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BUTANOLIDE, TYROSOL ESTER AND ACETOPHENONE DERIVATIVES FROM TERRESTRIAL *STREPTOMYCES* SPP.

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ABSTRACT

Chemical investigations of the crude extracts from the terrestrial derived streptomycete isolates Ank150, 174, and 179, yielded individually to four new compounds, namely 2-hydroxy-1-(4-hydroxy-3-methoxy-phenyl)-ethanone (1), 1-(3,4-dimethoxy-phenyl)- 2-hydroxy-ethanone (2), acetic acid 2-(4-hydroxy-phenyl)-ethyl ester (5) and N-acetyltyrosol (7). In addition, the polyhydroxybutyric acid (PHB), reductomycin, virginiae butanolide B and tyrosol were identified. The structures of the new compounds 1, 2, 5, and 7 were determined on the basis of spectroscopic analysis (1D and 2D) and mass spectra and comparison with related structures.

KEYWORDS: terrestrial streptomycetes, butanolides, acetophenones, natural products.

INTRODUCTION

Natural products are naturally derived metabolites and/or by-products from microorganisms, plants or animals.[1-5] Microorganisms particulary streptomycetes have been good resources of many diversity of secondary metabolites. Many reported butanolides have been shown to induce antibiotic biosynthesis e.g virginiae.[6-8] Based on traditional medicine, these products have been exploited for human use and represent a rich source of biologically active compounds, a good source of novel and clinically important drugs and are an example of molecular diversity with recognized potential in drug discovery and development.[9-11]

In our going to exploration of metabolite from microorganism, the crude extracts of the terrestrial *Streptomyces* spp. Anke150, 174 and 179, exhibited activity against, *Bacillus*

subtilis, *Escherichia coli*, *Staphylococcus aureus*, *Streptomyces viridochromogenes (Tü57)*, *Mucor miehei and Candida Albicans*, In the chemical screening the extract showed fluorescent (366 nm) and UV absorbing (254 nm) bands, Which turned light green on spraying with anisaldehyde/sulphuric acid reagent, while others turned red. Separation of the obtained crude extracts by silica gel chromatography led to the isolation of four compounds among which one was new (7) and the other three compounds (1, 2 and 5) were isolated from bacteria for the first time. Moreover, 4 known compounds were isolated: polyhydroxybutyric acid (PHB).[12] Virginiae butanolide B.[6-8] reductomycin.[13,14] and tyrosol which was reported to be antibiotically weakly active against *Saccharomyces cerevisiae* (>100), *Nematospora corlyi* (> 100), moderately phytotoxic and antifungal.[15] The structure of the known compounds has been easly identified by sub-structure searches in AntiBase data.[15] and were confirmed by comparison of their masses and NMR spectra with reference data from our collection of spectral data and with the literature.

MATERIALS AND METHODS

General

NMR spectra were measured on Varian Unity 300 and Varian Inova 600 spectrometers. Electron spray ionization mass spectrometry (ESI HRMS): Finnigan LCQ ion trap mass spectrometer coupled with a Flux Instruments (Basel, Switzerland) quaternary pump Rheos 4000 and a HP 1100 HPLC (nucleosil column EC 125/2, 100-5, C 18) with a Diode Array Detector (Finnigan Surveyor LC System). High resolution mass spectra (HRMS) were recorded by ESI MS on an Apex IV 7 Tesla Fourier-Transform Ion Cyclotron Resonance Mass Spectrometer (Bruker Daltonics, Billerica, MA, USA). EI mass spectra were recorded on a Finnigan MAT 95 spectrometer (70 eV), Thermo Electron Corp., Bremen, Germany; with perfluorkerosene as reference substance for EI HRMS. UV-VIS spectra were recorded on a Perkin-Elmer Lambda 15 UV/VIS spectrometer. Flash chromatography was carried out on silica gel (230-400 mesh). R_f-values were measured on Polygram SIL G/UV_{254} (Macherey-Nagel & Co.). Size exclusion chromatography was done on Sephadex LH-20 (Pharmacia).

Fermentation, Extraction and Isolation

A) Terrestrial *Streptomyces* sp. isolate Ank174.

