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Structure, Conformation and Absolute Configuration of New Antifeedant Dolabellanes from *Trichilia trifolia*☆

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Abstract—The structures of three new dolabellane diterpenoids, (1*R*,3*E*,7*Z*,11*S*,12*S*)-dolabella-3,7,18-trien-17-oic acid (**1**), (1*R*,3*E*,6*R*,7*Z*,11*S*,12*S*)-dolabella-3,7,18-trien-6,17-olide (**2**) and (1*R*,3*S*,4*R*,7*Z*,11*S*,12*S*)-3-hydroxydolabella-7,18-dien-4,17-olide (**3**), isolated from the wood of *Trichilia trifolia*, were elucidated by 1D and 2D NMR spectroscopy. The stereochemistry of **1** and **2** was confirmed by X-ray diffraction analysis, while that of **3** was ascertained from NOESY data. Comparison between experimental and calculated $^{1}H-^{1}H$ vicinal coupling constants and the analysis of molecular mechanics structures revealed that the 11-membered ring of **1** and **2** exists in a conformational equilibrium in solution, while in **3** this ring possesses a more rigid structure. The absolute configuration of **3** was established from its Cotton effects. Dolabellanes **1**–**3** caused significant feeding reduction by the rice weevil *Sitophilus oryzae*. © 2000 Published by Elsevier Science Ltd.

Introduction

The genus *Trichilia*, which consists of about 230 species mainly distributed in lowland tropical America, is distinguished by the production of structurally diverse limonoids, many of which are biologically active against insects.[1–4] According to a recent review,[2] the probability of finding additional botanical insecticides from *Trichilia* appears high. Therefore, as a continuation of a program directed toward the discovery of insecticidal agents from Mexican Meliaceae,[5] we have now investigated the wood of *Trichilia trifolia* L., locally known as 'huesito'.[6] Previous chemical studies on the seeds of this plant led to the isolation of several limonoids.[7] In addition, it was demonstrated that an EtOH extract of this species significantly reduced the larval weight of the variegated cutworm, *Peridroma saucia*.[8] As a part of the present investigation, it was found that the initial CH_2Cl_2 extract prepared from the wood of this species induced significant feeding deterrence against the rice weevil (*Sitophilus oryzae*). Subsequently, fractionation of the active extract led to the isolation of three novel dolabellane diterpenes, namely, (1*R*,3*E*,7*Z*,11*S*,12*S*)-dolabella-3,7,18-trien-17-oic acid (**1**), (1*R*,3*E*,6*R*,7*Z*,11*S*,12*S*)-dolabella-3,7,18-trien-6,17-olide (**2**) and (1*R*,3*S*,4*R*,7*Z*,11*S*,12*S*)-3-hydroxydolabella-7,18-dien-4,17-olide (**3**). In this paper, we describe the structure elucidation, the absolute configuration, detailed conformational analysis guided by molecular mechanics and the feeding deterrence activity against the rice weevil *S. oryzae* of compounds **1**–**3**.

Results and Discussion

Compound **1** was isolated as colorless crystals. Its molecular formula was determined as $C_{20}H_{30}O_2$ by HRMS in conjunction with the NMR data. Upon treatment with diazomethane, **1** readily formed the methyl ester **4**. This result, as well as the strong absorption at 1681 cm^{-1} in the IR spectrum, was consistent with the presence of an α,β-unsaturated carboxylic acid. The UV absorption bands and the ^{13}C NMR signal at δ_C 173.4 supported the conjugated functionality. The ^{1}H and ^{13}C NMR spectra of **1**, shown in Tables 1 and 2, respectively, were quite similar to those of edunol[9] and related compounds[10] suggesting that **1** possessed the same basic dolabellane skeleton. The structure of dolabellane **1** was ascertained from the correlations found in the $^{1}H-^{1}H$ COSY, HMQC and HMBC contour

☆ Taken in part from the Ph.D. thesis of María del Carmen Ramírez.

Keywords: dolabellanes; diterpenes; *Trichilia trifolia*; conformation; configuration; X-ray crystal structures; antifeedants.

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Table 1. ^{1}H NMR chemical shifts (measured in ppm at 500 MHz from $CDCl_3$ solutions with TMS as the internal standard), multiplicities and coupling constants (coupling constants are in Hz) of dolabellanes **1**–**3** (assignments are supported by COSY, HMQC and HMBC experiments)

