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Isolation and Structures of Arvenins from *Anagallis arvensis* L. (Primulaceae). New Cucurbitacin Glucosides

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Four new cucurbitacin glucosides (named arvenin I, II, III and IV) were isolated as bitter principles of *Anagallis arvensis* L. (Primulaceae). On the basis of the spectral studies involving carbon-13 nuclear magnetic resonance spectra and the chemical transformations, the structures of these bitter glucosides have been established as 2-O-β-D-glucopyranosyl cucurbitacin B for arvenin I, 2-O-β-D-glucopyranosyl 23,24-dihydrocucurbitacin B for arvenin II, 2-O-β-D-glucopyranosyl cucurbitacin D for arvenin III and 2-O-β-D-glucopyranosyl cucurbitacin R for arvenin IV, respectively. This is the first isolation of cucurbitacin glucosides from the family Primulaceae.

Keywords——*Anagallis arvensis* L.; Primulaceae; arvenin I, II, III and IV; cucurbitacin glucosides; ^{13}C-NMR

The dry whole plant of *Anagallis arvensis* L., which belongs to the family Primulaceae, is used as a herb for the liver complaint in Taiwan, and shows remarkable bitterness. The presence of an oleanane series of triterpenoid (anagalligenone B),[2] flavones[3] and saponins[4] in this plant was previously reported. However isolation of the bitter substance has not been reported. In the course of our study on the chemical constituents of *Anagallis arvensis* L., we have isolated four new cucurbitacin glucosides (named arvenin I, II, III and IV) showing remarkable bitterness, in addition to the several known cucurbitacin triterpenoids.[5] The present paper deals with the full accounts of the isolation and the structural study on these arvenins. Preliminarily the structure elucidation of arvenin I and II by means of carbon-13 nuclear magnetic resonance (^{13}C–NMR) spectra was reported.[6]

The isolation of arvenins was performed according to the procedure as shown in Fig. 1. The dry whole plants of *Anagallis arvensis* L. were extracted with methanol. After removal of the *n*-hexane soluble part from the methanol extract, the residue was further extracted with ethyl acetate. The ethyl acetate insoluble part showed strong bitterness, while the ethyl acetate soluble part, from which several cucurbitacin aglycones were isolated,[5] showed slightly bitterness. The ethyl acetate insoluble part was then passed through a charcoal column using methanol, and the eluate was chromatographed on silica gel to give two strongly bitter fractions by elution with $CHCl_3$–MeOH–H_2O (13:2:1, lower layer). Careful silica gel chromatography of the formerly eluted major bitter fraction using AcOEt–MeOH (100:1) gave two strongly bitter substances as colourless powder: the initially eluted bitter substance was named as arvenin II, and the subsequently eluted one was named as arvenin I. Similarly two bitter substances were isolated as colourless powder from the later eluted minor bitter

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2) S. Heitz, D. Billet, T. Staron, and D. Raulis, *C.R. Acad. Sci. Paris Ser. D*, **268**, 2279 (1969).
3) S.I. Tsitsin, *Tr. Bot. Sadov. Akad. Nauk. Kaz. SSR*, **11**, 111 (1969).
4) M.M. Mukhamedziev and P.K. Alimbaeva, *Izu. Akad. Nauk. Kirg. SSR*, **1970**, 54.
5) Y. Yamada, K. Hagiwara, K. Iguchi, Y. Takahashi, and H.Y. Hsu, *Phytochemistry*, **17**, 1798 (1978).
6) Y. Yamada, K. Hagiwara, K. Iguchi, and S. Suzuki, *Tetrahedron Lett.*, **1977**, 2099.

