Amorphane Sesquiterpenes from a Marine *Streptomyces* sp.[⊥,1]

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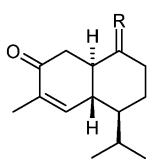
The chemical investigation of the crude extract of the marine-derived *Streptomyces* sp. M491 yielded three new sesquiterpenes, namely, 10α,11-dihydroxyamorph-4-ene (**4**), 10α,15-dihydroxyamorph-4-en-3-one (**6**), and 5α,10α,-11-trihydroxyamorphan-3-one (**7**). In addition, the known compounds 10α-hydroxyamorph-4-en-3-one (**2**), *o*-hydroxyacetanilide, genistein, prunetin, and indole-3-carbaldehyde and the macrolide antibiotic chalcomycin A were identified. The structures were determined on the basis of spectroscopic analysis, especially 1D and 2D NMR data. This is the first report of these sesquiterpenes from bacteria.

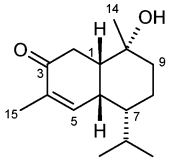
In our ongoing investigation of metabolites from microorganisms, the crude extract of the marine *Streptomyces* sp. M491 from the Qingdao coast (China) exhibited activity against *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus* and a weak inhibition of *Streptomyces viridochromogenes* (Tü 57) in biological screening. Chemical screening indicated nonpolar zones, which showed no significant UV absorption, but turned violet to blue with anisaldehyde/sulfuric acid. Separation of the crude extract by silica gel chromatography led to the isolation of four sesquiterpenes, among which three were new and the fourth was isolated from bacteria for the first time. Additionally, indole-3-carbaldehyde,[2] the isoflavanones genistein[3] and prunetin,[4] *o*-hydroxyacetanilide,[5] and a macrolide, chalcomycin A,[6] were obtained and identified with AntiBase data.[7] The macrolide was responsible for the antibiotic activity of the crude extract.

Only very few sesquiterpenes have been isolated from bacteria so far, e.g., africantriol,[8] 4*S*,7*R*-germacra-1*E*,5*E*-dien-11-ol,[9] cadin-4-en-1-ol,[10] sesquiterpenol,[11] geosmin,[12] methylisoborneol,[13] and pentalenolactones,[14] although they are common metabolites of plants, fungi,[15−18] soft corals, and sponges. Some are inhibitors of leukotriene biosynthesis[16] or show selectivity for human colon tumor cell lines;[19] others are known to be responsible for the earthy or musty off-smells in public water supplies.[11]

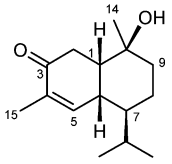
Chromatography of the crude extract and isolation of metabolites were performed as usual (see Experimental Section and Supporting Information). From fraction B_1, a colorless sesquiterpene with the molecular formula $C_{15}H_{24}O_2$ (from HR (+)-ESIMS) was isolated, which had, according to the HSQC and HMBC NMR data, a decalin skeleton. A detailed analysis of the ^{1}H and 2D NMR spectra revealed the alternative structures of a *trans*-fused 10α- or 10β-hydroxycadin-4-en-3-one (**1a/b**) or the *cis*-fused 10α-hydroxyamorph-4-en-3-one (**2**) and 10β-hydroxy-4-muurolen-3-one (**3**), respectively. In the latter two compounds, the olefinic proton (H-5) shows a significant coupling (6.4 Hz) with the bridgehead proton H-6,[20] as it did for the compound discussed here (δ 7.08, dq, J = 6.4, 1.2 Hz), whereas in the *trans*-fused **1a** and **1b**,[21] the olefinic proton appears as a broad singlet.[22] The *cis* orientation was further confirmed by the coupling pattern of H-1, which appeared in methanol as a dt signal with J = 14.7 and 4.5 Hz. Both coupling constants were repeated in the ABX signal of CH_2-2 (in $CDCl_3$, CH_2-2 gives a 2H doublet (J = 10.3 Hz)), so that H-6 must contribute the second small coupling constant for the triplet splitting and stands therefore in a *cis* position with respect to H-1. The strong NOE between H-8 and H-2$_\alpha$ demands a *cis* fusion as well. No NOE was visible between Me-14 and H-11 or between Me-14 and Me-12/13; however, there was a strong signal between Me-14 and H-7, which indicated clearly a *trans* orientation of the methyl and isopropyl group. Our NMR data were identical with values published for 10α-hydroxyamorph-4-en-3-one (**2**) within narrow limits ($\Delta\delta = \pm 0.1$ for ^{1}H, and $\Delta\delta = -0.5$ to -1.1 for ^{13}C) and confirmed the identity with the latter.



