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Fungal Metabolites XXVI (°): the Structure of Saponaceolides B, C and D, New C-30 Terpenoids from *Tricholoma saponaceum*

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Abstract: The structures of saponaceolides B, C and D, new C-30 terpenoids from *Tricholoma saponaceum*, have been established, including their absolute configuration High cytotoxic activity was detected for saponaceolides B and C

INTRODUCTION

In the course of our screening program of Italian Basidiomycetes for new biologically active compounds,[1] we found that an AcOEt extract of *Tricholoma saponaceum*, while exhibiting no antibacterial activity (plate diffusion method), strongly inhibits the growth of P 388 mouse leukemia cells Subsequent separation of the extract gave the principal metabolite named saponaceolide A (**1**) (1.1% content on the crude extract) whose structure was established by NMR and X-ray studies [2] The interest for this compound is elicited by its high anticancer activity on a human colon adenocarcinoma cell line (line LoVo)[3] and the unprecedented structure **1**, which seems to be assembled according to an hitherto unknown biosynthetic pathway (Fig 1).

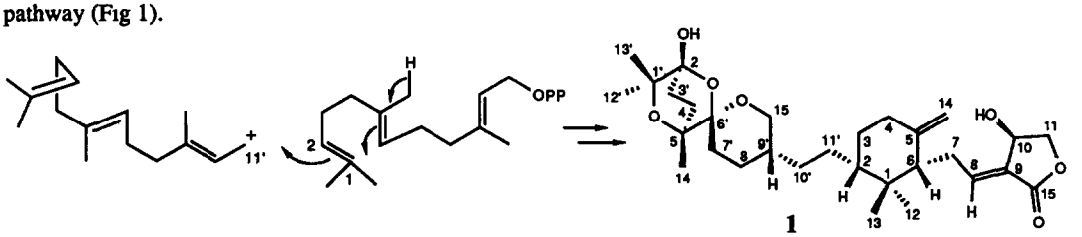

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Fig 1 Biosynthetic pathway proposed for saponaceolide A (**1**)

According to the proposed scheme, the C_{30} triterpenoid carbon skeleton of compound **1** can formally be dissected at C(2)-C(11') into two C_{15} sequences, each following the Biogenetic Isoprene Rule However, the

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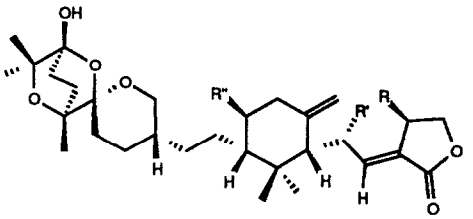
This paper is dedicated to Professor Paolo Grünanger on the occasion of his 65th birthday.
(°) Part XXV, Bosetti, A , Fronza, G , Vidari, G., Vita-Finzi, P., Norlactarane and lactarane sesquiterpenes from *Lactarius scrobiculatus*, *Phytochemistry* **1989**, *28*, 1427

two farnesyl units cannot be linked by a tail to tail coupling, as it is found in the biosynthesis of squalene, the common precursor of triterpenes.[4] It appears, instead, that alkylation-cyclization of methylenecyclohexane of saponaceolide A (**1**) must proceed by a concerted mechanism promoted by electrophilic addition of an active CH_2 group (a formal carbon cation) at C(11') on the terminal C(1)-C(2) double bond of the right half of the molecule To our knowledge a C-C bond formation of this kind has no precedent in all terpenoid biosynthetic pathways Only few examples for comparable reactions are known or postulated, such as the formation of methyl hopanoids[5] in microorganisms and cycloiridals in *Iris* plantlets [6]

The singularity of structure **1**, associated with its interesting anticancer activity, prompted us to investigate whether minor saponaceolides accompany saponaceolide A in the extract of *Tricholoma saponaceum* In this paper we detail the isolation (see the Experimental part) and structure determination of three novel saponaceolides, B (**2**),[7] C (**3**) and D (**4**) along with the determination of the absolute configuration of saponaceolide A (**1**).



2 R = R' = R" = H
3 R = R' = OH, R" = H
4 R = R" = OH, R' = H

These new findings show that more than an oddity, saponaceolides represent a characteristic new class of secondary metabolites in higher mushrooms [8] Basidiomycetes indeed continue to be a source of novel natural compounds not isolable from other living organisms [9]

RESULTS AND DISCUSSION

Structure of saponaceolide B (2)

