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Nambiscalarane, a novel sesterterpenoid comprising a furan ring, and other secondary metabolites from bioluminescent fungus *Neonothopanus nambi*

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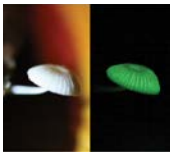
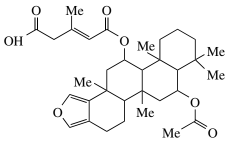
In the course of isolation of fungal luciferin from bioluminescent fungus *Neonothopanus nambi*, structures of novel nambiscalarane, Aurisin Z, *trans*-α-hydroxy-γ-phenylbutyrolactone, methyl 4-butyramidobenzoate as well as known nambinones A and C and polyhydroxylated sterol were identified.





Nambiscalarane

Bioluminescence is a fascinating phenomenon attracting researchers' interest since ancient times.[1] The light emitted during bioluminescence is produced by oxidation of a small organic molecule, luciferin, with atmospheric oxygen. The development of isolation and structure elucidation techniques during the last 50 years made it possible to structurally characterize seven different natural luciferins: coelenterazine and its derivatives (used by many taxonomically unrelated species), luciferins of fireflies, bacteria, *Dinoflagellata*, crustacean *Cypridina*, limpet *Latia* and a worm *Diplocardia longa*. Recently we have determined the chemical structures of eighth luciferin[2] and several of its natural analogues[3] found in a worm *Fridericia heliota*.

Many other luciferins exist in nature, however, their chemical structures are still unknown.[1] Among the least understood is the mechanism of fungal bioluminescence. All known bioluminescent fungi are mushroom-forming species belonging to the *Agaricales* lineage of the *Basidiomycota*.[4] A recent hypothesis states that all luminous fungi share the same mechanism of bioluminescence.[5] Over the years many unsuccessful attempts to isolate fungal luciferins have been reported,[6] yet only recently our group has effectively elucidated the structure of this elusive luciferin.[7] Newly discovered fungal luciferin, 3-hydroxyhispidin, is biosynthesized by oxidation of its precursor hispidin, which is a well known member of a styrylpyrone chemotype of fungal and plant secondary metabolites. Herein we report that in the course of isolation and purification of fungal luciferin seven prevalent compounds were isolated from mycelium of bioluminescent fungus *Neonothopanus nambi (N. nambi)*. Their structures were established by spectroscopic methods.

Along with luciferin, methanolic extracts[†] of mycelium of the fungus contained several predominant compounds.

Substance **1** designated as nambiscalarane was determined to be a member of a small family of pentacyclic sesterterpenoids bearing a furan ring and named scalaranes. Scalaranes are mainly found in marine organisms (predominantly in sponges), various fungi and rarely in plants and insects.[8] To identify the structure of the nambiscalarane **1** the following spectra were acquired: ^{1}H, ^{13}C, ^{13}C-DEPT-135, 2D COSY, 2D TOCSY, 2D ^{13}C-HSQC, 2D ^{13}C-HMBC (J_{long} = 7 Hz), 2D NOESY (τ_{m} = 200, 400 ms), 2D H2BC and 2D HSQC-TOCSY experiments (for details, see Online Supplementary Materials).

^{1}H NMR spectrum of compound **1** showed significant overlap of high-multiplicity signals in low field. ^{13}C NMR identified 33 carbon chemical shifts. Comparison of 1D ^{13}C with ^{13}C DEPT-135 spectra identified 7 carbons in methyl groups, 8 carbons in CH_2 groups, 8 CH carbons (3 olefinic) and 10 carbons without attached protons (3 carboxylic, 3 olefinic and 4 saturated). The connectivities in ^{1}H NMR in the range 1.0–1.5 ppm are ambigous in 2D COSY NMR spectrum. The structure elucidation was started from analysis of HSQC, H2BC and HSQC-TOCSY experiments, resulted in identification of four spin systems: (i) CH_2–CH_2–CH_2 chain, carbon atoms C^1–C^3, (ii) CH–CH(O)–CH_2 chain, carbon atoms C^5–C^7, (iii) CH–CH(O)–CH_2 chain, carbon atoms C^9–C^{11}–C^{12} and (iv) CH–CH_2–CH_2 chain, carbon atoms C^{14}–C^{16}. All other protons in NMR spectrum are singlets or signals with very small constants (H^{24}, H^{25}, $H^{2'}$, $H^{6'}$), namely: six methyls, one acetyl, one methylene with redundant chemical shifts and three aromatic singlets. The analysis of 2D HMBC spectrum stated numerous HMBC connectivities between spin systems (i)–(iv), five methyls and two aromatic singlets. Assuming that three-bond rule should explain the observed connectivities, the scalarane skeleton with two carbon atoms in furan cycle (C^{24} and C^{25}) was unambiguously identified. The other signals in NMR spectra are those of acetyl and 3-methyl glutaconic acid, which are connected to scalarane *via* ester bond to C^6 and C^{11} carbon atoms, respectively, as concluded from HMBC data. NOESY spectrum of compound **1** failed to provide stereochemical information because of signal overlap in low field region. Therefore, the structure of compound **1** was assigned as (*E*)-5-(7-acetoxy-5b,8,8,11a,13a-pentamethyl-4,5,5a,5b,6,7,7a,8,9,10,11,11a,11b,12,13,13a-hexadecahydrochryseno[1,2-*c*]furan-12-yloxy)-3-methyl-5-oxopent-3-enoic acid.

