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GYMNOPILINS, BITTER PRINCIPLES OF THE BIG-LAUGHTER MUSHROOM GYMNOPILUS SPECTABILIS

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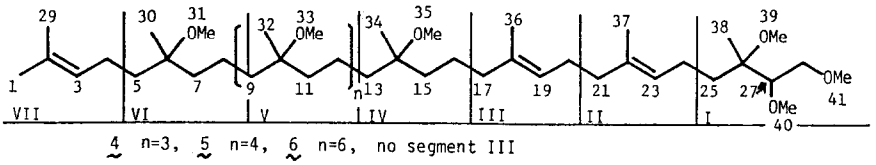
Abstract: Planar structures of main bitter principles of Gymnopilus spectabilis have been determined to be 1, 2 and 3, mainly on the basis of spectroscopic evidence.

We report here the isolation and characterization of bitter principles of hitherto unknown type, for which we suggest the name gymnopilins, from the wild, very bitter mushroom Gymnopilus spectabilis, called in Japan o-waraitake (big-laughter mushroom).[1]

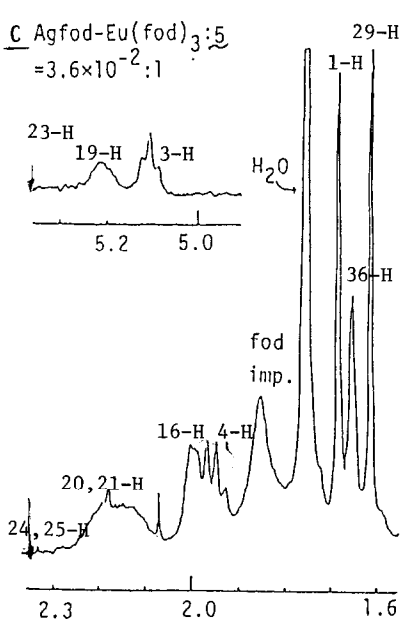
Gymnopilins were obtained as amorphous powder in an 0.5% yield from fresh fruiting bodies though successive MeOH extraction, separation of a polar fraction and careful chromatography of the fraction on sephadex LH-20 (ethyl acetate). Although ^{13}C and ^{1}H nmr spectra of gymnopilins indicated that they were practically homogeneous, tlc behavior (Fertigplatten Kieselgel $60F_{254}$) suggested that they were a mixture of very closely related compounds. The ^{13}C spectrum of the mixture in CD_3OD displayed dominant peaks at 18.8(t), 26.7(q), 42.9(t) and 72.9(s) indicating the presence of oligoisoprenoid partial structure A (formula 1), together with many weak peaks involving those due to an ester carbonyl group and an acid carbonyl group at 172.6 and 179.9 ppm respectively. Upon hydrolysis gymnopilins afforded a single carboxylic acid [mp 109 °C, δ_C (D_2O) 28.18(q), 50.19(t), 72.01(s), 181.67(s)] and a mixture of amorphous, tasteless polyols named gymnoprenols. The acid was identified with 3-hydroxy-3-methylglutaric acid (mmp, ^{1}H and ^{13}C nmr), the biosynthetic precursor of isoprenoids. The ^{13}C nmr spectrum (CD_3OD) of the alcoholic moiety was almost identical with that of the mixture of gymnopilins, except for the absence of the peaks due to the acid moiety and appearance of new peaks at 63.8(t) and 77.9(d), instead of 67.0(t) and 75.8(d). These deacylation shifts showed clearly the presence of segment B in gymnopilins.

