A New Polyoxygenated Triterpene and Two New Aeginetic Acid Quinovosides from the Roots of *Rehmannia glutinosa*

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A new minor polyoxygenated triterpene named glutinolic acid (1) and two new aeginetic acid quinovosides (2, 3) were isolated from the roots of *Rehmannia glutinosa* Libosch. (Scrophulariaceae) cultivated in Gunwi-gun, Korea. The structures of these compounds were established as 3α,19α,20β,24,30-pentahydroxyurs-12-en-28-oic acid (1, glutinolic acid), aeginetic acid 5-*O*-β-D-quinovoside (2) and aeginetoyl ajugol 5″-*O*-β-D-quinovoside (3) on the basis of chemical and spectroscopic evidence.

Key words *Rehmannia glutinosa*; Scrophulariaceae; polyoxygenated triterpene; glutinolic acid; aeginetic acid quinovoside

Rehmanniae Radix is the fresh or dry root of *Rehmannia glutinosa* Libosch. (Scrophulariaceae). As officially listed in the Korean Pharmacopoeia, this herbal drug is to be used in dried form or after processing.[1)] The dry roots of *R. glutinosa* have been used in traditional Chinese medicine as an antipyretic and hemostatic.[2,3)] A number of chemical constituents with diverse structures, including iridoids,[4—13)] ionone glucosides,[14,15)] sesquiterpenes,[16,17)] phenylethanoid glycosides,[18,19)] norcarotenoids,[20,21)] cerebrosides,[12)] carbohydrates, and others,[22—26)] have been isolated from this plant. During our ongoing studies of bioactive constituents used in traditional Chinese medicines, we characterized the 70% EtOH extracts of the roots of *Paeonia lactiflora*[27—31)] and *Astragalus membranaceus*,[32—35)] and the aerial parts of *Lonicera japonica*,[36—41)] and several constituents have been isolated and characterized from these extracts. In the continuing search for new chemical and biomarkers from the medicinal plant *R. glutinosa* for quality control studies of related herbal medicines, a new polyoxygenated triterpene and two aeginetic acid quinovosides, together with other known components, were isolated. In the present investigation, we report the isolation and structure elucidation of a minor polyoxygenated triterpene named glutinolic acid (**1**) and two new aeginetic acid quinovosides (**2**, **3**) from the roots of *R. glutinosa* cultivated in Korea.

Results and Discussion

The dried roots of *R. glutinosa* were crushed and extracted with 70% EtOH. The concentrated extract was suspended in H_2O and successively extracted with EtOAc and BuOH. The EtOAc extract was added to 90% aqueous MeOH and extracted with hexane to give hexane and 90% MeOH extracts. The 90% MeOH extract was subjected to sequential column chromatography over silica gel, MCI gel and RP-18 gel to yield a new minor polyoxygenated triterpene named glutinolic acid (**1**) and two new aeginetic acid quinovosides (**2**, **3**).

