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# Sesquiterpene Lactones from *Cichorium endivia* L. and *C. intybus* L. and Cytotoxic Activity

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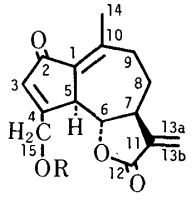
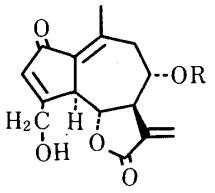
Four new sesquiterpene lactones, cichoriolide A and cichoriosides A, B and C, have been isolated from *Cichorium endivia* L. and *C. intybus* L., together with nine known sesquiterpene lactones. The structures of the new compounds were established on the basis of chemical and spectral data. Further, the cytotoxic activities of related glycosides and the aglycones were compared in the L-5178Y cultured cell system.

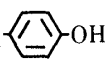
**Keywords**——*Cichorium endivia*; *Cichorium intybus*; Compositae; sesquiterpene glycoside; cichoriolide; cichorioside; cytotoxic activity

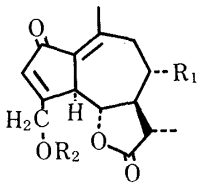
In connection with a study on the sesquiterpene glycosides of some plants in Compositae, we have also investigated *Cichorium endivia* L. and *C. intybus* L., whose leaves are used as green vegetables. From the methanolic extract of the two plants, a new eudesmane-type sesquiterpene lactone, cichoriolide A (**10**), and three new glycosides, cichoriosides A (**11**), B (**12**) and C (**13**), have been isolated together with six known guaiane-type sesquiterpene lactones, 8-deoxylactucin (**1**), crepidiaside A (**2**), lactucin (**3**), lactucopicrin (**4**), crepidiaside B (**5**) and 11$\beta$,13-dihydrolactucin (**6**), two known germacrane-type sesquiterpene glycosides, picriside B (**7**) and sonchuside A (**8**), and a eudesmane-type sesquiterpene glycoside, sonchuside C (**9**). 8-Deoxylactucin (**1**), crepidiaside B (**5**), sonchuside A (**8**) and cichoriolide A



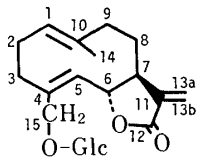
**1** : R=H
**2** : R=Glc



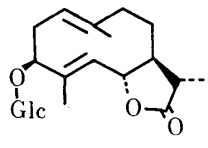
**3** : R=H
**4** : R=CO-CH$_2$-  -OH



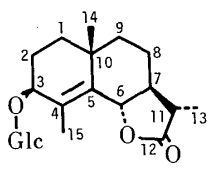
**5** : R$_1$=H, R$_2$=Glc
**6** : R$_1$=OH, R$_2$=H



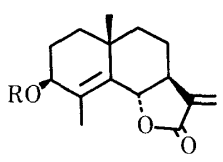
**7**



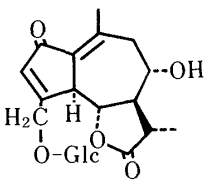
**8**



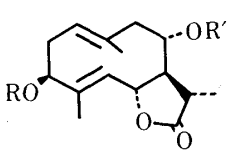
**9**



**10** : R=H
**11** : R=Glc



**12**



**13** : R=Glc
**13a** : R=H
**13b** : R=R'=Ac

Chart 1

(**10**) were isolated from both plants. The structures of the new compounds were determined on the basis of some chemical transformations and spectroscopic studies.

Nine known sesquiterpene lactones were identified by direct comparison [proton nuclear magnetic resonance ($^1$H-NMR) and infrared (IR) spectra] with authentic samples.[1-6]

Cichoriolide A (**10**), $C_{15}H_{20}O_3$, $[\alpha]_D$ +88°, was obtained as an amorphous powder. The IR spectrum suggested the presence of hydroxyl groups (3590 cm$^{-1}$), and a $\gamma$-lactone ring (1790 cm$^{-1}$). The $^1$H-NMR spectrum was similar to that of the aglycone of **9**[6] except for the appearance of two doublet signals at $\delta$ 5.50 (1H, d, $J$=3.1 Hz) and 6.19 (1H, d, $J$=3.3 Hz) instead of a methyl signal due to C-13. The circular dichroism (CD) spectrum of **10** showed a negative Cotton effect $[\theta]_{250}$ −3500, suggesting that the $\gamma$-lactone ring fusion is 6$\alpha$,7$\beta$-*trans*.

