156. New Antifungal Chromenyl Ketones and their Pentacyclic Dimers from *Hypericum revolutum* VAHL[1])

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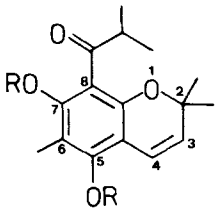
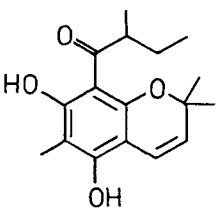
Two new 2*H*-1-benzopyranyl ketones **1** and **2** and three new pyrano[3,2-*c*:4,5,6-*d'e'*]di[1]benzopyrandiyl diketones **3**, **4a/4b**, and **5** have been isolated from the leaves and twigs of *Hypericum revolutum* VAHL (Guttiferae). The structure of **3** (hyperevoline) was established by X-ray analysis as 1,1'-[1,13,13a,13b-tetrahydro-5,8,10-trihydroxy-2,2,6,9,13,13-hexamethyl-2*H*,7a*H*-pyrano[3,2-*c*:4,5,6-*d'e'*]di[1]benzopyran-4,11-diyl]bis[2-methyl-1-propanone]. The structures of the isolated compounds were established by spectroscopic (UV, IR, EI-MS, ^{1}H- and ^{13}C-NMR) and chemical (acetylation and acidic dimerization) methods.

Introduction. – *Hypericum revolutum* VAHL (Guttiferae) is a shrub native to South-East Africa, growing at high altitude in open mountain grassland, along streams, and at the margins of evergreen forest. As early as 1943, it was demonstrated that extracts of a number of species of the genus *Hypericum* were active against *Staphylococcus aureus* [1], among them *Hypericum perforatum* [2]. There was some evidence that two compounds, hyperesin 1 and 2, exhibited activity against gram-positive microorganisms [3]. Other phytochemical investigations of the genus *Hypericum* for antibiotic [4] and antifungal



1 R = H
1a R = Ac



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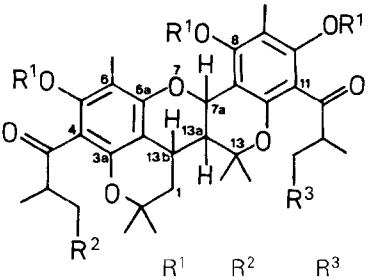


	R^1	R^2	R^3
3	H	H	H
3a	Ac	H	H
4a	H	H	CH_3
4b	H	CH_3	H
5	H	CH_3	CH_3

[1]) Presented in part at the 34th Annual Congress on Medicinal Plant Research, Hamburg, FRG, 22–27 September, 1986.

[5][6] activities led to the isolation of hyperforin, a prenylated phloroglucine antibiotic [7][8]. In addition, antibiotics containing phoroglucinol and filicinic-acid moieties were characterized: uliginosin A and B [9][10], which display *in vitro* inhibitory activity against *S. aureus* and *Trichophyton mentagraphytes*, but lack *in vivo* activity against gram-positive infections in rats [11]. More recently, sarothralin [12] and sarothralen A and B [13] were shown to possess significant inhibitory activity against various microorganisms.

In the course of a chemical and biological screening of African plants, it was observed that the light petroleum ether extract of *Hypericum revolutum* was fungicidal against *Cladosporium cucumerinum* in a TLC bioassay. Two new 2*H*-1-benzopyranyl ketones **1** and **2** were responsible for this activity. In addition, the new pyrano[3,2-*c*:4,5,6-*d'e'*]di[1]benzopyrandiyl diketones **3**, **4a/4b**, and **5** were isolated from the plant and characterized. However, these compounds showed no antifungal activity.

Results. – Leaves and twigs of *Hypericum revolutum* collected in Malawi were extracted with light petroleum ether. This extract showed antifungal properties in a TLC bioassay using *Cladosporium cucumerinum* [14]. In order to isolate the active compounds, the extract was subjected to fractionation by various chromatographic techniques to afford an antifungal yellow oil. Analytical HPLC on *RP18* using a photodiode-array detector showed the antifungal oil to be a mixture of two compounds with identical UV spectra. Semi-preparative HPLC on *RP18* ($MeOH/H_2O$) yielded yellow crystals of **1** (EI-MS: $M^{+\cdot}$ at 276) and **2** as a yellow oil (EI-MS: $M^{+\cdot}$ at 290). The structures of **1** and **2** were established by their EI-MS, ^{1}H- and ^{13}C-NMR spectra and the data of the acetylation product **1a**.

The EI-MS of both **1** and **2** showed a similar fragmentation pattern, with an easy loss of a CH_3 group from $M^{+\cdot}$ characteristic for 2,2-dimethyl-2*H*-1-benzopyrans to give the base-peak ion [9]. The 5-OH substitution of **1** was confirmed by comparison of its ^{1}H-NMR data with those of its acetylation product **1a**. Acetylation of OH–C(5) of 2*H*-1-benzopyran-5-ol is known to cause a marked diamagnetic shift of H–C(4) and a slight paramagnetic shift of H–C(3) in the ^{1}H-NMR spectrum [15]. For **1**, the *d* (J = 10 Hz) of H–C(4) and H–C(3) appear at 6.56 and 5.44 ppm, respectively, whereas for **1a** they are centred at 6.22 ($\Delta\delta$ = –0.34 ppm) and 5.64 ppm ($\Delta\delta$ = +0.2 ppm), respectively. The rest of the substitution pattern was deduced from the observation that the *sept.* of $(CH_3)_2CHCO$–C(8) of **1** appears at relatively low field (3.87 ppm). After acetylation, the signal appears at 3.17 ppm. This phenomenon (also observed in compound **3**, the structure of which was established by X-ray analysis) suggested a deshielding by the O-atom of the benzopyran ring and confirmed position 8 as the location of the isobutyryl group in **1**. The signal of OH–C(7) appears at 14.1 ppm, due to a strong H-bond with the carbonyl group of the isobutyryl moiety. The ^{13}C-NMR spectrum of **1** shows a signal for CH_3–C(6) at high field (7.1 ppm), confirming the position of the CH_3 group between two aromatic OH groups.

