An Unusual Nitrogenous Terphenyl Derivative from Fruiting Bodies of the Basidiomycete *Sarcodon leucopus*

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A new nitrogenous metabolite with a *p*-terphenyl core, sarcodonin (**3**), has been isolated from fruiting bodies of the basidiomycete *Sarcodon leucopus*. The structure of **3** was determined through a careful study of its peracetate **4**, by use of an array of spectroscopic techniques (including ^{15}N NMR, gradient-enhanced, and reverse-detected 2D NMR experiments) and chemical degradation. ROESY data and molecular mechanics (MM+) calculations led to assignment of the absolute configuration of **4**. Compound **3** exhibited moderate cytotoxicity against KB and P-388 tumor cell cultures.

Chemical investigations of the basidiomycete genus *Sarcodon* are few, a recent example being the isolation of the diterpenoid scabronine A from *S. scabrosus*.[1] Isolation of the *p*-terphenyl derivatives **1** and **2** from fruiting bodies of *Sarcodon leucopus* (Pers.) (Hydnaceae) has been reported earlier.[2] *p*-Terphenyl derivatives of fungal origin occur most often as 2,5-diphenyl-1,4-benzoquinones. The less common aromatic *p*-terphenyls include a number of polyoxygenated metabolites isolated from the fruiting bodies of basidiomycetes (for instance *Hydnum aurantiacum*,[3] *Peniophora gigantea*,[4] *Boletopsis leucomelaena*,[5] and *Anthracophyllum* and *Paxillus* spp.[6]). Several bioactive *p*-terphenyls have also been obtained from the ascomycete *Aspergillus candidus*, among them terphenyllin and deoxyterphenyllin, active toward HeLa cells,[7] and terprenin, a highly potent IgE antibody suppressant.[8,9] Three new *p*-terphenyls have been recently isolated from the sclerotia of *Penicillium raistrickii*, one of them displaying mild antiinsect and antibacterial activity.[10]

Compound **1** was highly active against KB cells, and **2** was less potent.[11] These results prompted us to search for additional metabolites from *S. leucopus*. An extraction process based on a water-free protocol was developed, to minimize possible hydrolytic reactions during extraction. Preliminary TLC comparison of the ethyl acetate extract with the original aqueous acetone extract showed a marked difference: **1** was not detected, **2** appeared as a minor constituent, and a new metabolite was predominant. The isolation and structure determination of this component, sarcodonin (**3**), is described.

Results and Discussion

The ethyl acetate extract obtained from *S. leucopus*, subjected to chromatography under low-pressure nitrogen flow, using acetyl polyamide or DIOL Si gel as solid phases, to minimize degradation, afforded **3**. Positive FABMS performed in glycerol or NBA matrix gave, in both cases, a *quasi*-molecular ion $[M + H]^+$ at *m/z* 723. Elemental analysis indicated nitrogen, and combination of these data suggested the formula $C_{36}H_{38}O_{14}N_2$, with 19 degrees of unsaturation.

Preliminary ^1H and ^{13}C NMR analysis of **3**, as well as IR and UV data, showed marked similarity with *p*-terphenyl derivatives **1** and **2**. Because **3** was unstable to prolonged NMR analysis, peracetate **4** was prepared. Complete high-field NMR analysis (500 MHz for ^1H), including gradient-enhanced and reverse-detected two-dimensional experiments (DQF−COSY, TOCSY, HSQC, HMBC) was performed on **4**. These data, aided by one-dimensional 135° DEPT and GATED decoupled ^{13}C NMR, allowed the complete signal assignments reported in the Experimental Section (see Table 1 in Supporting Information for 2D NMR data). The NMR data for **3** were assigned largely by comparison with the data determined for **4** or those reported for **2**,[2] aided by DEPT and GATED decoupling experiments. Table 2 in Supporting Information lists NMR data in $(CD_3)_2CO$ solvent for both **3** and **4**.

