Bioactive and Other Sesquiterpenes from *Chiloscyphus rivularis*

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Bioassay-directed fractionation of the methyl ethyl ketone extract of *Chiloscyphus rivularis* yielded five new sesquiterpenes, 12-hydroxychiloscyphone (**2**), chiloscypha-2,7-dione (**3**), 12-hydroxychiloscypha-2,7-dione (**4**), chiloscypha-2,7,9-trione (**5**), and rivulalactone (**6**) in addition to the known sesquiterpenes, 4-hydroxyoppositan-7-one (**7**), chiloscyphone (**1**), and isointermedeol (**8**). The structure and stereochemistry of rivulalactone, a novel trinorsesquiterpene, was confirmed by its synthesis starting from **1**. Compound **2** showed selective bioactivity in our yeast-based DNA-damaging assay and cytotoxicity to human lung carcinoma cells.

The systematic search for anticancer agents from plants has now been carried out for more than 35 years, since its beginnings in 1960 under the auspices of Dr. Jonathan Hartwell at the National Cancer Institute.[2] This search has been remarkably successful, with clinical drugs such as Taxol and the camptothecin analogue Topotecan as evidence of its success; in one recent evaluation, 62% of available anticancer drugs are natural products or modeled on natural products, and 7 of the 87 are plant or plant-derived products.[3] Although much of the effort has focused on the higher plants, cogent arguments have been advanced for the investigation of bryophytes,[4] and we initiated such an investigation some years ago.[5]

As part of our systematic search for potential anticancer agents from plants,[6] with a special focus on bryophytes, we collected a sample of the liverwort *Chiloscyphus rivularis* (Schrad.) Hazlinsky (Hepaticae, Lophocoleaceae). The taxonomy and nomenclature of this species are not fully resolved. Some authorities on the taxonomy of liverworts include *C. rivularis* under *C. polyanthos* (L.) Corda,[7] while others recognize it as a variety [*C. polyanthos* var. *rivularis* (Schrader) Nees].[8,9] *C. rivularis* grows submersed in water, in contrast with *C. polyanthos*, which generally grows on wet soil near streams. The genus name has also been spelled *Chiloscyphos* or *Cheiloscyphos*, and the authority for the species name *C. rivularis* has been credited to Loeske instead of Hazlinsky, who appears to be the earliest author for the name based on references given for synonyms in Schuster;[8] we follow the International Code of Botanical Nomenclature in our choice of spelling and authorities for names. The results reported in this paper suggest that *C. rivularis* is chemically distinct from *C. polyanthos*. Testing of *C. rivularis* in a yeast-based assay for DNA-damaging agents[6] indicated that it had reproducible activity, with an IC_{12} value of 1800 μg/mL against the yeast strain RS322. This result suggested that it contained a nonspecific DNA-damaging agent, and work was initiated to isolate and characterize the active agent(s).

Previous work on liverworts of the *Chiloscyphus* genus has yielded an assortment of sesquiterpenoids and other compounds. Perhaps the most interesting compound isolated to date is the sesquiterpenoid chiloscyphone, obtained initially from a Japanese collection of *C. polyanthos*. Originally assigned a *cis*-decalin structure,[10] its structure was reassigned based on synthetic[11] and spectroscopic[12] studies to the novel ring-contracted structure **1**, and it has given its name to the class of chiloscyphane sesquiterpenoids.[13] Its absolute stereochemistry has been determined by an X-ray crystallographic study.[14] Other compounds from *Chiloscyphus* sp. include chiloscypholone,[12] 11,12-epoxychiloscypholone,[13] and *ent*-(5*R*,6*S*,9*R*)-4α-hydroxyoppositan-10-one[13] from *C. pallescens*, (+)-α-selinene[15] (enantiomeric with the α-selinene from *Acorus calamus*), the *ent*-7,8-eudesmanolides diplophyllolide,[16,17] 7α-hydroxydiplophyllolide,[16−18] diplophyllin,[16] 3-oxodiplophyllin,[16] small amounts of other sesquiterpenoids,[18] and carotenoids[19] from *C. polyanthos* and (*E*)-dec-2-enal from a *Chiloscyphus* sp.[20] The only previous work reported on *C. rivularis* is a study of its reported photosynthetic characteristics and the observation that its thalli had high concentrations of chlorophylls a and b and carotenoids.[21]

Results and Discussion

Solvent−solvent partitioning of an MEK extract of *C. rivularis* between hexane and 80% aqueous MeOH, dilution of the aqueous MeOH fraction to 60% aqueous MeOH, and extraction of this with $CHCl_3$ yielded a $CHCl_3$ fraction showing bioactivity in the rad52 DNA repair-deficient yeast strain RS322.[6] Further bioassay-directed fractionation of the $CHCl_3$ fraction by centrifugal partition chromatography, Si gel chromatography, and preparative TLC afforded bioactive compound **2** as well as inactive compounds **3**−**7**. Chromatographic fractionation of the hexane fraction gave chiloscyphone (**1**) and compound **8**.

