Triterpenoids from *Hippocratea excelsa.* The Crystal Structure of 29-Hydroxytaraxerol

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The root bark of *Hippocratea excelsa* afforded a new derivative of β-amyrin, which was identified as its ferulate, together with components new in this species. They were identified as the rare 29-hydroxytaraxerol, 29-hydroxyglutinol, 29-hydroxyfriedelin and the sterol 6β-hydroxystigmast-4-en-3-one. The known triterpene quinone methides pristimerin and tingenone characteristics of this genus, β-sitosterol, *trans*-polyisoprene, squalene, β-amyrin, and the alditol galacticol characteristic of the Celastraceae were also isolated. The structures were established on the basis of spectral analysis, including homo- and heteronuclear correlation NMR experiments (COSY, DEPT, HMQC and HMBC) and by comparison with data reported in the literature. The structure of 29-hydroxytaraxerol was confirmed by X-ray diffraction. The antimicrobial and antifungal activities of the compounds were studied, but no significant activity was found.

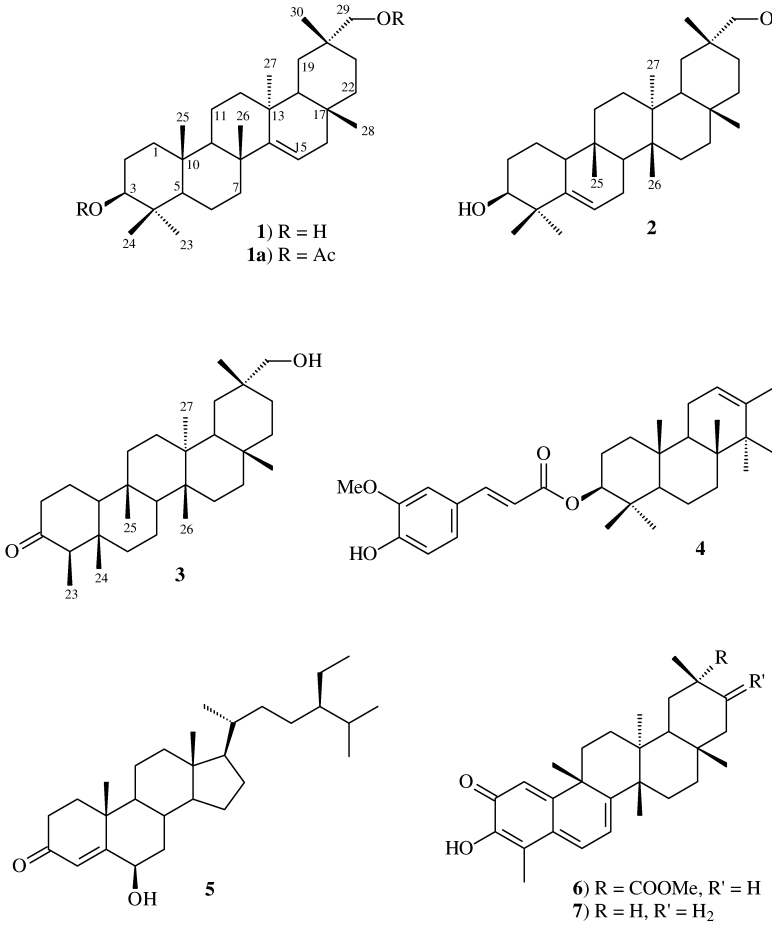
Key words: Hippocratea, Triterpenoids, NMR

The genus *Hippocratea* with about 115 species is distributed mainly in tropical areas of America, Africa and Asia. *Hippocratea excelsa* H. B. K. is a medicinal and insecticidal species. Its root bark is commonly known in the central part of México as "cancerina" due to its popular use in the treatment of cancer, and "mata piojo" in Yucatán, Michoacán and Guerrero, because of its use against lice and mites [1]. Other uses of this plant include as a sedative, anti-inflammatory and cicatrizing agent and against dysentery, gastritis, gastric ulcers [2]. The species is restricted to México and Central America. In México it can be located in the states of Chiapas, Oaxaca, Guerrero, Michoacán, Jalisco, México, Morelos, Durango and Yucatán [1].

