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ALEPPOSIDES, CARDENOLIDE OLIGOGLYCOSIDES FROM *ADONIS ALEPPICA*

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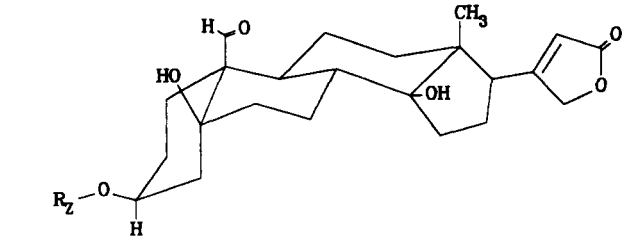
ABSTRACT.—The structures of novel oligoglycosidic cardenolides, alepposide A ($C_{55}H_{86}O_{23}$) [**1**] and alepposide B ($C_{48}H_{74}O_{20}$) [**2**], have been deduced mainly by nmr methods. Based on homonuclear (^{1}H and ^{13}C nmr, ^{1}H COSY) and proton-detected heteronuclear shift correlation experiments [HMQC both for ^1J(C,H) and for long-range couplings], alepposide A [**1**] was shown to have the structure strophanthidin-3-*O*-β-glucopyranosyl-(1↦4)-*O*-β-diginopyranosyl-(1↦4)-*O*-β-oleandropyranosyl-(1↦4)-*O*-β-digitoxopyranosyl-(1↦4)-*O*-β-digitoxopyranoside. The structure of alepposide B [**2**] was established as strophanthidin-3-*O*-β-glucopyranosyl-(1↦4)-*O*-β-oleandropyranosyl-(1↦4)-*O*-β-digitoxopyranosyl-(1↦4)-*O*-β-digitoxopyranoside.

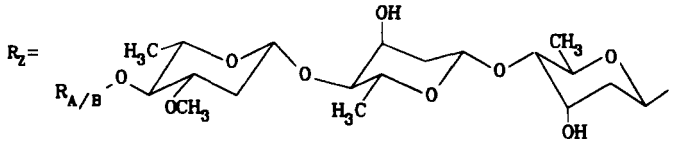
Earlier examination of the annual *Adonis aleppica* Boiss. (Ranunculaceae), a close relative of the perennial *Adonis vernalis*, led to the isolation of 3-*epi*-periplogenine, periplorhamnoside, and strophanthidin-diginoside (1). Upon renewed investigation the isolation of uzarigenin-3-*O*-sulfate, a 5-α-cardenolide, and aleppotrioloside, an aliphatic alcohol glycoside, was reported (2,3). Early studies showed *A. aleppica* to contain a cardenolide named a_6 with a pentameric sugar moiety and a bluish-grey color-reaction with vanillin/H_2SO_4 (1), a color typically occurring with 19-methylcardenolides. Considering the aldehyde resonance in the ^{1}H nmr (δ_H = 10.0 ppm), a_6 was believed to be derived from a new 19-aldehyde aglycone (1).

Because they are rarely found in nature, oligoglycosidic cardenolides are of special interest regarding the phytochemistry of cardenolides in general and the chemotaxonomy of the genus *Adonis* in particular. The present paper deals with the isolation and characterization of two novel cardenolides, named alepposides A [**1**] and B [**2**], with long-chained sugar moieties. High field nmr measurements were applied to deduce the complete structure and stereochemistry of these complex oligosaccharides without resorting to derivatization/degradation. Employment of proton-detected heteronuclear correlation spectroscopy [HMQC both for ^1J(C,H) and long-range couplings] with increased sensitivity allowed the acquisition of CH correlations using small samples.

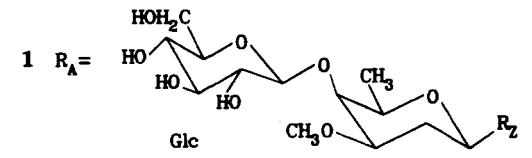
RESULTS AND DISCUSSION

In order not to omit phenolic substances and to avoid degradation of genuine glycosides, the isolation of alepposides A and B from the organic layer [$CHCl_3$-iPrOH (3:2)] of the MeOH/H_2O extract was carried out without the usual precipitation with Pb^{++}-acetate. Therefore, extensive pre-purification by gel filtration (Sephadex LH-20), liquid chromatography (lc) on Amberlite XAD-2, and droplet counter-current chromatography (dccc) was necessary. Further lc separation with Si gel and reversed-phase middle pressure liquid chromatography (rp-mplc) gave pure compounds **1** and **2**.