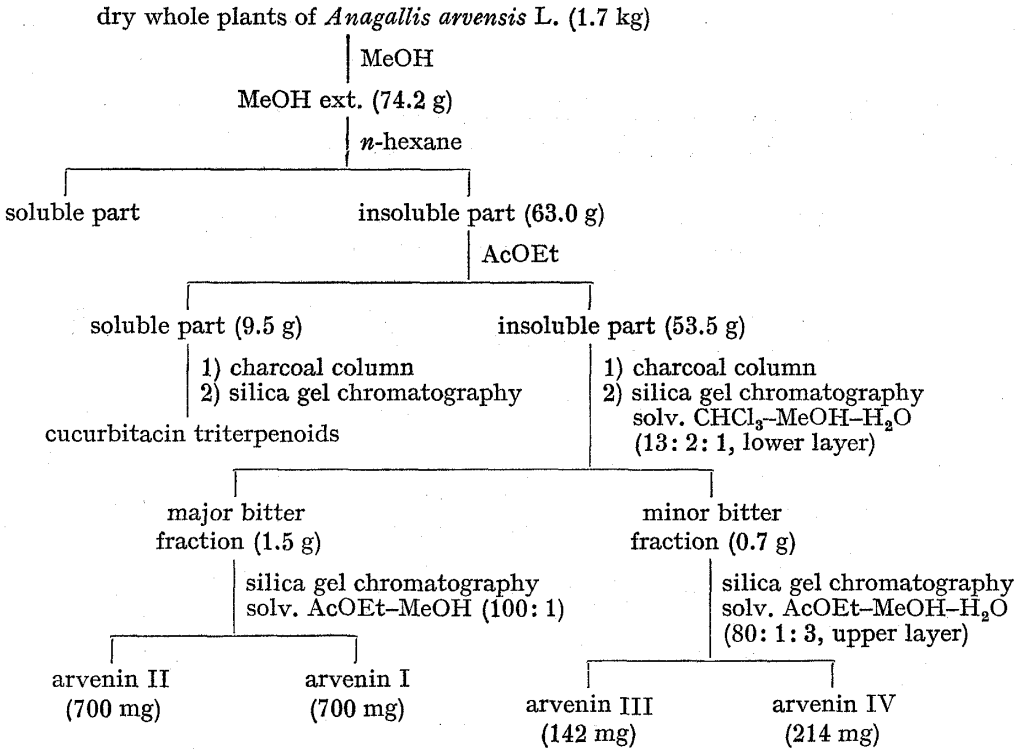


Fig. 1. Isolation of Arvenins and Cucurbitacin Triterpenoids

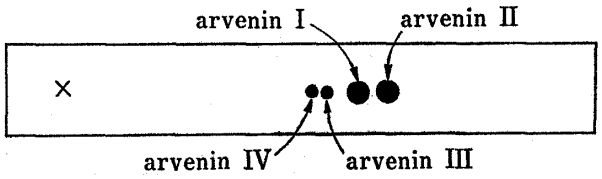


Fig. 2. Thin-Layer Chromatogram of Arvenins

Plate; Kieselgel 60 F_{254}(Merck),
Solvent system; AcOEt-MeOH-H_2O(10: 1: 3, developed five times),
Detection; 20% H_2SO_4.

fraction by silica gel chromatography using AcOEt–MeOH–H_2O (80: 1: 3, upper layer). These substances were named as arvenin III and IV, respectively, in order of elution. Thin-layer chromatogram (TLC) of these arvenins are illustrated in Fig. 2.

The physical properties of arvenins are shown in Table I. Each arvenin contains many hydroxyl and carbonyl groups as shown by the infrared (IR) spectra. The presence of an α,β-unsaturated ketone in arvenin I and III was indicated by the ultraviolet (UV) and the proton nuclear magnetic resonance (^{1}H-NMR) spectra, while this chromophore

