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Tricholomenyns C, D, and E, Novel Dimeric Dienyne Geranyl Cyclohexenones from the Fruiting Bodies of *Tricholoma acerbum* +

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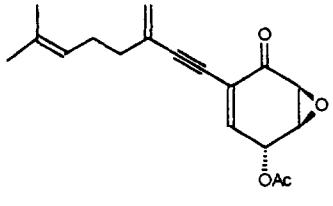
Abstract - Tricholomenyns C, D, and E, isolated from the fruiting bodies of *Tricholoma acerbum* and other species of the genus *Tricholoma*, are the first naturally occurring dimeric dienyne geranyl cyclohexenones.

Recently, in the course of our phytochemical studies on *Tricholoma acerbum* (Bull. Fr.) Quel. (Basidiomycetes) we have isolated two new compounds, called tricholomenyns A (**1**), and B, [1] which are the first members of a new class of highly oxygenated natural cyclohexanoid compounds. Indeed, the semiquinone moiety of tricholomenyns is a characteristic structural feature of several metabolites isolated from antibiotic producing microorganisms; [2] however, the dienyne geranyl side chain represents an unprecedented substitution. This paper deals with the structure elucidation of tricholomenyns C (**2**), D (**3**), and E (**4**) which, to our knowledge, are the first examples of natural dimeric cyclohexenoid structures..



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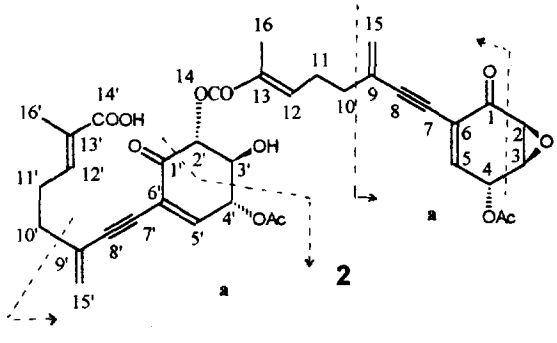

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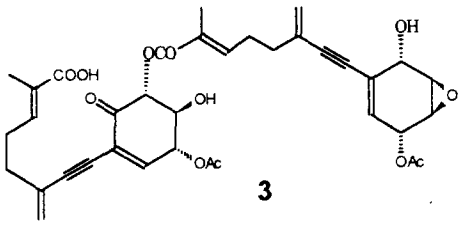
In the course of the initial chromatographic fractionation of an EtOAc extract of *T. acerbum*, tricholomenyns C - E were eluted in mixtures more polar than tricholomenyns A and B.[1] Pure samples of **2** - **4** were eventually obtained by multiple low and medium pressure liquid chromatographic separations on flash SiO_2 and reversed-phase RP-18 columns, and centrifugal radial preparative chromatography (Chromatotron). Compounds **2** - **4** are not stable on prolonged contact with silica gel and on storing, even at - 20° C, slowly decompose to untractable resinous material. Therefore, due to severe losses occurring during

+ This paper constitutes part 41 of the series "Fungal Metabolites", and is dedicated to Professor Paolo Grünanger on the occasion of his 70th birthday

extraction and isolation processes, the contents of **2-4** in fresh fruiting bodies (1.1 kg) are actually much higher than the amounts isolated in this investigation: 130, 2.5, and 2.2 mg, respectively.

Tricholomenyn C (**2**) [3] had molecular formula $C_{36}H_{36}O_{12}$ on the basis of elemental analysis, DCIMS (NH_3) data (*m z* 678, $M+NH_4^+$) and the number of carbon signals in the NOE-suppressed ^{13}C - NMR spectrum. Moreover, the UV absorption maximum at 280 nm indicated a cross-conjugated enone-dienyne chromophore identical to **1**. Analysis of the ^1H and ^{13}C NMR data of **2** (Table 1) revealed several couples of signals possessing nearly identical chemical shifts and coupling constants. They could be attributed to two identical moieties (see the partial structure **a** in formula **2**) comprising a γ-acetoxy enone group linked to a conjugated enyne system as in tricholomenyn A (**1**). The occurrence of an oxirane ring in **2** was firmly established by two oxygenated sp^3 methine carbons at δ_c 52.6 and 54.6 ppm[1] which had the typical $^1J_{CH}$ coupling constant (188 Hz) of three membered rings measured by INEPT experiments. Moreover, the proton at δ 5.87 ppm, geminal to an acetoxy group, was part of an AMX system of three oxygenated methine (H-2', H-3' and H-4'), each exhibiting *trans*-diaxial vicinal coupling constants.



