Sarcodonins and Sarcoviolins, Bioactive Polyhydroxy-*p*-terphenyl Pyrazinediol Dioxide Conjugates from Fruiting Bodies of the Basidiomycete *Sarcodon leucopus*

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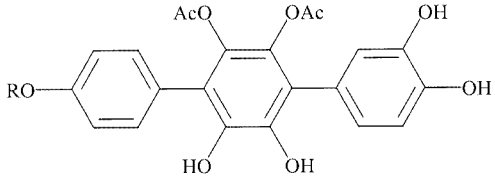
Six new polyhydroxy-*p*-terphenyl pyrazinediol dioxide conjugates (**4**–**9**) related to sarcodonin (**3**) have been isolated from the EtOAc extract of the fruiting bodies of the basidiomycete *Sarcodon leucopus* and we established their structures by spectral analysis and chemical conversions. Three of them, named sarcodonins α (**4**), β (**5**), and γ (**6**), afforded the same peracetate **12** upon acetylation. Compounds **7**, **8**, and **9** gave peracetate **13** and were characterized as the *N*-oxide epimers of **3**–**5**, respectively, and are named, accordingly, episarcodonin, episarcodonin α, and episarcodonin β. From the EtOH extract, we obtained a mixture of two violet pigments. Chemical and spectroscopic data allowed their structures to be established as the *p*-terphenyl *ortho*-quinones related to the sarcodonins, namely sarcoviolin α (**10**) and episarcoviolin α (**11**). Compounds **3**, **4**, **6**, and **7** and the mixture of **10** and **11** were found to be active in assays against tumor cell cultures.

Introduction

The basidiomycete genus *Sarcodon* rarely has been the subject of chemical studies. A number of cyathane diterpenoids have been obtained from the inedible fruiting bodies of *S. scabrosus*, including scabronines A−G, which are stimulators of nerve growth factor (NGF) synthesis.[1−3] From the fruiting bodies of *S. leucopus* (*S. laevigatum* = *Hydnum laevigatum*) (Pers.) (Hydnaceae), we previously isolated two polyhydroxy-*p*-terphenyls (**1** and **2**),[4] one of them endowed with high cytotoxic activity against KB tumor cells,[5] and, more recently, sarcodonin (**3**), an unique polyhydroxy-*p*-terphenyl pyrazinediol dioxide conjugate that is moderately active against KB and P-388 cells.[5] Compounds with a *p*-terphenyl core are relatively rare among natural products and are restricted essentially to the Kingdom of Fungi.[6] Until recently, fully aromatic polyhydroxy-*p*-terphenyls have been reported less frequently than their related terphenylquinones, which are well-known as fungal pigments. Recent additions to this group include promising bioactive metabolites such as the potent immunoglobulin *E* antibody suppressant terprenin,[7,8] obtained from cultures of *Aspergillus candidus*, the anti-insect and cytotoxic compounds obtained from the sclerotia of *A. arenarius*[9] and *Penicillium raistrickii*,[10] as well as the benzofuranoid terphenyls Bl-I to Bl-V from fruiting bodies of *Boletopsis leucomelas* that possess lipoxygenase inhibitory activity,[11] and leucomentins 2 and 4, which have been identified in *Paxillus panuoides* and exhibiting strong inhibitory activity against lipid peroxidation in rat liver microsomes.[12] These encouraging results prompted us to carry out further work on *Sarcodon leucopus* and we report here the isolation of six new sarcodonins from the ethyl acetate extract. Three of these compounds (**4**−**6**, sarcodonins α, β, and γ, respectively) differ from **3** in the acetylation pattern of the hydroxyl groups, and the remaining ones (**7**−**9**) are 1β-epimers of metabolites **3**−**5**. The deep-violet ethanol extract contained two terphenylquinone pigments related to sarcodonins, namely sarcoviolin α (**10**) and episarcoviolin α (**11**). Their structures were established on the basis of spectroscopic and chemical evidences, as detailed below.



1 R = H
2 R = Ac

Results and Discussion

As previously reported, studying the fruiting bodies of *Sarcodon leucopus* is complicated by the observed degradation of the main metabolites during extraction and chro-

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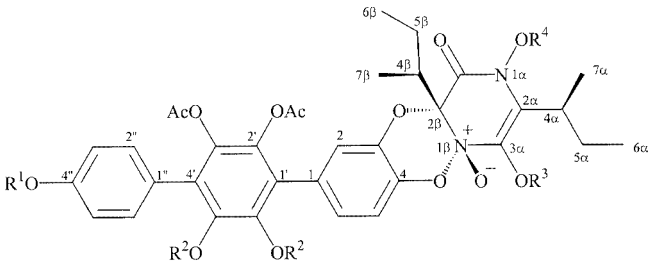
matography on a column of silica gel. Chromatography on acetylated polyamide and Diol Si gel has allowed the isolation of **3**, which has been studied mainly as its more-stable peracetate **12**.[5] During our present reinvestigation of the ethyl acetate extract, we isolated the components, without preliminary acetylation, by flash-column chromatography under nitrogen pressure using polyamide and Diol Si gel as stationary phases.



3 R^1 = Ac R^2 = H R^3 = H R^4 = H
4 R^1 = H R^2 = H R^3 = H R^4 = H
5 R^1 = H R^2 = Ac R^3 = H R^4 = H
6 R^1 = Ac R^2 = H R^3 = Ac R^4 = H
12 R^1 = Ac R^2 = Ac R^3 = Ac R^4 = Ac

Following TLC analysis (UV light and chromogenic spray reagents), three main fractions (B, C, and D; see Exp. Sect.) were obtained in the range of polarity of **3** and appeared to be similar to **3** on the basis of their UV activity and chromogenic reactions. Fraction B, which was purified further using Diol Si gel, afforded sarcodonin γ (**6**), which in the FAB mass spectrum showed an $[M + H]^+$ peak at m/z = 765 and an $[M + Na]^+$ peak at m/z = 787. The ^1H and ^{13}C NMR spectra (see Tables 1 and 2) suggested a close correlation with **3**. In particular, the typical low-field signals of the *p*-terphenyl core and the majority of the signals of the side chains in the aliphatic moiety closely resemble the corresponding ones in the spectrum of **3**.[5] The main differences are a singlet at δ = 2.18 ppm in the ^1H NMR spectrum and peaks at δ = 169.1 and 19.4 ppm in the ^{13}C NMR spectrum, which we attribute to an additional acetyl group, the presence of which was confirmed by the molecular weight (m/z = 764) that is 42 amu higher than that of **3**. This information strongly indicates an acetyl derivative of sarcodonin. Acetylation of **6** afforded the known peracetate **12**, which, thus, confirmed that both compounds have identical basic structures. Comparison of the chemical shifts for the acetyl groups in the ^1H NMR spectrum of **6** with those observed for **3** and **12** indicated that three OAc groups were located at the same positions as those in **3**, namely C-2′, C-3′ (resonating at δ = 1.97 and 1.98 ppm, typical values for acetyl units in the central ring), and C-4′′ (resonating at δ = 2.33 ppm, a typical value for an acetyl unit in the outer ring).[4,5] The additional OAc group appears as a sharp singlet at δ = 2.18 ppm, which is similar in value and shape to the signal of the C-3α OAc unit in **12** and is different from the broad signal of the N-1α OAc unit in the same peracetate (δ = 2.23 ppm). The location of this OAc group at C-3α was corroborated by the observation that in the ^{13}C NMR spectrum only the signals assigned to C-3α (δ = 164.5 ppm) and C-4α (δ = 36.0 ppm) in **6** (see Table 2) show significant variations with respect to those in **3** ($\Delta\delta = \delta_{\mathbf{6}} - \delta_{\mathbf{3}}$ = 5.5 and 2.4 ppm, respectively), and they have values very similar to those observed for the corresponding carbon atoms in **12**.

