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3,4-β-EPOXY-8-DEOXYCUMAMBRIN B, A SESQUITERPENE LACTONE FROM *TANACETUM PARTHENIUM*

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Key Word Index—*Tanacetum parthenium*; Compositae; feverfew; 3,4-β-epoxy-8-deoxycumambrin B; epoxysantamarine; sesquiterpene lactones.

Abstract—From feverfew, *Tanacetum parthenium*, collected in South America, the guaianolide 3,4-epoxy-8-deoxycumambrin B and the eudesmanolide epoxysantamarine were isolated. The structures were derived by means of spectroscopic methods. The relative stereochemistry of 3,4-epoxy-8-deoxycumambrin B was established by chemical conversion and NOESY experiments. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Feverfew, *Tanacetum parthenium*, has been the subject of much interest because of its sesquiterpene constituents [1–8]. Some of the detected sesquiterpene lactones are known to exhibit biological activities like cytotoxiciy, growth regulating, antimicrobial effects and allergic contact dermatitis in Man [9–15]. An exocyclic α-methylene function of the sesquiterpene lactones, which may react with sulphydryl groups of proteins by a Michael addition [9–11], is responsible for these activities. In this paper, a new guaianolide, 3,4-β-epoxy-8-deoxycumambrin B (**1a**), and its structural elucidation, are presented. The C-10 epimer of this lactone (**1b**) was previously described as a constituent of *Stevia grisebachiana* by Sigstad *et al.* [16]. In addition, we report on the eudesmanolide epoxysantamarine (**2**) [3,4-epoxy-1-hydroxy-1(13)-eudesmen-12,6-olide] [17]. Compound **1a** is described for the first time as a natural product, while **2** has not been isolated previously from *T. parthenium*.

RESULTS AND DISCUSSION

In addition to the lactones (**1a**) and epoxysantamarine (**2**), the germacranolide parthenolide (**3**) and the eudesmanolides reynosin (**4**) and santamarine (**5**) were identified as constituents of the flowers. The occurrence of the latter compound is probably specific for the provenance of the plant, which was collected in South America. Former investigations have shown that the occurrence of different sesquiterpene lactones depends on the habitat of the plant [2–4, 6, 7]. A list of known lactones from *T. parthenium* and their habitats is given in Table 1.

The ^{1}H NMR data of **1a** resemble those for guaianolide **1b** from *S. grisebachiana* [16]. Compared with these data the H-6 proton signal is shifted to higher field while the the H-7 proton clearly shifts downfield. We attribute these differences to a change of configuration at C-10 resulting in an α-orientated hydroxyl group. This reversion, deduced from the chemical shifts of the H-6 and H-7 protons was reported previously for 8-deoxycumambrin B (**6**) [18]. Extensive NMR studies, including ^{1}H, ^{13}C NMR, (^{13}C–^{1}H) COSY, (^{1}H–^{1}H) COSY, COLOC and NOESY, confirmed both the structure of **1a** and the reversed configuration at C-10.

The ^{1}H NMR and (^{13}C, ^{1}H) COSY spectra revealed the position of the epoxy group at C-3 and C-4, and allowed us to assign a methyl group at C-4. A W-coupling between H-1 and H-9a is in agreement with the configuration at these positions. The *cis*-configuration of protons H-1 and H-9a was further established by a positive NOE between H-9b and H-6. Significant positive NOEs were also observed between H-5 and H-7, H-5 and H-1 and between H-5 and the methyl group at C-4. With the absence of a NOE between H-5 and H-6 it became clear that H-5, H-1 and H-7 are *cis* to each other. These results obviously indicate a *trans*-lactone and also the orientation of the epoxy group *trans* to the methyl group at C-4 and to H-5 and H-1. The NOESY data, however, give no indication of the configuration at C-10. After acetylation of the tertiary hydroxyl group at C-10 [19] and NOESY experiments (Table 2) on the acetylation product, a positive NOE between H-7 and the acetylmethyl group was observed. Thus, the α-orientation of the hydroxy group at C-10 was confirmed.

The ^{1}H and ^{13}C NMR data for **2** agree with those

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Table 1. Sesquiterpene lactones from different plant origins and authors

