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CIS-EUDESMAN-12,6-OLIDES FROM *CALOSTEPHANE DIVARICATA*

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Key Word Index—*Calostephane divaricata;* Compositae; Inuleae; sesquiterpene lactones; eudesmanolides; germacranolide, *seco*-eudesmanolide.

Abstract-The reinvestigation of *Calostephane* diuaricata, collected in Namibia, resulted in the isolation of 19 eudesmanolides, called calostephanolides and divaricatolides, respectively, a germacranolide and a seco-eudesmanolide. The structures and the stereochemistry of these lactones were elucidated by high field NMR techniques and a few chemical transformations. The results are similar to those of a Transvaal collection but the stereochemistry was in part different. However, all eudesmanolides were 6,7-*cis*-lactones.

INTRODUCTION

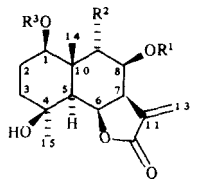
Calostephane diuaricata Benth. has been studied previously [1]. A collection from Transvaal afforded five eudesman-12,6β-olides. As the small genus *Calostephane* is proposed to be closely related to *Anisopappus* [2], we have collected material in Namibia. The results are discussed in this paper.

RESULTS AND DISCUSSION

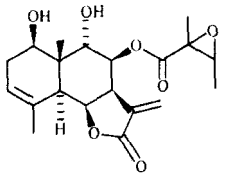
Careful separation of the polar fractions of the aerial parts of C. *diuaricata* finally gave the eudesmanolides I-19, the germacranolide 20 and the seco-eudesmanolide 23 which was purified as its diacetate 24.

The main product was the lactone 1. The molecular formula ($C_{25}H_{34}O_9$) followed from the mass spectrum. The nature of the nine oxygens could be deduced from the fragmentation ions [M $-$ 2 x H_2O, $-C_4H_7CO_2H$, $-C_4H_7OCO_2H$), from the typical ^1H NMR signals of the ester groups and the signals of a methylene lactone (Table 1). Furthermore, the presence of an eudesmanolide was likely as two methyl singlets at δ 1.35 and 1.39 were visible. As only one tertiary hydroxy group was present a guaianolide could be excluded. However, the observed couplings did not agree with any known sesquiterpene lactone type. Spin decoupling allowed an assignment of all signals and as H-14 showed a W-coupling with H-l the whole sequence could be determined. The chemical shift of H-l required a hydroxy group at C-l. Accordingly, the ester groups were at C-8 and C-9. However, the relative position of these groups and the stereochemistry of the lactone was not clear. NOE difference spectroscopy allowed the assignment of the stereochemistry at all chiral centres. Clear effects were observed between H-9 and H-14 (5%), H-2β (5%) and H-9 (7%), between H-15, H-5 (4%) and H-6 (6%), between H-7, H-5 (3%) and H-8 (6%), between H-6, H-5 (6%) and H-15 (5%) as well as between the ester protons H-5' and H-13 (1%). The last effect was an indication that the epoxyangelate may be at C-8. This was established by ^{13}C NMR techniques. The signals were assigned by a COSY spectrum and the relative position of the ester groups followed from the long range C/H-couplings which allowed the assignment of the carbonyl carbons and consequently the position of the ester groups from a COLOC spectrum. The ^{13}C NMR data are shown in Table 2.

The spectral data of lactone 2 (Tables 1 and 2) indicated the presence of an isomer of **1.** The identical relative position of the ester groups was determined by an INEPT spectrum which indicated long range couplings between H-8 and H-S with the carbonyl carbon of the epoxyangelate. As in both lactones a NOE between H-3' and H-5' of the epoxy esters was observed the presence of an epoxy tiglate could be excluded. Accordingly, the lactones differed in the absolute configuration of the epoxyangelate. Inspection of models indicated that the observed shift differences of H- 13 can be best explained if in both cases a preferred conformation of the epoxyangelate groups is proposed. This was supported by the observed NOE's. While in the case of 1 an effect between H-S and H-13 was present, a NOE between H-S and H-9 was observed in the case of 2. The necessary conformations would explain a downfield shift of H-13 in the isomer 1 by the epoxy group leading to a preliminary assignment of the configurations of the epoxy esters in **1** and 2 and related lactones (see below). This was further supported by the ^1H NMR spectra of the 1 1α,13-dihydro derivatives of the l-o-acetates **3a** and **4a** obtained by boranate reduction (see below). Again H-13 was shifted downfield in the case of **3a** while in the dihydro derivative 4a H-9 was deshielded. Inspection of models supported the proposed preferred conformations of the two epimeric epoxyangelate residues. To help with the comparison of the chemical shifts the dihydro derivative 5Aca was prepared by reduction of **5Ac** (see below). A similar pair of epimeric epoxymethylbutyrates has been reported from *Helianthus pumilus* [3] where, however, the shifts differences are less pronounced probably due to a less crowded situation. The shifts of the 4'-methyl group agreed with our results which may support our assignment as in one case the configuration is established by X-ray analysis [4]. Finally, the observed differences in the optical rotation of **1** and 2 as well as of 3 and 4 supports the proposed assignment



	1	2	3†	4†	5	5Ac†	6	7	8	9	10	10a	11	11a	12
R^1	E*	E'*	E	E'	Ang	Ang	Ang	E	E	Tigl	Ang	Ang	Tigl	Tigl	Ang
R'	OAng	OAng	OAng	OAng	OAng	OAng	OTigl	OTigl	OTigl	OTigl	O H	OH	OH	OH	H
R^3	H	H	Ac	Ac	H	Ac	H	H	Ac	H		H	Ac	H	Ac	H



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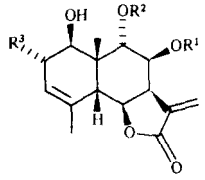
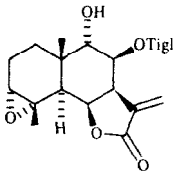


	14	15	Ib	17	18
R^1	E	E'	E	E'	Ang
R^2	Ang	Ang	E	E	H
R^3	OH	OH	H	H	OH



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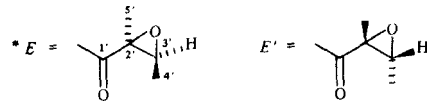


† 3a, 4a and 5Aca are the 11α,13dihydroderivatives

as the 2*R*,3*R*-enantiomer of the methyl esters shows a positive rotation [5] in agreement with the observed lower negative rotation of the isomers 2 and 4, respectively.

