200. New Cell Growth-Inhibitory Cyclohexadienone Derivatives from *Hypericum calycinum* L.[1])

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The crude petroleum-ether extract of the aerial parts of *Hypericum calycinum* L. (Guttiferae) exhibited *in vitro* growth-inhibitory activity against the Co-115 human colon carcinoma cell line. Bioassay-guided fractionation of this extract allowed the isolation of the cyclohexadienone derivatives **1**–**5**, four of which are previously undescribed compounds. The structures of the known chinesin II (**1**) and of **2** (hypercalin A) were established by ^{1}H- and ^{13}C-NMR spectroscopy and were confirmed by X-ray analysis of their crystalline mixture which revealed the complete relative configuration of both compounds. The structure of **3** (hypercalin B) was elucidated by means of extensive 1D- and 2D-NMR experiments, including DQ-COSY, HETCOR and LR-HETCOR. The structure of compound **4** (hypercalin C) was established by ^{1}H- and ^{13}C-NMR spectroscopy and confirmed by X-ray analysis to be the 3,5-dihydroxy-4-{[(1*R**,2*S**,5*S**)-2-hydroxy-2-methyl-5-(1-methylethenyl)cyclopentyl]methyl}-6,6-bis-(3-methylbut-2-enyl)-2-(2-methylpropanoyl)cyclohexa-2,4-dien-1-one. The structures of the higher isomeric homologues **5a**/**5b** were deduced by comparison of their UV, ^{1}H-, and ^{13}C-NMR spectra with those of **4**. The isolated compounds appeared to be related to chinesin I and II previously isolated from *Hypericum chinense* L. and were responsible for the growth-inhibitory activity of the extract against the Co-115 human carcinoma cell line. Moreover, **1**/**2** and **3** showed molluscicidal activity against the schistosomiasis-transmitting snail *Biomphalaria glabrata*.

Introduction. – There is a growing interest in the constituents of the genus *Hypericum* (Guttiferae) since some of them have been found to possess very interesting biological properties. Among them, hypericin which is a red dianthrone derivative isolated from St John's wort (*Hypericum perforatum* L.) was first mentioned as the main active component responsible for the photosensibilizing effect of the plant [1]. Furthermore, the extracts of *Hypericum perforatum* L. have been claimed in popular medecine to possess an antidepressive activity which has been demonstrated *in vivo* [2]: this activity was first explained by the presence of hypericin which was shown to inhibit *in vitro* type A and B monoamine oxydase [3]. However, newest investigations have demonstrated that hypericin does not display any inhibition of monoamine oxidase *in vitro* [4]. Recently, it has been found that hypericin and pseudohypericin possess high antiretroviral activity *in vitro* and *in vivo*, suggesting that these compounds may be of interest in the search of new tools against the acquired immune disease syndrom [5].

[1]) Presented in part at the 36th Congress of the Society of Medicinal Plant Research, Freiburg im Breisgau, FRG, 12–16th September, 1988, and at the Meeting of the Swiss Chemical Society, Bern, 21st October, 1988.

Other phytochemical investigations of the genus *Hypericum* have led to the isolation of chinesin I and II from *Hypericum chinense* L. These two compounds display interesting antimicrobial activities against various microorganisms. In addition, chinesin I was tested *in vitro* against *HeLa* cells and was shown to have a relatively strong cytotoxicity [6].

During our biological and chemical investigations of African medicinal plants, new antifungal chromenyl ketones and their pentacyclic dimers have been isolated from the aerial parts of the South-East African plant *Hypericum revolutum* VAHL [7]. More extensive investigations of this plant have shown the lipophilic extract of the root bark to possess growth-inhibitory activity against the Co-115 human colon carcinoma cell line. Fractionation of this extract resulted in the isolation of hyperevolutin A and B which were found to be the main active components [8].

Extending our biological screening to some European species of *Hypericum,* we have shown that the light petroleum ether extract of *Hypericum calycinum* L. possessed a significant growth-inhibitory activity against the same cell line [9]. *Hypericum calycinum* L. (Guttiferae) originates from South-Eastern Europe and Asia Minor but is now found as a horticultural plant in parks and gardens all over Europe. Only a few reports have appeared on its constituents, namely on the tannin content [10] and on xanthone derivatives [11]. Bioassay-guided fractionation of the light petroleum ether extract of *Hypericum calycinum* L. led to the isolation of five cyclohexadienone derivatives **1**–**5**. One of them, compound **1**, was found to be identical with chinesin II previously isolated from *Hypericum chinense* L. [6]. The crystalline mixture of **1** and **2**, compound **3**, and the crystalline mixture of **4** and **5a**/**5b** were shown to be responsible for the activity of the extract in the Co-115 growth-inhibition bioassay. All isolated compounds appear to possess some structural similarities with colupulone isolated from *Humulus lupulus* L. [12].

Results. – The powdered aerial parts of *Hypericum calycinum* L. (collected in Champéry VS, Switzerland) were extracted with light petroleum ether. The extract was included in a general biological screening of *Hypericum* species and subjected to growth-inhibitory testing against the Co-115 human colon carcinoma cell line. It was found to be active with an ED_{50} of 2.7 μg/ml. Preliminary fractionation of the extract was performed by droplet counter-current chromatography (DCCC) using light petroleum ether/EtOH (96%)/ AcOEt/H_2O 83:67:33:17 (ascending mode) as solvent system, which allowed a good localization of the activity into 3 fractions.

The major fraction (ED_{50} = 0.34 μg/ml) was crystallized from hexane yielding a crystalline component which was found to be responsible for the activity of this fraction. The DCI-MS of this apparently pure compound (ED_{50} = 0.37 μl/ml) showed, however, two ion peaks at *m/z* 445 and 431 suggesting a mixture of homologues. Anal. HPLC of the crystals was performed on *RP-18* (photodiode array detector). With MeOH/H_2O 85:15, no separation occurred (elution with the solvent front). Addition of 0.1% AcOH to the solvent system caused a complete change of the elution pattern and gave a base-line separation of the two compounds **1** and **2**. Separation of **1** and **2** on a semi-prep. scale was possible with the same solvent system. Compound **1**, an unstable transparent oil, had a mol. wt. of 430 and ^{1}H- and ^{13}C-NMR data (see *Table 1*) in good accordance with those given for chinesin II previously isolated from *Hypericum chinense* L. [6]. The major compound **2** (mol. wt. 444) which we call hypercalin A crystallized from hexane as white transparent needles. The ^{1}H- and ^{13}C-NMR spectra of hypercalin A (**2**) showed some

Table 1. *^{13}C-NMR Data* **1–5**[a])

