Phytochemistry, Vol 24, No 5, pp 1021–1026, 1985
Printed in Great Britain

0031–9422/85 $3 00+0 00
Pergamon Press Ltd

PSEUDOGUAIANOLIDES AND CARABRONE DERIVATIVES FROM *LOXOTHYSANUS SINUATUS*

FERDINAND BOHLMANN, LAXMI N MISRA and JASMIN JAKUPOVIC

Institute for Organic Chemistry, Technical University of Berlin, D-1000 Berlin 12, West Germany

(*Revised received* 15 *September* 1984)

Key Word Index—*Loxothysanus sinuatus*, Compositae, sesquiterpene lactones, pseudoguaianolides, carabrone derivatives, guaianolides

Abstract—The aerial parts of *Loxothysanus sinuatus* afforded in addition to known compounds nine new pseudoguaianolides, three guaianolides and five carabrone derivatives The structures were elucidated by spectroscopic methods The chemotaxonomy is discussed briefly The configurations of two guaianolides were corrected

INTRODUCTION

The Mexican genus *Loxothysanus* was traditionally placed in the unnatural tribe Helenieae The genera of this tribe have since been realigned into other tribes with which they might seem best related [1] The position of *Loxothysanus* is unclear We therefore have studied the constituents of *L sinuatus* (Less) B L Robinson which may support a possible placement The results will be discussed in this paper

RESULTS AND DISCUSSION

The aerial parts of *Loxothysanus sinuatus* afforded germacrene D, caryophyllene, *ent*-kaurenic acid, its 15α-isobutyryloxy derivative, the pseudoguaianolides mexicanin [2], helenalin [3], helenalin tiglate [4], helenalin iso-valerate [4], mexicanin C [5], mexicanin C-tiglate [6], 6β-acetoxyamarilin [7], α-cyclocostunolide [8] and β-cyclocostunolide [9] as well as nine new pseudoguaianolides (**1**–**9**), the guaianolides **10**–**12** and the carabrone derivatives **13**–**17**

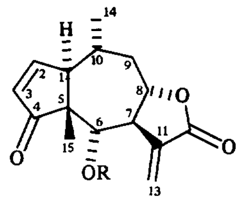
The structure of **1** was deduced from the ^{1}H NMR spectrum (Table 1) and by NOE difference spectroscopy All signals could be assigned by spin decoupling and clear NOEs between H-15 and H-6, H-8 and H-10 as well as between H-14 and H-1, 2, 9α and 9β, between H-7 and H-9α and between 6-OH and H-13′ established the stereochemistry at all chiral centres Accordingly, **1** is an 8-epimer of helenalin (**4**) The ^{1}H NMR spectral data of **2** (Table 1) were close to those of **1** However, the presence of a tiglate residue caused the expected downfield shift of the H-6 signal and also a slight difference in the coupling $J_{6\ 7}$ indicating a small change in the conformation The NOEs established that the configurations were still the same (H-15 with H-6, H-8 and H-10) As observed previously, the presence of 8α,12-olides also followed from the clear shift differences of H-8 in **1** and helenalin, its ^{1}H NMR having been added for comparison in Table 1

Table 1 ^{1}H NMR spectral data of **1**, **2** and helenalin (400 MHz, $CDCl_3$, TMS as internal standard)

	1	**2**	Helenalin
H-1	2 98 *ddd*	3 08 *ddd*	3 05 *ddd*
H-2	7 76 *dd*	7 71 *dd*	7 68 *dd*
H-3	6 13 *dd*	6 08 *dd*	6 09 *dd*
H-6	4 14 *dd*	5 68 *d*	4 45 *dd*
H-7	2 91 *dddd*	3 12 *dddd*	3 55 *br dddd*
H-8	4 52 *ddd*	4 64 *ddd*	4 97 *ddd*
H-9α	1 49 *ddd*	1 56 *ddd*	1 82 *ddd*
H-9β	2 58 *ddd*	2 58 *ddd*	2 26 *ddd*
H-10	2 04 *dddq*	2 03 *dddq*	2 07 *dddq*
H-13	6 23 *d*	6 22 *d*	6 38 *d*
H-13′	6 01 *d*	5 97 *d*	5 78 *d*
H-14	1 24 *d*	1 28 *d*	1 25 *d*
H-15	1 15 *s*	1 20 *s*	0 90 *s*
OR		6 70 *qq* 1 73 *br d*	
OH	3 07 *d*	1 72 *br s*	2 32 *d*

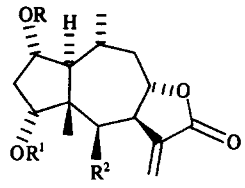
J (Hz) 1,2 = 2, 1,3 = 3, 1,10 = 11, 2,3 = 6, 7,8 = 10 5, 7,13 = 7,13′ = 8,9β = 3, 8,9α = 11 5, 9α,9β = 13, 9,10 = 4 5, 9′,10 = 13, 10,13 = 7, compound **1** 6,7 = 9, 6,OH = 2, compound **2** 6,7 = 7 5, helenalin 1,2 = 2, 1,3 = 3, 1,10 = 12, 2,3 = 6, 6,7 = 1 5, 6,OH = 4, 7,8 = 7 5, 7,13 = 3, 7,13′ = 3, 8,9α = 2 5, 8,9β = 9, 9α,9β = 15, 9α,10 = 6 5, 9β,10 = 4, 10,14 = 7

