Secondary mould metabolites. Part 59.[1] Sesquiterpene illudanes: semi-synthesis of new illudins, structures and biological activity

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Owing to their unacceptable toxicity natural illudins are of limited interest as antitumor agents. In an attempt to exploit their cytotoxic potential several derivatives have been synthesized to obtain compounds with an improved therapeutic index. In this paper we describe some alkylation and oxidation reactions giving rise in some cases to less cytotoxic derivatives. Structures and relative stereochemistries have been determined on the basis of ^{1}H and ^{13}C NMR evidence. All the compounds are less cytotoxic than illudin M and S but still very active.

Basidiomycetes, including the genus *Omphalotus*, are known to produce a series of active compounds when grown in pure cultures; the majority of these compounds belong to the illudane class, including the toxic illudins M and S and related compounds with antibacterial and antitumor activity.[2] Recently it was observed that *Omphalotus olearius* when grown on natural, poor substrates (leaves or wood) produces only illudin S, whereas with rich artificial substrates (glucose, amino acids) illudins M and S[3] and a cyclic peptide, omphalotin, are obtained.[4] We observed too, with longer growth times in liquid medium (malt extract–peptone–glucose, MPG), the production of a series of new derivatives (illudins A–E) besides illudin M.[5] These results may be an effect of a different response of the fungus to the different substrates and to non-competitive laboratory conditions or competitive natural situations with other fungi or predators. Recently, from *Omphalotus nidiformis*, a species strictly related to the Euro-American species *Omphalotus olearius*, a new series of oxygenated illudins (F–H) were isolated,[6] that are isomers of the compounds described by us.[5]

Owing to their high toxicity and poor selectivity, natural illudins are not suitable for development as antitumor agents. Therefore in the last few years a series of analogues of illudins with improved therapeutical profile have been prepared.[7–11]

Results and discussion

Continuing our search to obtain larger amounts of illudin M for chemical transformations into possibly less toxic chemotherapeutic agents, we subjected *O. olearius* to metabolic stress conditions recycling the biomass (mycelium) on liquid medium for longer times. Under these conditions illudin M **1** was obtained in amounts of 40 mg l^{-1} of solution for each recycle, and was isolated from the less polar fractions from chromatography of the EtOAc extracts. Larger amounts of illudins M and S were obtained from *Pleurotus japonicus* grown on a liquid medium containing glucose–corn steep liquor.

McMorris suggested that the toxicity of illudins may be related to their ability to behave as bifunctional alkylating agents with respect to thiol-containing enzymes of the target organisms.[7] In particular he observed that the α,β-unsaturated carbonyl moiety of illudins may react with nucleophiles in a Michael reaction. To reduce the toxicity of illudins McMorris prepared a series of illudin derivatives unable to undergo a Michael addition and therefore less toxic.[7]

Table 1 IC_{50}-Values for illudin analogs and some known compounds when tested on A2780 cell line

Compound	IC_{50} (μM)
GSH	260
1	0.0028
2	9.9
3	6.8
4	27.6
5a	63.6
13	24
14	1.7

With the ability to inactivate illudins toward the Michael reaction we treated illudin M with diazomethane in the presence of copper. Cyclopropanation of the double bond α to the carbonyl group gave the isomeric compounds **2** and **3**; moreover methyl derivative **4** was obtained as a by-product (Scheme 1).

Illudin S **9** underwent the same reaction, but the immediate products **10a**–**12a** were separated as the diacetates **10b**–**12b** due to the difficulty in obtaining them as pure compounds (Scheme 2). As shown in Table 1 compounds **2**–**4** are still active but 3 orders of magnitude less cytotoxic to A2780 cell lines than is the parent compound illudin M **1**.

To further modify illudins we employed some oxidizing agents. In particular, treatment of illudin M **1** with *m*-chloroperbenzoic acid (MCPBA) gave rise to the formation of compound **5a**, an isomer of illudin G previously isolated from shaken liquid cultures of *Omphalotus nidiformis*.[6] The reaction mechanism may be explained by formation of the expected more electrophilic epoxide at positions 4–5,† followed by migration of the double bond to open the epoxide (Scheme 3).

Analogously, using the catalytic epoxidation method with oxone (see Experimental section), the same compound **5a** was

† We are grateful to a Referee for suggesting this mechanism.

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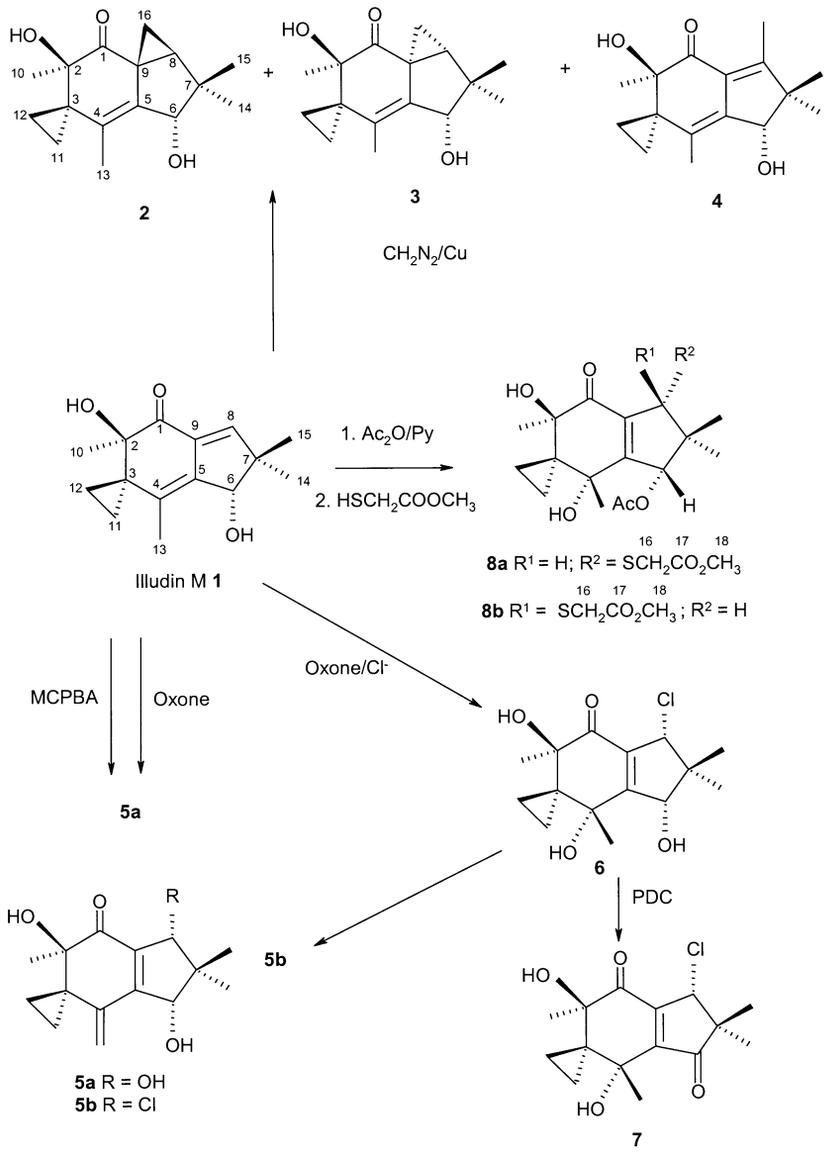