Plant origin	Sesquiterpene lactones
U.K. [3] (L, S)	Parthenolide (**3**), Costunolide (**7**)
U.K. [2] (L)	1β-Hydroxy-10,14-dehydro-1,10*H*-parthenolide (**10**), Canin (**13**), Artecanin (**12**), Tanaparthin-α-peroxide (**17**), Tanaparthin-β-peroxide (**16**), *seco*-Tanapartholide B (**19**)
North America [4] (L, S)	Parthenolide (**3**), Artecanin (**12**), Santamarine (**5**), Reynosin (**4**)
U.K. [5] (L)	Parthenolide (**3**), Canin (**13**), Artecanin (**12**), *seco*-Tanapartholide A (**18**), 3β-Hydroxyparthenolide (**3a**)
Germany [1] (S)	Parthenolide (**3**) 8α-Hydroxyestafiatin (**15**), 8α-iso-Butyryloxyestafiatin (**15a**), 8-α-Angeloyloxyestafiatin (**15b**) 3β-Hydroxycostunolide (**7a**), Reynosin (**4**), 3β-Hydroxycostunolide (**3a**), artemorin (**9**), 3β-Hydroxy-anhydroverlotorin (**8**), 1β-Hydroxy-10, 14-dehydro-1,10-H-parthenolide (**10**), Anhydroverlotorin-4α,5β-epoxide (**11**), Canin (**13**), Artecanin (**12**), 10-epi-Canin (**14**), Tanaparthin-β-peroxide (**16**), Tanaparthin-α-peroxide (**17**), *seco*-Tanapartholides A and B (**18**, **19**)
Mexico [6] (L, F)	Santamarine (**5**)
Mexico [7] (no declaration)	Santamarine (**5**), Canin (**13**), Artecanin (**12**), Reynosin (**4**)

F = flowers; L = leaves; S = seeds.

reported for epoxysantamarine [17], except for a difference in the signal of the H-3 proton, a broad singlet. This could be an indication for a β-orientation of the epoxide ring. Further investigations to confirm its structure failed because of the minute amount of sample.

EXPERIMENTAL

NMR spectroscopy. ^{1}H NMR measurements were performed with a Bruker DRX 500 (500 MHz) instrument using TMS as int. standard. The multiplicity of the ^{13}C NMR signals was determined by DEPT experiments.

GC-MS. EI and CI measurements (isobutane) were carried out with a Hewlett-Packard HP 5890 gas chromatograph coupled with a VG Analytical 70-250 S mass spectrometer.

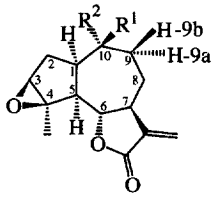
Table 2. Positive NOESY signals of compound **1a** and the acetylation product **1c**

	1a	**1c**
H-1	H-14	H-14
H-2a	H-14	H-14
H-2b	H-6, H-9b	H-6, H-9a, H-14
H-3	H-15	H-15
H-5	H-7, H-15	H-7, H-15
H-6	H-2b, H-9b	H-2b, H-9a,b, H-15
H-7	H-5	H-5, AcMe
H-8a	—	H-14
H-8b	—	—
H-9a	H-14	H-2b, H-6, H-14
H-9b	H-2b, H-6, H-14	H-6
H-14	H-1, H-2a, H-9a,b	H-1, H-2a,b, H-8a, H-9a, Ac-Me
H-15	H-5	H-5, H-6
AcMe	—	H-7, H-14

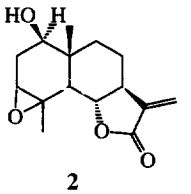
Plant material. Blossoms of *T. parthenium* were collected in South America.

Extraction. Dried blossoms (33 g) were extracted under reflux with $CHCl_3$ for 18 hr. The crude extract was evapd at red. pres. and the residue (3 g) was subjected to CC over silica gel (150 g) using *n*-heptane with a gradient of EtOAc (10–100%). Four frs (1A–4A) were collected and frs 2A–4A were further sepd by silica gel CC. Fr. 1A yielded 80 mg (**3**) after evapn at red. pres. Fr. 2A was subjected to CC over silica gel (60 g, EtOAc/*n*-heptane, 2:1), and afforded 5 frs (1B–5B). Fr. 2B gave 49 mg **4.** Rechromatography (toluene/EtOAc, 2:1) of fr. 1B yielded 14 mg **5**. Fr. 3B after final purification by silica gel CC (toluene/EtOAc acetate 5:2) yielded 16 mg **1a**. Fr. 4B was subjected to silica gel CC (toluene/EtOAc, 1:1) and gave 1 mg of an unidentified lactone. Fr. 3A after further sepn (toluene/EtOAc, 1:1) afforded 1 mg **2**.

