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Triterpenoids from *Turraea holstii*

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Abstract

Investigations of the stem and root bark of *Turraea holstii* (Meliaceae) have yielded three novel triterpenoids, holstinone A (21*R*,23*R*-epoxy-7α,24*S*-dihydroxy-21α,25-dimethoxyapotirucalla-1,14-dien-3-one), holstinone B (21*S*,23*R*-epoxy-7α,24*S*,25-trihydroxy-21β-methoxyapotirucalla-1,14-dien-3-one) and holstinone C (21*R*,23*R*-epoxy-7α,24*S*,25-trihydroxy-21α-methoxyapotirucalla-1,14-dien-3-one).

Keywords: *Turraea holstii*; Meliaceae; Triterpenoid; Protolimonoid; Holstinone A; Holstinone B; Holstinone C

1. Introduction

Turraea holstii Gurke is a member of the Meliaceae growing in East Africa. *Turraea* species have yielded a range of interesting compounds including simple triterpenoids such as niloticin from *Turraea nilotica* (Mulholland & Taylor, 1988), melianone and glabretal-type compounds from *Turraea obtusifolia* (Ackermann, 1991; Mulholland & Monkhe, 1993), the rings A–D intact limonoids from the bark of *Turraea floribunda* (Akinniyi, Connolly, Mulholland, Rycroft & Taylor, 1986) the ring B-opened limonoids, the turraflorins from the seed of *Turraea floribunda* (Fraser, Mulholland & Nair, 1994) and the highly complex prieurianin-type compound, nymania 1, from the seed of *Turraea obtusifolia* (Fraser, Mulholland & Taylor, 1995).

2. Results and discussion

The methanol extract of the root bark of *Turraea holstii* yielded holstinone A. HRMS indicated a molar mass of 498.3353 g mol^{-1} indicating a molecular formula of $C_{31}H_{46}O_5$. However, examination of the NMR spectra showed that a molecule of methanol had been lost. Thus, the correct molecular formula was $C_{32}H_{50}O_6$. The compound was a dimethoxy compound with methoxy group proton resonances occurring at δ 3.34 and δ 3.21. The presence of a 7α-hydroxyapotirucalla-1,14-dien-3-one skeleton was deduced as follows. Resonances at δ 7.11 and δ 5.80 (J 10.2 Hz) in the ^{1}H NMR spectrum, ascribable to H-1 and H-2, respectively, and resonances at δ 158.2 d, δ 125.5 d and δ 205.2 s, ascribable to C-1, C-2 and C-3, respectively, in the ^{13}C NMR spectrum indicated an α,β-unsaturated ring A ketone. Resonances ascribed to H-7β and H-15 occurred at δ 3.96 and δ 5.46, respectively, and C-7, C-14 and C-15 resonances at δ 71.5 d, δ 161.6 s and δ 119.6 d, respectively. The sidechain was found to be the 21,25-dimethoxy analogue of the melianodiol sidechain (Mulholland & Taylor, 1988), commonly found in the Meliodeae subfamily to which *Turraea* belongs. Resonances ascribed to H-21, H-23 and H-24 occurred at δ 4.79 (d, J 3.6 Hz), δ 4.21 (m) and δ 3.34 (d, J 6.7 Hz), respectively. Thus, structure **1** is assigned to holstinone A. Sarrett's oxidation (CrO_3/py) yielded **1A**, the 3,7-diketo-derivative, and Jones'

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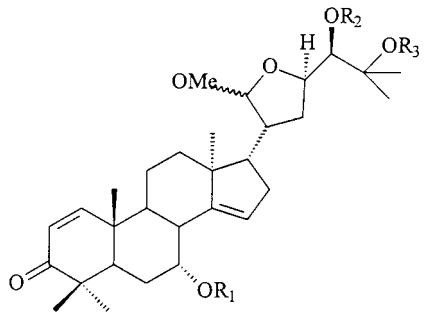