TABLE I. $^1$H-NMR Chemical Shifts and Coupling Constants

| Proton No. | **10**[a] | **11**[b] | **12**[b] |
|---|---|---|---|
| 3 | 3.55 (1H, br t, $J$=9 Hz) | | 7.01 (1H, br s) |
| 6 | 4.58 (1H, br d, $J$=12 Hz) | | |
| 13a | 5.50 (1H, d, $J$=3.1 Hz) | Overlapped | 1.67 (3H, d, $J$=7 Hz) |
| 13b | 6.19 (1H, d, $J$=3.3 Hz) | 6.21 (1H, d, $J$=3.1 Hz) | |
| 14 | 1.11 (3H, s) | 1.13 (3H, s) | 2.47 (3H, s) |
| 15 | 1.88 (3H, br s) | 1.88 (3H, br s) | |
| Anomeric | | 4.94 (1H, d, $J$=7 Hz) | 4.97 (1H, d, $J$=7 Hz) |

| Proton No. | **6**[b] | **13**[b] | **13a**[a] |
|---|---|---|---|
| 1 | | | 4.92 (1H, dd, $J$=11, 5 Hz) |
| 3 | 6.99 (1H, br s) | | 4.23 (1H, dd, $J$=10, 6 Hz) |
| 5, 6 | | | 4.64 (2H, overlapped) |
| 8 | | | 3.90 (1H, dt, $J$=10, 3 Hz) |
| 13 | 1.68 (3H, d, $J$=7 Hz) | 1.77 (3H, d, $J$=7 Hz) | 1.42 (3H, d, $J$=7 Hz) |
| 14 | 2.50 (3H, s) | 1.45 (3H, br s) | 1.47 (3H, br s) |
| 15 | 4.74 (1H, br d, $J$=19 Hz) | 1.98 (3H, br s) | 1.79 (3H, br s) |
| 15′ | 5.34 (1H, br d, $J$=19 Hz) | | |
| Anomeric | | 4.87 (1H, d, $J$=7 Hz) | |

| Proton No. | **13b**[c] |
|---|---|
| 1 | 4.47 (1H, br d, $J$=12 Hz) |
| 2 | 2.03 (1H, dd, $J$=18, 11 Hz) |
| 2′ | Overlapped |
| 3 | 5.12 (1H, dd, $J$=11, 6 Hz) |
| 5 | 4.34 (1H, br d, $J$=9 Hz) |
| 6 | 4.09 (1H, t, $J$=9 Hz) |
| 8 | 4.78 (1H, dt, $J$=10, 2 Hz) |
| 9 | Overlapped |
| 9′ | 1.91 (1H, t, $J$=10 Hz) |
| 11 | 2.12 (1H, dq, $J$=7, 11 Hz) |
| 13 | 1.35 (3H, d, $J$=7 Hz) |
| 14 | 1.27 (3H, br s) |
| 15 | 1.36 (3H, br s) |
| OAc | 1.53 (3H, s) |
| | 1.74 (3H, s) |

*a*) Run at 89.55 MHz in $CDCl_3$ solution. *b*) Run at 89.55 MHz in pyridine-$d_5$ solution. *c*) Run at 399.65 MHz in $C_6D_6$ solution.

A comparison of the carbon-13 nuclear magnetic resonance ($^{13}$C-NMR) spectrum of **10** with that of the aglycone of **9** showed an upfield shift by 8.3 ppm in the C-12 signal (lactone carbonyl), suggesting that the methyl group at the $\gamma$-lactone ring had been converted to an exocyclic methylene. The reduction product of **10** with $NaBH_4$ was identified by direct comparison ($^1$H-NMR) with the aglycone of **9**. These results led us to conclude that cichoriolide A has the structure **10**.

