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ISOLATION AND STRUCTURE OF WIGHTIONAL AND WIGHTIOLIDE FROM *ANDROGRAPHIS WIGHTIANA*

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Key Word Index—*Andrographis wightiana*; Acanthaceae; diterpenes; wightional, wightiolide and wightionolide; ^{1}H and ^{13}C NMR spectra.

Abstract—Two new *ent*-labdane-type diterpenoids, wightional and wightiolide have been isolated from the leaves of *Andrographis wightiana*. Their structures were determined on the basis of extensive ^{1}H and ^{13}C, ^{1}H homonuclear COSY, and HMBC NMR spectroscopic studies. To correlate the newly isolated diterpenes with wightionolide, a number of derivatives of wightionolide have been prepared and characterized.

INTRODUCTION

Andrographis wightiana is a widely distributed herb found in the western Ghats, the hills of Travancore and the Annamalais Hills of India [1]. Isolation and structural determination of wightionolide (**1**), the only example of an *ent*-labdane-type diterpene lactone having geminal hydroxymethyl groups at the C-4 position, was reported earlier [2]. The structure of **1** was derived from chemical and degradation studies and an X-ray crystal diffraction analysis of 13,14-dibromo-17,19,20-tri-*O*-acetylwightionolide. The major diterpene in the leaves of *A. wightiana* is **1**. We report in this communication, on the structural determination of two new minor diterpenes, wightional (**2**) and wightiolide (**7**), isolated from the same plant. Some bicyclic *ent*-labdane-type diterpenoids related to **1** and **2** are andrograpanin [3], nivenolide [4], andrographolide [5, 6] and 14-deoxyandrographolide [5, 7].

RESULTS AND DISCUSSION

From a chloroform extract of the aerial parts of *A. wightiana* Arn. ex Nees (family Acanthaceae) collected in the Annamalais Hills, the diterpenoids **2** and **7** were isolated by chromatographic separation. Compound **2**, mp 99–100°, was homogeneous by TLC, and its EI mass spectrum and elemental analysis were consistent with the molecular formula $C_{20}H_{30}O_5$ $[M]^+$ m/z 350. The mass spectral fragments at m/z 322 $[M-28]^+$ and m/z 319 $[M-31]^+$ indicated that it contains a carbonyl and a hydroxymethyl group. The IR spectrum of **2** showed the presence of hydroxyl groups (3520 and 3450 cm^{-1}), an α,β-unsaturated γ-lactone (1730 cm^{-1}) and an aldehyde (1720 cm^{-1}). The presence of the α,β-unsaturated γ-lactone was also supported by a positive Legal colour reaction [8], the signals for a vinyl proton at δ 7.15 (1H, *d*, J = 1 Hz) and the signals for an adjacent methylene at δ 4.85 (2H, *s*) in its ^{1}H NMR spectrum. The signal at δ 9.54 (1H, *d*, J = 5 Hz) indicated the presence of a secondary aldehydic group at C-8. Its occurrence along with **1**, and the ^{1}H NMR doublet, suggested that the aldehydic group may be located at C-8 and not at C-18 or C-19. The ^{1}H and the ^{13}C NMR spectra of wightional (Table 1) are consistent with the proposed structure **2**. The ^{1}H–^{1}H COSY correlations are given in Table 2. The ^{13}C NMR spectrum of **2** showed nearly similar signals for most of the carbons, indicating that the diterpene is a mixture of two epimers, **2A** and **B**, in an approximate ratio of 3:2. The two epimers are most probably due to the aldehydic group, and purification by chromatographic methods failed to separate the epimers.

As accurate ^{1}H and ^{13}C NMR assignments for **1** were not made in the earlier work [2], ^{1}H, ^{13}C, DEPT, ^{1}H–^{1}H COSY and HMBC NMR experiments were performed (Table 1). Table 2 shows the significant ^{1}H and ^{13}C long-range correlations observed in the HMBC spectrum and the ^{1}H–^{1}H COSY correlations. The ^{13}C NMR data provide important information in deriving the structures of newly isolated diterpenoids. Some of the earlier ^{13}C NMR spectral data for labdanic diterpenoids depended on multiplicities in the SFORD spectra, making use of the changes produced by substituent(s) and by comparison of the shifts produced

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Table 1. ^{1}H and ^{13}C NMR spectral data for compounds **1** ($CDCl_3$), **2** ($CDCl_3$ + 1 drop of DMSO-d_6) and **7** ($CDCl_3$), δ (ppm); multiplicity (J in Hz)

