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松橄榄中的麦角甾醇类过氧化物

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摘要 从松橄榄(*Cryptoporus volvatus*)子实体中首次分离到4个麦角甾醇类化合物，其中3个是过氧化物；这些化合物的碳谱和氢谱信号通过二维谱技术及与文献报道的有关数据比较得到了指定，另外还提出了它们的一个可能的生源途径。

关键词 松橄榄，多孔菌科，麦角甾醇过氧化物 隐孔菌

ERGOSTEROL PEROXIDES FROM CRYPTOPORUS VOLVATUS

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Abstract Four ergosterols, among which three are peroxides were isolated from the methanol extract of the fungus *Cryptoporus volvatus*. Assignments of their ^{1}H and ^{13}C NMR resonance were achieved by the aid of 2D NMR experiments as well as comparison with reported data. And a possible biogenetic pathway for these compounds was proposed.

Key words *Cryptoporus volvatus*, Polyporaceae, Ergosterol, Peroxides

Cryptoporus volvatus Hubbard. (Polyporaceae), a widely distributed fungus in eastern Asia, is used as an antiinflammatory, antiviral, antiphlogistic and antiseptic agent by the native minority peoples in Yunnan province of China. There have been some reports about its chemical constituents on dealing with ergosterol [1] and sesqiterpenoids [2]. Further investigation of the methanol extracts of this fungus led to the isolation of three ergosterol peroxides and cerevisterol. We briefly describe the structure elucidation and the possible biogenetic pathway of these compounds.

Compound 1 gave a deep green colour in Liebermann-Burchard test. Its EI-MS spectrum showed a molecular ion perk at m/z 430[M, $C_{28}H_{46}O_3$]$^+$and fragment ions at 412[M-H_2O]$^+$, 394[M-2H_2O]$^+$, 376[M-3H_2O]$^+$, 305[M-C_9H_{17}(side chain)]$^+$, 269[M-2H_2O-C_9H_{17}]$^+$and 251[M-3H_2O-C_9H_{17}]$^+$](base peak), indicating that it should be a ergosterol dervative. All of the ^{1}H and ^{13}C NMR spectra data (see Tables 1 and 2) were fully assigned with those of cerevisterol [3,4].

Compouds 2, gave positive Liebermann-Burchard reaction. Its molecular fomula was determined as $C_{28}H_{44}O_3$by EI-MS spectrum. And fragment ions showed at m/z 410[M-H_2O]$^+$, 396[M-O_2]$^+$(base peak), and 303[M-C_9H_{19}(side chain)]$^+$, among those the peak of [M-O_2]$^+$was characteristic of ergosterol peroxide [4]. The ^{1}H and ^{13}C NMR data were identical with those of 3β-hydroxy-5α,

