Available online at www.sciencedirect.com
SCIENCE DIRECT®
Phytochemistry 66 (2005) 633–638
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Clerodane diterpenoids from *Pulicaria wightiana* ☆

Biswanath Das *, M. Ravinder Reddy, R. Ramu, N. Ravindranath, H. Harish, K.V.S. Ramakrishna, Y. Koteswar Rao, K. Harakishore, U.S.N. Murthy

Indian Institute of Chemical Technology, Organic Chemistry-1, Tarnaka, Hyderabad 500 007, India

Received 8 November 2004; accepted 2 February 2005

Dedicated to Oishika (daughter of the corresponding author) with great affection on the occasion of her 10th birthday

Abstract

Five clerodane diterpenoids have been isolated from the aerial parts of *Pulicaria wightiana* along with 3′5,6-trihydroxy-3,4′,7-trimethoxyflavone and 2-methyl-5-hydroxy-chroman-4-one. The structures and stereochemistry of the compounds were established from spectral (mainly 1D and 2D NMR) studies. The last two compounds were not reported earlier from this plant. The antibacterial activity of the diterpenoids were studied.

Keywords: *Pulicaria wightiana*; Compositae; Clerodanes; Antibacterial activity

1. Introduction

Various species of *Pulicaria* are distributed in different parts of India and are known for their medicinal properties (Annonymus, 1969). Earlier chemical investigations on different *Pulicaria* species afforded several sesquiterpenoids (Bohlman et al., 1979), diterpenoids (Rustaiyan et al., 1981; Nurmukhamedova et al., 1986) and flavonoids (Mansour et al., 1990). From *Pulicaria wightiana* an isopimarane was previously reported (Chiplunkar and Nagasampagi, 1992). We have recently isolated five new clerodane diterpenoids, **1**–**5**, together with 3′,5,6-trihydroxy-3,4′,7-trimethoxyflavone and 2-methyl-5-hydroxy-chroman-4-one from the aerial part of the plant. Here, we report the isolation of the constituents and the characterization of the new compounds.

2. Results and discussion

Compound **1** was isolated as white crystals. Its molecular formula was suggested to be $C_{21}H_{30}O_5$ from its elemental analysis and ^1H and ^{13}C NMR spectra (indicating the presence of 30 protons and 21 carbons, respectively). The mass spectra (EIMS and LSIMS) did not show the molecular ion peak but a significant peak at *m*/*z* 330 ($M^{+\cdot}$ − MeOH) was observed in EIMS. The IR spectrum revealed the presence of hydroxyl group as well as unsaturated γ-lactone and ester function in the molecule. The ^1H and ^{13}C NMR spectral data (Tables 1 and 2, respectively), suggested that the compound is related to the clerodane diterpenoid, (−)-hardwickiic acid (Misra et al., 1979). The assignment of protons and carbons was made with the help of 2D NMR (HSQC, HMBC and NOESY) (Fig. 1) and APT experiments. The ^1H NMR signals of the olefinic protons (δ 6.72, 1H, *t*, *J* = 3.5 Hz, H-3), two tertiary methyls (δ 1.24, 3H, *s*, Me-19 and 0.78, 3H, *s*, Me-20) and a secondary methyl (δ 0.86, 3H, *d*, *J* = 7.0 Hz, Me-17) are similar to those of (−) hardwickiic acid and related

☆ Part 47 in the series, "Studies on phytochemicals". IICT Communication No. 050221.

* Corresponding author. Tel.: +91 40 27173874; fax: 91 40 27160512.
E-mail address: biswanthdas@yahoo.com (B. Das).

0031-9422/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2005.02.004

Table 1
^{1}H NMR spectral data (δ in ppm) of compounds **1**–**5**[a]

