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FURTHER INEUPATOROLIDE-LIKE GERMACRANOLIDES FROM *INULA CUSPIDATA**

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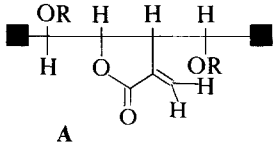
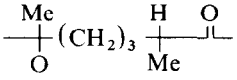
Key Word Index—*Inula cuspidata*; Compositae; sesquiterpene lactones; germacranolides; hydroxygermacrene; acetylenic sulfoxides.

Abstract—The aerial parts of *Inula cuspidata* afforded in addition to known compounds five germacranolides closely related to ineupatorolide A. Furthermore, a hydroxygermacrene and two isomeric acetylenic sulfoxides were present. The structures were elucidated by spectroscopic methods.

While most sequiterpene lactones so far found in *Inula* species (Compositae, tribe Inuleae) are relatively simple [1], from *I. eupatorioides* recently some more complex ones have been reported [2]. We now have investigated *I. cuspidata* C. B. Clarke, which contained similar lactones. The aerial parts afforded β-farnesene, geranyl linalol [3], squalene, the tricyclic alcohol **8** [4], the acetylenic compounds **9** and **10** [5] and the alcohol **7**, which was identical with an alcohol isolated previously [6], but proposed to be the 3-hydroxy derivative. The structure followed from the ^{1}H NMR data (Experimental), which were partly close to those of germacrene D. The olefinic methyl signal was replaced by signals of exomethylene protons and a double doublet at 3.76 ppm indicating the presence of a secondary hydroxyl group. The position of the latter followed from the chemical shift of H-14 and spin decoupling, while the β-orientation was proposed from biogenetic consideration, since **7** most probably is formed via germacrene D-1,10-epoxide (**6**) (see Scheme). Inspection of models showed that a β-hydroxy group would agree with the couplings observed; however, the flexibility of the 10-membered ring also allowed a conformation where the couplings would be in agreement with an α-hydroxy group. The polar fractions contained several sesquiterpene lactones, which could be separated in part by HPLC only (reversed phase). Even so, a mixture of isomeric diesters could not be separated. The ^{1}H NMR data (Table 1) led to the structures **1**–**5**, which are closely related to that of ineupatorolide A, though additional oxygen functions were present. The ^{1}H NMR data of **1**–**5** clearly showed that all compounds were methylene lactones. If the data of **1** and **5** were compared the presence of 8,12-lactones were very likely. **1** (incaspitolide A) obviously was a diisobutyrate with an additional hydroxy and a keto group. Careful spin decouplings starting with the H-7 signal led to the sequence **A**.



A



B

The remaining signals, a methyl singlet at 1.12 ppm, a methyl doublet at 1.01 ppm, a doublet doublet quartet at 3.03 ppm and multiplets at 1.75 (2 H) and 1.55 (4 H) ppm would agree with a sequence **B**; thus leading to structure **1**. The stereochemistry was deduced from the couplings observed and from inspection of models. As both, $J_{6,7}$ and $J_{8,9}$ were large, only a *trans*-orientation at C-6–C-9 was likely. In a conformation being in agreement with the couplings of H-6–H-9, those of H-4 required a β-position of the 4-methyl group. As the ^{1}H NMR spectrum indicated a relatively rigid conformation, a hydrogen bridge between 10-OH and the keto group was likely. This, however, required a 10β-hydroxy group. Therefore the stereochemistry at C-4 and C-7–C-10 was the same as that of ineupatorolide A, where the configuration is established by X-ray [2]. As in the latter case a decision whether **1** is a 8,12- or a 6,12-lactone, was not possible without knowing the absolute configuration. The ^{1}H NMR data (Table 1) of the mixture of **2** and **3** (incaspitolide B and C) clearly showed that these compounds only differed from **1** by the ester residues. The typical signals of isobutyrates, isovalerate and 2-methylbutyrates clearly could be identified. However, as a separation was not possible, no decision can be given where the different ester groups should be placed. As the signals of the tertiary protons of the isobutyrate moieties were different in the spectrum of **1**, more than two compounds probably were present. One unusual feature was remarkable in the ^{1}H NMR spectrum of the mixture: the α-protons of the isovalerate showed two double doublets probably due to a restricted free rotation of this ester residue. The ^{1}H NMR data of **4** (incaspitolide D) (Table 1) indicated that the substitution pattern was different from that of **1**. Spin decoupling using the H-7 signal as point of departure established the whole sequence H-2–H-9. The position of ester groups was

