Resin Glycosides. XXIII. Two Novel Acylated Trisaccharides Related to Resin Glycoside from the Seeds of *Cuscuta chinensis* [1)]

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In a continuing study on resin glycosides of crude drugs originated from Convolvulaceae plants, we examined the seeds of *Cuscuta chinensis*. Two novel acylated trisaccharides named cus-1 and cus-2 were isolated, together with a mixture of resin glycoside-like compounds. Their structures were defined as α-L-rhamnopyranosyl-(1→3)-[2-*O*-(11*S*)-11-hydroxytetradecanoyl]-[4-*O*-(2*R*,3*R*)-3-hydroxy-2-methylbutyryl]-α-L-rhamnopyranosyl-(1→2)-[6-*O*-acetyl]-D-glucopyranose and α-L-rhamnopyranosyl-(1→3)-[2-*O*-(11*S*)-11-hydroxyhexadecanoyl]-[4-*O*-(2*R*,3*R*)-3-hydroxy-2-methylbutyryl]-α-L-rhamnopyranosyl-(1→2)-[6-*O*-acetyl]-D-glucopyranose, respectively. They are considered to be closely related to so-called resin glycosides in terms of structure and biosynthesis.

Key words *Cuscuta chinensis*; acylated trisaccharide; resin glycoside; Convolvulaceae; cus-1; cus-2

Cuscutae Semen, seeds of the parasitic plants, *Cuscuta chinensis* LAM. and *C. japonica* CHOISY,[2)] are a well-known traditional Chinese medicine, used as a tonic,[3)] and are considered to have antitumour activity in the Unani system of medicine in India.[4)] Sano reported that the seeds of *C. japonica* contained a resin glycoside which resembled convolvulin of Jalapae Tuber, the tuber of *Ipomoea purga* HAYNE,[5)] but Kawasaki *et al.* found no resinous glycoside analogous to pharbitin, the resin glycoside of the seeds of *Pharbitis Nil* CHOISY, or convolvulin.[6)] Recently, Nohara *et al.* reported compound 15, which seemed to be a resin glycoside, along with a new alkaloid, cuscutamine, two new lignans, cuscutosides A and B, and eleven known phenolic compounds from Cuscutae Semen commercially obtained in Japan.[7)]

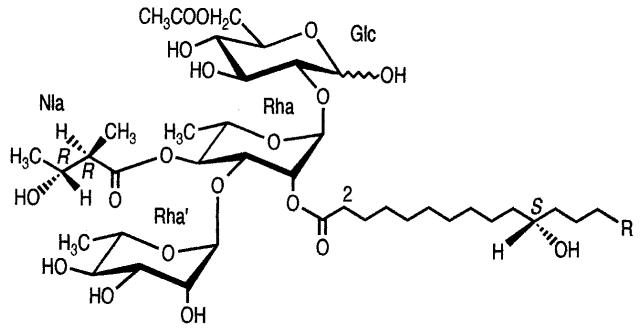
We investigated the seeds of *C. chinensis* collected in inner Mongolia and isolated the resin-related compound (compound 15)[7)] and new compounds named cus-1 and cus-2, together with a mixture of ether-insoluble resin glycoside-like compounds. This paper describes the isolation and structural characterization of cus-1 (**1**) and cus-2 (**2**).

Powdered seeds of *C. chinensis* were percolated successively with *n*-hexane and $CHCl_3$. The $CHCl_3$ extractive was chromatographed over Sephadex LH-20 to afford two fractions, fr. A (a mixture of resin glycosides) and fr. B. Fraction B was chromatographed successively over silica gel and octadecyl silica gel (ODS), then subjected to preparative HPLC to furnish **1** (0.019%, compound 15)[7)] and **2** (0.0015%).

Cus-1 (**1**), white powder, $[\alpha]_D$ −22.4° (MeOH), exhibited six anomeric and two acetoxy methyl proton signals in the ^{1}H-NMR spectrum, but showed no significant peaks on FD- and FAB-MS. It presented two spots (*Rf* 0.60, 0.63) on silica gel TLC, and could be separated into two fractions, but each fraction came to equilibrium in the ratio of about 5:3 within a few hours at room temperature.

On alkaline hydrolysis with 3% K_2CO_3, **1** furnished a hydroxy fatty acid, colorless needles, mp 46—47 °C, $[\alpha]_D$ +2.5°, an organic acid mixture and an oligosaccharide, white powder, mp 148—154 °C, $[\alpha]_D$ −47.8°.

