Structures of New Sesquiterpenes and Hepatoprotective Constituents from the Egyptian Herbal Medicine *Cyperus longus*

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Six new sesquiterpenes, cyperusols A_1 (**1**), A_2 (**2**), B_1 (**3**), B_2 (**4**), C (**5**), and D (**6**), together with two monoterpenes and 13 sesquiterpenes were isolated from an Egyptian herbal medicine, the whole plants of *Cyperus longus*. The stereostructures of the new sesquiterpenes were determined on the basis of chemical and physicochemical evidence. In addition, the principal constituents were found to exhibit inhibitory activity on D-galactosamine-induced cytotoxicity in primary cultured mouse hepatocytes.

Cyperus longus L. (Cyperaceae) is widely distributed in Mediterranean, western and central Europe, tropical Africa, and western and central Asia. The whole plant of *C. longus* has been used as a diuretic and tonic in Egyptian traditional medicine. Several flavonoids and alkaloids from this natural medicine have been reported.[1,2] However, its bioactive constituents have not yet been characterized. In the course of our studies on the bioactive constituents from Egyptian herbal medicines,[3−9] we previously reported the structure elucidation of a new nor-stilbene dimer with a tropilene ring, longusone A, three stilbene dimers, longusols A−C, and the radical-scavenging activities of the phenolic constituents from the dried whole plants of *C. longus*.[7] In this paper, we describe the isolation and structure elucidation of six new sesquiterpenes, cyperusols A_1 (**1**), A_2 (**2**), B_1 (**3**), B_2 (**4**), C (**5**), and D (**6**), two monoterpenes, and 13 sesquiterpenes, and the inhibitory effects of both the extracts and pure compounds on D-galactosamine (D-GalN)-induced cytotoxicity in primary cultured mouse hepatocytes.

Results and Discussion

The methanolic extract from the dried whole plants of *C. longus* originating in Egypt was partitioned into a mixture of ethyl acetate (EtOAc) and water to furnish the EtOAc-soluble and H_2O-soluble fractions. As indicated in Table S1, the methanolic extract [inhibition (%): 56.4 ± 5.0 at 30 μg/mL] and the EtOAc-soluble fraction [63.9 ± 5.7% at 30 μg/mL] showed hepatoprotective activities. The active EtOAc-soluble fraction was subjected to normal-phase and reversed-phase silica gel column chromatographies and repeated HPLC to give cyperusols A_1 (**1**), A_2 (**2**), B_1, and B_2 as an inseparable mixture (**3** and **4**), C (**5**), and D (**6**), together with two monoterpenes, (−)-1-*p*-menthene-7,8-diol[10] (**7**) and sobrerol[11] (**8**), and 13 sesquiterpenes, caryolane 1,9β-diol[12] (**9**), 3,7-epoxycaryophyllane-5α,15-diol[13] (**10**), 7,15-epoxycaryophyllane-3,5β-diol[13] (**11**), clovanediol[12] (**12**), tricyclohumuladiol[14] (**13**), 1,5,8,8-tetramethyl-8-bicyclo[8.1.0]undecene-2,9-diol[15] (**14**), **15**,[16] **16**,[17] 1β-hydroxy-10β*H*-guaia-4,11-dien-3-one[18] (**17**), guaidiol[19] (**18**), **19**,[20] mandassidione[21] (**20**), and ligucyperonol[22] (**21**).

Cyperusol A_1 (**1**) was isolated as a colorless oil with positive optical rotation ($[\alpha]_D^{23}$ +12.5°). Its IR spectrum showed absorption bands at 3459, 3351, 1694, 1642, and 914 cm^{-1}due to hydroxyl, conjugated carbonyl, and olefinic methylene functions. In the UV spectrum of **1**, an absorption maximum was observed at 234 nm (log ϵ 4.02), suggestive of an enone moiety. The EIMS of **1** showed a molecular ion at *m*/*z* 250 [M^+] in addition to fragment ions at *m*/*z* 235 [base peak] and 217. The molecular formula $C_{15}H_{22}O_3$ of **1** was determined by HREIMS measurement. The ^1H (pyridine-d_5) and ^{13}C NMR (Table 1) spectra[23] of **1** showed signals assignable to three tertiary methyls [δ 1.47, 1.59, 1.69 (all s, H_3-15, 14, 13)] and an olefinic methylene [δ 4.75, 4.77 (both br s, H_2-12)] together with four methylenes (H_2-3, 6, 8, 9), a methine (H-7), and six quaternary carbons (C-1, 2, 4, 5, 10, 11). The $^1H-^1H$ COSY experiment on **1** indicated the presence of a partial structure (C-6−9) shown as a bold line in Figure 1. In the HMBC experiment, long-range correlations were observed between the following protons and carbons of **1** (H_2-3 and C-1, 2; H_2-6 and C-1, 5; H_2-8 and C-11; H_2-9 and C-10; H_2-12 and C-7, 11, 13; H_3-13 and C-7, 11, 12; H_3-14 and C-1, 9, 10; H_3-15 and C-3−5), thus clarifying the connectivities of the quaternary carbons in **1**. This information confirmed the structure of **1** as 2-oxo-1(5),11(12)-guaiadiene-4,10-diol. The relative configuration of **1** was determined by a NOESY experiment, in which correlations were observed between the following protons (H-3β and H_3-15; H-6α and H-7; H-6β and H_3-15; H-7 and H-9α; H-9α and H_3-14) (Figure 1). Finally, the absolute configuration of **1** was determined by application of the CD excitation chirality method for the allylic benzoate (**1b**) as shown in Scheme 1.[24−26] Thus, **1** was treated with sodium borohydride ($NaBH_4$) in the presence of cerium chloride ($CeCl_3$) to give **1a** as the major product. Treatment of **1a** with benzoyl chloride in the presence of 4-(dimethylamino)pyridine (4-DMAP) afforded the 2-*O*-benzoate (**1b**). The configuration at C-2 in **1b** was determined by a NOESY experiment in which correlations were observed between H-2 and H-3β and between H-3β and H_3-15 (Scheme 1). The 2-*O*-benzoate (**1b**) showed a negative Cotton effect [229 nm ($\Delta\epsilon$ −5.95)], which indicated the C-2*S* absolute configuration in **1b**.

Cyperusol A_2 (**2**) was also isolated as a colorless oil with positive optical rotation ($[\alpha]_D^{23}$ +32.1°). The molecular formula $C_{15}H_{22}O_3$, which was the same as that of **1**, was determined from the EIMS and by HREIMS analysis. That is, the molecular ion was observed at *m*/*z* 250 [M^+] together with fragment ions at *m*/*z* 235 and 217 [base peak]. The IR spectrum of **2** showed absorption bands at 3432, 3351, 1686, 1636, and 912 cm^{-1} assignable to hydroxyl, conjugated carbonyl, and olefinic methylene functions, whereas the UV spectrum showed an absorption maximum at 235 nm (log ϵ 3.87), which was suggestive of an enone moiety. The proton and carbon signals in the ^1H and ^{13}C NMR

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Chart 1

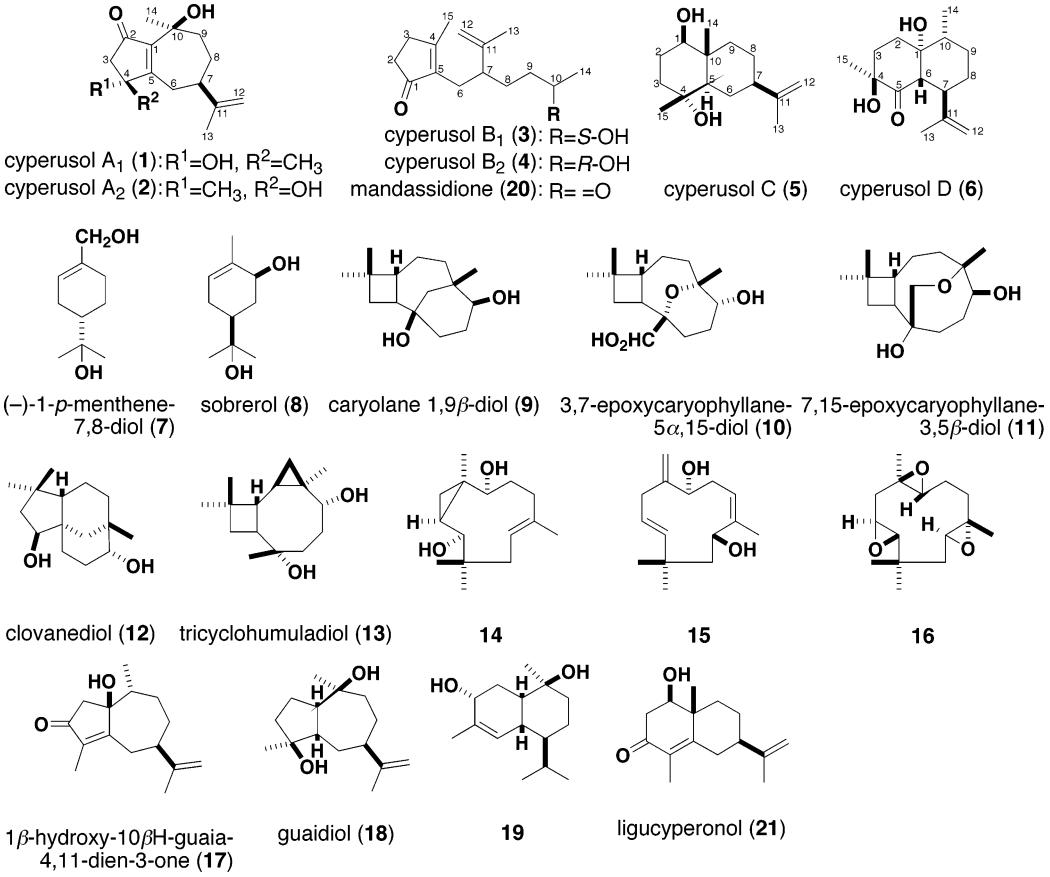


Table 1. ^{13}C NMR Data of Cyperusols A_1 (**1**), A_2 (**2**), B_1 (**3**), B_2 (**4**), C (**5**), and D (**6**)

