Pergamon

PII: S0031-9422(97)00464-0

Phytochemistry, Vol. 46, No. 8, pp. 1369–1373, 1997

Printed in Great Britain
0031-9422/97 $17.00+0.00

SESQUITERPENE LACTONES AND FLAVANONES IN *SCALESIA* SPECIES

OTMAR SPRING,* NORBERT HEIL and BERNHARD VOGLER†

Universität Hohenheim, Institut für Botanik; † Institut für Chemie, Garbenstr. 30, 70599 Stuttgart, Germany

(*Received* 3 *March* 1997)

Key Word Index—*Scalesia* spp; Heliantheae; Asteraceae; sesquiterpene lactones; flavanones; chemotaxonomy.

Abstract—Trichome microsampling and HPLC analysis revealed the presence of sesquiterpene lactones and flavonoids in capitate glandular trichomes from the leaf surface of various species of *Scalesia*. The paucity in plant material of these plants, endemic to the Galapagos Islands, necessitated the use of micropreparative techniques including LC-NMR and LC-MS for structure elucidation. Leaf extracts of *Scalesia baurii* ssp. *hopkinsii*, *S. stewartii* and a combined sample of several species of the genus afforded two eudesmanolides, four guaianolides, four xanthanolides and three flavanones. This is the first report on the terpenoid chemistry of *Scalesia* and the chemotaxonomic aspects are discussed. © 1997 Elsevier Science Ltd

INTRODUCTION

The genus *Scalesia* Arn., as recently delineated by Eliasson [1], comprises 18 taxa in 14 species. It is the largest of four Compositae genera of the Galapagos Islands and consists of woody shrubs and trees with a chromosome number of 4x = 68. Some of its members are endemic to single islands of the archipelago and are regarded as extremely endangered. No phytochemical data of *Scalesia* have so far appeared in the literature. In continuation of our chemotaxonomical studies of genera of the Helianthinae (cf. [2]), we were interested in investigating representatives of the genus for comparison of its relationship with other taxa of the subtribe. Since the accessibility of plant material from the Galapagos Islands is very difficult, we were dependent on herbarium specimens of the University of Goeteborg, collected by U. Eliasson for his monograph on *Scalesia* [1]. Two leaves of each specimen belonging to 15 different taxa were provided for chemical analysis. By means of a recently described microsampling technique for the detection of sesquiterpene lactones in epidermal trichomes [3], we screened the plant material and selected *S. baurii* ssp. *hopkinsii* (Rob.) Eliasson and *S. stewartii* Riley as the most promising representatives for preparative chemical investigation. The airdried leaf tissue of both species consisted of only 257 and 791 mg, respectively. A third sample was prepared from tissue of the remaining taxa (4.28 g) in order to obtain spectroscopic information of minor compounds, not present in sufficient quantities in a single plant.

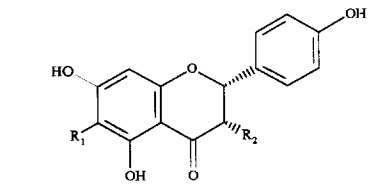
RESULTS AND DISCUSSION

The extract of *S. baurii* ssp. *hopkinsii* afforded the flavanone naringenin (**1**) as the major compound. Its structure was identified by comparison of its spectral data with those given in the literature [4]. From a slightly more polar fraction a second flavanone (**2**) was isolated. Its ^{1}H NMR spectrum differed from that of naringenin in the presence of a methoxy signal at δ 3.86 and the loss of one proton of the A-ring. The ^{1}H NMR and mass spectroscopic data ($[M]^+ = m/z$ 302 for $C_{16}H_{14}O_6$) were identical with those of 6-methoxynaringenin [5]. From the less polar fractions of the HPLC separation, two sesquiterpene lactones of the xanthanolide-type were isolated. The minor compound (**5**) was identified as tomentosin, a constituent that we had recently found in species of the South American genus *Pappobolus* [6]. The major sesquiterpene lactone (**6**) was identical in all spectroscopic data with 4-dihydrotomentosin [7].

