Tetrahedron Vol. 44, No 6, pp 1597 to 1602, 1988
Printed in Great Britain.

0040-4020/88 $3.00+.00

PANAL: A POSSIBLE PRECURSOR OF FUNGAL LUCIFERIN

Hideshi Nakamura and Yoshito Kishi*
Department of Chemistry, Harvard University
12 Oxford Street, Cambridge, MA 02138, U.S.A.

Osamu Shimomura
Marine Biological Laboratory
Woods Hole, MA 02543, U.S.A.

(*Received in USA* 4 *December* 1987)

Abstract: A new sesquiterpene named panal was isolated from the luminous mushroom Panellus stipticus. Upon treatment with salts of ammonia or primary amines, panal yields a substance(s) which emits light in the presence of Fe^{2+}, H_2O_2, and surfactants. On the basis of chemical and spectroscopic data, the structure 1 was assigned to panal, excluding the absolute configuration.

The emission of light by certain fungi has drawn much attention for centuries.[1-3] Nonetheless, the chemistry of fungal bioluminescence is still hidden in a veil. We have recently observed that a crude aqueous extract of the luminous mushroom Panellus stipticus yields, upon treatment with salts of ammonia or primary amines, a substance(s) which emits light in the presence of Fe^{2+}, H_2O_2, and surfactants.[4] This suggests a possibility that the crude aqueous extract may contain the precursor of the light-emitting principle (luciferin) for the bioluminescence of fungi. Thus, we used the chemiluminescent activity for monitoring the "active" substance, resulting in the isolation of a new sesquiterpene, which we have named panal. In this paper, we would like to report the structure of panal.

Panal (1; α_D -17°) was isolated as an amorphous solid. The molecular formula ($C_{15}H_{18}O_5$) was established from the high-resolution mass spectrum. A broad IR absorption at 1700 cm^{-1}, coupled with a UV absorption at 218 nm (ε 15,300), suggested the presence of one or more conjugated carbonyl functionalities. The ^{13}C-NMR spectrum exhibited 15 signals, which were assigned to the following groups: 1 x CO (208.58 ppm, s), 1 x CHO (193.34, d), 1 x CO_2H (170.74, s), 1 x CH=C (141.24, d and 127.30, s), 1 x CH_2=C (138.11, t and 145.80, s), 1 x CH(OH) (63.83, d), 4 x CH (50.55, d, 42.09, d, 38.31, d, and 32.00, d), 2 x CH_2 (47.81, t and 33.08, t), and 1 x CH_3 (19.25, q). On the basis of these spectroscopic data, it is evident that panal contains the functional groups summarized in Diagram 1. In addition, extensive homo-nuclear spin-decoupling experiments established the proton spin coupling systems summarized in Diagram 2.

Diagram 1

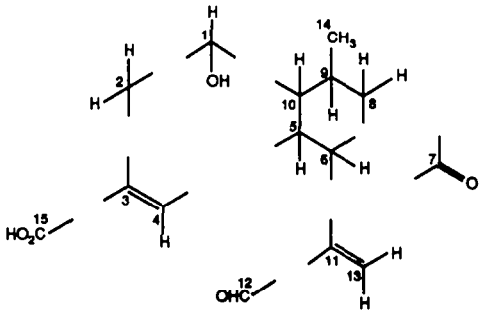
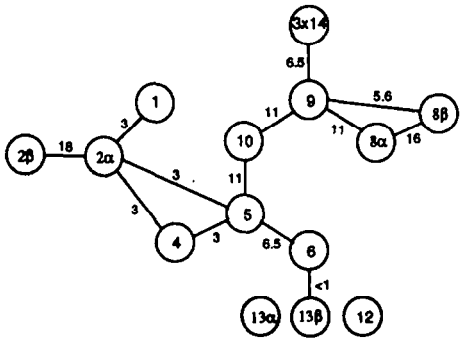