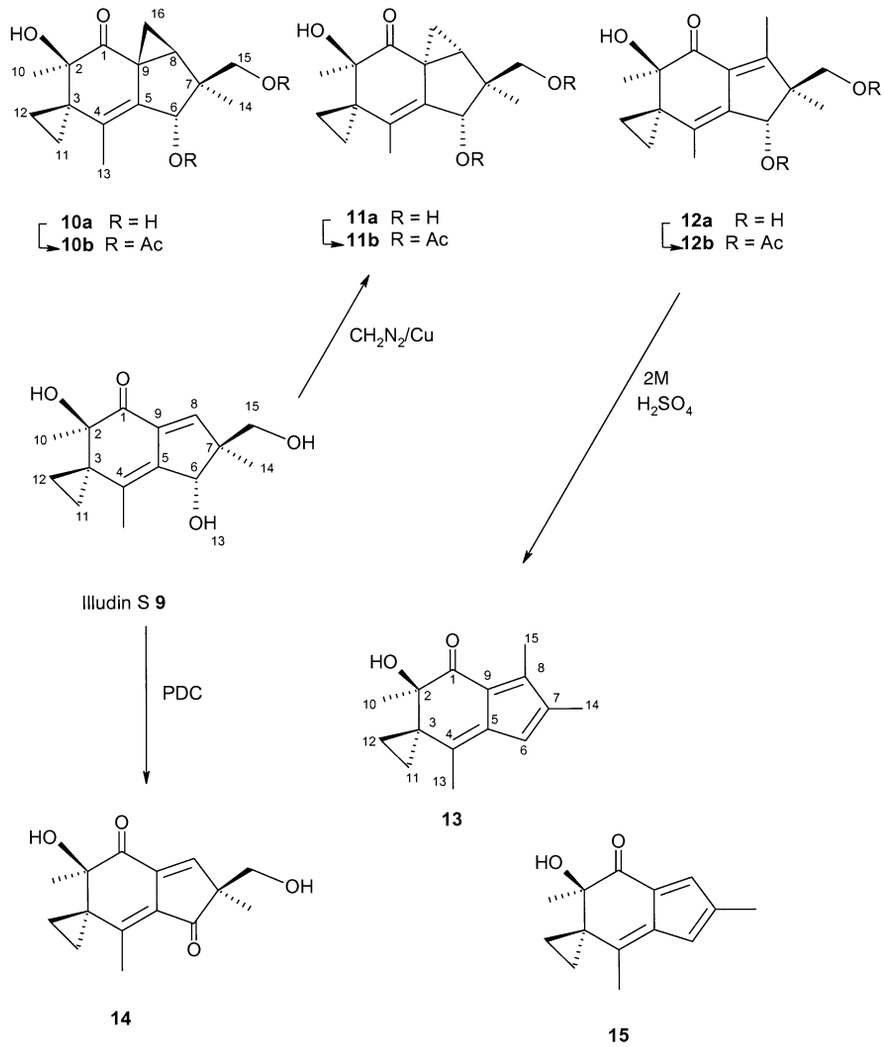
Scheme 1 Reactions of illudin M **1**.

obtained, whereas using oxone in the presence of chloride ions the chlorinated compound **6** was obtained, which was subsequently oxidized to the ketone **7**. By treatment of illudin S **9** with pyridinium dichromate (PDC) only the secondary hydroxy group was oxidized, to give dehydroilludin S **14**, analogously to what happens for illudin M.[7] Dehydroilludins are less reactive and therefore less toxic than the parent illudins.[7]