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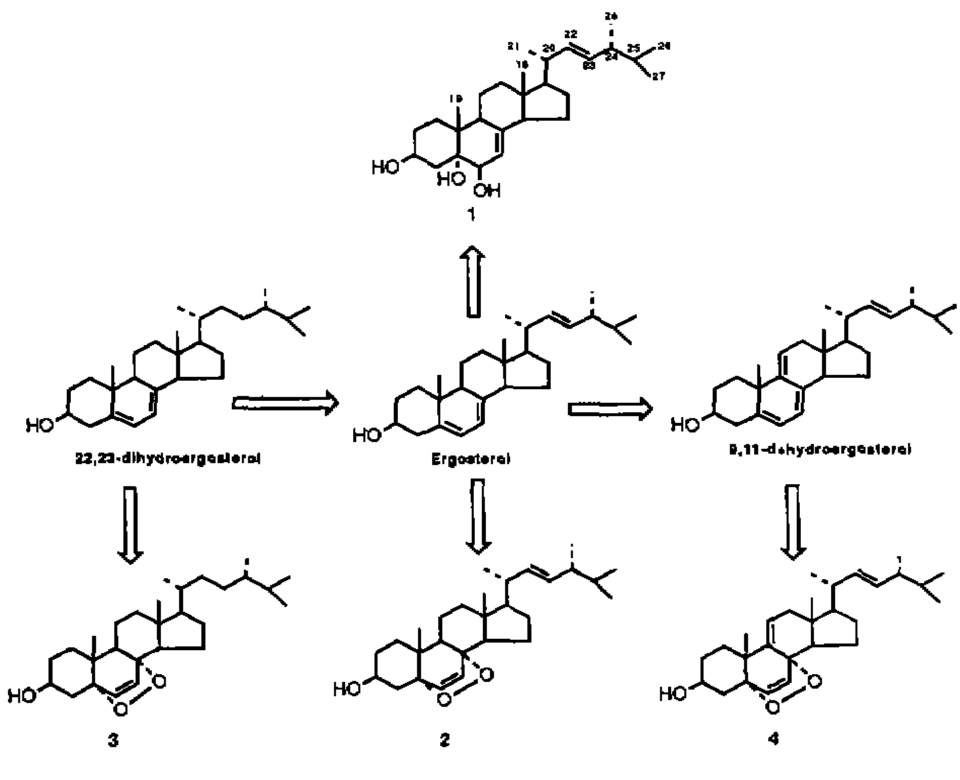
8α–epidioxyergosta–6, 22–diene [4]. But the assignments of the signals of C–11 and C–15 in reference [4] should be reversed according to 2D NMR experiments of ^1H–^1H and ^1H–^{13}C COSY and COLOC, for the carbon signal of C–11 at δ23.45 showed a clear cross peak with the proton signal of Me–19 in the COLOC spectrum. This compound has been found to occur in lower terrestrial organisms such as fungi and lichens as well as marine organisms [5].

Compound 3 gave a deep green colour in the Liebermann–Burchard test. Based on EI–MS spectrum, its molecular formula was $C_{28}H_{46}O_3$ which had only two hydrogen atoms more than compound 2, and fragment ion peaks showed at m / z 412$[M-H_2O]^+$and 398$[M-O_2]^+$. As the ^1H and ^{13}C NMR signals of the A–D rings agreed with those of compound 2, while the signals of the side chain were identical with those of an ergosterol derivertive in which the double bond between C–22 and C–23 were hydrogenated [6]. Thus compound 3 was shown to be 3 β–hydroxy–5α,8α–epidioxyergosta–6–ene. It was reported to occur in marine organisms [5].



Possible biogenetic pathway of compounds 1-4 in *Crytoporus volvatus*

Compound 4, $C_{28}H_{42}O_3$($M=426$) also gave positive Liebermann–Burchard reaction and characteristic fragment ion of ergosterol peroxide at m / z 394$[M-O_2]^+$(base peak). The ^1H and ^{13}C NMR signals of 4 revealed the presence of an aditional double bond when compared against compound 2. This double bond was determined to be located between C–9 and C–11 by the analysis of ^1H and ^{13}C NMR signals and comparison of its EI–MS fragment ions with those reported data [5], and its ^1H and ^{13}C NMR signals were assigned as shown in Tables 1 and 2. Accordingly, the structure of 4 was shown to be

3β-hydroxy-5α,8α-epidioxyergosta-6,9,22-triene. It was also reported to occur in marine organisms [5]

It is noted that ergosterol peroxides have been confirmed to be naturally occuring compounds by recent studies [5] . According to the research result of Nes and his co-workers [7] , we proposed a possible biogenetic pathway for compounds 1— 4 in *Cryptoporus volvatus* shown in the Figure. 22, 24-Dihydroergosterol and ergosterol are considered to be their precusors.

Table 1 ^{13}C NMR spectral data for compounds 1—4 in pyridine-d_5(ppm)

