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Zeinosides A–D: pregnane glycosides from *Caralluma adenensis*

Ola E. Abdel-Sattar,[a] Manal M. Sabry,[b] Riham A. El-Shiekh,[b] Ali M. El-Halawany,[b] Othman S. S. Al-Hawshabi,[f] Mustafa Abdullah Yilmaz,[cd] Ali Riza Tufekci,[e] Meselhy R. Meselhy[b] and Essam Abdel-Sattar *[b]

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Four undescribed pregnane glycosides, zeinosides A–D, were isolated from the methanolic extract of *Caralluma adenensis* (Family Apocyanaceae). This plant is native to Yemen and traditionally used for its antidiabetic, anti-ulcer, anti-inflammatory, antiparasitic, and antipyretic properties. The structures of these compounds were elucidated using comprehensive spectroscopic techniques, including 1D and 2D NMR, LC-ESI-IT-TOF-MS, and IR spectroscopy. The structures of zeinosides A–D are determined as a polyhydroxylated C-21 steroidal skeleton with benzoyl esters at C-15 and/or C-20, except for zeinoside C, which has a ketone at C-20. The sugar moieties consist of β-D-digitalose and β-D-glucose units, with zeinoside A containing two sugars and zeinosides B–D each bearing three. The stereochemistry was determined through NOESY correlations and comparison with literature data. These findings add to the chemical diversity of the *Caralluma* genus and suggest that zeinosides A–D could be promising candidates for future studies, potentially supporting the plant's traditional uses in medicine.

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Introduction

The genus *Caralluma* (family Apocynaceae, subfamily Asclepiadaceae) comprises more than 120 leafless, succulent perennial herbs that are represented widely in arid and semi-arid regions of Africa, the Arabian Peninsula, and Asia,[1] thriving in harsh environments like stony plains and rocky outcrops. Yemen has a highly diverse group of approximately 25 species, many of which are near endemic and endangered.[2,3] *Caralluma* species have a long history of traditional use in treating diabetes, gastrointestinal and rheumatic disorders, skin conditions, and inflammation.[4] In Yemen and other Asian countries, decoctions or raw plant material is routinely used as tonics, antiseptics, and for liver disease, hypertension, and wound healing.[4–6] Phytochemically, *Caralluma* plants are rich in C-21 steroidal compounds, particularly pregnane glycosides with oxygenated aglycones and sugar chains composed of deoxyhexoses and hexoses.[2,7] Previous studies on several *Caralluma* species collected from Saudi Arabia and Yemen; *C. arabica*,[8] *C. awdeliana*,[9] *C. russelliana*,[10,11] *C. tuberculata*,[12] and *C. penicillata*,[13,14] have resulted in the isolation of several new pregnane glycosides. These findings suggest that the genus *Caralluma* is a potential source of bioactive natural compounds.

Caralluma adenensis (Defl) A. Berg. is a succulent herb found in rocky escarpments and wadis at elevations ranging from 500–2000 m in Yemen and other parts of the Arabian Peninsula.[2,15] *C. adenensis* is traditionally used as antidiabetic, anti-ulcer, anti-inflammatory, antiparasitic, and anti-pyretic agent.[16]

In continuation of our research to investigate the chemical diversity of *Caralluma* species growing in Yemen, we report herein the isolation and structure elucidation of four previously undescribed pregnane glycosides, zeinosides A–D from the deffated methanolic extract of *C. adenensis* collected near Abyan, Yemen. This communication not only fills a gap into the established pregnane glycoside profile of the genus but also provides an impetus for testing their potential pharmacological applications.

Results and discussion

The methanolic extract fraction (MR) was chromatographed over normal and/or RP-18 gel columns to afford four previously undescribed pregnane glycosides (**1–4**) (Fig. 1). Compounds **1–4** gave positive tests for sterols (Liebermann–Bucrchard test) and sugars and/or glycosides (Molish test). Analysis of the 1D (Tables 1 and 2) and 2D NMR spectral data of **1–4** (SI Fig. S1–S33) indicated that they are derivatives of C/D-*cis*-polyoxypregnanes (with signals due to a benzoyl group in **1**, **3**, and **4**,

[a]*Herbal Department, Egyptian Drug Authority, Giza, Egypt*
[b]*Pharmacognosy Department, Faculty of Pharmacy, Cairo University, 11562 Cairo, Egypt. E-mail: essam.abdel-sattar@pharma.cu.edu.eg*
[c]*Department of Analytical Chemistry, Faculty of Pharmacy, Dicle University Science and Technology Research and Application Center, Diyarbakir, 21280, Turkiye*
[d]*Dicle University Science and Technology Research and Application Center (DUBTAM), Diyarbakir, 21280, Turkiye*
[e]*Department of Chemistry, Faculty of Science, Çankırı Karatekin University, Çankırı, Turkiye*
[f]*Department of Biology, Faculty of Science, University of Aden, Aden, Yemen*

