Calopins and Cyclocalopins − Bitter Principles from *Boletus calopus* and Related Mushrooms

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Dedicated to Professor Lutz F. Tietze on the occasion of his 60th birthday

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Boletus calopus and closely related mushrooms of the *Boletus* section *Calopodes* are characterized by their bitter taste, which is caused mainly by *O*-acetylcyclocalopin A (**6a**), a member of a series of unique δ-lactone derivatives. Besides the simple lactone calopin (**1b**) and its *O*-acetyl derivative **1a**, the more complex cyclocalopins **6**–**12** have been isolated and their structures elucidated. Cyclocalopin D (**10a**) is an unprecedented α-glucoside in which the sugar residue is bound to an enolic hydroxy group. The chemical conversion of **6b** into **1a** established the same stereochemistry for the calopin and cyclocalopin types of metabolites.

Introduction

The bitter taste of several boletes is well known to mushroom hunters. In the case of *Tylopilus felleus* (Bull.: Fr.) Karst. (German: Gallenröhrling), a single specimen can spoil a mushroom dinner. The compounds responsible for the bile-like bitterness of this species are still unknown. In this publication we report on the isolation and structural elucidation of the bitter principles from *Boletus calopus* Fr. (German: Schönfuss-Röhrling) and related species. The occurrence of these metabolites in the European toadstools *B. calopus* Fr. and *B. radicans* Pers.: Fr., as well as in the North American *B. coniferarum* Dick and Snell, *B. peckii* Frost, and *B. rubripes* Thiers supports the incorporation of these boletes in the section *Calopodes* Fr.[1]

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Supporting information for this article is available on the WWW under http://www.eurjoc.org or from the author.

Results and Discussion

Isolation of the Metabolites

The isolation of the metabolites from the bitter *Boletus* species was achieved by exhaustive extraction of the freeze- or air-dried, defatted fruit bodies with chloroform, followed by separation of the components by high-speed countercurrent chromatography (HSCCC). The enriched fractions were then purified by reversed-phase HPLC with a methanol/water gradient to yield *O*-acetylcalopin (**1a**) and calopin (**1b**), as well as varying amounts of *O*-acetylcyclocalopin A (**6a**) and cyclocalopin A (**6b**). The cyclocalopins B−F were isolated from the ethyl acetate extract of freeze-dried *B. radicans*.

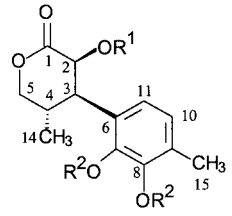
O-Acetylcalopin and Calopin

The major component of the extracts from *Boletus calopus, B. coniferarum*, and *B. radicans* was *O*-acetylcalopin (**1a**), which possesses the molecular formula $C_{15}H_{18}O_6$. From the NMR spectroscopic data (Table 1) and the HMBC correlations, the presence of a 2,3-dihydroxy-4-methylphenyl ring was easily discernible. In addition, the coupling pattern of the aliphatic protons indicated the presence of an $-OCH_2-CH_A(CH_3)-CH_B(Ar)-CH_C(OAc)-$ system, which, according to the ^{13}C NMR spectroscopic data and the associated HMBC correlations, formed part of a δ-lactone ring. Since H_B of this moiety exhibited HMBC correlations to the *ortho*-carbon atoms of the trisubstituted phenyl ring, structure **1a** can be proposed for *O*-acetylcalopin.

Table 1. ^1H and ^{13}C NMR data for *O*-acetylcalopin (**1a**) and calopin (**1b**) in $CDCl_3$, recorded at 600 MHz and 150.9 MHz, respectively

Pos.	**1a**		**1b**	
	δ(^{13}C)	δ(^{1}H)	δ(^{13}C)	δ(^{1}H)
1	170.1		173.9	
2	67.7	5.66 (d, 1 H)	69.0	4.71 (d, 1 H)
3	45.6	3.60 (dd, 1 H)	44.9	3.53 (dd, 1 H)
4	34.1	2.58 (m, 1 H)	31.9	2.76 (m, 1 H)
5α	71.7	4.15 (dd, 1 H)	71.3	4.02 (dd, 1 H)
5β		4.35 (dd, 1 H)		4.51 (dd, 1 H)
6	122.1		123.9	
7	142.9		144.1[a]	
8	142.0		141.8[a]	
9	124.2		123.9	
10	121.8	6.60 (d, 1 H)	119.2[b]	6.71 (d, 1 H)
11	122.4	6.49 (d, 1 H)	123.3[b]	6.53 (d, 1 H)
12	169.9			
13	20.3	1.77 (s, 3 H)		
14	15.8[c]	1.02 (d, 3 H)	15.4	1.11 (d, 3 H)
15	15.6[c]	2.22 (s, 3 H)	17.3	2.22 (s, 3 H)

[a] [b] [c] Assignments may be interchanged. Coupling constants [Hz]: **1a**: $J_{2\text{-}3} = 9.3$; $J_{3\text{-}4} = 9.3$; $J_{4\text{-}5\alpha} = 11.5$; $J_{4\text{-}5\beta} = 4.4$; $J_{5\alpha\text{-}5\beta} = 11.5$; $J_{4\text{-}14} = 6.7$; $J_{10\text{-}11} = 7.8$. **1b**: $J_{2\text{-}3} = 7.6$; $J_{3\text{-}4} = 5.2$; $J_{4\text{-}5\alpha} = 11.6$; $J_{4\text{-}5\beta} = 6.6$; $J_{5\alpha\text{-}5\beta} = 11.6$; $J_{4\text{-}14} = 6.8$; $J_{10\text{-}11} = 8.0$.



	1a	**1b**	**1c**	**1d**	**1e**
	12 13				
R^1	$COCH_3$	H	Ac	Ac	H
R^2	H	H	Ac	-CO-	$-C(CH_3)_2-$

Calopin (**1b**), $C_{13}H_{16}O_5$, lacks the *O*-acetyl group, with the result that the signal due to 2-H (δ_H = 4.71 ppm) appeared at a significantly higher field than its counterpart in **1a** (δ_H = 5.66 ppm). As expected, both compounds yielded the same crystalline triacetate **1c** on acid-catalyzed acetylation. Treatment of *O*-acetylcalopin with phosgene afforded the cyclic carbonate **1d**.

The stereochemical arrangement of the substituents on the δ-lactone ring of **1a** followed from the large vicinal coupling constants $^3J_{2,3} \approx {}^3J_{3,4} \approx 10$ Hz, in combination with NOESY measurements (Figure 1). A strong cross-peak between 2-H and 3-H indicated a *cis* relationship between these protons, whereas the cross-peak between 3-H and the methyl group at C-4 suggested a *trans* arrangement of 3-H and 4-H. The large coupling constants between the ring protons could only be explained if the δ-lactone ring were to adopt a boat or twist-boat conformation. This was supported by the NOESY correlations shown in Figure 1. Of special relevance was a strong cross-peak between 5-H_α and 2-H, both of which occupy flagpole positions. The tendency of δ-lactones to adopt boat or twist-boat conformations is well documented.[2,3]

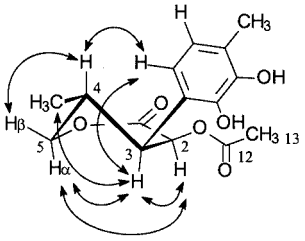


Figure 1. Conformation of *O*-acetylcalopin (**1a**) as determined by NOESY correlations

The high-field NMR modification of Mosher's method[4] was applied for the determination of absolute configuration. To achieve selective acylation of calopin (**1b**) at the secondary hydroxy group, the catechol group was protected by heating **1b** with 2,2-dimethoxypropane.[5] Treatment of the resulting acetal **1e** with either (*R*)-(−)- or (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA-Cl) and (4-dimethylamino)pyridine (DMAP) yielded the (*S*)- and (*R*)-MTPA esters $\mathbf{2_S}$ and $\mathbf{2_R}$, respectively. Analysis of the shift differences (Δδ) between the diastereomeric esters (Figure 2) allowed the (2*S*,3*R*,4*S*) configuration to be assigned to calopin (**1b**). Importantly, in the case of $\mathbf{2_R}$ the methoxy group of the MTPA residue was shielded by the neighboring phenyl group, causing a strongly positive Δδ value of 180 Hz. This complemented the conclusions drawn from the negative Δδ values of the lactone and aryl protons, which placed them on the left-hand side of the MTPA plane. Very similar Δδ values were obtained with synthetic (2*S*,3*R*,4*S*)-2-hydroxy-4-methyl-3-phenyl-δ-lactone (**3**),[6] supporting the assignment of absolute configuration established with natural calopin.

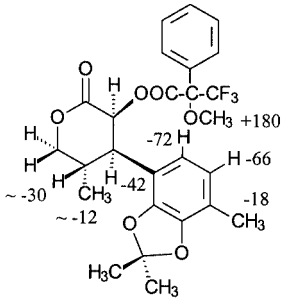
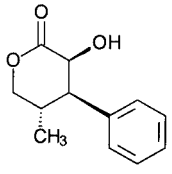


Figure 2. Δδ values, in Hz, obtained for the MTPA esters **2** [Δδ = δ(*S*-MTPA ester) − δ(*R*-MTPA ester)][4]



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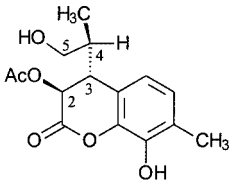
Interestingly, during the HPLC separation procedure described above, a less polar form of *O*-acetylcalopin with the

same UV spectrum could be detected. It yielded **1a** after evaporation of the solvent. The conversion of this unstable compound into the more stable *O*-acetylcalopin was catalyzed by acids. By collecting the corresponding HPLC fraction in a pre-cooled flask at −78° C we were able to obtain the ^{1}H NMR spectrum of the unstable form (“*O*-acetylisocalopin”, see Exp. Sect.). This displayed the same sequence of proton resonances as **1a**, but with an upfield shift of the diastereotopic protons 5a-H/5b-H to δ_H = 3.03/3.67 ppm (**1a**: δ_H = 4.15/4.35 ppm), indicating a free CH_2OH group. From the coupling constants $^3J_{2,3}$ = 10 and $^3J_{3,4}$ = 4 Hz, the dihydrocoumarin structure **4** can be proposed for *O*-acetylisocalopin. On acetylation with acetic anhydride in pyridine, lactone **4** underwent ring-opening and yielded the tetraacetate **5**.



