Journal of Natural Products
Vol. 58, No. 4, pp. 483-494, April 1995

ADOLIGOSES, OLIGOSACCHARIDES OF RARE SUGARS FROM *ADONIS ALEPPICA*

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ABSTRACT.—Five novel tri-, tetra-, and penta-saccharides named adoligoses A–E [**1–5**], consisting of rare didesoxy sugars and their 3-*O*-Me ethers, have been isolated from *Adonis aleppica*. Their structures were determined by dcims and nmr measurements. Full establishment of the proton spin-systems was based on 2D nmr data, spectral simulation procedures, and force-field calculations. Sarmentose and cymarose moieties were found in both anomeric configurations and represent a major difference from alepposides A–D, biogenetically related cardenolide oligoglycosides isolated previously from the same plant source. This is the first report on complex, long-chained oligosaccharides of 2,6-didesoxy sugars found typically in cardenolide and pregnane glycosides.

Naturally occurring cardenolides are cardioactive steroidal glycosides containing rare desoxy sugars. In addition to the common hexoses and pentoses, 6-desoxyhexoses and 2,6-didesoxyhexoses and the corresponding 3-*O*-Me ethers are typical structural units. From both the pharmacological and biosynthetic points of view, the role of the carbohydrate moiety of cardenolides is still unclear. However, the results of recent studies show the distinct influence of the sugar portions and of their stereochemistry on the inotropic activity of cardenolides (1–3) and related pregnanes (4,5). Furthermore, there are gaps in our knowledge regarding the biosynthesis and formation of the glycosidic linkage of the cardenolides (6).

Cardenolide glycosides have been characterized by enzymatic or acid-catalyzed cleavage of the intact glycosides and subsequent chromatographic identification (paper chromatography, tlc, gc, and gc-ms after derivatization) of the degradation products (7). Recently, the structure elucidation of intact glycosidic natural products has been gaining more prominence. In this regard, the application of nmr and ms spectroscopic methods is significant. The occurrence of four complex cardenolide glycosides with 4–6 sugars, called alepposides A–D in *Adonis aleppica* Boiss. (Ranunculaceae), has recently been demonstrated by our group. Characterization of these compounds was carried out by extensive applications of various nmr methods including HMQC and HMBC experiments (8,9).

Because precise structural determination is desirable in biosynthetic, chemotaxonomic and structure-activity relationship (SAR) studies, the enhancement of methods for compound identification seems desirable. For this purpose we have used high-field ^{1}H-nmr and ms techniques as powerful and sensitive tools to form the basis of a facile method of identification of certain complex natural products (8). Further phytochemical investigation of *A. aleppica* has now yielded five novel oligosaccharides, adoligoses A–E [**1–5**], which were found to constitute tri-, tetra-, and penta-saccharides of rare desoxy sugars. The discovery of these compounds allowed an extension of our previous ^{1}H-nmr studies because of the lack of interference by resonances of the steroid nucleus. This paper deals with the isolation and structure elucidation of the adoligoses with unambiguous nmr assignments of the protons of didesoxy sugars having been made for the first time. Detailed information about the stereochemistry, sugar sequence, long-range substituent chemical shifts, and molecular conformation of the adoligoses is presented.

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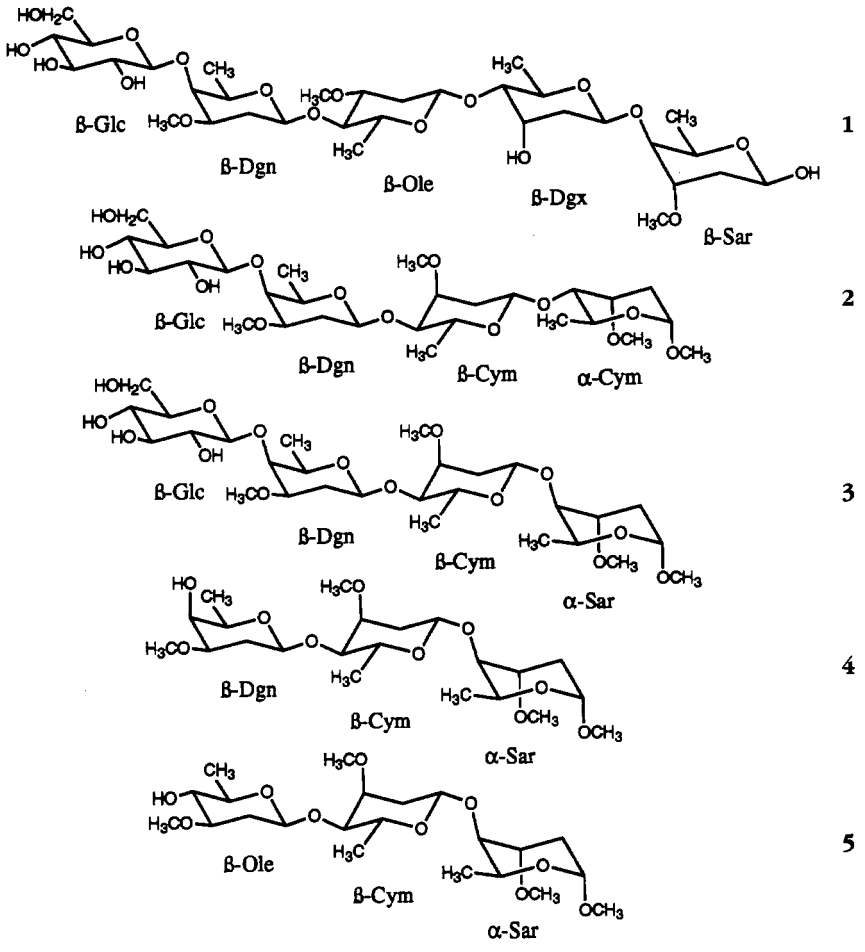
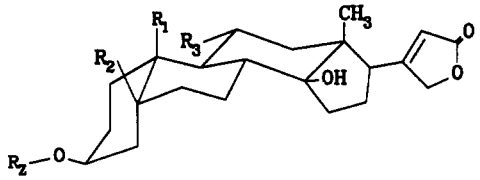


