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Retymicin, Galtamycin B, Saquayamycin Z and Ribofuranosyllumichrome, Novel Secondary Metabolites from *Micromonospora* sp. Tü 6368

II. Structure Elucidation

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Abstract A detailed screening of the secondary metabolite pattern from *Micromonospora* sp. strain Tü 6368 resulted in the isolation of ten compounds belonging to five different structural families. The structures of the novel compounds 1-(α-ribofuranosyl)-lumichrome (**3**), retymicin (**7**), galtamycin B (**11**) and saquayamycin Z (**14**) were assigned by spectroscopic methods and chemical transformations. This strain fits our hypothesis that the metabolite analysis of biosynthetically talented strains leads readily to novel compounds.

Keywords structure elucidation, *Micromonospora*, angucyclinones, tetracenequione glycosides, saquayamycin Z, lumichromes

Introduction

The well established HPLC-DAD-screening resulted in the discovery of several secondary metabolites produced by *Micromonospora* sp. strain Tü 6368 under different cultivation conditions. Four of the isolated compounds turned out to be new: 1-(α-ribofuranosyl)-lumichrome (**3**), retymicin (**7**), galtamycin B (**11**) and saquayamycin Z (**14**), the others were known: two lumichromes (**1** and **2**), three angucyclinones (**4**~**6**) and one tetracenequinone (**9**). In the previous paper the taxonomy and fermentation as well as the isolation, characterisation and biological activities of the secondary metabolites were described [1]. In this part we present the structure elucidation, which was done mainly by NMR analysis.

Lumichromes

Lumichrome (**1**) and 1-methyllumichrome (**2**) were identified by comparison of UV, HREI-MS as well as ^{1}H and ^{13}C-NMR data with literature values (Fig. 1) [2]. The third yellow fluorescent compound of this family exhibited the molecular formula $C_{17}H_{18}N_4O_6$, determined by HRESI-MS (m/z=375.12972 $[M+H]^+$), and differs from **1** by a C_5-unit. Comparison of the ^{1}H and ^{13}C NMR spectra of **3** and **1** led to the assumption that a sugar moiety is added to the lumichrome skeleton. In conjunction with the ^{1}H, ^{1}H COSY spectrum the additional signals could be assigned to a pentofuranose. To determine the linkage of the sugar (position 1 or 3) an HMBC experiment with **3** was carried out and revealed interactions between C-2 and 1′-H and 2′-H, respectively, an expected cross signal between C-10a and 1′-H was missing. Nevertheless we assumed that the sugar is linked to N-1 due to the substitution pattern of 1-methyllumichrome (**2**). To identify the sugar moiety, NOE experiments were carried out. Selective irradiations revealed correlations between 1′-H and 2′-H as well as 2′-H and 3′-H. The signals of 3′-H and 4′-H overlapped, thus no determination of a correlation was possible. Four possibilities are left for the configuration of the pentofuranose: α-D- and α-L-ribofuranose as well as β-D-

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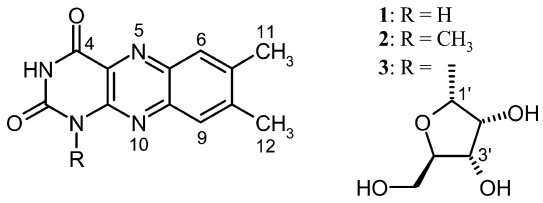

Fig. 1 Structural formulae of lumichromes (**1**~**3**).

and β-L-lyxofuranose. From the ^{1}H coupling constants between 1′-H/2′-H, 2′-H/3′-H (each with ^3J=5.5 Hz) and 3′-H/4′-H (^3J=9.0 Hz) a ribofuranose was suggested. This was confirmed by GC-MS analysis after methanolysis of **3** followed by silylation. The Rt-values of the sugar peaks were compared with those of D-(−)-ribose and D-(−)-lyxose after derivatisation under the same conditions. As a result 1-(α-ribofuranosyl)-lumichrome (**3**) was identified, which has not been described in the literature so far (Fig. 1). The D- or L-configuration has not yet been determined. However 1-(β-D-ribofuranosyl)-, 3-(β-D-ribofuranosyl)-, and 1,3-di-(β-D-ribofuranosyl)-lumichromes are already known [3] as synthetic compounds.

Angucyclinones

The known angucyclinones, rabelomycin (**4**) [4], 3-deoxyrabelomycin (**5**) [5] and dehydrorabelomycin (**6**) [6] were identified by comparison of HREI-MS, ^{1}H and ^{13}C NMR data with literature values (Fig. 2). The fourth compound, the main compound of the crude extract, was named retymicin (**7**), its molecular formula $C_{18}H_{16}O_6$ was determined by HRESI-MS (m/z=329.10259 $[M+H]^+$). The ^{1}H and ^{13}C NMR spectra show signals for one methyl, two methylene, one aliphatic and five aromatic methine groups as well as nine quaternary carbon atoms. Two of these indicate oxygenated aliphatic carbon atoms (δ_C=78.4 and 80.4) and two others at δ_C=176.8 and 209.3 point to a carbonyl group and a ketone, respectively. The ^{1}H NMR spectrum showed similarities to the rings A and D of 3-deoxyrabelomycin (**5**), therefore we assumed an angucyclinone variant. The ^{1}H, ^{1}H COSY experiment confirmed a 1,2,3-substituted aromatic system (ring D) and a 2,3,5-substituted cyclohexanone moiety (ring A). Additionally, a Z configurated double bond (δ_H=6.47 and 6.52, ^3J=10.0 Hz) was established. Due to the lack of a third carbonyl group in the ^{13}C NMR spectrum, retymicin (**7**) is not a quinone in ring C. In addition, ring B is not aromatic, the ^{13}C NMR spectrum reveals signals at δ_C=78.4 and 80.4, which belong to ring B. Furthermore there are three signals at δ_C=146.3, 148.9 and 162.1 typical for oxygenated quaternary aromatic carbon atoms. Consequently, there are only a carbonyl group and an oxygen atom remaining for ring C. The structural elements could be unambiguously combined with the help of an HMBC experiment resulting in formula **7** (Fig. 2). Thus retymicin (**7**) is a xanthone derivative, which emerges biosynthetically from an angucyclinone such as ochracenomicin A (**8**) [7], compound **7** has not been described so far. The stereochemistry is shown according to known angucyclinones and is not yet proven.