C	1	2a	2b	7	H	1	2	7
1	38.2 *t*	38.6	39.8 *t*	38.6 *t*	1	1.80 *m*	1.80 *m*	1.90 *m*
						0.85 *m*	0.90 *m*	1.1 *m*
2	17.6 *t*	—	17.6 *t*	18.3 *t*	2a	1.50 *m*	1.55 *m*	1.57 *m*
3	30.0 *t*	30.2	30.7 *t*	30.0 *t*	3a	2.00 *m*	2.00 *m*	1.90 *m*
					3b	1.00 *m*	0.90 *m*	1.00 *m*
4	41.7 *s*	41.9	42.2 *s*	42.2 *s*	4	—	—	—
5	51.3 *d*	51.9	52.4 *d*	52.8 *d*	5	1.00 *m*	1.11 *m*	1.05 *m*
6	26.7 *t*	26.4	26.9 *t*	24.5 *t*	6a	1.60 *m*	1.71 *m*	2.00
					6b	1.25 *m*	1.35 *m*	1.30
7	30.7 *t*	—	36.5 *t*	38.3 *t*	7a	1.85 *m*	1.71 *m*	1.58 *m*
					7b	1.10 *m*	1.12 *m*	
8	52.1 *d*	53.4	53.7 *d*	147.1 *s*	8	0.80 *m*	2.25 *m*	—
9	41.3 *d*	50.4	50.6 *d*	56.4 *d*	9	1.38 *m*	1.20 *m*	1.96 *m*
10	37.7 *s*	37.5	38.1 *s*	39.6 *s*	10	—	—	—
11	21.4 *t*	20.4	22.2 *t*	21.8 *t*	11a	1.60 *m*	1.75 *m*	1.75 *m*
					11b	1.40 *m*	1.40 *m*	1.80 *m*
12	26.9 *t*	26.9	27.2 *t*	24.7 *t*	12a	2.35 *m*	2.40 *m*	2.42 *m*
					12b	2.22 *m*	2.20 *m*	2.12 *m*
13	134.3 *s*	133.9	134.2 *s*	134.8 *s*	13	—	—	—
14	144.3 *d*	144.4	144.5 *d*	144.1 *d*	14	7.14 *t*	7.15 *d*	7.09 *s*
						(J = 1)	(J = 1)	
15	70.0 *t*	70.1	70.2 *t*	70.2 *t*	15	4.77 *dd*	4.85 *s*	4.76 *dd*
						(J = 2, 1)		(J = 3, 1)
16	174.3 *s*	174.1	174.5 *s*	174.5 *s*	16	—	—	—
17	65.1 *t*	—	204.8 *d*	107.1 *t*	17a	3.63 *dt*	9.54 *d*	4.85 *d*
						(J = 10, 5)	(J = 5.4)	(J = 0.5)
					17b	3.55 *dt*		4.57 *d*
						(J = 10, 5)		(J = 0.5)
					17-OH	2.82 *t*	—	—
						(J = 5)		
18	63.8 *t*		63.8 *t*	64.3 *t*	18a	3.86 *ddd*	3.85 *dd*	3.86 *d*
						(J = 11, 6, 1)	(J = 11, 4)	(J = 11)
					18b	3.70 *ddd*	3.72 *dd*	3.67 *dd*
						(J = 11, 6, 1)	J = 11, 4)	(J = 11, 0.5)
					18-OH	3.60 *dd*		
						(J = 6.5, 5)		
19	72.3 *t*	70.1	73.1 *t*	73.5 *t*	19a	3.86 *dd*	3.80 *dd*	3.88 *dd*
						(J = 11, 5)	(J = 11, 2)	(J = 11, 0.5)
					19b	3.32 *dd*	3.40 *dd*	3.35 *dd*
						(J = 11, 5)	(J = 11, 2)	(J = 11, 0.5)
					19-OH	3.63		
20	14.4 *q*	14.6	15.0 *q*	15.1 *q*	20	0.76 *s*	0.8, 0.82 *s*	0.63 *s*

by closely related compounds [9, 10]. The assignments for C-6 and C-11 made earlier [9, 10] in the labdane diterpenoids were reversed in later studies [3, 11] of andrographolides. The ^{13}C NMR assignments of some andrographolides have been made recently on the basis of 2D COSY and COLOC spectral data [12].

Attempts were made to prepare **2** from **1**. The hydroxymethyl groups at C-4 were protected by formation of an acetonide (**3**) by the treatment of **1** with acetone and 70% $HClO_4$. Similarly, the dihydroacetonide (**4**) was prepared from 13,14-dihydrowightionolide (**5**) [2]. Oxidation of **3** by the Pfitzner–Moffatt procedure [13] furnished the aldehyde (**6**), mp 136–138°. Several attempts to hydrolyse the acetonide–aldehyde (**6**) under different acidic conditions gave a gum which showed a TLC spot corresponding to **2**, but which failed to give **2**. In a model experiment, we were able to cleave the acetonide by heating **3** with 5% HCl at 90° for 15 min to afford **1** in *ca* 86% yield. However, the acetonide group of the aldehyde (**6**) could not be cleaved under a variety of acidic conditions to afford the desired product, probably because of condensation of the aldehyde with the generated alcohol under the acidic conditions.