The terrestrial isolate Ank174 was pre-cultivated on M_2 agar plates at 28 °C for 3 days. With pieces of a well-grown agar subculture, a 25 L cultivated in a 25-liter scale on M_2 medium at

28°C for 7 days on a linear shaker (110 rpm). The culture broth was mixed with *ca.* 1.5 kg Celite and filtered under pressure. The water phase was extracted with a XAD-16, the resin washed with 20 L water and eluted with 15 L methanol, while the mycelium was extracted firstly with ethyl acetate (3 times) and then acetone (1 time). Both extracts were combined in the view of TLC, evaporated to dryness affording 1.9 g yellowish-brown crude extract. Chromatography of the obtained crude extract (1.9 g) was performed using silica gel with a MeOH/CH_2Cl_2 gradient delivering four fractions A-D as in (fig. 1). Fraction A was identified as fat acids on the view of TLC and spraying with anisaldehyde/H2SO4 reagent. Purification of fraction B on Sephadex LH-20 (MeOH) and RP18 (30% MeOH) delivered 2-hydroxy-1-(4-hydroxy-3-methoxy-phenyl)-ethanone (1; 3 mg) and 1-(3, 4-dimethoxy-phenyl)-2-hydroxy-ethanone (2; 5 mg). Triturating of fraction C with methanol delivered polyhydroxy butyric acid; PHB (400 mg) as white solid and insoluble material. Chromatography of fraction D using Sephadex LH-20 (MeOH), followed by RP18 afforded virginiae butanolide B (8 mg) as colourless oil; (Figure S1).

B) Terrestrial *Streptomyces* sp. isolate ANK150.

The terrestrial *Streptomyces* strain Ank150 was cultivated in the same way on M_2 medium. The obtained extract (2.5 g) from a 25 L shaker culture delivered by the same treatment four fractions A-D. Fractions A and B are the fats but treatment of fraction C with Sephadex LH-20 (MeOH) followed by purification in RP18 afforded two new compounds: 2-hydroxy-1-(4-hydroxy-3-methoxy-phenyl)-ethanone (1; 5 mg) and acetic acid 2-(4-hydroxy-phenyl)-ethyl ester (**5**; 3 mg). Purification of fraction D delivered tyrosol (4 mg); (Figure S2).

C) Terrestrial *Streptomyces* sp. isolate ANK179.

Similarly, the terrestrial *Streptomyces* strain Anke 179 was cultivated on M_2 medium and the obtained extract (2.1 g) from 25 L culture delivered also four fractions A-D after silica gel column chromatography. Fraction A and B were identified as fats. Fraction C further purified on Sephadex LH-20 (MeOH) followed by RP18 to yield 3-(2-Oxo-tetrahydro-furan-3-yl)-propionic acid (7; 5 mg). Finally, purification of fraction D resulted in the known reductomycin (yellow needles, 120 mg); (Figure S3).

2-Hydroxy-1-(4-hydroxy-3-methoxy-phenyl)-ethanone (1)

Colourless solid, greenish-blue coloration with anisaldehyde/sulfuric acid spraying reagent.

R_f: 0.52 (CH_2Cl_2/3% MeOH). UV/VIS: λ_{max} (log ε) (20 µg/ml MeOH): (MeOH) 301 (3.93), 276 (4.02), 229 (4.17) 204 (4.19); (MeOH/HCl): 302 (3.93), 276 (4.05), 229 (4.16), 204 (4.19); (MeOH/NaOH): 344 (4.32), 327 (4.80), 210 ^{1}H NMR (300 MHz, CD_3OD): see Table 1. ^{13}C NMR (125 MHz, CD_3OD): see Table 1. (-)-ESI MS m/z (%) 385.2 ($[2M-2H+Na]^-$, 100), 181.2 ($[M-H]^-$, 100). ESIHR MS: *m/z* $[M+H]^+$ calcd for $C_9H_{11}O_4$: 183.0651853; found 183.0651660. *m/z* $[M+Na]^+$ calcd for $C_9H_{10}O_4Na$: 205.0471353, found 205.0471060. *m/z* $[M-H]^-$ calcd for $C_9H_9O_4$: 181.05062466, found 181.0505740.