Proton	1	2	3	4	5
H-1α	1.68–1.56 (*m*)	1.78 (*m*)	1.74 (*m*)	1.78–1.42 (*m*)	1.88–1.50 (*m*)
H-1β	1.68–1.56 (*m*)	1.68–1.54 (*m*)	1.63–1.44 (*m*)	1.78–1.42 (*m*)	1.88–1.50 (*m*)
H-2α	2.40–2.21 (*m*)	2.41 (*m*)	2.56 (*m*)	2.32 (*m*)	2.48–2.06 (*m*)
H-2β	2.21–2.04 (*m*)	2.28–2.04 (*m*)	2.40–2.18 (*m*)	2.24–2.16 (*m*)	2.48–2.06 (*m*)
H-3	6.72 (*t*, 3.5)	6.84 (*t*, 3.5)	6.48 (*t*, 3.5)	6.78 (*t*, 3.5)	6.78 (*t*, 3.5)
H-6β	3.64 (*dd*, 10.0,3.0)	3.48 (*d*, 3.5)	3.61 (*d*, 3.5)	3.62 (*dd*, 10.0,3.0)	3.62 (*dd*, 10.0,3.0)
H-7α	1.68–1.56 (*m*)	–	4.42 (*t*, 3.5)	1.78–1.42 (*m*)	1.88–1.50 (*m*)
H-7β	1.68–1.56 (*m*)	3.98 (*t*, 3.5)	–	1.78–1.42 (*m*)	1.88–1.50 (*m*)
H-8β	1.68–1.56 (*m*)	1.68–1.54 (*m*)	1.63–1.44 (*m*)	1.78–1.42 (*m*)	1.88–1.50 (*m*)
H-10	1.30 (*dd*, 10.0,3.0)	1.32 (*dd*, 10.0,3.0)	1.42 (*dd*, 10.0,3.0)	1.30 (*dd*, 10.0,3.0)	1.39 (*dd*, 10.0,3.0)
H-11α	1.68–1.56 (*m*)	1.68–1.54 (*m*)	1.63–1.44 (*m*)	1.78–1.42 (*m*)	1.88–1.50 (*m*)
H-11β	1.68–1.56 (*m*)	1.68–1.54 (*m*)	1.63–1.44 (*m*)	1.78–1.42 (*m*)	1.88–1.50 (*m*)
H-12α	2.40–2.21 (*m*)	2.28–2.04 (*m*)	2.40–2.18 (*m*)	2.24–2.16 (*m*)	2.48–2.06 (*m*)
H-12β	2.21–2.04 (*m*)	2.28–2.04 (*m*)	2.40–2.18 (*m*)	2.04 (*m*)	2.48–2.06 (*m*)
H-14	5.84 (*s*)	5.83 (*s*)	5.86 (*s*)	6.84 (*brs*)	6.21 (*brs*)
H-15	–	–	–	6.08 (*brs*)	7.18 (*brs*)
H-16α	4.72 (*s*)	4.76 (*s*)	4.76 (*s*)	–	7.34 (*s*)
H-16β	4.72 (*s*)	4.76 (*s*)	4.76 (*s*)	–	
Me-17	0.86 (*d*, 7.0)	1.10 (*d*, 7.0)	1.18 (*d*, 7.0)	0.82 (*d*, 7.0)	0.92 (*d*, 7.0)
Me-19	1.24 (*s*)	1.45 (*s*)	1.36 (*s*)	1.21 (*s*)	1.24 (*s*)
Me-20	0.78 (*s*)	1.08 (*s*)	1.08 (*s*)	0.78 (*s*)	0.78 (*s*)
–COOMe	3.76 (*s*)	3.76 (*s*)	–	3.78 (*s*)	3.79 (*s*)
OH-6	4.86 (*brs*)	6.14 (*brs*)	2.88 (*brs*)	5.10 (*brs*)	4.88 (*brs*)
OH-7	–	2.97 (*brs*)	–	–	–
OH-15	–	–	–	3.94 (*brs*)	–

[a] Multiplicity and coupling constant (*J* in Hz) are in parenthesis; the assignments were made on the basis of ^{1}H–^{1}H COSY, NOESY and HMBC data.

compounds (Rustaiyan et al., 1981; Misra et al., 1979). However, the isolated compound contains no furan ring at C-12 but instead of it an α,β-unsaturated-γ-lactone moiety (δ 5.84, 1H, *s*, H-14 and 4.72, 2H, *s*, H_2-16) is present. The compound is also not a free acid but a methyl ester (δ 3.76, 3H, s). Additionally, the new compound contains a hydroxyl group (δ 4.86, 1H, *brs*). The ^{13}C NMR spectrum of **1** showed the signals characteristic of clerodane diterpenoids (Rustaiyan et al., 1981).