Wightiolide **7**, obtained as a colourless crystalline compound, mp 155–157°, analysed for the molecular formula $C_{20}H_{30}O_4$ (EI mass spectrum, $[M]^+$ m/z 334). The IR spectrum showed the presence of hydroxyl (3415) and α,β-unsaturated γ-lactone (1720 cm^{-1}) groupings. The ^{1}H NMR spectrum exhibited signals at δ 7.09 (1H, *s*, H-14) and 4.76 (2H, *dd*, J = 3 and 1 Hz, H-15). The presence of an exocyclic methylene was

Table 2. ^1H–^1H COSY and ^1H–^{13}C HMBC NMR spectral data for compounds **1** and **7**

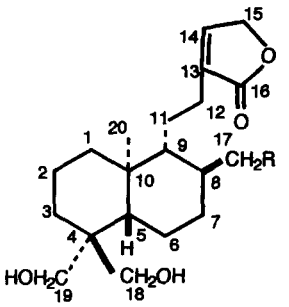
	1		**7**	
Position	COSY ^1H–^1H	HMBC $^1H \rightarrow ^{13}C$	COSY ^1H–^1H	HMBC $^1H \rightarrow ^{13}C$
1a	H-1b, H-2, H-3a	C-2, C-3, C-5, C-10, C-20	H-2, H-3a, H-3b	C-2, C-5, C-10, C-20
2	H-1a, H-3a	C-1, C-3, C-4, C-10	H-1, H-3a	C-1, C-4, C-10
3a	H-1, H-2, H-3b	C-1, C-2, C-4, C-5, C-18	H-1, H-2, H-3b	—
5	H-6a	C-4, C-7, C-10, C-18, C-19, C-20	H-6b, H-19b, CH_3-20	C-1, C-4, C-10
6a	H-5, H-6b, H-7a	C-5, C-7, C-10	H-6b, H-7a, H-7b	C-1, C-4, C-5, C-9
7a	H-6a, H-6b, H-7b, H-8	C-8, C-9, C-17	H-6, H-7a, H-17a	C-8
8	H-9	C-6, C-7, C-9, C-10, C-17, C-20	—	—
9	H-8, H-11a	C-7, C-8, C-10	—	C-5, C-8, C-10, C-17, C-20
11a	H-11b, H-12b, H-17a, H-17b	C-9, C-10, C-12	H-12a, H-12b, CH_3-20	C-8, C-12, C-13
12a	H-11a, H-11b, H-12b, H-14	C-13, C-14, C-16	H-11a, H-11b, H-12b, H-14	C-9
12b	H-11a, H-11b, H-12a, H-14	C-13, C-14, C-16	H-11a, H-11b, H-12a, H-14	C-13, C-14
14	H-12a, H-15	C-12, C-13, C-15, C-16	H-12a, H-12b, H-15	C-13, C-15, C-16
15	H-14	C-12, C-13, C-14, C-16	H-12a, H-12b, H-14	C-13, C-14, C-16
17a	H-11a, H-17b, OH-17	C-7, C-8, C-9	H-7a, H-7b, H-17b	C-6, C-7, C-9
17b	H-11a, H-17a, OH-17	C-7, C-8, C-9	H-7a, H-7b, H-17a	C-7, C-8, C-9
17-OH	H-17a, H-17b	—	—	—
18a	H-18b, OH-18	C-3, C-4, C-5, C-19	H-18b	C-3, C-4, C-5
18b	H-18a	C-3, C-4, C-5	H-18a	C-3, C-4, C-5
18-OH	H-18a	—	—	—
19a	H-19b, OH-19	C-3, C-4, C-5, C-18	H-5, H-19b	—
19b	H-19a	C-3, C-4, C-5	H-19a	C-3, C-4, C-5
20	—	C-1, C-5, C-10	H-5, H-11	C-1, C-5, C-9, C-10