The hydroxy fatty acid was methylated with diazomethane to give colorless plates, mp 33—34 °C, whose field desorption–mass spectrum (FD-MS) showed the $[M+H]^+$ ion peak at *m/z* 259, and fragment ion peaks corresponding to $[M-CH_3(CH_2)_2]^+$ and $[CH_3(CH_2)_2CHOH]^+$ at *m/z* 215 and 73, respectively, indicating it to be methyl 11-hydroxytetradecanoate. On GC analysis, it was identified as the methyl ester of convolvulinolic acid, a characteristic component hydroxy fatty acid of so-called



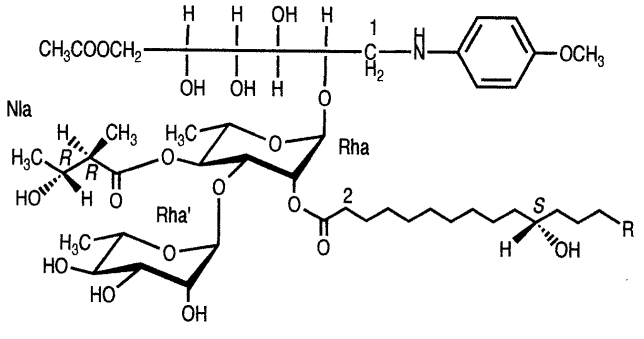


Fig. 1. Structures of **1**, **2**, **1a** and **2a**

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resin glycosides.[8] Its absolute configuration was determined to be *S* by Mosher's method.[9] The organic acid mixture was consisted of acetic acid and nilic acid (3-hydroxy-2-methylbutyric acid) as determined by GC analyses. The latter was concluded to have 2*R*,3*R* configuration from a comparison of its ^{1}H-NMR spectrum and the specific rotation of the *p*-bromophenacyl ester, mp 71—72 °C, $[\alpha]_D$ −15.1°, with those of an authentic sample.[10]

The oligosaccharide exhibited the quasi-molecular ion at *m/z* 495 $[M+Na]^+$ in the positive ion FAB-MS, and the $[M-H]^-$ ion at *m/z* 471 in the negative ion FAB-MS. On acidic hydrolysis, it furnished rhamnose and glucose, which were concluded to be L- and D-form, respectively, by GC analyses according to Hara *et al.*[11] The oligosaccharide is, therefore, a trisaccharide composed of 2 mol of L-rhamnose and 1 mol of D-glucose. These findings suggested that **1** is an acylated oligosaccharide composed of 1 mol each of the trisaccharide, acetic acid, (2*R*,3*R*)-nilic acid and (11*S*)-convolvulinolic acid, and that **1** has a reducing terminal and exists as an equilibrium mixture of α- and β-anomers.

In order to determine the molecular weight of **1** and to confirm the above suggestions, **1** was converted into aminoalditol derivative (**1a**) by one-pot reaction with *p*-anisidine and $NaBH_3CN$. **1a**, mp 53—55 °C, $[\alpha]_D$ −49.8°, showed a quasi-molecular ion peak $[M-H]^-$ at *m/z* 946, and fragment ion peaks at *m/z* 904 and 720 assignable to $[M-H-C_2H_2O(\text{acetyl}-H)]^-$ and $[M-H-C_{14}H_{26}O_2(\text{hydroxytetradecanoyl}-H)]^-$, respectively, in the negative ion FAB-MS. Thus, the molecular weight of **1** was estimated to be 840.

Compound **1a** exhibited two anomeric (δ, 99.3, 104.0) and three ester carbonyl (δ 171.1, 173.0, 175.3) carbon signals in the ^{13}C-NMR spectrum, and the signals ascribable to nonequivalent 1-CH_2 of the aminoalditol moiety (δ 3.70, 3.91, each dd), two anomeric protons (δ 5.48, 5.84), an acetoxy methyl protons (δ 1.92, 3H, s) and 2-H of the nilic acid moiety (δ 2.75, 1H, dq) in the ^{1}H-NMR spectra.

The ^{1}H- and ^{13}C-signals of **1a** except for those of methylene in the convolvulinolic acid moiety were assigned from the ^{1}H–^{1}H correlation (COSY) and ^{1}H–^{13}C COSY spectra (Table 1). In a comparison of the carbon signals due to the sugar moiety with those of reported for methyl pyranosides,[12] a glycosylation shift of 2.6 ppm was observed at 3-C of one (Rha) of the two rhamnopyranosyl units, so the rhamnopyranosyl-(1→3)-rhamnopyranose unit is linked to the aminoglucitol unit derived from the glucose moiety in **1**. Further, compared

Table 1. NMR Spectral Data for **1** and **1a** (in Pyridine-d_5)