The ^{1}H NMR spectra of **3**–**8** (Table 2) indicated that all compounds must be very similar, only those of **7** and **8** showed that an additional hydroxy group was present Spin decoupling showed that this one had to be placed at C-6 Accordingly, a pair of double doublets in the spectra of **3**–**6**, which could be assigned to H-6 by decoupling, were replaced by a new low field signal at δ4 95 In the spectrum of **7** this signal was also a double doublet, but in that of **8** a broadened doublet was observed In both cases sharp doublets were visible after deuterium exchange Additional oxygen functions were at C-2 and C-4, as followed from the result of spin decoupling Starting with the H-8 signal, which could be assigned by irradiation of H-7, the whole sequence could be established Although

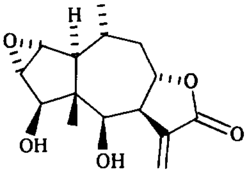


1 R = H

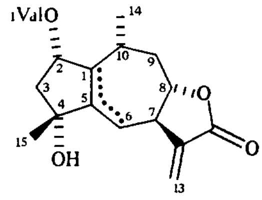
2 R = Tigl



	3	**4**	**5**	**6**	**7**	**8**
R	iVal	Ac	iVal	Tigl	iVal	Ac
R^1	Ac	Ac	H	H	H	H
R^2	H	H	H	H	OH	OH

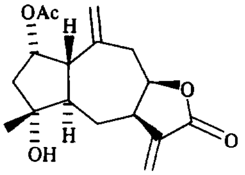


9

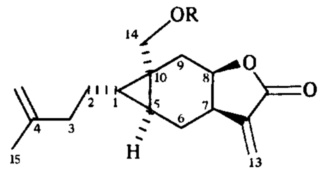


10 Δ5

11 Δ1(5)



12



	13	**14**	**15**	**16**	**17**
X	αOH,H	βOH,H	αOH,H	βOH,H	=O
R	H	H	Ac	Ac	Ac

already the couplings observed indicated the stereochemistry at C-1, C-2, C-4, C-6 and C-8, final proof was necessary NOE difference spectroscopy clearly established the proposed configurations of all chiral centres Irradiation of H-15 gave clear NOEs of H-2, H-3β, H-4, H-8 and H-10 Further NOEs were obtained between H-14, H-9α and H-2, between H-7 and H-1 and between H-1 and H-7

In the ^{1}H NMR spectrum of **7** the typical signals of an isovalerate were visible, but in that of **8** these signals were replaced by an acetate singlet while all other signals were nearly identical

The spectra of **3** and **4** again differed only in the signals of the ester groups While **4** was a diacetate, **3** showed in addition to an acetate singlet the typical isovalerate signals All signals could be assigned by spin decoupling which clearly showed that the oxygen functions were again at C-2 and C-4 The couplings were identical with those of **7** and **8**, thus indicating the same stereochemistry The relative position of the ester groups in **3** could be established by acetylation of **5**, where the position of the isovalerate residue could be deduced from the chemical shift of H-2

The ^{1}H NMR spectrum of **6** clearly showed that in this lactone the isovalerate residue was replaced by a tiglate as followed from the typical signals of this ester group

The ^{1}H NMR spectrum of **9** (Table 3) was close to that of 6β-acetoxyamarilin [7] However, the signal of H-6 was shifted up field We have tried to transform **9** to 6β-acetoxyamarilin, but this was not successful In addition to the diacetate only the 4-*O*-acetate was obtained The position of the acetate groups easily could be deduced from the ^{1}H NMR spectra (Table 3)

The ^{1}H NMR spectrum of **10** (Table 4) was close to that of a guaianolide isolated from *Helenium puberulum* [10] The acetate residue, however, was replaced by an isovaleryloxy group Biogenetic considerations suggest that both compounds have the same stereochemistry at C-2 and C-4 and therefore we have again studied this point NOE difference spectroscopy showed that indeed both oxygen functions at C-2 and C-4 were α-orientated, as clear NOEs were observed between H-15 and H-2 and H-3β, between H-14 and H-2, between H-7 and H-1 as well as between H-10 and H-8 Therefore the configuration of puberolide [10] has to be corrected to 2α-acetoxy-4α-hydroxy-1α,10βH-guaia-5,11(13)-dien-8α,12-olide The ^{1}H NMR spectrum of **11** (Table 4) indicated that an isomer of **10** was present The low field H-6 signal was replaced by a pair of double doublets which collapsed to doublets on irradiation at 2 64 (H-7) All signals were close to those of a guaianolide from a *Geigeria* species [11] Again the acetate singlet was replaced by the signals of an isovalerate Careful inspection of the ^{1}H NMR spectral data showed that also here a 2α-ester group at C-2 was more likely Thus the configuration of the lactone from *Geigeria* [11] should be changed to 2α-acetoxy-4α-hydroxy-10βH-guaia-1(5),11(13)-dien-8α,12-olide

The ^{1}H NMR spectrum of **12** (Table 4) was close to

Table 2 ^{1}H NMR spectral data of **3–8** (400 MHz, $CDCl_3$, TMS as internal standard)