* Part 379 in the series "Naturally Occurring Terpene Derivatives". For Part 378 see Bohlmann, F. and Zdero, C. (1982) *Phytochemistry* **21**, (in press).

Table 1. ^{1}H NMR spectral data of compounds **1**–**4**, **5a**, **5b**, **5c** and **5d** (400 MHz, TMS as int. standard)

	1	**2/3**	**4** (60°)*	**5a**	**5c**	**5d**
H-1	1.55 *m*	1.55 *m*		4.11 *dd*	5.11 *dd*	5.19 *brd*
H-2			2.29 *dd*	1.95 *m*	1.73 *dddd*	
			3.71	1.32 *dddd*	1.43 *m*	†
H-3	1.75 *m*	1.75 *m*	1.85 *m*	1.45 *dddd*	1.48 *dddd*	†
				1.71 *m*	1.89 *dddd*	
H-4	3.03 *ddq*	3.03 *m*	2.20 *m*	1.97 *m*	3.21 *ddq*	3.24 *ddq*
H-5	—		5.03 *dd*		—	
H-6	4.84 *d*	4.85 *d*, 4.83 *d*	4.29 *br dd*	5.06 *d*	4.82 *d*	4.78 *d*
H-7	3.45 *dddd*	3.46 *dddd*	2.99 *dddd*	3.24 *dddd*	3.46 *br d*	3.55 *br d*
H-8	4.66 *dd*	4.66 *dd*	4.69 *dd*	5.10 *dd*	4.58 *dd*	4.48 *dd*
H-9	4.62 *d*	4.63 *d*, 4.61 *d*	5.32 *br d*	5.01 *br d*	4.78 *d*	4.77 *d*
H-13	6.40 *d*	6.39 *d*	6.44 *d*	6.35 *d*	6.43 *d*	6.43 *d*
H-13	5.92 *d*	5.91 *d*	5.61 *d*	5.73 *d*	5.97 *d*	5.98 *d*
H-14	1.12 *s*	1.12 *s*	1.30 *s*	1.07 *s*	1.10 *s*	1.42 *s*
H-15	1.01 *d*	1.01 *d*	0.99 *d*	1.13 *d*	1.03 *d*	1.03 *d*
OCOR	2.66, 2.62 *qq*	2.31, 2.27 *dd*	2.69, 2.66 *qq*	2.69, 2.55 *qq*	2.66, 2.64 *qq*	2.66, 2.64 *qq*
	1.21, 1.18 *d*,	2.1 *m*, 0.95 *d*,	1.26, 1.24, 1.23,	1.24, 1.21,	1.22, 1.21,	1.20, 1.19,
	(*i*Bu)	0.94 *d* (*i*Val)	1.21 *d*	1.18, 1.14 *d*	1.20, 1.19 *d*	1.18 *d*
		2.66, 2.62 *qq*, 1.21 *d*,			2.14 *s* (OAc)	2.17 *s*, 2.12 *s* (OAc)
		1.20 *d*, 1.19 *d*, 1.18 *d*,				
		(*i*Bu), 2.46 *tq*, 0.87 *t*,				
		1.19 *d* (Mebu)				

* OH 1.81 *d*; † obscured multiplets.