As part of our search for new biologically active compounds from plants used in traditional Mexican medicine, we have initiated a study of Celastraceae species growing in the Yucatán Peninsula. In this paper we describe our results on the study of *Hippocratea excelsa* from Yucatán. Previous studies of the petroleum ether extract of the root bark of this species resulted in the isolation of friedelin and its 28-oxidized derivatives canophyllol, canophyllal and canophyllic acid together with the triterpenoid quinone methides pristimerine, tingenone, celastrol and excelsin [3]. *H. excelsa* collected in Yucatan did not afford the 28-oxidized derivatives of friedelin. Instead, 29-hydroxyfriedelin (**3**), 29-hydroxyglutinol (**2**), the rare 29-hydroxytaraxerol (**1**), the new ferulate of β-amyrin (**4**), and the sterol 6β-hydroxy-estigmast-4-en-3-one (**5**) were isolated as new natural products in *H. excelsa*. The known characteristic quinone methides of *H. excelsa* pristimerin (**6**) and tingenone (**7**) were also obtained together with the ubiquitous β-sitosterol, β-amyrin, squalene, *trans*-polyisoprene and the alditol galacticol, which are all characteristic of the Celastraceae family.

All compounds were identified by extensive analysis of spectroscopic data and comparison with data reported in the literature. The antimicrobial and antifungal activities are described.

Compound **1**, $[\alpha]_D + 23.5^\circ$, was obtained as colorless crystals from CH_2Cl_2 with m.p. 300 °C. Its IR spectrum showed absorption for hydroxyl groups at 3375 cm^{-1}. The HR-FABMS gave an accurate molecular ion peak at m/z 442.3809, corresponding to the molecular formula $C_{30}H_{50}O_2$ (calcd. 442.3811) which was also in good agreement with the ^{13}C NMR and DEPT spectral data. The ^{13}C NMR spectrum and



DEPT experiments of **1** indicated the presence of 30 carbon atoms, which accounted for seven methyl groups, eleven methylenes, one of them bearing a hydroxyl group, five methines including one olefinic and one hydroxymethine, and seven quaternary carbons, one of them being olefinic.

The ^{1}H NMR spectrum confirmed the presence of seven tertiary methyl groups. It also showed signals for one vinyl proton at $\delta = 5.54$ as a doublet of doublets ($J = 8.0$, 3.0 Hz), ($\delta_{\mathrm{C}} = 116.85$), one hydroxymethine proton (1H, $\delta = 3.19$ dd, $J = 11.0$, 4.5 Hz, $\delta_{\mathrm{C}} = 79.01$) and two methylene protons (2H, $\delta = 3.31$ brs, $\delta_{\mathrm{C}} = 73.9$) attached to a carbon bearing a hydroxyl group. The mass spectrum showed intense peaks at m/z 302 and 220 which must be due to retro Diels Alder fragmentation, indicating the presence of a double bond at C14-C15. Therefore we must be dealing with a pentacyclic triterpene of the taraxerane type with a primary hydroxyl group. Comparison between the ^{13}C NMR spectral data of **1** with those reported for taraxerol [4] and 28-hydroxytaraxerol (myricadiol) [5], showed differences in the chemical shifts for the carbon atoms of rings D and E, mainly for carbon atoms 19, 20 and 21. Therefore the primary hydroxyl group must be at C-29 or C-30. The HMBC spectrum showed correlations between the H-3 methine signal at $\delta = 3.19$ and two methyl signal at $\delta = 27.8$ and 15.5 (C-23 and C-24, respectively), and between the methylene protons signal ($\delta = 3.31$) and a methyl signal (C-30, $\delta = 24.6$).

The 29-hydroxytaraxerol and its diacetate had been isolated only from *Lithocarpus cornea* (Fagaceae), but no NMR data were published for the diol because it was insoluble in $CDCl_3$ [6]. Nevertheless the proton chemical shifts published for its diacetate derivative were similar with those obtained for the diacetate derivative of **1**. The molecular structure of **1** was confirmed by X-ray diffraction as 29-hydroxytaraxerol (**1**).

Table 1. NMR spectral data for **1**, and **1a** in $CDCl_3$.