Tetracenequinone Glycosides

Galtamycinone (**9**) could be identified by HREI-MS, ^{1}H and ^{13}C NMR data [8], structure **9** represents a *C*-glycosylated tetracenequinone and is the aglycone of galtamycin (**10**) [9]. The second isolated red compound of this structural family exhibited a similar UV spectrum as **9**

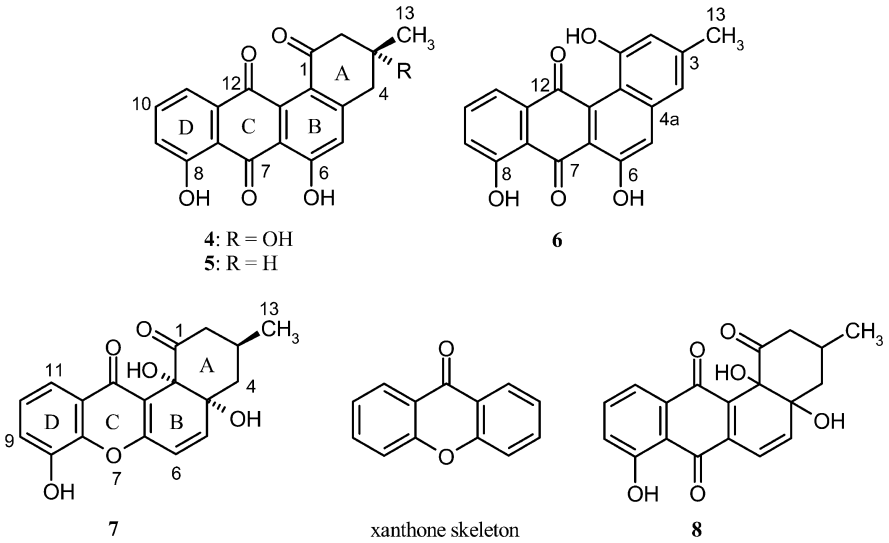

Fig. 2 Structural formulae of rabelomycins (**4**~**6**), retymicin (**7**) and related compounds.

and **10**. Its molecular formula was assigned as $C_{43}H_{48}O_{15}$ by HRESI-MS (m/z=803.29204 $[M-H]^-$) in conjunction with its ^{13}C NMR spectrum. There is a difference of four mass units compared with **10** ($C_{43}H_{52}O_{15}$), which is an *O*-glycoside of **9** with two α-L-rhodinoses and one β-D-olivose as building blocks. By comparison of the ^{13}C NMR data (Table 1) with **9**, it clearly turned out that **9** is the aglycone of the novel compound, named galtamycin B (**11**). It is noteworthy that four of the carbons of **11** could not be detected in spectra measured in pyridine-d_5 and three were not seen, if $CDCl_3$ was used as a solvent.

Additionally to the ^{13}C NMR signals of **9** the signals of a trisaccharide moiety are visible (Table 1). Among these we identify signals for an α,β-unsaturated ketone (δ_C=127.3, 144.3, 197.0), three anomeric carbon atoms (δ_C=95.6, 99.0, 99.2), three methyl groups (δ_C=15.5, 16.4, 17.4), six oxygenated methine groups and three methylene groups. These data suggested three deoxy sugars, the structure of which could be determined with 2D NMR experiments (^1H, ^1H COSY, HSQC and HMBC) and by comparison with literature values of 2-deoxyfucose, rhodinose and aculose. The HMBC experiment gave the linkage of the sugars to the β-D-olivose of the aglycone [$CDCl_3$: 1A-H (δ_H=5.09) to C-4′ (δ_C=89.5)] and to each other [$CDCl_3$: 1B-H (δ_H=4.90) to C-4A (δ_C=79.3); 1C-H (δ_H=5.23) to C-4B (δ_C=76.3)]. The anomeric configurations of the three *O*-glycosidically bound sugars followed from the $^3J_{H,H}$ coupling constants (=3.5 Hz) and turned out to be α. Considering that an α-*O*-glycosidic bond reveals a L-sugar we assigned the sequence of the trisaccharide as L-aculosyl-(1→4)-α-L-rhodinosyl-(1→4)-α-L-2-deoxyfucosyl, which is connected to 4′-*O* of the aglycone. Thus formula **11** represents galtamycin B, which is a novel tetracenequinone glycoside (Fig. 4).

Aculose is a rather reactive sugar and adds easily methanol to the double bond under acidic conditions resulting in 2-methoxycinerulose A (Fig. 3) [10, 11]. If methanol was used in the work-up procedure and the following chromatographic steps, we isolated the methanol adduct **12** instead of **11**. The adduct **12** differs slightly in the Rf value from **11** ($CHCl_3$ - MeOH 9 : 1: 0.68 and 0.62, respectively) and exhibits the molecular formula $C_{44}H_{52}O_{16}$ confirmed by HRESI-MS (m/z=835.31881 $[M-H]^-$). Significant in the ^{13}C NMR spectra of **12** compared with **11** is the loss of two olefinic carbon atoms, the shift of the carbonyl group from δ_C=197.0 to 207.6 and the additional methoxy (δ_C=57.1), methine (δ_C=78.9) and methylene groups (δ_C=39.8). ESI-MS^n experiments with **12** revealed the successive cleavage of 2-methoxycinerulose A, rhodinose, 2-deoxyfucose and olivose. These data proved the sequence of the sugar moieties of **12** (Fig. 4).