J (Hz): compounds **1**–**3**: 3,4 = 5; 3′4 = 9.5; 4,15 = 7; 6,7 = 11; 7,8 = 7,13 ~ 2; 8,9 = 6; compound **4**: 2,2′ = 18; 2,3 = 10; 2,3′ = 5; 2′,3 = 2′,3′ = 5; 4,5 = 1.5; 4,15 = 7; 5,6 = 11; 6,7 ~ 1; 6.OH = 10; 7,8 = 6; 7,13 = 3; 7,13′ = 2.7; 8,9 = 10; compound **5a**: 1,2α = 5.5; 1,2β = 12; 2α,2β = 2β,3α = 12.5; 2β,3β = 2.5; 3α,3β = 13; 4,15 = 7; 6,7 = 10; 7,8 = 4.5; 7,13 = 2.5; 8,9 = 8.5; compound **5c**: 1,2α = 4 (at 50°2), 1,2β = 6 (at 50° 8.5); 2α,2β = 3α,3β = 13; 3α,4 = 4; 3β,4 = 10.5; 4,15 = 7; 6,7 = 10; 7,8 = 2; 7,13 = 1.5; 8,9 = 8.5; compound **5d**: 1,2α ~ 1; 1,2β = 10; 4,15 = 7; 6,7 = 10; 7,8 = 7,13 = 1.5; 8,9 = 8; O*i*Bu 2′,3′ = 2′,4′ = 7; O*i*Val 2′,2′ = 16; 2′,3′ = 3′,4′ = 3′,5′ = 7; OMebu 2′,3′ = 2′,5′ = 3′,4′ = 7.

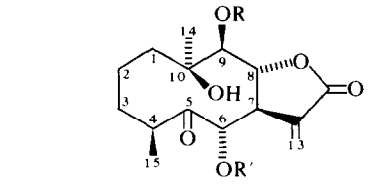
deduced from the chemical shifts, as the signal of H-6 was at higher field as that in the spectrum of **1** and therefore a free hydroxyl at C-6 was very likely. The presence of a keto group at C-1 was supported by the chemical shifts of H-2 and their couplings. Though the stereochemistry at C-4 and C-10 could not be determined with certainty, it is likely to be the same as in **1**–**3**. The configurations at C-5–C-9 followed from the couplings observed, if models were considered. Obviously the conformation of **4** was different from that of **1**, as the angles between H-6 and H-7 were nearly 90°, probably due to a hydrogen bond between the 6-OH group and the ester group at C-5. The structure of **5a** (incaspitolide E) caused some difficulties, since acetylation afforded the monoacetate **5c** and the diacetate **5d**. The ^{1}H NMR data (Table 1) showed that **5c** and **5d** obviously were secondary acetates with a keto group at C-5, while the data of **5a** indicated that the keto group was masked as the H-4 signal was at much higher fields. Furthermore, the H-1 signal was a sharp double doublet, unusual for a proton near a hydroxy group. Therefore the presence of a hemi-ketal was very likely, which, however, could be in equilibrium with the ketone **5b**, which could be shifted by acetylation. Probably the acetylation of the tertiary hydroxyls at C-5 and C-10 were less favoured, no trace of 5-*O*-acetate being obtained. The ^{1}H NMR data were in good agreement with this assumption. Those of **5c** clearly showed that the substitution at C-4–C-10 was the same as in **1**. The additional acetoxy group only could be placed at C-1 as the corresponding signal of the proton under the acetoxy group was a clear double doublet. If a model was considered, a hydrogen bridge between the 10-hydroxy and the keto group was very likely. Then, however, only an α-orientated hydroxy would agree with the couplings observed. All other angles would be in good agreement with the couplings observed for the other signals. Inspection of a model of **5a** also showed that the observed couplings would agree with the proposed stereochemistry. In the spectrum of **5d** the downfield shift of H-14 clearly