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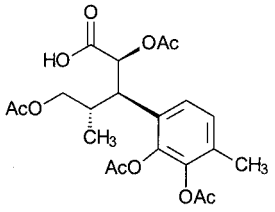

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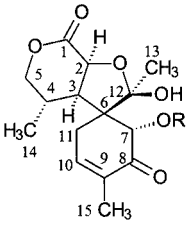
O-Acetylcyclocalopin A and Cyclocalopin A

O-Acetylcyclocalopin A (**6a**) was identified as the main bitter principle of *Boletus calopus* and the other members of this group of boletes. It was accompanied by the slightly bitter cyclocalopin A (**6b**), which could be transformed into **6a** by treatment with acetic anhydride and catalytic amounts of DMAP.



6a, R = Ac
6b, R = H

The molecular formulae of *O*-acetylcyclocalopin A (**6a**) and its deacetyl derivative **6b** were determined by high-resolution EIMS as $C_{17}H_{22}O_7$ and $C_{15}H_{20}O_6$, respectively. The IR spectrum (KBr) of **6a** exhibited three carbonyl absorptions, at $\tilde{\nu}$ = 1745, 1725, and 1690 cm^{-1}, whereas in the case of **6b** only bands at $\tilde{\nu}$ = 1740 and 1670 cm^{-1} were visible. The IR absorption at $\tilde{\nu} \approx$ 1740 cm^{-1} and the characteristic signals in the ^{1}H and ^{13}C NMR spectra of **6a** and **6b** (Table 2) indicated that these compounds contained the same δ-lactone moiety as the calopins. In contrast, both compounds lacked the signals required for an aryl residue. Instead, signals for an enone moiety [−CH(OR)−CO−C(CH$_3$)=CH−CH$_2$−] could be identified, in accordance with the carbonyl absorption at $\tilde{\nu} \approx$ 1690 cm^{-1}. The double bond was connected to a CH_2 group, the diastereotopic protons of which, together with the olefinic proton and a methine proton [δ_H = 5.47 ppm (δ_C = 77.2 ppm)], showed HMBC correlations with a quaternary carbon atom (δ_C = 55.1 ppm) (see Figure 3). Since the corresponding methine proton signal in the ^{1}H NMR spectrum of cyclocalopin A (**6b**) was observed at δ_H = 4.27 ppm (δ_C = 76.0 ppm), it had to belong to a carbinol group, acetylated in the case of **6a**. On comparison of cyclocalopin A (**6b**) with its acetyl derivative **6a**, the large downfield shift of the enone carbonyl signal, from δ_C = 193.2 to 199.3 ppm, pointed to close proximity of the carbinol and the carbonyl groups. Connection of both ends of the partial structure with the quaternary carbon atom (δ_C = 55.1 ppm) then produced a cyclohexenone ring. The HMBC correlation (Figure 3) between δ_H = 2.43 ppm (3-H) and the methine carbon atom of the cyclohexenone ring at δ_C = 77.2 ppm allowed the connection of C-3 of the δ-lactone to the quaternary carbon atom at δ_C = 55.1 ppm of the cyclohexenone ring. The carbon signal at δ_C = 107.2 ppm was assigned to an acetal carbon atom substituted with a methyl group (δ_H = 1.47 ppm). HMBC correlations between 2-H of the lactone ring and the acetal carbon atom (δ_C = 107.2 ppm), as well as between the methyl group (δ_H = 1.47 ppm) and the quaternary carbon atom (δ_C = 55.1 ppm), allowed structures **6a** and **6b** to be assigned to *O*-acetylcyclocalopin A and cyclocalopin A, respectively.

Table 2. ^{1}H- and ^{13}C-NMR data of *O*-acetylcyclocalopin A (**6a**) and cyclocalopin A (**6b**) recorded in $CDCl_3$ at 600 and 150.9 MHz, respectively

Pos.	**6a**		**6b**	
	δ_C	δ_H	δ_C	δ_H
1	172.4		172.6	
2	73.7	4.65 (d, 1 H)	73.7	4.59 (d, 1 H)
3	47.4	2.43 (dd, 1 H)	46.2	2.21 (m, 1 H)
4	30.8	2.28 (m, 1 H)	30.6	2.21 (m, 1 H)
5α	72.2	3.86 (dd, 1 H)	72.2	3.83 (dd, 1 H)
5β		4.10 (dd, 1 H)		4.07 (dd, 1 H)
6	55.1		56.3	
7	77.2	5.47 (s, 1 H)	76.0	4.27 (s, 1 H)
7-OH				3.90 (br. s, 1 H)[a]
8	193.2		199.3	
9	134.3		133.0	
10	143.1	6.68 (m, 1 H)	144.5	6.72 (m, 1 H)
11α	28.0	2.76 (ddq, 1 H)	27.8	2.72 (ddm, 1 H)
11β		2.58 (ddd, 1 H)		2.55 (ddd, 1 H)
12	107.2		107.6	
12-OH		2.67 (br. s, 1 H)[a]		2.98 (br. s, 1 H)[a]
13	21.3	1.47 (s, 3 H)	21.7	1.67 (s, 3 H)
14	16.2	0.81 (d, 3 H)	16.4	0.77 (d, 3 H)
15	15.2	1.84 (m, 3 H)	15.1	1.85 (m, 3 H)
16	169.9			
17	20.8	2.21 (s, 3 H)		

[a] δ_H dependent on concentration. Coupling constants [Hz]: **6a**: $J_{2\text{-}3}$ = 10.5; $J_{3\text{-}4}$ = 10.5; $J_{4\text{-}5\alpha}$ = 11.2; $J_{4\text{-}5\beta}$ = 3.5; $J_{5\alpha\text{-}5\beta}$ = 11.2; $J_{4\text{-}14}$ = 6.4; $J_{10\text{-}11\alpha}$ = 4.3; $J_{10\text{-}11\beta}$ = 2.6; $J_{11\alpha\text{-}11\beta}$ = 20.8; $J_{11\alpha\text{-}15}$ = 1.5. **6b**: $J_{2\text{-}3}$ = 9.7; $J_{4\text{-}5\alpha}$ = 11.0; $J_{4\text{-}5\beta}$ = 2.9; $J_{5\alpha\text{-}5\beta}$ = 11.0; $J_{4\text{-}14}$ = 6.4; $J_{10\text{-}11\alpha}$ = 5.7; $J_{10\text{-}11\beta}$ = 2.6; $J_{11\alpha\text{-}11\beta}$ = 20.8.

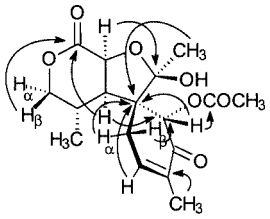


Figure 3. Selected HMBC correlations for *O*-acetylcyclocalopin (**6a**)

The relative stereochemistry of cyclocalopin A (**6b**) was determined unambiguously by single-crystal X-ray analysis (Figure 4). This showed that the stereochemistry about the lactone ring was the same as in calopin and that this ring adopted a boat conformation. A comparison of the ^{1}H NMR shifts and coupling constants observed for *O*-acetylcyclocalopin A (**6a**) with those of **6b** showed the same relative configuration for both compounds. This was supported by the NOESY experiments summarized in Figure 5 and by the close agreement of the ^{13}C NMR shifts for the acetal carbon atoms (C-12) in cyclocalopin A and its acetyl derivative.

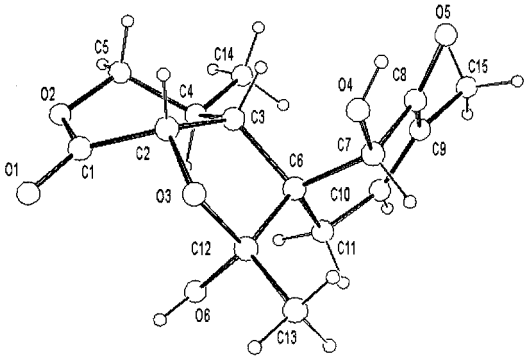


Figure 4. X-ray structure of cyclocalopin A

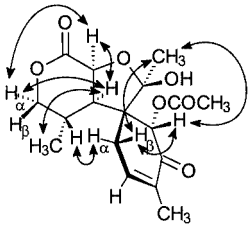
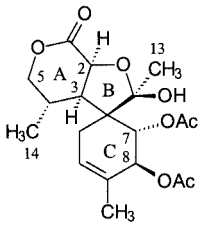


Figure 5. Selected NOESY correlations for **6a**

Cyclocalopin B and Cyclocalopins C_1/C_2

Cyclocalopin B (**7**) exhibited NMR signals and HMBC and NOESY correlations that indicated that rings A and B were identical with those of cyclocalopin A (**6b**). Again, a correlation between 2-H and 5α-H in the NOESY spectrum suggested a twist-boat conformation for the δ-lactone ring. In ring C the carbonyl function at C-8 had been transformed into an acetoxy group. The stereochemistry of ring C was defined by NOESY correlations between 7-H and 13-CH_3 and between 8-H, 3-H, and 14-CH_3. The *trans*-diequatorial relationship of the two vicinal acetoxy groups also followed from the magnitude of the coupling constant $^3J_{7,8}$ = 8.7 Hz.