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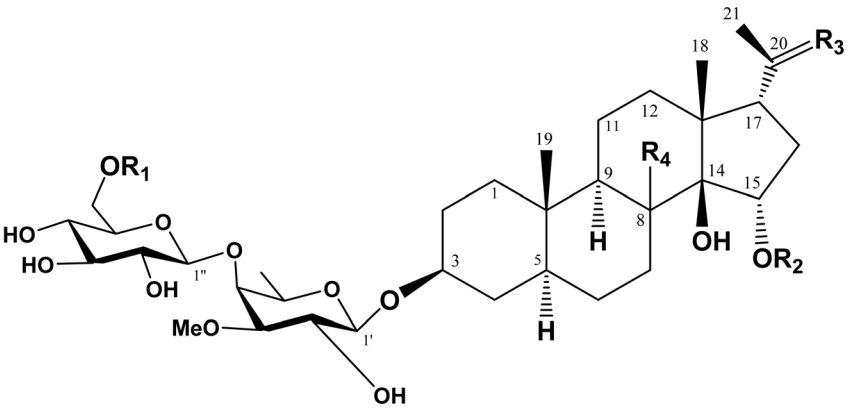

	R_1	R_2	R_3	R_4
Zeinoside A (1)	H	benzoyl	-H and OH	OH
Zeinoside B (2)	Glc	benzoyl	-H and *O*-benzoyl	OH
Zeinoside C (3)	Glc	benzoyl	=O	OH
Zeinoside D (4)	Glc	benzoyl	-H and OH	OH
Desmiflavaside A (5)	Glc	benzoyl	-H and OH	H

Fig. 1 Structures of zeinosides A–D (**1–4**) isolated from *Caralluma adenensis* and desmiflavaside A (**5**).

and two benzoyl groups in **2**), and their skeletons do not contain any double bonds.[8,13,14,17] Placing the benzoyl esters at C-15 and/or C-20 was confirmed based on the downfield shift of the respective protons and carbons[8,17] and the long-range correlation observed in HMBC (Fig. 2 and SI Fig. S1–S33) between the signal of carbonyl carbon of the benzoyl groups and H-15 and/or H-20 of the aglycones[8,13,17] (Fig. 1 and 2). Accordingly, benzoyl esters were placed at C-15 and/or C-20 of **1**, **2**, and **4**, while a ketonic group was placed at C-20 of **3**.[17] In addition, two sugar units were indicated in the structure of **1**, while three sugar units were in **2**, **3**, and **4**.

Compound **1** was isolated as a white powder, $[\alpha]_{24}^{D}$ −31.7° (*c*. 0.0036), the LCMS-IT-TOF analysis showed a pseudo molecular ion peak at *m*/*z* 817.3929 $[M + Na]^+$ in positive mode (Fig. S8), assigned for the molecular formula $C_{41}H_{62}O_{15}$. IR spectrum of **1** showed absorption bands for hydroxyl (3410 cm^{-1}), ester (1712 cm^{-1}), and aryl (11 450, 713 cm^{-1}) groups.

The NMR spectra of compound **1** displayed signals corresponding to two sugar moieties, including one 6-deoxy sugar, alongside those indicative of a C_{21}-steroidal skeleton. The ^1H and ^{13}C NMR data of compound **1** (Tables 1 and 2) were indistinguishable from those of desmiflavaside A (Tables 1, 2 and Fig. 1)[17] except for the presence of two sugar units in compound **1** and three in desmiflavaside A, and the presence of OH group at C-8 in **1**. The ^1H, ^{13}C NMR, and DEPT data (Tables 1 and 2) revealed four methyls, one methoxy group, twenty two methines, nine methylenes, and five quaternary carbons, totaling 41 carbon atoms with 21 attributed to the C_{21} steroidal skeleton. Signals for two angular methyls of the pregnane backbone appeared as two singlets at δ_H 1.17 and 0.89 (δ_C 17.40 and 13.31), assigned to Me-18 and Me-19, respectively. The ^1H–^1H COSY and TOCSY correlations clearly established the spin systems in the aglycone. Furthermore, the HMBC correlations between Me-19 and C-10, C-1, C-5 and C-9; Me-18 with C-13, C-12, C-14 and C-17; H-20 with C-13, C-16, and C-21; H-15 with C-13, C-14, C-16 and C-17 confirmed the 3, 8, 14, 15, 20-pentaoxygenated pregnane skeleton (Fig. 2).