Diagram 2



As expected, upon $NaBH_4$ reduction followed by CH_2N_2 treatment, panal yielded the triol methyl ester 2 ($C_{16}H_{24}O_5$). The ^{1}H-NMR spectrum of 2 provided several crucial pieces of structural information. First, the C.7 proton appeared at 3.96 ppm, spin-coupled with the C.6 as well as C.8 protons ($J_{6,7}$ = 5.4 Hz, $J_{7,8\alpha}$ = 3.6 Hz, $J_{7,8\beta}$ = 12.0 Hz), which allowed placement of the ketone at the C.7 position. Secondly, the proton signals due to the exo methylene group exhibited approximately 1.1 ppm upfield shifts on the reduction, suggesting the presence of a H_2C=C-CHO group in 1. This assignment was further supported by the C.13 and C.11 chemical shifts[5] observed in the ^{13}C-NMR spectrum of 1 and also by the spin-spin coupling between the C.11 carbon and C.12 proton.[6] A spin-decoupling experiment proved the spin-spin coupling between one of the C.13 protons (6.42 ppm) and the C.6 proton, establishing that this functional group is connected to the C.6 carbon. Coupled with this information, the chemical shifts observed for the C.3 and C.4 carbons and for the C.4 proton allowed us to conclude that a HO_2C-C=CH group was present in 1.[7]

Table 1 ^{1}H- and ^{13}C-NMR data of Panal.[a,b,c]

No.	^{1}H	^{13}C
1	4.42 (br s)	63.83(d)
2	2.43 (α-H, dq, J = 18, 3 Hz)	47.81(t)
	2.60 (β-H, br d, J = 18 Hz)	
3	--	127.30(s)
4	6.78 (br t, J = 3 Hz)	141.24(d)
5	3.12 (br m)	38.31(d)
6	4.00 (br d, J = 6.5 Hz)	50.55(d)
7	--	208.58(s)
8	2.31 (α-H, dd, J = 16 and 11 Hz)	33.08(t)
	2.68 (β-H, br dd, J = 16 and 5.6 Hz)	
9	2.16 (tqd, J = 11, 6.5 and 5.6 Hz)	32.00(d)
10	1.59 (t, J = 11 Hz)	42.09(d)
11	--	145.80(s)
12	9.46 (s)	193.34(d)
13	6.23 (α-H, s)	138.11(t)
	6.42 (β-H, br s)	
14	1.12 (d, J = 6.5 Hz)	19.25(q)
15	--	170.74(s)

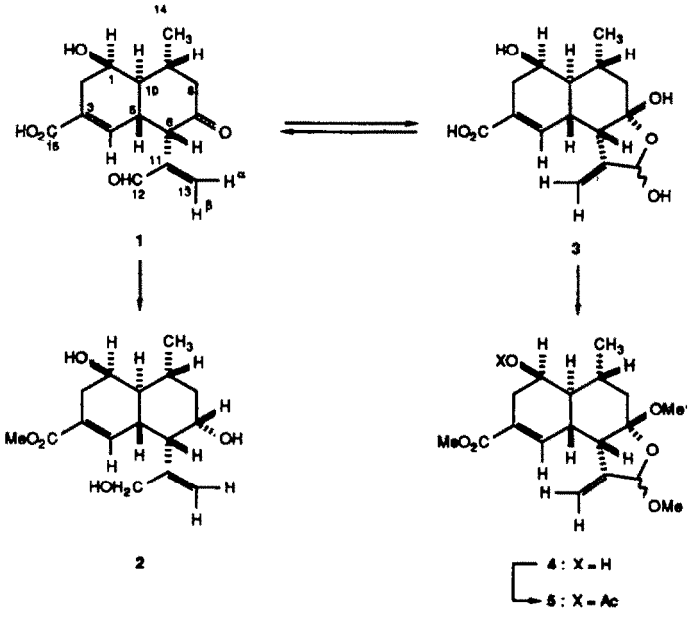
a) The ^{1}H-NMR spectrum was recorded in $CDCl_3$ on a Bruker AM 500 spectrometer (500 MHz) and the ^{13}C-NMR on a Bruker AM 250 spectrometer (62.5 MHz).
b) Chemical shifts are given in ppm from TMS.
c) The multiplicity of the carbon signals was determined from the off-resonance decoupling experiments. The assignment of carbons bearing protons was made on the basis of selective proton decoupling experiments.