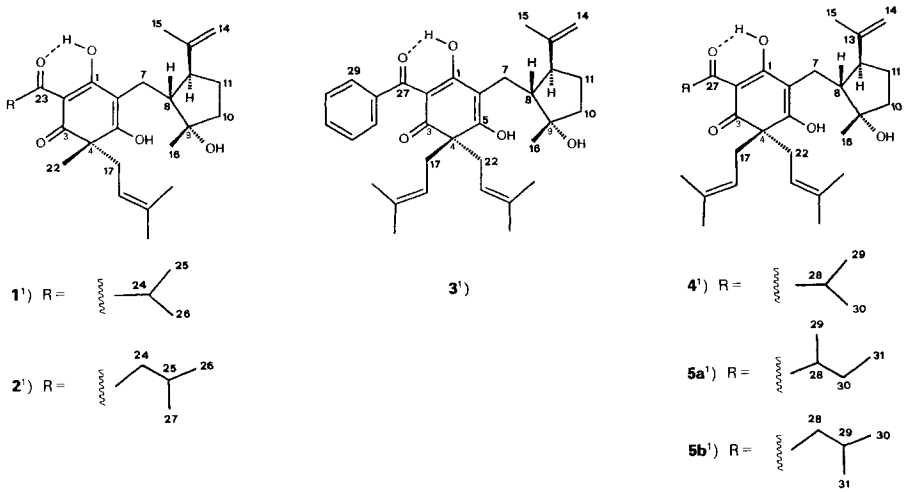
	1[b])	**2**[b])	**3**[c])	**4**[b])	**5a**[b])[c])	**5b**[b])[d])
C(1)	189.8	189.7	189.0	190.3	190.4	190.2
C(2)	105.7	107.2	107.6	107.1	108.5	107.8
C(3)	196.5	196.9	195.4	195.9	196.2	196.0
C(4)	53.1	53.0	57.8	57.7	57.64	57.57
C(5)	174.4	174.6	176.2	174.2	174.3	174.1
C(6)	108.2	108.4	109.5	109.7	109.8	
C(7)	21.1	21.1	21.6	21.6	21.6	
C(8)	50.0	50.0	50.1	49.9	49.9	
C(9)	81.5	81.3	80.7	81.2	81.2	
C(10)	43.6	43.5	43.2	43.8	43.7	
C(11)	28.5	28.5	28.8	28.8	28.8	
C(12)	54.1	54.1	54.2	54.4	54.3	
C(13)	146.1	146.2	146.2	146.1	146.2	
C(14)	111.7	111.7	111.6	111.8	111.7	
C(15)	18.5	18.5	18.5	18.4	18.5	
C(16)	29.1	29.1	29.2	29.2	29.23	29.15
C(17)	37.6	37.7	37.3	37.3	37.5	37.3
C(18)	118.8	118.7	119.6	119.5	119.6	119.5
C(19)	134.6	134.6	134.0	133.9	133.92	133.89
C(20)	17.9	17.9	18.0	17.9	17.9	
C(21)	25.8	25.8	25.85	25.8	25.8	
C(22)	24.6	24.5	38.4	38.8	38.9	38.6
C(23)	207.9	203.0	118.3	118.1	118.2	118.0
C(24)	35.6	48.3	134.7	134.8	134.9	134.7
C(25)	18.7	25.7	25.87	25.9	25.9	
C(26)	19.1	22.8	18.1	18.0	18.0	
C(27)		22.6	196.6	207.4	207.0	202.5
C(28)			139.5	35.6	42	48.4
C(29)			127.7	18.8	16.6	25.9
C(30)			127.6	18.9	26.4	22.7
C(31)			130.6		11.9	22.8
C(32)			127.6			
C(33)			127.7			

[a]) Arbitrary numbering. [b]) 50.1 MHz, $CDCl_3$. [c]) 100.6 MHz, $CDCl_3$. [d]) Signals for C(1) to C(5), C(16) to C(19), and C(22) to C(24) may be attributed to the other isomer.

differences compared with those given for chinesin I: the signals of the 2-methylbutanoyl group of chinesin I were replaced by those of a 3-methylbutanoyl chain in **2**. An interesting feature of compounds **1** and **2** is the possible tautomerism of the enol protons implicating the presence of a second minor form in which the enolic OH group is located at C(3) and the keto group at C(1)[2]). Preliminary observations in the ^{1}H-NMR spectra suggest the existence, in $CDCl_3$ solution, of such a minor tautomer for both **1** and **2**.

In the ^{1}H-NMR spectrum of **2**, CH_2(24)[2]) appeared as 2 *dd* (J = 14, 7 Hz) centred at 3.04 and 2.79 ppm, whereas the signals of CH_3(26) and CH_3(27) appeared as 2 *d* (J = 6.5 Hz) at 0.98 and 0.96 ppm. The signal of H–C(25) could be detected as an obscured complex *m* at *ca.* 2.1 ppm by selective decoupling experiments on CH_2(24). The ^{13}C-NMR spectrum of **2** gave further evidence for the structure of the side chain, with one CH_2 at 48.2 ppm, one *C*H at 25.7 ppm, and two CH_3 at 22.8 and 22.6 ppm. In both **1** and **2**, a minor ^{1}H-NMR signal of an

[2]) Arbitrary numbering, as shown in the structures and *Table 1*.



enolic proton appeared at 18.35 ppm next to the major one at 19.2 ppm. Furthermore, in the 200-MHz ^{1}H-NMR spectra ($CDCl_3$), some additional signals of the H-bonded ketonic side chain could be observed at 25°: next to the main signal of H–C(24) of **1** at 4.01 ppm (*sept.*, $J = 6.8$ Hz), a minor signal was observed as a *sept.* at 4.26 ppm; additional signals were also observed for CH_2(24) of **2**.

In order to confirm the relative configuration of the cyclopentane ring and to establish the relative configuration at C(4), the crystalline mixture of the homologues chinesin II (**1**) and hypercalin A (**2**) was subjected to single-crystal X-ray analysis which confirmed the overall connectivity deduced by the NMR analysis for the major compound **2** and revealed the relative configuration at C(4) *(Fig. 1a)*. Nevertheless, this X-ray analysis does not justify a detailed discussion of the geometry of **2** due to the poor quality of the crystals. Crystals of pure **2** were found to be unsuitable for X-ray analysis.

The second active fraction (260 mg; $ED_{50} = 0.7$ µg/ml) showed, on silica-gel TLC, one major UV-active compound which was further purified by low-pressure chromatography

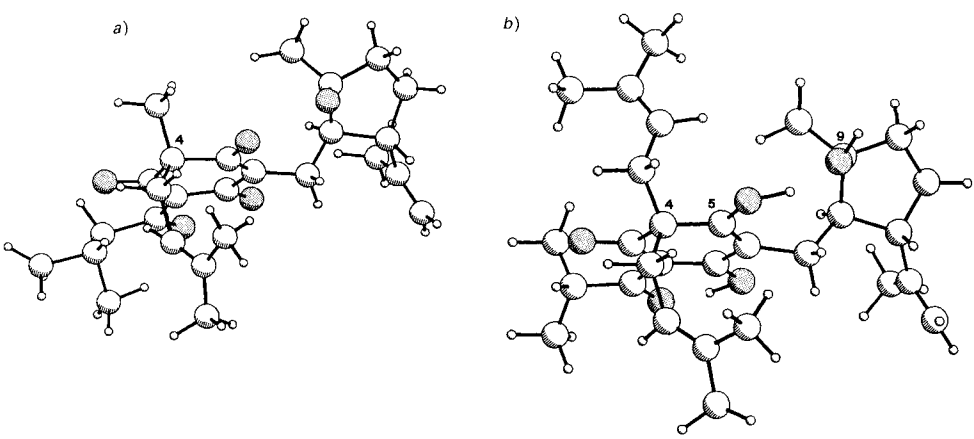


Fig. 1. *PLUTO* [13] *plots of* a) *hypercalin A* (**2**) *and* b) *of hypercalin C* (**4**).
O-atom, C-atom, —o : H-atom.

