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ISOLATION AND STRUCTURE OF GYMNOPRENOLS, A NOVEL TYPE OF POLYISOPRENEPOLYOLS FROM GYMNOPILUS SPECTABILIS

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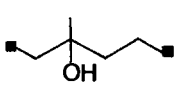
Summary: Gymnoprenols, a novel type polyisoprenepolyols, have been isolated from the fruiting body of an hallucinogenic mushroom, Gymnopilus spectabilis. The structures of these polyols which occur as a mixture of isoprene homologues have been elucidated by chemical degradations and spectroscopic analyses.

In the course of investigation of the constituents of the toxic mushroom we have found that the fruiting bodies of Gymnopilus spectabilis[1], which has been known as an hallucinogenic mushroom[2], contain a substance possessing the structure of a novel type of polyisoprene chain, occurring as a mixture of isoprene homologues with 45 to 60 carbon atoms. In the present paper, we describe the isolation and structural elucidation of these polyisoprenepolyols which have been named as gymnoprenols.

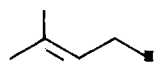
The dried fruiting bodies of Gymnopilus spectabilis collected at Yamagata Pref. were extracted with methanol and the extracts were concentrated to leave a gummy residue which was partitioned between ethyl acetate and water. The compounds extracted with ethyl acetate were then passed through a Florisil column prewashed with methanol and then eluted with a mixture of ethyl acetate and methanol to yield crude polyisoprenepolyols. The crude polyols were then rechromatographed on a Florisil column eluting with chloroform-methanol to give fractions containing the three main components. Each constituent was further purified by means of preparative HPLC using a Lichroprep RP-8 column, to afford gymnoprenol-A, -B and -F as a mixture of homologous compounds[4].

Gymnoprenol-A, an oily substance showing a retention time of 9.2 min[3] at HPLC analysis, exhibits molecular ion peaks at m/e 777$(M+Na)^+$ and m/e 863$(M+Na)^+$ in a FAB-mass spectrum indicating that these compounds have molecular formulae of $C_{45}H_{86}O_8$ and $C_{50}H_{96}O_9$. The nmr spectrum of gymnoprenol-A (1) shows intense

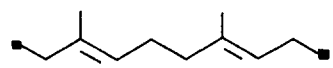
signals at 1.14 and 1.41 in a ratio of 1:2 which were assignable to the protons of a tertiary methyl group bearing an oxygen atom and three methylene groups, respectively[5]. The integral ratio of the signals reveals the presence of five or six repeated structural units such as (i) in the molecule. This was also supported by the cmr spectrum of 1 which exhibits prominent signals at 27.0(q), 73.5(s),19.4(t) and 43.5(t), the latter two signals appearing in a ratio of 1:2. The 400MHz nmr spectrum of 1 revealed the presence of three trisubstituted double bonds, one of which is located at a terminal position as in (ii): 1.59 (3H,s), 1.62 (3H,s), 1.63 (3H,s), 1.67 (3H,s), 1.99 (6H,m), 2.04 (4H,m) and 5.04 (3H,m). The cmr spectrum of 1 also shows signals due to three trisubstituted double bonds at 16.0(q), 16.1(q), 17.7(q), 25.9(q), 125.5(d), 125.9(d), 126.0(d), 131.9(s), 135.8(s) and 136.0(s). Comparison of the allylic methylene carbon signals which, could be assigned by INEPT and a selective decoupling experiment in the cmr spectrum of 1 with those of gymnoprenol-B (2) (vide infra), revealed that there are two allylic carbons located between two double bonds such as in (iii). The signals at 3.46 (1H, dd, J=3.5, 8.0Hz), 3.53 (1H, dd, J=8.0, 11.5Hz), 3.78 (1H, dd, J=3.5, 11.5Hz) in the nmr spectrum of 1 shift to 5.04 (dd,J=2.0, 9.0Hz), 4.10 (dd, J=9.0, 11.5Hz), 4.50 (dd, J=2.0, 11.5Hz) in the spectrum of the diacetate 3 which was obtained by the acetylation of 1. A decoupling experiment on these signals indicated the presence of vicinal primary and secondary alcohols in adjacent position of tertiary carbinol as in the partial structure (iv).