The C.4-C.5 connectivity was established from a nuclear Overhauser effect (NOE) observed between the C.4 and C.6 protons in 1 (10%). This observation is consistent with the fact that a spin-spin coupling, although small (3 Hz), was observed between the C.4 and C.5 protons. The C.1, C.2, and C.3 connectivity was determined from the ^{1}H-NMR spectrum of 1, which showed a long-range spin-spin coupling (J = 3 Hz) between the C.2α and C.4 protons. In addition, an NOE (1.8%) was detected between the C.14 and C.1 protons.

Based on the evidence presented above, the gross-structure 1 is assigned to panal. The spin-spin coupling constants $J_{5,10} = J_{9,10} = 11$ Hz, $J_{1,10} \simeq 0$ Hz, and $J_{5,6} = 6.5$ Hz established the relative stereochemistry depicted in 1, but its absolute stereochemistry remains unknown at this time.

When kept in wet chloroform, panal (1) formed a diastereomeric mixture of hydrated substances (3), which precipitated from chloroform as a white amorphous solid. Upon aqueous acid treatment, panal (1) was regenerated from 3. Treatment of 3 with p-TSA/CH_3OH, followed by esterification (CH_2N_2), yielded a 1:1 diastereomeric mixture of methyl ketals 4. Acetylation of 4 furnished a 1:1 diastereomeric mixture of the corresponding acetates 5. These observations are well explained by the proposed structure.

Scheme 1



Sesquiterpenes with a 1,4-dialdehyde functionality have been isolated from fungi and shown to be related to the chemical defense system.[8,9] Interestingly, polygodial, a 1,4-dial isolated from a plant source, reacts with an amine to yield a pyrrole derivative. This reaction may account for the observed biological activity of polygodial.[10] By analogy, the 1,4-ketoaldehyde functionality of panal may react with salts of ammonia or primary amines to yield a compound(s) responsible for the chemiluminescence and possibly bioluminescence.[4] We are currently engaged in the isolation and structure elucidation of the compound(s) formed upon treatment of panal with amine salts.

Table 2 500 MHz ^{1}H-NMR data of the methyl ester 2[a,b]

No.	H
1	4.36 (br s)
2	2.46 (α-H, dtd, J = 18, 3, and 2 Hz)
	2.54 (β-H, br d, J = 18 Hz)
4	6.78 (br s)
5	2.71 (dddd, J = 12, 5.4, 3 and 2 Hz)
6	3.13 (br t, J = 5.4 Hz)
7	3.96 (ddd, J = 12, 5.4 and 3.6 Hz)
8	1.45 (α-H, q, J = 12 Hz)
	1.86 (β-H, dt, J = 12 and 3.6 Hz)
9	1.70 (tqd, J = 12, 6.2 and 3.6 Hz)
10	1.36 (br t, J = 12 Hz)
12	4.07 (dd, J = 12 and 0.5 Hz)
	4.11 (dd, J = 12 and 0.5 Hz)
13	5.09 (α-H, s)
	5.34 (β-H, br s)
14	1.03 (d, J = 6.2 Hz)
15-OCH_3	3.72 (s)

a) Recorded in $CDCl_3$ on a Bruker AM 500 spectrometer.
b) Chemical shifts are given in ppm from TMS.

Acknowledgements: Financial assistance from the National Science Foundation (CH 86-105050) to Harvard University and to Marine Biological Laboratory (DMB 87-03463) is gratefully acknowleged.

