EXPERIMENTAL

The air dried plant material (250 g) (collected in summer 1982 in West Virginia) was extracted for 12 hr at room temp. with 500 ml Et_2O–petrol (1:2) and the extract obtained was separated by CC (SiO_2). The fraction obtained with Et_2O and Et_2O–MeOH, 10:1, was further separated first by TLC (SiO_2 PF 254, C_6H_6–Me_2CO, 4:1) affording two bands (visible under UV light, 255 nm). The less polar band (R_f ~ 0.7) gave on repeated TLC (C_6H_6–Me_2CO, 3:1) a band (R_f ~ 0.7) containing **5**–**7**, 12 mg **2** (R_f ~ 0.65) and 20 mg **1** (R_f ~ 0.60). The mixture of **5**–**7** was separated by HPLC (RP 8, MeOH–H_2O, 7:3, detector: UV and refractometer) affording 3 mg **5** (R_t 5.5 min.), 3 mg **6** (R_t 5.7 min.) and 3 mg **7** (R_t 6.0 min.). **7**: IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1770 (γ-lactone), 1720 (C=CCO_2R); MS m/z (rel. int.): 360.194 $[M]^+$ (1) ($C_{21}H_{28}O_5$), 246 $[M - RCO_2H]^+$ (2), 228 $[246 - H_2O]^+$ (1.5), 97 $[C_5H_9CO]^+$ (100).

The more polar band of the polar CC-fraction (see above, R_f ~ 0.6) gave by HPLC (RP 8, MeOH–H_2O, 3:2, UV detector and refractometer) 5 mg **3** [R_t 4.5 min; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600 (OH), 1775 (γ-lactone), 1740 (OAc); MS m/z (rel. int.): 228.115 $[M - HOAc, H_2O]^+$ (10) ($C_{15}H_{16}O_2$), 55 $[C_4H_7]^+$ (100); CI (isobutane): 307 $[M+1]^+$ (64), 247 $[307 - HOAc]^+$ (56), 203 $[247 - CO_2]^+$ (100); $[\alpha]_D$ ~ +30° ($CHCl_3$; c 0.3)] and 3 mg **4** [R_t 4.9 min; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600 (OH), 1770 (γ-lactone), 1720 (C=CCO_2R); MS m/z (rel. int.): 246.126 $[M - Tigl\,OH]^+$ (3.5) ($C_{15}H_{18}O_3$), 228 $[246 - H_2O]^+$ (3), 83 $[C_4H_7CO]^+$ (100), 55 $[83 - CO]^+$ (89)]. Compounds **3**, **4** and **7** could not be induced to crystallize though they were homogeneous by TLC in several solvent systems and by HPLC. Compounds **1**, **2**, **5** and **6** were identified by comparing the ^1H NMR spectral data with those in the lit. [1] or with those of authentic samples. Furthermore the ^1H NMR signals of **1**, **2** and **5** were fully assigned by spin decoupling.

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FIVE LABDANE DERIVATIVES FROM *KOANOPHYLLON CONGLOBATUM*

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Key Word Index—*Koanophyllon conglobatum*; Compositae; diterpenes; labdane derivatives; 7,8-*seco*-labdane derivative.

Abstract—A re-investigation of the aerial parts of *Koanophyllon conglobatum* afforded six new diterpenes, five labdanes and one *seco*-labdane, as well as two hydroperoxides which may be artefacts.

The large genus *Koanophyllon* is placed in the tribe Eupatorieae, subtribe Critoniinae [1]. So far, only two species have been studied chemically [2]. Both afforded diterpenes, some of them being characteristic. We have now re-investigated *Koanophyllon conglobatum* (DC.) K. et R.

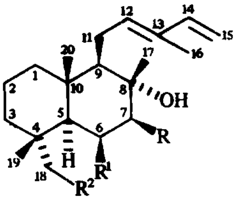
The aerial parts afforded several widespread compounds together with koanophyllic acids B and D [2], the labdane derivatives **1**–**5**, the *seco* derivative **6**, and the epimeric hydroperoxides **7** and **8**. The ^1H NMR spectral data of **1**–**5** (Table 1) clearly showed that the presence of abienol derivatives with additional oxygen functions similar to diterpenes which had been isolated from *Austroeupatorium inulaefolium* [3]. The position and configuration of the hydroxyl groups in **1** followed from the chemical shifts of the methyl groups and from the observed couplings of the low-field double-doublet at δ 4.53, which was coupled to a pair of double-doublets at δ 2.01 and 1.68. These were obviously the signals of H-7. The downfield shifts of H-17, H-19 and H-20, when compared with the shifts in the spectrum of **2**, required axial methyl groups at C-4, C-8 and C-10, which were deshielded by the 6β-hydroxyl group. The ^1H NMR spectral data of **2** clearly showed that a 7β-acetoxy group was present.