Table 2
^{13}C NMR spectral data (δ in ppm) of compounds **1**–**5**[a]

Carbon	1	2	3	4	5
C-1	17.1	16.7	17.6	17.5	17.3
C-2	26.8	27.2	27.2	27.1	27.1
C-3	138.2	140.7	129.6	140.6	138.3
C-4	140.5	141.7	140.4	140.9	141.4
C-5	44.1	44.5	41.8	44.8	44.8
C-6	74.8	75.0	72.9	74.3	74.4
C-7	35.2	75.4	88.0	35.9	36.2
C-8	34.3	36.7	36.8	34.0	34.0
C-9	38.1	38.2	39.5	38.6	38.6
C-10	45.1	45.6	46.0	45.5	45.5
C-11	33.4	37.8	41.7	35.7	38.6
C-12	22.1	22.3	23.1	18.6	17.9
C-13	169.8	170.1	169.7	138.5	125.3
C-14	114.2	115.4	115.5	142.9	110.8
C-15	173.3	171.3	170.0	96.7	142.7
C-16	72.2	72.9	72.2	171.5	138.9
C-17	15.2	12.4	11.9	15.5	15.6
C-18	170.8	173.8	173.6	171.7	171.7
C-19	16.3	18.2	17.2	16.5	16.4
C-20	17.2	19.6	20.3	17.1	17.6
–COOMe	52.2	52.5	–	52.4	52.2

[a] The assignments were made on the basis of APT, HSQC and HMBC data.

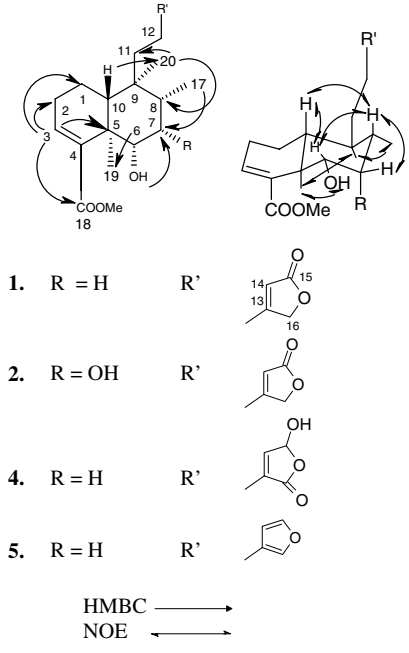

Fig. 1. Selected HMBC and NOE correlations of **1**, **2**, **4** and **5**.

The molecule was found to contain 21 carbons including two carbonyls (lactone and ester) and four double bonded carbons along with two quarternary, three tertiary, six secondary and four primary carbons. The hydroxyl group in **1** was placed at C-6 as the H-6 (δ 3.64, 1H, *dd*, J = 10.0, 7.0 Hz) showed clear correlation with C-19 (δ 16.3) in the HMBC spectrum (Fig. 1). The H-3 was related to C-1, C-2, C-5 and –COOMe and H-10 to C-20. The spectrum also showed the correlation between the proton of OH-6 and C-7 and the latter with Me-17. The NOESY experiment (Fig. 1) suggested the compound **1** to be of ent-clerodane type (Rustaiyan et al., 1981). The H-10 did not show any correlation with Me-19 but it showed correlation with H-8 (δ 1.62, 1H, *m*), which was again related to H-6. The proton of OH-6 was also related to Me-19. These correlation indicated that the hydroxyl at C-6 is with α-configuration. Me-19 was also correlated with Me-20 and the latter with Me-17. All these spectral data clearly confirmed the structure and stereochemistry proposed for the compound **1**.