evident from its ^1H NMR spectrum, δ 4.57 and 4.85 (1H each, *d*, $J = 0.5$ Hz), and the ^{13}C NMR resonances at δ 147.1 (*s*, C-8) and 107.1 (*t*, C-17). In addition, the ^1H NMR spectrum of **7** showed the presence of a *tert*-methyl group at δ 0.63, two methylenes attached to hydroxyl groups at δ 3.67 and 3.88 (each 1H, *dd*, $J = 11$ and 0.5 Hz), and 3.35 and 3.86 (each 1H, *dd*, $J = 11$ and 0.5 Hz). Its occurrence in the plant along with **1** suggested structure **7** for the diterpene. The ^1H and the ^{13}C NMR data (Table 1) are in conformity with the proposed structure. The ^{13}C NMR signals were analysed by the aid of a DEPT spectrum. Table 2 gives the ^1H–^{13}C long-range COSY connectivities observed in the HMBC experiment, and the ^1H–^1H correlations. To transform **1** into **7**, the acetonide (**3**) was converted to the 17-acetate (**8**), the 17-benzoate (**9**), the 17-*p*-toluenesulphonate (**10**) and the 17-*p*-bromobenzensulphonate (**11**). All attempts to form the alkene from these esters under pyrolytic conditions failed to give the desired product. The acetonide (**3**) on treatment with $POCl_3$ in pyridine afforded 17-ω-chlorowightionolide (**12**). The *p*-toluenesulphonyl ester (**10**) on refluxing with NaI gave the corresponding 17-ω-iodo derivative (**13**). Several attempts to dehydrohalogenate under a variety of conditions failed to give the desired olefin. The acetonide **13** on treatment with methanol-5% HCl gave 17-ω-iodowightionolide (**14**), which also failed to give **7**. Functionalization of the hydroxymethyl group at C-17 posed no problems; however, the compounds **12** and **13** failed to dehydrohalogenate to give the olefin, probably due to steric hindrance of the lactonic side chain. The structures of **2** and **7** are unusual in that there are no other *ent*-labdane-type diterpenoids, except **1**, which have both geminal methyls at the C-4 position being hydroxylated.

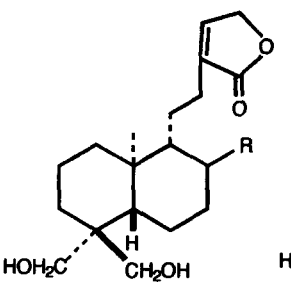
EXPERIMENTAL

General. Mps were determined by open capillary method and are uncorr. IR spectra were recorded on Perkin-Elmer Infracord Spectrophotometer. EIMS were run on an Atlas-Varian Mat CH-7 instrument. ^1H and ^{13}C NMR spectra were recorded on Varian XL-400, at 400 MHz for ^1H and 100 MHz for ^{13}C spectrometers unless stated otherwise.

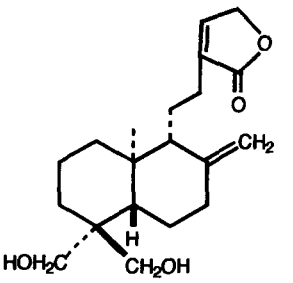
Plant material. The aerial parts of *A. wightiana* were collected in the Annamalias Hills in 1964–1965. The plant was identified by (the late) Mr T. S. N. Rao and a voucher specimen is deposited in the Herbarium of the



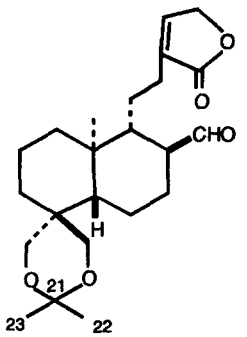
1 Wightionolide R = OH
5 13,14-Dihydrowightionolide R = OH
12 ω-Chlorowightionolide R = Cl
14 ω-Iodowightionolide R = I



2a Wightional R = α-CHO
2b Wightional R = β-CHO



7 Wightiolide



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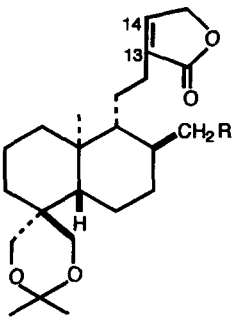

3 R = OH
4 R = OH (13,14-dihydro)
8 R = OAc
9 R = OBz
10 R = OSO_2-(Me-substituted phenyl)
11 R = OSO_2-(Br-substituted phenyl)
13 R = I

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Isolation of wightionolide (**1**), *wightional* (**2**) *and wightiolide* (**7**). The leaves of *A. wightiana* (10 kg) were extracted with $CHCl_3$ (25 l × 2) at 25°, and the extract was evapd *in vacuo* to a thick syrup, diluted with EtOAc and kept overnight. The ppt. was collected (30 g) and chromatographed over silica gel (900 g), eluted with $CHCl_3$–5% MeOH, and the sepn was monitored by TLC. The initial frs afforded **1** (11.5 g). For ^1H and ^{13}C NMR spectra: see Table 1.

Further elution and purification by prep. TLC afforded **7** (45 mg; 0.00045%), mp 155–157° (TLC: $CHCl_3$–5% MeOH, R_f 0.65); Found: C, 71.6; H, 9.1. $C_{20}H_{30}O_4$ requires: C, 71.8; H, 9.0%. MS (direct inlet): *m/z* 334 (M^+, 60%), 316 ($M^+ - H_2O$, 30), 303 ($M^+ - CH_2OH$, 35), 298 (316 − H_2O, M* 281, 30), 286 (316 − OH, M* 245, 100), 285 (45), 271 (40), 258 (70). IR (nujol): ν_{max} 3415, 1712, 1625, 1300, 1210, 1158, 1145, 1095, 1075, 1062, 1053, 1038, 1027, 1010, 995, 980, 943, 886, 869, 855, 842, 830, 720 cm^{-1}. For ^1H and ^{13}C NMR spectra: see Table 1.