Compound **2** had the composition $C_{15}H_{22}O_2$ as determined by HREIMS. Its UV and IR spectra indicated the presence of an α,β-unsaturated ketone (224 nm and 1659 cm^{-1}). Its ^{1}H-NMR spectrum showed signals for two olefinic methylene protons (δ 5.95, 6.10), a methine proton α to a carbonyl group (δ 3.52, d, J = 7.6 Hz), and a two-proton eight-line pattern at δ 4.25 suggestive

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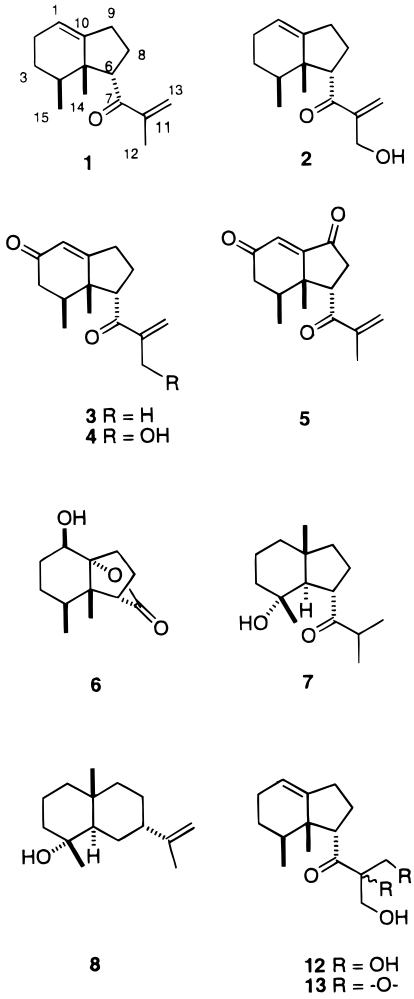


of a CH_2OH group. A DQ COSY spectrum indicated that this latter signal was coupled only to a multiplet at δ 2.50, and when the spectrum was obtained in the presence of added D_2O, the eight-line pattern was simplified to an AB quartet (J = 15.6 Hz). The presence of the OH group was confirmed by an absorption at 3558 cm^{-1} in the IR spectrum. These data thus indicated the presence of structural unit **a** in compound **2**, (Figure 1), and this was confirmed by an HMBC spectrum, which gave the correlations shown (arrows).

The remaining signals in the ^{1}H-NMR spectrum of **2** were less easily assigned, consisting of complex multiplets in the δ 1.4−2.5 region, together with a signal for a vinyl proton at 5.40 (1H, m) and for two methyl groups at 0.96 (3H, s) and 0.83 (3H, d, J = 6.4). The presence of an additional double bond was confirmed by the ^{13}C-NMR spectrum, which gave signals for two olefinic carbons at δ 117.4 and 146.2, in addition to the carbons of the α,β-unsaturated ketone system at δ 207.2, 147.8, and 124.8. The presence of three unsaturations demanded that compound **2** be bicyclic.

The structure could be elucidated further starting from the methine proton at 3.52 ppm α to the carbonyl group. Because this proton appeared as an apparent doublet, it must be flanked by a quaternary carbon and by a carbon carrying one or possibly two protons. The DQ COSY spectrum showed a correlation between the proton at 3.52 ppm and a proton at 2.00 ppm, and the latter proton was correlated with a proton at 1.69 ppm and a signal at 2.50 ppm. A combination of HETCOR and DEPT spectra showed clearly that both sets of

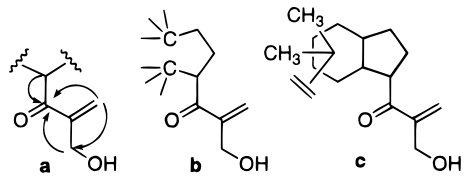


Figure 1. Structural units of compound **2**. HMBC correlations (H to C) are shown by arrows.

Table 1. ^{1}H− and ^{13}C-NMR Data for Compound **2**[a]

position	δ_H[b]	δ_C[c,d]	COSY	HMBC
1	5.40 m	117.4 (1)	H-2	
2a,b	1.94 m	25.3 (2)	H-1, H-3	
3a,b	1.38 m	26.9 (2)	H-2	
4	1.36 m	32.9 (1)	H-15	
5		50.0 (0)		
6	3.52 d 7.6	52.8 (1)	H-8a	C-5, C-7, C-9, C-10, C-14
7		207.2 (0)		
8a	2.00 m	26.0 (2)	H-6, H-8b, H-9	C-7
8b	1.69 m		H-8a, H-9	C-7
9a,b	2.50 m	29.0 (2)	H-8a, H-8b	
10		146.2 (0)		
11		147.8 (0)		
12a,b	4.25 dq 15.6, 6.0	63.1 (2)	12-OH	C-7, C-11, C-13
13a	6.10 br s	124.8 (2)		C-7, C-12
13b	5.95 br s			C-7, C-12
14	0.96 (3H) s	20.6 (3)		
15	0.83 (3H) d 6.4	17.6 (3)	H-4	C-3, C-4, C-5
12-OH	2.50 m		H-12	

[a] Data recorded in $CDCl_3$ at 400 MHz (^{1}H) and 100 MHz (^{13}C). [b] Multiplicities and coupling constants (in Hz) are listed. [c] Assignments were determined by HETCOR and DEPT experiments. [d] Carbon type as determined by DEPT spectra: 0 = quaternary, 1 = methine, 2 = methylene, 3 = methyl.

protons at 1.69/2.00 and 2.50 ppm were due to methylene groups, and the COSY spectrum showed no additional couplings to the protons at 2.50 ppm. These data thus allow the expansion of structural unit **a** to unit **b**, where both terminal carbons are quaternary (Figure 1). This fact, together with the presence of two methyl groups and an HMBC correlation between H-6 and C-10, demands the expansion of the partial structure to **c** (Figure 1), where two methyl groups and one double bond remain to be located.