The structure of the higher homologue **2** was deduced by comparison of its ^{1}H- and ^{13}C-NMR spectra with those of **1**. The signal of $CH_3CH_2CH(CH_3)CO$–C(8) appears as a *sext.* at 3.77 ppm, and the signals of the 2 CH_3 groups of the acyl chain appear as a *d* (J = 6.5 Hz) and a *t* (J = 7.5 Hz), respectively, at 1.16 and 0.90 ppm. The signals of the 2 CH_3–C(2) are slightly shifted and give rise to 2*s* at 1.48 and 1.47 ppm. Two additional signals are also present in the ^{1}H-NMR spectrum of **2**, appearing as 2 *m* at 1.85 and 1.44 ppm (together 2 H), corresponding to the CH_2 group of the 2-methylbutyryl moiety.

The fast-running non-fungicidal fraction obtained by low-pressure liquid chromatography (see *Exper. Part*) was rechromatographed on silica gel to afford a crystalline product which was subjected to single-crystal X-ray analysis. Although the results of the analysis suggested structure **3** with a mol. wt. of 552, the EI-MS showed the presence of peaks at *m/z* 552, 566, and 580 with a difference of 14 amu in each case, indicating a mixture of **3** with higher homologues. Analytical HPLC using a photodiode-array detector showed the crystals to be a mixture of at least three compounds with identical UV

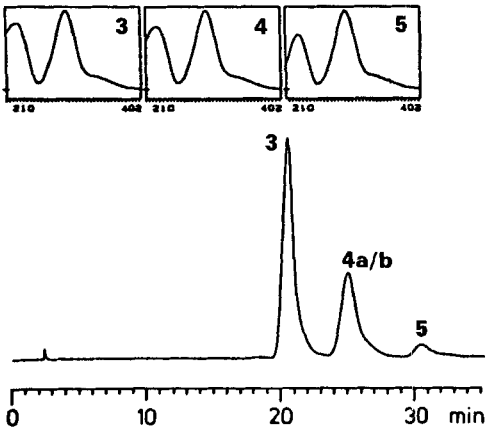


Fig. 1. *Analytical HPLC of hyperevoline* (**3**) *and its higher homologues* **4a/4b** *and* **5** *on* RP18 *with MeOH/H_2O 86:14.* H_3PO_4 was added to the H_2O (5 ml/l); photodiode-array detection at 254 nm; flow-rate 1.5 ml/min.

spectra (*Fig. 1*). Semi-preparative HPLC of a part of the crystalline mixture on *LiChrosorb RP18* with CH_3CN/H_2O allowed the isolation of compounds **3**, **4a/4b**, and **5** whose EI-MS gave molecular ions at 552, 566, and 580, respectively. HPLC of another portion of the crystalline mixture on *μBondapak RP18* with $MeOH/H_2O$ gave an improved separation [16].

The main compound, hyperevoline (**3**), was the lowest homologue (pale yellow crystals from hexane/Et_2O). Its ^1H- and ^{13}C-NMR and EI-MS data were in accordance with the structure deduced from X-ray analysis (see *Fig. 2* and *Exper. Part*). The molecule is composed of five fused rings (A–E). Benzene ring A has a flat boat conformation with atoms C(3a), C(5), C(6), and C(13c) in a plane (planar to within 0.0008(30) Å). Atoms C(4) and C(6a) are displaced from this plane by 0.063(3) and 0.048(2) Å, respectively.

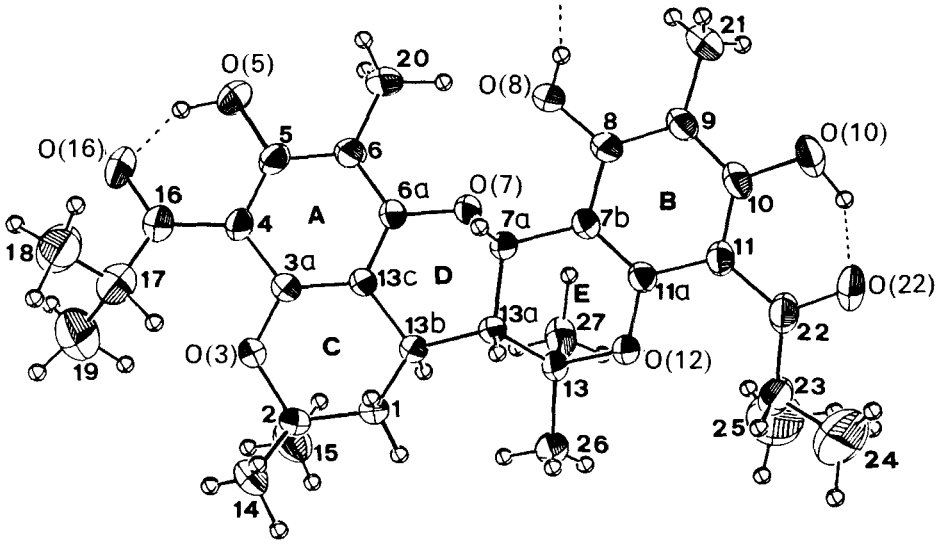


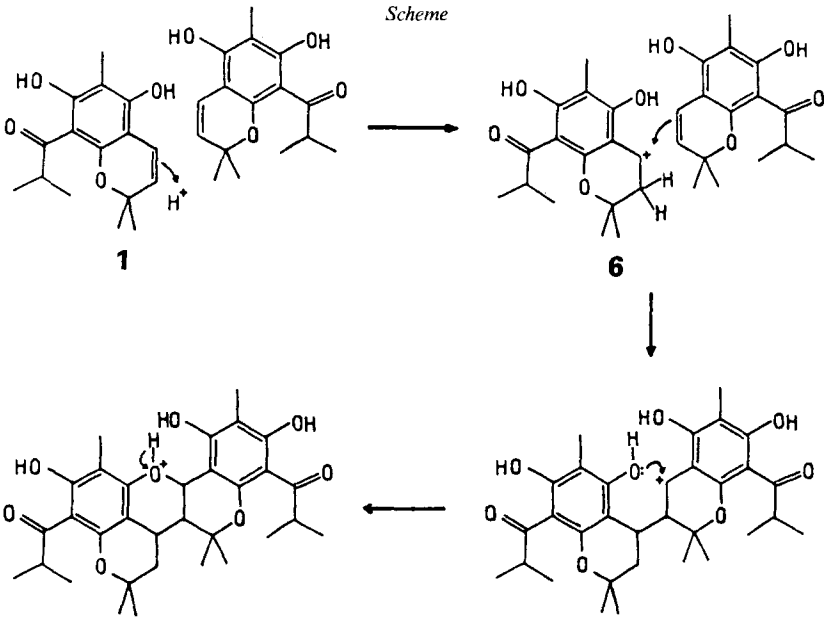
Fig. 2. *View of hyperevoline* (**3**) *showing the atomic-numbering scheme and the vibrational ellipsoids* (50% probability level)