on a diol support using gradient elution with hexane/AcOEt 95:5 → 90:10. This yielded a pale yellow amorphous compound, hypercalin B (**3**), with $ED_{50} = 0.34$ μg/ml. It gave a $[M + H]^+$ ion peak at m/z 519 by DCI-MS (NH_3, positive ions), and its IR, ^{1}H-NMR, and ^{13}C-NMR spectra had some similarities with the ones of **1** and **2**. The structure of hypercalin B (**3**) was established by extensive 1D- and 2D-NMR experiments.

The ^{1}H-NMR spectrum of **3** showed the degenerated signals of 5 aromatic protons at 7.42–7.33 ppm, indicating the presence of 1 Ph ring. Compared to **2**, no signals due to a 3-methylbutanoyl side chain could be observed, and the *s* of CH_3(22) which appeared at 1.35 ppm in **2** was replaced by the characteristic signals of the protons of an additional prenyl chain at 4.83 ppm (*t*), 2.56, and 2.51 ppm (2*dd*, J = 14, 8 Hz). The position of this second prenyl moiety was first deduced by comparison with the ^{13}C-NMR of colupulone which is structurally related to **3**: a strong signal for the quaternary C-atom carrying the geminal alkenyl groups appears at 57.1 ppm in colupulone [14]. This high-intensity signal was also observed for **3** (at 57.8 ppm) and helped greatly identification. Extensive 2D-NMR experiments, including DQ-COSY, HETCOR [15], and LR-HETCOR allowed the assignments of the signals of the H- and C-atoms (see *Table 2*) and gave further evidence of the structure of **3**, particularly of its preferential enolic form. Interestingly, a 400-MHz LR-HETCOR of **3** in $CDCl_3$ (delays optimized for J(C,H) = 7 Hz) revealed long-range correlations *(Fig. 2)* between the two enolic C-atoms C(1) and C(5) at 189.0

Table 2. *NMR Data of Hypercalin B* (**3**)[a]) (400 MHz, $CDCl_3$; δ [ppm])

	^{13}C-NMR[b])	^{1}H-NMR (J [Hz]; HETCOR J(C,H) = 140 Hz)	DQ-COSY	LR-HETCOR[b]) (delays optimized for J(C,H) = 7 Hz)
C(1)	189.0			*C*(1)/O*H*–C(1), H_a–C(7), H_b–C(7)
C(2)	107.6			*C*(2)/O*H*–C(1)
C(3)	195.4			*C*(3)/CH_2(17)
C(4)	57.8			*C*(4)/CH_2–C(22)
C(5)	176.2			*C*(5)/H_a–C(7), H_b–C(7), CH_2(22)
C(6)	109.5			*C*(6)/H_a–C(7), H_b–C(7), *H*–C(8), O*H*–C(1)
CH_2(7)	21.6	2.12 (*dd*, J = 15, 12, H_a)	H_a–C(7)/H_b–C(7), H–C(8)	*C*(7)/*H*–C(8), *H*–C(12)
		2.65 (*dd*, 15, 3, H_b)	H_b–C(7)/H_a–C(7), H–C(8)	
H–C(8)	50.1	1.78 (*m*)	H–C(8)/H_a–C(7), H_b–C(7), H–C(12)	*C*(8)/CH_3(16), *H*–C(12), H_a–C(7), H_b–C(7)
C(9)	80.7			*C*(9)/H_b–C(7), H_a–C(10), CH_3(16)
CH_2(10)	43.2	1.64 (*m*, H_a)	H_a–C(10)/H_b–C(10), H_a–C(11), H_b–C(11),	*C*(10)/CH_3(16)
		1.84 (*m*, H_b)	H_b–C(10)/H_a–C(11), H_b–C(11), H_a–C(10)	
CH_2(11)	28.8	1.44 (*m*, H_a)	H_a–C(11)/H_b–C(11), H–C(12), H_a–C(10), H_b–C(10)	*C*(11)/*H*–C(12)
		1.74 (*m*, H_b)	H_b–C(11)/H_a–C(11), H–C(12), H_a–C(10), H_b–C(10)	
H–C(12)	54.2	2.37 (*ddd*, J = 11, 11, 7, 1H)	H–C(12)/H_a–C(11), H_b–C(11), H–C(8)	*C*(12)/CH_2(14)

Table 2 (cont.)

	^{13}C-NMR[b])	^{1}H-NMR (J [Hz]; HETCOR J(C,H) = 140 Hz)	DQ-COSY	LR-HETCOR[b]) (delays optimized for J(C,H) = 7 Hz)
C(13)	146.2			C(13)/CH_3(15), H_a–C(11), H_b–C(11), H–C(12)
CH_2(14)	111.6	4.86 (unresolv. *qd*, H_a) 4.83 (unresolv. *qd*, H_b)	CH_2(14)/CH_3(15)	C(14)/CH_3(15)
CH_3(15)	18.5	1.75 (*s*, 3H)	CH_3(15)/H_a–C(14), H_b–C(14)	C(15)/H_a–C(14), H_b–C(14)
CH_3(16)	29.2	1.23 (*s*, 3H)		C(16)/H–C(8)
CH_2(17)	37.3	2.60 (*m*, 2H)	CH_2(17)/$(CH_3)_2$C(19)	
H–C(18)	119.6	4.92 (*t*, J = 7)	H–C(18)/$(CH_3)_2$C(19), CH_2(17)	C(18)/CH_3(20) *or* CH_3(21)
C(19)	134.0			C(19)/CH_3(21)
CH_3(20)	18.0	1.53 (*s*, 3H)	CH_3(20)/CH_2(17)	C(20)/CH_3(21)
CH_3(21)	25.85	1.57 (*s*, 3H)	CH_3(21)/CH_2(17)	C(21)/CH_3(20), H–C(18)
CH_2(22)	38.4	2.51, 2.56 (2*dd*, J = 14, 8, 2H)	CH_2(22)/$(CH_3)_2$C(24)	
H–C(23)	118.3	4.83 (*m*, 1H)	H–C(23)/$(CH_3)_2$C(24), CH_2(22)	C(23)/CH_3(25) *or* CH_3(26)
C(24)	134.7			C(24)/CH_3(25), CH_2(22)
CH_3(25)	25.87	1.57 (*s*, 3H)	CH_3(25)/CH_2(22)	C(25)/CH_3(26), H–C(23)
CH_3(26)	18.1	1.55 (*s*, 3H)	CH_3(26)/CH_2(22)	C(26)/CH_3(25),
C(27)	196.6			C(27)/H–C(29), H–C(33), OH–C(1)
C(28)	139.5			C(28)/H–C(30), H–C(32)
H–C(29)	127.7	7.42 (*m*)		C(29)/arom. H's
H–C(30)	127.6	7.33 (*m*)		C(30)/arom. H's
H–C(31)	130.6	7.42 (*m*)		C(31)/arom. H's
H–C(32)	127.6	7.33 (*m*)		C(32)/arom. H's
H–C(33)	127.7	7.42 (*m*)		C(33)/arom. H's
OH–C(1)		18.17 (*s*)		

[a]) Arbitrary numbering, as shown in the structure.