Of the two methyl groups, the carbon of the one giving rise to a singlet at 0.96 ppm in the ^{1}H-NMR spectrum showed an HMBC correlation with the methine proton at 3.52 ppm; this evidence thus demands the location of this carbon at C-5. The other methyl group appeared as a doublet at 0.83 ppm, and its protons gave an HMBC correlation to the quaternary carbon at C-5; this methyl group is thus located at C-4. The double bond must be located at the 1(10) position to account for the chemical shift of the H-9 protons.

The above data thus indicate that compound **2** has the structure shown, a conclusion that is confirmed by the HMBC correlations given in Table 1 and shown in Figure 2. The mass spectral fragmentations of **2** offer strong corroborative evidence for this conclusion.

The stereochemistry of **2** was assigned with the aid of a NOESY spectrum, which showed NOE correlations between the protons of H_3-14 and H-6 and between the protons of H_3-15 and H-6. These correlations require that H-6, CH_3-14, and CH_3-15 all be cis to each other, and thus that the C-6 side chain have the α-orientation. The absolute stereochemistry of **2** was assigned as that

Table 2. ^{1}H-NMR Data for Compounds **3**–**6**[a,b]

position	3	4	5	6
1	6.01 s	5.88 s	6.41 s	4.18 dd 2.3, 2.6
2a				1.95 m
2b				1.73 m
3	2.18 (2H) m	2.22 (2H) m	2.33 (2H) m	a 1.53 m
				b 1.32 m
4	2.16 m	2.22 m	2.16 m	1.73 m
6	3.68 d 7.4	3.66 d 8.0	3.98 d 7.6	2.59 d 3.8
8a	2.15 m	2.18 m	2.73 dd 18.4, 7.6	2.10 m
8b	1.82 m	1.86 m	2.43 d 18.4	1.73 m
9	a 2.85 m	2.83 (2H) m		a 2.53 m
	b 2.79 m			b 1.73 m
12	1.86 (3H) s	4.31 (2H) br, s	1.88 (3H) s	
13a	5.88 br, s	6.18 br, s	6.13 br, s	
13b	5.84 br, s	6.08 br, s	5.98 br, s	
14	1.13 (3H) s	1.15 (3H) s	1.28 (3H) s	0.97 (3H) s
15	0.90 (3H) d 6.0	0.91 (3H) d 5.6	1.02 (3H) d 6.7	0.89 (3H) d 6.7
12-OH		2.22 m		

[a] Data recorded in $CDCl_3$ at 400 MHz. [b] Multiplicities and coupling constants (in Hz) are listed.

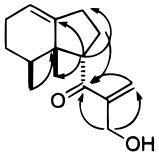


Figure 2. Selected HMBC correlations (H to C) for compound **2**.

of chiloscyphone (**1**) on the basis of their very similar CD spectra, with both showing negative Cotton effects at about 348 nm. The structure and relative and absolute stereochemistries of **2** can thus be assigned as those of 12-hydroxychiloscyphone. Complete ^{1}H- and ^{13}C-NMR assignments for **2** were made by a combination of DQ COSY, HETCOR, and HMBC experiments and are given in Table 1.

Compound **3** had the molecular composition $C_{15}H_{20}O_2$ as determined by HREIMS, and had NMR spectra that showed some similarities to those of **2**. One major difference between the spectra of **2** and **3** was that in the spectra of **3** the signals for the $-CH_2OH$ group of **2** (δ_H 4.25; δ_C 63.1) were replaced by the signals for a vinyl methyl group (δ_H 1.86, 3H; δ_C 17.6). A second major difference was that the ^{13}C-NMR spectrum of **3** contained signals for two carbonyl carbons (δ_C 199.0 and 204.7).

The first carbonyl group of **3** was clearly still conjugated to a double bond in the side chain, because the H_2-13 protons had the downfield shift (δ_H 5.84, 5.88) characteristic of β-protons in an α,β-unsaturated carbonyl system. The other carbonyl group was assigned to C-2 because the ^{1}H-NMR signal of H-1 changed from a multiplet of 5.40 ppm in **2** to a sharp singlet at 6.01 ppm in **3**. These data indicated that compound **3** had the structure shown. The stereochemistry of **3** was confirmed by the observation of NOE correlations between H_3-14 and H-6 and between H_3-15 and H-6, showing that it had the same relative stereochemistry as **2**. The structure and stereochemistry of **3** were thus assigned as chiloscypha-2,7-dione, a new sesquiterpenoid.