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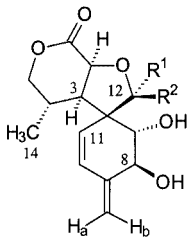


8, R^1 = OH, R^2 = CH_3
9, R^1 = CH_3, R^2 = OH

The cyclocalopins C_1 (**8**) and C_2 (**9**), $C_{15}H_{20}O_6$, were obtained as an inseparable 2:1 mixture of epimers. Their gross structures were determined by their ^{1}H and ^{13}C NMR spectra and HMBC correlations. Both compounds contained a conjugated diene system with an exocyclic methylene group in ring C. Two vicinal hydroxy groups in ring C were in a *trans*-diequatorial arrangement, in agreement with the coupling constant $^3J_{7,8} \approx$ 10.5 Hz. Epimers **8** and **9** differed in their configurations at the hemiacetal unit. The ^{13}C NMR shift for the C-12 signal of the main component **8** (δ_C = 110.3 ppm) contrasted with that of cyclocalopin A (δ_C = 107.6 ppm), whereas the minor component **9** was in good agreement (δ_C = 107.9 ppm). The configuration at C-12 had a strong influence on the chemical shifts in the ^{1}H and ^{13}C NMR spectra. Thus, in cyclocalopin C_2 (**9**), the OH group of the hemiacetal group induced a downfield shift of the signal of the neighboring vinyl proton 11-H to δ = 5.84 ppm, compared to δ = 5.10 ppm in **8**. On the other hand, the angular proton H-3 was deshielded in epimer **8** (δ_H = 3.49 ppm) relative to the same proton in **9** (δ_H = 2.76 ppm). In the ^{13}C NMR spectra, the C-3 signals in **8** and **9** appeared at δ = 44.4 and 48.2 ppm, respectively, a manifestation of the γ-effect.[7] As in the NOESY spectrum of cyclocalopin B (**7**), the 7-H and the methyl protons (13-H) of both epimers correlated to one another, whereas 8-H showed correlations to 3-H and the methyl protons (14-H) at the lactone ring. This supported the stereochemical assignments given in formulas **8** and **9**.

Cyclocalopins D, E, and F

The crystalline cyclocalopin D (**10a**) exhibited a peak for [M^+ + Na] at *m*/*z* = 539 in the ESI HRMS, corresponding to the molecular formula $C_{23}H_{32}O_{13}$. In the ^{1}H and ^{13}C NMR spectra, the typical signals of the A and B rings of a cyclocalopin could be recognized, together with those of a hexose moiety. The sugar was identified as α-glucose by its ^{1}H-^{1}H coupling constants and the ^{13}C-^{1}H coupling ^1J = 173.2 Hz at the anomeric carbon atom. α-Hexopyranoses in the $^4C_1$ conformation exhibit values of around 170 Hz for $^1J_{C\text{-}1,H}$, whereas values around 160 Hz are typical for β-hexopyranoses.[8] After subtraction of the glucose moiety, the molecular composition of the aglycon was $C_{17}H_{22}O_8$,

corresponding to seven double bond equivalents. Given the presence of a C=C double bond (δ_{C-9} = 118.6 ppm; δ_{C-8} = 152.4 ppm) and two carboxyl groups (δ_{C-1} = 176.0 ppm; $\delta_{acetate}$ = 172.9 ppm), the aglycon of cyclocalopin D had to contain four rings. From the HMBC correlations indicated in Figure 6, it was possible to deduce structure **10a** for cyclocalopin D. The position of the sugar unit followed from the cross-peak between the anomeric proton 1′-H and the olefinic C-8.

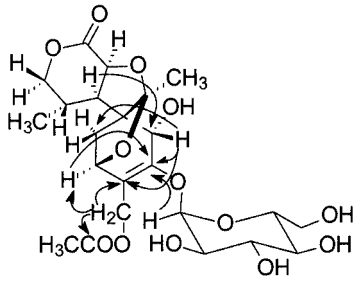
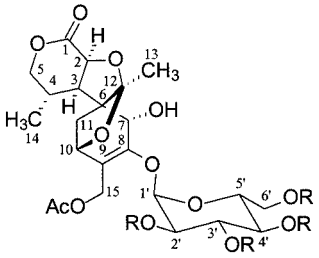


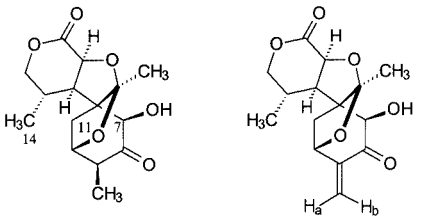
Figure 6. Selected HMBC correlations for cyclocylopin D (**10a**)



10a, R = H
10b, R = Ac

The angular methyl group (C-13) showed NOE relationships to 2-H, 3-H, and 7-H. This and additional NOESY correlations between 3-H and 7-H were in agreement with the stereochemistry given in formula **10a**. On treatment of **10a** with acetic anhydride in pyridine containing a catalytic amount of DMAP, the tetraacetate **10b** was formed. Biosynthetically, cyclocalopin D could be produced from cyclocalopin A (**6b**) by internal conjugate addition of the OH group at C-12 to C-10, followed by glucosidation of the resulting enol, hydroxylation of the allylic methyl group, and acetylation. Cyclocalopin D is unusual amongst secondary metabolites in that it incorporates an α-glucoside moiety attached to an enol group.[9]

Two further ether-bridged metabolites are cyclocalopin E (**11**) and cyclocalopin F (**12**). The latter compound could only be obtained in admixture with **11**. The structures of these compounds followed from a detailed analysis of the ^1H and ^{13}C NMR spectra, including HMBC, HMQC, and NOESY correlations. In contrast to that in **10a**, the 7-OH group in **11** and **12** occupied an equatorial β-position, resulting in an NOESY correlation between 7-H and the methyl group (C-14) at the lactone ring. The disappearance of the deshielding effect of the axial OH group caused a remarkable upfield shift of the axial 11α-proton signal from δ = 2.09 ppm in **10a** to δ = 1.89 ppm in **11**.



11 **12**

Conversion of Cyclocalopin A into *O*-Acetylcalopin and Biosynthetic Considerations

Oxidation of cyclocalopin A (**6b**) with bismuth oxide in acetic acid[10] yielded *O*-acetylcalopin (**1a**). In this reaction sequence, the α-hydroxy ketone should first be converted into 1,2-diketone **13**, which would afford **1a** by retroaldol cleavage to acetate **14** and subsequent aromatization (Figure 7).

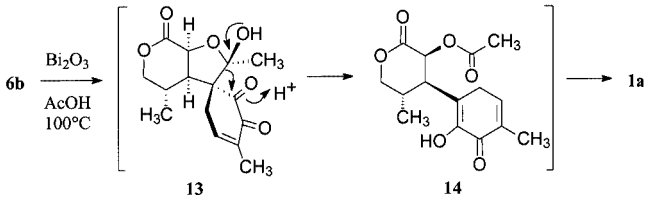


Figure 7. Conversion of cyclocalopin A (**6b**) into *O*-acetylcalopin A (**1a**)

This result suggests the probable role of cyclocalopin A (**6b**) as the biosynthetic precursor of *O*-acetylcalopin (**1a**). Since **1a** and **6b** are both C_{15} compounds, a terpenoid origin for these metabolites appears plausible. If one assumes the tetrahydroxylated acorenedione **15** as a hypothetical precursor, oxidative cleavage could give the oxo acid **16**, which on lactonization and hemiacetal formation would yield cyclocalopin A (**6b**) directly (Figure 8).[11] Highly oxidized acorenones have been isolated from cultures of the basidiomycetes *Hemimycena cucullata*[12] and *Rhodotus palmatus*.[13]

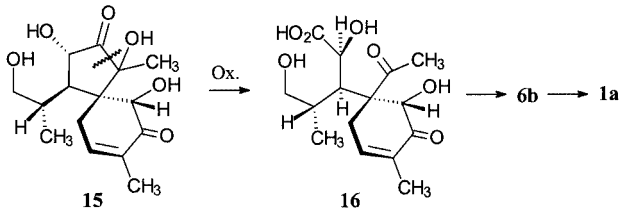


Figure 8. Proposal for the biosynthesis of **6b** and **1a** from a hypothetical acorenedione precursor **15**

Unfortunately, feeding experiments with young fruit bodies of *Boletus radicans* have so far been unsuccessful; ^{13}C-labeled glucoses, acetate, mevalolactone, fatty acids, tyrosine, and alanine all failed to be incorporated into the calopins and cyclocalopins at detectable levels.

