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HELIANGOLIDES AND GERMACROLIDES FROM *DISYNAPHIA MULTICRENULATA*

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Abstract—Investigation of *Disynaphia multicrenulata* afforded, in addition to known *ent*-kaurene derivatives and some widespread sesquiterpenes, several sesquiterpene lactones Six of them had not been isolated previously, two germacrolides and two pairs of epimeric heliangolides Their structures were elucidated by high field ^{1}H NMR spectroscopy The chemotaxonomic situation is discussed briefly

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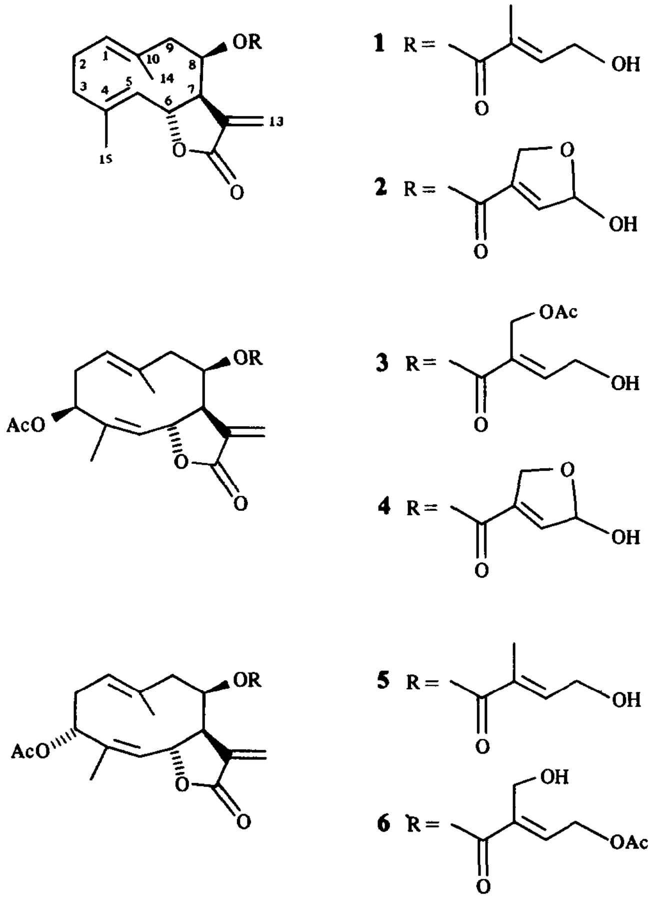
INTRODUCTION

The genus *Disynaphia* (tribe Eupatorieae) is placed in the subtribe Disynaphiinae [1] So far only one species has been studied chemically [2] Several sesquiterpene lactones were isolated, which in part were closely related to those from *Grazielia*, which is placed in the same subtribe We now have investigated the constituents of a further species from Paraguay, *D multicrenulata* (Sch Bip ex Baker) K et R The results are discussed in this paper.

RESULTS AND DISCUSSION

While the roots of *Disynaphia multicrenulata* afforded only known compounds (see Experimental), the aerial parts gave in addition to known compounds a complex mixture of sesquiterpene lactones Separation of this



mixture by column chromatography, TLC and finally HPLC afforded 8β-(5-hydroxytiglinoyloxy)costunolide [3], eupatoriopicrin [4], 9β-hydroxy-8β-*O*-tigloyl costunolide [5], hiyodorilactone C [6], chromolaenide [7], eucannabinolide [4], eupaformonin [8], eupaformosanin [9] and 3-epi-chromolaenide [10] as well as six new lactones, the germacranolides **1** and **2** and the heliangolides **3**, **4**, **5** and **6** The structure of **1** easily could be deduced from the ^{1}H NMR spectrum (Table 1) which was very close to that of 8β-(5-hydroxytiglinoyloxy)-costunolide, differing only in the signals of the ester residue Also the ^{1}H NMR signals of **2** differed only in those of the ester group The presence of the semiacetal of a 5-hydroxy-4-oxo-tiglate could be deduced from the typical ^{1}H NMR signals (Table 1) [11] The ^{1}H NMR spectra (Table 1) of the lactones **3** and **4** showed that heliangolides were present Most signals were nearly identical with those of eucannabinolide The downfield shift of the signal of H-20 and the presence of two acetate methyl singlets in the spectrum of **3** clearly indicated that this compound simply was the 20-*O*-acetate of eucannabinolide The typical signals of the ester residue of **4** showed that this lactone had the same oxygen function at C-8 as **2** The relative position of the ester groups in **3** and **4** followed from the chemical shifts of H-8α which was nearly the same in the spectra of similar heliangolides The ^{1}H NMR spectra of **5** and **6** (Table 1) were close to that of eupaformosanin Especially the changed multiplicity of the H-3 signal clearly showed that these two lactones had a 3α-acetoxy group, while the nature of the ester residue at C-8 could be deduced from the characteristic ^{1}H NMR signals Again the relative position of the ester groups followed from the unaltered chemical shift of H-8α in the spectra of known compounds [9, 10]