Table I. Physical Data of Arvenins

	Arvenin I	Arvenin II	Arvenin III	Arvenin IV
mp	141—146°	140—143°	169—170°	160—161°
$[\alpha]_D^{20}$	+40.6° (c 1.6 EtOH)	+31.7° (c 1.7 EtOH)	+16.7° (c 0.6 EtOH)	+37.5° (c 0.7 EtOH)
λ_{max}^{EtOH} mμ	228 (ε 10900)	End absorption	228 (ε 8700)	End absorption
ν_{max}^{KBr} cm^{-1}	3400, 1720, 1690 1630	3400, 1715, 1690	3370, 1720, 1690 1630	3400, 1715, 1690
^{1}H-NMR	$\delta_{ppm}^{CDCl_3}$ 0.96 (3H, s)	$\delta_{ppm}^{CDCl_3}$ 0.97 (3H, s)	$\delta_{ppm}^{CD_3OD}$ 0.98 (3H, s)	$\delta_{ppm}^{CD_3OD}$ 0.93 (3H, s)
	1.07 (3H, s)	1.08 (3H, s)	1.10 (3H, s)	1.05 (3H, s)
	1.27 (6H, s)	1.30 (6H, s)	1.34 (3H, s)	1.19 (6H, s)
	1.35 (3H, s)	1.48 (12H, s)	1.38 (9H, s)	1.28 (3H, s)
	1.44 (3H, s)	1.99 (3H, s)	1.46 (6H, s)	1.33 (3H, s)
	1.54 (3H, s)	5.75 (1H, m)	5.85 (1H, m)	1.39 (6H, s)
	1.56 (3H, s)		6.82 (1H, d, J=15)	5.81 (1H, m)
	2.02 (3H, s)		6.97 (1H, d, J=15)	
	5.75 (1H, m)			
	6.52 (1H, d, J=16)			
	7.02 (1H, d, J=16)			

lacked in case of arvenin II and IV. The ^{1}H–NMR spectra also showed the presence of an acetyl group in arvenin I and II, whereas no acetyl signal was observed in arvenin III and IV. Furthermore the ^{1}H–NMR spectra showed eight tertiary methyl signals, one olefinic proton signal and complex signals due to sugar moiety in every compound. These spectral data strongly suggested that arvenins were triterpenoid glycosides.

Hydrogenation of arvenin I over Pd-C gave arvenin II, and similar hydrogenation of arvenin III furnished arvenin IV. Upon alkaline hydrolysis, arvenin II was transformed into arvenin IV. These chemical transformations (shown in Fig. 3), coupled with the spectral data described above, provided the evidence that arvenin II and IV were a dihydro derivative of arvenin I and III, respectively, and arvenin III and IV were a desacetyl derivative of arvenin I and II, respectively.

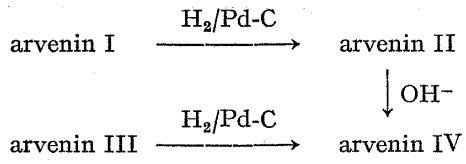


Fig. 3. Chemical Transformations of Arvenins

Acetylation of arvenin I yielded an acetate [mp 108—112°, $[\alpha]_D$ −32.1°]. The ^{1}H–NMR spectrum showed six acetyl signals (δ 1.88—2.14 ppm), one of which was present originally in arvenin I. Acid-catalyzed hydrolysis of arvenin I gave a crystalline bitter aglycone [$C_{30}H_{44}O_7$, mp 142—143°, $[\alpha]_D$ +50.0°, UV λ_{max}^{EtOH} 230 (ε 11300) mμ] in addition to D-glucose. The spectroscopic properties of the aglycone suggested that the compound was a cucurbitacin triterpenoid, and this compound was identified as cucurbitacin D (6)[7] by comparison of the physical data with those of the authentic sample of cucurbitacin D. However it is noticed that the acetyl group present in arvenin I was lost during the acid-catalyzed hydrolysis. From these facts, it was deduced that the structure of arvenin I consisted of three structural units; cucurbitacin D, D-glucose and an acetyl group. The manner and the cites of the linkage of these units were elucidated by examination of ^{13}C–NMR spectrum.

