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COMPONENTS OF THE ETHER-INSOLUBLE RESIN GLYCOSIDE-LIKE FRACTION FROM *CUSCUTA CHINENSIS**

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Key Word Index—*Cuscuta chinensis*; Convolvulaceae; resin glycoside; glycosidic acids; cuscutic acids A-D; short chain organic acids; trisaccharide.

Abstract—A trisaccharide and four new glycosidic acids, named cuscutic acids A–D, along with known organic acids, acetic acid, propionic acid, (2*S*)-2-methylbutyric acid, tiglic acid, (2*R*, 3*R*)-nilic acid, (11*S*) convolvulinolic acid and (11*S*)-jalapinolic acid have been isolated from the alkaline hydrolysate of the ether-insoluble resin glycoside-like fraction of the seeds of *Cuscuta chinensis*. The compounds were characterized on the basis of chemical and physical data. © 1998 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

Cuscuta Semen, which is a crude drug prepared from the seeds of *Cuscuta chinensis* LAM., appears as an upper grade drug in Shen Nung's Herbal and is one of the most important traditional Chinese medicines for use as a tonic liver and kidney [1]. Nowadays, the seeds of *C. australis* and *C. japonica* are used as substitutes because of the decline in the production capacity of those of *C. chinensis*.

In our systematic survey of resin glycosides, we have examined the plants *Cuscuta* which is the unique genus having parasitic feature in the Convolvulaceae family. In the previous paper [2], we reported two novel acylated trisaccharides, cus-1 (**1**) and cus-2 (**2**) which are closely related with so-called resin glycosides, from the seeds of *C. chinensis*. During the study, an ether-insoluble resin glycoside-like fraction was obtained and tried to isolate homogeneous compounds from the fraction, but all attempts were unsuccessful. We investigated the component organic and glycosidic acids which are regarded to be the essential components of resin glycoside [3]. This paper deals with the results of isolation and characterization of the components obtained from the alkaline hydrolysate of the fraction.

RESULTS AND DISCUSSION

The fraction under investigation seemed to be a complicated mixture of ether-insoluble resins (Mayer's "convolvulin" [4]), as judged by its insolubility in Et_2O, TLC properties and matrix assisted laser desorption ionization time of flight (MALDI TOF) mass spectrum, which showed many peaks around *m*/*z* 1831, 1957, 2797, 2941, 2925, 3775 and 3911.

Saponification of the fraction afforded Et_2O-soluble and H_2O-soluble portions. The former afforded an oil and a solid. GC analysis of the oil revealed the presence of acetic, propionic, 2-methylbutyric (**3**), tiglic (**4**) and nilic (**5**, 3-hydroxy-2-methylbutyric) acids. Among them, **3** was proved to have the *S*-configuration by Helmchen's method [5]. The absolute configuration of **5** was defined as 2*R*, 3*R* from a comparison of its ^1H NMR spectrum and the specific rotation of its *p*-bromophenacyl ester with those of an authentic sample [6].

Methylation of the hydroxy fatty acid fraction with CH_2N_2 provided the methyl ester (**6a**) of convolvulinolic acid (**6**, 11-hydroxytetradecanoic acid) and the methyl ester (**7a**) of jalapinolic acid (**7**, 11-hydroxyhexadecanoic acid) (GC and FD MS analysis). Further, their absolute configurations at C-11 were determined to be *S* by Mosher's method [7].

The H_2O-soluble portion was subjected to reversed phase chromatography on MCI gel CHP-20P, to furnish monosaccharide and glycosidic acid fractions. The former afforded an oligosaccharide (**8**) by HPLC purification.

Compound **8**, on acidic hydrolysis, furnished rham-

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*Part XXVI in the series "Resin Glycosides". For part XXV: Ono, M., Honda, F., Karahashi, A., Kawasaki, T., *Chem. Pham. Bull.*, 1977, **45**, 1955–1960.