From the ^{1}H NMR spectra of 3 and 4 (Table 1), it was evident that the l-O-acetates of **1** and 2, respectively, were present. This was established by partial acetylation of the diols 1 and 2. The ^{1}H NMR spectra of 5 and of the acetylation product **5Ac** (Table 1) showed that the corresponding diangelate was present. The spectrum of 6 (Table 3) indicated that this lactone was a mixed diester with an angelate and a tiglate group. Comparison of the chemical shifts of the protons of the ester moieties showed that the angelate group was at C-8 as in all cases a downfield shift of the ester protons was observed in the 9-acyloxy derivatives. The structure of 7 followed from the ^{1}H NMR spectrum (Table 3) as it was very similar to that of 1, only the angelate signals being replaced by those of a tiglate. Similarly, the spectrum of 8 showed that the corresponding l-O-acetate was present. Again this was established by partical acetylation of 7. The chemical shift of H-13 indicated that the epoxy ester was identical with that of lactone 1.

The presence of a corresponding ditiglate followed from the ^{1}H NMR spectrum of 9 (Table 3) while that of 10 and that of its acetate 10s showed that no ester group was present at C-9. Accordingly, the H-l signal was shifted downfield. In agreement with the presence of a 6,7-*cis*-lactone [6], a positive Cotton-effect was observed. The spectral data of **11** and of its l-O-acetate **11a**, obtained by partial acetylation, indicated the presence of the corresponding 8-0-tiglate while that of the angelate 12 required the absence of the oxygen function at C-9 (Table 3). Accordingly, a pair of double doublets at 62.30 and 1.45 (H-9) was visible and the H-8 signal was now a doublet of triplets. The 8-desacyl derivative of 12 we have named calostephanolide.

The ^{1}H NMR spectrum of 13 (Table 4) differed markedly from those of 1-12 though most of the couplings were similar. The molecular formula ($C_{20}H_{26}O_7$) and the typical ^{1}H NMR signals of an epoxyangelate together with the signal of an olefinic methyl group indicated the presence of a lactone where one hydroxy group was eliminated. Spin decoupling allowed the assignment of all signals and the observed couplings required the proposed stereochemistry which differed at C-9 from that reported

Table 1. ^{1}H NMR spectral data of compounds **1–5, 3a, 4a, 5Aca** and **5Ac** (400 MHz, CDCl$_3$, δ-values)

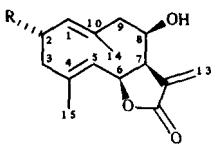
H	1	2	3	4	3a	4a	5	5Ac	5Aca	Multiplicity
1	3.20	3.23	4.64	4.61	4.60	4.64	3.21	4.69	4.65	*dd*
2α	1.85		1.85		1.85		1.84	1.85	1.84	*ddd*
2β	2.12		2.09		2.07		2.11		2.07	*ddd*
3a	1.65		1.73		1.73		1.63	1.73	1.72	*br d*
3β	1.40		1.45		1.49		1.38	1.50	1.49	*ddd*
5	1.57		1.71	1.76	1.70		1.56	1.77	1.70	*d*
6	4.88	4.86	4.88	4.86	4.81		4.86	4.87	4.79	*dd*
7	3.47	3.41	3.44	3.38	2.81	2.19	3.44	3.41	2.78	*br dd*
8	5.39	5.45	5.31	5.41	5.24	5.09	5.39	5.43	5.18	*dd*
9	5.03	5.12	4.95	5.04	5.00	5.28	5.14	5.06	5.16	*d*
11					2.90	2.88	—	—	2.84	*dq*
13	6.41	6.28	6.39	6.26	1.25 *d*	1.15 *d*	6.31		1.15 *d*	*d*
13'	5.79	5.74	5.78	5.73			5.72			*d*
14	1.35	1.48	1.49	1.55	1.46	1.52	1.33	1.52	1.48	s
15	1.39	1.34	1.36	1.35	1.37	1.37	1.40	1.36	1.36	s
8-OCOR	3.04	3.03	3.04	3.02	3.08	3.11	6.12 *qq*	6.14 *qq*	6.14 *qq*	*q*
	1.32	1.26	1.33	1.27	1.41	1.34	1.96 *dq*	1.99 *dq*	2.00 *dq*	*d*
	1.44	1.49	1.44	1.48	1.53		1.77 *dq*	1.79 *dq*	1.85 *dq*	s
9-OCOR	6.30	6.29	6.17		6.16	6.14	6.26	6.16	6.18	*qq*
	2.05		1.97		1.98		2.04	1.99	2.05	*dq*
	1.92		1.89		1.88		1.91		1.88	*dq*
OAc	—	—	2.01	2.11	2.01	1.94	—	2.00	1.99	s

J [Hz]: 1, 2α = 2α, 3α = 2β, 3β = 4; 1, 2β = 11.5; 2α, 2β = 3α, 3β = 13; 2α, 3β = 3; 2β, 3α = 12; 5, 6 = 3.5; 6, 7 = 6; 7, 8 = 7; 7, 13 = 7, 13′ = 1; 8, 9 = 3; compounds **3a**, **4a** and **5Aca** 7, 11 = 11, 13 = 7.

