Secondary metabolites of *Astragalus danicus* Retz. and *A. inopinatus* Boriss.

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The chemical composition of the above-ground parts of *Astragalus danicus* and *A. inopinatus* collected in the Baikal region (Eastern Siberia) was studied for the first time. From *A. danicus*, pentacyclic triterpene saponins were isolated and identified, *viz.*, 3-*O*-(β-glucuronopyranosyl)-3β,22β,24-trihydroxyolean-12-ene, 3-*O*-[*O*-(β-D-xylopyranosyl)-(1→2)-(β-glucuronopyranosyl)]-3β,22β,24-trihydroxyolean-12-ene, 3-*O*-[*O*-(β-D-glucopyranosyl)-(1→2)-(β-glucuronopyranosyl)]-3β,22β,24-trihydroxyolean-12-ene, 3-*O*-[*O*-(α-L-rhamnopyranosyl)-(1→2)-*O*-(β-D-xylopyranosyl)-(1→2)-(β-glucuronopyranosyl)]-3β,22β,24-trihydroxyolean-12-ene, 3-*O*-[*O*-(α-L-rhamnopyranosyl)-(1→2)-*O*-(β-D-glucopyranosyl)-(1→2)-(β-glucuronopyranosyl)]-3β,22β,24-trihydroxyolean-12-ene, 3-*O*-methyl-D-*chiro*-inositol, and linolenic acid. In *A. inopinatus*, the same saponins were identified as well as tricosan-1-ol and tetracosan-1-ol, 5,7,4′-trihydroxyflavon (apigenin), and a tetracyclic triterpenoid, 20(*R*),24(*S*)-epoxycyclolanost-9(11)-ene-3β,6α,16β,25-tetrol (cycloastragenol). All reported compounds from the both genus of *Astragalus* were isolated for the first time. Methanolic extracts of *A. danicus* and *A. inopinatus* exhibited low inhibitory activity with respect to the growth of HeLa cells. The chloroform fraction of *A. danicus* showed a strong antimicrobial activity against *Staphylococcus aureus* and a strong cytotoxic activity against HeLa cells.

Key words: *Astragalus,* saponins, cycloastragenol, *O*-methylinositol, apigenin, higher alcohols, linolenic acid, biological activity.

The genus *Astragalus* (Fabaceae family) comprises about 2000 species; of these, about 80 species produce saponins. Biological activities of many saponins have been established. The chemical composition of *Astragalus inopinatus* and triterpene substances *A. danicus* has not been studied previously. *A. danicus* is used in alternative and oriental medicine as a tonic and in treatment of gynaecological disorders and hypertension;[1] no published data on the use of *A. inopinatus* have been found.

Results and Discussion

We studied the chemical composition of the above-ground parts of the species under consideration collected in the blooming period in the Baikal region, Eastern Siberia. Methanolic extracts of air-dry finely ground plant material were treated successively with chloroform and *n*-butanol. Five pentacyclic triterpene glycosides were isolated from the butanolic extract of *A. danicus*, namely, one monoside (**1**), two biosides (**2** and **3**), and two triosides (**4** and **5**). From the butanolic extract of *A. inopinatus*, glycosides **2**—**5** were isolated. *O*-Methylcyclitol ((+)-pinitol) (**6**) and linolenic acid (**7**) were found in the chloroform extract of *A. danicus.* Two saturated alcohols, *viz.*, tricosan-1-ol (**8**) and tetracoosan-1-ol (**9**), a flavon (apigenin) (**10**), and a tetracyclic triterpenoid, *viz.*, cycloastragenol (**11**) were obtained from a similar fraction of *A. inopinatus.* These compounds were identified by chemical and spectral methods, namely, 1D and 2D NMR spectroscopy (DEPT, COSY, HETCOR, HMBC), high-resolution mass spectrometry (HR MS), and EI MS, FAB MS (positive and negative ions), and GC/MS.

The compounds were isolated by column chromatography on silica gel; in addition, reversed-phase preparative HPLC was used to purify compounds **2**—**5** and **7**.

Compound **1** has the formula $C_{36}H_{58}O_9$ (HR FAB MS). Acid hydrolysis of monoside **1** yielded glucuronic acid (GlcA) (identified by TLC with an authentic sample*) and a genin $C_{30}H_{50}O_3$ (HR FAB MS). The ^{1}H and ^{13}C NMR spectra of glycoside **1** confirm the identification of GlcA and attest to the pyranose form of its oxide ring and to the β-configuration of the glycosidic bond (δ_H 4.91, d, H(1), J = 7.8 Hz; δ_C 105.9, C(1)). In terms of its physicochemical parameters and ^{1}H and

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* The absolute configuration (D- or L-) of the glucuronic acid residues in compounds **1**—**5** has not been determined; this monosaccharide is depicted in the D-form for convenience.