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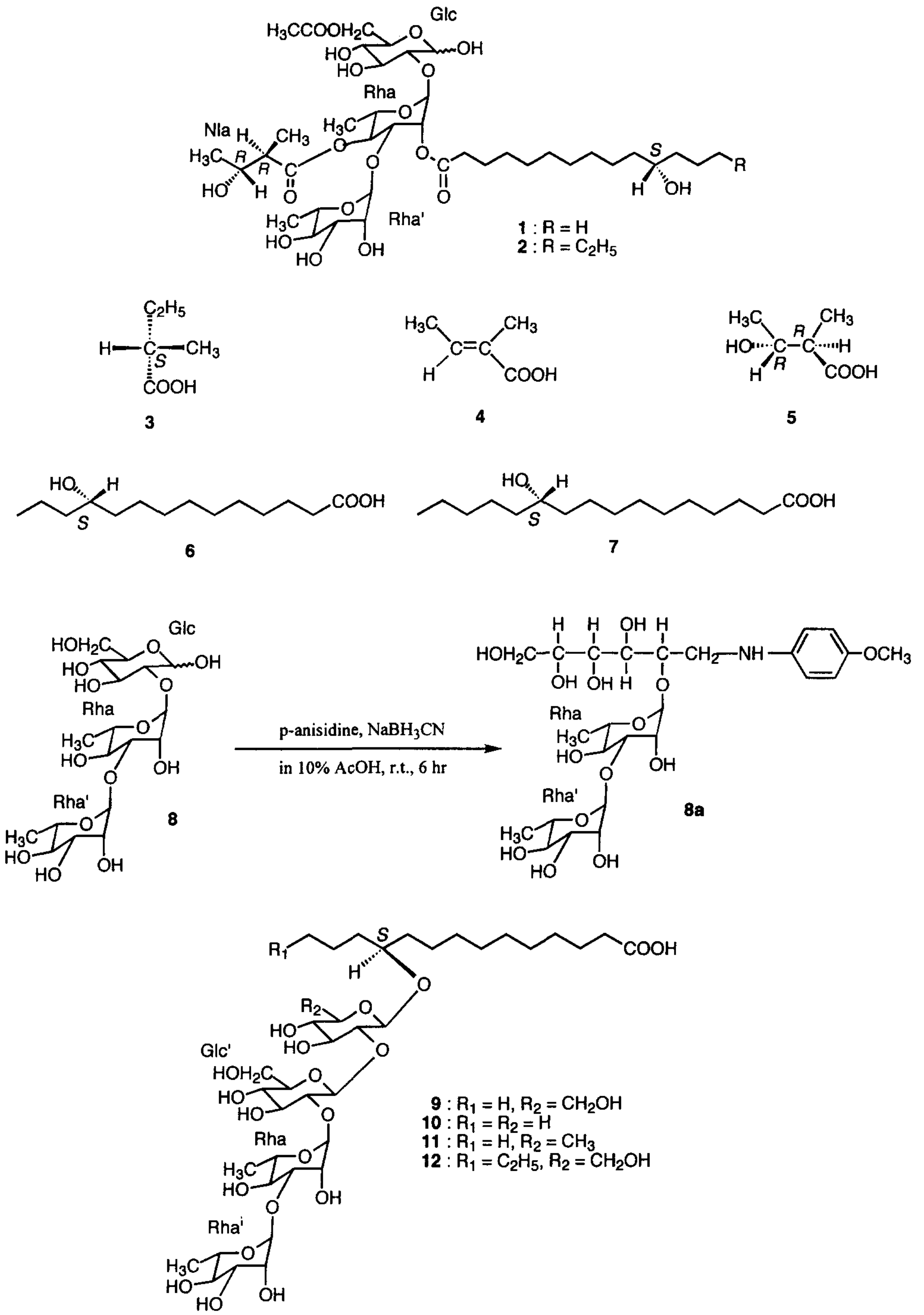

Scheme 1.

nose and glucose, whose absolute configurations were defined as L and D, respectively, by the Hara method [8]. The quasi-molecular ion peak at *m/z* 471 [M−H]$^-$ in the negative ion FAB mass spectrum indicated that **8** consisted of 2 mol of L-rhamnose and 1 mol of D-glucose. However, the ^{1}H NMR spectrum showed six anomeric proton signals, suggesting that **8** was an equilibrium mixture of α- and β-anomers of a trisaccharide with a reducing terminal. When treated with *p*-anisidine and $NaBH_3CN$ [2], **8** afforded an aminoalditol derivative (**8a**). The negative FAB MS of **8a** showed the [M-H]$^-$ ion peak at *m/z* 578 together with fragment ion peaks at *m/z* 432 [578−146 (methylpentose unit)]$^-$, 286 [432−146]$^-$ and 164 [286−122]$^-$, indicating that the glucose unit (Glc) was located at the reducing end. Inspection of the ^{13}C NMR spectra of **8** and **8a** revealed glycosylation shifts [9] for C–3 of the inner rhamnose unit (Rha) and C–2 of the glucose unit (Glc). These findings suggested that **8** corresponded to the sugar part of cus-1 (**1**) and cus-2 (**2**) [2]. Thus the aminoalditol derivative of **1** was deacylated, and the product was identified with **8a** by ^{13}C NMR comparison. Accordingly, **8** is α-L-rhamnopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-D-glucopyranose.