Compound **4** showed an $[M + H]^+$ peak at *m/z* 891 in the FABMS, accounting for the introduction of four acetate groups. Elemental analysis confirmed the molecular formula $C_{44}H_{46}O_{18}N_2$, with 23 degrees of unsaturation.

From comparison of the proton and carbon NMR spectra of **4** with those of **5** (protoleucomelone)[12] obtained in previous work,[2] it was apparent that **4** and **3** contained a *p*-terphenyl nucleus bearing oxygenated functions at the same positions as in **5**. In addition to the *p*-terphenyl resonances, **4** showed signals attributable to an aliphatic moiety, and to seven acetoxy groups. Five of the acetoxy groups were located at C-2′, C-6′, C-3′, C-5′, and C-4″. This was confirmed by the observed HMBC correlations of the oxygen-bearing quaternary carbons with the acetate methyls. Further signals attributable to two acetoxy groups were observed at δ 2.23 and 2.14 (^1H NMR) and at δ 167.8, 167.3, 19.4, and 18.0 (^{13}C NMR). A ^{13}C chemical shift comparison between **3** and **4** showed a negligible acetylation shift ($\Delta\delta = \delta_4 - \delta_3 < 0.3$) for C-3 and C-4, in contrast with the marked shift observed for C-5′ ($\Delta\delta = +5.6$) and C-6′ ($\Delta\delta = +5.5$). This indicated that the two new acetoxy groups were not at C-3, C-4 (as in **5**) and that they had to be located on the aliphatic part of **4**.

Four methyls, two methylenes, two methines, and four quaternary carbons were assigned to the aliphatic part of the molecule on the basis of the DEPT experiment. DQF−COSY, TOCSY, and HMBC correlations established the presence of two distinct units, herewith indicated as α and β, both possessing a 3-methylpentane skeleton. The un-

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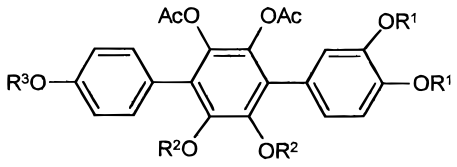
ambiguous assignment of the four quaternary carbons C-2α, C-3α, C-2*β*, and C-3*β* was based on the integrated analysis of HMBC and GATED decoupled data. The deshielded chemical shifts of these quaternary carbons indicated their direct connection with one (or more) heteroatoms. The three lower-field signals (*δ* 163.4, 163.3, and 159.8) were clearly due to sp^2-hybridized carbons, while the fourth (*δ* 91.1) was evidently a strongly deshielded sp^3-hybridized carbon.[13]

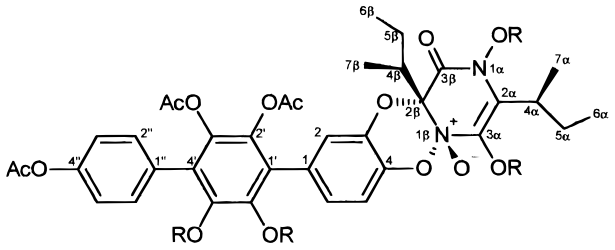
The proton-decoupled ^{15}N NMR spectrum of **4** showed two signals at *δ* 187.2 and 367.0, indicating very different shielding effects for the two nuclei. The ^{15}N NMR literature excluded protonated nitrogens and suggested that the lower-field resonating nitrogen could bear a positive charge.[14,15]

These data did not provide the complete structure of **4**, and efforts to obtain crystals suitable for X-ray diffraction analysis were unsuccessful. Thus, controlled degradative reactions were attempted.

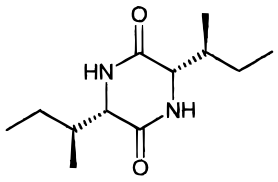
Peracetate **4** was maintained under hydrogen atmosphere in the presence of Pd/C for 40 h, and the two main products, **6** and **7**, were isolated. Compound **6** displayed an $[M + H]^+$ peak at *m/z* 553 (FABMS). The ^1H and ^{13}C NMR spectra, strongly resembling those of **2**, indicated **6** to be a hydrogenolysis product with a *p*-terphenyl structure bearing five acetoxy groups; four on the central C-1′−C-6′ ring and the fifth on the outer C-1″−C-6″ ring. As further confirmation, **6** was easily converted into peracetate **5** by conventional acetylation.



