55. New Hyperforin Derivatives from *Hypericum revolutum* VAHL with Growth-Inhibitory Activity against a Human Colon Carcinoma Cell Line[1])

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The crude petroleum-ether extract of the root bark of *Hypericum revolutum* VAHL (Guttiferae) exhibited *in vitro* growth-inhibitory activity against the Co-115 human colon carcinoma cell line. Activity-guided fractionation of this extract resulted in the isolation of two new hyperforin derivatives **1** and **2**. The structure of **1** (hyperevolutin A) was established by X-ray analysis as the 4-hydroxy-8-*exo*-methyl-5,7-*exo*-bis(3-methylbut-2-enyl)-1-(2-methyl-1-oxopropyl)-8-*endo*-(4-methylpent-3-enyl)bicyclo[3.3.1]non-3-ene-2,9-dione. The structure of the homologue **2** was deduced by comparison of its UV and ^{1}H- and ^{13}C-NMR spectra with those of **1**.

Introduction. – The antibiotic properties of extracts of *Hypericum* species (Guttiferae) are well established [1–3]. One active constituent, designated hyperforin, was isolated from *Hypericum perforatum* L. and first characterized in 1971 [4] [5]. A number of reports have appeared on this novel antibiotic, particularly on its controversial absolute configuration [6–9], but finally X-ray analysis of its 3,5-dinitrobenzoate [10] and *p*-bromobenzoate [11] allowed direct proof of its absolute configuration.

Recent phytochemical investigations of various *Hypericum* species for new bioactive products led to the isolation of saroaspidine A, B, and C from *Hypericum japonicum* THUNB. [12] and of chinesin 1 and 2 from *Hypericum chinense* L. [13]. All isolated compounds were shown to display interesting antimicrobial activities against various microorganisms. In addition, chinesin 1 had relatively strong activity in a cytotoxicity test against *HeLa* cells.

Finally, in the course of our systematic chemical and biological screening studies of African plants, antifungal chromenyl ketones have been isolated from the aerial parts of *Hypericum revolutum* VAHL [14]. *Hypericum revolutum* is a shrub native to South-East Africa, growing at altitude along the margins of evergreen forests. More extensive investigations showed that the petroleum-ether extract of the root bark of *Hypericum revolutum* displayed significant growth-inhibitory activity against the Co-115 human colon carcinoma cell line. Hyperevolutin A (**1**) and B (**2**) were shown to be the main active

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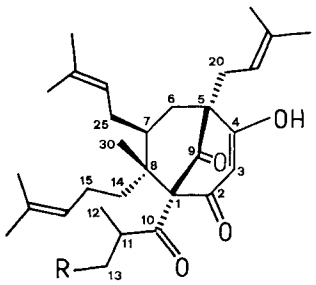

1 R = H[2])
2 R = CH_3(31)[2])

components [15]. Both compounds appeared to be related to the antibiotic hyperforin isolated from *Hypericum perforatum* L.

Results. – The root bark of *Hypericum revolutum* collected in Malawi was extracted with petroleum ether. This extract showed growth-inhibitory activity against Co-115 colon tumour cells (ED_{50} = 3.4 μg/ml). In order to localize the activity, the light petroleum-ether extract was subjected to bioassay-guided fractionation by droplet countercurrent chromatography (DCCC) with a H_2O-containing solvent system (see *Exper. Part*) giving an enriched fraction (ED_{50} = 1.4 μg/ml) which contained a compound that was crystallized from hexane. The crystalline product was the major active constituent of the extract with an ED_{50} of 0.7 μg/ml and was subjected to single-crystal X-ray analysis.