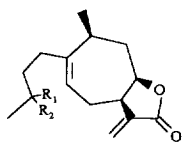
In the extract of *S. stewartii*, tomentosin (**5**) was the major compound, while 4-dihydrotomentosin (**6**) was present in trace amounts only. Several other fractions afforded sesquiterpene lactones and a flavonoid of unknown nature, but in amounts of less than 0.1 mg, thus precluding further structure elucidation.

For that reason and in spite of the shortage in plant material of the other species in the genus, we decided to combine the remaining leaf samples of all specimens

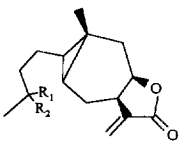
* Author to whom correspondence should be addressed.



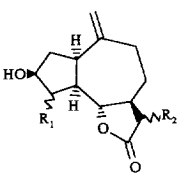
	R_1	R_2
1	H	H
2	OMe	H
3	OMe	OMe
4[8]	OMe	βOH



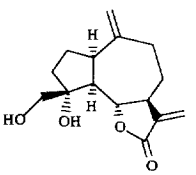
	R_1	R_2
5	=O	
6	H	OH



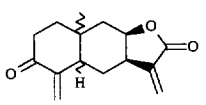
	R_1	R_2
7	=O	
8	H	OH



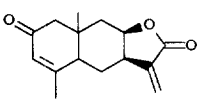
	R_1	R_2
9	$=CH_2$	$=CH_2$
10	CH_2OH	$=CH_2$
11	CH_2OH	CH_3



12



13



14

for extraction. This extract was supposed to provide chemical information on minor compounds for a subsequent indirect peak assignment [cf. 3] of HPLC elution diagrams of the taxa (Spring *et al.* in preparation).

LC-^{1}H NMR measurements of the crude extract revealed the presence of the flavanones **1** and **2** as major compounds accompanied by an additional flavonoid, **3**, and several sesquiterpene lactones in minor amounts. Compound **3** which showed peak overlapping with **2** was separated by repeated HPLC. The ^{1}H NMR signals of the A- and B-ring were unchanged when compared with 6-methoxynaringenin. Two protons, however, at δ 6.09 (*d*, $J = 3.3$ Hz, H-3) and δ 5.42 (*d*, H-2) and an additional methoxy signal at δ 3.49 (*s*) indicated the presence of a flavonol methoxylated in position 3. This was confirmed by mass spectroscopic measurements which showed $[M]^+$ at *m/z* 332 for $C_{17}H_{16}O_7$, followed by the loss of a fragment with *m/z* 150 ($C_9H_{10}O_2$). The small amount of the compound did not allow additional experiments to establish the absolute configuration. However, comparison with data published for (2*R*, 3*R*)-2,3-dihydro-5,7,4′-trihydroxy-6-methoxyflavonol (**4**), isolated from *Hymenoxis turneri* [8], confirmed a different stereochemistry at C-2 and C-3 as indicated by the low coupling constant of H-2 and H-3 ($J = 3.3$ Hz instead of 12 Hz [9]). The absolute stereochemistry of the two optically active carbons could not be determined due to the small amount of the compound.