	1				**1a**	
	α-Anomer		β-Anomer			
	^{1}H[a]	^{13}C[b]	^{1}H[a]	^{13}C[b]	^{1}H[c]	^{13}C[d]
Glc-1	5.91 d (3.2)	92.9	5.24 d (7.8)	96.9	3.70 dd (7.0, 13.5)	46.6
					3.91 dd (3.5, 13.5)	
2	4.13 dd (3.2, 9.5)	81.8	4.19 dd (7.8, 9.9)	80.2	4.80 ddd (7.0, 3.5, 7.0)	79.9
3	4.79 dd (9.5, 9.5)	73.5	4.24 dd (9.9, 9.9)	78.5	4.90 dd (7.0, 1.5)	71.6
4	4.05 dd (9.5, 9.2)	72.4	3.96 dd (9.9, 9.9)	71.8	4.33 dd (1.5, 8.0)	72.3
5	4.80 ddd (9.2, 6.3, 1.8)	70.4	3.98 ddd (9.9, 6.2, 1.8)	75.1	4.67 ddd (8.0, 2.5, 7.0)	70.7
6	4.76 dd (6.3, 11.0)	65.2	4.69 dd (6.2, 11.5)	64.8	4.74 dd (2.5, 11.0)	68.1
	4.98 dd (1.8, 11.0)		4.93 dd (1.8, 11.5)		5.02 dd (7.0, 11.0)	
Rha-1	5.80 d (1.6)	100.4	6.28 d (1.6)	98.8	5.84 d (1.5)	99.3
2	6.10 dd (1.6, 3.2)	72.7	6.13 dd (1.6, 3.5)	72.9	6.05 dd (1.5, 3.2)	72.9
3	4.79 dd (3.2, 9.9)	75.8	4.89 dd (3.5, 10.1)	75.5	4.75 dd (3.2, 9.9)	75.3
4	5.75 dd (9.9, 9.9)	73.4	5.84 dd (10.1, 10.1)	73.8	5.73 dd (9.9, 9.9)	73.5
5	4.70 dq (9.9, 6.2)	67.6	5.16 dq (10.1, 6.5)	67.5	4.60 dq (9.9, 6.4)	67.8
6	1.55 d (6.2)	17.9	1.63 d (6.5)	18.1	1.46 d (6.4)	18.1
Rha'-1	5.42 d (1.6)	104.1	5.55 d (1.6)	104.3	5.48 d (1.2)	104.0
2	4.52 dd (1.6, 3.3)	72.4	4.57 dd (1.6, 3.3)	72.5	4.54 dd (1.2, 3.5)	72.4
3	4.42 dd (3.3, 9.2)	72.7	4.46 dd (3.3, 9.2)	72.6	4.42 dd (3.5, 9.3)	72.5
4	4.20 dd (9.2, 9.2)	73.7	4.23 dd (9.2, 9.2)	73.8	4.20 dd (9.3, 9.3)	73.7
5	4.31 dq (9.2, 6.4)	70.7	4.39 dq (9.2, 6.1)	70.7	4.34 dq (9.3, 6.0)	70.6
6	1.46 d (6.4)	18.5	1.46 d (6.1)	18.6	1.60 d (6.0)	18.5
Con-1		173.1		173.2		173.0
2	2.26 ddd (7.0, 8.0, 15.2)	34.4	2.27 ddd (7.0, 8.0, 15.2)	34.5	2.22 ddd (8.0, 8.0, 16.0)	34.5
	2.30 ddd (7.0, 8.0, 15.2)		2.30 ddd (7.0, 8.0, 15.2)		2.28 ddd (8.0, 8.0, 16.0)	
11	3.83 dddd (3.9, 3.9, 7.8, 7.8)	70.7	3.83 dddd (3.9, 3.9, 7.8, 7.8)	70.6	3.84 dddd (4.0, 4.0, 7.0, 7.0)	70.7
14	0.96 t (7.1)	14.5	0.96 t (7.1)	14.5	0.97 t (7.0)	14.5
Nla-1		175.5		175.4		175.3
2	2.69 dq (7.0, 7.0)	48.8	2.71 dq (7.0, 7.0)	48.9	2.75 dq (7.0, 7.0)	48.9
3	4.20 dq (7.0, 7.0)	69.3	4.22 dq (7.0, 7.0)	69.3	4.22 dq (7.0, 7.0)	69.2
4	1.30 d (6.5)	21.4	1.30 d (6.5)	21.3	1.32 d (7.0)	21.3
5	1.19 d (7.2)	14.1	1.20 d (7.2)	14.0	1.23 d (7.0)	13.9
Ac-1		170.9		170.8		171.1
2	1.96 s	20.8	1.92 s	20.7	1.92 s	20.8

δ in ppm from TMS; coupling constants (J_{H-H}) in Hz are given in parentheses; *a*) 600 MHz; *b*) 150 MHz; *c*) 400 MHz; *d*) 100 MHz. Assignments are based on ^{1}H–^{1}H COSY, NOESY and ^{13}C–^{1}H COSY spectra. Con, convolvulinolic acid group; Nla, nilic acid group.

with the carbon signals (δ 48.6—74.5) of an authentic *p*-methoxyphenylamino-D-glucitol, the signal due to 2-C appeared at δ 79.9, so the sugar moiety of **1** was concluded to be L-rhamnopyranosyl-(1→3)-L-rhamnopyranosyl-(1→2)-D-glucose.