Saponaceolide B (**2**) was obtained as colorless crystals, m p 134-136 °C, $[\alpha]_D^{20}$ + 17 91°, after crystallization (hexane-Me_2CO) of the chromatographic fractions eluted before saponaceolide A [2] The NMR signals indicated for **2** the molecular formula $C_{30}H_{46}O_6$, which was confirmed by the ions observed at m/z 502 (M^+) and 520 ($M + NH_4^+$) in the CIMS (NH_3) spectrum. On the contrary, the easy loss of a molecule of H_2O precluded detection of molecular ion in the LREIMS spectrum of compound **2**, the highest signal being observed at m/z 484 ($M - H_2O^+$) The IR spectral data of saponaceolide B (**2**) were characteristic for alcoholic, lactone and olefinic functions The presence of one trisubstituted and one 1,1-disubstituted double bond was established by the multiplicity of the four olefinic ^{13}C NMR signals (Table 1) and the splitting of the vinylic ^1H NMR signals Moreover, the ^{13}C NMR spectrum of **2** revealed the presence of four additional quaternary carbons, two being of hemiacetal type (δ_C 96 5 and 101 1) and two linked only to one oxygen (δ_C 72 7 and 77 5) Finally a total of five methyls gave rise to as many well separated singlets in the ^1H NMR spectrum of compound **2** These data were clearly consistent with the presence of an α-alkyliden-γ-lactone ring and the integrity of the spiroketal tricyclic moiety typical of saponaceolide A (**1**) Notably most features of the ^{13}C NMR spectrum of compound **2** (Table 1) were very similar to those of saponaceolide A,[2] the main difference being a new CH_2 signal replacing the C(10)-OH signal of compound **1**. Thus saponaceolide B (**2**) corresponds to 10-dehydroxysaponaceolide A Homonuclear ^1H decoupling and NOE difference experiments confirmed structure **2**, including the E stereochemistry for the C(8)-C(9) double bond

Table 1 ^{13}C Magnetic Resonance Spectral Assignments for Saponaceolides B (2) and C (3) *

Carbon number	2	3	Carbon number	2	3
1	39 5(0)	39 4(0)	1'	72 7[c](0)	72 8[c](0)
2	35 6(1)	35 7(1)	2'	96 5[d](0)	96 7[d](0)
3	28.4[a](2)	28 6[a](2)	3'	29 9[e](2)	29 3[e](2)
4	37 0(2)	36 4(2)	4'	29 1[e](2)	29 0[e](2)
5	147 9(0)	145 2(0)	5'	77 5[c](0)	77 6[c](0)
6	53 3(1)	59 4(1)	6'	101 1[d](0)	101 3[d](0)
7	27.6[a](2)	70 4(1)	7'	31 5[e](2)	31 7[e](2)
8	142 1(1)	148 0(1)	8'	24 7(2)	24 8(2)
9	124.4(0)	129 5(0)	9'	47 9(1)	48 3(1)
10	25 1[a](2)	65 9(1)	10'	26 6[a](2)	26 9[a](2)
11	65 2(2)	73 0(2)	11'	27 8[a](2)	28 0[a](2)
12	20 7[b](3)	20 9[c](3)	12'	26 3[b](3)	27 3[c](3)
13	14 6(3)	18 4(3)	13'	25 8[b](3)	25 9[c](3)
14	107 5(2)	111 2(2)	14'	22 3[b](3)	22 5[c](3)
15	171 2(0)	170 6(0)	15'	65 8(2)	65 9(2)

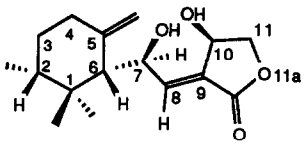
* Recorded in $CDCl_3$ at 75 47 MHz - Chemical shifts are reported as δ (ppm) values from internal TMS The number (in parentheses) of protons attached to each carbon was established by DEPT technique
a,b,c,d,e = assignments can be interchanged in each vertical column

Structure of saponaceolide C (3)

Silica gel CC elution of fractions more polar than saponaceolide A (**1**) gave, in the order, the two isomeric saponaceolides C (**3**) and D (**4**) The former, m p 152-154 °C, $[\alpha]_D^{20}$ + 31 55° was assigned the formula $C_{30}H_{46}O_8$ from NMR data and the ions observed at m/z 552 ($M + NH_4^+$) and 534 ($M + NH_4^+ - H_2O \equiv M^+$) in the CIMS ($NH_3$) spectrum. With respect to the formula of saponaceolide A (**1**), structure **3** contains one additional oxygen atom This was attributed to the presence of an OH group at C(7), on the firm ground of decoupling experiments and careful comparison of NMR spectral data of **1** and **3** In particular the ^{13}C NMR spectrum of compound **3** shows a new *C*H-OH signal at δ_c 70 4, replacing the signal of a methylene carbon (δ_c 27 7) for compound **1** Moreover, in the ^1H NMR spectrum of **3**, the signal for H-8 at δ 7 0 is splitted as a doublet of doublets, due to one vicinal (4 0 Hz) and one allylic (2 0 Hz) coupling constant This clearly indicated loss of one of the two vicinal coupling constants shown by the same proton for structure **1** [2] The coupling constants between H-7 and the vicinal hydrogen atoms H-6 and H-8 ($J_{6-7} \cong J_{7-8}$ = 4 0 Hz) might allow the stereochemical assignment of the secondary OH at C(7) if coupled with conformational analysis of the two possible diastereoisomers (7R)-**3** and (7S)-**3**