position	**1**[a]	**1**[b]	**2**[a]	**2**[b]	**3**[b]	**4**[b]	**5**[b]	**6**[a]	**6**[b]
C-1	143.7	143.8	143.5	143.5	209.6	209.5	79.3	79.1	79.2
C-2	207.6	207.0	206.9	206.8	34.3	34.3	28.5	32.5	31.4
C-3	52.7	51.7	52.7	51.6	31.6	31.6	40.8	37.9	36.9
C-4	75.0	75.9	75.4	76.2	170.8	170.7	71.6	75.1	75.9
C-5	177.0	174.7	176.3	173.5	139.2	139.2	52.9	211.5	210.3
C-6	28.5	28.0	29.8	28.4	27.9	27.8	25.7	56.4	55.5
C-7	43.8	43.3	44.3	43.6	45.7	46.0	45.7	39.4	38.7
C-8	28.0	27.3	27.9	26.5	28.8	29.0	26.4	33.5	32.3
C-9	37.6	36.1	37.9	36.0	36.9	37.2	40.5	30.5	29.9
C-10	71.6	71.7	71.8	72.1	68.0	68.3	38.9	42.0	40.9
C-11	150.2	148.8	150.3	148.8	146.9	147.0	150.3	151.3	149.8
C-12	109.9	110.1	110.4	110.3	112.0	111.9	108.3	107.9	107.5
C-13	19.9	19.9	20.2	20.5	18.2	18.2	21.0	22.8	22.3
C-14	27.5	27.1	27.8	27.3	23.7	23.4	13.0	15.6	14.9
C-15	26.6	26.4	26.9	26.4	17.6	17.6	22.7	24.5	23.7

[a] Measured in pyridine-d_5 at 125 MHz. [b] Measured in $CDCl_3$ at 125 MHz.

(Table 1) spectra[23] of **2** were similar to those of **1**, except for the signals around the 4-position. ^{1}H−^{1}H COSY and HMBC data of **2** are shown in Figure 1. The relative configuration of **2** possessing a 4α-methyl group was also determined by a NOESY experiment, which showed correlations between the following protons (H-3α and H_3-15; H-6α and H-7, H_3-15; H-7 and H-9α; H-9α and H_3-14). The circular dichroic (CD) spectra of **1** and **2** showed similar Cotton effects [**1**: 243 nm ($\Delta\epsilon$ +4.26), 326 (−0.32); **2**: 244 (+4.15), 326 (−0.34)], so that the stereostructure of **2** was elucidated as the diastereoisomer of **1** at the 4-position.

Esterification of the mixture (ca. 1:1) of cyperusols B_1 (**3**) and B_2 (**4**) by (*R*)-2-methoxy-2-trifluoromethylphenylacetic acid [(*R*)-MTPA] in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) and 4-DMAP followed by HPLC separation [chiral column: Seramospher chiral RU-1; mobile phase: MeOH; detection: UV (254 nm)] furnished the (*R*)-MTPA esters (**3a** and **4a**) in a ca. 1:1 ratio. Treatment of **3a** and **4a** with 1.0% sodium methoxide (NaOMe)−methanol yielded **3** and **4**, respectively, which were obtained as colorless oils with positive optical rotation (**3**: $[\alpha]_D^{25}$ +32.2°; **4**: $[\alpha]_D^{25}$ +45.8°) (Scheme 2). The EIMS of **3** and **4** showed a common molecular ion at *m*/*z* 236 [M^+] together with a fragment ion at *m*/*z* 110 [base peak], and HREIMS analysis revealed the molecular formula of **3** and **4** to be $C_{15}H_{24}O_2$. The IR spectra of **3** and **4** showed absorption bands due to hydroxyl, unsaturated carbonyl, and olefinic methylene functions (**3**: 3451, 1700, 1644, 888 cm^{-1}; **4**: 3453, 1700,

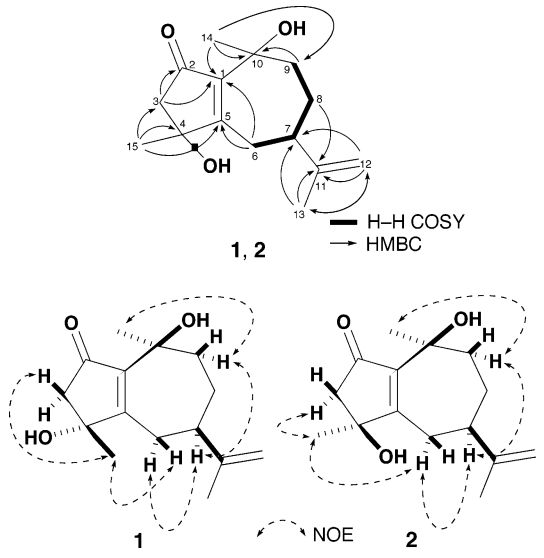
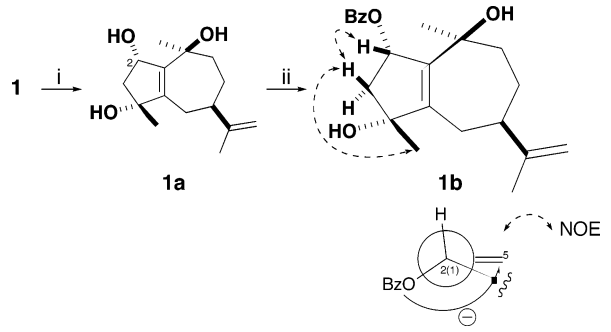


Figure 1.

Scheme 1[a]



[a] (i) $NaBH_4$, $CeCl_3$−MeOH, 0 °C; (ii) BzCl, 4-DMAP−pyridine, rt.

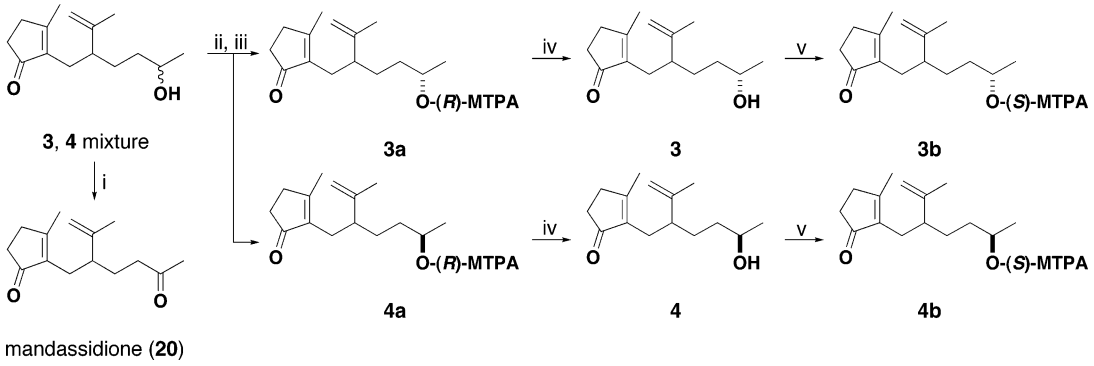
1644, 889 cm^{-1}), whereas their UV spectra showed an absorption maximum [**3**: 241 nm (log ϵ 3.78); **4**: 241 nm (3.89)], which was suggestive of the presence of an enone moiety. The ^1H ($CDCl_3$) and ^{13}C NMR (Table 1) spectra[23] of **3** and **4** showed signals assignable to three methyls [δ **3**: 1.16 (d, J = 6.1 Hz, H_3-14), 1.63 (br s, H_3-13), 2.02 (s, H_3-15); **4**: 1.17 (d, J = 6.1 Hz, H_3-14), 1.63 (br s, H_3-13), 2.02 (s, H_3-15)], a methine bearing an oxygen function [δ **3**: 3.76 (m, H-10); **4**: 3.75 (m, 10-H)], and an olefinic methylene {δ **3**: [4.58 (br s), 4.68 (m), H_2-12]; **4**: [4.58 (br s), 4.68 (m), H_2-12]} together with five methylenes (H_2-2, 3, 6, 8, 9), a methine (H-7), and four quaternary carbons (C-1, 4, 5, 11). The proton and carbon signals in the ^1H and ^{13}C NMR (Table 1) were superimposable on those of mandassidione (**20**),[21] except for the signals around the 10-hydroxyl group. The $^1H-^1H$ COSY experiments on **3** and **4** indicated the presence of a common partial structure shown as bold lines in Figure 2, whereas the connectivities of the quaternary carbons were elucidated by an HMBC experiment. Finally, the mixture (ca. 1:1) of **3** and **4** was oxidized with pyridinium chlorochromate (PCC) to give **20**.[21] On the basis of this evidence, the structures of **3** and **4** were elucidated to be the dihydro derivatives of **20** at the 10-position.

