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Limonoids from *Turraea floribunda* (Meliaceae)

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Abstract

Six novel limonoids and limonoid derivatives, turraflorins D–I along with the known turraflorins A and B have been isolated from seed of the South African *Turraea floribunda* (Meliaceae).

Keywords: *Turraea floribunda*; Meliaceae; Limonoids; Turraflorins A, B, D, E, F, G, H and I

1. Introduction

Turraea floribunda (Hochstetter), commonly known as the wild honeysuckle tree, is a member of the Meliodeae subfamily of the Meliaceae family. The species is distributed throughout East Africa, being locally found along the east coast of South Africa stretching from the Transkei coast up to Mozambique (Moll, 1981). *T. floribunda* is a deciduous shrub growing in open woodland or among rocks, in forests and riverine bushland and can reach up to 13 m under ideal conditions. It has a white flower which is powerfully scented, especially at night. In local traditional medicine, rheumatism, dropsy and heart disease are treated with an infusion made from the roots and bark, while the bark alone is used by witchdoctors to induce a trance prior to performing divining dances. An overdose is reputed to be poisonous (Coates-Palgrave, 1984; Hutchings et al., 1996).

Previous analysis of the bark and rootbark has led to the isolation of havanensin-class limonoids (Akinniyi et al., 1986; Torto et al., 1995; Torto et al., 1996) and the seeds have previously yielded turraflorins A–C which are ring B opened limonoids of the turraflorin class (Mulholland et al., 1998; Fraser et al., 1994). The current re-investigation of the seeds was undertaken to re-isolate turraflorin A which was required as a starting material for a synthetic project. However, in addition to turraflorin A, **1**, and B, **2**, an additional three limonoids and four tetranortriterpenoids with oxidized furan rings were isolated. In previous work the complete assignment of the NMR spectra of turraflorins A and B could not be made, but has now been possible (Tables 1 and 2). Physical data for turraflorins A and B not reported previously are given in the experimental section. Turraflorins A, B, G and I, the compounds present in largest amounts, were submitted for screening in the NCI 60 cancer cell line screen but no significant activity was found.

2. Results and discussion

The minced seed of *T. floribunda* was extracted successively with hexane and dichloromethane in a soxhlet apparatus. Thin layer chromatography indicated that the two extracts were similar and they were combined prior to compounds being separated by means of column chromatography. Eight compounds were isolated, turraflorins A, **1**, and B, **2**, which had been isolated previously from this source and turraflorins D–I (**3–8**) which have not been reported previously.

Turraflorin D differed from turraflorin A, **1**, only in the presence of a 23-hydroxy-21,23-butenolide ring instead of the typical limonoid furan ring. This compound occurred as a mixture of inseparable epimers as is

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Table 1
^{1}H NMR data for Turraflorins A, B and D–I (400 MHz, $CDCl_3$) (values in square brackets relate to the minor epimer)