The presence of one benzoyl unit was confirmed [^1H-NMR signals at δ_H 8.0 (2H, d, J = 7.7, Hz, H-2/H-6), 7.62 (t, J = 7.5 Hz, H-4), and 7.5 (2H, t, J = 7.5 Hz, H-3/H-5)][12,13,17] (Fig. S1) and a ^{13}C-NMR signal (Fig. S2) at δ_C 165.79 for the ester carbonyl[10,17] and the HMBC (Fig. S7) correlation observed between the carbonyl signal and H-15 (δ_H 5.39), confirmed benzoylation at C-15.[17]

In the ^1H- and ^{13}C-NMR spectra of **1**, the signals corresponding to the sugar moiety closely resembled those reported for desmiflavaside A,[17] penicilloside A,[13] awdelioside A,[9] and arabincoside A,[8] and confirmed the presence of one D-glucose and one D-digitalose unit; signals for two anomeric protons at δ_H 4.12 (d, 7.4) and 4.29 (d, 7.6), with their respective carbon signals at δ_C 101.10 and 103.73. D-Digitalose was established as inner sugar unit based on HMBC correlations and the β-configuration of both sugar units was inferred from the large coupling constants of their anomeric protons. From the aforementioned findings, compound **1** was identified as 15-*O*-benzoyl-pregnane 3β, 8β, 14β, 15α, 20*R* pentaol 3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-digitalopyranoside and named zeinoside A.

Compound **2** was isolated as a white powder, $[\alpha]_{24}^{D}$ −31.7° (*c*. 0.0011), the LCMS-IT-TOF analysis showed a pseudo molecular ion peak at *m*/*z* 1083.4754 $[M + Na]^+$ in positive mode (Fig. S17), assigned for the molecular formula $C_{54}H_{76}O_{21}$. The spectral data obtained for compound **2**, along with comparison to reported pregnane glycosides, clearly support its identification as a polyhydroxylated, and benzoylated pregnane derivative. The ^1H- (Fig. S9) and ^{13}C-NMR data of compound **2** (Tables 1, 2 and

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Table 1 ^{1}H-NMR (600 MHz) and ^{13}C-NMR (125 MHz) analysis of aglycones of zeinosides A–D (**1–4**) and desmiflavaside A (**5**)