C	**1**	HSQC	HMBC J^2, (J^3)	**1a**	HSQC	HMBC J^2, (J^3)
1	37.8	1.64	(H-25)	37.7	1.64, 1.68	H-2, (H-25), (H-3)
2	27.1	1.55		23.4	1.61	
3	79.0	3.19	(H-23), (H-24)	81.0	4.46	(H-23), (H-24)
4	38.7	–	H-23, H-24	37.7	–	H-23, H-24, H-3, (H-4)
5	55.5	0.77	(H-23), (H-24), (H-25)	55.6	0.88	(H-3), (H-23), (H-24), (H-25)
6	18.8	1.67		18.7	1.62	
7	41.3	2.03	(H-26)	41.2	2.03, 1.36	(H-5)
8	38.9	–	H-26	39.0	–	H-26
9	49.2	1.45	(H-25), (H-26)	49.1	1.43	(H-25), (H-26)
10	38.0	–	H-25	37.9	–	H-5, H-25
11	17.5	1.39		17.4	1.43	
12	37.7	1.09	(H-27), (H-18)	33.6		H-11, (H-18), (H-27)
13	36.0	–	H-27	37.6	–	H-27
14	158.0	–	(H-26), (H-27)	157.8	–	(H-16), (H-26), (H-27)
15	116.9	5.52		117.0	5.54	H-16
16	33.6	1.92, 1.62	(H-28)	37.3	1.92, 1.60	
17	37.6	–	H-18, H-28, (H-19)	35.9	–	H-28
18	48.0	0.99	(H-27), (H-28)	47.9	1.02	H-19, (H-27), (H-28)
19	31.1	1.38	(H-29), (H-30)	31.3	1.40	(H-21)
20	33.8	–	H-29	32.4	–	H-30
21	28.1	1.40, 1.24	(H-29), (H-30)	28.4	1.42, 1.25	(H-29), (H-30)
22	35.3	1.27, 1.80	(H-28)	35.1	1.38	H-21, (H-28)
23	28.0	0.98	(H-24)	28.0	0.85	(H-24)
24	15.5	0.80	(H-23), (H-5)	16.6	0.87	(H-3), (H-23)
25	15.4 s	0.92	(H-5)	15.5	0.95	
26	25.9	1.09		25.9	1.09	(H-9)
27	21.1	0.92	(H-12)	21.1	0.90	(H-12)
28	29.9	0.84	(H-18), (H-22)	29.8	0.83	
29	73.9	3.31	OH, (H-30)	74.4	3.76	(H-19), (H-21), (H-30)
30	24.6	0.94	(H-29)	24.9	0.95	(H-21), (H-29)
MeCO (3)	–	–		21.0, 171.0	2.08	(H-3)
MeCO (29)				21.3, 171.6	2.04	

Total assignments of proton and carbon resonances of **1** and its diacetate **1a** (Table 1) achieved by NMR studies including 1D and 2D homo- and heteronuclear experiments, are reported. This is the second report of the occurrence of **1** in nature.

The structure of **1** contains five fused six-membered rings, A/B/C/D/E, as a taraxerol skeleton, a hydroxyl substituent, equatorially attached to ring A and a hydroxymethylene substituent bisectionally attached to ring E (Fig. 1). In the taraxerol skeleton, rings D and E are *cis*-fused and other junctions show *trans*-fusion. The cyclohexane A and B rings adopt chair conformations, while the cyclohexane ring C, the cyclohexene ring D the cyclohexane ring E resemble twisted boat conformations [7]. The bond lengths and angles in the taraxerol skeleton are comparable with the corresponding values in taraxerol acetate [8]. The C14–C15 distance of 1.338 (5) Å confirms its double-bond character. The hydroxymethylene substituent is attached bisectionally to ring E and the C19–C20–C29–O2 torsion angle of $-59.0(4)$ indicates a (-)-synclinal conformation.

The diol **2** was isolated as a crystalline compound with m. p. 274 – 275 °C. The mass spectrum gave a molecular ion peak at *m/z* 442, in agreement with a molecular formula $C_{30}H_{50}O_2$, which was the same to that of **1**. As in case of compound **1**, the ^{13}C NMR indicated the presence 30 carbon signals corresponding to seven methyls, eleven methylenes, five methines and seven quaternary carbon atoms in accord with DEPT experiments. The ^1H NMR spectrum was very similar to that of **1**. It also showed the corresponding proton signals for one hydroxymethine and a hydroxymethylene at $\delta = 3.44$ (1H, brs, $\delta_C = 76.31$) and 3.25 (2H, AB, $J = 10.4$ Hz, $\delta_C = 74.43$), which was assigned at positions 3 and 29, respectively. Mass spectral fragments observed at *m/z* 290 and 259 (290-CH_2OH) were attributed to the *retro*-Diels-Alder fragmentation of the pentacyclic triterpene of the glutinane type and the subsequent loss of 31 mass units of one hydroxymethy-