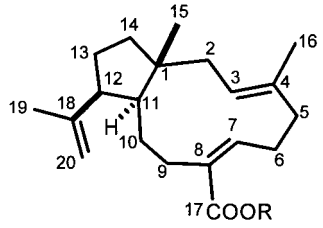
Proton	1	2	3
2a	2.22 dd (12.0, 13.0)	2.09 dd (14.0, 8.0)	1.98 br d (15.5)
2b	1.73 dd (13.0, 4.0)	1.91 dd (14.0, 8.0)	1.50 dd (15.5, 6.5)
3	5.23 br dd (11.5, 3.0)	5.10 tq (8.0,1.0)	4.68 br d (6.5)[a]
5a	2.31 m	2.35 dd (14.5, 4.0)	1.90 m (14.2, 6.3, 2.1)[b]
5b	2.18 m	2.78 dd (14.5, 3.3)	2.44 td (14.2,13.3, 5.5)[b]
6a	3.32 m	5.22 tdd (3.8, 2.3, 1.5)	2.48 m (14.1, 13.3, 6.3, 5.4)[b]
6b	2.31 m	–	2.16 m (14.1, 8.3, 5.5, 2.1)[b]
7	5.93 dd (11.8, 3.5)	6.82 br t (1.5)	6.14 br dd (8.3, 5.4)[b]
9a	2.46 br ddd (13.1, 7.6, 5.2)	1.95 ddd (13.0, 10.5, 6.0)	2.56 tdt (13.5, 3.0, 1.5)
9b	2.16 m	2.46 dtdd (13.0, 3.5, 2.5,1.5)	2.25 br ddd (13.5, 3.8, 2.5)
10a	1.46 m	1.39 m	1.68 m
10b	1.29 ddd (14.6, 7.4, 4.5)	1.39 m	1.45 m
11	1.73 m	1.69 m	1.72 m
12	2.69 br q (7.5)	2.63 br td (9.5, 8.0)	3.02 br td (11.0, 8.5)
13a	1.60 m	1.60 m	1.64 m
13b	1.60 m	1.60 m	1.64 m
14a	1.48 m	1.42 m	1.37 td (12.5, 7.5)
14b	1.55 m	1.46 m td (12.0, 8.0)	1.44 m
15	1.07 s	0.87 s	0.95 s
16	1.57 s	1.61 s	1.33 s
19	1.64 s	1.78 s	1.64 s
20a	4.67 m	4.75 dquint (2.5, 1.0)	4.74 dquint (2.5, 1.0)
20b	4.80 dq (2.5, 1.2)	4.90 dq (2.5, 1.2)	4.88 dq (2.5,1.2)

[a] Upon D_2O addition.
[b] Data obtained by spectral simulation with a correlation coefficient of 0.56 Hz.

plots. Altogether, this evidence suggested that compound **1** might possess a dolabella-3,7,18-trien-17-oic acid structure. Subsequently, an X-ray crystallographic analysis confirmed the structure and stereochemistry as depicted in Fig. 1. Compound **1** crystallizes with two chemically identical but crystallographically different molecules, which differ mainly in the orientation of the carboxylic acid moiety. The 5- and 11-membered rings are joined in a *trans* fashion and the stereochemistry of the double bonds at C-3 and C-7 is *E* and *Z*, respectively. The conformation in the solid state results in the 5-membered ring adopting an envelope conformation with the C-12 atom lying out of the plane. The conformation of the cycloundecadiene ring is such that the vinylic methyl group (C-16) and the carboxyl group (C-17) are both oriented towards the same side of the molecule, with their directions varying from being parallel by 29.7°. The structure shows significant strain in the crystalline lattice as indicated by deviation from planarity of the double bonds. The C–C=C–C torsion angles are $\Delta(3,4)=169°$, $\Delta(7,8)=168°$. The molecules are packed in the crystal forming centrosymmetric dimers as frequently found in compounds containing a carboxylic acid unit.

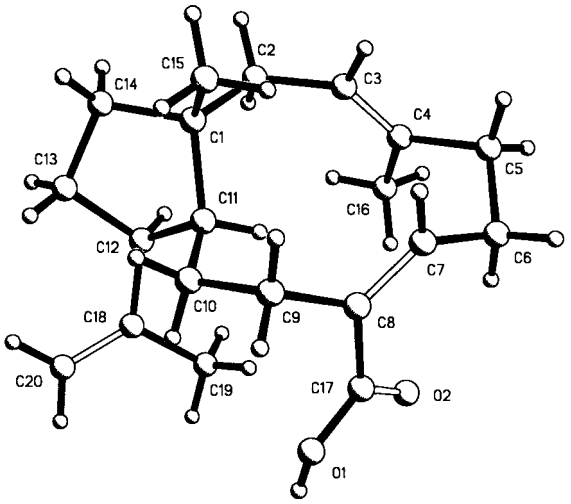
Table 2. ^{13}C NMR chemical shifts of dolabellanes **1**–**3**. (Measured in ppm at 125.7 MHz from $CDCl_3$ solutions with TMS as the internal standard. Assignments are supported by DEPT, HMQC and HMBC experiments)