Treatment of gymnoprenols with CH_3I/NaH (THF, reflux) afforded permethyl ethers, which were able to be separated by semipreparative HPLC (μPORASIL, Hex-AcOEt 6:4) to give three main components, gymnoprenol (G) A_9 methyl ether 4 (FDMS M^+ 866), G A_{10} methyl ether 5 (FDMS M^+ 966) and G B_{11} methyl ether 6 (FDMS M^+ 1098) in a ratio of 1:3:1. The ^{1}H nmr spectrum of the major ether 5 ($CDCl_3$,

400 MHz, Fig 1) closely resembled that of the mixture of G methyl ethers. Presence of the isoprenoid terminal moiety VII in 5 was suggested by the characteristic olefinic methyl peaks at δ 1.61 (29-H) and 1.68 (1-H), methylene peaks at around δ 1.95 (overlapped by other peaks) and olefinic proton peaks centered at 5.11. Since the secondary ethereal methine proton resonated at a rather high field and exhibited a dd peak (δ 3.32, J=7, 2 Hz), the group connected to segment B was supposed to be an sp^3 carbon,bearing a methyl (δ 1.07), a methylene (δ 1.40) and a methoxyl (δ 3.13) group, on the basis of biogenetic ground. The other teminal group is therefore expressed by partial formula I. Low-field line position of the two-proton quartet at δ 2.09 (20-H, J=7 Hz) suggested connection of segments II to III and the reasoning turned out to be correct by the pseudo-INDOR technique (Fig 1B). Moreover measurements of nmr spectra by the addition of increasing small amounts of Ag(fod)-Eu(fod)$_3$ complex[2] revealed that segment II was connected further with segment I, since as shown in Fig 1C, by this procedure (a) peaks due to methyl and methoxyl groups in segment I rapidly shifted to low field or broadened to disappear (b) peaks due to olefinic methyl and olefinic hydrogen in segment II moved to low field and then broadened to disappear (c) peaks assignable to olefinic methyl and olefinic hydrogen in segment III showed Eu^{3+} induced shift, although to a lesser extent than those in segment II, while (d) peaks responsible to those in the terminal segment VII (δ 1.61, 1.68 and 5.11) remained almost unchanged. The presence of segment V with n=6 was ascertained by the strong signals at δ 1.10, 1.25, 1.40 and 3.37, and their intensities. Therefore formula 5 composed of ten isoprene units was inferred for G A_{10} methyl ether. Supporting evidence for this formulation was obtained from ozonolysis of the mixture of G methyl ethers, which afforded trimethoxy carboxylic acid 7 [m/z M+1 221.1412, $C_{10}H_{21}O_5$, δ_c ($CDCl_3$, 100 MHz) 19.5(q), 28.4 (t), 29.3(t), 49.9(q), 59.1(q), 59.8(q), 73.5(t), 77.6(s), 84.8(d), 179.3(s); δ_H ($CDCl_3$, 400 MHz) 1.08 (3H, s), 1.98 (2H, nine lines J=7.8 Hz), 2.41 (2H, dd, J= 7.4, 8.2 Hz), 3.20 (3H, s), 3.27 (1H, dd, J=2, 7 Hz), 3.36 (3H, s), 3.44 (1H, dd, J=7, 10 Hz), 3.49 (3H, s) and 3.69 (1H, dd, J=2, 10 Hz)] and a mixture of methyl ketones exhibiting the ^1H nmr spectrum consistent with formula 8, in more than 80% yields. Conclusive evidence for formula 5 was provided by the ^{13}C nmr spectrum of a synthetic model (2SR, 3RS)-1,2,3-trimethoxy compound 9,[3] [δ_c ($CDCl_3$) 15.97(q), 17.66(q), 18.44(q), 21.55(t), 25.71(q), 26.52(t), 34.48(t), 39.80(t), 49.15(q), 58.96(q), 59.67(q), 74.34(t), 77.85(s), 83.44(d), 124.41(d), 131.09(s), 134.86(s)]. The ^{13}C spectrum of 9 was essentially superimposable with that of 5 (Fig 2), except for the absence of the peaks due to segments IV, V, VI, C-4 and C-17. The assignment of the carbon signals of 5 was made by combination of the INEPT technique (Fig 2A) and comparison with the spectrum of 9. Since the ^{13}C nmr spectrum of the C-11 isomeric model 10[3] [δ_c ($CDCl_3$) 19.87(q), 49.80(q), 74.08(t), 78.24(s), 85.06(d) instead of the underlined peaks of 9.