Glutinolic acid (**1**) was isolated as an amorphous powder. Its molecular formula was established as $C_{30}H_{48}O_7$ from the $[M-H]^-$ peak at *m*/*z* 519.3354 (Calcd for $C_{30}H_{47}O_7$ 519.3322) in the high resolution (HR) (−)-FAB-MS. The IR absorption bands at 3357, 1700, 1670, and 1033 cm^{-1} implied the presence of hydroxyl, carbonyl, and double bond functionalities. The electron impact mass spectrum (EI-MS) displayed a dehydrated molecular ion peak at *m*/*z* 502 $[M-H_2O]^+$. The characteristic retro Diels–Alder fragment peaks at *m*/*z* 278 [D/E ring–H_2O]$^+$ and *m*/*z* 224 [A/B ring]$^+$ indicated a double bond located at C-12 and C-13; dioxygenated substitution on rings A/B; and trioxygenated and acid substitution on rings D/E.[42)] Its ^{1}H-NMR spectrum showed the presence of five singlet methyl signals (δ 0.79, 0.92, 1.04, 1.23, 1.32), one triplet-like olefinic proton signal (δ 5.28), one triplet-like oxygenated methine proton signal (δ 3.77, $W/2=6.6$ Hz), and two pairs of oxygenated methylene protons (δ 3.74 and 3.55, $J=11.0$ Hz each; δ 3.69 and 3.39, $J=11.3$ Hz each). The characteristic H-18 and H-16α signals for the urs-12-ene type triterpenoid with C-19 hydroxyl group on the α-face were observed at δ 2.96 (1H, br s) and 2.68 (1H, ddd, $J=4.2$, 13.2, 13.2 Hz), respectively.[43)] The ^{13}C-NMR data for **1** revealed the presence of one oxymethine carbon at δ 71.3, two oxymethylene carbons at δ 68.7 and 66.3, two oxygenated quaternary carbons at δ 74.9 and 76.6, a carboxylic acid carbon at δ 183.0, and an olefinic double bond (δ 129.5, 139.5) supporting a pentahydroxy-urs-12-en-oic acid skeleton bearing an α-hydroxyl at C-19. Regarding the two hydroxyl groups and a carboxylic acid group on the D/E rings, two methyl groups on the D/E rings were transformed into the COOH and one CH_2OH group, respectively. The latter group and the remaining hydroxyl group on the D/E rings were assigned at C-20 due to its quaternary nature. A comparison of the ^{13}C-NMR spectra of **1** and 3β,19α,23,30-tetrahydroxyurs-12-en-28-oic acid (30-hydroxyrotundic acid) 28-*O*-glucoside[44)] showed the expected downfield shifts for absorption due to C-20 (+28.4 ppm) and C-30 (+3.8 ppm), and upfield shifts for C-18 (−4.8 ppm), C-22 (−5.0 ppm), and C-29 (−4.7 ppm) due to the γ-gauche effect exerted by the β-oriented hydroxyl group at C-20.[45—47)] The NMR data of rings C/D/E in **1** were similar to those of kudinolic acid (3β,19α,20β-trihydroxyurs-12-en-28-oic acid) isolated from the leaves of *Ilex kudincha*.[48)] This was further corroborated by the heteronuclear multiple bond correlation (HMBC) spectrum, in which the proton signal at δ 2.96 (H-18) correlated with C-12 (δ 129.5), C-13 (δ 139.5), C-17 (δ 48.4), C-20 (δ 76.6), and C-28 (δ 183.0). The HMBC correlations of the methyl signal at δ 1.23 (H-29) with C-19 (δ 74.9) and C-20 (δ 76.6), and of the hydroxymethyl signals at δ 3.74 and 3.55 (H-30) with C-19 (δ 74.9), C-20 (δ 76.6), and C-21 (δ

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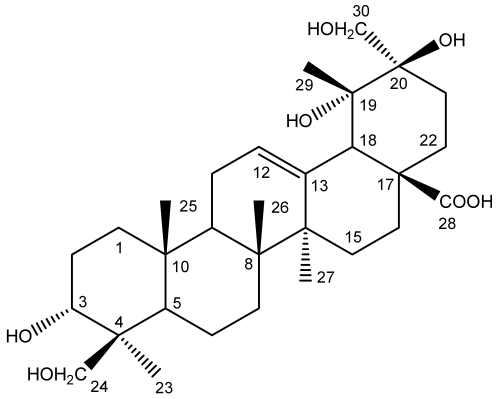
Table 1. NMR Data of Glutinolic Acid (**1**) in MeOH-d_4

No.	^{1}H	^{13}C	No.	^{1}H	^{13}C
1	1.38—1.44 1.29—1.32	34.2	16	1.52—1.58 2.68 (ddd, 4.2, 13.2, 13.2)	26.9
2	1.52—1.58 1.38—1.44	26.1	17	—	48.4
3	3.77 (t-like, $W/2=6.6$)	71.3	18	2.96 (br s)	49.6
4	—	44.0	19	—	74.9
5	1.38—1.44	50.7	20	—	76.6
6	1.52—1.58 1.38—1.44	19.6	21	2.25 (ddd, 3.8, 13.4, 13.4) 1.52—1.58	27.2
7	1.55—1.58 1.29—1.32	34.6	22	2.04 (ddd, 4.0, 13.4, 13.4) 1.55—1.58	32.7
8	—	41.1	23	1.04 (s)	22.9
9	1.84 (dd, 6.6, 10.4)	48.4	24	3.69 (d, 11.3) 3.39 (d, 11.3)	66.3
10	—	38.0	25	0.92 (s)	16.2
11	1.86 2.04 (ddd, 4.0, 13.4, 13.4)	24.8	26 27	0.79 (s) 1.32 (s)	17.5 24.4
12	5.28 (t-like)	129.5	28	—	183.0
13	—	139.5	29	1.23 (s)	22.8
14	—	42.6	30	3.74 (d, 11.0) 3.55 (d, 11.0)	68.7
15	0.98—1.03 1.77 (ddd, 3.8, 13.4, 13.6)	29.6			



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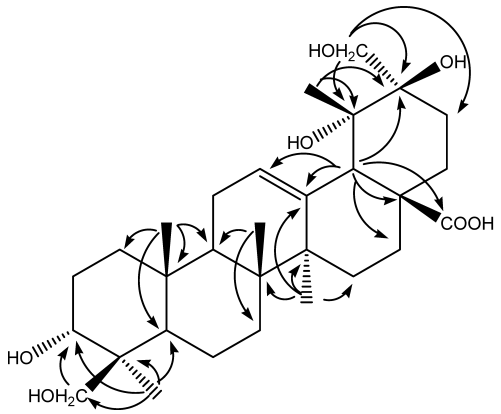