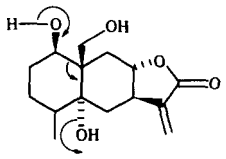
Table 2. ^{13}C NMR signals of compounds 1 and 2 (CDCl$_3$, δ-values)

C	1	2
1	71.4	71.7
2	24.5	24.1
3	38.2	38.4
4	71.2	71.2
5	43.6	43.6
6	75.0	75.4
7	40.1	40.6
8	68.2	68.3
9	71.8	72.4
10	40.3	40.7
11	134.6	135.0
12	168.6	168.7
13	125.0	124.7
14	14.1	14.7
15	29.5	29.7
OEpang 1'	168.2	168.5
2	59.4	60.4
3'	59.8	59.2
4	13.4	13.4
5'	20.6	20.7
OAng 1'	166.5	166.7
2	125.8	126.0
3'	142.6	142.8
4	16.1	16.3
5	18.6	19.2

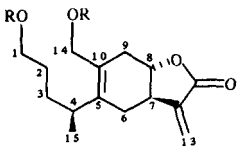
Assignment by 2D techniques (COSY)



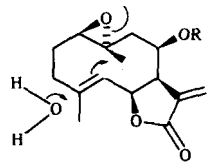
20 R = OH
21 R = H



22



23 R = H
24 R = Ac



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for the diesters isolated previously from this plant [1] where also the opposite configuration at C-S and C-10 was proposed [7]. Accordingly, some couplings were different in the two series.

The ^{1}H NMR spectra of 14-18 (Table 4) indicated that another type of eudesmanolide was present. In the case of

Table 3. ^{1}H NMR spectral data compounds **6–12, 10a** and **11a** (400 MHz, $CDCl_3$, δ-values)

H	6	7	**8**	9	**10**	**10a**	11	**11a**	12	Multiplicity
1	3.21	3.20	4.66	3.20	3.91	4.94	3.91	4.93	3.26	*dd*
2α	1.85	1.85	1.85	1.85	1.82	1.89	*1.83*	*1.89*	1.86	*ddd*
2β	2.13	2.12	2.12	2.13	2.06	2.33	*2.07*	*2.33*	*2.03*	ddd
3α	1.65	1.64	1.65	1.64	1.66	1.59	1.67	1.59	1.65	*br d*
3β	1.38	1.40	1.40	1.40	1.46	1.48	1.47	1.49	1.47	*ddd*
5	1.57	1.57	1.79	1.57	1.82	1.95	*1.80*	*1.96*	1.30	*d*
6	4.89	4.90	4.91	4.89	4.82	4.81	4.84	4.82	4.81	*dd*
7	3.47	3.51	3.46	3.46	3.58	3.53	*3.56*	*3.52*	*3.33*	*br* dd
8	5.41	5.40	5.39	5.38	5.32	5.47	5.32	5.45	5.55 *dt*	*dd*
9	5.12	5.00	4.93	5.11	3.94	3.36	*3.97*	*3.36*	2.30 dd	*d*
13	6.33	6.41	6.41	6.30	6.29	6.28	6.28	6.25	6.31	*d*
13'	5.74	5.81	5.78	5.72	5.72	5.70	*5.70*	*5.68*	*5.73*	*d*
14	1.35	1.36	1.39	1.36	1.27	1.31	1.27	1.36	1.33	*s*
15	1.42	1.40	1.46	1.42	1.33	1.35	1.34	1.30	1.34	*s*
8-OCOR	6.13 *qq*	3.05 *d*	3.05 *d*	6.78 *qq*	6.09 qq	6.09 *qq*	6.77 99	6.75 *qq*	6.07 *qq*	
	1.97 *dq*	1.31 *d*	1.24 *d*	1.85 *br d*	1.95 *dq*	1.97 *dq*	1.77 *dq*	1.78 *dq*	1.97 *dq*	
	1.79 *dq*	1.45 *s*	*1.50 s*	1.87 *br s*	1.76 *dq*	1.77 *dq*	1.74 *dq*	1.74 *dq*	1.79 *dq*	
9-OCOR	6.91 *br q*	6.91 qq	6.79 *qq*	6.91 99						
	1.87 *br d*	1.87 *br s*	1.84 *br s*	1.79 *br s*						
	1.88 *br s*			1.75 *br s*						
OAc			1.98 s			2.11 s		2.10 s		

.I [Hz]: see Table 1; compound 12: 8, 9 = 3.5; 8, 9′ = 4; 9, 9′ = 15

Table 4. ^{1}H NMR spectral data of compounds **13–19** (400 MHz, $CDCl_3$, δ-values)