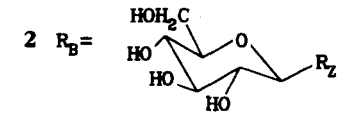




Ole Dgx Dgx



Glc Dgn



Glc

The ^{1}H-nmr spectrum of **1** shows signals due to five anomeric protons: one doublet (d) at 4.56 ppm and four doublets of doublets (dd) corresponding to 2-desoxy sugars with 1β configuration. The latter are shown to be 2,6-didesoxy sugars by the presence of four methyl doublets ($J = 6.3$ Hz) between 1.2 and 1.3 ppm. Two of the 2,6-didesoxy sugars are methyl ethers (OMe absorptions at 3.40 and 3.39 ppm), which gives a marked upfield shift to the anomeric protons (δ_H 4.67 and 4.62 ppm, respectively) compared with the unmethylated sugars (δ_H 4.91 ppm, 2H). The latter anomeric proton signals are partially overlapped with the signals belonging to the AB(X) spin system of H-21 (δ^A_H 5.02 and δ^B_H 4.90 ppm). This behavior is typical of digitoxose (Dgx) (4). Together with H-22 (δ 5.89 ppm, dd) the presence of a γ-butenolide ring system is confirmed; thus the aglycone of **1** may be assumed to be a cardenolide.

One methyl singlet at δ 0.84 ppm and an aldehyde absorption at δ 10.04 ppm are assigned to H-18 and H-19. The signal of H-17α appears as a typical multiplet at δ 2.82 ppm. A broad singlet at δ 4.13 ppm indicates an equatorial proton at the C-3 position, which can be associated with a 3β,5β-diOH- or a 3α,5α-diOH-steroid skeleton.

Because of the multiple signals between 3.0 and 4.3 ppm, evidence for the hydroxylation pattern of the aglycone moiety could not be obtained directly from the ^{1}H-nmr spectra. ^{13}C-nmr data were therefore obtained and comparisons made with data of cardenolides isolated in our laboratory, namely convallatoxin. From the 55 carbon signals

of **1**, 23 could be unambiguously assigned to the skeleton of strophanthidin (Table 1). The 3-*O*-linkage of the sugar unit, as usually found in cardenolides, was ascertained by glycosidation shift effects of the A-ring carbons (C-3 +8.28 ppm, C-4 −1.75 ppm, C-2 −1.63 ppm).

The main information obtained from the dci-NH_3 ms of **1** is given in Table 2. A quasi-molecular ion at *m/z* 1132 $[M+NH_4]^+$ is in agreement with the presence of strophanthidin (*m/z* 422), four 2,6-didesoxy sugars (mol wt 148↦130 in sugar chains), two OMe groups (+*m/z* 14 each) and one hexose moiety (mol wt 180↦162 in sugar chains) deduced from the ^{1}H-nmr spectra. Dci-NH_3 ms shows a stepwise degradation of both the "intact" glycoside and the oligomeric sugar moiety: Glycoside fragmentation starts with the terminal sugar (Glc in **1** and **2**), while oligoglycosides formed by cleavage of the relatively weak sugar–3β-*O*-aglycone bonds are degraded from the anomeric ends (Dgx in **1** and **2**). From this fragmentation pattern, the sequential arrangement of the monosaccharide units in **1** can be determined: the two Dgx units are attached to the aglycone moiety and to one another, followed by two 2,6-desoxy-3-OMe sugars and a terminally linked hexose. Thus, besides the arrangement of the 3-

TABLE 1. Nmr Data[a] for the Aglycone Moieties of Alepposides A [**1**] and B [**2**] in Comparison with the ^{13}C-nmr Data of the Strophanthidin Portion of Convallatoxin (=Strophanthidin-rhamnoside).