3,4-β-*Epoxy*-8-*deoxycumambrin B* (**1a**). ^{1}H NMR (C_6D_6): δ 1.87 (1H, *dddd*, $J_{1,2a}$ = 6.9 Hz; $J_{1,2b}$ = 11.7 Hz; $J_{1,5}$ = 7.4 Hz; $J_{1,9a}$ = 1.6 Hz, H-1), 1.54 (1H, *dd*, $J_{2a,1}$ = 7.7 Hz; $J_{2a,2b}$ = 13.2 Hz, H-2a), 0.80 (1H, *dd*, $J_{2b,1}$ = 11.8 Hz; $J_{2b,2a}$ = 13.3 Hz, H-2b), 2.68 (1H, *br s*, H-3), 2.09 (1H, *dd*, $J_{5,1}$ = 7.3 Hz; $J_{5,6}$ = 11.2 Hz, H-5), 3.00 (1H, *dd*, $J_{6,5}$ = 11.2 Hz; $J_{6,7}$ = 9.2 Hz, H-6), 3.19 (1H, *ddddd*, $J_{7,6}$ = 9.1 Hz; $J_{7,8a}$ = 7.1 Hz; $J_{7,8b}$ = 10.6 Hz; $J_{7,13a}$ = $J_{7,13b}$ = 0.5 Hz, H-7), 0.84 (1H, *dddd*, $J_{8a,7}$ = 6.5 Hz; $J_{8a,8b}$ = 13.5 Hz; $J_{8b,9a}$ = 8.3 Hz; $J_{8b,9b}$ = 10.3 Hz, H-8a), 1.84 (1H, *dddd*, $J_{8b,7}$ = 10.1 Hz; $J_{8b,8a}$ = 13.3 Hz; $J_{8b,9a}$ = 1.6 Hz; $J_{8b,9b}$ = 8.0 Hz, H-8b), 1.16 (1H, *ddt*, $J_{9a,1}$ = $J_{9a,8b}$ = 1,5 Hz; $J_{9a,8a}$ = 8.2 Hz; $J_{9a,9b}$ = 14.3 Hz, H-9a), 1.03 (1H, *ddd*, $J_{9b,8a}$ = 10.9 Hz; $J_{9b,8b}$ = 7.8 Hz; $J_{9b,9a}$ = 14.3 Hz, H-9b), 6.09 (1H, *d*, $J_{13a,13b}$ = 3.6 Hz, H-13a), 4.90 (1H, *d*,



1a R_1 = Me, R_2 = OH
1b R_1 = OH, R_2 = Me
1c R_1 = Me, R_2 = OAc



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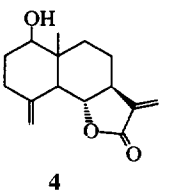

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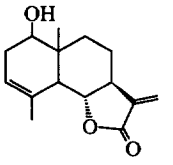

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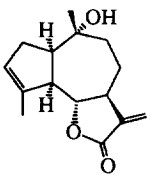

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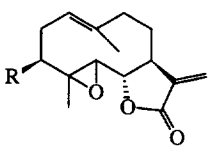
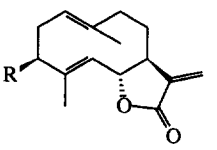
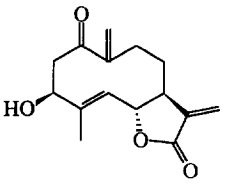


3 R = H
3a R = OH



7 R = H
7a R = OH



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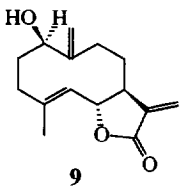

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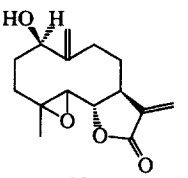

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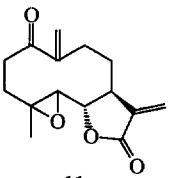

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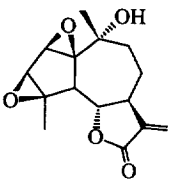

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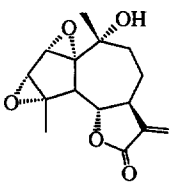

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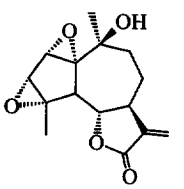

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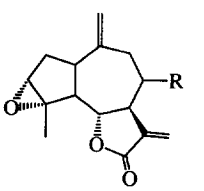

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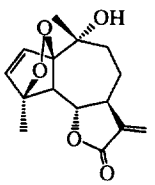

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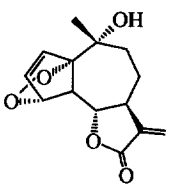

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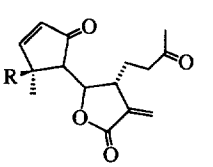

18 R = H
19 R = OH

$J_{13b,13a}$ = 3.3 Hz, H-13b), 0.73 (3H, *s*, H-14), 1.67 (3H, *s*, H-15). ^{13}C NMR (100.6 MHz, C_6D_6): δ 48.59 (*d*, C-1), 31.00 (*t*, C-2), 61.20 (*d*, C-3), 65.30 (*s*, C-4), 49.72 (*d*, C-5), 79.60 (*d*, C-6), 43.35 (*d*, C-7), 24,63 (*t*, C-8), 30.52 (*t*, C-9), 73.15 (*s*, C-10), 169.69 (*s*, C-11), 142.53 (*s*, C-12), 118.33 (*t*, C-13), 33.91 (*q*, C-14), 20.02 (*q*, C-15). MS (CI, *i*-butane), *m/z* (rel. int.): 265 ([M + 1]$^+$, 15), 247 (100), 229 (47), 219 (11), 201 (17). MS (EI), *m/z* (rel. int.): 264 ([M]$^+$, 29), 249 (41), 231 (27), 221 (12), 203 (23), 175 (27), 161 (24), 137 (46), 121 (34), 97 (100), 81 (51), 67 (53). $[\alpha]_D^{22}$ −9 (*c* = 0.86, $CHCl_3$).