We performed molecular mechanics calculations, using Allinger's MM2(85) program,[10] on model compounds **5** and **6** in which a large part of the structure **3**, i e. the substituent at C(2), is replaced by a methyl group It is expected, in fact, that the substituent at C(2) does not affect significantly the conformational mobility around the C(6)-C(7) and C(7)-C(8) bonds

Table 2 reports the calculated relative energies and selected geometrical data for those conformers of **5** and **6** which contribute for at least 1% to the entire populations A cluster of conformers usually exists for the same arrangement of the C(6)-C(7) and C(7)-C(8) bonds due to rotation of the two OH groups, only the conformers with the lowest energy in each cluster has been reported in Table 2 Compound **5** exists for ca 90% in only one conformation (**5A**), whereas the stereoisomeric structure **6** has a higher conformational



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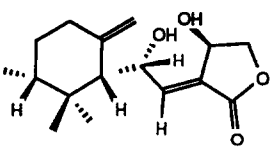

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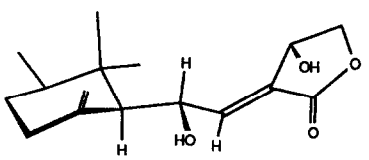
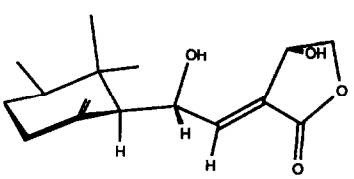
Table 2 Calculated Relative Energies (kcal/mol), Equilibrium Percentages and Selected Geometrical Data for Conformers of Compounds **5** and **6**

Conformer	E_{rel}	equil percent	torsional angles 5-6-7-8	6-7-8-9	9-10-11-11a	9-10-O-H	6-7-O-H
5A	0 00	90 0	166	146	-12	-62	70
5B	1.68	5 3	175	-84	10	-84	-170
5C	1 97	3 2	170	-65	-25	-78	-173
5D	2 41	1 5	54	-151	-18	-65	-89
6A[a]	0 00	40 7	153	133	-13	-69	45
6B	0.10	34.4	40	57	-10	-77	-178
6C	0 81	10 4	46	-150	-16	-71	-73
6D	0 92	8 6	155	169	6	-78	-83
6E	1 34	4 2	147	-90	-10	-80	179
6F	1.89	1 7	-75	-149	-16	-76	-80

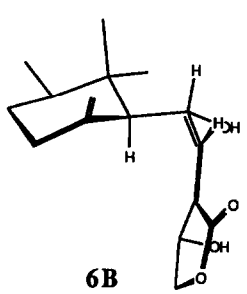
[a] 1 78 kcal/mol relative to **5A**.



5A



6A



6B

mobility, the two conformers **6A** and **6B** accounting for ca 75% of the entire population This conformational dishomogenity should clearly be reflected in the values of the vicinal coupling constants associated with the two single bonds C(6)-C(7) and C(7)-C(8) The experimental values of the coupling constants J_{6-7} and J_{7-8} for saponaceolide C (**3**) were compared with the average values for model compounds **5** (J_{6-7} = 10 3 Hz, J_{7-8} = 10 1 Hz) and **6** (J_{6-7} = 3 9 Hz, J_{7-8} = 5 4 Hz), calculated employing the Altona[11]and Garbish[12] equations, respectively A much better agreement exists between **3** and compound **6** than with the alternative structure **5**, therefore the stereocenter C(7) of saponaceolide C (**3**) must have the same stereochemistry as in formula **6**

Structure of saponaceolide D (4)