	3	**4**	**5**	**6**	**7**	**8**
H-1	2 32 *dd*	2 32 *dd*	2 32 *dd*	2 39 *dd*	2 24 *dd*	2 24 *dd*
H-2	5 01 *ddd*	5 04 *ddd*	5 00 *ddd*	4 99 *ddd*	5 00 *ddd*	5 00 *ddd*
H-3α	1 50 *dd*	1 52 *dd*	1 50 *dd*	1 56 *dd*	1 54 *dd*	1 56 *dd*
H-3β	2 69 *ddd*	2 70 *ddd*	2 64 *ddd*	2 66 *ddd*	2 63 *ddd*	2 62 *ddd*
H-4	4 78 *d*	4 78 *d*	3 71 *br dd*	3 71 *br dd*	3 87 *br dd*	3 88 *br d*
H-6α	2 39 *dd*	2 39 *dd*	2 85 *m*	2 88 *m*	4 95 *br dd*	4 95 *br d*
H-6β	1 19 *dd*	1 19 *dd*	1 14 *dd*	1 16 *dd*	—	—
H-7	2 89 *m*	2 89 *m*	2 87 *m*	2 90 *m*	3 01 *dddd*	3 00 *dddd*
H-8	4 12 *ddd*	4 17 *ddd*	4 18 *dd*	4 20 *ddd*	4 62 *ddd*	4 62 *ddd*
H-9α	1 46 *ddd*	1 47 *ddd*	1 42 *ddd*	1 43 *ddd*	1 37 *ddd*	1 38 *ddd*
H-9β	2 35 *ddd*	2 35 *ddd*	2 35 *ddd*	2 36 *ddd*	2 40 *ddd*	2 39 *ddd*
H-10	1 92 *m*	1 93 *m*	1 91 *m*	1 95 *m*	1 98 *m*	1 99 *m*
H-13	6 18 *d*	6 17 *d*	6 18 *d*	6 18 *d*	6 40 *d*	6 40 *d*
H-13′	5 40 *d*	5 40 *d*	5 48 *d*	5 48 *d*	5 61 *d*	5 60 *d*
H-14	1 03 *d*	1 03 *d*	1 01 *d*	1 01 *d*	1 02 *d*	1 01 *d*
H-15	0 97 *s*	0 97 *s*	0 88 *s*	0 90 *s*	0 87 *s*	0 86 *s*
OR	2 16 *d*	—	2 18 *d*	6 85 *qq*	2 16 *d*	—
	2 10 *m*	—	2 11 *m*	1 79 *br d*	2 11 *tqq*	—
	0 93 *d*	—	0 93 *d*	1 82 *br s*	0 94 *d*	—
OAc	2 08 *s*	2 10 *s*	—	—	—	2 05 *s*
		2 04 *s*				
OH				1 95 *br d*	1 98 *br d*	
					1 66 *br d*	

J (Hz) 1,2 = 7, 1,10 = 11, 2,3α = 2, 2,3β = 9 5, 3α,3β = 16, 3β,4 = 4 5, 7,8 = 9, 7,13 = 7, 13′ = 3 5, 8,9α = 12, 8,9β = 3, 9α,9β = 13, 9α,10 = 12 5, 9β,10 = 4, 10,14 = 7, compounds **3–6** 6α,6β = 15 5, 6β,7α = 12 5, compound **6** 4,OH = 5, compounds **7** and **8** 6α,7 = 3 5, compound **7** 4,OH = 5 5, 6, OH = 3

Table 3 ^{1}H NMR spectral data of **9** and the acetates (400 MHz, $CDCl_3$, TMS as internal standard)

	9	4-*O*-Acetate	Diacetate
H-1	1 65 *br d*	1 82 *d*	1 80 *d*
H-2	3 43 *br d*	3 47 *br s*	3 46 *br d*
H-3	3 39 *br d*		3 34 *d*
H-4	3 90 *br s*	4 86 *s*	4 80 *br s*
H-6	4 30 *br d*	4 41 *dd*	5 53 *d*
H-7	2 92 *dddd*	2 93 *dddd*	3 05 *dddd*
H-8	4 63 *ddd*	4 58 *ddd*	4 57 *ddd*
H-9α	1 30 *ddd*	1 30 *ddd*	1 38 *ddd*
H-9β	2 49 *ddd*	2 49 *ddd*	2 51 *ddd*
H-10	2 09 *m*	2 10 *m*	2 10 *m*
H-13	6 38 *d*	6 31 *d*	6 22 *d*
H-13′	5 55 *d*	5 53 *d*	5 41 *d*
H-14	1 23 *d*	1 24 *d*	1 28 *d*
H-15	1 15 *s*	1 07 *s*	1 15 *s*
OAc	—	2 16 *s*	2 07 *s*
OH	—	2 02 *d*	—

J (Hz) 1, 10 = 11 5, 2, 3 = 3, 6, 7 = 4 5, 7, 8 = 9, 7, 13 = 7, 13′ = 3 5, 8, 9α = 12 5, 8, 9β = 3, 9α, 9β = 13 5, 9α, 10 = 13, 9β, 10 = 5 5, 4-*O*-acetate 6, OH = 3 5

that of florilenalin [12] However, the acetate methyl singlet indicated the presence of florilenalin 2-*O*-acetate as the H-2 signal was significantly shifted to lower fields The couplings of H-1 and H-2 clearly showed that the stereochemistry was that of florilenalin, where the absolute configuration was established by X-ray [12] Also the ^{13}C NMR spectrum nicely agreed with the structure (see Experimental) The acetate **12** already was prepared from florilenalin, but no full ^{1}H NMR data were reported [12]