Another major component isolated from the methanolic extract of *N. nambi* was compound **2**. ESI HRMS of pure aurisin Z **2**

[†] For procedures and NMR data, see Online Supplementary Materials.

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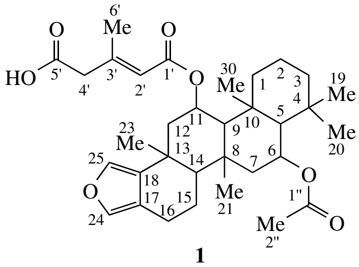

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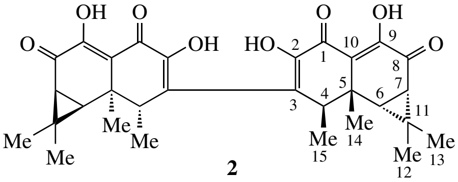

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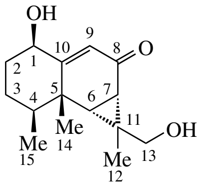

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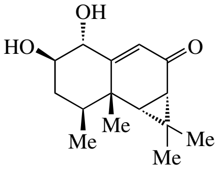

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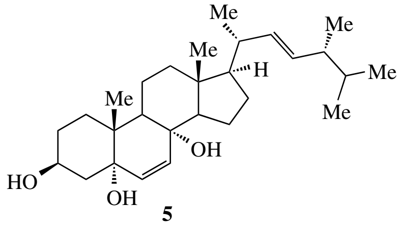

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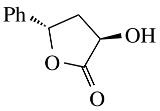

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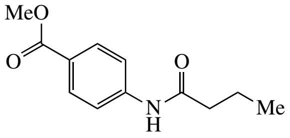

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provided a molecular ion with *m/z* 523.2332 corresponding to a molecular formula of $C_{30}O_8H_{35}^+$ (calc. *m/z* 523.2326). The structure and atom connectivities of compound **2** were not directly evident from combination of ^{1}H and ^{13}C NMR spectral data due to the presence of eight quaternary carbon atoms out of fifteen visible in 1D ^{13}C NMR spectrum. ^{1}H NMR spectrum of aurisin Z only showed the presence of four methyl and three methine groups, whereas ^{13}C NMR spectrum confirmed the presence of 15 carbon atoms, of which two are unsaturated carbons connected to hydroxyl groups (^{13}C chemical shifts 142.2 and 157.4 ppm), four carbon atoms in double bonds and two ketone groups (186.2 and 190.6 ppm).

The fraction of aurisin Z also contained two aditional compounds **3** and **4**, whose ^{1}H and ^{13}C NMR spectra were identical to those of previously described nambinones A and C, isolated from *N. nambi* in 2012 by a group of researchers in Thailand.[9] Similarities between NMR spectral data of known compounds **3** and **4** and novel compound **2** allowed us to determine structure and relative stereochemistry of aurisin Z – (1a*S*,1'a*S*,7*R*,7a*S*,7b*R*,7'*R*,7'a*S*,7'b*R*)-3,3',5,5'-tetrahydroxy-1,1,1',1',7,7a,7',7'a-octamethyl-7,7a,7',7'a-tetrahydro-1*H*,1'*H*-[6,6'-bi(cyclopropa[*a*]naphthalene)]-2,2',4,4'(1a*H*,1'a*H*,7b*H*,7'b*H*)-tetraone – a dimeric polycyclic nonaromatic hydrocarbon comprising three fused rings – two hexatomic and one triatomic, which places it into aristolane family of sesquiterpenes.

Compound **5** was isolated as a white solid, and its molecular formula $C_{28}H_{46}O_3$ was deduced from HRMS (*m/z* 431.3520 $[M+H]^+$). It was instantly recognised as a polyoxygenated steroid, ergosta-6,22-diene-3β,5,8α-triol, from ^{1}H and ^{13}C NMR spectra. The same steroid **5** was first isolated from shiitake mushrooms (*Lentinus edodes*),[10] and its ^{1}H and ^{13}C NMR spectra coincided with those of our compound **5** isolated from fungi *N. nambi*.