[b]) Signals of the corresponding C-atoms of the two prenyl side chains (C(17) to C(21) and C(22) to C(26)) can be exchanged.

and 176.2 ppm, respectively, and the 2 H–C(7) at 2.65 ppm (*dd*, J = 15, 3 Hz) and 2.12 ppm (*dd*, J = 15, 12 Hz). Another long-range coupling could be observed between the ketonic C-atom C(3) (195.4 ppm) and the CH_2 protons of the prenyl chains, confirming the preferential tautomeric form of **3** in $CDCl_3$ solution.

The third fraction which similarly displayed activity in the Co-115 growth inhibition bioassay (ED_{50} = 0.5 μg/ml) contained one major component which was purified by crystallization from CH_2Cl_2/MeOH. This component, a mixture of hypercalin C (**4**; major) and its higher homologues **5a**/**5b** (minor) as shown by DCI-MS (mol. wt. 484 and 498, resp.), was recrystallized from MeCN and subjected to direct X-ray analysis. Although performed with a mixture of homologues (*cf.* **1**/**2**), the X-ray analysis allowed the

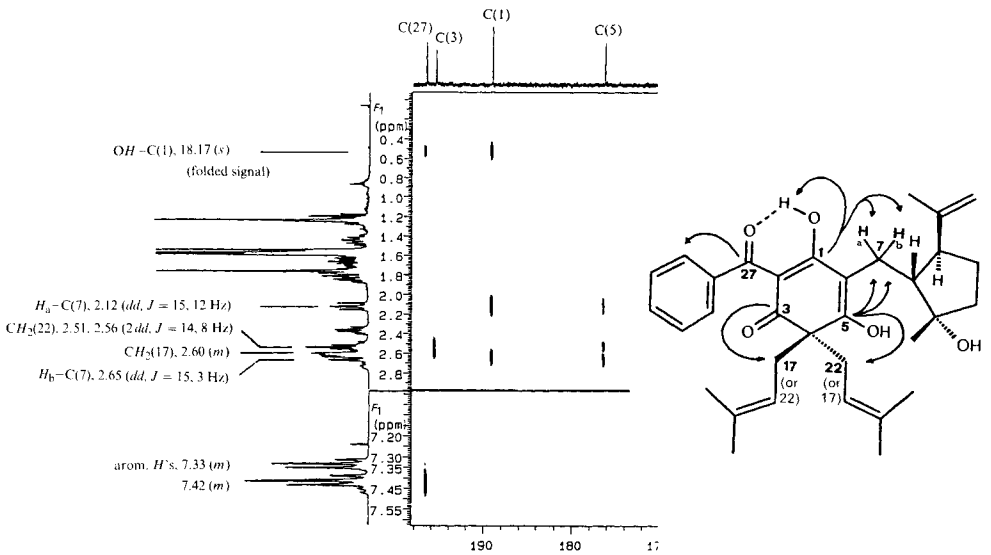


Fig. 2. *Partial long-range HETCOR* ($CDCl_3$, 400 MHz) *of hypercalin* B^2) (**3**). Delays optimized for J(C,H) = 7 Hz. For complete ^1H,^{13}C long-range correlations data, see *Table 2*.

complete structure elucidation of the major constituent, hypercalin C(**4**). Preparative low-pressure liquid chromatography of the mother liquors still containing **4**/**5** on a diol support (hexane 100% → hexane/AcOEt 95:5) enabled the isolation of more **4**/**5** (one single spot on silica-gel TLC (light petroleum ether/AcOEt 1:1 + 0.5% AcOH)). This sample was subjected to growth inhibition testing and was found to be active with ED_{50} = 0.3 μg/ml. Finally, semi-prep. HPLC of the crystalline material (obtained from an other batch) on *RP-18* ($MeOH/H_2O$ 85:15 + 0.1% AcOH) allowed the separation of pure hypercalin C (**4**) from its higher isomeric homologues **5a**/**5b**.

In the case of hypercalin C (**4**), the X-ray analysis was much more precise than in the case of hypercalin A (**2**) and, apart from confirming the connectivity deduced from NMR, allows a detailed discussion of its geometry (*Figs. 1b* and *3*). The six-membered ring (atom C(1)^i) to C(6)) is best described as having a half-chair conformation. Atoms C(4) and C(5) are displaced by −0.052(5) and 0.071(5) Å, respectively, from the best plane through atoms C(1), C(2), C(3), and C(6) (planar to within 0.005(3) Å). The five-membered ring (atoms C(8) to C(12)) has an envelope conformation, with atom C(12) displaced by 0.60(1) Å from the best plane through the remaining four atoms (planar to within 0.014(3) Å). The two aforementioned planes are inclined to one another by 130.8°, with torsion angle C(8)–C(7)–C(6)–C(1) and C(9)–C(8)–C(7)–C(6) being −82.4 and −67.8°, respectively. In the six-membered ring, the average bond length involving atom C(2) is 1.436(4) Å which indicates a high degree of delocalization involving bond C(1)=C(2), the hydroxy O(1) and carbonyl groups C(3)=O(3) and C(27)=O(27).

There are two H-bonds in molecule **4**. That involving H–O(1) and carbonyl O(27) is stronger than that involving H–O(5) and hydroxy O(9), see *Table 3*. Interestingly, the existence of a H-bond between *H*–O(5) and O(9) was suggested by *Nagai* and *Tada* after NOESY experiments on the methyl ether of chinesin I [6]. In the crystal, molecules related