Table 1. ^1H NMR spectroscopic data (500 MHz in $CDCl_3$ solution) of compounds **4**−**9**

Position	m (*J*)	**4**	**5**	**6**	**7**	**8**	**9**
2	d (2.0)	7.06	7.07	7.12	7.11	7.11	6.96
5	d (8.5)	7.13	7.13	7.16	7.15	7.15	7.13
6	dd (8.0, 2.0)	7.03	7.06	7.05	7.02	7.03	7.01
2′′, 6′′	d (N = 8.5)[a]	7.19	7.18	7.41	7.38	7.21	7.17
3′′, 5′′	d (N = 8.5)[a]	6.84	6.86	7.19	7.18	6.87	6.85
2′ $COCH_3$	s	1.94[b]	1.99[b]	1.97[b]	1.94[b]	1.95[b]	1.99
3′ $COCH_3$	s	1.97[b]	2.01[b]	1.98[b]	1.97[b]	1.97[b]	1.99
5′ $COCH_3$	s		2.01[b]				1.99
6′ $COCH_3$	s		1.99[b]				1.99
4′′ $COCH_3$	s			2.33	2.32		
4α	m	3.03	3.07	3.04	2.48	2.48	2.48
5α	m	1.39	1.42	1.46	1.39	1.43	1.38
5′α	m	1.61	1.62	1.64	1.61	1.59	1.61
6α	t (7.5)	0.89	0.89	0.92	0.88	0.89	0.89
7α	d (7.0)	1.07	1.10	1.12	1.09	1.10	1.10
4β	m	2.47	2.51	2.57	2.48	2.48	2.48
5β	m	1.39	1.33	1.39	1.44	1.36	1.41
5′β	m	1.81	1.82	1.86	1.84	1.82	1.80
6β	t (7.5)	0.99	1.04	1.01	1.02	1.02	1.02
7β	d (7.5)	1.28	1.29	1.29	1.32	1.33	1.32
3α $COCH_3$	s			2.18			

[a] AA′BB′ system ($N = J_{AB} + J_{AB'}$ with $J_{AB'} < 0.5$ Hz). [b] Values with identical superscripts within each column may be interchanged.

Preliminary ^1H NMR spectroscopic analysis of fraction D suggested that it was a mixture of compounds closely related to **3**. This situation was confirmed by acetylation, which afforded two peracetates. Following preparative acetylation and purification, one of them was readily identified as **12** by MS and NMR spectroscopic analysis, while the other one appeared as a related, but different, compound, **13**. Its FAB-MS contained a peak for $[M + H]^+$ at m/z = 891, which is identical to that of **12**, and its ^1H NMR spectrum closely resembles that of **12**, except that it displays several broadened peaks (see Exp. Sect. and Figure 1 in Supporting Information). At this point, flash chromatography of fraction D, in which the subfractions were not pooled on the basis of TLC profiles, but according to their ^1H NMR spectra and micro-acetylation, gave pure samples of **4** and **8**. Upon acetylation, these compounds yielded peracetates **12** and **13**, respectively.

Compound **4** showed a FAB-MS peak for $[M + H]^+$ at m/z = 681, which is 42 amu less than that observed for sarcodonin. Its ^1H NMR spectrum closely resembles that of **3**, with the main differences being the absence of the 4′′-OAc peak at δ = 2.30 ppm and a marked upfield shift of the H-3′′/H-5′′ signals (see Table 1). The ^{13}C NMR spectrum confirmed the similarity with sarcodonin and it clearly

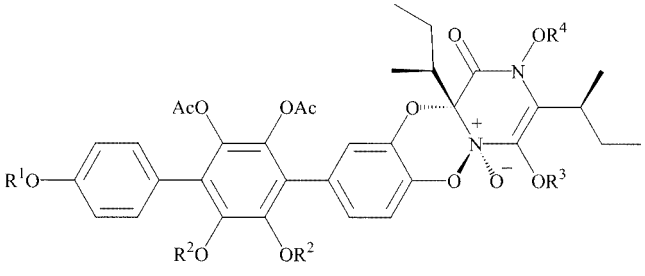
Table 2. ^{13}C NMR spectroscopic data (125 MHz in $CDCl_3$ solution) of compounds **4**−**9**

Position	m	**4**[a]	**5**	**6**	**7**	**8**	**9**
1	s	128.4	127.9	128.5	128.3	128.4	127.9
2	d	119.1	118.8	119.0	119.7	119.6	118.6
3	s	140.9[a]	141.1[a]	141.0[a]	141.6[a]	141.4[a]	141.1[a]
4	s	140.8[a]	140.8[a]	140.8[a]	141.1[a]	141.0[a]	140.8[a]
5	d	116.9	116.8	117.1	117.2	117.0	116.8
6	d	125.2	124.9	125.1	125.6	125.6	125.1
1′	s	122.5	123.1	121.5	121.7	122.4	123.1
2′	s	139.8[b]	139.0[b]	139.6[b]	139.7[b]	139.8[b]	139.0[b]
3′	s	139.8[b]	139.5[b]	139.4[b]	139.4[b]	139.5[b]	139.5[b]
4′	s	120.8	121.1	121.2	121.2	121.0	121.1
5′	s	133.6[c]	139.5[b]	133.7[c]	133.9[c]	133.6[c]	139.5[b]
6′	s	133.9[c]	139.0[b]	133.8[c]	133.7[c]	133.7[c]	139.0[b]
1′′	s	122.7	128.6	128.9	128.6	122.5	128.6
2′′, 6′′	d	131.2	131.0	131.3	131.2	131.2	131.0
3′′, 5′′	d	115.9	115.3	122.0	122.2	116.0	115.3
4′′	s	155.9	155.9	150.8	150.8	156.2	155.9
2′ *C*OCH$_3$	s	169.7[d]	167.7	168.6[d]	168.8[d]	169.6[d]	167.7
3′ *C*OCH$_3$	s	169.9[d]	167.7	168.7[d]	169.6[d]	170.2[d]	167.7
5′ *C*OCH$_3$	s		167.7				167.7
6′ *C*OCH$_3$	s		167.7				167.7
2′ CO*C*H$_3$	q	20.1	20.0	20.1	20.2[e]	20.1[e]	20.0
3′ CO*C*H$_3$	q	20.1	20.0	20.1	19.9[e]	20.2[e]	20.0
5′ CO*C*H$_3$	q		20.0				20.0
6′ CO*C*H$_3$	q		20.0				20.0
4′′ *C*OCH$_3$	s			169.3	170.1		
4′′ CO*C*H$_3$	q			21.1	21.1		
2α	s	166.2	166.2	165.8	165.8	166.1	165.8
3α	s	159.2	158.7	164.5	159.0	158.8	158.7
4α	d	33.7	33.4	36.0	37.7	37. 8	37.7
5α	t	25.8	25.7	26.3	25.7	25.8	25.7
6α	q	12.0	11.5	12.0	11.4	11.4	11.3
7α	q	16.3	16.5	16.7	17.4	17.1	16.5
2β	s	91.4	91.0	91.5	90.6	90.7	91.0
3β	s	159.8	159.4	159.3	159.6	159.7	159.4
4β	d	41.9	41.7	41.7	41.8	42.0	42.0
5β	t	23.3	23.1	23.3	23.0	23.1	23.1
6β	q	12.3	12.3	12.3	12.5	12.5	12.3
7β	q	13.9	13.8	13.8	13.8	13.9	13.8
3α *C*OCH$_3$	s			169.1			
3α CO*C*H$_3$	q			19.4			

[a] Values with identical superscripts within each column may be interchanged.

indicates the absence of the acetyl unit at C-4′′ (see Table 2). Thus, structure **4** was assigned to sarcodonin α.

