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# Potential Anticancer Agents. V.[1] Cardiac Glycosides of *Asclepias albicans* (Asclepiadaceae)

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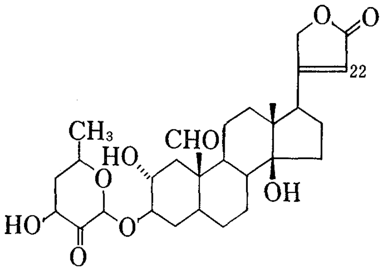
The cytotoxic activity of extracts of *Asclepias albicans* (Asclepiadaceae) was traced to uzarigenin (2), desglucouzarin (3) and a new cardenolide glycoside, uzarigenin 3-O-β-D-glucopyranosyl (1→4)-β-D-glucopyranoside(4). The latter compound also displayed significant antitumor activity against P-388 lymphocytic leukemia.

**Keywords**——cardiac glycosides; *Asclepias albicans*; Asclepiadaceae; structure elucidation; cytotoxicity; antitumor activity

The milkweeds of the genus *Asclepias* (Asclepiadaceae)[3] are well known for their folkloric use in the treatment of many cancerous states; indeed the genus name is derived from the Greek "God of Healing".[4] Some years ago Kupchan and co-workers[5] obtained calotropin (**1**) as the major cytotoxic principle of *A. curassavica*, but no subsequent reports have appeared on the anticancer principles of *Asclepias* species.

As part of a continuing search for anticancer agents from higher plants we have examined extracts of the Californian plant *Asclepias albicans* S. Wats. (Asclepiadaceae).

A 50% aqueous ethanol extract of *A. albicans* showed consistent activity in the Eagles carcinoma of the nasopharynx (KB) test system in cell culture,[6] and solvent partition of a



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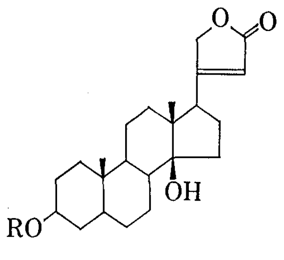

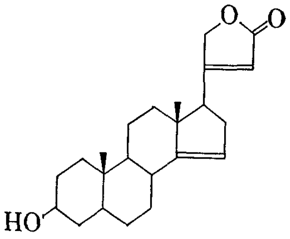

**5**

**2**: R=H
**3**: R=β-D-glucopyranosyl
**4**: R=β-D-glucopyranosyl (1→4)-β-D-glucopyranosyl
**6**: R=β-D-glu $(OAc)_4$
**7**: R=β-D-glu $(OAc)_3$-(1→4)-β-D-glu $(OAc)_4$

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[1] Part IV: M. Ogura, G.A. Cordell, and N.R. Farnsworth, *Lloydia*, 40, 157 (1977).
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[3] J.L. Hartwell, *Lloydia*, **30**, 379 (1967).
[4] W. Bakarjiew, *Ther. Hung.*, **17**, 144 (1969) [*Biol. Abstr.*, **51**, 64241 (1970)].
[5] S.M. Kupchan, J.R. Knox, and J.E. Kelsey, *Science*, **146**, 1685 (1964).
[6] R.I. Geran, N.H. Greenberg, M.M. MacDonald, A.M. Schumacher, and B.J. Abbott, *Cancer Chemother. Rep.*, 3 (2), 1 (1972).

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methanol extract of the plant indicated the activity to be located in the chloroform and water soluble fractions.

Chromatography of the chloroform-soluble fractions on silica gel, monitoring with the KB test, afforded uzarigenin (**2**) as the principal cytotoxic constituent. Uzarigenin (**2**) is a well-established constituent of *Asclepias* species, having previously been obtained from *A. curassavica*,7–8) *A. glaucophylla*,9–10) *A. lilacina*,11) *A. swynnertonii*,12) *and A. syriaca*.13–15)

From chromatography of the water-soluble fraction on silica gel, again monitoring biologically active fractions, two cardenolide glycosides were obtained as active constituents. One of these, desglucouzarin (**3**), is also an established constituent of *Ascelpias*, having previously been isolated from *A. mellodora*16–17) and *A. syriaca*.13,14–17)

The second glycoside is new and on the basis of the following evidence was assigned the structure uzarigenin 3-O-$\beta$-D-glucopyranosyl (1→4)-$\beta$-D-glucopyranoside (**4**).