Carbon	1	2	3
1	46.0	46.3	43.3
2	43.4	41.3	48.0
3	125.4	129.1	70.7
4	135.2	128.9	85.0
5	39.8	40.4	35.6
6	25.9	80.4	23.4
7	149.4	148.0	133.3
8	129.3	134.3	140.3
9	32.5	26.3	34.8
10	25.5	21.9	27.2
11	42.0	42.2	49.3
12	50.8	50.3	51.0
13	28.4	28.5	27.7
14	42.2	41.1	42.8
15	24.0	25.4	19.8
16	15.5	20.1	22.6
17	173.4	173.6	171.9
18	146.8	145.9	147.0
19	23.2	23.4	22.9
20	111.3	112.3	113.6

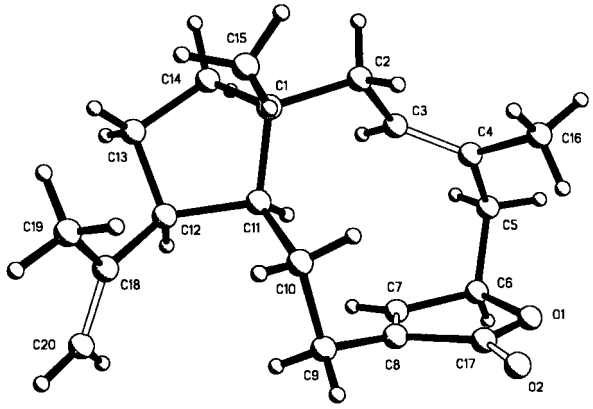


1 : R = H
4 : R = Me

The molecular formula of **2** was established as $C_{20}H_{28}O_2$ by HRMS. The IR spectrum showed the presence of an α,β-unsaturated γ-lactone. The ^{1}H and ^{13}C NMR spectra of **2** (Tables 1 and 2) were similar to those of **1**. ^{1}H–^{1}H COSY, HMQC and HMBC experiments confirmed this assumption. The structure of **2** was unambiguously assigned as (1*R*,3*E*,6*R*,7*Z*,11*S*,12*S*)-dolabella-3,7,18-trien-6,17-olide by X-ray crystallography (Fig. 2). Compound **2** also crystallizes with two chemically identical but crystallographically different molecules, the conformations of which are similar but very different from those of **1** (Fig. 1).

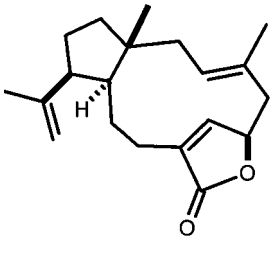


1



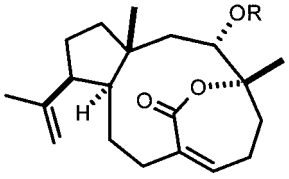
2

Figure 1. X-Ray diffraction structures of **1** and **2**.