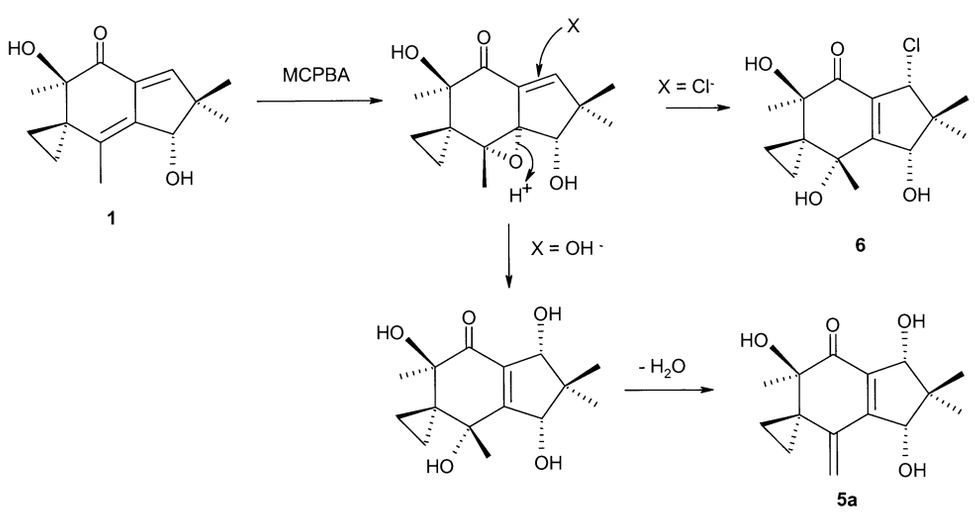
McMorris examined the reaction of illudins with various nucleophiles and found that at low pH illudin M **1** behaves as a bifunctional alkylating agent. On the other hand at neutral pH thiols such as thioglycolates or glutathione added to the α,β-unsaturated carbonyl group, producing the unstable intermediate **16** which rapidly underwent opening of the cyclopropane ring by water as nucleophile and loss of the tertiary hydroxy group to give a mixture of the stable indanetriol adducts **17** (Scheme 4). Analogously, illudin S **9** reacts with methyl thioglycolate to give a mixture of inseparable aromatic adducts.[8] In 1996, reactions of illudin S **9** with cysteine and cysteine-containing peptides were reported to give the corresponding indanol–cysteine adducts.[9] In all the reported reactions only indanols were isolated. Therefore with the aim of isolating intermediates of the above-mentioned reaction, we treated illudin M monoacetate **1a** with methyl thioglycolate as solvent; from the reaction mixture two diastereomeric tetrahydroindanols **8a**,**b** were isolated and their absolute configuration determined by NOE experiments. The reaction may be explained by the addition of the thiol to the unsaturated carbonyl group to give the unstable intermediate **18**, followed by addition of water to the double bond and oxidation to the diastereomeric compounds **8a**,**b** (see Scheme 4).

By reaction of several illudin S derivatives with dilute sulfuric acid (reverse Prins reaction), McMorris obtained a new series of active compounds, the acylfulvenes; these compounds are less toxic than illudin S **9** and this property may be related to their lower reactivity with thiols.[8] We too obtained a new substituted acylfulvene **13** by reaction of illudin S derivative **12a** (*in situ*) with dilute sulfuric acid.

The structures of compounds **2**–**8** and **10**–**14** were established by ^{1}H and ^{13}C NMR studies (Table 2 and Experimental section) while the absolute configurations of the newly formed carbon atoms in compounds **2**, **3**, **5**–**8**, **10**, and **11** were determined by NOE difference experiments having assumed that the C-6 methine proton is β-disposed as in the starting compounds **1** and **9**. In particular, the mutual NOE enhancements observed between H-6β and H-16b (4–8%) in compounds **2** and **10b**, but not in compounds **3** and **11b**, indicate that these protons are on the same β-face of the molecule. In compounds **5a**,**b**, **6** and **8a** the larger NOE enhancements observed for both H-6β and H-8 by irradiation of H_3-15 with respect to H_3-14 require that H-8 is β-oriented too; in contrast, the smaller NOE enhancements observed in compound **8b** for H-8 with respect to H-6β by



Scheme 2 Reactions of illudin S **9**.



Scheme 3 A possible mechanism of formation of compounds **5a** and **6** from illudin M **1**.

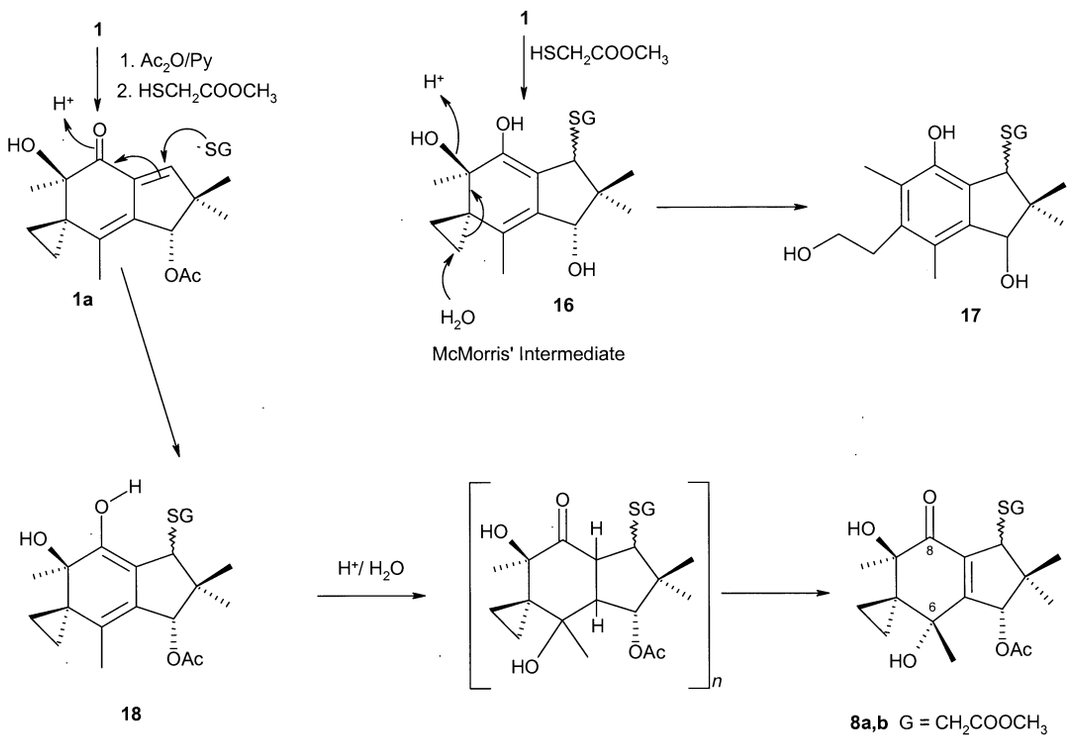
irradiation of H_3-15 imply that H-8 is α-oriented. Finally, irradiation of H_3-13 in compounds **6** and **8a**,**b** resulted, as in illudin B,[5] in a large NOE enhancement of H-6β, this fact suggesting that CH_3-13 is β-oriented.