Next, the absolute configurations at the 10-position in **3** and **4** were determined by the application of the modified Mosher's method.[27] Treatment of **3** and **4** with (*S*)-MTPA in the presence of EDC·HCl and 4-DMAP yielded 10-(*S*)-MTPA esters (**3b**, **4b**), respectively. As shown in Figure 2, the protons attached to the 6-, 7-, 8-, 9-, 12-, 13-, and 15-carbons in the 10-(*S*)-MTPA ester (**3b**) resonated at lower fields compared with those of the 10-(*R*)-MTPA ester (**3a**) [$\Delta\delta$: positive], while the protons of the 14-carbon in **3b** appeared at higher field compared with those of **3a** [$\Delta\delta$: negative]. In addition, the C-14 protons of **4b** resonated at lower field compared to those of **4a** [$\Delta\delta$: positive], while the protons at the 6−9-, 12-, 13-, and 15-carbons in **4b** were observed at higher fields compared to those of **4a** [$\Delta\delta$: negative]. Consequently, the absolute configurations at the 10-position of **3** and **4** are *S* and *R*, respectively.

Cyperusol C (**5**) was isolated as a colorless oil with negative optical rotation ($[\alpha]_D^{24}$ −42.3°). The EIMS of **5** showed a molecular ion at *m*/*z* 238 [M^+]. The molecular formula $C_{15}H_{26}O_2$ of **5** was determined from the molecular ion and by HREIMS measurement. The IR spectrum of **5** showed absorption bands at 3389, 1644, and 890 cm^{-1} ascribable to hydroxyl and olefinic methylene functions. The ^1H and ^{13}C NMR ($CDCl_3$, Table 1)[23] spectra of **5** showed signals assignable to three tertiary methyls [δ 0.89, 1.11, 1.75 (all s, H_3-14, 15, 13)], a methine bearing the oxygen function [δ 3.32 (dd, J = 4.4, 11.2 Hz, H-1)], and an olefinic methylene [δ 4.72 (m, H_2-12)] together with five methylenes (H_2-2, 3, 6, 8, 9), two methines (H-5, 7), and three quaternary carbons (C-4, 10, 11). As shown in Figure 3, the $^1H-^1H$ COSY experiment indicated the presence of two partial structures (C-1−3 and C-5−9), and in the HMBC experiment, long-range correlations were observed between the following proton and carbon pairs (H-5 and C-4; H_2-12 and C-7, 13; H_3-13 and C-7, 11, 12; H_3-14 and C-1, 5, 9, 10; H_3-15 and C-3−5).

The relative configuration of **5** was determined by a NOESY experiment, in which correlations were observed

Scheme 2[a]



[a] (i) PCC−CH_2Cl_2, 0 °C; (ii) (*R*)-MTPA, EDC·HCl, 4-DMAP−CH_2Cl_2, rt; (iii) HPLC separation, column: Ceramospher Chiral RU-1, mobile phase: MeOH, detection: UV (254 nm); (iv) 1.0% NaOMe−MeOH, rt; (v) (*S*)-MTPA, EDC·HCl, 4-DMAP−CH_2Cl_2, rt.

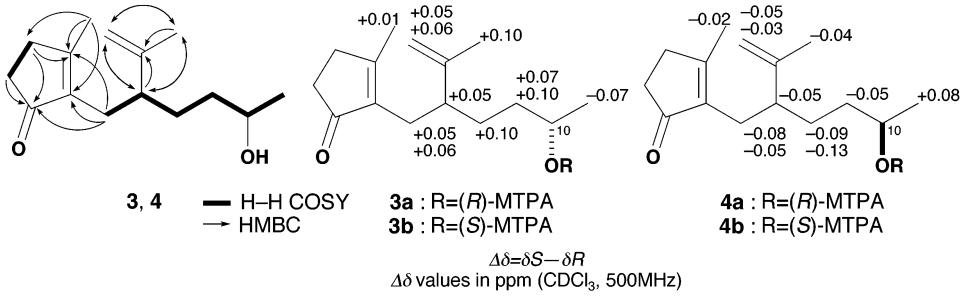


Figure 2.

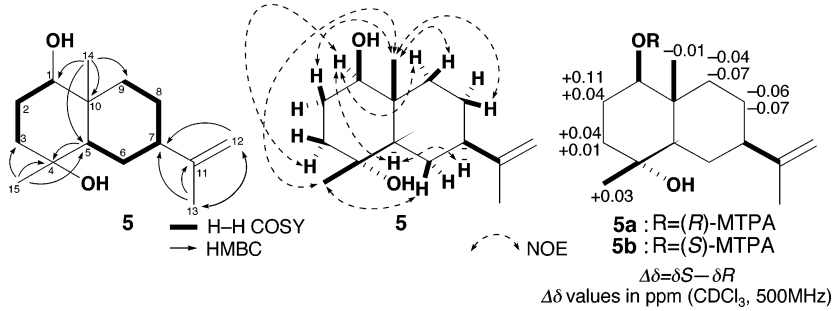


Figure 3.

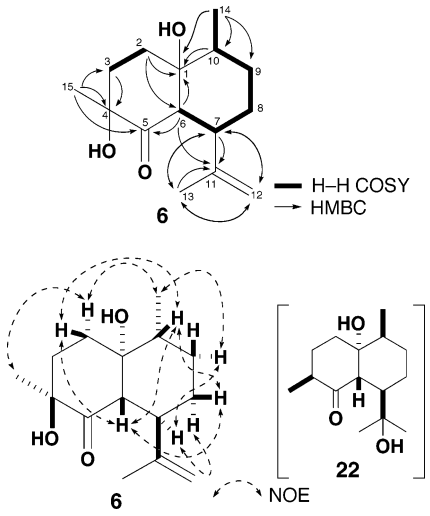


Figure 4.

between the 1- and 5-protons, 5- and 7-protons, and 14- and 15-protons. Furthermore, the absolute configuration of **5** was determined by a modified Mosher's method.[27] As shown in Figure 3, the protons at the 2-, 3-, and 15-carbons of the (*S*)-MTPA ester (**5b**) resonated at lower fields than those of the (*R*)-MTPA ester (**5a**) [$\Delta\delta$: positive], while the protons at the 8-, 9-, and 14-carbons of **5b** were observed at higher fields compared to those of **5a** [$\Delta\delta$: negative]. Consequently, the absolute configuration at C-1 in **5** is *R*.

Cyperusol D (**6**) was also obtained as a colorless oil with positive optical rotation ($[\alpha]_D^{22}$ +35.8°). The molecular formula $C_{15}H_{24}O_3$ was determined from the EIMS and by HREIMS analysis. That is, a molecular ion was observed at *m/z* 252 [M^+] in addition to a fragment ion at *m/z* 148 [base peak]. The IR spectrum of **6** showed absorption bands at 3496, 1717, 1647, and 884 cm^{-1} assignable to hydroxyl, carbonyl, and olefinic methylene functions. The ^1H and ^{13}C NMR ($CDCl_3$, Table 1) spectra[23] of **6** showed signals assignable to three methyls [δ 0.92 (d, J = 6.4 Hz, H_3-14), 1.30, 1.79 (both s, H_3-15, 13)] and an olefinic methylene {δ [4.60 (m), 4.66 (br s), H_2-12]} together with four methylenes (H_2-2, 3, 8, 9), three methines (H-6, 7, 10), and four quaternary carbons (C-1, 4, 5, 11). The $^1H-^1H$ COSY experiment indicated the presence of the two partial structures shown as bold lines in Figure 4 (C-2−3, C-6−10−14). In the HMBC experiment, long-range correlations were observed between the following protons and carbons: H_2-2 and C-1, 6; H_2-3 and C-4; H-6 and C-1, 5, 11; H-7 and C-11−13; H-10 and C-1; H_2-12 and C-7, 13; H_3-13 and C-7, 11, 12; H_3-14 and C-1; H_3-15 and C-3−5 (Figure 4). This information confirmed the structure of **6** as 5-oxo-11(12)-cadinene-1,4-diol.

