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DIOSPHENOLS FROM *SPIROSTACHYS AFRICANA*

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Key Word Index—*Spirostachys africana*; Euphorbiaceae; heartwood; diterpenes; diosphenols; stachenol.

Abstract—Three new diosphenols, *ent*-2-hydroxy-norbeyer-1,4,15-trien-3-one, *ent*-2,6β-dihydroxy-norbeyer-1,4,15-trien-3-one and *ent*-2,6α-dihydroxy-norbeyer-1,4,15-trien-3-one, together with stachenol, were isolated and identified spectroscopically from the heartwood of *Spirostachys africana*. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

In a previous report [1], we described two diterpenoids and a norditerpenoid from *Spirostachys africana*. We now report on three new diosphenols of the stachene series which are related to the already known *ent*-2-hydroxy-beyer-1,15-dien-3-one [2]. Diosphenols obtained from other species have insect antifeedant activity [3], which is also one of the traditional uses of *S. africana*. Stachenol, previously synthesised from stachenone [2], is also present in the plant.

RESULTS AND DISCUSSION

Chromatography of the carbonate-washed latex from the heartwood of *S. africana*, gave the three crystalline diosphenols **1**–**3**, in addition to stachenol. The three diosphenols were easily classified as belonging to the beyerane series from the characteristics of the 15(16)-ene system, IR absorption (*ca* 750 cm^{-1}), ^1H NMR signals (two doublets between δ 5.6 and 6.3) and ^{13}C NMR signals (two peaks between δ 134 and 138) data [1, 4]. Each compound had four other olefinic carbons indicating extra degrees of unsaturation. The three compounds showed similar UV maxima near 260 nm. Although the UV maxima of diosphenols reduces substantially [5, 6] on acetylation, this was not the case with these compounds, leading to the conclusion that there were at least two moieties absorbing at nearly the same wavelength. IR absorption near 1660 cm^{-1} was attributed to an α,β-unsaturated keto-enol system.

The HR mass spectrum of diosphenol **1** showed a $[M]^+$ at m/z 284.1786 (calculated for $C_{19}H_{24}O_2$ is 284.1776). Its ^1H NMR spectrum, showed only three methyls peaks (δ 1.05, 1.11 and a doublet at 1.99). The absence of the fourth methyl peak at δ less than 1.0, when compared with other beyeranes [1, 4], suggested that ring A was modified. The methyl peak at δ 1.99 belonged to the unsaturated system and this was assigned to the methyl at position 18. The two doublets of the doublets at δ 2.79 and 2.36 were assigned to the C-6 methylene group. Deutrium-exchange and acetylation procedures showed that the hydroxyl proton was a sharp singlet at δ 6.42; this did not shift at different concentrations confirming that the hydroxyl proton was chelated. COSY and NOESY experiments on both **1** and its acetate **1a** enabled the unambiguous assignment of all protons. Thus, diosphenol **1** was *ent*-2-hydroxy-norbeyer-1,4,15-trien-3-one.

Diosphenols **2** and **3** had an identical m/z of 300 corresponding with the molecular formula, $C_{19}H_{24}O_3$. Both had chelated hydroxyl protons and only one proton at position C-6 when compared with diosphenol **1**. In **2**, the C-6 proton was equatorially oriented at δ 5.22 as a doublet of doublets (J = 3.6 and 2.9 Hz) and, thus, was *ent*-2,6β-dihydroxy-norbeyer-1,4,15-trien-3-one. In diosphenol **3**, H-6 (δ 4.89, J = 12.2 and 4.3) was in the axial position and, therefore, was *ent*-2,6α-dihydroxy-norbeyer, 1,4,15-trien-3-one.