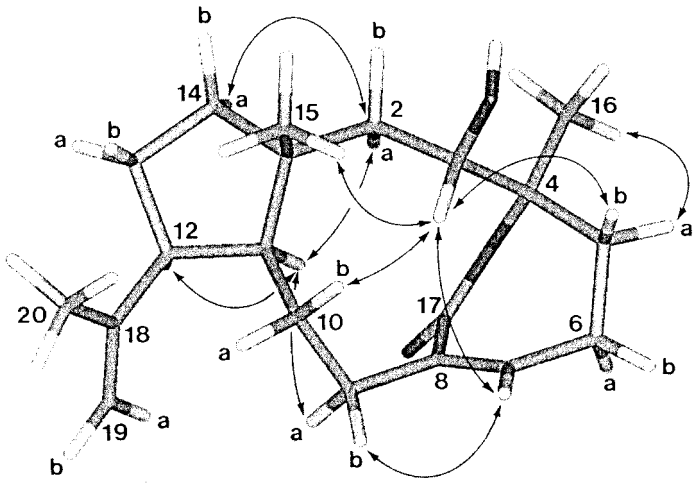
2

Dolabellane **3** had the molecular formula $C_{20}H_{30}O_3$, as determined by HRMS and ^{13}C NMR, signifying six sites of unsaturation. A direct comparison between the NMR spectra of **3** (Tables 1 and 2) with those of **1** and **2** indicated the structural similarity of these compounds and revealed the presence of two features unique to **3**, namely, a secondary carbinol functionality and an α,β-unsaturated ϵ-lactone moiety. The carbinol group was supported by the presence of one oxygen-bearing methine at δ_H 4.68 (br m), which became a broad doublet upon addition of D_2O. In the HMQC spectra, this signal correlated with that at δ_C 70.7. The IR absorption band at 3624 cm^{-1} and the formation of the monoacetyl derivative **5** confirmed the presence of a free hydroxyl group. The ^{13}C NMR signals at δ_C 85.0 and 171.9 as well as the IR band at 1702 cm^{-1} were consistent with the presence of an α,β-unsaturated ϵ-lactone moiety. The dolabellane skeleton in **3** and the allocation of the functional groups were confirmed by an HMBC experiment. Thus, an unusual four-bond correlation was found between C-17 and the protons of Me-16, which is feasible due to the perfect W-type arrangement present in the C(17)–O(4)–C(4)–C(16)–H(16) moiety. This correlation, together with those of C-4/H-2a,H-3,H-5b,H-6b,H-16 and C-17/H-9a,H-9b indicated that the ϵ-lactone was positioned between C-4 and C-17. On the other hand, the correlations C-1/H-2a,H-2b; C-4/H-2a,H-3; C-3/H-2a,H-2b,H-5a,H-5b,H-16; and C-5/H-3,H-16 were in agreement with the disposition of the secondary hydroxyl at C-3. The stereochemistry and conformation in solution of **3** was determined taking into account the correlations found in its NOESY spectrum in combination with a molecular mechanics[11] model (Fig. 2; E_{MMX}=51.9 kcal/mol), which was validated by comparing the calculated[12,13] and the observed vicinal coupling constants (Table 3). As represented in Fig. 2, the correlations H-3/H-5b,H-7,H-10b,H-15 and H-7/H3,H9b indicated that these atoms were located in the β-side of the molecule, supporting the *Z* configuration of the C-7 double bond and the stereochemistry at C-3 and C-4. The strong NOE interactions H-11/H-2a,H-9a,H-12 located these atoms in the α-side of the structure, revealing not only the *trans* stereochemistry between C-15 and H-11, but also the *cis* relationship between C-15 and the isopropenyl side chain, as in **1** and **2**.



3 : R = H
5 : R = Ac

The conformation in solution of **1** and **2** were also analyzed employing the same methodology. For dolabellane **1**, the strong NOESY interactions between H-3/H-7,Me-15; H-7/H-9b,Me-15; and H-9b/Me-15, together with the *trans*-diaxial couplings $J_{2a,3}$=12.0 and $J_{6a,7}$=11.8 Hz, indicated that the C(2)–C(3)–C(4)–C(5)–C(6)–C(7) fragment of **1** was quite rigid, in contrast with the rest of the structure, where the averaged couplings (ca. 7.5 Hz) observed for H-9a, H-10b and H-12 evidenced the presence of a dynamic conformational behavior. The search for the minimum energy conformations by means of molecular mechanics indicated the presence of an equilibrium between two relevant conformations (**1a** and **1b**, Fig. 3), whose geometry in the C(2)–C(3)–C(4)–C(5)–C(6)–C(7)–C(8) fragment is



3

Figure 2. Minimum energy structure of **3**, showing relevant NOESY correlations.

identical. The minimum energy conformation **1a** (E_{MMX}=37.8 kcal/mol) was in close agreement with the conformation found in the solid state. The presence of the second minimum conformation **1b** at (E_{MMX}=38.7 kcal/mol) was experimentally supported by strong NOESY interactions between H-7/H10b and H-10b/Me-15 (Fig. 3).

In the case of dolabellane **2**, its NOESY spectrum displayed the correlations H-3/H-5a,H-7,H-11 (located in the α-side of the molecule) and H-3/H-5b,H-15 (located on the β-side). These data, together with the coupling constants $J_{2a,3}$=$J_{2b,3}$=8.0 Hz, clearly indicated the presence of a conformational equilibrium. This was in agreement with the conformational search, also achieved by molecular mechanics, which exhibited the presence of two relevant conformers represented in Fig. 3. Structure **2a**, which was very similar with that found in the solid state, corresponded to the most stable conformation in solution with E_{MMX}=47.8 kcal/mol, while **2b**, obtained by simultaneous rotation of the C(2)–C(3) and C(4)–C(5) bonds, corresponded to the second minimum conformation (E_{MMX}=48.5 kcal/mol).

Table 3. Selected dihedral angles (ϕ_{MMX} in degrees), calculated coupling constants (J_{calc} in Hz) and observed coupling constants (J_{obs} in Hz) for dolabellane **3**

H(x)–C–C–H(y) x,y	ϕ_{MMX}	J_{calc}	J_{obs}
2a,3	−107	2.8	2.5[a]
2b,3	+139	6.1	6.5
5a,6a	−51	4.7	6.3[b]
5a,6b	+65	2.1	2.1[b]
5b,6a	−164	12.7	13.3[b]
5b,6b	−48	5.4	5.5[b]
6a,7	+131	6.5	5.4[b]
6b,7	+16	6.3	8.3[b]
9a,10a	+69	1.6	3.0
9a,10b	+180	13.3	13.5
9b,10a	−46	5.6	3.5
9b,10b	+65	2.2	2.5
11,12	−4	10.3	11.0
12,13a	+38	6.8	8.5
12,13b	+160	11.2	11.0
13a,14a	−46	5.7	7.5
13b,14b	−166	12.7	12.5

[a] Estimated from $W_{1/2}$.
[b] Determined by spectral simulation.