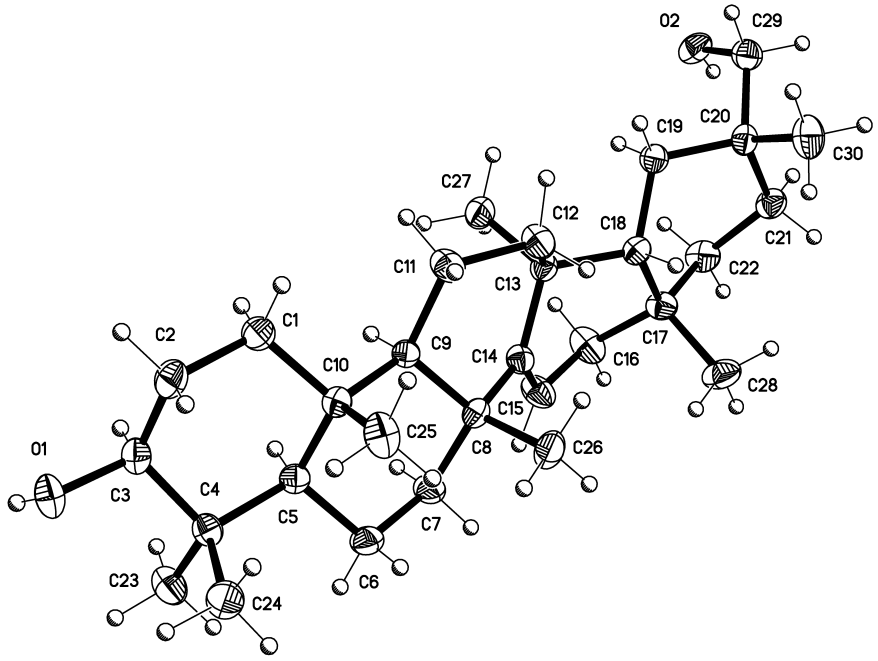


Fig. 1. Numbering scheme and ellipsoids at the 30% level. H atoms are represented by circles of arbitrary radius.

lene. Comparison of the ^{13}C and ^{1}H NMR data with those published for 29-hydroxyglutinol isolated from *Elaeodendron balae* (Celastraceae) [9] and *Maytenus ilicifolia* (Celastraceae) [10] confirmed the structure of compound **2**. The total assignments of proton chemical shifts are reported.

Compound **3** has the same molecular formula $C_{30}H_{50}O_2$ as compounds **1** and **2**, as determined by HR-EIMS. The ^{13}C NMR spectrum confirmed the presence of 30 carbon atoms. The major differences between **3** and the triterpenes **1** and **2** been in the number of methylene and methine groups, the presence of a carbonyl group at $\delta = 213.3$ (IR absorption at 1703 cm^{-1}) and a secondary methyl group according with the ^{1}H NMR spectrum. All these data indicated the presence of a triterpenoid of the friedelane type, which was identified as 29-hydroxyfriedelin in accord with HMBC long range couplings between the hydroxymethylene protons ($\delta = 3.26$) and C-19, C-20, C-21 and C-30 at $\delta = 20.7$, 33.1, 27.7 and 25.8, respectively. Comparison of the ^{1}H and ^{13}C NMR data with those published for 29-hydroxyfriedelin isolated from *Maytenus obtusifolia* (Celastraceae) [11] confirmed the above assumption.

Compound **4** was isolated as a crystalline compound with m.p. 199 – 200 °C and a molecular formula of $C_{40}H_{58}O_4$, as determined by HR-FABMS. IR absorptions at 3693, 3537 and 1696, 1634, 1601 cm^{-1}, indicated de presence of hydroxyl group and an aromatic α,β-unsaturated ester. The ^{13}C NMR and DEPT experiments accounted for nine methyls, ten methylenes, ten methines including three aromatic and three olefinic ones, and eleven quaternary carbon atoms, one of them a carbonyl group and four aromatic or olefinic ones. The ^{1}H NMR spectrum of compound **4** showed the presence of signals typical of a 1,2,4-substituted aromatic ring, together with an AB *trans* olefinic proton system, one methoxyl singlet and a phenolic OH signal, which suggested the presence of a feruoyl moiety in the molecule. The high field profile of the spectrum together with a vinyl proton signal at $\delta = 5.16$ resembles the β-amyrin spectrum. The mass spectral fragments observed at *m/z* 218 and 384 in the EIMS are in agreement with the *retro*-Diels-Alder fragments of a β-amyrin type triterpene with a ferulic ester or its positional isomer. The relative positions of the OH and OMe substituents in the aromatic ring were confirmed by the long range HMBC correlation between the OH proton and C-8' ($\delta = 114.6$). Therefore the structure of compound **4** must correspond to the ferulic ester of β-amyrin.