Carbon	Compound 1	Proton	Compound 1	Compound 2	Convallatoxin
	δ_C		δ_H	δ_C	δ_C
C-1	25.91[b]	H-1α, -1β	2.13 m, 1.31 m[b]	25.94	25.97
C-2	25.18	H-2α, -2β	1.60 m, 1.93 m	25.18	25.23
C-3	76.26	H-3α	4.14 br s	76.28	75.32
C-4	36.77	H-4α, -4β	1.62 m, 2.18 m	36.82	36.21
C-5	75.29			75.32	74.89
C-6	37.11	H-6α, -6β	1.64 m, 1.48 m	37.22	37.26
C-7	18.94[b]	H-7α, -7β	1.76 m, 2.10 m[b]	18.96	19.08
C-8	42.56	H-8β	1.97 m	42.60	42.63
C-9	40.48	H-1α	1.44 m	40.54	40.48
C-10	56.09			56.12	56.11
C-11	23.26	H-11α, -11β	1.53 m, 1.20 m	23.25	23.27
C-12	40.35	H-12α, -12β	1.52 m, 1.68 m	40.40	40.34
C-13	50.71			50.74	50.72
C-14	85.91			85.93	85.92
C-15	32.42	H-15α, -15β	1.65 m, 2.16 m	32.46	32.46
C-16	27.93	H-16α, -16β	2.12 m, −[c]	27.95	27.95
C-17	51.71	H-17α	2.82 m	51.77	51.76
C-18	16.18	H-18	0.84 s	16.16	16.15
C-19	209.90	H-19	10.04 s	209.93	209.73
C-20	177.14			177.17	177.19
C-21	75.21	H_A-21, H_B-21	5.02 dd, 4.90 dd, $J_{A,B}=18.4$ and $J_{21,22}=1.5$	75.22	75.20
C-22	117.92	H-22	5.89 dd	177.97	177.93
C-23	178.15			178.14	178.14

[a]In CD_3OD at 300 K. Chemical shifts are relative to solvent shift: $\delta_H=3.30$ ppm, $\delta_C=49.00$ ppm; *J* given in Hz.

[b]Opposite assignment is more consistent with glycosidation effects calculated for several strophanthidin glycosides.

[c]Could not be assigned.

TABLE 2. Dci-NH_3 Ms Data of Alepposide A [**1**].

m/z	Ion	Composition
Cardenolide glycoside		
1132	$[M+NH_4]^+$	Str-Dgx-Dgx-Ole-Dgn-Glc
970	$[M+NH_4]-(Glc-H_2O)]^+$	Str-Dgx-Dgx-Ole-Dgn
826	$[M+NH_4-((Glc\text{-}Dgn)-H_2O)]^+$	Str-Dgx-Dgx-Ole
682	$[M+NH_4-((Glc\text{-}Dgn\text{-}Ole)-H_2O)]^+$	Str-Dgx-Dgx
552	$[M+NH_4-((Glc\text{-}Dgn\text{-}Ole\text{-}Dgx)-H_2O)]^+$	Str-Dgx
422	$[M(aglycone)+NH_4]^+$	Str
Sugar moiety (S)		
728	Dgx-Dgx-Ole-Dgn-Glc	$S+NH_4^+-H_2O$
598	Dgx-Ole-Dgn-Glc	$S+NH_4^+-Dgx$
468	Ole-Dgn-Glc	$S+NH_4^+-2\,Dgx$
324	Dgn-Glc	$S+NH_4^+-2\,Dgx\text{-}Ole$

OMe sugars, the sequence of the oligoglycosidic portion of **1** could be deduced from the ms data.

The ^{1}H-nmr (1D and 2D) spectra of alepposide B [**2**] are remarkably similar to those of **1**. The tetrasaccharide nature of its sugar moiety followed from the occurrence of four glycosidically linked anomeric H resonances, of which two (δ 4.91 ppm, 2H) suggest the presence of two Dgx moieties (as in **1**). One main difference from **1** is the absence of one 2,6-didesoxy-3-OMe sugar, while the four remaining sugars and the aglycone moiety are identical. The structure of a tetrameric sugar portion for **2** is supported by the dci-NH_3 ms data (Table 3): The two Dgx units are attached to the aglycone moiety, followed by one 2,6-desoxy-3-*O*-Me sugar and a terminally linked hexose.

Corroborative evidence for the molecular structures of the sugar moieties was obtained from an inverse-detected direct (one-bond) heteronuclear correlation experiment (HMQC) of **1**. This led to the full CH assignment of the strophanthidin portion (Table 1). Further reliable identification of the sugars as β-digitoxose (Dgx I, Dgx II), β-diginose (Dgn), β-oleandrose (Ole), and β-glucose (Glc) was achieved.