Proton	A	B	D	E	F	G	H	I
1	6.76 (*d*, 10.4)	6.76 (*d*, 10.4)	6.75 (*d*, 10.4) [6.73 (*d*, 10.4)]	6.68 (*d*,10.8) [6.71 (*d*, 11.9)]	6.74 (*d*, 10.4)	4.00 (*dd*, 9.3, 6.7)	4.42 (*brs*)	5.03 (brs, $W_{1/2} = 8$ Hz)
2	6.05 (*d*, 10.4)	5.91 (*d*, 10.4)	6.11 (*d*, 10.4)	6.03 (*d*, 10.4) [6.07 (*d*, 10.4)]	5.98 (*d*, 10.4)	2.70 (*m*)	α 2.40 (*m*)[a]	2.12 (*m*)[b]
							β 2.92 (*dd*, 15.5, 2.4)	2.03 (*m*)[b]
3	–	–	–	–	–	–	–	4.75 (brs, $W_{1/2} = 8$ Hz)
5	3.10 (*dd*, 7.3, 2.9)	3.24 (*m*)	3.14 (*dd*, 8.2,3.4)	3.17 (*dd*, 7.6, 3.1)	3.31 (*dd*, 7.3, 3.6)	2.75 (*m*)	2.79 (*m*)	2.57 (*m*)
6α	2.30 (*dd*, 16.6, 2.9)	2.40 (*m*)	2.36 (*dd*, 17.1, 2.4)	2.40 (*m*)	2.40 (*m*)	α 2.80 (*m*)	α 2.60 (*m*)[a]	α 2.60
β	2.38 (*dd*, 16.6, 7.7)		2.43 (*dd*, 17.2, 8.0)			β 2.38 (*m*)	β 2.40 (*m*)[a]	β 2.26
9	2.75 (*d*, 6.4)	2.74 (*d*, 6.3)	2.80 (*d*, 6.4) [2.79 (*d*, 6.4)]	2.84 (*m*)	2.78 (*d*, 6.6)	3.15 (*d*,8.2)	3.25 (*d*, 6.6)	3.57 (*d*, 6.8)
11	5.38 (*dd*, 11.0, 6.4)	5.26 (*dd*, 10.6, 6.2)	5.43 (*dd*, 10.8, 6.4)	5.51 (*m*) 5.44 (*dd*, 10.8, 6.2)	5.32 (*dd*, 10.6, 6.4)	4.18 (*dd*, 9.6, 8.0)	5.31 (*dd*, 11.3, 6.4)	5.23 (*dd*, 11.5, 6.7)
12	5.71 (*d*, 11.0)	4.24 (*d*, 10.6)	5.71 (*d*, 11.0)	5.63 (*m*) 5.51 (*m*)	4.32 (*d*, 10.6)	5.26 (*d*, 9.4)	5.88 (*d*, 11.3)	5.76 (*d*, 11.5)
15	5.65 (*brs*)	5.62 (*brs*)	5.67 (*brs*)	5.71 (*m*)	5.62 (*brs*)	5.75 (*brs*)	5.65 (*brs*)	5.76 (*dd*, 3.4, 2.3)
16α	2.38 (*m*)	2.45 (*m*)	2.91 (*ddd*, 17.1, 10.4, 2.2)	2.78 (*m*) [2.52]	2.50 (*m*)	2.34 (*m*)	2.40 (*m*)[a]	2.42 (*ddd*, 16.4, 11.0, 2.4)
β	2.60 (*ddd*, 16.5, 8.3, 2.8)	2.62 (*m*)	2.56 (*ddd*, 17.0, 9.9, 3.0) [2.68]	2.78 (*m*) [2.42]	2.65 (*m*)	2.58 (*ddd*, 16.6,8.4,3.1)	2.60 (*m*)[a]	2.61
17	3.29 (*dd*, 10.4, 8.4)	3.24 (*m*)	3.29 (*dd*, 18.0, 8.7)	3.34 (*m*) [3.28]	3.10 (*m*)	3.24 (*dd*, 10.6, 8.3)	3.30 (*m*)	3.23 (*dd*, 10.4, 8.4)
18	0.81 (*s*)	0.69 (*s*)	0.98 (*s*)	1.13 (*s*) [0.94 (*s*)]	0.72 (*s*)	0.77 (*s*)	0.87 (*s*)	0.84 (*s*)
19	0.95 (*s*)	0.94 (*s*)	0.99 (*s*)	0.99 (*s*)	0.97 (*s*)	0.90 (*s*)	1.19 (*s*)	1.33 (*s*)
21	7.14 (*brs*)	7.27 (*brs*)	–	5.89 (*m*)	–	7.14 (*brs*)	7.20 (*brs*)	7.18 (*s*)
22	6.17 (*brs*)	6.45 (*brs*)	6.94 (*brs*)	5.96 (*m*)	7.31 (*brs*)	6.20 (*brs*)	6.22 (*brs*)	6.22 (*brs*)
23	7.25 (*brs*)	7.27 (*brs*)	6.08 (*brs*)	–	4.86 (*brs*)	7.29 (*brs*)	7.30 (*brs*)	7.30 (*brs*)
28	0.93 (*s*)	0.97 (*s*)	0.97 (*s*)	0.98 (*s*)	1.03 (*s*)	0.94 (*s*)	1.05 (*s*)	0.81 (*s*)
29	1.01 (*s*)	1.02 (*s*)	1.06 (*s*)	1.05 (*s*)	1.06 (*s*)	1.03 (*s*)	1.10 (*s*)	1.02 (*s*)
30A	4.91 (*brs*)	4.88 (*d*, 1.3)	4.98 (*brs*)	5.05 (*brs*) [5.01]	4.93 (*d*, 1.6)	5.07 (*brs*)	5.07 (*brs*)	4.95 (*d*, 2.0)
B	5.21 (*d*, 1.3)	5.17 (*d*, 1.5)	5.27 (*brs*)	5.33 (*brs*) [5.29]	5.20 (*d*, 1.5)	5.35 (*brs*)	5.11 (*brs*)	5.13 (*d*, 1.8)
OCH_3	3.60 (*s*)	3.61 (*s*)	3.65 (*s*)	3.66 (*s*)	3.65 (*s*)	3.69 (*s*)	3.64 (*s*)	3.66 (*s*)
C-11 *Ac*	1.89 (*s*)	2.02 (*s*)	1.95 (*s*)	1.97 (*s*)	2.03 (*s*)	–	2.02 (*s*)	1.94 (*s*)
C-12 *Ac*	1.71 (*s*)	–	1.89 (*s*)	1.91 (*s*)	–	1.92 (*s*)	1.80 (*s*)	1.77 (*s*)
C-1 *Ac*	–	–	–	–	–	–	–	1.98 (*s*)
C-3 *Ac*	–	–	–	–	–	–	–	2.03 (*s*)

