2-Alkyl-3,4-dihydroxy-5-hydroxymethylpyridine Derivatives: New Natural Vitamin B_6 Analogues from a Terrestrial *Streptomyces* sp.

Rajendra P. Maskey[a], Felix Huth[a], Iris Grün-Wollny[b], and Hartmut Laatsch[a]

[a] Department of Organic and Biomolecular Chemistry, University of Göttingen, Tammannstraße 2, D-37077 Göttingen, Germany
[b] Labor Grün-Wollny, Versaillerstraße 1, D-35394 Gießen, Germany

Reprint requests to Prof. Dr. H. Laatsch. Fax: +49(0)551- 399660. E-mail: hlaatsc@gwdg.de

Z. Naturforsch. **60b,** 63 – 66 (2005); received June 3, 2004

Dedicated to Prof. Hiriyakkanavar Ila on the occasion of her 60th birthday

The ethyl acetate extract of the strain *Streptomyces* sp. GW23/1540 has yielded four new 2-alkyl-5-(hydroxymethyl)pyridine-3,4-diols, 5-hydroxymethyl-2-isopropyl-pyridine-3,4-diol (**1a**), 5-hydroxymethyl-2-propyl-pyridine-3,4-diol (**1b**), 2-*sec*-butyl-5-hydroxymethyl-pyridine-3,4-diol (**1c**), and 5-hydroxymethyl-2-isobutyl-pyridine-3,4-diol (**1d**). Similarly, the strain *Streptomyces* sp. GW63/1571 afforded 2-*sec*-butyl-5-hydroxymethyl-pyridine-3,4-diol (**1c**) and another new natural product, (3a*S*, 7a*R*)-3a-hydroxy-3a,4,7,7a-tetrahydro-1-benzofuran-2(3*H*)-on e (**3**), together with anthranilic acid, anthranilamide, and phenylacetamide. The new natural products were inactive against three micro algae, the fungus *Mucor miehei*, the yeast *Candida albicans*, and the bacteria *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Streptomyces viridochromogenes*.

Key words: Streptomyces sp., Pyridine Derivatives

Introduction

About 300 simple pyridine derivatives are known as metabolites from bacteria and fungi [1]. Two thirds of them exhibit molecular weights of less than 500, and their inherent polarity is increased in most cases by hydroxy and carboxy groups. Important examples are the nikkomycins [2], the α-alkylpyridines with unsaturated side chain (*e.g.* piericidins [3]) and the α,α-bipyridyls (collismycins [4], caerulomycins, *etc.*). Biological activities are very widespread and include antibiotics, insecticides, enzyme inhibitors, and antitumor drugs. Vitamin B_6 (**2**) is a pyridine derivative of general importance.

In our search for bioactive microbial metabolites, we isolated recently various new quinazolinones and several simple acids and amides from the terrestrial *Streptomyces* sp. GW63/1571 [5]. In addition we have now identified from the same strain and a related *S.* sp. GW23/1540 a number of weakly UV absorbing polar compounds, namely 5-hydroxymethyl-2-isopropyl-pyridine-3,4-diol (**1a**), 5-hydroxymethyl-2-propyl-pyridine-3,4-diol (**1b**), 2-*sec*-butyl-5-hydroxymethyl-pyridine-3,4-diol (**1c**) and 5-hydroxymethyl-2-isobutyl-pyridine-3,4-diol (**1d**). In this paper, we report the isolation and structure elucidation of these new pyridine derivatives, and of the benzofuranone **3**.

Results and Discussion

The pyridine derivatives were observed on TLC as very weakly UV absorbing bands which drew our attention because of a violet to red colour reaction with anisaldehyde/sulphuric acid. In contrast to the similarly reacting indole derivatives [6] and phenylalkyl amides [7], some of them being isolated from the same strain, the violet to red colouration did not change even after standing over night on silica gel. The compounds **1a/1b** and **1c/1d** were obtained from the strain GW23/1540 as mixtures and were not separable by column chromatography and PTLC on silica gel, or HPLC on RP18. The strain GW63/1571, however, produced **1c** free of isomers which facilitated the structure elucidation.