The sterol **5**, showed a molecular ion at *m/z* 428 corresponding to a molecular formula $C_{29}H_{48}O_2$, in agreement with the ^{13}C NMR and DEPT spectra. The

^{1}H NMR spectrum showed the typical methyl signals of a sterol of the stigmastane type, two singlets, three doublets and a triplet at $\delta = 0.73, 1.36, 0.91, 0.82, 0.80$ and 0.83, respectively. It also showed a vinyl proton singlet and a hydroxymethine triplet at $\delta = 5.8$ and 4.33 ($\delta_C = 129.2$ and 73.2), respectively. These signals together with a carbonyl and a vinyl quaternary carbon at $\delta = 200.6$ and 168.7 indicated the presence of an α,β-unsaturated ketone with an allylic alcohol, which can be placed at A and B rings (C3-C6) of the steroidal skeleton. Therefore the structure of compound **5** must correspond to that of 6β-hydroxystigmast-4-en-3-one. Comparison of the ^{1}H and ^{13}C NMR with those published for the compound isolated from the aquatic fern *Azolla nilotica* [12] and *Mangifera indica* [13], confirmed the above assumption.

As a continuation of a program oriented towards the discovery of bioactive natural products, the crude extract and isolated compounds were evaluated for their antimicrobial and antifungal activities. The hexane extract showed a moderate activity against *B. subtilis* and *K. pneumoniae*, but the tested compounds showed no significant activity.

Experimental Section

General experimental procedures

Melting points were determined on a Fisher-Jones type apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ solutions on a Rudolph Research Autopol IV polarimeter. IR spectra were recorded on KBr disks on a Nicolet Magna 750 Fourier transform IR spectrometer. EIMS were obtained on a Hewlett-Packard 5970 series II gas chromatograph as injection system. HREIMS were done on a VG Micromass LTD-ZAB-2F spectrometer at 70 eV. NMR spectra were recorded on Bruker Avance 400 and Varian Unity Plus 500 spectrometers in $CDCl_3$ solutions with TMS as internal standard; chemical shifts are recorded in δ values.

Plant material

Hippocratea excelsa H. B. K. was collected on the road Thul-Becanchén (3 km) south of Yucatán (México) in January 1999 and authenticated by Salvador Flores. A voucher specimen (J. S. Flores 12350) has been deposited at the Herbarium Alfredo Barrera Marin, Universidad Autónoma de Yucatán (UADY), Mérida, Yuc., México.

Extraction and isolation

Dried and ground root bark of *H. excelsa* (3.0 kg) were extracted at room temperature with hexane (5 l, 5×), CH_2Cl_2 (5 l, 7×) and MeOH (5 l) successively. The hexane extracts were concentrated *in vacuo* (500 ml), treated with MeOH (500 ml) and filtered to give 5.7 g of a gummy white solid, identified by NMR as *trans*-polyisoprene. The hexane-methanolic solution was concentrated at reduced pressure to give 34.2 g of crude residue. The CH_2Cl_2 extracts were treated in the same fashion giving 152 g of polyisoprene (m.p. 97 °C) and 55.9 g of crude residue. The crude residues were combined to give 90.1 g of total residue, since TLC of both residues indicated similar components.

The crude residue was chromatographed on a silica gel column (600 g), eluted with hexane, mixtures of hexane-EtOAc and EtOAc-MeOH of increasing polarity. Eluates (59) were collected, monitored by TLC, and combined in 15 major fractions (A1-A15).

GC-MS and ^{1}H NMR of low polar fractions indicated the presence of squalene. Fractions A8, A9 and A10 crystallized spontaneously giving 1.1 g of β-amyrin, 1.2 g of β-sitosterol and 14 mg 29-hydroxytaraxerol (**1**), respectively. Mother liquor of fraction A9 (16.1 g) was rechromatographed (chromatography B) on a column packed with 100 g of silica gel, which had been deactivated with triethyl amine. Deeply red fractions B3 and B4 contained the triterpene quinone pristimerin (**6**). Fraction B4 (6.9 g) was successively chromatographed on sephadex LH-20 (150 g) using a mixture of hexane-CH_2Cl_2-MeOH (2:1:1 v/v/v) to give the triterpene quinone pristimerin (**6**). Rechromatography of fraction B5 (3.2 g) on sephadex LH-20, as described above, gave two crystalline triterpenes identified as 29-hydroxytaraxerol (**1**) and 29-hydroxyglutinol (**2**) (12 mg). Mother liquor of fraction A10 (4.9 g) was successively subjected to Sephadex LH-20 column chromatography eluted with a mixture of hexane-$CHCl_3$-MeOH (2:1:1 v/v/v) to give tingenone (**7**), β-amyrin ferulate (**4**) and a sterol identified as 6β-hydroxystigmast-4-ene-3-one (**5**).