Table 1 reports the IC_{50}-values for the *in vitro* inhibition of growth of A2780 tumor cell lines. Direct comparison with McMorris' acylfulvene **15** (see Scheme 2) was not possible because this compound was never tested on the A2780 cell line. However, in general, the cytotoxicity values of the tested compounds are similar or less than one order of magnitude lower than the range of cytotoxicity (0.9–4.2 μM) shown by **15** on a series of mulliding-resistant (MDR) cell lines.[8] In compounds **2** and **3** the elimination of the conjugated enone system of illudin M **1** due to the cyclopropanation leads to the expected reduction in cytotoxic activity, according to the mechanism proposed by McMorris.[7] More surprising is the scarce activity of **4**: in this case a possible explanation is that methylation at position 8 introduces enough steric hindrance to

Table 2 ^{1}H NMR chemical shifts for compounds **2**–**14**

Proton[a,b]	**2**[c]	**3**[c]	**4**[c]	**5a**[c]	**6**[c]	**7**[c]	**8a**[c]	**8b**[c]	**10b**[c]	**11b**[c]	**12b**[d]	**13**[d]	**14**[d]
6	4.04	4.07	4.32	4.23	4.61		5.67	5.92	5.54	5.63	5.87	6.42	
8	1.97	2.13		4.35	4.75	5.11	3.77	3.90	2.10	2.29			6.81
10	1.34	1.44	1.28	1.27	1.51	1.53	1.52	1.42	1.37	1.44	1.34	1.35	1.36
13a	1.73	1.52	1.66	5.81	1.27	1.38	1.19	1.27	1.46	1.52	1.51	2.08[e]	2.07
13b				5.28									
14	0.97	1.13	1.02[e]	1.07	1.20	1.27[e]	1.13	1.10	1.09	1.07	1.06	1.92[e]	1.21
15a	1.11	0.85	1.14[e]	0.93	1.14	1.23[e]	1.15	1.18	4.10	3.91	4.07	2.39[e]	3.77
15b									4.00	3.83	3.98		3.66
16a	1.63	2.04	2.13				3.54	3.57	1.71	1.74	2.15		
16b	1.17	1.12					3.38	3.40	1.42	1.27			
OH-2	3.82	3.92	3.94	3.15	3.95	4.25	3.98[e]	4.12[e]	3.85	3.94	3.65	4.08	2.30
OH-4					3.95	4.25	3.73[e]	3.73[e]					
OR-6	3.99	4.03	3.90	3.15	3.95		2.06	2.10	2.12[e]	2.05[e]	2.08[e]		
OH-8				3.15									
OR-15									2.02[e]	1.98[e]	1.98[e]		2.30

[a] The protons of the cyclopropane rings resonate as complex multiplets between δ 1.45 and δ 0.06. [b] In compounds **8a**,**b** H_3-18 resonate at δ 3.72; in compounds **2**, **3**, **10b** and **11b** $J_{8,16a}$ and $J_{8,16b}$ range between 5.1 and 8.6 Hz, and $J_{16a,16b}$ ranges between 3.1 and 4.2 Hz. [c] In acetone-d_6. [d] In $CDCl_3$. [e] Assignments within each column may be interchanged.



Scheme 4 A possible mechanism of formation of compounds **8a**,**b** from illudin M **1**.

strongly reduce the addition to the double bond. Probably for the same reason the cytotoxicity of the acylfulvene **13** is lower than that of McMorris acylfulvene **15** although data are not exactly comparable. In compound **5** there is still the extended conjugation system, but different from that of **1**, and here again the steric hindrance of the cyclopropyl group at position 3 might reduce the addition. In compound **14** the electron-withdrawing force of the carbonyl group, instead of an OH group, at position 6 could modify the electrophilicity of the double bond. In conclusion, the biological activity of the unique arrangement of the reactive conjugated system of illudins M and S seems very sensitive to small structural changes.

Experimental

Flash-column chromatography was performed with Merck silica gel (0.04–0.06 mm), and TLC with Merck HF_{254} and RP-18 F_{254}. IR spectra were determined on a Perkin-Elmer 177 spectrophotometer; MS on a Finnigan-MAT-TSQ70 spectrometer. NMR spectra were recorded on a Bruker AC 250L spectrometer operating at 250.1 MHz for ^{1}H and 62.9 MHz for ^{13}C; chemical shifts are in ppm (δ) from $SiMe_4$ (TMS) as internal standard. Compounds were detected on the plates with Pancaldi's reagent [cerium(IV) sulfate–phosphomolybdic acid–sulfuric acid].

Isolation and purification of illudins M and S

Strains of *Clitocybe illudens* Sacc. [*Omphalotus olearius* Singer (CBS 164.51 and ATCC 11719)] and *Pleurotus japonicus* (ATCC 20195) were used. All strains were maintained on MPGA (malt, peptone, glucose, agar; 20 : 4 : 30 : 15 g l^{-1}).

Strain *O. olearius* CBS 164.51 was sub-cultured in a stationary Erlenmeyer flask containing a liquid medium MPG (2 l) for one month at 24 °C; the culture filtrates separated from mycelium were extracted twice with EtOAc containing 1% of methanol and the extracts were dried (Na_2SO_4) and evaporated to yield a mixture (400 mg) of metabolites. The mixture was

chromatographed on a column of flash silica gel with hexane–EtOAc (1 : 1) as eluent to yield in order of decreasing R_f-value: (3′*S*,6′*R*)-2′,3′-dihydro-3′,6′-dihydroxy-2′,2′,4′,6′-tetramethylspiro(cyclopropane-1,5′-[5*H*]inden)-7′(6′*H*)-one **1** (illudin M; 40 mg) and (2′*S*,3′*R*,6′*R*)-2′,3′-dihydro-3′,6′-dihydroxy-2′-hydroxymethyl-2′,4′,6′-trimethylspiro(cyclopropane-1,5′-[5*H*]inden)-7′(6′*H*)-one **9** (illudin S; 30 mg).