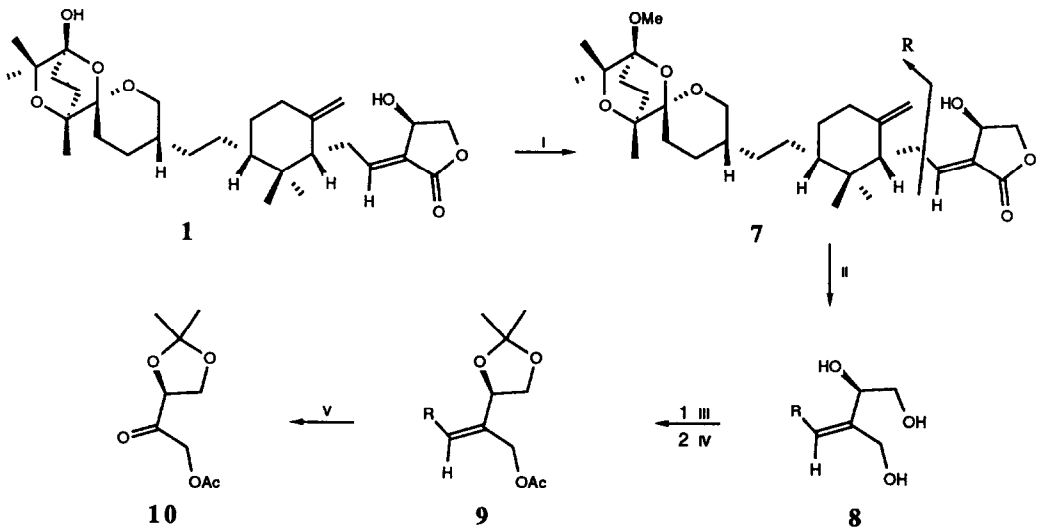
Because of the tiny amounts isolated, melting point and specific rotation of saponaceolide D could not be determined with confidence. The structure was established as **4** on the basis of ^{1}H NMR and MS spectra Pseudomolecular ions at m/z 552 ($M + NH_4^+$) and m/z 534 ($M + NH_4^+ - H_2O$), corresponding to the formula $C_{30}H_{46}O_8$ were detected in the MS spectrum of **4** only resorting to DCI technique (NH_3 as ionizing gas) In fact, in the normal CIMS (NH_3) spectrum, operating either in positive or negative ion mode, the highest peak occurred at m/z 516 ($M - H_2O^+$) ^{1}H NMR decouplings and a COSY spectrum at 500 MHz revealed coupled units such as (a) H-6, H_2-7, H-8 with allylic coupling to H-10, (b) H-10, H-11α and H-11β, (c) H-9', H-15'α and H-15'β, (d) H-2, H-3, H-4α and H-4β The saponaceolide skeleton easily accommodated the spin coupled units, most of the spectral features being almost identical with those of saponaceolide A (**1**)

With respect to the latter compound, saponaceolide D contains an additional secondary OH, which must be placed β either at C(8') or at C(3), as the geminal proton at δ 3 43, with J values of 11 0, 11 0 and 5 0 Hz, must be an axial proton (α-oriented) with two large trans diaxial couplings and a smaller axial-equatorial coupling Moreover, as two of the vicinal couplings of this proton were with allylic protons at δ 2 2 and δ 2 6, the structure 3β-hydroxysaponaceolide A (**4**) was assigned to saponaceolide D

Absolute configuration of saponaceolide A (1)

By NMR and X-ray studies only relative configuration could be assigned to the many stereogenic centres of saponaceolide A (**1**) [2] Chemical correlation (Scheme 1) of compound **1** with 1-O-acetyl-3,4-O-isopropylidenerythrulose (**10**)[13] allows now to establish the absolute stereochemistry Saponaceolide A (**1**) was first protected as 2'b-O-methylacetal (**7**) and then reduced with DIBAL to triol **8**

Protection of the 1,2-diol system as cyclic ketal and primary alcohol as acetate smoothly afforded intermediate **9**, which was immediately submitted to ozonolysis reaction Compound **10**, obtained in this way, shows a negative value for specific rotation, thus being the L-enantiomer of erythrulose [13] Configuration 2R, 6R, 10R, 2'S, 5'S, 6'S, 9'R was therefore assigned to saponaceolide A as depicted in formula **1** Biosynthetic considerations suggest this absolute stereochemistry as the most likely also for saponaceolides **2-4**



Scheme 1 *Reagents* I = MeOH-pTsOH, II = $(iBu)_2AlH$; III = Me_2CO-$Me_2C(OMe)_2$-pTsOH, IV = Ac_2O-Py, V = O_3-Ph_3P.

Biological activities of saponaceolides 1-3

Saponaceolide A (**1**) has no *in-vitro* anti-HIV activity (NCI test) Furthermore saponaceolides A (**1**) and B (**2**) are devoid of any antifungal and antibacterial activity (tested with the Kirby-Bauer growth inhibition method) However, the high cytotoxic activity displayed by the extract of *Tricholoma saponaceum* against tumoral cells[2] is retained in saponaceolides Saponaceolide B (**2**) has an ID_{50} of 163 ± 45 ng/ml on a human colon adenocarcinoma cell line (line LoVo)[3] which represents an activity even greater than that of saponaceolide A (**1**) (ID_{50} = 450 ± 112 ng/mL)[2] Additional data indicative of cytotoxic activity were obtained by bioassaying on *Artemia salina* (brine shrimps assay)[14] The LD_{50} (μg/mL) values (95% confidence) found for saponaceolides A(**1**), B(**2**) and C(**3**) were 0 27 (0 6-0 1) 0 04 (0 07-0 02) and 1 22 (4 2-0 7), respectively. Thus it appears that enhancing hydrophylicity of the right part of the saponaceolide skeleton is detrimental to the cytotoxic activity of these compounds