RESULTS AND DISCUSSION

During the isolation of alepposides A–D, vanillin/H_2SO_4 was used as a convenient tlc detection reagent (8,9). Compounds **1–5** appeared as additional bluish-grey spots, suggesting them to be closely related to the cardenolides. While compound **1** was isolated by means of mplc from the same fraction containing the polar cardenolide strophanthidin-diginosido-glucoside [**9**] (8), compounds **2–5** exhibited a lower polarity by tlc. Furthermore, compounds **2**/**3** and **4**/**5** were found to comprise pairs of epimers. This was suggested from the dcims data, which showed only slight differences in signal intensities. Base peaks observed at *m/z* 482 due to quasi-molecular ions $[M+NH_4]^+$ and their ^{13}C-nmr spectra (22 carbon atoms) showed **4**/**5** to consist of three 2,6-didesoxy-3-*O*-Me hexoses and one further OMe group; thus they are Me-trisaccharides ($C_{22}H_{40}O_{10}$). Compounds **2**/**3** were characterized by analogy as Me-tetrasaccharides differing from **4**/**5** by an additional hexose moiety (*m/z* 644 $[M+NH_4]^+$, $C_{28}H_{50}O_{15}$). In contrast to **2–5**, the ms of **1** showed a weak quasimolecular ion (*m/z* 760) and a more complex fragmentation pattern. Combining this information with the ^{13}C-nmr data (33 carbon atoms), however, it was possible to deduce that **1** is a pentasaccharide with a free anomeric hydroxyl function. Thus, **1** is a hemiacetal showing fragmentation from the anomeric sugar moiety along with the usual sequence cationic ions of the sugar chain. The molecular formula of **1**, deduced as $C_{33}H_{58}O_{18}$, was consistent with the presence of



Strophanthidin glycosides (R_1=CHO, R_2=OH, R_3=H)

6 β-glc-β-dgn-β-ole-β-dgx-β-dgx-=R_Z
7 β-glc^{1}-$_{6}$β-glc-β-dgn-β-ole-β-dgx-β-dgx-=R_Z
8 β-dgn-β-can-β-dgx-β-dgx-=R_Z
9 β-glc-β-dgn-=R_Z
11 β-glc^{1}-$_{4}$β-glc-β-cym-=R_Z
12 β-cym-=R_Z

Sarmentogenin glycoside (R_1=CH_3, R_2=H, R_3=OH)

10 β-cym-=R_Z

one hexose, three 2,6-didesoxy-3-*O*-Me hexoses, and one 2,6-didesoxy hexose moiety.

The major compound **1** (32 mg) was studied intitally and basic information concerning the identity of the individual sugar units was obtained from its ^{1}H-nmr spectrum (see Table 4). Comprehensive nmr studies of the alepposides (8,9) led to the assignment of typical proton signals ('fingerprint signals') due to several (didesoxy-) hexoses.[2]

Determination of anomeric configuration (α/β) could easily be achieved from the coupling constants of the anomeric protons. By analogy, the following monomers were identified in **1**: β-glucose (=glc→H-6=AB(M), H-2, dd at highest field), β-diginose (=dgn→H-4, br d at lowest field, H-5, dq around 3.50 ppm), β-oleandrose (=ole→H-4, t at highest field, H-2_{eq} ddd around 2.33 ppm), β-digitoxose (=dgx→H-3, ddd at lowest field with only small *J* values) and α-sarmentose (=sar→H-5, dq at lowest field). In a similar manner, the monomers of the other compounds were identified as follows: **2**—β-glc, β-dgn, β-cym, α-cym; **3**—β-glc, β-dgn, β-cym, α-sar; **4**—β-dgn, β-cym, α-sar; **5**—β-ole, β-cym, α-sar. Further structural information including the sugar sequences was derived mainly from the ^{13}C-nmr data. The ^{13}C-nmr resonances provide a sensitive tool for the determination of both sugar stereochemistry and linkage by observation of long-range substituent chemical shifts. Important preconditions were the constant use of a single solvent (preferably MeOH-d_5) and the availability of appropriate reference data (8,9).

When comparing the ^{13}C-nmr shift values of **1** with those of alepposides A [**6**, terminal-ole-dgn-glc trisaccharide (9)] and C [**7**, terminal-ole-dgn-glc$_{6}$-^{1}glc tetrasaccharide (8)], excellent agreement was apparent for the β-glc (terminal), β-dgn, and β-ole moieties. As shown in Table 1, deviations of δ values were less than 0.15 ppm, indicating the presence of a terminal glc-dgn-ole-fragment in **1**. Because of the congruence of chemical shifts assigned to ole C-1 through C-3 and 3-OMe, the preceding sugar moiety could not be sar but was actually dgx; the linkage of sar bearing an axial hydroxyl at C-4 (equatorial in dgx) would cause a noticeable substituent chemical shift to the ole ^{13}C skeleton. Corroborative evidence was provided by comparison of the δ values with those of analogous fragments in the alepposides as listed in Table 1. The digitoxose moiety in **1** is very similar to an in-chain DGX moiety in an -ole-DGX-dgx- fragment. Although the ^{13}C-nmr shifts were closely comparable for the C-3 through C-

[2]Abbreviations for the didesoxy sugars are used as follows: cym=cymarose, dgx=digitoxose, dgn=diginose, ole=oleandrose, sar=sarmentose.

TABLE 1. ^{13}C-Nmr Data[a] (δ values) for the Glucose[b], Diginose, Digitoxose[c], and Oleandrose Moieties of Oligosaccharides **1–5** in Comparison with Cardenolides[d] **6–9** (8,9).