[a] Resonances superimposed, not possible to determine *J*.

[b] Not possible to assign as α or β.

Table 2
^{13}C NMR data for turraflorins A, B and D–I (400 MHz, $CDCl_3$) (data for minor epimer is given in brackets)

Carbon	A	B	D	E	F	G	H	I
1	152.3	153.1	152.2 [152.1]	151.7	152.8	82.1	73.4	75.8
2	125.3	124.4	125.5	125.3	124.7	41.5	42.3	24.9
3	203.8	204.0	204.0 [203.9]	203.4 [203.6]	203.8	213.7	214.3	75.1
4	46.3	46.1	46.4	46.2	46.2	47.4	48.8	39.1
5	44.1	43.6	44.1	43.6	43.8	49.0	45.3	38.6
6	31.5	31.2	31.6	31.3	31.5	30.9	32.5	33.4
7	174.2	173.9	174.3	174.0	173.9	174.4	174.1	174.5
8	139.1	139.3	138.6 [138.5]	137.5 [138.0]	139.3	140.0	139.2	139.1
9	52.5	52.1	52.5	52.0	51.9	55.9	50.5	50.5
10	42.3	42.0	42.4	42.3 [42.1]	42.1	48.2	46.1	47.1
11	72.4	75.3	72.3	71.1 [72.1]	73.6	79.9	73.8	74.0
12	77.0	77.2	76.9	77.2 [77.5]	76.2	77.3	76.3	76.9
13	52.3	52.7	53.5 [53.2]	52.9	54.0	51.6	52.2	52.2
14	148.7	149.5	147.8 [148.0]	148.9 [148.0]	149.6	148.8	149.7	149.9
15	123.6	122.6	123.6 [123.3]	122.6	121.4	122.7	123.0	123.6
16	37.9	36.1	34.9 [36.1]	37.1	35.4	37.8	37.3	37.7
17	48.3	49.3	48.0 [47.5]	48.7 [49.5]	48.2	46.8	48.5	48.8
18	15.7	13.8	15.8 [15.6]	15.4 [14.2]	14.3	15.1	15.0	15.2
19	21.6	21.4	21.7	21.5	21.5	19.2	21.0	19.3
20	123.9	124.4	136.7 [137.1]	167.6	133.8	124.3	123.7	123.9
21	140.3	139.9	171.1 [170.8]	99.7 [98.9]	176.1	139.8	140.0	140.3
22	111.2	111.9	147.3 [146.3]	119.7 [117.0]	147.5	111.1	111.0	111.3
23	142.6	142.3	96.0 [96.4]	170.4	70.8	142.3	142.4	142.6
28	23.3	22.9	23.3	23.0	23.1	27.0	26.4	27.8
29	22.9	22.6	22.9	22.7 [22.6]	22.7	19.6	24.2	24.6
30	119.1	118.3	119.8 [120.0]	120.6 [120.1]	118.8	118.0	120.1	121.4
OCH_3	52.3	52.0	52.4	52.2	52.1	52.0	51.8	51.9
C-11 CH_3COO	170.0	170.7	170.1	169.6	170.2	171.1	169.9	170.9
C-11 CH_3COO	20.9	21.0	21.0	20.9	21.1	21.2	21.2	21.6
C-12 CH_3COO	170.5	–	170.4	172.1	–	–	170.8	170.8
C-12 CH_3COO	20.8	–	20.9	20.8	–	–	20.8	21.0
C-1 CH_3COO	–	–	–	–	–	–	–	170.3
C-1 CH_3COO	–	–	–	–	–	–	–	21.6
C-3 CH_3COO	–	–	–	–	–	–	–	170.7
C-3 CH_3COO	–	–	–	–	–	–	–	21.5