The ^{1}H NMR spectral data of **17** (Table 5) clearly showed that a carabrone derivative was present as most signals were close to those of the cyclopropane derivative However, the methyl singlet of the carabrone spectrum was replaced by a pair of doublets at δ4 10 and 3 89 An additional singlet at δ2 14 (3H) showed that obviously an acetoxymethylene group was present All data therefore nicely agreed with the structure **17** which was also supported by the fragmentation in the mass spectrum After elimination of acetic acid a McLafferty reaction (loss of acetone) occurred. Furthermore a loss of methyl ethyl ketone can be observed These fragments are not visible in the MS of carabrone itself The spectral data of the epimeric mixture of **15** and **16** (Table 5), which could not be separated by TLC or HPLC indicated that these lactones were corresponding 4-hydroxy derivatives of **17** Accordingly, the singlet of the methyl ketone was replaced by a doublet and a triplet quartet at 3 78 was due to the H-4 proton. While the chemical shifts of H-14 were nearly the same as that of **17**, in the spectrum of **13** and **14** which also could not be separated, these doublets were shifted up field and the acetate signal was missing All data therefore showed that we were dealing with the epimeric diols **13** and **14**

As the concentrations of the epimers in **13**, **14**, **15** and **16**

Table 4 ^{1}H NMR spectral data of **10–12** (400 MHz, $CDCl_3$, TMS as internal standard)

	10	**11**	**12**
H-1	2 58 *br ddd*	—	2 10 *br d*
H-2	5 05 *ddd*	5 57 *br d*	5 22 *br dd*
H-3α	2 41 *dd*	2 37 *dd*	2 15 *dd*
H-3β	1 71 *dd*	1 88 *br d*	1 88 *br d*
H-5	—	—	2 15 *br dd*
H-6α	5 99 *dd*	2 85 *dd*	2 07 *br dd*
H-6β		2 91 *br dd*	1 47 *ddd*
H-7	3 38 *br dddd*	2 64 *ddddd*	3 20 *m*
H-8	3 82 *ddd*	4 02 *ddd*	4 58 *ddd*
H-9α	1 69 *ddd*	1 65 *ddd*	2 67 *br dd*
H-9β	2 45 *ddd*	2 30 *ddd*	2 28 *dd*
H-10	1 58 *m*	2 50 *m*	—
H-13	6 26 *d*	6 23 *d*	6 25 *d*
H-13′	5 68 *d*	5 57 *d*	5 64 *d*
H-14	1 05 *d*	1 20 *d*	5 01 *br s*
H-14′			4 82 *br s*
H-15	1 32 *s*	1 32 *s*	1 15 *s*
OR	2 18 *d*	2 17 *d*	
	2 10 *m*	2 10 *m*	
	0 94 *d*	0 94 *d*	

J (Hz) Compound **10** 1,2 = 2,3α = 2 4, 1,6 = 3, 1,10 = 10; 2,3β = 9, 3α,3β = 12 5, 6,7 = 4, 7,8 = 9, 7,13 = 7,13′ = 3 5, 8,9α = 11, 8,9β = 9β,10 = 3, 9α,9β = 13 5, 9α,10 = 13 5, 10,14 = 7, compound **11** 2,3α = 7, 3α,3β = 15, 6α,6β = 16, 6α,7 = 7,13 = 7,13′ = 3, 6β,7 = 11 5, 7,8 = 10, 8,9α = 12, 8,9β = 9β,10 = 3, 9α,9β = 12 5, 9α,10 = 11, 10,14 = 7, compound **12** 1,2 = 2 5, 1,5 = 12, 2,3α = 5 5, 3α,3β = 16, 5,6β = 12, 6α,6β = 14, 6α,7 = 5, 6β,7 = 10; 7,8 = 9 5, 7,13 = 7,13′ = 2 5, 8,9α = 3 5, 8,9β = 11 5, 9α,9β = 13

were different, the signals of the epimers could be assigned Inspection of models showed that the observed shift differences in the spectra of **13** and **14** may be explained if hydrogen bonds between the hydroxy groups would lead to fixed conformations Most likely in both cases the methyl at C-4 was equatorial In the main epimer a clear NOE between H-5 and H-14 and between H-14′ and H-8 led to a conformation where a deshielding effect on H-9α could be expected This would indicate that the main compound was that with a 4-S-configuration

Loxothysanus is a genus of problematical relationships It has been related variously to such distantly related genera, among others, as *Bahia* [13], *Perityle* [14], *Hymenothrix* [15] and *Hymenopappus* [16] All of these have at one time or another been included in the classically constituted tribe Helenieae [1] In spite of recent subdivisions of the latter in which each of the above mentioned genera are placed in different subtribes [14], the chemistry of *Loxothysanus* does not support inclusion into any of the suggested groupings Rather the chemical data suggest a position in the subtribe Gaillardiinae, which is a primitive element which links the mass of the classically circumscribed chaffless Helenieae to the tribe Heliantheae Faced with the chemical data presented here, one is inclined to agree with the comment of Turner [15], "*Loxothysanus* is an old relict group whose origin is not easily traced via extant relatives"

Table 5 ^{1}H NMR spectral data of **13–17** (400 MHz, $CDCl_3$, TMS as internal standard)