The remaining HPLC fractions afforded sesquiterpene lactones of the xanthanolide, guaianolide and eudesmanolide type. Besides **5** and **6**, two additional xanthanolides, carabrone (**7**) and 4-dihydrocarabrone (**8**), were identified by comparison of their ^{1}H NMR and mass spectral data with those of authentic samples from previous investigations [6]. Fractions between 8 and 13 min (50% MeOH, flow rate of 1 ml min^{-1}) afforded the known guaianolide **9** (zaluzanin C) and its derivatives **10**–**12**. Compound **10** and **11** were obtained as an inseparable mixture of two very similar structures which showed signal overlap for most protons, except for H-13a,b. Peak integration revealed a ratio of 2:1. The ^{1}H NMR spectrum of **10** differed from **9** in the lack of the third pair of methylene protons. Instead, a pair of protons at δ 3.77 and 3.55 with a geminal coupling constant of $J = 11.1$ Hz indicated the presence of a CH_2OH. The position of this function in C-4 was concluded from the complex signal of H-5 and the upfield shift of H-3, while the pattern of H-9a,b was unchanged and indicated a quaternary carbon at C-10. The absolute configuration at C-4 could not be deduced, due to signal overlap of H-15 with H-3. The proposed structure was in agreement with the mass spectrum which showed $[M]^+ = m/z$ 264 for $C_{15}H_{20}O_4$ and a base peak of *m/z* 233 for $[M\text{-}CH_2OH]^+$. In compound **11**, the exocyclic methylene function of the lactone ring, present in the previous compound, was reduced to a methyl group which gave a doublet at δ 1.44 ($J = 7$ Hz, H-13) with three proton intensity and showed coupling with a multiplet at δ 2.21 (H-11). This coincided with the mass spectrum which showed $[M]^+ = m/z$ 266 for $C_{15}H_{22}O_4$. An attempt was made to separate the mixture of compound **10** and **11** by adding an excess of L-cysteine in order to form an adduct with the exocyclic methylene function of **10**. In LC-MS measurements (APCI+Q1MS; gradient 30–100% MeOH in 30 min) this resulted in a strong decrease of the UV-absorption of the expected sample peak and the loss of the mass spectral signals previously belonging to compound **10** whereas those of **11** remained unaffected. Furthermore, a peak appeared at higher polarity which showed fragment patterns of the adduct of **10** with L-cysteine. Compound **12** had a $[M]^+ = m/z$ 264 for $C_{15}H_{20}O_4$. Its ^{1}H NMR spectrum revealed a close relationship with the previous guaianolide **10**, except for the secondary hydroxyl function present in the latter compound. Instead, the coupling pattern of H-5 in compound **12** indicated only two neighbouring protons (H-6 and H-1) and a quaternary carbon at C-4. COSY experiments showed vicinal coupling of the protons H-2a/b with a pair of protons at C-3. Therefore, C-4 had to be substituted with a ternary hydroxyl group. Due to the downfield shift of H-1 in comparison to **9** and **10** and with respect to the downfield shift of H-5 when

compared to **10**, we assume that the 4-hydroxyl function should be in the α-position.

From the polar parts of the chromatogram two eudesmanolides, **13** and **14** were isolated. The presence of a 7,8-*cis*-lactone was shown by the coupling pattern of H-8. For **13**, mass spectroscopic measurements showed $[M]^+ = m/z$ 246 for $C_{15}H_{18}O_3$. The olefinic part of the ^1H NMR spectrum consisted of two pairs of exocyclic methylene protons, H-13a,b and H-15a,b. An additional proton at δ 4.57 accounted for H-8 and revealed coupling with a geminal pair of protons at δ 2.91 (*dd*, H-9a) and 2.46 (*dd*, H-9b) according to COSY experiments. Multiplicity of H-9a,b indicated a quaternary carbon at C-10. The position of H-6a at δ 1.94 and H-6b at δ 1.44 was deduced from the coupling with H-7 (δ 3.03). The remaining signals at δ 2.30, 2.10, 1.97 and 1.88 showed coupling to each other as expected for two vicinal CH_2 groups. This left only two possibilities, either at C-1 or C-3, for the carbonyl function which was required according to the mass spectrum. The strong downfield shift of H-15a,b favoured the conjugated position of the carbonyl and suggested a close structural relationship with 3-oxoisoalantolactone [9](= telekin, iso dehydro [10]). However, significant differences appeared in the shift values of H-14 (δ 1.34), H-15a (δ 5.40) and H-9a/b (δ 2.91 and 2.46) in comparison with the data given in the literature. This is most probably due to stereochemical differences, but the absolute configuration of the molecule could not be elucidated during this study.