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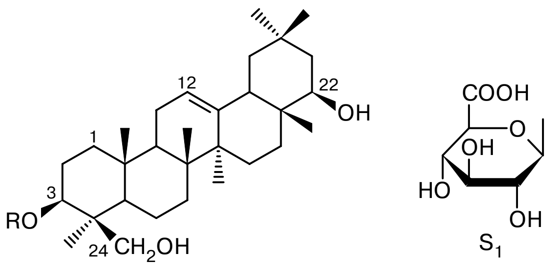
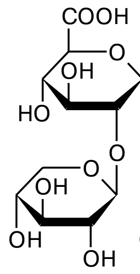
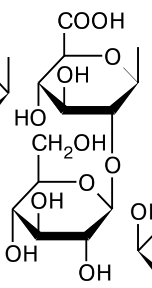
^{13}C NMR spectroscopy, the genin is 3β,22β,24-trihydroxyolean-12-ene; it was identified as soyasapogenin B (**12**).[2,3]



R = S_1 (**1**), S_2 (**2**), S_3 (**3**), S_4 (**4**), S_5 (**5**), H (**12**)



S_2



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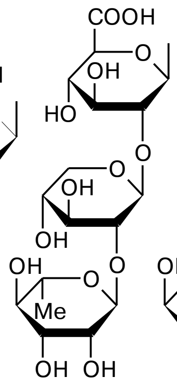
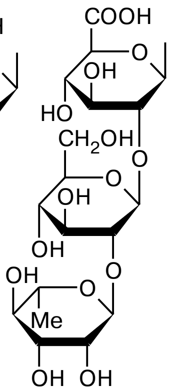


S_4



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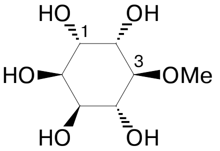

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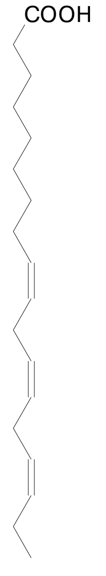

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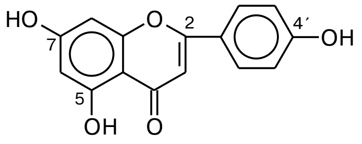
$Me(CH_2)_{21}CH_2OH$

8

$Me(CH_2)_{22}CH_2OH$

9



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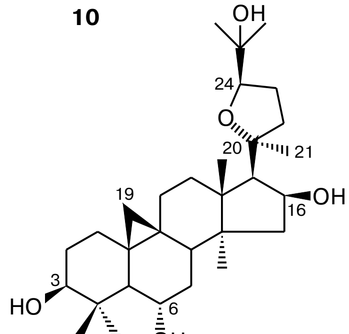

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Table 1. ^{13}C NMR spectral data of triterpene fragments of compounds **1**—**5**, **11**, and **12** (δ, **1** in C_5D_5N; **2**—**5** and **11** in CD_3OD; **12** in $CDCl_3$)

C atom	**12**	**1**	**2**	**3**	**4**	**5**	**11**
1	38.4	39.1	39.4	39.1	40.0	40.0	33.6
2	27.7	26.8	26.9	26.8	27.0	27.0	31.2
3	80.9	89.4	91.1	91.5	92.3	92.3	79.5
4	42.8	44.7	44.7	44.2	45.0	44.7	42.8
5	55.9	57.0	56.9	56.7	57.7	57.6	54.6
6	18.4	19.2	19.3	19.0	19.6	19.6	69.9
7	33.1	33.7	33.8	33.7	34.5	34.5	34.2
8	39.7	40.4	40.4	40.4	40.9	40.9	46.0
9	47.7	48.2	48.2	48.2	47.6	47.6	22.2
10	36.7	36.9	37.0	36.9	37.6	37.6	31.0
11	23.7	24.5	24.4	24.4	25.0	25.0	26.8
12	122.5	122.9	122.8	122.8	138.9	138.8	32.5
13	143.5	145.2	145.2	145.2	145.3	135.3	47.0
14	42.1	42.8	42.8	42.8	43.5	43.5	47.2
15	25.9	26.8	26.9	26.8	27.1	23.2	47.0
16	28.2	29.1	29.1	29.1	30.1	30.1	74.7
17	37.4	38.4	38.4	38.4	38.7	38.7	59.2
18	44.7	45.7	45.8	45.8	46.9	46.9	22.0
19	46.2	47.2	47.2	47.2	47.6	47.6	29.0
20	30.5	31.3	31.2	31.2	31.5	31.5	88.5
21	41.5	42.7	42.7	42.7	42.4	42.4	28.6
22	76.6	75.9	76.0	76.0	77.1	77.1	35.6
23	22.3	23.7	23.2	23.0	23.2	23.3	26.9
24	64.5	63.5	63.3	63.7	64.1	64.4	82.8
25	16.1	16.0	16.0	16.0	16.3	16.5	72.6
26	16.9	17.5	17.5	17.4	17.7	17.7	26.8
27	25.4	26.2	26.1	26.1	25.5	25.5	27.1
28	28.2	29.1	29.1	29.0	29.2	29.2	27.8
29	32.8	33.7	33.7	33.6	32.7	32.7	16.0
30	20.0	21.6	21.5	21.5	20.5	20.5	20.7

Note. The signals presented in Tables 1 and 2 were assigned based on 2D NMR spectroscopy (DEPT, COSY, HETCOR, ROESY, HMBC).