Determining the structure of compound **8** was not so easy. Its FAB mass spectrum showed an $[M + H]^+$ peak at $m/z = 681$, which indicates that **8** is an isomer of **4**. This situation was confirmed by spectral analysis (see Tables 1 and 2). The proton NMR spectrum at 500 MHz was almost superimposable with that of **4**, with the only signal showing a $\Delta\delta$ ($\delta_4 - \delta_8$) ≥ 0.5 ppm being that at $\delta = 2.48$ ppm, which is easy to attribute to H-4α on the basis of a careful analysis aided by a COSY 2D NMR spectroscopy experiment. Analogously, the carbon NMR spectrum shows chemical shift differences > 1 ppm only for the signal assigned to C-4α. These data clearly indicate that the *p*-terphenyl moiety is identical in both compounds and the structural modifications in **8** should concern the aliphatic portion. The large signal broadening observed in the ^1H NMR spectrum of peracetate **13** did not allow us to obtain satisfactory 2D NMR spectra, in particular ROESY spectra. This problem precluded the possibility of determining the structure of **13** on the basis of integrating 2D NMR spectroscopic data with Molecular Mechanics study, as had been carried out previously for **12** to overcome the difficulties in obtaining crystals of a sarcodonin derivative suitable for X-ray analysis.[5] During this study, we observed fortuitously that a sample of **4** that had been maintained in a refrigerator (−4 °C) contained significant amounts of **8**. Analysis of a sample of **8** stored under the same conditions revealed that the reverse process did not occur. In subsequent experiments, pure samples of both compounds, dissolved separately in benzene, were heated under reflux for 5 h. ^1H NMR spectra showed that **8** was unmodified, whereas **4** had been completely converted into **8**. In parallel experiments, peracetates **12** and **13** were recovered unchanged. To establish if acetylation of both hydroxyl groups in the aliphatic portion was required to block thermal conversion, we also submitted sarcodonin γ (**6**) to reflux in benzene for 5 h. Recovery of unmodified **6** proved that acetylation at only position C-3α results in thermal isomerization being avoided. To acquire more information on the positions of the hydroxyl groups, the ^1H NMR spectra of both **4** and **8** were recorded in [D_6]acetone so that the signals of protons on heteroatoms could be observed (see Exp. Sect.). In both compounds, the signals of phenol hydroxyl groups exhibited only negligible differences. On the basis of previously reported data,[5] we attribute the peaks at $\delta = 7.68$ (1 H) and 7.71 (1 H) ppm in the ^1H NMR spectrum of **4**, and at $\delta = 7.68$ ppm (2 H) in that of **8**, to the C-5′ and C-6′ hydroxyl groups. For both compounds, the signal at $\delta = 8.49$ ppm (1 H) was assigned to the C-4′′ hydroxyl group. The hydroxyl groups on the diazine ring appear at $\delta = 9.57$ and 10.39 ppm for **4** and $\delta = 9.08$ and 10.20 ppm for **8**. We assigned the lower-field signal in **4** to the N−OH group (N-1α) and, consequently, the other one to the C-3α hydroxyl group. Interestingly, isomerization affects the N−OH signal less ($\Delta\delta = 0.19$ ppm) than it does the C-3α-OH signal ($\Delta\delta = 0.49$ ppm). To corroborate these assignments, we obtained a ^1H NMR spectrum of **6** in [D_6]acetone (see Exp. Sect.); it shows a single



7 R^1 = Ac R^2 = H R^3 = H R^4 = H
8 R^1 = H R^2 = H R^3 = H R^4 = H
9 R^1 = H R^2 = Ac R^3 = H R^4 = H
13 R^1 = Ac R^2 = Ac R^3 = Ac R^4 = Ac

low-field signal at δ = 10.33 ppm, which we attribute to the N−OH group. Thus, we confirmed that only a slight modification of the diazine ring occurs in **8** with respect to **4**.

At this point, we considered a hypothesis that is compatible with the available spectral and chemical data. Since the tautomers quickly interconvert and generally cannot be separated (in particular, fast tautomeric processes are typical of protons on heteroatoms[13]), we ruled out tautomerism involving the hydroxamic acid function (N-1α, C-3β), which could convert them into an *N*-oxide (**4a**, as reported in Supporting Information, Figure 2). Further support came from the slight difference, mentioned above, between the N-OH chemical shifts of **4** and **8**, which indicates, at least, that the N-1α/C-3β portion was not modified. We also discarded a slow prototropism for the enol function (C-2α, C-3α), which, in principle, could change into a keto form (**4b**, as reported in Supporting Information, Figure 2). In fact, we did not observe in both the ^{1}H and ^{13}C spectra of **8** signals attributable to the presence of a C-2α methine unit and the ^{13}C NMR spectral chemical shift for C-3α was almost identical in both compounds. In addition, the keto form should afford a peracetate accounting for six OAc functions (instead of the seven deducible from the FAB-MS of **13**). Turning to stereoisomerism, one should consider, in principle, all the structures generated by inversion at the stereogenic 1β, 2β, 4α, and 4β centers, but inversion at the three last centers is hard to justify by a reasonable mechanism which is completely blocked upon acetylation at C-3α. At this point, we investigated the stereostructures **4** and **8** (the latter generated by inversion at 1β in **4**) by a Molecular Mechanics study, carried out using the Molecular Dynamics methodology and assuming the obtained energy minima as the preferred conformations in solution. Examination of stereomodels (see Supporting Information, Figures 3 and 4) showed that an almost-planar five-membered cycle is present in **4**, as a result of an intramolecular hydrogen bond, with the distance between the hydroxyl proton and the oxygen atom of the *N*-oxide function (OH···ON) being 2.32 Å. The situation in **8** is different: it lacks the planar cycle and has an OH···ON distance of 3.36 Å. This finding explains satisfactorily why the bridge proton in **4** is more deshielded than it is in **8**. A possible mechanism to explain the role of the bridge proton in the *N*-epimerisation of **4** to **8** could resemble the Cope β-elimination[14] of *N*-oxides, followed by a stereoselective retro-Cope[15] of the unstable intermediate, to give the isomer **8** as the final stable product. In this regard, it is worth noting that some retro-Cope cyclizations occur with high stereoselectivity.[16] On the basis of the discussion above, we consider structure **8** to be established and, consequently, we have assigned this compound the trivial name episarcodonin α.