Diosphenols **2** and **3** were obtained as a single TLC spot co-crystalline mixture, with **2** as the major component (75%). The mixture of acetates of the two diosphenols, obtained by boiling in acetic anhydride, was resolved by flash chromatography to give acetates **2a** and **3a**. But a better way of resolving the mixture

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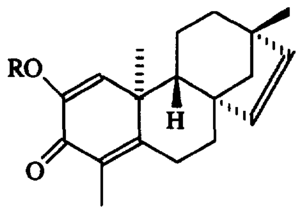

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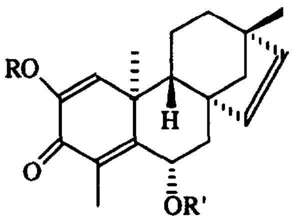
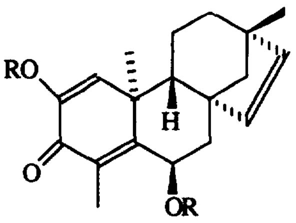


2 R = R' = H

2a R = R' = Ac

2b R = Ac, R' = H



3 R = H

3a R = Ac

of diosphenols was through kinetic acetylation with pyridine, as a base, followed by chromatography to give the more mobile monoacetate **2b** and the less mobile acetate mixture rich in **3a**. Monoacetate **2b** had a triplet signal at δ 5.20 for H-6. The difficulty in acylating the axial hydroxyl in **2** is due to 1,3-diaxial hindrance from the 19-methyl group and the 15,16-ene system.

Stachenol was readily identified by comparing its melting point, IR data and chemical reactions with those reported [2]. The proton at δ 3.20 was assigned to the axial proton at H-3, which shifted to δ 4.28 on acetylation. The ^{13}C NMR data are shown in Table 3. Stachenol is being reported for the first time as a plant product, having been previously prepared from *ent*-3-beyer-15-enone (stachenone).

EXPERIMENTAL

^{1}H and ^{13}C NMR: 200 and 50 MHz, respectively; COSY and NOESY at 500 MHz; CDCl$_3$ solns with TMS as int. standard. VLC: silica gel 60 G$_{254}$; visualisation by UV at 254 nm and vanillin–H$_2$SO$_4$. Optical rotations were measured in CHCl$_3$, unless otherwise stated.

A slurry of the latex (60 g) on silica gel (100 g) was chromatographed using vacuum liquid chromatography (VLC) on silica gel (150 g) eluting with hexane (1l), 5% EtOAc in hexane, 25% EtOAc in hexane and 40% EtOAc in hexane. A total of 30 frs (200 ml) was collected. Further VLC of pooled frs 3–12, eluted with 2% EtOAc in hexane, led to the isolation of diosphenol **1**. Later frs gave stachenol.

ent-*2-Hydroxy-norbeyer-*1,4,15-*trien*-3-*one* (*diosphenol* **1**). Yield 2.05 g, recrystallised from hexane, mp 125–126°. $[\alpha]_D + 14°$ (c 0.004). HRMS m/z: 284.1786 $[M]^+$ (calcd. for $C_{19}H_{24}O_2$, 284.1776). UV λ_{max}^{MeOH} nm (log ε): 262 (4.16). IR ν_{max}^{KBr} cm^{-1}: 3402, 3339 (OH); 1678 (CO), 1632, 1621 (C=C), 1578, 746. NMR: Tables 1 and 2.

ent-*2-Acetyl-norbeyer-*1,4,15-*trien*-3-*one* (*acetate* **1a**). Recrystallised from MeOH and H$_2$O, mp 59–63°. $[\alpha]_D - 19°$ (c 1.250). HRMS m/z: 326.1888 $[M]^+$ (calc.d for $C_{19}H_{24}O_2$, 326.1881). UV λ_{max}^{MeOH} nm (log ε): 250 (4.11). IR ν_{max}^{KBr} cm^{-1}: 1769, 1649 (CO); 1674, 1614 (C=C), 750. NMR: Tables 1 and 2.