The attachment of GlcA in glycoside **1** to the O(3) atom of genin **12** is indicated by the downfield shift of the signal of C(3) in the ^{13}C NMR spectrum of monoside **1** by 11.6 ppm with respect to that in the spectrum of genin **12** (Table 1) and by the presence of a cross peak between H(1) of the GlcA residue (δ_H 4.91) and C(3) of the genin (δ_C 89.4) in the HMBC NMR spectrum of the monoside. Thus, compound **1** is 3-*O*-β-glucuronide of soyasapogenin B. Previously,[2] a derivative of this glycoside at the carboxy group has been isolated.

Biosides **2** and **3** have the molecular formulas $C_{41}H_{66}O_{13}$ and $C_{42}O_{68}O_{14}$ (HR FAB MS) and characteristic ions with *m/z* 633 $[(M - H) - 132]^-$, 457 $[(M - H) - 132 - 176)]^-$ and 633 $[(M - H) - 162]^-$, 457 $[(M - H) - 162 - 176)]^-$, respectively, which points to the presence of a pentose and a uronic acid in compound **2** and a hexose and a uronic acid in compound **3**. Partial acid hydrolysis of biosides yielded one progenin identical with monoside **1**. According to ^1H and ^{13}C NMR spectroscopy (Table 2), the pentose was

Table 2. ^{13}C NMR spectrl data of the carbohydrate parts of glycosides **1**—**5** (δ, **1** in C_5D_5N; **2**—**5** in CD_3OD)

Carbohydrate residue, C atom	**1**	**2**	**3**	**4**	**5**
GlcA					
1	105.9	105.2	105.2	105.6	105.5
2	75.4	81.2	82.4	78.8	78.8
3	78.5	78.5	78.5	76.9	76.9
4	73.9	73.5	73.5	74.2	74.1
5	77.0	78.2	78.2	78.0	78.0
6	175.3	176.0	176.0	176.0	176.0
Glc					
1			104.5		102.4
2			75.8		79.5
3			78.2		79.0
4			70.5		71.2
5			78.3		79.3
6			62.0		61.9
Xyl					
1		105.1		103.2	
2		75.8		79.3	
3		77.1		78.6	
4		71.0		70.5	
5		67.1		66.8	
Rha					
1				102.3	102.1
2				72.4	72.4
3				72.4	72.4
4				74.4	74.4
5				69.8	69.8
6				18.4	18.4

identified as xylose, and the hexose was identified as glucose having the pyranose forms of the oxide rings and the β-configurations of anomeric centers (δ_H 4.50, J = 8.0 Hz, H(1), δ_C 105.0 C(1) Xyl; δ_H 4.12, J = 7.5 Hz, H(1), δ_C 103.2, C(1) Glc). The attachment of the Xyl residue in compound **2** and the attachment of Glc in **3** to the O(2) atom of the glucuronic acid residues is indicated by the downfield shift of the signals of C(2) of the GlcA residues in the ^{13}C NMR spectra of these biosides with respect to that in monoside **1** (see Table 2). The above-considered results were confirmed by analysis of the ^1H—^{13}C HMBC NMR spectra of biosides **2** and **3**. These spectra contain cross peaks between the H(1) atoms of the Xyl residue (δ_H 4.64) in compound **2** (and H(1) of the Glc residue (δ_H 4.78) in **3**) and the C(2) atoms of the GlcA residues (δ_C 81.2 and 82.4 in **2** and **3**, respectively). Since the terminal xylose and glucose residues were fouind to have β-configurations, we assigned them to the D-series on the basis of Klyne's calculations of molecular rotation.[4] For compound **2**: $[M]_D$(**2**) − $[M]_D$(**1**) = −26.0 (*cf.* Ref. 5: $[M]_D$ (methyl β-D-xylopyranoside) −108.1, $[M]_D$ (methyl α-D-xylopyranoside +251.2). For compound **3**: $[M]_D$(**3**) − $[M]_D$(**1**) = −96.9 (*cf.* Ref. 5: $[M]_D$ (methyl β-D-glucopyranoside) −66.3, $[M]_D$ (methyl α-D-glucopyranoside) +308.3).

Thus, biosides **2** and **3** were identified as 3-*O*-[*O*-(β-D-xylopyranosyl)-(1→2)-(β-glucuronopyranosyl)]-3β,22β,24-trihydroxyolean-12-ene and 3-*O*-[*O*-(β-D-glucopyranosyl)-(1→2)-(β-glucuronopyranosyl)]-3β,22β,24-trihydroxyolean-12-ene, respectively. Previously,[6] a bioside **2** derivative has been isolated and characterized; bioside **3** has been isolated[7] from *Galega officinalis*.