In addition, the relative configuration of **6** was clarified by NOESY experiment as shown in Figure 4, except for the 1-position. The C-1 configuration was examined by pyridine-d_5-induced solvent shift in the ^1H NMR spectrum.[12,28] Namely, the signals due to the 7α-axial proton [δ 2.35 (ddd, J = 3.5, 12.0, 13.0 Hz)] and 9α-axial proton [δ 1.34 (dddd, J = 3.5, 13.0, 13.5, 16.5 Hz)] in the $CDCl_3$ solution were markedly deshielded in the pyridine-d_5 solution at δ 3.03 (ddd, J = 4.0, 12.0, 13.0 Hz) and 1.80 (dddd, J = 4.0, 13.0, 13.0, 16.5 Hz), respectively. On the basis of these findings, the orientation of the 1-hydroxyl group in **6** is α-axial.

Finally, the absolute configuration of **6** was deduced by comparison of the CD spectra with those of a structurally similar cadinane-type sesquiterpene (**22**).[29] The CD spectra of **6** and **22** showed similar Cotton effects at [**6**: 301 nm ($\Delta\epsilon$ +1.06); **22**: 284 nm ($[\theta]$ +4456[29] = $\Delta\epsilon$ +1.35)], so that the absolute configuration was elucidated as shown.

The inhibitory effects of the 20 compounds on D-GalN-induced cytotoxicity in the primary cultured mouse hepatocytes were examined. The monoterpene, (−)-1-*p*-menthene-7,8-diol (**7**, IC_{50} = 90 μM), and five sesquiterpenes, caryolane 1,9β-diol (**9**, ca. 100 μM), 1,5,8,8-tetramethyl-8-bicyclo[8.1.0]-undecene-2,9-diol (**14**, 70 μM), 2,10,10-trimethyl-6-methylene-2,8-cycloundecadiene-1,5-diol (**15**, 27 μM), 1,6,6,10-tetramethyl-4,9,14-trioxatetracyclotetradecane (**16**, 95 μM), and 1β-hydroxy-10βH-guaia-4,11-dien-3-one (**17**, 83 μM), showed inhibitory activity as shown in Table 2. The hepatoprotective activity of **15** tended to be stronger than that of silybin (IC_{50} = 41 μM), which shows potent hepatoprotective activity.[8,30,31]

Experimental Section

General Experimental Procedures. The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l = 5 cm); CD spectra, JASCO J-720WI spectrometer; UV spectra, Shimadzu UV-1600 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrometer; EIMS and HREIMS, JEOL JMS-GCMATE mass spectrometer; FABMS and HRFABMS, JEOL JMS-SX 102A mass spectrometer; ^1H NMR spectra, JEOL JNM LA-500 (500 MHz) spectrometer; ^{13}C NMR spectra, JEOL JNM LA-500 (125 MHz) spectrometer in $CDCl_3$ unless otherwise stated with tetramethylsilane as an internal standard; HPLC detector, Shimadzu RID-6A refractive index and SPD-10A UV−vis detectors.

The following experimental conditions were used for chromatography: normal-phase silica gel column chromatography, silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150−350 mesh); reversed-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100−200 mesh); TLC, precoated TLC plates with silica gel 60F_{254} (Merck, 0.25 mm) (normal-phase) and silica gel RP-18 $F_{254}S$ (Merck, 0.25 mm) (reversed-phase); reversed-phase HPTLC, precoated TLC plates with silica gel RP-18 $WF_{254}S$ (Merck, 0.25 mm); detection was achieved by spraying with 1% $Ce(SO_4)_2$−10% aqueous H_2SO_4 followed by heating.

Plant Material. The whole plants of *Cyperus longus* were purchased in Cairo, Egypt, in February 2000, and identified

Table 2. Inhibitory Effects of Constituents from *C. longus* on D-GalN-Induced Cytotoxicity in Primary Cultured Mouse Hepatocytes[a]

	inhibition (%)					IC_{50}
	0 μM	3 μM	10 μM	30 μM	100 μM	(μM)
cyperusol A_1 (**1**)	0.0 ± 3.2	2.2 ± 3.5	5.6 ± 4.7	9.5 ± 4.2	16.5 ± 2.3*	
cyperusol B_1 (**3**)	0.0 ± 2.8	3.0 ± 2.4	20.4 ± 3.0**	22.7 ± 2.6**	34.7 ± 4.9**	
cyperusol B_2 (**4**)	0.0 ± 5.9	4.4 ± 5.9	6.5 ± 4.6	−1.4 ± 3.1	19.9 ± 2.9*	
cyperusol C (**5**)	0.0 ± 1.2	6.3 ± 3.9	8.1 ± 6.1	28.4 ± 2.9**	29.9 ± 3.7**	
cyperusol D (**6**)	0.0 ± 4.9	−3.6 ± 2.6	2.6 ± 7.3	31.9 ± 7.0*	46.1 ± 3.1**	
(−)-1-*p*-menthene-7,8-diol (**7**)	0.0 ± 1.5	−1.5 ± 1.9	1.7 ± 2.2	16.7 ± 0.9**	53.3 ± 4.7**	90
sobrerol (**8**)	0.0 ± 3.0	5.5 ± 1.4	8.9 ± 1.8	15.9 ± 3.6*	23.7 ± 3.7**	
caryolane 1,9β-diol (**9**)	0.0 ± 4.1	6.3 ± 1.3	16.8 ± 4.9**	26.7 ± 2.4**	51.7 ± 3.2**	ca. 100
3,7-epoxycaryophyllane-5α,15-diol (**10**)	0.0 ± 1.3	5.7 ± 3.9	10.4 ± 2.6	19.9 ± 3.2**	45.9 ± 1.6**	
7,15-epoxycaryophyllane-3,5β-diol (**11**)	0.0 ± 2.6	12.5 ± 3.8*	14.3 ± 1.4**	24.8 ± 2.4**	36.4 ± 2.7**	
clovanediol (**12**)	0.0 ± 4.8	3.6 ± 3.3	13.6 ± 3.2	32.3 ± 3.7**	40.2 ± 4.2**	
tricyclohumuladiol (**13**)	0.0 ± 4.9	10.9 ± 0.5	16.1 ± 0.9*	22.1 ± 4.0**	37.7 ± 4.6**	
14	0.0 ± 5.3	5.9 ± 6.3	16.6 ± 7.4	37.3 ± 4.1**	54.8 ± 2.1**	70
15	0.0 ± 1.7	11.6 ± 2.7	25.6 ± 4.5**	47.5 ± 6.6**	80.8 ± 6.2**	27
16	0.0 ± 3.8	6.9 ± 3.4	9.7 ± 2.0	25.7 ± 5.8**	51.1 ± 1.8**	95
1β-hydroxy-10βH-guaia-4,11-dien-3-one (**17**)	0.0 ± 3.1	−4.5 ± 5.1	−1.1 ± 1.8	13.5 ± 2.7*	58.0 ± 5.6**	83
guaidiol (**18**)	0.0 ± 2.7	2.6 ± 2.0	10.4 ± 1.4	20.3 ± 1.7**	35.4 ± 3.7**	
19	0.0 ± 2.4	−2.3 ± 5.6	3.7 ± 5.5	9.3 ± 5.5	15.2 ± 0.3**	
mandassidione (**20**)	0.0 ± 3.1	−0.1 ± 2.1	7.7 ± 1.8	14.7 ± 1.9*	21.6 ± 2.2**	
ligucyperonol (**21**)	0.0 ± 1.8	5.5 ± 3.2	13.9 ± 3.3*	12.6 ± 2.4*	46.7 ± 1.8**	
silybin[b]	0.0 ± 0.3	4.8 ± 1.1	7.7 ± 0.7	45.2 ± 8.8**	77.0 ± 5.5**	41

[a] Each value represents the mean ± SEM ($N = 4$). Significantly different from the control, *$p < 0.05$, **$p < 0.01$. [b] Commercial silybin was purchased from Funakoshi Co., Ltd. (Tokyo, Japan).

by Prof. Gisho Honda of the Graduate School of Pharmaceutical Sciences, Kyoto University. A voucher of the plant is on file in our laboratory.