Benzene ring B has a flat twist conformation with C(7b) and C(10) lying on the 2-fold axis and C(8), C(9), C(11), and C(11a) displaced by 0.052(3), −0.050(3), 0.043(3), and −0.044(3) Å, respectively, from the best plane through all six atoms. Pyran rings C and E have sofa conformations. Atom C(1) is displaced by 0.615(3) Å from the best plane through the remaining five atoms in ring C (planar to within 0.038(3) Å), while C(13) is displaced by 0.714(3) Å from the best plane through the remaining five atoms in ring E (planar to within 0.043(3) Å). Pyran ring D has a twist conformation with atoms C(6a) and C(13a) lying on the 2-fold axis and atoms O(7), C(7a), C(13b), and C(13c) displaced by −0.388(3), 0.343(3), −0.232(3), and 0.240(3) Å, respectively, from the best plane through all six atoms. There are two strong intramolecular H-bonds between O(5) and O(16) and O(10) and O(22) and a weaker intermolecular H-bond linking O(8) to O(16) of a symmetry-related molecule (see *Table 2* in *Exper. Part*).

The homologous compounds **4a/4b** which were similarly crystallized as pale yellow crystals from hexane/Et_2O contain one *sec*-butyl and one isopropyl group in the acyl moieties at C(4) and C(11). As some signals of the ^{13}C- and ^1H-NMR spectra are split, it seems reasonable to suggest that **4a** and **4b** are position isomers with the acyl chains interchangeable at C(4) and C(11). In the ^1H-NMR spectrum, the terminal CH_3 group of the 2-methylbutyryl moiety is clearly visible as a split *t* centred at 0.90 ppm. Compound **5**, the highest homologue, contains two *sec*-butyl groups in the acyl moieties. When compared with **3**, 2 additional signals were observed at 27.1 ppm and 26.7 ppm in the ^{13}C-NMR spectrum, assigned to the 2 CH_2 groups of the 2-methylbutyryl moieties by DEPT experiments.

Although compounds **3**, **4a/4b**, and **5** can be considered as 'dimers' of **1** and **2**, they are probably not artefacts formed during the isolation procedure because two-dimensional TLC analysis of the original petroleum-ether extract on silica gel showed the

Scheme



presence of a UV-active spot corresponding to the mixture of **3–5** and giving a similar red colour after treatment with *Godin* reagent [17]. The presence of the 3 pentacyclic dimers was also confirmed by TLC analysis of a petroleum-ether extract of another batch of *Hypericum revolutum*. However, previous observations [18] of the reactivity of 2*H*-1-benzopyrans show that **1** is capable, under acidic conditions, to form the carbocation **6**, resulting in various dimerization reactions. Indeed, we obtained the pentacyclic dimer **3** by acidic treatment of **1** (see *Exper. Part*). A possible mechanism for its formation is shown in the *Scheme*.

Discussion. – During our systematic screening studies of African medicinal plants for biologically active substances, a series of 5 new benzopyran derivatives (**1–5**) has been isolated from *Hypericum revolutum* VAHL. Compounds **1** and **2** are biogenetically related to the antibiotics uliginosin B and sarothalen B isolated from other species of the genus *Hypericum*, having the same 2*H*-1-benzopyran moiety in their structure. The presence of a higher homologue of uliginosin B was mentioned by *Parker* and *Johnson* [9], but they were unable to remove the $M + 14$ impurity. However, in the case of **1** and **2**, semi-preparative HPLC on *RP18* was found to be suited for the isolation of homologues which are generally difficult to separate. More details of the separation procedure are given elsewhere [16].

Various quantities of **1** and **2** have been tested against *Cladosporium cucumerinum* in a TLC bioassay [14]. The minimum quantity of both compounds required to show activity in the bioassay was 5 μg. On acetylation of **1**, its antifungal activity was lost.

Experimental Part

General. TLC: silica gel precoated Al sheets (*Merck*); toluene/AcOEt 93:7; detection: 254 mm and *Godin* reagent [17]. Prep. low-pressure liquid chromatography: *Lobar*® silica-gel column (40–63 μm; 2.5 cm × 27 cm; *Merck*) equiped with a *Duramat 80* pump (*Chemie und Filter*, Regensdorf). HPLC: *LiChrosorb-RP18* column (7 μm; 25 cm × 4.6 mm i.d.; *Knauer*); *Spectra Physics 8700* pump; the chromatogram at 254 nm and the UV/VIS spectra were recorded with a photodiode-array detector *HP 1040A* (*Hewlett Packard*). Semi-prep. HPLC: *μBondapak-C18* column (30 cm × 7.8 mm i.d.; *Waters*); *Waters 6000 A* pump coupled with a *Waters* solvent-delivery system (automatic gradient controller); detection at 254 nm with a *Pye Unicam LC-UV* detector. M.p.: *Mettler FP 80/82* hot stage apparatus; uncorrected. 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 MHz and 50.1 MHz, resp.; $CDCl_3$ solns.; chemical shifts in δ (ppm) relative to TMS. EI-MS: *Nermag R 1030* spectrometer.

Plant Material. Twigs and leaves of *Hypericum revolutum* were collected on Zomba Plateau, Malawi, in September 1984. A voucher specimen is deposited at the Herbarium, Chancellor College, University of Malawi, Zomba.