1 $R^1 = R^2 = R^3 = H$
2 $R^1 = R^2 = H$, $R^3 = Ac$
5 $R^1 = R^2 = R^3 = Ac$
6 $R^1 = H$, $R^2 = R^3 = Ac$



3 R = H
4 R = Ac



7

Compound **7** crystallized from a subfraction of the reaction mixture. It did not show UV absorption, and an $[M + H]^+$ peak at *m/z* 227 appeared in its FABMS. The ^1H and ^{13}C NMR spectra suggested a symmetrical structure, whose resonances were clearly related to those of the alkyl chains in **4**. Typical amide absorptions were observed in the IR spectrum (3640−3400 and 1670 cm^{-1}). These data suggested a cyclodipeptide, and a literature search showed good agreement of physical data obtained for **7** (including $[\alpha]_D$)[16] with those reported for cyclo-L-isoleucyl-L-isoleucine obtained by one-step synthesis[17] or isolated from the culture broth of *Ustilago cynodontis*.[18]

These results confirmed the *p*-terphenyl moiety of **4**, as well as the junction positions (C-3, C-4) with the nitrogenous aliphatic part and indicated a modified cyclodipeptide derived from L-isoleucine for the latter. Consequently, the absolute configuration *S* for both positions 4α and 4*β* was established.

In addition to the cited acetoxy groups and the 1,4-diazine ring, three further degrees of unsaturation remained in the aliphatic part of **4**, and these were assigned to two double bonds and one further ring. One double bond (C-2α/C-3α) is included within the α-chain and bears an acetoxy group at C-3α, as demonstrated by the HMBC correlation between the carbon peak at *δ* 163.4 and the acetyl signal at *δ* 2.14. The presence of a single sp^2-hybridized carbon atom in the *β* chain (C-3*β*) excluded a C=C double bond, while a −C(OAc)=N− group was ruled out on the basis of a ^{13}C acetylation shift analysis for the quaternary carbons in the diazine ring. In fact, an acetylation shift from **3** to **4** was observed for C-3α ($\Delta\delta = +4.4$) and C-2α ($\Delta\delta = -2{,}8$), while the other two carbons experienced a negligible shift (C-2*β*: $\Delta\delta = -0.4$; C-3*β*: $\Delta\delta = 0$). This indicated that the *β* chain was not involved in the acetylation. Consequently, the second acetoxy group (*δ* 2.23, broad) had to be at N-1α, thus establishing the −CO−N(OAC)− portion of the structure. The third unsaturation is evidently due to the ring junction of the C-2*β* and N-1*β* atoms with the *p*-terphenyl moiety at C-3,C-4. The alternative hypothesis would require a further double bond and a single junction point and was rejected on the basis of the above-discussed data.

The molecular formula of **4** required a further oxygen atom, and this could only be justified by an *N*-oxide function at N-1*β*, in agreement with the observed downfield ^{15}N NMR signal. A peak at *m/z* 707 in the FABMS of **3**, attributable to the loss of an oxygen atom $[M + H - 16]^+$, corroborated this assumption.

Regardless of the stereochemistry, two distinct joining modes between the *p*-terphenyl moiety and the diazine ring may be considered, indicated in Supporting Information as **4A** (with the *β* chain close to ring C-1′−C-6′ of the *p*-terphenyl) and **4B** (with the *β* chain in the outer position). Each structure, **4A** or **4B**, has four possible diastereomers, and hence, eight different stereostructures (Supporting Information) have to be considered, namely **4Aa**−**d** and **4Ba**−**d**. To discriminate among these possibilities, as well as to establish the configurations at N-1*β* and C-2*β*, we resorted to an integrated use of ROESY data and a molecular mechanics study of the eight diastereomers.