Sugar		Compound								
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
β-Glucose	1	104.36	104.51	104.51			104.30	105.04		104.30
	2	75.95	75.95	75.95			75.95	75.87		75.93
	3	78.13	78.15	78.14			78.13	78.10		78.15
	4	71.84	71.80	71.79			71.80	71.82		81.86
	5	77.93	77.92	77.92			77.89	77.90		77.78
	6	63.02	62.99	62.98			62.99	62.99		63.04
β-Diginose	1	101.33	101.43	101.42	103.58		102.34	102.27	102.38	100.71
	2	33.58	33.19	33.17	33.02		33.59	33.57	32.78	33.33
	3	80.70	80.59	80.58	79.25		80.69	80.64	79.06	80.57
	4	74.22	74.47	74.49	67.75		74.08	74.36	67.36	74.02
	5	71.92	71.80	71.79	72.01		71.91	71.86	72.35	71.98
	6	17.67	17.76	17.75	17.25		17.69	17.91	16.90	17.59
	OMe	56.61	56.60	56.60	55.86		56.60	56.72	55.97	56.60
β-Oleandrose	1	102.13				101.45	102.34	102.27		
	2	37.25				37.37	33.59	33.57		
	3	80.29				81.59	80.27	80.28		
	4	84.07				76.94	84.07	84.03		
	5	72.32				73.24	72.29	72.32		
	6	18.80				18.41	18.80	18.80		
	OMe	57.23				57.40	57.21	57.29		
β-Digitoxose	1	102.32					100.52	100.52	100.52	
	2	38.41					38.71	38.71	38.72	
	3	68.30					68.28	68.28	68.29	
	4	83.67					83.51	83.51	83.57	
	5	69.28					69.42	69.42	69.46	
	6	18.48					18.49	18.49	18.49	

[a]In CD_3OD; chemical shifts are relative to solvent shift: δ_C=49.00 ppm.

[b]Terminal glucose unit in alepposide C [**7**].

[c]Digitoxose shift values due to an in-chain digitoxose moiety [DGX in -ole-DGX-dgx- fragments, see Pauli (8)].

[d]Structures given in formula drawings.

6 region ($\Delta\delta$ <0.20 ppm), distinct chemical shift differences of −0.34 and +1.80 ppm were noted for C-2 and C-1, respectively. This is readily explained by the influence of sar in an -ole-dgx-sar- fragment. Comparison of the ^{13}C-nmr data of **1** with those of authentic sarmentosides (sarmentocymarin [**10**] and sarveroside, see Table 2) confirmed the identification of sar and exhibited typical glycosylation shifts at C-3 through C-5 in **1**. As also indicated by the chemical shift value of sar C-1 (93.24 ppm) and the ms data mentioned above, **1** has a terminal reducing sugar (free anomeric end). Finally, the proton and carbon nmr assignments of **1** (Tables 1–4) were verified by an HMQC nmr experiment. Definite evidence for the sugar linkages also came from the cross-peaks in the HMBC experiment, which clearly showed the ^3J connectivities between the anomeric protons and the attached C-4 carbons of the preceding sugars: glc H-1→dgn C-4, dgn H-1→ole C-4, ole H-1→dgx C-4, and dgx H-1→sar C-4. Thus, **1** is β-glc-β-dgn-β-ole-β-dgx-β-sar.

Apart from **1**, ole was only present in compound **5** of the compounds obtained in this investigation. The high-field triplet due to H-4_{ax} at 2.96 ppm is not only a specific sign of all equatorial hydroxylated 2,6-didesoxyhexoses [canarose (can)=2,6-didesoxyglucose and the corresponding 3-*O*-Me ether oleandrose], but its chemical shift also provides useful information concerning the sugar sequence. While glycosidation

TABLE 2. ^{13}C-Nmr Data[a] (δ values) for the Sarmentose and Cymarose Moieties of Oligosaccharides **1–5** in Comparison with Cardenolides[b] **10**, **11** (13), and **12**[c] (14).

Sugar		Compound							
		1	**2**	**3**	**4**	**5**	**10**	**11**	**12**
β-Cymarose	1		103.39	103.38	102.82	102.82		98.4	
	2		35.52	35.59	36.00	36.13		36.6	
	3		78.24	78.24	78.48	78.48		78.6	
	4		83.88	83.86	83.89	83.92		83.6	
	5		69.88	69.91	70.16	69.88		70.4	
	6		18.61	18.60	18.54	18.54		18.9	
	OMe		57.61	57.94	58.50	58.50		58.6	
α-Sarmentose	1	93.24		99.27	99.27	99.27	97.53		
	2	33.64		29.25	29.95	29.25	31.63		
	3	70.11		62.79	67.79	62.79	68.70		
	4	76.44		76.94	76.94	76.94	70.38		
	5	77.38		77.20	77.19	77.19	80.23		
	6	16.67		16.76	16.75	16.75	17.52		
	OMe	57.27		57.27	57.26	57.26	57.22		
α-Cymarose	1		100.89						97.6
	2		32.22						36.4
	3		76.53						78.7
	4		79.10						74.0
	5		68.88						71.1
	6		16.62						18.9
	OMe		57.31						58.1
anomeric OMe	OMe		56.53	55.30	55.31	55.31			

[a]In CD_3OD; chemical shifts are relative to solvent shift: δ_C=49.00 ppm; data from Winkler (13) in CD_3OD, data from Yamauchi and Abe (14) in pyridine-d_5.

[b]Structures given in formula drawings.

[c]Containing a terminal cymarose in the β-configuration.

with hexoses and didesoxy hexoses gives rise to marked downfield shifts (H-4_{ax} 3.28 ppm for glc-ole- and 3.16 ppm for dgn-ole- fragments), H-4_{ax} of a terminal ole moiety like in oleandrin resonated at δ 3.03 ppm. The lack of a ^{13}C-nmr glycosidation effect centered at ole C-4 was inferred by comparison with nmr data of alepposide A (**6**, see Table 1) and with literature values (10,11), and gave further evidence for the terminal arrangement of ole in **5**.