Table 3. HPLC analysis of *Boletus* species from the section *Calopodes*

	B. calopus	*B. radicans*	*B. coniferarum*	*B. rubripes*	*B. peckii*
O-Acetylcalopin (**1a**)	+++	+++	++	+++	+++
Calopin (**1b**)	+	+++	+++	+++	++
O-Acetylcyclocalopin A (**6a**)	+++	+	+	+	+
Cyclocalopin A (**6b**)	+++	+++	++	+++	++
Cyclocalopin B (**7**)	+	++	+	+	+
Cyclocalopin C_1, C_2 (**8**, **9**)	−	+	−	−	−
Cyclocalopin D (**10a**)	+++	++	+	+	+++
Cyclocalopin E (**11**)	+	++	+	+	+

Distribution of Calopins and Cyclocalopins in Boletus, Section Calopodes

The European species *Boletus calopus* and *B. radicans* and the North American *B. coniferarum*, *B. rubripes*, and *B. peckii* are placed in the section *Calopodes* Fr.[1] HPLC analysis of the $CHCl_3$ and EtOAc extracts from dried fruit bodies of this section (Table 3) have demonstrated the presence of calopins and cyclocalopins in all species so far investigated. The cyclocalopins are responsible for the bitter taste of the fruit bodies, a common feature of the section *Calopodes*. In all species, *O*-acetylcalopin (**1a**) and cyclocalopin A (**6b**) are major components. The glycoside cyclocalopin D (**10a**) occurs in larger quantities in *B. calopus*, *B. radicans*, and *B. peckii*, while cyclocalopins C_1 (**8**) and C_2 (**9**) were only found in *B. radicans*. Interestingly, no calopins could be detected in the extracts of the bitter bolete *Tylopilus felleus*.

Experimental Section

General: Melting points (uncorrected): Reichert Thermovar hotstage. Optical rotations: Perkin−Elmer 241. IR spectra: Perkin−Elmer 1420 Ratio Recording Infrared Spectrometer (KBr). Intensity of the bands: ss (very strong), s (strong), m (medium), w (weak). UV/Vis and CD spectra: Jobin Yvon Instruments S. A. CD-6-Dichrograph. NMR: Bruker WM 400, Bruker ARX 300, Bruker AMX 600; chemical shifts δ in ppm are referenced to the residual solvent signal ($CDCl_3$: δ_H = 7.26 ppm, δ_C = 77.0 ppm; CD_3OD: δ_H = 3.31 ppm, δ_C = 49.0 ppm). For the ^{1}H-coupled ^{13}C NMR spectrum the decoupler was gated on for 2 s and gated off for 2 s during the acquisition (Bruker Pulse Sequence zggd). Multiplets due to ^1J couplings are given in capital letters, ^3J and other couplings in small letters. MS: Finnigan MAT 90, 95 Q (direct inlet, 70 eV). X-ray diffraction: Enraf−Nonius CAD4 diffractometer at 293(2) K with Mo-K_α (λ = 0.71069 Å) radiation. TLC: 60 F_{254} silica gel (0.25 mm) on aluminium foil (Merck) with various mixtures of ethyl acetate (EtOAc) and petroleum ether (40−60 °C) (PE). Preparative HPLC separations: Waters-Millipore with M680gradient controller, two M 590 EF pumps and U 6 K injector equipped with a Knauer variable-wavelength monitor with a superpreparative flow cell. A Nucleosil 100 C-18 (7 μm) pre-packed HPLC column (250 × 20 mm) with a pre-column (Macherey & Nagel, 30 × 20 mm) and gradient systems with MeOH/H_2O mixtures were used. Retention times, R_T, were determined with an HPLC column (250 × 4 mm; Nucleosil 100 C-18, 5 μm; Macherey & Nagel) with MeOH/H_2O, 1:9 (solvent system A) and MeOH/H_2O, 9:1 (solvent system B) as eluents; solvent gradient: 0 min: 100% A → 45 min 25% A and 75% B; flow rate: 1 mL/min. High-speed countercurrent chromatography (HSCCC) was performed with an apparatus from P. C. Inc., Potomac, MD, USA, consisting of a multi-layer coil, a counter-weight/triple coil, and a Rainin's Dynamax® SD-200 pump. The numbering of the carbon skeleton follows biogenetic considerations and is indicated in formulae **1a** and **6a**.

Fungal Collections: *Boletus calopus* was collected in August/September 1996 and 1997 near Sonthofen, Allgäu, Germany (leg. et det. E. Elstner), *B. radicans* in late summer 1997 and 1998 at the Gerolfinger Fort near Ingolstadt (leg. et det. N. Arnold), Germany, *B. coniferarum* and *B. rubripes* in October 1994 at the Barlow Pass, near Granite Falls, Cascade Mountains, WA, USA (leg. W. and R. Steglich, det. J. Ammirati), and *B. peckii* in August 1997 near Coweeta Hydrological Station, Macon County, NC, USA (leg. W. Steglich, det. N. Arnold). Voucher specimens are kept in the herbarium of the Department Chemie, Universität München.

Isolation of the Calopins and Cyclocalopins: Workup of fresh or freeze-dried (*Boletus radicans* or *B. calopus*) and air-dried (*B. coniferarum*, *B. rubripes*, *B. peckii*) material for isolation of the metabolites was performed in portions of up to 50 g dry weight. The amounts of metabolites varied, depending on the species and the time of storage, as well as on the age and quality of the fruit bodies (for an approximate quantification see Table 3).

Extraction with MeOH: Air-dried fruit bodies of *B. coniferarum* (30 g) were pulverized and defatted with petroleum ether (60−80 °C). Extraction of the material with a mixture of MeOH (1 L), 2 M HCl (1 mL), and ascorbic acid (0.5 g) in 250-mL portions (4 ×) and evaporation of the combined extracts in vacuo yielded a residue, which was distributed between acidified water (pH = 3) and EtOAc. The organic layers were dried ($MgSO_4$), and the metabolites were separated by preparative HPLC (Nucleosil 100 C-18, 7 μm, 250 × 20 mm, UV detection at 220 nm; solvent A: MeOH/H_2O, 1:9, solvent B: MeOH/H_2O, 9:1; solvent gradient: 0 min: 90% A and 10% B → 80 min: 50% A and 50% B, 100 min: 50% A and 50% B; flow rate 6.75 mL/min) to afford 16 mg of *O*-acetylcyclocalopin A (**6a**, R_T = 20.9 min), 12 mg of calopin (**1b**, R_T = 21.7 min), and 55 mg of *O*-acetylcalopin (**1a**, R_T = 35.2 min).

Extraction with $CHCl_3$: The dried and pulverized mushrooms (*B. calopus*, *B. coniferarum*, *B. radicans*) were defatted with petroleum ether (40−60 °C) and then exhaustively extracted with $CHCl_3$. Fresh fungi were extracted directly with $CHCl_3$. After concentration of the combined extracts, the pale brown residue was purified by HSCCC [mobile phase: $CHCl_3$, 3 volume parts, stationary phase: H_2O/MeOH, 5:2 volume parts; 230 mL column, "re-

verse" rotation mode, 600 rpm, flow: 2 mL/min]. The compounds **6b**, **6a**, **1b**, and **1a** were eluted in the given order with the mobile phase. The final purification of the enriched fractions was achieved by preparative HPLC on RP-18.

Extraction with EtOAc: The lyophilized, pulverized, and defatted fruit bodies of *B. radicans* were exhaustively extracted with EtOAc in an ultrasonic bath. After concentration of the combined extracts, the brown residue was dissolved in $CHCl_3$ (5 mL) and purified by HSCCC as described above. The substances were eluted in the order **11/12**, **7**, **8/9**, and **10a**. The purification was achieved by preparative HPLC. In the case of glucoside **10a**, a different solvent gradient was used (0 min: 100% solvent system A → 45 min: 50% A and 50% B).

***O*-Acetylcalopin (1a):** Oil; R_f (TLC) = 0.62 (PE/EtOAc, 1:2), 0.41 (toluene/acetone, 7:3); R_T (analytical HPLC) = 29.4 min. $[\alpha]_D^{26}$ = +129.8 (*c* = 0.11, $CHCl_3$). UV/Vis (CH_3CN): λ_{max} (lg ε) = 203 nm (3.422), 224 (2.881, sh), 276 (2.201). CD (CH_3CN): λ_{max} (Δε) = 230 (0), 240 (−0.6), 262 (−0.2), 275 (−0.7), 293 (0). IR (KBr): $\tilde{\nu}$ = 3438 cm^{-1} (s, br.), 2966 (m), 2931 (m), 1744 (ss), 1626 (m), 1474 (m), 1438 (m), 1404 (m), 1382 (m), 1274 (m, sh), 1238 (s, br.), 1101 (m), 1046 (m), 1022 (w, sh), 989 (w), 954 (w), 782 (w), 757 (w). ^1H and ^{13}C NMR see Table 1. EIMS: *m*/*z* (%) = 295 (8) [M^+ + H], 294 (43) [M^+], 253 (14), 252 (90), 234 (24), 207 (14), 206 (100), 191 (19), 178 (7), 177 (33), 175 (8), 163 (7), 159 (12), 148 (8), 137 (23), 136 (7). $C_{15}H_{18}O_6$: calcd. 294.1103; found 294.1107.

Calopin (1b): Oil; R_f (TLC) = 0.43 (PE/EtOAc, 1:2), 0.31 (toluene/acetone, 7:3); R_T (analytical HPLC) = 23.7 min. $[\alpha]_D^{23}$ = −14.2 (*c* = 0.002 in $CHCl_3$). $[\alpha]_D^{23}$ = +24.9 (*c* = 0.002 in MeOH). UV/Vis (CH_3CN): λ_{max} (lg ε) = 203 nm (3.828), 227 (3.091, sh), 278 (2.383). CD (CH_3CN): λ_{max} (Δε) = 218 nm (+4.2), 231 (sh, +1.4), 242 (0), 248 (−0.4), 279 (−0.4), 287 (0). ^1H and ^{13}C NMR see Table 1. EIMS: *m*/*z* (%) = 253 (16) [M^+ + H], 252 (97) [M^+], 234 (28), 207 (19), 206 (100), 191 (20), 177 (44), 164 (18), 163 (15), 161 (20), 159 (24), 149 (14), 147 (20), 146 (14), 145 (13), 136 (53), 135 (27), 131 (13), 123 (16), 121 (10), 91 (15). $C_{13}H_{16}O_5$: calcd. 252.0998; found 252.1002.