Fig. 1. Key HMBC Correlations for **1**

27.2) confirmed the above deduction. The axial α-orientation of the secondary hydroxyl group in rings A/B was confirmed by the ^{1}H-NMR data of **1**. The width at half height of the H-3 signal in the ^{1}H-NMR spectrum (δ 3.77, $W/2=6.6$ Hz) showed the axial position of the hydroxyl group.[49] Furthermore, the ^{13}C-NMR spectrum exhibited signals at δ 71.3 for C-3 and δ 66.3 for C-24, which were similar to those of the related compounds.[50,51] The relative configuration of H-3, H-23 and H-24 and other special information regarding **1** were further supported by nuclear Overhauser effect spectroscopy (NOESY) experiments, wherein nuclear Overhauser effects (NOEs) were observed between equatorial H-3 (δ 3.77) and equatorial H-23 (δ 1.04); between H-24 (δ 3.39, 3.69) and H-25 (δ 0.92); between H-12 (δ 5.28) and H-18 (δ 2.96)/H-29 (δ 1.23); and between equatorial H-29 (δ 1.23) and equatorial H-30 (δ 3.55, 3.74). Full ^{1}H- and ^{13}C-NMR data obtained in detailed two-dimensional (2D) experiments are listed in Table 1. Thus, compound **1** was identified as 3α,19α,20β,24,30-pentahydroxyurs-12-en-28-oic acid (glutinolic acid), which, to the best of our knowledge, is a new compound.

Compound **2**, $C_{21}H_{34}O_8$ by HR-FAB-MS, showed UV maximum at 260 (4.47) nm and IR bands for OH (3398 cm^{-1}), α,β-unsaturated C=O (1686 cm^{-1}), double bond (1610 cm^{-1}), and glycosidic C–O (1067, 1004 cm^{-1}) functionalities. The ^{1}H-NMR spectrum displayed resonances attributable to three tertiary CH_3 (δ 0.81, 1.14, 1.18), an olefinic CH_3 (δ 2.28, d, $J=0.7$ Hz), two *trans* olefinic proton signals (δ 6.81, d, $J=16.2$ Hz; 6.36, d, $J=16.2$ Hz), an olefinic proton signal (δ 5.76, s), resonances for three partially overlapping methylenes between δ 1.16 and 2.10, and oxygenated methines and a secondary CH_3 (δ 1.21, d, $J=6.1$ Hz) due to a sugar moiety. The ^{1}H–^{1}H correlation spectroscopy (COSY) experiment with **2** allowed sequential assignments of the monosaccharide and aglycon moieties. The coupling constants allowed the identification of a β-quinovosyl (6-deoxyglucosyl) unit. Furthermore, homo-nuclear coupling correlations for –CH_2–CH_2–CH_2– and –CH=CH–C(CH_3)=CH– were observed, the latter of which were in good agreement with those of the side chain, (2*E*,4*E*)-3-methylpentadienoic acid.[20] The ^{13}C-NMR spectrum of **2** gave 21 carbon resonances that were attributed to three CH_3, three CH_2, and three quaternary C (two oxygen-bearing, δ 80.3, 83.6) for the dihydroxy-trimethylcyclohexyl group; a methyl and five oxygenated CH for the quinovose moiety; and six carbon signals for the side chain, (2*E*,4*E*)-3-methylpentadienoic acid. The heteronuclear multiple quantum correlation (HMQC) spectroscopic data analysis of **2** furnished assignments of the proton-bearing carbon and corresponding proton resonances in the NMR spectra (Table 2). The HMBC correlations of the geminal CH_3 signals at C-1 with C-2 (δ 37.1) and an oxygenated quaternary C-6 (δ 80.3), and a CH_3 signal at an oxygenated quaternary C-5 (δ 83.6) with the same C-6 supported the 5,6-dihydroxy-1,1,5-trimethylcyclohexyl fragment of **2**. Furthermore, long-range heteronuclear correlations for H-7 and C-5 and C-6, and anomeric H and C-5 (Fig. 2) supported that the side chain and quinovose were linked at the C-6 and C-5 positions, respectively, of the cyclohexyl moiety. Therefore, the aglycon should be aeginetic acid.[20,21,52—55] Acid hydrolysis of **2** with 5% HCl gave an aglycon, aeginetic acid,[21] and D-quinovose as the sugar component identified on TLC and GC analyses by comparison with authentic sample. Given the above ob-

Table 2. NMR Data for Aeginetic Acid 5-*O*-*β*-D-Quinovoside (**2**) and Aeginetoyl Ajugol 5″-*O*-*β*-D-Quinovoside (**3**)