Compound **2** was isolated as a viscous mass. Its molecular formula was decided to be $C_{21}H_{30}O_6$ from its elemental analysis and ^{1}H and ^{13}C NMR spectra (indicating the presence of 30 protons and 21 carbons, respectively). The EIMS was not informative about the molecular ion peak and the fragmentation pattern but LSIMS showed the peak at *m/z* 379 ($M^{+\cdot}$ + 1). The IR spectrum revealed the presence of hydroxyl group and ester and γ-lactone functionalities. The ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2) suggested that the structure of **2** is similar to that of **1** but the former contains an additional –OH group. This is also supported by a comparison of the molecular formula of **2** with that of **1**. The ^{1}H NMR spectrum showed the presence of two hydroxyl groups (δ 6.14, 1H, *brs* and 2.97, 1H, *brs*) along with three methyls (δ 1.10, 3H, *d*, J = 7.0 Hz, Me-17; 1.45, 3H, *s*, Me-19 and 1.08, 3H, *s*, Me-20), one methyl ester (δ 3.76, *s*, 3H) and two olefinic protons (δ 6.84, 1H, *t*, J = 3.5 Hz, H-3 and 5.86, 1H, *s*, H-14). The number of carbon atoms in both **1** and **2** were same but the latter possesses four tertiary carbons while the former three. The HMBC experiment (Fig. 1) decided the positions of the two hydroxyl groups at C-6 and C-7 as H-6 (δ 3.48, *d*, J = 3.5 Hz) showed correlation with Me-19 while H-7 (δ 3.98, *t*, J = 3.5 Hz) with Me-17, H-10 was related to Me-20 and H-3 to C-1, C-2 and –COOMe. In the NOESY experiment (Fig. 1). H-10 (δ 1.32, *dd*, J = 10.0, 3.0 Hz) was not correlated to Me-19 but it was related to H-8 (δ 1.68–1.54, *m*) which was again related to H-6 and H-7. The two hydroxyl groups at C-6 and C-7 are thus with α-configuration. Me-20 was also related to Me-19 and Me-17, all having similar α-configuration. The structure and stereochemistry of **2** was thus completely decided.

Compound **3** was obtained as white crystals. Its molecular formula was assigned to be $C_{20}H_{26}O_5$ from its elemental analysis, ^{1}H and ^{13}C NMR spectra (indicating the presence of 26 protons and 20 carbons) and LSIMS [*m/z* 347 ($M^{+\cdot}$ + 1)]. The EIMS was not informative. The IR spectrum indicated the presence of hydroxyl group and unsaturated γ and δ lactones in the molecules. The ^{1}H and ^{13}C NMR spectral data of **3** (Tables 1 and 2) suggested that structurally the compound is similar to that of **1** and **2** but the former contains no ester function. The ^{1}H NMR spectrum showed the presence of one hydroxyl (δ 2.88, 1H, *brs*) and three methyl groups (δ 1.18, 3H, *d*, J = 7.0 Hz, Me-17; 1.36, 3H, *s*, Me-19 and 1.08, 3H, *s*, Me-20). The downfield shift of H-7 (δ 4.42, *t*, J = 3.5 Hz) and the absence of ester moiety decided that three contains a δ-lactone function involving C-4 and C-7. The ^{13}C NMR spectrum (Table 2) also suggested the presence of this δ-lactone. The HMBC experiment (Fig. 2) showed that H-6 (δ 3.61, *d*, J = 3.5 Hz) was related to C-19 (δ 17.2) and C-8 (δ 36.8) while H-7 to C-5 (δ 41.8) and C-17 (δ 11.9). The hydroxyl group was thus placed at C-6. In the NOESY experiment (Fig. 2) H-10 (δ 1.42, *dd*, J = 10.0, 3.0 Hz) did not show correlation with Me-19 but showed correlation with H-6 and H-8 (δ 1.63–1.44, *m*). H-7 was found to be related to H-6 and Me-17. Inspection of the structure of **3** made by molecular model suggested that such NOE correlations are expected when lactone ring is at the β position and H-6, H-7 and H-8 are with β, α, and β configurations, respectively. If H-7 were with β orientation it could not show NOE correlation with Me-17. However, the coupling constant of H-7 was only 3.5 Hz possibly due to the distortion of the B ring having the lactone ring at the β position. All the spectral data are thus in agreement with the structure and stereochemistry of **3**.

Compound **4** was isolated as viscous mass. Its molecular formula was deduced to be $C_{21}H_{30}O_6$ from its elemental analysis and ^{1}H and ^{13}C NMR spectra

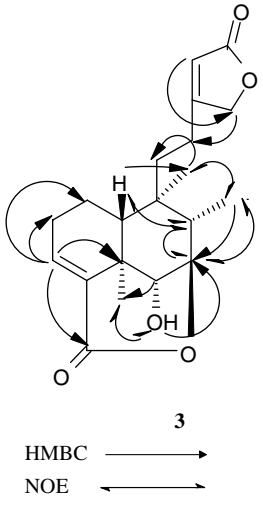

Fig. 2. Selected HMBC and NOE correlations of **3**.