Acetylation of 1a and 1b: Compound **1b** (6 mg) was dissolved in acetic anhydride (1 mL), and, after addition of 1 drop of concd. H_2SO_4, the mixture was stirred for 3 d at room temperature. The mixture was distributed between $CHCl_3$ and water, and the organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography of the residue on silica gel 60 with PE (60−80 °C)/EtOAc, 1:1, yielded the pure triacetate **1c** (3.5 mg, 39%). The same procedure was used for the conversion of **1a** into **1c**. Colorless crystals; m.p. 206−208 °C; R_f (TLC) = 0.28 (PE/EtOAc, 2:3). $[\alpha]_D^{26}$ = +96 (*c* = 0.29, $CHCl_3$). IR (KBr): $\tilde{\nu}$ = 3436 cm^{-1} (m, br, H_2O), 2928 (w), 2855 (w), 1775 (ss), 1752 (ss, sh), 1629 (w), 1501 (w), 1459 (w), 1431 (w), 1408 (w), 1369 (m), 1236 (s), 1207 (s), 1171 (s), 1106 (m), 1060 (m), 1041 (m), 849 (w). ^1H NMR (300 MHz, $CDCl_3$): δ = 1.04 (d, *J* = 6.7 Hz, 3 H, 14-H), 1.92 (s, 3 H, 13-H), 2.17 (d, *J* = 0.5 Hz, 3 H, 15-H), 2.28 (s, 3 H, 17-H or 19-H), 2.29 (s, 3 H, 17-H or 19-H), 2.27−2.30 (m, 1 H, 4-H), 3.47 (dd, *J* = 9.7, 7.9 Hz, 1 H, 3-H), 4.14 (dd, *J* = 11.7, 11.7 Hz, 1 H, 5α-H), 4.31 (dd, *J* = 11.7, 4.9 Hz, 1 H, 5β-H), 5.67 (d, *J* = 9.7 Hz, 1 H, 2-H), 6.96 (d, *J* = 8.1 Hz, 1 H, 11-H), 7.11 (dq, *J* = 8.1, 0.5 Hz, 1 H, 10-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 16.1 (2C), 20.3, 20.4, 20.5, 36.5 (C-4), 66.9 (C-1), 70.7 (C-5), 124.0, 126.4, 128.4, 131.8, 141.4 (C-7 or C-8), 141.7 (C-7 or C-8), 167.5, 168.2, 168.6, 170.1 (C-1) ppm. EIMS: *m*/*z* (%) = 378 (1) [M^+], 337 (4), 336 (22), 295 (13), 294 (85), 276 (5), 253 (13), 252 (100), 234 (9), 207 (5), 206 (37), 177 (10), 137 (6). $C_{19}H_{22}O_8$: calcd. 378.1315; found 378.1291.

Treatment of 1a with Phosgene: Pyridine (0.2 mL) and phosgene in toluene (20%, 5 drops) were added, under argon, to **1a** (8.3 mg) in benzene (3 mL). The mixture was stirred for 3 h at room temperature and the solvent was then evaporated under reduced pressure. The residue, still containing pyridine hydrochloride, was used directly for measurement of the ^1H NMR spectrum and MS of the unstable cyclic carbonate **1d**, R_f (TLC) = 0.69 (toluene/acetone, 7:3). ^1H NMR (400 MHz, $CDCl_3$): δ = 1.12 (d, *J* = 6.8 Hz, 3 H, 14-H), 1.93 (s, 3 H, 13-H), 2.34 (s, 3 H, 15-H), 2.53 (m, 1 H, 4-H), 3.59 (dd, *J* = 8.5, 7.0 Hz, 1 H, 3-H), 4.20 (dd, *J* = 11.5, 11.5 Hz, 1 H, 5α-H), 4.39 (dd, *J* = 11.5, 5.2 Hz, 1 H, 5β-H), 5.74 (d, *J* = 8.5 Hz, 1 H, 2-H), 6.90 (d, *J* = 8.3 Hz, 1 H, 11-H), 7.00 (d, *J* = 8.3 Hz, 1 H, 10-H) ppm. EIMS: *m*/*z* (%) = 320 (5) [M^+], 278 (8), 261 (6), 260 (48). $C_{16}H_{16}O_7$: calcd. 320.0896; found 320.0893.

***O*,*O*-Isopropylidenecalopin (1e):** A solution of **1b** (6 mg) and 2,2-dimethoxypropane (6 μL, 2 equiv.) in dry toluene (2 mL) was heated for 4 h at 50 °C and then stirred for 24 h at room temperature. After removal of the solvent, the residue was dissolved in a small volume of MeOH, filtered through a short RP-18 pre-column, and purified by preparative HPLC (RP-18, solvent system A: MeOH/H_2O, 1:9, B: MeOH/H_2O, 9:1; solvent gradient: 0 min: 100% A → 60 min: 50% A and 50% B, 90 min: 50% A and 50% B; flow rate 7 mL/min) to yield **1e** (4.6 mg, 65%), R_T = 89.8 min, as a colorless solid, R_f (TLC) = 0.71 (PE/EtOAc, 1:2). ^1H NMR (600 MHz, $CDCl_3$): δ = 1.08 (d, *J* = 6.9 Hz, 3 H, 14-H), 1.63, 1.67 [each s, 3 H, $C(CH_3)_2$], 2.17 (s, 3 H, 15-H), 2.42 (m, 1 H, 4-H), 2.85 (d, *J* = 4.9 Hz, 1 H, 2-OH), 3.33 (dd, *J* = 9.5, 8.0 Hz, 1 H, 3-H), 4.09 (dd, *J* = 11.5, 11.5 Hz, 1 H, 5α-H), 4.31 (dd, *J* = 11.5, 5.0 Hz, 1 H, 5β-H), 4.59 (dd, *J* = 9.5, 4.9 Hz, 1 H, 2-H), 6.46 (d, *J* = 8.0 Hz, 1 H, 11-H), 6.58 (d, *J* = 8.0 Hz, 1 H, 10-H) ppm. ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 14.5 (C-15), 16.4 (C-14), 25.7, 26.0 [each $C(CH_3)_2$], 35.4 (C-4), 45.9 (C-3), 66.5 (C-2), 70.8 (C-5), 117.6, 117.7 [each C-6 or $C(CH_3)_2$], 118.1 (C-9), 121.2 (C-11), 123.0 (C-10), 145.1 (C-7), 145.5 (C-8), 174.9 (C-1) ppm. EIMS: *m*/*z* (%) = 292 (100) [M^+], 277 (62), 261 (14), 203 (35), 189 (18), 177 (44), 159 (84), 145 (13), 131 (21), 115 (9), 91 (19), 83 (51), 77 (10). $C_{16}H_{20}O_5$: calcd. 292.1311; found 292.1334.

(*R*)-MTPA Ester of *O*,*O*-Isopropylidenecalopin (2_R): Compound **1e** (1.2 mg), (*S*)-(+)-MTPA-Cl (1.6 mg, 1.5 equiv.), and a catalytic amount of DMAP were stirred in pyridine (25 μL) for 72 h at room temperature. After removal of the volatile compounds under reduced pressure, ^1H NMR analysis of the residue revealed the presence of a mixture of 17% $\mathbf{2_R}$ and 83% unchanged **1e**. Selected ^1H NMR spectroscopic data (600 MHz, $CDCl_3$): δ = 2.14 (s, 3 H, 15-H), 3.12 (s, 3 H, OCH_3), 3.43 (dd, *J* = 9.6, 8.4 Hz, 1 H, 3-H), 3.92 (dd, *J* = 11.3, 11.2 Hz, 1 H, 5α-H), 4.33 (dd, *J* = 11.3, 4.9 Hz, 1 H, 5β-H), 5.86 (d, *J* = 9.6 Hz, 1 H, 2-H), 6.42 (d, *J* = 8.0 Hz, 1 H, 11-H), 6.51 (d, *J* = 8.0 Hz, 1 H, 10-H) ppm.

(*S*)-MTPA Ester of *O*,*O*-Isopropylidenecalopin (2_S): Treatment of **1e** and (*R*)-(−)-MTPA-Cl, carried out as described above, yielded a mixture of 13% $\mathbf{2_S}$ and 87% **1e**. Selected ^1H NMR spectroscopic data (600 MHz, $CDCl_3$): δ = 2.11 (s, 3 H, 15-H), 3.42 (s, 3 H, OCH_3), 3.36 (dd, *J* = 8.3, 6.7 Hz, 1 H, 3-H), 4.33 (dd, *J* = 11.3, 4.9 Hz, 1 H, 5β-H), 5.83 (d, *J* = 9.6 Hz, 1 H, 2-H), 6.30 (d, *J* = 8.0 Hz, 1 H, 11-H), 6.40 (d, *J* = 8.0 Hz, 1 H, 10-H) ppm.