The molecular formulas of triosides **4** and **5** are $C_{47}H_{76}O_{17}$ and $C_{48}H_{78}O_{18}$ (HR FAB MS). The negative-ion FAB mass spectra contain fragment ions with *m/z* 765 $[(M - H) - 146]^-$, 633 $[(M - H) - 146 - 132]^-$ and 457 $[(M - H) - 146 - 132 - 176)]^-$ for compound **4** and fragment ions with *m/z* 795 $[(M - H) - 146]^-$, 633 $[(M - H) - 146 - 162]^-$ and 457 $[(M - H) - 146 - 162 - 176)]^-$ for compound **5**. These data indicate that trioside **4** contains a deoxyhexose, a pentose, and a uronic acid, and trioside **5** contains a deoxyhexose, a hexose, and a uronic acid. In the products of partial acid hydrolysis of both glycosides, one progenin (^1H and ^{13}C NMR, negative ion FAB MS) identical with monoside **1** was found. In turn, biosides **2** and **3** are progenins of triosides **4** and **5**; this follows from comparison of the ^1H and ^{13}C NMR spectra of the biosides and triosides, respectively (see Table 2). According to the data from ^1H and ^{13}C NMR spectroscopy, the deoxyhexose in both triosides was identified as rhamnopyranose. In establishing the configuration of the rhamnosidic bond, the chemical shifts of C(3) and C(5) (δ_C 72.5 and 69.4 for α-configuration and δ_C 75.4 and 73.5 for β-configuration) rather than the chemical shift of the anomeric C atom are the characteristic values in the ^{13}C NMR spectrum.[8] In compounds **4** and **5**, the chemical shifts of C(3) are 72.4 ppm and those of C(5) are 69.8 ppm (see Table 2); hence, rhamnose has the α-configuration of the glycosidic center in both cases. The binding of this residue to Xyl in glycoside **4** and to Glc in glycoside **5** at the O(2) atoms is judged by the downfield shifts of signals for the C(2) atoms, equal to 3.5 ppm and 3.7 ppm in triosides **4** and **5**, respectively, relative to the positions of these signals in the spectra of biosides **2** and **3** (see Table 2). The same is indicated by the cross peaks between the H(1) atoms of rhamnose (δ_H 5.19 in **4** and δ_H 5.22 in **5**) and the C(2) atoms of Xyl (δ_C 79.3) and Glc (δ_C 79.5) in the HMBC NMR spectra of compounds **4** and **5**, respectively.

The assignment of terminal α-rhamnopyranosides in compounds **4** and **5** to the L-series was performed by the Klyne method.[4] For compound **4**: $[M]_D$(**4**) − $[M]_D$(**2**) = −247.3; for compound **5**: $[M]_D$(**5**) − $[M]_D$(**3**) = −272.4 (*cf.* Ref. 5: $[M]_D$ (methyl α-L-rhamnopyranoside) −111.3, $[M]_D$ (methyl β-L-rhamnopyranoside) +169.8).

Thus, triosides **4** and **5** were identified as 3-*O*-[*O*-(α-L-rhamnopyranosyl)-(1→2)-*O*-(β-D-xylopyranosyl)-(1→2)-(β-glucuronopyranosyl)]-3β,22β,24-trihydroxyolean-12-ene and 3-*O*-[*O*-(α-L-rhamnopyranosyl)-(1→2)-*O*-(β-D-glucopyranosyl)-

(1→2)-(β-glucuronopyranosyl)]-3β,22β,24-trihydroxyolean-12-ene, respectively. Compound **4** was first isolated from *A. membranacea*,[6] and compound **5** has been isolated from *Medicago hispida*.[9]

O-Methylinositol[10] (**6**) and linolenic acid[11] (**7**) were isolated from the chloroform extract of *A. danicus* by flash chromatography on silica gel. *O*-Methylinositol (**6**) was identified as 3-*O*-methyl-D-*chiro*-inositol ((+)-pinitol), acid **7** was identified using the data from HR MS, ^{1}H and ^{13}C NMR spectra, and GC/MS of the methyl ester.

From a similar fraction from *A. inopinatus,* two saturated alcohols **8** and **9**, flavon (**10**), and tetracyclic triterpenoid (**11**) were isolated by chromatography on silica gel. The alcohols were identified by GC/MS and HR MS in the form of *O*-acetates as tricosan-1-ol (**8**) and tetracosan-1-ol (**9**).[12] The compounds **10**, **11** were identified as apigenin[13] and cycloastragenol,[14] using physicochemical constants and NMR, MS spectra, respectively. Triterpenoid **11** was isolated for the first time from the products of acid hydrolysis of saponins from *A. membranaceus* Bunge roots.[6]

Aqueous and methanolic extracts of both types of astragal and the chloroform and *n*-butanolic fractions of the methanolic extracts of *A. danicus* were assayed *in vitro* for cytotoxic (HeLa cells), fungistatic (*Candida albicans*), and antimicrobial (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) activities. The aqueous extracts were found to be inactive in all of the assays listed above. The methanolic extracts of *A. danicus* and *A. inopinatus* inhibit the growth of the HeLa cells by 30% (here and below, the concentration used is 1000 μg mL^{-1}); the chloroform fraction of the methanolic extract of *A. danicus* inhibits it by 95%. In addition, the same fraction suppresses the growth of Gram-positive bacteria *Staphylococcus aureus* by 82%.