Although the result of the X-ray analysis suggested structure **1** with a mol. wt. of 468, the DCI-MS (reactant gas NH_3) of the crystalline material showed the presence of peaks at *m/z* 483 and 500, in addition to the expected peaks at *m/z* 469 ($[M + H]^+$) and 486 ($[M + NH_4]^+$). This difference of 14 amu suggested the presence of a higher homologue of **1** with a mol. wt. of 482. Anal. HPLC on *RP-18* using a photodiode-array detector showed the crystals, active in the Co-115 assay, to be a mixture of two compounds with identical UV spectra. A base-line separation of the two products occurred only after addition of 0.1% AcOH to the MeOH/H_2O mixture (87:13). Semi-prep. HPLC of the

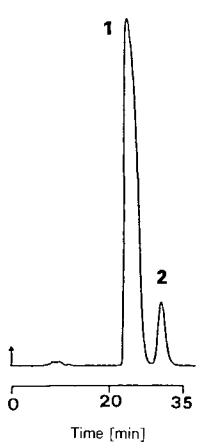

Fig. 1. *Semi-prep. HPLC of hyperevolutin A* (**1**) *and B* (**2**). *μBondapak C18* (30 cm × 7.8 mm i.d.); MeOH/H_2O 83:17 (0.1% AcOH added to the solvent mixture); UV detection at 275 nm; flow-rate 5 ml/min; 120-mg sample in 2.4 ml of MeOH; injection of 150-μl portions.

[2]) Arbitrary numbering, see *Fig. 2*.

crystalline material ($MeOH/H_2O$ 83:17, 0.1% AcOH; see *Fig. 1*) yielded hyperevolutin A (**1**; DCI-MS: 469 ($[M + H]^+$), 486 ($[M + NH_4]^+$)) and a minor compound, hyperevolutin B (**2**), with a mol. wt. of 482. The main compound **1** was the lowest homologue whose ^1H- and ^{13}C-NMR and DCI-MS data were in accordance with the structure deduced from X-ray analysis (see *Fig. 2* below, and *Exper. Part*).

The overall geometry of **1** is very similar to that observed for the *p*-bromobenzoate of hyperforin [11]. Both molecules consist of a bicyclic tetraketone in its enol form with three and four side chains, respectively, terminating in an isobutenyl group. As in hyperforin [11], the saturated six-membered ring moiety has a chair conformation. The unsaturated six-membered ring moiety has an envelope conformation with C(9) displaced by 0.60(1) Å from the best least-squares plane through the remaining five atoms. An interesting feature of both compounds is the presence of three carbonyl groups attached to C(1) (see *Fig. 2*), two of which being part of a ring and one of a side chain. Molecules related by the screw axis in the *a* direction are linked by a H-bond involving O*H*–C(4) and C(2)=*O* (see *Table 2, Exper. Part*). There are no further short intermolecular contacts involving non-H atoms.

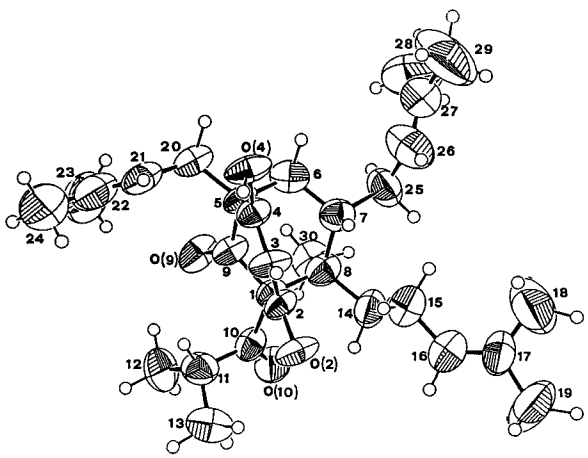

Fig. 2. *View of hyperevolutin A* (**1**) *showing the crystallographic atomic numbering scheme*[2]) *and the vibrational ellipsoids* (50% probability level)

The structure of the closely related compound **2** was deduced mainly by comparison of its ^1H- and ^{13}C-NMR spectra with those of **1**.