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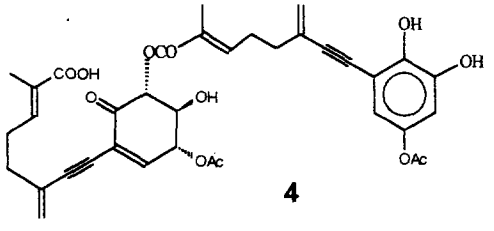

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These data allowed the construction of two cyclohexenone subunits each containing a pattern of structural elements present in tricholomenyns A (**1**) and B, respectively, [1] included the configuration of the two couples of three contiguous stereocenters. To complete the structure of tricholomenyn C one had to accomodate four sp^3 methylene carbons and two methacrylic moieties, including one free carboxylic acid group (δ_C 172.4 ppm), which explained the long tailing of **2** on TLC silica gel plates and the positive reaction to the Bromocresol green spray reagent. Final verification of the key connectivities came from ^1H - ^1H and ^1H - ^{13}C COSY spectra, ^1H - ^1H decoupling experiments, NOESY and COLOC ($^n J_{CH}$ = 10 Hz) spectra. In particular, NOE enhancements allowed assignment of the *E* stereochemistry to either methacrylic group while the key correlation from H-2' to C-14 put the ester bond at C-2'. Consequently, the structure **2** was assigned to tricholomenyn C.

Comparison of the NMR spectra of tricholomenyns D and E with those of **1** and **2** indicated dimeric structures which could derive from **2** by modifications within the right-hand cyclohexenone moiety; by contrast, the left-hand part of **2**, terminating with the free carboxylic acid group, was also occurring in tricholomenyns D and E. The signals of a secondary allylic alcohol (δ_C 65.2, δ_H 4.51 ppm) replacing those of the enone group indicated that tricholomenyn D (**3**), [4] $C_{36}H_{38}O_{12}$ (*m z* 680, $M+NH_4^+$ in the DCIMS (NH_3) spectrum), was the 1-dihydroderivative of **2**. The relative configuration of the stereogenic centers in formula **3** was deduced from the ^1H NMR coupling constants with the aid of Dreiding models: the nearly identical coupling constants between H-1 and H-2 (2.6 Hz), and H-3 and H-4 (2.0 Hz) demand similar dihedral angles, while a long range coupling between the protons H-1 and H-4 (2.0 Hz) suggests that the OH group at C-1

and the acetoxy substituent at C-4 are both pseudoaxially oriented on the "closed boat" conformation of the 7-oxabicyclo[4.1.0]hept-3-ene ring system. The spectroscopic data reported for related molecules [5] support our conclusions.

Table 1 - ^{1}H * and ^{13}C nmr$^{\Delta, +}$ Data ($CDCl_3$) for Tricholomenyns C (**2**), D (**3**), and E (**4**)