No.	**2**		No.	**3**	
	δ_H	δ_C (DEPT)		δ_H	δ_C (DEPT)
1	—	39.9 (C)	1″	—	39.9 (C)
2	1.16 (overlap) 1.69 (t, 13.4)	37.1 (CH_2)	2″	1.13 (overlap) 1.67 (overlap)	37.1 (CH_2)
3	1.28 (m) 2.09 (qt, 3.4, 13.4)	18.7 (CH_2)	3″	1.27 (dt, 3.2, 13.4) 2.10 (tt, 3.4, 13.4)	18.7 (CH_2)
4	1.59—1.73 (m)	32.9 (CH_2)	4″	1.73 (overlap) 1.61 (dd, 3.8, 14.2)	32.9 (CH_2)
5	—	83.6 (C)	5″	—	83.6 (C)
6	—	80.3 (C)	6″	—	80.4 (C)
7	6.81 (d, 16.2)	140.9 (CH)	7″	6.83 (d, 16.2)	141.3 (CH)
8	6.36 (d, 16.2)	133.7 (CH)	8″	6.36 (d, 16.2)	133.6 (CH)
9	—	154.3 (C)	9″	—	154.7 (C)
10	5.76 (br s)	119.0 (CH)	10″	5.81 (br s)	118.7 (CH)
11	—	170.9 (C)	11″	—	168.7 (C)
12	2.28 (d, 0.7)	14.4 (CH_3)	12″	2.30 (s)	14.6 (CH_3)
13	1.18 (s)	25.9 (CH_3)	13″	1.18 (s)	26.0 (CH_3)
14	0.81 (s)	27.6 (CH_3)	14″	0.80 (s)	27.6 (CH_3)
15	1.14 (s)	21.9 (CH_3)	15″	1.13 (s)	21.9 (CH_3)
1′	4.41 (d, 7.6)	98.0 (CH)	1‴	4.41 (d, 7.6)	98.0 (CH)
2′	3.20 (dd, 7.6, 9.0)	75.8 (CH)	2‴	3.20 (t, 7.9)	75.7 (CH)
3′	3.28 (t, 9.8)	78.8 (CH)	3‴	3.30 (overlap)	78.8 (CH)
4′	3.22 (t, 9.3)	72.6 (CH)	4‴	3.21 (t, 8.8)	72.5 (CH)
5′	3.00 (t, 9.0)	77.0 (CH)	5‴	3.00 (t, 9.1)	77.0 (CH)
6′	1.21 (d, 6.1)	18.4 (CH_3)	6‴	1.21 (d, 6.1)	18.5 (CH_3)

* Data of the ajugol moiety: δ_H 5.47 (d, 2.1, H-1), 6.20 (dd, 2.1, 6.7, H-3), 4.98 (dd, 2.1, 6.7, H-4), 2.87 (dd, 2.1, 9.3, H-5), 4.85 (m, H-6), 1.95 (dd, 4.2, 14.1, H-7a), 2.21 (dd, 6.5, 14.1, H-7b), 2.53 (dd, 2.1, 9.3, H-9), 1.36 (s, 10-CH_3), 4.66 (d, 7.9, H-1′), 3.19 (t, 9.1, H-2′), 3.37 (t, 8.8, H-3′), 3.27 (t, 8.7, H-4′), 3.30 (overlap, H-5′), 3.66 (dd, 5.4, 11.8, H-6′a), 3.88 (dd, 1.6, 11.8, H-6′b); δ_C 93.4, 140.9, 104.8, 39.3, 79.7, 48.0, 79.1, 51.5, 26.0 (aglycon C-1—C-10), 99.3, 74.8, 78.0, 71.7, 78.2, 62.8 (glucose C-1—C-6).

servations, the structure of compound **2** was aeginetic acid 5-*O*-*β*-D-quinovoside.