common when a hemiacetal ring is present instead of the furan ring (Cheplogoi and Mulholland, 2003). The NMR data for the major epimer is reported in the text, but the NMR data for the minor epimer is also given in Tables 1 and 2. Turraflorin D is a ring B opened limonoid with a carbomethoxy group at C-7 indicated by a resonance at δ 174.3 (C-7) and a resonance due to the carbon of the methoxy group at δ 52.4 in the ^{13}C NMR spectrum and a three proton methoxy group proton resonance at δ 3.65 in the ^{1}H NMR spectrum. As is usual in ring B-opened limonoids, a 8,30-double bond was present with the non-equivalent H-30 protons occurring as broad singlets at δ 5.27 and δ 4.98 in the ^{1}H NMR spectrum and C-8 and C-30 occurring at δ 138.6 and 119.8, respectively, in the ^{13}C NMR spectrum. An α,β-unsaturated ketone was present in ring A with H-1 and H-2 occurring as a pair of doublets at δ 6.75 and δ 6.11 ($J = 10.4$ Hz) and C-1, C-2 and C-3 occurring at δ 152.2, δ 125.5 and δ, 204.0, respectively. Ring D contained a 14,15-double bond with resonances due to C-14 and C-15 occurring at δ 147.8 and δ 123.6, respectively, and H-15 occurring at δ 5.67 in the ^{1}H NMR spectrum. Acetate esters were present at C-11β and C-12α as in turraflorin A. The COSY spectrum indicated coupling between H-9 (δ 2.80, *d*, 6.4 Hz), H-11 (δ 5.43, *dd* 10.8, 6.4 Hz), and H-12 (5.71, *d*, 11.0 Hz). Literature investigations (Mulholland et al., 1998) have shown that when ring B is open, the C-11β,C-12α-substitution pattern is indicated by large coupling constants ($J_{9,11} = 6–8$ Hz; $J_{11,12} = 7–11$ Hz) and with the C-11α, C-12α-substitution pattern, the coupling constants are each less than 4 Hz. The 11β,12α-substitution pattern for turraflorin D was confirmed by use of the NOESY spectrum. The H-12β resonance was shown to correlate with the H-17β and the 3H-19 resonances indicating that the ester at C-12 must have the α-orientation. Likewise, the H-11α resonance showed correlations with the H-9 and 3H-18 (which are both α) resonances. The methyl group resonances were assigned using the HMBC spectrum. The C-12, 13, 14 and 17 resonances showed correlations with the resonance at δ 0.98 which was assigned to 3H-18; the C-1, 5, 9 and 10 resonances showed correlations with the resonance at δ 0.99 which was assigned to 3H-19 and the C-3, 4 and 5 resonances showed correlations with resonances at δ 0.97 and δ 1.06 which could be assigned to 3H-28 and 3H-29, respectively, due to a correlation seen in the NOESY spectrum between the 3H-28 and H-5 resonances.