	2		**3**		**4**	
assignment	^{13}C	$^{1}H^{\neq}$	^{13}C	$^{1}H^{\#}$	^{13}C	$^{1}H^{\nabla}$
1	189.4 (0)	-	65.2[a] (1)	4.51m	144.4[a] (0)	-
2	52.6 (1)	3.60 dd	52.8 (1)	3.63 ddd	142.2[a] (0)	-
3	54.6 (1)	3.78 ddd	53.7(1)	3.48 dt	115.5 (1)	6.64[a] d
4	63.8 (1)	5.84 dt	64.5[a] (1)	5.97 dt	143.5[a] (0)	-
5	140.7 (1)	6.80 dd	127.5 (1)	5.54 bd	110.4 (1)	6.66[a] d
6	124.4[a] (0)	-	125.4 (0)	-	109.8 (0)	-
7	82.3 (0)	-	86.5 (0)	-	83.0 (0)	-
8	94.6 [b] (0)	-	92.8 [b] (0)	-	95.8 [b] (0)	-
9	129.2 (0)	-	129.9 [c] (0)	-	129.7 [c] (0)	-
10	35.3 [c] (2)	2.35 m	35.5 (2)	2.35 m	35.5 (2)	2.35 m
11	27.3 [d] (2)	2.48 m	28.3 [d] (2)	2.45 m	27.8[d] (2)	2.45 m
12	142.8 [e] (1)	6.85[a] tq	143.5[e] (1)	6.85 [a] tq	143.1[e] (1)	6.87 [b] tq
13	127.2 [f] (0)	-	127.2 [f] (0)	-	127.5 [f] (0)	-
14	166.7 (0)	-	167.2 (0)	-	167.0 (0)	-
15	123.8 [g] (2)	5.36 [b] d 5.38 [b] d	123.6 [g] (2)	5.35 [b] d 5.36 [b] d	123.3 [g] (2)	5.37 [c] d 5.42 [c] d
16	12.2 [h] (3)	1.92 [c] d	12.4 [h] (3)	1.90 [c] d	12.5 [h] (3)	1.92 [d] d
1'	188.0 (0)	-	187.9 (0)	-	188.1 (0)	-
2'	76.7 (1)	5.54 d	76.7 (1)	5.53 d	76.6 (1)	5.45 d
3'	72.5 (1)	4.25 dd	72.8[i] (1)	4.21 dd	72.7 [i] (1)	4.18 dd
4'	72.3 (1)	5.87 dd	72.6[i] (1)	5.84 dd	72.6 [i] (1)	5.78 dd
5'	146.9 (1)	6.97 d	146.8 (1)	6.93 d	146.9 (1)	6.92 d
6'	124.2 [a] (0)	-	124.6 (0)	-	124.5 (0)	-
7'	81.5 (0)	-	81.7 (0)	-	81.6 (0)	-
8'	94.9 [b] (0)	-	95.0 [b] (0)	-	95.0 [b] (0)	-
9'	129.2 (0)	-	129.5 [c] (0)	-	129.4 [c] (0)	-
10'	35.2 [c] (2)	2.35 m	35.5 (2)	2.35 m	35.5 (2)	2.35 m
11'	27.1 [d] (2)	2.48 m	27.5 [d] (2)	2.45 m	27.5 [d] (2)	2.45 m
12'	142.6 [e] (1)	6.91 [a] tq	143.0 [e] (1)	7.0 [a] tq	143.0 [e] (1)	6.99 [b] tq
13'	127.6 [f] (0)	-	127.6 [f] (0)	-	127.6 [f] (0)	-
14'	172.4 (0)	-	172.5 (0)	-	171.8 (0)	-
15'	124.0 [g] (2)	5.48 [b] d 5.49 [b] d	124.0 [g] (2)	5.41 [b] d 5.45 [b] d	124.1 [g] (2)	5.46 [c] d 5.51 [c] d
16'	11.9 [h] (3)	1.81 [c] d	12.1 [h] (3)	1.83 [c] d	12.1 [h] (3)	1.84 [d] d
$\underline{C}OCH_3$	170.3 (0) 169.5 (0)	-	170.4 (0) 169.0 (0)	-	170.5 (0) 169.8 (0)	-
$CO\underline{CH_3}$	20.5 (3) 20.3 (3)	2.15 s 2.20 s	20.7 (3) 20.7 (3)	2.10 s 2.18 s	20.7 (3) 20.9 (3)	2.19 s 2.26 s
O<u>H</u>	-	2.65 s	-	2.65 s 3.04 s	-	

* 300 MHz. $^{\Delta}$ 75.7 MHz. $^{+}$ The number in parentheses indicates the number of hydrogens attached to the corresponding carbon and was determined from DEPT experiments. $^{\neq}$ *J*'s (Hz) for **2** : 12,16 = 12', 16' = 1.0; 2, 4 = 1.3; 2, 3 = 3.5; 3, 5 = 4', 5' = 2.5; 3', 4' = 9.0; 2', 3' = 11.5; 15a, 15b = 15'a, 15'b = 3, 4 = 1.5; 4, 5 = 5.0; 11, 12 = 11', 12' = 7.0. $^{\#}$ *J*'s for **3**: 12, 16 = 12', 16' = 1.0; 2, 3 = 3.9; 1, 4 = 3, 4 = 2.0; 3, 5 = 1.8; 2, 5 = 0.8; 1, 2 = 2.6; 2', 3' = 11.5; 3', 4' = 8.6; 1, 5 = 1.3; 15a, 15b = 15'a, 15'b = 1.5; 4, 5 = 4.5; 4', 5' = 2.5; 11, 12 = 11', 12' = 7.0. $^{\nabla}$ *J*'s for **4**: 12,16 = 12', 16' = 1.2; 2', 3' = 11.5; 3', 4' = 8.7; 15a, 15b = 15'a, 15'b = 1.5; 4', 5' = 3, 5 = 2.5; 11, 12 = 11', 12' = 7.0. [a-i] Assignments in each vertical column may be interchangeable.