The ^{1}H-NMR spectra showed acylation shifts at 2-H (1.58 ppm) and 4-H (1.55 ppm) of Rha, and 6-H_2 (0.51, 0.48 ppm) of the aminoglucitol moiety (Table 1). The acyl groups were therefore placed at 2- and 4-OH of Rha, and 6-OH of the glucitol moiety. The positions of the acyl groups could not be determined by means of long-range ^{1}H–^{13}C COSY, but were elucidated from the heteronuclear multiple-bond correlation spectroscopy (HMBC) spectrum of **1** obtained with a 600 MHz NMR instrument.

The ^{1}H-NMR signals at δ 5.91, 5.80, 5.42, 5.24, 6.28 and 5.55 were assigned to the anomeric protons of the glucopyranosyl unit (Glc), inner rhamnopyranose (Rha) and nonreducing rhamnopyranose (Rha′) of the α- and β-anomers in **1**, respectively, from the COSY, nuclear Overhauser and exchange (NOESY) and homonuclear Hartmann–Hahn (HOHAHA) spectra (Table 1). The HMBC spectrum showed cross peaks between the signals of 2-H_3 (δ 1.96), 1-C (δ 170.9) of the acetyl group and 6-H_2 (δ 4.76, 4.98) of the α-anomer, and between the signals of 2-H_3 (δ 1.92) and 1-C (δ 170.8) of the acetyl group and 6-H_2 (δ 4.69, 4.93) of the β-anomer, indicating the acetyl group to be at 6-OH of Glc. On the other hand, the signals of 1-C (δ 173.1) and 2-H_2 (δ 2.26, 2.30) of the convolvulinolic acid group were correlated with 2-H (δ 6.10 (α-anomer) and 6.13 (β-anomer)) of Rha, respectively. Therefore, acetic acid, convolvulinolic acid and nilic acid groups were concluded to be attached at 6-OH of Glc, and 2-OH and 4-OH of Rha, respectively, though no correlation peak between 1-C of the nilic acid group and 4-H of Rha was observed.

Further, the chemical shifts and $^3J_{H-H}$ for sugar moieties of **1** (Table 1) and the $J_{1\text{-}C-1\text{-}H}$ values of 170 (α-Glc), 155 (β-Glc), 170 (Rha) and 170 Hz (Rha′) indicated that all the monosaccharide moieties are in pyranose form, and that Glc exists as both α- and β-anomers at equilibrium in the $^4C_1$ conformation, and that both Rha and Rha′ take the α configuration in $^1C_4$ conformation.

Accordingly, the structure of cus-1 (**1**) was defined as α-L-rhamnopyranosyl-(1→3)-[2-*O*-(11*S*)-11-hydroxytetradecanoyl]-[4-*O*-(2*R*,3*R*)-3-hydroxy-2-methylbutyryl]-α-L-rhamnopyranosyl-(1→2)-[6-*O*-acetyl]-D-glucopyranose.

Cus-2 (**2**), white powder, $[\alpha]_D$ −21.2° (MeOH), showed similar behavior to **1** on TLC and FAB-MS. On alkaline hydrolysis, **2** yielded jalapinolic acid (11-hydroxyhexadecanoic acid), acetic acid, nilic acid and a trisaccharide which was identical with that of **1**. The absolute configuration of jalapinolic acid was established to be 11*S* and those of nilic acid to be 2*R*, 3*R*, in the same ways as in the case of **1**.

The *p*-methoxyphenylaminoalditol derivative (**2a**) of **2**, white powder, $[\alpha]_D$ −45.5°, exhibited the $[M-H]^-$ ion peak at *m/z* 974, 28 mass units more than that of **1a**, in the negative ion FAB-MS, and $[M+H]^+$ and $[M+Na]^+$ at *m/z* 976 and 998, respectively, in the positive ion FAB-MS. The ^{1}H- and ^{13}C-NMR spectra of **2** and **2a** were superimposable on those of **1** except for the signals due to the jalapinolic acid moiety.