2-Hydroxy-1-(3, 4-dimethoxy-phenyl)-ethanone (2)

Coulourless solid, greenish-blue coloration with anisaldehyde/sulfuric acid spraying reagent. R_f = 0.64 (CH_2Cl_2/3% MeOH). UV/VIS: λ_{max} (log ε) (20 µg/ml MeOH): (MeOH) 301 (3.55), 273(3.68), 226(3.89), 202(3.94); (MeOH/HCl): 300 (3.50), 273 (3.64), 227 (3.85), 202 (3.89); (MeOH/NaOH): 304(3.52), 273 (3.62), 210 (3.74), 207 (3.79) nm. ^{1}H NMR (300 MHz, CD_3OD): see Table 1. ^{13}C NMR (125 MHz, CD_3OD): see Table 1. EI MS (70 eV): *m/z* (%) 196.2 ($[M]^{+\cdot}$, 18), 165.1 (100), 137.1 (8), 79.0 (12), 77.1 (10), 51.0 (10) ESIHR MS: *m/z* $[M+Na]^+$ calcd for $C_{10}H_{12}O_4Na$: 219.0627853; found 219. 0627120.

Acetic acid 2-(4-hydroxy-phenyl)-ethyl ester (5):

Coulourless solid, reddish-brown coloration with anisaldehyde/sulfuric acid spraying reagent. R_f = 0.52 (CH_2Cl_2/3% MeOH). ^{1}H NMR (600 MHz, CD_3OD): see Table 2. ^{13}C NMR (125 MHz, CD_3OD): see Table 2. –(+)-ESI MS *m/z* = 203.1 $[M+Na]^+$.

3-(2-Oxo-tetrahydro-furan-3-yl)-propionic acid (7)

Colourless oil, violet coloration with anisaldehyde/sulfuric acid spraying reagent.
R_f = 0.20 (CH_2Cl_2/3% MeOH). – $[\alpha]_D^{20}$ = 0° (1 mg/1 ml MeOH). UV/VIS: λ_{max} (log ε) (20 µg/ml MeOH): (MeOH) 203 (3.40), 201(3.41); (MeOH/NaOH): 207 (3.33), 205(3.35) ^{1}H NMR (300 MHz, CD_3OD): see Table 2. ^{13}C NMR (125 MHz, CD_3OD): see Table 2. ESI MS *m/z* = 157.3 $[M-H]^-$. HRESI MS: *m/z* $[M-H]^-$ calcd for $C_7H_9O_4$, 157. 050625; found: 157.0506320.

RESULTS AND DISCUSSIONS

Cultivation of the *Stryptomyces* spp. Ank150, 174, and 179 in M_2 medium.[16] at 28 °C in a 25-liter shaker culture scale delivered a yellowish-brown culture broth which was worked up in the usual manner. TLC-directed work-up of the resulting crude extract was separated by a sequence of chromatographic steps (fig. 1-3).

Compound **1** was isolated as UV absorbing (254 nm), blue fluorescence (366 nm) colourless solid which turned to greenish-blue on spraying with anisaldehyde/sulphuric acid reagent. The ESI MS of compound **1** indicated a molecular weight of *m/z* 182, and ESI HRMS confirmed the molecular formula as $C_9H_{10}O_4$ entailing five double bond equivalences.