VLC of pooled frs 15–21 gave a mixt. of diosphenols **2** and **3**, recrystallised from Et$_2$O and hexane (870 mg), mp 185–193°. Boiling the mixt. in Ac$_2$O for 2 hr gave a mixt. of acetates. Flash CC of this mixt., using hexane–EtOAc (4:1), gave *ent*-2,6α-diacetyl-norbeyer-1,4,15-trien-3-one(diosphenol diacetate **3a**). Recrystallised from hexane, mp 132–133°. $[\alpha]_D + 19°$ (c 0.002). HRMS m/z: 384.1922 $[M]^+$ (calcd. for $C_{23}H_{28}O_5$, 384.1937). UV λ_{max}^{MeOH} nm (log ε): 252 (4.10). IR ν_{max}^{KBr} cm^{-1}: 1769, 1744 and 1655 (CO), 1683 and 1615 (C=C), 752. ^{1}H NMR partial: δ 1.06 (3H, s, H-17), 1.12 (1H, d, J = 9.8 Hz, H-14e), 1.30 (3H, s, 20-H), 2.07 (3H, d, J = 0.8 Hz, H-18), 2.14 (3H, s, OAc-6), 2.27 (3H, s, OAc-2), 5.68 (1H, d, J = 5.8 Hz, 16-H), 5.91 (1H, d, J = 5.8 Hz, 15-H), 6.61 (1H, s, H-1). *ent*-2,6β-Diacetyl-norbeyer-1,4,15-trien-3-one (diosphenol diacetate **2a**). $[\alpha]_D + 52°$ (c 0.430). HRMS m/z: 384.1920 $[M]^+$ (calcd for $C_{19}H_{24}O_3$, 384.1937). UV λ_{max}^{MeOH} nm (log ε): 267 (4.09). IR ν_{max}^{KBr} cm^{-1}: 1772, 1742 and 1658 (CO), 1678 and 1609 (C=C), 768. ^{1}H NMR partial: δ 1.06 (3H, s, H-17), 1.06 (1H, d, J = 9.8 Hz, H-14e), 1.30 (3H, s, 20-H), 2.05 (1H, dt, J = 3.1 Hz, H-7e), 2.09 (3H, s, OAc-6), 2.11 (3H, s, 18-H), 2.27 (3H, s, OAc-2), 5.67 (1H, d, J = 5.8 Hz, 16-H), 6.11 (1H, d, J = 5.8 Hz, 15-H), 6.66 (1H, s, H-1).

The mixt. of diosphenols was acetylated using pyridine–Ac$_2$O overnight. Flash CC of the mixt., eluting with hexane–EtOAc (5:1), gave crystalline monoacetate **2b** and a syrupy diacetate mixt. **2a** and **3a** rich in **3a**.

ent-*2-Acetyl-6β-hydroxy-norbeyer-*1,4,15-*trien*-3-*one* (*monoacetate* **2b**). Recrystallised from hexane, mp 205–207°. $[\alpha]_D + 46$ (c 0.001). HRMS m/z: 342.1842 $[M]^+$ (calcd for $C_{21}H_{26}O_4$, 342.1831). UV λ_{max}^{MeOH} nm (log ε): 254 (4.10). IR ν_{max}^{KBr} cm^{-1}: 3501 (OH) 1767 and 1642 (CO), 1670 and 1601 (C=C), 758. NMR: Tables 1 and 2.

ent-*2,6β-Dihydroxy-norbeyer-*1,4,15-*trien*-3-*one* (*diosphenol* **2**). Complete deacylation with NaOMe–MeOH of **2a** or **2b**, gave **2**, recrystallised from Et$_2$O–hexane, mp 194–197° $[\alpha]_D + 22°$ (c 0.009). HRMS m/z: 300.1719 $[M]^+$ (calcd for $C_{19}H_{24}O_3$, 300.1725). UV λ_{max}^{MeOH} nm (log ε): 262.5 (4.10). IR ν_{max}^{KBr} cm^{-1}: 1772, 1742 and 1658 (CO), 1678 and 1609 (C=C), 768. NMR: Tables 1 and 2.