Compound **4** had the composition $C_{15}H_{20}O_3$ as determined by HREIMS. Its ^{1}H- and ^{13}C-NMR spectra (Tables 2 and 3) could readily be assigned by noting that the signals for the protons and carbons of the ring system were very similar to those of compound **3**, while the signals for the protons and carbons of the side chain

Table 3. ^{13}C-NMR Data for Compounds **3**–**6**[a,b]

position	3	4	5	6
1	124.9	121.5	122.9	66.5
2	199.0	199.0	199.4	29.9
3	41.2	41.2	41.9	23.6
4	33.4	33.4	35.2	32.7
5	51.0	51.2	48.3	51.5
6	51.8	52.2	47.1	51.8
7	204.7	205.1	204.3	177.8
8	26.7	26.7	41.1	21.5
9	29.9	29.9	203.6	28.1
10	177.9	177.6	160.0	92.5
11	145.2	147.4	145.0	
12	17.6	62.5	17.3	
13	121.4	125.6	126.7	
14	18.2	18.3	18.7	17.1
15	16.6	16.8	16.0	11.3

[a] Data recorded in $CDCl_3$ at 100 MHz. [b] Assignments made with the aid of HETCOR and DEPT spectra.

matched those of compound **2**. Compound **4** could thus be assigned as the new sesquiterpenoid 12-hydroxychiloscypha-2,7-dione.

Compound **5** had the composition $C_{15}H_{18}O_3$ as determined by HRCIMS. Its NMR spectra (Tables 2 and 3) indicated that it had the same side chain and A-ring as compound **3**, but its B-ring contained a carbonyl group (δ_C 203.6) in place of a methylene group. The carbonyl group was assigned to C-9 on the basis of the coupling observed in a DQ COSY spectrum between H-6 and H_2-8; the latter protons appeared downfield at 2.43 and 2.73 ppm, providing further evidence of the adjacent carbonyl group. Compound **5** was thus assigned the new structure chiloscypha-2,7,9-trione.

Compound **6** had the composition $C_{12}H_{18}O_3$ as determined by HREIMS, indicating it to be a trisnorsesquiterpenoid. Analysis of its NMR data (Tables 2 and 3) and its DQ COSY, HETCOR, and HMBC spectra indicated the presence of two $-CH_2CH_2-$ units. One of these units was bounded by a quaternary carbon at one end and by a methine group α to a carbonyl group at the other end; the quaternary carbon had an unusually large chemical shift of 92.5 ppm for an sp^3 carbon. The other $-CH_2CH_2-$ group showed coupling to a methine group at each end. One of these groups was oxygen bearing (δ_H 4.18, δ_C 66.5) and was also connected to the same quaternary carbon with the unusually large chemical shift. The other methine group (δ_H 1.73) carried a methyl group (δ_H 0.89, J = 6.7 Hz) and a quaternary carbon (δ_C 51.5).

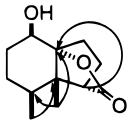


Figure 3. Selected HMBC correlations (H to C) for compound **6**.

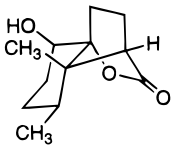


Figure 4. Stereostructure of compound **6**.

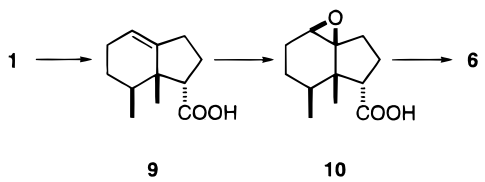
These spectroscopic data can only be accommodated by the structure **6**, an unusual trisnorsesquiterpenoid of the chiloscyphane class; the key HMBC correlations that confirm this structure are shown in Figure 3. The unusual downfield shift of C-10 is explicable based on its substitution by an acyloxy function and by the presence of a β-hydroxyl group. The stereochemistry at C-1 was assigned by a consideration of the coupling constants of H-1, which appeared as a doublet of doublets with $J = 2.3$ and 2.6 Hz. These data indicated that H-1 must be equatorial, and thus the C-1 OH group must be β-axial. Compound **6** thus has the structure and stereochemistry shown (Figure 4), and is assigned the name rivulalactone.

Compounds **1**, **7**, and **8** were identified as chiloscyphone,[10] 4-hydroxyoppositan-7-one (**7**),[13] and isointermedeol (**8**)[22] by a comparison of their ^{1}H-NMR, ^{13}C-NMR, and mass spectral data with those in the literature.

Synthetic chemistry studies were initiated for two reasons. In the first place, the structure of rivulalactone (**6**) appeared to be derivable, both synthetically and possibly biosynthetically, from chiloscyphone (**1**), and it was of interest to carry out this conversion. Second, the fact that only 12-hydroxychiloscyphone (**2**) of all the compounds isolated showed any biological activity (see below) was intriguing and prompted an investigation of structure−activity relationships in this area with the aim of preparing additional active compounds.

Conversion of chiloscyphone (**1**) to rivulalactone (**6**) was proposed to proceed through the steps of side-chain degradation to the trisnor acid (**9**), followed by epoxidation to the epoxyacid **10**, which would be expected to undergo intramolecular cyclization to **6** (Scheme 1).