Typical infrared (IR) absorptions were observed for several hydroxyl groups (3520—3240 $\text{cm}^{-1}$) and a butenolide (1780 $\text{cm}^{-1}$) group, and the latter was also evident from the ultraviolet (UV) spectrum ($\lambda_{\text{max}}$ 217 nm). The compound did not give a molecular ion under conditions of electron impact, but in the field ionization (F.I.) spectrum a molecular ion at *m/e* 698 and fragment ions at *m/e* 374 and *m/e* 356 were observed, suggesting an uzarigenin disaccharide derivative.7–15)

Acetylation afforded a heptaacetate derivative, as indicated by the PMR spectrum, which showed seven three-proton singlets, showing a molecular ion-60 (by the field ionization technique) at 932 mass units. Acidic hydrolysis gave an anhydroaglycone identical with anhydrouzarigenin (**5**) and it therefore remained to deduce the nature and stereochemistry of the gylcone portion. Emulsin hydrolysis18) gave uzarigenin (**2**) and desglucouzarin (**3**), identical with authentic samples.

The sugar portions from acidic and enzymatic hydrolysis were found to consist solely of glucose by thin-layer chromatography (TLC) of the free sugar and gas-liquid chromatography (GLC) of the trimethyl silyl ether derivative in comparison with standard samples. From these data it was inferred that the compound was an uzarigenin diglucoside; the location and stereochemistry of the linkage remained to be determined.

The PMR spectrum of **4** in pyridine-$d_5$ showed two singlet methyl groups at 0.56 and 0.69 ppm and sugar protons in the region of 3.4—4.7 ppm. In this solution, part of the region containing the anomeric protons overlapped with the protons at C-21, but it was possible to discern the anomeric protons as two doublets (4.98 and 5.12 ppm, $J$=8 Hz). In a deuterochloroform–methanol-$d_4$ mixture the anomeric protons overlapped completely and were observed as a two-proton doublet ($J$=7 Hz) at 4.44 ppm. The anomeric protons appeared as two separated doublets (4.99 and 5.22 ppm, $J$=7 and 6 Hz, respectively) in the pyridine-$d_5$ spectrum of the hydrogenated product.

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7) R. Tschesche, D. Forstmann, and V.H.M. Rao, *Chem. Ber.*, **91**, 1204 (1958).
8) B. Singh and R.P. Rastogi, *Indian J. Chem.*, **7**, 1105 (1969).
9) J.M. Nascimento, Ch. Tamm, H. Jaeger, and T. Reichstein, *Helv. Chim. Acta*, **47**, 1775 (1964).
10) J.M. Nascimento, *Rev. Port. Quim.*, **6**, 97 (1964) [*Chem. Abstr.*, **64**, 5449*i* (1966)].
11) L. Sawlewicz, E. Weiss, and T. Reichstein, *Helv. Chim. Acta* **50**, 504 (1967).
12) K. Jäggi, H. Kaufmann, W. Stöcklin, and T. Reichstein, *Helv. Chim. Acta.*, **50**, 2457 (1967).
13) S. Bauer, L. Masler, O. Bauerova, and D. Sikl, *Experientia*, **17**, 15 (1961).
14) L. Masler, S. Bauer, O. Bauerova, and D. Sikl, *Coll. Czech. Chem. Commun.*, **27**, 872 (1962).
15) H. Mitsuhashi, K. Hayashi, and K. Tomimoto, *Chem. Pharm. Bull.*, **18**, 828 (1970).
16) J. Petrivic, *Naturwissenschaften*, **53**, 332 (1966).
17) J. Petrivic, *Farm. Glas.*, 23, 3 (1967) [*Chem. Abstr.*, **67**, 8686*g* (1967)].
18) On treatment with $\alpha$-glucosidase (Sigma) at room temperature for 2 hours the isolate was converted to desglucouzarin. The $\alpha$-glucosidase activity was confirmed using several $\alpha$-linked disaccharides and the same hydrolysis time as for **4**. $\beta$-Linked disaccharides were not hydrolyzed under these conditions. However, this result should be considered in conjunction with other findings.

These PMR spectral data indicated that the two sugar linkages in **4** were β, and this was supported by the application of Klyne's rule[19]: $[M]_D$ (**4**)$-[M]_D$ (**3**)$=+10$; $[M]_D$ (**3**)$-[M]_D$ (uzarigenin)$=-176$; $[M]_D$ (methyl α-D-glucopyranoside)$=+355$[14]; $[M]_D$ (methyl β-D-glucopyranoside)$=-65$.[14]

Exhaustive methylation with NaH/$CH_3I$ in dimethyl sulfoxide (DMSO) followed by acid hydrolysis (methanol–HCl) afforded a mixture of methylated sugars identical (GLC) with the mixtures obtained by methylation/hydrolysis of the 1→4 linked disaccharides cellobiose (β-linked) and maltose (α-linked).

