH	3.38	4	70.18	CH	3.53
17	43.08	C	—	5	77.51	CH	3.71
18	41.70	CH	3.45	6	69.38	CH_2	4.18, 3.87
19	41.96	CH_2	1.10, 1.76				
20	40.80	C	—	Glc II 1	103.43	CH	4.41, *d*, J = 7.9 Hz
21	28.41	CH_2	1.82, 2.45	2	76.78	CH	3.71
22	22.06	CH_2	1.88, 1.94	3	76.80	CH	3.55
23	28.39	CH_3	0.99, *s*	4	70.56	CH	3.49
24	16.11	CH_3	0.87, *s*	5	72.77	CH	3.73
25	16.41	CH_3	0.79, *s*	6	61.95	CH_2	3.62, 3.25
26	16.99	CH_3	0.78, *s*				
27	27.53	CH_3	1.07, *s*	Glc III 1	104.33	CH	4.49 *d*, J = 8.0 Hz
28	81.43	CH_2	3.48, 3.24	2	76.44	CH	3.48
29	28.79	CH_3	1.20, *s*	3	77.69	CH	3.36
30	177.61	C	—	4	70.94	CH	3.22
				5	74.39	CH	3.18
				6	62.13	CH_2	3.52, 3.40

* Chemical shifts (ppm) are relative to internal TMS.
† Assignments based on 2D-COSY, HETCOR and NOESY spectra.

δ 124.25. These facts suggested that the aglycone had a pentacyclic triterpene structure with a double bond [δ 124.24 (CH), 140.90 (C)] and a carbonyl group (δ 177.61). A comparison of these findings with the results in the literature [7–9] revealed that the aglycone had the characteristics of an olean-12-ene triterpene.

The appearance of one carbonyl carbon instead of the seventh methyl singlet in the ^{1}H and ^{13}C NMR spectra was evident from the relative downfield shift of C-20 (δ 40.80) in comparison with methyl oleanolate ($\sim\delta$ 30.6, [7, 9]. This suggested that one methyl group at C-20 of oleanolic acid had been replaced by a carboxyl group [2, 5, 7]. However, in the ^{1}H NMR spectrum we did not see a carboxyl proton, and a carbonyl carbon (δ 177.61) shifted upfield in the ^{13}C NMR spectrum. The chemical shift of C-28 (CH_2) in the ^{13}C NMR spectrum was δ 81.43. If C-28 was the free alcohol, its peak would be more shifted upfield by at least $\sim$10–15 ppm [9]. In the related literature, it has been indicated that the pentacyclic triterpenes feature a C-28 hydroxyl [6, 9] and a C-20 carboxyl [7–9] group, and likewise there were C-28 carboxyl and C-20 hydroxyl groups [7, 9]. Lactone rings have also been reported to exist between C-28 (C=O) and C-20 (—O—) [6, 10]. In view of this, the lactonization of the aglycone of compound **1** from C-28 (CH_2OH) to C-20 (—COO—) is not an unexpected variant. Thus, the C-28 (CH_2, δ 81.43) and C-30 (C=O, δ 177.61) signals confirmed the presence of a 7-membered lactone ring. Therefore, these chemical shifts strongly suggested that compound **1** had a new ε-lactone ring at the C-30/C-28 position.

The ^{1}H and ^{13}C NMR spectra of compound **1** showed that the five sugars were present in one saccharide unit [2, 6], attached to C-3 of the aglycone. The individual proton spin systems for each sugar residue were delineated by homonuclear correlations as detected from the COSY spectrum. The ^{13}C–^{1}H