Regarding the absolute configuration of the dollabellanes isolated in the present study, compound **3** was selected for the configurational analysis because, as revealed by the conformational analysis reported herein, it possesses a rigid structure and also an inherently dissymmetric chromophore, since the C=C–C=O moiety is not planar. This molecular arrangement was also evidenced by the low molar absorptivity of the *K*- and *R*-bands in the UV spectrum. According to Snatzke,[14] the rotatory strength of such chromophores can assume values approximately 100 times greater than inherently symmetric but dissymmetrically perturbed chromophores. In addition, he stated that the rules for unsaturated ketones can be applicable to unsaturated lactones.[14] The CD spectrum of **3** displayed the *K*-band absorption at 245 nm with a positive value of $\Delta\epsilon$=+2.0×10^5, while the *R*-band was observed at 300 nm with $\Delta\epsilon$=−4.6×10^3. Therefore, according to the rules for C=C–C=O systems,[14,15] these values correspond to a positive chirality as shown by the minimum energy structure of **3**, where the C=C–C=O dihedral angle is Φ=+136°.

Compounds **2** and **3** might be biogenetically derived from acid **1**. Thus, hydroxylation of **1** would give (1*R*,3*E*,6*R*,7*Z*,11*S*,12*S*)-6-hydroxydolabella-3,7,18-trien-17-oic acid, which upon internal lactonization would lead ultimately to compound **2**. On the other hand, the lactone ring in compound **3** can be envisaged as arising via nucleophilic attack of the acid group on to the epoxide moiety in intermediate (1*R*,3*S*,4*S*,7*Z*,11*S*,12*S*)-3,4-epoxydolabella-7,18-dien-17-oic acid, biosynthesized by epoxidation of the C-3/C-4 double bond of compound **1**. According to the molecular model of this intermediate, it is possible that the *Z* configuration of the C-7/C-8 double bond could facilitate and also direct the lactonization process. Therefore,