From the above prep. TLC, **2** was isolated; (220 mg; 0.0022%), mp 99–100°; $[\alpha]_D$ −12.6° (*c* 0.38, MeOH), (TLC: $CHCl_3$–5% MeOH, R_f 0.7). Found: C, 68.1; H, 9.0. $C_{20}H_{30}O_5$ requires: C, 68.5; H, 8.6%. MS: *m/z* 350 (M^+, 5%), 332 ($M^+ - H_2O$, 20), 322 ($M^+ - CO$, 30), 319 ($M^+ - CH_2OH$, 16), 301 (319 − H_2O, 44), 286 (301 − CH_3, 44), 283 (46), 274 (90), 271 (60). IR (nujol): ν_{max} 3520, 3450, 2720, 1730, 1720, 1640, 1300, 1270, 1248, 1203, 1171, 1149, 1130, 1098, 1080, 1052, 1038, 1020, 1000, 975, 960, 942, 895, 868, 848, 830, 803, 770, 720 cm^{-1}. For ^1H and ^{13}C NMR spectra: see Table 1.

17-*Hydroxy*-18,19-*isopropylidenedioxy*-ent-*labd*-13-*en*-16,15-*olactone* (**3**). A soln of **1**, (5 g) in Me_2CO (300 ml) was stirred with 70% $HClO_4$ (5 ml) at room temp. for 1.5 hr. $NaHCO_3$ (5 g) was added and stirred for 10 min. The solvent was evapd *in vacuo* and H_2O (100 ml) was added. The mixt. was extracted with $CHCl_3$, the organic layer washed with H_2O and dried over Na_2SO_4. Removal of the solvent gave a gum which on crystallization from EtOAc afforded colourless crystals (**3**, 4 g), mp 130–131°. ^1H NMR (250 MHz, $CDCl_3$): δ 7.10 (1H, *s*, H-14), 4.74 (2H, *s*, H-15), 3.75, 3.15 (2H, AB, *J* = 11.4 Hz, H-19), 3.63 (2H, *m*, H-17), 3.62 (2H, *m*, H-18), 1.33, 1.29 (each 3H, *s*, $-CMe_2$), 0.67 (3H, *s*, Me-20). ^{13}C NMR (62.9 MHz, $CDCl_3$): 38.3 (C-1), 17.6 (C-2), 33.3 (C-3), 38.2 (C-4), 53.2 (C-5), 26.9 (C-6), 30.6 (C-7), 51.0 (C-8), 41.4 (C-9), 38.0 (C-10), 21.5 (C-11), 27.0 (C-12), 134.4 (C-13), 144.4 (C-14), 70.8 (C-15), 174.6 (C-16), 65.6 (C-17), 62.0 (C-18), 70.1 (C-19), 14.4 (C-20), 98.6 (C-21), 21.6 (C-22), 25.9 (C-23).

Hydrolysis of **3** *to wightionolide* (**1**). A soln of **3** (40 mg) in MeOH (5 ml) and 5% HCl (40 ml) was warmed on a water-bath at *ca* 90° for 15 min. The solvent was evapd *in vacuo* and the residue crystallized from EtOAc to give **1** (30 mg), mp 176–177°.

17-*Hydroxy* - 18,19 - *isopropylidenedioxy*-ent-*labd* -

13,14 - *dihydro* - 16,15 - *olactone* (**4**). A soln of **5** (100 mg) in Me_2CO (200 ml) was stirred with 70% $HClO_4$ (0.2 ml) for 30 min. $NaHCO_3$ (1 g) was added and the solvent evapd *in vacuo*. The mixt. was added to H_2O and extracted with $CHCl_3$, washed with H_2O and dried (Na_2SO_4). Removal of the solvent *in vacuo* gave a viscous liquid which was sublimed at 150°/10^{-3} mm to afford **4** as an oil which was homogeneous by TLC. Found C, 70.2; H, 10.0. $C_{23}H_{38}O_5$ requires: C, 70.0; H, 9.7%.