Compound **3** had the molecular formula $C_{36}H_{56}O_{16}$ based on the (+)-HR-FAB-MS. The UV spectrum exhibited absorption maximum at 269 (4.73) nm, suggesting the presence of acyclic dienones.[56] The IR spectrum showed absorption bands for OH (3409 cm^{-1}), *α*,*β*-unsaturated C=O (1700 cm^{-1}), double bond (1612 cm^{-1}), and glycosidic C–O (1070, 1005 cm^{-1}) functionalities. An inspection of the ^{1}H- and ^{13}C-NMR spectra of the compound suggested the presence of aeginetic acid quinovoside and an ajugol moiety of a 6-*O*-acylated ajugol derivative, 6-*O*-(4″-*O*-*α*-L-rhamnopyranosyl)vanilloyl ajugol, which was also isolated from the same plant, suggesting that **3** is an aeginetoyl ajugol quinovoside.[20] Positive FAB-MS of **3** gave the quasimolecular ions at *m*/*z* 767 $[M+Na]^+$ and 745 $[M+H]^+$ followed by other fragment ion peaks at *m*/*z* 397 $[C_{20}H_{33}O_6C{\equiv}O^+]$, due to the acylium ion of aeginetic acid quinovoside and *m*/*z* 251 $[(C_{20}H_{33}O_6C{\equiv}O^+)-146]^+$, which revealed the elimination of one methyl hexosyl moiety. This result was confirmed by alkaline hydrolysis of **3** with 2% NaOH in MeOH, which furnished methyl aeginetate 5-*O*-*β*-D-quinovoside and ajugol. The linkage position of the 5-*O*-*β*-D-quinovosyl aeginetoyl group to the ajugol moiety was ascertained by long-range correlation from the H-6 of ajugol at *δ* 4.85 to the carbonyl carbon resonance of the 5-*O*-*β*-D-quinovosyl aeginetoyl moiety at *δ* 168.7 in the HMBC spectrum of **3** (Fig. 3). From the above spectroscopic and chemical data, the structure of **3** was established as aeginetoyl ajugol 5″-*O*-*β*-D-quinovoside.

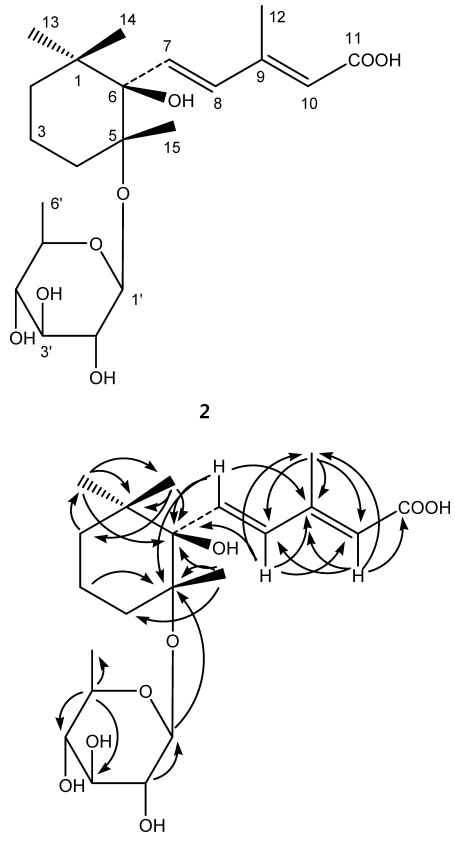


Fig. 2. Key HMBC Correlations for **2**

This paper constitutes the first isolation of a triterpenoid from the genus *Rehmannia*. Although aeginetic acid was previously identified from dried roots[17] and from steamed roots,[21] aeginetic acid quinovoside and its derivative have been isolated for the first time.

Three new isolates were examined for their ability to inhibit NO production in LPS-treated RAW 264.7 cells as previously described.[57] None of the compounds tested displayed inhibitory effects against NO production (IC_{50} >200 *μ*M).

Experimental

General The optical rotations were determined on a JASCO P-1020 polarimeter. The IR and UV spectra were recorded on a JASCO FT/IR-5300 and Hitachi JP/U3010 spectrometer, respectively. The EI-MS was performed on a Hewlett Packard 5989B mass spectrometer. The high-resolution FAB mass spectrum was obtained in a 3-nitrobenzyl alcohol matrix in positive-ion mode on a JEOL JMS-700 MStation. The NMR spectra were measured in CD_3OD on a Bruker Avance 400 (400 MHz) or a Bruker Avance 500 (500 MHz), and the chemical shifts were referenced to the solvent signals (δ_H: 3.31; δ_C: 49.00). GC analysis was performed with a Younglin YL 6100 gas chromatograph equipped with an H_2 flame ionization detector. The column was an HP-5 capillary column (30 m×0.32 mm×0.25 mm): column temperature, 200 °C; injector and detector temperature, 290 °C; and He flow rate, 1 ml/min. TLC was performed on silica gel 60 F_{254} (Merck) and cellulose plates (Art no. 5716, Merck). All of the chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO, U.S.A.).

Plant Material The roots of *R. glutinosa* were cultivated at U-bo-myeon, Gunwi-gun, Gyeongbuk province, Korea, harvested in 2008, and authenticated by Dr. J.-H. Lee, one of the authors. A voucher specimen (09F1001–A01BXX0811) was deposited in the herbarium of the College of Oriental Medicine, Dongguk University.