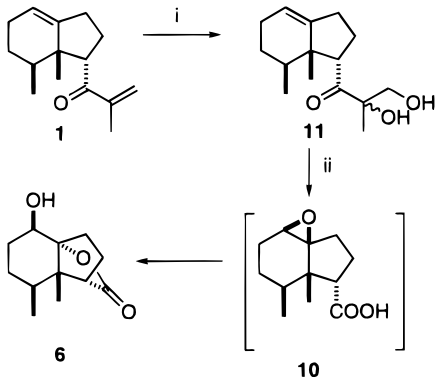
Scheme 1. Proposed Semisynthesis of Rivulalactone (**6**)



9 **10**

In the event, epoxidation and side-chain degradation could be carried out in a single step (Scheme 2). Chiloscyphone (**1**) was first selectively hydroxylated with osmium tetroxide in Me_2CO at −10 to −20° C to yield the diol **11** by reaction of the less hindered of the two double bonds. Treatment of **11** with *m*-chloroperbenzoic acid then converted **11** in a clean reaction into rivulalactone (**6**), identical with the natural product in all respects. This conversion of chiloscyphone (**1**), with

Scheme 2. Semisynthesis of Rivulalactone (**6**)



i OsO_4/NMO, acetone:H_2O(8:1), -10 - -20°C, 60 min
ii *m*-CPBA/CH_2Cl_2, RT, 60 min

a known structure and stereochemistry, into **6** offers independent confirmation of the structure and stereochemistry of rivulalactone (**6**); in particular, the stereochemistry of the C-1 hydroxyl group is established by this synthesis.

The mechanism of the conversion of the diol **11** to **6** is of some interest. Presumably the double bond is epoxidized in the normal way, to the β-epoxide, while the α-hydroxycarbonyl group of the side chain is also attacked by the peracid. The resulting Baeyer−Villiger reaction would then give an unstable hemiacetal intermediate that would fall apart to the epoxyacid **10** and thence to rivulalactone (**6**). Alternatively, oxidative cleavage could occur via a cyclic peroxide intermediate.

Because the bioactivity of **2**, as compared with the related sesquiterpenes **1** and **3**−**6**, appeared to be related in part to the presence of the hydroxyl group at C-12, it was of interest to determine the effect of additional oxidation on the bioactivity of compound **2**. This compound was thus subjected to hydroxylation and to epoxidation to give the triol **12** and the epoxy alcohol **13**, respectively. The structures of compounds **12** and **13** followed directly from their methods of synthesis and were confirmed by the spectroscopic data in the Experimental Section.

All the compounds isolated were tested against RS322 (rad52) yeast strain, and compound **2** was also tested against the RS188N (RAD^+) and RS321(rad52.top1) strains. Compound **2** had IC_{12} values of 75 and 88 μg/mL in RS321 and RS322, respectively, but was inactive (IC_{12} > 1000 μg/mL) in the repair-proficient strain RS188N. These data are characteristic of a selective DNA-damaging agent that does not act as a topoisomerase I or topoisomerase II inhibitor. Compound **2** was cytotoxic to the human lung carcinoma A-549 cell line, with an IC_{50} of 2.0 μg/mL. Compound **2** was also tested in the National Cancer Institute's 60-cell line cytotoxicity panel.[23] In this test, it did not show any significant selectivity toward one or more cell lines, but instead showed a rather uniform cytotoxicity (GI_{50} value) of approximately 10 μM.

Although several of the other compounds isolated were structurally very similar to compound **2**, surprisingly none of them showed any activity against the repair-deficient yeast strain RS322 (IC_{12} values all greater than 500 μg/mL). The two semisynthetic analogues **12** and **13** were also inactive in this assay.

Experimental Section

General Experimental Procedures. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were taken in $CHCl_3$ solution with a Perkin-Elmer model 241 polarimeter, and CD spectra were obtained on a JASCO J720 spectropolarimeter. The ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ solution, unless otherwise stated, on a Varian Unity 400 spectrometer at 400 and 100.57 MHz, respectively. ^{1}H−^{1}H COSY, DEPT, and ^{1}H−^{13}C HETCOR NMR experiments were performed on the same spectrometer, using standard Varian pulse sequences. Mass spectra were taken on a VG 7070 E-HF instrument. Chromatography was performed using Si gel Merck G60 (230−400 mesh), preparative TLC with Si gel GF_{254} plates (Analtech, 500 μm, 20 × 20 cm), and reversed-phase preparative TLC with Whatman PLKC18F linear K reversed-phase (500 μm, 20 × 20 cm) plates.

Plant Material. *Chiloscyphus rivularis* (5 kg) was collected in June 1994, on submersed rocks in Oregon as SPJ-13165B (WBA-2731). A voucher specimen is preserved at the U. S. National Herbarium.

Isolation of Sesquiterpenes. Plant material (500 g) was extracted with MEK, and the MEK extract (10.3 g) was partitioned between hexane and 80% aqueous MeOH, the hexane removed, and H_2O added until a 60% aqueous MeOH mixture was achieved. This was extracted thoroughly with $CHCl_3$. The $CHCl_3$ extract was dried under vacuum to yield 3.1 g of active material. This extract was chromatographed using centrifugal partition chromatography (CPC) with hexanes−EtOAc−MeOH−H_2O (1:1:1:1) as a solvent system to yield eight fractions. The active fraction 3 was loaded onto a Si gel column and eluted with a gradient of EtOAc in hexane to give seven fractions. Fraction 3 eluted from this column with hexanes−EtOAc, 7:3, was found to be the most active fraction and was further purified by preparative TLC (hexanes−EtOAc, 8:2) to give pure **2** (190 mg). Fraction 2 from this column was purified by preparative TLC ($CHCl_3$−Me_2CO, 9:1) to give compound **7** (10 mg).

Fraction 4 from CPC was chromatographed on a Si gel column and eluted with a gradient of EtOAc in hexane. Further purification of fraction 2 eluted from this column with hexanes−EtOAc, 8:2, by preparative TLC ($CHCl_3$−Me_2CO, 97:3) gave compound **3** (30 mg). Fraction 6 from CPC was purified by preparative TLC ($CHCl_3$−Me_2CO, 8:2) and reversed-phase preparative TLC (C-18, MeOH−H_2O, 8:2) to give compounds **5** (1 mg) and **6** (2.5 mg).