	1*	1	2*	2	3	4
C-1	32 75	33.85	34.80	35.49	35.51	33.35
2	30.43	32.60	30.12	31.27	30.90	31.71
3	67.26	67 61	66.46	65.86	65 86	65.69
4	39 24	41.91	37.05	28 29	38.27	37.37
5	75.88	76.18	82.17	82.32	82.32	83.01
6	73.18	74.28	135.47	135.53	136.20	136.46
7	117.48	120.46	130.77	130.93	130.95	130.97
8	143.37	141.61	79.43	79.27	79.31	78.80
9	43.18	43.81	51.27	52.00	52 00	144.18
10	36.99	38.10	37.05	37.50	37.50	38.63
11	22.84	23.50	23.45	23 71	23.72	119.19
12	38.95	38.97	39.46	39 65	39.77	41 49
13	43.62	43 81	44.63	44.72	44.89	43.85
14	54.68	55.28	51.77	52.18	52.00	48.80
15	21.92	22.45	20.67	21.16	21.15	21.36
16	27.82	28.45	28.59	29.01	28.44	28.95
17	55.99	56.27	56 35	56 43	56.55	56 10
18	12.14	12.55	12.91	13.03	12.80	13.19
19	18.24	18.79	18.17	18.39	18.35	25 74
20	40.27	40.79	39.64	39.97	35.84	40.09
21	20.97	21.43	20.90	21.13	19.00	20.90
22	135.41	136.21	135.24	135.78	33.85	135.75
23	132.06	132.00	132.41	132.37	31.24	132.51
24	42.76	43.12	42.84	43.09	39.40	43.06
25	33.01	33.39	33.12	33.37	31.80	33.35
26	19.78	20.16	19.93	20.17	17.85	20.12
27	19.48	19.87	19.63	19.87	20.66	19.83
28	17.44	17.86	17.56	17.85	15.70	17.80

*Measured in $CDCl_3$.

EXPERIMENTAL

Mps. uncorr. ^1H and ^{13}C NMR spectra were recorded with Brucker AM-400 using TMS as int.standard. EIMS spectra were taken at 20eV or 70eV accelerating voltage on Finnigan-4510 spectrometer.

Extraction and isolation

Air-dried, finely powdered fruit bodies of *C. volvatus* (2500 g) collected in Xishuangbanna, Yunnan province were extracted with MeOH. The MeOH extract was concentrated under reduced pressure below 60℃ to give brownish viscous syrup(720 g), which was dissolved in 1000 mL H_2O and then extracted with ether. The combined ether extracts were extracted with saturated aqueous Na_2CO_3 to remove the phenol constituents. The neutral ether layer was washed with H_2O and then concertrated to dryness to afford brownish powder(30 g), which was chromatographed on silic gel eluting with $CHCl_3$ to give several fractions. Fr. 2 was repeatedly chromatographed on reverse-phase silica gel(RP-18) eluting with MeOH-H_2O(8 : 2) to furnish compounds 1(20 mg; yield: 0.001%), 2(50 mg; yield: 0.002%), 3(30 mg; yield: 0.0012%) and 4(40 mg; yield: 0.0016%).

Table 2 ^{1}H NMR spectral data for compounds 1—4 in pyridine-d_5(ppm)*

	1**	1	2**	2	3	4
H-3	3.86 m	3.99 m	3.95 m	4.35 m	4.39 m	4.39 m
6	3.43 d	3.56 d	6.23 d	6.30 d	6.34 d	6.34 d
	(4.9)	(5.2)	(8.3)	(8.4)	(8.4)	(8.4)
7	5.17 dd	5.27 dd	6.49 d	6.52 d	6.55 d	6.55 d
	(4.9,2.2)	(5.2,2.2)	(8.3)	(8.1)	(8.4)	(8.5)
11						5.49 m
18	0.47 s	0.64 s	0.83 s	0.77 s	0.77 s	0.78 s
19	0.92 s	1.06 s	0.88 s	0.89 s	0.88 s	1.18 s
21	0.89 d	1.04 d	1.00 d	1.00 d	1.00 d	1.03 d
	(6.6)	(6.6)	(6.6)	(6.6)	(6.6)	(6.6)
22	5.04 dd	5.19 dd	5.15 dd	5.18 dd		5.20 dd
	(15.4,7.4)	(15.4,7.2)	(15.3,7.2)	(15.3,8.0)		(15.3,8.2)
23	5.07 dd	5.27 dd	5.23 dd	5.26 dd		5.29 dd
	(15.3,8.0)	(15.4,7.8)	(15.3,7.2)	(15.3,7.0)		(15.3,7.4)
26	0.74 d	0.83 d	0.84 d	0.86 d	0.83 d	0.83 d
	(6.4)	(6.8)	(6.4)	(6.6)	(6.3)	(6.3)
27	0.74 d	0.81 d	0.82 d	0.85 d	0.83 d	0.82 d
	(6.4)	(7.1)	(6.4)	(6.5)	(6.5)	(6.2)
28	0.69 d	0.93 d	0.91 d	0.95 d	0.87 d	0.97 d
	(6.8)	(6.8)	(6.6)	(6.8)	(6.4)	(6.8)