Table 1 Comparison of the ^{13}C NMR data of galtamycinone (**9**), galtamycin B (**11**) and the methanol adduct **12**

C-atom	**9**[a] δ_C	**11**[a] δ_C	**11**[b] δ_C	**12**[b] δ_C
C-1	157.7	157.8	154.1	154.1
C-2	117.1	117.2	116.7	116.8
C-3	142.1	142.3	141.3	141.3
C-4	115.5	115.6	n.d.	n.d.
C-4a	129.9	130.2[c]	129.2	129.1
C-5	164.0	n.d.	163.5	163.2
C-5a	109.9	110.0	109.4	109.3
C-6	187.0	n.d.	186.9	186.9
C-6a	133.3	n.d.	n.d.	n.d.
C-7	118.9	119.0	118.8	118.8
C-8	133.4	133.4	132.9	132.9
C-9	138.6	138.2	137.1	137.0
C-10	159.6	159.7	159.4	159.4
C-10a	116.9	117.1	116.6	116.6
C-11	188.4	188.7[c]	187.9	187.9
C-11a	126.4	n.d.	126.3	126.2
C-12	118.0	118.1[c]	n.d.	n.d.
C-12a	125.9	126.1	124.6	124.6
C-13	22.2	22.2	22.2	22.2
C-1′	72.2	71.9	71.2	71.2
C-2′	41.1	40.6	38.4	38.4
C-3′	73.5	71.6	71.4	71.4
C-4′	78.7	86.4	89.5	89.5
C-5′	77.5	75.9	74.4	74.3
C-6′	19.0	19.0	18.6	18.6
C-1A	—	99.0	99.6	99.6
C-2A	—	33.9	32.3	32.2
C-3A	—	66.8	66.8	66.8
C-4A	—	79.4	79.3	79.1
C-5A	—	64.5	64.6	64.5
C-6A	—	16.4	16.1	16.1
C-1B	—	99.2	100.0	100.0
C-2B	—	24.9	24.4	24.5
C-3B	—	25.0	24.6	24.6
C-4B	—	76.9	76.3	75.6
C-5B	—	67.2	67.1	67.2
C-6B	—	17.4	17.1	17.2
C-1C	—	95.6	95.3	100.6
C-2C	—	144.3	142.9	78.9
C-3C	—	127.0	127.4	39.8
C-4C	—	197.0	196.7	207.6
C-5C	—	70.9	70.6	72.0
C-6C	—	15.5	15.1	14.9
C-7C	—	—	—	57.1

[a] pyridine-d_5, [b] $CDCl_3$

[c] chemical shift assignments were made on the bases of C–H chemical shift correlation experiments

n.d.=not detectable

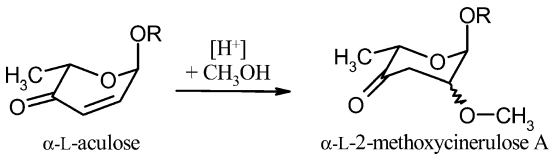

Fig. 3 Addition of methanol to L-aculose of galtamycin B (**11**) and saquayamicin Z (**14**) resulting in L-2-methoxycinerulose A of the methanol adducts **12** and **15**, respectively.

Saquayamycin Z

The comparison of the UV spectrum of the orange compound with the HPLC-UV/VIS database showed similarities to saquayamycin B [12], an angucycline with aquayamycin (**13**) [13] as aglycone. Looking for the molecular mass and formula of the compound by ESI-MS, the result was rather striking ($C_{73}H_{102}O_{29}$, m/z=1465.63952 $[M+Na]^+$) and leads to the largest angucycline ever reported [14, 15]. Thus we named the metabolite saquayamycin Z (**14**). The ^{13}C NMR data of the aglycone were in full agreement with those of other saquayamycins. Compared with **13**, additional signals for eight anomeric carbon atoms (δ_C=92.6∼101.4) and eight methyl groups (δ_C=15.1∼18.0) were detected pointing to typical angucycline deoxy sugars. Three of these sugars belong to a trisaccharide *O*-glycosidically connected to 4′-*O* of the *β*-D-olivose of the aglycone [1A-H (δ_H=5.08) to C-4′ (δ_C=89.3)]. A detailed 2D NMR analysis showed that this sugar side chain is identical with that of galtamycin B (**11**) and is terminated by L-aculose with its typical enone system (δ_C=127.3, 142.9, 196.7). The remaining five sugars are part of a pentasaccharide *O*-glycosidically connected to 3-*O* of the aglycone [1D-H (δ_H=5.24) to C-3 (δ_C=82.4)]. The position is the same as in saquayamycin A and B. The five sugars could be confirmed by 2D NMR experiments (^1H, ^1H COSY, HSQC, HMBC and 2D TOCSY) as three rhodinoses and two olivoses. The sequence was determined by an HMBC experiment and by ESI-MS/MS experiments of the methanol adduct **15** (Fig. 5). The HMBC experiment reveals the connection of the sugars to each other [1B-H (δ_H=4.90) to C-4A (δ_C=79.0); 1C-H (δ_H=5.24) to C-4B (δ_C=76.2); 1E-H (δ_H=4.48) to C-4D (δ_C=75.8); 1F-H (δ_H=4.94) to C-3E (δ_C=80.4); 1G-H (δ_H=4.48) to C-4F (δ_C=75.5); 1H-H (δ_H=4.96) to C-