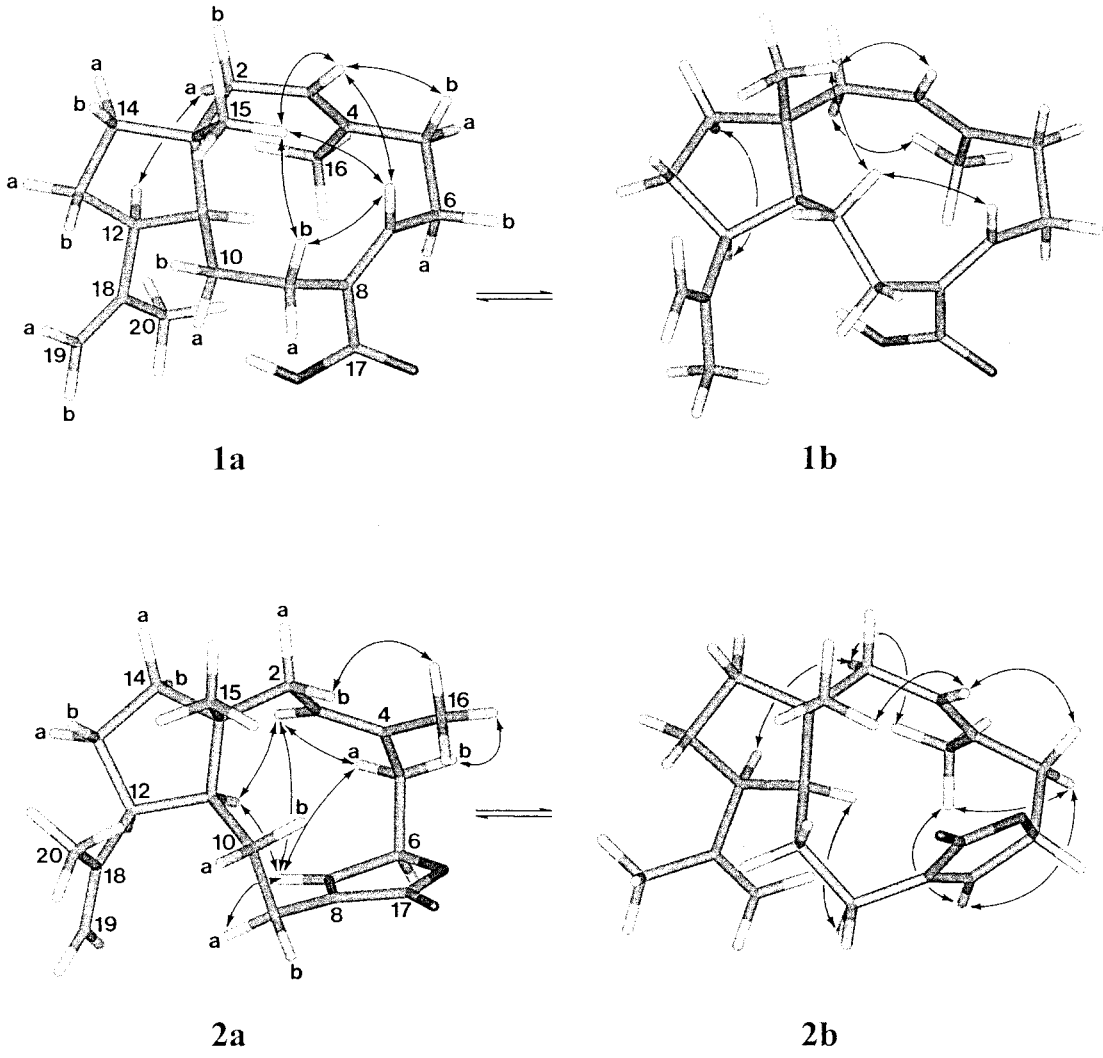


Figure 3. Minimum energy structures of **1** and **2**, showing the conformational equilibrium and relevant NOESY correlations.

based on biogenetic considerations, **1** and **2** should have the same absolute configuration as **3**. It is relevant to mention that this is the first description of the absolute configuration of higher plant dolabellanes. Interestingly, our results revealed that higher plants, liverworts and algae dolabellanes possess the same absolute configuration. On the other hand, there seems to be an antipodal relationship between coelenterate and higher plant dolabellanes. Compounds **1**–**3** are the first dolabellanes reported so far[16] to have the isopropenyl group at C-12 in the β-configuration. From a chemotaxonomic point of view, the results of the present investigation represent the first report on the presence of dolabellane diterpenes in a member of the Meliaceae family and the second from higher plants.

Finally, based on the flour-disk bioassay,[17] compounds **1**–**3** showed significant (P=0.05) feeding reduction with *S. oryzae* (Table 4). However, compounds **4** and **5** were inactive suggesting that the carboxyl and hydroxyl functionalities in **1** and **3**, respectively, are important structural requirements for the observed activity. The bioassay results suggest that these compounds may have a defensive role in the host plant.

Table 4. Antifeedant activity of the extract from the wood of *T. trifolia* and dolabellanes **1**–**5**. Means followed by * are significantly different from control in Bonferroni contrasts. Values in parentheses indicate standard deviation. P (significance level)=0.05

Treatment	Mean diet consumed (mg/day)
Control	7.61 (1.59)
Extract	1.62 (0.37)*
1	1.17 (0.28)*
2	3.36 (1.42)*
3	1.03 (0.53)*
4	6.74 (0.68)
5	6.61 (0.20)

Experimental

General methods

Melting points were measured on a Fisher-Johns apparatus and are uncorrected. Optical rotations were taken on a JASCO DIP-360 polarimeter. UV spectra were obtained on a Shimadzu 160 UV spectrophotometer in MeOH solutions. CD spectra were measured on a JASCO 720 spectropolarimeter at 25°C in MeOH. IR spectra were recorded

using KBr discs on a Nicolet FT-5X-IR spectrophotometer. ^{1}H (500 MHz) and ^{13}C (125 MHz) and 2D NMR spectra (all in $CDCl_3$) were recorded either on a Bruker DMX500 or on a Varian VXR-300S instrument using TMS as the internal standard. Mass spectra were recorded on a JEOL JMS-AX505 HA instrument, at an ionization energy of 70 eV. HPLC was carried out with a Waters HPLC instrument equipped with a Waters 996 UV photodiode array detector set at 209–214 nm, using a silica gel column (19 mm i.d.×300 mm) at a flow rate of 6.7 mL/min. Control of the equipment, data acquisition, processing, and management of chromatographic information were performed by the Millennium 2000 software program (Waters). Column chromatography: silica gel 60 (Merck, 70–230 mesh). TLC: silica gel 60 F_{254} (Merck).

Plant material

The wood of *T. trifolia* was collected in Yucatán, México in June 1996. A voucher specimen (J.C. Tun 643) is deposited at the Herbarium of the Universidad Autónoma de Yucatán (UADY), México.

Extraction and isolation

The air-dried and milled wood (6.2 kg) was extracted with CH_2Cl_2 (12 L×3). Evaporation of the solvent in vacuo afforded 130 g of a brown residue. The concentrated extract was subjected to Si gel (1300 g) column chromatography using 10:0→0:10 gradient mixtures of hexane–EtOAc as the eluent. Fractions were pooled based on their TLC profile to yield 14 primary fractions, designated as I-XIV. Fraction VII (2.15 g), eluted with hexane–EtOAc (95:5) was further chromatographed on Si gel (44 g) using hexane–EtOAc (97:3) as eluent. Five tertiary fractions were obtained (VIIA–VIIE). Fractions VIIB (133 mg) and VIID (431 mg) were purified by HPLC [hexane–*i*-PrOH–MeOH (90:5:5)] to yield **1** (304 mg) and **2** (15 mg), respectively. Primary fraction XII (4 g), eluted with hexane–EtOAc (8:2), was subjected to column chromatography on Si gel (60 g) using a gradient of hexane–EtOAc (10:0→0:10) to yield twelve fractions XIIA–XIIG. Fraction XIIG (360 mg) gave 10 mg of pure vanillin after purification by HPLC (hexane–*i*-PrOH–MeOH 90:5:5). Fraction XIIH (316 mg) was repeatedly chromatographed on HPLC (hexane–*i*-PrOH–MeOH 90:5:5) to give **3** (51 mg).