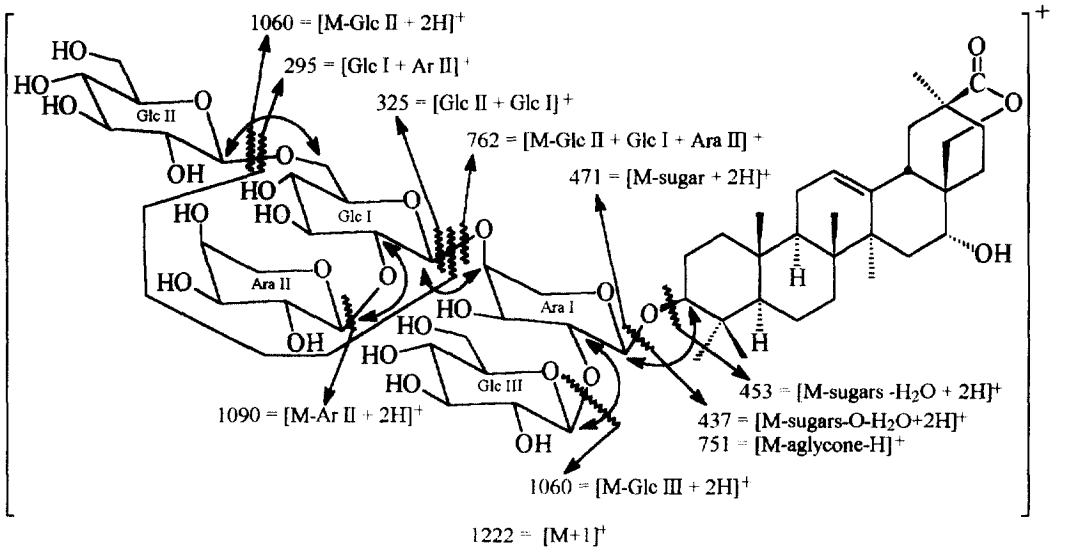

Fig. 1. Mass spectral fragmentations and interglycosidic NOE's of coumoside A for the elucidation of the sugar sequence and linkage sites.

COSY spectrum allowed the assignments of sugar carbon resonances to each individual sugar. Formation of a glycosidic bond is known as a result of a 4 to 8 ppm downfield shift of the resonance due to carbon atoms involved in the glycosidic linkage, a fact clearly reflected by ^{13}C chemical shift data of coumoside A. Four interglycosidic linkages (δ 69.38 (CH_2), 78.70, 79.27, 84.59 (CH)) were present in the sugar moiety of compound **1** (Table 1) [2, 5, 11].

The sequence and the linkage sites of the sugar chain were subsequently confirmed by the observation of interresidue NOE's between the linkage position protons across the glycosidic bonds to the anomeric protons of the next sugar unit or the aglycone (Fig. 1). The NOE's of Glc II H-1 with both Glc I H-6a and Glc I H-6b, Glc I H-1 with Ara I H-4, Ara II H-1 with Glc I H-2, Glc III H-1 with Ara I H-2, and Ara I H-1 with aglycone C-3 were observed in the NOESY experiment suggesting that the saccharide chain had the structure: Glc II (1→6), [Ara II (1→2)] Glc I (1→4), [Glc III (1→2)] Ara I– and this chain was attached to the C-3 of the aglycone through a glycosidic linkage. The NOESY spectrum also displayed a dipolar interaction between Ara I H-1 and the aglycone C-3, connecting this arabinose to the aglycone C-3 position [2, 5].

The ^{13}C NMR spectrum of the sugar moiety of compound **1** was remarkably similar to that reported in the literature [2, 5, 12]. Compound **1** contained a pentasaccharide chain and glycosidic linkages similar to isocyclamin [2]. The only difference was xylose displaced by arabinose II. The sequence of the sugars was also established through the positive ion FAB-mass spectrum which exhibited the molecular ion peak at *m/z* 1222 $[M+H]^+$ and fragment ions at *m/z* 1090 $[M-Ara\ II+2H]^+$, 1060 $[M-Glc\ II+2H]^+$, 762 $[M-Glc\ II-Glc\ I-Ara\ II]^+$, 751 $[M-Aglycone-H]^+$, 471 $[M-sugar\ moiety+2H]^+$, 453 $[M-sugar\ moiety-H_2O+2H]$, 437 $[M-sugar\ moiety-O-H_2O+2H]^+$, 325 $[Glc\ II+Glc\ I]^+$ and 295 $[Glc\ II+Ara\ II]^+$, respectively (Fig. 1).

The inspection of the NOE data for the aglycone part of the molecule (Fig. 1) revealed that the relative stereochemistry at the common centers were identical with those of reported triterpenes [2, 5]. A NOESY experiment on compound **1** showed the presence of characteristic cross-peak correlation of H-16 (δ 3.38) with H-18β (δ 3.45) and H-28β (δ 3.48). Thus, the hydroxyl group at C-16 had the α-configuration.