Although dgn was apparent in **4** from its ^{1}H-nmr data, no equivalent ^{13}C-nmr data sets exist for **4** for comparison with compound **1**, alepposide A [**6**], or alepposide C [**7**] (see Table 1). In particular, no C-4 resonance around δ 74.3 ppm due to a C-4-substituted dgn was found in **4**, thus suggesting dgn to be the terminal sugar in **4**. In fact, equivalent shift values of dgn in alepposide D (**8**, terminal dgn unit) can be assigned in **4** because the preceding sugar causes only small Δδs below 0.4 ppm, except at 1.20 ppm for C-1. Accordingly, the terminal sugars of the trisaccharides **4** and **5** are dgn and ole, respectively. In order to deduce the linkage of cym and sar in **4** and **5**, the ^{13}C-nmr spectral data of **2** and **3** were assigned first, starting again with inferences from the signals due to dgn.

The seven carbon resonances of dgn also appeared in the ^{13}C-nmr spectra of **2** and **3**. In contrast to **4**, they showed absolute congruence with the dgn δ values of alepposides A [**6**] and B [**7**] (see Table 1). Because glc must also be terminal (δ 62.99 for C-6), glc-dgn- fragments like those of **1** can be determined as the terminal disaccharides of both **2** and **3**. Consequently, the four sugars in **2** must be arranged in the order glc-dgn-cym-cym. Comparison of the dgn δ values in **2** and **3** showed no relevant differences (Δδ

TABLE 3. ^{1}H-Nmr Data[a] for β-Glucose, β-Diginose, β-Oleandrose, and β-Digitoxose Moieties in Oligosaccharides **1–5**.

Hexose Proton		1	2		3	4	5	6_A	6_B
β-Glucose[b]	Mult. . .	d	dd		t	t	ddd	dd	dd
	J [1]. . .	7.7	9.2		9.0	9.6	1.8	11.8	5.7
	δ_H [1]. .	4.566	3.213		3.355	3.264	3.236	3.863	3.648
	δ_H [2]. .	4.558	3.208		3.35	3.28	3.24	3.87	3.666
	δ_H [3]. .	4.556	3.211		3.35	3.28	3.27	3.89	3.65
Didesoxyhexose Proton(s)		1	2_{ax}	2_{eq}	3	4	5	6	OCH_3
β-Diginose[b]	Mult. . .	dd	ddd	dddd[c]	ddd	d(d)	dq	d	s
	Coupl.[d].	1, 2_{eq}/1, 2_{ax}	2_{ax}, 3	2_{gem}	3, 2_{eq}	4, 3	5, 4	6, 5	
	J [1]. . .	2.2/9.9	12.1	12.1	4.4	2.8	1.1	6.4	—
	δ_H [1]. .	4.674	1.754	1.996	3.448	3.989	3.504	1.304	3.395
	δ_H [2]. .	4.522	1.80	2.01	3.44	3.989	3.525	1.295	3.397
	δ_H [3]. .	4.566	1.741	1.996	3.454	3.987	3.524	1.295	3.397
	δ_H [4]. .	4.543	1.677	1.902	3.393	3.866	3.474	1.271	3.367
β-Oleandrose	Mult. . .	dd	ddd	ddd	ddd	t	dq	d	s
	J [1]. . .	2.0/9.7	11.6	12.6	5.3	9.0	8.8	6.1	—
	J [5]. . .	1.9/9.9	11.5	12.6	4.6	8.9	9.4	6.1	—
	δ_H [1]. .	4.631	1.459	2.325	3.395	3.166	3.358	1.285	3.397
	δ_H [5]. .	4.593	1.349	2.326	3.472	2.960	3.208	1.273	3.413
β-Digitoxose	Mult. . .	dd	ddd	ddd	ddd	dd	dq	d	—
	J [1]. . .	2.2/9.9	3.1	13.8	3.5	2.8	9.6	6.3	—
	δ_H [1]. .	4.852	1.684	2.060	4.222	3.258	3.804	1.219	—

[a]In CD_3OD; chemical shifts are relative to solvent shift: δ_C=3.300 ppm, J given in Hz; mutual coupling constants are only given once at first occurrence in the table.

[b]Full analysis of the proton spin-systems and determination of J in β-glucose and β-diginose was carried out using **1** as a model compound (also see discussion).

[c]Apart from vicinal and geminal couplings, H-2_{eq} showed long-range coupling behavior with J=1 Hz.

[d]The order of the given J values refers to all didesoxyhexoses in this table.

≤0.02 ppm), indicating the linkage of the same (=cym) moiety to C-1 of dgn in both compounds. Thus, the sequence of **3** was determined to be glc-dgn-cym-sar, since the ^{13}C-nmr spectra of **3** and **2** exhibited two equivalent sets of seven resonances due to cym (see Table 2). The remaining set of seven ^{13}C-nmr resonances of **3** could be assigned to sar. With respect to glycosidation effects and differences in the anomeric configuration, the nmr data showed good agreement with those of sarveroside, sarmentocymarin [**10**], and **1** (see Table 2).

The seven carbon resonances of **3** assigned to sar were apparent in both trisaccharides **4** and **5**. Furthermore, the ^{13}C-nmr signals of a cym moiety could also be assigned and were in good agreement when compared with **2** and **3** (see Table 2). Consequently, sar and cym must be linked together in **4** and **5**, forming the disaccharide moiety -cym-sar-OMe, since congruent δ values of this fragment also appeared in **3**. The small δ differences within the cym moiety of **4** and **5** were used to indicate the linkage of the terminal sugars dgn and ole, respectively, as shown above. From these spectral properties, the structure of **4** was determined as β-dgn-β-cym-α-sar-OMe (=α-Me adoligose D), while **5** is β-ole-β-cym-α-sar-OMe (=α-Me adoligose E).

The presence of two cym moieties in **2** was concluded from the occurrence of two typical ^{1}H-nmr signals due to H-3 (=ddd at lowest field with only small J values, δ 3.88 and 3.77 ppm, respectively). Omitting the thirteen ^{13}C-nmr resonances of the terminal glc-dgn- sequence, the two remaining sets of seven carbon shifts showed distinct

TABLE 4. ^{1}H-Nmr Data[a,b] for Sarmentose and Cymarose Moieties in Oligosaccharides **1–5**.