The new isolate is therefore derived by the union of cellobiose and uzarigenin (**2**) and has the structure uzarigenin 3-O-β-D-glucopyranosyl (1→4)-β-D-glucopyranoside (**4**).

Each of the isolates was cytotoxic in the 9 KB test system and in addition, **4**, showed significant inhibitory activity against the P-388 lymphocytic leukemia system at doses in the range of 15—60 mg/kg.

## Experimental

Melting points were determined by means of a Kofler hot plate and are uncorrected. The UV spectra were obtained with a Beckman DB G grating spectrophotometer. PMR spectra were determined with a Varian model T60A instrument. Tetramethylsilane was used as an internal standard and chemical shifts are reported in δ units. Low resolution mass spectra were obtained with a Hitachi-Perkin Elmer RMU-6D single-focusing spectrometer operating at 70 eV. Optical rotations were obtained using a Carl Zeiss polarimeter. GLC was carried out with a Perkin-Elmer 881 gas chromatograph. Silica gel $PF_{254}$ and Merck precoated silica gel G plates were used for chromatography except where otherwise indicated. Solvent systems were as follows: (A) benzene–MeOH, (3: 1), (B) $CHCl_3$–MeOH (3: 1), (C) $CHCl_3$–MeOH (20: 1), and (D) isoamyl alcohol–saturated water.

**Extraction and Fractionation**——The stem, flower and fruit parts of *Asclepias albicans* (5 kg), collected in March 1972 in California, were extracted with petroleum ether and the marc was extracted with MeOH. The MeOH extract was concentrated *in vacuo* and the residue was partitioned between $H_2O$ (2 l) and $CHCl_3$ (4×2 l). Concentration of the dried ($Na_2SO_4$) $CHCl_3$ fraction afforded 60 g of $CHCl_3$-soluble material.

**Biological Activity of the Fractions**——The petroleum ether, $CHCl_3$ and aqueous fractions were evaluated for their cytotoxicity in the Eagles carcinoma of the nasopharynx test system in cell culture.[6] The $CHCl_3$ and aqueous fractions exhibited approximately equal activities ($ED_{50}$ 3 μg/ml) and each was further fractionated to obtain the active components.

**Chromatography of the $CHCl_3$-soluble Fraction**——The chloroform-soluble fraction (12 g) was chromatographed on 700 g of Silica gel $PF_{254}$ packed in chloroform. Fractions eluted with $CHCl_3$–MeOH (85: 15) were combined ($ED_{50}$ 2.4 μg/ml) and the residue (3.3 g) was chromatographed on 300 g of Silica gel $PF_{254}$. Fractions eluted with $CHCl_3$–MeOH (8: 1) were combined ($ED_{50}$ 0.6 μg/ml) and the residue (1.65 g) was chromatographed on 100 g of Silica gel $PF_{254}$, eluting with EtOAc (20 ml fractions). Fractions 18—32 gave crystals (536 mg) from EtOAc, mp 246°, identified as uzarigenin (**2**). $[\alpha]_D^{25}$ $-15.9°$ ($c=0.98$, pyridine) (Lit.[18]) $-19.1°$ (pyridine), $+23.5°$ ($c=0.85$, MeOH). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3480, 2930, 2850, 1795, 1735, 1640. UV $\lambda_{max}^{MeOH}$ nm (ε): 217 (19500). NMR δ ($CDCl_3$): 0.78 and 0.88 (3H each, s, 18 and 19-$CH_3$), 3.58 (1H, m, 3-H), 4.88 (2H, m, 21-$H_2$), 5.87 (1H, bs, 22-H). MS *m/e*: 374 ($M^+$), 356 (M-$H_2O$, 100%), 341, 338, 323, 264, 228, 203. *Rf* 0.54 (A), 0.30 (C).

**Chromatography of the Water-soluble Fraction**——The aqueous phase obtained above was extracted with EtOAc: MeOH (9: 1) (3×2 l). The organic phase was concentrated to give 20.7 g of residue, which was chromatographed on 600 g of Silica gel $PF_{254}$, eluting with EtOAc–MeOH (4: 1) and collecting 50 ml fractions. Fractions 10—13 deposited 423 mg of crystals of desglucouzarin (**3**) and fractions 27—33 deposited 369 mg of **4**. Additional **4** (85.4 mg) was obtained from fractions 34—37.