EXPERIMENTAL

Melting points were determined on a Fisher-Johns hot-stage apparatus and are uncorrected IR spectra were recorded as KBr pellets or neat oils on a Perkin-Elmer 197 spectrophotometer Mass spectra were obtained on a Finnigan MAT 8222 instrument NMR spectra were recorded on a Bruker WP80SY, a Bruker CPX300 or a Bruker 500 MHz instruments in $CDCl_3$ Chemical shifts are reported in δ units with Me_4Si as internal standard, the abbreviations s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet and b=broad are used throughout Optical rotations were determined with a digital Perkin-Elmer 241 polarimeter Column chromatography was performed on Kieselgel 60 (Merck), 0 04-0 06 mm, slurry packed Analytical TLC plates (250 μm) were obtained from Merck All solvents were purified and dried by standard techniques just before use All reactions were routinely carried out under an inert atmosphere of dry argon All organic solutions were washed with brine, then dried over $MgSO_4$ and filtered prior to rotary evaporation at water aspirator pressure Residual solvent was removed under vacuum, usually at less than 1 torr

Isolation of saponaceolides B (2), C (3) and D (4)

Extraction of *Tricholoma saponaceum* with AcOEt and separation of the extract by column chromatography on silica gel (column A) has already been reported in a previous paper[2] 34 groups of fractions were collected, saponaceolide A (**1**) occurring in all fractions 22-29[2] Saponaceolide B (**2**) occurred in fractions 16-20, mainly in 18-20 ones. These were pooled together (1 23 g) and chromatographed on Al_2O_3 (activity III) to free saponaceolide B (**2**) from free fatty acids Elution with CH_2Cl_2 yielded compound **2** (37 mg) which was further purified by crystallization from Me_2CO-hexane Free fatty acids were recovered by eluting the above column with MeOH-AcOH, 90 10 Fractions 28-29 (0.370 g) of column A were further separated on two consecutive silica gel columns (eluent hexane-AcOEt gradient) to give crystalline saponaceolide C (**3**) (29 mg) A small amount of saponaceolide C (**3**) was also isolated from fraction 30 of column A Fractions 31 and 32 of column A were separately chromatographed on silica gel (column B and C, respectively) Elution with an hexane-AcOEt gradient gave 4 fractions (I-IV) from column B and 5 fractions (V-IX) from column C Further column chromatography of fractions II and VI (43 2 mg) on reverse phase RP18 (25-40 μm) (eluent H_2O-MeOH gradient) yielded saponaceolide D (**4**) (1 7 mg)

Saponaceolide B (2) Mp 134-136 °C, $[\alpha]_D^{20}$ + 17 91° (CH_2Cl_2, c = 1 8) Found C, 71 75, H, 9 30 $C_{30}H_{46}O_6$ requires. C, 71 68; H, 9 22% $\bar{\nu}_{max}$ (KBr). 3420, 2960, 2925, 2850, 1755, 1675, 1640, 1445, 1375, 1315, 1185, 1135, 1120, 1070, 1030, 995, 970, 955, 930, 900, 880, 840 cm^{-1}, ^{13}C NMR spectrum is reported in Table 1, ^1H NMR (300 MHz) δ 0 58, 1 02, 1 09, 1,21 and 1 29 (5 s's, 3H each, C(12)H_3, C(13)H_3, C(12')H_3, C(13')H_3 and C(14')H_3), 1 0-2.08 (m's, all CH_2 and CH proton signals but those assigned), 1 93 (bt, 1H, H-6), 2 2-2 5 (m's, 4H, C(4)H_2 and C(7)H_2), 2.88 (m, 2H, C(10)H_2, 3 59 (ddd, 1H, $J_{15'\alpha\text{-}15'\beta}$ = 10 8 Hz, $J_{15'\beta\text{-}9'}$ = 4 5 Hz, $J_{15'\beta\text{-}8'\beta}$ = 2 0 Hz, H-15'β), 3 70 (t, 1H, $J_{15'\alpha\text{-}15'\beta} = J_{15'\alpha\text{-}9'}$ = 10 8 Hz, H-15'α), 4 39 (t, 2H, J = 7 5 Hz, C(11)H_2), 4 40 (q, 1H, J's ≅ 1.2 Hz, H-14a), 4.84(q, 1H, J's ≅ 1 4 Hz, H-14b), 6 72 (m, 1H, $J_{8\text{-}7a} + J_{8\text{-}7b}$ = 13 7 Hz, $J_{8\text{-}10a} + J_{8\text{-}10b}$ = 6 0 Hz, H-8), LREIMS (70 eV, DIS) m/z (rel intensity) 484(M-H_2O,

3), 426(16), 386(9), 221(15), 147(11), 142(62), 141(100), 140(13), 135(10), 125(11), 123(22), 120(17), 109(14), 107(14), 105(11), 99(11), 97(14), 95(21), 81(22), 79(13), 69(27), 67(14), 55(28), 43(60), CIMS (NH_3) m/z:520 (M + NH_4^+), 502(M^+), 410, 392, 316, 258, 177, 160, 147, 130, 117.