Didesoxyhexose proton(s)		1 (α-OMe)	2_{ax}	2_{eq}	3	4	5	6	OCH_3
β-Sarmentose	Mult...	dd	ddd	dddd[c]	ddd	dd	dq	d	s
	Coupl.[d]	$1,2_{eq}/1,2_{ax}$	2_{ax}, 3	2_{gem}	3, 2_{eq}	4, 3	5, 4	6, 5	—
	J [**1**] ..	2.2/9.9	3.1	13.6	3.3	3.0	1.5	6.4	—
	δ_H [**1**] .	4.848	1.680	1.822	3.779	3.351	3.879	1.181	3.366
α-Sarmentose	Mult...	br dd	ddddd[e]	dt	ddd	br s	dq	d	s
	J [**5**] ..	3.8/1.3	3.3	15.0	3.3	3.2	1.3	6.7	—
	δ_H [**3**] .	4.607 (3.279)	1.824	1.930	3.66	3.43	4.092	1.149	3.348
	δ_H [**4**] .	4.608 (3.279)	1.793	1.956	3.680	3.448	4.093	1.148	3.348
	δ_H [**5**] .	4.607 (3.279)	1.784	1.954	3.677	3.448	4.090	1.148	3.347
β-Cymarose	Mult...	dd	ddd	ddd	ddd	dd	dq	d	s
	J [**4**] ..	1.8/9.9	2.7	13.9	3.2	2.8	9.6	6.2	—
	J [**5**] ..	1.9/9.9	2.4	13.9	3.1	2.8	9.6	6.4	—
	δ_H [**2**] .	4.782	1.56	2.235	3.83	3.29	3.84	1.213	3.411
	δ_H [**3**] .	4.852	1.558	2.231	3.84	3.29	3.84	1.211	3.413
	δ_H [**4**] .	4.791	1.566	2.203	3.869	3.278	3.815	1.207	3.431
	δ_H [**5**] .	4.787	1.575	2.185	3.858	3.213	3.814	1.212	3.429
α-Cymarose	Mult...	br d	ddd	ddd	ddd	dd	dq	d	—
	J [**2**] ..	≈4	2.8	◇	3.3	2.8	◇	6.6	—
	δ_H [**2**] .	4.599 (3.361)	1.66	1.80	3.771	3.29	3.84	1.197	3.408

[a]In CD_3OD; chemical shifts are relative to solvent shift: δ_C=3.300 ppm, *J* given in Hz; mutual coupling constants are only given once at first occurrence in this table.

[b]Full analysis of the α-sarmentose and α/β-cymarose proton spin-systems and determination of *J* refers to compounds **4**/**5** (also see discussion).

[c]Apart from vicinal and geminal couplings H-2_{eq} shows a long-range coupling behavior leading to a fourth sharp splitting of the corresponding signal (1.1 Hz).

[d]The order of the given *J* values refers to all didesoxyhexoses in this table.

[e]The signal appears as a 'ddddd' leading to two long-range couplings of sar H-2_{ax} (both *J*s 1.3 Hz).

[◇]Not determined because of extensive signal overlap.

differences (see Table 2). While one set was identical with cym in the glc-dgn-cym-sar-sequence of **3**, all remaining ring carbons were shifted 1–5 ppm upfield. These substituent chemical shifts could not be explained by the relatively weak influences of different substituents at the C-1 and C-4 positions (**2**↔**3**: cym instead of dgn and OMe instead of sar, respectively), but more likely were the result of different anomeric configurations. An analogous observation can be made when comparing the shift values of ole in oleandrin (→α-ole) with those of digifolein [**10**] and glaucoside A [**11**] (→β-ole). In fact, the two cymaroses in **2** differ in C-1 stereochemistry as ascertained from the *J* ($1,2_{ax}$) values: 4 Hz in α-cym and 9.9 Hz in β-cym. As cym was in the β-configuration in **3** and the corresponding ^{13}C-nmr shifts of **4** and **5** showed very good agreement with those in **2**, the complete structure of the latter must be formulated as β-glc-β-dgn-β-cym-α-cym-OMe (=α-Me adoligose B). Consequently, compound **3** is the C-4 epimer, namely, β-glc-β-dgn-β-cym-α-sar-OMe (=α-Me adoligose C).

To summarize all structural information, the free adoligoses were shown to have the following structures: adoligose A [**1**]=β-glucopyranosyl(1→4)-*O*-β-diginopyranosyl(1→4)-*O*-β-oleandropyranosyl(1→4)-*O*-β-digitoxopyranosyl(1→4)-*O*-β-sarmentose; adoligose B [**2**]=β-glucopyranosyl(1→4)-*O*-β-diginopyranosyl(1→4)-*O*-β-cymaropyranosyl(1→4)-*O*-α-cymarose; adoligose C [**3**]=β-glucopyranosyl(1→4)-*O*-β-diginopyranosyl(1→4)-*O*-β-cymaropyranosyl(1→4)-*O*-α-sarmentose; adoligose D [**4**]=β-diginopyranosyl(1→4)-*O*-β-sarmentose; and adoligose E [**5**]=β-oleandropyranosyl(1→4)-*O*-β-cymaropyranosyl(1→4)-*O*-α-sarmentose. Consequently, compounds **2**–**5** are the 1-*O*-Me derivatives of adoligoses B–E, respectively, and the two

Me-tetra- and trisaccharides are epimeric pairs (cym/sar in **2/3**, dgn/ole in **4/5**). Assignment to the D/L-series according to Klyne's rule (12) was not attempted since exceptions have been found (e.g., scilliglaucosidine) and reference data of all diastereomeric D/L- and α/β-didesoxy sugars are not available.

Some aspects of the ^{13}C-nmr spectra that provide further support for the deduced sugar sequences of compounds **1–5** should also be mentioned. One of them concerns the ^{13}C-nmr δ values of the anomeric OMe functions, which were exactly the same for **2**, **4**, and **5** (Table 2), thus proving the identical linkage (α) and stereochemistry of the next sugar (sar). Another detail refers to the resonances of the anomeric carbons. Although the chemical shift of C-1 in **1** (93.24 ppm) is due to a free anomeric hydroxyl function, the C-1 signals of **3**, **4**, and **5** at 99.27 ppm, as well as that of **2** at 100.89 ppm are consistent with anomeric (β-)OMe groups. The signals of the latter appeared at higher field compared with those of the C-3-OMe groups, with the chemical shift values between 55.0 and 57.0 ppm apparently indicating the presence of an (α-oriented?) anomeric OMe group, whereas 3-OMe carbons usually resonate between 57.0 and 59.0 ppm in MeOH-d_4.