	Zeinoside A (**1**)		Zeinoside B (**2**)		Zeinoside C (**3**)		Zeinoside D (**4**)		Desmiflavaside A (**5**)[17]	
No.	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	39.3	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR
1	37.9	1.06 (1H, m) 1.61 (1H, m)	37.5	0.88 (1H, m) 1.61 (1H, m)	30.4	1.68 (1H, m) 1.13 (1H, m)	37.9	1.08 (1H, m) 1.64 (1H, m)	39.3	1.76 (m), 1.02 (m)
2	29.0	1.47 (1H, m) 1.32 (1H, m)	28.5	1.67 (1H, m) 1.37 (1H, m)	79.4	1.60 (1H, m) 1.34 (1H, m)	29.0	1,34 (1H, m) 1.50 (1H, m)	30.4	1.85 (m), 1.49 (m)
3	76.3	3.44 (1H, m)	76.8	3.44 (1H, m)	35.2	3.44 (1H, m)	76.6	3.47 (1H, m)	79.4	3.58 m
4	34.5	1.97 (2H, m)	34.0	1.89 (2H, m)	45.6	1.80 (2H, m)	34.5	1.90 (2H, m)	35.2	1.63 (m), 1.29 (m)
5	44.9	1.05 (1H, m)	44.3	0.84 (1H, m)	21.9	0.80 (1H, m)	44.9	0.89 (1H, m)	45.6	1.00 (m)
6	25.0	1.52 (2H, m)	24.4	1.48 (2H, m)	30.0	1.42 (2H, m)	25.0	1.50 (2H, m)	21.9	1.56 (m), 1.30 (m)
7	33.8	1.76 (2H, m)	33.3	1.50 (2H, m)	41.4	1.48 (1H, m) 1.37 (1H, m)	33.8	1.61 (1H, m) 1.80 (1H, m)	30.0	1.32 (m), 1.20 (m)
8	75.5	—	75.9	—	50.5	—	75.5	—	41.4	1.72 (m)
9	50.2	1.09 (1H, m)	50.2	1.05 (1H, m)	37.1	1.03 (1H, m)	50.2	1.08 (1H, m)	50.5	1.06 (m)
10	36.5	—	36.1	—	28.4	—	36.0	—	37.1	—
11	17.9	1.66 (2H, m)	17.4	1.30 (2H, m)	40.1	1.07 (1H, m)	17.9	1.97 (1H, m) 1.4.1 (1H, m)	28.4	2.09 (m), 1.03 (m)
12	39.5	1.40 (1H, m) 1.17 (1H, m)	41.1	1.37 (1H, m) 1.62 (1Hb, m)	48.0	2.46 (1H, m)	39.5	1.67 (1H, m) 1.24 (1H, m)	40.1	1.46 (m), 1.41 (m)
13	48.9	—	46.7	—	82.4	—	47.8	—	48.0	—
14	83.1	—	82.7	—	77.3	—	82.1	—	82.4	—
15	74.9	5.39 (1H, m)	75.5	5.39 (1H, t, J = 8.7 Hz)	27.3	5.45 (1H, t, J = 8.3 Hz)	74.6	5.39 (1H, t, J = 8.5, Hz)	77.3	5.60 (m)
16	27.3	1.57 (1Ha, m) 2.43 (1Hb, m)	31.3	1.59 (1H, m) 2.48 (1H, m)	54.8	2.49 (1H, m) 1.85 (1H, m)	27.3	1.60 (1Ha, m) 2.42 (1Hb, m)	27.3	2.50 (m), 1.75 (m)
17	53.7	1.42 (1H, m)	50.71	1.90 (1H, m)	15.6	2.67 (1H, m)	53.7	1.45 (1H, m)	54.8	1.62 (m)
18	17.4	1.17 (3H, s)	18.9	1.17 (s, 3H)	12.8	1.05 (s, 3H)	17.6	1.08 (3H, s)	15.6	1.11 (s)
19	13.3	0.89 (3H, s)	12.9	0.89 (s, 3H)	66.0	0.85 (s, 3H)	13.3	0.92 (3H, s)	12.8	0.84 (s)
20	64.6	3.90 (1H, q, J = 6.5 Hz)	73.9	5.22 (1H, dq, J = 12.5, 6.1 Hz)	21.9	—	64.6	3.65 (1H, dq, J = 11.6, 5.7)	66.0	4.03 (m)
21	22.1	0.93 (3H, d, J = 6.5 Hz)	16.9	1.20 (3H, d, J = 6.2 Hz)		2.16 (3H, s)	22.1	0.97 (3H, d, J = 6.2 Hz)	21.9	1.07 (d, 6.6)
Bz at C-15										
C=O	165.8	—	165.2	—	131.7	—	165.8	—	167.8	
1	130.6	—	128.6	—	130.7	—	130.6	—	131.7	
2, 6	129.2	8.0 (2H, d, J = 7.7 Hz)	130.3	8.14 (2H, m)	129.5	8.02 (2H, d, J = 7.5 Hz)	129.2	7.90 (2H, d, J = 7.5 Hz)	130.7	8.09 (dd, 2.0,4 7.0)
3, 5	129.7	7.50 (2H, t, J = 7.5 Hz)	129.0	7.55 (2H, m)	134	7.50 (2H, t, J = 7.58 Hz)	129.7	7.54 (2H, t, J = 7.5 Hz)	129.5	7.47 (t, 7.0)
4	133.7	7.62 (1H, t, J = 7.5 Hz)	133.2	7.67 (1H, m)	133.9	7.63 (1H, t, J = 7.58 Hz)	133.7	7.66 (1H, t, J = 7.5 Hz)	134.2	7.60 (t, 7.0)
Bz at C-20										
C=O			164.9	—						
1			128.8	—						
2, 6			129.7	7.90 (2H, m)						
3, 5			129.7	7.55 (2H, m)						
4			133.4	7.67 (1H, m)						

Fig. S10) were indistinguishable from those of compound **1** except for the presence of three sugar units and the presence of two benzoyl units at C-15 and C-20 in compound **2**. In addition, the NMR data of compound **2** (Tables 1, 2, Fig. 1, and S9–S17) were similar from those of desmiflavaside A,[17] except for the presence of two benzoyl units in compound **2** (relative to one in desmiflavaside A), and the presence of a OH group at C-8 in **2** (absent in desmiflavaside A).[17] The presence of two benzoyl units was additionally confirmed by the presence of signals for ten aromatic protons and two ester carbonyls at δ_C 165.2 and 164.9 in the NMR spectra of **2**.[18,19] The long range HMBC (Fig. S14) correlation observed between the carbonyl carbon