H	**13**	**14**	**15**	**16**	**17**	**18**	**19**
1	4.08 *dd*	*3.98 d*	*4.00 d*	*4.24 dt*		4.02 *d*	α 1.87 *dt*; β 1.50 *dd*
2	2.35 *br d*; 2.03 *br d*	4.12 *br d*	4.12 *br d*	2.55 *br d*; 2.49 *br* dd		4.13 *ddq*	α *1.62* brdd; β 1.99 *dddd*
3	5.41 *br s*	5.52 dq	5.52 *dq*	5.45 *br s*		5.47 *dq*	3.58 *br s*
5	2.69 *br s*	1.93 *d*	1.93 *d*	1.86 *d*		1.82 *d*	2.66 *d*
6	4.81 *dd*	*4.69 dd*	*4.70 dd*	*4.57 dd*		*4.69 dd*	*4.90 dd*
7	*3.61 br dd*	*3.78 ddr*	*3.84 ddt*	*3.81 ddt*		*3.81 ddt*	*3.81 ddt*
8	*5.39 dd*	*5.77 dd*	*5.73 dd*	*5.69* dd	5.75 *dd*	*5.67 dd*	*5.46* dd
9	3.94 *d*	5.42 *d*	5.46 *d*	*5.44 d*	5.47 *d*	3.74 *d*	3.87 *d*
13	6.33 *d*	6.49 *d*	6.46 *d*	6.46 *d*	6.44 *d*	6.36 *d*	6.36 *d*
13'	5.81 *d*	6.31 *d*	6.17 *d*	6.25 *d*	6.13 *d*	6.06 *d*	5.59 *d*
14	0.99 *s*	1.05 *s*	1.05 *s*	1.00 *s*		1.17 *s*	1.33 *s*
15	1.84 *hr s*	1.91 *br s*	1.91 *br s*	1.85 *br s*		1.89 *br s*	1.23 s
8-OCOR	3.05 9	3.02 9	3.08 9	3.11 *q*	3.06 *q*	6.17 99	6.74 99
	1.29 *d*	1.24 *d*	1.24 *d*	1.29 *d*	1.278 *d*	2.01 *dq*	1.76 *dq*
	1.49 *s*	1.49 s	1.47 *s*	1.54 *s*		1.93 *dq*	1.73 *dq*
9-OCOR		6.26 *qq*	6.26 *q q*	3.13 *q*			
		2.06 *dq*	2.05 *dq*	1.35 *d*			
		1.88 *dq*	1.88 *dq*	1.54 *s*			

J [Hz]: Compound 13: I, 2α = 7; 1, 2β = 10; 2α, 2β = 17; 5, 6 = 3; 6, 7 = 6; 7, 8-7; 7, 13 = 7, 13′ = 1; 8, 9 = 3.5; compounds **14–18**: 1, 2 = 7.5; 2, 3 = 2, 15 = 3, 15 ~ 1.5; 5, 6 = 9.5; 6, 7 = 8; 7, 8 = 6; 7, 13 = 3.5; 7, 13′ = 3; 8, 9 = 10; compound **19**: 1α, 1β = 1α, 2β = 2α, 2β = 13; 1α, 2α = 1β, 2β = 4; 2α, 3 ~ 1; 2β, 3 = 3; 3, 15-l; 5, 6 = 2.5; 6, 7 = 8; 7, 8 = 9; 7, 13 = 2; 7, 13′ = 1.7; 8, 9 = 3.5.

18 careful spin decoupling allowed the assignment of all signals. As a homoallylic coupling between H-2 and H-5 was observed the whole sequence was established. As already followed from the molecular formula five oxygen functions were present. The chemical shifts of H-l, H-2 and H-9 indicated that the corresponding carbons were hydroxylated while at C-8 an angelate group had to be placed as followed from the typical signals. The observed couplings of H-5-H-9 differed markedly from those of 13. NOE difference spectroscopy allowed the assignment of the stereochemistry. Especially clear effects were observed on saturation of H-14 with H-S (7%), H-9 (4%) and H-2 (4%). Inspection of models indicated that these findings required a cis-decalin derivative with an annelated *cis*-lactone moiety. The observed couplings agreed well with the expected ones. The 2,9-bisdesoxy derivative with a free 8-hydroxy group we have named divaricatolide.

Inspection of the ^{1}H NMR spectra of 14 and 15 (Table 4) showed that these lactones were also divaricatolides which differed only in the stereochemistry of the epoxy ester at C-8. Its presence and that of an angelate followed from the typical ^{1}H NMR signals. The observed chemical shifts of H-13 indicate that in lactone 14 as in 1 a 2*S*,3*S*-epoxide was most likely.

The lactones 16 and 17 could not be separated. The ^{1}H NMR spectrum (Table 4) showed that again a mixture of epimeric 8-*O*-2,3-epoxymethyl butyrates was present with one hydroxy group at C-l and a second epoxy ester residue at C-9. The observed couplings indicated identical stereochemistry with that of 14 and 15.

The ^{1}H NMR spectrum of 19 (Table 4) showed some similarities to that of 13. However, the double bond was replaced by an epoxide as followed from a methyl singlet at δ 1.23 and a broadened singlet at δ 3.58. Furthermore, the l-hydroxy group was absent. Accordingly, additional signals for H-l (δ 1.87 *dt* and 1.50 *dd*) were visible. The chemical shift of H-5 (6 2.66 *d*) required a 3α,4α-epoxide.

In the ^{1}H NMR spectrum of 20 (see Experimental), the signals could only be assigned by spin decoupling at elevated temperature. Accordingly, the presence of a germacranolide was very likely. Comparison of the data with those of germacran-12,6β-olides [7] indicated that a 2α,8β-dihydroxy derivative of such a lactone was present.

The last lactone was isolated as its diacetate 24. In the ^{1}H NMR spectrum in deuteriobenzene (Table 5), all signals could be assigned by spin decoupling. The resulting sequences showed that a derivative of eriolangin [8] was present. However, the 6-angeloyloxy group was absent and the configuration at C-8 was different. The observed couplings required a 7,8-*trans*-lactone. Remarkable is the large homoallylic coupling between H-6β and H-9 (3 Hz). The configuration at C-4 could not be determined, the proposed one is that of eriolangin.

Most likely the precursor of the lactones l-19 is the germacranolide 21 which by oxidation can be transformed to 20 or to the epoxide 25. As shown in Scheme 1, the latter then could lead to the isolated lactones. The seco derivative 23 is probably formed by fragmentation of 22 (Scheme 1) which, however, is not related to 21.

It is remarkable that the two collections from Transvaal and Namibia led to the isolation of very similar lactones which, however, differ in the stereochemistry at C-9. In the extract of the Namibia collection no trace of the isomers could be detected.