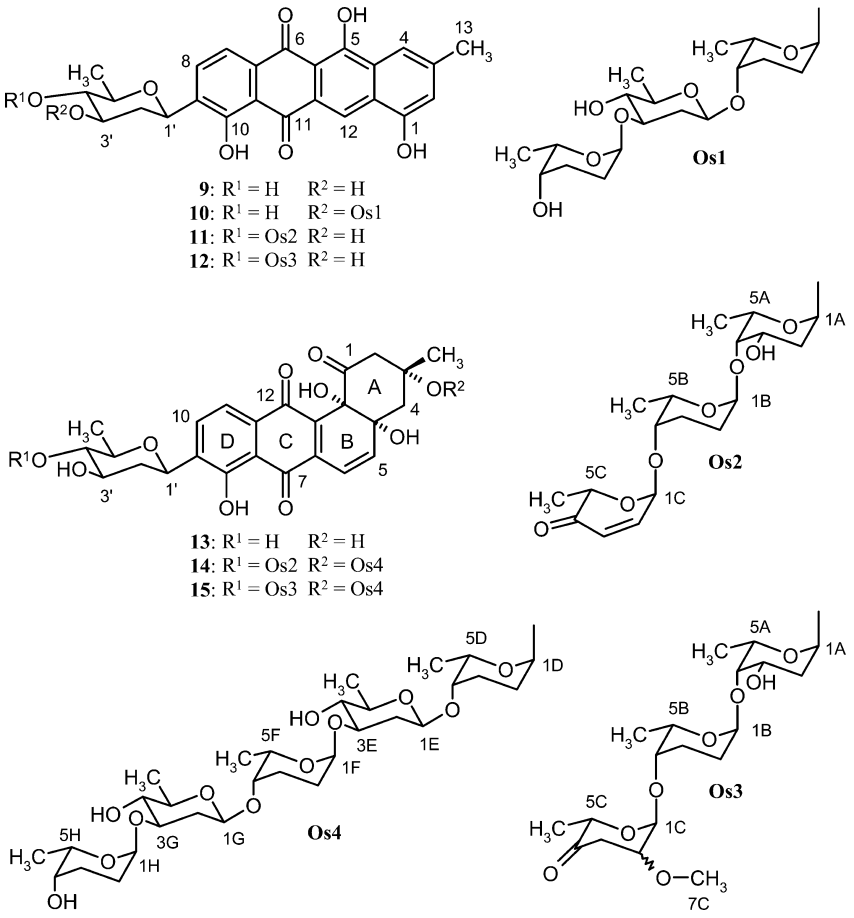

Fig. 4 Structural formulae of galtamycin B (**11**), saquayamycin Z (**14**) and related compounds.

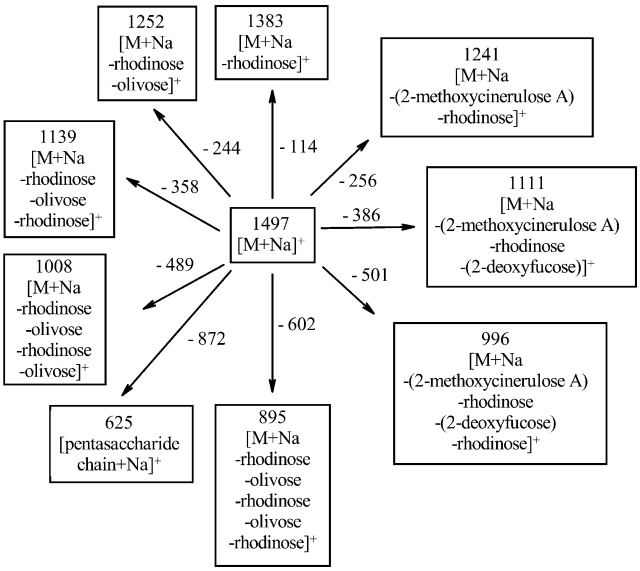

Fig. 5 Fragmentation pattern of ESI-MS/MS experiments (positive mode) of the methanol adduct **15**.

3G (δ_C=80.7)]. Thus saquayamycin Z (**14**) contains a α-L-rhodinosyl-(1→3)-β-D-olivosyl-(1→4)-α-L-rhodinosyl-(1→3)-β-D-olivosyl-(1→4)-α-L-rhodinosyl side chain with an alternating sequence rhythm. A coupled HSQC experiment proved the assumed configurations of the anomeric carbon atoms of both sugar side chains: $^1J_{C-H}$ coupling constants of 156 Hz (C-1E and C-1G) were typical for β-D-olivoses, 174 Hz (C-1A) for α-L-2-deoxyfucose, 174 Hz (C-1C) for α-L-aculose and 166~174 Hz (C-1B, C-1D, C-1F and C-1G) for α-L-rhodinoses. Both oligosaccharide side chains together led to formula **14** of saquayamycin Z (Fig. 4).

Discussion

From the cultures of *Micromonospora* sp. strain Tü 6368 altogether ten secondary metabolites were isolated by performing two fermentations under different conditions. The lumichromes **1** to **3** were produced without XAD supplementation, the others, belonging to angucycline/angucyclinone family (**4**~**7**, **9**, **11** and **14**) need this supplementation for optimum yields.

The lumichromes show a tricyclic chromophoric system similar to that of riboflavin, which carries an open chain D-ribose moiety non glycosidically attached to N-10, whereas 1-(α-ribofuranosyl)-lumichrome (**3**) is the first natural product with a α-ribofuranosyl moiety glycosidically attached to N-1.

Angucyclinones are PKS II polyketides, which can be *C*-glycosylated, *e.g.* by D-olivose, at position 9 of the angular core structure. The core structure itself can be varied in the late biosynthesis in different ways: (i) bishydroxylation at C-4a/C-12b reversing the aromaticity of ring B resulting in aquayamycin (**13**), (ii) oxidative fission of the quinone ring C, decarboxylation and recyclisation resulting in the xanthone skeleton of retymicin (**7**) and (iii) rearrangement of the angular aquayamycin skeleton resulting in the fully aromatic linear tetracenequinone skeleton of galtamycinone (**9**) [13].