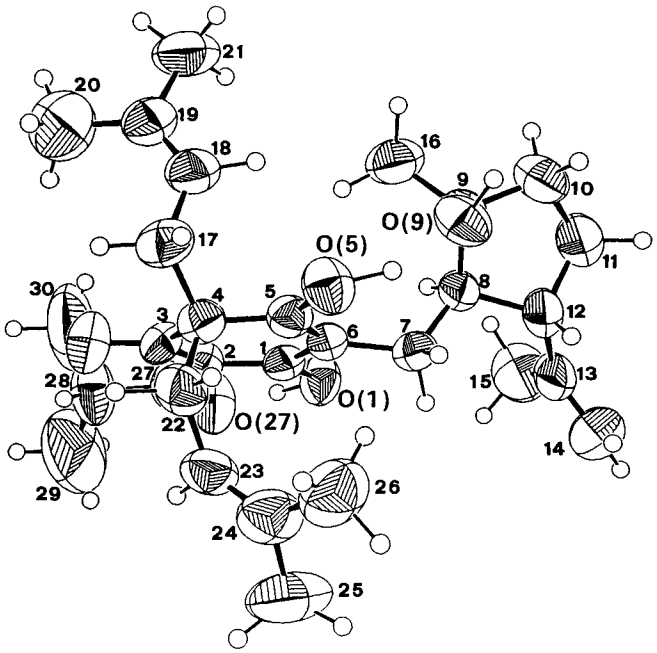


Fig. 3. *View of hypercalin C*[1]) (**4**) *showing the atomic-numbering scheme and the vibrational ellipsoids* (50% probability level)

by the 2-fold screw axis in the *c* direction are linked by a H-bond involving H–O(9) and carbonyl O(3), see *Table 3*. In the poorly analyzed structure of hypercalin A (**2**), the same overall geometry and the same inter- and intramolecular H-bonding system is observed.

The two higher isomeric homologues **5a**/**5b** could not be separated by semi-prep. HPLC on *RP-18* and crystallized together from hexane. Their structures were elucidated by comparison of their ^{1}H- and ^{13}C-NMR spectra with those of hypercalin C (**4**).

The ^{1}H-NMR spectrum (200 MHz, $CDCl_3$) of **5a**/**5b** revealed the presence of 1 *sext.* at 3.93 ppm (J = 6.8 Hz; H–C(28) of isomer **5a**) and 2*dd* at 3.0 and 2.85 ppm (J = 14, 7 Hz; CH_2(28) of isomer **5b**), providing evidence of their structural difference as compared to **4**: the 2-methylpropanoyl side chain of compound **4** was replaced by a 2-methylbutanoyl moiety in the higher homologue **5a** and by a 3-methylbutanoyl side chain in its isomer **5b**. The characteristic signal of both acyl side chains could also be observed in the ^{13}C-NMR spectrum of **5a**/**5b**: 1 *C*H (42 ppm), 1 CH_2 (26.4 ppm), and 2 CH_3 (16.6 and 11.9 ppm) for **5a**, and 1 CH_2 (48.4 ppm), 1 *C*H (25.9 ppm), and 2 CH_3 (22.7 and 22.8 ppm) for **5b**.

Discussion. – During our biological and chemical screening of *Hypericum* species, bioassay-guided fractionation of the light petroleum ether extract of *Hypericum calycinum* L. led to the isolation of compounds **1**–**5**. Although the isolated compounds have similar R_f on silica-gel TLC (see *Exper. Part*), they were separated into 3 active fractions in one single operation by means of droplet counter-current chromatography (DCCC) [17]. Compounds **1**–**5** were shown to be the active constituents of the extract in the Co-115 growth inhibition assay. The complete details of the biological assay have been given elsewhere [16]. In our test, all these compounds display similar activity (ED_{50} = *ca.* 0.35 μg/ml), which was comparable to that of 5-fluorouracil, a synthetic drug used in the

Table 3. *Distances* [Å] *and Angles* [°] *in Hypercalin C* (**4**)

Distances			
C(1)–O(1)	1.294(6)	C(9)–C(16)	1.526(9)
C(1)–C(2)	1.425(7)	C(10)–C(11)	1.498(9)
C(1)–C(6)	1.418(7)	C(11)–C(12)	1.530(9)
C(2)–C(3)	1.449(8)	C(12)–C(13)	1.513(9)
C(2)–C(27)	1.433(8)	C(13)–C(14)	1.309(13)
C(3)–O(3)	1.248(6)	C(13)–C(15)	1.471(12)
C(3)–C(4)	1.515(7)	C(17)–C(18)	1.496(9)
C(4)–C(5)	1.495(7)	C(18)–C(19)	1.303(9)
C(4)–C(17)	1.559(8)	C(19)–C(20)	1.496(12)
C(4)–C(22)	1.582(8)	C(19)–C(21)	1.529(11)
C(5)–O(5)	1.332(6)	C(22)–C(23)	1.529(10)
C(5)–C(6)	1.354(7)	C(23)–C(24)	1.296(9)
C(6)–C(7)	1.531(7)	C(24)–C(25)	1.512(12)
C(7)–C(8)	1.530(8)	C(24)–C(26)	1.521(11)
C(8)–C(9)	1.557(8)	C(27)–O(27)	1.270(7)
C(8)–C(12)	1.532(8)	C(27)–C(28)	1.486(9)
C(9)–O(9)	1.458(7)	C(28)–C(29)	1.460(15)
C(9)–C(10)	1.537(8)	C(28)–C(30)	1.436(16)
O(1)···O(27)	2.383(5)	O(5)···O(9)	2.646(5)
H(O1)··O(27)	1.31	H(O5)··O(9)	1.66
O(9)···O(3^i))[a])	2.714(6)		
H(O9)··O(3^i))[a])	1.71(7)		
Angles			
O(1)–C(1)–C(2)	118.5(5)	O(9)–C(9)–C(10)	110.4(4)
O(1)–C(1)–C(6)	116.5(4)	O(9)–C(9)–C(16)	109.6(5)
C(2)–C(1)–C(6)	125.0(5)	C(10)–C(9)–C(16)	113.9(5)
C(1)–C(2)–C(3)	116.6(4)	C(9)–C(10)–C(11)	107.8(5)
C(1)–C(2)–C(27)	119.3(5)	C(10)–C(11)–C(12)	105.3(5)
C(3)–C(2)–C(27)	124.0(5)	C(8)–C(12)–C(11)	102.5(5)
C(2)–C(3)–O(3)	123.6(5)	C(8)–C(12)–C(13)	115.1(5)
C(2)–C(3)–C(4)	120.8(5)	C(11)–C(12)–C(13)	116.6(6)
O(3)–C(3)–C(4)	115.6(5)	C(12)–C(13)–C(14)	119.1(9)
C(3)–C(4)–C(5)	114.6(4)	C(12)–C(13)–C(15)	118.7(7)
C(3)–C(4)–C(17)	110.3(5)	C(14)–C(13)–C(15)	122.2(8)
C(3)–C(4)–C(22)	106.5(4)	C(4)–C(17)–C(18)	114.1(5)
C(5)–C(4)–C(17)	111.3(5)	C(17)–C(18)–C(19)	127.5(6)
C(5)–C(4)–C(22)	107.5(4)	C(18)–C(19)–C(20)	125.6(7)
C(4)–C(5)–O(5)	113.8(4)	C(20)–C(19)–C(21)	113.4(8)
C(4)–C(5)–C(6)	123.4(4)	C(4)–C(22)–C(23)	112.9(5)
O(5)–C(5)–C(6)	122.7(5)	C(22)–C(23)–C(24)	127.3(6)
C(1)–C(6)–C(5)	118.7(4)	C(23)–C(24)–C(25)	121.3(7)
C(1)–C(6)–C(7)	119.2(5)	C(23)–C(24)–C(26)	125.5(7)
C(5)–C(6)–C(7)	122.0(5)	C(25)–C(24)–C(26)	113.1(7)
C(6)–C(7)–C(8)	117.7(4)	C(2)–C(27)–O(27)	118.4(5)
C(7)–C(8)–C(9)	116.4(5)	C(2)–C(27)–C(28)	125.1(6)
C(7)–C(8)–C(12)	110.6(5)	O(27)–C(27)–C(28)	116.4(5)
C(9)–C(8)–C(12)	104.0(4)	C(27)–C(28)–C(29)	114.1(8)
C(8)–C(9)–O(9)	105.7(4)	C(27)–C(28)–C(30)	108.7(8)
C(8)–C(9)–C(10)	104.5(5)	C(29)–C(28)–C(30)	109.5(12)
C(8)–C(9)–C(16)	112.3(4)		
O(1)–H(O1)··O(27)	156	H(O5)–O(5)··O(9)	148
O(9)–H(O9)··O(3^i))[a])	167(1)		