Extraction and Isolation. Dried whole plants of *C. longus* L. (3.2 kg) were finely cut and extracted three times with MeOH under reflux for 3 h. Evaporation of the solvent under reduced pressure gave the methanolic extract (589 g, 18.4%). The MeOH extract (570 g) was partitioned in an EtOAc−H_2O (1:1, v/v) mixture, and removal of the solvent in vacuo from the EtOAc- and H_2O-soluble fractions yielded 104 g (3.4%) and 466 g (15.0%) of residues, respectively. The EtOAc-soluble fraction (94.0 g) was subjected to ordinary-phase silica gel column chromatography [2.0 kg, *n*-hexane−EtOAc (10:1−5:1−2:1−1:1, v/v)−$CHCl_3$−MeOH−H_2O (10:3:1, lower layer−6:4:1, v/v/v)−MeOH] to give seven fractions [Fr. 1 (37.54 g), Fr. 2 (6.93 g), Fr. 3 (14.92 g), Fr. 4 (19.45 g), Fr. 5 (6.25 g), Fr. 6 (2.30 g), Fr. 7 (6.61 g)]. Fraction 2 (6.93 g) was subjected to reversed-phase silica gel column chromatography [210 g, MeOH−H_2O (30:70−50:50−75:25, v/v)−MeOH] to give seven fractions [Fr. 2-1 (1.17 g), Fr. 2-2 (0.95 g), Fr. 2-3 (0.85 g), Fr. 2-4 (0.76 g), Fr. 2-5 (1.12 g), Fr. 2-6 (0.59 g), Fr. 2-7 (1.49 g)]. Fraction 2-3 (0.85 g) was further separated by HPLC [YMC-Pack ODS-A, 20 × 250 mm, i.d., CH_3CN−H_2O (25:75, v/v)] to give cyperusols A_1 (**1**, 31 mg, 0.0011%) and D (**6**, 30 mg, 0.0011%). Fraction 2-5 (1.12 g) was further purified by HPLC [CH_3CN−H_2O (40:60, v/v)] to give ligucyperonol (**21**, 40 mg, 0.0014%). Fraction 2-6 (0.59 g) was also purified by HPLC [CH_3CN−H_2O (40:60, v/v)] to give 1β-hydroxy-10βH-guaia-4,11-dien-3-one (**17**, 52 mg, 0.0019%). Fraction 3 (12.98 g) was subjected to ordinary-phase silica gel column chromatography [400 g, *n*-hexane−EtOAc (2:1, v/v)−$CHCl_3$−MeOH−H_2O (10:3:1, lower layer−6:4:1, v/v/v)−MeOH] to give nine fractions [Fr. 3-1 (0.33 g), Fr. 3-2 (1.57 g), Fr. 3-3 (7.86 g), Fr. 3-4 (0.85 g), Fr. 3-5 (0.21 g), Fr. 3-6 (0.12 g), Fr. 3-7 (0.95 g), Fr. 3-8 (0.87 g), Fr. 3-9 (0.22 g)]. Fraction 3-2 (1.57 g) was subjected to reversed-phase silica gel column chromatography [50 g, MeOH−H_2O (30:70−50:50−70:30, v/v)−MeOH] and finally HPLC [MeOH−H_2O (50:50 or 60:40, v/v)] to give cyperusols A_2 (**2**, 7 mg, 0.0003%) and C (**5**, 7 mg, 0.0003%) and clovanediol (**12**, 8 mg, 0.0003%). Fraction 3-3 (7.86 g) was also subjected to reversed-phase silica gel column chromatography [240 g, MeOH−H_2O (30:70−50:50−60:40−75:25, v/v)−MeOH] and HPLC [MeOH−H_2O (60:40, v/v) or CH_3CN−H_2O (25:75, v/v)] to furnish **5** (54 mg, 0.0022%), (−)-1-*p*-menthene-7,8-diol (**7**, 15 mg, 0.0006%), sobrerol (**8**, 41 mg, 0.0017%), caryolane 1,9β-diol (**9**, 266 mg, 0.011%), 3,7-epoxycaryophyllane-5α,15-diol (**10**, 5 mg, 0.0002%), 7,15-epoxycaryophyllane-3,5β-diol (**11**, 47 mg, 0.0019%), **12** (94 mg, 0.0039%), tricyclohumuladiol (**13**, 244 mg, 0.010%), **14** (9 mg, 0.0004%), guaidiol (**18**, 23 mg, 0.0009%), and **19** (46 mg, 0.0019%). Fraction 4 (19.45 g) was subjected to reversed-phase silica gel column chromatography [600 g, MeOH−H_2O (25:75−50:50−70:30, v/v)−MeOH] to give 10 fractions [Fr. 4-1 (3.09 g), Fr. 4-2 (1.45 g), Fr. 4-3 (0.76 g), Fr. 4-4 (1.74 g), Fr. 4-5 (1.52 g), Fr. 4-6 (0.85 g), Fr. 4-7 (2.03 g), Fr. 4-8 (2.73 g) Fr. 4-9 (1.68 g), Fr. 4-10 (3.60 g)]. Fraction 4-3 (0.76 g) was further separated by HPLC [MeOH−H_2O (45:55, v/v)] to give **16** (22 mg, 0.0008%). Fraction 4-5 (1.52 g) was further separated by HPLC [MeOH−H_2O (55:45, v/v)] to give **15** (23 mg, 0.0008%). Fraction 4-6 (0.85 g) was purified by HPLC [MeOH−H_2O (55:45, v/v)] to give cyperusols B_1 and B_2 mixture (**3** and **4** mixture, 62 mg, 0.0022%). Fraction 4-7 (2.03 g) was also purified by HPLC [MeOH−H_2O (60:40, v/v)] to give mandassidione (**20**, 47 mg, 0.0017%).

The known compounds were identified by comparison of their physical data ($[\alpha]_D$, UV, IR, ^{1}H NMR, ^{13}C NMR, MS) with reported values[10,11,13−22] or authentic samples.[12]

Cyperusol A_1 (1): colorless oil; $[\alpha]_D^{23}$ +12.5° (*c* 0.50, MeOH); CD (MeOH) λ_{max} ($\Delta\epsilon$) 219 (−4.25), 243 (+4.26), 326 (−0.32); UV (MeOH) λ_{max} (log ϵ) 234 (4.02); IR (film) ν_{max} 3459, 3351, 1694, 1642, 1231, 1065, 914 cm^{-1}; ^{1}H NMR (pyridine-d_5) δ 1.47, 1.59 (3H each, both s, H_3-15, 14), 1.69 (3H, br s, H_3-13), 1.74, 1.82 (1H each, both m, Hβ, Hα-8), 1.89 (1H, ddd, J = 4.3, 4.6, 13.4 Hz, Hα-9), 2.31 (1H, ddd, J = 6.1, 12.8, 13.4 Hz, Hβ-9), 2.41 (1H, m, H-7), 2.68 (1H, dd, J = 11.3, 16.5 Hz, Hβ-6), 3.01 (1H, br d, J = ca. 17 Hz, Hα-6), 2.77, 2.98 (1H each, both d, J = 18.0 Hz, Hβ, Hα-3), 4.75, 4.77 (1H each, both br s, H_2-12); ^{1}H NMR δ 1.46, 1.49 (3H each, both s, H_3-15, 14), 1.76 (3H, br s, H_3-13), 1.67, 1.87 (1H each, both m, Hβ, Hα-8), 1.72 (1H, m, Hα-9), 2.07 (1H, ddd, J = 5.8, 13.7, 13.8 Hz, Hβ-9), 2.39 (1H, m, H-7), 2.50 (1H, dd, J = 11.0, 16.8 Hz, Hβ-6), 2.61 (2H, s, H_2-3), 2.64 (1H, br d, J = ca. 17 Hz, Hα-6), 4.75 (2H, m, H_2-12); ^{13}C NMR data, see Table 1; EIMS (%) *m*/*z* 250 [M^+, 5], 235 [base peak], 232 [$M^+ - H_2O$, 35], 217 [49]; HREIMS *m*/*z* 250.1568 (calcd for $C_{15}H_{22}O_3$ [M^+], 250.1569).

Cyperusol A_2 (2): colorless oil; $[\alpha]_D^{23}$ +32.1° (*c* 0.30, MeOH); CD (MeOH) λ_{max} ($\Delta\epsilon$) 218 (−4.10), 244 (+4.15), 326 (−0.34); UV (MeOH) λ_{max} (log ϵ) 235 (3.87); IR (film) ν_{max} 3432, 3351, 1686, 1636, 1236, 1051, 912 cm^{-1}; ^{1}H NMR (pyridine-d_5) δ 1.57, 1.70, 1.72 (3H each, all s, H_3-15, 14, 13), 1.85 (1H, m, Hα-9), 1.88 (2H, m, H_2-8), 2.27 (1H, ddd, J = 6.1, 12.5, 13.2 Hz, Hβ-9), 2.50 (1H, m, H-7), 2.78 (1H, dd, J = 2.8, 16.4 Hz, Hα-6), 2.84, 2.93 (1H each, both d, J = 18.0 Hz, Hβ, Hα-3), 2.98 (1H, dd, J = 10.0, 16.4 Hz, Hβ-6), 4.80, 4.97 (1H each, both br s, H_2-12); ^{1}H NMR δ 1.47, 1.47, 1.77 (3H each, all s, H_3-15, 14,

13), 1.73 (1H, ddd, J = 4.3, 4.6, 14.0 Hz, Hα-9), 1.81 (2H, m, H_2-8), 2.07 (1H, ddd, J = 5.8, 13.4, 14.0 Hz, Hβ-9), 2.39 (1H, m, H-7), 2.52 (1H, dd, J = 3.1, 16.5 Hz, Hα-6), 2.60 (2H, s, H_2-3), 2.63 (1H, dd, J = 9.2, 16.5 Hz, Hβ-6), 4.78 (2H, m, H_2-12); ^{13}C NMR data, see Table 1; EIMS (%) m/z 250 [M^+, 5], 235 [39], 232 [$M^+ - H_2O$, 50], 217 [base peak]; HREIMS m/z 250.1570 (calcd for $C_{15}H_{22}O_3$ [M^+], 250.1569).

