Phytochemistry, Vol. 21, No. 10, pp. 2475–2479, 1982.
Printed in Great Britain.

0031-9422/82/102475-05$03.00/0

SESQUITERPENE LACTONES OF *EUPATORIUM SCABRIDUM**

WERNER HERZ and PALIANAPPAN KULANTHAIVEL
Department of Chemistry, The Florida State University, Tallahassee, FL 32306, U.S.A.

(*Received* 18 *February* 1982)

Key Word Index—*Eupatorium scabridum*, Compositae; Eupatorieae; sesquiterpene lactones; guaianolides; germacradienolides.

Abstract—*Eupatorium scabridum*, a hybrid biotype presumed to arise by hybridization of *E. rotundifolium* and *E. semiserratum* furnished anti-tumor sesquiterpene lactones characteristic of the putative parents.

INTRODUCTION

As part of our continuing study[1, 2] of *Eupatorium* species *sensu stricto* which elaborate sesquiterpene lactones with cytotoxic and anti-tumor activity, we have examined the hybrid derivative biotype *E. scabridum* Ell. This taxon, a predominantly triploid member of what has sometimes been called the *E. rotundifolium* complex, is found in the coastal plain of northern Florida, southern Georgia, south Carolina to Louisiana and north into Arkansas and reflects hybridization between *E. rotundifolium* L. and *E. semiserratum* DC.[3]. Our material came from Arkansas where relatively large populations occur.

RESULTS AND DISCUSSION

Eight previously known sesquiterpene lactones, as well as β-amyrin acetate and plant sterol glucosides, were isolated from *E. scabridum* and were identified spectroscopically (^{1}H NMR, mass spectra) or by comparison with known samples. The major lactone constituents were eupatundin (**1a**) [4, 5] and eupaserrin (**2a**) [6, 7]; smaller amounts of euparotin (**3a**) [6, 8, 9], eupatoroxin (**4**)[4, 5], eupachloroxin (**5**)[4, 5], deacetyleupaserrin (**2b**)[6, 7, 10], lactone **2c**[7] and lactone **6b** were also isolated.† Lactone **6b** is the angelate analog of a substance (**6a**) from *E. mohrii*[11] and is identical with a lactone isolated from '*E. rotundifolium* ssp. *ovatum*'‡ which was originally[12] assumed to have an α-orientated 9-hydroxyl group; we concur[11] with the recent revision[13] of its stereochemistry to **6b** because of the observed value of $J_{8,9}$(2 Hz). 270 MHz ^{1}H and ^{13}C NMR spectra of those compounds for which such data are not available in the literature are listed in Tables 1 and 2. Oxidation with Jones' reagent converted **1a** to **7** (dehydroeupatundin)[6]; oxidation of **1a** with pyridinium dichromate, pyridinium chlorochromate or Collins reagent was accompanied by double bond migration to **8a** (dehydroiseupatundin) whose spectral properties were similar to those of the recently reported guevariolide **8b**[14].

E. scabridum contains sesquiterpene lactones found in *E. rotundifolium* and in *E. semiserratum* separately; thus the phytochemistry reflects the putative parentage of this hybrid biotype[3]. *E. rotundifolium* characteristically elaborates 1, 5-dihydroxy-8-acyloxy-3, 4, 10, 14-guaidienolides, their epoxidation products or slight modifications thereof; thus *E. rotundifolium* from Florida gave[4, 6] **1a**, **3a**, **3b**, **4a**, **5**, **9a**, **9b** and **10a** and a collection from Guatemala§ furnished **10b** and **10c** in addition to unspecified 'previously isolated lactones from this species', but no germacradienolides[15]. On the other hand *E. semiserratum* contains only the germacradienolides **2a** and **2b**[6, 10] which we have now also found in *E. scabridum*. Lactone **2c** has been isolated previously from *E. mikanioides*[7].

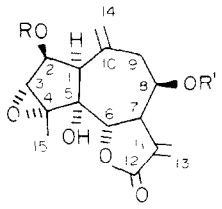
As expected the flavones to which we have ascribed responsibility for the characteristic yellow secretion which is observed when stems of *E. semiserratum* are broken[10] were not present in the extract of our *E. scabridum* which lacked this property.