The EI and ESI mass spectra of the mixture **1a/1b** showed the molecular weight to be $m/z = 183$ and that of **1c/1d** to be $m/z = 197$. EI HRMS of the molecular peaks indicated the molecular formulae $C_9H_{13}NO_3$ and $C_{10}H_{15}NO_3$ for **1a/1b** and **1c/1d**, respectively. The

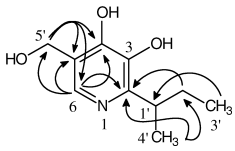
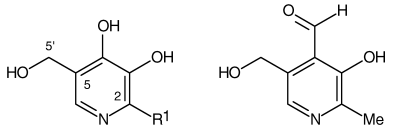


Fig. 1. Structure of **1b** derived by H,H COSY (not shown), HMQC (not shown) and HMBC (→) couplings.

proton NMR spectra of the mixtures of **1a/1b** and **1c/1d** and of pure **1c** showed only one low field shifted aromatic singlet at $\delta = 7.4$ (in acetone), respectively, indicating the compounds to possess an electron deficient aromatic system. They further depicted three acidic proton signals between $\delta = 4.9$ and 7.9. The ^{13}C NMR spectra of **1a/1b** and **1c/1d** showed five sp^2 carbon signals and the residual carbon atoms were aliphatic. The interpretation of the 2D NMR couplings (Fig. 1) of pure **1c** gave the final structure. The structure of the mixtures **1a/1b** and **1c/1d** were determined by comparison of the NMR data with those of **1c** and by interpretation of their 2D correlation spectra.



1a: R^1 = *iso*-Pr
1a: R^1 = *n*-Pr
1b: R^1 = *sec*-Bu
1c: R^1 = *iso*-Bu

2

The 2-alkyl-5-hydroxymethyl-pyridine-3,4-diols (**1a**–**d**) show some similarity with the structure of vitamin B_6, which was reported first from rice bran and yeast [8, 9] and acts as a cofactor in protein metabolism. 2-Alkyl-5-hydroxymethyl-pyridine-3,4-diols (**1a**–**d**) are rare in nature. A related compound is 2-methyl-5-hydroxymethyl-pyridine-3,4-diol (**2**), a synthetic product with anticoccidial activity [10]. The isolated compounds did not exhibit antialgal, antifungal or antibacterial activity in the agar diffusion test at concentrations of 40 μg/paper disk.

Compound **3** was obtained as a colourless solid and was neither detectable under UV light nor gave it a colour reaction with anisaldehyde/sulphuric acid. EI HRMS of the molecular peak at $m/z = 154$ indicated the molecular formula $C_8H_{10}O_3$. The proton NMR spectrum displayed a 2H multiplet at $\delta = 5.67$, which was assigned to olefinic protons. An OH signal at $\delta = 5.47$, a doublet of doublet of a methine group connected to oxygen at $\delta = 4.42$ and various multiplets were detected between $\delta = 2.60$ and 2.15. The ^{13}C NMR spectrum depicted eight signals as required

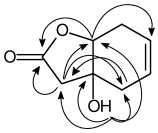
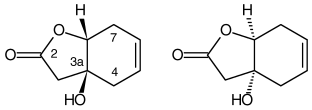


Fig. 2. Structure of **3** derived by H,H COSY (not shown), HMQC (not shown) and HMBC (→) couplings.

by the molecular formula. The signal at $\delta = 175.2$ was assigned to a carbonyl carbon of a carboxylic acid, an ester or a lactone. Signals at $\delta = 125.0$ and 123.8 belong to methine carbon atoms of a double bond. A methine carbon at $\delta = 82.7$ and a quaternary carbon at $\delta = 73.0$ must be connected to oxygen atoms due to their chemical shift. In addition, 3 methylene signals were identified. By interpretation of the ^{1}H, ^{13}C and 2D NMR data, the structure **3** was derived (Fig. 2).