Saponaceolide C (3). Mp 152-154°C, $[\alpha]_D^{20}$ + 31 6° (MeOH, c = 1.4). Found C,67 42, H, 8 71 $C_{30}H_{46}O_8$ requires: C, 67,39, H, 8.67%. $\bar{\nu}_{max}$ (KBr): 3391, 2971, 2941, 2862, 1721, 1665, 1465, 1448, 1383, 1367, 1320, 1220, 1186, 1157, 1135, 1120, 1102, 1076, 1039, 994, 956, 932, 909, 890, 838, 816, 802, 752, 705, 682, cm^{-1}; ^{13}C NMR spectrum is reported in Table 1, ^1H NMR (300 MHz) δ 0 92, 1 09, 1 13, 1 21 and 1 29 (5s's, 3H each, C(12)H_3, C(13)H_3, C(12')H_3, C(13')H_3 and C(14')H_3), 1 0-2 05 (m's, all CH_2 and CH proton signals but those assigned, 2 08 (bd, 1H, $J_{6\text{-}7}$ = 4.2 Hz, H-6), 2 16 (ddd, 1H, $J_{4\alpha\text{-}4\beta}$ = 13 0 Hz, $J_{4\beta\text{-}3\alpha}$ = 10 8 Hz, $J_{4\beta\text{-}3\beta}$ = 1 6 Hz, H-4β), 2.30 (dt, 1H, $J_{4\alpha\text{-}4\beta}$ = 13.0 Hz, $J_{4\alpha\text{-}3\alpha}$ = $J_{4\alpha\text{-}3\beta}$ = 4 3 Hz, H-4α), 2.43 (s, 1H, OH), 2.58 (m, 1H, OH), 3.58 (ddd, 1H, $J_{15'\alpha\text{-}15'\beta}$ = 11.0 Hz, $J_{15'\beta\text{-}9'}$ = 4 5 Hz, $J_{15'\beta\text{-}8'\beta}$ = 2 0 Hz, H-15'β), 3 73 (t, 1H, $J_{15'\alpha\text{-}15'\beta}$ = $J_{15'\alpha\text{-}9'}$ = 11.0 Hz, H-15'α), 4 27 (dd, 1H, $J_{11\alpha\text{-}11\beta}$ = 10 0 Hz, $J_{11\beta\text{-}10}$ = 3 0 Hz, H-11β), 4 48 (dd, 1H, $J_{11\alpha\text{-}11\beta}$ = 10 0 Hz, $J_{11\alpha\text{-}10}$ = 6 5 Hz, H-11α), 4 90 (bs, 1H, H-14a), 5 01 (bs, 1H, H-14b), 5 12 (bt, 1H, $J_{6\text{-}7}$ $J_{7\text{-}8}$ = 4.0 Hz, H-7), 5.19 (bd, 1H, $J_{10\text{-}11\alpha}$ = 6 5 Hz, H-10), 7.0 (dd, 1H, $J_{7\text{-}8}$ = 4 0 Hz, $J_{8\text{-}10}$ = 2 0 Hz, H-8), CIMS (NH_3) m/z:552 (M + NH_4^+), 534 (M^+), 518, 410, 392 (M - $C_6M_6O_4$ = ion arising from rupture of the C(6)-C(7) bond) 375, 177, 161, 144.