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

therapy of colon cancer (ED_{50} = 0.23 μg/ml in EtOH, in our assay). Tests are underway to evaluate the activity of hypercalin derivatives on other solid tumor cell lines and to subject these compounds to additional bioassays.

Since compounds **1**–**5** possess some structural similarities to calofloride, a molluscicidal neoflavonoid isolated from *Calophyllum verticillatum* (Guttiferae) [18] [19], they have been tested against the schistosomiasis-transmitting snail *Biomphalaria glabrata*. Whereas the homologous mixture **4**/**5** which was poorly soluble in water was found to be inactive, hypercalin B (**3**) and the crystalline mixture chinesin II (**1**)/hypercalin A (**2**) were molluscicidal at a minimum concentration of 20 and 10 ppm, respectively.

Experimental Part

General. See [8]. In addition or deviating from [8]: Prep. low-pressure liquid chromatography: *Lobar*® diol column (40–63 μm; 2.5 cm × 31 cm; *Merck*) equipped with a *Duramat 80* pump (*Chemie und Filter*, Regensdorf). Anal. HPLC: 7 μm *Lichrosorb RP-18* column (25 cm × 4.6 mm i.d.; *Knauer*). Semi-prep. HPLC: 7 μm *LiChrosorb RP-18* column (25 cm × 16 mm; *Knauer*). IR spectra: *Philips PU 9716*. 2D-NMR spectra: *Varian VXR 400* using a computer switchable 5-mm multinuclear probe at 400 MHz (^{1}H) and 100 MHz (^{13}C), resp.

Plant Material. Aerial parts of *Hypericum calycinum* L. were collected in September 1987 and September 1988 in Champéry (Valais), Switzerland. A voucher specimen is deposited at the Institute of Pharmacognosy and Phytochemistry, School of Pharmacy, University of Lausanne.

Extraction and Isolation. The powdered aerial parts (200 g) of *Hypericum calycinum* (collected in September 1987) were extracted at r.t. with light petroleum ether: 7.7 g of extract (ED_{50} = 2.7 μg/ml) which was fractionated by DCCC (light petroleum ether/96% EtOH/AcOEt/H_2O 83:67:33:17, ascending mode) to afford 3 fractions (*I*, *II*, and *III*) with enhanced activity in the Co-115 growth inhibitory bioassay. The main *Fraction II* (1020 mg, ED_{50} = 0.34 μg/ml) contained virtually one component which was subjected to crystallization from hexane, yielding 325 mg of white crystalline mixture **1**/**2**. The crystals were subjected to growth-inhibition testing (ED_{50} = 0.37 μg/ml) and to X-ray analysis from which only the overall connectivity deduced by NMR analysis were obtained for the new product **2**. A 60-mg portion (in 0.5 ml $CHCl_3$ + 1 ml MeOH) was separated by semi-prep. HPLC (injection volume 80 μl) on *LiChrosorb RP-18* with MeOH/H_2O 85:15 (0.1% AcOH added to the solvent) to afford 7 mg of *chinesin II* (**1**) and 40 mg of *hypercalin A* (**2**). Low-pressure chromatography of the enriched *Fraction III* (260 mg; ED_{50} = 0.7 μg/ml) was performed on a *Lobar*® diol column (hexane/AcOEt 95:5 → 90:10) to afford 60 mg of a pale yellow powder, *hypercalin B* (**3**), which was active with an ED_{50} = 0.34 μg/ml. *Fraction I* which similarly displayed growth-inhibitory activity (ED_{50} = 0.5 μg/ml) yielded, on standing in CH_2Cl_2/MeOH, crystals consisting of **4**/**5a**/**5b**, which were recrystallizated from MeCN and subjected to X-ray analysis. The mother liquor (160 mg) was subjected to *Lobar*® low-pressure chromatography on diol (hexane 100% → hexane/AcOEt 95:5) which allowed the obtention of an additional quantity (25 mg) of **4**/**5a**/**5b** which was shown to inhibit the Co-115 human colon carcinoma cell line with an ED_{50} = 0.30 μg/ml.

From 350 g of another batch of the aerial parts of *H. calycinum* (collected in 1988), it was possible to obtain 13.5 g of light petroleum ether extract, 10 g of which yielded 740 mg of the crystalline mixture **1**/**2**, 220 mg of hypercalin B (**3**), and 110 mg of the crystalline mixture **4**/**5a**/**5b**, using the same isolation procedure, with an additional final filtration on *Sephadex LH 20* (light petroleum ether/CH_2Cl_2/MeOH 10:10:1). Semi-prep. HPLC (*LiChrosorb RP-18*, MeOH/H_2O 85:15 + 0.1% AcOH) of **4**/**5a**/**5b** (60 mg in 0.8 ml $CHCl_3$ + 1 ml MeOH, injection volume 100 μl) allowed the separation of 20 mg of pure *hypercalin C* (**4**) from its higher isomeric homologues **5a**/**5b** (11 mg).

(6R)-3,5-Dihydroxy-4-{[(1S*,2R*,5R*)-2-hydroxy-2-methyl-5-(1-methylethenyl)cyclopentyl]methyl}-6-methyl-6-(3-methylbut-2-enyl)-2-(2-methylpropanoyl)cyclohexa-2,4-dien-1-one* (= *Chinesin II*; **1**). Colorless oil. TLC (SiO_2, light petroleum ether/AcOEt 1:1 + 0.5% AcOH): same as **2**. UV: as for **2**. ^{1}H-NMR (200 MHz, $CDCl_3$): 19.2 (*s*, OH–C(1)); 18.35 (minor *s*); 9.92 (br. *s*, OH–C(5)); 4.87, 4.83 (*2m*, CH_2(14)); 4.77 (*m*, *J* = 7.5, 1.5, H–C(18)); 4.26 (minor *sept.*); 4.01 (*sept.*, *J* = 6.8, H–C(24)); 2.69 (*dd*, *J* = 14.5, 3 H_b–C(7)); 2.65–2.50 (*m* (lines at 2.64, 2.55, 2.52), CH_2(17)); 2.47 (obscured *ddd*, only 2 *J* measured, *J* = 11, 7, H–C(12)); 2.13 (*dd*, *J* = 15, 12 H_a–C(7)); 2.05–1.79 (*m*); 1.77 (*s*, CH_3(15)); 1.75–1.60 (*m*); 1.56 (*s*, CH_3(20), CH_3(21)); 1.54–1.37 (*m*); 1.36 (*s*,

$CH_3(22)$); 1.26 (*s*, $CH_3(16)$); 1.13, 1.12 (2*d*, $J = 6.8$ $CH_3(25)$, $CH_3(26)$). ^{13}C-NMR (50.1 MHz, $CDCl_3$): *Table 1*. DCI-MS (reactant gas NH_3): 431 ($[M + H]^+$).