The ^1H NMR COSY experiments on the eudesmanolide **14** established the sequences H-13a,b to H-9a,b and H-7 to H-5 (δ 2.75). Of the remaining signals, two methyl groups were assigned at δ 1.36 (H-14) and 1.57 (H-15), and an olefinic proton at δ 5.85 showed long range coupling with a pair of protons at δ 2.41 and 2.46. Together with the mass spectroscopic data $[M]^+ = m/z$ 246 for $C_{15}H_{18}O_3$) the results indicated structural identity with pinnatifidin [11].

So far, from the taxonomical point of view, there was general agreement that *Scalesia* belongs in the subtribe Helianthinae [1]. This was based mainly upon morphological and caryological characters (basic chromosome number of x = 17). A recent study on cpDNA of *S. pedunculata* [12] placed this species near *Pappobolus*, a genus endemic to the Andes of Colombia, Ecuador, and Peru. This would make sense for geographical reasons, but the chemical data do not support a direct relationship. *Pappobolus* is entirely dominated by heliangolides and xanthanolides [6, 13] while *Scalesia* contains xanthanolides in combination with guaianolides and eudesmanolides. Since *Scalesia* is a tetraploid genus, it may be speculated that interspecific hybridization contributed to its chemical constitution which so far is unique in the subtribe.

EXPERIMENTAL

Plant material. All samples of *Scalesia* used in this study were collected by U. Eliasson during a field trip in the Galapagos and vouchers are deposited at the University of Göteborg.

Extraction and structure elucidation. Screening for sesquiterpene lactones was performed in the usual manner [3] using extracts of glandular trichomes for HPLC analysis (Hypersil ODS, 5 μm; 4 × 250 mm; 50% MeOH or 30% MeCN alternatively used as solvents; UV detection simultaneously at 225 and 265 nm; dimethylphenol as int. standard). For structure elucidation the plant material was extracted with CH_2Cl_2 and compounds were sepd on HPLC as given above.

LC-^1H NMR measurements were carried out as onflow spectra on a Varian unity *inova*; 500 MHz, detector type 9050 equipped with a NMR flow cell of 60 μl detectable vol. LC-MS experiments were performed on a Finnigan TSQ 700 under atmosphere pres. chemical ionization (APCI). For testing the Michael addition of compound **10** with nucleophiles, a portion of the mixt. of compound **10** and **11** was mixed with L-cysteine (5 μl, 0.1 M in H_2O) and incubated for 30 min prior to injection into the LC-MS system.

Table 1. ^1H NMR spectral data of compounds **2**–**3** (500 MHz, $CDCl_3$) and compound **4**

H	**2**	**3**	**4** [8]*
2β	5.41 *dd*	6.09 *d*† (3.3)	4.92 *d* (12)
3α	3.08 *dd* (12.7, 17.1)	5.42 *d*† (3.3)	4.12 *d*
3β	2.83 *dd* (3.1, 17.1)		
8	6.14 *s*	6.14 *s*	6.07 *s*
2′	7.35 *d* (8.6)	7.35 *d* (8.6)	7.30 *br d* (8)
3′	6.90 *d*	6.90 *d*	6.78 *br d*
5′	6.90 *d* (8.6)	6.90 *d* (8.6)	6.78 *br d* (8)
6′	7.35 *d*	7.35 *d*	7.30 *br d*
C6-OMe	3.86 *s*	3.86 *s*	3.67 *s*
C3-OMe		3.49 *s*	

* data from ref. [8] measured in CCl_4.

† signals interchangeable.