The global minimum energy conformation of each stereostructure was searched by exploring its conformational space using the molecular dynamics (MD)[19] simulated annealing[20,21] approach. Analysis of all structures within 5 kcal/mol from the global minima (> 97% population) revealed that they could be derived by simple rotations of acetoxy, biphenyl, and aliphatic chains bonds, while no relevant conformational changes were observed for the two heterocyclic rings. Therefore, the lowest-energy geometry for each stereoisomer was used as the preferred conforma-

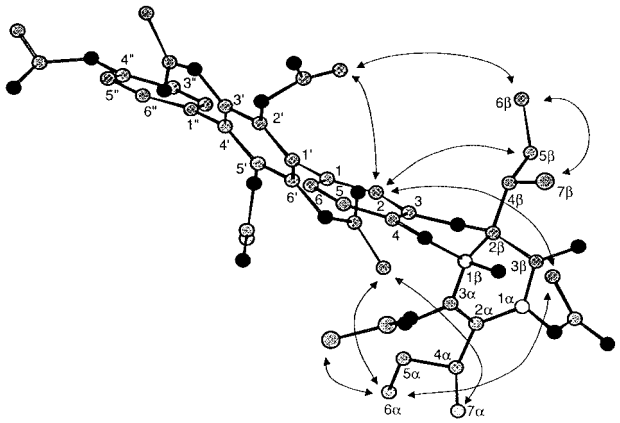


Figure 1. Perspective view of the lowest-energy conformer of (1*βR*,2*βR*)-**4Aa** (H atoms omitted) showing relevant ROE correlations (arrows).

tion in order to compare interproton distances with experimental ROE interactions (Table 3 in Supporting Information).

It is important to note ROE interactions among protons of the terphenyl moiety with those of the aliphatic portion. In particular, a ROE correlation between H-2 and H-5*β* indicated the closeness of *β*-chain to terphenyl-ring B and hence a **4A**-like junction. In addition ROE correlations were observed between C-2′−OAc or C-6′−OAc and H_3-6*β*, H_3-6α, and H_3-7α, as well as between H-2 and N-1α−OAc, which were adequately satisfied with 1*βR*,2*βR* stereochemistry (Figure 1). In fact, all other stereostructures have at least one (frequently two) pertinent interproton distance larger than 8 Å, which does not account for these critical ROE interactions. If the mean value of interproton distances is considered, the preference for the (1*βR*,2*βR*)-**4A** stereostructure is even more evident, this being the only one with a mean H/H value lower than 5 Å. Thus, sarcodonin peracetate has been assigned structure **4**, and the absolute configuration at the chiral centers was established as 1*βR*, 2*βR*, 4α*S*, and 4*βS*.

The parent metabolite **3** bears three acetoxy groups: analysis of NMR data in comparison with data of **1** and **2**,[2] indicated that they are at C-4″, C-2′, and C-3′ of the *p*-terphenyl. The ^{1}H NMR spectrum of **3** in $(CD_3)_2CO$ showed four 1H low-field, D_2O exchangeable singlets, (*δ* 7.72, 7.87, 9.50, and 10.26). By comparison with the ^{1}H NMR spectrum of **2** (OH signals at *δ* 7.66, 7.76, 8.02, 8.16), the two higher field signals were assigned to the phenolic hydroxyls on the central ring of the *p*-terphenyl moiety. The other unusually deshielded signals are due to "mobile" protons located in the aliphatic part of the molecule. Literature data suggested assignment of the lowest field resonance to the N−OH function,[22] thus supporting the presence of N−OH and C=C−OH groups in the diazine ring.