Extraction and Isolation. The powdered twigs and leaves (80 g) were extracted at r.t. with light petroleum ether: 7.9 g of extract. A 4-g portion was fractionated by flash chromatography on silica gel (63–200 μm; *Merck*) with a gradient of toluene and AcOEt (99:1 → 95:5; flow rate 30 ml/min) to afford 970 mg of material containing the antifungal compounds. Repetitive chromatography of this antifungal fraction on a *Lobar*® silica-gel column with $CHCl_3$/hexane/MeOH 80:8:0.3 yielded 210 mg of **1/2**. A soln. of 120 mg of **1/2** in 2 ml of MeOH was subjected to repetitive semi-prep. HPLC on *μBondapak C18* with MeOH/H_2O 63:17 to give 53 mg of **1** (yellow crystals from hexane) and 37 mg of **2** (yellow oil), after filtration on silica gel (63–200 μm; *Merck*) with $CHCl_3$ in order to remove the oxidation products formed on evaporation of MeOH/H_2O.

The fast running non-fungicidal fraction (580 mg) obtained from the repetitive *Lobar*® low-pressure chromatography was injected onto a silica-gel column (63–200 μm; *Merck*) with CH_2Cl_2/hexane 95:5 to afford 235 mg of **3/4a/4b/5**. After crystallisation from hexane/Et_2O 1:1, 158 mg of pale yellow crystals were obtained. A 36-mg

portion in 0.4 ml of $CHCl_3$ and 1 ml of CH_3CN was separated by repetitive semi-prep. HPLC (injection volume 250 µl) on a *LiChrosorb-RP18* (7 µm) column (25 cm × 16 mm; *Knauer*) with CH_3CN/H_2O 95:5 to afford 20 mg of **3**, 12 mg of **4a/4b**, and 3 mg of **5** (peak tailing). A better separation was obtained by semi-prep. HPLC (injection volume 125 µl) on a *µBondapak-C18* column (30 cm × 7.8 mm i.d.; *Waters*) with CH_3OH/H_2O 82:18 using 70 mg of **3/4a/4b/5** in 0.8 ml of $CHCl_3$ and 2 ml of CH_3CN: 41 mg of **3**, 23 mg of **4a/4b**, and 5 mg of **5**.

*1-(5,7-Dihydroxy-2,2,6-trimethyl-2*H*-1-benzopyran-8-yl)-2-methyl-1-propanone* (**1**). Yellow prisms. M.p. 79–81°. TLC (SiO_2, toluene/AcOEt 93:7): R_f 0.41; grey-brown with *Godin* reagent. UV (MeOH): 270 (sh), 285 (22400). UV (MeOH/$AlCl_3$): unchanged. UV (MeOH/$AlCl_3$/HCl): unchanged. UV (MeOH/NaOMe): 292, 335. UV (MeOH/NaOAc): 292, 335. UV (MeOH/NaOAc/H_3BO_3): 282, 355. IR (KBr): 3380, 2960, 1640, 1590, 1570, 1220, 1150, 1130, 910, 840, 730, 710. ^{1}H-NMR (200 MHz, $CDCl_3$): 14.14 (*s*, OH–C(7)); 6.56 (*d*, *J* = 10, H–C(4)); 5.44 (*d*, *J* = 10, H–C(3)); 5.42 (*s*, OH–C(5)); 3.87 (*sept.*, *J* = 6.5, $(CH_3)_2CH$); 2.03 (*s*, CH_3–C(6)); 1.47 (*s*, 2 CH_3–C(2)); 1.18 (*d*, *J* = 6.5, $(CH_3)_2CH$). ^{13}C-NMR (50.1 MHz, $CDCl_3$): 210.7 (C=O); 163.8 (C(5)); 155.4 (C(7)); 154.1 (C(8a)); 124.9 (C(4)); 116.5 (C(3)); 105.1 (C(8)); 102.3, 101.3 (C(4a), C(6)); 77.7 (C(2)); 39.3 ($(CH_3)_2CH$); 27.7 (2 CH_3–C(2)); 19.5 ($(CH_3)_2CH$); 7.1 (CH_3–C(6)). EI-MS: 276 ($M^{+\cdot}$), 261 (100, $M^{+\cdot} - CH_3$), 243, 233, 191.

*1-(5,7-Dihydroxy-2,2,6-trimethyl-2*H*-1-benzopyran-8-yl)-2-methyl-1-butanone* (**2**). Yellow oil. TLC (SiO_2, toluene/AcOEt 93:7): R_f 0.41. UV data: as for **1**. ^{1}H-NMR (200 MHz, $CDCl_3$): 14.18 (*s*, OH–C(7)); 6.56 (*d*, *J* = 10, H–C(4)); 5.45 (*d*, *J* = 10, H–C(3)); 5.39 (*s*, OH–C(5)); 3.77 (*sext.*, *J* = 6.5, $CH_3CH_2CH(CH_3)$); 2.04 (*s*, CH_3–C(6)); 1.85 (*m*, 1 H, $CH_3CH_2CH(CH_3)$); 1.48, 1.47 (2*s*, 2 CH_3–C(2)); 1.44 (*m*, 1 H, $CH_3CH_2CH(CH_3)$); 1.16 (*d*, *J* = 6.5, $CH_3CH_2CH(CH_3)$); 0.9 (*dd*, *J* = 7.5, 7.5, $CH_3CH_2CH(CH_3)$). ^{13}C-NMR (50.1 MHz, $CDCl_3$): 210.9 (C=O); 163.7 (C(5)); 155.3 (C(7)); 154.2 (C(8a)); 125.0 (C(4)); 116.5 (C(3)); 105.6 (C(8)); 102.2, 101.4 (C(4a), C(6)); 77.7 (C(2)); 46.1 ($CH_3CH_2CH(CH_3)$); 27.7 (2 CH_3–C(2)); 26.9 ($CH_3CH_2CH(CH_3)$); 16.9 ($CH_3CH_2CH(CH_3)$); 12.0 ($CH_3CH_2CH(CH_3)$); 7.1 (CH_3–C(6)). EI-MS: 290 ($M^{+\cdot}$), 275 (100, $M^{+\cdot} - CH_3$), 257, 233, 191.