	R_1	R_2	R_3	21
1	OH	OH	Me	αOMe
1A	=O	OH	Me	αOMe
1B	=O	=O	Me	αOMe
1C	OAc	OAc	Me	αOMe
2	OH	OH	H	βOMe
3	OH	OH	H	αOMe

oxidation (CrO_3/acetone) yielded **1B**, the 3,7,24-triketo-derivative. Acetylation (Ac_2O/py) yielded **1C**, the 7α,24-diacetate as expected. NOE difference experiments were performed on the diacetate. Irradiation of the H-21 resonance at δ 4.77 gave an enhancement of the methoxy group proton signal at δ 3.34 and vice versa. Irradiation of the H-24 resonance at δ 4.99 gave an enhancement of the H-23α resonance at δ 4.18, the C-25 methoxy group proton signal at δ 3.18 and one of the C-25 methyl group proton resonances at δ 1.20. Irradiation of H-23α gave enhanced signals for H-24, both methoxy group proton resonances, the upfield acetate group methyl proton resonance (δ 1.91) and the proton resonance of the other methyl group at C-25 at δ 1.23. Irradiation of the C-25 methoxy group proton resonance at δ 3.18 resulted in enhancement of the H-24, 3H-26 and 2H-27 resonances. The enhancement of the C-21 methoxy group proton resonance on irradiation of H-23α implies that the methoxy group is in the α orientation.

The methanol extract of the stem bark yielded holstinone B and holstinone C. The highest peak in the mass spectrum of holstinone B occurred at m/z 466.3081 indicating a molecular formula of $C_{30}H_{42}O_4$. However, the NMR spectra indicated that molecules of methanol and water had been lost giving a molecular formula of $C_{31}H_{48}O_6$. Holstinones B and C differed from holstinone A in having only one methoxy group which was placed at C-21. The C-21 resonance occurred at about δ 110 in all three compounds indicating the methoxy group was present at C-21 in all three, but C-25 occurred at δ 77.2 in holstinone A but significantly further upfield at δ 72.3 and δ 73.1 in the other two compounds indicating a hydroxy group at C-25. It was found that holstinones B and C differed only from each other only in the stereochemistry at C-21. The ^{1}H NMR spectrum of holstinone B showed that the C-26 and C-27 methyl group proton resonances occurred unexpectedly downfield at δ 1.63 and δ 1.54 but these resonances occurred at δ 1.29 and δ 1.25 in the ^{1}H NMR spectrum of holstinone C. A model showed that hydrogen bond formation between the hydroxy group at C-25 and the acetal oxygen is highly favoured. The model shows that of the two

Table 1
^{1}H NMR data for **1**, **1A**, **1B**, **1C**, **2** and **3** ($CDCl_3$, 300 MHz)

C	**1**	**1A**	**1B**	**1C**	**2**	**3**
1	7.11 (d, 10.2)	7.12 (d, 10.3)	7.12 (d, 10.3)	7.14 (d, 10.3)	7.11 (d, 10.2)	7.12 (d, 10.2)
2	5.80 (d, 10.2)	5.87 (d, 10.3)	5.88 (d, 10.3)	5.82 (d, 10.3)	5.80 (d, 10.2)	5.80 (d, 10.2)
7	3.96 (brs, $W_{1/2}$ 6.6)			5.21 (m)	3.96 (t, 2.6)	3.96 (brs)
15	5.46 (dd, 1.6, 3.5)	5.92 (dd, 1.8, 3.5)	5.89 (m)	5.21 (m)	5.46 (dd, 1.6, 15)	5.47 (m)
21	4.79 (d, 3.6)	4.79 (d, 3.7)	4.96 (d, 3.7)	4.77 (d, 3.5)	4.80 (d, 3.7)	4.78 (d, 3.6)
23	4.21 (m)	4.17 (m)	5.03 (dd, 5.3, 10.1)	4.18 (m)	4.42 (m)	4.23 (m)
24	3.34 (d,6.7)	3.33 (d, 7.6)		4.99 (d, 3.4)	3.51 (m)	3.22 (d, 10.0)
CH_3	1.22	1.35	1.36	1.23	1.63	1.29
	1.14	1.32	1.32	1.20	1.54	1.25
	1.14	1.21	1.32	1.15	1.14	1.14
	1.13	1.12	1.30	1.14	1.14	1.14
	1.10	1.12	1.13	1.05	1.10	1.10
	1.07	1.08	1.09	1.05	1.07	1.07
	1.03	1.00	1.01	1.01	1.04	1.04
OCH_3	3.34 (s)	3.33 (s)	3.36 (s)	3.34 (s)	3.34 (s)	3.35 (s)
	3.21 (s)	3.20 (s)	3.23 (s)	3.18 (s)	–	
$OCOCH_3$				1.90		
				2.11		
OH	2.56 (d, 6.7)				2.77 (brs)	2.51 (d, 10.0)