**Desglucouzarin (3)**——Prisms from MeOH–$CHCl_3$, mp 262—265°. $[\alpha]_D^{25}$ $-44.1°$ ($c=1.16$, pyridine), $-18.1°$ ($c=0.21$, MeOH). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3500—3280, 2940, 1730, 1625. UV $\lambda_{max}^{MeOH}$ nm (ε): 218 (14300). NMR δ (pyridine-$d_5$): 0.66 and 1.00 (3H each, s, 18 and 19-$CH_3$), 4.0—4.8 (sugar protons), 5.04 (1H, d, $J=$ 7 Hz, sugar 1H), 5.16 (2H, m, 21-$H_2$), 6.19 (1H, bs, 22-H). MS (F.I.) *m/e*: 536 ($M^+$), 518 ($M^+-H_2O$), 492 ($M^+-CO_2$), 374 (genin), 356, (genin-$H_2O$). *Rf* 0.26 (A), 0.68 (B), 0.44 (D). *Anal.* Calcd for $C_{29}H_{44}O_9$: C, 64.92; H, 8.22. Found: C, 64.65; H, 8.35.

**Acetylation of Desglucouzarin (3)**——Compound **3** (70 mg) was dissolved in 2 ml of $Ac_2O$–pyridine (1: 1) and the mixture was allowed to stand at room temperature for 48 hr. The solvents were removed *in vacuo* and the residue was crystallized from acetone–ether to give 66 mg of **6** as needles, mp 162—163°. IR $\nu_{max}^{KBr}$

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[19] W. Klyne, *Biochem. J.*, **47**, xii (1950).

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cm$^{-1}$: 3500—3430, 2940, 1760—1740, 1625. NMR ($CDCl_3$): 0.78 and 0.88 (3H each, s, 18 and 19-$CH_3$), 2.00, 2.02, 2.03, 2.07 (3H each, s, 4×OAc), 3.70—4.10 (2H, m, 3-H and sugar 5-H), 4.17 (2H, m, 2×sugar 6-H), 4.59 (1H, d, $J$=7.5 Hz, sugar 1-H), 4.88 (2H, bs, 21-$H_2$), 4.90—5.60 (3H, m, sugar methine protons), 5.87 (1H, bs, 22-H). MS (F.I.) *m/e*: 704 ($M^+$), 686 (M-$H_2O$), 644 (M-AtOH), 602 (M-AcOH-$CH_2CO$), 446 (M-$(AcOH)_4$–$H_2O$), 373 (genin-H), 357 (genin-OH), 330 (acetyl sugar –$H_2O$). *Anal.* Calcd for $C_{37}H_{52}O_{13}$: C, 63.05; H, 7.44. Found: C, 62.45; H, 7.47.

**Acid Hydrolysis of Desglucouzarin (3)**——Compound 3 (104 mg) and 40 ml of 2% HCl in MeOH were allowed to stand for 14 hr at room temperature. After refluxing for 30 min, the solution was concentrated *in vacuo* to afford 18 mg of anhydrouzarigenin (**5**), recrystallized from MeOH as needles, mp 259—262°. Additional quantities of **5** were obtained by $CHCl_3$ extraction of the residue after the removal of MeOH. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3450, 3120, 3070, 2980—2840, 1810, 1740, 1630. MS *m/e*: 356 ($M^+$), 341 (M-$CH_3$), 338 (M-$H_2O$), 323 (M-$CH_3$-$H_2O$). *Rf* 0.65 (A), 0.65 (C). *Anal.* Calcd for $C_{23}H_{32}O_3$: C, 77.54; H, 9.00. Found: C, 77.30; H, 9.29.

**Enzymic Hydrolysis of 3**——A mixture of 100 mg of 3 and 5 drops of toluene in 100 ml of $H_2O$ was stirred with 100 mg of $\beta$-glucosidase at room temperature for 7 days. The reaction mixture was extracted with $CHCl_3$ fraction and the residue crystallized from MeOH to afford uzarigenin (**2**) as needles, identical (mp, mixed mp, IR, NMR, TLC) with an authentic sample. Desglucozarin (**3**) was unchanged by reaction with $\alpha$-glucosidase under similar conditions.