Thus, eleven compounds were isolated and identified from *A. danicus* and *A. inopinatus.* Compounds **1** and **2** have not been isolated previously in the native state; their physicochemical parameters and spectral characteristics were determined for the first time. None of the compounds described have been isolated previously from both types of astragal. It was found that the chloroform fraction of *A. danicus* exhibits high cytotoxic and antimicrobial activities.

Experimental

Melting points were determined on a Bristoline stage; optical rotations were measured on a Perkin—Elmer 241 instrument. IR spectra were recorded on a Mattson Polaris spectrometer in pellets with KBr and the NMR spectra were run at ambient temperature on a Varian VXR-500 spectrometer (500 MHz for ^{1}H; 125 MHz for ^{13}C). The FAB and HR mass spectra were recorded on a JEOL SX102A instrument with double focusing (glycerol or thioglycerol as the matrix, xenon as the ionizing gas); GC/MS and EI MS were run on the same instrument. HPLC analysis was carried out on a Rainin/Varian Dynamax SD-200 chromatograph (C(18); 5 μm; a 4.6×250 mm analytical column, and a 21.4×250 mm preparative column). Low-pressure column chromatography was carried out using silica gel 200—400 mesh (Fisher); GPC was carried out on Sephadex G-25 and ion exchange chromatography was done on Amberlite IRA-68 (OH$^-$) and Amberlite IRC-50 (H$^+$) ion exchange resins; TLC was performed on Whatman Al SilG/UV silica gel. The substances on chromatograms were visualized by spraying the plates with a 0.5% solution of vanillin in 50% H_3PO_4. For column and thin layer chromatography, the following solvent systems were used (in systems (1) and (2), the proportion of the polar solvent increased from 0 to 15% (v/v)): (1) hexane—acetone; (2) chloroform—methanol; chloroform—methanol—water: (3) 70 : 15 : 1; (4) 70 : 23 : 1; (5) 70 : 23 : 4; (6) 80 : 36 : 7.

Plant material. The above-ground parts of the plants *Astragalus danicus* Retz. *and A. inopinatus* Boriss. were gathered during the blooming period (July 1998, Buryatia, Tunkinskii region). The herbarium specimens are deposited with the M. G. Popov Academic Herbarium of the Siberian Institute of Physiology and Biochemistry of Plants of the Siberian Branch of the RAS (Irkutsk).

Extraction and fractionation of the extract. Air-dry finely ground raw material of *A. danicus* and *A. inopinatus* (0.5 kg each) was extracted successively with H_2O and MeOH (3×3 L). The methanolic extract was concentrated to 1/4 initial volume and treated successively with chloroform (3×250 mL) and *n*-butanol (5×300 mL).

A. danicus. The chloroform extract (2.1 g) was subjected to flash chromatography on silica gel in system (1). The fraction containing component **6** was eluted with 5% acetone in hexane, and the fraction containing component **7**, with 15% acetone in hexane**.** Re-chromatography on silica gel in system (2) (5%) gave individual compound **6**.

Preparative HPLC (MeCN—H_2O, 45 : 55 → 55 : 45 (v/v)) gave linolenic acid[11] (**7**) identified from the data from NMR spectroscopy and GC/MS of the corresponding methyl ester carried out after treatment with the MethPrep I reagent (Alltech).

The butanolic extract (3.5 g) was chromatographed on silica gel using systems (3)—(6) for elution. The use of system (4) gave a fraction containing monoside **1**. This fraction was chromatographed once again on silica gel in systems (3) and (4) and then successively purified on Sephadex G-25 and the Amberlite IRC-50 cation exchange resin using gradient elution with the H_2O—MeOH system. This gave compound **1** (10 mg).

Further washing of the column with system (5) yielded a fraction containing a mixture of biosides **2** and **3** (~250 mg) and elution with system (6) afforded a mixture of triosides **4** and **5** (~320 mg). Compounds **2—5** were isolated by preparative HPLC in the MeCN—H_2O system (45 : 55 (v/v)).