A combination of reverse- and normal-phase HPLC of the glycosidic acid fraction provided four new homogeneous glycosidic acids named cuscutic acids A (**9**), B (**10**), C (**11**) and D (**12**).

Compound **9**, on complete hydrolysis, gave (11*S*)-convolvulinolic acid (**6**) L-rhamnose and D-glucose. The negative FAB mass spectrum of **9** showed the $[M-H]^-$ ion peak at *m/z* 859 along with fragment peaks at *m/z* 713 $[859-146]^-$, 567 $[713-146]^-$, 405 [567–162 (hexose unit)]$^-$ and 243 $[405-162]^-$ (convolvulinolic acid-H)]$^-$ suggesting that the sugar moiety of **9** was a linear tetraglycoside having the sequence of rhamnosyl-rhamnosyl-glucosyl-glucoside. The ^1H and ^{13}C NMR spectra of **9** showed four anomeric proton and carbon signals at δ_H 4.99, 5.69, 6.27 and 5.95, and δ_C 102.4, 102.3, 102.2 and 104.0. In addition a terminal methyl and equivalent 2-methylene protons of the aglycone were observed at δ_H 0.87 (t, $J = 7.4$ Hz), δ_C 14.5 and δ_H 2.51 (t, $J = 7.5$ Hz), δ_C 35.0, respectively (Table 2). The NOESY spectrum of **9** showed cross peaks between H-1 of Rha′ and H-3 of Rha, H-1 of Rha and H-2 of Glc′, H-1 of Glc′ and H-2 of Glc, and between H-1 of Glc and H-11 of the aglycone. Comparing the ^{13}C chemical shifts of **9** and those of methyl pyranosides reported in the literature [9], glycosylation shifts were observed at C-2 of Glc (+5.7 ppm), C-2 of Glc′ (+4.2 ppm) and C-3 of Rha (+7.6 ppm). Further, the coupling constants of the anomeric and methine proton signals as well as the ^{13}C chemical shifts due to the sugar moiety [9] indicated that all the monosaccharide units were pyranoses, and that the modes of glycosidic linkage of Glc and Glc′, were β in the $^4C_1$ conformation and those of Rha and Rha′ were α in the $^1C_4$ conformation.

Accordingly, the structure of **9** was (11*S*)-convolvulinolic acid 11-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside.

Compound **10** gave, on acidic hydrolysis, **6** along with L-rhamnose, D-glucose and D-xylose (ca 2:1:1). The negative FAB mass spectrum of **10** exhibited the $[M-H]^-$ peak at *m/z* 829 and fragment ion peaks at *m/z* 683 $[829-146]^-$, 537 $[683-146]^-$, 375 $[537-162]^-$ and 243 [375–132 (pentose unit)]$^-$, indicating that **10** was also a tetraglycoside which consisted of 1 mol each of **6**, D-xylose, D-glucose and 2 mol of L-rhamnose, and that the inner glucose of **9** was probably substituted by xylose (Xyl) in **10**. The NOESY spectrum of **10** showed the correlations between H-1/H-3 of Rha, H-1 of Rha/H-2 of Glc′, and H-1 of Glc′/H-2 of Xyl. Moreover, in its ^{13}C NMR spectrum (Table 1), glycosylation shifts of 6.6, 3.8 and 7.0 ppm were observed for C-2 of Xyl, C-2 of Glc′, and C-3 of Rha, as well as one of 8.4 ppm for C-11 of **6**, in comparison with methyl convolvulinolate (δ_C 71.7). The modes of glycosidic linkages of Rha and Rha′ were ascertained to be α, and those of Glc and Xyl to be β, respectively, in the same way as above (Table 2).

From these data, **10** was characterized as (11*S*)-convolvulinolic acid 11-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-xylopyranoside.

Compound **11** liberated, on complete acidic hydrolysis, **6**, along with L-rhamnose, D-glucose and D-quinovose in the ratio of about 2:1:1. Its negative FAB mass spectrum showed the $[M-H]^-$ peak at *m/z* 843 and fragment ions at *m/z* 697 $[843-146]^-$, 551 $[697-146]^-$, 389 $[551-162]^-$, and 243 $[389-146]^-$, suggesting that the sugar moiety of **11** was also a linear tetrasaccharide in which D-xylose in **10** was replaced by D-quinovose (Qui). The ^1H and ^{13}C NMR spectra demonstrated that the glycosidic linkages of **11** were placed at 2-OH of Qui, 2-OH of Glc′, 3-OH of Rha and OH of the convolvulinolic acid moiety, and that the glycosidic linkages of rhamnose units were α in the $^1C_4$ conformation and those of glucose and quinovose units were β in the $^4C_1$ conformation (Table 2). The arrangements of the sugar linkages were confirmed by the NOESY spectrum of **11**, which showed cross peaks between H-1 of Rha′/H-3 of Rha, H-1 of Rha/H-2 of Glc′, and H-1 of Glc′/H-2 of Qui.