The UV spectra (MeOH) of **1** displayed four strong bands at λ_{max} = 204, 229, 277 and 302 nm in neutral solution. Under basic methanol conditions, the latter band showed a bathochromic shift to λ_{max} = 344 nm. The ^{1}H and ^{13}CNMR data were listed in Table 1. The ^{1}H NMR spectrum in CD_3OD exhibited a doublets of two ortho-coupled protons each of 1H at δ 7.50 (dd, *J* = 8.2, 2.0 Hz) and 6.85 (d, *J* = 8.2 Hz) along with a meta-coupled proton at δ 7.52 (d, *J* = 2.0 Hz) of 1, 2, 4-tri-subistituted benzene ring. In the high field region, it showed a resonance of two oxygenated groups, one of 2H for oxy-methylene group at δ 4.83, the remaining one of 3H for the methoxy group at δ 3.90 was displayed. Furthermore, the ^{1}HNMR spectrum displayed two exchangeable proton signals each of 1H, one as triplet for OH at δ 3.54, and broad singlet at δ 6.14 when the ^{1}HNMR spectrum was measured in $CDCl_3$. The ^{13}C NMR and HMQC spectrum of **1** indicated the presence of 9 carbon signals, of which a carbonyl group at δ 198.5, six sp^2 carbons of the benzene ring (two oxygenated carbons which were down field C-3 at δ 149.5 and C-4 at δ 154.6), three methine carbons C-2, C-5 and C-6 at δ 111.4, 116.2 and 124.0 respectively, and the last carbon C-1 at δ 127.2 which directly attached to carbonyl group. Additionally, in the sp^3 region displayed signals of oxy-methylene and oxy-methyl groups at δ 65.8 and 56.4 respectively. From the reveled spectral data, there are three structure possibilities 1, 3 and 4. Subjecting the compound for HMBC measurement, a ^{3}J correlation from the methoxy group (δ 3.90) to carbon-3 (δ 149.5) and also a ^{3}J correlation was observed from the doublet proton of C-5 (δ_H 6.85) to C-3 (δ 149.5) were observed, so structure 3 was excluded. On the other hand, structure 4 was also excluded because there was a ^{3}J correlation from H-2 (δ_H 7.52) and H-6 (δ_H 7.50) with the carbonyl carbon (δ_C 198.5). Furthermore, a correlation was observed from the methylen protons to the carbonyl carbon. This finally confirmed the structure of 1 as 2-hydroxy-1-(4-hydroxy-3-methoxy-phenyl)-ethanone, Figure 2.

A second new compound 2 with a lower polarity than that of 1 was obtained as colorless solid, showing the same color reaction as acetophenone derivative 1. The molecular weight was deduced by EI MS as *m/z* 196, and HRESI MS resulted in the molecular formula

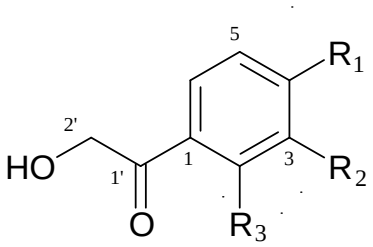
$C_{10}H_{12}O_4$ with Δm of 14 au from compound 1. The ^{1}H and ^{13}C NMR spectrum of 2 (Table 1) displayed a pattern as in 1, except in the up field region, it displayed an additionally methoxy group at δ 3.90 (δ_C 56.5). The OH group in case of compound 1 could be methylated in compound 2. A complete 2D correlation confirming structure 2 as 1-(3, 4-dimethoxy-phenyl)-2-hydroxy-ethanone. Figure 3.

Compound 5 was obtained as colourless solid from fraction III of strain ANK150 after further fractionation on Sephadix LH20 (MeOH) and finally purified on RP-18 (MeOH/H_2O). It showed a reddish-brown coloration when exposed to anisaldehyde/H_2SO_4 spraying reagent. The molecular weight of 5 was deduced by ESI MS as *m/z* 180, with the molecular formula of $C_{10}H_{12}O_3$ entailing five double bond equivalences. The ^{1}HNMR spectrum displayed two ortho-coupled proton each of 2H doublets at δ 7.03 (*J* = 8.5 Hz) and 6.70 (*J* = 8.5 Hz) in the aromatic region, indicating the presence of 1, 4-disubistituted benzene ring. In the aliphatic region, it showed a resonance of two triplet methylens at δ 4.18 (*J* = 7.1 Hz) of oxy-methylen and 2.81 (*J* = 7.0 Hz), which could be connected to sp^2 carbon. Additionally, a singlet of 3H for methyl signals was observed at δ 1.96 with a typical sp^2 connection. Furthermore, the ^{13}C NMR and HMQC spectrum delivered signals for one carbonyl group at δ 172.9, 6 sp^2 carbons in the range of δ 157.1-116.2 of which an oxygenated one at δ 157.1 for a benzene ring, oxy-methylene at δ 66.6, further CH_2 group at δ 35.2 along with a methyl carbon at δ 20.8. According two the above spectral data, two possible structures 5 and 6 for compound 5 were delivered. Based on HMBC experiments (Figure 4), structure 6 was excluded, according to the observed ^{3}J correlation from CH_2-2' to the carbonyl signal C-4' (172.9). Finally, the structure of **5** was confirmed as acetic acid 2-(4-hydroxy-phenyl)-ethyl ester.