1a: R = α-OH, β-Me
1b: R = β-OH, α-Me



2



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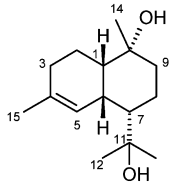

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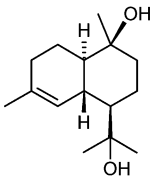

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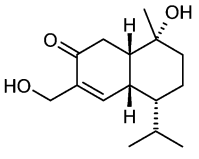
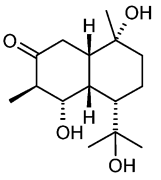


6



7

10α-Hydroxyamorph-4-en-3-one (**2**) is reported here for the first time from microorganisms; however, it was previously isolated from the plant *Taiwania cryptomerioides* Hayata, It exhibited moderate activity in the brine shrimp lethality test.[22]

Compound **4** was isolated as a white solid with the formula $C_{15}H_{26}O_2$ and properties similar to those for **2**. The ^{13}C NMR spectra of **4** and **2** differed by the presence of an additional methylene group and a quaternary carbon signal at δ 76.6 instead of the ketone carbonyl and the isopropyl methine in **2**. The ^{1}H NMR spectrum

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Table 1. ^{1}H NMR Data ($CDCl_3$) of Compounds **2**, **4** (300 MHz), **6**, and **7** (600 MHz)

H	2[a]	4	6	7
1	2.10 (td, 14.7; 4.5 Hz)	1.59 (m)	2.13 (m)	2.07 (m)
2	2.50, 2.31 (ABX, 14.7, 17.3, 4.7 Hz)	1.60 (m)	2.40 (dd, 17.2; 9.6 Hz) 2.38 (dd, 17.2; 2.5 Hz)	2.28, 2.24 (ABX, 12.9, 5.5, $J_{AX} < 1$ Hz)
3		2.03 (m)		
4				2.43 (qui, 7.3 Hz)
5	7.08 (dq, 6.4, 1.2 Hz)	5.75 (dq, 6.2, 1.5 Hz)	7.11 (br d, 6.3 Hz)	3.71 (t, 7.2 Hz)
6	2.72 (m)	2.48 (m)	2.77 (m)	2.68 (m)
7	1.56 (m)	1.48 (m)	1.50 (m)	1.99 (m)
8	1.58 (m)	1.60 (m)	1.20−1.60 (m)	1.56 (m)
9	1.40−1.52 (m)	1.54 (m)	1.20−1.60 (m)	1.56 (m) 1.70 (m)
10				
11	1.90 (m)		1.88 (sept, 6.7 Hz)	
12	0.92 (d, 6.9 Hz)	1.17 (s)	0.92 (d, 6.9 Hz)	1.35 (s)
13	0.92 (d,6.9 Hz)	1.24 (s)	0.90 (d, 6.9 Hz)	1.09 (s)
14	1.14 (s)	1.22 (s)	1.17 (s)	1.17 (s)
15	1.75 (t, 1.2 Hz)	1.67 (s)	4.25, 4.22 (AB, 13.2 Hz)	1.08 (d, 6.5 Hz)

[a] Data measured in MeOH-d_4 at 300 MHz.

showed similarities to that of **2** as well and displayed an olefinic proton signal at δ 5.75 instead of 7.08 in **2**, in agreement with the absent ketone carbonyl. Besides four multiplets of methine and methylene groups in the aliphatic region, four methyl singlets at δ 1.67, 1.22, 1.24, and 1.17 indicated that the isopropyl methine of **2** was substituted. A literature search[23] resulted in the cadinene derivative trichotomol[24] (**5**); however, comparison of the published data with those of **4** showed some shift differences, indicating that our compound may be a diastereomer of **5**.