Cichorioside A (**11**), $[\alpha]_D$ +172°, was obtained as an amorphous powder. The fast atom bombardment mass spectrum (FAB-MS) of **11** showed ion peaks at *m/z* 411 $(C_{21}H_{30}O_8+H)^+$ and 433 $(C_{21}H_{30}O_8+Na)^+$. The $^1$H-NMR spectrum was similar to that of **10** except for the region of the sugar moiety including the anomeric proton at $\delta$ 4.94 (1H, d, $J$=7 Hz). In the $^{13}$C-NMR spectrum, twenty-one signals including six signals due to a glucopyranosyl moiety were observed, and the remaining fifteen signals were also similar to those of **10** except for a signal at $\delta$ 83.3 due to C-3. Acid hydrolysis of **11** afforded glucose as the sugar moiety and enzymatic hydrolysis with cellulase afforded **10** as an aglycone. From these results, we concluded that **11** was a glucoside of **10** at C-3.

Cichorioside B (**12**), $[\alpha]_D$ −48°, was obtained as an amorphous powder. The FAB-MS showed ion peaks at *m/z* 441 $(C_{21}H_{28}O_{10}+H)^+$ and 463 $(C_{21}H_{28}O_{10}+Na)^+$. The $^1$H-NMR spectrum exhibited a doublet methyl signal at $\delta$ 1.67 (3H, d, $J$=7 Hz), a vinyl methyl signal at $\delta$ 2.47 (3H, s), an anomeric proton signal at $\delta$ 4.97 (1H, d, $J$=7 Hz) and an olefinic proton signal at $\delta$ 7.01 (1H, br s). The $^{13}$C-NMR spectrum was similar to that of **6** except for the signals at $\delta$ 169.9 due to C-4, 69.3 due to C-15 and six signals due to the sugar moiety. Enzymatic hydrolysis of **12** afforded **6** as an aglycone. In the $^{13}$C-NMR spectrum, C-15 was shifted downfield by 6.8 ppm and C-4 was shifted upfield by 5.3 ppm compared with those of **6**. Therefore the structure of cichorioside B was decided to be **12**.

TABLE II. $^{13}$C-NMR Chemical Shifts

| Carbon No. | **10**[a] | **11**[b] | **12**[b] | **13**[b] |
|---|---|---|---|---|
| Aglycone moiety | | | | |
| 1 | 27.0[c] | 23.8[f] | 132.7 | 127.1 |
| 2 | 33.2[d] | 33.5[g] | 195.2 | 33.1 |
| 3 | 77.6 | 83.3[h] | 132.7 | 83.1 |
| 4 | 128.9 | 130.6 | 169.9 | 141.0 |
| 5 | 126.2 | 125.6 | 49.7[k] | 128.3 |
| 6 | 83.0 | 83.7[h] | 81.5 | 77.6 |
| 7 | 49.6 | 50.1 | 61.8 | 60.5 |
| 8 | 23.0[c] | 23.1[f] | 69.1[l] | 71.7 |
| 9 | 38.3[d] | 38.7[g] | 49.3[k] | 53.6 |
| 10 | 41.9 | 41.9 | 147.7 | 135.2 |
| 11 | 139.0 | 140.1 | 42.1 | 41.2 |
| 12 | 170.2 | 170.3 | 178.1 | 179.9 |
| 13 | 118.3 | 118.1 | 16.1 | 18.4[m] |
| 14 | 19.6[e] | 19.8[i] | 21.6 | 17.5[m] |
| 15 | 18.4[e] | 19.6[i] | 69.3[l] | 12.7 |
| Sugar moiety | | | | |
| 1 | | 102.3 | 104.4 | 102.8 |
| 2 | | 75.3 | 75.4 | 75.4 |
| 3 | | 78.8[j] | 78.6 | 78.6 |
| 4 | | 72.2 | 71.7 | 72.0 |
| 5 | | 78.5[j] | 78.6 | 78.6 |
| 6 | | 63.3 | 62.8 | 63.0 |

Run at 22.5 MHz *a*) in $CDCl_3$ and *b*) in pyridine-$d_5$ solution. *c*—*m*) Assignments may be interchanged in each column.