Table 2. ^{1}H NMR data of compounds **11** and **12**

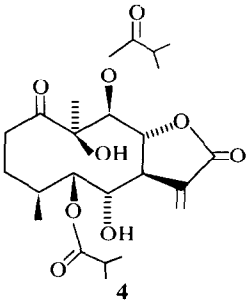
	11*		**12***	
	$CDCl_3$	C_6D_6	$CDCl_3$	C_6D_6
H-1	3.99 *br d*	3.61 *m*	3.99 *br d*	3.61 *m*
H-1′	3.42 *ddd*	2.92 *ddd*	3.42 *ddd*	2.92 *ddd*
H-4	4.63 *dd*	4.68 *m*	4.58 *dd*	4.68 *m*
H-5	3.79 *ddd*	3.50 *m*	3.79 *ddd*	3.50 *m*
H-6	5.92 *dd*	6.07 *dd*	5.92 *dd*	5.97 *dd*
H-7	6.77 *d*	7.05 *d*	6.81 *d*	7.09 *d*
H-9	6.34 *br s*	6.71 *br s*	6.32 *br s*	6.72 *br s*
H-14	2.05 *d*	1.36 *d*	2.04 *d*	1.35 *d*
SOMe	2.64 *s*	2.18 *s*	2.66 *s*	2.12 *s*
OAc	2.01 *s*	1.67 *s*	2.05 *s*	1.74 *s*

* H-2 2.05 *m*, H-2′ 1.74 *m*, H-3 2.17 *m*, H-3′ 1.06 *m* ($CDCl_3$).

J (Hz): 1,1′ = 1′,2 = 13; 1′,2′ = 4; 3,4 = 4.5; 3,4 = 3,5 = 10; 5,6 = 6.5; 6,7 = 15; 9,14 = 1.3.



	1	2	3
R	*i*Bu	*i*Bu	*i*Val, Mebu
R′	*i*Bu	*i*Val, MeBu	*i*Bu



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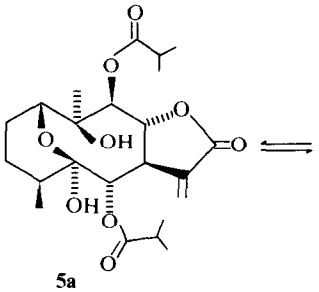
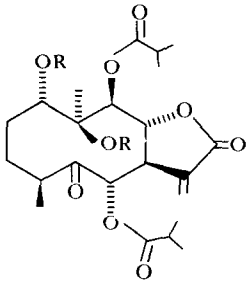
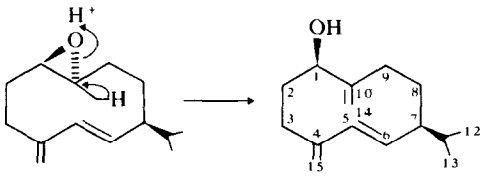


5a



5b R = R′ = H
5c R = Ac, R′ = H
5d R = R′ = Ac



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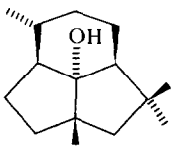

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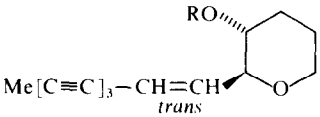
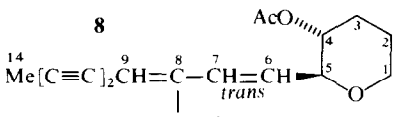
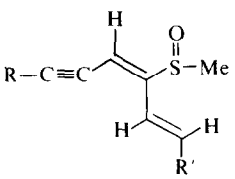