In conclusion, it seems to be clear that at least the chemistry gives no indications of relationships between

Table 5. ^{1}H NMR spectral data of cotnpounds 23 and 24 (400 MHz, a-values)

H	23* ($CDCl_3$)	24 (C_6D_6)	24 ($CDCl_3$)
1, 1'	3.67 ABX,	4.02 *dt* 3.95 *dt*	4.02 ABX,
2		1.28 *tt*	1.50 *m*
3	1.45 *m*	1.06 *tt*	1.40 *m*
4	2.95 *tq*	2.52 *tq*	2.79 *tq*
6α	2.55 *m*	2.03 *ddddd*	*2.60 m*
68	2.00 m	*1.47 dddddd*	1.96 *br dd*
	2.59 m	1.90 *ddddd*	*2.60 m*
8	3.93 *dt*	*3.34 dt*	*3.93 dt*
9a	2.44 *br* ddd	*2.12 dddddd*	*2.47 br ddd*
98	2.88 ddd	*2.40 ddd*	*2.72 ddd*
13	6.18 *d*	*6.06 d*	*6.19 d*
13'	5.49 d	*4.89 d*	*5.50 d*
14	4.35 *d*	*4.44 br d*	*4.65 br d*
14	3.92 *d*	*4.39 br d*	*4.58 br d*
15	1.03 *d*	*0.78 d*	1.04 *d*
OAc		*1.75, 1.74 s*	*2.07, 2.04 s*

*Taken from the crude sample.

J [Hz]: 1, 1′ ~ 12; 1, 2 = 2, 3 = 3, 4 = 4, 15 ~ 7; 6α, 6β = 17; 6α, 7 = 5; 6α, 9α = 6α, 9β = 6α, 14 = 6α, 14′ = 6β, 14 = 6β, 14′ ~ 1; 6β, 7 = 7, 8 = 8, 9α = 11; 6β, 9α = 3; 6β, 9β = 1.7; 7, 13 = 3.5; 7, 13′ = 3; 8, 9β = 5; 9α, 9β = 15.5; 14, 14′ = 13.

Anisopappus, *Antiphiona* and *Calostephane* which have been proposed from the morphology [2]. 6,7-*cis*-Eudesmanolides seem to be rare in Compositae. A few compounds with a 10α-methyl group are reported from *Montanoa* [8] and *Pegolettia* [9] species. The latter genus is placed as *Calostephane* in the Inula group of the subtribe Inulinae [2]. Perhaps the co-occurrence of 6,7-*cis* lactones is an indication that these genera are related. Further studies are needed to clarify the complex situation in the subtribe Inulinae.

EXPERIMENTAL

The air-dried aerial parts (140 g) were collected near Tsumeb, Namibia, in March 1988, voucher 88/87, deposited in the SW African Herbarium at Windhoek. They were worked-up and separated as reported previously [10]. CC gave two crude fractions (1: Et_2O–petrol, 1: 9; 2: Et_2O and Et_2O–MeOH, 9 : 1). TLC of fraction 1 gave 30 mg taraxasteryl acetate and 20 mg lupeylacetate, identified by comparing the ^{1}H NMR spectra with those of authentic material. Fraction 2 was separated by medium pressure chromatography (silica gel, ϕ 30–60 μ, starting with Et_2O–petrol, 1: 3, with increasing amounts of Et_2O and finally Et_2O–MeOH, 9: 1) affording six crude fractions (2/1–2/6). HPLC (always RP 8 or RP 18, flow rare 3 ml/min, *ca* 100 bar) with MeOH–H_2O (7: 3, RP 8) gave 22 mg 19 (*R*, 3.1 min), 50 mg of a mixture of 3 and 4 (*R*, 4.0 min, 2/1/2) and 42 mg 5 (*R*, 5.0 min). HPLC of 2/1/2 (MeOH–H_2O, 13 : 7, RP 18) gave 25 mg 3 (*R*, 6.7 min) and 17 mg 4 (*R*, 7.3 min). TLC of 2/2 (Et_2O–petrol, 9 : 1, 3 ×) gave 5 mg of a mixture of 16 and 17 (ca 3 : 2, R_f 0.35), 1 mg 6 (R_f 0.50) and a mixture which gave by HPLC

(MeOH–H_2O, 13 : 7, RP 18), 2 mg **8** (*R*, 5.7 min) and 3 mg 9 (*R*, 6.4 min). Fraction 2/3 was a mixture of 400 mg **1** and 2 (*ca* 2 : 1). Fraction 2/4 gave by HPLC (MeOH-HzO, 3 : 2, RP 18) 6 mg 7 (*R*, 13.8 min), 23 mg 1 (*R*, 15.8 min) and 21 mg 2 (*R*, 18.1 min). Fraction 2/5 gave by HPLC (MeOH–H_2O, 3 : 2, RP 8) 2 mg 20 (*R*, 1.1 min), 7 mg 13 (*R*, 2.4 min), 8 mg 18 (*R*, 4.8 min) and three mixtures (2/5/4–2/5/6). TLC of 2/5/4 (Et_2O–MeOH, 50: 1, 2 ×) gave 30 mg 10 (R_f 0.55) and 5 mg 11 (R_f 0.48) and TLC of 2/5/5 (Et_2O–petrol, 3: 1 with 0.5% MeOH, 7 x) afforded 6 mg 14 (R, 0.70) and 4 mg 15 (*R*, 0.65). Fraction 2/5/6 could not be separated as such. After acetylation (Ac_2O, 1 hr, 70") TLC (Et_2O–petrol, 3 : 1) gave 6 mg 24 (*R*, 0.75), 5 mg **10a** (*R*, 0.58) and 4 mg 1 **1a** (R_f 0.50). HPLC of fraction 2/6 (MeOH–H_2O, 3 : 2, RP 8) gave 2 mg 12 (R, 2.5 min).