Cyperusol B_1 (3): colorless oil; $[\alpha]_D^{25}$ +32.2° (c 0.20, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 241 (3.78); IR (film) ν_{max} 3451, 1700, 1644, 1442, 1387, 1075, 888 cm^{-1}; ^{1}H NMR δ 1.16 (3H, d, J = 6.1 Hz, H_3-14), 1.37 (2H, m, H_2-8), 1.45 (2H, m, H_2-9), 1.63 (3H, br s, H_3-13), 2.23 (1H, br d, J = ca. 7 Hz, H_2-6), 2.31 (1H, m, H-7), 2.34 (2H, m, H_2-2), 2.48 (2H, m, H_2-3), 3.76 (1H, m, H-10), [4.58 (1H, br s), 4.68 (1H, m), H_2-12]; ^{13}C NMR data, see Table 1; EIMS m/z 236 [M^+, 9], 110 [base peak]; HREIMS m/z 236.1773 (calcd for $C_{15}H_{24}O_2$ [M^+], 236.1776).

Cyperusol B_2 (4): colorless oil; $[\alpha]_D^{25}$ +45.8° (c 0.30, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 241 (3.89); IR (film) ν_{max} 3453, 1700, 1644, 1445, 1385, 1075, 889 cm^{-1}; ^{1}H NMR δ 1.17 (3H, d, J = 6.1 Hz, H_3-14), 1.35 (2H, m, H_2-8), 1.47 (2H, m, H_2-9), 1.63 (3H, br s, H_3-13), 2.23 (1H, br d, J = ca. 7 Hz, H_2-6), 2.27 (1H, m, H-7), 2.34 (2H, m, H_2-2), 2.49 (2H, m, H_2-3), 3.75 (1H, m, H-10), [4.58 (1H, br s), 4.68 (1H, m), H_2-12]; ^{13}C NMR data, see Table 1; EIMS m/z 236 [M^+, 12], 110 [base peak]; HREIMS: m/z 236.1779 (calcd for $C_{15}H_{24}O_2$ [M^+], 236.1776).

Cyperusol C (5): colorless oil; $[\alpha]_D^{24}$ −42.3° (c 1.10, MeOH); IR (film) ν_{max} 3389, 1644, 1385, 1169, 1075, 890 cm^{-1}; ^{1}H NMR δ 0.89, 1.11, 1.75 (3H each, all s, H_3-13, 15, 14), 1.13 (1H, ddd, J = 4.0, 13.0, 13.5 Hz, Hα-9), 1.90 (1H, ddd, J = 3.5, 3.5, 13.5, Hβ-9), 1.26, 1.84 (1H each, both m, Hβ, Hα-6), 1.28 (1H, m, H-5), 1.38 (1H, dddd, J = 3.5, 13.0, 13.5, 17.0 Hz, Hβ-8), 1.61 (1H, m, Hα-8), 1.52 (1H, ddd, J = 3.5, 12.0, 13.5 Hz, Hα-3), 1.62, 1.72 (1H each, both m, Hβ, Hα-2), 1.79 (1H, ddd, J = 3.0, 3.5, 12.0 Hz, Hβ-3), 1.94 (1H, m, H-7), 3.32 (1H, dd, J = 4.4, 11.2 Hz, H-1), 4.72 (2H, m, H_2-12); ^{13}C NMR data, see Table 1; EIMS m/z 238 [M^+, 19], 72 [base peak]; HREIMS m/z 238.1929 (calcd for $C_{15}H_{26}O_2$ [M^+], 238.1933).

Cyperusol D (6): colorless oil; $[\alpha]_D^{22}$ +35.8° (c 0.45, MeOH); CD (MeOH) λ_{max} ($\Delta\epsilon$) 245 (−0.17), 301 (+1.06); IR (KBr) ν_{max} 3496, 1717, 1647, 1456, 1375, 1092, 884 cm^{-1}; ^{1}H NMR (pyridine-d_5) δ 1.10 (3H, d, J = 6.4 Hz, H_3-14), 1.33 (1H, dddd, J = 4.0, 13.0, 13.0, 16.5 Hz, Hβ-8), 1.44 (1H, dddd, J = 4.0, 6.5, 6.5, 16.5 Hz, Hβ-9), 1.56, 1.90 (3H each, both s, H_3-15, 13), 1.72 (1H, m, H-10), 1.80 (1H, dddd, J = 4.0, 13.0, 13.0, 16.5 Hz, Hα-9), 1.93 (1H, dddd, J = 4.0, 4.0, 6.5, 16.5 Hz, Hα-8), 2.11 (1H, m, Hα-2), 2.38 (1H, ddd, J = 4.0, 13.0, 13.5 Hz, Hβ-2), 2.18, 2.33 (1H each, both m, Hα, Hβ-3), 3.03 (1H, ddd, J = 4.0, 12.0, 13.0 Hz, H-7), 3.95 (1H, d, J = 12.0 Hz, H-6), [4.73 (1H, m), 4.92 (1H, br s), H_2-12]; ^{1}H NMR δ 0.92 (3H, d, J = 6.4 Hz, H_3-14), 1.18 (1H, dddd, J = 3.5, 13.0, 13.0, 16.5 Hz, Hβ-8), 1.30, 1.79 (3H each, both s, H_3-15, 13), 1.34 (1H, dddd, J = 3.5, 13.0, 13.5, 16.5 Hz, Hα-9), 1.49 (1H, dddd, J = 3.5, 7.0, 7.0, 16.5 Hz, Hβ-9), 1.67 (1H, m, H-10), 1.79 (1H, m, Hα-8), 1.84 (2H, m, H_2-2), 1.92 (2H, m, H_2-3), 2.35 (1H, ddd, J = 3.5, 12.0, 13.0 Hz, H-7), 3.48 (1H, d, J = 12.0 Hz, H-6), [4.60 (1H, m), 4.66 (1H, br s), H_2-12]; ^{13}C NMR data, see Table 1; EIMS m/z 252 [M^+, 7]; 148 [100]; HREIMS m/z 252.1723 (calcd for $C_{15}H_{24}O_3$ [M^+], 252.1725).

$NaBH_4$−$CeCl_3$ Reduction of Cyperusol A_1 (1). A solution of **1** (4.0 mg, 0.016 mmol) in MeOH (2.0 mL) was treated with sodium borohydride ($NaBH_4$, 1.0 mg, 0.028 mmol) in the presence of cerium chloride ($CeCl_3$, 10.0 mg), and the mixture was stirred at 0 °C for 1.5 h. The reaction mixture was quenched with acetone, and then removal of the solvent under reduced pressure yielded a reduction mixture. The reaction mixture was poured into ice−water and extracted with EtOAc. The EtOAc extract was washed with brine, dried over $MgSO_4$ powder, and filtered. Removal of the solvent under reduced pressure furnished a residue, which was purified by silica gel column chromatography [0.5 g, n-hexane−EtOAc (2:1, v/v)] to give **1a** (3.7 mg, 92%).

1a: colorless oil; ^{1}H NMR (pyridine-d_5) δ 1.43, 1.68, 1.72 (3H each, all s, H_3-15, 14, 13), 1.83 (2H, m, H_2-8), [1.95 (1H, ddd, J = 4.3, 4.6, 14.1 Hz), 2.45 (1H, m), H_2-9], 2.40 (1H, m, H-7), [2.55 (1H, dd, J = 2.8, 15.9 Hz), 2.63 (1H, dd, J = 10.0, 15.9 Hz), H_2-6], [2.47 (1H, dd, J = 2.8, 12.2 Hz), 2.78 (1H, dd, J = 6.7, 12.2 Hz), H_2-3], 4.74, 4.90 (1H each, both m, H_2-12), 5.24 (1H, dd, J = 2.8, 6.7 Hz, H-2); ^{13}C NMR (pyridine-d_5) δ_C 141.7 (C-1), 74.2 (C-2), 51.6 (C-3), 74.1 (C-4), 145.1 (C-5), 27.9 (C-6), 45.5 (C-7), 27.8 (C-8), 39.2 (C-9), 79.7 (C-10), 150.8 (C-11), 109.3 (C-12), 20.1 (C-13), 27.7 (C-14), 26.6 (C-15); positive-ion FABMS (%) m/z 275 [M + Na]$^+$; HRFABMS m/z 275.1628 (calcd for $C_{15}H_{24}O_3Na$ [M + Na]$^+$, 275.1623).

Benzoylation of 1a. A solution of **1a** (3.7 mg, 0.015 mmol) in dry pyridine (1.0 mL) was treated with benzoyl chloride (50 μL) in the presence of 4-(dimethylamino)pyridine (4-DMAP, 4.0 mg), and the mixture was stirred at room temperature for 4 h. The reaction mixture was poured into ice−water and extracted with EtOAc. The EtOAc extract was successively washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$, and brine, then dried over $MgSO_4$ powder and filtered. After removal of the solvent under reduced pressure, the residue was purified by normal-phase silica gel column chromatography [0.5 g, n-hexane−EtOAc (3:1, v/v)] to furnish **1b** (3.5 mg, 67%).