(1*R*,3*E*,7*Z*,11*S*,12*S*)-dolabella-3,7,18-trien-17-oic acid (1). colorless crystals; mp 100–103°C; $[\alpha]_D$=−131 (*c*=1, MeOH); UV (MeOH) λ_{max} (log ϵ) 204 (4.14), 225 (3.92) nm; CD (MeOH) $\Delta\epsilon$ (nm) -7.3×10^5 (213), $+2.6\times10^4$ (245), -1.8×10^4 (263); IR (KBr) ν_{max} 3420, 3082, 2920, 1681, 1641, 1271, 882 cm^{-1}; ^{1}H and ^{13}C NMR (Tables 1 and 2); EIMS *m/z* 302 [M^+(78)], 287 (8), 259 (23), 257 (8), 233 (11), 219 (9), 191 (44), 189 (18), 164 (91), 136 (89), 135 (100), 121 (98), 93 (48), 79 (33), 67 (31), 41 (29); HRMS *m/z* 302.2248 (calcd for $C_{20}H_{30}O_2$, 302.2246).

(1*R*,3*E*,6*R*,7*Z*,11*S*,12*S*)-dolabella-3,7,18-trien-4,17-olide (2). colorless crystals; mp 83–86°C; $[\alpha]_D$=+32 (*c*=1, MeOH); UV (MeOH) λ_{max} (log ϵ) 204 (3.86) nm; CD (MeOH) $\Delta\epsilon$ (nm)+3.5×10^5 (213); IR (KBr) ν_{max} 3621, 2976, 1750, 1046 cm^{-1}; ^{1}H and ^{13}C NMR (Tables 1 and 2); EIMS *m/z* 300 [M^+(28)], 273 (4), 257 (8), 232 (26), 206 (6), 205 (6), 189 (7), 165 (15), 136 (100), 121 (68), 93 (90), 79 (18), 67 (11), 55 (8); HRMS *m/z*300.2085 (calcd for $C_{20}H_{28}O_2$, 300.2089).

(1*R*,3*S*,4*R*,7*Z*,11*S*,12*S*)-3-hydroxydolabella-7,18-dien-4,17-olide (3). colorless crystals; mp 196–197°C; $[\alpha]_D$=+16 (*c*=1, MeOH); UV (MeOH) λ_{max} (log ϵ) 204 (3.82), 228 (3.74) nm; CD (MeOH) $\Delta\epsilon$ (nm) $+2.0\times10^5$ (245), -4.6×10^3 (300); IR (KBr) ν_{max} 3624, 2976, 1702, 1389, 1046, 887 cm^{-1}; ^{1}H and ^{13}C NMR (Tables 1 and 2); EIMS *m/z* 318 (15), 300 (20), 285 (12), 274 (63), 257 (30), 246 (35), 231 (21), 216 (30), 204 (34), 191 (30), 173 (32), 161 (40), 146 (70), 135 (100), 121 (79), 107 (79), 93 (79), 79 (52), 67 (52), 43 (20); HRMS *m/z*318.2192 (calcd for $C_{20}H_{30}O_3$, 318.2195).

(1*R*,3*E*,7*Z*,11*S*,12*S*)-dolabella-3,7,18-trien-17-oic acid methyl ester (4). Methylation of **1** (20 mg) with an excess of ethereal diazomethane afforded **4** (12 mg); mp 122–124°C; $[\alpha]_D$=−152 (*c*=1, MeOH); UV (MeOH) λ_{max} (log ϵ) 204 (4.06), 227 (3.81) nm; IR (KBr) ν_{max} 3077, 2924, 2862, 1713, 1195, 884; ^{1}H NMR ($CDCl_3$) δ 1.07 (3H, s), 1.29 (1H, ddd, *J*=14.6, 7.4, 4.5 Hz), 1.39 (1H, m), 1.46 (1H, m), 1.53 (1H, m), 1.55 (3H, s), 1.59 (2H, m), 1.62 (3H, s), 1.73 (1H, m), 1.73 (1H, dd, *J*=13.0, 4.0 Hz), 2.16 (1H, m), 2.18 (1H, m), 2.22 (1H, dd, *J*=12.0, 13.0 Hz), 2.31 (1H, m), 2.46 (1H, br ddd, *J*=13.1, 7.6, 5.2 Hz), 2.69 (1H, br q, *J*=7.5 Hz), 3.25 (1H, m), 3.69 (3H, s), 4.65 (1H, m), 4.80 (1H, m), 5.22 (1H, br dd, *J*=11.5, 3.0 Hz), 5.76 (1H, dd, *J*=11.8, 3.5 Hz); ^{13}C NMR ($CDCl_3$) δ 15.4 (C-16), 23.2 (C-19), 24.0 (C-15), 25.5 (C-6), 25.6 (C-10), 28.4 (C-13), 32.7 (C-9), 39.8 (C-5), 41.8 (C-11), 42.2 (C-14), 43.4 (C-2), 46.0 (C-1), 50.7 (OMe), 50.9 (C-12), 111.1 (C-20), 125.4 (C-3), 129.9 (C-8), 135.2 (C-4), 146.4 (C-7), 146.9 (C-18), 174.0 (C-17); HRMS *m/z* 316.2406 (calcd for $C_{21}H_{32}O_2$, 316.2402).