3-*O*-Methyl-D-*chiro*-inositol (**6**), m.p. 186—187 °C (MeOH), $[\alpha]_D^{23}$ +57 (*c* 1.09; H_2O) (*cf.* Ref. 10: m.p. 188 °C; $[\alpha]_D^{23}$ +59). IR, ν/cm^{-1}: 3409 (OH), 1070, 1040 (C—O—). Found, *m/z*: 195.0862 $[M + H]^+$. $C_7H_{15}O_6$. Calculated: $[M + 1]^+$ 195.0869. ^{1}H NMR (CD_3OD), δ: 3.84 (m, 2 H, H(1), H(6)); 3.64 (dd, 1 H, H(2), *J* = 2.4, 9.7 Hz); 3.59 (dd, 1 H, H(5), *J* = 2.4, 9.7 Hz); 3.48 (t, 1 H, H(4), *J* = 9.7 Hz); 3.18 (t, 1 H, H(3), *J* = 9.7 Hz).

Linolenic acid (**7**). Found, *m/z*: 277.2245 $[M - H]^-$. $C_{18}H_{29}O_2$. Calculated: $[M - 1]^-$ 277.2251. ^{1}H NMR ($CDCl_3$), δ: 5.36 (m, 6 H, CH=CH); 2.80; 2.35; 2.05; 1.62 (all m, each 2 H, CH_2); 1.32 (m, 12 H, CH_2); 0.96 (t, 3 H, Me). ^{13}C NMR ($CDCl_3$), δ: 180.5; 131.9; 130.2; 128.3; 128.2; 127.7; 127.1; 34.0; 29.5; 29.1; 29.0; 28.9; 27.2; 25.6; 25.5; 24.6; 20.5; 14.5. Methyl linolenate: $C_{19}H_{32}O_2$. MS (EI, 70 eV), *m/z* (I_{rel} (%)) : 292 $[M]^+$ (13), 261 $[M - 31]^+$ (10), 121 (27), 95 (65), 79 (100), 55 (45).

3-*O*-(β-Glucuronopyranosyl)-3β,22β,24-trihydroxyolean-12-ene (1). White plates, m.p. 242—244 °C (MeOH), $[\alpha]_D^{23}$ +25.7 (*c* 0.5; MeOH). IR, ν/cm^{-1}: 3400 (OH), 1600 br (COO$^-$), 1068, 1040 (C—O—). Negative-ion FAB MS, *m/z*: 633 $[M - H]^-$, 457 $[(M - H) - 176)]^-$. Found, *m/z*: 633.3990 $[M - H]^-$. $C_{36}H_{57}O_9$. Calculated: $[M - 1]^-$ 633.4003. ^{1}H NMR (C_5D_5N), δ: 5.40 (s, 1 H, H(12)); 4.40 and 3.65 (both d, each 1 H, C$\underline{H}_2$OH, *J* = 10.7 Hz); 3.75 (m, 1 H, H(22)); 3.65 (m, 1 H, H(3)); 4.91 (d, 1 H, H(1) GlcA, *J* = 7.8 Hz); 4.31 (m, 1 H, H(5) GlcA); 4.26 (m, 1 H, H(4) GlcA); 4.13 (m, 1 H, H(3) GlcA); 3.95 (m, 1 H, H(2) GlcA). The ^{13}C NMR spectral data are listed in Tables 1 and 2.

3-*O*-[*O*-(β-D-Xylopyranosyl)-(1→2)-(β-glucuronopyranosyl)]-3β,22β,24-trihydroxyolean-12-ene (2). White plates, m.p. 220—222 °C (MeOH), $[\alpha]_D^{23}$ +17.9 (*c* 0.6; MeOH). IR, ν/cm^{-1}: 3410 (OH), 1600 br (COO$^-$), 1070, 1045 (C—O—). Negative-ion FAB MS, *m/z*: 765 $[M - H]^-$, 633 $[(M - H) - 132]^-$ and 457 $[(M - H) - 132 - 176)]^-$. Found, *m/z*: 765.4420 $[M - H]^-$. $C_{41}H_{65}O_{13}$. Calculated: $[M - 1]^-$ 765.4425. ^{1}H NMR (the signals for the anomeric protons of carbohydrates, CD_3OD), δ: 4.50 (d, 1 H, H(1) GlcA, *J* = 7.1 Hz); 4.64 (d, 1 H, H(1) Xyl, *J* = 8.0 Hz). The ^{13}C NMR spectral data are listed in Tables 1 and 2.

3-*O*-[*O*-(β-D-Glucopyranosyl)-(1→2)-(β-glucuronopyranosyl)]-3β,22β,24-trihydroxyolean-12-ene (3). White plates, m.p. 283—285 °C (MeOH), $[\alpha]_D^{23}$ + 8.3 (*c* 0.5; MeOH) (*cf.* Ref. 7: m.p. 285—287 °C, for $C_{42}H_{68}O_{14} \cdot 2H_2O$: $[\alpha]_D^{25}$ + 27.5 (*c* 0.2; MeOH)). IR, ν/cm^{-1}: 3410 (OH), 1600 br (COO$^-$), 1072, 1068, 1035 (C—O—). Negative-ion FAB MS, *m/z*: 795 $[(M - H)]^-$, 633 $[(M - H) - 162]^-$ and 457 $[(M - H) - 162 - 176)]^-$. Found, *m/z* : 795.4542 $[M - H]^-$. $C_{42}H_{67}O_{14}$. Calculated: $[M - 1]^-$ 795.4531. ^{1}H NMR (the signals for the anomeric protons of carbohydrates, CD_3OD), δ: 4.50 (d, 1 H, H(1) GlcA, *J* = 7.1 Hz); 4.78 (d, 1 H, H(1) Glc, *J* = 7.5 Hz). The ^{13}C NMR spectral data are listed in Tables 1 and 2.