18,19 - *Isopropylidenedioxy* - 17 - *oxo* - ent - *labd* - 13 - *en* - 16,15 - *olactone* (**6**). To a stirred soln of **3**, (500 mg) in DMSO (5 ml), C_6H_6 (5 ml) and pyridine (5 ml) was added dicyclohexylcarbodiimide (800 mg). The soln was stirred for 1 hr and kept at room temp. for 40 hr. To the mixt. was added C_6H_6 (30 ml) and a soln of oxalic acid (500 mg) in MeOH (95 ml). This was stirred for 30 min and filtered to remove dicyclohexylurea. The filtrate was washed with 10% $NaHCO_3$ (50 ml) and then with H_2O, and the organic layer dried over Na_2SO_4. The solvent was removed *in vacuo* to give a gummy residue (450 mg). This was chromatographed over silica gel in C_6H_6 and the major fr. crystallized from C_6H_6–Et_2O to afford colourless needles (**6**, 140 mg), mp 136–138°. Found: C, 70.9; H, 9.1. $C_{23}H_{34}O_5$ requires: C, 70.7; H, 8.8%. IR (nujol): ν_{max} 1740, 1690, 1640 cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$): δ 9.60 (1H, *d*, $J = 4$ Hz, CHO-17), 7.10 (1H, *t*, H-14), 4.75 (2H, *q*, $J = 1.5$ Hz, H-15), 3.10–3.90 (4H, *m*, OCH_2-18, 19), 1.35 (6H, *s*, $-CM_2$), 0.75 (3H, *s*, Me-20). ^{13}C NMR (DMSO-d_6 + $CDCl_3$): δ 38.1 (C-1), 17.4 (C-2), 33.6 (C-3), 37.8 (C-4), 52.8 (C-5), 26.5 (C-6), 37.5 (C-7), 53.2 (C-8), 49.2 (C-9), 38.1 (C-10), 20.3 (C-11), 26.9 (C-12), 132.9 (C-13), 145.0 (C-14), 70.4 (C-15), 173.6 (C-16), 204.1 (C-17), 61.5 (C-18), 70.0 (C-19), 14.3 (C-20), 98.3 (C-21), 21.7 (C-22), 26.0 (C-23).

Acetoxy-18,19-*isopropylidenedioxy*-ent - *labd* - 13 - *en* - 16,15 - *olactone* (**8**). To a soln of the acetonide (**3**, 180 mg), in HOAc (5 ml) was added Ac_2O (6 ml) at 5°, and the mixt. was kept at room temp. for 16 hr, when it was poured over crushed ice and extracted with $CHCl_3$. The organic layer was washed with $NaHCO_3$ and then with H_2O and dried over Na_2SO_4. Removal of the solvent gave a gum which crystallized from EtOAc–hexane to afford colourless needles (**8**, 125 mg), mp 114–115°. Found: C, 69.2; H, 9.2. $C_{25}H_{38}O_6$ requires: C, 69.1; H, 8.8%. IR (nujol): ν_{max} 1740, 1260, 1230 cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$): δ 7.12 (1H, *s*, H-14), 4.80 (2H, *m*, H-15), 3.20–4.20 (6H, *m*, $-OCH_2$-17, 18, 19), 2.05 (3H, *s*, OAc-17), 1.40 (6H, *s*, $-CMe_2$), 0.72 (3H, *s*, Me-20).

17-*Benzoyloxy* - 18,19 - *isopropylidenedioxy* - ent - *labd* - 13 - *en* - 16,15 - *olactone* (**9**). To a soln of **3**, (500 mg) in pyridine (1.5 ml) was added benozyl chloride (1.5 g) at 0° and the mixt. was kept at room temp. for 16 hr. The mixt. was poured over crushed ice and extracted with $CHCl_3$, washed with 10% HCl and H_2O and the organic layer dried (Na_2SO_4). Removal of the solvent *in vacuo* gave a gum which crystallized from EtOAc–hexane as colourless needles, mp 176–177°. Found: C, 72.7; H, 8.3. $C_{30}H_{40}O_6$ requires: C, 72.6; H, 8.1%. IR (nujol): ν_{max} 1740 α,β-unsaturated γ-lactone), 1720 (ester), 1455, 1375, 1350, 1310, 1280, 1225 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 8.00 (2H, *m*, 2′,6′-Ar-H), 7.58 (1H, *m*, 4′-Ar-H), 7.53 (2H, *m*, 3′,5′ Ar-H), 7.03, (1H, *s*, H-14), 4.70 (2H, *s*, H-15), 3.20, 3.65 (2H, AB, $J = 11.3$ Hz, H-19), 3.65 (2H, AB, $J = 11.7$ Hz, H-18), 2.21 (2H, *m*, H-12), 0.85, 2.15 (2H, *m*, H-7), 1.31, 1.69 (2H, *m*, H-6), 0.88, 1.80 (2H, *m*, H-1), 1.75 (1H, *m*, H-9), 1.51 (2H, *m*, H-2), 1.30 (2H, *m*, H-11), 1.30, 1.32 (each 3H, *s*, $-C(Me)_2$), 0.89 (1H, *m*, H-5), 0.88 (1H, *m*, H-8), 0.72 (3H, *s*, Me-20). ^{13}C NMR (62.9 MHz, $CDCl_3$): 38.297 *t* (C-1), 17.6 *t* (C-2), 31.1 *t* (C-3), 38.3 *s* (C-4), 52.0 *d* (C-5), 26.6 *t* (C-6), 33.3 *t* (C-7), 53.4 *d* (C-8), 38.9 *d* (C-9), 38.3 *s* (C-10), 21.5 *t* (C-11), 27.2 *t* (C-12), 134.1 *s* (C-13), 144.3 *d* (C-14), 70.8 *t* (C-15), 174.1 *s* (C-16), 68.1 *t* (C-17), 62.0 *t* (C-18), 70.0 *t* (C-19), 14.4 *q* (C-20), 98.7 *s* (C-21), 21.5 *q* (C-22), 25.8 *q* (C-23), 166.6 *s* (–O–CO), 130.2 *s* (C-1′), 129.5 *d* (C-2′, C-6′), 128.4 *d* (C-3′, C-5′), 133.0 *d* (C-4′). The assignments are based on HETCOR and DEPT spectra.