Taken together the above results showed that **11** is (11*S*)-convolvulinolic acid 11-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)-β-D-quinovopyranoside.

Compound **12** yielded (11*S*)-jalapinolic acid, D-glucose and L-rhamnose by acidic hydrolysis. In the negative FAB mass spectrum, **12** showed peaks at *m/z* 887 $[M-H]^-$, 741 $[887-146]^-$, 595 $[741-146]^-$, 433 $[595-162]^-$ and 271 $[433-162]^-$, all of which are 28 amu less than those of **9**, suggesting that **12** has the same sugar moiety as that of **9**. Its ^1H and ^{13}C NMR spectra were superimposable on those of **9** except for the signals due to the aglycone.

Consequently, the structure of **12** was defined as (11*S*)-jalapinolic acid 11-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside.

Among the components found in the alkaline hydrolysate of the resin glycoside-like fraction in this study, acetic acid, propionic acid, (2*S*)-2-methylbutyric acid, (2*R*, 3*R*)-nilic acid, (11*S*)-convolvulinolic acid, (11*S*)-jalapinolic acid and trisaccharide are also found in cus-1 and cus-2. On taking this and the presence of four new glycosidic acids into account, it is anticipated that the components found in this study as well as cus-1 and cus-2 in the previous report combine with one another via ester linkage to form new types of resin glycosides.

Table 1. NMR spectral data for compounds **8** and **8a** (pyridine-d_5, 600 MHz for ^{1}H and 150 MHz for ^{13}C)

	8				**8a**	
	α-anomer		β-anomer			
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
Glci-1					3.64 *dd* (7.8, 12.9)	46.9
					3.89 *dd* (4.2, 12.9)	
2					4.70 *ddd* (4.2, 4.2, 7.8)	80.4
3					4.82 *dd* (4.2, 1.6)	71.6
4					4.39 *dd* (1.6, 7.8)	72.7
5					4.48 *ddd* (7.8, 5.3, 4.2)	73.3
6					4.29 *dd* (5.3, 11.1)	65.1
					4.41 *dd* (11.1, 4.2)	
Glc-1	5.98 *d* (3.4)	93.4	5.23 *d* (7.5)	97.4		
2	4.16 *dd* (3.4, 9.2)	81.8	4.21 *dd* (7.5, 9.3)	80.7		
3	4.78 *dd* (9.2, 9.2)	74.1	4.23 *dd* (9.3, 9.3)	78.2		
4	4.17 *dd* (9.2, 9.2)	72.7	4.07 *dd* (9.3, 9.3)	72.2		
5	4.71 *ddd* (9.2, 5.4, 2.4)	73.2	3.89 *ddd* (9.3, 5.6, 2.6)	79.3		
6	4.37 *dd* (5.4, 11.6)	63.3	4.28 *dd* (5.6, 11.7)	62.9		
	4.48 *dd* (11.6, 2.4)		4.47 *dd* (11.7, 2.6)			
Rha-1	5.81 *d* (1.6)	104.1	6.28 *d* (1.6)	104.0	5.79 *d* (1.6)	103.2
2	4.91 *dd* (1.6, 3.2)	72.3	4.93 *dd* (1.6, 3.4)	72.4	4.86 *dd* (1.6, 3.2)	72.1
3	4.73 *dd* (9.3, 3.2)	80.6	4.82 *dd* (9.5, 3.4)	80.3	4.61 *dd*(3.2, 9.5)	79.8
4	4.34 *dd* (9.3, 9.3)	74.2	4.46 *dd* (9.5, 9.5)	73.8	4.35 *dd* (9.5, 9.5)	72.8
5	4.90 *dq* (9.3, 6.2)	71.9	5.07 *dq* (9.5, 6.2)	70.2	4.47 *dq* (9.5, 6.3)	70.7
6	1.52 *d* (6.2)	18.4	1.66 *d* (6.2)	18.5	1.55 *d* (6.3)	18.5
Rha′-1	5.87 *d* (1.6)	104.4	5.95 *d* (1.6)	102.4	5.92 *d* (1.4)	103.9
2	4.67 *dd* (1.6, 3.3)	72.3	4.72 *dd* (1.6, 3.3)	72.6	4.68 *dd* (1.4, 3.6)	72.3
3	4.52 *dd* (3.3, 9.3)	72.8	4.56 *dd* (3.3, 9.3)	72.9	4.51 *dd* (3.6, 9.3)	72.7
4	4.22 *dd* (9.3, 9.3)	74.0	4.23 *dd* (9.3, 9.3)	74.2	4.18 *dd* (9.3, 9.3)	74.2
5	4.58 *dq* (9.3, 6.2)	70.0	4.63 *dq* (9.3, 6.1)	69.9	4.57 *dq* (9.3, 6.3)	70.0
6	1.44 *d* (6.2)	18.5	1.51 *d* (6.1)	18.7	1.53 *d* (6.3)	18.6