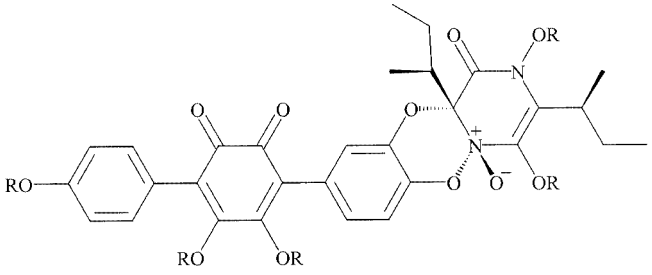
To establish whether **8** preexists in the mushroom or is formed during workup, we carried out a rapid extraction with EtOAc of a freshly collected sample of *S. leucopus*. The extract was immediately fractionated on polyamide under nitrogen and the subfractions containing **4** as the main component (TLC) were submitted to acetylation, which afforded a mixture of peracetates **12** and **13**. Therefore, we feel that **8** is normally present in the mushroom.

To investigate the causes of signal broadening observed in both the ^{1}H and ^{13}C NMR spectra of peracetate **13**, we obtained ^{1}H NMR spectra at various temperatures. Low-temperature spectra were run in $CDCl_3$ starting at 25 °C and repeating the scans at 10, −10, −25, and −50 °C. At room temperature, all the signals in the high-field region of the NMR spectrum appeared broadened, with the exception of two sharp singlets at δ = 1.96 (6 H) and 2.31 (3 H) ppm, which we assign, respectively, to the isochronous OAc groups in C-3′ and C-5′ and to the C-4′′−OAc group. Additionally, the aromatic signals attributable to 2-H, 5-H, and 6-H show severe broadening, but the AA′BB′ system at lower fields does not. Lowering the temperature, each broad signal became progressively sharper and at the same time split into two signals. Thus, for example, the original broad signal centered at δ = 0.89 ppm (6α-Me) at −25 °C (see Figure 5 in Supporting Information) gives rise to two peaks, which at −50 °C are resolved into two triplets at δ = 0.85 and 0.91 ppm. At low temperatures, several signals due to OAc groups, which are relatively sharp but low in intensity, were observed in the range δ = 1.99−2.02 ppm. This evidence strongly suggests that **13** undergoes a conformational process at room temperature that is slow in the NMR spectroscopic time scale. The separate signals that appearing at low temperatures may be attributed to at least two main conformers. This slow conformational motion does not involve the outer ring and the two OAc groups far from the aliphatic portion. Interestingly, a broadening was observed at −50 °C for the signals of the AA′BB′ system, which indicates that slower rotation of the outer ring occurs at this temperature. These assumptions were reinforced by experiments at high temperatures (25, 40, 80, and 120 °C) that we carried out in [D_5]nitrobenzene. In this solvent, at the initial temperature, extensive broadening of the signals complicates the interpretation of the ^{1}H NMR spectra. Raising the temperature progressively, we observed an appreciable sharpening of the peaks, in particular for the acetate signal at δ = 2.26 ppm that we attribute to the N−OAc group. All these data indicate a different conformational mobility of peracetate **13** with respect to **12**, since no discernible broadening is observed in the NMR spectra of the latter. Examination of the Molecular Mechanics stereomodels of both peracetates (available from previous work;[5] see Supporting Information, Figures 6 and 7) showed that in stereostructure **13** the "β" side chain is closer to the acetoxy group in C-2′ than it is in the stereostructure **12**. In particular, the measured distance between 7β-Me and 2′-OAc is 3.70 Å in **13** and 8.03 Å in **12**. This finding suggests that hindered mobility in **13** is a possible reason for the observed broadening in the NMR spectra.

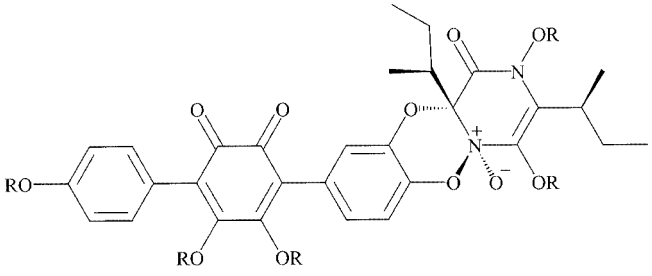
Repeated chromatography of fraction C on Diol Si gel under nitrogen pressure allowed the isolation of four metabolites (**3**, **5**, **7**, and **9**), which we subjected to FAB MS and ^{1}H and ^{13}C NMR spectroscopic analysis and acetylation. Spectral analysis allowed the immediate identification of **3** as the known sarcodonin, which, of course, afforded per-

acetate **12** upon acetylation. The same peracetate was obtained from compound **5**, whose FAB-MS indicated a molecular weight of 764 amu. ^{1}H and ^{13}C NMR spectra (see Tables 1 and 2) supported the analogy with sarcodonin and suggested **5** is a tetraacetate having the same skeleton as sarcodonin. The main differences in the NMR spectra, with respect to **3**, were observed for the *p*-terphenyl core. The signals in the ^{1}H NMR spectrum that are due to the AA′BB′ system of ring C-1′′−C-6′′ were shifted to higher fields with respect to **3** and close to the values observed for **4**. In addition, the signals of the acetyl groups at δ = 1.99 (6H) and δ = 2.01 (6H) ppm strongly suggest the lack of an OAc group at C-4′′ and the presence of a fully acetylated central ring. ^{13}C NMR spectroscopic analysis corroborated these assumptions and allowed us to assign structure **5** to sarcodonin β. Compounds **7** and **9** display ^{1}H and ^{13}C NMR spectra (see Tables 1 and 2) similar to those of **3** and **5**, respectively, but show the same Δδ at position 4α observed for **8** with respect to **4**. Upon acetylation, both compounds afforded peracetate **13**. On the basis of FAB-MS, NMR spectroscopic data, and acetylation, **7** and **9** were established as the 1β-epimers of **3** and **5** and, accordingly, are named episarcodonin (**7**) and episarcodonin β (**9**).