For stereochemical reasons, the annellation between a five- and a six-membered ring as in (3a*S*, 7a*R*)-3a-hydroxy-3a,4,7,7a-tetrahydro-1-benzofuran-2(3*H*)-one (**3**) can only be *cis*-configured. This was confirmed by the clear NOE enhancement between the OH proton at $\delta = 5.47$ and the methine proton 7a-H at $\delta = 4.42$. The absolute stereochemistry of **3** was determined by comparison of the calculated CD spectrum with that of the measured one. The CD spectrum of the stable conformer of **4** was calculated at 0 K in the gas phase using *ab-initio*-methods [11] without taking solvent effects into account. The calculated CD spectrum showed a positive Cotton effect in the short UV region, while the measured spectrum displayed a negative Cotton effect starting at *ca.* 200 nm. We conclude that the compound under investigation must be the enantiomer of **4**, *i. e.* **3**.



3 **4**

The skeleton of **3** is often incorporated in complex natural products like longilactone [12] or saluenolide A [13]. 5,7-Dibromo-3a-hydroxy-6-methoxy-3a,7a-dihydro-3*H*-benzofuran-2-one isolated from a sponge [14], has the same carbon skeleton as **3**. Phyllanthurinolactone [15] is a glycoside of similar structure which is a leaf-movement factor of a nyctinastic plant, *Phyllanthus urinaria* L.

Experimental Section

General methods and materials have been described earlier [6]. M_2 medium: Malt extract (10 g), yeast extract (4 g)

and glucose (4 g) were dissolved in 1 l tap water. Before sterilisation, the pH was adjusted to 7.8.

Taxonomic studies on the producing strains

The taxonomic description and fermentation of the strain GW23/1540 have been discussed previously [5]. The actinomycete strain GW63/1571 was obtained from the strain collection of bioLeads in Heidelberg, Germany, and was gram-positive, non-acid fast, grew aerobically, and differentiated into substrate and aerial mycelium. The sparse aerial mycelium showed short straight hyphae with no particular morphological features. The aerial spore mass colour was light brown on yeast extract-malt agar, and oatmeal agar. The substrate mycelium was dark brown on all media. A brown diffusible pigment was formed on yeast extract-malt extract agar, and on soil extract agar. Melanin pigments were produced on tyrosine agar.

Fermentation of strain GW63/1571 and isolation of the metabolites

The strain GW 63/1571 was inoculated on 10 agar plates with M_2 medium, which were then incubated at 28 °C for 72 h. Those agar cultures were used to inoculate 60 one-litre Erlenmeyer flasks each with 160 ml of the M_2 medium and shaken at 28 °C for three days at 120 rpm. The culture broth was extracted using our standard method [7] to yield 1.6 g of brown crude extract.

The extract was dissolved in MeOH (50 ml) and defatted by extracting with cyclohexane (2×50 ml). The methanolic phase was then separated on Sephadex LH 20 (3×70 cm, MeOH) into four fractions. Purification of fraction 2 and 4 by column chromatography (silica gel), PTLC and HPLC delivered phenylacetamide, 2-aminobenzamide, anthranilic acid, 2-*sec*-butyl-5-hydroxymethyl-pyridine-3,4-diol (**1c**) and (3a*S*, 7a*R*)-3a-hydroxy-3a,4,7,7a-tetrahydro-1-benzofuran-2(3*H*)-one (**3**).