A conceivable biosynthetic way to **3** is a biological Diels−Alder reaction, where a *p*-terphenyl ortho-quinone precursor plays the diene role, with *syn* addition to a cycloisoleucine-derived precursor as dienophile. Fungal metabolites related to the latter are well-known and include aspergillic acid and pulcherriminic acid[23−25] both related to cyclo-L-isoleucyl-L-isoleucine. In these compounds the hydroxamic acid function (predominant in solution) is in tautomeric equilibrium with an *N*-oxide function. These metabolites may be considered oxidized forms of biosynthetic dioxopiperazines.[26] Pulcherriminic acid, by reductive treatments, affords leucine anhydride.[25] Diels−Alder additions are, in principle, easily reversible reactions, and hydrogenolysis of **4** may be considered a *retro*-Diels−Alder reaction followed by reduction.

Sarcodonin (**3**) was moderately active toward KB (ED_{50} = 10.0 *μ*g/mL) and P-388 (ED_{50} = 27.0 *μ*g/mL) cultured tumor cells. The higher cytotoxic activity of **1** with respect to **3**,[11] coupled with mild antibacterial activity[2] and literature data on biologically active fungal *p*-terphenyls,[7] suggest that **1** could play a role in the chemical defense of the mushroom. Hydroxamic acid derivatives also have been extensively studied in relation to their defensive action against insects in the Gramineae.[27]

Experimental Section

General Experimental Procedures. Elemental analyses were performed using a Perkin-Elmer 240 instrument. FABMS were recorded on a Kratos MS 50 instrument using glycerol and 3-nitrobenzyl alcohol (NBA) as matrixes. 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 499.86 (^{1}H) and 125.70 MHz (^{13}C) and equipped with a gradient-enhanced, reverse-detection probe or on a Bruker AC-250 spectrometer operating at 250.13 (^{1}H), 62.89 (^{13}C), and 25.35 MHz (^{15}N). Chemical shifts (*δ*) are indirectly referred to TMS using solvent signals for ^{1}H and ^{13}C NMR spectra and based on external reference (saturated aqueous solution of $^{15}NH_4NO_3$, 20.68 ppm) for the ^{15}N NMR spectrum. Broad-band decoupled (Waltz) NMR spectra were run for ^{15}N and ^{13}C nuclei. In addition, GATED decoupled and 135° DEPT ^{13}C NMR experiments were registered. All NMR experiments, including two-dimensional spectra (i.e., DQF−COSY, TOCSY, HSQC, HMBC, and ROESY) were performed using software supplied by the manufacturers and acquired at constant temperature (298 K). Molecular mechanics and molecular dynamics studies of the eight possible stereoisomers of compound **4** were performed using the HyperChem release 3 program.[28] TLC was carried out using precoated Si gel F_{254} plates (Merck). Freshly prepared $FeCl_3$ solution as well as phosphomolybdic acid were used as spray reagents. Column chromatography employed LiChroprep Si-60, LiChroprep DIOL 25−40 (Merck), and MN-Polyamid SC 6-Ac (Macherey-Nagel).

Fungal Material. Fruiting bodies of *S. leucopus* were collected on the slopes of Mount Etna near Catania in November 1996, cut into small pieces, and freeze-dried. The mushroom was identified by Dr. M. Dollo, Associazione Micologica Bresadola (Catania). A voucher specimen (CAT.ed) is deposited in the Mycological Herbarium, Dipartimento di Botanica, Università di Catania (Prof. P. Signorello).

Extraction and Isolation. Freeze-dried fruiting bodies of *S. leucopus* (360 g) were extracted with hexane (4 L, 1 h) to remove lipid material. The residue was then extracted with EtOAc (5 L, 21 h × 2). The EtOAc extracts were pooled, dried over Na_2SO_4, and taken to dryness yielding 8.4 g of crude extract. This was chromatographed on acetyl polyamide eluting with a gradient from 10% to 70% EtOAc in hexane. The eluates, analyzed by TLC, were pooled in four fractions (A−D). The most $FeCl_3$-reactive fraction C (1.03 g) was chromatographed by MPLC over LiChroprep DIOL with an elution gradient (35% to 70% of EtOAc in hexane) to afford three subfractions (C_1−C_3). The main constituent, **3**, was eluted in subfraction C_2 (250 mg) in mixture with **2**. Further chromatographic purification of subfraction C_2 (gradient 55% to 70% of EtOAc in hexane) gave 220 mg of pure **3** (yield 2.62% of the crude EtOAc extract and 0.061% of the dried mushroom) and **2** (18 mg).