In the ^1H-NMR spectrum, the signals of the isobutyryl group in **1** with 1 *sept.* ($J = 6.5$ Hz) at 2.31 ppm for H–C(11), 1 *d* at 1.16 ppm ($J = 6.5$ Hz) for CH_3(12), and 1 further obscured *d* at 1.07 ppm for CH_3(13) were replaced in **2** by those corresponding to a 2-methylbutyryl group in which CH_3(12) appeared at 1.14 ppm (*d*, $J = 6.7$ Hz) and the terminal CH_3(31) at 0.83 ppm (*dd*, $J = 7.4$, 7.4 Hz)[2]). Comparison of the ^{13}C-NMR spectra gave further evidence of the structural difference between **1** and **2**. The signals of the isobutyryl group in **1** with 2 CH_3 (21.5 and 20.7 ppm for C(12) and C(13) and 1 CH group (41.9 ppm for C(11)) were replaced in **2** by characteristic signals confirming the 2-methylbutyryl moiety: 1 CH (48.7 ppm, C(11)), 1 CH_2 (27.6 ppm, C(13)), and 2 CH_3 groups (16.6 and 11.5 ppm, C(12) and C(31), resp.)[2]). As expected, the UV spectra of **1** and **2** were identical as the chromophore is not affected by the introduction of an additional CH_2 group in **2**.

As in the case of hyperforin [7], the UV spectrum of hyperevolutin A (**1**) depended on the pH (see *Exper. Part*) and on the concentration. A $6 \cdot 10^{-5}$ M solution of **1** in MeOH

had a maximum absorption at 273 nm, whereas a more dilute MeOH solution ($2.6 \cdot 10^{-5}$M) absorbed at higher wavelength (285 nm). These observations indicated the ionogenic nature of its chromogenic grouping which, according to the ^{1}H- and ^{13}C-NMR spectra in $CDCl_3$, should exist predominantly in the enolic form in $CDCl_3$. Preliminary observations in the ^{1}H-NMR spectrum of **1** in dilute $CDCl_3$ solutions suggested the existence of an equilibrium between the enol form **1** and its diketo tautomer.

The ^{1}H-NMR ($CDCl_3$) of a 0.09M solution of **1** showed 1 olefinic *s* at 6.16 ppm for H–C(3) and 1 broad signal at 7.04 ppm, obviously belonging to the enolic OH–C(4). At this concentration, the ^{13}C-NMR spectrum of **1** revealed only the presence of the enolic form with 2 quaternary C-atoms at 187.9 and 185.2 ppm (C(4) and C(2)) and 1 CH group at 109.7 ppm (C(3)). However, careful examination of the ^{1}H-NMR ($CDCl_3$) of a 0.03M solution of **1** showed some concentration-dependant variations which probably could be explained by the presence of the minor diketo tautomer of **1**: 2 small *d* coupled to each other (J = 19.2 Hz) appeared at 3.72 and 3.47 ppm, providing evidence for the geminal CH_2(3) group of the diketo tautomer. Furthermore, the 2 broad *m* (2.51 and 2.48 ppm) of $CH_2(20)^2$) of **1** in a 0.09M solution whose assignments were deduced by comparison with soulattrone A, a related bicyclic terpenoid isolated from *Calophyllum soulattri* (Guttiferae) [16], changed into 2 unresolved *dd* on dilution (0.03M).

Discussion. – During our systematic screening of African plants for biologically active substances, two new growth-inhibitory compounds, hyperevolutin A (**1**) and B (**2**), have been isolated from the root bark of *Hypericum revolutum* VAHL. Both compounds possess an enolic proton, thus explaining their irregular behaviour on solid supports, especially silica gel, to which they have the tendency of irreversibly adsorbing. In that respect, the use of a liquid-liquid chromatography technique, DCCC, was essential to isolate these two biologically active compounds [17]. It is shown here that this method is suitable for the fractionation of lipophilic constituents, even with the use of a H_2O-containing solvent system.