(indicating the presence of 30 protons and 21 carbons, respectively). The molecular ion peak was not observed in the mass spectra (EIMS and LSIMS) but the peaks at *m/z* 265 and 113 arising from the fragment at C-11 to C-12 appeared in the EIMS. The molecular formula of **4** was similar to that of **2**. The IR spectrum indicated the presence of hydroxyl, ester and γ-lactone functions. The ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2) suggested that the compound **4** is structurally related to **1** and **2**. The functionalities present in both the rings A and B of **4** and **1** were exactly similar. However, the former contains an additional –OH group which was reasonably placed at C-15 and the carbonyl group of the lactone moiety at C-16 as H-15 and H-14 appeared at δ 6.08 (1H, *brs*) and 6.84 (1H, *brs*), respectively. The ^{13}C NMR spectrum (Table 2) revealed the presence of four tertiary carbons among which two are oxygenated. The HMBC experiment (Fig. 1) clearly showed that H-6 (δ 3.62, *dd*, $J = 10.0$, 3.5 Hz) was related to C-19 (δ 16.5) while H-15 to C-13 (δ 138.5) indicating the presence of two hydroxyl groups at C-6 and C-15. H-10 (δ 1.30, *dd*, $J = 10.0$, 3.0 Hz) showed correlation with C-19 (δ 16.5) and C-20 (δ 17.1). However, the NOE spectrum (Fig. 1) displayed that this proton was not related to Me-19 (δ 1.21, 3H, *s*) and Me-20 (δ 0.78, 3H, *s*) but related to H-6 and H-8 (δ 1.78–1.42, *m*) suggesting that the protons at C-6, C-8 and C-10 are all with β-configuration. The proton of HO-6 (δ 5.10, *brs*) was also related to Me-19. The structure and stereochemistry of **4** were thus clearly settled.

Compound **5** was obtained as viscous mass. Its elemental analysis, ^{1}H and ^{13}C NMR spectra (indicating the presence of 30 protons and 21 carbons, respectively), and LSIMS [*m/z* at 347 ($M^{+\cdot} + 1$)] suggested the molecular formula to be $C_{21}H_{30}O_4$. The IR spectrum of the compound showed the presence of hydroxyl and unsaturated ester groups as well as furan moiety. The NMR (1D and 2D) spectra suggested that the structure and stereochemistry of **5** are similar to those of **1** except the former contains a furan ring at C-12 instead of a lactone moiety present in **1**. In the downfield region of the ^{1}H NMR spectrum four signals (δ 7.34, 1H, *brs*, H-16; 7.18, 1H, *brs*, H-15; 6.74, *t*, $J = 3.8$ Hz, H-3 and 6.21, 1H, *brs*, H-14) were observed. The spectrum also revealed the presence of methyl ester group (δ 3.79, 3H, *s*), three methyls (δ 1.24, 3H, *s*, Me-19; 0.92, 3H, *d*, $J = 7.0$ Hz, Me-17 and 0.78, 3H, *s*, Me-20) and a hydroxyl group (δ 4.88, 1H, *brs*). The ^{13}C NMR spectrum showed the signals for 21 carbons including those for a furan ring (δ 125.3, C-13; 110.8, C-14; 142.7, C-15 and 138.9, C-16) and a methyl ester (δ 171.7 and 52.2). The H-6 (δ 3.62, *dd*, $J = 10.0$, 7.0 Hz) showed clear correlation with C-19 (δ 16.4) in the HMBC experiment (Fig. 1) but not with Me-19 in the NOESY experiment (Fig. 1) indicating 6α position of the –OH group. The proton of this hydroxyl group also showed NOE corre-

Table 3
Antibacterial activity of the compounds **1**–**5**[A]

Compound	*Bacillus subtilis*		*Bacillus sphaericus*		*Staphylococcus aureus*		*Klebsiella aerogenes*		*Chromobacterium violaceum*	
	a	b	a	b	a	b	a	b	a	b
1	8	12	8	11	7	9	7	12	7	9
2	7	9	8	12	8	11	6	9	8	12
3	7	11	7	9	6	9	6	8	7	9
4	8	11	8	12	7	10	7	9	8	12
5	7	12	6	9	6	9	6	9	7	10
Penicillin G streptomycin	15		14		12		23		24	

[a] Compound with a concentration of 30 μg/ml was tested against the organism.
[b] Compound with a concentration of 100 μg/ml was tested against the organism.
[A] Results were counted after 24 h of treatment and inhibitory zone diameters were measured in mm.

lation (Fig. 1) with Me-19. The NOESY experiment also proved that the stereochemistry of other chiral centers (C-5, C-8, C-9 and C-10) of **5** are similar to that of **1**. The structure of **5** with stereochemistry was thus properly deduced.