Based upon the above observations, the structure of the coumoside A was established as 3β-*O*-{β-D-glucopyranosyl-(1-6)-[α-L-arabinopyranosyl-(1-2)]-β-D-glucopyranosyl-(1-4)-[β-D-glucopyranosyl-(1-2)]-α-L-arabinopyranosyl}-16α-hydroxy-30,28β-lactone-olean-12-ene which is a novel natural product.

EXPERIMENTAL

General

NMR spectra were recorded on a Bruker NMR at 400 MHz instrument in DMSO-d_6 using TMS as internal standard. (+) FAB was recorded on a Zab-spec MS instrument. The melting point was determined on a Gallenkamp apparatus and is uncorr. The optical rotation was measured on a full-circle model of Polyscience Polarimeters using 20 × 1 cm cell. Flash column chromatography was performed on a silica gel 60 (230–400 mesh) and reversed-phased silica gel RP-18 and preparative TLC was performed with pre-coated silica gel F_{254} (20 × 20 cm, 0.2 mm) plates. A voucher specimen of *Cyclamen coum* has been deposited in a deepfreezer at the Department of Chemistry, Karadeniz Technical University, Turkey.

Isolation of compound **1**

Specimens of the *Cyclamen coum* were collected at Giresun Yağlıdere region, in the north of Turkey in March 1995. The chopped wet plants (~1500 g) were extracted with cold MeOH (1.5 l, 3×, 24 h each). The total aq. MeOH extract was filtered, and the filtrate was concentrated on a rotary evaporator at 30°. The aq. extract thus obtained (400 ml) was extracted with $CHCl_3$, (150 ml, 3×). The portion of aq. phase (100 ml) was evaporated *in vacuo* at 30–35° until dryness. The crude mixture (2.5 g) obtained was chromatographed on a Kieselgel 60 (60 g, 230–400 mesh) flash column chromatography. Elution with $CHCl_3$ (100 ml), then discontinuous gradient elution with $CHCl_3$–CH_3OH (300 ml) (5:1–5:2), $CHCl_3$ and then discontinuous gradient elution with $CHCl_3$–CH_3OH–H_2O (400 ml) (3:3:0.3–5:4:1) and finally $CHCl_3$–CH_3OH–H_2O (100 ml) (2:5:1) gave 8 fractions (ca 75–100 ml each). The second fraction (0.120 g) was rechromatographed on a flash reversed-phase silica gel RP-18 column eluted with discontinuous gradient with CH_3COCH_3–H_2O (60 ml) (9:1–6:4), then finally with CH_3COCH_3 to give 23 fractions. The fractions 13–15 were combined after the analyses of TLC to give compound **1** (11.2 mg). The TLC analysis with CH_3COCH_3–H_2O (reversed-phase silica gel RP-8 $F_{254}S$, 1.3:1), R_f: 0.75) of compound **1** showed that it was pure. Colorless crystals, mp 282° (dec); $[\alpha]_D$ +17° (95% EtOH; *c* 0.0004); ^1H NMR (DMSO-d_6, 400 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm) see Table 1; positive FAB-mass (MNBA) *m/z* rel. int: 1222 (35) $[M+H]^+$, 1090 (4) [M–Ara II + 2H]$^+$, 1060 (5) [M–Glc II + 2H]$^+$, 762 (6) [M – Glc II – Glc I – Ara II]$^+$, 751 (6) [M – Aglycone – H]$^+$, 471 (100) [M – sugar moiety + 2H]$^+$, 453 (85) [M – sugar moiety–H_2O + 2H]$^+$, 437 (13) [M–sugar moiety–O–H_2O + 2H]$^+$, 325 (10) [Glc II + Glc I]$^+$ and 295 (68) [Glc II + Ara II]$^+$.

Acknowledgments—This study was supported by a grant from Karadeniz Technical University of Turkey. Thanks to TUBITAK for recording the NMR spectra and (+) FAB-mass spectrum. Thanks also to Dr Mahir Küçük for his help with plant collection and identification. We are grateful to Dr İnci Akşahin for 400 MHz NMR measurements.

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