Table 2. ^{1}H NMR spectral data of compounds **10**–**14** (500 MHz, $CDCl_3$)

H	10	11	12	13	14
1a	2.90 *m*	3.15 *ddd*	3.22 *ddd*	1.97 *m*§	2.41
1b				1.88 *m*§	2.46
2a	~2.0	†	1.95 *m*	2.10 *m*§	
2b	~2.0	†	1.80 *m*	2.30 *m*§	
3a	3.80 *m*	3.65 *m*	2.10 *m*		5.58 *bs*
3b			1.76		
4	~2.0	†			
5	2.23 *m*	1.95 *m*	2.41 *dd*	2.24 *m*	2.75 *bd*
6*a*	4.23 *dd*	4.03 *dd*	4.05 *dd*	1.95 *m*	2.07 *m*
6b				1.44 *m*	1.40 *m*
7	2.80 *m*	†	2.79 *m*	3.03 *m*	3.39 *m*
8a	2.28 *m*	†	2.25 *dq*	4.57 *ddd*	5.14 *ddd*
8b	1.43 *m*	†	1.47 *m*		
9a	2.63 *m*	2.58 *m*	2.57 *ddd*	2.91 *dd*	2.35 *dd*
9b	1.95 *m*		2.10 *m*	2.46 *dd*	1.80 *dd*
11		~2.21			
13a	6.23 *d*	1.44 *d*‡	6.26 *d*	6.26 *d*	6.28 *d*
13b	5.53 *d*		5.55 *d*	5.66 *d*	5.60 *d*
14a	5.07 *br s*	5.00 *br s*	5.05 *bs*	1.34 *s*‡	1.36 *s*
14b	4.97 *br s*	5.00 *br s*	4.95 *bs*		
15a	3.77 *br d*	†	3.82 *dd*	5.40 *bs*	1.52 *s*
15b	3.55 *br d*	†	3.57 *dd*	5.12 *bs*	

J (Hz): **10**: 5,6 = 10.8; 6,7 = 9.3; 7,13a = 3.4; 7,13b = 3.2; 9a,b = 12.8; 15a,b = 11.1; **12**: 5,1 = 11.1; 5,6 = 11.3; 6,7 = 9.0; 7,13a = 3.4; 7,13b = 3.1; 15a,4 = 15b,4 = 1; 15a,b = 9.8; **13**: 7,13a = 1.8; 7,13b = 1.6; 8,9a = 4.8; 8,9b = 8.2; 9a,b = 13.4; **14**: 1a,b = 14.2; 6a,b = 13.7; 7,8 = 8.1; 8,9a = 4.1; 8,9b = 11.8; 9a,b = 13.9.

~ signal overlapped.

† signal obscured.

‡ three proton intensity.

§ signal interchangeable.

6-*Methoxynaringenin* (**2**). $C_{16}H_{14}O_6$, EIMS 70 *m/z* (rel. int.): 302 $[M]^+$ (18), 287 $[M-CH_3]^+$ (15), 182 $[M-120]^+$ (100), 167 $[182-CH_3]^+$ (80); UV λ_{max}^{MeOH} nm: 291, 330 sh.

2,3-*Dihydro*-5,7,4′-*trihydroxy*-3,6-*dimethoxy-flavonol* (**3**). $C_{17}H_{16}O_7$, EIMS 70 *m/z* (rel. int.): 332 $[M]^+$ (18), 302 $[M-OCH_3]^+$ (45), 182 $[M-150]^+$ (100), 167 $[182-CH_3]^+$ (75).

15-*Hydroxyzaluzanin C* (**10**). $C_{15}H_{20}O_4$, EIMS 70 *m/z* (rel. int.): 264 $[M]^+$ (4), 246 $[M-H_2O]^+$ (5), 233 $[M-CH_2OH]^+$ (100), 215 $[233-H_2O]^+$ (30); APCI+Q1MS; grad. 30–100% MeOH in 30 min (rel. int.): 297 $[M+H+MeOH]^+$ (100); 265 $[M+H]^+$ (3); 247 $[265-H_2O]^+$ (5).