The crystalline mixture **1**/**2** was tested against the Co-115 human carcinoma cell line. The complete details of the biological assay have been given elsewhere [18]. In our test, the activity of the crystalline mixture **1**/**2** (ED_{50} = 0.7 µg/ml, $1.5 \cdot 10^{-6}$M reported on the mol. wt. of **1**) was comparable to that of 5-fluorouracil, a synthetic drug used in the therapy of colon cancer (ED_{50} = 0.23 µg/ml, $1.8 \cdot 10^{-6}$M in EtOH). Tests are underway to evaluate the activity of hyperevolutin A (**1**) on other solid tumor cell lines and to subject the compound to additional bioassays. Preliminary results showed hyperevolutin A and B to have no antifungal activity against *Cladosporium cucumerinum* fungi in a TLC bioassay [19].

Experimental Part

General. TLC: silica gel precoated Al sheets *(Merck)*; detection: 254 nm and *Godin* reagent [20]. Droplet counter current chromatography (DCCC): *Büchi-670*-DCC chromatograph, 580 tubes (length 40 cm, 290 tubes with 3 mm i.d. and 290 tubes with 2.7 mm i.d.). Anal. HPLC: *µBondapak C-18* (30 cm × 3.9 mm i.d.; *Waters*); *Spectra Physics 8700* pump; the chromatogram at 275 nm and the UV/VIS spectra were recorded with a photodiode-array detector *HP 1040A (Hewlett-Packard)*. Semi-prep. HPLC: *µBondapak-C-18* column (30 cm × 7.8 mm i.d.; *Waters)*; *Waters 6000A* pump coupled with a *Waters* solvent-delivery system (automatic gradient controller); detection at 275 nm with a *LKB 2238 Uvicord S II* detector. M.p.: *Mettler FP 80/82* hot stage apparatus; uncorrected. $[\alpha]_D$: *Perkin-Elmer-241* polarimeter. UV spectra: *Perkin-Elmer-Lambda-3* spectrophotometer. IR spectra: *Perkin-Elmer 681*. ^{1}H- and ^{13}C-NMR spectra: *Varian VXR 200* equipped with a switchable 5-mm probe at 200 and 50.1 MHz, resp.; $CDCl_3$ solns.; chemical shifts in δ (ppm) rel. to TMS as internal standard. DCI-MS: *Nermag R 1030* spectrometer.

Plant Material. Root bark of *Hypericum revolutum* VAHL were collected on Zomba plateau, Malawi, in April 1988. A voucher specimen is deposited at the Herbarium, Chancellor College, University of Malawi, Zomba. Another batch of *Hypericum revolutum* was collected in May 1988 near Nelspruit, in E. Transval (South Africa) by the Head of the Lowveld Botanical Garden.

Extraction and Isolation. The powdered root bark (104 g) was extracted at r.t. with light petroleum ether: 3.25 g of extract (ED_{50} = 3.4 µg/ml). A 2.5-g portion was fractionated by DCCC (light petroleum ether/94% EtOH/AcOEt/H_2O 83:67:33:17, ascending mode, flow-rate *ca.* 25 ml/h) to afford 410 mg of the enriched fraction (ED_{50} = 1.4 µg/ml) which was subjected to crystallization from hexane: 205 mg of white crystalline **1/2**. The crystals were subjected to growth-inhibition testing (ED_{50} = 0.7 µg/ml) and to single-crystal X-ray analysis. A 120-mg portion in 2.4 ml MeOH was separated by semi-prep. HPLC (injection volume 150 µl) on a *µBondapak C18* column (30 cm × 7.8 mm i.d., *Waters*) with MeOH/H_2O 83:17 (0.1% AcOH added to the solvent) to afford 110 mg of hyperevolutin A (**1**) and 7 mg of hyperevolutin B (**2**).

The crystalline material obtained from the petroleum-ether extract of the batch of *Hypericum revolutum* collected in South Africa appeared to have a slightly higher content of **2**. Semi-prep. HPLC on a *µBondapak C18* column (30 cm × 7.8 mm i.d., *Waters*) of 120 mg of the crystalline material in 2.4 ml of MeOH (injection volume 200 µl) using MeOH/H_2O 80:20 (0.1% AcOH added to the solvent system) yielded 93 mg of **1** and 17 mg of **2**.