Isolation of *O*-Acetylisocalopin (4): The EtOAc phase from the MeOH extraction of the air-dried fruit bodies of *B. coniferarum* or *B. radicans* (see above) was separated by HPLC [Nucleosil 100 C-18, 7 μm (Macherey & Nagel), column 20 × 250 mm; solvent system A: MeOH/H_2O, 1:9, B: MeOH/H_2O, 9:1; solvent gradient: 0 min 90% A and 10% B → 60 min: 50% A and 50% B, 90 min:

50% A and 50% B; flow rate 6.75 mL/min]. The fraction containing **4** (R_T = 75 min) was collected in a pre-cooled flask at −78° C. The sample was lyophilized and investigated by NMR spectroscopy. Compound **4**: yellowish solid, R_T (analytical HPLC) = 32.2 min. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 0.80 (d, *J* = 6.9 Hz, 3 H, 14-H), 2.18 (s, 3 H, 13-H), 2.22 (s, 3 H, 15-H), 2.41 (m, 1 H, 4-H), 3.03 (dd, *J* = 10.9, 10.9 Hz, 1 H, 5a-H), 3.67 (dd, *J* = 10.9, 4.6, 1 H, 5b-H), 3.89 (dd, *J* = 10.0, 4.0, 1 H, 3-H), 5.45 (d, *J* = 10.0 Hz, 1 H, 2-H), 6.58 (d, *J* = 8.0 Hz, 1 H, 11-H), 6.66 (d, *J* = 8.0 Hz, 1 H, 10-H) ppm. ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 21.1 (C-13), 33.5 (C-4), 37.5 (C-3), 65.7 (C-5), 73.4 (C-2), 122.6 (C-10), 124.4 (C-11) ppm (other signals not determined).

Acetylation of 4: A mixture of **1a** and **4** (10 mg) was dissolved in acetic anhydride (2 mL). After addition of pyridine (1 mL), the mixture was stirred for 2 h at room temperature under argon. The mixture was concentrated under reduced pressure and the residue was purified by HPLC [Lichrosorb RP-18, 7 μm (Merck); column 250 × 16 mm; solvent system A: acetonitrile/H_2O, 1:9, B: acetonitrile/H_2O, 9:1; solvent gradient: 0 min: 90% A and 10% B → 45 min: 25% A and 75% B; flow rate 6.75 mL/min] to yield **1c** (5.4 mg, R_T = 12.5 min) and **5** (5.3 mg, R_T = 38.5 min) as colorless solids. Compound **5**: m.p. 140 °C; R_f (TLC) = 0.49 (PE/EtOAc, 3:2). $[\alpha]_D^{27}$ = −13.0 (*c* = 0.1, $CHCl_3$). UV/Vis (CH_3CN): λ_{max} (lg ε) = 213 nm (2.66), 238 (1.90), 258 (1.83). IR (KBr): $\tilde{\nu}$ = 3437 cm^{-1} (s, br.), 2930 (m), 2855 (w), 1778 (ss, sh), 1743 (ss), 1630 (w), 1461 (m), 1433 (m), 1374 (s), 1209 (ss, br.), 1168 (ss), 1130 (w), 1085 (m), 1054 (m), 1016 (m), 955 (w), 915 (w), 869 (w). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 0.84 (d, *J* = 6.8 Hz, 14-H), 2.06, 2.15, 2.17, 2.28, 2.32 (each s, 3 H), 2.48 (m, 4-H), 3.49 (dd, *J* = 7.3, 6.5 Hz, 3-H), 3.97 (dd, *J* = 11.3, 5.7 Hz, 5b-H), 4.05 (dd, *J* = 11.3, 5.6 Hz, 5a-H), 5.27 (d, *J* = 6.5 Hz, 2-H), 7.06 (d, *J* = 7.9 Hz, 10-H or 11-H), 7.09 (d, *J* = 7.9 Hz, 10-H or 11-H) ppm. ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 14.8, 16.1, 20.27, 20.31, 20.7, 20.9, 33.3 (C-4), 40.9 (C-3), 67.0 (C-5), 73.5 (C-2), 125.7 (C-10 or C-11), 128.0 (C-10 or C-11), 129.5 (C-6 or C-9), 131.3 (C-6 or C-9), 141.6 (2C, C-7 and C-8), 167.6, 167.8, 169.6, 170.0, 170.8 ppm.

***O*-Acetylcyclocalopin A (6a):** Colorless crystals; m.p. 190 °C; R_f (TLC) = 0.54 (PE/EtOAc, 1:2); R_T (analytical HPLC) = 23.0 min. $[\alpha]_D^{25}$ = −29.1 (*c* = 0.35, $CHCl_3$). UV/Vis (CH_3CN): λ_{max} (lg ε) = 238 nm (2.51), 332 (1.12). UV/Vis (EtOH): λ_{max} (lg ε) = 218 nm (2.37), 273 (2.64). CD (CH_3CN): λ_{max} (Δε) = 220 nm (+1.69), 245 (−3.58), 326 (+1.08). CD (EtOH): λ_{max} (Δε) = 216 nm (+3.32), 246 (−1.52), 321 (+0.48). IR (KBr): $\tilde{\nu}$ = 3380 cm^{-1} (s, br.), 2960 (w), 2920 (m), 2900 (sh), 2850 (w), 1745 (s), 1725 (s), 1690 (s), 1650 (w), 1390 (m), 1370 (m), 1335 (w), 1235 (s, br.), 1205 (sh), 1085 (m). ^{1}H and ^{13}C NMR: see Table 2. EIMS (70 eV): *m*/*z* (%) = 321 (1) [M^+ + H − H_2O], 320 (0.8) [M^+ − H_2O], 278 (6), 237 (15), 236 (44), 219 (9), 218 (39), 207 (28), 191 (5), 173 (5), 162 (19), 161 (100), 149 (10), 147 (9), 125 (8), 121 (19); CIMS (isobutane): *m*/*z* (%) = 339 (0.4) [M^+ + H], 322 (19) [M^+ + 2 H − H_2O], 321 (100) [M^+ + H − H_2O], 279 (9), 278 (11), 237 (12), 236 (21), 218 (20), 207 (8), 161 (25). +FABMS (glycerol): *m*/*z* (%) = 339 (< 0.5) [M^+ + H], 338 (0.8) [M^+], 322 (19) [M^+ + 2 H − H_2O], 321 (100) [M^+ + H − H_2O], 279 (11). HR EIMS $C_{17}H_{20}O_6$ [M^+ − H_2O]: calcd. 320.1260; found 320.1256.

Cyclocalopin A (6b): Colorless crystals; m.p. 165 °C; R_f (TLC) = 0.24 (PE/EtOAc, 1:2), 0.17 (toluene/acetone 7:3); R_T (analytical HPLC) = 18.8 min. $[\alpha]_D^{25}$ = −34.5 (*c* = 0.20, $CHCl_3$). UV/Vis (CH_3CN): λ_{max} (lg ε) = 200 nm (2.51), 241 (2.65). UV/Vis (EtOH): λ_{max} (lg ε) = 211 nm (2.57), 243 (2.80). CD (CH_3CN): λ_{max} (Δε) = 217 nm (+5.88), 247 (−5.33), 318 (+9.74). CD (EtOH): λ_{max} (Δε) = 216 nm (+6.17), 246 (−5.32), 322 (+1.02). IR (KBr): $\tilde{\nu}$ = 3600 cm^{-1} (w), 3400 (m, br.), 2980 (w), 2920 (w), 2880 (w), 2820 (w), 1740 (s), 1670 (s), 1630 (w), 1385 (m), 1320 (m), 1210 (s), 1160 (s), 1140 (m), 1100 (s), 1080 (m), 1040 (s), 1010 (m). ^{1}H and ^{13}C NMR (150.9 MHz, $CDCl_3$): see Table 2. EIMS: *m*/*z* (%) = 296 (1.6) [M^+], 279 (7), 278 (19) [M^+ − H_2O], 254 (9), 237 (14), 236 (77), 219 (10), 218 (48), 208 (12), 207 (33), 191 (4), 179 (9), 162 (19), 161 (100), 151 (17), 149 (23), 125 (48), 121 (23). $C_{15}H_{20}O_6$: calcd. 296.1260; found 296.1264. $C_{15}H_{18}O_5$ [M^+ − H_2O]: calcd. 278.1154; found 278.1114.

Acetylation of 6b: Acetic anhydride (4 μL) and pyridine (3 μL) in dichloromethane (3 mL) and a catalytic amount of DMAP were added to **6b** (5.5 mg) in dichloromethane (10 mL). The solution was stirred overnight at room temperature and then washed with 2.5% citric acid (2 ×) and water (1 ×). The aqueous phases were extracted with dichloromethane, the combined organic layers were dried (Na_2SO_4), and the solvents were evaporated to yield **6a** (2.7 mg, 44%). The ^{1}H NMR and MS data of **6a** are identical with those of the natural product.

Conversion of 6b into 1a: A suspension of Bi_2O_3 (1.5 mg) in acetic acid (0.5 mL) was added to **6b** (1 mg). The mixture was stirred for 1 h at 100 °C, another portion of Bi_2O_3 (0.5 mg) was added, and the stirring was continued for 1 h. The yellow solution was concentrated under reduced pressure, and the residue was dissolved in MeOH and purified by filtration through a short RP-18 column. The formation of **1a** was established by HPLC analysis and co-injection with authentic **1a**.