1b: colorless oil; $[\alpha]_D^{22}$ +27.6° (c 0.30, MeOH); CD (MeOH) λ_{max} ($\Delta\epsilon$) 229 (−5.95); UV (MeOH) λ_{max} (log ϵ) 229 (4.03), 273 (3.32); IR (film) ν_{max} 3496, 1680, 1645, 1620, 1436, 1218, 1084 cm^{-1}; ^{1}H NMR δ 1.33, 1.42, 1.76 (3H each, all s, H_3-15, 14, 13), 1.73 (2H, m, H_2-8), 1.78, 2.05 (1H each, both m, H_2-9), [2.08 (1H, dd, J = 2.8, 16.5 Hz), 2.29 (1H, dd, J = 11.6, 16.5 Hz), H_2-6], 2.38 (1H, m, H-7), [2.42 (br d, J = ca. 16 Hz), 2.55 (1H, dd, J = 7.8, 15.6 Hz), H_2-3], 4.71, 4.76 (1H each, both br s, H_2-12), 6.10 (1H, br d, J = ca. 8 Hz, H-1), 7.47 (2H, dd, J = 7.6, 8.2 Hz, H-3′, 5′), 7.58 (1H, br t, J = ca. 8 Hz, H-4′), 8.06 (2H, dd, J = 1.2, 8.2 Hz, H-2′, 6′); ^{13}C NMR δ_C 133.6 (C-1), 77.8 (C-2), 46.5 (C-3), 82.3 (C-4), 141.7 (C-5), 28.1 (C-6), 44.3 (C-7), 28.4 (C-8), 39.7 (C-9), 71.9 (C-10), 149.9 (C-11), 109.3 (C-12), 20.1 (C-13), 27.7 (C-14), 25.9 (C-15), 130.2 (C-Bz-1), 129.7 (C-Bz-2,6), 128.5 (C-Bz-3,5), 133.2 (C-Bz-4), 167.0 (C-Bz-7); EIMS (%) m/z 356 [M^+, 2], 105 [base peak]; HREIMS m/z 356.1995 (calcd for $C_{22}H_{28}O_4$ [M^+], 356.1987).

PCC Oxidation of a Mixture of 3 and 4. A solution of the mixture of **3** and **4** (3.4 mg, 0.014 mmol) in dry CH_2Cl_2 (1.0 mL) was treated with pyridinium chlorochromate (PCC, 10.0 mg, 0.046 mmol), and the mixture was stirred at 0 °C for 1 h. The reaction mixture was poured into ice−water and extracted with EtOAc. The EtOAc extract was washed with saturated aqueous $NaHCO_3$ and brine, then dried over $MgSO_4$ and filtered. Removal of the solvent under reduced pressure gave a crude product, which was purified by silica gel column chromatography [0.5 g, n-hexane−EtOAc (3:1, v/v)] to furnish mandassidione (**20**, 2.7 mg, 80%).[21]

Preparation of the (R)-MTPA Esters (3a, 4a). A solution of the mixture of **3** and **4** (19.1 mg, 0.081 mmol) in dry CH_2Cl_2 (2.0 mL) was treated with (*R*)-2-methoxy-2-trifluoromethylphenylacetic acid [(*R*)-MTPA, 91.5 mg, 0.40 mmol] in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl, 70.0 mg, 0.40 mmol) and 4-DMAP (20.0 mg, 0.16 mmol), and the mixture was stirred at room temperature for 24 h. The reaction mixture was poured into ice−water and extracted with EtOAc. The EtOAc extract was successively washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$, and brine, then dried over $MgSO_4$ powder and filtered. Removal of the solvent under reduced pressure furnished a residue, which was purified by HPLC [Shiseido Ceramospher Chiral RU-1, 6.0 × 250 mm, i.d., MeOH] to give **3a** (14.1 mg, 39%) and **4a** (15.8 mg, 43%), respectively.

3a: colorless oil; ^{1}H NMR δ 1.24 (2H, m, H_2-8), 1.30 (3H, d, J = 5.6 Hz, H_3-14), 1.38, 1.51 (1H each, both m, H_2-9), 1.47 (3H, br s, H_3-13), 1.99 (3H, s, H_3-15), [2.12 (1H, dd, J = 7.0, 13.5 Hz), 2.16 (1H, dd, J = 7.5, 13.5 Hz), H_2-6], 2.25 (1H, m, H-7), 2.32 (2H, m, H_2-2), 2.46 (2H, m, H_2-3), 3.56 (3H, s, −OCH_3), [4.52 (1H, br s), 4.63 (1H, m), H_2-12], 5.11 (1H, m, H-10), [7.39 (1H, m), 7.41 (2H, dd-like), 7.54 (2H, dd-like), Ph-H].

4a: colorless oil; ^{1}H NMR δ 1.24 (3H, d, J = 6.1 Hz, H_3-14), 1.31, 1.40 (1H each, both m, H_2-8), 1.52 (2H, m, H_2-9), 1.60 (3H, br s, H_3-13), 2.00 (3H, s, H_3-15), [2.19 (1H, dd, J = 7.2, 13.5 Hz), 2.23 (1H, dd, J = 7.6, 13.5 Hz), H_2-6], 2.26 (1H, m,

H-7), 2.32 (2H, m, H_2-2), 2.46 (2H, m, H_2-3), 3.53 (3H, s, $-OCH_3$), 4.60, 4.69 (1H each, both br s, H_2-12), 5.09 (1H, m, H-10), [7.38 (1H, m), 7.40 (2H, dd-like), 7.51 (2H, dd-like), Ph-H].

Deacylation of 3a and 4a. A solution of **3a** (9.8 mg, 0.022 mmol) in 1.0% NaOMe−MeOH (3.0 mL) was stirred at room temperature for 24 h. The reaction mixture was neutralized with Dowex HCR-W2 (H^+ form), and the resin was removed by filtration. Evaporation of the solvent under reduced pressure furnished a residue, which was purified by silica gel column chromatography [0.5 g, *n*-hexane−EtOAc (2:1, v/v)] to give **3** (3.6 mg, 70%). Using a similar procedure, **4** (3.8 mg, 77%) was obtained from **4a** (9.4 mg).

Preparation of the (*S*)-MTPA Esters (3b, 4b). A solution of **3** (3.6 mg, 0.015 mmol) in dry CH_2Cl_2 (1.0 mL) was treated with (*S*)-MTPA (9.0 mg, 0.039 mmol) in the presence of EDC·HCl (7.5 mg, 0.039 mmol) and 4-DMAP (2.7 mg, 0.022 mmol), and the mixture was stirred at room temperature for 24 h. The reaction mixture was poured into ice−water and extracted with EtOAc. The EtOAc extract was successively washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$, and brine, then dried over $MgSO_4$ powder and filtered. Removal of the solvent under reduced pressure furnished a residue, which was purified by silica gel column chromatography [0.5 g, *n*-hexane−EtOAc (5:1, v/v)] to give **3b** (5.1 mg, 74%). Using a similar procedure, **4b** (4.9 mg, 67%) was obtained from **4** (3.8 mg, 0.016 mmol) using (*S*)-MTPA (9.0 mg), EDC·HCl (7.5 mg), and 4-DMAP (2.7 mg).

3b: colorless oil; ^{1}H NMR δ 1.23 (3H, d, $J = 6.1$ Hz, H_3-14), 1.34 (2H, m, H_2-8), 1.45, 1.61 (1H each, both m, H2−9), 1.57, 2.00 (3H each, both s, H_3-13, 15), [2.17 (1H, dd, $J = 7.0$, 13.5 Hz), 2.22 (1H, dd, $J = 7.5$, 13.5 Hz), H_2-6], 2.30 (1H, m, H-7), 2.32 (2H, m, H_2-2), 2.46 (2H, m, H_2-3), 3.56 (3H, s, $-OCH_3$), [4.58 (1H, br s), 4.68 (1H, m), H_2-12], 5.11 (1H, m, H-10), [7.40 (1H, m), 7.42 (2H, dd-like), 7.53 (2H, dd-like), Ph-H].

4b: colorless oil; ^{1}H NMR δ 1.22, 1.27 (1H each, both m, H_2-8), 1.32 (3H, d, $J = 6.2$ Hz, H_3-14), 1.47 (2H, m, H_2-9), 1.56 (3H, br s, H_3-13), 1.98 (3H, s, H_3-15), [2.11 (1H, dd, $J = 7.2$, 13.5 Hz), 2.17 (1H, dd, $J = 7.8$, 13.5 Hz), H_2-6], 2.21 (1H, m, H-7), 2.32 (2H, m, H_2-2), 2.46 (2H, m, H_2-3), 3.53 (3H, s, $-OCH_3$), [4.55 (1H, br s), 4.66 (1H, m) H_2-12], 5.09 (1H, m, H-10), [7.38 (1H, m), 7.40 (2H, dd-like), 7.52 (2H, dd-like), Ph-H].