When the residue from the ethyl acetate extract of fruiting bodies of *Sarcodon leucopus* was extracted with ethanol, a deep-violet solution was obtained, which suggested the presence of terphenylquinone pigments related to the quinones previously isolated from basidiomycetes.[6] Since the pigments were adsorbed strongly on the polyamide stationary phase, for its purification we resorted to a chromatographic protocol based on combining the use of Diol Si gel and Sephadex LH-20 as stationary phases (see Exp. Sect.). This process yielded a violet pigment that gives a dark-green spot on silica gel TLC. Preliminary ^{1}H NMR spectroscopic analysis indicated that it is a mixture of two strictly related compounds, but preparative column chromatography did not allow their separation and so we carried out spectral analysis on the whole mixture. ^{1}H and ^{13}C NMR spectra (see Exp. Sect.) show marked analogies with those of the sarcodonins, which indicates the presence of a terphenyl core and the same aliphatic portion. Nevertheless, the lack of acetate signals and the presence of a new carbonyl peak at δ = 176.1 ppm (C-2′, C3′) is evident in the ^{13}C NMR spectrum, which suggests a quinone structure related to a deacetylated sarcodonin. This assignment was corroborated by IR spectroscopy bands at 1646 and 1632 cm^{-1}.[17] Many signals in the ^{13}C NMR spectrum appear doubled, which indicates a mixture of two very similar compounds. The ^{1}H NMR spectrum contains two clearly separated signals at δ = 2.98 and 2.55 ppm, with the former attributed to the 4α-H signal of **10** and the latter to the overlapped signals of 4β-H of **10** and 4α-H/4β-H of **11**. These assignments were based on the features we had observed for the epimeric pairs of sarcodonins, whose ^{1}H NMR spectra showed separate signals only for 4α-H, with a value of $\Delta\delta$ ($\delta_4 - \delta_8$) = ca. 0.55 ppm (see Table 1). On the basis of integrations, we estimated the mixture **10/11** to be in an approximate ratio of 2:1. The FAB-MS spectrum of the mixture did not contain pseudo-molecular ion peaks for the expected quinone structures of **10** or **11**, but we rationalized the two neat peaks at *m*/*z* = 617 and 633 as the Na and K adducts for a mixture of isomers having molecular weights of 594, which is compatible with a quinone structure. A negative-ion ESI mass spectrum of the mixture was also recorded to give the $[M - H]^-$ molecular ion peak at *m*/*z* = 593. To corroborate this evidence, the mixture of **10** and **11** was acetylated to afford two peracetates, **14** and **15**. These compounds also could not be separated, but, nevertheless, the FAB-MS of the whole mixture gave the expected $[M + H]^+$ ion at 805 amu. It is worth noting here that previously reported terphenyl metabolites having a *para*-quinone core, like the flavomentins A−D from *Paxillus atrotomentosus* and *P. panuoides*, are orange pigments, while *ortho*-quinones, like the spiromentins A−D[18] from *P. atrotomentosus*, or the hydroxylated phlebiarubrone derivatives from *Phlebia strigosozonata*, are violet pigments.[19] The λ_{max} values in the UV-Vis spectrum of the pigment are very close to those of spiromentins A−D and phlebiarubrone derivatives.[19] All these data strongly suggest that the structures of the constituents in the mixture of epimeric *ortho*-quinones are related to sarcodonins and episarcodonins, namely sarcoviolin α (**10**) and episarcoviolin α (**11**). As further confirmation, the mixture of **10** and **11** was subjected to reductive acetylation with Zn/Ac_2O, according to a standard procedure for fungal terphenylquinones.[20] This procedure afforded a mixture of the "leuco" peracetates **12** and **13**, which were identified by TLC and ^{1}H NMR spectroscopic analysis.



10 R = H
14 R = Ac



11 R = H
15 R = Ac

Compounds **3** (sarcodonin), **4** (sarcodonin α), **6** (sarcodonin γ), and **7** (episarcodonin) and the mixture of **10** and **11** (sarcoviolins) were tested in the three-cell line panel

High Throughput PreScreen (one-dose primary anticancer assay) carried out at National Cancer Institute (Bethesda, USA). Fully aromatic terphenyls proved cytotoxic at a concentration of 5×10^{-5} M against NCI-H460 (Lung), MCF7 (Breast), and SF-268 (CNS). In particular, **3**, **6**, and **7** show the highest cytotoxicity towards SF-268 cells, with 96, 93, and 95% of cells killed, respectively. Sarcoviolins significantly reduced the growth of all cell lines at 10^{-4} M (MCF7 totally blocked). A mixture of sarcodonins was tested for *anti*-HIV activity at the Virology Laboratory of London Hospital (London, UK) and showed $EC_{50} = 5$ μg/mL (the concentration at which the viral antigen p24 or progeny virus in infected cell cultures is reduced by 50%). In conclusion, sarcodonins and sarcoviolins are a new group of polyhydroxy-*p*-terphenyl pyrazinediol dioxide conjugates having an unusual *N*-oxide function and promising biological activity.

Experimental Section

General: High-resolution positive-ion fast atom bombardment mass spectra (HR FAB-MS) were recorded on a Fisons ZAB S2E instrument using 3-nitrobenzyl alcohol (NBA) as the matrix. Negative-ion electrospray ionization mass spectra (ESI-MS) were recorded on an Agilent 1100 Series ESI/MSD spectrometer, using a capillary voltage of 3.5 kV, a cone voltage of 100 V, a vaporizer temperature of 300 °C, and a carrier gas flow (nitrogen) of 10 L/min. IR spectra were measured on a Perkin−Elmer 684 spectrophotometer. UV spectra were recorded using a Hewlett−Packard 8452 spectrophotometer. Optical rotations were measured at 25 °C on a Jasco 135 instrument. NMR spectra were run on a Varian Unity Inova spectrometer operating at 499.9 (^{1}H) and 125.7 MHz (^{13}C) and equipped with a gradient-enhanced, reverse-detection probe. Chemical shifts (δ) are indirectly referred to TMS using solvent signals. Broadband-decoupled (Waltz) NMR spectra were run for ^{1}H and ^{13}C nuclei. All NMR spectroscopy experiments, including two-dimensional spectra, i.e., COSY, HSQC, and ROESY, were performed using software supplied by the manufacturers and acquired at constant temperature (25 °C). Variable-temperature ^{1}H NMR spectroscopy experiments were carried out by obtaining a sequence of ^{1}H NMR spectra of peracetate **13** in $CDCl_3$ at 10, −10, −25, and −50 °C and in $C_6D_5NO_2$ at 25, 40, 80, and 120 °C. Molecular Mechanics studies were performed using the HyperChem program (release 5).[21] TLC was carried out using pre-coated silica gel F254 plates (Merck). Freshly prepared $FeCl_3$ solution, as well as phosphomolybdic acid, was used as a spray reagent. Sil G-25 UV_{254} silica gel plates (Macherey−Nagel) were used in the TLC analysis of sarcoviolins **10** and **11**. Column chromatography employed Polyamid CC-6 (Macherey−Nagel), Sephadex LH-20 (Sigma−Aldrich), LiChroprep Si-60, and LiChroprep DIOL 25−40 (Merck) as stationary phases.

Fungal Material: Fruiting bodies of *Sarcodon leucopus* were collected on the slopes of Mount Etna near Catania in October 2001 and cut into small pieces. A small portion was immediately extracted with EtOAc, whereas the rest was freeze-dried and stored in a freezer under a nitrogen atmosphere. The mushroom was identified by Dr. M. Dollo, Associazione Micologica Bresadola (Catania). A voucher specimen (CAT.ed) has been deposited in the Mycological Herbarium, Dipartimento di Botanica, Università di Catania (Prof. P. Signorello).