*2-sec-Butyl-5-hydroxymethyl-pyridine-3,4-diol (**1c**)*

Colourless solid, $R_f = 0.45$ (CH_2Cl_2/10% MeOH). – ^{1}H NMR ([D$_6$]-DMSO, 300 MHz): $\delta = 7.83$ (s br, H/D exchangeable, 1 H, OH), 7.50 (s, 1 H, 6-H), 7.14 (s br, H/D exchangeable, 1 H, OH), 5.66 (s br, H/D exchangeable, 1 H, OH), 4.39 (s, 2 H, 5'-H$_2$), 3.51 (sext, $^3J = 7.2$ Hz, 1 H, 1'-H), 1.56 (pent, $^3J = 7.2$ Hz, 2 H, 2'-H$_2$), 1.15 (d, $^3J = 7.2$ Hz, 3 H, 4'-H$_3$), 0.76 (t, $^3J = 7.2$ Hz, 3 H, 3'-H$_3$). – ^{13}C NMR ([D$_6$]-DMSO, 125.7 Hz): $\delta = 165.0$ (C$_q$-3), 163.5 (C$_q$-2), 138.8 (CH-6), 123.1 (C$_q$-4), 115.9 (C$_q$-5), 53.9 (CH$_2$-5'), 32.6 (CH-1'), 27.9 (CH$_2$-2'), 18.5 (CH$_3$-4'), 11.7 (CH$_3$-3'). – MS (EI, 70 eV): *m/z* (%) = 197 (6) [M$^+$], 164 (100), 150 (31), 116 (14), 91 (13), 84 (16), 57 (18), 44 (22). – MS (DCI, NH$_3$): *m/z* (%) = 215 (31) [M+NH$_4^+$], 198 (50) [M+H$^+$], 102 (100). – HRMS (EI): $m/z = 197.1052$ (calcd. 197.1051 for $C_{10}H_{15}NO_3$).

*(3aS,7aR)-3a-Hydroxy-3a,4,7,7a-tetrahydro-1-benzofuran-2(3H)-one (**3**)*

Colourless solid. – UV/vis (MeOH): end absorption; IR (KBr): $\nu = 3550 - 3200$, 2925, 2850, 1780, 1650, 1205, 1075, 1017 cm^{-1}. – $[\alpha]_D^{20} = -17^\circ$ (c 0.0255, MeOH). – ^{1}H NMR ([D$_6$]-DMSO, 300 MHz): $\delta = 5.67$ (m, 2 H, 5-H, 6-H), 5.47 (s br, H/D exchangeable, 1 H, OH), 4.42 (dd, $^3J = 7.0$, 5.0 Hz, 1 H, 7a-H), 2.60 (d, $^2J = 16.0$ Hz, 1 H, 3-H$_\alpha$), 2.56 (m, 1 H, 7-H), 2.41 (d, $^2J = 16.0$ Hz, 1 H, 3-H$_\beta$), 2.39 (m, 1 H, 4-H), 2.24 (m, 1 H, 4-H), 2.15 (m, 1 H, 7-H). – ^{13}C NMR ([D$_6$]-DMSO, 125.7 Hz): $\delta = 175.2$ (C$_q$-2), 125.0 (CH-5), 123.8 (CH-6), 82.7 (CH-7a), 73.0 (C$_q$-3a), 42.3 (CH$_2$-3), 34.5 (CH$_2$-4), 28.8 (CH$_2$-7). – MS (EI, 70 eV): *m/z* (%) = 154 (20) [M$^+$], 136 (11), 125 (7), 100 (100), 72 (27), 55 (22). – HRMS (EI): $m/z = 154.0629$ (calcd. 154.0629 for $C_8H_{10}O_3$).

The isolation of the following compounds from strain GW23/1540 has been described previously [5].

*5-Hydroxymethyl-2-isopropyl-pyridine-3,4-diol (**1a**) and 5-hydroxymethyl-2-propyl-pyridine-3,4-diol (**1b**)*