Strain *O. olearius* (ATCC 11719) was sub-cultured in stirred (250 rpm) Erlenmeyer flasks containing 50 ml of a liquid medium containing glucose (40 g l^{-1}), corn-steep liquor (5 g l^{-1}), KH_2PO_4 (1 g l^{-1}), KCl (0.5 g l^{-1}), $MgSO_4 \cdot 7H_2O$ (0.5 g l^{-1}), dry yeast (5 g l^{-1}), and monosodium glutamate (10 g l^{-1}) for one month. From 17 flasks 400 mg of crude extracts were obtained and from these pure illudin M (80 mg) and illudin S (30 mg) were separated.

Strain *P. japonicus* (ATCC 20195) was sub-cultured in a stationary Erlenmeyer flask containing 2 l of the same liquid medium as in the preceding cultivation for one month. Extraction of the culture filtrates gave 2.0 g of crude extracts, from which illudin M (500 mg) and illudin S (700 mg) were obtained.

Cyclopropanation of illudin M 1

Into a 50 ml round-bottomed flask were placed 50 mg of pure copper powder and 300 mg (1.2 mmoles) of illudin M **1**, dissolved in 20 cm^3 of diethyl ether. The solution was cooled at 0 °C and a solution containing 1.5 mmol of diazomethane in 10 cm^3 of diethyl ether was added dropwise with magnetic stirring. After 5 h the solution was filtered and the solvent removed; subsequent chromatography on a silica gel column, using hexane–ethyl acetate (80 : 20) as eluent gave (1a*S*,3*S*,6*R*,7a*R*)-1,1a,2,3-*tetrahydro*-3,6-*dihydroxy*-2,2,4,6-*tetramethylspiro*(5*H*-*cyclopropa*[*c*]*indene*-5,1′-*cyclopropan*)-7(6*H*)-*one* **2** which was isolated as a white solid (30 mg; R_f 0.23), mp 100–102 °C; the ^{1}H NMR data are reported in Table 2; selected NOE experiments ‡ ([$^2H_6$]acetone): {H-6} enhanced H_3-15 (1%), H-16b (4%); {H_3-10} enhanced H-11a (1.5%), H_3-14 (1%), OH-2 (2%); {H_3-13} enhanced H-6 (3.5%), H-11b (5%), H-12b (2%), OH-6 (3%); {H_3-14} enhanced H-6 (1%), H-8 (5.5%), H_3-10 (1%), OH-6 (2.5%); {H_3-15} enhanced H-6 (6%), H-8 (2%), OH-6 (2.5%); {H-16b} enhanced H-6 (5%), H-16a (15%); δ_C([$^2H_6$]acetone) 212.22 (s, C-1), 134.83 and 130.81 (s, C-4 and -5), 78.43 (d, ^1J 142.5 Hz, C-6), 75.58 (s, C-2), 44.02 (d, ^1J 172 Hz, C-8), 43.14, 35.92 and 30.94 (s, C-3, -7 and -9), 25.02 (q, ^1J 127.5 Hz, C-10), 24.18 and 23.63 (q, ^1J 125 Hz, C-14 and -15), 22.57 (t, ^1J 164 Hz, C-16), 13.83 (q, ^1J 127 Hz, C-13), 7.83 and 4.68 (t, ^1J 162 and 161 Hz, C-11 and -12) (Found: C, 73.05; H, 8.4. $C_{16}H_{22}O_3$ requires C, 73.25; H, 8.45%); EIMS, *m*/*z* 262 (M^+, 40%), 247 (40), 229 (100), 201 (76), 178 (91).

The residual mixture was separated by RP_{18} (reversed-phase) PLC using methanol–water (50 : 50) as eluent to yield (1a*R*,3*S*,6*R*,7a*S*)-1,1a,2,3-*tetrahydro*-3,6-*dihydroxy*-2,2,4,6-*tetramethylspiro*(5*H*-*cyclopropa*[*c*]*indene*-5,1′-*cyclopropan*)-7(6*H*)-*one* **3** (60 mg; R_f 0.15), mp 97–99 °C and (3′*S*,6′*R*)-2′,3′-*dihydro*-3′,6′-*dihydroxy*-1′,2′,2′,4′,6′-*pentamethylspiro*(*cyclopropane*-1,5′-[5*H*]*inden*)-7′(6′*H*)-*one* **4** (30 mg; R_f 0.11), mp 127–129 °C; the ^{1}H NMR data are reported in Table 2. For **3** selected NOE experiments ([$^2H_6$]acetone): {H-6} enhanced H_3-13 (1.5%), H_3-15 (1%); {H_3-10} enhanced H-11a (2%), OH-2 (3%); {H_3-13} enhanced H-6 (5.5%), H-11b (5.5%), H-12b (3%); {H_3-15} enhanced H-6 (6%), H-8 (4%), H_3-14 (1%); δ_C([$^2H_6$]acetone) 210.42 (s, C-1), 135.75 and 131.25 (s, C-4 and -5), 77.63 (d, C-6), 76.66 (s, C-2), 43.01, 38.11 and 31.67 (s, C-3, -7 and -9), 38.75 (d, C-8), 28.29, 26.20 and 20.62 (q, C-10, C-14 and -15), 26.69 (t, C-16), 13.92 (q, C-13), 7.66 and 6.21 (t, C-11 and -12) (Found: C, 73.15; H, 8.30%); EIMS, *m*/*z* 262 (M^+, 50%), 247 (52), 229 (100), 201 (92), 178 (91).

For **4** (Found: C, 73.18; H, 8.35%); EIMS, *m*/*z* 262 (M^+, 91%), 247 (100), 229 (47), 201 (75), 173 (60).