**Uzarigenin 3-O-β-D-Glucopyranosyl (1→4)-β-D-Glucopyranoside (4)**——Prisms from MeOH, mp 300—305°. $[\alpha]_D^{24}$ $-12.1°$ ($c$=3.05, MeOH), $-32.4°$ ($c$=1.05, pyridine). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3520—3240, 2940—2860, 1780, 1745, 1640. UV $\lambda_{max}^{MeOH}$ ($\varepsilon$): 217 (14600). NMR $\delta$ (pyridine-$d_5$): 0.65 and 1.00 (3H each, s, 18 and 19-$CH_3$), 4.0—4.8 (sugar protons), 4.98 (1H, d, $J$=8 Hz, sugar 1-H), 5.12 (1H, d, $J$=8 Hz, sugar 1-H), 5.27 (2H, m, 21-$H_2$), 5.61 (1H, bs, 22-H); NMR $\delta$ ($CDCl_3$-methanol-$d_4$):(20) 0.83 and 0.89 (3H each, s, 18 and 19-$CH_3$), 3.4—4.0 (sugar protons), 4.44 (2H, d, $J$=7 Hz, sugar 1-H×2), 4.95 (2H, m, 21-$H_2$), 5.87 (1H, bs, 22-H). MS (F.I.) *m/e*: 698 ($M^+$), 680 (M-$H_2O$), 654 (M-$CO_2$), 636 (M-$CO_2$-$H_2O$), 536 (M-162), 518 (M-162-$H_2O$), 496 (M-162-$CO_2$), 474 (M-162-$CO_2$-$H_2O$), 374 (genin), 356 (genin-$H_2O$), 338 (genin-$H_2O$-$H_2O$), 166, 144, 126. *Rf* 0.08 (A), 0.23 (B), 0.15 (D). *Anal.* Calcd for $C_{35}H_{54}O_{14}$: C, 60.17; H, 7.73. Found: C, 59.57; H, 8.05.

**Acetylation of 4**——Compound 4 (40 mg) in 1 ml of $Ac_2O$–pyridine (1: 1) was allowed to stand at room temperature for 36 hr. After concentration *in vacuo* the residue was dissolved in 5 ml of $CHCl_3$ and the organic phase was successively washed with 3 ml of 0.1 N HCl, 3 ml of 0.1 N $Na_2CO_3$ and 3 ml of satd. NaCl, dried over ($Na_2SO_4$) and concentrated. The product **7** (25 mg) was obtained from ether, containing a trace of $(CH_3)_2CO$, as a microcrystalline powder, mp 160—165°. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3500, 2940—2860, 1760—1750, 1630, NMR ($CDCl_3$): 0.79 and 0.89 (3H each, s, 18 and 19-$CH_3$), 2.00, 2.03, 2.04, 2.05, 2.06, 2.10, 2.13 (3H each, s, 7×OAc), 5.90 (1H, bs, 22-H). MS (F.I.) *m/e*: 932 (M-AcOH), 848 (M-AcOH-2×$CH_2CO$), 830 (M-2×AcOH-$CH_2CO$), 806 (M-AcOH-3×$CH_2CO$), 788 (M-2×AcOH-2×$CH_2CO$), 746 (M-2×AcOH-3×$CH_2CO$), 644 (M-330-$H_2O$), 602 (M-330-AcOH), 583 (M-330-AcOH-$H_2O$), 560 (M-330-AcOH-$CH_2CO$), 374 (genin), 356 (genin-$H_2O$).

**Hydrogenation of 4**——The isolate 4 (200 mg) was hydrogenated in glacial acetic acid (5 ml), using platinum oxide ($PtO_2$) as a catalyst, for 2 days at room temperature. The reaction mixture was filtered through Celite to afford a pale yellow solid after concentration *in vacuo*. Purification by preparative TLC, eluting with $CHCl_3$–MeOH (3: 1), afforded the hydrogenated product which was recrystallized from MeOH to yield crystals, mp 285—286° (dec.), IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3420, 1800 (saturated $\gamma$-lactone). UV, no absorption. NMR $\delta$ (pyridine-$d_5$) 0.56 and 0.69 (3H each, s, 18 and 19-$CH_3$), 3.7 —4.7 (sugar protons), 4.99 (1H, d, $J$= 7 Hz, sugar 1-H), 5.22 (1H, d, $J$=6 Hz, sugar 1-H); NMR $\delta$ ($CDCl_3$-methanol-$d_4$):(20) 0.72 and 0.85 (3H each, s, 18 and 19-$CH_3$), 3.5 and 4.1 (sugar protons), 4.95 (2H, d, $J$=7 Hz, sugar 1-H×2).