The methanol extract was concentrated under vacuo (600 ml) to give the alditol galacticol (m.p. 190 – 191 °C, EIMS *m/z* 183 $[M+H]^+$).

*29-Hydroxytaraxerol (**1**)*. Colorless crystals, m. p. 300 °C. – $[\alpha]_D^{25} + 23.5°$ (c 0.088 mg/ml), $CHCl_3$). – IR ($CHCl_3$): $\tilde{\nu} = 3375, 1572, 1465, 1372$ cm^{-1}; – MS (HR-FAB) *m/z* 442.3809 (calcd. for $C_{30}H_{50}O_2$, 442.3811). – MS (EI, 70 eV): *m/z* (%) = 442 (40) $[M]^+$, 427 (18) $[M\text{-}Me]^+$, 411 (22) $[M\text{-}CH_2OH]^+$, 302 (100) $[M\text{-}C_9H_{16}O$ (Ring E)$]^+$, 287 (18) $[M\text{-Ring E-Me}]^+$, 269 $[M\text{-Ring E-Me-}H_2O]^+$, 220 (74) $[C_{15}H_{24}O]^+$, 187 (29) $[C_{15}H_{24}O\text{-Me-}H_2O]^+$, 135 (29). – ^{1}H and ^{13}C NMR (see Tables 1 and 2).

Crystals suitable for X-ray analysis were grown from CH_2Cl_2 solution by slow evaporation. The data were collected on a Siemens P4/PC diffractometer using the program XSCANS [14], with graphite monochromated Cu-K_α. The accurate unit cell dimensions were obtained by the least-squares fit of setting angles of 38 reflections ($10.65° < \theta <$

Table 2. NMR spectral data for **2** and **4** in $CDCl_3$.

C	**2**	HSQC	HMBC J^2, (J^3)	**4**	HSQC	HMBC J^2, (J^3)
1	18.2	1.54, 1.46		38.3	1.65, 1.61	H-2
2	27.8	1.85, 1.67		23.5	1.91, 1.69	H-1
3	76.3	3.44	(H-23), (H-24)	80.8	4.62	(H-1), H-2
4	40.8	–		37.9	–	(H-2)
5	141.6	–	(H-6)	55.2	0.87	(H-1), (H-7)
6	122.0	5.61		18.3	1.55, 1.43	H-7
7	23.5	1.96, 1.74		32.6	1.52, 1.32	H-6
8	47.8	1.48	(H-6), (H-25), (H-26)	39.8	–	H-7, H-9, (H-6), (H-11), (H-15)
9	34.8	–	(H-25)	47.5	1.56	H-11, (H-1)
10	49.6	1.96	H-25	36.8	–	H-1, H-9, (H-2), (H-6), (H-11)
11	34.6	1.50, 1.34	(H-25)	23.7	1.86, 1.63	H-9
12	30.5	1.36, 1.33	(H-27)	121.6	5.17	H-11, (H-18)
13	37.7	–	(H-26), H-27	145.2	–	H-18, (H-11), (H-19), (H-27)
14	39.5	–		41.7	–	H-15, (H-9), (H-18)
15	32.5	1.52, 1.29	(H_26)	26.1	1.74, 0.95	H-16
16	35.7	1.52, 1.28	(H-28)	26.9	1.98, 0.77	(H-18)
17	30.5	–	H-28	32.5	–	H-16, H-18, H-22, (H-19)
18	41.9	1.62	(H-27), (H-28)	47.2	1.94	H-19
19	39.3	1.37, 0.94	(H-30)	46.7	1.65, 1.00	H-18
20	33.1	–	H-30	31.1	–	H-19 (H-22)
21	29.5	1.45, 1.15	(H-30)	34.7	1.32, 1.07	H-22
22	27.9	1.64, 1.33	(H-28)	37.1	1.41, 1.20	H-21
23	28.9	1.02	(H-24)	28.1	0.90	
24	25.4	1.12	(H-23)	16.8	0.96	
25	16.1s	0.82		15.6	0.97	(H-1), (H-8)
26	18.1	1.01		16.9	0.93	(H-7), (H-9)
27	20.4	0.97		26.0	1.12	(H-15)
28	32.0	1.19		28.4	0.81	(H-16), (H-18), (H-22)
29	74.4	3.25	(H-30)	33.3	0.86	(H-19)
30	26.0	0.99	(H-21), (H-29)	23.7	0.85	
1’				167.1	–	(H-2’), H-3’), (H-3)
2’				116.2	6.27	(H-3’)
3’				144.6	7.57	(H-5’), (H-9’)
4’				127.1	–	H-3’, H-9, (2’), (H-8’)
5’				109.2	7.02	(H-3’), (H-9’)
6’				146.8	–	H-5’, (H-8’), (O-H)
7’				147.8	–	H-8’, (H-5’), (H-9’)
8’				114.6	6.89	(O-H)
9’				123.0	7.06	(H-3’), (H-5’)
OMe				55.9	3.91	(H-6’)
OH				–	5.08	