Cyclocalopin B (7): Colorless oil; R_T (analytical HPLC) = 39.0 min. $[\alpha]_D^{33}$ = +18.7 (*c* = 0.10, $CHCl_3$). UV/Vis (CH_3CN): λ_{max} (lg ε) = 201 nm (3.088). CD (CH_3CN): λ_{max} (Δ ε) = 205 nm (+6.21), 210 (+5.47), 216 (+5.82). IR (KBr): $\tilde{\nu}$ = 3436 cm^{-1} (m, br.), 2925 (m), 2854 (m), 1744 (ss), 1668 (m), 1454 (m), 1436 (m), 1375 (m), 1334 (m), 1245 (s), 1154 (m), 1141 (m), 1098 (m), 1076 (m), 1051 (m, sh), 1036 (m), 917 (m), 866 (m), 753 (w). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 0.95 (d, *J* = 6.6 Hz, 3 H, 14-H), 1.46 (s, 3 H, 13-H), 1.63 (br. s, 3 H, 15-H), 2.01 (s, 3 H, 7-CH_3CO), 2.07 (s, 3 H, 8-CH_3CO), 2.20 (dm, *J* = 19.9 Hz, 1 H, 11β-H), 2.32 (m, 1 H, 4-H), 2.41 (dm, *J* = 19.9 Hz, 1 H, 11α-H), 2.69 (dd, *J* = 10.6, 10.6 Hz, 1 H, 3-H), 3.94 (dd, *J* = 11.1, 11.1 Hz, 1 H, 5α-H), 4.14 (dd, *J* = 11.1, 4.3 Hz, 1 H, 5β-H), 4.61 (d, *J* = 10.6 Hz, 1 H, 2-H), 5.27 (d, *J* = 8.7 Hz, 1 H, 7-H), 5.56 (br. "s", 2 H, 8-H, 10-H) ppm. ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 15.6 (C-14), 18.0 (C-15), 20.7 (8-*C*H_3CO), 21.0 (C-13), 21.2 (7-*C*H_3CO), 27.3 (C-11), 31.0 (C-4), 48.1 (C-3), 52.9 (C-6), 72.3 (C-5), 73.5 (C-8), 73.9 (C-2), 74.0 (C-7), 108.3 (C-12), 124.6 (C-10), 130.8 (C-9), 170.1 (7-CH_3*C*O), 171.0 (8-CH_3*C*O), 172.8 (C-1) ppm. EIMS (70 eV): *m*/*z* (%) = 366 (17) [M^+ + H − OH], 365 (100) [M^+ − OH], 323 (6), 305 (7), 280 (4), 263 (6), 245 (5), 221 (11), 220 (9), 202 (11). $C_{19}H_{24}O_7$ [M^+ − OH]: calcd. 364.1522; found 364.1543.

2:1 Mixture of Cyclocalopin C_1 (8) and C_2 (9): Colorless oil. $[\alpha]_D^{33}$ = +168.6 (*c* = 0.12; $CHCl_3$). UV/Vis (CH_3CN): λ_{max} (lg ε) = 228 nm (3.182), 240 (2.946, sh). ^{1}H NMR (600 MHz, $CDCl_3$): **8/9**: δ = 1.15/0.91 (d, *J* = 6.6/6.5 Hz, 3 H, 14-H), 1.48/1.50 (s, 3 H, 13-H), 2.02/2.30 (m, 1 H, 4-H), 3.49/2.76 (dd, *J* = 10.2, 10.2/10.8, 10.8 Hz, 1 H, 3-H), 3.76/3.71 (d, *J* = 10.7/10.3 Hz, 1 H, 7-H), 3.93/3.87 (dd, *J* = 11.6, 11.6/9.9, 9.9 Hz, 1 H, 5α-H), 4.15/4.14 (dd, *J* = 11.6, 4.2/9.9, 3.5 Hz, 1 H, 5β-H), 4.31/4.23 (d, *J* = 10.7/10.3 Hz, 1 H, 8-H), 4.68/4.59 (d, *J* = 10.2/10.8 Hz, 1 H, 2-H), 5.10/5.84 (d, *J* = 10.2/10.2 Hz, 1 H, 11-H), 5.15/5.16 (s, 1 H, 15b-H), 5.46/5.46 (s, 1 H, 15a-H), 6.32/6.35 (d, *J* = 10.2/10.2 Hz, 1 H, 10-H) ppm. ^{13}C NMR (150.9 MHz, $CDCl_3$): **8/9**: δ = 15.9/15.5 (C-14), 23.0/22.4 (C-13), 29.1/30.4 (C-4), 44.4/48.2 (C-3), 57.5/56.4 (C-6), 70.05/69.95 (C-8),

71.6/72.7 (C-5), 72.1/73.5 (C-2), 72.6/74.4 (C-7), 110.3/107.9 (C-12), 113.4/113.2 (C-15), 125.7/125.4 (C-11), 131.4/132.1 (C-10), 141.5/142.5 (C-9), 172.2/172.5 (C-1) ppm. EIMS (70 eV): *m/z* (%) = 296 (2) [M^+], 279 (32), 278 (17) [$M^+ - H_2O$], 249 (10), 237 (17), 236 (100), 235 (39), 219 (10), 218 (48), 197 (41), 194 (25), 162 (22), 161 (92), 160 (21), 159 (34), 145 (40), 121 (22), 120 (39), 107 (55), 105 (22), 91 (30), 77 (20). $C_{15}H_{20}O_6$: calcd. 296.1260; found 296.1241.

Cyclocalopin D (10a): Colorless crystals; m.p. 110 °C; R_T (analytical HPLC) = 14.1 min. $[\alpha]_D^{25}$ = +109.7 (*c* = 0.26 in MeOH). UV/Vis (CH_3OH): λ_{max} (lg ε) = 212 nm (3.014), 244 (1.906). UV/Vis (CH_3CN): λ_{max} (lg ε) = 210 nm (3.305), 257 (2.330). CD (CH_3OH): λ_{max} (Δε) = 211 nm (−0.14), 233 (+0.06), 262 (−0.08), 318 (+0.01). CD (CH_3CN): λ_{max} (Δε) = 196 nm (+1.16), 215 (−0.39), 236 (+0.14), 254 (−0.04). IR (KBr): $\tilde{\nu}$ = 3434 cm^{-1} (ss, br.), 2930 (m), 1739 (s), 1672 (m), 1453 (m), 1383 (m), 1235 (s), 1168 (m), 1099 (m), 1073 (s), 1046 (s), 1018 (s), 953 (m), 919 (m), 899 (w), 864 (w), 835 (w), 779 (w). ^{1}H NMR (600 MHz, CD_3OD): δ = 1.26 (d, *J* = 6.6 Hz, 3 H, 14-H), 1.35 (s, 3 H, 13-H), 1.68 (dd, *J* = 11.3, 5.1 Hz, 1 H, 11β-H), 2.02 (s, 3 H, 15-CH_3CO), 2.05 (m, 1 H, 4-H), 2.09 (d, *J* = 11.3 Hz, 1 H, 11α-H), 2.81 (dd, *J* = 10.7, 10.7 Hz, 1 H, 3-H), 3.44 (dd, *J* = 9.3, 9.3 Hz, 1 H, 4′-H), 3.53 (dd, *J* = 9.3, 3.5 Hz, 1 H, 2′-H), 3.72 (m, 1 H, 5′-H), 3.73 (m, 1 H, 6′a-H), 3.75 (m, 1 H, 6′b-H), 3.78 (dd, *J* = 9.3, 9.3 Hz, 1 H, 3′-H), 4.03 (dd, *J* = 11.5, 11.5 Hz, 1 H, 5α-H), 4.15 (dd, *J* = 11.5, 4.4 Hz, 1 H, 5β-H), 4.40 (br. s, 1 H, 7-H), 4.62 (d, *J* = 5.1 Hz, 1 H, 10-H), 4.70 (d, *J* = 12.3 Hz, 1 H, 15a-H), 4.77 (d, *J* = 10.7 Hz, 1 H, 2-H), 4.80 (d, *J* = 12.3 Hz, 1 H, 15b-H), 5.75 (d, *J* = 3.5 Hz, 1 H, 1′-H) ppm. ^{13}C NMR (150.9 MHz, CD_3OD): δ = 17.2 (Qm, *J* = 128.1 Hz, C-14), 20.8 (Q, *J* = 129.5 Hz, 15-*C*H_3COO), 24.4 (Q, *J* = 127.5 Hz, C-13), 32.4 (Dm, *J* = 131.9 Hz, C-4), 33.1 (Tm, *J* = 148.2 Hz, C-11), 50.7 (Dm, *J* = 131.1 Hz, C-3), 61.1 (Td, *J* = 148.2, 2.9 Hz, C-15), 61.9 (m, C-6), 62.0 (Tm, *J* = 141.3 Hz, C-6′), 69.4 (Dm, *J* = 147.4 Hz, C-7), 71.1 (Dm, *J* = 143.4 Hz, C-4′), 72.6 (Tm, *J* = 143.2 Hz, C-5), 73.1 (Dm, *J* = 143.2 Hz, C-2′), 74.6 (Dm, *J* = 143.7 Hz, C-3′), 74.6 (Dd, *J* = 150.4, 5.0 Hz, C-2), 75.0 (Dm, *J* = 142.0 Hz, C-5′), 77.9 (Dm, *J* = 157.4 Hz, C-10), 97.0 (D, *J* = 173.2 Hz, C-1′), 117.8 (m, C-12), 118.6 (m, C-9), 152.4 (m, C-8), 172.9 (m, 15-CH_3*C*OO), 176.0 (ddd, *J* = 7.4, 7.4, 2.3 Hz, C-1) ppm. ESI MS (pos.): *m/z* (%) = 1056.8 (100) [2 M^+ + Na], 539.5 (7) [M^+ + Na]. $C_{23}H_{32}NaO_{13}$ [M^+ + Na]: calcd. 539.1741; found 539.1748.