With respect to the seven typical cucurbitacin aglycones, we carried out the measurements of ^{13}C–NMR spectra and the assignments[8] of the most of the signals were done.[9] The ^{13}C–NMR spectra[10] of arvenin I—IV were also measured and the signals are summarized in Table II as compared with those of the aglycones. When the spectrum of arvenin I was compared with those of the aglycones, it was revealed that the chemical shifts of the signals due to the aglycone moiety in arvenin I were closely related to those of cucurbitacin B except for the signals underlined in the Table. Therefore it is clear that the acetyl unit in arvenin I exists on cucurbitacin aglycone; the acetyl group linked with cucurbitacin D at C-25 position (cucurbitacin B is same as 25-O-acetyl cucurbitacin D). The remarkable low field shift of the C-2 signal (+6.2 ppm) and the high field shifts of both C-1 (−1.9 ppm) and C-3 (−1.8 ppm) signals, comparing with the corresponding signals of cucurbitacin B, are undoubtedly attributed to the effect by glucosidic linkage (so-called glucosidation shift).[11] This showed that glucopyranosyl unit linked with cucurbitacin B at C-2 position. The stereochemistry at anomeric position of glucose was assigned to be β-configuration on the basis of the ^{13}C

7) S.M. Kupchan, C.W. Sigel, L.J. Guttman, R.J. Restivo, and R.F. Bryan, *J. Am. Chem. Soc.*, **94**, 1353 (1972).

8) Y. Yamada, K. Hagiwara, K. Iguchi, Y. Takahashi, and S. Suzuki, *Chemistry Letters*, **1978**, 319.

9) Further detailed investigation on the ^{13}C-H coupling constant and the coupling pattern showed that the signals due to C-8 and C-10 carbons in the previous paper[8] should be reversed each other in every aglycone. The full accounts on the assignments will be described elsewhere.

10) The values of 20.4 ppm (methyl carbon) of arvenin I and 49.3 ppm (methylene carbon) of arvenin II in the preliminary communication[6] should be replaced by 21.8 and 35.4 ppm, respectively, by comparison with the signals of cucurbitacin aglycones and arvenin III and IV which have been newly isolated.

11) R. Kasai, M. Suzuo, J. Asakawa, and O. Tanaka, *Tetrahedron Lett.*, **1977**, 175; K. Tori, S. Seo, Y. Yoshimura, H. Arita, and Y. Tomita, *Tetrahedron Lett.*, **1977**, 179.

chemical shift (104.0 ppm).[12] From these results, the structure of arvenin I was unambiguously represented to be 2-O-β-D-glucopyranosyl cucurbitacin B (1).

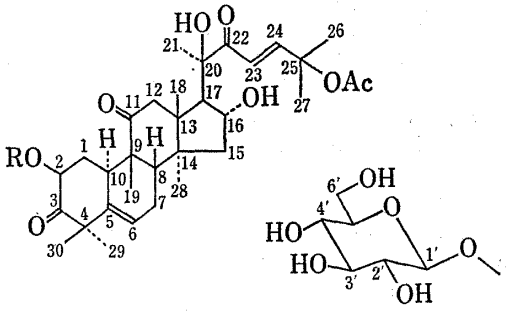
Table II. ^{13}C-NMR Chemical Shifts[a] of Arvenins