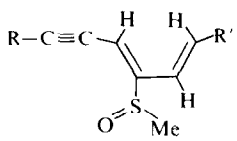
9 R = H
10 R = Ac



11 *cis* SOMe
12 *trans*



11



12

indicated that the second acetoxy group was at C-10. The coupling of H-1 again was altered, indicating that the conformation was changed, probably due to the missing hydrogen bridge. At elevated temperature in the spectrum of **5c** the H-1 couplings were close to those in **5d**, showing that the hydrogen bridge may be very weak. Consequently also the chemical shifts in the spectrum of **5c** at higher temperature were altered. The polar fractions further contained minute amounts of the isomeric sulfoxides **11** and **12**, which, however, could not be separated. The molecular formula and the ^{1}H NMR data clearly showed that methyl sulfoxides derived from **10** were present. Spin decoupling allowed the assignment of all signals which were in part very similar to those of **10**. The position of the sulfoxide group followed from the chemical shifts of H-7 and from the coupling of the olefinic proton with the acetylenic methyl group. The observed magnitude is characteristic for this situation. Surprising, however, were the small chemical shift differences of H-9 in the two isomers. Normally in such sulfoxides the *cis*-olefinic proton is more deshielded [7]. Perhaps due to steric hindrance the 7,8-bond was in a S-*cis*-conformation, where the H-9 proton would be deshielded by the 6,7-double bond (see Scheme). The assignment of the

configurations of the two isomers caused difficulties. However, the stronger Eu(fod)$_3$ induced shift of the acetate signal would support the proposed stereochemistry, if the assumed configurations were valid.

The acetylenes of types **9**–**12** have been isolated for the first time from a member of the tribe Inuleae. Though several acetylenic sulfoxides were reported previously [7], no derivatives of **10** were known.

EXPERIMENTAL

The air-dried aerial parts (250 g), collected north of Delhi (voucher deposited in Dehradun, Botanical Survey of India), was extracted with Et_2O–petrol (1:2) and the extract was separated by CC (Si gel) and further by repeated TLC (Si gel). The less polar fractions gave 20 mg *β*-farnesene, 7 mg geranyl linalol, 5 mg **7** (Et_2O–petrol, 1:3), 15 mg **8**, 17 mg **9** and 7 mg **10**. The polar parts (Et_2O and Et_2O–MeOH, 20:1) afforded 20 mg **1**, 6 mg **2** and **3**, 5 mg **4** and 7 mg **5a**, which were separated by HPLC (reversed phase, MeOH–H_2O, 3:2). Furthermore, 2 mg **11** and **12** (Et_2O–MeOH, 50:1) were isolated as a mixture, which could not be separated.

Incaspitolide A (**1**). Colourless crystals, mp 158–159°; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (OH), 1785 (lactone), 1745 (CO_2R), 1720 (C=O); MS *m/z* (rel. int.): 351.181 [M − OCOR]$^+$ (2) ($C_{19}H_{27}O_6$), 263 [351 − RCO_2H]$^+$ (5), 245 [263 − H_2O]$^+$ (3), 71 [C_5H_7CO]$^+$ (100); CI (isobutane): 439 [M + 1]$^+$ (8), 421 [439 − H_2O]$^+$ (100).

$$[\alpha]_{24}^{\lambda} = \underset{+42}{589} \quad \underset{+51}{578} \quad \underset{+60}{546} \quad \underset{+158}{436\,nm} \quad (CHCl_3;\ c\ 0.2).$$

Incaspitolide B and C (**2** *and* **3**). Colourless crystals, mp 147–149°; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3580 (OH), 1780 (lactone), 1750 (CO_2R), 1720 (C=O); MS *m/z* (rel. int.): 452 [M]$^+$ (0.1), 351.181 [M − RCO_2]$^+$ (2) ($C_{19}H_{27}O_6$), 263 [351 − RCO_2H]$^+$ (7), 245 [263 − H_2O]$^+$ (4), 85 [C_4H_9CO]$^+$ (64), 71 [C_3H_7CO]$^+$ (100).

$$[\alpha]_{24}^{\lambda} = \underset{+38}{589} \quad \underset{+40}{578} \quad \underset{+49}{546} \quad \underset{+133}{436\,nm} \quad (CHCl_3;\ c\ 1.2).$$

Incaspitolide D (**4**). Colourless crystals, mp 229–231°; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3560 (OH), 1770 (lactone), 1740 (CO_2R), 1720 (C=O); MS *m/z* (rel. int.): 454 [M]$^+$ (0.1), 366.168 [M − RCO_2H] (0.2) ($C_{19}H_{26}O_7$), 278 [366 − RCO_2H]$^+$ (1), 260, [278 − H_2O]$^+$ (1), 71 [C_3H_7CO]$^+$ (100).