ent-*2,6α-Dihydroxy-norbeyer-*1,4,15-*trien*-3-*one*

Table 1. ^{1}H NMR data for diosphenols (δ) in $CDCl_3$

Proton	**1**		**1a**		**2**		**2b**		**3**	
H-1	6.28 *s*		6.65 *s*		6.31 *s*		6.67 *s*		6.22 *s*	
H-6a	2.36 *dddd*	(14.3, 13.3, 4.3, 0.9)	2.34 *dddd*	(14.0,m 13.5, 4.3, 0.8)	—	—	—	—	4.89 *ddd*	(12.3, 4.3, 0.9)
H-6e	2.79 *ddd*	(13.3, 4.0, 3.1)	2.78 *ddd*	(13.5, 3.9, 3.0)	5.22 *dd*	(3.5, 2.9)	5.20 *dd*	(3.5, 2.9)	—	—
H-7a	1.41 *ddd*	(14.3, 13.3, 4.0)	1.44 *ddd*	(14.0, 13.5, 3.9)	1.62 *dd*	(14.9, 3.5)	1.65 *dd*	(14.9, 3.6)	1.79 *dd*	(12.3, 12.2)
H-7e	1.86 *ddd*	(13.3, 4.3, 3.1)	1.84 *ddd*	(13.5, 4.3, 3.0)	2.08 *dd*	(14.9, 2.9)	2.07 *dd*	(14.8, 2.9)	2.06 *dd*	(12.2, 4.3)
H-9a	1.24 *dd*	(12.1, 4.8)	1.38 *dd*	(12.0, 4.9)	1.33 *dd*	(12.0, 4.8)	1.46 *dd*	(11.8, 5.0)	1.22 *dd*	(12.1, 4.8)
H-11a	1.63 *dddd*	(13.2, 12.1, 12.0, 6.4)	1.61 *dddd*	(13.1, 12.0, 11.8, 6.4)	1.71 *dddd*	(13.1, 11.8, 6.5, 4.8)	1.71 *dddd*	(13.0, 11.7, 6.4, 5.0)	1.56 *dddd*	(12.9, 12.0, 6.4, 4.8)
H-11e	1.80 *dddd*	(13.2, 5.8, 4.8, 1.5)	1.72 *dddd*	(13.1, 5.7, 4.9, 1.5)	1.83 *dddd*	(13.1, 5.8, 4.8, 1.8)	1.76 *dddd*	(13.0, 5.8, 5.0, 2.0)	1.78 *dddd*	(12.9, 5.6, 4.8, 2.0)
H-12a	1.27 *ddd*	(12.8, 12.0, 5.8)	1.28 *ddd*	(12.8, 11.8, 5.7)	1.27 *ddd*	(12.8, 11.8, 5.8)	1.30 *ddd*	(13.0, 11.7, 5.8)	1.26 *ddd*	(12.0, 11.8, 5.6)
H-12e	1.35 *dddd*	(12.8, 6.4, 2.8, 1.5)	1.33 *dddd*	(12.8, 6.4, 2.7, 1.5)	1.37 *dddd*	(12.8, 6.5, 3.0, 1.8)	1.37 *dddd*	(13.0, 6.4, 2.7, 2.0)	1.33 *dddd*	(11.8, 6.4, 2.7, 2.0)
H-14a	0.99 *d*	(9.8)	1.02 *d*	(10.0)	1.00 *d*	(9.7)	1.04 *d*	(9.9)	1.06 *d*	(9.7)
H-14e	1.57 *dd*	(9.8, 2.8)	1.57 *dd*	(10.0, 2.7)	1.63 *dd*	(9.7, 3.0)	1.62 *dd*	(9.9, 2.7)	1.64 *dd*	(9.7, 2.7)
H-15	5.91 *d*	(5.8)	5.90 *d*	(5.8)	6.22 *d*	(5.8)	6.23 *d*	(5.8)	5.85 *d*	(5.8)
H-16	5.65 *d*	(5.8)	5.64 *d*	(5.8)	5.61 *d*	(5.8)	5.62 *d*	(5.8)	5.66 *d*	(5.8)
Me-17	1.05 *s*		1.04 *s*		1.05 *s*		1.06 *s*		1.05 *s*	
Me-18	1.99 *d*	(0.9)	1.94 *d*	(0.8)	2.05 *s*		2.01 *s*		2.31 *d*	(0.9)
Me-20	1.11 *s*		1.15 *s*		1.36 *s*		1.40 *s*		1.15 *s*	
OH/OAc-2	6.42 *s*		2.26 *s*		6.27 *s*		2.27 *s*		6.42 *s*	