All assignments are based on ^{1}H-^{1}H COSY, NOESY and ^{1}H-^{13}C COSY spectra. Coupling constants (*J*) are in parentheses. Glci, glucitol residue; Glc, glucopyranosyl; Rha, rhamnopyranosyl.

EXPERIMENTAL

General

The instruments and materials generally used were as cited in the preceding report [2]. MALDI TOF mass spectra were obtained with a Perceptive Voyager Elite spectrometer (laser 451 nm, accelerating voltage 30 kV). Optical rotation was determined at 20°.

Plant material

Seeds of *Cuscuta chinensis* were collected in the central section of Inner Mongolia and conserved in the Laboratory of Natural Products and Instrumental Analysis, Faculty of Pharmaceutical Sciences, Setsunan University, 573-01 Osaka, Japan.

Extraction and purification of the ether-insoluble mixture of resin glycoside-like compounds

Fr. A (16.01 g) described in the preceding paper [2] was repeatedly chromatographed over Sephadex LH-20 (MeOH) to yield a resin glycoside-like fraction (15.98 g, 0.33%); white powder, MALDI TOF MS (matrix; sinapic acid) *m*/*z*: 1831, 1957, 2797, 2941, 2925, 3775 and 3911.

Saponification of the resin glycoside-like fraction

The resin glycoside-like fraction (3.5 g) was dissolved in 3% K_2CO_3 (100 ml) and heated at 95° for 4 hr. After cooling, the reaction mixture was adjusted to pH 4.0 with 1 M HCl and shaken with Et_2O (100 ml × 3). The combined Et_2O layer was washed with H_2O, dried over Na_2SO_4, and concentrated to give an oil (370 mg, organic acid fraction) and a ppt (580 mg, hydroxyfatty acid fraction).

A part of the oil was analyzed by GC [conditions: 2 m × 3.2 mm packed with Unisol 30T (5%); isothermal 110°; N_2 at 0.5 kgcm^{-2}] R_t (min): 2.09 (acetic acid), 3.59 (propionic acid), 5.83 (2-methylbutyric acid), 13.81 (tiglic acid)]. Another part of the oil was esterified with CH_2N_2 in the usual way and the product was examined by GC under the same conditions as above except for the column temp. 100°, R_t (min): 7.38 (methyl nilate).

A part of the precipitate (15 mg) was methylated with CH_2N_2 followed by chromatography over silica gel (*n*-hexane-EtOAc, 5:1) to afford **6a** (11 mg) and **7a** (3 mg).

6a. Colourless plates, mp 33–34°. FD MS *m*/*z*: 259 $[M+H]^+$, 215 $[M\text{-}CH_3(CH_2)]^+$, 73 $[CH_3(CH_2)_2CH(OH)]^+$. Identical with an authentic sample of methyl convolvulinolate by GC [conditions 2 m × 3 mm packed with silicone OV-17; isothermal 240°; N_2 at 1.0 kgcm^{-2}] R_t (min): 4.83. A soln of **6a** (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 temp. overnight. The solvent was removed under a stream of N_2 and the residue was chromatographed over silica gel, *n*-hexane-EtOAc (10:1) to give the (-)-MTPA ester (3 mg) of **6a** as a colorless oil. ^1H NMR (600 MHz, $CDCl_3$): δ 0.850 (3H, *t*, $J=7.3$ Hz, H_3-14), 2.301 (2H, *t*, $J=7.5$ Hz, H_2-2), 3.553 (3H, *q*, $J=1.2$ Hz, OCH_3), 3.666 (3H, *s*, $COOCH_3$), 5.099 (1H, *tt*, $J=5.3$, 7.0 Hz, H-11). The ^1H NMR spectrum was superimposable on that of an authentic sample of the (-)-MTPA ester of methyl (11*S*)-convolvulinolate [7].