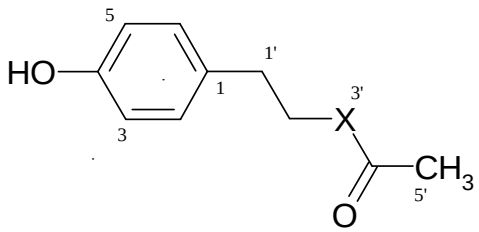
In addition to compounds 1, 2 and 5, compound 7 was obtained from the extracts of *Streptomyces* sp. isolate Ank179 as colorless non-UV absorbing oil with medium polarity, which turned violet with anisaldehyde/sulfuric acid on TLC. ESI MS delivered a molecular weight of *m/z* 158, and ESI HRMS established the molecular formula as $C_7H_{10}O_4$. The ^{1}H and ^{13}C NMR spectra showed an oxy-methylene group (δ_H 4.35 ddd, 4.20 m; δ_C 68.2) and one methine multiplet (δ_H 2.68; δ_C 39.8). Additionally, one methylene triplet was observed at δ 2.44 (*J* = 7.6 Hz). Finally, two methylene multiplets were observed in the regions of δ 2.43-1.72. The ^{13}C NMR and HMQC spectra confirmed these groups and showed in addition two carbonyl signals at δ_C 181.7 and 177.0 of an acids, esters and/or amides. According to three double bond equivalents and only one methine carbon, the compound must be a lactone.

The structure was completely assigned by 2D couplings (Figure 5). The oxy-methylene, multiplets methylenes and triplet one of 7 showed cross signals confirming the partial structure, [-OCH_2-CH_2-CH-CH_2-CH_2-CO-] in the H^1-H^1-COSY and HMBC experiments. The oxy-methylene (H_2-4) displayed a ^3J coupling to the carbonyl signal at δ_C 177.0, confirming the attachment *via* the methine carbon C-2 (δ 39.8). The chemical shift of methine carbon (δ_C 39.8) and the methylene one (δ_C C 32.7) confirming their attachments at an sp^2 carbon which in this case the carbonyl one Correlation from H_2-2' to C-3' and C-2, from H_2-1' to C-3', C-1 and C-3, from H_2-3 to C-2, C-4, C-1' was detected. Finally, a coupling from H_2-4 to C-1 was observed, confirming the lactonization form of compound 7. The missing correlation between the methine and the carbonyl was also found in other γ-lactones.[17,18] This established compound **7** as 3-(2-oxo-tetrahydro-furan-3-yl)-propionic acid.

Figure Legends.



5, X = O,



7, X = N

1 R_1 = OH, R_2 = OCH_3, R_3 = H

2 R_1 = OCH_3, R_2 = OCH_3, R_3 = H

3 R_1 = OCH_3, R_2 = OH, R_3 = H

4 R_1 = OH, R_2 = H, R_3 = OCH_3

Figure 1: structure of the isolated compounds.

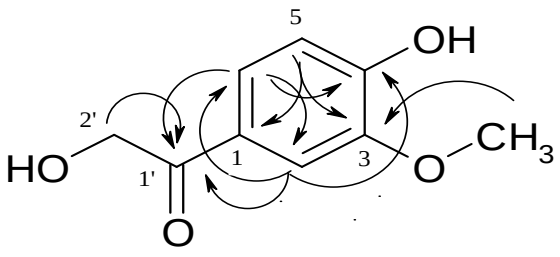


Figure 2: Selected HMBC (→) correlations of 2-hydroxy-1-(4-hydroxy-3-methoxy-phenyl)-ethanone (1).