The relative stereochemistry of **4** could not be fully deduced from the NOESY spectrum due to signal overlap. The fact that the olefinic proton in the cadinene derivative **5** appears as a singlet as in **1a**/**1b** but compound **4** gave a doublet as in **2**/**3** indicated again that it possessed *cis*-fused rings, and an NOE between H-6 and Me-14 indicated their *cis* orientation as well. Thus, terpene **4** is most likely 10α,11-dihydroxyamorph-4-ene (6-epi-trichotomol), which agrees with the negative sign of the optical rotation.

HREIMS of the colorless solid **6** revealed the formula $C_{15}H_{24}O_3$. The ^{13}C NMR spectrum showed again signals of an α,β-unsaturated ketone as in **2**, in addition to four methines, four methylenes instead of three, and three methyl groups instead of four as in **2**. The major difference was the presence of a methylene group connected to oxygen (δ 62.2) and the absence of a methyl signal, which was interpreted as an oxidation of one of the methyl groups of **2**. In the ^{1}H NMR spectrum, the olefinic methyl signal in **2** (δ 1.75) was substituted by an AB signal of an oxygen-bound methylene group (δ 4.25, 4.22), which showed an allylic coupling with the double bond proton in the ^{1}H−^{1}H COSY spectrum. The doublet splitting of H-5 indicated again *cis*-fused rings, which identified compound **6** as the new terpenoid 10α,15-dihydroxyamorph-4-en-3-one.

Compound **7** was isolated as an oil with physical and spectroscopic properties similar to those of **2**, **4**, and **6**. EIMS delivered a mass of 252 Da and (+)-ESIMS confirmed the formula $C_{15}H_{24}O_3$. However, the NMR data of **7** and especially the absence of double-bond signals in the spectra required a formula of $C_{15}H_{26}O_4$, so that a loss of water must have occurred during MS. In addition, the ketone carbonyl signal appeared now at δ 210.7 and an oxymethine proton at δ 3.71 gave a triplet. Instead of a methyl signal at δ ~1.7, a high-field-shifted methyl doublet was located at δ 1.08. The proton spectrum showed three methyl singlets; however, signals due to an isopropyl group were missing as in **4**. The ^{13}C NMR spectrum indicated the presence of 15 carbon signals, i.e., signals of four methyls, three methylenes, five methines, and three quaternary C atoms, including the ketone. The major difference was again the absence of the double-bond signals.

The relative stereochemistry at positions C-4 and C-5 was deduced by the positive NOE of the H-5 signal on Me-15 and H-6. The latter proton showed coupling constants of 6.3, 7.2, and 12.5 Hz, whereas H-5 had a coupling constants of 7.2 and 8.0 Hz, and H-1 had small values as well. The 12.5 Hz coupling indicated therefore a *syn*- or *anti*-periplanar orientation of H-6 and H-7. Irradiation into the signal at δ 2.68 (H-6) showed, however, an NOE on H-1, H-7, and Me-14: **7** was therefore deduced as the new sesquiterpene 5α,10α,11-trihydroxyamorphan-3-one. Compounds **2**, **4**, **6**, and **7** are the first amorphane derivatives isolated from bacteria.

Compounds **2**, **4**, **6**, and **7** were inactive against *Bacillus subtilis*, *Streptomyces viridochromogenes* (Tü 57), *Staphylococcus aureus*, and *Escherichia coli*, the microalgae *Chlorella vulgaris*, *Chlorella sorokiniana*, and *Scenedesmus subspicatus*, and the fungi *Mucor miehei* and *Candida albicans* at a concentration of 80 μg/paper disk, as well as against brine shrimp at a concentration of 10 μg/mL.