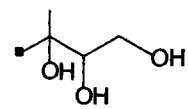
(i)



(ii)



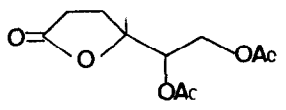
(iii)



(iv)

To determine the arrangement of the building units (i)~(iv), oxidative degradation of the diacetate was carried out. Diacetate 3 was treated with OsO_4 (pyridine, rt) followed by $NaIO_4$ (aq dioxane, rt) to afford the products 4 and 5 after purification by HPLC. Since the product 4 was found to be a mixture of hemiacetal isomers (pmr, 5.52; cmr, 98.9, 99.7), this product was transformed to γ-lactone 6 by Jones oxidation. The structure of 6 was elucidated from its spectral properties; ms(FD), m/e 245 $(M+1)^+$ for $C_{11}H_{16}O_6$: ir($CHCl_3$), 1770, 1740 cm^{-1} :pmr($CDCl_3$), 1.46 (3H,s), 1.97 (1H, ddd, J=7.0, 9.5, 12.5Hz),2.06 (3H,s), 2.12 (3H,s), 2.40 (1H, ddd, J=7.0, 9.5, 12.5Hz), 2.64 (2H, ddd, J=7.0, 9.5, 13Hz), 4.14 (1H,dd), 4.43 (1H,dd), 5.21 (1H,dd): cmr($CDCl_3$), 20.6(q), 20.64(q), 23.0(q), 28.4(t), 30.0(t), 62.1(t), 73.9(d), 84.7(s), 169.7(s), 170.4(s), 175.5(s). Another hemiacetal 5 was also transformed to γ-lactone 7 which showed

molecular ion peaks at 552 $(M+1+Na)^+$ and 638 $(M+1+Na)^+$ in FD mass spectrum indicating that this product was a mixture of isoprene homologues with molecular formulae of $C_{30}H_{56}O_7$ and $C_{35}H_{66}O_8$ (n=4 and 5 in 7). The products 7a and 7b which could be separated by HPLC (Lichrosorb RP-18) showed ir bands at 3400 (-OH), 1750 (γ-lactone) and 1705 cm^{-1} (methyl ketone) and pmr signals at 1.14, 1.41, 2,13 (3H,s), 2.48 (2H,t, J=6.0Hz) to support the structures shown. From the structures of the degradated products two possible structures 1a and 1b were proposed for gymnoprenol-A.



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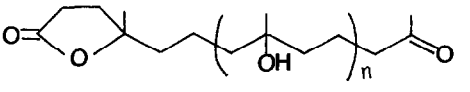
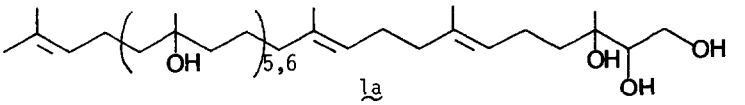
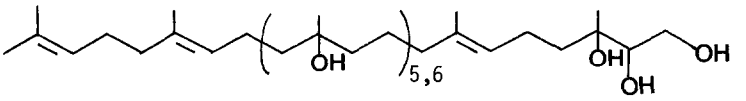


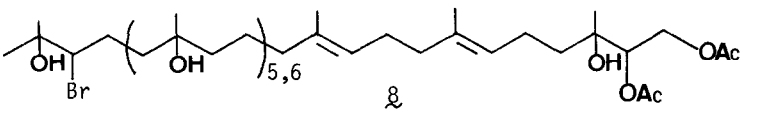
7a: n=4, 7b: n=5



1a



1b



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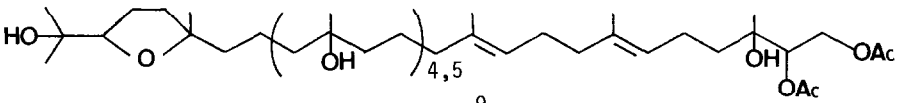