Oxidation of illudin M 1 with MCPBA

To a solution of illudin M **1** (50 mg, 0.2 mmol) in Et_2O at 0 °C was added a solution of MCPBA (70%) (70 mg, 0.2 mmol) in 10 ml of Et_2O. The reaction mixture was stirred for 12 h at 20 °C, diluted with Et_2O, washed with aq. $NaHCO_3$, dried over Na_2SO_4, and evaporated *in vacuo*. The residual mixture was separated by PLC using hexane–ethyl acetate (1 : 1) as eluent to give (1′*R*,3′*R*,6′*R*)-1,2′,3′,4′-tetrahydro-1,3′,6′-trihydroxy-2′,2′,6′-trimethyl-4′-methylenespiro(cyclopropane-1,5′-[5*H*]inden)-7′(6′*H*)-one **5a** (40 mg; R_f 0.10) as an oil; the ^{1}H NMR data are reported in Table 2; selected NOE experiments ([$^2H_6$]acetone): {H-6} enhanced H-13a (4%), H_3-14 (0.5%), H_3-15 (1.5%); {H-8} enhanced H_3-14 (0.5%), H_3-15 (1.5%); {H-13a} enhanced H-6 (5%), H-13b (20%); {H_3-14} enhanced H-6 (2%), H-8 (2%); {H_3-15} enhanced H-6 (8%), H-8 (8%); δ_C([$^2H_6$]acetone) 201.60 (s, C-1), 158.62 (s, C-5), 144.21 and 137.15 (s, C-4 and -9), 114.78 (t, C-13), 83.18 and 79.22 (d, C-6 and -8), 75.35 (s, C-2), 46.64 (s, C-7), 31.97 (s, C-3), 26.97 (q, C-15), 25.41 (q, C-10), 17.32 (q, C-14), 13.76 and 4.86 (t, C-11 and -12); EIMS, *m*/*z* 264 (M^+, 25%), 246 (74), 231 (61), 203 (100), 185 (51), 175 (57); HREIMS, *m*/*z* 264.1345 (Calc. for $C_{15}H_{20}O_4$: *M*, 264.1362).

Oxidation of illudin M 1 with oxone

Into a 100 ml round-bottomed flask were placed phosphate buffer (20 ml; pH 7.8), methylene dichloride (20 ml), illudin M **1** (100 mg, 0.40 mmol) and n-$Bu_4^+HSO_4^-$ (130 mg). The stirred solution was cooled to 0 °C and an aqueous solution of oxone (potassium peroxymonosulfate, 2.45 g, 4 mmol) in 20 ml of water was added dropwise. After 3 h the organic layer was separated, washed, and concentrated *in vacuo*. The residual mixture was separated by PLC, using hexane–ethyl acetate (1 : 1) as eluent. The previously described compound **5a** was obtained (30 mg).

Oxidation of illudin M 1 with oxone in the presence of KCl

Under the same conditions as the above-described reaction but with a buffer containing KCl. The residual mixture was separated by PLC with hexane–ethyl acetate (1 : 1), and (1′*R*,3′*R*,4′*S*,6′*R*)-1′-chloro-1′,2′,3′,4′-tetrahydro-3′,4′,6′-trihydroxy-2′,2′,4′,6′-tetramethylspiro(cyclopropane-1,5′-[5*H*]inden)-7′(6′*H*)-one **6** was obtained (25 mg; R_f 0.30) as an oil; the ^{1}H NMR data are reported in Table 2; selected NOE experiments ([$^2H_6$]-acetone + D_2O): {H_3-10} enhanced H-11a (2%), H-12a (0.5%); {H_3-13} enhanced H-6 (2%), H-11b (3%), H-12b (4.5%); {H_3-14} enhanced H-8 (2.5%), H-6 (2.5%); {H_3-15} enhanced H-6 (8.5%), H-8 (8.5%); δ_C([$^2H_6$]acetone) 200.02 (s, C-1), 162.18 (s, C-5), 134.59 (s, C-9), 82.51 (d, C-6), 75.46 (s, C-2), 71.37 (s, C-4), 67.72 (d, C-8), 47.84 (s, C-7), 33.76 (s, C-3), 26.52 (q, C-10), 26.52 (q, C-15), 24.57 (q, C-13), 19.45 (q, C-14), 7.12 (t, C-12), 4.00 (t, C-11); CIMS (isobutane), *m*/*z* 301 (MH^+, 56%), 283 (84), 265 (100), 247 (95), 229 (47); HREIMS, *m*/*z* 300.1115 (Calc. for $C_{15}H_{21}ClO_4$: *M*, 300.1128).

In the NMR tube compound **6** gave rise to (1′*R*,3′*R*,6′*R*)-1′-chloro-1′,2′,3′,4′-tetrahydro-3′,6′-dihydroxy-2′,2′,6′-trimethyl-4′-methylenespiro(cyclopropane-1,5′-[5*H*]inden)-7′(6′*H*)-one **5b**; δ_H([$^2H_6$]acetone) 5.80 (1H, br s, H-13a), 5.39 (1H, br s, H-13b), 4.78 (1H, br s, H-8), 4.43 (1H, br s, H-6), 4.00 (2H, br s, OH-2 and -6), 1.31 (3H, s, H_3-10), 1.29 (3H, s, H_3-14), 1.10, 1.05, 0.92, 0.13 (4H, m, H_2-11 and -12), 1.06 (3H, s, H_3-15); selected NOE experiments ([$^2H_6$]acetone + D_2O): {H-6} enhanced H_3-13a (5%), H_3-14 (0.5%), H_3-15 (1.5%); {H-8} enhanced H_3-14 (0.5%), H_3-15 (1.5%); {H-13a} enhanced H-6 (7.5%), H-13b (22.5%); {H-13b} enhanced H-11b (5%), H-13a (28%); {H_3-14} enhanced H-6 (3.5%), H-8 (3.5%); {H_3-15}

‡ Locants given for NOE experiments are based on the illudane ring system, and are presented in the Schemes.

enhanced H-6 (11.5%), H-8 (11.5%); δ_C([$^2H_6$]acetone) 199.38 (s, C-1), 159.00 (s, C-5), 143.80 and 135.95 (s, C-4 and -9), 116.04 (t, C-13), 82.90 (d, C-6), 75.43 (s, C-2), 67.95 (d, C-8), 46.37 (s, C-7), 30.72 (s, C-3), 27.93 (q, C-15), 25.05 (q, C-10), 20.06 (q, C-14), 13.88 and 4.87 (t, C-11 and -12).