Colourless solid. – IR (KBr): $\nu = 3360$, 3190, 2978, 2936, 2877, 1666, 1618, 1558, 1426, 1386, 1246, 1126, 1042, 1005, 987, 967, 769, 708, 570 cm^{-1}. – ^{1}H NMR ([D$_6$]-acetone, 300 MHz): $\delta = 7.94$ (s br, H/D exchangeable, 2 H, **1a**, **1b**), 7.40 (s, 2 H, 6-H, **1a**, **1b**), 6.59 (s br, H/D exchangeable, 2 H, OH, **1a**, **1b**), 4.97 (s br, H/D exchangeable, 2 H, OH, **1a**, **1b**), 4.54 (s, 4 H, 4'-H$_2$, **1a**, **1b**), 3.82 (hept, $^3J = 7.1$ Hz, 1 H, 1'-H, **1a**), 2.93 (t, $^3J = 7.5$ Hz, 2 H, 1'-H$_2$, **1b**), 1.65 (sext, $^3J = 7.5$ Hz, 2 H, 2'-H$_2$, **1b**), 1.20 (d, $^3J = 7.1$ Hz, 6 H, 2'-H$_3$, 4'-H$_3$, **1a**), 0.90 (t, $^3J = 7.5$ Hz, 3 H, 3'-H$_3$, **1b**). – ^{13}C NMR ([D$_6$]-acetone, 75.5 Hz): $\delta = 166.4$ (C$_q$-3, **1a**), 166.3 (C$_q$-3, **1b**), 166.2 (C$_q$-2, **1a**), 162.3 (C$_q$-2, **1b**), 139.7 (CH-6, **1b**), 139.6 (CH-6, **1a**), 124.6 (C$_q$-4, **1a**), 124.3 (C$_q$-4, **1b**), 115.6 (C$_q$-5, **1a**), 115.6 (C$_q$-5, **1b**), 55.4 (CH$_2$-4', **1a**), 55.4 (CH$_2$-4', **1b**), 29.9 (CH$_2$-1', **1b**), 27.4 (CH-1', **1a**), 22.1 (CH$_2$-2', **1b**), 21.1 (CH$_2$-2', C-3', **1a**), 14.0 (CH$_3$-3', **1b**). – MS ((+)-ESI): *m/z* (%) = 389 (100) [2M+Na$^+$], 206 (24) [M+Na$^+$]. – HRMS (ESI): $m/z = 184.09678$ (calcd. 184.09736 for $C_9H_{14}NO_3$), 206.09876 (calcd. 206.07931 for $C_9H_{13}NO_3Na$).

*2-sec-Butyl-5-hydroxymethyl-pyridine-3,4-diol (**1c**) and 5-hydroxymethyl-2-isobutyl-pyridine-3,4-diol (**1d**)*

Colourless solid. – IR (KBr): $\nu = 3345$, 3158, 2966, 2930, 2869, 1682, 1624, 1596, 1561, 1461, 1420, 1380, 1295, 1126, 1055, 1007, 978, 794, 760, 722, 617, 565 cm^{-1}. – ^{1}H NMR ([D$_6$]-acetone, 300 MHz): $\delta = 7.96$ (s br, H/D exchangeable, 2 H, OH **1c**, **1d**), 7.41 (s, 2 H, 6-H, **1c**, **1d**), 6.66 (s br, H/D exchangeable, 2 H, OH, **1c**, **1d**), 5.07 (s br, H/D exchangeable, 2 H, OH, **1c**, **1d**), 4.54 (s, 4 H, 5'-H$_2$,