While the sugar sequences of adoligoses **1–5** have been deduced mainly from their ^{13}C-nmr data, interesting observations can be made by detailed analysis of the proton spin-systems. Although high-field nmr affords sufficient resolution for signals of protons with geminal OH groups (sugar protons), evaluation of the C-2 methylene protons of the didesoxy hexoses has remained difficult in natural glycosides such as cardenolides and pregnanes. The reason is the strong signal overlap between 1 and 3 ppm of the ^{1}H-nmr spectra. The present availability of free sugar chains and non-steroidal derivatives of those sugars in the form of adoligoses A–E [**1–5**] permits closer examination than previously possible. Therefore, adoligose A [**1**] has been chosen as a model compound and nmr studies have been performed and interpreted in the following respects: complete assignment of all proton resonances of the single sugars supported by their ^{1}H-^{1}H-COSY data; and determination of the coupling constants by direct analysis of the experimental signals assuming the presence of nuclei first order spin-systems. The ^{1}H-nmr data of compounds **2–5** were examined in an analogous way and the complete results are listed in Tables 3 and 4. Delving further into the proton coupling behavior, the presence of non-first order spin-systems cannot be ignored. This follows from the appearance of additional weak absorption lines and shifts in line intensities, e.g., sar H-3 with its vicinal neighbors H-4, H-2(ax), and H-2(eq) forming the M-part of an AMXY spin-system. Furthermore, the protons H-1 to H-4 of both α- and β-sarmentose are involved in several long-range couplings. Thus, the corresponding signals—besides the vicinal and geminal coupling—show small splittings of ca. 1 Hz and/or are significantly broadened (e.g., signals of H-1 in α-sar and H-4 in α/β-sar). Consequently, in the case of model compound **1**, spectral simulation has also been carried out in order to refine the J values (J [**1**] in Tables 3 and 4) and to verify the assignments (signal shape). Figure 1 shows the complete coupling pathway for β-dgn in **1** and demonstrates the good agreement of measured and simulated ^{1}H-nmr signals. Besides the usual vicinal couplings, H-4 was shown to have an additional long-range connectivity in **1** (see Table 1), thus indicating its non-terminal position as postulated in Ref. (8).

Force-field calculations were also performed on the trisaccharide **4** as a model compound. The search for the lowest energy conformation was carried out by a combination of subsequent molecular dynamic and minimization procedures. Considering the Karplus equation, the dihedral angles of the calculated molecule are in excellent agreement with the experimentally found J values. Thus, evaluation of the proton spin-systems and the results of the molecular modelling calculations both led to

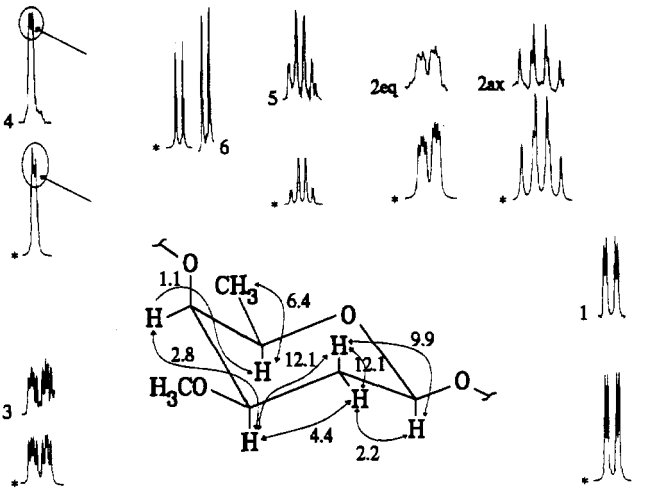


FIGURE 1. Intraresidual coupling pathways of β-diginose in adoligose A [**1**] together with experimental (numbered) and calculated (*) ^{1}H-nmr signals. In the case of H-4, consideration of a long-range coupling with the anomeric proton of the following sugar (β-glc) was necessary to fit with the shape of the measured signal (see arrows).

identical conformations of the sugar units. Calculations on conformers due to rotation along the axis of the glycosidic C-O-C bonds indicated preference for quasi-linear conformations. The calculated lowest energy molecular conformation of Me-adoligose D [**4**] is given in Figure 2.

A final observation is that ^{1}H-nmr coupling constants deviate slightly from one another when comparing one sugar with different linkages. For example, two sets of *J* values are found for the β-ole protons in **1** and **5**, as expected from their different substitution pattern (see Table 3): Ole is the terminal sugar in **5**, but is followed by glc-dgn- in **1**. Conversely, the *J* values of sugars with a comparable "chemical environment" (considering their position in the sugar-chain and configuration of the preceding and the following sugar) show very good agreement. Thus, in Tables 3 and 4 they are only given once for β-glucose, β-diginose, and α-sarmentose. The results show that both the exact ^{1}H-nmr shift values and the coupling constants are able to supply important structural information concerning sugar linkages, which up to now has been a domain of ^{13}C- and/or 2D nmr methods. In order to achieve general rules for this purpose, further oligosaccharides will be the subject of continuing research.