3-*O*-[*O*-(α-L-Rhamnopyranosyl)-(1→2)-*O*-(β-D-xylopyranosyl)-(1→2)-(β-glucuronopyranosyl)]-3β,22β,24-trihydroxyolean-12-ene (4). White needles, m.p. 223—225 °C (MeOH), $[\alpha]_D^{23}$ −11.2 (*c* 1.2; MeOH) (*cf.* Ref. 6: m.p. 223—224 °C, $[\alpha]_D^{23}$ −12.1 (*c* 1.0; MeOH)). IR, ν/cm^{-1}: 3420 (OH), 1600 br (COO$^-$), 1070, 1045 (C—O—). Negative-ion FAB MS, *m/z*: 911 $[M - H]^-$, 765 $[(M - H) - 146]^-$, 633 $[(M - H) - 146 - 132]^-$ and 457 $[(M - H) - 146 - 132 - 176]^-$ Found, *m/z*: 911.4992 $[M - H]^-$. $C_{47}H_{75}O_{17}$. Calculated: $[M - 1]^-$ 911.5004. ^{1}H NMR (the signals for the anomeric protons of carbohydrates, CD_3OD), δ: 5.19 (d, 1 H, H(1) Rha, *J* = 1.5 Hz); 4.78 (d, 1 H, H(1) Xyl, *J* = 8.0 Hz); 4.40 (d, 1 H, H(1) GlcA, *J* = 7.0 Hz). The ^{13}C NMR spectral data are listed in Tables 1 and 2.

3-*O*-[*O*-(α-L-Rhamnopyranosyl)-(1→2)-*O*-(β-D-glucopyranosyl)-(1→2)-(β-glucuronopyranosyl)]-3β,22β,24-trihydroxyolean-12-ene (5). White needles, m.p. 244—246 °C (MeOH), $[\alpha]_D^{23}$ −21.9 (*c* 0.6; MeOH) (*cf.* Ref. 9: m.p. 245—247 °C, $[\alpha]_D$ −22.5 (c 0.42; MeOH)). IR: 3420 (OH), 1600 (br, COO$^-$), 1070, 1045 (C—O—). Negative-ion FAB MS, *m/z*: 941$[M - H]^-$, 795 $[(M - H) - 146]^-$, 633 $[((M - H) - 146 - 162)]^-$ and 457 $[(M - H) - 146 - 162 - 176)]^-$. Found, *m/z*: 941.5112 $[M - H]^-$. $C_{48}H_{77}O_{18}$. Calculated: $[M - 1]^-$ 941.5110. ^{1}H NMR (the signals of anomeric protons of carbohydrates, CD_3OD), δ: 5.22 (d, 1 H, H(1) Rha, *J* = 1.5 Hz); 4.92 (d, 1 H, H(1) Glc, *J* = 7.5 Hz); 4.42 (d, 1 H, H(1) GlcA, *J* = 7.0 Hz). The ^{13}C NMR spectral data are listed in Tables 1 and 2.

Acid hydrolysis. Monoside **1** (3 mg) (or 7 mg of a mixture of biosides **2** and **3**, or 9 mg of a mixture of triosides **4** and **5**) in 5 mL of a 6% H_2SO_4—dioxane mixture (1 : 1) was heated for 5 h at 100 °C. The reaction mixture was neutralized with the Amberlite IRA-68 (OH$^-$) anion exchange resin, the solution was concentrated, and the residue was dissolved in water and extracted with 3×7 mL of $CHCl_3$. TLC of the aqueous layer in solvent systems (6) and (7) allowed identification (by comparison with authentic samples) of GlcA in monoside **1**; Xyl (or Glc) and GlcA in biosides **2** and **3**; and Xyl (or Glc), Rha, and GlcA in triosides **4** and **5**.

Partial acid hydrolysis. A mixture of saponins **2** and **3** (50 mg) or triosides **4** and **5** (57 mg) in 7 mL of a 3% H_2SO_4—dioxane mixture (1 : 1) was heated on a water bath for 4 h at 80 °C. The reaction mixture was neutralized with the Amberlite IRA-68 (OH$^-$) anion exchange resin, the solution was concentrated, and the residue was dissolved in water and extracted with Bu^{n}OH (3×7 mL). The extract was concentrated and the residue was chromatographed on a column with SiO_2. Elution with system (2) (1—5% MeOH in $CHCl_3$) gave fractions (1) and (2) containing genin **12** and monoside **1**, respectively. Re-chromatography of fraction (1) in system (2) (3% MeOH in $CHCl_3$) yielded 12 mg of genin **12**; flash chromatography of fraction 2 in system (3) afforded 11 mg of monoside **1**.