Compound **6** was isolated as a white solid, and its molecular formula $C_{10}H_{10}O_3$ was deduced from HRMS (*m/z* 179.0703 $[M+H]^+$). ^{1}H and ^{13}C NMR spectral data allowed us to identify compound **6** as a member of a vast and widely spread in nature class of substituted butyrolactones, *trans*-α-hydroxy-γ-phenylbutyrolactone. However, this particular butyrolactone has not been isolated previously from natural sources. Most butyrolactones are highly cytotoxic and therefore butyrolactone **6** may have a protective role in fungi.

Compound **7** was determined to be a disubstituted amine containing benzoic acid moiety derived from *p*-aminobenzoic acid (PABA). PABA is a growth factor in many bacterial species. Methyl 4-butyramidobenzoate **7** might be a primitive form of antibiotic, similar in action to sulfanilamide, produced by fungi to inhibit the methabolism of PABA in bacteria. Compound **7** has never been found before in natural sources. We presume that it could exist in nature in acidic form and it might have been esterified during extraction process.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mencom.2016.04.003.

References

1 O. Shimomura, *Bioluminescence: Chemical Principles and Methods*, World Scientific Publishing Co, Singapore, 2006.

2 V. N. Petushkov, M. A. Dubinnyi, A. S. Tsarkova, N. S. Rodionova, M. S. Baranov, V. S. Kublitski, O. Shimomura and I. V. Yampolsky, *Angew. Chem. Int. Ed.*, 2014, **53**, 5566.

3 (*a*) M. A. Dubinnyi, A. S. Tsarkova, V. N. Petushkov, Z. M. Kaskova, N. S. Rodionova, S. I. Kovalchuk, R. H. Ziganshin, M. S. Baranov, K. S. Mineev and I. V. Yampolsky, *Chem. Eur. J.*, 2015, **21**, 3942; (*b*) A. S. Tsarkova, M. A. Dubinnyi, M. S. Baranov, V. N. Petushkov, N. S. Rodionova, M. B. Zagudaylova and I. V. Yampolsky, *Mendeleev Commun.*, 2015, **25**, 99.

4 D. E. Desjardin, A. G. Oliveira and C. V. Stevani, *Photochem. Photobiol. Sci.*, 2008, **7**, 170.

5 A. G. Oliveira, D. E. Desjardin, B. A. Perry and C. V. Stevani, *Photochem. Photobiol. Sci.*, 2012, **11**, 848.

6 (*a*) S. Kuwabara and E. C. Wassink, in *Bioluminescence in Progress*, eds. F. H. Johnson and Y. Haneda, Princeton University Press, Princeton, NJ, 1966, pp. 233–245; (*b*) M. Endo, M. Kajiwara and K Nakanishi, *J. Chem. Soc. D*, *Chem. Commun.*, 1970, 309; (*c*) M. Isobe, D. Uyakul and T. Goto, *J. Biolumin. Chemilumin.*, 1987, **1**, 181; (*d*) M. Isobe, D. Uyakul and T. Goto, *Tetrahedron Lett.*, 1988, **44**, 1169.

7 K. V. Purtov, V. N. Petushkov, M. S. Baranov, K. S. Mineev, N. S. Rodionova, Z. M. Kaskova, A. S. Tsarkova, A. I. Petunin, V. S. Bondar, E. K. Rodicheva, S. E. Medvedeva, Yuichi Oba, Yumiko Oba, A. S. Arseniev, S. Lukyanov, J. I. Gitelson and I. V. Yampolsky, *Angew. Chem. Int. Ed.*, 2015, **54**, 8124.

8 (*a*) J. R. Hanson, *The Chemistry of Fungi*, The Royal Society of Chemistry, Cambridge, 2008; (*b*) H. N. Kamel, Y. B. Kim, J. M. Rimoldi, F. R. Fronczek, D. Ferreira and M. Slattery, *J. Nat. Prod.*, 2009, **72**, 1492; (*c*) M. A. Gonzalez, *Curr. Bioact. Compd.*, 2010, **6**, 178.

9 S. Kanokmedhakul, R. Lekphrom, K. Kanokmedhakul, C. Hahnvajanawong, S. Bua-art, W. Saksirirat, S. Prabpai and P. Kongsaeree, *Tetrahedron*, 2012, **68**, 8261.

10 A. Rivera, O. L. Benavides and J. Rios-Motta, *Nat. Prod. Res.*, 2009, **23**, 293.

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