*1,1'-[1,13,13a,13b-Tetrahydro-5,8,10-trihydroxy-2,2,6,9,13,13-hexamethyl-2*H*,7a*H*-pyrano[3,2-c:4,5,6-d'e']di[1]benzopyran-4,11-diyl]bis[2-methyl-1-propanone]* (= *Hyperevoline*; **3**). Pale yellow prism from hexane/Et_2O 1:1. M.p. 206–210°. TLC (SiO_2, toluene/AcOEt 93:7): R_f 0.51, red with *Godin* reagent. UV (MeOH):226 (33000), 295 (36200). UV (MeOH/$AlCl_3$): unchanged. UV (MeOH/$AlCl_3$/HCl): unchanged. UV (MeOH/NaOMe): 305 (sh), 330. UV (MeOH/NaOAc): 300 (sh), 330. UV (MeOH/NaOAc/H_3BO_3): 295, 330. IR (KBr): 3400, 2960, 1610, 1290, 1250, 1200, 1130, 970, 870. ^{1}H-NMR (200 MHz, $CDCl_3$): 14.26, 13.83 (2*s*, OH–C(5), OH–C(10)); 6.82 (*s*, OH–C(8)); 4.68 (*d*, *J* = 5, H–C(7a)); 3.82 (*m*, 2 $(CH_3)_2CH$); 2.58 (*ddd*, *J* = 12, 4.5, 3.5, H–C(13b)); 2.16 (*dd*, *J* = 5, 4.5, H–C(13a)); 2.10, 2.08 (2*s*, CH_3–C(6), CH_3–C(9)); 2.05 (*m*, 1H–C(1)); 1.88 (*dd*, *J* = 12, 12, 1H–C(1)); 1.57, 1.53 (2*s*, 2 CH_3–C(13)); 1.46 (*s*, 2 CH_3–C(2)); 1.18 (4*d*, *J* = 7, 2 $(CH_3)_2CH$). ^{13}C-NMR (50.1 MHz, $CDCl_3$): 210.9, 210.2 (2 C=O); 166.0, 162.6, 160 (C(5), C(8), C(10)); 158.0, 154.1, 153.4, (C(3a), C(6a), C(11a)); 106.4, 105.7, 105.3, 104.6, 100.4, 97.8 (C(4), C(6), C(7b), C(9), C(11), C(13c)); 78.4, 77.4 (C(2), C(13)); 70.3 (C(7a)); 47.5 (C(13b)); 42.2 (C(1)); 39.6, 39.5 (2 $(CH_3)_2CH$); 30.1 (CH_3–C(13)); 28.0, 26.8 (2 CH_3–C(2)); 24.4 (C(13a)); 20.3 (CH_3–C(13)); 19.7, 19.6, 19.4, 19.3 (2 $(CH_3)_2CH$); 7.4, 7.3 (CH_3–C(6), CH_3–C(9)). EI-MS: 552 ($M^{+\cdot}$), 537, 277 (100), 261, 243, 233, 205.

X-Ray Analysis of **3**. Suitable crystals were grown from hexane/Et_2O 1:1. Crystal data: $C_{32}H_{40}O_8$, M_r = 552, space group $P2_1/n$, a = 12.408(1), b = 18.151 (1), c = 13.207 (1) Å, β = 100.73(1)°, V = 2922.4 Å^3, $F(000)$ = 1184, Z = 4, D_x = 1.254 gcm^{-3}, Mo$K\alpha$, λ = 0.71073 Å, μ = 0.53 cm^{-1}. A crystal of dimensions 0.27 × 0.27 × 0.57 mm was used for data collection. Preliminary *Weissenberg* and precession photographs indicated the crystals to be monoclinic, space group $P2_1/n$. Intensity data with index limits h –14 to 14, k 0 to 21, and l 0 to 15 with θ_{max} = 25° were measured on a *Stoe Siemens AED2* four-circle diffractometer (graphite-monochromated Mo$K\alpha$ radiation) using the ω/θ scan mode. There was no significant intensity variation for 5 standard reflections measured every h. Of 4945 unique reflections measured, 3306 were considered observed ($F_o > 6\sigma(F_o)$). Cell parameters from ± ω values of 14 reflections and their equivalents in the range 30° < 2θ < 40°. No absorption or extinction corrections applied. The structure was solved by direct methods using the SHELX-76 system [19] which was used for all further calculations. In the final cycles of least-squares refinement, the majority of H-atoms located from difference maps were included and refined isotropically. The protons $(CH_3)_2CH$ were included in idealized positions and treated as 'rigid groups' as the C-atoms undergo considerable thermal motion, probably due to a small degree of rotational disorder (C–H = 1.08 Å, H–C–H = 109.5° with an overall U_{iso} refined value 0.153). Weighted anisotropic blocked (374 + 114 parameters) full-matrix least-squares refinement for 3306 reflections converged at R = 0.061, R_w = 0.071; $w^{-1} = \sigma^2(F_o)+0.01323(F_o)^2$. Average parameters shift/e.s.d. < 0.1. Heights in final difference map, ρ_{max} = 0.77 ρ_{min} = – 0.33 eÅ^{-3}. The ρ_{max} was observed in the region of the $(CH_3)_2CH$ groups and indicated (from distances and angles) a small degree of rotational disorder of these groups; no attempt was made to include this disorder in the model. Atomic scattering factors were taken from 'International Tables for X-Ray Crystallography'

Table 1. *Final Positional Parameters and Equivalent Isotropic Thermal Parameters* ($\times 10^4$) *for* **3** *with e.s.d.'s in Parentheses.* $U_{eq} = 1/3\,\Sigma_i\,\Sigma_j\,U_{ij}\,a_i^*a_j^*(\bar{a}_i\,\bar{a}_j)$.