1c, **1d**), 3.65 (m, 1 H, 1'-H, **1c**), 2.85 (t, $^3J = 7.2$ Hz, 2 H, 1'-H_2, **1d**), 1.61 (m, 3 H, 2'-H, 2'-H_2, **1c**, **1d**), 1.18 (d, $^3J = 7.1$ Hz, 3 H, 4'-H_3, **1c**), 0.88 (d, $^3J = 6.6$ Hz, 6 H, 3'-H_3, 4'-H_3, **1d**), 0.79 (t, $^3J = 7.4$ Hz, 3 H, 3'-H_3, **1c**). – ^{13}C NMR ([D_6]-acetone, 75.5 Hz): $\delta = 166.5$ (C_q-3, **1c**), 166.5 (C_q-3, **1d**), 165.5 (C_q-2, **1c**), 1621.8 (C_q-2, **1d**), 139.7 (CH-6, **1c**), 139.7 (CH-6, **1d**), 124.6 (C_q-4, **1d**), 124.2 (C_q-4, **1c**), 117.5 (C_q-5, **1d**), 116.9 (C_q-5, **1c**), 55.3 (CH_2-5', **1c**), 55.3 (CH_2-5', **1d**), 36.6 (CH_2-1', **1d**), 34.0 (CH-1', **1c**), 29.1 (CH_2-2', **1c**), 28.9 (CH_2-2', **1a**), 22.6 (CH_3-3', C-4', **1d**), 19.0 (CH_3-4', **1c**), 12.2 (CH_3-3', **1c**). – MS ((+)-ESI): *m/z* (%) = 417 (100) [2M+Na^+], 220 (27) [M+Na^+]. – HRMS (ESI): $m/z = 198.11245$ (calcd. 198.11301 for $C_{10}H_{16}NO_3$), 220.09446 (calcd. 220,09496 for $C_{10}H_{15}NO_3Na$).

Acknowledgements

We thank H. Frauendorf and R. Machinek for the spectral measurements, L. Lissy and N. Sieck for assistance in microbiological work and isolation. This investigation was financed by a grant from the Bundesministerium für Bildung und Forschung (BMBF, grant 03F0346A).

[1] H. Laatsch, AntiBase 2003, A Natural Products Database for Rapid Structure Determination. Chemical Concepts, Weinheim 2003; see Internet http://www.gwdg.de/ ucoc/laatsch/.
[2] H. Hagenmaier, A. Keckeisen, W. Dehler, H. P. Fiedler, Liebigs Ann. Chem. 1018 (1981).
[3] H. Nishioka, T. Sawa, Y. Takahashi, H. Naganawa, M. Hamada, T. Takeuchi, J. Antibiot. **46**, 564 (1993).
[4] M. Stadler, F. Bauch, T. Henkel, A. Mühlbauer, H. Müller, F. Spaltmann, K. Weber, Arch. Pharm. **334**, 143 (2001).
[5] R. P. Maskey, M. Shaaban, I. Grün-Wollny, H. Laatsch, J. Nat. Prod. **67**, 1131 (2004).
[6] R. Veluri, I. Oka, I. Wagner-Döbler, H. Laatsch, J. Nat. Prod. **66**, 1520 (2003).
[7] R. P. Maskey, R. N. Asolkar, E. Kapaun, I. Wagner-Döbler, H. Laatsch, J. Antibiot. **55**, 643 (2002).
[8] P. György, J. Am. Chem. Soc. **60**, 983 (1938).
[9] O. Isler, G. Brubacher, S. Ghisla, B. Kräutler, Vitamine II, p. 193ff., Georg Thieme Verlag, Stuttgart (1988).
[10] Y. Morisawa, M. Kataoka, T. Watanabe, N. Kitano, T. Metsuzawa, J. Med. Chem. **17**, 1235 (1974).
[11] M. Carnell, S. Grimme, S. D. Peyerimhoff, Chem. Physics **179**, 385 (1994).
[12] H. Morita, E. Kishi, K. Takeya, H. Itokawa, O. Tanaka, Chem. Lett. **5**, 749 (1990).
[13] Y. Zhao, P. Wang, S. Parsons, D. W. H. Rankin, F. Gueritte, T. Sevenet, H. Yu, Y. N. He, X. J. Hao, Chinese Chem. Lett. **13**, 333 (2002).
[14] a) P. Ciminiello, C. Dell'Aversano, E. Fattorusso, S. Magno, M. Pansini, J. Nat. Prod. **62**, 590 (1999); b) P. Ciminiello, E. Fattorusso, M. Forino, S. Magno, M. Pansini, Tetrahedron **53**, 6565 (1997).
[15] M. Ueda, T. Shigemori-Suzuki, S. Yamamura, Tetrahedron Lett. **36**, 6267 (1995).