Oxidation of compound 6 with pyridinium dichromate

A mixture of compound **6** (20 mg) and PDC (100 mg) in CH_2Cl_2 (6 ml) was stirred under N_2 at room temperature for 20 h. The reaction mixture was filtered and purified by PLC with hexane–ethyl acetate (1 : 1) as eluent; (1′*R*,4′*R*,6′*R*)-1′-chloro-1′,4′-dihydro-4′,6′-dihydroxy-2′,2′,4′,6′-tetramethylspiro(cyclopropane-1,5′-[5*H*]indene)-3′,7′(2′*H*,6′*H*)-dione **7** was obtained (15 mg; R_f 0.40). The ^{1}H NMR data are reported in Table 2; δ_C([$^2H_6$]acetone) 208.72 (s, C-6), 200.90 (s, C-1), 152.88 and 151.60 (s, C-5 and -9), 76.40 and 69.54 (s, C-2 and -4), 61.78 (d, C-8), 50.51 (s, C-7), 34.33 (s, C-3), 25.76 and 22.85 (q, C-10 and -13), 25.70 and 21.44 (q, C-14 and -15), 7.38 (t, C-12), 3.73 (t, C-11); CIMS (isobutane), *m*/*z* 301 (MH^+, 26%), 281 (100), 263 (30); HREIMS, *m*/*z* 298.0940 (Calc. for $C_{15}H_{19}ClO_4$: *M*, 298.0972).

Reaction of illudin M monoacetate 1a with methyl thioglycolate

Illudin M 6-monoacetate **1a** (100 mg), prepared as described in the literature,[7] was dissolved in 0.4 ml of methyl thioglycolate and the reaction mixture was stirred at room temperature for 4 h. The crude mixture was purified by PLC, using hexane–ethyl acetate (2 : 1), to afford isomers (1′*R*,3′*R*,4′*S*,6′*R*)-3′-*acetoxy*-1′,2′,3′,4′-*tetrahydro*-4′,6′-*dihydroxy*-1′-*methoxycarbonylmethylthio*-2′,2′,4′,6′-*tetramethylspiro*(*cyclopropane*-1,5′-[5*H*]-*inden*)-7′(6′*H*)-*one* **8a** (30 mg; R_f 0.17) and (1′*S*,3′*R*,4′*S*,6′*R*)-3′-*acetoxy*-1′,2′,3′,4′-*tetrahydro*-4′,6′-*dihydroxy*-1′-*methoxycarbonylmethylthio*-2′,2′,4′,6′-*tetramethylspiro*(*cyclopropane*-1,5′-[5*H*]*inden*)-7′(6′*H*)-*one* **8b** (20 mg; R_f 0.11) as oils; EIMS *m*/*z* 394 ($M^+ - H_2O$, 6%), 352 (53), 247 (97), 229 (100) (Found: C, 58.10; H, 6.80; S, 7.60. $C_{20}H_{28}O_7S$ requires C, 58.24; H, 6.84; S, 7.77%). The ^{1}H NMR data are reported in Table 2.

For **8b** selected NOE experiments ([$^2H_6$]acetone): {H-6} enhanced H_3-13 (1%), H_3-14 (<0.5%); {H-8} enhanced H_3-14 (1.5%), H_3-15 (0.5%); {H_3-13} enhanced H-6 (11%), H-11b (5%), H-12b (5.5%); {H_3-14} enhanced H-6 (2%), H-8 (13%); {H_3-15} enhanced H-6 (11%), H-8 (2.5%), H_2-16 (1.5%); δ_C ([$^2H_6$]acetone) 199.67 (s, C-1), 171.56 (s, C-19), 170.99 (s, C-17), 160.65 (s, C-5), 137.88 (s, C-9), 82.33 (d, C-6), 76.07 (s, C-2), 70.72 (s, C-4), 56.84 (d, C-8), 52.48 (q, C-18), 47.73 (s, C-7), 34.73 (t, C-16), 34.34 (s, C-3), 25.23 and 25.11 (q, C-10 and -13), 23.04 and 22.49 (q, C-14 and -15), 21.00 (q, C-20), 6.59 and 5.00 (t, C-11 and -12).

For **8a** selected NOE experiments ([$^2H_6$]acetone): {H-6} enhanced H_3-13 (1.5%), H_3-14 (0.5%), H_3-15 (1.5%); {H-8} enhanced H_3-14 (0.5%), H_3-15 (1.5%); {H_3-13} enhanced H-6 (14%), H-11b (8.5%) and H-12b (9.5%); {H_3-14} enhanced H-6 (7%), H-8 (7%); {H_3-15} enhanced H-6 (13%), H-8 (13%); δ_C([$^2H_6$]acetone) 201.03 (s, C-1), 171.29 (s, C-19), 170.50 (s, C-17), 157.50 (s, C-5), 139.60 (s, C-9), 82.43 (d, C-6), 75.60 (s, C-2), 70.72 (s, C-4), 57.57 (d, C-8), 52.38 (q, C-18), 45.64 (s, C-7), 35.55 (t, C-16), 34.50 (s, C-3), 28.46 (q, C-15), 26.60 and 24.85 (q, C-10 and -13), 21.08 (q, C-20), 19.94 (q, C-14), 7.27 and 4.14 (t, C-11 and -12).

Cyclopropanation of illudin S 9

Into a 50 ml round-bottomed flask were placed 15 mg of pure copper powder and 100 mg (0.4 mmol) of illudin S **9**, dissolved in 20 ml of diethyl ether. The magnetically stirred solution was cooled at 0 °C and a solution containing 0.5 mmol of diazomethane in 10 ml of diethyl ether was added dropwise. After 5 h the solution was filtered and the solvent removed; subsequently, the crude mixture was dissolved in pyridine (1 ml), acetic anhydride (2 ml) was added at 0 °C, the solution was kept overnight, reactants were removed under reduced pressure, and the residue was purified by PLC, using hexane–EtOAc (4 : 1) (three runs), to give compounds **10b**–**12b**.