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Table 2 ^{1}H NMR (600 MHz) and ^{13}C NMR (125 MHz) of sugar moieties of zeinosides A–D (**1–4**) and desmiflavaside A (**5**)

	Zeinoside A (**1**)		Zeinoside B (**2**)		Zeinoside C (**3**)		Zeinoside D (**4**)		Desmiflavaside A (**5**)[17]	
No.	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR
Dig at C-3										
1′	101.1	4.12 (1H, d, J = 7.4 Hz)	101.2	4.14 (1H, d, J = 7.5 Hz)	101.2	4.11 (1H, d, J = 7.5 Hz)	101.2	4.14 (1H, d, J = 7.9 Hz)	102.8	4.30 (d, 7.8)
2′	68.8	3.32 (1H, m)	69.7	3.35 (1H, m)	69.7	3.30 (1H, m)	68.7	3.35 (1H, m)	71.3	3.55 (m)
3′	84.4	3.04 (1H, m)	84.6	3.03 (1H, m)	84.5	3.00 (1H, m)	84.5\6	3.03 (1H, m)	85.7	3.18 (m)
4′	74.8	3.9 (1H, m)	73.9	4.01 (1H, d, J = 2.8 Hz)	73.9	3.95 (1H, m)	73.9	4.01 (1H, m)	74.8	4.15 (m)
5′	69.4	3.43 (1H, m)	69.1	3.43 (1H, m)	69.4	3.39 (1H, m)	69.2	3.43 (1H, m)	71.6	3.60 (m)
6′	17.4	1.09 (3H, d, J = 6.3 Hz)	17.1	1.11 (3H, d, J = 6.0 Hz)	17.5	1.06 (3H, d, J = 6.0 Hz)	17.1	1.11 (3H, d, J = 6.06 Hz)	17.5	1.26 (d, 6.0)
OCH_3	58.2	3.33 (3H, s)	57.7	3.35 (3H, s)	58.1	3.30 (3H, s)	58.2	3.35 (3H, s)	58.5	3.49 (s)
Glc at C-4′										
1″	103.7	4.29 (1H, d, J = 7.6 Hz)	103.5	4.34 (1H, d, J = 7.6 Hz)	103.5	4.31 (1H, d, J = 7.6 Hz)	103.5	4.34 (1H, d, J = 7.7 Hz)	104.1	4.57 (d, 7.8)
2″	74.9	2.95 (1H, m)	74.0	2.97 (1H, m)	74.0	2.91 (1H, m)	74.1	2.95, (1H, m)	75.8	3.19 (m)
3″	77.1	3.41 (1H, m)	77.1	3.34 (1H, m)	76.6	3.25 (1H, m)	76.1	3.34, (1H, m)	77.8	3.33 (m)
4″	70.7	3.05 (1H, m)	70.9	3.05 (1H, m)	70.8	2.98 (1H, m)	70.9	3.05, (1H, m)	71.8	3.26 (m)
5″	77.5	3.12 (1H, m)	77.3	3.12 (1H, m)	77.1	3.09 (1H, m)	76.3	3.12, (1H, m)	77.4	3.43 (ddd, 2.0, 6.0, 8.3)
6″	61.8	3.50 (1H, m)	69.4	3.53 (1H, brd, J = 12.0)	69.1	3.52 (1H, m)	69.1	3.57 (1H, brd, J = 11.8 Hz)	70.3	4.12 (dd, 2.0, 12.0)
		3.62 (1H, brd, J = 11.5 Hz)		3.96 (1H, d, J = 12.0 Hz)		3.92 (1H, d, J = 12.0 Hz)		3.87 (1H, d, J = 12.0 Hz)		3.76 (dd, 6.0, 12.0)
Glc at C-6″										
1‴			103.9	4.28 (1H, d, J = 7.8 Hz)	103.9	4.23	103.9	4.27 (1H, d, J = 7.7 Hz)	105.0	4.38 (d, 7.8)
2‴			74.6	2.92 (1H, m)	74.6	2.92 (1H, m)	74.6	2.95 (1H, m)	75.1	3.16 (m)
3‴			76.5	3.40 (1H, m)	77.2	3.41 (1H, m)	76.9	3.40 (1H, m)	78.0	3.32 (m)
4‴			70.5	3.02, (1H, m)	70.5	2.98 (1H, m)	70.5	3.02, (1H, m)	71.6	3.25 (m)
5‴			76.8	3.12, (1H, m)	77.1	3.10(1H, m)	76.8	3.12, (1H, m)	78.0	3.24 (m)
6‴			61.1	3.43 (1H, m)	61.5	3.37 (1H, m)	61.5	3.45 (1H, m)	62.0	3.85 (dd, 2.4, 12.0)
				3.66 (1H, dd, J = 11.6, 5.1 Hz)		3.62 (1H, m)		3.67 (1H, d, J = 11.7 Hz)		3.64 (dd, 5.4, 12.0)