***p*-Terphenyl Derivative (2):** MS and ^{1}H NMR [$CDCl_3$−CD_3OD (7:3), 250.13 MHz] data are in agreement with those reported.[2]

Sarcodonin (3): white amorphous powder; UV (EtOH) λ_{max} (ϵ) 202 (40 452), 256 (15 226) nm; IR ($CHCl_3$) ν_{max} 3680, 3550,

3360, 3045, 2970, 1770, 1680, 1520, 1465, 1375, 1200, 1030 cm^{-1}; ^1H NMR ($CDCl_3$, 250.1 MHz) δ 7.35 and 7.13 (each 2H, AA′BB′ system, $J_{AB} + J_{AB'} = 8.5$ Hz with $J_{AB'} < 0.5$ Hz, H-2″, H-6″ and H-3″, H-5″), 7.08 (1H, d, $J = 8.5$ Hz, H-5), 7.07 (1H, d, $J = 2.0$ Hz, H-2), 6.99 (1H, dd, $J = 8.5$, 2.0 Hz, H-6), 3.03 (1H, m, H-4α), 2.42 (1H, m, H-4β), 2.30 (3H, s, 4″-COCH_3), 1.95, 1.93 (each 3H, s, 2′-COCH_3, 3′-COCH_3), 1.80 (1H, m, H-5′β), 1.58 (1H, m, H-5′α), 1.38 (1H, m, H-5α), 1.23 (3H, d, $J = 7.0$ Hz, H-7β), 1.20 (1H, m, H-5β), 1.03 (3H, d, $J = 7.0$ Hz, H-7α), 0.96 (3H, t, $J = 7.5$ Hz, H-6β), 0.86 (3H, t, $J = 7.5$ Hz, H-6α); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 169.8 (s, 4″-*C*OCH$_3$), 169.4, 169.0 (s, 2′-*C*OCH$_3$, 3′-*C*OCH$_3$), 166.1 (s, C-2α), 159.8 (s, C-3β), 159.0 (s, C-3α), 150.5 (s, C-4″), 141.0, 140.7 (s, C-3, C-4), 139.8, 139.7 (s, C-2′, C-3′), 133.6 (s, C-5′, C-6′), 131.2 (d, C-2″, C-6″), 128.9 (s, C-1″), 128.3 (s, C-1), 125.1 (d, C-6), 122.0 (s, C-1′), 121.8 (d, C-3″, C-5″), 121.2 (s, C-4′), 119.0 (d, C-2), 117.0 (d, C-5), 91.5 (s, C-2β), 42.0 (d, C-4β), 33.6 (d, C-4α), 25.7 (t, C-5α), 23.2 (t, C-5β), 21.1 (q, 4″-COCH_3), 20.1 (q, 2′-COCH_3, 3′-COCH_3), 16.3 (q, C-7α), 13.8 (q, C-7β), 12.2 (q, C-6β), 12.0 (q, C-6α); FABMS (glycerol) *m/z* 723 $[M + H]^+$, 707, 563, 521, 479, 384, 342 FABMS (3-nitrobenzyl alcohol) *m/z* 761 $[M + K]^+$, 745 $[M + Na]^+$, 723 $[M + H]^+$; *anal.* C 59.68%, H 5.14%, N 3.71%, calcd for $C_{36}H_{38}O_{14}N_2$, C 59.83%, H 5.26%, N 3.87%.

Acetylation of 3. A solution of **3** (120 mg) in pyridine (5 mL) and acetic anhydride (5 mL) was stirred for 6 h at room temperature. After workup and flash chromatography on a LiChroprep DIOL column, eluting with a gradient from 2% to 4% of EtOAc in hexane, the peracetate **4** (146 mg) was obtained.