Tricholomenyn E (**4**),[6] $C_{36}H_{36}O_{12}$ (*m z* 678, $M+NH_4^+$ in the DCIMS (NH_3) spectrum), showed two *meta* coupled aromatic protons at δ 6.64 and 6.66 ppm and was obtained from **1** upon standing in $CHCl_3$ containing cat. HCl; therefore, it was assigned the catechol structure **4**.

It appears that biosynthesis of tricholomenyns C - E proceeds *via* a not yet isolated intermediate arising from the regiospecific oxidation of **1** at the C-14 methyl group. Therefore, the absolute configuration of **2 - 4** should correspond to that assigned to **1** on the basis of its CD spectrum.[1] Coupling of two molecules of the postulated epoxy-acid precursor would likely occur *via* the stereo- and regiospecific intermolecular opening of the epoxy ring of one monomer by the carboxylic group of another molecule. Since the epoxy ring of **1** was stable in a solvent (EtOAc - cat. AcOH) resembling the extraction medium, we believe that dimers **2 - 4** are genuine metabolites in *T. acerbum* and not artefacts arising in the course of the extraction of fungal fruiting bodies.

Tricholomenyn C (**2**) was detected not only in the fruiting bodies of *T. acerbum*, but also in *T. ustaloides* Romagn., *T. vaccinum* Kummer, *T. albobrunneum* Kummer, and *T. imbricatum* Kummer; therefore, this metabolite may be a useful chemotaxonomic marker for a number of species within the large genus *Tricholoma*.

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References and Notes

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2. (a) G. P. Orezzi, E. Arlandini, M. Ballabio, G. Cassinelli, E. Di Matteo, M.L. Garofano, A. Inventi-Solari, and F. Arcamone *Kangshengsu* **11**, 474 (1986) [C. A. **106**: 210630 (1987)]
   (b) W. Turner and D. Aldridge, "Fungal Metabolites II" Academic Press, London, 1983.
3. Physical and spectral data for **2**: $[\alpha]^{20}_D$ = -55.2 (*c* = 0.5, CH_2Cl_2); ^1H and ^{13}C NMR data are reported in Table 1. DCIMS (NH_3) *m z* (rel. int.) [M + NH_4^+] 678 (3.5), [M^+] 660 (0.4), 348 (97), 290 (16), 190 (24), 181 (100), 151 (83), 134 (91); IR 3480, 3015, 2920, 220, 1710, 1640, 1615, 1420, 1369, 1222, 1065, 1025, 907 cm^{-1}. UV (CH_2Cl_2) λ_{max} (log ε) 228 (4.31), 280 sh (3.83) nm.
   Anal. Calcd for $C_{36}H_{36}O_{12}$: C, 65.45 H, 5.49; found: C, 65.53 H, 5.42.
4. Physical and spectral data for **3**: $[\alpha]^{20}_D$ = -32.8 (*c* = 0.2, CH_2Cl_2); ^1H and ^{13}C NMR data are reported in Table 1. DCIMS (NH_3) *m z* (rel. int.) [M + NH_4^+] 680; IR 3420, 2935, 2202, 1711, 1646, 1433, 1369, 1224, 1072, 910 cm^{-1}. UV (CH_2Cl_2) λ_{max} (log ε) 227 (4.41), 275 sh (4.12) nm.
5. (a) A. Mühlenfeld and H. Achenbach *Phytochemistry* **27**, 3853 (1988) and references 6-8 cited therein.
   (b) T. Kamikubo and K. Ogasawara *Tetrahedron Lett.* **36**, 1685 (1995).
6. DCIMS (NH_3) *m z* (rel. int.) [M + NH_4^+] 678 (4.2), [M^+], 660 (0.8), 390 (17), 348 (100), 308 (20), 290 (26), 134 (16), 116 (12). The limited amount of material available has precluded an unambiguous determination of the specific rotation of compound **4**.

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