signal at δ_C 165.2 and H-15 (δ_H 5.39), and between the carbonyl carbon signal at δ_C 165.2 and H-20 (δ_H 5.22), together with the downfield shifts of the corresponding proton (H-15 and H-20) and carbon signals confirmed benzoylation at both C-15 and C-20.[17–19] Furthermore, a trisaccharide sugar moiety at C-3 (δ_H 3.44/δ_C 76.76) was identified as 3-*O*-β-D-glucopyranosyl-(1 → 6)-D-glucopyranosyl-(1 → 4)-β-D-digitalopyranoside.[8,11,13,17] From the foregoing evidence, the structure of compound **2** was identified as 15, 20 di-*O*-benzoyl-pregnane 3β, 8β, 14β, 15α, 20*R* pentaol 3-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl-(1 → 4)-β-D-digitalopyranoside and named zeinoside B.

Compound **4** was isolated as an amorphous powder, $[\alpha]_{24}^{D}$ −32.7° (*c*. 0.0025); the LCMS-IT-TOF analysis showed a pseudo molecular ion peak at *m*/*z* 979.4510 $[M + Na]^+$ in positive mode (Fig. S33), assigned for the molecular formula $C_{47}H_{72}O_{20}$. Compound **4** showed similar aglycone to those of compound **1** (Tables 1 and 2), and similar oligosaccharide moiety at C-3 resembled to compound **2**, identified as 3-*O*-β-D-glucopyranosyl-(1 → 6)-D-glucopyranosyl-(1 → 4)-β-D-digitalopyranoside (Tables 1, 2, Fig. 1, and S25–S32) and data reported in the literature.[8,11,13,17] In compound **4**, the assignments of the aglycone skeleton, and the sequential assignments of the proton and carbon resonances of the sugar moiety, as well as their connectivity to each other, were determined using 1D NMR (^{1}H-, ^{13}C-NMR, and DEPT) and 2D NMR experiments (COSY, TOCSY, HMQC, and HMBC) (Fig. S25–S32). From the above-mentioned data, compound **4** was identified as 15-*O*-benzoyl-pregnane 3β, 8β, 14β, 15α, 20*R* pentaol 3-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl-(1 → 4)-β-D-digitalopyranoside and named zeinoside D.

Compound **3** was obtained as an amorphous powder, $[\alpha]_{24}^{D}$ −24.3° (*c*. 0.0027). LCMS-IT-TOF analysis revealed a pseudomolecular ion peak at *m*/*z* 977.4318 $[M + Na]^+$ in positive mode (Fig. S24), consistent with the molecular formula $C_{47}H_{70}O_{20}$. The ^{1}H (Fig. S18) and ^{13}C NMR (Fig. S19), along with DEPT data for compound **3** (Tables 1 and 2), indicated that the sugar moiety at C-3 resembled those of compounds **2** and **4**, identified as 3-*O*-β-D-glucopyranosyl-(1 → 6)-D-glucopyranosyl-

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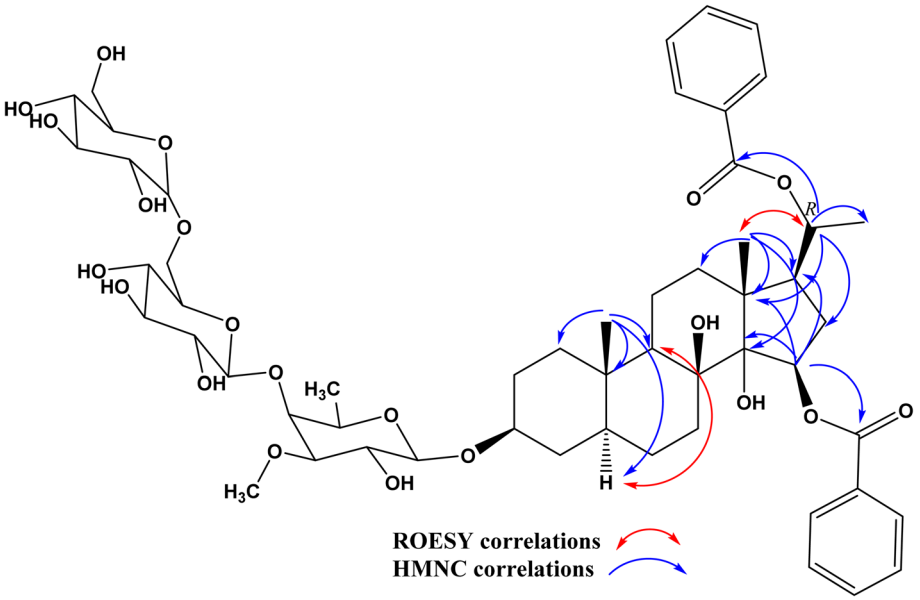