	13 and **14**	**15** and **16**	**17**
H-1	0 52 (0 62) *m*	0 69 *m*	0 67 *ddd*
H-2	1 65–1 45 *m*	1 60–1 40 m	1 66 *ddt*
H-2′			1 57 *ddt*
H-3			2 53 *dd*
H-3′			
H-4	3 92 (3 81) *tq*	3 78 *tq*	—
H-5	0 52 (0 62) *m*	0 69 *m*	0 72 *ddd*
H-6	2 34 *ddd*	2 34 *ddd*	2 32 *ddd*
H-6′	0 96 *ddd*	0 97 *ddd*	0 93 *ddd*
H-7	3 10 *dddd*	3 10 *ddddd*	3 07 *ddddd*
H-8	4 85 *ddd*	4 83 *ddd*	4 80 *ddd*
H-9	2 85 (2 77) *dd*	2 62 *dd*	2 59 *dd*
H-9′	0 90 (0 92) *dd*	0 99 *dd*	0 94 *dd*
H-13	6 24 *d*	6 24 *d*	6 23 *d*
H-13′	5 56 *d*	5 59 *d*	5 57 *d*
H-14	3 62 (3 59) *d*	4 08 (4 04) *d*	4 10 *d*
H-14′	3 38 (3 51) *d*	3 96 (3 94) *d*	3 89 *d*
H-15	1 21 (1 19) *d*	1 15 *d*	2 07 *s*
R	—	2 08 *s*	2 14 *s*

J (Hz) 5,6 = 6,7 = 7, 5,6′ = 9, 6,6′ = 14 5, 6′,7 = 13, 7,8 = 8 5, 7,13 = 7,13′ = 2 5, 8,9 = 7, 8,9′ = 11, 9,9′ = 14 5, 14,14′ = 12, compounds **13–16** 3,4 = 4,15 = 6 5, compound **17** 1,2 = 1,2′ = 2,3 = 2′,3 = 7, 1,5 = 5, 2,2′ = 14 5

EXPERIMENTAL

The air dried plant material (voucher Turner 15472, TEX, collected in Mexico) was worked-up in the usual fashion [17] The CC fractions of the extract of the aerial parts (400 g) were as follows 1 (petrol), 2 (Et_2O–petrol, 1 9), 3 (Et_2O–petrol, 1 4 and Et_2O–petrol, 1 1), 4 (Et_2O), 5 (Et_2O), 6 (Et_2O–MeOH, 9 1), 7 (Et_2O–MeOH, 4 1) and 8 (Et_2O–MeOH, 1 1) TLC (petrol) of fraction 1 gave 150 mg germacrene D and 5 mg caryophyllene and fraction 2 contained 850 mg *ent*-kaurenic acid Fraction 3 also contained acids and was therefore first treated with CH_2N_2 TLC (Et_2O–petrol, 3 7) gave 500 mg methyl-*ent*-kaurenoate and 500 mg of the 15α-isobutyrate TLC of fraction 4 (Et_2O–petrol, 7 3, three developments) gave 150 mg *ent*-kaurenic acid, 8 mg mexicanin C-tiglate, 10 mg **4** and a mixture which afforded by HPLC (MeOH–H_2O, 13 7) 15 mg **9** (R_t 8 min) further a mixture which was separated again by HPLC (MeOH–H_2O, 1 1) yielding 2 mg mexicanin C-tiglate (R_t 10 5 min), 5 mg helenalin (R_t 11 min) and 4 mg helenalin isovalerate (R_t 13 min) TLC (Et_2O–petrol, 1 1) of fraction 5 gave four bands (5/1–5/4) 5/1 was pure mexicanin C-tiglate (2 mg) 5/2 was separated by HPLC (MeOH–H_2O, 1 1) affording 4 mg **4** (R_t 10 5 min), 2 mg mexicanin C-tiglate (R_t 11 min), 4 mg **2** (R_t 12 5 min), a mixture which was purified by TLC (CH_2Cl_2–C_6H_6–Et_2O, 1 1 3) to give 2 mg **6** (R_f 0 45) and 8 mg **5** (R_t 23 min) 5/3 was separated by HPLC (MeOH–H_2O, 1 1) to give eight fractions (5/3/1–5/3/8) 5/3/1 contained 16 mg **1** (R_t 4 5 min), 5/3/2 contained 10 mg 6β-acetoxyamarilin (R_t 5 0 min), 5/3/4 13 mg α-cyclocostunolide (R_t 8 8 min) 5/3/5 (R_t 12 0 min) was separated by TLC (CH_2Cl_2–C_6H_6–Et_2O, 1 1 3) to get 3 mg **2** (R_f 0 58) and a band which by HPLC (MeOH–H_2O, 9 11) gave 4 mg β-cyclocostunolide (R_t 20 min) and 2 mg helenalin (R_t 23 min), 5/3/6 (R_t 13 min) gave by TLC (CH_2Cl_2–C_6H_6–Et_2O, 1 1 3) 3 mg helenalin, 5/3/7 (R_t 13 5 min) gave by TLC (CH_2Cl_2–C_6H_6–Et_2O, 1 1 3) 2 mg **6** (R_f 0 5) and 3 mg **11**

(R_f 0 42) and 5/3/8 (R_t 24 0 min) gave 2 5 mg **10** 5/4 contained 90 mg **17** (R_f 0 15)

Fraction 6 was separated by TLC (CH_2Cl_2–C_6H_6–Et_2O, 1 1 3) to give two bands The first one (R_f 0 55) contained 5 mg **17** and the second one (R_f 0 40) was separated by HPLC (MeOH–H_2O, 1 1) affording 10 mg **17** (R_t 8 2 min), 500 mg **15** and **16** (R_t 10 0 min) and a mixture (R_t 5 5 min) which was separated again by HPLC (MeOH–H_2O, 2 3) to give a fraction (R_t 10 0 min) which was treated by CH_2N_2 to remove impurities of acid material and then again separated by HPLC (MeOH–H_2O, 1 1) to give 2 mg mexicanin C (R_t 8 min)