The methyl esters **1**, **2**, **4** and **5** are not artifact as MeOH was not used in the extraction, isolation and purification of these compounds. The original extract also showed (TLC) the presence of these compounds.

Along with the isolated dieterpenoids (**1**–**5**) two other known constituents, 3′,5,6-trihydroxy-3,4′,7-trimethoxyflavone (Wilson et al., 1968) and 2-methyl-5-hydroxy-chroman-4-one (Anderson et al., 1983) were also isolated. These two compounds were not reported earlier from the title species.

A fresh sample of the plant material was extracted under nitrogen atmosphere. The extract was observed (TLC) to contain all the diterpenoids (**1**–**5**) along with flavonoid and the chromanone discussed above indicating that any one of these compounds was not an air-oxidation product.

The antibacterial activity of the new constituents **1**–**5** was evaluated (Table 3) by following the Agar cup bioassay method employed (Srinivas et al., 2003) earlier by us. The compounds showed moderate activity against the Gram-positive organisms, *Bacillus subtilis*, *Bacillus sphaerius* and *Staphylococeus aureus*. However, they were less active against the Gram-negative organisms, *Kelbsiella aerogenes* and *Chromobacterium violaceum* and inactive against *Pseudomonas aeruginosa*.

3. Experimental

3.1. General

Melting points were measured in a Buchi-510 apparatus and are uncorrected. The IR spectra were recorded on a Perkin–Elmer RX1 FT-IR spectrophotometer, the NMR spectra on a Varian Gemini-200 MHz spectrometer and the mass spectra on a VG-Micromass 7070H and Finnigan-MAT 1020 instruments. Optical rotations were measured on a JASCO DIP-360 polarimeter. Column chromatography was performed with silica gel (BDH, 100–200 mesh) and TLC with silica gel GF_{254}. Antibacterial activity was studied following our reported method (Srinivas et al., 2003).

3.2. Plant material

The aerial parts of *P. wightiana* were collected from Tirumala hill, Andhra Pradesh in September, 2002 and identified by Professor C. Rajugopal, Department of Botany, Osmania University, Hyderabad. A voucher specimen (No. PW-AP-2) is preserved in our laboratory and another voucher specimen (No. IICP 101002) in IICT herbarium.

3.3. Extraction and isolation

The shade dried plant material (6 kg) was powdered and extracted with hexane (12 l) for 72 h at room temperature. The defatted plant material was subsequently extracted thrice with $CHCl_3$, each extraction was continued for 72 h with 12 l of solvent. The total extract was concentrated to afford a thick brown mass (42.2 g). The residue (42 g) was subjected to column chromatography over silica gel using hexane–EtOAc as eluent. Separation of the components in the mixture was monitored by TLC. The following compounds were eluted according to the increasing order of polarity: chromanone (15 mg), compound **5** (12 mg), compound **1** (98 mg), flavonoid (17 mg), compound **4** (14 mg), compound **2** (14 mg) and compound **3** (18 mg) (the compounds were eluted with 3%, 7%, 15%, 20%, 25% and 30% EtOAc in hexane, respectively).

3.4. Compound *1*

White crystals, m.p. 200–201 °C (MeOH), $[\alpha]_D^{25}$ −34.9 (*c* 1.08, $CHCl_3$); IR ν_{max} (KBr) cm^{-1}: 3422, 1751 (*br*), 1677, 1634, 1018; ^1H and ^{13}C NMR: Tables 1 and 2; EIMS: *m/z* (rel. int.) 330 (42), 313 (18), 216 (12), 117(100), 111(16). Anal. Calcd. for $C_{21}H_{30}O_5$: C, 69.61; H, 8.29%. Found: C, 69.73; H, 8.21%.

3.5. Compound *2*

Viscous, $[\alpha]_D^{25}$ −31.1 (*c* 0.6, $CHCl_3$); IR ν_{max} (KBr) cm^{-1}: 3460, 1752 (*br*), 1638, 1220; ^1H and ^{13}C NMR: Tables 1 and 2; LSIMS: *m/z* (rel. int.) 379 ($M^{+\cdot}$ + 1) (2%). Anal. Calcd. for $C_{21}H_{30}O_6$: C, 66.67; H, 7.94%. Found: C, 66.52; H, 8.02%.