Carbon	Arvenin I (1)	Arvenin II (3)	Arvenin III (5)	Arvenin IV (7)
C-1	35.0	35.4	35.0	35.0
2	78.2	78.1	78.0	78.1
3	211.3	211.1	211.3	211.5
4	48.5[b]	48.6[b]	48.7[b]	48.7[b]
5	140.8	140.7	140.9	140.9
6	120.4	120.4	120.5	120.5
7	24.2[c]	24.2[c]	24.2[c]	24.1[c]
8	42.9	42.8	42.8	42.9
9	48.9[b]	48.9[b]	48.9[b]	48.9[b]
10	34.4	34.3	34.4	34.4
11	212.7	212.6	212.6	212.8
12	49.1[c]	49.1[c]	49.2[c]	49.1[c]
13	51.0[d]	50.8[d]	51.1[d]	51.0[d]
14	51.5[d]	51.5[d]	51.5[d]	51.6[d]
15	46.1	46.0	46.7	46.2
16	70.8	70.4	70.5	70.5
17	59.6	58.9	59.0	58.7
20	79.8	80.0	79.1	80.1
22	204.0	214.7	204.0	215.9
23	122.5	32.1	120.8	32.5
24	150.0	35.4	155.5	38.3
25	79.8	81.7	70.3	69.2
26	26.3[e]	26.0	29.7	29.8
27	26.6[e]	26.0	29.7	29.8
28	25.3	25.5	25.4	25.4
29	28.7	28.7	28.9	28.9
The other CH_3	21.8	21.7	21.8	21.8
(18, 19, 21, 30)	20.4	20.1	20.3	20.2
	19.9	19.9	19.9	19.8
	18.9	18.8	18.9	18.9
$-O\underline{C}OCH_3$	169.7	170.0		
$-OCO\underline{C}H_3$	21.8	22.2		
C-1′	104.0	103.9	104.0	104.1
2′	75.6	75.5	75.6	75.7
3′	78.2	78.1	78.3	78.1
4′	71.4	71.4	71.5	71.5
5′	78.2	78.1	78.3	78.1
6′	62.6	62.6	62.7	62.7

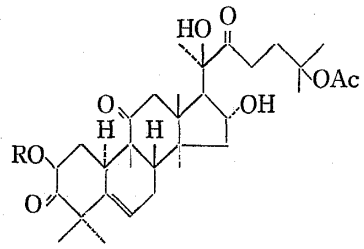
a) The spectra were taken with Varian NV-14 spectrometer (15.1 MHz) at 51—52° in C_5D_5N with TMS as an internal reference using 5 mm tubes.
b—*e*) Assignments may be reversed in each column.

The structure of arvenin II which was a dihydro derivative of arvenin I was elucidated by comparison of its ^{13}C-NMR spectrum with that of arvenin I as shown in Table II. On going from arvenin I to arvenin II, the olefinic carbon signals at C-23 and C-24 positions (122.5 and 150.0 ppm) in arvenin I disappeared. Alternatively the methylene carbon signals (32.1 and 35.4 ppm) underlined in the Table were newly arisen and both adjacent carbon signals (C-22 and C-25) were somewhat deshielded by saturation of the double bond. The other signals remained almost unshifted. This observation clearly indicated that arvenin II

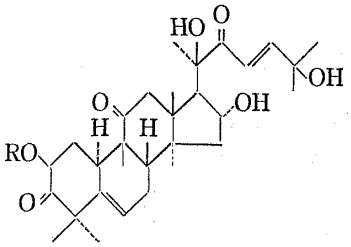
12) J.B. Stothers, "Carbon-13 NMR Spectroscopy," Academic Press, New York, 1972.



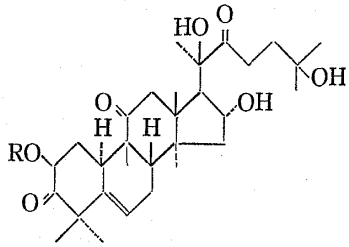
1: R=glucose arvenin I
2: R=H cucurbitacin B



3: R=glucose arvenin II
4: R=H 23,24-dihydrocucurbitacin B



5: R=glucose arvenin III
6: R=H cucurbitacin D



7: R=glucose arvenin IV
8: R=H cucurbitacin R

Fig. 4

is 23,24-dihydroarvenin I (3) which possesses 23,24-dihydrocucurbitacin B as an aglycone; ^{13}C–NMR signals of arvenin II also showed a good accordance with those of 23,24-dihydrocucurbitacin B except for the signals due to the sugar moiety. Therefore the structure of arvenin II was established as 2-O-β-D-glucopyranosyl 23,24-dihydrocucurbitacin B (3).