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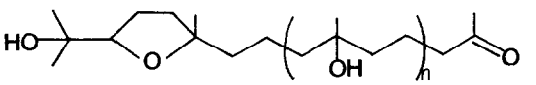
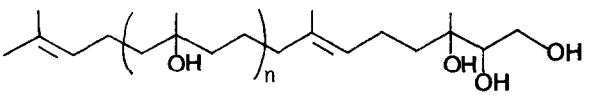


10a: n=4, 10b: n=5



2 (n=7,8,9)

To confirm the position of the central double bond, the diacetate 3 was transformed to a bromohydrin (e.g. 8) by treatment with NBS in t-BuOH[6]. The double bonds of the compound 9, which was derived from the bromohydrin, were oxidatively cleaved ($OsO_4/NaIO_4$) to afford degradation products 10a, 10b and 4 after HPLC separation. The products 10a and 10b show molecular ion peaks at m/e

595$(M+Na)^+$ and 681$(M+Na)^+$, respectively, in the FD mass and the latter exhibits a peak at m/e 550.4718(M^+-6H_2O), in high resolution mass and the prominent peaks at m/e 43, 143, 279, 347, 415, 483 and 509 in EI mass, indicating that 10a and 10b possess the molecular formulae $C_{33}H_{64}O_7$ and $C_{38}H_{74}O_8$, respectively. The nmr spectra of both 10a and 10b show the signal at 3.76 (1H,m) due to a proton on the tetrahydrofuran ring along with signals at 2.11 (methyl ketone), 1.14 (methyl groups attached on the carbon bearing oxygen) and 1.41 (methylene groups). From this result the structure of gymnoprenol-A is conclusively deduced as 1a.

Gymnoprenol-B (2), a minor constituent of the polyisoprenepolyols possesses a similar structural units to those of 1 excepting that the former contains only two trisubstituted double bonds; pmr, 1.62 (6H,s), 1.67 (3H,s), 1.99 (4H,m), 2.11 (2H,m), 3.46 (1H,dd), 3.53 (1H,dd), 3.78 (1H,dd), 5.08 (2H,m): cmr, 16.0(q), 17.7(q), 25.9(q), 64.0(t), 78.1(d), 125.8(d), 126.0(d), 132.0(s), 135.9(s). Oxidative cleavage of diacetate of 2 under the same conditions as described for 1 afforded the products 4 and 5c~e, the latter were oxidized to γ-lactone 7c~e which show molecular ion peaks at m/e 723$(M+Na)^+$, 810$(M+1+Na)^+$and 896$(M+1+Na)^+$, respectively, in the FD mass. Thus the structure of gymnoprenol-B was deduced as 2 (n=7, 8, 9).

The structures of such polyisoprenepolyols, which formally correspond to a hydrated form of the common polyprenyl chain, have never been encountered in naturally occurring polyisoprene derivatives[7]. The stereochemistry of the chiral centers in 1 and 2, biological activities, and biosynthesis of these compounds are under investigation.

References and Notes

1) G.M.Hatfield, L.R.Brady, J. Pharm. Sci., 58, 1298 (1970);G.M.Hatfield, L.R.Brady, Lloydia, 34, 2 (1971);G.M.Hatfield, L.J.Valdes, A.H.Smith, Lloydia, 41, 140 (1978).

2) R.G.Benedict, "Mushroom toxins other than Amanita", S.Kadis, A.Ciegler, S.J.Ajl (Eds), Microbial toxins Vol 8, p.281, Academic Press, New York, 1972.

3) HPLC conditions; column:4mmφx250mm,Lichrosorb RP-8, MeOH-H_2O=80:20 v/v%, flow rate: 1.0 ml/min. pressure:190kg/cm^2 .

4) Isoprene homologues in 1 where n=5 and 6 might be called as gymnoprenol-A_9 and A_{10} respectively according to the numbers of the isoprene units.

5) Nmr spectra were taken in CD_3OD solution unless otherwise stated.

6) R.P.Hanzlik, Org. Syn., 56, 112 (1977).

7) Caparrapidiol and caparrapitriol, sesquiterpene diol and triol possessing similar structures, were obtained from Ocotea caparrapi; J.B.Castillo, C.J.W.Brooks, M.M.Campbell, Tetrahedron Lett., 3731 (1966).

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