In Table 4 are summarized ^{1}H- and ^{13}C-nmr data of the oligosaccharide portion of alepposide A [**1**]. With moderate signal overlap in the 3.1–4.2 ppm region, coupling constants could be determined and assignment of ring proton resonances within individual monosaccharide units was available from ^{1}H,^{1}H-COSY and HMQC experiments. Thus, besides the anomeric protons, typical proton signals due to each sugar could be determined from the ^{1}H-nmr spectra (1D,2D) of **1** and **2**.

TABLE 3. Dci-NH_3 Ms Data of Alepposide B [**2**].

m/z	Ion	Composition
Cardenolide glycoside		
988	$[M+NH_4]^+$	Str-Dgx-Dgx-Ole-Glc
826	$[M+NH_4]-(Glc-H_2O)]^+$	Str-Dgx-Dgx-Ole
682	$[M+NH_4-((Glc\text{-}Ole)-H_2O)]^+$	Str-Dgx-Dgx
552	$[M+NH_4-((Glc\text{-}Ole\text{-}Dgx)-H_2O)]^+$	Str-Dgx
422	$[M(aglycone)+NH_4]^+$	Str
Sugar moiety (S)		
584	Dgx-Dgx-Ole-Glc	$S+NH_4^+-H_2O$
454	Dgx-Ole-Glc	$S+NH_4^+-Dgx$
324	Ole-Glc	$S+NH_4^+-2\,Dgx$

TABLE 4. Nmr Data[a] for the Oligosaccharide Moiety of Alepposide A [1].

Unit[b]		δ_C	δ_H	$J_{H,H}$
Dgx I	1′	98.24	4.90_9	$J_{1,2eq}=1.8, J_{1,2ax}=8.5$
	2′	38.71	$1.98\,(2_{eq})$	$J_{2eq,3}=5.5, J_{2eq,2ax}=13.6$
			$1.68\,(2_{ax})$	$J_{2ax,3}=2.9$
	3′	68.28	4.22_2	$J_{3,4}=2.9$
	4′	83.51	3.21_5	$J_{4,5}=9.6$
	5′	69.42	3.80	$J_{5,6}=6.3$
	6′	18.46	1.20	
Dgx II	1″	100.52	4.90_6	$J_{1,2eq}=1.8, J_{1,2ax}=8.5$
	2″	38.54	$2.03\,(2_{eq})$	$J_{2eq,3}=5.5, J_{2eq,2ax}=13.6$
			$1.72\,(2_{ax})$	$J_{2ax,3}=2.9$
	3″	68.28	4.22_8	$J_{3,4}=2.9$
	4″	83.51	3.26_5	$J_{4,5}=9.6$
	5″	69.57	3.84	$J_{5,6}=6.3$
	6″	18.49	1.21	
Ole	1‴	102.16	4.62	$J_{1,2eq}=1.8, J_{1,2ax}=9.6$
	2‴	37.11	$2.33\,(2_{eq})$	$J_{2eq,3}=5.2, J_{2eq,2ax}=12.5$
			$1.46\,(2_{ax}$	$J_{2ax,3}=11.4$
	3‴	80.27	3.35_8	$J_{3,4}=9.2$
	4‴	84.07	3.16	$J_{4,5}=9.2$
	5‴	72.29	3.35_0	$J_{5,6}=6.3$
	6‴	18.80	1.28	
	OMe‴	57.21	3.40_2	
Dgn	1⁗	102.34	4.67	$J_{1,2eq}=2.2, J_{1,2ax}=9.9$
	2⁗	33.59	$2.00\,(2_{eq})$	$J_{2eq,3}=4.4, J_{2eq,2ax}=11.8$
			$1.75\,(2_{ax})$	$J_{2ax,3}=12.5$
	3⁗	80.69	3.44	$J_{3,4}=2.6$
	4⁗	74.08	3.99	$J_{4,5}=1.3$
	5⁗	71.91	3.50	$J_{5,6}=6.3$
	6⁗	17.69	1.30	
	OMe⁗	56.60	3.39_5	
Glc	1⁗′	104.30	4.56	$J_{1,2}=7.7$
	2⁗′	75.95	3.21_7	$J_{2,3}=9.2$
	3⁗′	78.13	3.35_8	$J_{3,4}=9.2$
	4⁗′	71.80	3.26_8	$J_{4,5}=9.6$
	5⁗′	77.89	3.23	$J_{5,6a}=2.2, J_{5,6b}=5.5$
	6⁗′	62.99	$3.86\,(6_a)$	$J_{6a,6b}=11.8$
			$3.65\,(6_b)$	

[a]In CD_3OD at 300 K. Chemical shifts are relative to solvent shift: $\delta_H=3.30$ ppm, $\delta_C=49.00$ ppm.
[b]Dgx = digitoxose, Dgn = diginose, Glc = glucose, Ole = oleandrose; mutual coupling constants are given only once, at their first occurrence in the table.