The low-field signals in the spectrum of **3** indicated a 6β,7β-dihydroxyabienol since the couplings of H-6 and H-7 were small. Accordingly, **3** was an isomer of austrofolin [3] which differed in the stereochemistry of the 12,13-double bond and in that at C-6. In agreement with this

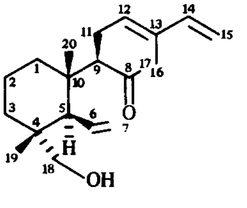
Table 1. ^{1}H NMR spectral data of compounds 1–6 (400 MHz, $CDCl_3$, TMS as internal standard)

	1	**2**	**3**	**4**	**5**	**6**
H-5	0.98 *m*				1.05 *d*	2.28 *d*
H-6	4.53 *br dd*	1.84 *ddd*	4.42 *br dd*	4.51 *br dd*	4.38 *br dd*	5.84 *ddd*
H-7	2.01 *dd*	4.75 *dd*	3.42 *dd*	2.02 *dd*	4.77 *d*	5.11 *dd*
H-7′	1.68 *dd*					5.27 *dd*
H-9						2.78 *dd*
H-11	2.43 *ddd*	2.43 *ddd*		2.46 *ddd*	2.49 *ddd*	2.49 *ddd*
H-11′	2.27 *ddd*	2.18 *ddd*		2.28 *ddd*	2.26 *ddd*	2.27 *m*
H-12	5.62 *br dd*	5.56 *br dd*	5.59 *br dd*	5.62 *br dd*	5.58 *br dd*	5.25 *dd*
H-14	6.35 *dd*	6.34 *dd*	6.34 *dd*	6.36 *dd*	6.33 *dd*	6.29 *dd*
H-15*t*	5.07 *d*	5.05 *d*	5.07 *d*	5.07 *d*	5.06 *d*	5.07 *br d*
H-15*c*	4.91 *d*	4.90 *d*	4.93 *d*	4.92 *d*	4.90 *d*	4.92 *br d*
H-16	1.80 *br s*	1.77 *d*	1.82 *br s*	1.83 *d*	1.79 *br s*	1.69 *br s*
H-17	1.42 *s*	1.17 *s*	1.39 *s*	1.47 *s*	1.39 *s*	2.05 *s*
H-18	0.98 *s*	0.79 *s*	1.03 *s*	3.55 *d*	0.96 *s*	3.30 *d*
H-18′				3.20 *d*		3.11 *d*
H-19	1.18 *s*	0.86 *s*	1.24 *s*	1.20 *s*	1.20 *s*	0.90 *s*
H-20	1.22 *s*	0.87 *s*	1.25 *s*	1.28 *s*	1.23 *s*	1.04 *s*
OAc		2.09 *s*	—	—	2.17 *s*	—

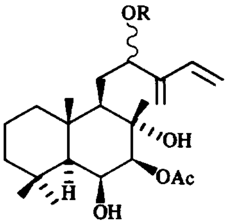
J (Hz): 11, 12 ~ 7.5; 14, 15*t* = 17; 14, 15*c* = 11; compound **1**: 6, 7 = 6, 7′ ~ 3; 7, 7′ = 14; compound **2**: 5, 6 = 2; 6, 6′ = 13; 6, 7 = 5; 6′, 7 = 12; compounds **3** and **5**: 5, 6 = 6, 7 = 7, OH ~ 3; compound **4**: 6, 7 = 6, 7′ ~ 3; 7, 7′ = 14; 18, 18′ = 10; compound **6**: 5, 6 = 10.5; 6, 7 = 17; 6, 7′ = 10; 7, 7′ = 2.5; 9, 11 = 11.5; 9, 11′ = 3; 11, 11′ = 14.



	1	**2**	**3**	**4**	**5**
R	H	OAc	OH	H	OAc
R^1	OH	H	OH	OH	OH
R^2	H	H	H	OH	H



6



7 R = OH
8 12 epi, R = OH
9 R = H

assumption, the chemical shifts of H-17 and H-20, as well as those of the olefinic protons, differed in the expected way. The corresponding 6-desoxy derivative from a *Nidorella* species has the same stereochemistry [4].

The ^{1}H NMR spectral data of **4** showed that this triol was a 18-hydroxy derivative of **1**. Accordingly, one of the methyl singlets was replaced by a pair of doublets at δ3.55 and 3.20. The other signals were close to those of **1**. Inspection of the ^{1}H NMR spectrum of **5** showed that this diterpene was simply the 7-*O*-acetate of **3**. Accordingly, the H-7 signal was shifted downfield in the spectrum of **5**.