(6R)-3,5-Dihydroxy-4-{[(1S*,2R*,5R*)-2-hydroxy-2-methyl-5-(1-methylethenyl)cyclopentyl]methyl}-6-methyl-2-(3-methylbutanoyl)-6-(3-methylbut-2-enyl)cyclohexa-2,4-dien-1-one* (= *Hypercalin A*; **2**). White needles from hexane. M.p. 76–80°. TLC (SiO_2, light petroleum ether/AcOEt 1:1 +0.5% AcOH): R_f 0.53; violet carmine color with *Godin* [20] reagent. $[\alpha]_D^{22} = +70$ ($c = 0.5$, $CHCl_3$). UV (96% EtOH): 354, 224. UV ($CHCl_3$): 328 (8500), 273 (5100), 244 (7300). IR (KBr): 3320, 2960, 2870, 1630, 1570, 1510, 1450, 1370, 1340, 1305, 1260, 1240, 1200, 1120, 1110, 895. ^{1}H-NMR (200 MHz, $CDCl_3$): 19.15 (*s*, OH–C(1)); 18.35 (minor *s*); 10.06 (br. *s*, OH–C(5)); 4.88, 4.83 (2 br. *s*, $CH_2(14)$); 4.76 (*t*, $J = 7$, H–C(18)); 3.04, 2.79 (2*dd*, $J = 14$, 7 $CH_2(24)$); 2.73, 2.67 (2*m*, H_b–C(7)); 2.65–2.51 (*m* (lines at 2.65, 2.61, 2.56, 2.53), $CH_2(17)$); 2.47 (*ddd*, $J = 11$, 11, 7, H–C(12)); 2.24–2.02 (*m*, H_a–C(7), H–C(25)); 2.02–1.79 (*m*); 1.78 (*s*, $CH_3(15)$); 1.75–1.58 (*m*); 1.55 (*s*, $CH_3(20)$, $CH_3(21)$); 1.35 (*s*, $CH_3(22)$); 1.26 (*s*, $CH_3(16)$); 0.98, 0.96 (2*d*, $J = 6.5$ $CH_3(26)$, $CH_3(27)$). ^{13}C-NMR (50.1 MHz, $CDCl_3$): multiplicities from DEPTGL experiments, *Table 1*. DCI-MS (reactant gas NH_3): 445 ($[M + H]^+$).

X-Ray Analysis of **2**: Suitable crystals, hexagonal rods, were grown from light petroleum ether/$CHCl_3$. Crystal data: $C_{27}H_{40}O_5$, $M = 444$, space group $P6_322$, $a = b = 19.469(5)$, $c = 28.41(3)$ Å, $V = 9325.9$ Å^3, $F(000) = 2904$, $D_m(KI/H_2O) = 1.10(1)$ g·cm^{-3}, $Z = 12$ $D_x = 0.948$ g·cm^{-3}, MoK_α, $\lambda = 0.71073$ Å, $\mu = 0.36$ cm^{-1}. Preliminary *Weissenberg* and precession photographs indicated the crystals to be hexagonal, with diffraction symmetry $P6/mmm$, space group $P6_322$. Intensity data with index limits h 0 to 18, k 0 to 17, l 0 to 30, and $\theta_{max} = 22.5°$ were measured on a *Stoe Siemens AED2* four-circle diffractometer (graphite-monochromated MoK_α radiation) using the ω/θ scan mode. Two crystals were used for data collection: the first (0.46 × 0.42 × 0.27 mm) for $2\theta \leqslant 40°$, with 6% intensity variation for 2 standard reflections measured every h; a second larger crystal with a poor mosaic spread (0.76 × 0.42 × 0.19 mm) for the region $30° < 2\theta < 45°$, with a 2% intensity variation of 2 standard reflections measured every h. A total of 3600 unique reflections ($R_{int} = 0.038$) were measured of which only 1409 could be considered observed with $F_o > 6\sigma(F_o)$. Cell parameters from $\pm\omega$ values of 25 reflections in the range $14° < 2\theta < 19°$. The structure was solved by direct methods using the program SHELXS-86 [21]. The program SHELX-76 [22] was used for all further calculations. 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]. No OH protons could be located. Weighted anisotropic full-matrix least-squares refinement for 1409 reflections converged at $R = 0.101$, $R_w = 0.092$; $w^{-1} = \sigma^2(F_o) + 0.00036(F_o^2)$. Average parameter shift/e.s.d. < 0.40. Heights in final difference map $\Delta\rho_{max} = 0.49$, $\Delta\rho_{min} -0.35$ eÅ^{-3}. The high R factor is due to the poor quality of the crystals which did not diffract well much beyond 30° in 2θ. The region of the C(2) side chain was poorly resolved, and during refinement, two C-atoms remained non-positive definite. A small amount of residual density was observed in the region of the C(2) side chain, but the model proposed seems to refine the best. An attempt to carry out a low-temperature analysis failed as the crystal appears to undergo some kind of phase transition below −20° [23]. Atomic scattering factors were taken from 'International Tables for X-Ray Crystallography' [24]. Final positional and equivalent isotropic thermal parameters and bond distances have been deposited with the *Cambridge Crystallographic Data Centre*.

2-Benzoyl-3,5-dihydroxy-4-{[(1R,2S*,5S*)-2-hydroxy-2-methyl-5-(1-methylethenyl)cyclopentyl]methyl}-6,6-bis(3-methylbut-2-enyl)cyclohexa-2,4-dien-1-one* (= *Hypercalin B*; **3**). Pale yellow amorphous powder. M.p. 54–57°. TLC (SiO_2, light petroleum ether/AcOEt 1:1 + 0.5% AcOH): R_f 0.52; violet-brown with *Godin* reagent. $[\alpha]_D^{22} = +147$ ($c = 0.5$, $CHCl_3$). UV ($CHCl_3$): 360 (12400), 285 (3700), 244 (6600). IR (KBr): 3370, 3060, 2960, 2920, 2860, 1635, 1585, 1550, 1500, 1430, 1450, 1375, 1315, 1260, 1180, 1105, 930, 890, 800, 770, 730, 700. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. DCI-MS (reactant gas NH_3): 519 ($[M + H]^+$).