TABLE III. Yields

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Cichorium endivia* L. (yield, $10^{-4}$%) | 24 | 4.8 | 4.8 | 6.3 | 6.3 | 3.2 | 6.3 | 4.0 | | 16 | 2.4 | | |
| *Cichorium intybus* L. (yield, $10^{-4}$%) | 17 | | | | 100 | | | 7.0 | 2.0 | 2.0 | | 6.0 | 8.0 |

TABLE IV. Cytotoxic Activity of Sesquiterpene Lactones

| Compound | $ID_{50}$ (μg/ml) | $ID_{50}$ (μg/ml) of the aglycone |
|---|---|---|
| Guaianolide | | |
| Ainsliaside A[11] | 25.9 | 0.4 |
| Crepiside A[12] | 6.3 | |
| Crepiside H[12] | 16.5 | 1.3 |
| Crepiside I[12] | 6.5 | 0.03 |
| Crepidiaside A[2] | >100 | 1.7 |
| 8-Epidesacylcynaropicringlucoside[13] | 11.0 | 0.03 |
| Glucozaluzanin C[14] | 20.6 | 0.4 |
| Ixerin D[15] | >100 | 0.1 |
| Ixerin M[13] | 17.8 | 4.7 |
| Ixerin N[13] | 15.0 | 1.3 |
| Ixerin U[16] | 7.3 | 0.1 |
| Lactucopicriside[4] | >100 | 1.2 |
| Macrocliniside A[17] | 21.4 | 0.09 |
| Macrocliniside C[17] | 16.6 | 1.5 |
| Picriside A[5] | >100 | 4.7 |
| Prenantheside A[18] | 4.0 | 0.4 |
| Youngiaside A[19] | >100 | |
| Youngiaside B[19] | >100 | >10 |
| Youngiaside C[19] | >100 | |
| Germacranolide | | |
| Picriside B[5] | >100 | >10 |
| Picriside C[5] | >100 | >10 |
| Sonchuside B[6] | 6.5 | 0.1 |
| Melampolide | | |
| Ainsliaside B[11] | 39.0 | >10 |
| Ixerin B[20] | 91.8 | |
| Ixerin C[20] | 73.7 | 0.4 |
| Ixerin G[15] | 48.8 | |

All aglycones were obtained from their glycosides by enzymatic hydrolysis.

Cichorioside C (**13**), $[\alpha]_D$ +101°, was obtained as an amorphous powder. The FAB-MS showed ion peaks at *m/z* 429 $(C_{21}H_{32}O_9+H)^+$ and 451 $(C_{21}H_{32}O_9+Na)^+$. The IR spectrum suggested the presence of hydroxyl groups (3400 $cm^{-1}$), a γ-lactone ring (1760 $cm^{-1}$) and double bonds (1665, 1640 $cm^{-1}$). The $^1$H-NMR spectrum was similar to that of **8** except for the doublet methyl signal at δ 1.77 (3H, d, $J$=7 Hz) due to C-13.

In a comparison of the $^{13}$C-NMR spectrum of **13** with that of **8**, various signals showed shifts; C-6, C-10 and C-11 (each γ to C-8) at δ 77.6 (Δ −3.1 ppm), 135.2 (Δ −2.6 ppm) and 41.2 (Δ −1.1 ppm), respectively, C-7 and C-9 (each β to C-8) at δ 60.5 (Δ +6.1 ppm), 53.6 (Δ +12.3 ppm), respectively, and C-9 (α-position) at δ 71.7 (Δ +43.1 ppm). Thus, **13** was assumed to be a sonchuside A (**8**) analog having a hydroxyl group at C-8.

Acid hydrolysis of **13** afforded glucose as the sugar moiety, and enzymatic hydrolysis

with cellulase afforded an aglycone (**13a**). The mass spectrum (MS) of **13a** showed a molecular ion peak at *m/z* 266 in agreement with the molecular formula $C_{15}H_{22}O_4$. The $^1$H-NMR spectrum of **13a** also exhibited three methyl signals at $\delta$ 1.42 (3H, d, $J=7$ Hz), 1.47 (3H, br s) and 1.79 (3H, br s), olefinic proton signals at $\delta$ 4.92 (1H, dd, $J=11$, 5 Hz) due to H-1 and 4.64 (1H, overlapped) due to H-5, two carbinyl proton signals at $\delta$ 4.23 (1H, dd, $J=10$, 6 Hz) and 3.90 (1H, dt, $J=10$, 3 Hz), and a lactonic proton signal at $\delta$ 4.64 (1H, overlapped).