*Supported in part by a grant from the U.S. Public Health Service (CA-13121) through the National Cancer Institute.

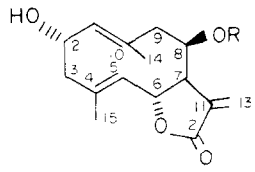
†Lactones **1a**, **2a**, **2b**, **3a**, **4** and **5** were reported to be cytotoxic [5, 6]. Only **2a**, **2b** and **3** were tested *in vivo*; **2a**, **2b** exhibited activity against Walker 256 carcinoma[6] and **3a** exhibited activity against P-388 leukemia in the mouse[5]. Previously untested compounds obtained in the present work have been submitted to the anti-cancer screen of the Division of Cancer Treatment, National Cancer Institute.

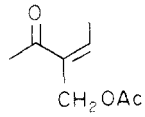
‡Collections of so-called *E. rotundifolium* L. ssp. *ovatum* (Bigel.) Montg. and Fairbr. which gave [12, 16] **1a**, **1b**, **3b**, **4**, **6b**, **11a**, **11b**, **11a**, **11b** and **12** actually were[11] *E. cordigerum* Fernald, a putative hybrid of diploid *E. perfoliatum* L. (pollen parent) and triploid *E. rotundifolium* (female parent)[3].

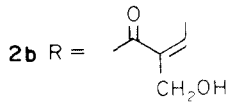
§The source given for this collection is surprising. The range of *E. rotundifolium* does not extend into Central America and the species is not listed, even as an adventive, by Williams, L. O. (1976) *Flora of Guatemala, Eupatorieae, Helenieae, Senecioneae, Fieldiana: Botany*, Vol. 24, Part XII.