7a. Colourless plates, mp 43–44°. EIMS *m*/*z*: 286 $[M]^+$, 255 $[M\text{-}OCH_3]^+$, 215 $[CH(OH)(CH_2)_9COOCH_3]^+$, 101 $[CH_3(CH_2)_4(OH)]^+$, which was identical with an authentic sample of methyl (11*S*)-jalapinolate by GC analysis under the same condition as for **6a**, R_t (min): 7.51. Under the same conditions as used for **6a**, **7a** was treated with (-)-MTPA chloride to afford a colourless oil. ^1H NMR (600 MHz, $CDCl_3$) δ: 0.839 (3H, *t*, $J=6.9$ Hz, H_3-16), 2.299 (2H, *t*, $J=7.5$ Hz, H_2-2), 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, H-11). These data were identical with those of an authentic (-)-MTPA ester of methyl (11*S*)-jalapinolate [7].

The H_2O layer was chromatographed on MCl gel CHP 20P ($H_2O \rightarrow$ MeOH). The H_2O and MeOH eluates were evaporated to afford a white powder (2.03 g, sugar fraction) and a white powder (470 mg, glycosidic acid fraction), respectively.

Isolation and characterization of **8**

A part of the sugar fraction (350 mg) was desalted on a Sephadex LH-20 column (MeOH). The product was successively subjected to silica gel chromatography ($CHCl_3$-MeOH-H_2O, 6:4:0.1) and HPLC (Nucleosil 5-NH_2, 300 mm × 10 mm; 80% MeCN) to give a white powder (**8**, 288 mg), mp 151–154°, $[\alpha]_D$-47.8° ($c=2.5$, MeOH). Negative FAB MS *m*/*z*: 471 $[M-H]^-$. Found: C, 44.01; H, 7.08, $C_{18}H_{32}O_{14}\cdot H_2O$ requires: C, 44.08; H, 6.99. ^1H (600 MHz, pyridine-d_5) and ^{13}C NMR (150 MHz, pyridine-d_5) δ: Table 1.

Compound **8** (5 mg) in 1M H_2SO_4 (1 ml) was heated at 95° for 0.5 hr. The mixture was neutralized with $Ba(OH)_2$, and the precipitates were filtered off. The filtrate was subjected to TLC analysis [Avicel SF; *n*-BuOH-pyridine-H_2O, 6:2:3 top layer + pyridine (1)] R_f: 0.41 (glucose), 0.65 (rhamnose). This mixture was subjected to GC analysis after conversion into the TMSi ethers of methyl thiazolidine-4 (*R*)-carboxylate derivatives according to Hara *et al* [8]. [condition: GL Sciences OV-17, 50 m × 0.25 mm capillary column; isothermal 220°; He at 1.5 kgcm^{-2}] R_t (min): 19.53 (L-rhamnose), 26.86 (D-glucose).

Preparation of aminoalditol derivative **8a**

A soln of **8** (60 mg) in 10% AcOH-EtOH (10 ml) was treated with *p*-anisidine (50 mg) and $NaBH_3CN$ (30 mg), and the reaction mixture was allowed to stand at room temp for 3 hr. After removal of the solvent, the residue was desalted by chromatography over MCI gel CHP 20P ($H_2O \rightarrow$ MeOH). The MeOH eluate was concentrated *in vacuo* and the residue was chromatographed over silica gel ($CHCl_3$-MeOH-H_2O, 6:4:0.2) to give **8a** (76.8 mg) as an amorphous powder, mp 109–112°. Negative ion FAB MS (TEA) *m*/*z*: 578, 432, 286, 164. ^1H (600 MHz, pyridine-d_5); ^{13}C NMR (150 MHz, pyridine-d_5): Table 1.

Alkaline hydrolysis of aminoalditol derivative of **Cus-1**.

The aminoalditol derivative of cus-1 (23 mg) [2] was dissolved in 3% K_2CO_3 (2 ml) and heated at 95° for 4 hr. After cooling, the reaction mixture was adjusted to pH 4.0 with 1 M HCl and shaken with Et_2O (2 ml × 3). The lower layer was desalted by chromatography over MCI gel HP 20P($H_2O \rightarrow$ MeOH). The MeOH eluate was concentrated *in vacuo* and the residue was chromatographed over silica gel ($CHCl_3$-MeOH-H_2O, 6:4:0.2) to afford an amorphous powder (12 mg), mp 110-112°. Its ^{13}C NMR spectrum (150 MHz, pyridine-d_5) was superimposable on that of **8a**.