Extraction and Isolation The dried roots (16 kg) were chopped into

small pieces and percolated with 70% EtOH for 5 d at room temperature, 8 times. The 70% EtOH extract was evaporated to dryness under reduced pressure and then partitioned between H_2O and EtOAc and then BuOH (778 g). The EtOAc extract was added to 90% aqueous MeOH and extracted with hexane to give hexane (100 g) and 90% MeOH (106 g) extracts. The 90% MeOH extract (106 g) was fractionated by column chromatography over silica gel with CH_2Cl_2/MeOH (gradient) to yield 50 subfractions (Fr. 90M01—90M50). Fraction 90M32 (5.6 g) was further purified on a silica gel column with EtOAc saturated with water to yield 45 subfractions (Fr. 90M32-01—90M32-45). Fraction 90M32-28 (500 mg) was chromatographed on a silica gel column with MeOH/H_2O (10 : 1) to yield compounds **1** (2 mg) and **2** (50 mg) from 90M32-28-15. Fraction 90M32-40 (2 g) was chromatographed on a silica gel column with EtOAc saturated with water/MeOH (95 : 5) to afford subfraction 90M-32-40-45 (800 mg), which was further purified on an RP-18 column with 50% MeOH to yield compound **3** (100 mg).

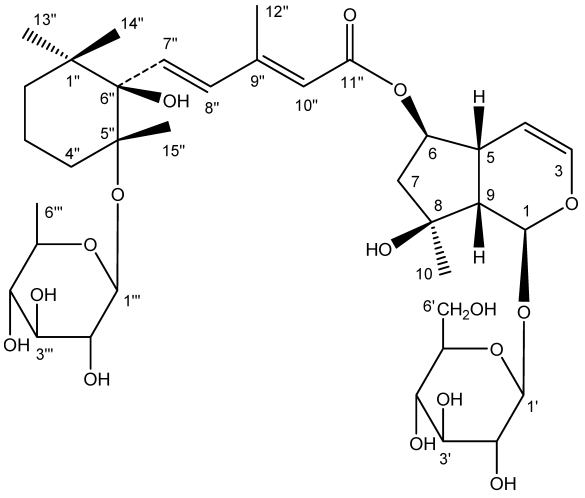
Glutinolic Acid (**1**): Amorphous white powder. $[\alpha]_D^{27}$ +9.0° (c=0.5, MeOH); UV λ_{max} (MeOH) nm (log ε): 202 (3.47); IR (KBr) ν_{max} cm^{-1}: 3357 (OH), 1700 (C=O), 1670 (CH=CH), 1456, 1251, 1150, 1033, 1004, 860; EI-MS *m/z* (rel. int., %): 502 $[M-H_2O]^+$ (6), 484 $[M-2H_2O]^+$ (3), 469 $[M-(3H_2O+CH_3)]^+$ (2), 466 $[M-3H_2O]^+$ (2), 453 $[M-(2H_2O+CH_2OH)]^+$ (4), 440 $[M-(H_2O+2CH_2OH)]^+$ (7), 278 [D/E ring (a)$-H_2O]^+$ (66), 263 $[a-(H_2O+CH_3)]^+$ (33), 224 [A/B ring (b)]$^+$ (11), 223 $[b-H]^+$ (19), 217 $[a-(H_2O+COOH+H)]^+$ (22), 206 $[b-H_2O]^+$ (58), 175 $[b-(H_2O+CH_2OH)]^+$ (100), 146 (32), 133 (44), 119 (43), 105 (49), 81 (38); (HR)-(−)-FAB-MS *m/z*: 519.3354. Calcd for $C_{30}H_{47}O_7$: 519.3322; HR-EI-MS *m/z*: 502.3299. Calcd for $C_{30}H_{46}O_6$ $[M-H_2O]^+$: 502.3294; (−)-FAB-MS *m/z*: 519 $[M-H]^-$; ^{1}H-NMR (400 MHz, CD_3OD) and ^{13}C-NMR (100 MHz, CD_3OD) data: Table 1.

Aeginetic Acid 5-*O*-β-D-Quinovoside (**2**): Amorphous white powder. $[\alpha]_D^{23}$ −80.1° (c=1.0, MeOH); UV λ_{max} (MeOH) nm (log ε): 260 (4.47); IR (KBr) ν_{max} cm^{-1}: 3398 (OH), 1686 (C=O), 1610 (CH=CH), 1375, 1245, 1168, 1067, 1004 (glycosidic C–O), 866; HR-FAB-MS *m/z*: 437.2166. Calcd for $C_{21}H_{34}O_8Na$: 437.2151; FAB-MS *m/z*: 437 $[M+Na]^+$, 419 $[(M+Na)-H_2O]^+$; ^{1}H-NMR (400 MHz, CD_3OD) and ^{13}C-NMR (100 MHz, CD_3OD) data: Table 2.