9α-*Angeloyloxycalostephanolide*-8-O-[2S, 3S-*epoxy*-2-*methylbutyrate]* (1). Colourless gum; IR $\nu_{max}^{CHCl_3}$ cm-': 3560, 3490 (OH), 1770 (y-lactone), 1740 (CO_2R), 1705, 1640 (C=CCO_2R); MS m/z (rel. int.): 478.220 $[M]^+$ (0.05) (calc. for $C_{25}H_{34}O_9$: 478.220), 463 $[M-Me]^+$ (I), 460 $[M-H_2O]^+$ (0.3), 445 $[463-H_2O]^+$ (0.3), 362 [M-RCO,H]+ (2), 360 $[460-RCO_2H]^+$ (2), 347 [362 -Me]+ (3), 262 $[362-RCO_2H]^+$ (6), 244 $[262-H_2O]^+$ (5), 162 (39), 109 (60), 99 $[RCO]^+$ (15), 83 $[RCO]^+$ (100), 71 $[99-CO]^+$ (32) 55 [83-CO]' (64); $[\alpha]_D^{24}$ -48 (CHCI,; c 2.19). Acetylation (Ac_2O, 1 hr, 70") afforded 3, identical with the natural product.

9α-*Angeloyloxycalostephanolide*-8-O-[2R,3R-*epoxy*-2-*methylbutyrate] (2).* Colourless gum; IR $\nu_{max}^{CHCl_3}$ cm-': 3560, 3490 (OH), 1770 (y-lactone), 1740 (CO_2R), 1705, 1640 (C=CCO_2R); MS m/z (rel. int.): 478.220 [M] $^+$ (0.05) (calc. for $C_{25}H_{34}O_9$: 478.220), 463 (0.6). 460 (0.4), 445 (0.6), 362 (2), 360 (3), 347 (1), 262 (5), 244 (8), 162 (40). 109 (44), 99 (15), 83 (100), 71 (10), 55 (61); $[\alpha]_D^{24^\circ}$ -33 (CHCI,; *c* 2.06).

l-O-Acetate of 1 (3). Colourless crystals, mp 169"; IR $\nu_{max}^{CCl_4}$ cm-': 3590 (OH), 1780 (y-lactone), 1760, 1245 (OAc), 1730 (CO_2R), 1720, 1640 (C=CCO_2R); MS ***m/z*** (rel. int.): 520.231 [M]' (0.4) (calc. for $C_{27}H_{36}O_{10}$: 520.231), 505 [M-Me]+ (0.5), 460 [M $-$HOAc] + (2), 442 $[460-H_2O]^+$ (OS), 405 [M $-$OCOR]$^+$ (1) 404 $[M-RCO_2H]^+$ (0.6), 362 [404 $-$ ketene]$^+$ (2.2), 262 $[362-RCO_2H]$ + (2.7). 99 [RCO] + (10), 83 [RCO] + (100); $[\alpha]_D^{24^\circ}$ -44 (CHCI,; c 0.26). To 20 mg 3 in 2 ml MeOH, 10 mg $NaBH_4$ and after 2 min dil. H_2SO_4 were added. After TLC (Et_2O) 17 mg 3a were obtained; colourless gum; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3580 (OH), 1795 (y-lactone), 1755, 1245 (OAc), 1730 (C =CCO_2R); MS m/z (rel. int.): 522.247 [M]' (0.2) (calc. for $C_{27}H_{38}O_{10}$: 522.247), 507 (0.5), 489 (0.1), 479 (1), 462 (1), 423 (0.5), 405 (0.5), 363 (1.3), 247 (9), 229 (7), 83 (100).

l-O-Acetate of 2 (4). Colourless crystals, mp 156"; IR $\nu_{max}^{CCl_4}$ cm-': 3590 (OH), 1780 (y-lactone), 1755, 1245 (OAc), 1730 (CO_2R), 1720, 1640 (C=CCO_2R); MS m/z (rel. int.): 520.231 $[M]^+$ (0.3) (calc. for $C_{27}H_{36}O_{10}$: 520.231), 505 (0.6), 460 (2), 404 (0.7) 362 (2), 262 (3.5), 99 (12), 83 (100); $[\alpha]_D^{24^\circ}$ -27 (CHCI,; *c* 0.15): Boranate reduction (s.a.) gave 4a; colourless crystals, mp 165"; IR $\nu_{max}^{CCl_4}$ cm^{1} · 3580 (OH), 1795 (y-lactone), 1745 (OAc), 1730 (C=CCO_2R); MS ***m/z*** (rel. int.): 522.247 $[M]^+$ (0.7) (calc. for $C_{27}H_{38}O_{10}$: 522.247), 507 (1), 479 (1), 462 (2), 363 (3), 247 (21), 83 (100).

9α-*Angeloyloxycalostephanolide*-8-O-*angelate (5).* Colourless gum; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3595, 3520 (OH), 1785 (y-lactone), 1735, 1645 (C=CCO_2R); MS m/z (rel. int.). 462.225 [M]' (1) (calc. for $C_{25}H_{34}O_9$: 462.225), 447 [M-Me] $^+$(2), 444 $[M-H_2O]^+$(3), 362 [M $-RCO_2H]^+$(3), 263 $[362-OCOR]^+$(4.5), 262 $[362-RCO_2H]^+$(3.5), 244 $[262-H_2O]^+$(7), 83 $[RCO]^+$(100), 55 [83-CO]+ (58); $[\alpha]_D^{24^\circ}$ -42 ($CHCl_3$; c 4.03). Acetylation (s.a.) afforded **5Ac**; colourless gum; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3580 (OH), 1785 (γ-lactone), 1740, 1240 (OAc), 1720, 1640 (C=CCO_2R); MS ***m/z*** (rel. int.): 504.236 [M]' (0.4) (calc. for $C_{27}H_{36}O_9$, 504.236), 489 (0.4), 486 (0.2), 444 (2), 387 (2), 386 (1), 345 (5), 344 (2.5), 245 (26), 244 (7), 83 (100). Boronate reduction (s.a.) of **5Ac** gave the dihydro derivative **5Aca**; colourless gum; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3580 (OH), 1795 (y-lactone), 1730 (CO_2R); MS m/z (rel. int.): 506.251 $[M]^+$ (1) (calc. for $C_{27}H_{38}O_9$: 506.251), 491 (1), 446 (10), 347 (10), 346 (8), 247 (10), 246 (3), 83 (100).