18,19 - *Isopropylidenedioxy* - 17 - p - *toluenesulphonyloxy* - ent - *labd* - 13 - *en* - 16,15 - *olactone* (**10**). To a soln of **3** (1.5 g) in pyridine was added *p*-toluenesulphonyl chloride (1.3 g) at 0° and the mixt. was kept at room temp. for 3 hr. The mixt. was poured over crushed ice, the crude product collected by filtration and crystallized from CH_2Cl_2–EtOH to afford colourless needles (**10**, 1.5 g), mp 165–166°. Found: C, 66.2; H, 8.0. $C_{30}H_{42}O_7$ requires: C, 65.9; H, 7.7%. IR (nujol) ν_{max} 1740, 1630, 1590 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 7.74 (2H, *d*, $J = 7.5$ Hz, 2′,6′-Ar-H), 7.10 (1H, *br s*, H-14), 7.30 (2H, *d*, $J = 7.5$ Hz, 3′,5′ Ar-H), 4.73 (2H, *s*, H-15), 3.98 (2H, *m*, H-18), 3.15–3.68 (2H, AB, $J = 9.5$ Hz, H-19), 3.56 (2H, *t*, $J = 9.4$ Hz, H-17), 2.44 (3H, *s*, Ar-Me), 2.14 (2H, *m*, H-12), 1.30, 1.32 (each 3H, *s*, $-CMe_2$), 0.73 (3H, *s*, Me-20). ^{13}C NMR (62.9 MHz, $CDCl_3$): 38.9 (C-1), 17.6 (C-2), 30.3 (C-3), 39.1 (C-4), 50.4 (C-5), 26.4 (C-6), 33.2 (C-7), 53.1 (C-8), 39.0 (C-9), 39.1 (C-10), 21.7 (C-11), 26.7 (C-12), 132.7 (C-13), 144.8 (C-14), 70.8 (C-15), 174.1 (C-16), 70.1 (C-17), 62.0 (C-18), 73.4 (C-19), 14.2 (C-20), 98.7 (C-21), 21.7 (C-22), 25.6 (C-23), 133.0 (C-1′), 129.9 (C-2′, C-6′), 127.9 (C-3′, C-5′), 133.8 (C-4′).

17-p-*Bromobenzenesulphonyloxy* - 18,19 - *isopropylidenedioxy* - ent - *labd* - 13 - *en* - 16,15 - *olactone* (**11**). To a soln of **3** (500 mg) in pyridine (1 ml) was added *p*-bromobenzenesulphonyl chloride (600 mg) at 0° and the mixt. was kept at room temp. for 3 hr. The mixt. was poured over crushed ice, the ppt. collected by filtration (600 mg) and crystallized from 80% EtOH to afford colourless plates, mp 148–150°. Found: C, 57.1; H, 6.8. $C_{29}H_{39}BrO_7S$ requires: C, 57.0; H, 6.4%.

17-ω-*Chlorowightionolide* (**12**). To a soln of **3** (120 mg) in pyridine (0.5 ml), was added $POCl_3$ (0.5 ml) at 0° and kept for 14 hr. The mixt. was poured over

crushed ice, extracted with $CHCl_3$, washed with 10% HCl and H_2O, dried over Na_2SO_4 and the solvent removed *in vacuo*. The residue was chromatographed over silica gel and crystallized from EtOAc to afford colourless plates (**12**, 25 mg), mp 190–191°. Found, C, 65.0; H, 8.6. $C_{20}H_{31}ClO_4$ requires: C, 64.8; H, 8.4%.