Glucose (Glc) was identified by the coupling pattern of its all axial protons and H-6 [δ_H 3.86 and 3.65 ppm, respectively, AB(M) spin system]. H-2 characteristically appears at highest field as a non-overlapped double of doublets (δ_H 3.22 ppm).

The mostly deshielded proton of digitoxose (Dgx) is H-3eq (ddd, only small J's) forming an AMXY spin system together with the H-4ax and H-2 methylene protons. The H-5 signal (dq) shows a large coupling ($J=9.6$) to H-4, indicating that the latter is axial. Compound **1** gives two sets of parallel proton signals due to the two Dgx moieties, which in case of H-3 and H-5 are distinguishable.

Diginose (Dgn) exhibits three typical non-overlapped absorptions. The signal of H-4 appears at lowest field showing only small J's. A coupling constant of 1.3 Hz extracted from the H-5 signal (dq) demonstrates the equatorial orientation of H-4, while H-3 must be axial since $J_{2eq,3}=12.5$ Hz.

Oleandrose (Ole) could be shown to be present in **1** and **2**, while diginose was absent in **2**. Thus according to the ms fragmentation pattern the monosaccharides of alepposide B are arranged as -Dgx-Dgx-Ole-Glc. In oleandrose the C-2 methylene protons form part of a nuclei first-order spin system (AMXY) resonating at extremely different shift values ($\Delta\delta = 1.17$ ppm) with H-2eq unusually deshielded at 2.33 ppm. The detection of H-3/H-5 Ole protons is hindered by extensive signal overlap in the 3.1–3.5 ppm region. However, information about the sequential arrangement of the monosaccharide units in **1** came from the shift value of Ole H-4: If the terminal disaccharide of **1** is -Ole-Glc, H-4 representing the geminal proton of the obligatory position of glycosidation should have equal shift values in **1** and **2**. However, H-4 is shifted more upfield in **1** which should be caused by a weaker glycosidation effect of a 2,6-didesoxy sugar (Dgn) compared with that of (terminal) glucose in **2**. Analogous observations can be made comparing the shift values of the anomeric glucose protons ($\Delta\delta = 0.122$ ppm). Thus, the terminal trisaccharide in **1** is suggested to be -Ole-Dgn-Glc.

Definitive evidence for the composition of **1** was obtained from an inverse-detected long-range heteronuclear correlation experiment (long-range variation of the HMQC experiment). Long-range couplings between anomeric and H-4 "sugar protons" could be detected in both directions (H-1↦H-4 and H-4↦H-1) proving the sequential arrangement of the monosaccharide units in **1** to be -Dgx-Dgx-Ole-Dgn-Glc and confirming their all (1↦4) glycosidic linkage. A particularly meaningful section of this map is given in Figure 1. Interestingly F 1 cross-sections ($^1H\mapsto{}^{13}C$) in some cases allow the identification of sugar ring carbons proving multiple long-range coupling behavior

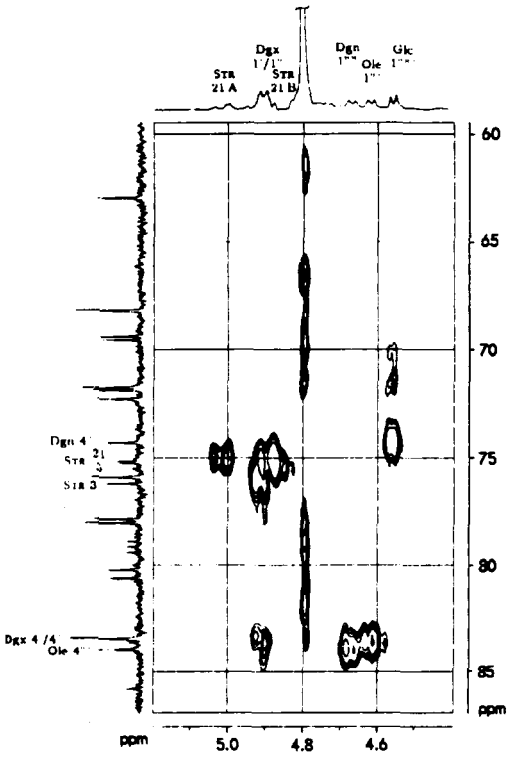


FIGURE 1. Section of the long-range HMQC spectrum of alepposide A [**1**], providing proof of the sequential arrangement of the sugar units.

within the sugar units. For example, Ole and Dgn ring carbons can be detected from the corresponding Ole-H-2eq and Dgn-H-4 cross-sections. Moreover the structure of **2** could be confirmed by ^{13}C-nmr measurements (Tables 1 and 5) which gave further evidence for the identity of the sugar units as well as for the strophanthidin portion.