HPLC of fraction 7 (MeOH–H_2O, 1 1) gave 12 mg mexicanin (R_t 5 7 min), 60 mg **12** (R_t 7 8 min) and 100 mg **15** and **16** (R_t 10 min) TLC of fraction 8 (Et_2O–MeOH, 20 1) gave three bands (8/1–8/3) 8/1 (R_f 0 75) was separated again by TLC (Et_2O–$CHCl_3$, 1 1, two developments) to give 100 mg **15** and **16** (R_f 0 20) and 9 mg **7** (R_f 0 08) 8/2 was separated by HPLC (MeOH–H_2O, 1 1) to give 13 mg mexicanin (R_t 6 min), 12 mg **8** (R_t 6 9 min), 40 mg **12** (R_t 8 min) and 40 mg **15** and **16** (R_t 5 0 min) and a fraction (R_t 6 8 min) which by TLC (Et_2O–MeOH, 9 1, three developments) yielded 8 mg **8** (R_f 0 80), 8 mg **13** and **14** (R_f 0 73) and 40 mg **12** (R_f 0 60)

Known compounds were identified by comparison of the 400 MHz ^1H NMR spectra with those of authentic material, by co-TLC or by rigorous structure elucidation by ^1H NMR, MS and IR spectroscopy and by comparison with the data from literature All compounds were pure by TLC and HPLC as well as by ^1H NMR spectroscopy though some could not be induced to crystallize

8-*Epihelenalin* (**1**) Colourless crystals, mp 179°, IR $\nu^{\mathrm{CCl_4}}_{\mathrm{max}}$ cm^{-1} 3600 (OH), 1780 (γ-lactone), 1705 (C=CC=O), MS *m/z* (rel int) 262 121 $[M]^+$ (18) (calc for $C_{15}H_{18}O_4$ 262 121), 244 $[M-H_2O]^+$ (6), 234 $[M-CO]^+$ (5), 216 $[244-CO]^+$ (4), 196 (12), 178 (22), 124 (100),

$$[\alpha]^{\lambda}_{24^\circ} = \frac{589\quad 578\quad 546\quad 436\,\mathrm{nm}}{-7\quad -7\quad -9\quad -26}\;(c\ 0\ 84,\ \mathrm{CHCl_3})$$

8-*Epihelenalin tiglate* (**2**) Colourless oil, IR $\nu^{\mathrm{CHCl_3}}_{\mathrm{max}}$ cm^{-1} 1770 (γ-lactone), 1710 (C=C–C=O, C=C–CO_2R), MS *m/z* (rel int) 344 162 $[M]^+$ (3) (calc for $C_{20}H_{24}O_5$ 344 162), 262 $[M-O{=}C{=}C(Me)CH{=}CH_2]^+$ (2), 244 $[M-RCO_2H]^+$ (17), 216 $[244-CO]^+$ (7), 188 $[216-CO]^+$ (10), 83 $[C_4H_7CO]^+$ (100), 55 $[83-CO]^+$ (39)

4-O-*Acetyl-pulchellin-2-O-isovalerate* (**3**) Colourless oil, IR $\nu^{\mathrm{CCl_4}}_{\mathrm{max}}$ cm^{-1} 1780 (γ-lactone), 1750, 1250 (OAc), 1750 (CO_2R), MS *m/z* (rel int) 392 220 $[M]^+$ (1) (calc for $C_{22}H_{32}O_6$ 392 220), 290 $[M-RCO_2H]^+$ (5), 230 $[290-AcOH]^+$ (49), 85 $[C_4H_9CO]^+$ (84), 57 $[85-CO]^+$ (100),

$$[\alpha]^{\lambda}_{24^\circ} = \frac{589\quad 578\quad 546\quad 436\,\mathrm{nm}}{-31\quad -31\quad -36\quad -64}\;(c\ 1\ 0,\ \mathrm{CHCl_3})$$

Pulchellin diacetate (**4**) Colourless oil, IR $\nu^{\mathrm{CCl_4}}_{\mathrm{max}}$ cm^{-1} 1775 (γ-lactone), 1740, 1240 (OAc), MS *m/z* (rel int) 350 173 $[M]^+$ (1) (calc for $C_{19}H_{26}O_6$ 350 173), 290 $[M-AcOH]^+$ (4), 248 $[290-\mathrm{ketene}]^+$ (21), 230 $[290-AcOH]^+$ (100), 215 $[230-Me]^+$ (8), $[\alpha]^{24^\circ}_{D}$ −12, $CHCl_3$, *c* 0 5

Pulchellin-2-O-isovalerate (**5**) Colourless oil, IR $\nu^{\mathrm{CCl_4}}_{\mathrm{max}}$ cm^{-1} 3600 (OH), 1775 (γ-lactone), 1735 (CO_2R), MS *m/z* (rel int) 350 209 $[M]^+$ (2 5) (calc for $C_{20}H_{30}O_5$ 350 209), 248 $[M-RCO_2H]^+$ (32), 230 $[248-H_2O]^+$ (24), 85 $[C_4H_9CO]^+$ (98), 57 $[85-CO]^+$ (100), $[\alpha]^{24^\circ}_{D}$ −13, $CHCl_3$, *c* 0 43 8 mg **5** in 0 1 ml Ac_2O were heated for 1 hr at 70° TLC (Et_2O–petrol, 2 1) gave 6 mg **3** (R_f 0 45), identical with the natural acetate (^1H NMR and co-TLC)

Pulchellin-2-O-tiglate (**6**) Colourless oil, IR $\nu^{\mathrm{CCl_4}}_{\mathrm{max}}$ cm^{-1} 3600 (OH), 1775 (γ-lactone), 1710 (C=CCO_2R), MS *m/z* (rel int) 348 194 $[M]^+$ (0 4) (calc for $C_{20}H_{28}O_5$ 348 194), 248 $[M-RCO_2H]^+$ (12), 230 $[248-H_2O]^+$ (8), 83 $[C_4H_7CO]^+$ (100), 55 $[83-CO]^+$ (44).