15-*Hydroxy*-11,13-*dihydro-zaluzanin C* (**11**). $C_{15}H_{22}O_4$, EIMS 70 *m/z* (rel. int.): 266 $[M]^+$ (2), 248 $[M-H_2O]^+$ (6), 235 $[M-CH_2OH]^+$ (100), 217 $[235-H_2O]^+$ (40); APCI+Q1MS; grad. 30–100% MeOH in 30 min (rel. int.): 299 $[M+H+MeOH]^-$ (100); 267 $[M+H]^+$ (4); 249 $[267-H_2O]^+$ (8).

Scalesin (**12**). $C_{15}H_{20}O_4$, APCI+Q1MS; grad. 40–100% MeOH in 40 min (rel. int.): 297 $[M+H+MeOH]^+$ (12); 279 $[297-H_2O]^+$ (30), 265 $[M+H]^+$ (10); 247 $[265-H_2O]^+$ (100); 229 $[247-H_2O]^+$ (90).

3-*oxo-isoalantolactone* (**13**). $C_{15}H_{18}O_3$, APCI+Q1MS, 50% MeOH isocrat. (rel. int.): 279 $[M+H+MeOH]^+$ (25), 247 $[M+H]^+$ (20); 229 $[247-H_2O]^+$ (100).

Pinnatifidin (**14**). $C_{15}H_{18}O_3$, APCI+Q1MS, 50% MeOH isocrat. (rel. int.): 279 $[M+H+MeOH]^+$ (15), 247 $[M+H]^+$ (40); 229 $[247-H_2O]^+$ (100).

Acknowledgements—We wish to thank Mrs Klaiber and Mr Walter, Universität Hohenheim, Institut für Chemie, for MS measurements and Dr W. Hiller, Varian, Darmstadt, for LC-^{1}H NMR measurements. We are especially grateful to Dr. U. Eliasson, University of Goteborg, for providing leaf material from his herborium specimens.

REFERENCES

1. Eliasson, U., *Opera Botanica*, 1974, **36**, 1.
2. Spring, O. and Buschmann, H., in *Compositae: Systematics. Proceedings of the International Compositae Conference, Kew* 1994, Vol. 1, ed. D. J. N. Hind and H. J. Beentje. Royal Botanical Gardens, Kew, 1996, p. 307.
3. Spring, O., in *Modern Phytochemical Methods*, ed.

N. H. Fischer, M. B. Isman and A. H. Stafford. Plenum Press, New York, 1991, p. 319.
4. Balza, F. and Towers, G. H. N., *Phytochemistry*, 1984, **23**, 2333.
5. Wollenweber, E., Mann, K., Arriaga-Giner, F. J., Roitman, J. N. and West, J. G., *Phytochemistry*, 1993, **33**, 871.
6. Spring, O., Vargas, D. and Fischer, N. H., *Phytochemistry*, 1991, **30**, 1861.
7. Bohlmann, F., Mahanta, P. K., Jakupovic, J., Rastogi, R. C. and Natu, A. A., *Phytochemistry*, 1978, **17**, 1165.
8. Gao, F., Wang, H., Mabry, T. J., Kinghorn, A. and Douglas, A., *Phytochemistry*, 1990, **29**, 2865.
9. Herz, W., Subramaniam, P. S. and Geissman, T. A., *Journal of Organic Chemistry*, 1968, **33**, 3743.
10. Herz, W. and Kumar, N., *Phytochemistry*, 1979, **18**, 1743.
11. Herz, W., Mitra, R. B., Rabindran, K. and Viswanathan, N., *Journal of Organic Chemistry*, 1962, **27**, 4041.
12. Schilling, E. E., Panero, J. L. and Eliasson, U. H., *American Journal of Botany*, 1994, **81**, 248.
13. Spring, O., Panero, J. L. and Schilling, E. E., *Biochemical Systematics and Ecology*, 1992, **20**, 671.