Consequently, the structure of cus-2 (**2**) was defined as α-L-rhamnopyranosyl-(1→3)-[2-*O*-(11*S*)-11-hydroxyhexadecanoyl]-[4-*O*-(2*R*,3*R*)-3-hydroxy-2-methylbutyryl]-α-L-rhamnopyranosyl-(1→2)-[6-*O*-acetyl]-D-glucopyranose.

Many Convolvulaceae plants contain so-called resin glycosides, which are composed of glycosidic acids (oligosides of hydroxy fatty acids) and characteristic organic acids (acetic, tiglic, nilic, 2-methylbutyric, isovaleric acids, *etc.*) in general.[8] Cus-1 and -2 are the first acylated trisaccharides which consist of the components, rhamnose, glucose, acetic, nilic, convolvulinolic and jalapinolic acids, common with those of the resin glycosides. They may be classified as a new group of resin glycosides, because they correspond to the hydrolysis products of the glycosidic linkage between the aglycone and sugar moieties of so-called jalapins,[13] which have a variety of intramolecular macrocyclic ester structures.

Experimental

General Procedures All melting points (mp) were determined on a Yanaco MP-S3 apparatus and are uncorrected. ^{1}H-, ^{13}C- and two dimensional (2D)-NMR spectra were recorded on JEOL JNM GSX-400 and GE Omega 600 spectrometers. Spectra were taken for 0.5—2% (w/v) solutions in pyridine-d_5 at 26 °C with tetramethylsilane (TMS) as an internal reference. EI-, FD- and FAB-MS were obtained on a JEOL JMS DX-300 spectrometer. Analytical GC was carried out on a Shimadzu GC-8A gas chromatograph for organic and hydroxy fatty acids and a Hitachi G-3000 gas chromatograph for sugars, with flame ionizing detectors. Optical rotations were determined with a JASCO DIP-140 polarimeter. Preparative HPLC separation was run on a JASCO TWINCLE equipped with a Shodex SE-11 differential refractometer. TLC was carried out on Silica gel 60 precoated Al sheets (Merck, Art 5554) and Avicel SF (Funakoshi Pharm. Co.). For column chromatography, Silica gel 60 (Merck Art 9385), MCI gel CHP 20P (100—200 mesh, Mitsubishi Chemical Industries) and Sephadex LH-20 (25—100 mesh, Pharmacia Fine Chemicals) were used.

Isolation of 1 and 2 Crushed seeds of *C. chinensis* (4.9 kg) collected in Inner Mongolia, China in September 1992 were percolated successively with *n*-hexane (5 l) and $CHCl_3$ (15 l) at room temperature, and each extract was evaporated under reduced pressure to afford a yellow syrup (233.1 g) and a solid (24.5 g). The solid was treated with MeOH (40 ml) and the soluble portion was concentrated *in vacuo* to give a pale yellow powder (23.2 g), which was chromatographed over Sephadex LH-20 (MeOH) to afford fr. A (16.01 g, a mixture of resin glycoside-like compounds) and fr. B (6.54 g). Fraction B was subjected to column chromatography on silica gel ($CHCl_3$–MeOH, 93:7→90:10), giving three fractions, fr. 1 (3.08 g), fr. 2 (2.48 g) and fr. 3 (0.92 g). Reversed-phase chromatography over Fuji ODS G3 (80% MeOH) of fr. 2 furnished fr. 4 (1.98 g), and fr. 5 (493 mg). Fraction 4 was separated by preparative HPLC on Inertsil ODS (GL sciences, 20 mm i.d. × 250 mm, 83% MeOH) to give fr. 6 (1.90 g) and fr. 7 (76.5 mg).

Fraction 6 (942 mg) was further purified by silica gel chromatography ($CHCl_3$–MeOH–H_2O, 8:1:0.1) to yield a white powder (**1**, 939 mg), mp 79—81 °C, $[\alpha]_D^{28}$ −22.4° (c=2.08, MeOH). Silica gel TLC ($CHCl_3$–MeOH–H_2O, 8:2:0.2) *Rf*: 0.60, 0.63. *Anal.* Calcd for $C_{39}H_{68}O_{19}\cdot 1/2H_2O$: C, 55.11; H, 8.18. Found: C, 54.92; H, 8.28. ^{1}H-NMR (py-d_5, 600 MHz) δ: see Table 1. ^{13}C-NMR (py-d_5, 150 MHz) δ: see Table 1, 19.5, 25.3, 26.4, 29.3, 29.5, 29.7, 30.0, 30.2, 34.5 (2-C), 38.5, 40.7, (CH_2 of convolvulinolic acid moiety of α-anomer); 19.5, 25.4, 26.4, 29.3, 29.5, 29.7, 30.0, 30.2, 34.5 (2-C), 38.5, 40.7, (CH_2 of the convolvulinolic acid moiety of the β-anomer).