The structure of the oxidized furan ring remained to be determined. The ^{13}C NMR spectrum indicated resonances due to the presence of a lactonic carbonyl group at δ 171.1, a hemiacetal carbon resonance at δ 96.0 and a trisubstituted double bond with a fully substituted carbon resonance at δ 138.6 and a methine carbon resonance at δ 147.8. There are two possible structures which could fit this data: a 20,22-double bond with a C-23 lactone and C-21 hemiacetal carbon, or a structure with a 20, 22-double bond with a C-21 lactone and C-22 hemiacetal carbon. Compounds containing both these types of sidechains have been isolated from *Turraea parvifolia* (Cheplogoi and Mulholland, 2003). An analysis of the NMR data for compounds from *T. parvifolia* shows that in the former case the C-21 hemiacetal carbon resonance occurs at approximately δ 99 and C-23 occurs at δ 171 and, in the latter case, with C-23 being the hemiacetal group carbon, C-23 occurs at δ 96 and C-21 at δ 170. Thus the hemiacetal carbon resonance may be used to distinguish between the two structures. In turraflorin D, the hemiacetal carbon resonance occurs at δ 96.0 indicating that turraflorin D has the second possible structure, i.e. a 23-hydroxy-21,23-butenolide ring. Coupling between the H-22 and H-23 resonances in the COSY spectrum is very weak, however, strong correlations between the H-22 and H-23 resonances in the NOESY spectrum and a correlation between the C-21 carbonyl carbon and H-17 support this structure. Thus structure **3** is assigned to turraflorin D.

The molecular ion was not seen in the HRMS, but fragment ions due to the loss of one and two molecules of acetic acid were detected at *m/z* 510.2215 and 450.2053. The base peak in the spectrum occurred at *m/z* 210, due to $C_{12}H_{18}O_3$ which arose due to cleavage of the C-9, C-10 bond.

Turraflorin E, **4**, was found to be the 21-hydroxy-23, 21-butenolide isomer of turraflorin D with the hemiacetal carbon resonance occurring at δ 99.7 (C-21) and the lactone carbonyl carbon (C-23) at δ 170.4 in the ^{13}C NMR spectrum. Turraflorin E was also synthesized by oxidizing turraflorin A with selenium dioxide and H_2O_2. The aim of the synthesis was to attempt oxidation at C-16 of turraflorin A, however, this reaction did not occur, but the furan ring became oxidized.

The structure of turraflorin F was similar to those of **3** and **4**, except that it was a monacetate and that the sidechain ring was different. No pairing of peaks occurred in the ^{13}C NMR spectrum, indicating that no hemiacetal group was present. The COSY spectrum indicated coupling between H-9 (δ 2.78, *d*, 6.6 Hz), H-11α (δ 5.32, *dd*, 10.6, 6.4 Hz) and H-12β (δ 4.32, *d*, 10.6 Hz). The chemical shift of H-12, in comparison to **3** and **4**, indicated that a hydroxyl group instead of an acetoxy group was present in turraflorin F. The sidechain ring consisted of a C-20,22-double bond (δ 133.8, δ 147.5) an oxymethylene carbon (δ 70.8) and a lactone carbonyl carbon (δ 176.1). The H-22 resonance (δ 7.31) was seen to be strongly coupled to the two proton methylene proton resonance at δ 4.86 in the COSY and NOESY spectra, indicating that the methylene carbon resonance must be assigned to C-23 and the lactone carbonyl carbon to C-21. Thus structure **5** is proposed for turraflorin F. The highest peak in the mass spectrum occurred at *m/z* 452.2199. This was due to the loss of an acetic acid molecule from the molecular ion.

Turraflorin G was found to be a limonoid with resonances ascribable to the β-substituted furan ring carbons at δ 142.3, 139.8, δ 124.3 and δ 111.1. Ring B was again opened to give a carbomethoxy group at C-7 and the 8, 30; 14, 15-diene was present. The compound was a monoacetate. The COSY spectrum indicated coupling between H-9 (δ 3.15, *d*, 8.2 Hz), H-11 (δ 4.18, *dd*, 9.6, 8.0 Hz) and H-12 (δ 5.26, *d*, 9.4 Hz) and the HMBC spectrum showed a correlation between the corresponding C-12 resonance (δ 77.3) and the acetate methyl group proton resonance, confirming that the acetate group was present at C-12. No 1, 2-double bond was present, but a keto group was present at C-3. The C-3 resonance occurred at δ 213.7, considerably downfield of its position in the α,β-unsaturated ketones described previously. The HMBC spectrum showed a correlation between the C-3 resonance and an oxymethine resonance at δ 4.00 which was assigned to H-1. Two structures were possible: a 1,11-dihydroxy compound or a 1,11-oxide. A HMBC correlation between the C-1 resonance at δ 82.1 and the H-11 resonance suggested that a 1,11-oxide was present and this was supported by the facts that no hydroxyl group stretch was present in the IR spectrum and that acetylation attempts were unsuccessful. The stereochemistry at C-1 and C-11 was confirmed as C-1α, C-11β by correlations in the NOESY spectrum between the H-1 and the 3H-19 resonances and between the H-11 and H-9 resonances. The HRMS spectrum did not show the molecular ion, but a peak at 436.2264 indicated the loss of an acetic acid molecule. Thus structure **6** was assigned to turraflorin G.