(1*R*,3*S*,4*R*,7*Z*,11*S*,12*S*)-3-acetyloxydolabella-7,18-dien-4,17-olide (5). Acetylation of **3** (5 mg) using Ac_2O–pyridine at 25°C for 24 h gave **5** (3 mg); mp 162–164°C; $[\alpha]_D$=−40 (*c*=1, MeOH); UV (MeOH) λ_{max} (log ϵ) 204 (4.12), 232 (3.52) nm; IR (KBr) ν_{max} 2923, 2851, 1734, 1717, 1237, 1025, 800; ^{1}H NMR ($CDCl_3$) δ 0.74 (3H, s), 1.34 (3H, s), 1.37 (1H, td, *J*=12.5, 7.5 Hz), 1.62 (3H, s), 1.93 (1H, br d *J*=15.5 Hz), 2.07 (3H, s), 2.16 (1H, m), 2.32 (1H, m), 3.04 (br q, *J*=10.7 Hz), 4.73 (1H, dq, *J*=2.5, 1.0 Hz), 4.86 (1H, dq, *J*=2.5, 1.2 Hz), 6.16 (1H, br d, *J*=6.5 Hz), 6.28 (1H, dd, *J*=8.4, 5.7 Hz); ^{13}C NMR ($CDCl_3$) δ 18.3 (C-15), 21.2 (OAc), 22.7 (C-16), 22.7 (C-19), 22.7 (C-6), 26.0 (C-10), 27.7 (C-13), 33.9 (C-9), 37.0 (C-5), 41.9 (C-14), 44.0 (C-1), 47.7 (C-2), 48.8 (C-11), 50.2 (C-12), 70.0 (C-3), 85.0 (C-4), 113.3 (C-20), 133.2 (C-7), 140.2 (C-8), 147.0 (C-18), 170.2 (OAc), 171.0 (C-17); HRMS *m/z* 360.2305 (calcd for $C_{22}H_{32}O_4$, 360.2300).

X-Ray crystallographic analysis of 1 and 2[18]

X-Ray data were collected on a Siemens P4/PC diffractometer using graphite monochromated MoKα. Data sets were corrected for Lorentz-polarization effects but not absorption correction was applied, space groups were assigned on the basis of systematic absences. For both

compounds the structures were solved by direct methods using the program SIR92[19] and the structure refinements were performed by full-matrix least-squares with anisotropic temperature factors for the non-hydrogen atoms and isotropic temperature factors for the hydrogen atoms, using the SHELXL-97 program.[20]

Molecular modeling calculations

Minimum energy structures were generated using the MMX force-field as implemented in the PCMODEL molecular modeling program V 6.00 (Serena Software, Box 3076, Bloomington, IN 47402-3076). The systematic conformational search for the 5- and 11-membered rings was carried out considering dihedral angle rotations of ca. 20° in those bonds which allowed such movement, according to a Dreiding model. The E_{MMX} values as well as the comparison between the observed and calculated $^1H-^1H$ vicinal coupling constants were used as the convergence criterion. The π-system calculations were set for the Restricted Hartree–Fock and Full Self Consistent Field options.

Feeding inhibition assay on the weevil rice *S. oryzae*

The antifeedant activity of the extract and pure compounds was determined by a choice test using *S. oryzae* as previously described.[17] The extract and compounds were dissolved in 70% ethanol and added to wheat flour disks[17] to give final concentrations of 0.5% or 0.05 (w/w). The disks were allowed to dry. Then 10 weighed disks were placed in a Petri dish with 25 adult insects (2–7 days post emergence). After 72 h the disks were re-weighed. Four replicates were prepared for each concentration. The same procedure was used for negative controls containing only solvent. The results were analyzed by the Systat version 4.1 statistical software (Analytical software, PO Box 12185 Tallahassee FL).

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