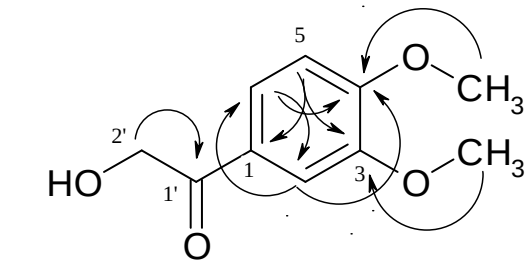
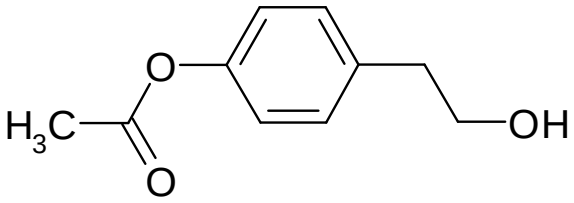


Figure 3: Selected HMBC (→) correlations of 1-(3,4-dimethoxy-phenyl)-2-hydroxy-ethanone (2).



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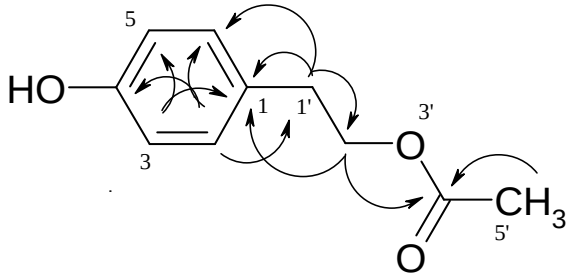

Figure 4: Selected HMBC (→) acetic acid 2-(4-hydroxy-phenyl)-ethyl ester (5).

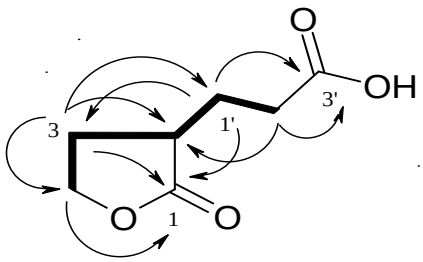

Figure 5: ^{1}H-^{1}H COSY (▬) and HMBC (→) couplings of 3-(2-Oxo-tetrahydro-furan-3-yl)-propionic acid (7).

Table 1: ^{1}H and ^{13}C NMR assignments of compounds 1 and 2 (*J* in Hz).

Position	Compound (1)			Compound (2)	
	δ_C [a,b]	δ_H [a,c]	δ_H [c,d]	δ_C [a,b]	δ_H [a,c]
1	127.2	-	-	128.6	-
2	111.4	7.52 (d, 2.0)	7.54 (d, 2.1)	111.3	7.52 (d, 2.0)
3	149.5	-	-	150.7	-
3-OCH_3	56.4	3.90 (s)	3.98 (s)	56.5	3.87 (s)
4	154.6	-	-	155.5	-

4-OCH_3	-	-	-	56.5	3.90 (s)
4-OH	-	-	6.14 (brs)	-	-
5	116.2	6.85 (d, 8.2)	6.98 (d, 8.2)	111.9	7.03 (d, 8.4)
6	124.0	7.50(dd, 8.2, 2.0)	7.45 (dd, 8.3, 2.0)	123.6	7.60 (dd, 8.4, 2.0)
1’	198.5	-	-	198.8	-
2’	65.8	4.83 (s)	4.83 (d, 4.7)	66.0	4.85 (s)
2’-OH	-	-	3.54 (t, 4.6)	-	-

[a]CD_3OD; [b](125 MHz); [c](300 MHz); [d]($CDCl_3$);.

Table 2: ^1H and ^{13}C NMR assignments of 5 and 7 in CD_3OD ((*J* in Hz).

No.	Compound 5		No.	Compound 7	
	δ_C[a,b]	δ_H[a,b]		δ_H[c,d]	δ_C[a,b]
1	129.9	-	1	181.7	-
2, 6	130.9	7.03 (d, 8.5)	2	39.8	2.68 (m)
3, 5	116.2	6.70 (d, 8.5)	3	29.6	2.43 (m, H_a), 1.96 (m, H_b)
4	157.1	-	4	68.2	4.35 (ddd, 17.5, 8.7, 2.5, H_a) 4.20 (m, H_b)
1'	35.2	2.81 (t, 7.0)	1'	26.8	2.10 (m, H_a), 1.72 (m, H_b)
2'	66.6	4.18 (t, 7.1)	2'	32.7	2.44 (t, 7.6)
4'	172.9	-	3'	177.0	-
5'	20.8	1.96 (s)	-	-	-

[a](125 MHz); [b](600 MHz); [c](300 MHz).

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