Fraction 7 was purified under similar conditions to those used for fr. 6 to give a white powder (**2**, 75 mg), mp 78—80 °C, $[\alpha]_D^{25}$ −21.2° (c=1.15, MeOH). Silica gel TLC ($CHCl_3$–MeOH–H_2O, 8:2:0.2) *Rf*; 0.63, 0.66. *Anal.* Calcd for $C_{41}H_{72}O_{19}$: C, 56.66; H, 8.35. Found: C, 56.68; H, 8.61.

^{1}H-NMR (py-d_5, 600 MHz) δ: superimposable on that of **1**, except for the signals of the CH_2 region of the jalapinolic acid moiety. ^{13}C-NMR (py-d_5, 150 MHz) δ: superimposable on that of **1** except for the signals due to the jalapinolic acid moiety; α-anomer: 14.2(16-C), 23.0, 25.3, 26.0, 26.3, 29.3, 29.5, 29.7, 30.0, 30.2, 32.4, 34.4(2-C), 38.4, 38.5, 70.7(11-C), 173.1(1-C); β-anomer: 14.2(16-C), 23.0, 25.3, 26.0, 26.3, 29.3, 29.5, 29.7, 30.0, 30.2, 32.4, 34.5(2-C), 38.4, 38.5, 70.6(11-C), 173.1(1-C).

Alkaline Hydrolysis of 1 and 2 A solution of **1** (126 mg) in 3% K_2CO_3 (10 ml) was heated at 95 °C for 1 h, and then adjusted to pH 4.0 with 1 N HCl. Precipitates were collected by filtration (the filtrate was saved) and purified by crystallization from *n*-hexane to furnish colorless needles (36 mg), mp 46—47 °C, $[\alpha]_D^{25}$ +2.5° (c=2.4, $CHCl_3$). This product was methylated with diazomethane followed by crystallization from *n*-hexane–AcOEt to afford colorless plates (38 mg), mp 33—34 °C, $[\alpha]_D^{21}$ +2.7° (c=0.6, $CHCl_3$). FD-MS *m/z*: 259 $[M+H]^+$, 215 $[M-CH_3(CH_2)_2]^+$, 73 $[CH_3(CH_2)_2CH(OH)]^+$. It was identical with an authentic sample of methyl convolvulinolate by GC (condition 1: column, 3 mm i.d. × 2 m glass column packed with silicone OV-17; carrier N_2, 1 kg/cm^2; column temperature 240 °C); t_R (min); 4.45. A mixture of methyl convolvulinolate (2 mg), (−)-1-methoxy-1-trifluoromethylphenylacetyl (MTPA) chloride (4 mg), dicyclohexylcarbodiimide (6 mg) and 4-dimethylaminopyridine (2 mg) in Et_2O (1.5 ml) was stirred at room temperature overnight. The solvent was removed under a stream of N_2 and the residue was chromatographed over silica gel (*n*-hexane–Et_2O, 10:1) to give the (−)-MTPA ester (3 mg) as a colorless oil. ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 0.850 (3H, t, J=7.3 Hz, 14-H_3), 2.301 (2H, t, J=7.5 Hz, 2-H_2), 3.553 (3H, q, J=1.2 Hz, OCH_3), 3.666 (3H, s, $COOCH_3$), 5.099 (1H, J=5.3, 7.0 Hz, 11-H). The ^{1}H-NMR spectrum was superimposable on that of an authentic sample of the (−)-MTPA ester of methyl (11*S*)-convolvulinolate.

The filtrate was extracted with Et_2O (10 ml × 3). The combined Et_2O layer was washed with H_2O, dried over Na_2SO_4 and evaporated to give an oil (9 mg, organic acid fraction), which was analyzed by GC [condition 2: GL Sciences Unisol 30T (5%) glass column, 4 mm i.d. × 2 m, column temperature, 120 °C; carrier gas N_2, 0.5 kg/cm^2]; t_R (min): 2.81 (acetic acid). After methylation of the oil with diazomethane in the usual way, the product was examined by GC under the same conditions as described above except that the column temperature was 100 °C, t_R (min): 7.53 (methyl nilate).

The lower layer was desalted on a Sephadex LH 20 column (90% MeOH), and the product was purified by chromatography on silica gel ($CHCl_3$–MeOH–H_2O, 6:4:1) to give a white powder (36 mg), mp 148—154 °C, $[\alpha]_D^{18}$ −47.8° (c=1.4, MeOH). Positive ion FAB-MS *m/z*: 495 $[M+Na]^+$. Negative ion FAB-MS *m/z*: 471 $[M-H]^-$.