Acetylation of **13a** afforded **13b**. The CD spectrum of **13b** showed a positive Cotton effect, $[\theta]_{217}$ +119000, which is typical of germacranolides in which the homoconjugated 1(10)- and 4, 5-*trans*-double bonds have a chiral arrangement.[7] Therefore in this conformation, both C-14 and C-15 are oriented above the plane of the medium ring. In the $^1$H-NMR spectrum of **13b**, a nuclear Overhauser effect (NOE) was observed in the signal of H-6 (*ca.* 12%) on irradiating $H_3$-15, so H-6 was $\beta$-oriented. The orientations of H-8 and H-11 were decided to be both $\beta$ from the coupling constants ($J_{6-7}=9$ Hz, $J_{7-8}=10$ Hz, $J_{7-11}=11$ Hz) and a comparison of the chemical shifts of 8$\alpha$- and 8$\beta$-hydroxy type germacranolides.[8,9] These results led us to conclude that cichorioside C has the structure **13**.

The cytotoxic activities of related glycosides and the aglycones having the $\alpha$-methylene-$\gamma$-lactone structure, which is thought to be a cytotoxic activator, were compared in the L-5178Y cultured cell system.[10] The results are summarized in Table IV. These data suggested that the glycosides of sesquiterpene lactones have lower cytotoxic activity towards L-5178Y cells as compared with the aglycones. Though these two plants contain many sesquiterpene glycosides, they should not be hazardous if used as green vegetables because of the lower activity.

## Experimental

Optical rotations were determined with a JASCO DIP-140 digital polarimeter. IR spectra were run on a JASCO A-202 IR spectrometer. MS and FAB-MS were measured on JEOL D-100 and JEOL DX-303 instruments, respectively. CD spectra were recorded on a JASCO 20A spectropolarimeter. $^1$H- and $^{13}$C-NMR spectra were recorded on a JEOL FX-90Q (89.55 and 22.5 MHz, respectively) spectrometer. Chemical shifts are given on the $\delta$ scale with tetramethylsilane as an internal standard (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad). Gas chromatography (GC) was done on a Hitachi K 53 gas chromatograph. High-performance liquid chromatography (HPLC) was run on a Kyowa Seimitsu model K 880 instrument.

**Isolation**——Fresh roots of *Cichorium endivia* L. (6.3 kg) cultivated in Shizuoka were extracted twice with methanol under reflux. The extract was concentrated under reduced pressure and the residue was suspended in water. The suspension was extracted with ether. The water layer was passed through an Amberlite XAD-2 column and the resin was eluted with methanol after being washed with water. The ether layer (13 g) gave **10** (100 mg) and the methanol eluate (21 g) gave **1** (150 mg), **2** (30 mg), **3** (30 mg), **4** (40 mg), **5** (40 mg), **6** (20 mg), **7** (40 mg), **8** (250 mg), **10** (100 mg) and **11** (15 mg) after repeated column chromatographies on silica gel and preparative HPLC on Develosil ODS. Fresh roots of *C. intybus* L. (10 kg) were treated in the same way and gave **1** (170 mg), **5** (1 g), **8** (70 mg), **9** (20 mg), **10** (20 mg), **12** (60 mg) and **13** (80 mg).

**8-Deoxylactucin (1)**[1]——Amorphous powder. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3425, 2950, 1770, 1670, 1610. $^1$H-NMR ($CDCl_3$) $\delta$: 2.48 (3H, s, $H_3$-14), 5.53 (1H, d, $J=3.1$ Hz, H-13a), 6.22 (1H, d, $J=3.3$ Hz, H-13b), 6.48 (1H, br s, H-3).

**Crepidiaside A (2)**[2]——Amorphous powder. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3420, 2940, 1770, 1685, 1640, 1620. $^1$H-NMR (pyridine-$d_5$) $\delta$: 2.48 (3H, s, $H_3$-14), 4.97 (1H, d, $J=7$ Hz, anomeric H), 5.41 (1H, d, $J=3.1$ Hz, H-13a), 6.21 (1H, d, $J=3.3$ Hz, H-13b), 6.99 (1H, br s, H-3).