The angucyclinones and derived aglycones are glycosylated in different positions, *e.g.* 3′-OH, 4′-OH, 3-OH or 12b-OH [14, 15]. Only the 4′-OH in the case of galtamycin B (**11**) and in the case of saquayamycin Z (**14**) both the 4′-OH and 3-OH are involved. The trisaccharide of **11** and **14** is striking because of the presence of α-L-2-deoxyfucose, which is not a usual sugar constituent of the angucyclines, whereas the pentasaccharide of **14** in an alternating sequence rhythm contains β-D-olivose and α-L-rhodinose, which are common sugar moieties of this group of antibiotics. Furthermore, saquayamycin Z (**14**) is the largest angucycline so far with a molecular formula of $C_{73}H_{102}O_{29}$ and a molecular weight of M_r=1443 and is assembled by the aquayamycin aglycone and eight additional deoxy sugars. Within the hitherto existing angucyclines the landomycins A and C with molecular weights of M_r=1087 and 1071 [16, 17], respectively, were the largest, each of them possesses six *O*-glycosidically bound sugar moieties.

Thus, *Micromonospora* sp. strain Tü 6368 seems to be an interesting candidate for studying the available glycosyl transferases in the light of their regio-, stereo- and substrate specifity. They may become valuable tools for genetically based combinatorial methods in the biosynthesis of biological active glycosides [18].

Experimental

General

NMR spectra were measured with Varian Inova-600 (600 MHz), Varian Mercury-300 (300 MHz) and Varian Unity-300 (300 MHz) instruments. Chemical shifts are expressed in δ values with solvents as internal standards. HRESI mass spectra were taken by Bruker Apex-Q III (7 Tesla) and DCI mass spectra by Finnigan MAT 95 (200 eV, NH_3 as reactant gas). IR spectra of samples in pressed KBr discs, were recorded on a Perkin Elmer FT-IR 1600 spectrometer, the UV spectra, on a Varian Cary 3E spectrophotometer, the CD spectra, on a Jasco J 500 spectrometer and the optical rotation values, with a Perkin Elmer 343 polarimeter. TLC was carried out on silica gel 60 F_{254}

plates (Merck, 0.25 mm), compounds were viewed under a UV lamp at 254 and 366 nm.

1-(α-Ribofuranosyl)-lumichrome (3)

Yellow solid; Rf 0.22 ($CHCl_3$ - MeOH, 9 : 1); $[\alpha]_{20}^{D}$ +319° (c=0.1, MeOH); UV λ_{max}^{MeOH} nm (log ε) 214 (4.45), 254 (4.55), 340 (3.85); CD λ_{max}^{MeOH} nm ($[\theta]^{20}$) 259 (+43864), 240 (−5393), 226 (+7147); IR ν_{max} (KBr) cm^{-1} 3425, 1768, 1755, 1693, 1682, 1558, 1430, 1401, 1355, 1228, 1129, 1039; ^{1}H NMR (300 MHz, CD_3OD) δ 2.54 (s, 6H, 11-H_3, 12-H_3), 3.69 (dd, J=12.5, 4.0 Hz, 1H, 5′-H_A), 3.91 (dd, J=12.5, 2.0 Hz, 1H, 5′-H_B), 4.18 (dd, J=9.0, 5.5 Hz, 1H, 3′-H), 4.22 (ddd, J=9.0, 4.0, 2.0 Hz, 1H, 4′-H), 5.13 (t, J=5.5 Hz, 1H, 2′-H), 6.61 (d, J=5.5 Hz, 1H, 1′-H), 7.81 (s, 1H, 9-H), 7.93 (s, 1H, 6-H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 20.4*[1] (C-11), 20.6*[1] (C-12), 60.9 (C-5′), 71.6 (C-3′), 79.4 (C-2′), 80.9 (C-4′), 89.8 (C-1′), 128.1 (C-9), 129.2 (C-6), 139.7 (C-5a), 141.6 (C-9a), 142.7*[2] (C-4a), 142.8*[2] (C-10a), 143.2 (C-7), 145.1 (C-8), 157.0 (C-2), 169.2 (C-4), *[1,*2] assignments bearing the same superscript may be interchanged; ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.48 (s, 6H, 11-H_3, 12-H_3), 3.47 (dd, J=12.5, 4.0 Hz, 1H, 5′-H_A), 3.67 (dd, J=12.5, 2.0 Hz, 1H, 5′-H_B), 4.01 (m, 2H, 3′-H, 4′-H), 4.68 (br s, 1H, 5′-OH), 5.04 (t, J=5.5 Hz, 1H, 2′-H), 5.54 (br s, 1H, 3′-OH), 6.41 (d, J=5.5 Hz, 1H, 1′-H), 7.56 (br s, 1H, 2′-OH), 7.82 (s, 1H, 9-H), 7.90 (s, 1H, 6-H), 8.09 (br s, 1H, 3-NH); ^{13}C NMR (150.8 MHz, DMSO-d_6): δ=19.8*[1] (C-11), 19.9*[1] (C-12), 59.3 (C-5′), 69.6 (C-3′), 77.5 (C-2′), 79.6 (C-4′), 87.8 (C-1′), 126.7 (C-9), 127.6 (C-6), 137.6 (C-5a), 139.3 (C-9a), 141.2*[2] (C-4a), 141.5*[2] (C-10a), 142.7 (C-7), 143.0 (C-8), 154.5 (C-2), 166.1 (C-4),[1,*2] assignments bearing the same superscript may be interchanged; HRESI-MS m/z 375.12972 $[M+H]^+$, calculated for $C_{17}H_{19}N_4O_6$ and found.