The ^{13}C signals of arvenin III which is a desacetyl derivative of arvenin I are closely related to those of arvenin I, except for the signals underlined in Table II. The remarkable high field shift of the C-25 signal (−9.5 ppm) and the low field shifts of the C-24 (+5.5 ppm), C-26 (+3.4 ppm) and C-27 (+3.1 ppm) signals are apparently due to the lack of the acetyl group[13] at C-25 position in arvenin III, indicating that the aglycone of arvenin III is desacetylcucurbitacin B [cucurbitacin D (6)]. The structure of arvenin III was thus reasonably represented to be 2-O-β-D-glucopyranosyl cucurbitacin D (5).

Similar comparison of the ^{13}C signals of arvenin IV which is a desacetyl derivative of arvenin II led to the conclusion that the aglycone of arvenin IV is desacetyl-23,24-dihydrocucurbitacin B (8) (cucurbitacin R), and the structure of arvenin IV is assigned to be 2-O-β-D-glucopyranosyl cucurbitacin R (7).

Arvenin I—IV are the first example of cucurbitacin glycosides isolated from the plant Primulaceae.

Experimental

All melting points were uncorrected. Optical rotations were taken with a JASCO DIP-SL automatic polarimeter. IR and UV spectra were obtained with a Hitachi 215 and a Hitachi 323 spectrometers, respectively. ^{1}H-NMR spectra were measured at 100 MHz on a JEOL PS-100 spectrometer and chemical shifts are given on δ (ppm) scale with tetramethylsilane as an internal standard. Mass spectra were recorded on a Hitachi RMU-7L spectrometer. Column chromatography was carried out with Kieselgel (70—230 mesh, Merck). Paper partition chromatography was conduced on Toyo Roshi No. 51 using AcOEt–pyridine–H_2O (10: 4: 3) as the solvent and $AgNO_3$ as the spray reagent. TLC was performed on Kieselgel 60 PF_{254} (Merck), and detection was made by spraying 20% H_2SO_4 followed by heating.

13) S.W. Pelletier, Z. Djarmati, and C. Pape, *Tetrahedron*, **32**, 995 (1976).

Isolation of Arvenin I, II, III and IV——Dry whole plants (1.7 kg) of *Anagallis arvensis* L., which were obtained in Taiwan, were extracted with 8 l of MeOH for 21 days at room temperature. The MeOH extract (74.2 g) was suspended in *n*-hexane (400 ml) and the *n*-hexane extract was removed by filtration. The residue (63.0 g), which was strongly bitter, was further extracted with AcOEt (500 ml). The AcOEt insoluble part (53.5 g) which showed stronger bitterness than the AcOEt extract was passed through charcoal column by elution with MeOH. The eluate was chromatographed on silica gel (dry column, 100 g, 40×330 mm) by elution with $CHCl_3$–MeOH–H_2O (13: 2: 1, lower layer). The major bitter fraction was further submitted to the dry column chromatography on silica gel (200 g, 30×490 mm) by elution with AcOEt (saturated with H_2O)–MeOH (100: 1) to give arvenin II (700 mg, fr. 82—93, colourless powder) and arvenin I (700 mg, fr. 101—120, colourless powder), successively. Both compounds showed strong bitterness. The minor bitter fraction was also submitted to the dry column chromatography on silica gel (150 g, 25×520 mm) by elution with AcOEt–MeOH–H_2O (80: 1: 3, upper layer), to give arvenin III (142 mg, fr. 131—175, colourless powder) and arvenin IV (214 mg, fr. 281—341, colourless powder), successively. These compounds also showed strong bitterness.

Physical properties of arvenins are shown in Table I.