A solution of this product (3 mg) in 2 N H_2SO_4 (0.5 ml) was heated at 95 °C for 0.5 h. The mixture was neutralized with $Ba(OH)_2$, and the precipitates were filtered off. The filtrate was evaporated under reduced pressure to give a syrup (3 mg). The syrup exhibited two spots identical with those of authentic samples of L-rhamnose and D-glucose on Avicel SF TLC [BuOH–pyridine–H_2O (6:2:3) top layer + pyridine (**1**)] *Rf*: 0.62 (L-rhamnose), 0.37 (D-glucose). The mixture was separated by silica gel chromatography ($CHCl_3$–MeOH–H_2O, 6:4:1, bottom layer) to afford a syrup (1.5 mg), and another syrup (0.7 mg). The former product (1.5 mg) was derived to the trimethylsilyl ether of the thiazolidine derivative according to Hara *et al.*[11] and was examined by GC (condition 3: column, GL Sciences, OV-17, capillary column, 0.25 mm i.d. × 50 m, column temperature 220 °C, carrier gas; He, 1.9 kg/cm^2); t_R (min): 18.87, which was identical with that of an authentic sample derived from L-rhamnose. The latter fraction (0.7 mg) was also examined in the same manner; t_R (min): 26.16 (D-glucose).

Compound **2** (68 mg) was hydrolyzed with 3% K_2CO_3 (6 ml) and treated in the same way as described for **1**. The precipitates were purified by recrystallization from *n*-hexane to furnish colorless needles (16 mg), mp 63 °C, EI-MS *m/z*: 272 $[M^+]$. This product was methylated with diazomethane followed by crystallization from *n*-hexane–AcOEt to afford colorless plates (15 mg), mp 43—44 °C, $[\alpha]_D^{25}$ +0.8° (c=1.2, $CHCl_3$), EI-MS *m/z*: 286 $[M]^+$, 255 $[M-OCH_3]^+$, 215 $[CH(OH)(CH_2)_9COOCH_3]^+$, 101 $[CH_3(CH_2)_4CH(OH)]^+$. GC (condition 1) t_R (min): 7.18, identical with that of an authentic sample of methyl jalapinolate. In the same manner as described above, the methyl ester was derived to the (−)-MTPA ester to afford a colorless oil. ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 0.839 (3H, t, J=6.9 Hz, 16-H_3), 2.299 (2H, t, J=7.5 Hz, 2-H_2), 3.558 (3H, q, J=1.2 Hz, OCH_3), 3.665 (3H, s, $COOCH_3$), 5.083 (1H, tt, J=5.5, 6.7 Hz, 11-H). These data were identical with those of authentic (−)-MTPA ester of methyl (11*S*)-jalapinolate.

The filtrate was worked up and analyzed by GC in the same manner as described for **1** to identify acetic acid, nilic acid, L-rhamnose and D-glucose.

Determination of the Absolute Configuration of Nilic Acid The organic acid fraction (8 mg from **1** or 6 mg from **2**) was dissolved in dry acetone (5 ml) and neutralized with triethylamine, then *p*-bromophenacyl bromide (10 mg) was added. The mixture was left to stand at room temperature for 1 h, then concentrated *in vacuo* and diluted with water (5 ml). The solution was extracted with Et_2O (5 ml × 3). The extractive was subjected to silica gel chromatography (*n*-hexane–AcOEt, 8:1→7.5:1) to give a white powder (*p*-bromophenacyl acetate), mp 65—68 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 2.22 (3H, s, CH_3COO–), 5.28 (2H, s, –CH_2–CO-ph), 7.65 (2H, ddd, J=8.5, 2.0, 2.0 Hz, arom H), 7.77 (2H, ddd, J=8.5, 3.0, 3.0 Hz, arom H), and colorless needles (*p*-bromophenacyl nilate) (5 mg from **1**, $[\alpha]_D^{25}$ −14.1° (c=0.5, $CHCl_3$), and 3 mg from **2**, $[\alpha]_D^{25}$ −13.9° (c=0.3, $CHCl_3$)), which were separately examined by ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.25 (3H, d, J=7.0 Hz, 2-CH_3), 1.30 (3H, d, J=7.0 Hz, 2-CH_3), 2.62 (1H, dq, J=7.0, 7.0 Hz, 2-H), 3.97 (1H, dq, J=7.0, 6.5 Hz, 3-H), 5.33 (1H, d, J=16.5 Hz, 2-H_2), 5.43 (1H, d, J=16.5 Hz, 2-H_2), 7.63 (3H, ddd, J=8.5, 2.3 Hz, arom H), 7.79 (3H, ddd, J=8.5, 2.3 Hz, arom H). These data were identical with those of an authentic sample of *p*-bromophenacyl (2*R*,3*R*)-nilate.