Experimental Section

General Experimental Procedures. The optical rotations were measured on a Perkin-Elmer polarimeter (model 241). UV/vis spectra were recorded on a Perkin-Elmer Lambda 15 UV/vis spectrometer. IR spectra were recorded on a Perkin-Elmer 1600 Series FT-IR spectrometer with KBr pellets. NMR spectra were measured on a Varian Inova 600 (599.740 MHz) and a Varian Unity 300 (300.145 MHz) spectrometer. ESI mass spectra were recorded on a Quattro Triple Quadrupol mass spectrometer, with a Finnigan TSQ 7000 with nano-ESI API ion source. ESIHR mass spectra were measured on a Micromass LCT mass spectrometer coupled with a HP 1100 HPLC with a diode array detector. EIMS was performed on a Finnigan MAT95 (70 eV), and perfluorokerosene was used as reference substance in HREIMS. Flash chromatography was carried out on silica gel (230−400 mesh). Thin-layer chromatography (TLC) was performed on Polygram SIL G/UV_{254} (Macherey-Nagel & Co.). R_f values were measured on Polygram SIL G/UV_{254} (Macherey-Nagel & Co.). Size exclusion chromatography was done on Sephadex LH-20 (Pharmacia).

Taxonomy of the Producing Strain. The strain M491 was derived from sediment obtained from Jiaozhou Bay in China. It was isolated on Gause's starch medium with incubation at 28 °C. The pure culture was maintained on Gause's starch agar medium with $K_2Cr_2O_7$ at 4 °C. The strain forms a white vegetative mycelium and a green aerial mycelium. The substrate mycelium does not have transverse septa or fragments. The aerial mycelium has many branches. The strain forms straight sporophores, and the spores are rectangle or square with a smooth surface. Melanin pigment is not produced on tyrosine agar, and water-soluble pigment is not produced on other media. The strain can utilize starch, glucose, and esculin as carbon sources. The strain does not produce pyocyanin and fluorochrome and does not peptonize or coagulate milk. Gelatin is degraded and hydrogen sulfide is not produced. The strain is catalase positive, lipase positive, and nitrate reductase negative. Due to its physiology and morphological features as well as the 16S rRNA (GenBank accession number DQ184668),

Table 2. ^{13}C NMR Data of Compounds **2**, **4** (at 75 MHz), **5** (125 MHz), **6**, and **7** (150 MHz) in $CDCl_3$

C no.	2	4	5[a]	6	7
1	46.9	46.7	49.8	43.1	44.3
2	37.9	20.4	22.7	35.4	39.7
3	201.7	30.9	30.6	200.0	210.7
4	135.7	136.1	134.3	137.3	50.5
5	153.2	124.9	124.7	151.8	83.5
6	36.9	34.1	40.8	37.2	41.0
7	44.4	50.1	53.0	45.4	45.9
8	20.5	24.1	27.1	19.3	21.4
9	34.6	34.7	42.3	34.1	35.0
10	71.7	72.0	72.1	71.2	71.9
11	29.1	76.6	74.2	27.8	82.1
12	21.7	29.9	24.1	21.3	30.0
13	16.1	24.7	32.1	15.7	24.1
14	28.6	29.0	20.7	28.8	28.7
15	16.0	23.5	24.1	62.2	11.4

[a] From ref 24.

the strain can be assigned to the genus *Streptomyces*. The strain is deposited in the culture collection of marine actinomycetes at the Institute of Oceanology, Chinese Academy of Sciences, Nanhai Road 7, 266071 Qingdao, China.

Fermentation of Streptomyces sp. Isolate M491 and Workup. The marine *Streptomyces* sp. M491 was cultivated in 100 × 1 L Erlenmeyer flasks each containing 250 mL of SM medium[25] at 28 °C, while shaking for 5 days on a linear shaker. The culture broth was mixed with ca. 1.5 kg of Celite and filtered under pressure. The water phase was extracted with XAD-16, the resin was washed with distilled water and eluted with methanol, while the mycelium was extracted first with ethyl acetate and then acetone. The extracts were combined and separated on silica gel (column 50 × 3 cm, CH_2Cl_2/MeOH gradient), yielding four fractions, A−D. Fraction A contained fats and was discarded. Purification of fraction B on Sephadex LH-20 (MeOH) gave three subfractions, B_1−B_3. From B_1 and B_2, *o*-hydroxyacetanilide (4.4 mg), indole-3-carbaldehyde (2 mg), and 10α-hydroxyamorph-4-en-3-one (**2**, 3 mg) were obtained after PTLC and HPLC. Fraction B_3 contained prunetin [R_f = 0.60 (CH_2Cl_2/7% MeOH), 1.6 mg]. Fraction C was purified by chromatography on Sephadex LH-20 (CH_2Cl_2/50% MeOH) and PTLC (CH_2Cl_2/5% MeOH) and delivered genistein (25 mg) and 10α,11-dihydroxyamorph-4-ene (**4**, 11 mg). Fraction D was separated on Sephadex LH-20 (CH_2Cl_2/40% MeOH) into subfractions D_1 and D_2. Purification of D_1 by PTLC delivered chalcomycin A (34 mg). Subfraction D_2 was separated on Sephadex LH-20 (MeOH) and by PTLC (CH_2Cl_2/7% MeOH) followed by HPLC (20% MeCN/H_2O) to yield 10α,15-dihydroxyamorph-4-en-3-one (**6**, 1 mg) and 5α,10α,11-trihydroxyamorphan-3-one (**7**, 3 mg).