Peracetate (4): white amorphous powder; $[\alpha]^{25}_D$ −20.4° (*c* 1.6, $CHCl_3$); UV (EtOH) λ_{max} (ϵ) 206 (66 471), 252 (21 471) nm; IR ($CHCl_3$) ν_{max} 3050, 2950, 1780, 1680, 1520, 1460, 1370, 1200, 1030 cm^{-1};^1H NMR ($CDCl_3$, 499.8 MHz) δ 7.30 and 7.12 (each 2H, AA′BB′ system, $J_{AB} + J_{AB'} = 8.5$ Hz with $J_{AB'} < 0.5$ Hz, H-2″, H-6″ and H-3″, H-5″), 7.10 (1H, d, $J = 8.5$ Hz, H-5), 6.97 (1H, dd, $J = 8.5$, 2.0 Hz, H-6), 6.94 (1H, d, $J = 2.0$ Hz, H-2), 2.94 (1H, m, H-4α), 2.30 (3H, s, 4″-COCH_3), 2.23 (3H, br s, 1α-COCH_3), 2.17 (1H, m, H-4β), 2.14 (3H, s, 3α-COCH_3), 1.97 (6H, s, 2′-COCH_3, 6′-COCH_3), 1.94 (6H, s, 3′-COCH_3, 5′-COCH_3), 1.82 (1H, m, H-5′β), 1.60 (1H, m, H-5′α), 1.49 (1H, m, H-5α), 1.34 (3H, d, $J = 7.0$ Hz, H-7β), 1.32 (1H, m, H-5β), 1.07 (3H, d, $J = 7.0$ Hz, H-7α), 0.99 (3H, t, $J = 7.5$ Hz, H-6β), 0.88 (3H, t, $J = 7.5$ Hz, H-6α); ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ 168.9 (s, 4″-*C*OCH$_3$), 167.8 (s, 1α-*C*OCH$_3$), 167.6 and 167.4 (s, each 2C; 2′-*C*OCH$_3$, 6′-*C*OCH$_3$ and 3′-*C*OCH$_3$, 5′-*C*OCH$_3$), 167.3 (s, 3α-*C*OCH$_3$), 163.4 (s, C-3α), 163.3 (s, C-2α), 159.8 (s, C-3β), 150.7 (s, C-4″), 141.0 (s, C-4), 140.5 (s, C-3), 139.2 and 139.1 (s, each 2C; C-2′, C-6′ and C-3′, C-5′), 130.7 (d, C-2″, C-6″), 130.0 (s, C-4′), 129.5 (s, C-1′), 128.8 (s, C-1″), 128.2 (s, C-1), 124.8 (d, C-6), 121.3 (d, C-3″, C-5″), 119.1 (d, C-2), 116.3 (d, C-5), 91.1 (s, C-2β), 43.4 (d, C-4β), 36.3 (d, C-4α), 26.3 (t, C-5α), 23.7 (t, C-5β), 21.1 (q, 4″-COCH_3), 20.0 and 19.9 (q, each 2C; 2′-COCH_3, 6′-COCH_3 and 3′-COCH_3, 5′-COCH_3), 19.4 (q, 3α-COCH_3), 18.0 (q, 1α-COCH_3), 16.4 (q, C-7α), 14.0 (q, C-7β), 12.3 (q, C-6β), 12.1 (q, C-6α); ^{15}N NMR ($CDCl_3$, 25.35 MHz) δ 367.0, (s, N-1β), 187.2 (s, N-1α); FABMS (glycerol) *m/z* 891 $[M + H]^+$; *anal.* C 59.32%, H 5.16%, N 3.14%, calcd for $C_{44}H_{46}O_{18}N_2$, C 59.40%, H 5.23%, N 3.16%.