Fig. 2 Key HMBC and ROESY correlations of zeinoside B (**2**).

(1 → 4)-β-D-digitalopyranoside. The NMR spectra of the aglycone parts (Fig. S18–S23) of **3** and those of compounds **1** and **4** were nearly similar, except for some differences in the pregnane skeleton section. The most main differences are in the signals for the methyl doublet of H-21 (δ_H 0.93–0.97, d, J = 6.5); (δ_C ~22.0) and oxymethine multiplet of H-20 [δ_H 3.65–3.90, m; (δ_C ~64.5)] present in compounds **1** and **4** (ref. 17) that are absent and replaced by downfield shifted methyl singlet signal [δ_H 2.16 (s, H-21); δ_C 32.10)] and a ketone signal (δ_C 215.02) in the ^{13}C NMR spectra of compound **3** (Fig. S19). Further differences were evident by comparison of HMBC correlations (Fig. S23); Me-21 to C-17 and C-20; H-17 to C-20 and C-21. Based on the above findings and the spectral data of related compounds (**1**, **2**, and **4**), the structure of **3** was identified as 15-*O*-benzoyl-pregnan-20-one-3β, 8β, 14β, 15α tetraol 3-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl-(1 → 4)-β-D-digitalopyranoside and named zeinoside C.

The stereochemistry of the groups at C-3, C-8, C-14, C-15, C-17, C-18, and C-19 of compounds **1–4** was determined on compound **2** (zeinoside B) through interpretation of ROESY correlations (Fig. 2 and S16) and coupling constants, and by comparison with data reported in literature. ROESY (Fig. S16) correlations between H-20 (δ_H 5.22) and both H-18 (δ_H 1.17) and H-16b (δ_H 1.59); as well as between H-16a (δ_H 2.48) and H-17 (δ_H 1.90), indicated that all these protons are on the same side of the molecule, *i.e.*, β-oriented. The presence of ROESY correlation between H-5 and H-9 and along with their absence with each of H-19 and H-18, indicated that H-5 (δ_H 0.85) and H-9 (δ_H 1.05) are in the α-position.[9,13,14,17,20]

Regarding the absolute configuration of C-20, the ^{13}C chemical shift values observed for C-16 and C-20 showed significant differences compared to those reported in the literature for various 20*R*- and 20*S*-pregnane compounds, which were prepared by the reduction of both epimers. Therefore, the absolute configuration at C-20 was deduced to be *R*-configuration, supported by the ROESY correlation between H-18 (δ_H 1.17) and H-20 (δ_H 5.22).[9,13,14,17,20,21]

Conclusion

In this study, we successfully isolated and characterized four previously undescribed pregnane glycosides (zeinosides A–D) from the methanolic extract of *Caralluma adenensis,* a plant native to Yemen that's long been used in traditional medicine. These compounds were identified through detailed spectroscopic analyses including NMR and mass spectrometry, having polyhydroxylated pregnane skeleton with benzoyl groups at positions C-15 and/or C-20, along with oligosaccharides consist of β-D-digitalose and β-D-glucose units. Compound **1** (zeinoside A) has two sugars, while compounds **2–4** each has three, with a ketone at C-20 in compound **3** (zeinoside C), free hydroxy groups at C-20 for compounds **1**, **4** (zeinosides A and D) and two benzoyl groups in compound **2** (zeinoside B).

Our findings enhance the established chemical profile of the *Caralluma* genus, underscoring its potential as a source of bioactive natural products. Considering the plant's traditional uses for treating diabetes, inflammation, ulcers, and infections, zeinosides A–D (**1–4**) present promising opportunities for further research. Future studies investigating their pharmacological properties could reveal new therapeutic applications, contributing to the development of natural remedies inspired by the Yemen's rich biodiversity.