Isolation and determination of the absolute configuration of 2*-methylbutyric acid* (**3**) *and nilic acid* (**4**)

The organic acid fraction (190 mg) was dissolved in dry Me_2CO (10 ml) and neutralized with triethylamine, then *p*-bromophenancylbromide (280 mg) was added. The mixture was left to stand at room temp. overnight. After removal of the solvent, the mixture was diluted with H_2O (10 ml). The soln was extracted with Et_2O (10 ml × 3) and the extractive was chromatographed over silica gel (*n*-hexane-EtOAc (6:1)$\rightarrow$EtOAc) to give Fr. 1 (63 mg), Fr. 2 (21 mg), Fr. 3 (58 mg) and Fr. 4 (37 mg). Fr. 1 was further purified by HPLC [Kusano CIG Si gel, 100 mm × 22 mm, *n*-hexane-EtOAc (8:1)] to give a white powder. This (28 mg) was heated with 1 M KOH (1 ml) at 95° for 1 hr and the mixture was adjusted to pH 3 with 1 M HCl, diluted with H_2O (3 ml), then extracted with Et_2O

Table 2. NMR spectral data for compounds **9-12** (pyridine-d_5, 600 MHz for ^{1}H and 150 MHz for ^{13}C)

	9		**10**		**11**		**12**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
Glc-1	4.99 d (7.5)	102.4					5.01 d (7.6)	102.5
2	4.27 dd (7.5, 9.4)	80.6					4.29 dd (7.6, 9.3)	80.6
3	4.50 dd (9.4, 9.4)	79.2					4.50 dd (9.3, 9.3)	79.2
4	4.03 dd (9.4, 9.4)	71.9					4.03 dd (9.3, 9.3)	71.9
5	3.91 ddd (9.4, 5.2, 2.6)	77.9					3.92 ddd (9.3, 5.1, 2.5)	77.9
6	4.32 dd (5.2, 11.6)	62.9					4.33 dd (5.1, 11.5)	62.9
	4.47 dd (11.6, 2.6)						4.47 dd (11.5, 2.5)	
Xyl-1			5.04 d (6.5)	102.5				
2			4.26 dd (6.5, 8.3)	81.2				
3			4.44 dd (8.3, 8.3)	78.0				
4			4.18 ddd (8.3, 9.1, 5.4)	70.8				
5			3.73 dd (9.1, 11.3)	66.5				
			4.31 dd (11.3, 5.4)					
Qui-1					4.89 d (7.5)	102.2		
2					4.25 dd (7.5, 9.1)	80.6		
3					4.39 dd (9.1, 9.1)	78.9		
4					3.61 dd (9.1, 9.1)	76.9		
5					3.72 dq (9.1, 6.0)	72.5		
6					1.55 d (6.0)	18.5		
Glc′-1	5.69 d (7.4)	102.3	5.60 d (7.2)	102.8	5.71 d (7.3)	102.0	5.70 d (7.3)	102.4
2	4.24 dd (7.5, 9.1)	79.1	4.23 dd (7.2, 9.3)	78.7	4.23 dd (7.3, 9.0)	79.0	4.24 d (7.3, 9.1)	79.1
3	4.19 dd (9.1, 9.1)	79.0	4.20 dd (9.3, 9.3)	79.1	4.19 dd (9.0, 9.0)	79.1	4.20 dd (9.1, 9.1)	79.0
4	4.08 dd (9.1, 9.1)	72.6	4.12 dd (9.3, 9.3)	72.4	4.08 dd (9.0, 9.0)	72.6	4.08 dd (9.1, 9.1)	72.7
5	3.82 ddd (9.1, 5.6, 3.0)	77.3	3.83 ddd (9.3, 5.6, 3.0)	77.5	3.83 ddd (9.0, 5.8, 3.0)	77.4	3.82 ddd (9.1, 5.5, 3.0)	77.3
6	4.25 dd (5.6, 11.5)	63.3	4.29 dd (5.6, 11.5)	63.1	4.26 dd (5.8, 11.3)	63.2	4.25 dd (5.5, 11.5)	63.4
	4,43 dd (11.5, 3.0)		4.43 dd (11.5, 3.0)		4.43 dd (11.3, 3.0)		4.44 dd (11.5, 3.0)	
Rha-1	6.27 d (1.8)	102.2	6.31 d (1.8)	102.1	6.28 d (1.8)	102.2	6.28 d (1.6)	102.2
2	4.93 dd (1.8, 3.2)	71.9	4.93 dd (1.8, 3.2)	71.9	4.93 dd (1.8, 3.2)	71.8	4.94 dd (1.6, 3.0)	71.9
3	4.79 dd (3.2, 9.4)	80.1	4.82 dd (3.2, 9.5)	79.5	4.79 dd (3.2, 9.2)	79.9	4.80 dd (3.0, 9.3)	80.1
4	4.42 dd (9.4, 9.4)	72.9	4.42 dd (9.5, 9.5)	73.0	4.43 dd (9.2, 9.2)	73.0	4.43 dd (9.3, 9.3)	72.9
5	5.01 dq (9.4, 6.2)	69.9	5.02 dq (9.5, 6.3)	69.8	5.01 dq (9.2, 6.3)	69.8	5.02 dq (9.3, 6.2)	69.9
6	1.75 d (6.2)	18.9	1.73 d (6.3)	18.9	1.75 d (6.3)	18.8	1.76 d (6.2)	18.9