Extraction and Isolation: Freeze-dried fruiting bodies of *S. leucopus* (310 g) were extracted with EtOAc (5 L, 5 h × 2). The residue was then extracted with EtOH (3.5 L, 10 h × 2). The EtOAc extract was dried with Na_2SO_4 and evaporated to dryness to yield the crude material (8.2 g). This material was chromatographed on Polyamid CC-6 eluting with a gradient from 40 to 90% $CHCl_3$ in *n*-hexane and, subsequently, with a gradient from 5 to 20% MeOH in $CHCl_3$. The eluates, which were analyzed by TLC, were pooled into four fractions (A−D). Fraction A (4.9 g) was discarded from further study. Fractions B, C, and D, which were rich in sarcodonin-related constituents, were further subjected to flash chromatography over LiChroprep DIOL under nitrogen pressure. Fraction B (376 mg) was eluted with increasing amounts of CH_2Cl_2 in *n*-hexane (from 70 to 100% CH_2Cl_2), affording three subfractions (B_1−B_3). The main constituent **6** (sarcodonin γ) was eluted in subfraction B_2 (43 mg, 0.52% of the crude EtOAc extract and 0.014% of the dried mushroom weight). Fraction C (1.9 g) was purified by flash chromatography over LiChroprep DIOL eluting with a gradient of EtOAc in hexane (from 40 to 100% EtOAc) to give five subfractions (C_1−C_5). Further flash chromatography of subfraction C_2 (elution: from 80 to 100% CH_2Cl_2 in *n*-hexane followed by 0.5% MeOH/CH_2Cl_2) over LiChroprep DIOL afforded compound **7** (episarcodonin; 127 mg, 1.55% of EtOAc extract and 0.041% of dry weight) and pure **3** (sarcodonin; 89 mg, 1.09% of the crude extract and 0.029% of dry weight). Subfraction C_4 was also eluted under nitrogen pressure on LiChroprep DIOL (elution from 80 to 100% CH_2Cl_2 followed by 1% MeOH/CH_2Cl_2) yielding **9** (episarcodonin β; 43 mg, 0.52% of EtOAc extract and 0.014% of fruiting bodies) and **5** (sarcodonin β; 18 mg, 0.22% of crude extract and 0.006% of dry weight). Fraction D (986 mg) was subjected to flash chromatography on LiChroprep DIOL eluting with increasing amounts of MeOH in CH_2Cl_2 (from 100% CH_2Cl_2 to 5% MeOH). This process afforded two subfractions of **8** (episarcodonin α, 235 mg; 2.87% of EtOAc extract and 0.076% of dry weight) and **4** (sarcodonin α, 202 mg; 2.46% of EtOAc extract and 0.065% of dry weight). The EtOH extract (7.1 g) was subjected to chromatography on LiChroprep DIOL under nitrogen pressure eluting with a gradient from 5 to 10% of MeOH in EtOAc, affording four fractions (E−H). Fraction F (372 mg) was further analyzed by isocratic flash chromatography on Sephadex LH-20 elutiong with acetone, affording an inseparable mixture of two violet pigments, **10** and **11** (19 mg; 0.27% of EtOH extract, 0.006% of dry weight; ratio 2:1).

A minor amount (10 g) of the fresh fruiting bodies was extracted with EtOAc (400 mL, 2 h). After drying with Na_2SO_4, the EtOAc extract was concentrated to give a solid residue (258 mg). The crude extract was purified under nitrogen pressure on polyamide CC-6 eluting with a gradient of $CHCl_3$ in *n*-hexane (from 40% to 90%) followed by MeOH in $CHCl_3$ (from 5 to 20%). This procedure gave five subfractions (A′−D′). Subfraction D′ (31 mg), containing **4** as the main constituent, was acetylated by exposure overnight to vapors of pyridine and acetic anhydride to yield a mixture of two peracetates, **12** and **13**.

Sarcodonin α (4): White amorphous powder. $[\alpha]_D^{25} = -9.9$ (c = 1.22, $CHCl_3$). R_f (TLC) = 0.55 (10% MeOH/CH_2Cl_2). UV (EtOH): λ_{max} (lg ε) = 210 (4.66), 270 (4.24) nm. IR ($CHCl_3$): $\tilde{\nu}$ = 3299, 1771, 1524, 1370, 1273, 1200 cm^{-1}. For ^{1}H and ^{13}C NMR spectral data recorded in $CDCl_3$, see Tables 1 and 2. ^{1}H NMR [499.9 MHz, $(CD_3)_2CO$, 25 °C]: δ = 0.85 (t, J = 7.5 Hz, 3 H, 6α-H), 0.87 (d, J = 7.0 Hz, 3 H, 7α-H), 1.02 (t, J = 7.5 Hz, 3 H, 6β-H), 1.30 (d, J = 7.0 Hz, 3 H, 7β-H), 1.35 (m, 1 H, 5α-H), 1.39 (m, 1 H, 5β-H), 1.63 (m, 1 H, 5′α-H), 1.89 (m, 1 H, 5′β-H), 1.95 and 1.97 (3H each, s, 2′-$COCH_3$, 3′-$COCH_3$), 2.58 (m, 1 H, 4β-H), 3.10 (m, 1 H, 4α-

H), 7.04 (d, J = 2.0 Hz, 1 H, 2-H), 7.06 (dd, J = 8.0, 2.0 Hz, 1 H, 6-H), 7.15 (d, J = 8.0 Hz, 1 H, 5-H), 7.19 and 6.90 (2H each, AA′BB′ system, $J_{AB} + J_{AB'}$ = 8.5 Hz with $J_{AB'} < 0.5$ Hz, 2′′-H, 6′′-H and 3′′-H, 5′′-H), 7.68 and 7.71 (1H each, br. s, 5′-OH, 6′-OH), 8.49 (br. s, 1 H, 4″-OH), 9.57 (br. s, 1 H, 3α-OH), 10.39 (br. s, 1 H, 1α-OH) ppm. HR positive-ion FAB MS: m/z = 681.6719 calcd. for $C_{34}H_{37}N_2O_{13}$ found 681.4992 $[M + H]^+$.

Sarcodonin β (5): White amorphous powder. $[\alpha]_D^{25} = -41.7$ (c = 0.12, $CHCl_3$). R_f (TLC) = 0.54 (5% MeOH/CH_2Cl_2). For ^1H and ^{13}C NMR data, see Tables 1 and 2. HR positive-ion FAB MS: m/z = 765.7463 calcd. for $C_{38}H_{41}N_2O_{15}$ found 765.7213 $[M + H]^+$.

Sarcodonin γ (6): White amorphous solid. $[\alpha]_D^{25} = -11.7$ (c = 0.24, $CHCl_3$). R_f (TLC) = 0.64 (3% MeOH/CH_2Cl_2). For ^1H and ^{13}C NMR spectral data recorded in $CDCl_3$, see Tables 1 and 2. ^1H NMR [499.9 MHz, $(CD_3)_2CO$, 25 °C]: δ = 0.89 (t, J = 7.5 Hz, 3 H, 6α-H), 0.98 (t, J = 7.5 Hz, 3 H, 6β-H), 0.99 (d, J = 7.0 Hz, 3 H, 7α-H), 1.28 (m, 1 H, 5β-H), 1.29 (d, J = 7.0 Hz, 3 H, 7β-H), 1.42 (m, 1 H, 5α-H), 1.61 (m, 1 H, 5′α-H), 1.87 (m, 1 H, 5′β-H), 1.93 and 1.96 (3H each, s, 2′-$COCH_3$, 3′-$COCH_3$), 2.18 (s, 3 H, 3α-$COCH_3$), 2.29 (s, 3 H, 4′′-$COCH_3$), 2.78 (m, 1 H, 4β-H), 3.11 (m, 1 H, 4α-H), 7.04 (d, J = 2.0 Hz, 1 H, 2-H), 7.06 (dd, J = 8.0, 2.0 Hz, 1 H, 6-H), 7.17 (d, J = 8.0 Hz, 1 H, 5-H), 7.19 and 7.38 (2 H each, AA′BB′ system, $J_{AB} + J_{AB'}$ = 8.5 Hz with $J_{AB'} <$ 0.5 Hz, 2′′-H, 6′′-H and 3′′-H, 5′′-H), 7.89 and 7.99 (1H each, br. s, 5′-OH, 6′-OH), 10.33 (br. s, 1 H, 1α-OH) ppm. HR positive-ion FAB MS: m/z = 765.7463 calcd. for $C_{38}H_{41}N_2O_{15}$ found 765.6986 $[M + H]^+$.