From the structural information, alepposide A [**1**] has the structure strophanthidin-3-*O*-β-glucopyranosyl-(1→4)-*O*-β-diginopyranosyl-(1→4)-*O*-β-oleandropyranosyl-(1→4)-*O*-β-digitoxopyranosyl-(1→4)-*O*-β-digitoxopyranoside, while alepposide B [**2**] is strophanthidin-3-*O*-β-glucopyranosyl-(1→4)-*O*-β-oleandropyranosyl-(1→4)-*O*-β-digitoxopyranosyl-(1→4)-*O*-β-digitoxopyranoside.

For **1** and **2** "sugar protons" the spin systems are non-first-order. Additionally, multiple long-range couplings ($J \leq 1.0$ Hz), resulting in slight signal broadening, make difficult the direct determination of coupling constants. Therefore spectral simu-

TABLE 5. Nmr Data[a] for Alepposide B [**2**].

Unit[b]		δ_H	$J_{H,H}$	δ_C
Str	3α	4.14 br s		
	17α	2.82 m		
	18 CH_3	0.84 s		
	19 CHO	10.04 s		
	21_A	5.01 dd	$J_{A,B}=18.4, J_{21,22}=1.5$	
	21_B	4.90 dd		
	22	5.89 dd		
Dgx I	1′	4.90	$J_{1,2eq}=1.5, J_{1,2ax}=8.8$	98.28
	2′	1.96 (2_{eq})	$J_{2eq,3}=5.5, J_{2eq,2ax}=13.6$	38.75
		1.71 (2_{ax})	$J_{2ax,3}=2.6$	
	3′	4.23	$J_{3,4}=2.6$	68.30
	4′	3.25	$J_{4,5}=9.6$	83.56
	5′	3.80	$J_{5,6}=6.3$	69.46
	6′	1.22		18.45
Dgx II	1″	4.90	$J_{1,2eq}=1.5, J_{1,2ax}=8.8$	100.52
	2″	2.01 (2_{eq})	$J_{2eq,3}=5.5, J_{2eq,2ax}=13.6$	38.72
		1.72 (2_{ax})	$J_{2ax,3}=2.6$	
	3″	4.23	$J_{3,4}=2.6$	68.30
	4″	3.23	$J_{4,5}=9.6$	83.56
	5″	3.84	$J_{5,6}=6.3$	69.61
	6″	1.20		18.48
Ole	1‴	4.65	$J_{1,2eq}=1.8, J_{1,2ax}=9.6$	102.11
	2‴	2.33 (2_{eq})	$J_{2eq,3}=5.2, J_{2eq,2ax}=12.5$	37.53
		1.49 (2_{ax})	$J_{2ax,3}=11.4$	
	3‴	3.41	$J_{3,4}=9.2$	80.15
	4‴	3.28	$J_{4,5}=9.2$	83.50
	5‴	3.41	$J_{5,6}=6.3$	72.78
	6‴	1.36		18.77
	OMe‴	3.46		58.20
Glc	1⁗	4.44	$J_{1,2}=7.7$	104.13
	2⁗	3.16	$J_{2,3}=9.2$	75.60
	3⁗	3.33	$J_{3,4}=9.2$	78.27
	4⁗	3.22	$J_{4,5}=9.6$	71.82
	5⁗	3.24	$J_{5,6a}=1.8, J_{5,6b}=5.9$	78.11
	6	3.85 (6_a)	$J_{6a,6b}=11.8$	63.06
		3.62 (6_b)		

[a]In CD_3OD at 300 K. Chemical shifts are relative to solvent shift: $\delta_H = 3.30$ ppm; J given in Hz.

[b]Str = strophanthidin, Dgx = digitoxose, Glc = glucose, Ole = oleandrose; mutual coupling constants are given only once, at their first occurrence in the table.

lations have been carried out by the use of LAOCOON III. The results were in good agreement with the measured spectra. Coupling constants given are based on both the measured spectra and spectral simulations.