* Coupling constants are expressed in parenteses

** Measured in $CDCl_3$.

Compound 1. Needles, mp 249—252℃ {lit [4] mp.240—243℃}; $[\alpha]_D^{25}$−101.1° (c=0.147, pyridine){lit [3] $[\alpha]_D^{20}$−94° (c=0.29, pyridine)}.EIMS: m/z 430[M]$^+$, 412, 394, 376, 352, 333, 305, 269, 251, ^{1}H and ^{13}C NMR see Tables 1 and 2.

Compound 2. Needles, mp 168—173℃ {lit [4] mp 181—183℃; lit [8] mp 176—178℃} ,$[\alpha]_D^{25}$−27.0° (c=0.188, pyridine) {lit [8] $[\alpha]_D^{21.5}$−32.9° ($CHCl_3$)}. EIMS: m/z 428[M]$^+$, 410, 396, 376, 363, 303, 81, 69. ^{1}H and ^{13}C NMR see Tables 1 and 2.

Compound 3. Needles, mp 142—145℃, $[\alpha]_D^{25}$103.1° (c=0.153, pyridine). EIMS: m/z 430[M]$^+$, 410, 398, 303, ^{1}H and ^{13}C NMR see Table 1 and 2.

Compound 4. Needles, mp 165—170℃ {lit [8] mp 161—165℃}. $[\alpha]_D^{25}$−13.7° (c=0.125, pyridine). EIMS: m/z 426[M], 394, 376, 299, 251, 69. ^{1}H and ^{13}C NMR see Tables 1 and 2.

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11. 质谱须注明所用的方法，如(EIMS, CIMS, GC—MS, FABMS 等)及离解能，只须给出分子离子峰及重要的特征碎片峰(相对强度)，如：EIMS(70eV m/z(%): 386[M$^+$](36), 368[M—H_2O]$^+$(100), 275[M—111]$^+$(35) 等。高分辨质谱(HRMS)若有必要可多给一些信息。

12. 紫外光谱表示法，如 UVλ_{max}^{EtOH}nm(lgε): 203(4.17)。

13. 红外光谱表示法，如 IRν_{max}^{KBr}cm^{-1}: 1740。官能团的指定放在圆括号内，如: 1740(>C=O)。若要标明吸收带的强度，则采用以下缩写符号: w(弱), m(中等), v(可变), s(强), vs(很强)。

14. 有机化合物和无机化合物及有关的缩写符号须规范化(参考 CA)，如氘代溶剂 $CDCl_3$, DMSO—d_6, D_2O, pyridine—d_5 等。常见化学试剂在文中均以化学符号表示，如：MeOH, EtOH, n—BuOH, PrOH, iso—PrOH, PhOH(苯酚), petrol(石油醚), $CHCl_3$, CCl_4, C_6C_6, Et_2O, Me_2CO, HOAc, EtOAc, THF, Ac_2O, NaOMe, CH_2N_2, HCO_2H(甲酸), TCA(三氯乙酸), TFA(三氟乙酸), NaOAc, NaOH, HCl, H_2SO_4, CO_2, H_3BO_3, NH_3, N_2 等。

15. 制备薄层析须注明(1)薄层厚度；(2)样品的量；(3)确定带的方法；(4)从吸附剂上洗脱下化合物所用的溶剂。特殊 TLC 的吸附剂须注明，如: $AgNO_3$—硅胶(1∶9)。

16. 气相色谱(GC)须注明检测器(FID,EC 等)，载气及流速，操作温度，柱子情况等。

17. 高压液相(HPLC)须注明(1)柱子情况，如大小、型号；(2)压力及溶剂；(3)检测方法，如 UV 或折射率。

18. X—衍射只须给出立体结构图(最好有键长)及必要的数据，详细记录可指明在什么地方储存。