The molecular formula of **6** is $C_{20}H_{32}O_2$, which followed from the M + 1 peak obtained by chemical ionization. The IR spectrum indicated the presence of a hydroxyl and a keto group, while the ^{1}H NMR spectrum (Table 1) showed that a tertiary CH_2OH group, a methyl ketone and the same side chain as in **1**–**5** were present. A low-field double-doublet at δ2.78 was obviously due to a proton α to the keto group. Spin-decoupling showed that this proton was coupled to allylic protons. The latter gave rise to a three-fold doublet at δ2.49 and a signal at 2.27, which was overlapped by the H-5 signal; H-5 was coupled to a three-fold doublet of a monosubstituted vinyl group. All the data therefore agreed with the structure of the *seco*-labdane **6**, probably formed by fragmentation of **4**. Accordingly, the stereochemistry at C-5 and C-9 is probably the same as in **4**. We have named this ketone *seco*-koanolabda-12*E*,14-diene (**6**).

In the ^{1}H NMR spectra of **7** and **8** (Table 2), the olefinic methyl signals were replaced by exomethylene signals, showing that an additional oxygen function had been introduced into **5** by allylic rearrangement to an iso-abienol derivative; thus **7** and **8** are epimeric hydroperoxides. Accordingly, reaction of **7** with triphenylphosphine led to the carbinol **9**. The assignment of the relative stereochemistry of the epimers at C-12 was not possible.

Table 2. ^{1}H NMR spectral data of compounds **7–9** (400 MHz, $CDCl_3$, TMS as internal standard)

	7	**8**	**9**
H-6	4.40 *br dd*	4.43 *br dd*	4.43 *br dd*
H-7	4.77 *d*	4.79 *d*	4.76 *d*
H-11	1.92 *ddd*	2.14 *ddd*	2.03 *m*
H-11′	1.61 *m*	1.66 *m*	—
H-12	5.08 *dd*	4.54 *dd*	4.79 *dd*
H-14	6.37 *dd*	6.40 *dd*	6.34 *dd*
H-15*t*	5.55 *d*	5.46 *d*	5.55 *d*
H-15*c*	5.13 *br d*	5.15 *br d*	5.10 *br d*
H-16	5.27 *br s*	5.32 *br s*	5.31 *br s*
H-16′	5.24 *br s*	5.30 *br s*	5.19 *br s*
H-17	1.37 *s*	1.39 *s*	1.45 *s*
H-18	0.96 *s*	0.99 *s*	1.00 *s*
H-19	1.10 *s*	1.20 *s*	1.17 *s*
H-20	1.17 *s*	1.21 *s*	1.21 *s*
OAc	2.19 *s*	2.21 *s*	2.20 *s*
OH	8.34 *s*	8.30 *s*	—

J (Hz): 5, 6 = 6, 7 ~ 3; 10, 11 = 3; 11, 11′ = 14; 11, 12 = 10; 11′, 12 = 3; 14, 15*t* = 17.5; 14, 15*c* = 11; compound **9**: 11, 12 = 8; 11′, 12 = 5.

Neither were the absolute stereochemistries of all the diterpenes determined. The optical rotation of **3**, however, had the same sign as abienol and austrofolin. It is most likely, therefore, that these are all labdanes.

As mentioned above, similar labdane derivatives have been isolated from an *Austroeupatorium* species [3], but they are also present in *Stevia* species [5–7]; *Hofmeisteria fasciculata*, which contains 7β-hydroxyabienol (F. Bohlmann, unpublished results); and *Nidorella* species [4]. Outside the Compositae, similar labdanes occur in the hepatic genus *Porella* [8]. Accordingly, the chemotaxonomic relevance is limited.

EXPERIMENTAL

The air-dried aerial parts (collected in the province Bahia, Brazil; voucher No. RMK 8575) were extracted with Et_2O–petrol, 1:2 (12 hr room temp.), and the resulting extract was worked-up in the usual way. The CC fractions (100 ml) were as follows: 1 (petrol), 2 (Et_2O–petrol, 1:10), 3 (Et_2O–petrol, 1:3), 4 (Et_2O–petrol, 1:1), 5 (Et_2O) and 6 (Et_2O–MeOH, 10:1). Fraction 5 on repeated TLC (always SiO_2 PF 254; Et_2O–petrol, 1:1, several developments; detection by UV 255 nm) gave 5 mg **6** (R_f 0.35), 1 mg **2** (R_f 0.33), 7 mg **1** and 5 mg **5** (R_f 0.20). Fraction 6 on TLC (Et_2O–C_6H_6–CH_2Cl_2, 1:2:2) gave three fractions: 6a, 6b and 6c. Repeated TLC of 6a (same solvent) gave 5 mg **3** (R_f 0.32) 0.5 mg **7** (R_f 0.30) and 0.5 mg **8** (R_f 0.29). Repeated TLC of 6b (Et_2O–C_6H_6–CH_2Cl_2, 2:1:1, two developments) gave 1 mg koanophyllic acid D and 1 mg koanophyllic acid B, while TLC of 6c (Et_2O–C_6H_6–CH_2Cl_2, 2:1:1) gave 1.4 mg **4** (R_f 0.42) (always increasing polarity). Compounds **1–8** showed no impurities in the 400 MHz ^{1}H NMR spectra, and were homogeneous by TLC in different solvent mixtures. However, they could not be induced to crystallize.