9α-*Tigloyloxycalostephanolide*-8-O-*angelate (6).* Colourless gum; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3590, 3520 (OH), 1780 (γ-lactone), 1725, 1640 (C=CCO_2R); MS ***m/z*** (rel. int.): 462.225 $[M]^+$ (0.1) (calc. ***for*** $C_{25}H_{34}O_8$: ***462.225). 447*** (0.3), ***445*** (0.3), ***362*** (1). ***263*** (2), ***262*** (1), ***245*** (3), ***244*** (3), ***227*** (3.7), ***83*** (100).

9α-*Tigloyloxycalostephanolide*-8-O-[2S,3S-*epoxy*-2-*methyl hutyrate] (7). Colourless* gum IR $\nu_{max}^{CHCl_3}$ cm^{-1}: ***3560, 3480*** (OH), 1770 (y-lactone), 1705, 1640 (C=CCO_2R); MS m/z (rel. int.): 478.220 $[M]^+$ (0.05) (calc. for $C_{25}H_{34}O_9$ 478.220) 378 (0.2), 362 (3), 360 (2.5), 263 (3), 262 (5), 244 (10), 162 (34), 109 (41), 83 (100); $[\alpha]_D^{24^\circ}$ $-$54 (CHCI,; c 0.39).

l-O-Acetate ***of*** 7 (8). Colourless **gum;** IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3580 (OH), 1780 (y-lactone), 1755, 1255 (OAc), 1720, 1635 (C =CCO_2R); MS ***m/z*** (rel. int.): 520.231 [M]' (0.3) (calc. for $C_{27}H_{36}O_{10}$: 520.231), 505 (0.8). 460 (0.5), 405 (1), 389 (1.3). 289 (0.5), 245 (5), 244 (4.5), 162 (7). 99 (5), 83 (100).

9α-*Tigloyloxycalostephanolide*-8-O-*tiglate (9).* Colourless gum; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3580, 3510 (OH), 1780 (y-lactone), 1715, 1700, 1645 (C=CCO_2R); MS m/z (rel. int.): 362.173 [M $-RCO_2H]^+$ (1.5) (calc. for $C_{20}H_{28}O_5$ 362.173), 262 (3), 244 (4.3), 162 (41), 83 (100), 55 (40).

9α-*Hydroxycalostephanolide*-8-O-*angelate (10).* Colourless crystals, mp 222"; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3570 (OH), 1760 (y-lactone), 1700 (C=CCO_2R); MS ***m/z*** (rel. int.): 380.184 [M] $^+$(0.1) (calc. for $C_{20}H_{28}O_7$: 380.184), 365 (1), 362 (1), 280 (3), 263 (2.5), 245 (4), 227 (6), 162 (4), 83 (100); $[\alpha]_D^{24^\circ}$ $-$139 (CHCI,; *c* 0.31); CD (MeCN): $\Delta\varepsilon_{254}$ + 1.15. Acetylation (s.a.) afforded **10a**; colourless crystals, mp 196"; IR $\nu_{max}^{CCl_4}$ cm^{1} · 3580, 3470 (OH), 1780 (y-lactone), 1740, 1260 (OAc), 1715, 1640 (C=CCO_2R); MS ***m/z*** (rel. int.): 422.194 [M] $^+$(0.2) (calc. for $C_{22}H_{30}O_8$: 422.194), 407 (0.3), 404 (0.3). 386 (0.2), 362 (0.35), 344 (0.7), 322 (22), 244 (11), 83 (100), 55 (66).

9α-*Hydroxycalostephanolide*-8-O-*tiglate* (11). Colourless crystals, mp 201': IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3570, 3400 (OH), 1765 (y-lactone), 1700, 1630 (C=CCO_2R); MS m/z (ret. int.): 380.184 $[M]^+$ (0.2) (calc. for $C_{20}H_{28}O_7$: 380.184), 365 (2.5), 362 (0.6), 344 (0.7), 280 (9), 262 (3), 244 (3), 162 (6), 83 (100). Acetylation (s.a.) afforded **11a**; colourless crystals, mp 204°; IR $\nu_{max}^{CCl_4}$ cm-': 3580, 3470 (OH), 1780 (y-lactone), 1740, 1255 (OAc), 1720, 1645 (C =CCO_2R); MS m/z (rel. int.): 422.194 [M] $^+$ (0.1) (calc. for $C_{22}H_{30}O_8$ 422.194). 407 (I), 404 (0.4), 322 (19), 262 (4), 244 (11), 83 (100).

Calostephanolide-8-O-*angelate (12).* Colourless gum; IR $\nu_{max}^{CHCl_3}$ cm- ': 3560 (OH), 1765 (y-lactone), 1710 (C=CCO_2R); MS m/z (rel. int.): 364 $[M]^+$ (0.1), 349 [M-Me]+ (1.6), 346.178 $[M-H_2O]^+$ (2) (calc. for $C_{20}H_{26}O_5$: 346.178), 264 [M $-RCO_2H]^+$ (4), 247 (ll), 229 (10), 83 (100).