Atom	x/a	y/b	c/z	U_{eq} (Å^2)	Atom	x/a	y/b	c/z	U_{eq} (Å^2)
C(1)	5147(2)	2628(2)	907(2)	376(7)	C(13)	6169(3)	4206(1)	2263(2)	388(8)
C(2)	5384(2)	1834(2)	666(2)	373(7)	C(13a)	5911(2)	3947(1)	1140(2)	314(7)
O(3)	5818(2)	1806(1)	-289(2)	474(6)	C(13b)	6159(2)	3111(1)	1013(2)	316(7)
C(3a)	6538(2)	2330(1)	-475(2)	360(7)	C(13c)	6718(2)	2971(1)	108(2)	327(7)
C(4)	7108(3)	2195(2)	-1299(2)	408(8)	C(14)	4334(3)	1385(2)	426(3)	516(9)
C(5)	7931(3)	2711(2)	-1429(2)	406(8)	C(15)	6213(3)	1471(2)	1496(3)	553(10)
O(5)	8542(2)	2616(1)	-2165(2)	606(7)	C(16)	6835(3)	1590(2)	-2041(3)	530(10)
C(6)	8122(2)	3356(2)	-855(2)	395(8)	O(16)	7456(2)	1468(1)	-2666(2)	620(7)
C(6a)	7481(2)	3480(1)	-124(2)	328(7)	C(17)	5756(3)	1140(2)	-2177(3)	649(11)
O(7)	7577(2)	4129(1)	410(2)	339(5)	C(18)	5271(4)	1027(3)	-3299(4)	800(14)
C(7a)	6492(2)	4422(1)	454(2)	292(6)	C(19)	6014(6)	420(3)	-1630(5)	1058(20)
C(7b)	6606(2)	5217(1)	809(2)	324(7)	C(20)	8960(3)	3912(2)	-1068(3)	567(10)
C(8)	7077(2)	5740(2)	230(2)	358(7)	C(21)	7860(3)	7011(2)	31(3)	557(10)
O(8)	7367(2)	5491(1)	-646(2)	512(6)	C(22)	5675(3)	6512(2)	2762(3)	491(9)
C(9)	7231(3)	6473(2)	547(2)	384(7)	O(22)	5741(3)	7193(1)	2929(2)	862(10)
C(10)	6745(3)	6695(2)	1365(2)	383(8)	C(23)	5076(3)	6072(2)	3439(3)	587(10)
O(10)	6832(2)	7419(1)	1602(2)	575(7)	C(24)	4186(7)	6519(4)	3792(7)	1579(34)
C(11)	6192(2)	6212(2)	1934(2)	363(7)	C(25)	5887(7)	5811(4)	4378(5)	1173(23)
C(1a)	6202(2)	5451(1)	1654(2)	310(7)	C(26)	5494(4)	3813(2)	2931(3)	654(11)
O(12)	5786(2)	4970(1)	2261(2)	427(6)	C(27)	7394(3)	4202(2)	2742(2)	470(9)

[20]. Final positional and equivalent thermal parameters are given in *Table 1*, bond distances and angles in *Table 2*. The numbering scheme is apparent from *Fig. 2* prepared using ORTEP [21]. Supplementary material is available from *H. St.-E.*

*2-Methyl-1-(1,13,13a,13b-tetrahydro-5,8,10-trihydroxy-4-isobutyryl-2,2,6,9,13,12-hexamethyl-2*H,7*a*H-*pyrano*[3,2-c:4,5,6-d'e']*di*[1]*benzopyran-11-yl)-1-butanone*/*2-Methyl-1-(1,13,13a,13b-tetrahydro-5,8,10-trihydroxy-11-isobutyryl-2,2,6,9,13,13-hexamethyl-2*H,7*a*H-*pyrano*[3,2-c:4,5,6-d'e']*di*[1]*benzopyran-4-yl)-1-butanone* (**4a**/**4b**). Pale yellow prisms from hexane/Et_2O 1:1. M.p. 193–197°. TLC (SiO_2, toluene/AcOEt 93:7): R_f 0.51. UV data: as for **3**. ^{1}H-NMR ($CDCl_3$, 200 MHz): 14.31, 14.25, 13.84, 13.82 (OH–C(5), OH–C(10)); 6.82, 6.81 (H–C(8)); 4.70, 4.68 (split *d*, H–C(7a)); 3.83, 3.7 (*m*, $(CH_3)_2CH$, $CH_3CH_2CH(CH_3)$); 2.58 (*m*, H–C(13b)); 2.17 (*m*, H–C(13a)); 2.11, 2.08 (2*s*, CH_3–C(6), CH_3–C(9)); 2.05 (*m*, H–C(1)); 2.0–1.6 (br. *m*, 2 H, H–C(1), 1 H of $CH_3CH_2CH(CH_3)$); 1.57, 1.53 (2*s*, 2 CH_3–C(13)); 1.46 (*s*, 2 CH_3–C(2)); 1.42 (*m*, 1 H of $CH_3CH_2CH(CH_3)$); 1.2 (*m*, $(CH_3)_2CH$, $CH_3CH_2CH(CH_3)$); 0.9 (*m*, $CH_3CH_2CH(CH_3)$). ^{13}C-NMR (50.1 MHz): 210.9, 210.2 (2 C=O); 166.1, 162.6, 160.0 (C(5), C(8), C(10)); 158.0, 154.1, 153.5 (C(3a), C(6a), C(11a)); 106.5, 105.8, 105.4, 104.7, 100.6, 97.8 (C(4), C(6), C(7b), C(9), C(11), C(13c)); 78.6, 77.4 (C(2), C(13)); 70.4 (C(7a)); 47.6 (C(13b)); 46.3 ($CH_3CH_2CH(CH_3)$); 42.4 (C(1)); 39.6 ($(CH_3)_2CH$); 30.1 (CH_3–C(13)); 28.2 (CH_3–C(2)); 27.0 ($CH_3CH_2CH(CH_3)$); 26.8 (CH_3–C(2)); 24.4 (C(13a)); 20.4 (CH_3–C(13)); 19.7, 19.3 ($(CH_3)_2CH$); 16.8, 17.2 ($CH_3CH_2CH(CH_3)$); 12.0 ($CH_3CH_2CH(CH_3)$); 7.5, 7.3 (CH_3–C(6), CH–C(9)). EI-MS: 566 ($M^{+\cdot}$), 551, 291, 277 (100), 261, 233, 205.