**Lactucin (3)**[1]——Amorphous powder. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3370, 3275, 1765, 1670, 1630, 1610. $^1$H-NMR (pyridine-$d_5$) $\delta$: 2.54 (3H, s, $H_3$-14), 4.06 (1H, br t, $J=10$ Hz, H-8), 4.78 (1H, br d, $J=18$ Hz, H-15), 5.39 (1H, br d, $J=18$ Hz, H-15′), 6.44 (1H, dd, $J=3.1$, 1.5 Hz, H-13a), 6.64 (1H, dd, $J=3.3$, 1.5 Hz, H-13b), 7.05 (1H. br s, H-3).

**Lactucopicrin (4)**[4]——Amorphous powder. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3425, 1780, 1745, 1695, 1645, 1620. $^1$H-NMR (pyridine-$d_5$) $\delta$: 2.51 (3H, s, $H_3$-14), 5.58 (1H, d, $J=2.7$ Hz, H-13a), 6.22 (1H, d, $J=3.1$ Hz, H-13b), 7.02 (1H, br s, H-3), 7.27 (2H, d, $J=9$ Hz, H-3′, -5′), 7.52 (2H, d, $J=9$ Hz, H-2′, -6′).

**Crepidiaside B (5)**[2]——Amorphous powder. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3460, 1770, 1690, 1625. $^1$H-NMR (pyridine-$d_5$) $\delta$: 1.17 (3H, d, $J=6$ Hz, $H_3$-13), 2.48 (3H, s, $H_3$-14), 4.96 (1H, d, $J=7$ Hz, anomeric H), 6.98 (1H, br s, H-3).

**11$\beta$,13-Dihydrolactucin (6)**[3]——Amorphous powder. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3450, 1780, 1685, 1640, 1620. $^1$H-NMR (pyridine-$d_5$) $\delta$: 1.72 (3H, d, $J=7$ Hz, $H_3$-13), 2.54 (3H, s, $H_3$-14), 3.93 (1H, t, $J=9$ Hz, H-6), 4.78 (1H, br d, $J=19$ Hz, H-15), 5.38 (1H, br d, $J=19$ Hz, H-15′), 7.04 (1H, br s, H-3).

**Picriside B (7)**[5]——Amorphous powder. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400, 2940, 1770, 1670. $^1$H-NMR (pyridine-$d_5$) $\delta$: 1.38 (3H, br s, $H_3$-14), 4.98 (1H, d, $J$=7 Hz, anomeric H), 5.57 (1H, d, $J$=3.1 Hz, H-13a), 6.39 (1H, d, $J$=3.3 Hz, H-13b).

**Sonchuside A (8)**[6]——Amorphous powder. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3420, 2940, 1770, 1670, 1640. $^1$H-NMR (pyridine-$d_5$) $\delta$: 1.27 (3H, d, $J$=7 Hz, $H_3$-13), 1.38 (3H, br s, $H_3$-14), 1.97 (3H, br s, $H_3$-15), 4.82 (1H, d, $J$=7 Hz, anomeric H).

**Sonchuside C (9)**[6]——Amorphous powder. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400, 1770, 1635. $^1$H-NMR (pyridine-$d_5$) $\delta$: 1.15 (3H, s, $H_3$-14), 1.16 (3H, d, $J$=7 Hz, $H_3$-13), 1.88 (3H, br s, $H_3$-15), 4.94 (1H, d, $J$=7 Hz, anomeric H).

**Cichoriolide A (10)**——Amorphous powder. $[\alpha]_D^{25}$ +88° ($c$=0.36, $CHCl_3$). *Anal.* Calcd for $C_{15}H_{20}O_3$: C, 72.55; H, 8.11. Found: C, 72.37; H, 8.08. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3590, 1790, 1690. CD ($c$=0.18, MeOH) [$\theta$] (nm): −3500 (250). MS $m/z$: 248 ($M^+$, 38), 230 ($M^+ - H_2O$, 78), 215 (34), 204 (98), 191 (66), 163 (81). $^1$H- and $^{13}$C-NMR: Tables I and II.