Acetylation of Arvenin I——A solution of arvenin I in 1 ml of pyridine was treated with 1 ml of acetic anhydride, and the mixture was stirred at room temperature for 16 hr. The mixture was poured into ice-water, and the precipitate was collected by filtration and dried: mp 108—112°, $[\alpha]_D^{20}$ −32.1° (*c* 0.28 EtOH), UV λ_{max}^{EtOH} 228 (ε 12500) mμ, IR ν_{max}^{KBr} 3440, 1755, 1735, 1690, 1630 cm^{-1}, ^{1}H-NMR $\delta_{ppm}^{CDCl_3}$ 1.03, 1.10, 1.26, 1.28, 1.32, 1.43, 1.58, 1.60 (each 3H, s), 1.88 (3H, s), 2.03 (9H, s), 2.14 (6H, s), 2.72 (1H, d, J=7, 17-H), 2.76 (1H, d, J=14, 12-H), 3.29 (1H, d, J=14, 12-H), 3.64 (1H, br. d, J=9, 10-H), 4.53 (1H, dd, J=6, 13, H-2), 4.74 (1H, d, J=8, anomeric), 5.76 (1H, m, H-6), 6.44 (1H, d, J=16, 23-H), 7.16 (1H, d, J=16, 24-H).

Acid-catalyzed Hydrolysis of Arvenin I——A solution of arvenin I (179 mg) in 30 ml of 0.5 N H_2SO_4 (prepared by adding conc. H_2SO_4 to 50% aqueous EtOH solution) was heated at 79—84° for 7 hr. The reaction mixture was poured into 100 ml of water and extracted with 150 ml of AcOEt. The organic layer was washed successively with aqueous $NaHCO_3$ solution and water, and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave colourless residue which was chromatographed on silica gel by elution with $CHCl_3$–EtOH (100: 1) to give cucurbitacin D as colourless needles (8.5 mg); mp 142—143°, $[\alpha]_D^{20}$ +50.0° (*c* 0.2 EtOH), UV λ_{max}^{EtOH} 230 (ε 11300) mμ, IR $\nu_{max}^{CHCl_3}$ 3600, 3430, 1710, 1690, 1630 cm^{-1}, ^{1}H-NMR $\delta_{ppm}^{CDCl_3}$ 0.99 (3H, s), 1.09 (3H, s), 1.31 (3H, s), 1.37 (12H, s), 1.41 (3H, s), 2.56 (1H, d, J=7, 17-H), 2.70 (1H, d, J=14, 12-H), 3.30 (1H, d, J=14, 12-H), 4.35 (1H, t, J=7, 16-H), 4.45 (1H, dd, J=6, 13, 2-H), 5.80 (1H, m, 6-H), 6.64 (1H, d, J=16, 23-H), 7.15 (1H, d, J=16, 24-H), Mass spectrum *m/e* 516 (M^+).

The aqueous layer was passed through anion exchange resin (Dowex 2×8) by elution with H_2O. The eluate (8.5 mg, colourless powder, $[\alpha]_D$ +52°) showed a good accordance with the standard sample of D-glucose on paper partition chromatography.

Catalytic Hydrogenation of Arvenin I——To a solution of 15 mg of arvenin I in 5 ml of EtOH was added 5 mg of 10% Pd-C. The mixture was stirred under hydrogen for 45 min, and the reaction mixture was filtered. Evaporation of the solvent of the filtrate gave 15 mg of reduction products as white powder, which was chromatographed on silica gel (dry column) by elution with AcOEt (saturated with H_2O)–MeOH (100: 1) to give white powder (4 mg). This substance was identified as arvenin II.

Catalytic Hydrogenation of Arvenin III——To a solution of 20 mg of arvenin III in 5 ml of EtOH was added 10 mg of 10% Pd-C. The mixture was stirred under hydrogen for 1 hr and the reaction mixture was filtered. Evaporation of the solvent of the filtrate gave white powder (18 mg), which was identified as arvenin IV.

Alkaline Hydrolysis of Arvenin II——To a solution of 40 mg of arvenin II in 2 ml of EtOH was added 5% aqueous NaOH solution and the mixture was stirred at room temperature for 48 hr. The reaction mixture was extracted with AcOEt, and the organic layer was washed with water and dried over anhydrous Na_2SO_4. Evaporation of the solvent afforded white powder (20 mg), from which arvenin IV was obtained as white powder (16 mg) by preparative TLC (AcOEt–MeOH–H_2O=10: 1: 3).