Turraflorin H was found to be a limonoid whose structure differed from that of turraflorin G in that instead of a 1,11-oxide, an acetate group is present at C-11β as in turraflorins A, D, E and I and a hydroxyl group is present at C-1α. The H-1 resonance occurred as a broad singlet at δ 4.42 and the corresponding C-1 resonance at δ 73.4 showed correlations in the HMBC spectrum with the H-9 and 3H-19 resonances. The assignment of the stereochemistry of H-1 as β was again deduced by a correlation in the NOESY spectrum between the 3H-19 and H-1 resonances. The molecular ion was not detected in the HRMS. The highest peak in the spectrum occurred at *m/z* 541.2418, corresponding to the loss of a methyl group from the proposed structure. Other significant peaks occurred at *m/z* 481.2229 corresponding to the loss of a methyl group and a molecule of acetic acid and at *m/z* 436.2231, corresponding to the loss of two acetic acid molecules from the molecular ion. Thus structure **7** was assigned to turraflorin H.

Turraflorin I was found to be a limonoid which differed in the substitution pattern on ring A when compared to turraflorin H. The C-3 keto group carbon resonance was absent from the ^{13}C NMR spectrum and an additional oxymethine resonance occurred at δ 75.1. The molecule was a tetra-acetate, and acetate groups were placed at C-1, C-3, C-11 and C-12. The COSY spectrum showed coupling between the H-1 (δ 5.03), the two non-equivalent H-2 (δ 2.12, δ 2.03) and H-3 (δ 4.75) resonances. The stereochemistry at C-1 was confirmed to be the same as in turraflorin H, by a correlation seen between the H-1 and the 3H-19 resonances in the NOESY spectrum. The stereochemistry at C-3 could not be determined using the NOESY spectrum, however, the $W_{1/2}$ value of 8 Hz for the H-3 resonance indicated that the orientation of H-3 was β, and hence the acetate group was in the α-orientation (Ferguson et al., 1975). A HRMS could not be obtained for the sample as it appeared to decompose on analysis. However, a GCMS analysis showed the highest peak occurring at *m/z* 642, which corresponded to the molar mass of the proposed structure. Thus structure **8** is proposed for turraflorin I.

3. Experimental

Seeds of *T. floribunda* (291 g) were collected in Durban in September, 2000, and a voucher specimen retained (Fraser 01, NH). The seeds were dried and successively extracted with hexane, dichloromethane, ethyl acetate extract and methanol for 24 h each using a soxhlet apparatus. After removal of solvent, t.l.c. analysis of the hexane (15 g) and dichloromethane extracts (10 g) indicated that they were very similar, so they were combined and then separated using column chromatography over silica gel (Merck 9385) using a dichloromethane: ethyl acetate step gradient, starting with 100% dichloromethane and gradually increasing the amount of ethyl acetate to 15%. This yielded: turraflorin A, **1**; turraflorin B, **2**; turraflorin D, **3**; turraflorin E, **4**; turraflorin F, **5**; turraflorin G, **6**; turraflorin H, **7**; turraflorin I, **8.** NMR anaylsis of the crude ethyl acetate and methanol extracts showed no limonoids were present so these extracts were not investigated further.

Optical rotations were measured at room temperature in chloroform using a Perkin–Elmer 241 polarimeter with a 10 cm flow tube. IR spectra were recorded with a Nicolet Impact 400 D spectrometer on sodium chloride plates and calibrated against an air background. HRMS were obtained using a Kratos high resolution MS 9/50 spectrometer at the Cape Technikon. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Inova 400 MHz NMR spectrometer.