In summary, the oligosaccharides adoligoses A–E [**1–5**] constitute a new type of secondary natural product. Their isolation from *A. aleppica* was achieved using rapid and efficient isolation procedures (vlc, mplc) under gentle conditions to avoid the formation of artifacts. The observation of bluish-grey reaction products on spraying the adoligoses with vanillin/H_2SO_4 is of interest, and suggests that the unexpected bluish-green color of the alepposides may be due to both the aglycone (=strophanthidin, green color) and the sugar moiety after tlc (9). The structures of the adoligoses are very closely related to the saccharide units of alepposides A–E (8,9), although identical sugar sequences were not evident. In particular, the occurrence of α-/β-sarmentose and α-cymarose is a new observation for *Adonis* species and for plants in the Ranunculaceae in general. Indeed, the adoligoses could be possible precursors of oligomeric cardenolide glycosides like the alepposides. In addition, adoligoses A–E [**1–5**] can serve as model compounds for ^{1}H-

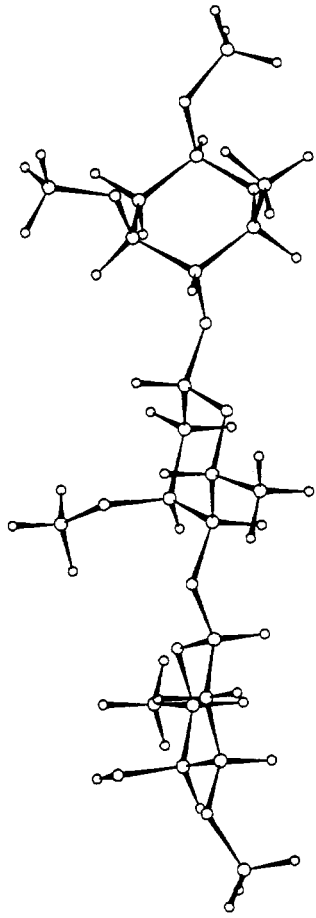


FIGURE 2. Molecular conformation of adoligose D [4] derived from forcefield calculations and ^{1}H-nmr measurements.

nmr-based structure determination of complex cardenolides and therefore should be able to contribute to future studies on the SAR of cardenolide sugar chains.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were recorded at 300° K on Bruker AC300, AC400, and AMX500 spectrometers using 5-mm tubes and solutions in CD_3OD (99.8% D). The solvent chemical shifts were used as internal standards (CD_3OD: δ_H 3.300, δ_C 49.00). ^{1}H-Nmr chemical shifts are given in three decimal places (see Tables 3 and 4) in order to make certain distinctions for signal assignments (digital resolution of measurements better than 0.0004 ppm). COSY nmr spectra were recorded in the absolute value mode using a spectral window of 3000 Hz, with 1 K complex data points in t_2 and 128 t_1 increments. Prior to Fourier transformation, the time domain and data matrices were multiplied with sine window functions in both dimensions. HMQC and HMBC spectra were recorded with 2 K complex data points in t_2 and 256 t_1 increments and were processed using shifted sine window functions in both dimensions. The long-range experiment was optimized for 5 Hz. Dcims were run on a Finnigan INCOS 50 System with NH_3 as reactant gas (emitter heating rate 10 mA sec^{-1}, calibration with FC 43). Optical rotations were measured with a Perkin-Elmer 241 polarimeter and uv spectra taken with a Beckman DB-G instrument. Mplc preparations were carried out on custom-built glass columns (20 cm×16 mm i.d.) with a Knauer hplc pump (Model 64) and a DuPont detector at 210 nm (additional tlc detection was also used). Gradient elution was performed using corresponding vessels as described by Pauli (8). Tlc separations were carried out on Merck precoated Si gel 60 plates (aluminum carrier, fluorescence indicator) developed in

$CHCl_3$-MeOH-H_2O mixtures (75:24:1, 70:29:1, unsaturated chambers). The molecular modeling studies were performed on a Silicon Graphics workstation using the Insight/Discover software package. For minimization, Steepest Descent and Conjugate Gradient procedures were used. Nmr spectral simulations were carried out with the program LAOCOON III implemented for Atari ST computers. Authentic samples of sarveroside and sarmentocymarin were obtained from the Reichstein collection and oleandrin was purchased from Roth Chemicals, Germany.

PLANT MATERIAL.—Authentic plant material of *Adonis aleppica* Boiss. was collected in April 1990, near Urfa, Turkey, and identified by the author. Voucher specimens are deposited at the Heinrich Heine-Universität, Düsseldorf, Germany.

EXTRACTION AND PURIFICATION.—Whole plants (3.5 kg, air-dried) were successively extracted with 23 liters $CHCl_3$, 49 liters MeOH, and 24 liters MeOH/H_2O (70%) with an Ultra-Turrax apparatus. The combined extracts (1.145 kg) were evaporated *in vacuo* (40°) to give a brown gummy residue, divided into 7 portions, re-dissolved in H_2O, and exhaustively extracted with $CHCl_3$-*i*-PrOH (3:2). The organic layers were combined, the solvent was removed *in vacuo*, and the residue (162 g) in three portions filtered over XAD-2 by stepwise elution with H_2O, MeOH, and Me_2CO. Vlc of the MeOH eluates (48 g) on cellulose (Avicel, Merck, stepwise gradient elution with petroleum ether/EtOAc/MeOH/H_2O) followed by gel filtration on 1.0 kg Sephadex LH-20 (MeOH) afforded 30 g of an enriched cardenolide mixture. The latter was fractionated by vlc on Si gel 60 (stepwise gradient elution with petroleum ether/EtOAc/MeOH/H_2O) to yield fractions *1a* (10.6 g) and *1b* (9.7 g).