Experimental

Isolation of panal (1): Fruiting bodies of *Panellus stipticus*, collected in the Beebe Wood area, Falmouth, Massachusetts, were ground and extracted repeatedly with 30% (v/v) methanol/water. The extracts were concentrated under reduced pressure to approximately 1/8 of the original volume, adjusted to pH 6.5 (dil. HCl), and partitioned with ethyl acetate. The ethyl acetate layer was discarded. The aqueous layer was then adjusted to pH 2.0 (dil. HCl) and extracted with ethyl acetate. The ethyl acetate extract was evaporated to dryness under reduced pressure. The residue was dissolved in 30% (v/v) methanol/water and adsorbed on an anion exchange column (Fractogel TSK DEAE 650M), which was eluted with gradient increase of NaCl concentration (0 to 1.0 M) in 30% (v/v) methanol/water. The eluate containing the chemiluminescent activity was further purified by two steps of HPLC: first, on an ODS column (Du Pont Zorbax) with 27% (v/v) acetonitrile/water (pH adjusted to 2.6 with H_3PO_4) and second, on an anion-exchange column (TSK DEAE-5PW) in 40% (v/v) acetonitrile/water containing 1 mM sodium acetate and 8 mM NaCl. After evaporation of the solvent, the salts were removed by using ethanol. Thus, 300 mg of panal was isolated from 100 g of fruiting bodies: $[\alpha]_D$ -17° (c 0.9, MeOH); ^{1}H- and ^{13}C-NMR, see Table 1; UV (MeOH) λ_{max} 218 nm (ε 15,300), IR ($CHCl_3$) ν_{max} 1700 cm^{-1}; EIMS m/z 278 (M^+, relative intensity 45%), 260 (M^+-H_2O, 95%), 232 (78%), 216 (100%), 201 (50%), 145 (67%), 117 (63%), 91 (86%); HREIMS, m/z 278.1157 (Calcd. for $C_{15}H_{18}O_5$ 278.1160).

Chemiluminescence assay of panal: $(NH_4)_2SO_4$ was added to a solution of panal in 30% (v/v) methanol/water (5-50 μl) until saturated. After 1 h, the mixture was diluted with 3 ml of 50 mM

Tris-HCl/0.18 mM EDTA buffer (pH 8.0), followed by addition of ca. 10 mg of $NaHCO_3$ and ca. 10 mg of cetyltrimethylammonium bromide. Chemiluminescence was initiated by addition of 20 µl of 0.1 M $FeSO_4$, followed by 30 µl of 10% H_2O_2.

Preparation of triol methyl ester 2: To a solution of panal 1 (2.0 mg) in MeOH (0.2 ml) at 0 °C was added sodium borohydride (1 mg), and the same amount of sodium borohydride was added again after 30 min. The solution was stirred for 30 min, acidified with acetic acid, and warmed to room temperature. The solvent was removed under reduced pressure, and the residue was dissolved in methanol and treated with an ethereal solution of diazomethane for 10 min at 0 °C. After removal of the solvent, the crude product was purified by silica gel tlc with 9:1 CH_2Cl_2/MeOH to yield methyl ester 2 (0.64 mg, 29%): ^{1}H-NMR, see Table 2; CIMS (isobutane) m/z 297 [$(M+H)^+$, relative intensity 19%], 279 (51%), 261 (100%), 247 (80%), 243 (54%), 229 (56%), 201 (25%), 183 (20%).

Conversion of hydrated panal 3 to panal 1: A suspension of 3 (8.22 mg, 0.0278 mmol) in water (1 ml) was acidified by adding a few drops of 0.5 N HCl. The resulting solution was partitioned with ethyl acetate and treated as described under the isolation of panal, to yield a ca. 4:1 mixture (6.22 mg, 80% yield) of panal 1 and hydrate 3.