3.6. Compound *3*

White crystals, m.p. 205–206 °C (MeOH), $[\alpha]_D^{25}$ −37.3 (*c* 0.8, $CHCl_3$); IR ν_{max} (KBr) cm^{-1}: 3411, 1740 (br), 1622, 1450,1133; ^1H and ^{13}C NMR: Tables 1 and 2; LSIMS: *m/z* (rel. int.) 347 ($M^{+\cdot}$ + 1) (2%). Anal. Calcd. for $C_{20}H_{26}O_5$: C, 69.36; H, 7.51%. Found: C, 69.43; H, 7.48%.

3.7. Compound *4*

Viscous, $[\alpha]_D^{25}$ −16.3 (*c* 0.4, $CHCl_3$); IR ν_{max} (KBr) cm^{-1}: 3423, 1762 (*br*), 1684, 1762 (*br*),1684,1438, 1256; ^1H and ^{13}C NMR: Tables 1 and 2; EIMS: *m/z* (rel. int.) 265 (2), 209 (6), 164 (32), 120 (90), 113 (4). Anal. Calcd. for $C_{21}H_{30}O_6$: C, 69.61; H, 8.29%. Found: C, 69.52; H, 8.34%.

3.8. Compound **5**

Viscous, $[\alpha]_D^{25}$ −33.8 (*c* 1.12, $CHCl_3$); IR ν_{max} (KBr) cm^{-1}: 3382, 1730, 1476, 1240, 955, 885; ^1H and ^{13}C NMR: Tables 1 and 2; LSIMS: *m/z* (rel. int.) 347 ($M^{+\cdot}$ + 1). Anal. Calcd. for $C_{21}H_{30}O_4$: C, 72.83; H, 8.67%. Found: C, 72.73; H, 8.74%.

Acknowledgements

The authors thank CSIR and UGC, New Delhi, for financial assistance. They are also thankful to the referees for their valuable positive comments.

References

Anderson, J.R., Edwards, R.L., Whalley, A.J.S., 1983. Metabolites of the higher fungi. Part 21. 3-Methyl-3,4-dihydroisocoumarins and related compounds from the Ascomycete family Xylariaceae. Journal of Chemical Society Perkin Transactions I, 2185–2192.

Annon., 1969. The Wealth of India: Raw Materials, vol. VIII, CSIR, New Delhi, p. 317.

Bohlman, F., Knoll, K.-H., El-Emary, N.A., 1979. Newartige sesquiterpene lactone aus *Pulicaria crispa*. Phytochemistry 18, 1231–1233.

Chiplunkar, Y.G., Nagasampagi, B.A., 1992. Isolation and structure elucidation of a new isopimarane from *Pulicaria wightiana*. Journal of Natural products 55, 1328–1329.

Mansour, R.M.A., Ahmed, A.A., Melek, F.R., Saleh, N.A.M., 1990. The flavonoids of *Pulicaria incisa*. Fitoterapia 61, 186–187.

Misra, R., Panday, R.C., Dev, S., 1979. Higher isoprenoids – VIII: diterpenoids from the oleoresin of *Hardwickia pinnata* part 1: hardwickiic acid. Tetrahedron 35, 2301–2310.

Nurmukhamedova, M.R., Abdullaeu, N.D., Sidyakin, G.P., 1986. Diterpenoids of *Pulicaria salviifolia*, II, structure of salvicin. Khimiya Prirodnykl Soedinenii, 299–301.

Rustaiyan, A., Simozar, E., Ahmadi, A., Grenz, M., Bohlmann, F., 1981. A hardwickiic acid derivative from *Pulicaria ghaphalodes*. Phytochemistry 20, 2772–2773.

Srinivas, K.V.N.S., Rao, Y.K., Mahender, I., Das, B., Rama Krishna, K.V.S., Hara Kishore, K., Murty, U.S.N., 2003. Flavonoids from *Caesalpinia pulcherima*. Phytochemistry 63, 789–793.

Wilson, R.G., Bowie, J.H., Williams, D.H., 1968. Solvent effects in NMR spectroscopy: solvent shifts of methoxyl resonance in flavones induced by benzene; an aid to structure elucidation. Tetrahedron 24, 1407–1414.