Aeginetoyl Ajugol 5″-*O*-β-D-Quinovoside (**3**): Amorphous white powder. $[\alpha]_D^{22}$ −120.0° (c=1.0, MeOH); UV λ_{max} (MeOH) nm (log ε): 269 (4.73); IR (KBr) ν_{max} cm^{-1}: 3409 (OH), 1700 (C=O), 1651, 1612 (CH=CH), 1237, 1157, 1070, 1005 (glycosidic C–O); HR-FAB-MS *m/z*: 767.3441. Calcd for $C_{36}H_{56}O_{16}Na$: 767.3466; FAB-MS *m/z*: 767 $[M+Na]^+$, 745 $[M+H]^+$, 621 $[(M+Na)-146]^+$, 603 $[(M+Na)-146-H_2O]^+$, 437 $[(M+H)-146-162]^+$, 419 $[(M+H)-146-162-H_2O]^+$, 401 $[(M+H)-146-162-(2\times H_2O)]^+$, 397 $[C_{20}H_{33}O_6C{\equiv}O^+]$, 379 $[(C_{20}H_{33}O_6C{\equiv}O^+)-H_2O]^+$, 251 $[(C_{20}H_{33}O_6C{\equiv}O^+)-146]^+$, 233 $[(C_{20}H_{33}O_6C{\equiv}O^+)-146-H_2O]^+$; ^{1}H-NMR (400 MHz, CD_3OD) and ^{13}C-NMR (100 MHz, CD_3OD) data: Table 2.

Acid Hydrolysis of 2 Compound **2** (30 mg) was refluxed with 5% HCl in 60% aqueous dioxane (5 ml) for 1 h. The reaction mixture was concentrated, added to crushed ice, and extracted with $CHCl_3$. The $CHCl_3$ extract was purified on a silica gel column with $CHCl_3$/MeOH/H_2O (7 : 1.5 : 0.5) solution to afford aeginetic acid, which was crystallized from MeOH to give fine needles. Aeginetic acid: mp 204—205°C, $[\alpha]_D^{27}$ −63.9° (c=1.0, MeOH); ^{1}H-NMR (400 MHz, CD_3OD) δ: 0.79 (3H, s, 1β-CH_3), 1.06 (3H, s, 5-CH_3), 1.20 (3H, s, 1α-CH_3), 2.30 (3H, br s, 9-CH_3), 5.78 (1H, br s, H-10), 6.41 (1H, d, J=16.0 Hz, H-8), 6.69 (1H, d, J=16.0 Hz, H-7); EI-MS *m/z* (rel. int., %): 268 $[M]^+$ (4.3), 250 $[M-H_2O]^+$ (3.9), 207 (37.5), 181 (10.2), 127 (63.3), 109 (100), 95 (57.8), 69 (53.1). The aqueous layer was neutralized with Ag_2CO_3, filtered and then concentrated to dryness *in vacuo* to give a residue that was subjected to RP-18 column chromatography with 40% aqueous MeOH to yield pure quinovose ($[\alpha]_D^{27}$ +48.3° (c=1.0, H_2O), *Rf* 0.51 with BuOH/HOAc/H_2O (4 : 1 : 2) on cellulose plate. The dried sugar (1 mg) was treated with pyridine (0.1 ml), and then the solution was added to a pyridine solution (0.1 ml) of L-cysteine methyl ester hydrochloride (2 mg) and warmed at 60 °C for 1 h. The solvent was evaporated under a N_2 stream and dried *in vacuo*. The residue was trimethylsilylated with TMS-HT (0.1 ml) at 60 °C for 30 min. After the addition of hexane and water, the hexane layer was removed and checked by GC. The retention times (t_R) of the peaks were 4.493 and 5.053 min. The t_R of the peaks of the authentic sample were 4.490 and 5.048 min (D-quinovose).