*4-Hydroxy-8-*exo*-methyl-5,7-*exo*-bis(3-methylbut-2-enyl)-1-(2-methyl-1-oxopropyl)-8-*endo*-(4-methylpent-3-enyl)bicyclo[3.3.1]non-3-ene-2,9-dione* (= *Hyperevolutin A*; **1**). White prisms from hexane. M.p. 128–131°. TLC (SiO_2, light petroleum ether/AcOEt/AcOH 70:30:3): R_f 0.27, orange with *Godin* reagent $[\alpha]_D^{25}$ = +84.4 (*c* = 0.5, MeOH). UV (MeOH, $6 \cdot 10^{-5}$ M): 273. UV (MeOH, $2.6 \cdot 10^{-5}$ M): 285. UV (MeOH, 0.1% AcOH): 269 (11400). UV (MeOH, 0.1% AcOH/$AlCl_3$) and UV (MeOH, 0.1% AcOH/$AlCl_3$/HCl): unchanged. UV (MeOH, 0.1% AcOH/NaOMe): 286. UV (MeOH, 0.1% AcOH/NaOAc) and UV (MeOH, 0.1% AcOH/NaOAc/H_3BO_3): 286. IR (KBr): 3430*w*, 2970, 2920, 2870, 1730, 1600, 1510, 1490, 1450, 1320, 1240. ^{1}H-NMR (200 MHz, $CDCl_3$, 35 mg/0.8 ml)[2]: 7.04 (br. *s*, OH–C(4)); 6.16 (*s*, H–C(3)); 5.02–4.99 (*m*, H–C(16), H–C(21), H–C(26)); 2.51, 2.48 (*m*, CH_2(20)); 2.31 (*sept.*, *J* = 6.5, H–C(11)); 2.14, 2.09, 1.95, 1.88, 1.82, 1.76 (br. *m*, 6 H); 1.67, 1.64, 1.56 (3 *s*, CH_3(18), CH_3(19), CH_3(23), CH_3(24), CH_3(28), CH_3(29)); 1.49, 1.44, 1.38 (*m*, 2 H); 1.16 (*d*, *J* = 6.5), 1.07 (obscured *d*, CH_3(12), CH_3(13)); 1.05 (*s*, CH_3(30)); at lower concentration (10 mg/0.8 ml, *ca.* 0.03M, $CDCl_3$): 3.72, 3.47 (2 *d*, *J* = 19.2, CH_2(3) of the minor diketo tautomer). ^{13}C-NMR (50.1 MHz, $CDCl_3$, 35 mg/0.8 ml; multiplicities from DEPTGL experiments): 210.2, 206.6 (C(9), C(10)); 187.9, 185.2 (C(2), C(4)); 135.0, 133.5, 131.3 (C(17), C(22), C(27)); 124.5, 122.2, 119.0 (C(16), C(21), C(26)); 109.7 (C(3)); 80.3, 60.0, 48.3 (C(1), C(5), C(8)); 42.3, 41.9 (C(7), C(11)); 39.4, 37.0, 29.2, 27.8, 24.8 (C(6), C(14), C(15), C(20), C(25)); 25.9, 25.8, 25.7 (C(19), C(24), C(29)); 21.5, 20.7 (C(12), C(13)); 18.1, 18.0, 17.7 (C(18), C(23), C(28)); 14.1 (C(30)). DCI-MS: 486 ($[M + NH_4]^+$), 469 ($[M + H]^+$).