9α-*Hydroxy*-5-epi-*divaricatolide*-8-O-[2S,3S-*epoxy*-2-*methylbutyrate] (13).* Colourless gum; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500 (OH) 1760 (y-lactone), 1730 (CO_2R); MS ***m/z*** (rel. int.): 378.168 [M]' (0.3) (calc. for $C_{20}H_{26}O_7$ 378.168), 360 (2.5), 342 (0.5), 262 (5), 244 (36), 229 (18), 226 (24), 83 (100); $[\alpha]_D^{24}$ -99 (CHCI,; *c* 0.53).

9α-*Angeloyloxy*-2α-*hydroxydivaricatolide*-8-O-[2S,3S-*epoxy*-2-*methylbutyrate*] *(14).* Colourless gum; IR $\nu_{max}^{CCl_4}$ cm-': 3580, 3440 (OH), 1780 (y-lactone), 1735 (CO_2R), 1720, 1640 (C =CCO_2R); MS *m/z* (rel. int.): 476.205 $[M]^+$ (1) (calc. for $C_{25}H_{32}O_9$: 476.205). 458 (1), 376 (2.5). 360 (2). 342 (4), 260 (3). 83 (100).

9α-*Angeloyloxy*-2α-*hydroxydivaricatolide*-8-O-[2R,3R-*epoxy*-2-*methylbutyrate*] *(15).* Colourless gum; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3580, 3450 (OH), 1780 (y-lactone), 1730 (CO_2R); MS m/z (rel. int.): 476.205 [M]' (0.7) (calc. for $C_{25}H_{32}O_9$: 476.205). 458 (1). 376 (3),

360 (2), 342 (5), 260 (4), 83 (100).

9α-[2,3-*Epoxy*-2-*methylbutyryloxy*]-*divaricatolide*-8-O-[2S,3S- and 2R,3R-*epoxy*-2-*methylbutyrate*] (16 and 17). Colourless gum; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3570 (OH), 1790 (γ-lactone), 1740 (CO_2R); MS m/z (rel. int.): 476.205 [M]$^+$ (0.5) (calc. for $C_{25}H_{32}O_9$: 376.205), 458 (3), 360 (7), 342 (0.8), 244 (8), 83 (100).

2α,9α-*Dihydroxydivaricatolide*-8-O-*angelate* (18). Colourless gum; IR $\nu_{max}^{CHCl_3}$ cm-^{1}: 3570, 3430 (OH), 1770 (γ-lactone), 1710, 1640 (C=CCO_2R); MS m/z (rel. int.): 378.168 [M]$^+$ (0.5) (calc. for $C_{20}H_{26}O_7$: 378.168), 360 (1), 342 (0.4), 278 (1.5), 261 (4), 260 (4), 83 (100); $[\alpha]_D^{24°}$ + 43 (CHCl$_3$; *c* 0.74).

9α-*Hydroxy*-3α,4α-*epoxy*-1-*desoxy*-5-epi-*divaricatolide*-8-O-*tiglate* (19). Colourless gum; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3620 (OH), 1770 (γ-lactone), 1710, 1645 (C=CCO_2R); MS m/z (rel. int.): 362.173 [M]$^+$ (2) (calc. for $C_{20}H_{26}O_6$: 362.173), 262 (6), 234 (0.5), 216 (OS), 83 (100); $[\alpha]_D^{24°}$ -47 (CHCl$_3$; c 2.04).

2α,8β-*Dihydroxygermacra*-1(10)E,4E-*diene*-12,6β-*olide* (20). Colourless gum; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3560 (OH), 1760 (γ-lactone); MS m/z (rel. int.): 264.136 [M]$^+$ (1.3) (calc. for $C_{15}H_{20}O_4$: 264.136), 246 (3), 95 (63), 69 (76), 55 (100); ^{1}H NMR (CDCl$_3$, 60°): δ 5.26 (*br d*, H-l), 4.63 (ddd, H-2), 2.55 (dd, H-3), 2.08 (t, H-3'), 5.83 (*br d*, H-5), 5.23 (dd, H-6), 3.19 (*br d*, H-7), 4.26 (ddd, H-8), 2.69 (*br* dd, H-9), 2.35 (dd, H-9'), 6.38 and 5.66 (*d*, H-13), 1.50 (*br s*, H-14), 1.78 (*d*, H-15); *J* [Hz]: 1,2 = 9; 2,3 = 5; 2,3' = 3,3' = 5,6 = 11; 5,15 = 1; 6,7 = 7; 7,8 = 1; 7,13 = 2.5; 7,13' = 2; 8,9 = 2.5; 8,9' = 4.5; 9,9 = 14.

6-*Desacyloxy*-8-epi-*eriolangin* (23). Isolated as its diacetate **24**; colourless gum; IR $\nu_{max}^{CCl_4}$ cm-^{1}: 1780 (γ-lactone), 1740, 1235 (OAc); MS *m/z* (rel. int.): 350.173 [M]$^+$ (9) (calc. for $C_{19}H_{26}O_6$: 350.173), 308 [M − ketene]$^+$ (12), 290 [M − HOAc]$^+$ (43), 230 [290 − HOAc]$^+$ (74), 215 [230 − Me]$^+$ (100), 202 [290 − HOAc, H_2C=CH_2, McLafferty]$^+$ (82), 189 [290 − $(CH_2)_3$OAc]$^+$ (95), 174 [202 − CO]$^+$ (71), 161 [189 − CO]$^+$ (73); $[\alpha]_D^{24°}$ +88 (CHCl$_3$; c 0.60); CD (MeCN): $\Delta\varepsilon_{263}$ +0.06.

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