3,4-*β-Epoxy*-10-*acetoxy*-8-*deoxycumambrin B* (**1c**). Compound **1** (5 mg, 19 mmol) was dissolved in 100 ml of toluene. *N,N*-Dimethylaminopyridine (1 mg, 8 mmol), 10 ml (94 mmol) Ac_2O, and 25 ml Et_3N were added and the mixt. was stirred for 2 days at room temp. Ac_2O (5 ml, 47 mmol) and 5 ml Et_3N were then added and the soln was stirred for a further 2 days. The reaction mixt. was treated with Et_2O and extracted with dilute HCl. The Et_2O phase was washed with $NaHCO_3$ and H_2O and dried over Na_2SO_4. After removal of solvent the product was purified by CC on silica gel (toluene–Me_2CO, 20:1) yielding 2 mg (34%) crystalline **1c**. $[\alpha]_D^{22}$ −14 (*c* 0.18, $CHCl_3$) ^{1}H-NMR (C_6D_6): δ 2.41 (1H, *m*, H-1), 1.56 (1H, *m*, H-2a), 0.77 (1H, *t*, H-2b), 2.83 (1H, *br s*, H-3), 2.07 (1H, *dd*, H-5), 2.84 (1H, *dd*, H-6), 2.63 (1H, *m*, H-7), 2.17 (1H, *dd*, H-8a), 1.81 (1H, *m*, H-8b), 1.06 (1H, *m*, H-9a), 0.71 (1H, *m*, H-9b), 6.05 (1H, *d*, H-13a), 4.82 (1H, *d*, H-13b), 1.26 (3H, *s*, H-14), 1.66 (3H, *s*, H-15), 1.55 (3H, *s*, Ac- Me). ^{13}C NMR (C_6D_6): δ 46.30 (*d*, C-1), 29.36 (*t*, C-2), 60.24 (*d*, C-3) 64.68 (*s*, C- 4), 49.08 (*d*, C-5), 78.49 (*d*, C-6), 43.44 (*d*, C-7), 24.40 (*t*, C-8), 26.84 (*t*, C-9), 77.60 (*s*, *C*-10), 169.41 (*s*, C-11), 141.62 (*s*, C-12), 118.30 (*t*, C-13), 28.47 (*q*, C-14) 21.70 (*q*, C- 15), 19.67 (*q*, Ac-Me). MS (EI), *m/z* (rel. int.): 263 ([M-CO-Me]$^+$, 1), 246 (46), 231 (54), 218 (11), 203 (24), 175 (23), 161 (20), 135 (31), 105 (36), 95 (100).

Epoxysantamarine (**2**). ^{1}H NMR ($CDCl_3$): δ 3.46 (1*H*, *dd*, H-1), 2.45 (1H, *m*, H-2a), 1.80 (1H, *m*, H-2b), 3.03 (1H, *d*, H-3), 1.91 (1H, *d*, H-5), 3.92 (1H, *t*, H-6), 2.47 (1H, *m*, H-7), 2.10 (1H, *m*, H-8a), 1.59 (1H, *m*, H-8b), 1.93 (1H, *m*, H-9a), 1.26 (1H, *m*, H-9b), 6.11 (1H, *d*, H-13a), 5.43 (1H, *d*, H-13b), 0.93 (3H, *s*, H-14), 1.50 (3H, *s*, H-15). ^{13}C NMR ($CDCl_3$): δ 74.3 (*d*, C-1), 31.6 (*t*, C-2), 61.35 (*d*, C-3), 53.15 (*d*, C-5), 81.2 (*d*, C-6), 51.1 (*d*, C-7), 21.6 (*t*, C-8), 34.8 (*t*, C-9), 139.57 (*s*, C-12), 117.9 (*t*, C-13), 12.34 (*q*, C-14), 22.2 (*q*, C-15). MS (EI), *m/z* (rel. int.): 264 ([M]$^+$, 6), 246 (34), 218 (69), 203 (53), 175 (64), 161 (37), 149 (56), 123 (100), 109 (47). $[\alpha]_D^{22}$ +40 (*c* 0.37, $CHCl_3$).

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