X-Ray Analysis of **1**. Suitable crystals, in the form of transparent blocks, were grown from hexane. Crystal data: $C_{30}H_{44}O_4$, M_r = 468.7, space groupe $P2_12_12_1$, *a* = 9.456(2), *b* = 15.256(4), *c* = 20.445(3) Å, *V* = 2949.4 Å^3, *F*(000) = 1024, *Z* = 4, D_x = 1.055 gcm^{-3}, Mo*Kα*, *λ* = 0.71073 Å, *µ* = 0.38 cm^{-1}. A crystal of dimensions 0.42 × 0.38 × 0.30 mm was used for data collection. Preliminary *Weissenberg* and precession photographs indicated the crystals to be orthorhombic, space group $P2_12_12_1$. Intensity data, with index limits *h* 0 to 10, *k* 0 to 16, *l* 0 to 22 and θ_{max} = 22.5°, were measured on a *Stoe Siemens AED2* four-circle diffractometer (graphite-monochromated Mo*Kα* radiation) using the ω/θ scan mode. There was no significant intensity variation for 4 standard reflections measured every h. Of the 2108 unique reflections measured, 1526 were considered observed ($F_0 > 3\sigma(F_0)$). Cell parameters from ± *ω* values of 20 reflections and their *Friedel* pairs in the range $15° < 2\theta < 22°$. No absorption or extinction correlations applied. The structure was solved by direct methods using the program SHELXS-86 [21]. The program SHELX-76 [22] was used for all further calculations. In the final cycles of least-squares refinement, the hydroxy proton H(O4) was located in a difference map and refined isotropically. The remainder of the H-atoms were included in idealized positions with the CH_3 groups treated as 'rigid groups' (C–H 1.08 Å, H–C–H 109.5°)[22]. Overall isotropic thermal parameters were assigned to the CH and CH_2, the C=CH, and the CH_3 protons (refined values 0.091, 0.246, and 0.257). Weighted anisotropic full-matrix least-squares refinement for 1524 reflections (2 reflections probably suffering from extinction were removed) converged at $R = 0.078$, $R_w = 0.077$; $w^{-1} = \sigma^2(F_0) + 0.00131(F_0^2)$. Average parameter shift/e.s.d. < 0.15. Heights in final difference map $\rho_{max} = 0.26$, $\rho_{min} = -0.21$ eÅ^{-3}. Atomic scattering factors were taken from [23]. Final positional and equivalent isotropic thermal parameters are given in *Table 1*, bond distances and angles in *Table 2*. The crystallographic numbering scheme is apparent from *Fig. 2* prepared using ORTEP-II [24]. Supplementary material is available from *H. St.-E.*

Table 1. *Final Positional and Equivalent Isotropic Thermal Parameters* ($\times 10^4$). E.s.d.'s in parentheses.

$$U_{eq} = 1/3 \sum_i \sum_j a_i^* \cdot a_j^* (\bar{a}_i \cdot \bar{a}_j).$$

Atom	x/a	y/b	z/c	U_{eq} [Å^2]
C(1)	–3(6)	3665(5)	3770(3)	555(26)
C(2)	–397(8)	3035(5)	4316(3)	591(28)
O(2)	601(6)	2682(4)	4615(3)	832(22)
C(3)	–1837(6)	2861(6)	4468(4)	650(30)
C(4)	–2889(6)	3267(5)	4137(4)	628(30)
O(4)	–4242(5)	3189(4)	4297(3)	825(22)
C(5)	–2658(6)	3849(5)	3560(3)	565(27)
C(6)	–2578(10)	4786(5)	3807(4)	766(33)
C(7)	–1388(9)	4926(6)	4310(4)	759(33)
C(8)	104(8)	4639(6)	4042(4)	770(34)
C(9)	–1190(8)	3693(5)	3268(4)	617(29)
O(9)	–955(6)	3693(4)	2695(3)	864(23)
C(10)	1346(9)	3287(6)	3442(4)	685(32)
O(10)	2417(6)	3696(4)	3412(3)	900(23)
C(11)	1271(9)	2363(6)	3157(5)	864(36)
C(12)	1611(13)	2420(8)	2417(4)	1174(48)
C(13)	2301(12)	1755(8)	3511(6)	1183(49)
C(14)	1236(10)	4701(6)	4595(4)	922(40)
C(15)	796(13)	4538(8)	5293(5)	1220(52)
C(16)	2080(17)	4534(10)	5727(6)	1513(67)
C(17)	2310(15)	4890(8)	6274(6)	1247(54)
C(18)	1323(25)	5469(13)	6599(8)	2161(106)
C(19)	3684(17)	4743(11)	6649(6)	1782(78)
C(20)	–3809(8)	3743(6)	3037(4)	776(34)
C(21)	–4063(9)	2799(8)	2825(5)	944(44)
C(22)	–3853(11)	2448(11)	2236(6)	1208(59)
C(23)	–3173(16)	2902(12)	1661(6)	1656(71)
C(24)	–4234(16)	1482(11)	2109(9)	1775(83)
C(25)	–1441(12)	5889(6)	4558(5)	1007(42)
C(26)	–2646(16)	6025(9)	5020(6)	1408(64)
C(27)	–3621(16)	6619(12)	5007(8)	1505(75)
C(28)	–3651(21)	7341(16)	4521(11)	2719(146)
C(29)	–4796(20)	6665(13)	5484(12)	2469(119)
C(30)	562(11)	5267(6)	3471(5)	988(40)