27.90°). The $\theta/2\theta$ scan method and a variable scan speed, depending on reflection intensity, were used. Three control reflections were measured after very 97 reflections and showed no systematic changes during data collection. Intensity data were corrected for the Lorentz and polarization effects [14]. The structures were solved by direct methods and refined by the full matrix least-squares method with the SHELXTL program [15]. Refinement was carried on F^2. Scattering factors incorporated in SHELXTL were used. The function $\Sigma w(|Fo|^2 \Delta |Fc|^2)^2$ was minimized with $w\Delta 1 = [\sigma^2(Fo)^2 + (0.0745P)^2 + 0.80P]$. All non-hydrogen atoms were refined with anisotropic thermal parameters. The coordinates of the hydrogen atoms involved in hydrogen bonds OH···O, were found in difference Fourier syntheses and their positional and were refined. The coordinates of the other hydrogen atoms were calculated from the geometry and refined as a riding model with their displacement parameters calculated as 1.2 times U_{eq} that of the carrier carbon atom. The crystallographic data, together with data collection and structure refinement details are listed in Table 3. The displacement ellipsoid representations of the molecule, together with the atomic numbering scheme are shown in Fig. 1. Crystallographic data for the structure have been deposited with the Cambridge Crystallographic Data Centre, CCDC-249572. Copies of the data can be obtained free of charge on application to The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: int.code+(1223)336-033; e-mail for inquiry: fileserv@ccdc.cam.ac.uk).

29-Hydroxytaraxeryl diacetate (**1a**). A sample of **1** (5 mg) was acetylated with Ac_2O/pyridine in the usual man-

Table 3. Crystal data, data collection and structure refinement for compound **1**.

Empirical formula	C_{30} H_{50} O_2
Formula weight	442.70
T [K]	293(2)
Wavelength [Å]	1.54178
Crystal system, space group	monoclinic, $P2_1$
Unit cell dimensions	
a [Å]	11.489(1)
b [Å]	7.6412(3)
c [Å]	15.104(1)
$\alpha = [^\circ]$	90
$\beta = [^\circ]$	98.583(4)
$\gamma = [^\circ]$	90
Volume [Å^3]	1311.1(2)
Z, Dx [mg/m^3]	2, 1.121
μ [mm^{-1}]	0.509
F(000)	492
θ Range for data collection [°]	2.96 to 56.74
hkl Range	$-12 \le h \le 12$
	$-8 \le k \le 8$
	$-16 \le l \le 16$
Reflections	
collected	4018
unique (R_{int})	3513 (0.0213)
observed ($I > 2\sigma(I)$)	3074
Data / restraints / parameters	3513 / 1 / 296
Goodness-of-fit on F^2	1.017
$R(F)(I > 2\sigma(I))$	0.0508
$wR(F^2)$ (all data)	0.1332
Absolute structure parameter	0.3(4)
Extinction coefficient	0.0068(7)
Max./min $\Delta\rho$ [Å^{-3}]	0.162/−0.142

ner, to give 4.6 mg of **1a** as gummy material. – MS (EI, 70 eV): *m/z* (%) 526 (30) $[M]^+$, 511 (10) [M-Me]$^+$, 466 (14) [M-AcOH]$^+$, 451 (15) [M-Me-AcOH]$^+$, 344 (100) [M-$C_{11}H_{18}O_2$ (Ring E)]$^+$, 329 (36) [M-Ring E-Me]$^+$, 262 (88) [M-Ring E-C_6H_8-2H]$^+$, 187 (26) [262-Me-AcOH]$^+$, 135 (26). – ^{1}H and ^{13}C NMR (see Tables 1 and 2).