**Cichorioside A (11)**——Amorphous powder, $[\alpha]_D^{22}$ +172° ($c$=0.18, MeOH). CD ($c$=0.27, MeOH) [$\theta$] (nm): −3100 (248). FAB-MS $m/z$: 411 $(C_{21}H_{30}O_8+H)^+$, 433 $(C_{21}H_{30}O_8+Na)^+$. $^1$H- and $^{13}$C-NMR: Tables I and II.

**Cichorioside B (12)**——Amorphous powder, $[\alpha]_D^{25}$ −48° ($c$=0.62, pyridine). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3450, 1770, 1675, 1635, 1615. FAB-MS $m/z$: 441 $(C_{21}H_{28}O_{10}+H)^+$, 463 $(C_{21}H_{28}O_{10}+Na)^+$. $^1$H- and $^{13}$C-NMR: Tables I and II.

**Cichorioside C (13)**——Amorphous powder, $[\alpha]_D^{25}$ +101° ($c$=0.79, MeOH). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400, 2940, 1760, 1665, 1640. FAB-MS $m/z$: 429 $(C_{21}H_{32}O_9+H)^+$, 451 $(C_{21}H_{32}O_9+Na)^+$. $^1$H- and $^{13}$C-NMR: Tables I and II.

**Sodium Borohydride Reduction of Cichoriolide A (10)**——A solution of cichoriolide A (**10**) (4 mg) in methanol (1 ml) was treated with sodium borohydride (10 mg) and the mixture was stirred for 20 min. The reaction mixture was acidified with acetic acid and diluted with water. The aqueous solution was extracted with ethyl acetate 3 times. Purification by HPLC (Develosil ODS-10) with water–acetonitrile (65 : 35) as the eluent provided **9** (0.8 mg) as an amorphous powder. The $^1$H-NMR spectrum was identical to that of sonchuside C.

**Enzymatic Hydrolysis of Cichorioside A (11)**——**11** (*ca.* 1 mg) was dissolved in water (1 ml) and the solution was treated with cellulase (Sigma type II) (1 mg) for 2 h at 38 °C with stirring, then the reaction mixture was extracted with ethyl acetate 3 times. The ethyl acetate extract was purified by HPLC to give the aglycone, which was shown to be identical with cichoriolide A (**10**) by HPLC. Conditions: column, Develosil ODS-7 (4.6 mm × 25 cm); eluent, $H_2O$–$CH_3CN$ (55 : 45); flow rate 1.3 ml/min; UV detector at 205 nm. $t_R$ 6.1 min (cichoriolide A).

**Enzymatic Hydrolysis of Cichorioside B (12)**——Cichorioside B (**12**) (12 mg) was hydrolyzed in the same way as **11** to give the aglycone **6** (1.6 mg) as an amorphous powder. MS $m/z$: 278 ($M^+$, 100), 260 ($M^+ - H_2O$, 3), 249 (10), 231 (28), 214 (19), 205 (25), 187 (54), 175 (23), 159 (58). $^1$H-NMR: Table I.

**Enzymatic Hydrolysis of Cichorioside C (13)**——Cichorioside C (**13**) (20 mg) was hydrolyzed in the same way as **12** to give the aglycone **13a** (3.3 mg) as an amorphous powder. MS $m/z$: 266 ($M^+$, 10), 248 ($M^+ - H_2O$, 3), 238 (3), 220 (14), 209 (11), 208 (11), 193 (43), 175 (19), 165 (15), 152 (36), 147 (53). $^{13}$C-NMR (pyridine-$d_5$) $\delta$: 12.4 (C-15), 17.3, 18.3 (C-13/C-14), 35.7 (C-2), 41.2 (C-11), 53.5 (C-9), 60.4 (C-7), 71.6 (C-8), 77.5, 77.7 (C-3/C-6), 125.2 (C-1). 127.7 (C-5), 134.6 (C-10), 143.4 (C-4), 179.7 (C-12).