Preparation of Aminoalditol Derivatives, 1a, 2a and Aminoglucitol *p*-Anisidine (30 mg) and $NaBH_3CN$ (30 mg) were added to a solution of compound **1** (26 mg) in 10% AcOH–EtOH (5 ml). The reaction mixture was allowed to stand at room temperature for 4 h. After removal of the solvent, the residue was desalted by chromatography over MCI gel CHP 20P (H_2O→MeOH). The MeOH eluate was concentrated *in vacuo* and the residue was chromatographed over silica gel ($CHCl_3$–MeOH–H_2O, 8:2:0.1) to afford **1a** (28 mg), a white powder, mp 53—55 °C, $[\alpha]_D^{25}$ −49.8° (c=2.0, MeOH). Negative ion FAB-MS (TEA) *m/z*: 946 $[M-H]^-$, 904 $[M-H-42(C_2H_2O)]^-$, 720 $[M-H-226(C_{14}H_{26}O_2)]^-$. Positive ion FAB-MS (glycerol) *m/z*: 970 $[M+Na]^+$, 948 $[M+H]^+$, 906 $[M+H-42]^+$. *Anal.* Calcd for $C_{46}H_{77}O_{19}N$: C, 58.27; H, 8.18; N, 1.47. Found: C, 58.37; H, 8.06; N, 1.33. ^{1}H-NMR (py-d_5, 400 MHz) δ: see Table 1. ^{13}C-NMR (py-d_5, 100 MHz) δ: see Table 1, 14.5(14-C), 19.5, 25.4, 26.4, 29.3, 29.5, 29.7, 30.0, 30.2, 34.5 (2-C), 38.5, 40.7, 70.7(11-C), 173.0(1-C) (convolvulinolic acid moiety).

Under the same conditions as above, compound **2** (20 mg) was derived in the aminoalditol derivative **2a** (22.1 mg), a white powder, mp 51—53 °C, $[\alpha]_D^{25}$ −45.5° (c=0.8, MeOH). Negative ion FAB-MS (TEA) *m/z*: 974 $[M-H]^-$, 932 $[M-H-C_2H_2O]^-$, 720 $[M-H-254(C_{16}H_{30}O_2)]^-$. Positive ion FAB-MS (glycerol) *m/z*: 998 $[M+Na]^+$, 976 $[M+H]^+$, 934 $[M+H-42(C_2H_2O)]^+$, 858 $[M+H-118(C_5H_{10}O_3)]^+$. ^{1}H-NMR (py-$d_5$, 600 MHz) δ: superimposable on the spectrum of **1a** except for the signals due to CH_2 of the aglycone moiety. ^{13}C-NMR (py-d_5, 150 MHz) δ: 14.3(16-C), 20.8, 25.4, 26.1, 26.4, 29.3, 29.5, 29.8, 30.0, 30.2, 32.4, 34.5(2-C), 38.5, 38.5, 70.6(11-C), 173.0(1-C) (jalapinolic acid moiety), and the other signals are same as those of **1a** (Table 1).

A solution of D-glucose (90 mg), *p*-anisidine (75 mg) and $NaBH_3CN$ (80 mg) in 10% AcOH–EtOH (10 ml) was left to stand at room temperature for 6 h. It was diluted with water (100 ml), then desalted over MCI gel CHP 20P (H_2O→MeOH). The MeOH eluate was concentrated *in vacuo* and the residue was purified by silica gel chromatography ($CHCl_3$–MeOH–H_2O, 6:4:1) to give a crystalline powder (112 mg, aminoglucitol), mp 102—103 °C, $[\alpha]_D^{26}$ −12.8° (c=1.8, MeOH). Negative ion FAB-MS (TEA) *m/z*: 286 $[M-H]^-$. ^{1}H-NMR (py-d_5, 600 MHz) δ: 3.619 (1H, dd, J=7.4, 12.5 Hz, 1-H_a), 3.78 (1H, dd, J=12.5, 4.6 Hz, 1-H_b), 4.62 (1H, ddd, J=4.6, 4.6, 7.4 Hz, 2-H), 4.67 (1H, dd, J=4.6, 2.2 Hz, 3-H), 4.44 (1H, dd, J=2.2, 7.5 Hz, 4-H), 4.54 (1H, ddd, J=7.5, 5.6, 4.0, 5-H), 4.32 (1H, dd, J=5.6, 10.8 Hz, 6-H_a), 4.43 (1H, dd, J=10.8, 4.0 Hz, 6-H_b). ^{13}C-NMR (py-d_5, 150 MHz) δ: 48.6(1-C), 72.8(2-C), 72.0(3-C), 74.3(4-C), 73.2(5-C), 65.0(6-C).

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