Retymicin (7)

Yellow solid; Rf 0.54 ($CHCl_3$ - MeOH, 9 : 1); $[\alpha]_{20}^{D}$ +54° (c=0.1, MeOH); UV log λ_{max}^{MeOH} nm (log ε) 215 (4.18), 268 (4.29), 303 sh, 328 sh; CD λ_{max}^{MeOH} nm ($[\theta]^{20}$) 348 (+7656), 309 (−11146), 268 (−35325), 219 (+59865); IR ν_{max} (KBr) cm^{-1} 3408, 1717, 1640, 1616, 1593, 1560, 1430, 1360, 1294, 1223, 1180, 1043; ^{1}H NMR (300 MHz, CD_3OD) δ 0.97 (d, J=6.5 Hz, 3H, 13-H_3), 1.57 (dd, J=14.0, 11.5 Hz, 1H, 4-H_A), 1.97 (dt, J=14.0, 3.0 Hz, 1H, 4-H_B), 2.20 (m, 1H, 3-H), 2.29 (dd, J=12.5, 11.0 Hz, 1H, 2-H_A), 2.54 (dt, J=11.0, 2.5 Hz, 1H, 2-H_B), 6.47 (d, J=10.0 Hz, 1H, 6-H), 6.52 (d, J=10.0 Hz, 1H, 5-H), 7.20 (dd, J=8.0, 2.0 Hz, 1H, 9-H), 7.24 (t, J=8.0 Hz, 1H, 10-H), 7.47 (dd, J=8.0, 2.0 Hz, 1H, 11-H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 21.8 (C-13), 31.4 (C-3), 43.6 (C-4), 47.4 (C-2), 78.4 (C-12b), 80.4 (C-4a), 116.0 (C-11), 117.8 (C-12a), 120.4 (C-6), 120.7 (C-9), 126.0 (C-11a), 126.9 (C-10), 146.3 (C-7a), 148.2 (C-8), 150.7 (C-5), 162.1 (C-6a), 176.8 (C-12), 209.3 (C-1); ^{1}H NMR (600 MHz, pyridine-d_5) δ 0.95 (d, J=6.5 Hz, 3H, 13-H_3), 1.77 (dd, J=14.0, 12.0 Hz, 1H, 4-H_A), 2.15 (dt, J=14.0, 3.0 Hz, 1H, 4-H_B), 2.61 (m, 1H, 3-H), 2.69 (dd, J=13.0, 12.0 Hz, 1H, 2-H_A), 2.92 (dt, J=12.0, 3.0 Hz, 1H, 2-H_B), 6.29 (d, J=10.0 Hz, 1H, 6-H), 6.69 (d, J=10.0 Hz, 1H, 5-H), 7.29 (t, J=8.0 Hz, 1H, 10-H), 7.45 (dd, J=8.0, 1.5 Hz, 1H, 9-H), 7.90 (dd, J=8.0, 1.5 Hz, 1H, 11-H); ^{13}C NMR (150.8 MHz, pyridine-d_5) δ 21.5 (C-13), 30.5 (C-3), 43.2 (C-4), 47.0 (C-2), 78.1 (C-12b), 79.6 (C-4a), 115.2 (C-11), 118.0 (C-12a), 119.3 (C-6), 120.1 (C-9), 125.8 (C-10), 125.9 (C-11a), 145.8 (C-7a), 148.4 (C-8), 150.4 (C-5), 160.2 (C-6a), 175.2 (C-12), 208.0 (C-1); HRESI-MS m/z 329.10259 $[M+H]^+$, calculated for $C_{18}H_{17}O_6$ and found.

Galtamycin B (11)

Red solid; Rf 0.62 ($CHCl_3$ - MeOH, 9 : 1); UV λ_{max}^{MeCN} nm (log ε) 249 sh, 264 (4.57), 294 sh, 485 (4.05); IR ν_{max} (KBr) 3447, 1700, 1636, 1560, 1458, 1437, 1384, 1288, 1261, 1087, 1040, 1014 cm^{-1}; ^{1}H NMR (600 MHz, pyridine-d_5) δ 1.29 (d, J=6.5 Hz, 3H, 6B-H_3), 1.42 (d, J=6.5 Hz, 3H, 6A-H_3), 1.47 (d, J=6.5 Hz, 3H, 6C-H_3), 1.54 (d, J=6.0 Hz, 3H, 6′-H_3), 1.76 (br d, J=12.5 Hz, 1H, 2B-H_A), 1.85 (q, J=12.0 Hz, 1H, 2′-H_A), 2.07 (br dd, J=13.0, 3.0 Hz, 1H, 3B-H_A), 2.15 (m, 1H, 2B-H_B), 2.20 (m, 2H, 2A-H_A, 3B-H_B), 2.40 (s, 3H, 13-H_3), 2.54 (dt, J=14.0, 3.5 Hz, 1H, 2A-H_B), 2.92 (br dd, J=12.5, 4.5 Hz, 1H, 2′-H_B), 3.56 (t, J=8.5 Hz, 1H, 4′-H) 3.74 (m, 2H, 5′-H, 4B-H), 3.87 (br dd, J=4.0, 2.0 Hz, 1H, 4A-H), 4.16 (m, 1H, 3′-H), 4.30 (q, J=6.5 Hz, 1H, 5B-H), 4.44 (br s, 1H, 3A-H), 4.80* (1H, 5C-H), 5.06 (br qd, J=6.5, 2.0 Hz, 1H, 5A-H), 5.13 (br s, 1H, 1B-H), 5.16 (d, J=11.5 Hz, 1H, 1′-H), 5.42 (d, J=3.5 Hz, 1H, 1C-H), 5.42* (1H, 1A-H), 6.18 (d, J=10.0 Hz, 1H, 3C-H), 6.98 (dd, J=10.0, 3.5 Hz, 1H, 2C-H), 7.25 (s, 1H, 2-H), 8.03 (s, 1H, 4-H), 8.07 (d, J=8.0 Hz, 1H, 8-H), 8.09 (d, J=8.0 Hz, 1H, 7-H), * chemical shift assignment were made on the base of a $^1J_{C-H}$ chemical shift correlation experiment; ^{13}C NMR (150.8 MHz, pyridine-d_5) see Table 1; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.19 (d, J=6.5 Hz, 3H, 6B-H_3), 1.28 (d, J=6.5 Hz, 3H, 6A-H_3), 1.36 (d, J=6.5 Hz, 3H, 3C-H_3), 1.39 (d, J=6.5 Hz, 3H, 6′-H_3), 1.46 (br d, J=12.5 Hz, 1H, 2′-H_A), 1.70 (br d, J=13.0 Hz, 1H, 2B-H_A), 1.91 (m, 2H, 2A-H_A, 3B-H_A), 2.00 (br dt, J=14.0, 4.0 Hz, 1H, 2B-H_B), 2.07 (br d, J=12.0 Hz, 1H, 3B-H_B), 2.28 (br dt, J=14.0, 3.5 Hz, 1H, 2A-H_B), 2.45 (br s, 3H, 13-H_3), 2.60 (br d, J=13.0 Hz, 1H, 2′-H_B), 3.09 (t, J=8.5 Hz, 1H, 4′-H), 3.50 (br s, 1H, 4A-H), 3.56 (m, 1H, 5′-H), 3.64 (br s, 1H, 4B-H), 3.86 (br s, 1H, 3′-H), 3.94 (br s, 1H, 3A-H), 3.97 (q, J=6.5 Hz, 1H, 5B-H), 4.50 (br q, J=6.5 Hz,