1a R = H, R' = Ang
1b R = Ac, R' = Ang

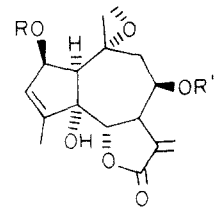


2a R = 

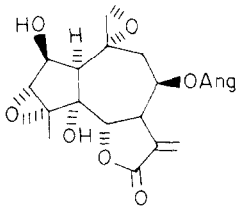


2b R =

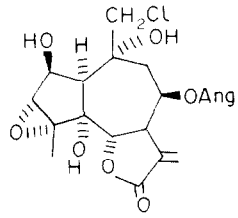
2c R = H



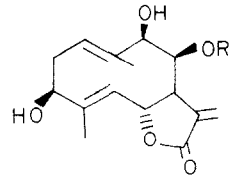
3a R = H, R' = Ang
3b R = Ac, R' = Ang



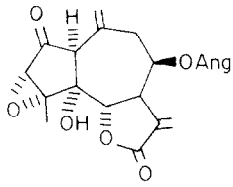
4



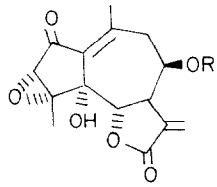
5



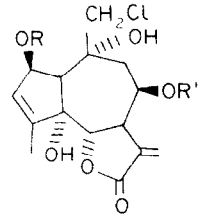
6a R = MeBu
6b R = Ang



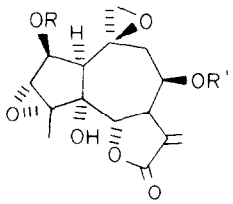
7



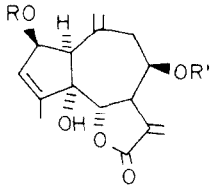
8a R = Ang
8b R = Tigl



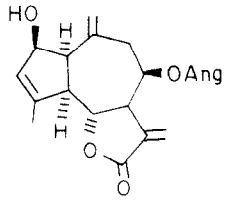
9a R = H, R' = Ang
9b R = Ac, R' = Ang



10a R = H, R' = Ang
10b R = Ac, R' = Ang
10c R = Ac R' = Tigl



11a R = Ac, R' = Ang or reverse
11b R = H, R' = Tigl



12

EXPERIMENTAL

Extractions from E. scabridum. Above ground material of *E. scabridum* Ell. (2.2 kg) collected by Dr. R. K. Godfrey and Mr. D. Gage on 1 September 1979 near route 19 just east of Delight, Arkansas (RKG 77202 on deposit in the Herbarium of Florida State University), was extracted with $CHCl_3$ and worked-up in the usual fashion[17]. The crude gum (115 g) was pre-absorbed on 200 g Si gel and chromatographed over 1 kg Si gel packed in C_6H_6. Fractions were collected as follows: fractions 1–12 (C_6H_6, 6 l.), 13–24 (C_6H_6–$CHCl_3$, 1:1, 6 l.), 25–36 ($CHCl_3$, 6 l), 37–40 ($CHCl_3$–MeOH, 99:1, 2 l), 41–50 ($CHCl_3$–MeOH, 49:1, 5 l), 51–65 ($CHCl_3$–MeOH, 19:1, 7–5 l.), 66–70 ($CHCl_3$–MeOH, 9:1, 2.5 l.) and 71–75 ($CHCl_3$–MeOH, 4:1, 2–5 l.).

Table 1. ^{1}H NMR spectra*

	1a	3a	4	5	6b	7	8a
H-1	2.72*d*(4.5)	~1.9‡	1.55*d*(4.5)	2.42*d*(4)	5.08*br dd* (12, 4)	3.72*br*	—
H-2a	4.42*d*(4.5)	4.76*dd* (2, 6)	4.32*d*(4.5)	4.40*d*(4)	2.49*ddd* (12, 6, 4)	—	—
H-2b	—	—	—	—	2.36*ddd* (12, 12, 10)	—	—
H-3	3.40	5.75*m*	3.38	3.38	4.74*brd*(10)	3.48	3.57
H-5	—	—	—	—	4.30*dd* (10.6)	—	—
H-6	4.91*d*(7.5)	4.82*d*(8.5)	5.00*d*(8)	4.92*d*(8)	5.15*dd* (8, 10)	4.54*d*(8)	4.34*d*(10.5)
H-7	3.79*m* (7.5, 4, 3.5, 3)	4.14*m* (8.5, 4, 3.5, 3)	4.24*m* (8, 4, 3.5, 3)	4.58*m* (8, 4, 3.5, 3)	2.97*m* (8, 3.5, 3)	3.77*m* (8, 4, 3.5, 3)	4.07*m* (10.5, 3.5, 3, 2,)
H-8	5.51*dt* (4, 8)	5.63*dt* (4, 8)	5.55*ddd* (8.5, 7.5, 4)	5.66*dt* (7.5, 4)	5.99*br d* (2)	5.53*dt* (4, 9)	5.7*br dd* (6.5, 2)
H-9a	3.03*dd* (14, 8)	2.72*dd* (15, 8)	2.90*dd* (15, 7.5)	2.49§	4.27*d*(2)	3.11*dd* (14, 9)	3.28*br d*(14)
H-9b	2.93*dd* (14, 8)	2.29*dd* (15, 8)	2.15*dd* (15, 8.5)	—	—	~1.9‡	2.71*dd* (14, 6.5)
H-13a	6.32*d*(3.5)	6.38*d*(3.5)	6.33*d*(3.5)	6.29*d*(3.5)	6.36*d*(3.5)	6.36*d*(3.5)	6.24*d*(3.5)
H-13b	5.54*d*(3)	5.63*d*(3)	5.58*d*(3)	5.52*d*(3)	5.77*d*(3)	5.61*d*(3)	5.51*d*(3)
H-14a	5.09*br*	2.74§ (5)	2.62§ (5)	3.61§ (12)	1.58*br*†	5.29*br*	2.23†
H-14b	5.07*br*						
H-15†	1.68*br*	1.96*br*	1.66*br*	1.66*br*	1.79*d*(1.5)	1.78*br*	1.80*br*
H-3′	6.06*br q*(7.5)	6.04*br q*	6.04*br q*	6.05 *br q*	6.12 br q	6.07*br q*	6.12*br q*
H-4′†	1.92*br d*(7.5)	1.89*br d*	1.91*br d*	1.91*br d*	1.98*br d*	1.89*br d*	1.90*br d*
H-5′†	1.80*br*	1.77*br*	1.80*br d*	1.78*br d*	1.89*br d*	1.77*br*	1.76*br*

*Run at 270 MHz in $COCl_3$. Unmarked signals are singlets. Frequencies in δ values downfield from TMS as internal standard. Coupling constants (in parentheses) in Hertz.