Table 2. *Bond Distances* [Å] *and Angles* [°]

C(1)–C(2)	1.518(10)	C(10)–O(10)	1.191(9)
C(1)–C(8)	1.589(11)	C(10)–C(11)	1.528(12)
C(1)–C(9)	1.523(10)	C(11)–C(12)	1.548(13)
C(1)–C(10)	1.553(10)	C(11)–C(13)	1.527(12)
C(2)–O(2)	1.247(8)	C(14)–C(15)	1.507(14)
C(2)–C(3)	1.423(10)	C(15)–C(16)	1.504(16)
C(3)–C(4)	1.353(10)	C(16)–C(17)	1.261(14)
C(4)–O(4)	1.327(8)	C(17)–C(18)	1.447(20)
C(4)–C(5)	1.493(10)	C(17)–C(19)	1.526(18)
C(5)–C(6)	1.517(11)	C(20)–C(21)	1.522(14)
C(5)–C(9)	1.529(10)	C(21)–C(22)	1.333(15)
C(5)–C(20)	1.535(10)	C(22)–C(23)	1.509(18)
C(6)–C(7)	1.540(11)	C(22)–C(24)	1.540(20)

Table 2 (cont.)

C(7)–C(8)	1.575(11)	C(25)–C(26)	1.495(15)
C(7)–C(25)	1.554(12)	C(26)–C(27)	1.293(17)
C(8)–C(14)	1.559(11)	C(27)–C(28)	1.484(24)
C(8)–C(30)	1.572(11)	C(27)–C(29)	1.479(21)
C(9)–O(9)	1.191(9)	O(4) · · · O(2^i)[a])	2.595(9)
		H(O4) · · · O(2^i)[a])	1.675(6)
C(2)–C(1)–C(8)	110.5(6)	C(14)–C(8)–C(30)	108.1(7)
C(2)–C(1)–C(9)	109.4(6)	C(5)–C(9)–O(9)	123.6(7)
C(2)–C(1)–C(10)	106.5(6)	C(1)–C(9)–O(9)	121.7(7)
C(8)–C(1)–C(9)	104.9(6)	C(1)–C(9)–C(5)	114.2(6)
C(8)–C(1)–C(10)	116.5(6)	C(1)–C(10)–O(10)	121.8(8)
C(9)–C(1)–C(10)	108.9(6)	C(1)–C(10)–C(11)	118.0(7)
C(1)–C(2)–O(2)	116.7(6)	O(10)–C(10)–C(11)	120.2(8)
C(1)–C(2)–C(3)	121.0(6)	C(10)–C(11)–C(12)	108.1(8)
O(2)–C(2)–C(3)	122.4(7)	C(10)–C(11)–C(13)	110.5(8)
C(2)–C(3)–C(4)	120.5(7)	C(12)–C(11)–C(13)	111.4(8)
C(3)–C(4)–C(5)	124.1(6)	C(8)–C(14)–C(15)	119.1(8)
C(3)–C(4)–O(4)	123.0(7)	C(14)–C(15)–C(16)	109.7(11)
C(5)–C(4)–O(4)	112.9(6)	C(15)–C(16)–C(17)	131.4(14)
C(4)–C(5)–C(6)	107.8(6)	C(16)–C(17)–C(18)	124.0(14)
C(4)–C(5)–C(9)	110.4(6)	C(16)–C(17)–C(19)	122.0(14)
C(4)–C(5)–C(20)	112.5(6)	C(18)–C(17)–C(19)	114.1(12)
C(6)–C(5)–C(9)	103.4(6)	C(5)–C(20)–C(21)	114.2(7)
C(6)–C(5)–C(20)	111.5(7)	C(20)–C(21)–C(22)	127.8(11)
C(9)–C(5)–C(20)	110.8(6)	C(21)–C(22)–C(23)	125.8(15)
C(5)–C(6)–C(7)	112.9(7)	C(21)–C(22)–C(24)	120.1(13)
C(6)–C(7)–C(8)	112.5(7)	C(23)–C(22)–C(24)	114.0(14)
C(6)–C(7)–C(25)	109.0(7)	C(7)–C(25)–C(26)	111.2(9)
C(8)–C(7)–C(25)	113.9(7)	C(25)–C(26)–C(27)	128.9(15)
C(1)–C(8)–C(7)	109.0(6)		
C(1)–C(8)–C(14)	110.8(7)	C(26)–C(27)–C(29)	123.7(19)
C(1)–C(8)–C(30)	109.1(6)	C(28)–C(27)–C(29)	113.0(16)
C(7)–C(8)–C(14)	110.3(7)	C(4)–O(4)–H(O4)	100.6(5)
C(7)–C(8)–C(30)	109.6(7)	O(4)–H(O4) · · · O(2^i)[a])	132.2(3)