1H, 5A-H), 4.56 (q, J=6.5 Hz, 1H, 5C-H), 4.89 (br d, J=10.5 Hz, 1H, 1′-H), 4.90 (br s, 1H, 1B-H), 5.09 (br t, J=3.0 Hz, 1H, 1A-H), 5.23 (d, J=3.5 Hz, 1H, 1C-H), 6.09 (d, J=10.5 Hz, 1H, 3C-H), 6.86 (dd, J=10.5, 3.5 Hz, 1H, 2C-H), 7.77 (br s, 1H, 2-H), 7.82 (br d, J=8.0 Hz, 1H, 7-H), 7.84 (br d, J=8.0 Hz, 1H, 8-H), 8.56 (br s, 1H, 12-H), 13.45* (br s, 1H, 10-OH), 14.52* (br s, 1H, 5-OH), 4-H was not detectable, * assignment may be interchanged; ^{13}C NMR (150.8 MHz, $CDCl_3$) see Table 1; HRESI-MS m/z 803.29204 $[M-H]^-$, calculated for $C_{43}H_{47}O_{15}$ and found.

Methanol adduct **12**: Red solid; Rf 0.68 ($CHCl_3$ - MeOH, 9 : 1); ^{13}C NMR (150.8 MHz, $CDCl_3$) see Table 1; HRESI-MS m/z 835.31881 $[M-H]^-$, calculated for $C_{44}H_{51}O_{16}$ and found.

Saquayamycin Z (14)