†Intensity of three protons.

‡Obscured signal.

§Intensity of two protons; center of AB system.

Table 2. ^{13}C NMR spectra*

	1a	3a	4	5	6b†	7	8a
1	58.09*d*‡	60.32*d*	57.24*d*	52.96*d*	122.90*d*	61.21*d*§	158.35
2	74.26*d*‡	74.40*d*	71.43*d*	70.16*d*	34.49*t*	204.21	194.48
3	64.32*d*‡	129.10*d*	64.10*d*	62.82*d*	74.81*d*§	62.14*d*§	64.64*d*
4	67.13	149.84	67.31	67.25	143.60	68.35	66.16
5	80.26	84.37	80.80	80.95	127.75*d*	77.70	‖
6	78.51*d*‡	82.51*d*	78.54*d*	79.38*d*	75.02*d*§	77.80*d*	‖
7	41.85*d*	42.20*d*	41.98*d*	41.10*d*	49.19*d*	41.41*d*	47.72*d*
8	68.70*d*‡	66.88*d*	67.81*d*	67.25*d*	78.50*d*	68.05*d*	66.43*d*
9	37.70*t*	36.99*t*	36.64*t*	36.53*t*	76.52*d*	35.91*t*	38.82*t*
10	139.15	55.68	55.17	74.01	137.04	133.22	133.98
11	134.47	134.14	134.63	135.19	136.30	134.56	134.74
12	169.42	169.44	169.53	169.67	168.74	168.85	167.94
13	122.43*t*§	123.27*t*	122.56*t*	121.60*t*	121.35*t*	124.76*t*	120.05*t*
14	121.72*t*§	54.78*t*	55.38*t*	55.57*t*	13.13*g*	123.78*t*	25.30*q*
15	15.83*q*	13.59*q*	15.70*q*	16.18*q*	11.77*q*	15.34*g*	15.82*q*
1′	167.27	166.94	167.13	167.27	165.88	166.89	166.66
2′	127.41	127.28	127.46	127.59	127.18	127.06	126.72
3′	138.69*d*	138.61*d*	138.42*d*	138.22*d*	137.21*d*	139.10*d*	140.10*d*
4′	20.52*q*	20.46*q*	20.54*q*	20.59*q*	20.07*q*	20.45*q*	20.48*q*
5′	15.83*q*	15.77*q*	15.80*q*	15.83*q*	15.30*q*	15.84*q*	15.82*q*

*Run in $CDCl_3$ at 67.9 MHz unless specified otherwise. Values are in δ values. Unmarked signals are singlets.

†Run in DMSO-d_6.

‡Assignments made by selective irradiation.

§Assignments may be interchanged.

‖Signals obscured by solvent signals.

Purification of fraction 6 (1.2g) by Si gel chromatography and recrystallization from a $CHCl_3$–MeOH mixture gave β-amyrin acetate.

Fractions 44–48 on trituration with Et_2O afforded 6.3 g pure eupaserrin (**2a**), identified by mp and NMR.

Fractions 52–59 were rechromatographed over Si gel. The fractions eluted with $CHCl_3$–MeOH, (49:1) predominantly gave a single compound (8 g) whose purification by TLC ($CHCl_3$–MeOH–EtOAc, 8:1:1) afforded pure eupatundin (**1a**). The ^1H and ^{13}C NMR data are listed in Tables 1 and 2. The combined fractions 61 and 62 on trituration with Et_2O gave 1.2 g euparotin (**3a**), identified by mp and NMR. ^1H and ^{13}C NMR data are listed in Tables 1 and 2.

Fractions 64–69 (21 g) were rechromatographed over 250 g Si gel. Fraction 7, eluted with $CHCl_3$–MeOH (19:1), upon trituration with $CHCl_3$–hexane predominantly gave a single compound (2.1 g). Purification by TLC ($CHCl_3$–MeOH–EtOAc, 8:1:1) gave two bands. The major band gave pure eupatoroxin (**4**) (1.25 g); the minor band was repurified by TLC ($CHCl_3$–MeOH–EtOAc, 7:1:2, developed twice) to yield 90 mg gummy **6b**. ^1H and ^{13}C NMR data are listed in Tables 1 and 2. Fractions 8 and 9 mainly showed two spots on TLC (EtOAc–hexane, 7:3). Purification by TLC with the above solvent system permitted isolation of 35 mg **2b** and **4**. Fractions 15–17 were purified by TLC (Et_2O–MeOH, 19:1) to yield crude eupachloroxin (**5**). Purification by TLC twice with the above solvent system yielded 80 mg pure **3**. Once again the major band was found to be **4**.