*1,1'-[1,13,13a,13b-Tetrahydro-5,8,10-trihydroxy-2,2,6,9,13,13-hexamethyl-2*H,7*a*H-*pyrano*[3,2-c:4,5,6-d'e']*di*[1]*benzopyran-4,11-diyl*]*bis*[2-*methyl-1-butanone*] (**5**). Pale yellow crystals from hexane/Et_2O 1:1. M.p. 170–173°. TLC (SiO_2, toluene/AcOEt 93:7): R_f 0.51. UV data: as for **3**. ^{1}H-NMR ($CDCl_3$, 200 MHz): 14.33, 13.87 (2*s*, OH–C(5), OH–C(10)); 6.84 (*s*, OH–C(8)); 4.69 (*d*, J = 5, H–C(7a)); 3.71 (*m*, 2 $CH_3CH_2CH(CH_3)$); 2.58 (*ddd*, H–C(13b)); 2.17 (*m*, H–C(13a)); 2.11, 2.10 (2*s*, CH_3–C(6), CH_3–C(9)); 2.05–1.78 (br. *m*); 1.58, 1.53 (2*s*, 2 CH_3–C(13)); 1.46, 1.44 (2*s*, 2 CH_3–C(2)); 1.42–1.35 (*m*); 1.16, 1.15 (2*d*, J = 6.8, 2 $CH_3CH_2CH(CH_3)$); 0.89 (*m*, 2 $CH_3CH_2CH(CH_3)$). ^{13}C-NMR ($CDCl_3$, 50.1 MHz): 210.8, 210.1 (2 C=O); 166.0, 162.5, 159.9 (C(5), C(8), C(10)); 158.0, 154.2, 153.5 (C(3a), C(6a), C(11a)); 105.8, 105.3, 105.1, 104.5, 100.5, 97.8 (C(4), C(6), C(7b), C(9), C(11), C(13c)); 78.4, 77.4 (C(2), C(13)); 70.3 (C(7a)); 47.5 (C(13b)); 46.3, 46.2 (2 $CH_3CH_2CH(CH_3)$); 42.3 (C(1)); 30.1 (CH_3–C(13)); 28.1 (CH_3–C(2)); 27.0 ($CH_3CH_2CH(CH_3)$); 26.8 (CH_3–C(2)); 26.7 ($CH_3CH_2CH(CH_3)$); 24.4 (C(13a)); 20.3 (CH_3–C(13)); 17.2, 16.7 (2 $CH_3CH_2CH(CH_3)$); 12.0 (2 $CH_3CH_2CH(CH_3)$); 7.5, 7.3 (CH_3–C(6), CH_3–C(9)). EI-MS: 580 ($M^{+\cdot}$), 565, 291 (100), 275, 233, 205.

Table 2. *Bond Distances* (Å) *and Angles* (°) *for* **3**

C(1)–C(2)	1.516(4)	C(7a)–C(7b)	1.516(4)	C(13a)–C(13b)	1.563(4)
C(1)–C(13b)	1.517(4)	C(7a)–C(13a)	1.525(4)	C(13b)–C(13c)	1.511(4)
C(2)–O(3)	1.462(4)	C(7b)–C(8)	1.413(4)	C(16)–O(16)	1.250(5)
C(2)–C(14)	1.518(4)	C(7b)–C(11a)	1.374(4)	C(16)–C(17)	1.550(6)
C(2)–C(15)	1.508(4)	C(8)–O(8)	1.351(4)	C(17)–C(18)	1.505(6)
O(3)–C(3a)	1.358(4)	C(8)–C(9)	1.397(4)	C(17)–C(19)	1.500(7)
C(3a)–C(4)	1.425(5)	C(9)–C(10)	1.391(5)	C(22)–O(22)	1.256(4)
C(3a)–C(13c)	1.390(4)	C(9)–C(21)	1.492(5)	C(22)–C(23)	1.497(6)
C(4)–C(5)	1.419(4)	C(10)–O(10)	1.349(4)	C(23)–C(24)	1.513(10)
C(4)–C(16)	1.468(4)	C(10)–C(11)	1.413(4)	C(23)–C(25)	1.520(7)
C(5)–O(5)	1.350(4)	C(11)–C(11a)	1.430(4)	H(05)...O(16)	1.67(4)
C(5)–C(6)	1.392(4)	C(11)–C(22)	1.471(5)	O(5)...O(16)	2.503(3)
C(6)–C(6a)	1.379(4)	C(11a)–O(12)	1.351(4)	H(010)...(022)	1.57(5)
C(6)–C(20)	1.512(5)	O(12)–C(13)	1.465(3)	O(10)...O(22)	2.441(4)
C(6a)–C(13c)	1.398(4)	C(13)–C(13a)	1.533(4)	H(08)...O(16)[a])	2.064(4)
C(6a)–O(7)	1.367(3)	C(13)–C(26)	1.505(5)	O(8)...O(16)[a])	2.889(3)
O(7)–C(7a)	1.459(3)	C(13)–C(27)	1.533(5)		