*6β-Hydroxy-*12E*-abienol* (**1**). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600 (OH), 1640, 1610 (C=C); MS *m/z* (rel. int.): 288.245 $[M-H_2O]^+$ (30) ($C_{20}H_{32}O$), 270 $[288-H_2O]^+$ (8), 255 $[270-Me]^+$ (6), 189 $[C_{14}H_{21}]^+$ (36), 134 $[C_{10}H_{14}]^+$ (57), 81 $[C_6H_9]^+$ (96), 69 $[C_5H_9]^+$ (100).

*7β-Acetoxy-*12E*-abienol* (**2**). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600 (OH), 1740, 1245 (OAc); MS *m/z* (rel. int.): 348.266 $[M]^+$ (6) ($C_{22}H_{36}O_3$), 330 $[M-H_2O]^+$ (5), 270 $[330-HOAc]^+$ (22), 189 $[C_{14}H_{21}]^+$ (41), 69 $[C_5H_9]^+$ (88), 55 $[C_4H_7]^+$ (100).

*6β,7β-Dihydroxy-*12E*-abienol* (**3**). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3590 (OH), 1620 (C=C); MS *m/z* (rel. int.): 304.240 $[M-H_2O]^+$ (4) ($C_{20}H_{32}O_2$), 286 $[304-H_2O]^+$ (6), 83 $[C_7H_{11}]^+$ (100).

$$[\alpha]_{24^\circ}^{\lambda} = \frac{589 \quad 578 \quad 546 \quad 436\text{ nm}}{+10 \quad +10 \quad +11 \quad +20} (CHCl_3;\ c\ 0.25).$$

*6β,18-Dihydroxy-*12E*-abienol* (**4**). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600 (OH), 1620 (C=C); MS *m/z* (rel. int.): 322 $[M]^+$ (0.5), 304.240 $[M-H_2O]^+$ (9) ($C_{20}H_{32}O_2$), 289 $[304-Me]^+$ (3), 286 $[304-H_2O]^+$ (4), 55 $[C_4H_7]^+$ (100).

*7β-Acetoxy-6β-hydroxy-*12E*-abienol* (**5**). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600 (OH), 1620 (C=C); MS *m/z* (rel. int.): 364.261 $[M]^+$ (0.7) ($C_{22}H_{36}O_4$), 346 $[M-H_2O]^+$ (18), 286 $[346-HOAc]^+$ (36), 271 $[286-Me]^+$ (8), 151 (94), 81 (100), 69 (71).

Seco-*koanolabda-*12E,14*-diene* (**6**). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3640 (OH), 1715 (C=O); MS *m/z* (rel. int.): 304 $[M]^+$ (2) ($C_{20}H_{32}O_2$), 286 $[M-H_2O]^+$ (4), 137 $[C_{10}H_{17}]^+$ (100); CI (isobutane): 305 $[M+1]^+$ (10), 287 $[305-H_2O]^+$ (22), 139 (100).

$$[\alpha]_{24^\circ}^{\lambda} = \frac{589 \quad 578 \quad 546 \quad 436\text{ nm}}{-9 \quad -9 \quad -11 \quad -26} (CHCl_3;\ c\ 0.5).$$

*7β-Acetoxy-6β-hydroxy-*12*-peroxiisoabienol* (**7**). MS *m/z* (rel. int.): 362.246 $[M-H_2O_2]^+$ (1.3) ($C_{22}H_{34}O_4$), 302 $[362-HOAc]^+$ (3), 69 $[C_5H_9]^+$ (100). To 0.5 mg **7** in 0.5 ml $CDCl_3$, 3 mg triphenylphosphine was added. After 5 min the ^{1}H NMR spectrum of **9** was visible.

Epimer **8**. MS *m/z* (rel. int.): 362.246 $[M-H_2O_2]^+$ (4) ($C_{22}H_{34}O_4$), 302 $[362-HOAc]^+$ (6), 69 $[C_5H_9]^+$ (100); for ^{1}H NMR data, see Table 2.

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