The stationary phase from CPC was evaporated, loaded onto a Si gel column, and eluted with a gradient of Me_2CO in $CHCl_3$. Fraction 5 from this column, eluted with $CHCl_3$−Me_2CO, 95:5, was further purified by preparative TLC ($CHCl_3$−Me_2CO, 9:1) to yield compound **4** (3 mg).

The hexane fraction (6.0 g) from the first partition was chromatographed on a Si gel column with a gradient of EtOAc in hexane. Fraction 1 obtained from this column was purified further by preparative TLC (hexanes−EtOAc, 9:1) to give compound **1** (160 mg). After purification by preparative TLC ($CHCl_3$−Me_2CO, 9:1) and reversed-phase preparative TLC (C-18, MeOH−H_2O, 9:1) fraction 3 from the same column yielded compound **8** (3 mg).

Chiloscyphone (1): oil; $[\alpha]_D$ −15.7° (*c* 0.91, $CHCl_3$); CD (MeOH) λ_{max} ($\Delta\epsilon$) 345 (−1.30) (lit.[10c] (dioxane) λ_{max} ($\Delta\epsilon$) 350 (−0.77)); ^{1}H NMR δ 0.83 (3H, d, J = 6.4), 0.95 (3H, s), 1.33−1.38 (3H, m), 1.68 (3H, m), 1.82 (3H, br s), 1.87−2.02 (3H, m), 2.50 (2H, m), 3.56 (1H, dd, J = 7.2, 1.6), 5.39 (1H, dd, J = 4.0, 2.4), 5.71 (1H, br s), 5.92 (1H, s); ^{13}C NMR δ 17.5, 17.7, 20.6, 25.3, 26.1, 27.0, 29.1, 33.0, 49.8, 52.6, 117.3, 123.7, 146.0, 146.4, 206.5; EIMS *m/z* 218 ($M^{\bullet+}$), 203, 175, 161, 149, 133, 122, 107, 93, 79, 69; all spectral data were identical with those in the literature.[10−12]

12-Hydroxychiloscyphone (2): colorless liquid; $[\alpha]_D$ −31.3° (*c* 0.3, $CHCl_3$); CD (MeOH) λ_{max} ($\Delta\epsilon$) 348 (−1.11); UV (MeOH) λ_{max} (log ϵ) 224 (3.65) nm; IR ($CHCl_3$) ν_{max} 3558, 3019, 2967, 2926, 2842, 1659, 1459, 1433, 1373, 1231, 1200, 1075, 1029, 992, 947 cm^{-1};^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m/z* 234 ($M^{\bullet+}$), 216 (14), 149 (69), 147 (93), 107 (100), 85 (49); HREIMS *m/z* 234.1619 (calcd for $C_{15}H_{22}O_2$, 234.1620).

Chiloscypha-2,7-dione (3): white crystals; mp 98 °C; $[\alpha]_D$ −73.3° (*c* 0.3, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 240 (4.23) nm; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m/z* 232 ($M^{\bullet+}$), 163 (66), 161 (100), 136 (57), 121 (53), 97 (21), 93 (16), 69 (50); HREIMS *m/z* 232.1461 (calcd for $C_{15}H_{20}O_2$, 232.1464).

12-Hydroxychiloscypha-2,7-dione (4): oil; $[\alpha]_D$ −40.5° (*c* 0.24, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 204 (3.50), 240 (3.75) nm; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m/z* 230 (3), 163 (44), 161 (32), 121 (76), 93 (23), 91 (43), 85 (20), 77 (43), 55 (100); HRCIMS *m/z* 249.1498 (M + 1$^+$) (calcd for $C_{15}H_{21}O_3$, 249.1490).

Chiloscypha-2,7,9-trione (5): white crystals; mp 179−181 °C; $[\alpha]_D$ −110° (*c* 0.13, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 228 (3.72), 270 (4.03) nm; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m/z* 246 ($M^{\bullet+}$, 8), 177 (13), 175 (21), 149 (57), 135 (51), 122 (73), 107 (53), 91-(52), 79 (100); HRCIMS *m/z* 247.1342 (M + 1$^+$) (calcd for $C_{15}H_{19}O_3$, 247.1334).

Rivulalactone (6): white crystals; mp 97−99 °C; $[\alpha]_D$ +20.4 (*c* 0.23, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 206 (3.17), 274 (2.83), 314 (2.62) nm; IR ($CHCl_3$) ν_{max} 3153, 2927, 1770, 1652, 1471, 1386, 1297 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m/z* 210 ($M^{\bullet+}$, 5), 195 (4), 192 (3), 182 (13), 122 (28), 107 (47), 95 (54), 81 (100), 67 (98); HREIMS *m/z* 210.1254 ($M^{\bullet+}$) (calcd for $C_{12}H_{18}O_3$, 210.1256).