Preparation of methyl ketals 4: A solution of 3 (3.8 mg) and p-toluenesulfonic acid monohydrate (0.5 mg) in a mixture of methanol (0.2 ml) and dimethoxypropane (0.02 ml) was stirred at room temperature for 2 h. The solution was then cooled to 0 °C and was treated with an ethereal solution of diazomethane for 30 min. Concentration in vacuo gave a residue, which was purified by silica gel tlc using 95:5 CH_2Cl_2/MeOH to afford methyl ketals 4 (2.82 mg, 66%) as a 1:1 mixture of diastereomers at C.12: ^{1}H-NMR ($CDCl_3$) δ 0.95 (H14, d, J = 6.5 Hz), 1.23 (H8α, t, J = 11 Hz), 1.32 (H10, t, J = 11 Hz), 1.53 (H8α, dd, J = 11, 12 Hz), 1.74 (H9, m), 1.78 (H9, m), 1.95 (H8β, ddd, J = 1.3, 3.2, 11 Hz), 1.98 (H8β, ddd, J = 1.5, 2.5, 11 Hz), 2.45 (H2β, dm, J = 17 Hz), 2.62 (H2α, d, J = 17 Hz), 2.81 (H5, br m), 3.15 (H6, m, half width 9 Hz), 3.36 (H6, br d, J = 4 Hz), 3.45, 3.47 (12-OMe, s), 3.39 (7-OMe, s), 3.74, 3.75 (CO_2Me, s), 4.35 (H1, br s), 5.04 (H13α, d, J = 2 Hz), 4.98 (H12, br s), 5.12 (H13α, dd, J = 2, 3 Hz), 5.19 (H13β, d, J = 2 Hz), 5.24 (H13β, dd, J = 1.5, 3 Hz), 5.32 (H12, br s), 7.04 (H4, br s).

Preparation of methyl ketal acetates 5: Compound 4 (1.0 mg) was treated with a mixture of acetic anhydride (0.01 ml), pyridine (0.1 ml), and dimethylaminopyridine (0.2 mg) for 3 h at room temperature. The solvent was removed under reduced pressure and the residue was purified by silica gel tlc to give compound 5 (1.1 mg, 98% yield) as a 1:1 mixture of diastereomers at C.12: ^{1}H-NMR ($CDCl_3$) δ 0.91 (H14, d, J = 6.5 Hz), 1.25-1.6 (H8β, H9, H10, complex), 1.95 (H8α, m), 2.02, 2.03 (OAc, s), 2.43 (H2α, dm, J = 18 Hz), 2.67 (H2β, br d, J = 18 Hz), 2.82 (H5, br m), 3.12 (H6, br), 3.38 (H6, br), 3.41 (7-OMe, s), 3.45, 3.47 (12-OMe, s), 3.72, 3.76 (CO_2Me, s), 4.99 (H12, br s), 5.05 (H13α, d, J = 2 Hz), 5.13 (H13α, dd, J = 2, 3 Hz), 5.20 (H13β, d, J = 2 Hz), 5.26 (H13β, dd, J = 1.5, 3 Hz), 5.32 (H12, br s), 5.46 (H1α, br s), 7.06 (H4, br s).

References and Footnotes

1. R. L. Airth, G. E. Foerster, and P. Q. Behrens, "Bioluminescence in Progress," Princeton University Press, Princeton, New Jersey, 1966, page 203 and references cited therein.
2. E. C. Wassink and S. Kuwabara, "Bioluminescence in Progress," Princeton University Press, Princeton, New Jersey, 1966, page 247.
3. M. Endo, M. Kajiwara, and K. Nakanishi, J. Chem. Soc., Chem. Commun., 309 (1970).
4. O. Shimomura, Photochem. Photobiol., submitted for publication.
5. The ^{13}C-NMR of methacrylaldehyde showed an aldehyde carbon signal at 193.8 ppm and olefinic carbon signals at 146.0 (=C-) and 133.2 ($=CH_2$) in $CDCl_3$.
6. For the value of spin couplings between ^{13}C-CHO, see: O. Yamamoto, M. Watanabe, and O. Kikuchi, Molec. Phys., 17, 249 (1969).
7. E-$CH_3CH=C(CH_3)CO_2H$ exhibited carbon resonances at 137.9 ppm (=CH-), 128.2 (=C), and 170.9 (CO_2H) and a proton resonance at 6.75 ppm: H. Brouwer and J. B. Stothers, Can. J. Chem., 50, 601 (1972).
8. O. Sterner, R. Bergman, C. Frauzen, and B. Wickberg, Tetrahedron Lett., 23, 3295 (1985).
9. W. A. Ayer and L. M. Browne, Tetrahedron, 37, 2199 (1981).
10. M. D'Ischia, G. Prota, and G. Sodano, Tetrahedron Lett., 23, 3295 (1981).