29-Hydroxyglutinol (**2**). Colorless crystals, m.p. 274 – 275 °C. – $[\alpha]_D^{25} + 92.76^\circ$ (c 0.08 mg/ml, $CHCl_3$). – IR ($CHCl_3$): $\tilde{\nu} = 3692, 3625, 1602, 1458, 1386$ cm^{-1}. – MS (EI, 70 eV): *m/z* (%) = 442 (12) $[M]^+$, 427 (3) [M-Me]$^+$, 424 (5) [M-H_2O]$^+$, 411 (5) [M-CH_2OH]$^+$, 290 (87) [M-$C_{10}H_{16}O$ (Ring A)]$^+$, 275 (44) [M-Ring A-Me]$^+$, 259 [M-Ring A-CH_2OH]$^+$, 152 (38) [$C_{10}H_{16}O$ (Ring A)]$^+$, 134 (28) [$C_{10}H_{16}O$-H_2O]$^+$. – ^{1}H and ^{13}C NMR (see Tables 1 and 2).

29-Hydroxyfriedelin (**3**). Colorless crystals, m.p. 255 – 257 °C. – $[\alpha]_D^{25}$ – 11.02° (c 0.182 mg/ml, $CHCl_3$). – IR ($CHCl_3$): $\tilde{\nu} = 3691, 3627, 1703, 1456, 1389$ cm^{-1}. – MS (EI, 70 eV): *m/z* (%) = 442 (100) $[M]^+$, 427 (15) [M-Me]$^+$, 411 (17) [M-CH_2OH]$^+$, 302 (43), 273 (59), 123 (98), 109 (67). – ^{1}H and ^{13}C NMR (see Tables 1 and 2).

β-Amyrin ferulate (**4**). Colorless crystals, m.p. 199 – 200 °C. – $[\alpha]_D^{25} + 76.6^\circ$ (c 0.3, $CHCl_3$). IR ($CHCl_3$): $\tilde{\nu} = 3693, 3537, 1696, 1634, 1601, 1513, 1463, 1384$ cm^{-1}. MS (HR-FAB): *m/z* 602.4322 (calcd. for $C_{40}H_{58}O_4$, 602.4335); MS (EI, 70 eV): *m/z* (%) = 602 (20) $[M]^+$, 384 (37) [M-$C_{16}H_{26}]^+$, 218 (100) [$C_{16}H_{26}$ (Rings D,E)]$^+$, 203 (33) [218-Me]$^+$, 177 (60). – ^{1}H and ^{13}C NMR (see Tables 1 and 2).

6*β-Hydroxystigmast-4-en-3-one* (**5**). Colorless crystals, m.p. 201 – 203 °C. – $[\alpha]_D^{25} + 32.21^\circ$ (c 0.432 mg/ml). IR ($CHCl_3$): $\tilde{\nu} = 3430, 1680, 1461, 1375$ cm^{-1}. – MS (EI, 70 eV): *m/z* (%) = 428 (58) $[M]^+$, 413 (9) [M-Me]$^+$, 287 (8) [M-$C_{10}H_{21}$ (side chain)]$^+$, 245 (17) [M-$C_{13}H_{26}$-H]$^+$, 227 (11) [245-H_2O]$^+$, 152 (33) [$C_9H_{12}O_2$]$^+$, 43 (100). – ^{1}H and ^{13}C NMR (see Tables 1 and 2).

Antimicrobial assay

Crude extracts were screened, using the agar-well diffusion method [16], against *Staphylococcus aureus* (6338), *Bacillus subtilis* (6633), *Streptococcus agalactiae* (260), *Escherichia coli* (10536), *Pseudomonas aeruginosa* (10231), *Klebsiella pneumoniae* (4209), *Shigella flexneri* serotype 4 (9748), *Candida albicans* (752), *Sacharomyces cerevisiae* (287) *Aspergillus niger* (16888) and *Tricophyton mentagrophytes* (4807); all of them were purchased from the American Type Culture Collection. A 5% (w/v) test solution of each dried extract was prepared in sterile dimethylsulfoxide. Amikacin (0.31 μg/μl), Nystatin (0.05 IU/μl) and Itraconazole (0.25 μg/μl) were used as positive control for bacteria, yeast and fungi, respectively. Dimethylsulfoxide was used as negative control. 100 μl of each solution or control were dropped in a 6 mm-diameter well. The plates were incubated for 24 h at 37 °C under aerobic conditions and the diameter of the inhibition zone around each well was then measured and recorded. All determinations were performed in triplicate.

Isolated compounds were screened using the microplate method [17] (Eloff, 1998). 100 μl of the DMSO test solution of each isolated compound (4 μg/μl) was serially diluted 50% with Mueller-Hinton broth and 100 μl of a culture of *B. subtilis* or *K. pneumoniae* and were added to 12 wells of a 96-well microplate. The microplates were incubated overnight at 37 °C.

To indicate bacterial growth, 40 μl of *p*-iodonitrotetrazolium violet dissolved in water were added to the microplates and incubate at 37 °C for 30 min.

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