The chemistry of this *Disynaphia* species differs from that of *Disynaphia halimifolia* [2] which contains more unusual sesquiterpene lactones, while no *ent*-kaurane derivatives were isolated, but a geranylgeraniol derivative Benzofurans are present in both species The investigation of further species seems to be necessary also from representatives of the other genera belonging to the subtribe Disynaphiinae to get a clear picture of the chemotaxonomy of this group

EXPERIMENTAL

The air dried plant material, collected in February 1982 in Paraguay near San Lorenzo, was extracted with Et_2O–petrol, 1 2, and with MeOH The resulting extracts were evaporated at low temperature The extract of the roots (280 g) gave on CC (silica gel) fractions (100 ml) as follows 1 (petrol), 2 (Et_2O–petrol, 10 1), 3 (Et_2O–petrol, 1 3), 4 (Et_2O–petrol, 1 1), 5 (Et_2O) and 6

Table 1 ^{1}H NMR spectral data of compounds **1**–**6** (400 MHz, $CDCl_3$, TMS as internal standard)

	1	**2**	**3**	**4**	**5**	**6**
H-1	4 91 *br dd*	4 92 *m*	5 30 *m*	5 30 *m*	5 10 *br d*	5 10 *br dd*
H-2α			2 75 *m*	2 75 *m*	2 78 *m*	2 77 *m*
H-2β			2 30 *m*	2 33 *m*	2 11 *m*	2 10 *m*
H-3α			5 95 *br d*	5 87 *br d*	—	—
H-3β			—	—	5 63 *dd*	5 61 *br dd*
H-5	4 81 *br d*	4 80 *br d*	5 22 *br d*	5 21 *br d*	5 22 *br d*	5 22 *br d*
H-6	5 17 *dd*	5 09 *dd*	5 30 *m*	5 30 *m*	5 28 *br d*	5 28 *br d*
H-7	2 95 *br ddd*	2 96 *br ddd*	2 97 *br s*	2 99 *br s*	2 98 *br s*	3 00 *br s*
H-8	5 84 *br d*	5 84 *br d*	5 27 *br dd*	5 28 *dd*	5 26 *br s*	5 26 *br s*
H-9α	2 87 *br dd*	2 85 *m*	2 74 *br d*	2 75 *m*	2 72 *dd*	2 74 *m*
H-9β	2 36 *m*	2 36 *m*	2 47 *br d*	2 50 *br d*	2 43 *dd*	2 44 *dd*
H-13	6 32 *d*	6 34 *d*	6 38 *d*	6 39 *d*	6 38 *d*	6 40 *d*
H-13′	5 64 *d*	5 66 *d*	5 81 *d*	5 81 *d*	5 79 *d*	5 78 *d*
H-14	1 51 *br s*	1 52 *br s*	1 81 *br s*	1 83 *br s*	1 94 *br s*	1 94 *br s*
H-15	1 80 *br s*	1 80 *br s*	1 84 *br s*	1 86 *br s*	1 83 *d*	1 84 *br s*
OAc	—	—	2 13 *s* 2 07 *s*	2 13 *s*		2 15 *s* 2 13 *s*
OCOR	6 78 *tq* 4 40 *dq* 1 85 *dt*	6 60 *br s* 6 23 *br s* 4 92 *br d* 4 72 *br d*	7 05 *t* 4 94 *d* 4 82 *d* 4 40 *dd* 4 34 *dd*	6 62 *br s* 6 17 *br s* 4 88 *br d* 4 69 *br d*	6 81 *tq* 6 36 *br s* 1 85 *dt*	6 78 *t* 4 87 *d* 4 40 *m*