Experimental

General procedures

1D- and 2D-NMR spectra were obtained on Agilent-Premium Compact (600 MHz, 14.1 Trsla) and on Bruker High

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Performance Avance III FTNMR spectrometer (^{1}H-NMR: 400 MHz and ^{13}C-NMR: 100 MHz) and using TMS as internal standard. The LC-MS/MS analysis was performed using a Shimadzu LC-ESI-IT-TOF-MS model ultrahigh-performance liquid chromatography (UHPLC) system, coupled with a high resolution ion tap-time of flight mass spectrometer. Optical rotations were measured on a Bellingham + Stanley ADP 440 + digital polarimeter (Bellingham & Stanley, Kent, UK). IR spectrophotometer, Shimadzu FT-IR Affinity-1 was used for recording IR spectra using KBr discs.

Plant material. The fresh aerial parts of *Caralluma adenensis* (Defl.) A. Berg. (Syn: *Ceropegia adenensis* (Defl.) Bruyns, *Caralluma rauhii* Lavranos; *Boucerosia adenensis* Defl.; *Crenulluma adenensis* (Defl.) Plowes, *Crenulluma rauhii* (Lavranos) Plowes; *Desmidorchis adenensis* (Defl.) Meve & Liede) were collected from Abyan, Yemen (13° 22′ 799″ N, 044° 38′ 110″ E), in April 2023. The plant material was collected and authenticated by Dr Othman S. S. Al-Hawshabi Associate Professor of Plant Taxonomy and Flora, Department of Biology, Faculty of Science, Aden University, Yemen. A voucher specimen (No. 6167) of the plant was deposited in the Department of Biology, Faculty of Science, University of Aden. The plant material was cut into small pieces, dried in the shade, ground, and stored in a tightly closed glass container until use.

Extraction and isolation. A sample (250 g) of the powdered aerial parts of *C. adenensis* was extracted with methanol (3 × 1.5 L) at room temperature by the aid of Ultraturrax. After filtration, the combined methanolic extract was evaporated under reduced pressure to yield a solid residue (30 g). Part of the methanolic extract (27 g) was suspended in H_2O–MeOH (4 : 1 v/v, 300 mL) and shacked with petroleum ether (60–80 °C) to give three grams of petroleum extract and 24 g of the remaining methanolic extract fraction (MR). Part of MR (18 g) was chromatographed over open column chromatography using Diaion HP20 column (5 × 20 cm) and elution was performed with water (1000 mL) and then MeOH (2000 mL). The methanolic fraction (DM) eluted from the Diaion column (9.5 g) was chromatographed on a flash Si gel column (7.5 × 15 cm) to give seven fractions (150 mL each). The major fractions were pooled together to give Fr-I (800 mg), Fr-II (2000 mg), Fr-III (900 mg), Fr-IV (850 mg), Fr-V (1000 mg), and Fr-VI (1000 mg). Part of Fr-I (250 mg) was chromatographed over a Si gel RP-18 column (2 × 24 cm). Elution with MeOH–H_2O (17 : 3) (flow rate 5 mL min^{-1}, and 10 mL each fraction) gave compound **1** (55 mg). Similarly, part of Fr-II (220 mg) was applied to a Si gel RP-18 column (2 × 24 cm) and eluted with MeOH–CH_3CN–H_2O; 10 : 5 : 8 (flow rate 5 mL min^{-1}) to give three subfractions (Fr-IIa to IIc) (10 mL each). Fr-IIc afforded compound **2** (140 mg), while Fr-IIb (70 mg) was further chromatographed on a flash silica gel column (2 × 15 cm) using CH_2Cl_2 : MeOH : H_2O (15 : 5 : 0.5) as the solvent system to yield compounds **3** (20 mg) and **4** (34 mg).

Author contributions

Ola E. Abdel-Sattar: methodology, investigation, writing – original draft. Manal M. Sabry: investigation, writing – original draft. Riham A. El-Shiekh: investigation, writing & editing writing original draft. Ali M. El-Halawany: conceptualization, supervision, writing – review & editing. Othman S. S. Al-Hawshabi: collecting and authentication of plant material, writing – review & editing. Mustafa Abdullah Yilmaz: methodology, compounds analysis, investigation, writing – review & editing. Dr Ali Riza Tufekci: compounds analysis, investigation, review & editing. Meselhy R. Meselhy: conceptualization, supervision, writing, editing original draft. Essam Abdel-Sattar: writing, review & editing.

Conflicts of interest

There are no conflicts of interest to declare.

Data availability

All data in the study are included in the article; further inquiries can be directed to the corresponding author.

Supplementary information: 1D- and 2D-NMR, IR, and MS spectra of the new compound. See DOI: **https://doi.org/10.1039/d5ra07188g**.

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