Cyclocalopin D Tetraacetate (10b): Compound **10a** (5 mg) in acetic anhydride (2 mL) and pyridine (1 mL) was stirred overnight at room temperature under argon. After concentration under reduced pressure, the crude product was purified by preparative TLC (diol phase, eluent EtOAc) to yield **10b** (3.5 mg, 50%) as a colorless oil; R_f (TLC) = 0.17 (toluene/HCO_2Et/HCO_2H, 10:5:3). $[\alpha]_D^{33}$ = +114.9 (*c* = 0.13 in MeOH). UV/Vis (CH_3CN): λ_{max} (lg ε) = 197 nm (3.096), 213 (2.997), 275 (1.831). CD (CH_3CN): λ_{max} (Δε) = 195 nm (+2.66), 220 (+0.40), 228 (+0.67), 287 (+0.10), 331 (+0.04). ^{1}H NMR (600 MHz, CD_3OD): δ = 1.24 (d, *J* = 6.6 Hz, 3 H, 14-H), 1.33 (s, 3 H, 13-H), 1.68 (dd, *J* = 11.2, 5.1 Hz, 1 H, 11β-H), 2.01, 2.03, 2.04 (each s, 3 H), 2.05 (s, 6 H), 1.95−2.05 (m, 2 H, 4-H and 11α-H), 2.82 (dd, *J* = 10.7, 7.7 Hz, 1 H, 3-H), 4.02 (dd, *J* = 11.7, 11.7 Hz, 1 H, 5α-H), 4.12 (dd, *J* = 12.8, 3.0 Hz, 1 H, 6′a-H), 4.14 (dd, *J* = 11.7, 4.9 Hz, 1 H, 5β-H), 4.23 (dd, *J* = 12.8, 3.0 Hz, 1 H, 6′b-H), 4.30 (s, 1 H, 7-H), 4.43 (ddd, *J* = 10.6, 3.0 Hz, 1 H, 5′-H), 4.48 (d, *J* = 12.5 Hz, 1 H, 15a-H), 4.64 (d, *J* = 5.1 Hz, 1 H, 10-H), 4.76 (d, *J* = 10.7 Hz, 1 H, 2-H), 5.01 (dd, 10.6, 3.6 Hz, 1 H, 2′-H), 5.05 (d, *J* = 12.5 Hz, 1 H, 15b-H), 5.13 (dd, *J* = 10.6, 10.6 Hz, 1 H, 4′-H), 5.56 (dd, *J* = 10.6, 10.6 Hz, 1 H, 3′-H), 6.06 (d, *J* = 3.6 Hz, 1 H, 1′-H) ppm. ^{13}C NMR (150.9 MHz, CD_3OD): δ = 17.1 (C-14), 20.4, 20.6 (2C), 20.7, 20.8, 24.2 (C-13), 32.4 (C-4 or C-10), 33.0 (C-4 or C-10), 50.5 (C-3), 60.8 (C-15), 62.0 (C-6), 62.9 (C-6′), 69.0 (C-7), 69.6 (C-4), 70.2 (C-5′), 71.1 (C-3′), 71.4 (C-2), 72.6 (C-5), 74.6 (C-2), 77.8 (C-10), 92.9 (C-1′), 117.7 (C-9 or C-12), 118.7 (C-9 or C-12), 151.0 (C-8), 171.3, 171.6, 171.7, 172.6, 173.0, 175.9 (C-1) ppm.

Cyclocalopin E (11): 50 mg (0.06%) from dried *B. radicans*. Colorless crystals; m.p 183 °C; R_f (TLC) = 0.13 (PE/EtOAc, 1:2; orange spot on heating with vanillin/sulfuric acid); R_T (analytical HPLC) = 19.8 min (RP-18). $[\alpha]_D^{33}$ = +38.0 (*c* = 1.11, CH_3CN). UV/Vis (CH_3CN): λ_{max} (lg ε) = 226 nm (2.186, sh), 283 (1.904). CD (CH_3CN): λ_{max} (Δε) = 201 nm (−0.90), 226 (+0.42), 253 (−0.16), 282 (+0.01). IR (KBr): $\tilde{\nu}$ = 3436 cm^{-1} (m, br.), 2979 (m), 2934 (m), 2856 (m), 1745 (ss), 1732 (ss), 1456 (m), 1388 (m), 1353 (w), 1236 (s), 1211 (s), 1170 (s), 1138 (m), 1114 (s), 1078 (m), 1048 (s), 1020 (s), 993 (m), 964 (m), 942 (w), 929 (m), 899 (w), 755 (w), 667 (w), 552 (w). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.20 (d, *J* = 6.8 Hz, 3 H, 14-H), 1.25 (d, *J* = 7.0 Hz, 3 H, 15-H), 1.26 (s, 3 H, 13-H), 1.89 (d, *J* = 12.2 Hz, 1 H, 11α-H), 2.05 (m, 1 H, 4-H), 2.40 (dd, *J* = 12.2, 6.1 Hz, 1 H, 11β-H), 2.49 (m, 1 H, 9-H), 3.09 (dd, *J* = 11.2, 10.6 Hz, 1 H, 3-H), 3.70 (br. s, 1 H, 7-OH), 3.96 (dd, *J* = 11.3, 11.2 Hz, 1 H, 5α-H), 4.19 (dd, *J* = 11.2, 3.8 Hz, 1 H, 5β-H), 4.29 (s, 1 H, 7-H), 4.45 (br. d, *J* = 6.1 Hz, 1 H, 10-H), 4.50 (d, *J* = 11.2 Hz, 1 H, 2-H) ppm. ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 11.2 (C-15), 15.8 (C-14), 21.6 (C-13), 32.0 (C-4), 37.4 (C-11), 43.0 (C-3), 50.6 (C-9), 58.8 (C-6), 71.0 (C-2), 71.7 (C-5), 76.2 (C-7), 81.8 (C-10), 116.2 (C-12), 171.8 (C-1), 209.3 (C-8) ppm. EIMS: *m/z* (%) = 296 (15) [M^+], 236 (14), 234 (12), 224 (14), 210 (38), 208 (25), 207 (17), 206 (21), 195 (30), 191 (11), 181 (60), 179 (12), 178 (11), 177 (17), 163 (26), 161 (21), 151 (19), 149 (17), 147 (20), 137 (23), 135 (24), 125 (16), 124 (33), 123 (28), 121 (19), 113 (15), 109 (17), 107 (16), 95 (27), 91 (15), 55 (25), 43 (100). $C_{15}H_{20}O_6$: calcd. 296.1260; found 296.1257.

Cyclocalopin F (12) (1:3 Admixture with 11): Yield 33 mg from dried *B. radicans*; R_f (TLC) ≈ 0.13 (PE/EtOAc, 1:2; overlap with **11**); R_T (analytical HPLC) ≈ 19.8 min (RP-18). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.19 (d, *J* = 6.8 Hz, 3 H, 14-H), 1.38 (s, 3 H, 13-H), 1.84 (d, *J* = 12.3 Hz, 1 H, 11α-H), ca. 2.06 (m, overlapping, 1 H, 4-H), 2.46 (dd, *J* = 12.3, 6.1 Hz, 1 H, 11β-H), ca. 2.46 (m, overlapping, 1 H, 9-H), 3.18 (dd, *J* = 11.4, 10.0 Hz, 1 H, 3-H), 3.59 (br. s, 1 H, 7-OH), 3.98 (dd, *J* = 11.4, 11.2 Hz, 1 H, 5α-H), 4.20 (dd, *J* = 11.2 Hz, overlapping, 1 H, 5β-H), 4.25 (s, 1 H, 7-H), 4.56 (d, *J* = 11.4 Hz, 1 H, 2-H), 4.93 (d, *J* = 6.1 Hz, 1 H, 10-H), 5.42 (s, 1 H, 15a-H), 5.98 (s, 1 H, 15b-H) ppm. ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 15.8 (C-14), 22.9 (C-13), 31.9 (C-4), 36.9 (C-11), 43.1 (C-3), ca. 58.8 (C-6, overlapping with signals from **11**), 71.5 (C-2), 71.6 (C-5), 76.5 (C-7), 80.5 (C-10), ca. 116.2 (C-12, overlapping), 122.2 (C-15), 144.7 (C-9), 171.8 (C-1, overlapping), 199.6 (C-8, from HMBC experiment) ppm. $C_{15}H_{18}O_6$: calcd. 294.1103; found 294.1095.

Crystallographic Data for Cyclocalopin A (6b): $C_{15}H_{20}O_6$, *M* = 296.32, crystal dimensions 0.30 × 0.30 × 0.43 mm, crystal system hexagonal, space group $P6_522$, unit cell dimensions and volume: *a* = 14.045(3), *b* = 14.045(3), *c* = 27.668(11) Å, *V* = 4726.6(24) $Å^3$, $D_{calcd.}$ = 1.330 Mg/m^3, radiation: Mo-K_α, wavelength λ = 0.71073 Å, *T* = 295(2) K, *F*(000) = 2011, μ = 0.107 mm^{-1}, ω-scan mode, 4992 reflections measured, 2207 unique with R_{int} = 0.0352 and 1485 observed with $I > 2\sigma(I)$, structure solution by SHELXS-86,[14] refinement by SHELXL-93,[15] 209 parameters. *R*1 ($2\sigma I$) = 0.0533, *wR*2 ($2\sigma I$) = 0.1096, *R*1 (all data) = 0.0891, *wR*2 (all data) = 0.1292; goodness of fit 1.121. Largest peaks in the final difference Fourier synthesis: 0.212 and −0.109 e·$Å^{-3}$. CCDC-185725 contains the supplementary crystallographic data

for this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK [Fax: (internat.) + 44-1223/336-033; E-mail: deposit@ccdc.cam.ac.uk].

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