10α,11-Dihydroxyamorph-4-ene (4): colorless solid, R_f = 0.46 (CH_2Cl_2/5% MeOH); $[\alpha]^{25}_D$ −26.4 (*c* 0.14, $CHCl_3$); IR (KBr) ν_{max} 3406, 2967, 2932, 2361, 2338, 1457, 1376, 1299, 1163, 1021 932, 906 cm^{-1}; NMR data, see Tables 1, 2; (+)-ESIMS *m*/*z* (%) 499 $[2M + Na]^+$ (28), 261 $[M + Na]^+$ (100); DCIMS (NH_3) *m*/*z* (%) 256 $[M + NH_4]^+$ (43), 238 $[M - H_2O + NH_4]^+$ (100), 221 $[M - H_2O + H]^+$ (20), 163 (74); (+)-HRESIMS *m*/*z* 261.1825160 (calcd for $[M + Na]^+$, $C_{15}H_{26}O_2$-Na, 261.182509).

10α,15-Dihydroxyamorph-4-en-3-one (6): colorless solid, R_f = 0.39 (CH_2Cl_2/5% MeOH); $[\alpha]^{25}_D$ −70.0 (*c* 0.07 $CHCl_3$); UV/vis (MeOH) λ_{max} (log ϵ) 237 (3.79); NMR data, see Tables 1, 2; EIMS *m*/*z* (%) 252 (3), 209 (4), 191 (25), 176 (10), 173 (8), 163 (4), 134 (3), 121 (5), 108 (20), 79 (9), 77 (10), 71 (14), 69 (24), 55 (16), 43 (100); (+)-HRESIMS *m*/*z* 253.1798790 (calcd for $[M + H]^+$, $C_{15}H_{25}O_3$, 253.179829).

5α,10α,11-Trihydroxyamorphan-3-one (7): colorless oil, R_f = 0.43 (CH_2Cl_2/5%MeOH); $[\alpha]^{25}_D$ −13.1 (*c* 0.13, $CHCl_3$); IR (KBr) ν_{max} 3438, 2960, 2930, 2369, 1700, 1458, 1385, 1194, 1119, 1045, 908 cm^{-1}; NMR data, see Tables 1, 2; EIMS *m*/*z* (%) 252 (86), 237 (19), 219 (21), 194 (13), 177 (25), 163 (100), 154 (36), 134 (32), 121 (24), 107 (21), 93 (31), 81 (23), 57 (21), 43 (94), 41 (38); DCIMS (NH_3) *m*/*z* (%) 522 $[2M - 2H_2O + NH_4]^+$ (1), 287 (2), 270.2 $[M - H_2O + NH_4]^+$ (100), (+)-HRESIMS *m*/*z* 275.1618800 (calcd for $[M - H_2O + Na]^+$, $C_{15}H_{24}O_3Na$, 275.161774).

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Article No. XXX on Marine Bacteria. For part XXIX, see: Kock, I.; Maskey, R. P.; Biabani, M. A. F.; Helmke, E.; Laatsch, H. *J. Antibiot.* **2005**, *58*, 530−534.
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(25) SM Medium: Defatted soybean powder (20 g) and mannitol (20 g) were dissolved in 500 mL of tap water and 500 mL of artificial sea water, and the medium was adjusted with 2 N NaOH, pH 7.8 and sterilized at 121 °C for 33 min.

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