Alkaline Hydrolysis of 3 A solution of **3** (12 mg) in 2% NaOH in MeOH (1 ml) was kept at room temperature for 3 h. The reaction mixture was added to water (5 ml) and extracted with CH_2Cl_2 (5 ml). The CH_2Cl_2 layer was concentrated and crystallized from MeOH to yield methyl aeginetate 5-*O*-β-D-quinovoside (5 mg) as an amorphous powder. Methyl aeginetate 5-*O*-β-D-quinovoside: ^{1}H-NMR (300 MHz, CD_3OD) δ: 0.80 (3H, s, 1β-CH_3), 1.13 (3H, s, 5-CH_3), 1.18 (3H, s, 1α-CH_3), 1.16 (1H, overlap, H-2a), 1.21 (3H, d, J=6.2 Hz, Qui 6-CH_3), 1.28 (1H, m, H-3a), 1.59—1.73 (3H, m, H-2b, 4), 2.11 (1H, qt, J=3.4, 13.4 Hz, H-3b), 2.29 (3H, d, J=0.9 Hz, 9-CH_3), 3.00 (1H, t, J=9.0 Hz, Qui H-5), 3.20 (1H, dd, J=7.1, 8.9 Hz, Qui H-2), 3.22 (1H, t, J=9.3 Hz, Qui H-4), 3.25 (1H, t, J=8.8 Hz, Qui H-3), 3.68 (3H, s, $COOCH_3$), 4.41 (1H, d, J=7.5 Hz, Qui H-1), 5.78 (1H, br s, H-10), 6.36 (1H, d, J=16.2 Hz, H-8), 6.83 (1H, d, J=16.2 Hz, H-7); ^{13}C-NMR (75.5 MHz, CD_3OD) δ: 39.9 (C-1), 37.1 (C-2), 18.7 (C-3), 32.9 (C-4), 83.6 (C-5), 80.4 (C-6), 141.4 (C-7), 133.5 (C-8), 154.6 (C-9), 118.2 (C-10), 169.4 (C-11), 51.4 ($COOCH_3$), 25.9 (1α-CH_3), 27.6 (1β-CH_3), 21.9 (5-CH_3), 14.4 (9-CH_3), 98.0 (Qui C-1), 75.8 (Qui C-2), 78.8 (Qui C-3), 72.6 (Qui C-4), 77.1 (Qui C-5), 18.4 (Qui C-6); FAB-MS *m/z*: 451 $[M+Na]^+$, 429 $[M+H]^+$, 283 $[(M+H)-146]^+$, 265 $[(M+H)-146-H_2O]^+$. The aqueous layer was neutralized with 10% HCl and then chromatographed on an MCI gel column with distilled water to afford ajugol as an amorphous powder. $[\alpha]_D^{27}$ −103.8° (c=0.5, MeOH); UV λ_{max} (MeOH) nm (log ε): 201 (3.63); IR (KBr) ν_{max} cm^{-1}: 3376 (OH), 1658 (CH=CH), 1077, 1005 (glycosidic C–O), 969, 946, 749; ^{1}H-NMR (500 MHz, CD_3OD) δ: 1.31 (3H, s, 10-CH_3), 1.78 (1H, dd, J=4.5, 13.4 Hz, H-7a), 2.03 (1H, dd, J=5.6, 13.4 Hz, H-7b), 2.54 (1H, br d, J=9.5 Hz, H-9), 2.72 (1H, br d, J=9.4 Hz, H-5), 3.19 (1H, t, J=8.9 Hz, H-2′), 3.26 (1H, t, J=9.6 Hz, H-4′), 3.36 (1H, t, J=9.8 Hz, H-3′), 3.65 (1H, dd, J=5.5, 11.8 Hz, H-6′a), 3.88 (1H, br d, J=11.8 Hz, H-6′b), 3.91 (1H, m, H-6), 4.63 (1H, d, J=7.9 Hz, H-1′), 4.90 (overlap with HDO, H-4), 5.45 (1H, br s, H-1), 6.15 (1H, br d, J=6.1 Hz, H-3); ^{13}C-NMR (125 MHz, CD_3OD) δ: 25.2 (C-10), 41.3 (C-5), 50.0 (C-7), 51.8 (C-9), 62.9 (C-6′), 71.7 (C-4′), 74.8 (C-2′), 77.8 (C-3′), 78.0 (C-5′), 78.2 (C-6), 79.4 (C-8), 93.7 (C-1), 99.4 (C-1′), 105.9 (C-4), 140.4 (C-3); FAB-MS *m/z*: 371 $[M+Na]^+$, 349 $[M+H]^+$.



3

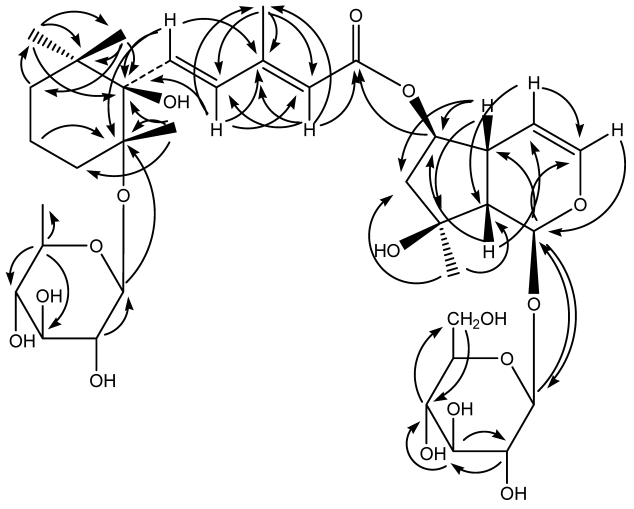


Fig. 3. Important HMBC Correlations for **3**

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