J (Hz) Compounds **1** and **2** 1, 2β = 12, 5, 6 = 9 5, 6, 7 = 8 5, 7, 8 ~ 1, 7, 13 = 3 5, 7, 13′ = 3, 8, 9α = 6, 9α, 9β = 14, compounds **3** and **4** 2, 3α = 11, 5, 6 = 10 5, 7, 13 = 2, 8, 9 = 3, 9α, 9β = 14, compounds **5** and **6** 1, 2 ~ 8, 2α, 3 = 11 5, 2β, 3 = 5, 5, 6 = 10 5, 5, 15 = 1, 7, 13 = 2, 8, 9 = 3, 9α, 9β = 14, $OCOC(Me)=CHCH_2OH$ 3, 4 = 6, 3, 5 = 4, 5 ~ 1, $OCOC(CH_2OAc)=CHCH_2OH$ 3, 4 = 5 5, 4, 4′ = 13, 5, 5′ = 12, $OCOC(CH_2OH)=CHCH_2OAc$ 3, 4 = 6, OCOR (**2** and **4**) 5, 5′ = 13 5

(Et$_2$O–MeOH, 20 1) TLC of fraction 1 (silica gel, AgNO$_3$ coated, petrol detection always by UV-light and KMnO$_4$ spray) afforded 88 mg germacrene D (R_f 0 38) and 20 mg caryophyllene (R_f 0 45), TLC of 10% of the fractions 2 and 3 after addition of CH$_2$N$_2$ (silica gel, Et$_2$O–petrol, 1 10) gave 0 4 g methyl-*ent*-kaurenoate (R_f 0 67), 35 mg *ent*-kaurenal (R_f 0 62), 134 mg methyl-15α-isovaleryloxy-*ent*-kaurenoate (R_f 0 45) and 15 mg *ent*-kauran-19-ol (R_f 0 15) and TLC of fraction 4 (silica gel, Et$_2$O–petrol, 1 1) afforded 55 mg 5-acetyl-2-[2-hydroxyisopropyl]-benzofuran [12] (R_f 0 52) and 150 mg 15α-hydroxy-*ent*-kaurenic acid (R_f 0 45) The extract of the aerial parts (300 g) gave on CC (silica gel) fractions (100 ml) as follows 1 (petrol), 2 (Et$_2$O–petrol, 1 10), 3 (Et$_2$O–petrol, 1 3), 4 (Et$_2$O–petrol, 1 1), 5 (Et$_2$O) and 6 (Et$_2$O–MeOH, 20 1) TLC of fraction 1 (silica gel, AgNO$_3$ coated, petrol) gave 75 mg germacrene D (R_f 0 35), 160 mg caryophyllene (R_f 0 45), 25 mg bicyclogermacrene (R_f 0 50), 50 mg squalene (R_f 0 30) and 2 mg α-humulene (R_f 0 33), TLC of 10% of fractions 2 and 3 after addition of CH$_2$N$_2$ (silica gel, Et$_2$O–petrol, 1 10) afforded 3 mg caryophyllene 1,10-epoxide (R_f 0 70), 145 mg methyl-*ent*-kaurenoate (R_f 0 6), 15 mg methyl-15α-isovaleryl-oxy-*ent*-kaurenoate (R_f 0 45), 10 mg 15α-angeloyloxy-*ent*-kaurenoate (R_f 0 42) and 30 mg spathulenol (R_f 0 39), TLC of 10% of fraction 4 after addition of CH$_2$N$_2$ (silica gel, Et$_2$O–petrol, 1 3) gave 50 mg spathulenol (R_f 0 62), 45 mg methyl-16α,17-epoxy-*ent*-kaurenoate (R_f 0 58), 70 mg methyl-15α-isovaleryloxy-*ent*-kaurenoate (R_f 0 55), 12 mg methyl-15α-angeloyloxy-*ent*-kaurenoate (R_f 0 53), 6 mg *ent*-kauren-19-ol (R_f 0 50), 45 mg methyl-15α-hydroxy-*ent*-kaurenoate (R_f 0 47) and 0 5 mg umbelliferone (R_f 0 41) Fractions 5 and 6 on repeated TLC (silica gel, CH$_2$Cl$_2$–C$_6$H$_6$–Et$_2$O, 3 3 4) afforded 900 mg 8β-(5-hydroxytigloyloxy)-costunolide (R_f 0 60), 860 mg eucannabinolide (R_f 0 55), 290 mg eupaformosanin (R_f 0 50) and mixtures of further sesquiterpene lactones which on HPLC (reversed phase, MeOH–H$_2$O, 3 2) and finally TLC (silica gel, C$_6$H$_6$–EtOAc, 3 2) gave 40 mg eupaformonin (R_f 0 72), 39 mg hiyodorilactone C (R_f 0 68), 11 mg 9β-hydroxy-8β-tigloyloxycostunolide (R_f 0 64), 3 mg **5** (R_f 0 60), 17 mg chromolaenide (R_f 0 59), 1 5 mg **2** (R_f 0 58), 1 mg **1** (R_f 0 55), 6 5 mg **4** (R_f 0 45), 5 5 mg **3** (R_f 0 40), 3 5 mg eupatoriopicrin (R_f 0 38), 3 mg **6** (R_f 0 37) and 6 5 mg epi-chromolaenide (R_f 0 34) Compounds **1–6** could not be induced to crystallize though they were homogeneous by TLC (different solvents) and by their 400 MHz ^{1}H NMR spectra Known compounds were identified by comparing the 400 MHz ^{1}H NMR spectra with those of authentic material, quantities were determined by weight