Table 2. ^{13}C NMR data of diosphenols (δ) in CDCl$_3$

Carbon	1	1a	2	2a	2b	3	3a
1	124.3	140.8	126.3	141.8	142.2	124.2	140.5
2	144.7	143.4	144.2	142.8	142.6	144.3	142.9
3	182.0	179.0	182.5	179.3	179.7	182.5	179.4
4	126.0	128.3	128.8	133.2	131.0	126.3	128.1
5	166.8	162.9	162.0	153.9	158.9	164.6	158.1
6	26.4	26.1	69.0	70.7	68.8	70.4	70.7
7	37.5	37.0	43.1	40.4	42.6	51.4	49.6
8	49.0	49.1	47.3	47.3	47.2	47.3	49.6
9	50.3	49.0	49.0	48.1	47.6	49.3	50.5
10	43.5	44.3	43.4	43.8	44.0	44.5	45.5
11	21.3	20.8	23.0	21.3	22.3	21.6	22.3
12	32.3	32.1	32.4	32.1	32.2	32.0	32.2
13	44.5	44.5	43.2	43.4	43.2	44.5	44.7
14	59.6	59.5	60.5	60.2	60.3	59.5	60.3
15	138.3	138.5	138.2	139.0	138.2	138.8	138.2
16	133.1	133.1	133.6	134.4	135.5	133.0	135.5
17	24.6	24.6	32.0	24.4	24.5	24.3	24.5
18	10.4	10.8	11.0	11.2	11.1	11.3	11.1
20	22.8	22.5	22.6	22.4	22.4	23.0	22.4
COCH$_3$	—	169.0	—	169.7	169.0	—	169.0
CH$_3$CO	—	20.6	—	20.6	20.6	—	20.6
				21.2			

Table 3. ^{13}C NMR data of stachenol and acetylstachenol (δ) in CDCl$_3$

Carbon	Stachenol	Acetyl stachenol
1	37.2	37.0
2	27.3	23.7
3	79.1	81.1
4	38.8	37.8
5	55.2	55.3
6	19.9	21.4
7	37.4	37.1
8	48.8	48.8
9	52.9	55.6
10	37.1	37.0
11	20.2	19.8
12	33.2	33.1
13	43.7	43.7
14	61.1	61.0
15	136.5	136.6
16	135.1	135.0
17	25.0	25.0
18	28.4	28.3
19	15.7	16.9
20	15.0	15.1
CH$_3$CO	—	20.4
CH$_3$CO	—	171.1

(*diosphenol* **3**). Deacylation of diacetate **3a** gave **3**, recrystallised from Et$_2$O–hexane, mp 172–175°. $[\alpha]_D + 9.2°$ (c 0.003). HRMS m/z: 300.1714 $[M]^+$ (calcd for $C_{19}H_{24}O_3$, 300.1725). UV λ_{max}^{MeOH} nm (log ε): 262.5 (4.10). IR ν_{max}^{KBr} cm^{-1}: 3520 and 3393 (OH), 1676 (CO), 1618 (C=C), 748. NMR: Tables 1 and 2.

ent-3*β*-*Beyer*-15-*enol* (*stachenol*). Yield 350 mg, recrystallised from hexane, mp 160–162° (lit. 164° [2]). $[\alpha]_D + 32°$, (c 0.025) (lit. +28° [2]). HRMS m/z: 288.2460 $[M]^+$ (calcd. for $C_{20}H_{32}O$, 288.2453). IR ν_{max}^{KBr} cm^{-1}: 3337 (OH), 748. ^{1}H NMR partial: δ 0.74 (3H, *s*, H-20), 0.79 (3H, *s*, 19-H), 0.99 (6H, *s*, 17-H and 18-H), 3.20 (1H, *dd*, J = 4.8 Hz, 11.0 Hz, H-3), 5.46 (1H, *d*, J = 5.7 Hz, 16-H), 5.68 (1H, *d*, J = 5.7 Hz, 15-H). ^{13}C NMR: Table 3.

ent-3*β*-*Acetyl-beyer*-15-*ene* (*stachenol acetate*). Recrystallised from EtOH–H$_2$O, mp 115–118°, (lit. 117.5–119.5° [2]). $[\alpha]_D + 13.5°$ (c 0.008) (lit. +12° [2]). HRMS m/z: 330.2533 $[M]^+$ (calcd for $C_{22}H_{34}O_2$, 330.2559). IR ν_{max}^{KBr} cm^{-1}: 1726 (CO), 743. ^{1}H NMR partial: δ 0.77 (3H, *s*, H-20), 0.86 (6H, *s*, 18-H and 19-H), 1.00 (3H, *s*, 17-H), 4.48 (1H, *dd*, J = 5.6 Hz, 10.6 Hz, H-3), 5.46 (1H, *d*, J = 5.6 Hz, 16-H), 5.67 (1H, *d*, J = 5.6 Hz, 15-H). ^{13}C NMR: Table 3.

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