C(2)–C(1)–C(13b)	112.4(2)	C(7b)–C(7a)–C(13a)	112.4(2)	C(7a)–C(13a)–C(13)	111.4(2)
C(1)–C(2)–O(3)	109.3(2)	C(7a)–C(7b)–C(8)	119.8(3)	C(7a)–C(13a)–C(13b)	111.1(2)
C(1)–C(2)–C(14)	111.2(3)	C(7a)–C(7b)–C(11a)	121.3(3)	C(13)–C(13a)–C(13b)	113.0(2)
O(3)–C(2)–C(14)	104.(2)	C(8)–C(7b)–C(11a)	118.8(2)	C(1)–C(13b)–C(13a)	113.3(2)
C(1)–C(2)–C(15)	113.3(2)	C(7b)–C(8)–O(8)	116.1(2)	C(1)–C(13b)–C(13c)	108.8(2)
O(3)–C(2)–C(15)	107.5(3)	C(7b)–C(8)–C(9)	121.7(3)	C(13a)–C(13b)–C(13c)	112.2(2)
C(14)–C(2)–C(15)	111.1(3)	O(8)–C(8)–C(9)	122.2(3)	C(3a)–C(13c)–C(6a)	118.5(3)
C(2)–O(3)–C(3a)	119.6(2)	C(8)–C(9)–C(10)	117.0(3)	C(3a)–C(13c)–C(13b)	122.0(3)
O(3)–C(3a)–C(4)	117.1(2)	C(8)–C(9)–C(21)	122.7(3)	C(6a)–C(13c)–C(13b)	119.4(2)
O(3)–C(3a)–C(13c)	121.9(3)	C(10)–C(9)–O(10)	120.3(3)	C(4)–C(16)–O(16)	118.6(3)
C(4)–C(3a)–C(13c)	121.1(3)	C(9)–C(10)–O(10)	115.8(3)	C(4)–C(16)–C(17)	124.0(3)
C(3a)–C(4)–C(5)	116.7(3)	C(9)–C(10)–C(11)	123.8(3)	O(16)–C(16)–C(17)	117.1(3)
C(3a)–(4)–C(16)	123.5(3)	O(10)–C(10)–C(11)	120.4(3)	C(16)–C(17)–C(18)	111.4(4)
C(5)–C(4)–C(16)	119.7(3)	C(10)–C(11)–C(11a)	115.7(3)	C(16)–C(17)–C(19)	107.5(4)
C(4)–C(5)–O(5)	121.1(3)	C(10)–C(11)–C(22)	119.1(3)	C(18)–C(17)–C(19)	111.4(4)
C(4)–C(5)–C(6)	122.9(3)	C(11a)–C(11)–C(22)	125.2(3)	C(11)–(C(22)–O(22)	118.2(3)
O(5)–C(5)–C(6)	115.9(3)	C(7b)–C(11a)–C(11)	122.1(3)	C(11)–C(22)–C(23)	125.4(3)
C(5)–C(6)–C(6a)	117.1(3)	C(7b)–C(11a)–O(12)	121.3(2)	O(22)–C(22)–C(23)	116.3(3)
C(5)–C(6)–C(20)	121.0(3)	C(11)–C(11a)–O(12)	116.6(3)	C(22)–C(23)–C(24)	111.7(4)
C(6a)–C(6)–C(20)	121.8(3)	C(11a)–O(12)–C(13)	116.9(2)	C(22)–C(23)–C(25)	109.1(4)
C(6)–C(6a)–C(13c)	123.3(3)	O(12)–C(13)–C(13a)	106.2(2)	C(24)–C(23)–C(25)	109.1(5)
C(6)–(6a)–O(7)	119.7(3)	O(12)–C(13)–C(26)	103.5(3)	O(5)–H(O5)...O(16)	149(7)
O(7)–C(6a)–C(13c)	117.1(3)	C(13a)–C(13)–C(26)	112.5(2)	O(10)–H(010).O(22)	149(7)
C(6a)–O(7)–C(7a)	109.9(2)	O(12)–C(13)–C(27)	107.6(2)	O(8)–H(08)...O(16)[a])	161(5)
O(7)–C(7a)–C(7b)	109.0(2)	C(13a)–C(13)–C(27)	114.2(3)		
O(7)–C(7a)–C(13a)	111.2(2)	C(26)–C(13)–C(27)	111.9(3)		

[a]) Symmetry operation $1.5 - x, 0.5 + y, -0.5 - z$.

Acetylations. The starting material was dissolved in 1 ml of Ac_2O/pyridine 1:1 and stirred at r.t. for 24 h for **1** and 36 h for **3**. The mixture was poured into ice/H_2O and the precipitate filtered off. The crude product was purified on silica gel (*Merck*; 0.063–0.2 mm) with $CHCl_3$.

Diacetate **1a** (4.2 mg) was obtained from **1** (5 mg). Colorless gum, TLC (SiO_2, toluene/AcOEt 93:7): R_f 0.20. ^{1}H-NMR ($CDCl_3$, 200 MHz):6.22 (*d*, J = 10, H–C(4)); 5.64 (*d*, J = 10, H–C(3)); 3.17 (*sept.*, J = 7, $(CH_3)_2CH$); 2.35, 2.22 (2*s*, 2 CH_3COO); 1.85 (*s*, CH_3–C(6)); 1.42 (*s*, 2 CH_3–C(2)); 1.14 (*d*, J = 7, $(CH_3)_2CH$). EI-MS: 360 ($M^{+\cdot}$), 318, 303, 275, 261 (100), 233.

Triacetate **3a** was obtained from **3** (5 mg) and recrystallized from hexane/Et_2O 1:1 to give 3.3 mg of white microneedles. M.p. 176–178°. TLC (SiO_2, toluene/AcOEt 93:7): R_f 0.07. ^{1}H-NMR: 4.4 (br. *d*, H–C(7a)); 3.15 (2

sept., J = 7, 2 $(CH_3)_2CH$); 2.55 (*m*, H–C(13b)); 2.23 (2*s*, 2 CH_3COO); 2.21 (*s*, CH_3COO); 2.11 (*m*, H–C(13a)); 1.99, 1.88 (2*s*, CH_3–C(6), CH_3–C(9)); 1.92 (*m*, CH_2(1)); 1.48, 1.43, 1.41 (3*s*, 2 CH_3–C(2), 2 CH_3–C(13)); 1.18, 1.15, 1.12 (*m*, 2 $(CH_3)_2CH$). EI-MS: 678 ($M^{+\cdot}$), 635, 593, 261, 233.

Acidic Dimerization. At r.t. **1** (4 mg) in 8 ml of MeOH/1N HCl 1:1 was stirred for 6 h and allowed to stand overnight. The mixture was evaporated to dryness, filtered through a small column of SiO_2 (*Merck*; 63–200 μm) with $CHCl_3$/hexane/MeOH 80:8:0.3 and the filtrate subjected to semi-prep. HPLC on *RP18* with MeOH/H_2O 94:6 to afford 2.1 mg of **3**. Crystallisation from hexane/Et_2O 1:1 gave pale yellow crystals of **3**, with identical retention time on *RP18*, m.p., UV, EI-MS, and ^{1}H-NMR as **3** from *Hypericum revolutum*.

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