20-Desoxyeupatoriopicrin (**1**) IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3600 (OH), 1770 (γ-lactone), 1725, 1650 (C=CCO$_2$R), MS m/z (rel int) 346 $[M]^+$ (0 5), 230 131 $[M - RCO_2H]^+$ (73) (C$_{15}$H$_{18}$O$_2$), 215 $[230 - Me]^+$ (17), 99 $[RCO]^+$ (100), 71 $[99 - CO]^+$ (75)

20-Dehydroeupatoriopicrin-semi acetal (**2**) MS m/z (rel int) 230 131 $[M - RCO_2H]^+$ (78) (C$_{15}$H$_{18}$O$_2$), 113 $[RCO]^+$ (36), 95 $[113 - H_2O]^+$ (80), 55 $[C_4H_7]^+$ (100)

Eucannabinolide-20-O-acetate (**3**) IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3460 (OH), 1760 (γ-lactone), 1735 (OAc, CO$_2$R), MS m/z (rel int) 462 189 $[M]^+$ (2) (C$_{24}$H$_{30}$O$_9$), 402 $[M - HOAc]^+$ (0 8), 228 $[402 - RCO_2H]^+$ (38), 157 $[RCO]^+$ (12), 97 $[157 - HOAc]^+$ (47), 69 $[97 - CO]^+$ (100)

$$[\alpha]_{24^\circ}^{\lambda} = \frac{589 \quad 578 \quad 546 \quad 436\,\text{nm}}{-72 \quad -74 \quad -86 \quad -156} (\text{CHCl}_3,\ c\ 0\ 45)$$

20-Dehydroeucannabinolide semi acetal (**4**) IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3600 (OH), 1760 (γ-lactone), 1740 (OAc, CO$_2$R), MS m/z (rel int)· 418 163 $[M]^+$ (7) (C$_{22}$H$_{26}$O$_8$), 400 $[M - H_2O]^+$ (2), 358 $[M - HOAc]^+$ (2), 228 $[358 - RCO_2H]^+$ (43), 113 $[RCO]^+$ (14), 95 $[113 - H_2O]^+$ (100)

$$[\alpha]_{24^\circ}^{\lambda} = \frac{589 \quad 578 \quad 546 \quad 436\,\text{nm}}{-126 \quad -128 \quad -144 \quad -261} (\text{CHCl}_3,\ c\ 0\ 36)$$

20-Desoxyeupaformasanin (**5**) IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3460 (OH), 1770 (γ-lactone), 1755 (OAc), 1715 (C=CCO$_2$R), MS m/z (rel int) 404 184 $[M]^+$ (7) (C$_{22}$H$_{28}$O$_7$), 344 $[M - HOAc]^+$ (2), 288 $[M - RCO_2H]^+$ (3 5), 246 $[288 - \text{ketene}]^+$ (27), 228 $[288 - HOAc]^+$ (32), 99 $[RCO]^+$ (100), 71 $[99 - CO]^+$ (69)

$$[\alpha]_{24^\circ}^{\lambda} = \frac{589 \quad 578 \quad 546 \quad 436\,\text{nm}}{-46 \quad -46 \quad -54 \quad -96} \text{CHCl}_3,\ c\ 0\ 17)$$

Eupaformosanin-19-O-acetate (**6**) IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3450 (OH), 1760 (γ-lactone), 1740 (OAc, CO$_2$R); MS m/z (rel int) 402 $[M - HOAc]^+$ (0 5), 228 $[402 - RCO_2H]^+$ (10), 157 $[RCO]^+$ (6), 97 $[157 - HOAc]^+$ (28), 69 $[97 - CO]^+$ (100)

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