possible hydrogen bond conformations (i.e. α or β to the acetal ring), the β one is very favourable but the α one barely possible. In the 21β-methoxy compound in which the methoxy group is co-planar with the cyclic oxygen and the new hydrogen bond, this will lead to a downfield shift in the 3H-26 and 3H-27 resonances. This suggested that the 21-methoxy group would have to be β in holstinone B to effect the downfield shifts of the methyl group proton resonances. Irradiation of H-23α resulted in an enhancement of the H-24 signal but not the C-21 methoxy group proton singlet as was the case in holstinone A, thus the β stereochemistry of the C-21 methoxy group was confirmed for holstinone B and, as no free rotation of the C-23, C-24 bond could occur as a result of the hydrogen bond formation, the configuration at C-24 could be established as *S*. In the C-21α-methoxy group case, as in holstinone C, where the methoxy group is no longer co-planar with the hydrogen bond, the downfield shift of the methyl proton resonances will not occur. Thus, structures **2** and **3** are assigned to holstinone B and C, respectively. This is the first report of the occurrence of these three compounds.

A previous investigation of the methanol extract of *Melia azedarach* (Whitehead, 1994) yielded an epimeric mixture of 21,25-dimethoxymelianodiols and it was believed that methylation might have occurred during the extraction process. As expected, no unusual downfield shifts were observed for either compound as the presence of the methoxy group at C-25 precludes hydrogen bonding. It is possible that holstinones A–C are artefacts of the extraction process.

3. Materials and methods

Stem bark (296 g) and root bark (360 g) of *Turraea holstii* Gurke were collected in the Wesu area, Taita Javeta District, Coast Province, Kenya. Plant material was identified by Mr. G. Mwachala of the National Museum of Kenya Herbarium and a voucher specimen retained at the herbarium (MSR1). Material was air dried and ground to a fine powder. These were allowed to stand in 2 l of MeOH at room temperature for 1 week. Extracts were decanted and the procedure repeated. Solvent was removed and the two extracts were sent to Durban, South Africa for analysis.

Extracts were separated into components using column chromatography over silica gel (Merck 9385) using a solvent system EtOAc:$MeCl_2$/90:10. Holstinone A was isolated from the root bark extract and holstinones B and C from the stem bark extract. Spectra were run on a Varian Gemini 300 NMR spectrometer. ^1H and ^{13}C NMR data for **1**, **1A**, **1B**, **1C**, **2** and **3** are listed in Tables 1 and 2.

3.1. Holstinone A, 21R,23R-epoxy-7α,24S-dihydroxy-21α,25-dimethoxyapotirucalla-1,14-dien-3one **1**

492 mg, m.p. 125–127°C, $[\alpha]_D = -30.1$ (*c* 1.1 $CHCl_3$), HRMS: *m/z* 498.3353 $[M–CH_3OH]^+$ ($C_{31}H_{46}O_5$ requires 498.3345), EIMS *m/z* 498 $[M–CH_3OH]^+$, 466 $[M–2CH_3OH]^+$, 395, 326. $IR_{\gamma max}^{NaCl}$ cm^{-1} 3467, 1677, 1091, 1045.

3.2. Sarrett's oxidation of **1**

Chromium trioxide (250 mg) was added to a stirred mixture of pyridine (10 ml) and CH_2Cl_2 (10 ml). The flask was fitted with a drying tube and stirring was continued for 15 min. A solution of **1** (40 mg) in CH_2Cl_2 was added and the mixture was stirred for 3 h at room temperature. The mixture was poured into water and the aqueous solution extracted with ether

Table 2
^{13}C NMR data for **1**, **1A**, **1B**, **1C**, **2** and **3** ($CDCl_3$, 75 MHz)