3,5-Dihydroxy-4-{[(1R,2S*,5S*)-2-hydroxy-2-methyl-5-(1-methylethenyl)cyclopentyl]methyl}-6,6-bis(3-methylbut-2-enyl)-2-(2-methylpropanoyl)cyclohexa-2,4-dien-1-one* (= *Hypercalin C*; **4**). White needles. M.p. 152–156°. TLC (SiO_2, light petroleum ether/AcOEt 1:1 + 0.5% AcOH): R_f 0.59; dark violet color with *Godin* reagent, $[\alpha]_D^{22} = +185$ ($c = 0.5$, $CHCl_3$). UV ($CHCl_3$): 331 (6800), 275 (6000), 244 (8200). IR (KBr): 3400, 2960, 2920, 2860, 1630, 1560, 1510, 1470, 1440, 1380, 1370, 1340, 1270, 1240, 1200, 1140, 1090, 890, 800. ^{1}H-NMR (200 MHz, $CDCl_3$): 19.21 (*s*, OH–C(1)); 18.26 (minor *s*); 9.87 (br. *s*, OH–C(5)); 4.88, 4.83 (2*m*, $CH_2(14)$); 4.80, 4.77, 4.74 (3*m*, H–C(18), H–C(22)); 4.26 (minor *sept.*); 4.04 (*sept.*, $J = 6.8$ H–C(28)); 2.75–2.45 (*m* (lines at 2.68, 2.67, 2.65, 2.61, 2.60, 2.58, 2.54, 2.50), H_b–C(7), $CH_2(17)$, $CH_2(22)$); 2.42 (obscured *ddd*, only 2 J measured, $J = 11$, 6.5, H–C(12)); 2.12 (*dd*, $J = 15$, 12, H_a–C(7)); 2.02–1.78 (*m*); 1.77 (*s*, $CH_3(15)$); 1.74–1.60 (*m*); 1.58, 1.54, 1.51 (3 br. *s*, $CH_3(20)$, $CH_3(21)$, $CH_3(25)$, $CH_3(26)$); 1.28 (*s*, $CH_3(16)$); 1.13, 1.12 (2*d*, $J = 6.8$, $CH_3(29)$, $CH_3(30)$). ^{13}C-NMR: *Table 1*; multiplicities from DEPTGL experiments. DCI-MS (reactant gas NH_3): 485 ($[M + H]^+$).

X-Ray Analysis of **4**: Suitable crystals, large hexagonal blocks were grown from CH_3CN. Crystal data: $C_{30}H_{44}O_5$, $M = 484$, space group $P2_12_12_1$, $a = 10.066(1)$, $b = 16.877(2)$, $c = 17.268(1)$ Å, $V = 2933.6$ Å^3, $F(000) = 1056$, $Z = 4$, $D_x = 1.096$ g·cm^{-3}, MoK_α, $\lambda = 0.71073$ Å, $\mu = 0.04$ mm^{-1}. A crystal of dimension

0.57 × 0.49 × 0.38 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 −9 to 9, k −16 to 16, l 0 to 16, and $\theta_{max} = 20.0°$ were measured on a *Stoe Siemens AED2* four-cycle diffractometer (graphite-monochromated MoK_α radiation) using the ω/θ scan mode. There was < 1% intensity variation for 3 standard reflections measured every h; 4838 measured reflections. Of the 1587 unique reflections ($R_{int} = 0.021$), 1509 were considered observed with $F_o > 4\sigma(F_o)$. Cell parameters from ± ω values of 18 reflections and their equivalents in the range $20° < 2\theta < 25°$. No absorption or extinction corrections were 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. 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_2, and the CH_3 protons (refined values 0.1098, 0.260, and 0.219). OH protons H(O1), H(O5), and H(O9) were located in difference maps, only atom H(O9) was refined, the others were held fixed with $U_{iso} = 0.10$ Å^2. Weighted anisotropic full-matrix least-squares refinement for 1499 reflections (10 reflections probably suffering from extinction were removed) converged at $R = 0.053$, $R_w = 0.061$; $w^{-1} = \sigma^2(F_o) + 0.00080(F_o^2)$. Average parameter shift/e.s.d. < 0.10. Heights in final difference map $\Delta\rho_{max} = 0.24$, $\Delta\rho_{min} = -0.16$ e Å^{-3}. Atomic scattering factors were taken from 'International Tables for X-Ray Crystallography' [24]. Final positional and equivalent isotropic thermal parameters have been deposited with the *Cambridge Crystallographic Data Centre*. Bond distances and angles are given in *Table 3* (no e.s.d.'s are given for the H-bonds involving H(O1) or H(O5) as these atoms were not refined). The crystallographic numbering scheme is apparent from *Fig. 3*, prepared using ORTEP [25]. Supplementary material is available from *H. St.-E.*

3,5-Dihydroxy-4-{[(1R,2S*,5S*)-2-hydroxy-2-methyl-5-(1-methylethenyl)cyclopentyl]methyl}-2-(2-methylbutanoyl)-6,6-bis(3-methylbut-2-enyl)cyclohexa-2,4-dien-1-one/3,5-Dihydroxy-4-{[(1R*,2S*,5S*)-2-hydroxy-2-methyl-5-(1-methylethenyl)cyclopentyl]methyl}-2(3-methylbutanoyl)6,6-bis(3-methylbut-2-enyl)cyclohexa-2,4-dien-1-one* (**5a**/**5b**). White prisms from hexane. M.p. 131–136°. TLC (SiO_2, light petroleum ether/AcOEt 1:1 + 0.5% AcOH); same as **4**. UV ($CHCl_3$): same as **4**. ^{1}H-NMR (200 MHz, $CDCl_3$): 19.27, 19.17, (2*s*, OH–C(1)); 4.89, 4.84 (2*m*, CH_2(14)); 4.79, 4.78, 4.75 (3*m*, H–C(18), H–C(23)); 3.93 (*sext.*, $J = 6.8$ H–C(28) of **5a**); 3.0, 2.85 (2*dd*, $J = 14$, 7 (CH_2(28) of **5b**); 2.75–2.48 (*m* (lines at 2.66, 2.63, 2.61, 2.60, 2.55, 2.51)); 2.43 (obscured *ddd*, 2 J measured, $J = 11$, 7, H–C(12)); 2.20–1.80 (*m*); 1.78 (*s*, CH_3(15)); 1.75–1.60 (*m*); 1.58, 1.54, 1.51 (3 br. *s*, CH_3(20), CH_3(21), CH_3(25), CH_3(26)); 1.47–1.30 (*m*); 1.29, 1.28 (2*s*, CH_3(16) of both isomers); 1.11 (*d*, $J = 6.8$ CH_3(29) of **5a**); 0.97, 0.96 (2*d*, $J = 6.7$ CH_3(30), CH_3(31) of **5b**); 0.91 (*dd*, $J = 7.5$, 7.5, CH_3(31) of **5a**). ^{13}C-NMR: *Table 1*. DCI-MS (reactant gas NH_3): 499 ($[M + H]^+$).

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