Because of their solubility, and following the relevant literature (4,6), the nmr spectra of **1** and **2** were initially recorded in $CDCl_3$ (400 MHz). Comparative studies in different solvents (pyridine-d_5, $CDCl_3$, CD_3OD) have shown enormous solvent effects especially concerning "sugar protons." Solutions in CD_3OD gave the best results with regard to reduction of signal overlap, resolution of small coupling constants, and stability of cardenolide solutions. However, only higher field nmr measurements (500 MHz) yield separated signals of H-21, Dgx anomeric protons, and HDO when CD_3OD is used.

To our knowledge, this is the first report of tetra- and pentaglycosidic C-19-aldehyde cardenolides. From a chemotaxonomical point of view, the findings of oligoglycosidic strophanthidin derivatives is an important tool for classification of the genus *Adonis*. The cardenolides isolated from *A. vernalis* as well as from other *Adonis* spp. are mostly derived from periplogenin, strophanthidin, 16-OH-strophanthidin, and adonitoxigenin, usually having mono- or disaccharide moieties. While cardenolides containing complex sugar chains are unknown in other *Adonis* spp. as summarized by Junginger (5), **1** and **2** appear in *A. aleppica* in considerable amounts. Additionally, cardenolide sulfates like uzarigenin-3-*O*-sulfate representing the major cardenolide of *A. aleppica* (2) have not been detected in the perennial *A. vernalis* and *Adonis amurensis* in the course of detailed studies (5). On the other hand, strophanthidin and its glycosides have been found in all the extracts of *Adonis* spp. studied, in annual as well as in perennial ones. Up to now, *A. aleppica*, due to the unusual way of conjugation/derivatization of the cardenolide aglycones (sulfatation and oligoglycosylation), seems to have an exceptional position within the genus *Adonis*. Further studies on the cardenolide complex of annual *Adonis* plants are necessary to determine whether or not other species show similar metabolic behavior.

Finally, alepposide A could be shown to be identical with compound a_6 isolated before from the same plant (1) by comparison of the ^1H- and ^{13}C-nmr data. However, a_6 was reported to give a bluish color reaction with vanillin/H_2SO_4, whereas the aglycone strophanthidin and its mono- and diglycosides produce green spots (5). Compounds **1** and **2** also did not give the typical green but gave a greenish-blue color. Although the mechanism of the vanillin/H_2SO_4 reaction is unknown, the sugar moieties of cardenolides could make contributions to the resulting color, especially in the case of extensive sugar chains. We have succeeded in the isolation of oligosaccharides which contain those 2,6-didesoxy, and/or 3-*O*-Me sugars typically found in cardenolides. These substances reveal bluish-grey colors after vanillin/H_2SO_4 detection, and their identification will be subject of future publication. With these findings, the "untypical" blue vanillin/H_2SO_4 reaction of alepposides should be interpreted as due to both the aglycone and the sugar moiety. Thus compound a_6 (=alepposide A) is not inevitably derived from a new 19-aldehyde cardenolide (1) but is actually a strophanthidin glycoside.

EXPERIMENTAL

INSTRUMENTATION.—Nmr spectra were recorded at 300° K on a Bruker AMX 500 spectrometer using solutions in CD_3OD (99.8% D). The solvent shifts were used as internal standard (CD_3OD: δ_H 3.30 ppm, δ_C 49.00 ppm). COSY spectra were recorded in the absolute value mode using a spectral window of 3000 Hz, 1K complex data points in t_2 and 128 t_1 increments. Prior to Fourier transformation, the time domain data matrices were multiplied with sine window functions in both dimensions. The inverse-detected heteronuclear shift correlation (HMQC) experiment was performed with a 13,000 × 3000 Hz spectral window, acquiring 1K complex data points in t_2 and 128 t_1 increments. Prior to Fourier transformation

this was multiplied with a sine window function in both dimensions and extended to yield a 1K × 1K frequency domain real matrix. Inverse-detected long-range heteronuclear shift correlation was carried out with the HMQC method using a delay of 50 msec between the first two pulses of the sequence, a 13,000 × 3000 Hz spectral window, and 1K complex data points in t_2 and 128 t_1 increments. The time domain data matrix was multiplied with sine (F2) and qsine (F1) window functions and extended to yield a 1K × 1K frequency domain real matrix. Dci mass spectra were run on a Finnigan INCOS 50 System with NH_3 as reactant gas (emitter heating rate 10 m A·sec^{-1}, calibration with FC43). Optical rotations were measured with a Perkin-Elmer 241 polarimeter; uv spectra were taken with a Beckmann DB-G instrument and ir with a Perkin-Elmer 297 photometer. Mplc preparations were carried out on a self-built glass column (20 cm × 16 mm i.d.) with a Knauer hplc pump (Model 64) and a DuPont detector at 217 nm.