C	1	1A	1B	1C	2	3
1	158.2 d	156.5 d	156.5 d	158.2 d	158.2 d	158.1 d
2	125.5 d	126.0 d	126.0 d	125.5 d	125.5 d	125.5 d
3	205.2 s	203.5 s	203.6 s	204.6 s	205.1 s	205.2 s
4	44.2 s[a]	44.7 s[a]	44.7 s[a]	42.7 s[a]	44.8 s[a]	44.7 s
5	46.1 d	52.5 d	52.6 d	46.2 d	45.9 d	45.8 d
6	24.2 t	36.2 t[b]	36.2t[b]	23.8 t	24.2 t	24.2 t
7	71.5 d	209.5 s	209.6 s	74.4 d	71.5 d	71.5 d
8	44.8 s[a]	52.6 s[a]	52.6 s[a]	44.2 s[a]	44.2 s[a]	44.2 s
9	36.7 d	44.8 d	44.8 d	38.3 d	36.7 d	36.7 d
10	40.2 s	39.6 s[a]	39.6 s[a]	39.9 s[a]	40.2 s[a]	40.1 s
11	16.3 t	17.3 t	17.4 t	16.5 t	16.3 t	16.3 t
12	34.8 t[b]	33.8 t	34.1 t	34.8 t[b]	34.7 t[b]	34.7 t[b]
13	46.9 s	47.4 s[a]	47.4 s[a]	46.8 s	46.9 s	46.8 s
14	161.6 s	152.8 s	152.7 s	159.2 s	161.6 s	161.6 s
15	119.6 d	126.0 d	126.0 d	118.6 d	119.6 d	119.6 d
16	35.5 t	35.5 t[b]	35.8 t[b]	35.1 t	35.8 t	33.7 t
17	57.8 d	57.9 d	57.1 d	57.8 d	57.7 d	57.6 d
20	44.5 d	44.7 d	47.1 d	46.1 d	44.5 d	44.4 d
21	109.2 d	109.1 d	108.9 d	108.4 d	109.5 d	109.5 d
22	32.5 t[b]	35.3t[b]	35.4 t[b]	33.2 t[b]	32.5 t[b]	32.6 t[b]
23	75.1 d	75.1 d	76.9 d	74.9 d	74.6 d	75.4 d
24	76.2 d	76.3 s	211.7 s	75.4 d	77.9 d	77.2 d
25	77.2 s	77.2 s	81.7 s	76.2 s	72.3 s	73.1 s
CH_3	27.5 q	27.9 q	27.8 q	27.3 q	30.0 q	27.5 q
	27.1 q	26.8 q	26.7 q	27.0 q	27.5 q	27.1 q
	21.6 q	21.6 q	22.5 q	22.6 q	27.1 q	26.5 q
	21.5 q	21.2 q	22.2 q	21.5 q	27.0 q	26.4 q
	20.1 q	21.0 q	21.4 q	21.3 q	21.5 q	21.5 q
	19.6 q	20.1 q	21.0 q	20.0 q	19.6 q	19.6 q
	18.9 q	18.4 q	18.5 q	19.0 q	18.9 q	18.9 q
OCH_3	55.5 q	55.5 q	55.6 q	55.4 q	55.6 q	55.6 q
	49.2 q	49.2 q	51.0 q	49.5 q	–	–
$OCOCH_3$	–	–	–	170.8 s	–	–
	–	–	–	170.2 s	–	–
$OCOCH_3$	–	–	–	21.2 q	–	–
	–	–	–	21.1 q	–	–

[a,b] Values in the same columns may be interchanged.

(3 × 15 ml). The ether fractions were combined and yielded **1A** (21*R*,23*R*-epoxy-24*S*-hydroxy-21α,25-dimethoxyapotirucalla-1,14-dien-3,7, dione), 27 mg.

3.3. Jones' oxidation of *1*

1 (40 mg) was dissolved in acetone (10 ml) and Jones' reagent (2 ml) was added to the mixture which was stirred for one hour and then extracted with chloroform to yield **1B** (21*R*,23*R*-epoxy-21α,25-dimethoxyapotirucalla-1,14-dien-3,7,24-trione), 29 mg.

3.4. Acetylation of *1*

1 (20 mg) was dissolved in pyridine (1 ml) and acetic anhydride (1 ml) was added. The mixture was left to stand overnight. MeOH was added to the reaction mixture and solvents were removed under vacuum to yield **1C** (21*R*,23*R*-epoxy-7α,24*S*-diacetoxy-2lα,25-dimethoxyapotirucalla-1,14-dien-3-one), 16.5 mg.

3.5. Holstinone B (21S,23R-epoxy-7α,24S,25-trihydroxy-21,β-methoxyapotirucalla-1,14-dien-3-one), *2*

80 mg, HRMS: *m/z* 466.3081 ($C_{31}H_{42}O_4$ req. 466.3082) $[M–CH_3OH–H_2O]^+$, EIMS: *m/z* 466 $[M–CH_3OH–H_2O]^+$, 395, 326. $IR_{\gamma_{max}}^{NaCl}$ cm^{-1} 3465, 1678, 1090, 1045.

3.6. Holstinone C (21R,23R-epoxy7α,24S,25-trihydroxy-21α-methoxy-apotirucalla-1,14-dien-3-one) *3*

30 mg, EIMS: *m/z* 466 $[M–CH_3OH–H_2O]^+$, 395, 326.

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