Preparation of the (*R*)- and (*S*)-MTPA Esters (5a, 5b) from Cyperusol C (5). A solution of **5** (5.6 mg, 0.024 mmol) in dry CH_2Cl_2 (1.0 mL) was treated with (*R*)-MTPA (11.0 mg, 0.047 mmol) in the presence of EDC·HCl (9.0 mg, 0.047 mmol) and 4-DMAP (3.5 mg, 0.028 mmol), and the mixture was stirred at room temperature for 12 h. The reaction mixture was poured into ice−water and extracted with EtOAc. The EtOAc extract was treated in the usual manner to give a residue, which was purified by silica gel column chromatography [0.5 g, *n*-hexane−EtOAc (3:1, v/v)] to give **5a** (2.9 mg, 61%) and **5** (3.1 mg, recovered). Using a similar procedure, (*S*)-MTPA ester **5b** (2.7 mg, 71%) and **5** (3.5 mg, recovered) were obtained from **5** (5.5 mg, 0.023 mmol) using (*S*)-MTPA (11.0 mg), EDC·HCl (9.0 mg), and 4-DMAP (3.5 mg).

5a: ^{1}H NMR δ 0.92, 1.14, 1.74 (3H each, all s, H_3-13, 15, 14), 1.22, 1.92 (1H each, both m, H_2-9), 1.34, 1.64 (1H each, both m, H_2-8), 1.63, 1.84 (1H each, both m, H_2-3), 1.65, 1.90 (1H each, both m, H_2-2), 1.94 (1H, m, H-7), 3.52 (3H, s, $-OCH_3$), 4.71 (2H, m, H_2-12), 4.81 (1H, dd, $J = 4.5$, 11.3 Hz, H-1), [7.39 (1H, m), 7.41 (2H, dd-like), 7.51 (2H, dd-like), Ph-H].

5b: ^{1}H NMR δ 0.92, 1.17, 1.73 (3H each, all s, H_3-13, 15, 14), 1.15, 1.88 (1H each, both m, H_2-9), 1.28, 1.57 (1H each, both m, H_2-8), 1.63, 1.84 (1H each, both m, H_2-3), 1.65, 1.90 (1H each, both m, H_2-2), 1.94 (1H, m, H-7), 3.52 (3H, s, $-OCH_3$), 4.71 (2H, m, H_2-12), 4.81 (1H, dd, $J = 4.5$, 11.3 Hz, H-1), [7.39 (1H, m), 7.41 (2H, dd-like), 7.51 (2H, dd-like), Ph-H].

Protective Effect on Cytotoxicity Induced by D-GalN in Primary Cultured Mouse Hepatocytes. The hepatoprotective effects of the constituents were determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay using primary cultured mouse hepatocytes. Hepatocytes were isolated from male ddY mice (30−35 g) by collagenase perfusion method. The cell suspension at 4×10^4 cells in 100 μL William's E medium containing fetal calf serum (10%), penicillin (100 units/mL), and streptomycin (100 μg/mL) was inoculated in a 96-well microplate and precultured for 4 h at 37 °C under a 5% CO_2 atm. The fresh medium (100 μL) containing D-GalN (2 mM) and a test sample were added, and the hepatocytes were cultured for 44 h. The medium was exchanged with 100 μL of the fresh medium, and 10 μL of MTT (5 mg/mL in phosphate-buffered saline) solution was added to the medium. After 4 h culture, the medium was removed, and 100 μL of 2-propanol containing 0.04 M HCl was then added to dissolve the formazan produced in the cells. The optical density (OD) of the formazan solution was measured by microplate reader at 562 nm (reference: 660 nm). Inhibition (%) was obtained by the following formula.

$$\text{inhibition (\%)} = [(\text{OD(sample)} - \text{OD(control)})/(\text{OD(normal)} - \text{OD(control)})] \times 100$$

Cytotoxic effects of the constituents were assessed by MTT colorimetric assay. Briefly, after 44 h incubation with a test sample in the absence of D-GalN, MTT assay was performed as described above.

Statistics. Values are expressed as means ± SEM. One-way analysis of variance followed by Dunnett's test was used for statistical analysis.

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Supporting Information Available: Table of data on D-GalN-induced cytotoxicity in primary mouse hepatocytes for the methanolic extract and its EtOAc- and H_2O-soluble fractions. This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Harborne, J. B. *Phytochemistry* **1971**, *10*, 1569−1574.
(2) Sadykov, Y. D.; Begovatov, Y. M. *Izv. Akad. Nauk. Tadzh. SSR, Otd. Fiz.-Mater. Khim. Geol. Nauk* **1990**, *4*, 31−34.
(3) Yoshikawa, M.; Murakami, T.; Shimada, H.; Yoshizumi, S.; Saka, M.; Matsuda, H. *Chem. Pharm. Bull.* **1998**, *46*, 1008−1014.
(4) Murakami, T.; Kishi, A.; Matsuda, H.; Yoshikawa, M. *Chem. Pharm. Bull.* **2000**, *48*, 994−1000.
(5) Yoshikawa, M.; Murakami, T.; Kishi, A.; Kageura, T.; Matsuda, H. *Chem. Pharm. Bull.* **2001**, *49*, 863−870.
(6) Murakami, T.; Kishi, A.; Yoshikawa, M. *Chem. Pharm. Bull.* **2001**, *49*, 974−978.
(7) Morikawa, T.; Xu, F.; Matsuda, H.; Yoshikawa, M. *Heterocycles* **2002**, *57*, 1983−1988.
(8) Yoshikawa, M.; Xu, F.; Morikawa, T.; Ninomiya, K.; Matsuda, H. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1045−1049.
(9) Yoshikawa, M.; Morikawa, T.; Xu, F.; Ando, S.; Matsuda, H. *Heterocycles* **2003**, *63*, 1787−1792.
(10) Ishida, T.; Asakawa, Y.; Takemoto, T.; Aratani, T. *J. Pharm. Sci.* **1981**, *70*, 406−415.
(11) Bettinetti, G.; Giordano, F.; Fronza, G.; Italia, A.; Pellegata, R.; Villa, M.; Ventura, P. *J. Pharm. Sci.* **1990**, *79*, 470−475.
(12) Yoshikawa, M.; Morikawa, T.; Murakami, T.; Toguchida, I.; Harima, S.; Matsuda, H. *Chem. Pharm. Bull.* **1999**, *47*, 340−345.
(13) Srinivasan, V.; Warnhoff, E. W. *Can. J. Chem.* **1976**, *54*, 1372−1382.
(14) Naya, Y.; Kotake, M. *Bull. Chem. Soc. J.* **1969**, *42*, 2405.
(15) Yang, X.; Deinzer, M. L. *J. Org. Chem.* **1992**, *57*, 4717−4722.
(16) Smith, R. J.; Mahiou, B.; Deinzer, M. L. *Tetrahedron* **1991**, *47*, 933−940.
(17) Hayano, K.; Mochizuki, K. *Heterocycles* **1997**, *45*, 1573−1578.
(18) Bohlmann, F.; Zdero, C.; King, R. M.; Robinson, H. *Phytochemistry* **1983**, *22*, 1201−1206.
(19) Syu, W.; Shen, C.; Don, M.; Ou, J.; Lee, G.; Sun, C. *J. Nat. Prod.* **1998**, *61*, 1531−1534.
(20) Kuo, Y.; Cheng, Y.; Lin, Y. *Tetrahedron Lett.* **1969**, 2375−2377.
(21) Nyasse, B.; Tih, R. G.; Sondengam, B. L.; Martin, M. T.; Bodo, B. *Phytochemistry* **1988**, *27*, 3319−3321.
(22) Naya, K.; Okayama, T.; Fujiwara, M.; Nakata, M.; Ohtsuka, T.; Kurio, S. *Bull. Chem. Soc. J.* **1990**, *63*, 2239−2245.
(23) The ^{1}H and ^{13}C NMR spectra of **1**−**6** were assigned with the aid of homo- and heterocorrelation spectroscopies (^{1}H−^{1}H, ^{13}C−^{1}H COSY), distortionless enhancement by polarization transfer (DEPT), and heteronuclear multiple bond connectivity (HMBC) experiments.
(24) Harada, N.; Iwabuchi, J.; Yokota, Y.; Uda, H.; Nakanishi, K. *J. Am. Chem. Soc.* **1981**, *103*, 5590−5591.

(25) Matsuda, H.; Morikawa, T.; Sakamoto, Y.; Toguchida, I.; Yoshikawa, M. *Bioorg. Med. Chem*. **2002**, *10*, 2527−2534.
(26) Morikawa, T.; Matsuda, H.; Toguchida, I.; Ueda, K.; Yoshikawa, M. *J. Nat. Prod*. **2002**, *65*, 1468−1474.
(27) Ohtani, I.; Kusumi, T.; Kashman, H.; Kakisawa, H. *J. Am. Chem. Soc*. **1991**, *113*, 4092−4096.
(28) Kitagawa, I.; Yoshikawa, M.; Yoshioka, I. *Tetrahedron Lett*. **1974**, *5*, 469−472.
(29) Winter, R. E. K.; Zehr, R. J.; Honey, M.; Arsdale, W. V. *J. Org. Chem*. **1981**, *46*, 4309−4312.
(30) Feher, J.; Deak, G.; Muzes, G.; Lang, I.; Niederland, V.; Nekam, K.; Karteszi, M. *Orv. Hetil*. **1989**, *130*, 2723−2727.
(31) Skottova, N.; Krecman, V. *Physiol. Res*. **1998**, *47*, 1−7.

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