Orange solid; Rf 0.52 ($CHCl_3$ - MeOH, 9 : 1); $[\alpha]_D^{20}$ −20° (c=0.05, AcCN); UV λ_{max}^{MeCN} nm (log ε) 217 (4.45), 316 (3.64), 429 (3.74); IR ν_{max} (KBr) cm^{-1} 3443, 1700, 1637, 1564, 1441, 1375, 1079, 1013; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.19 (d, J=6.5 Hz, 3H, 6D-H_3), 1.21 (d, J=6.5 Hz, 6H, 6F-H_3, 6H-H_3), 1.23 (d, J=6.5 Hz, 3H, 6B-H_3), 1.27 (d, J=6.5 Hz, 3H, 6A-H_3), 1.32*[1] (d, J=6.5 Hz, 3H, 6E-H_3), 1.33*[1] (d, J=6.5 Hz, 3H, 6G-H_3), 1.37 (d, J=6.5 Hz, 6H, 6′-H_3, 6C-H_3), 1.38 (m, 1H, 2′-H_A), 1.40 (s, 3H, 13-H_3), 1.40 (m, 1H, 2D-H_A), 1.54 (m, 1H, 2F-H_A) 1.57 (m, 1H, 2H-H_A), 1.68 (m, 2H, 2E-H_A, 2G-H_A), 1.70 (m, 1H, 2B-H_A), 1.76 (m, 3H, 3D-H_A, 3F-H_A, 3H-H_A), 1.83 (d, J=15.0 Hz, 1H, 4-H_A), 1.91 (m, 1H, 2A-H_A), 1.93 (m, 1H, 3B-H_A), 1.97 (m, 2H, 3D-H_B, 3F-H_B), 2.02 (m, 3H, 2B-H_B, 2H-H_B, 3H-H_B), 2.08 (m, 1H, 3B-H_B), 2.10 (m, 2H, 2D-H_B, 2F-H_B), 2.25 (br d, J=14.0 Hz, 1H, 4-H_B), 2.26 (m, 2H, 2E-H_B, 2G-H_B), 2.29 (m, 1H, 2A-H_B), 2.49 (d, J=13.0 Hz, 1H, 2-H_A), 2.52 (br dd, J=13.0, 5.0 Hz, 1H, 2′-H_B), 3.06 (m, 1H, 4′-H), 3.08 (m, 2H, 4E-H, 4G-H), 3.17 (d, J=13.0 Hz, 1H, 2-H_B), 3.24 (quint, J=6.5 Hz, 2H, 5E-H, 5G-H), 3.48 (m, 2H, 3E-H, 3G-H), 3.50 (m, 1H, 4A-H), 3.55 (m, 1H, 5′-H), 3.56 (br s, 2H, 4D-H, 4F-H), 3.62 (br s, 1H, 4H-H), 3.65 (br s, 1H, 4B-H), 3.83 (m, 1H, 3′-H), 3.94 (br s, 1H, 3A-H), 3.98 (q, J=6.5 Hz, 1H, 5B-H), 4.08 (q, J=6.5 Hz, 1H, 5D-H), 4.13 (q, J=6.5 Hz, 1H, 5F-H), 4.17 (q, J=6.5 Hz, 1H, 5H-H), 4.46 (br s, 1H, 4a-OH), 4.48 (br d, J=10.0 Hz, 2H, 1E-H, 1G-H), 4.48 (m, 1H, 5A-H), 4.56 (q, J=6.5 Hz, 1H, 5C-H), 4.58 (br s, 1H, 12b-OH), 4.86 (d, J=11.5 Hz, 1H, 1′-H), 4.90 (br s, 1H, 1B-H), 4.94 (br s, 1H, 4F-H), 4.96 (br s, 1H, 4H-H), 5.08 (br t, J=3.5 Hz, 1H, 1A-H), 5.24 (d, J=3.0 Hz, 1H, 1C-H), 5.24*[2] (1H, 1D-H), 6.09 (d, J=10.5 Hz, 1H, 3C-H), 6.44 (d, J=9.5 Hz, 1H, 5-H), 6.88 (dd, J=10.0, 3.0 Hz, 1H, 2C-H), 6.90 (d, J=9.5 Hz, 1H, 6-H), 7.59 (d, J=8.0 Hz, 1H, 11-H), 7.85 (d, J=8.0 Hz, 1H, 10-H), 12.28 (br s, 1H, 8-OH), *[1] assignment may be interchanged, *[2] chemical shift assignment were made on the base of a $^1J_{C-H}$ chemical shift correlation experiment; ^{13}C NMR (150.8 MHz, $CDCl_3$) δ 15.1 (C-6C), 16.0 (C-6A), 16.9*[1] (C-6D), 17.0*[1] (C-6F, C-6H), 17.1*[1] (C-6B), 18.0 (C-6E, C-6G), 18.4 (C-6′), 24.0 (C-2H), 24.3*[2] (C-2B), 24.4*[2] (C-3D), 24.5*[2] (C-3F), 24.6*[2] (C-3B, C-2D), 25.0 (C-2F), 25.3 (C-13), 25.4 (C-3H), 32.1 (C-2A), 36.9*[3] (C-2E), 37.0*[3] (C-2G), 38.6 (C-2′), 44.6 (C-4), 50.1 (C-2), 64.5 (C-5A), 66.6 (C-3A), 67.0 (C-5B, C-4H, C-5H), 67.4*[4] (C-5D), 67.5*[4] (C-5F), 70.5 (C-5C), 70.9 (C-1′), 71.1 (C-3′), 71.7 (C-5E, C-5G), 74.2 (C-5′), 75.5*[5] (C-4F), 75.6*[5] (C-4E, C-4G), 75.8 (C-4D), 76.2 (C-4B), 77.4 (C-12b), 79.0 (C-4A), 79.9 (C-4a), 80.4 (C-3E), 80.7 (C-3G), 82.4 (C-3), 89.3 (C-4′), 92.6 (C-1D), 95.2 (C-1C), 97.1 (C-1F), 97.4 (C-1H), 99.4 (C-1B), 99.9 (C-1A), 101.2*[6] (C-1E), 101.4*[6] (C-1G), 113.8 (C-7a), 117.4 (C-6), 119.6 (C-11), 127.3 (C-3C), 130.4 (C-11a), 133.4 (C-10), 138.1 (C-9), 138.7*[7] (C-6a), 138.8*[7] (C-12a), 142.9 (C-2C), 145.5 (C-5), 157.9 (C-8), 182.1 (C-12), 188.1 (C-7), 196.7 (C-4C), 204.8 (C-1), *[1,*2,*3,*4,*5,*6,*7] assignments bearing the same superscript may be interchanged; HRESI-MS m/z 1465.63952 $[M+Na]^+$, calculated for $C_{73}H_{102}O_{29}Na$ and found.

Methanol adduct **15**: Orange solid; Rf 0.59 ($CHCl_3$ - MeOH, 9 : 1); ^{13}C NMR (150.8 MHz, $CDCl_3$) δ 14.8 (C-6C), 16.0 (C-6A), 16.9*[1] (C-6D, C-6F), 17.1*[1] (C-6H), 17.2 (C-6B), 18.0 (C-6E, C-6G), 18.4 (C-6′), 24.0 (C-2H), 24.4*[2] (C-2B), 24.5*[2] (C-3D, C-3F), 24.6*[2] (C-3B, C-2D), 25.0 (C-2F), 25.2 (C-3H), 25.4 (C-13), 32.1 (C-2A), 36.9*[3] (C-2E), 37.1*[3] (C-2G), 38.6 (C-2′), 39.7 (C-3C), 44.7 (C-4), 50.1 (C-2), 57.0 (C-7C), 64.5 (C-5A), 66.7 (C-3A), 67.0*[4] (C-5H), 67.1*[4] (C-5B, C-4H), 67.4 (C-5D, C-5F), 70.9 (C-1′), 71.1 (C-3′), 71.7 (C-5E, C-5G), 71.9 (C-5C), 74.2 (C-5′), 75.5*[5] (C-4B, C-4F), 75.6*[5] (C-4E, C-4G), 75.8 (C-4D), 77.4 (C-12b), 78.8 (C-2C), 79.0 (C-4A), 79.9 (C-4a), 80.5 (C-3E), 80.8 (C-3G), 82.3 (C-3), 89.5 (C-4′), 92.6 (C-1D), 97.2 (C-1F), 97.5 (C-1H), 99.5 (C-1B), 100.0 (C-1A), 100.5 (C-1C), 101.2*[6] (C-1E), 101.4*[6] (C-1G), 113.9 (C-7a), 117.4 (C-6), 119.6 (C-11), 130.4 (C-11a), 133.4 (C-10), 138.1 (C-9), 138.7*[7] (C-6a), 138.8*[7] (C-12a), 145.5 (C-5), 157.9 (C-8), 182.1 (C-12), 188.1 (C-7), 204.8 (C-1), 207.5 (C-4C), *[1,*2,*3,*4,*5,*6,*7] assignments bearing the same superscript may be interchanged; ESI-MS m/z 1497 $[M+Na]^+$.

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