Episarcodonin (7): White amorphous solid. $[\alpha]_D^{25} = -5.4$ (c = 0.40, $CHCl_3$). R_f (TLC): 0.53 (5% MeOH/CH_2Cl_2). For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2. HR positive-ion FAB MS: m/z = 723.7091 calcd. for $C_{36}H_{39}N_2O_{14}$ found 723.6994 $[M + H]^+$.

Episarcodonin α (8): White amorphous powder. $[\alpha]_D^{25} = -1.8$ (c = 0.55, $CHCl_3$). R_f (TLC) = 0.55 (10% MeOH/CH_2Cl_2). UV (EtOH): λ_{max} (lg ε) = 210 (4.71), 270 (4.27) nm. IR ($CHCl_3$): $\tilde{\nu}$ = 3322, 1775, 1523, 1370, 1273, 1220 cm^{-1}. For ^1H and ^{13}C NMR spectral data recorded in $CDCl_3$, see Tables 1 and 2. ^1H NMR [499.9 MHz, $(CD_3)_2CO$, 25 °C]: δ = 0.85 (t, J = 7.5 Hz, 3 H, 6α-H), 0.97 (d, J = 7.0 Hz, 3 H, 7α-H), 1.02 (t, J = 7.5 Hz, 3 H, 6β-H), 1.31 (m, 1 H, 5β-H), 1.35 (d, J = 7.0 Hz, 3 H, 7β-H), 1.39 (m, 1 H, 5α-H), 1.53 (m, 1 H, 5′α-H), 1.88 (m, 1 H, 5′β-H), 1.95 and 1.99 (3H each, s, 2′-$COCH_3$, 3′-$COCH_3$), 2.38 (m, 1 H, 4α-H), 2.58 (m, 1 H, 4β-H), 7.09 (dd, J = 8.0, 2.0 Hz, 1 H, 6-H), 7.10 (d, J = 2.0 Hz, 1 H, 2-H), 7.15 (d, J = 8.0 Hz, 1 H, 5-H), 6.90 and 7.19 (2 H each, AA′BB′ system, $J_{AB} + J_{AB'}$ = 8.5 Hz with $J_{AB'} <$ 0.5 Hz, 2′′-H, 6′′-H and 3′′-H, 5′′-H), 7.68 (2 H each, br. s, 5′-OH and 6′-OH), 8.49 (br. s, 1 H, 4′′-OH), 9.08 (br. s, 1 H, 3α-OH), 10.20 (br. s, 1 H, 1α-OH) ppm. HR positive-ion FAB MS: m/z = 681.6719 calcd. for $C_{34}H_{37}N_2O_{13}$ found 681.5443 $[M + H]^+$.

Episarcodonin β (9): White amorphous powder. $[\alpha]_D^{25} = -28.0$ (c = 0.23, $CHCl_3$). R_f (TLC) = 0.55 (5% MeOH/CH_2Cl_2). For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2. HR positive-ion FAB MS: m/z = 765.7463 calcd. for $C_{38}H_{41}N_2O_{15}$ found 765.6432 $[M + H]^+$.

Acetylation of Compounds 3−9: Each compound (10−20 mg) was dissolved in pyridine (2 mL) and acetic anhydride (2 mL). Each solution was stirred for 12 h (room temp.) and, after standard workup, the crude product was purified on LiChroprep Si 60 using a gradient from 1 to 3% of EtOAc in CH_2Cl_2. Compounds **3**−**6** afforded peracetate **12** and compounds **7**−**9** gave peracetate **13**, with ca. 90% yields.

Thermal Treatment of Compounds 4, 6, 8, 12, and 13: Each compound (10−15 mg) was heated under reflux in benzene for 5 h and, after evaporation of the solvent, subjected to ^1H NMR spectroscopic analysis, which indicated the total conversion of **4** into **8**; **6** and **8** were recovered unmodified. Compounds **12** and **13** (10 mg) were not modified after 8 h under reflux.

Peracetate (12): MS and ^1H NMR spectroscopic data are in agreement with those previously reported.[5]

Peracetate (13): White amorphous powder. R_f (TLC) = 0.41 (8% EtOAc/CH_2Cl_2). UV (EtOH): λ_{max} (lg ε) = 206 (4.79), 252 (4.23) nm. IR ($CHCl_3$): $\tilde{\nu}$ = 3029, 2927, 1776, 1517, 1458, 1367 1276, 1192, 1104, 1023, 910 cm^{-1}. ^1H NMR (499.9 MHz, $CDCl_3$, 25 °C): δ = 0.89 (br. t, 3 H, 6α-H), 1.01 (t, J = 7.5 Hz, 3 H, 6β-H), 1.15 (br. d, 3 H, 7α-H), 1.33 (br. d, 3 H, 7β-H), 1.34 (m, 1 H, 5β-H), 1.51 (m, 1 H, 5α-H), 1.70 (m, 1 H, 5′α-H), 1.84 (m, 1 H, 5′β-H), 1.96 (s, 6 H, 3′-$COCH_3$, 5′-$COCH_3$), 2.31 (s, 3 H, 4′′-$COCH_3$), 2.45−2.62 (2 H, br. m, 4α-H and 4β-H), 6.97 (br. s, 1 H, 2-H), 7.01 (br. d, 1 H, 6-H), 7.14 and 7.31 (2H each, AA′BB′ system, 2′′-H, 6′′-H and 3′′-H, 5′′-H), 7.15 (d, J = 8.5 Hz, 1 H, 5-H) ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$, 25 °C): δ = 11.9 (br., C-6β), 14.0 (C-7β), 17.8 (C-7α), 19.1 (3α-CO*C*H$_3$), 19.9 (3′-, 5′-CO*C*H$_3$), 20.0 (2′-, 6′-CO*C*H$_3$), 21.1 (4′′-CO*C*H$_3$), 23.6 (C-5β), 26.6 (C-5α), 38.3 (br., C-4α), 42.7 (br., C-4β), 116.6 (br., C-5), 118.9 (br., C-2), 121.4 (C-3′′, C-5′′), 124.8 (br., C-6), 127.8 (C-1), 128.7 (C-1′′), 129.2 (C-1′), 130.4 (C-4′), 130.7 (C-2′′, C-6′′), 139.3 (C-3′, C-5′), 139.4 (C-2′, C-6′), 140.3 (br., C-3), 150.9 (C-4′′), 167.4 (2′-, 3′-, 5′-, 6′-*C*OCH$_3$), 168.9 (4′′-*C*OCH$_3$) ppm. HR positive-ion FAB MS: m/z = 891.8579 calcd. for $C_{44}H_{47}N_2O_{18}$ found 891.8256 $[M + H]^+$.