3β,22β,24-Trihydroxyolean-12-ene (12), m.p. 258—259 °C (EtOH), $[\alpha]_D^{23}$ +90.6 (*c* 0.7; $CHCl_3$) (*cf.* Ref. 2: m.p. 258—260 °C; $[\alpha]_D^{30.5}$ +92.4 ($CHCl_3$)). MS (EI, 70 eV), *m/z* (I_{rel} (%)): 458 $[M]^+$ (7), 440 $[M—H_2O]^+$ (5); C_{30} $H_{50}O_3$. ^{1}H NMR ($CDCl_3$), δ: 4.25, 3.35 (both d, each 1 H, C$\underline{H}_2$OH, *J* = 11.0 Hz); 3.43 (m, H(3)); 3.43 (m, H(2)); 1.25; 1.12; 1.04; 0.95; 0.91; 0.90; 0.87 (all s, 7 Me groups, each 3 H).[3] The ^{13}C NMR spectral data are listed in Table 1.

A. inopinatus. From the butanolic extract (3.2 g), saponins **2**—**5** were isolated under conditions described for a similar fraction of *A. danicus*. The chloroform extract of *A. inopinatus* (1.8 g) was subjected to flash chromatography on silica gel. Elution with system (1) (3% Me_2CO in C_6H_{14}) gave a fraction containing alcohols **8** and **9**. The mixture was acetylated (Ac_2O/Py, 50 °C) and the products were analyzed by GC/MS and HR MS; tricosan-1-ol **8** was identified as the corresponding acetate (Found, *m/z* 382.5020; $C_{25}H_{50}O_2$; Calculated: 382.5026) and tetracosan-1-ol **9** was identified as the acetate (Found, *m/z*: 396.5219; $C_{26}H_{52}O_2$; Calculated: 396.5227). Further elution of the column with system (1) (15% Me_2CO in C_6H_{14}) gave a fraction containing a mixture of components **10** and **11**. Flash chromatography of this fraction on silica gel in system (2) (3% and 5% MeOH in $CHCl_3$) yielded 17 mg of flavon **10** and 20 mg triterpenoid **11**.

5,7,4′-Trihydroxyflavon (apigenin) (10), m.p. 343—344 °C (EtOH) (*cf.* Ref. 13: m.p. 344 °C). IR, ν/cm^{-1}: 3426 (OH), 1655 (C=O), 1611 (C=C), 1489 (arom.), 830 (C—H). $C_{15}H_{10}O_5$. MS (EI, 70 eV), *m/z* (I_{rel} (%)): 270 $[M]^+$ (7), 214 $[M - 56]^+$ (17). ^{1}H NMR (CD_3OD), δ: 8.04 (d, 2 H, H(2′), H(6′), *J* = 8.9 Hz); 7.20 (d, 2 H, H(3′), H(5′), *J* = 8.9 Hz); 6.85 (s, 1 H, H(3)); 6.80 (d, 1 H, H(8), *J* = 2.4 Hz); 6.73 (d, 1 H, H(6), *J* = 2.4 Hz).

20(*R*),24(*S*)-Epoxycyclolanost-9(11)-ene-3β,6α,16β,25-tetrol (cycloastragenol) (11), m.p. 239—240 °C (EtOH), $[\alpha]_D^{23}$ +46.6 (*c* 0.8; MeOH) (*cf.* Ref. 14: m.p. 239—241 °C; $[\alpha]_D^{23}$ +50.6). IR, ν/cm^{-1}: 3450—3250 (OH), 3045 (>CH$_2$ of the cyclopropane ring). MS (EI, 15 and 70 eV), *m/z* (I_{rel} (%)): 490 $[M]^+$ (2), 472 $[M - H_2O]^+$ (18), 143 $[C_{18}H_{15}O_2]^+$ (100). Found, *m/z*: 489.3569 $[M - H]^-$. $C_{30}H_{49}O_5$. Calculated: $[M - H]^-$ 489.3571. ^{1}H NMR (CD_3OD), δ: 4.60 (dd, 1 H, H(16), J = 6.8, 7.8 Hz); 3.78 (t, 1 H, H(24), J = 6.4 Hz); 3.45 (td, 1 H, H(6), J = 2.9, 10.9 Hz); 3.21 (dd, 1 H, H(3), J = 4.4, 10.3 Hz); 0.54, 0.48 (both d, each 1 H, 2 H(19), J = 4.9 Hz). The ^{13}C NMR spectral data are listed in Table 1.

The cytotoxic activity studied by K. Metcalf (Laboratory of Biochemistry, Brigham Young University) and the bacteriostatic activity was assayed by J. Donaldson and L. Ford (Department of Botany, Brigham Young University).

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