17-*ω*-*Iodo*-18,19-*isopropylidenedioxy*-ent-*labd*-13-*en*-16,15-*olactone* (**13**). To a soln of **10** (1 g) in dry Me_2CO (200 ml) was added NaI (500 mg) and the mixt. heated under reflux for 22 hr. The residue was filtered, washed with Me_2CO and the filtrate evapd *in vacuo* and crystallized from EtOH to afford colourless plates (**13**, 600 mg), mp 149–150°. Found: C, 54.8; H, 7.3. $C_{23}H_{35}IO_4$ requires: C, 55.0, H, 7.0%. ^{1}H NMR (60 MHz, $CDCl_3$): δ 7.20 (1H, *t*, H-14), 4.85 (2H, *d*, $J = 2$ Hz, H-15), 3.10–3.90 (6H, *m*, $-OCH_2$-17, 18, 19), 1.50 (6H, *s*, $-CMe_2$), 0.80 (3H, *s*, Me-20).

17-*ω*-*Iodowightionolide* (**14**). A soln of **13** (300 mg) in MeOH (30 ml) and 5% HCl (20 ml) was heated on a water-bath at 80° for 10 min. The solvent was removed *in vacuo*, H_2O (25 ml) added and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O, dried over Na_2SO_4 and the solvent evapd to give a gum which crystallized from CH_2Cl_2–hexane to afford colourless needles (**14**, 165 mg), mp 134–135°. Found: C, 52.2; H, 6.8. $C_{20}H_{31}IO_4$ requires: C, 52.0; H, 6.8%. IR (nujol): ν_{max} 1740, 1455, 1452, 1370, 1260, 1220 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.12 (1H, *s*, H-14), 4.75 (2H, *s*, H-15), 3.72, 3.56 (2H, AB, $J = 11.5$ Hz, H-17), 3.15, 3.62 (2H, AB, $J = 11$ Hz, H-19), 3.26, 3.43 (2H, AB, $J = 9.4$ Hz, H-18), 1.33, 1.30 (each 3H, *s*, $-CMe_2$), 0.69 (3H, *s*, C-20). ^{13}C NMR (62.9 MHz, $CDCl_3$): 38.3 (C-1), 17.6 (C-2), 33.2 (C-3), 38.3 (C-4), 53.3 (C-5), 27.0 (C-6), 34.6 (C-7), 53.8 (C-8), 40.2 (C-9), 38.3 (C-10), 21.5 (C-11), 27.4 (C-12), 134.3 (C-13), 144.3 (C-14), 70.8 (C-15), 174.2 (C-16), 62.1 (C-17), 62.1 (C-18), 70.1 (C-19), 14.8 (C-20), 98.7 (C-21), 21.5 (C-22), 25.9 (C-23).

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REFERENCES

1. Gamble, J. S. (1956) *Flora of the Presidency of Madras*, Vol. II, p. 734. Botanical Survey of India, Calcutta, India.
2. Joshi, B. S., Kamat, V. N., Govindachari, T. R., Pai, B. R., Kartha, G. N. and Go, K. T. (1974) *J. Chem. Soc., Perkin* 1 2517.
3. Fujita, T., Fujitani, R., Takeda, Y., Takaishi, Y., Yamada, T., Kido, M. and Miura, I. (1984) *Chem. Pharm. Bull.* **32**, 2117.
4. Rojas, E. T. and Rodriguez-Hahn, L. (1978) *Phytochemistry* **17**, 574.
5. Cava, M. P., Chan, W. R., Haynes, L. J., Johnson, L. F. and Weinstein, B. (1962) *Tetrahedron* **18**, 397.
6. Cava, M. P., Chan, W. R., Stein, R. P. and Willis, C. R. (1965) *Tetrahedron* **21**, 2617.
7. Balamain, A. and Connoly, J. D. (1973) *J. Chem. Soc., Perkin 1* 1247.
8. Haynes, L. J. (1955) in *Modern Methods in Plant Analysis* (Peach, K. and Tracey, M. V., eds), Vol. II, p. 583. Springer, Berlin.
9. Buchwalter, A., Burfitt, I. R., Nagel, A. A., Wenkert, E. and Naf, F. C. (1975) *Helv. Chim. Acta* **58**, 1567.
10. Bastard, J., Duc, D. K., Fetizon, M., Francis, M. J., Grant, P. K., Weavers, R., Kaneko, C., Baddeley, G. V., Bernassau, J. M., Burfitt, I. R., Wovkulich, P. M. and Wenkert, E. (1984) *J. Nat. Prod.* **47**, 592.
11. Patra, A., Mitra, A. K., Biswas, S., Gupta, C. D., Basak, A. and Barua, A. K. (1981) *Org. Magn. Reson.* **16**, 75.
12. Fan, D., Wu, Y. and Qin, G. (1995) *Phytochem. Anal.* **6**, 262.
13. Pfitzner, J. G. and Moffatt, K. E. (1965) *J. Am. Chem. Soc.* **87**, 5661, 5670.