Fraction *1a* was rechromatographed by vlc (Si gel 60, petroleum ether-CH_2Cl_2-MeOH-H_2O gradient) to generate fractions *1ab* (5,980 mg) and *1ac* (4,870 mg). Droplet counter-current chromatography (dccc) of fraction *1ab* using CH_2Cl_2-*i*-PrOH-H_2O (5:6:4) in the descending mode yielded two fractions, *1aba* (5.2 g) and *1abb* (380 mg). While the latter was combined with fraction *1ac*, fraction *1aba* was purified by vlc on Si gel 60 (gradient elution in 38 steps with petroleum ether/EtOAc/MeOH/H_2O) to give 1.32 g of fraction *1abac* containing compounds **4** and **5**. Further purification was achieved by a three-step mplc procedure on RP-18 Si gel (LiChroprep, Merck) using MeCN/H_2O and MeOH/H_2O gradients, respectively, yielding two fractions *1abac/1* (7 mg) and *1abac/2* (15 mg). Compound **4** was isolated from fraction *1abac/1* by mplc on RP-18 Si gel with MeOH/H_2O gradient elution in a yield of 3.7 mg. Compound **5** was purified by prep. tlc of fraction *1abac/2* in solvent system $CHCl_3$-MeOH-H_2O (85:14:0.5) to give 6.6 mg of pure **5**. Compounds **4** and **5** both showed an R_f value of 0.62 [$CHCl_3$-MeOH-H_2O (84:14:0.5)]. Fraction *1ac* was submitted to further dccc using $CHCl_3$-*i*-PrOH-H_2O (5:6:4) in the descending mode, which afforded 1210 mg of fraction *1acb*, containing compounds **2** and **3**. Fraction *1acb* was then chromatographed in six steps by mplc on normal-phase Si gel 60 and RP-18 Si gel (LiChroprep, Merck) using *n*-hexane/EtOAc and MeOH/H_2O gradients, respectively. Prep. tlc in EtOAc-MeOH-H_2O (77:15:8), upon evaporation, afforded 10 mg of **2** and 12 mg of **3**, showing R_f values of 0.47 and 0.38 [EtOAc-MeOH-H_2O (77:15:8)], respectively.

Fraction *1b* was also rechromatographed by vlc in two steps [Si gel 60, EtOAc-MeOH-H_2O gradient, to produce fractions *1bb* (7,140 mg) and *1bbb* (4,270 mg), respectively] and dccc [$CHCl_3$-*i*-PrOH-H_2O (5:6:4), descending mode] to give 187 mg of fraction *1bbbd*. The latter was chromatographed twice by mplc on RP-18 Si gel (LiChroprep, Merck) using MeOH/H_2O gradients, which afforded 32 mg of **1** showing an R_f value of 0.69 [$CHCl_3$-MeOH-H_2O (75:24:1)].

Adoligose A [**1**].—Amorphous colorless solid: mp 136°; $[\alpha]^{20}D$ −20° (*c*=1.11, MeOH); uv λ max <210 nm; dci-NH_3-ms *m/z* 760 $[MNH_4]^+$ (20), 728 $[MNH_4-MeOH]^+$ (12), 598 $[MNH_4-(glc-H_2O)]^+$ (78), 580 $[MNH_4-glc]^+$ (6), 454 $[MNH_4-(glc-dgn-H_2O)]^+$ (8), 436 $[MNH_4-(glc-dgn)]^+$ (7), 324 $[MNH_4-(glc-dgn-H_2O)-(dgx-H_2O)]^+$ (7), 306 (9), 180 (72), 162 (46), 130 (78); ^{13}C-nmr data, see Tables 1 and 2; ^1H-nmr data, see Tables 3 and 4.

Me-adoligose B [**2**].—Amorphous colorless solid: mp 122°; $[\alpha]^{20}D$ −15° (*c*=0.60, MeOH); uv (MeOH) λ max <210 nm; dci-NH_3-ms *m/z* 644 $[MNH_4]^+$ (95), 612 $[MNH_4-MeOH]^+$ (3), 482 $[MNH_4-(glc-H_2O)]^+$ (2), 464 $[MNH_4-glc]^+$ (5), 338 $[MNH_4-(glc-dgn-H_2O)]^+$ (27), 324 (16), 320 (18), 306 (15), 162 (48), 127 (100); ^{13}C-nmr data, see Tables 1 and 2; ^1H-nmr data, see Tables 3 and 4.

Me-adoligose C [**3**].—Amorphous colorless solid: mp 150°; $[\alpha]^{20}D$ −36° (*c*=0.88, MeOH); uv (MeOH) λ max <210 nm; dci-NH_3-ms *m/z* 644 $[MNH_4]^+$ (43), 612 $[MNH_4-MeOH]^+$ (8), 482 $[MNH_4-(glc-H_2O)]^+$ (1), 464 $[MNH_4-glc]^+$ (3), 338 $[MNH_4-(glc-dgn-H_2O)]^+$ (19), 324 (16), 320 (6), 306 (30), 162 (100), 127 (45); ^{13}C-nmr data, see Tables 1 and 2; ^1H-nmr data, see Tables 3 and 4.

Me-adoligose D [**4**].—Amorphous colorless solid: mp 130°; $[\alpha]^{20}D$ −33° (*c*=0.36, MeOH); uv (MeOH) λ max <210 nm; dci-NH_3-ms *m/z* 482 $[MNH_4]^+$ (77), 450 $[MNH_4-MeOH]^+$ (43), 352 $[MNH_4-(dgn-H_2O)]^+$ (22), 338 (21), 324 (27), 306 (41), 178 (88), 162 (92), 127 (100); ^{13}C-nmr data, see Tables 1 and 2; ^1H-nmr data, see Tables 3 and 4.

Me-adoligose E [**5**].—Amorphous colorless solid: mp 108°, $[\alpha]^{20}D$ −71° (c=0.55, MeOH); uv (MeOH) λ max <210 nm; dci-NH_3-ms m/z 482 $[MNH_4]^+$ (72), 450 $[MNH_4-MeOH]^+$ (23), 352 $[MNH_4-(dgn-H_2O)]^+$ (5), 338 (18), 324 (42), 306 (33), 178 (78), 162 (79), 127 (100); ^{13}C-nmr data, see Tables 1 and 2; ^1H-nmr data, see Tables 3 and 4.

ACKNOWLEDGMENTS

The author is grateful to Prof. Dr. S. Berger, Philipps-Universität Marburg, for nmr measurements and helpful discussions, as well as to Dr. U. Matthiesen, Heinrich-Heine-Universität Düsseldorf, for dci mass spectral support. Many thanks are due to Prof. Dr. T. Reichstein, Basel, and Mr. R. Wandler, Sigma Chemicals, Milwaukee, WI, for supplying some of the authentic cardenolides. Furthermore, the reliable technical assistance of Mrs. S. Borstel, Heinrich Heine-Universität Düsseldorf, is gratefully acknowledged.

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Received 27 June 1994