4 n=3, 5 n=4, 6 n=6, no segment III



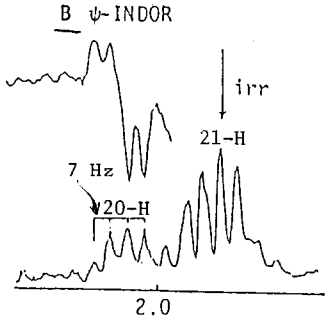


Fig 1 G A10 Methyl Ether

A ^{1}H nmr(400 MHz, $CDCl_3$)

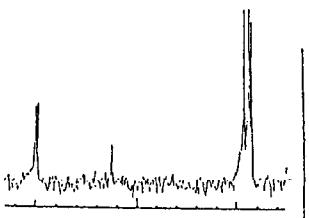


Fig 2A
INEPT
$\Delta = \frac{3}{4J}$, down: CH_2

Fig 2
G A10 Methyl Ether
^{13}C nmr(100 MHz)

Other δ_c's were practically identical] was clearly distinguishable from that of 9, the 2S,3R or 2R,3S configuration was assigned for 5.

Gymnoprenol A_9 methyl ether exhibited almost identical ^1H ($CDCl_3$, 400 MHz) and ^{13}C nmr ($CDCl_3$, 100 MHz) spectra with those of G A_{10} methyl ether, except for the intensity of the peaks due to segment V. Therefore formula 4 with nine isoprene units was assigned for G A_9 methyl ether. On the other hand ^1H nmr spectrum ($CDCl_3$, 400 MHz) of G B_{11} methyl ether indicated presence of two, rather than three olefinic protons and three olefinic methyls at δ 1.61(6H) and 1.68 (3H). The quartet at 2.09 was not observed. In the ^{13}C nmr spectrum, signals due to segment II (Fig 2, designated with *) were lacking, while other peaks appeared at the essentially same positions with those of G A_{10} methyl ether. Therefore planar structure 6 with eleven isoprene units and two double bonds was concluded for G B_{11} methyl ether. Planar structures of the bitter principles gymnopilin A_9, A_{10} and B_{11} are accordingly expressed by 1, 2 and 3 respectively.

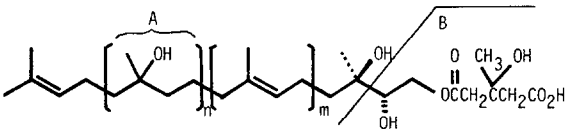
Our thanks are due to Professor S. Nozoe, Tohoku University, who reached the same conclusion independently from our work, for valuable discussions.

References and Note

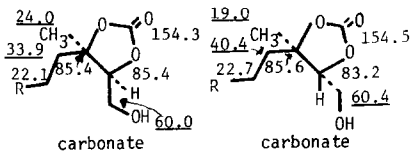
1) For biological activity see for example Jonathan Ott, "Hallucinogenic Plants of North America", Wingbow Press, Berkely, 1976, p 7.

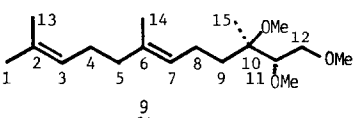
2) T. J. Wenzel and R. E. Sievers, J. Am. Chem. Soc., 102, 5903 (1980) and references therein.

3) Model compounds 9 and 10 were prepared from racemic nerolidol through successive epoxidation (^tBuOOH, $VO(acac)_2$, PhH), formolysis ($HClO_4$, DMF) to give a mixture of isomeric primary formates, formation of cyclic carbonates (ImC=OIm, PhH), separation of isomers, hydrolysis and methylation (CH_3I/NaH, THF). Stereochemistry was determined by comparing δ_c values of the epimeric triol carbonates.

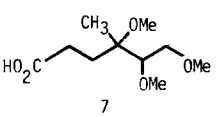


1 n=5 m=2 G A_9 3 n=8 m=1 G B_{11}
2 n=6 m=2 G A_{10}





9



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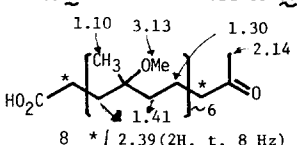

8 * { 2.39(2H, t, 8 Hz)
2.42(2H, t, 8 Hz)

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