Purification of fractions 23–26 by TLC yielded two bands. The upper band yielded **4**; the lower band on repurification by TLC ($CHCl_3$–MeOH–EtOAc, 15:2:3) yielded gummy **2c** (60 mg).

Fraction 73 on trituration with MeOH gave 220 mg of a mixture of β-sitosterol and stigmasterol-β-D-glucosides, identified by the ^1H NMR spectrum and MS of the tetraacetates and by the ^1H NMR spectrum and MS of the hydrolysed alcohols.

Oxidations of **2a**. (a) A soln of 100 mg **2a** in 15 ml Me_2CO was stirred with 1 ml Jones' reagent for 30 min at 0°. After addition of MeOH the soln was diluted with H_2O and extracted with Et_2O to yield 80 mg dehydroeupatundin (**7**) [6] as a gum. ^1H and ^{13}C NMR data are listed in Tables 1 and 2.

(b) A soln of 125 mg **2a** in 3 ml CH_2Cl_2 was stirred with 500 mg pyridinium dichromate for 32 hr. Work-up and purification by TLC (C_6H_6–EtOAc, 3:1) yielded 45 mg dehydroisoeupatundin (**8a**) as a gum. It was also obtained by oxidation of **2a** with pyridinium chlorochromate and Collins reagent. [Calc. for $C_{20}H_{22}O_7$: MW, 374.1365. Found: MW (MS), 374.1365.] ^1H and ^{13}C NMR data are listed in Tables 1 and 2.

REFERENCES

1. Herz, W., Govindan, S. V. and Kumar, N. (1981) *Phytochemistry* **20**, 1343.
2. Herz, W. and Kulanthaivel, P. (1982) *Phytochemistry* **21**, 2363.
3. Sullivan, V. I. (1972) Ph.D. dissertation. Florida State University.
4. Kupchan, S. M., Kelsey, J. E., Maruyama, M. and Cassady, J. M. (1968) *Tetrahedron Letters* 3517.
5. Kupchan, S. M., Kelsey, J. E., Maruyama, M., Cassady, J. M., Hemingway, J. C. and Knox, J. R. (1969) *J. Org. Chem.* **34**, 3876.
6. Kupchan, S. M., Fujita, T., Maruyama, M. and Britton, K. W. (1973) *J. Org. Chem.* **38**, 1260.

7. Herz, W., Kumar, N. and J. F. Blount (1980) *J. Org. Chem.* **45**, 489.
8. Kupchan, S. M., Hemingway, J. C., Cassady, J. M., Knox, J. R., McPhail, A. T. and Sim, G. A. (1967) *J. Am. Chem. Soc.* **89**, 465.
9. McPhail, A. T. and Sim, G. A. (1973) *Tetrahedron* **29**, 1751.
10. Herz, W., Govindan, S. V. and Kumar, N. (1981) *Phytochemistry* **20**, 1343.
11. Herz, W., Murari, R. and Govindan, S. V. (1979) *Phytochemistry* **18**, 1337.
12. Bohlmann, F., Mahanta, P. K., Suwita, A., Suwita, A., Natu, A. A., Zdero, C., Dorner, W., Ehlers, D. and Grenz, M. (1977) *Phytochemistry* **16**, 1973.
13. Bohlmann, F., Jakupovic, J., King, R. M. and Robinson, H. (1981) *Phytochemistry* **20**, 1613.
14. Bohlmann, F., Dahr, A. K., King, R. M. and Robinson, H. (1981) *Photochemistry* **20**, 1144.
15. Bohlmann, F., Zitzkowski, P., Suwita, A. and Fiedler, L. (1978) *Phytochemistry* **17**, 2101.
16. Bohlmann, F., Suwita, A., King, R. M. and Robinson, H. (1980) *Phytochemistry* **19**, 1233.
17. Herz, W. and Hogenauer, G. (1962) *J. Org. Chem.* **27**, 905.