COLLECTION OF PLANT MATERIAL.—Authentic plant material of *A. aleppica* was collected in April 1990 near Urfa (Turkey) and identified by the authors (GFP/PJ). Voucher specimens are deposited at the Heinrich-Heine-Universität, Düsseldorf, Germany.

EXTRACTION.—Whole plants (700 g, air-dried) were successively extracted with petroleum ether (bp 60–80°), MeOH, and MeOH/H_2O (50%) with an Ultra-Turrax apparatus. The combined MeOH and MeOH/H_2O extracts (115 g) were evaporated in vacuo to give a brown gummy residue, re-dissolved in H_2O, and exhaustively extracted with $CHCl_3$-iPrOH (3:2). The organic layers were combined, the solvent removed in vacuo, and the residue (26 g) re-dissolved in H_2O and extracted with CH_2Cl_2-petroleum ether (3:7). The material in the H_2O layer (22.6 g) was filtered over XAD-2 by stepwise elution with H_2O, MeOH, and Me_2CO. The MeOH eluates (4.3 g) were chromatographed on a Büchi 670 dcc chromatograph using $CHCl_3$-MeOH-H_2O (5:6:4) in descending mode. Fractions were monitored by tlc, and similar fractions were combined. One fraction (2.8 g) was further purified by lc on Sephadex LH 20 (118 g, MeOH) yielding a mixture of less polar cardenolides (2.1 g), which was submitted to lc on Si gel (100 g, 40–63 μm, tlc monitoring) with a discontinuous gradient of $CHCl_3$-MeOH-H_2O (100:0:0→75:24:1) to give crude fractions containing alepposides A and B. Convallatoxin (Table 1) was isolated from the same plant and identified by nmr measurements and comparison of the spectral data and physical properties with those of authentic convallatoxin (Fa. Merck, Germany).

ISOLATION OF ALEPPOSIDE A [**1**].—Upon evaporation, fractions 274–285 (10 ml each) gave an amorphous material (111 mg), from which **1** was isolated by mplc on RP-18 Si gel (24 g LiChroprep, 25–40 μm), eluting with a continuous MeOH/H_2O gradient (30 to 57% MeOH in 120 min, flow rate 5 ml·min^{-1}). The fractionation was monitored by uv detection and tlc and afforded **1** (25 mg) as an amorphous solid, showing R_f 0.70 on Si gel tlc using $CHCl_3$-MeOH-H_2O (80:19:1); $[\alpha]^{20}D$ +5.3° [c = 0.936, MeOH]; uv λ max (MeOH) nm (log ε) 216 (4.13); ir ν max (KBr) cm^{-1} 3440 (OH ν), 2930 (Me ν), 1760 and 1740 (butenolide), 1715 (C=O ν), 1620 (butenolide), 1460 (Me δ_{as}); dci-NH_3 ms see Table 2; ^1H and ^{13}C nmr see Tables 1 and 4.

ISOLATION OF ALEPPOSIDE B [**2**].—Evaporation of fractions 286–320 (10 ml each) gave an amorphous material (164 mg) which was submitted to mplc on RP-18 Si gel as above, affording 45 mg of an amorphous solid. Further purification was achieved by preparative tlc (Si gel F_{254}, 20 × 20 cm) using EtOAc-MeOH-H_2O (77:15:8). Compound **2** was obtained as an amorphous solid (8.5 mg), showing R_f 0.54 on Si gel tlc using $CHCl_3$-MeOH-H_2O (80:19:1): $[\alpha]^{20}D$ +7.7° [c = 0.850, MeOH]; uv λ max (MeOH) nm (log ε) 217 (4.16); ir ν max (KBr) cm^{-1} 3420 (OH ν), 2920 (Me ν), 1750 and 1740 (butenolide), 1705 (C=O ν), 1620 (butenolide); dci-NH_3 ms: see Table 3; ^1H and ^{13}C nmr see Tables 1 and 5.

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