Saponaceolide D (4). ^1H NMR (300 MHz). δ 0.61, 1.04, 1 09, 1.21 and 1 29 (5 s's, 3H each, C(12)H_3, C(13)H_3, C(12')H_3, C(13')H_3 and C(14')H_3, 0 9-1 9 (m's, all CH_2 and CH proton signals but those assigned), 1 9-2 2 (m, 2H, H-6 and H-4β), 2 5-2 7 (m, 2H, C(7)H_2), 2.64 (dd, 1H, $J_{4\alpha\text{-}4\beta}$ = 12 0 Hz, $J_{4\alpha\text{-}3\alpha}$ = 5 0 Hz, H-4α), 3.43 (td, 1H, $J_{2\beta\text{-}3\alpha}$ = $J_{3\alpha\text{-}4\beta}$ = 11.0 Hz, $J_{3\alpha\text{-}4\alpha}$ = 5 0 Hz, H-3α), 3.61 (dd, 1H, $J_{15'\alpha\text{-}15'\beta}$ = 11 0 Hz,, $J_{15'\beta\text{-}9'}$ = 4.5 Hz, H-15'β), 3.73 (t, 1H, $J_{15'\alpha\text{-}15'\beta}$ = $J_{15'\alpha\text{-}9'}$ = 11 0 Hz, H-15'α), 4 27 (dd, 1H, $J_{11\alpha\text{-}11\beta}$ = 10 5 Hz, $J_{11\beta\text{-}10}$ = 2.0 Hz, Hz, H-11β), 4 47 (dd, 1H, $J_{11\alpha\text{-}11\beta}$ = 10 5 Hz, $J_{11\alpha\text{-}10}$ = 6 0 Hz, H-11α), 4 70 (bs, 1H, H-14a), 5 0 (bs, 1H, H-14b), 5.06 (bd, 1H, $J_{11\alpha\text{-}10}$ = 6 0 Hz, $J_{11\beta\text{-}10}$ = 2 0 Hz, $J_{8\text{-}10}$ = 1 8 Hz, H-10), 6 99 (td, 1H, $J_{8\text{-}7}$ = 7 0 Hz, $J_{8\text{-}10}$ = 1 8 Hz, H-8), positive ions CIMS (NH_3) m/z. 534, 516, 408, negative ions CIMS (NH_3) m/z 516, DCIMS (NH_3) m/z: 552 (M + NH_4^+), 534(M^+), 516, 428, 426, 408, 391, 348, 316, 296, 279, 266, 259, 242, 232, 214, 192, 180, 163.

Degradation of saponaceolide A (1) to 1-O-acetyl-3,4-O-isopropylidenerythrulose (10)

A solution of saponaceolide A (1) (61 mg) and p-TsOH (2 mg) in dry MeOH was heated at 45°C for 90 min After the addition of 5% aq $NaHCO_3$, the solution was taken to dryness and the residue was chromatographed on silica gel (6.5 g) Elution with an hexane-AcOEt gradient (from 8 2 to 4 2) gave 2'b-O-methylsaponaceolide A (7) (52 mg, 83%), mp 170-172 °C; $\bar{\nu}_{max}$ 3413, 3084, 2942, 2868, 1733, 1671, 1644, 1451, 1373, 1191, 1140, 1118, 1083, 993, 956, 931, 903, 845, 734 cm^{-1}, ^1H NMR (300 MHz) δ 0 6, 1 05, 1 09, 1 15 and 1 25 (5s's, 3H each, C(12)H_3 C(13)H_3, C(12')H_3, C(13')H_3, and C(14')H_3), 3 4(s, 3H, OCH_3), 3 61 (dd, 1H, $J_{15'\alpha\text{-}15'\beta}$ = 11 0 Hz, $J'_{15'\beta\text{-}9'}$ = 3.5 Hz, H-15'β), 3 71 (t, 1H, $J_{15'\alpha\text{-}15'\beta}$ = $J_{15'\alpha\text{-}9'}$ = 11 0 Hz, H-15'α), 4 27 (dd, 1H, $J_{11\alpha\text{-}11\beta}$ = 10 5 Hz, $J_{11\beta\text{-}10}$ = 2 0 Hz, H-11β), 4 45 (dd, 1H, 1H, $J_{11\alpha\text{-}11\beta}$ = 10 5 Hz, $J_{11\alpha\text{-}10}$ = 6 0 Hz, H-11α), 4 59 (bs, 1H, H-14a), 4 9 (bs, 1H, H-14b), 5 05 (bd, 1H, H-10), 6 97 (td, $J_{7\text{-}8}$ = 7 0 Hz, $J_{8\text{-}10}$ = 2 5 Hz, H-8).

1 0 M DIBAL in hexane (210 μL) was added to compound 7 (50 mg, 0 094 mmol) in THF (2 0 mL) at -10 °C under an Ar atmosphere Stirring at 0 °C was continued for 45 min, and then excess hydride was quenched with H_2O (2 drops). The reaction mixture was diluted with CH_2Cl_2 and extracted with 10% NaOH The solvent was removed to give an oil which was purified by flash chromatography on silica gel (5g, Me_2CO-hexane, 4 1, then MeOH) to yield compound 8 (35 mg, 70%), mp 157-160 °C, $\bar{\nu}_{max}$ 3274, 2941, 2859, 1450, 1373, 1329, 1217, 1191, 1139, 1118, 1083, 1060, 1025, 995, 959, 934 cm^{-1}, ^1H NMR (300 MHz)δ 0 52, 0 98, 1 05, 1 12, 1 20 (5 s's, 3H each, C(12)H_3, C(13)H_3, C(12')H_3, C(13')H_3, C(14')H_3, 3 40 (s, 3H, OCH_3), 3,62 (dd, 1H, $J_{15'\alpha\text{-}15'\beta}$ = 11 0 Hz, $J_{15'\beta\text{-}9'}$ = 3.5 Hz, H-15'β), 3 71 (t, 1H, $J_{15'\alpha\text{-}15'\beta}$ = $J_{15'\alpha\text{-}9'}$ = 11 0 Hz, H-15'α), 3 69 (d, 2H, $J_{10\text{-}11}$ = 5 0 Hz, C(11)H_2), 4 17(ABq, 2H, J_{AB} = 12.0 Hz, C(15)H_2) 4 46 (s, 1H, H-14a), 4 80 (t, 1H, $J_{10\text{-}11}$ = 5 0 Hz, H-10), 4 86 (s, 1H, H-14b), 5 50 (t, 1H, $J_{8\text{-}7}$ = 6 5 Hz, H-8)