*3.1. Turraflorin A (**1**)*

White crystals (680 mg); m.p. 142–144 °C; EIMS: *m/z* 538 [M^{+}], 478 [M$^{+}-$CH$_3$COOH], 418 [M$^{+}-$2CH$_3$COOH], 403, 269, 227, 196(100), 81, 43; $[\alpha]_D = +17$ ($c = 0.071$, CHCl$_3$, 25°); IR: ν_{max} (NaCl) cm^{-1}: 2951 (C–H stretch), 1743 (C=O stretch, ester), 1683, (C=O stretch, α,β-unsaturated ketone), 1372, 1237 (C–O stretch, acetate ester) and 1043 (C–O stretch).

3.2. Oxidation of turraflorin A

Turraflorin A (58 mg) was dissolved in *tert*-butanol (8 ml) in a two-necked round-bottomed flask. A catalytic amount of SeO_2 (1 mg) was added. The flask was warmed to 40° and aq. H_2O_2 (50%, 1 ml) was added. The temperature was maintained at 45° and monitored using t.l.c. until all starting material had disappeared. The reaction mixture was worked up by adding benzene (5 ml), washing three times with saturated ammonium sulphate solution and drying over sodium sulphate before filtering and leaving to evaporate in the air. This yielded a colourless oil, which, after purification using column chromatography, yielded turraflorin E in a yield of 15%.

*3.3. Turraflorin B (**2**)*

White crystals (800 mg); m.p. 139–142 °C; EIMS: *m/z* (rel. int.): 436 [M$-$CH$_3$CO$_2$H]$^{+}$, 421 [M$-$CH$_3$CO$_2$H$-$CH$_3$]$^{+}$, 279, 210, 157, 129, 81, 43 (100); $[\alpha]_D = +89°$($c =$ 0.178, CHCl$_3$,25°); IR: ν_{max} (NaCl) cm^{-1}: 3469 (O–H stretch), 2977 (C–H stretch), 2927 (C–H stretch), 1743 (C=O stretch, ester), 1678 (C=O stretch; α,β-unsaturated ketone), 1446, 1370, 1236 (C–O stretch, acetate) and 1038 (C–O stretch).

*3.4. Turraflorin D (**3**)*

Amorphous (13.1 mg); HRMS: 510.2215 ($C_{31}H_{38}O_{10}$req. 510.2254; M$^{+}-$CH$_3$CO$_2$H); EIMS: *m/z* (rel. int.): 510.2215 (5) [M$-$CH$_3$CO$_2$H]$^{+}$, 450.2053 (15) [M$-$2× CH$_3$CO$_2$H]$^{+}$, 259.0987 (26), 241.0890 (58), 210.1255 (100), 149.0967 (45), 137.0959 (44); IR ν_{max} (NaCl) cm^{-1}: 3416 (O–H stretch), 2925 (C–H stretch), 2854 (C–H stretch), 1739 (C=O stretch, ester), 1683 (C=O stretch, α,β-unsaturated ketone), 1463, 1371, 1237 (C–O stretch, acetate) and 1045 (C–O stretch).

3.5. Turraflorin E (**4**)

Yellow amorphous (8.8 mg); EIMS : m/z (rel. int.) : 450 $[M-2\times CH_3CO_2H]^+$, 241, 207 (100), 165, 141, 73, 43; IR: ν_{max} (NaCl) cm^{-1}: 3437 (O–H stretch), 2977 (C–H stretch), 2951 (C–H stretch), 1747 (C=O stretch, ester), 1682 (C=O stretch, α,β-unsaturated ketone), 1371, 1237 (C–O stretch, acetate) and 1047 (C–O stretch).

3.6. Turraflorin F (**5**)

Pale yellow amorphous (11.1 mg); HRMS : 452.2199 ($C_{29}H_{36}O_8$– CH_3COOH req. 452.2199); EIMS m/z (rel. int.): 452.2199 (17) $[M-CH_3CO_2H]^+$, 434.2082 (3) $[M-CH_3CO_2H-H_2O]^+$, 392.1970 $[M-2CH_3CO_2H]^+$, 243.1020 (1 0 0) $[M-CH_3CO_2H- C_{12}H_{17}O_3]^+$, 225.0905 (28), 210.1246 (68), 149.0956 (45), 137.0968 (45); $[\alpha]_D = +97°$ ($c = 0.092$, $CHCl_3$, 25°); IR: ν_{max} (NaCl) cm^{-1}: 3453 (O–H stretch), 2978 (C–H stretch), 2929 (C–H stretch), 1738 (C=O stretch, ester), 1678 (C=O stretch, α,β-unsaturated ketone), 1436, 1372, 1233 (C–O stretch, acetate) and 1055 (C–O stretch).