Hydrogenolysis of 4. A solution of **4** (65 mg) in anhydrous EtOH (10 mL) was stirred at room temperature under 1.2 atm H_2 pressure and in the presence of Pd/C (5 mg). The reaction was monitored by TLC and stopped after 40 h. After filtration and solvent evaporation, the residue was submitted to flash chromatography (Lichroprep DIOL, 2.0% MeOH in CH_2Cl_2 as the eluent) affording compound **6** (26 mg), compound **7** (12 mg after crystallization from MeOH−$CHCl_3$), and a residual amount of **4** (8 mg).

Compound 6: white amorphous powder; FABMS (glycerol) *m/z* 553 $[M + H]^+$; ^1H NMR ($CDCl_3$−CD_3OD 7:3, 250.13 MHz) δ 7.30 and 7.14 (each 2H, AA′BB′ system, $J_{AB} + J_{AB'} = 8.5$ Hz with $J_{AB'} < 0.5$ Hz, H-2″, H-6″ and H-3″, H-5″), 6.83 (1H, d, $J = 8.5$ Hz, H-5), 6.74 (1H, d, $J = 2.0$ Hz, H-2), 6.63 (1H, dd, $J = 8.5$, 2.0 Hz, H-6), 2.32 (3H, s, 4″-COCH_3), 2.00 and 1.97 (each 6H, s, 2′-COCH_3, 6′-COCH_3 and 3′-COCH_3, 5′-COCH_3), ^{13}C NMR ($CDCl_3$−CD_3OD 7:3, 62.9 MHz) δ 170.3 (s, 4″-*C*OCH$_3$), 168.7 and 168.6 (s, each 2C; 2′-*C*OCH$_3$, 6′-*C*OCH$_3$ and 3′-*C*OCH$_3$, 5′-*C*OCH$_3$), 151.2 (s, C-4″), 145.7, 145.0 (s, C-3, C-4), 139.8 and 139.7 (s, each 2C: C-2′, C-6′ and C-3′, C-5′), 131.4, 129.6, 129.3 (s, C-1, C-1″, C-4′), 131.1 (d, C-2″, C-6″), 122.9 (s, C-1′), 121.9 (d, C-3″, C-5″), 121.8 (d, C-6), 116.8 (d, C-5), 115.5 (d, C-2), 21.2 (q, 4″-COCH_3), 20.2 and 20.1 (q, each 2C: 2′-COCH_3, 6′-COCH_3 and 3′-COCH_3, 5′-COCH_3).

Compound 7: white crystals (MeOH−$CHCl_3$); $[\alpha]^{25}_D$ −80° (*c* 0.15, EtOH) (lit.[15] $[\alpha]^{22}_D$ −85°); IR ($CHCl_3$) ν_{max} 3640, 3000, 1670, 1240, 1020 cm^{-1}; MS and NMR [performed in $(CD_3)_2$-SO] data are in agreement with those reported in the literature.[17]

Acetylation of 6. Acetic anhydride (0.5 mL) was added to a solution of **6** (22 mg) in pyridine (0.5 mL). The solution was stirred at room temperature for 2 h. Standard workup and flash chromatography (LiChroprep Si-60, 30% Me_2CO in hexane as the eluent) of the crude product afforded 26 mg of peracetate **5**, identified through ^1H and ^{13}C NMR spectral data.[2]

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Supporting Information Available: 2D NMR data for compound **4** in $CDCl_3$, ^1H and ^{13}C NMR data for compounds **3** and **4** in $(CD_3)_2$-CO, selected ROE correlations of **4** and related interproton distances measured for the lowest energy conformer of each stereostructure, partial drawing of the eight possible stereostructures of compound **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) Previously unpublished results. Cytotoxic activity against KB cells: ED_{50} = 2.2 μg/mL (**1**) and 22.0 μg/mL (**2**).
(12) A compound first named protoleucomelone (**5**) was obtained in Japan from the Basidiomycete *Boletopsis leucomelaena* (syn. *Polyporus leucomelas*);[29] subsequently, the structure of this metabolite was reassigned as cycloleucomelone.[5,6] Therefore, compound **5** is a semisynthetic derivative obtained previously.[2]
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