A solution of compound 8 (31 mg) and p-TsOH (2 mg) in Me_2CO (2 mL) and 2,2-dimethoxypropane (200 μL) was stirred at room temperature for 2 h After addition of 5% aq $NaHCO_3$ to neutrality, the reaction

mixture was diluted with CH_2Cl_2 and dried ($MgSO_4$) The solvent was removed to give an oil which was immediately treated with Ac_2O (0 3 mL) and pyridine (0 15 mL) Usual work up yielded an oil which was purified by flash chromatography (4 g, Hexane-AcOEt gradient) to give compound **9** (22 mg, 62% over two steps), $\bar{\nu}_{max}$ 3066, 2980, 2868, 1738, 1642, 1449, 1374, 1225, 1155, 1141, 1119, 1062, 1040, 1023, 996, 958, 931, 860 cm^{-1}, ^1H NMR (300 MHz) δ· 0 58, 1 01, 1.09, 1.17 and 1.25 (5 s's, 3H each, C(12)H_3, C(13)H_3, C(12')H_3, C(13')H_3 and C(14')H_3), 1.44 and 1.46 (2 s's, 3H each, $(CH_3)_2C$), 2.05 (s, 3H, CH_3COO), 3 40 (s, 3H, OCH_3), 3.62 (dd, 1H, $J_{15'\alpha\text{-}15'\beta}$ = 11.0 Hz, $J_{15'\beta\text{-}9'}$ = 3.4 Hz, H-15'β), 3.65-3 8 (m, 2H, H-15'α and H-11a), 4.1 (dd, $J_{11a\text{-}11b}$ = 8 0 Hz, $J_{11b\text{-}10}$ = 6.5 Hz, H-11b), 4.45 (s, 1H, H-14a), 4.50 (d, 1H, $J_{15a\text{-}15b}$ = 12 3 Hz, H-15a), 4 65 (d, 1H, $J_{15a\text{-}15b}$ = 12.3 Hz, H-15b), 4 86 (s, 1H, H-14b), 5.01 (t, 1H, $J_{10\text{-}11}$ = 7 Hz, H-10), 5 66 (t, 1H, $J_{8\text{-}7}$ = 6 3 Hz, H-8)

A slow stream of O_3 was passed through a CH_2Cl_2 solution of compound **9** (21 mg, 0.034 mmol) at -78 °C After ca 4 min. silica gel TLC indicated complete disappearance of starting material. Ph_3P (22 mg, 0 082 mmol) was then added to the reaction mixture at -78 °C and, after gradually warming to room temperature, stirring was continued overnight The solvent was removed to give a mixture which was purified by flash chromatography on silica gel (3g, 12·1 hexane-AcOEt) to yield ketone **10** (1.8 mg, 26%), $[\alpha]_D^{20}$ -55° ($CHCl_3$, c = O 1) $\bar{\nu}_{max}$ 2928, 2854, 1734, 1371, 1230, 1148, 1074, 843 cm^{-1}, ^1H NMR (300 MHz) δ: 1.40 and 1 52 (2 s's, 3H each, $(CH_3)_2C$), 2 18 (s, 3H, CH_3COO), 4.11 (dd, 1H, $J_{4\text{-}4'}$ = 8 9 Hz, $J_{4\text{-}3}$ = 5 4 Hz, H-4), 4 22 (dd, 1H, $J_{4\text{-}4'}$ = 8 9 Hz, $J_{4'\text{-}3}$ = 7 9 Hz, H-4'), 4.55 (dd, 1H, $J_{4\text{-}3}$ = 5 4 Hz, $J_{4'\text{-}3}$ = 7 9 Hz, H-3), 4.96 (ABq, 2H, $J_{1\text{-}1'}$ = 17 9 Hz, C(1)H_2); CIMS (CH_4) m/z 203 $(M + H)^+$

Other compounds from chromatographic separation of the ozonolysis mixture were not identified

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