3.7. Turraflorin G (**6**)

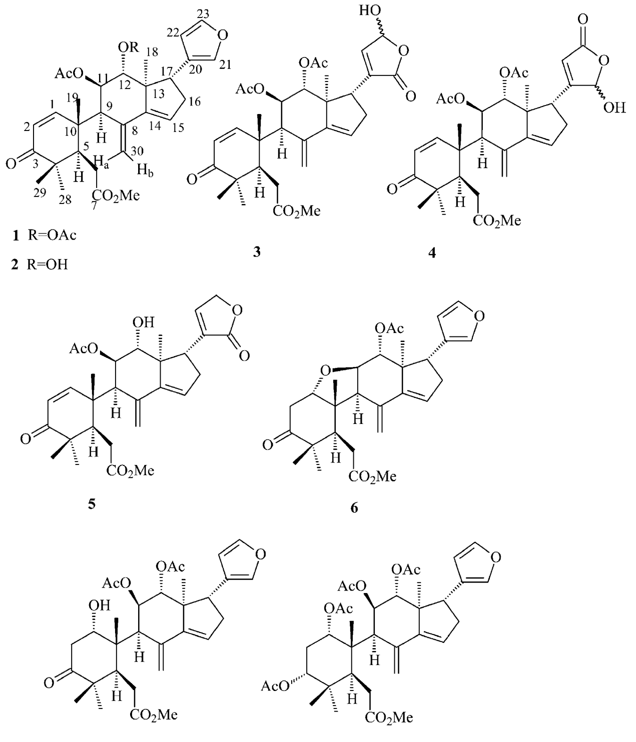
White crystalline (611 mg); m.p. 122–124 °C (CH_2Cl_2/ CH_3OH); HRMS : 436.2264 ($C_{29}H_{36}O_7$– CH_3COOH req. 436.2250); EIMS: m/z (rel. int.): 436.2264 (53) $[M-CH_3CO_2H]^+$, 421.2033 (1 0 0) $[M-CH_3CO_2H- CH_3]^+$, 279.1378 (51), 227.1066 (22), 210.1047 (61); $[\alpha]_D = +77°$ ($c = 0.218$, $CHCl_3$, 25°); IR: ν_{max} (NaCl) cm^{-1}: 2968 (C–H stretch), 2930 (C–H stretch), 1737 (C=O stretch, ester), 1370, 1235 (C–O stretch, acetate) and 1030 (C–O stretch).

3.8. Turraflorin H (**7**)

Fine white crystalline (13.2 mg); m.p. 134–135 °C; HRMS 541.2438 ($C_{31}H_{40}O_9$–CH_3 req. 541.2438); EIMS: m/z (rel. int.): 541.2438 (5) $[M-CH_3]^+$, 481.2229 (6) $[M-CH_3-CH_3CO_2H]^+$, 436.2231 (9) $[M-2\times CH_3CO_2H]^+$, 421.2056 (9), 404.1979 (8), 355.1844, 323.1608, 270.1245, 228.1137, 210.1091 (100), 195.0949, 147.9927, 97.1341, 57.7904; $[\alpha]_D = +23°$ ($c = 0.119$, $CHCl_3$, 25°) ; IR: ν_{max} (NaCl) cm^{-1}: 3468 (O–H stretch), 2978 (C–H stretch) and 2928 (C–H stretch), 1743 (C=O stretch, ester), 1372, 1236 (C–O stretch, acetate) and 1046 (C–O stretch).

3.9. Turraflorin I (**8**)

Fine white crystals (30 mg); m.p. 143–145 °C (CH_2Cl_2/CH_3OH); EIMS: m/z : 642 $[M^+]$, 534, 391, 179, 149, 136, 107; $[\alpha]_D = +14°$ ($c = 0.074$, $CHCl_3$, 25°); IR: ν_{max} (NaCl) cm^{-1}: 2951 (C–H stretch), 1743 (C=O stretch, ester), 1243 (C–O stretch, acetate) and 1051 (C–O stretch).



1 R=OAc

2 R=OH

3

4

5

6

7

8

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