4-Hydroxyopposita-7-one (7): white crystals; mp 46−48 °C; $[\alpha]_D$ +84° (*c* 0.31, $CHCl_3$). All spectral data were identical with those in the literature.[13]

Isointermedeol (8): white solid (hexanes−EtOAc) mp 36−38 °C; $[\alpha]_D$ −11.2° (*c* 0.27, $CHCl_3$). All spectral data were identical with those in the literature.[22]

Oxidation of Chiloscyphone (1). To a solution of chiloscyphone (**1**, 10 mg) in a mixture of Me_2CO and H_2O (8:1, 1.0 mL), osmium tetroxide (2.5 wt % solution in 2-methyl-2-propanol, 0.08 mL) and 4-methylmorpholine *N*-oxide (97%, 4 mg) were added at −10 to −20 °C. After 1 h the reaction was stopped by adding sodium sulfide. Excess EtOAc was added, and the solution was washed with H_2O, dried, and evaporated to dryness to yield crude product, which was purified by preparative TLC (Si gel, $CHCl_3$−Me_2CO, 8:2) to give 11,13-dihydroxychiloscyphone (**11**, 4.6 mg). Data for **11**: UV

(MeOH) λ_{max} (log ϵ) 210 (3.13) nm; IR ($CHCl_3$) ν_{max} 3426, 2945, 1701, 1456, 1350, 1040 cm^{-1};^{1}H NMR δ 0.85 (3H, d, J = 6.7), 0.93 (3H, s), 1.29 (3H, s), 1.36−1.56 (2H, m), 1.60−1.62 (1H, m), 1.83−1.86 (1H, m), 1.94−2.10 (4H, m), 2.32−2.43 (2H, m), 3.18 (1H, d, J = 6.8), 3.29 (1H, d, J = 9.6), 3.79 (1H, d, J = 9.6), 4.28 (1H, s), 5.39 (1H, br s); ^{13}C NMR δ 17.8 (CH_3-15), 20.3 (CH_3-14), 21.4 (CH_3-12), 25.3 (CH_2-2), 25.6 (CH_2-8), 27.1 (CH_2-3), 28.0 (CH_2-9), 31.8 (CH-4), 49.0 (C-5), 52.6 (CH-6), 67.1 (CH_2-13), 79.7 (C-11), 116.8 (CH-1), 146.1 (C-10), 214.7 (C-7); CIMS m/z 253 (M + 1^+), 237, 219, 149, 147, 107.

Conversion of 11 to Rivulalatone (6). *m*-CPBA (57−86%, 20 mg) was added to a solution of **9** (4 mg) in CH_2Cl_2 (1 mL). After 1 h (TLC control) excess EtOAc was added, and the solution was washed with saturated aqueous $NaHCO_3$, dried, and evaporated to afford the crude product. Separation of this by preparative TLC (Si gel, $CHCl_3$−Me_2CO, 7:3) gave synthetic rivulalactone **6** (2.5 mg). Data for synthetic **6**: white crystals, mp 100−103 °C, undepressed in admixture with naturally occurring **6**; $[\alpha]_D$ +22.8° (c 0.19, $CHCl_3$). All spectral data were identical with those of naturally occurring **6**.

Oxidation of 12-Hydroxychiloscyphone (2). To the solution of 12-hydroxychiloscyphone (**2**, 6 mg) in a mixture of Me_2CO and H_2O (8:1, 1.0 mL), osmium tetroxide (2.5 wt % solution in 2-methyl-2-propanol, 0.1 mL) and 4-methylmorpholine *N*-oxide (97%, 6 mg) were added. After 1 h the reaction was stopped by adding sodium sulfide. The reaction solution was diluted with H_2O, extracted with EtOAc (3 ×), and the combined extracts washed with H_2O, dried, and evaporated to dryness to yield crude product that was purified by preparative TLC (Si gel, 20% $CHCl_3$−MeOH, 80:20) to give 11,12,13-trihydroxychiloscyphone (**12**, 3.8 mg). Data for **12**: UV (MeOH) λ_{max} (log ϵ) 210 (3.23) nm; IR ($CHCl_3$) ν_{max} 3394, 2944, 1698, 1460, 1360, 1058 cm^{-1}; ^{1}H NMR (CD_3OD) δ 0.93 (3H, d, J = 6.8), 0.93 (3H, s), 1.40−1.45 (2H, m), 1.63−1.69 (1H, m), 1.77−1.82 (1H, m), 1.93−2.03 (4H, m), 2.32 (1H, m), 2.49 (1H, m), 3.57 (2H, dd, J = 6, 6), 3.65−3.71 (3H, m), 5.27 (1H, br s); ^{13}C NMR (CD_3OD) δ 18.0 (CH_3-15), 20.8 (CH_3-14), 26.3 (CH_2-2), 26.6 (CH_2-8), 28.3 (CH_2-3), 29.2 (CH_2-9), 33.3 (CH-4), 50.1 (C-5), 54.0 (CH-6), 64.9 (CH_2-12), 65.9 (CH_2-13), 84.3 (C-11), 117.4 (CH-1), 148.3 (C-10), 218.7 (C-7); CIMS m/z 269 (M + 1^+), 149, 107.