6β-Hydroxypulchellin-2-O-isovalerate (**7**) Colourless crystals, mp 127° IR $\nu^{\mathrm{CHCl_3}}_{\mathrm{max}}$ cm^{-1} 3620 (OH), 1770 (γ-lactone), 1720 (CO_2R), MS *m/z* (rel int) 366 204 $[M]^+$ (2 5) (calc for $C_{20}H_{30}O_6$ 366 204), 348 $[M-H_2O]^+$ (1 5), 264 $[M-RCO_2H]^+$ (48), 246 $[264-H_2O]^+$ (62), 85 $[C_4H_9CO]^+$ (64), 57 $[85-CO]^+$ (100), $[\alpha]^{24^\circ}_{D}$ +11, MeOH, *c* 0 47

6β-Hydroxypulchellin-2-O-acetate (**8**) Colourless crystals, mp 87°, IR $\nu^{\mathrm{KBr}}_{\mathrm{max}}$ cm^{-1} 3420 (OH), 1760 (γ-lactone), 1740 (OAc), MS *m/z* (rel int) 324 157 $[M]^+$ (2) (calc for $C_{17}H_{24}O_6$ 324 157), 264 $[M-AcOH]^+$ (20), 246 $[264-H_2O]^+$ (59), 228 $[246-H_2O]^+$ (10), 218 $[246-CO]^+$ (14), 203 $[218-Me]^+$ (12), 134 (56), 107 (52), 85 (100),

$$[\alpha]^{\lambda}_{24^\circ} = \frac{589\quad 578\quad 546\quad 436\,\mathrm{nm}}{+14\quad +16\quad +18\quad +26}\;(c\ 0\ 97,\ \mathrm{MeOH})$$

6β-Hydroxyamarilin (**9**) Colourless oil, IR $\nu^{\mathrm{CHCl_3}}_{\mathrm{max}}$ cm^{-1} 3600 (OH), 1770 (γ-lactone) 5 mg **9** was heated for 5 hr with 0 1 ml Ac_2O at 70° HPLC (MeOH–H_2O, 1 1) gave 3 mg 4-*O*-acetate (R_t 7 min) and 2 mg diacetate (R_t 11 5 min) 4-*O*-acetate colourless oil, IR $\nu^{\mathrm{CHCl_3}}_{\mathrm{max}}$ cm^{-1} 3610 (OH), 1770 (γ-lactone), 1740 (OAc), MS (CI, isobutane) 323 $[M+1]^+$ (100) (calc for $C_{17}H_{22}O_6+1$), 305 $[323-H_2O]^+$ (12), diacetate colourless oil, IR $\nu^{\mathrm{CHCl_3}}_{\mathrm{max}}$ cm^{-1} 1770 (γ-lactone), 1740, 1260 (OAc), MS *m/z* (rel int) 364 152 $[M]^+$ (9) (calc for $C_{19}H_{24}O_7$ 364 152), 30 $[M-OAc]^+$ (11), 304 $[M-OHAc]^+$ (6), 262 $[304-\mathrm{ketene}]^+$ (100), 244 $[304-HOAc]^+$ (22),

$$[\alpha]^{\lambda}_{24^\circ} = \frac{589\quad 578\quad 546\quad 436\,\mathrm{nm}}{+11\quad +14\quad +15\quad +25}\;(c\ 0\ 11,\ \mathrm{MeOH})$$

*4α-Hydroxy-2α-isovaleryloxy-1α,10β*H*-guaia-5,11(13)-dien-8α,12-olide* (**10**) Colourless oil, IR $\nu^{\mathrm{CCl_4}}_{\mathrm{max}}$ cm^{-1} 3600 (OH), 1775 (γ-lactone), 1735 (CO_2R), MS *m/z* (rel int) 348 194 $[M]^+$ (2) (calc for $C_{20}H_{28}O_5$ 348 194), 246 $[M-RCO_2H]^+$ (38), 228 $[246-H_2O]^+$ (24), 85 $[C_4H_9CO]^+$ (69), 57 $[85-CO]$ (100)

*4α-Hydroxy-2α-isovaleryloxy-10β*H*-guaia-1(5),11(13)-dien-8α,12-olide* (**11**) Colourless oil, IR $\nu^{\mathrm{CCl_4}}_{\mathrm{max}}$ cm^{-1} 3600 (OH), 1780 (γ-lactone), 1740 (CO_2R), MS *m/z* (rel int) 348 194 $[M]^+$ (2 5) (calc for $C_{20}H_{28}O_5$ 348 194), 330 $[M-H_2O]^+$ (1), 246 $[M-RCO_2H]^+$ (100), 231 $[246-Me]^+$ (12), 228 $[246-H_2O]^+$ (8), 85 $[C_4H_9CO]^+$ (34), 57 $[85-CO]^+$ (45)