Mixture of Sarcoviolin α (10) and Episarcoviolin α (11) (Approximate Ratio = 2:1): Amorphous violet solid. R_f [$(NH_4)_2SO_4$-impregnated silica gel plates] = 0.45 (C_6H_6/HCOOEt/HCOOH = 12:4:2), green spot. UV (MeOH): λ_{max} (lg ε) = 290 (4.08), 350 (3.52), 540 (2.71) nm. IR (KBr): $\tilde{\nu}$ = 3476, 2924, 2682, 1646, 1632, 1508, 1310 cm^{-1}. ^1H NMR (500 MHz, CD_3OD, 25 °C, number of hydrogen atoms not reported): δ = 0.84 (t, J = 7.5 Hz, 6α-H), 0.92 (t, J = 7.5 Hz, 6β-H), 1.02 (d, J = 7.0 Hz, 7α−H), 1.33 (d, J = 7.0 Hz, 7β-H), 1.42 (m, 5β-H), 1.44 (m, 5α-H), 1.56 (m, 5′α-H), 1.85 (m, 5′β-H), 2.55 (m, 4β-H in **10** and 4α-H + 4β-H in **11**), 2.98 (br. m, 4α-H in **10**), 6.77 and 7.31 (AA′BB′ system, $J_{AB} + J_{AB'}$ = 8.5 Hz, 2′′-H, 6′′-H and 3′′-H, 5′′-H), 6.99 (dd, J = 8.0 and 2.0 Hz 6-H), 7.26 (d, J = 2.0 Hz, 2-H), 7.28 (d, J = 8.0 Hz, 5-H) ppm. ^{13}C NMR (125.7 MHz, CD_3OD, 25 °C): δ = 11.9 and 12.4 (C-6α), 12.8 and 12.9 (C-6β), 14.4 (C-7β), 16.3 and 17.3 (C-7α), 24.5 (C-5α), 27.6 and 27.7 (C-5β), 34.7 and 38.9 (C-4α), 42.9 and 43.3 (C-4β), 92.4 and 92.9 (C-2β), 115.5 (C-3′′, C-5′′), 116.7 (C-5), 118.7 (C-2), 120.4 (C-4′), 121.3 (C-1′), 123.1 (C-1′′), 126.9 (C-6), 127.5 (C-1), 132.6 (C-5′, C-6′), 132.9 (C-2′′, C-6′′), 141.2 and 141.5 (C-4), 141.9 (C-3), 157.6 and 158.0 (C-4′′), 159.2 (C-3α), 161.2 (C-3β), 168.7 (C-2α), 176.1 (C-2′, C-3′) ppm. HR positive-ion FAB MS: m/z = 617.5560 calcd. for $C_{30}H_{30}N_2NaO_{11}$ found 617.4931 $[M + Na]^+$, 633.6641 calcd. for $C_{30}H_{30}KN_2O_{11}$ found 633.4995 $[M + K]^+$. Negative-ion ESI-MS: m/z = 593.5688 calcd. for $C_{30}H_{29}N_2O_{11}$ 593.2 found $C_{30}H_{29}N_2O_{11}$.

Acetylation of the Mixture of 10 and 11: A mixture of compounds **10** and **11** (10 mg) was acetylated following the procedure cited above for compounds **3**−**9**. This procedure gave a mixture of two peracetates, **14** and **15**. Positive-ion FAB MS (mixture of **14** and **15**): m/z = 805 $[M + H]^+$.

Reductive Acetylation: A mixture of compounds **10** and **11** (18 mg) was subjected to reductive acetylation with Zn/Ac_2O (250 mg/1.2 mL) in presence of pyridine (0.4 mL). The solution was stirred for 2 h (room temp.) and, after standard workup, the mixture was purified on LiChroprep Si 60 using a gradient from 15 to 50% of CH_2Cl_2 in hexane to afford peracetate **12** (14 mg) and **13** (6 mg), which were identified through TLC and ^1H NMR analyses.

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[1] T. Ohta, T. Kita, N. Kobayashi, Y. Obara, N. Nakahata, Y. Ohizumi, Y. Takaya, Y. Oshima, *Tetrahedron Lett.* **1998**, *39*, 6229−6232.
[2] T. Kita, Y. Takaya, Y. Oshima, T. Ohta, K. Aizawa, T. Hirano, T. Inakuma, *Tetrahedron* **1998**, *54*, 11877−11886.
[3] Y. Obara, N. Nakahata, T. Kita, Y. Takaya, H. Kobayashi, S. Hosoi, F. Kiuchi, T. Ohta, Y. Oshima, Y. Ohizumi, *Eur. J. Pharm.* **1999**, *370*, 79−84.
[4] C. Tringali, M. Piattelli, C. Geraci, G. Nicolosi, C. Rocco, *Can. J. Chem.* **1987**, *65*, 2369−2372.
[5] C. Geraci, P. Neri, C. Paternò, C. Rocco, C. Tringali, *J. Nat. Prod.* **2000**, *63*, 347−351.
[6] V. Calì, C. Spatafora, C. Tringali, in: *Studies in Natural Products Chemistry*, Volume 29: *Bioactive Natural Products, Part J* (Ed.: Atta-ur-Rahman), Elsevier Science, New York, **2003**, in press.
[7] K. Kawada, A. Arimura, T. Tsuri, M. Fuji, T. Komurasaki, S. Yonezawa, A. Kugimiya, N. Haga, S. Mitsumori, M. Inagaki, T. Nakatani, Y. Tamura, S. Takechi, T. Taishi, J. Kishino, M. Ohtani, *Angew. Chem. Int. Ed.* **1998**, *37*, 973−975.
[8] S. Yonezawa, T. Komurasaki, K. Kawada, T. Tsuri, M. Fuji, A. Kugimiya, N. Haga, S. Mitsumori, M. Inagaki, T. Nakatani, Y. Tamura, S. Takechi, T. Taishi, M. Ohtani, *J. Org. Chem.* **1998**, *63*, 5831−5837.
[9] H. Oh, J. B. Gloer, D. T. Wicklow, P. F. Dowd, *J. Nat. Prod.* **1998**, *61*, 702−705.
[10] G. N. Belofsky, K. B. Gloer, J. B. Gloer, D. T. Wicklow, P. F. Dowd, *J. Nat. Prod.* **1998**, *61*, 1115−1119.
[11] A. Takahashi, R. Kudo, G. Kusano, S. Nozoe, *Chem. Pharm. Bull.* **1992**, *40*, 3194−3196.
[12] B. S. Yun, I. K. Lee, J. P. Kim, I. D. Yoo, *J. Antibiot.* **2000**, *53*, 711−713.
[13] A. R. Katritzky, M. Karelson, P. A. Harris, *Heterocycles* **1991**, *32*, 329−369.
[14] A. C. Cope, N. A. Lebel, *J. Chem. Soc.* **1960**, *82*, 4656−4662.
[15] E. Giganek, J. M. Read, *J. Org. Chem.* **1995**, *60*, 5795−5802.
[16] A. R. Wheildon, D. W. Knight, M. P. Leese, *Tetrahedron Lett.* **1997**, *38*, 8553−8556.
[17] K. Nakanishi, *Infrared Absorption Spectroscopy*, Holden-Day, Inc., San Francisco, **1962**.
[18] H. Besl, A. Bresinsky, G. Geingenmüller, R. Herrmann, C. Kilpert, W. Steglich, *Liebigs Ann. Chem.* **1989**, 803−810.
[19] M. Gill, W. Steglich, *Fort. Chem. Org. Nat.* **1987**, *51*, 1−317.
[20] T. C. McMorris, M. Anchel, *Tetrahedron* **1967**, *23*, 3985−3991.
[21] *HyperChem. 5.0*, Hypercube Inc. **1996**.

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