**Acetylation of the Aglycone (13a) of Cichorioside C**——**13a** (3.3 mg) was acetylated in the usual manner using acetic anhydride and pyridine to give the acetate **13b** (1.8 mg). Amorphous powder. CD ($c$=0.0085, MeOH) [$\theta$] (nm): +119000 (217). $^1$H-NMR: Table I.

**Acid Hydrolysis of Cichoriosides A (11), B (12) and C (13)**——A solution of a glycoside (*ca.* 1 mg) in 10% $H_2SO_4$ (2 drops) was heated in a boiling water bath for 30 min. The solution was passed through an Amberlite IRA-45 column and concentrated to give a residue, which was reduced with $NaBH_4$ (*ca.* 1 mg) for 1 h at room temperature. The reaction mixture was passed through an Amberlite IR-120 column and the eluate was concentrated to dryness. Boric acid was removed by co-distillation with methanol and the residue was acetylated with acetic anhydride and pyridine (1 drop each) at 100 °C for 1 h. The reagents were evaporated off *in vacuo*. From each glycoside, glucitol acetate was detected by GC. Conditions: column, Spelco SPB 35 capillary column (0.75 mm × 30 m); column temperature, 200 °C; carrier gas, $N_2$; $t_R$, 12.0 min.

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## References and Notes

1) J. S. Pyrek, *Rocz. Chem.*, **51**, 2165 (1977).
2) S. Adegawa, T. Miyase, A. Ueno, T. Noro, M. Kuroyanagi and S. Fukushima, *Chem. Pharm. Bull.*, **33**, 4906 (1985).
3) T. M. Sarg, A. A. Omar, S. M. Khafagy, M. Grenz and F. Bohlmann, *Phytochemistry*, **21**, 1163 (1982).
4) K. Nishimura, T. Miyase, A. Ueno, T. Noro, M. Kuroyanagi and S. Fukushima, *Phytochemistry*, **25**, 2375 (1986).
5) K. Nishimura, T. Miyase, A. Ueno, T. Noro, M. Kuroyanagi and S. Fukushima, *Chem. Pharm. Bull.*, **34**, 2518 (1986).
6) T. Miyase and S. Fukushima, *Chem. Pharm. Bull.*, **35**, 2869 (1987).
7) F. C. Seaman and N. H. Fischer, *Phytochemistry*, **19**, 849 (1980).

8) B. Kh. Abduazimov, A. I. Yunusov, M. R. Yagudaev and G. P. Sidyakin, *Khim. Prir. Soedin.*, **3**, 314 (1984).
9) P. Singh, A. K. Sharma, K. C. Joshi, J. Jakupovic and F. Bohlmann, *Phytochemistry*, **24**, 2023 (1985).
10) M. Yoshida, A. Hoshi, K. Kurotani, T. Kanai and M. Ichino, *Gann*, **66**, 561 (1975).
11) T. Miyase and S. Fukushima, *Chem. Pharm. Bull.*, **32**, 3043 (1984).
12) T. Miyase, A. Ueno, T. Noro, M. Kuroyaagi and S. Fukushima, *Chem. Pharm. Bull.*, **33**, 4451 (1985).
13) K. Nishimura, T. Miyase, A. Ueno, T. Noro, M. Kuroyanagi and S. Fukushima, *Chem. Pharm. Bull.*, **33**, 3361 (1985).
14) S. Nagumo, K. Izawa, K. Higashiyama and M. Nagai, *Yakugaku Zasshi*, **100**, 427 (1980).
15) H. Asada, T. Miyase and S. Fukushima, *Chem. Pharm. Bull.*, **32**, 3036 (1984).
16) M. Seto, T. Miyase and S. Fukushima, *Chem. Pharm. Bull.*, **34**, 4170 (1986).
17) T. Miyase, K. Yamaki and S. Fukushima, *Chem. Pharm. Bull.*, **32**, 3912 (1984).
18) T. Miyase, M. Yamada and S. Fukushima, *Chem. Pharm. Bull.*, **35**, 1969 (1987).
19) S. Adegawa, T. Miyase and S. Fukushima, *Chem. Pharm. Bull.*, **34**, 3769 (1986).
20) H. Asada, T. Miyase and S. Fukushima, *Chem. Pharm. Bull.*, **32**, 1724 (1984).