For (1a*S*,2*S*,3*R*,6*R*,7a*R*)-3-acetoxy-2-acetoxymethyl-1,1a,2,3-tetrahydro-6-hydroxy-2,4,6-trimethylspiro(cyclopropa[*c*]-indene-5,1′-cyclopropan)-7(6*H*)-one **10b** (20 mg; R_f 0.16), the ^{1}H NMR data are reported in Table 2; selected NOE experiments ([$^2H_6$]acetone): {H-6} enhanced H_2-15 (2%), H-16b (7%); {H_3-14} enhanced H-6 (1%), H-8 (9.5%), H_3-10 (1%), H_2-15 (3%); {H_2-15} enhanced H-6 (2%), H_3-14 (0.5%); {H-16b} enhanced H-6 (8%), H_2-15 (1%), H-16a (14%); δ_C([$^2H_6$]acetone) 211.20 (s, C-1), 170.76 and 170.50 (s, 2 × Me*C*O_2), 132.15 and 130.45 (s, C-4 and -5), 75.27 (s, C-2), 74.08 (d, C-6), 69.01 (t, C-15), 46.84, 35.30 and 30.64 (s, C-3, -7 and -9), 40.05 (d, C-8), 24.70 (q, C-10), 22.70 (t, C-16), 20.82 and 20.66 (q, 2 × *Me*CO_2), 19.76 (q, C-14), 13.85 (q, C-13), 7.88 and 4.69 (t, C-11 and -12); HREIMS, *m*/*z* 362.1705 (Calc. for $C_{20}H_{26}O_6$: *M*, 362.1729).

For (1a*R*,2*S*,3*R*,6*R*,7a*S*)-3-acetoxy-2-acetoxymethyl-1,1a,2,3-tetrahydro-6-hydroxy-2,4,6-trimethylspiro(cyclopropa[*c*]-indene-5,1′-cyclopropan)-7(6*H*)-one **11b** (20 mg; R_f 0.16), the ^{1}H NMR data are reported in Table 2; selected NOE experiments ([$^2H_6$]acetone): {H-6} enhanced H_3-13 (2%), H_2-15 (2%); {H_2-15} enhanced H-6 (1.5%), H-8 (1%), H_3-14 (0.5%); δ_C([$^2H_6$]acetone) 209.02 (s, C-1), 170.76 and 170.10 (s, 2 × Me*C*O_2), 134.90 and 131.20 (s, C-4 and -5), 76.47 (s, C-2), 74.99 (d, C-6), 69.58 (t, C-15), 47.64, 38.40 and 31.97 (s, C-3, -7 and -9), 33.61 (d, C-8), 26.00 (q, C-10), 24.15 (t, C-16), 20.94 and 20.82 (q, 2 × *Me*CO_2), 16.00 (q, C-14), 14.23 (q, C-13), 8.24 and 6.74 (t, C-11 and -12); CIMS, *m*/*z* 363 [M^+ + 1]; HREIMS, *m*/*z* 362.1710 (Calc. for $C_{20}H_{26}O_6$: *M*, 362.1729).

For (2′*S*,3′*R*,6′*R*)-3′-acetoxy-2′-acetoxymethyl-2′,3′-dihydro-6′-hydroxy-1′,2′,4′,6′-tetramethylspiro(cyclopropane-1,5′-[5*H*]-inden)-7′(6′*H*)-one **12b** (15 mg; R_f 0.22), the ^{1}H NMR data are reported in Table 2; CIMS, *m*/*z* 363 [M^+ + 1]; HREIMS, *m*/*z* 362.1718 (Calc. for $C_{20}H_{26}O_6$: *M*, 362.1729).

Acylfulvene 13

100 mg of illudin S **9** were subjected to reaction with diazomethane (see above), the crude reaction mixture was evaporated under vacuum, and the residue was suspended in 28 ml of water, then 10 ml of 2 M H_2SO_4 were added and the resulting solution was stirred overnight, extracted with ethyl acetate (2 × 20 ml), washed with saturated aq. $NaHCO_3$, and the solvent was removed under reduced pressure. The crude extract was purified by PLC with hexane–EtOAc (2 : 1) as eluent, to give (6′*R*)-6′-hydroxy-1,2′,4′,6′-tetramethylspiro-(cyclopropane-1,5′-[5*H*]inden)-7′(6′*H*)-one **13** (20 mg; R_f 0.60). The ^{1}H NMR data are reported in Table 2; CIMS, *m*/*z* 231 [M^+ + 1]; HREIMS, *m*/*z* 230.1278 (Calc. for $C_{15}H_{18}O_2$: *M*, 230.1307).

Oxidation of illudin S 9 with pyridinium dichromate

A mixture of illudin S **9** (60 mg) and PDC (150 mg) in CH_2Cl_2 (20 ml) was stirred at room temperature for 4 h. The reaction mixture was filtered and was separated by PLC, using hexane–ethyl acetate (1 : 1) as eluent, to afford (2′*S*,6′*R*)-6′-hydroxy-2′-hydroxymethyl-2′,4′,6′-trimethylspiro(cyclopropane-1,5′-[5*H*]-indene)-3′,7′(2′*H*,6′*H*)-dione **14** (40 mg; R_f 0.35); EIMS, *m*/*z* 262 (M^+, 11%), 247 (10), 231 (23), 189 (20), 91 (25), 43 (100); HREIMS, *m*/*z* 262.1167 (Calc. for $C_{15}H_{18}O_4$: *M*, 262.1205).

in vitro Cytotoxicity test

The A2780 cells were cultured in RPMI-1640 containing 10% fetal calf serum. Cytotoxicity was assessed by growth inhibition assay after 1 h of drug exposure. Briefly, cells in the logarithmic

phase of growth were harvested and seeded in duplicate into 6-well plates. 24 h after seeding, cells were exposed to the drug and harvested 72 h after exposure and counted with a Coulter counter. IC_{50} is defined as the inhibitory drug concentration causing a 50% decrease of cell growth over that of the untreated control. All compounds are insoluble in water and were dissolved in dimethyl sulfoxide prior to dilution into the biological assay.

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