**Acid Hydrolysis of 4**——Compound 4 (152 mg) was dissolved in 1% MeOH–HCl and refluxed for 12 hr. Needles were obtained on cooling, and were recrystallized from MeOH to give 38 mg of anhydrouzarigenin (**5**), mp 259—262°. The compound was identical (mp, mmp, IR, NMR, MS and TLC) with the acid hydrolysis product of **3**.

The methanol phase was toncentrated and the residue was identified as glucose by TLC (cellulose, $n$-BuOH–AcOH–$H_2O$ (4: 1: 2), *Rf* 0.29) and by GLC examination of the TMS ether.

**Enzymic Hydrolysis of 4**——A mixture of 100 mg of 4 and 5 drops of toluene in 100 ml of $H_2O$ was stirred with 30 mg of $\beta$-glucosidase at room temperature overnight. The reaction mixture was extracted with EtOAc (3×80 ml), and the combined EtOAc extracts were concentrated. The residue was washed with $CHCl_3$ and recrystallized from MeOH to afford a crystalline powder identical with **3** by comparison of mmp, UV, IR, and TLC. The aglycone was obtained as needles from the $CHCl_3$-soluble fraction on recrystallization from MeOH and was identical with **2** by comparison of mmp, IR, MS and TLC.

b) Compound 4 (2 mg) was dissolved in 1% aq. MeOH (0.5 ml) with 2 mg of $\alpha$-glucosidase. After 2 hr at room temperature, the reaction mixture was heated at 100° for 1 hr and concentrated. The EtOAc soluble part of the residue was identified as **3** by mp, TLC and conversion to **6**. The water soluble fraction was identified as glucose by TLC and by GLC of the TMS ether.

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20) $CDCl_3$–methanol-$d_4$ (1: 1); $CF_3CO_2H$ was added to shift the water peaks.

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**Methylation and Hydrolysis of 4**——NaH (300 mg) in 10 ml of dry DMSO was stirred at 50—60° for 1 hr under $N_2$ and 50 mg of **4** in 10 ml of dry DMSO was added. The reaction mixture was stirred at room temperature for 2 hr under $N_2$. $CH_3I$ (3 ml) was added and the mixture was stirred at room temperature overnight. The total reaction mixture was added to 50 ml of $H_2O$ and extracted three times with 30 ml of $CHCl_3$. The $CHCl_3$ extract was dried ($Na_2SO_4$) and evaporated to dryness. Repetition of this process afforded a fully methylated product showing no OH absorption in the IR spectrum. The crude product was purified by preparative TLC on silica gel, eluting with EtOAc-EtOH (10: 1), to afford 40 mg of the methylated derivative.

The methylated compound (10 mg) was dissolved in methanolic HCl, sealed in a glass tube and heated at 100° for 3 hr. After cooling, the reaction mixture was neutralized with $Ag_2CO_3$ and filtered. The methylated sugars were analyzed by GLC, and gave a chromatogram identical with that of the product of a methylated 1→4-linked disaccharide such as maltose or cellobiose. The corresponding product from **3** was prepared in a similar manner and found to be identical with a product obtained from glucose in two chromatographic systems. GLC: i) Column, 3% SE 30 on Gas Chrom Q(100—120 mesh), 2 mm × 2 m; column temp., 130°; carrier gas, He, 2.6 kg/cm$^2$; $t_R$ (min), product from **4**, 7′48″, 9′30″, 11′48″; product from**3**, 7′48″, 9′42″. ii) Column, 15% ethylene glycol succinate polyester on Gas Chrom Q (60—80 mesh), 2mm × 2m; column temp., 165°; carrier gas, He, 2.6 kg/cm$^2$; $t_R$ (min), product from **4**, 2′00″,3′00″, 5′18″, 7′48″, product from **3**, 2′00″, 3′00″.

**Biological Activities of the Isolates**——Each of the isolates was examined for cytotoxicity in the Eagles carcinoma of the nasopharynx (KB) test system and for antitumor activity against P-388 lymphocytic leukemia in mice, according to established protocols.[6] Uzarigenin (**2**, NSC-277290), desglucouzarin (**3**, NSC-277288) and uzarigenin 3-O-$\beta$-D-glucopyranosyl (1→4)-$\beta$-D-glucopyranoside (**4**, NSC-277289) were active in the KB system at the 1—3.5 $\mu$g/ml level. Significant ($T/C > 130\%$) antitumor activity was observed with **4** in the dose range of 15—60 mg/kg.

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