Epoxidation of 12-Hydroxychiloscyphone (2). To a solution of 12-hydroxychiloscyphone (**2**, 3.9 mg) in CH_2Cl_2, H_2O_2 (30%, aqueous solution, 0.08 mL) and NaOH (1 N aqueous solution, 0.05 mL) were added. After 2 h the reaction solution was diluted with H_2O and extracted with EtOAc (3 ×), and the combined extracts were washed with H_2O, dried, and evaporated to dryness to yield the crude product, which was purified by preparative TLC (Si gel, hexanes−EtOAc, 70:30) to give 11,13-epoxy-12-hydroxychiloscyphone (**13**, 2.2 mg). Data for **13**: UV (MeOH) λ_{max} (log ϵ) 210 (3.27) nm; IR ($CHCl_3$) ν_{max} 3018, 2926, 1697, 1215 cm^{-1}; ^{1}H NMR δ 0.79 (3H, d, J = 7.2), 0.89 (3H, s), 1.40−1.46 (2H, m), 1.60−1.68 (1H, m), 1.83−1.88 (1H, m), 2.00−2.14 (3H, m), 2.35 (1H, m), 2.53 (1H, m), 2.86 (2H, d, J = 8), 3.02−3.07 (2H, m), 3.83−3.97 (2H, m), 5.37 (1H, br, s); ^{13}C NMR δ 17.6 (CH_3-15), 20.3 (CH_3-14), 25.4 (CH_2-2), 26.7 (CH_2-8), 27.9 (CH_2-3), 31.3 (CH_2-9), 33.4 (CH-4), 47.4 (CH-6), 48.7 (CH_2-13), 50.7 (C-5), 61.2 (C-11), 62.2 (CH_2-12), 116.4 (CH-1), 146.5 (C-10), 211.2 (C-7); CIMS m/z 251 (M + 1^+), 177, 149, 147.

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References and Notes

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(2) Wall, M. E.; Wani, M. C. *Cancer Research* **1995**, *55*, 753−760.
(3) Cragg, G. M.; Newman, D. J.; Snader, K. M. *J. Nat. Prod.* **1997**, *60*, 52−60.
(4) Zinsmeister, H. D.; Becker, H.; Eicher, T. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 130−147.
(5) Spjut, R. W.; Kingston, D. G. I.; Cassady, J. M. *Trop. Bryol.* **1992**, *6*, 193−202.
(6) (a) Gunatilaka, A. A. L.; Kingston, D. G. I.; Johnson, R. K. *Pure Appl. Chem.* **1994**, *66*, 2219−2222; (b) Gunatilaka, A. A. L.; Samaranayake, G.; Kingston, D. G. I.; Hofmann, G.; Johnson, R. K. *J. Nat. Prod.* **1992**, *55*, 1648−1654; (c) Heltzel, C. E.; Gunatilaka, A. A. L.; Glass, T. E.; Kingston, D. G. I. *Tetrahedron* **1993**, *49*, 6757−6762.
(7) Hong, W. S. *The Bryologist* **1993**, *96*, 592−597.
(8) Schuster, R. M. *The Hepaticae and Anthocerotae of North America;* Columbia University Press: New York, 1980; p 249.
(9) Conard, H. S. *How to Know the Mosses and Liverworts*; rev. by Redfern, P. L., Jr. W. C. Brown & Co.: Dubuque, IA, 1979.
(10) (a) Hayashi, S.; Matsuo, A.; Matsuura, T. *Tetrahedron Lett.* **1969**, 1599−1600; (b) Matsuo, A.; Hayashi, S. *Tetrahedron Lett.* **1970**, 1289−1292; (c) Matsuo, A. *Tetrahedron* **1972**, *28*, 1203−1209.
(11) Gras, T.-L. *J. Org. Chem.* **1981**, *46*, 3738−3741.
(12) Connolly, J. D.; Harrison, L. J.; Rycroft, D. S. *J. Chem. Soc., Chem. Commun.* **1982**, 1236−1238.
(13) Harrison, L. J.; Asakawa, Y. *Phytochemistry* **1991**, *30*, 3806−3807.
(14) Tori, M.; Hasebe, T.; Asakawa, Y.; Ogawa, K.; Yoshimura, S. *Bull. Chem. Soc. Jpn.* **1991**, *64*, 2303−2305.
(15) Andersen, N. H.; Shank, B.; Costin, C. R. *Experientia* **1973**, *29*, 645−647.
(16) Asakawa, Y.; Toyota, M.; Takemoto, T.; Suire, C. *Phytochemistry* **1979**, *18*, 1007−1009.
(17) Asakawa, Y. *Rev. Lationoam. Quim.* **1984**, *14*, 109−114.
(18) Asakawa, Y.; Matsuda, R.; Toyota, M.; Takemoto, T.; Connolly, J. D.; Phillips, W. R. *Phytochemistry* **1983**, *22*, 961−964.
(19) Czeczuga, B. *Phyto (Horn, Austria)* **1985,** *25*, 113−121.
(20) Toyota, M.; Asakawa, Y. *Flavour Fragrance J.* **1994**, *9*, 237−240.
(21) Farmer, A. M.; Boston, H. L.; Adams, M. S. *Verh.-Int. Ver. Theor. Angew. Limnol.* **1988**, *23*, 1912−1915.
(22) Thappa, R. K.; Dhar, K. L.; Atal, C. K. *Phytochemistry*, **1979**, *18*, 671−672.
(23) (a) Boyd, M. R. In *Cancer: Principles and Practice of Oncology Update*; DeVita, V. T., Hellman, S., Rosenberg, S. A., Eds.; Lippincott: Philadelphia, 1989, Vol. 3, pp 1−12; (b) Boyd, M. R.; Paull, K. D. *Drug Dev. Res.* **1995**, *34*, 91−109.

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