Florilenalin-2-O-acetate (**12**) Colourless crystals, mp 127°, IR $\nu^{\mathrm{KBr}}_{\mathrm{max}}$ cm^{-1} 3420 (OH), 1770 (γ-lactone), 1730 (OAc), MS *m/z* (rel int) 306 147 $[M]^+$ (3 5) (calc for $C_{17}H_{22}O_5$ 306 147), 246 $[M-AcOH]^+$ (42), 231 $[246-Me]^+$ (8), 228 $[246-H_2O]^+$ (24), 204 $[246-C_3H_6]^+$ (100), ^{13}C NMR ($CDCl_3$, C-1–C-15) 51 5*d*, 80 4*d*, 49 0*t*, 78 3*s*, 53 1*d*, 29 7*t*, 42 1*d*, 73 9*d*, 39 5*t*, 140 0*s*, 139 2*s*, 169 8*s*, 122 6*t*, 115 5*t*, 25 2*q* (OAc 170 6*s*, 21 2*q*)

$$[\alpha]^{\lambda}_{24^\circ} = \frac{589\quad 578\quad 546\quad 436\,\mathrm{nm}}{+79\quad +83\quad +94\quad +154}\;(c\ 5\ 28,\ \mathrm{MeOH})$$

14-*Hydroxy*-4H- *and* 4-*epi*-H-*carabrone* (**13** *and* **14**) Colourless oil, IR $\nu^{\mathrm{CHCl_3}}_{\mathrm{max}}$ cm^{-1} 3620 (OH), 1760 (γ-lactone), MS (CI, isobutane) *m/z* (rel int) 267 $[M+1]^+$ (5), 249 $[267-H_2O]^+$ (100), 231 $[249-H_2O]^+$ (25),

$$[\alpha]^{\lambda}_{24^\circ} = \frac{589\quad 578\quad 546\quad 436\,\mathrm{nm}}{+72\quad +77\quad +87\quad +150}\;(c\ 0\ 42,\ \mathrm{MeOH})$$

14-*Acetoxy*-4H- *and* 4-*epi*-H-*carabrone* (**15** *and* **16**) Colourless oil, IR $\nu^{\mathrm{CCl_4}}_{\mathrm{max}}$ cm^{-1} 3620 (OH), 1775 (γ-lactone), 1750, 1245 (OAc), MS (CI, isobutane) *m/z* (rel int) 249 $[M+1-AcOH]^+$

(100), 231 $[249-H_2O]^+$ (22),

$$[\alpha]^{\lambda}_{24^\circ} = \frac{589 \quad 578 \quad 546\,\text{nm}}{+89 \quad +95 \quad +108} \; (c\ 35\ 1,\ \text{MeOH})$$

14-*Acetoxycarabrone* (**17**) Colourless crystals, mp 83°, IR $\nu_{\max}^{CCl_4}$ cm^{-1} 1770 (γ-lactone), 1740, 1240 (OAc), 1720 (C=O), MS m/z (rel int) 306 147 $[M]^+$ (2 5) (calc for $C_{17}H_{22}O_5$ 306 147), 264 [M − ketene]$^+$ (12), 246 [M − AcOH]$^+$ (38), 188 [246 − Me_2CO, McLafferty]$^+$ (24), 164 [246 − MeCOEt]$^+$ (60), 85 (100)

$$[\alpha]^{\lambda}_{24^\circ} = \frac{589 \quad 578 \quad 546 \quad 436\,\text{nm}}{+79 \quad +83 \quad +93 \quad +153} \; (c\ 5\ 5,\ CHCl_3)$$

Acknowledgements—We thank the Deutsche Forschungsgemeinschaft for financial support and B L Turner, University of Texas at Austin, for collecting of the plant material and valuable discussions

REFERENCES

1 Turner, B L and Powell, A M (1977) *The Biology and Chemistry of the Compositae* (Heywood, V H, Harborne, J B and Turner, B L, eds), p 699 Academic Press, London

2 Dominguez, E and Romo, J (1963) *Tetrahedron* **19**, 1415

3 Herz, W, Romo de Vivar, J, Romo, J and Viswanathan, N (1963) *J Am Chem Soc* **85**, 19

4 Willuhn, G, Pottger, P M and Mathiesen, U (1983) *Planta Med* **49**, 226

5 Herz, W, Romo de Vivar, J, Romo, J and Viswanathan, N (1963) *Tetrahedron* **19**, 1359

6 Bohlmann, F, Zdero, C, King, R M and Robinson, H (1984) *Justus Liebigs Ann Chem* 503

7 Bohlmann, F, Misra, L N, Jakupovic, J, King, R M and Robinson, H (1985) *Phytochemistry* (in press)

8 Romo de Vivar, J and Jimenez, H (1965) *Tetrahedron* **21**, 1741

9 Yoshioka, H, Renold, W, Fischer, N H, Higo, A and Mabry, T J (1970) *Phytochemistry* **9**, 823

10 Bohlmann, F and Jakupovic, J (1979) *Phytochemistry* **18**, 131

11 Bohlmann, F, Zdero, C and Ahmed, M (1982) *Phytochemistry* **21**, 1679

12 Lee, K H, Ibuka, T, Kozuka, M, McPhail, A T and Onan, K D (1974) *Tetrahedron Letters* 2287

13 Rydberg, P A (1914) *N Am Fl* **34**, 1

14 Turner, B L (1962) *Brittonia* **14**, 101

15 Turner, B L (1974) *Wrightia* **5**, 45

16 Robinson, H (1981) *Smithsonian Contrib Botany* **51**, 78

17 Bohlmann, F, Zdero, C, King, R M and Robinson, H (1984) *Phytochemistry* **23**, 1979