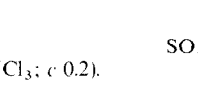
$$[\alpha]_{24}^{\lambda} = \underset{-95}{589} \quad \underset{-99}{578} \quad \underset{-116}{546} \quad \underset{-226}{436\,nm} \quad (CHCl_3;\ c\ 0.3).$$

Incaspitolide E (**5a**). Colourless gum; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (OH), 1775 (lactone), 1740 (CO_2R); MS *m/z* (rel. int.): 454 [M]$^+$ (0.1), 436 [M − H_2O]$^+$ (0.2), 366.168 [M − RCO_2H]$^+$ (0.5) ($C_{19}H_{26}O_7$), 278 [366 − RCO_2H]$^+$ (1), 71 [C_3H_7CO]$^+$ (100).

$$[\alpha]_{24}^{\lambda} = \underset{-5.1}{589} \quad \underset{-5.4}{578} \quad \underset{-6.2}{546} \quad \underset{-11.4}{436\,nm} \quad (CHCl_3;\ c\ 0.7).$$

To 3 mg **5a** in 0.5 ml $CHCl_3$ 5 mg 4-pyrrolidonopyridine [8] and 0.1 ml Ac_2O were added at room temp. After 15 min usual work-up and TLC (CH_2Cl_2–C_6H_6–Et_2O, 8:8:1) afforded 2 mg **5c** {MS(CI, isobutane) *m/z* (rel. int.): 497 [M + 1]$^+$ (14) ($C_{25}H_{37}O_{10}$), 497 [479 − H_2O]$^+$ (100)} and 1 mg **5d** {MS (CI, isobutane) *m/z* (rel. int.): 539 [M + 1]$^+$ (8) ($C_{27}H_{39}O_{11}$), 479 [539 − AcOH]$^+$ (100), 451 [M − RCO_2H]$^+$ (22), 419 [479 − AcOH]$^+$ (8)}.

1β-Hydroxy-1,10-dihydro-10,14-dehydrogermacrene D (**7**). Colourless oil; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600 (OH), 3080, 1655, 1610 (C=C); MS *m/z* (rel. int.): 220.183 [M]$^+$ (4) ($C_{15}H_{24}O$), 202 [M − H_2O]$^+$ (8), 159 [202 − C_3H_7]$^+$ (20), 55 [C_4H_7]$^+$ (100); ^{1}H NMR ($CDCl_3$): 3.76 *dd* (H-1), 2.04 *m* (H-2), 2.42 *ddd* and 2.18 *ddd* (H-3), 5.99 *br d* (H-5), 5.42 *dd* (H-6), 0.88 *d* (H-12), 0.80 *d* (H-13), 5.26 *br s* and 4.98 *br s* (H-14), 4.90 *br d* and 4.81 *br s* (H-15) [*J*(Hz): 1,2 = 12; 1,2′ = 4; 2,3 = 3,3′ = 12; 2,3′ = 4.5; 2′,3 = 5; 2′,3′ = 3; 5,6 = 16; 6,7 = 10; 11,12 = 11,13 = 7].

Sulfoxides **11** *and* **12**. Colourless gum, UV $\lambda_{max}^{Et_2O}$ nm 284, 300, 320; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2220 (C≡C), 1740 (OAc) 950 (CH=CH); MS *m/z* (rel. int.): 320.108 [M]$^+$ (8) ($C_{17}H_{20}O_4S$), 305 [M − Me]$^+$ (1), 245 [305 − AcOH]$^+$ (3), 197 [M − AcOH, SOMe]$^+$ (20), 143  (32), 71 (100).

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