[a]) Symmetry operation i: $-0.5 + x, 0.5 - y, 1 - z$.

*4-Hydroxy-8-*exo*-methyl-5,7-*exo*-bis(3-methylbut-2-enyl)-1-(2-methyl-1-oxobutyl)-8-*endo*-(4-methylpent-3-enyl)bicyclo[3.3.1]non-3-ene-2,9-dione* (= *Hyperevolutin B*; **2**). White powder. M.p. 132–137°. TLC (SiO_2, light petroleum ether/AcOEt/AcOH 70:30:3): R_f 0.27, orange with *Godin* reagent. UV same as for **1**. ^{1}H-NMR (200 MHz, $CDCl_3$): 6.01 (*s*, H–C(3)); 5.18–4.84 (br. *m*, H–C(16), H–C(21), H–C(26)); 3.72 (*d*, J = 19), 3.46 (*d*, J = 19, CH_2(3) of the minor diketo tautomer); 2.64–2.39 (*m*, CH_2(20)); 2.11–1.75 (br. *m*); 1.70, 1.68, 1.65, 1.58, 1.57, (CH_3(18), CH_3(19), CH_3(23), CH_3(24), CH_3(28), CH_3(29)); 1.53–1.21 (br. *m*); 1.14 (*d*, J = 6.7, CH_3(12)); 1.04 (*s*, CH_3(30)); 0.83 (*dd*, J = 7.4, 7.4, CH_3(31)). ^{13}C-NMR (50.1 MHz, $CDCl_3$): 210.1, 206.6 (C(9), C(10)); 185.7, 184.7 (C(2), C(4)); 135.7, 133.5, 131.4 (C(17), C(22), C(27)); 124.7, 122.4, 119.1 (C(16), C(21), C(26)); 110.0 (C(3)); 80.4, 59.1, 48.5 (C(1), C(5), C(8)); 48.7 (C(11)); 42.4 (C(7)); 39.0, 37.0, 29.3, 27.8, 27.6, 24.8 (C(6), C(13), C(14), C(15), C(20), C(25)); 26.0, 25.8, 25.7 (C(19), C(24), C(29)); 18.1, 18.0, 17.7 (C(18), C(23), (C(28)); 16.6 (C(12)); 14.2 (C(30)); 11.5 (C(31)). DCI-MS: 500 ($[M + NH_4]^+$), 483 ($[M + H]^+$).

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