Rha′-1	5.95 d (1.6)	104.0	5.96 d (1.6)	104.1	5.95 d (1.6)	103.9	5.95 d (1.5)	104.0
2	4.67 dd (1.6, 3.4)	72.4	4.68 dd (1.6, 3.4)	72.4	4.68 dd (1.6, 3.4)	72.4	4.68 dd (1.5, 3.3)	72.4
3	4.58 dd (3.4, 9.2)	72.7	4.59 dd (3.4, 9.3)	72.7	4.59 dd (3.4, 9.3)	72.7	4.59 dd (3.3, 9.2)	72.7
4	4.21 dd (9.2, 9.2)	74.2	4.21 dd (9.3, 9.3)	74.2	4.22 dd (9.3, 9.3)	74.2	4.22 dd (9.2, 9.3)	74.3
5	4.68 dq (9.2, 6.3)	70.0	4.69 dq (9.3, 6.2)	69.9	4.69 dq (9.3, 6.2)	70.0	4.69 dq (9.3, 6.2)	70.0
6	1.54 d (6.3)	18.5	1.56 d (6.2)	18.5	1.57 d (6.2)	18.5	1.55 d (6.2)	18.5
Ag-1		176.2		176.1		176.1		176.2
2	2.51 t (7.5)	35.0	2.51 t (7.5)	34.9	2.51 t (7.5)	34.9	2.51 t (7.5)	35.0
11	3.95 m	80.2	3.87 m	80.1	3.91 m	80.0	3.96 m	80.2
14	0.87 t (3H, 7.4)	14.5	0.91 t (3H, 7.2)	14.5	0.93 t (3H, 7.3)	14.4		
16							0.82 t (3H, 7.2)	14.3

All assignments are based on ^{1}H-^{1}H COSY, NOESY and ^{1}H-^{13}C COSY spectra. Coupling constants (*J*) are in parentheses. Glc, glucopyranosyl; Xyl, Xylopyranosyl; Qui, quinovopyranosyl; Rha, rhamnopyranosyl; Ag, aglycone (convolvulinolic acid or jalapinolic acid residue).

(5 ml × 3). The combined Et$_2$O layer was evaporated to furnish an oil (9 mg, 2-methylbutyric acid).

Two drops of SOCl$_2$ were added the oil (9 mg in dry benzene (3 ml), and the soln was refluxed for 15 min. (*S*)-(-)-1-Phenethylamine (30 mg) was added and the mixture was refluxed for another 30 min poured into ice water (3 ml) and extracted with EtOAc (5 ml). The EtOAc layer was washed with 0.1 M HCl (5 ml), 5% NaHCO$_3$ (5 ml) and H$_2$O (5 ml), successively. The EtOAc layer was concentrated and the residue chromatographed on silica gel (*n*-hexane-EtOAc), 5:1) to furnish colourless needles (3 mg), mp 85 . ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.25–7.32 (5H, *arom.* H), 5.66 (1H, br *s*, NH), 5.15 (1H, *dq*, $J=7.0$, 7.0 Hz, NCH), 2.08 (1H, *ddq*, $J=7.0$, 7.0, 7.0 Hz, H-2), 1.65, 1.42 (each 1H, *ddq*, $J=13.5$, 7.5, 7.0 Hz, H$_2$-3), 1.49 (3H, *d*, $J=7.0$ Hz, NHCHCH$_3$), 1.14 (3H, *d*, $J=7.0$ Hz, H$_3$-5), 0.86 (3H, *dd*, $J=7.5$, 7.5 Hz, H$_3$-4). HPLC [150 mm × 4.0 mm packed with Lichrosorb Si-60; UV (254 nm); *n*-hexane-EtOAc (4:1) at 1.5 ml/min] *Rt*: 5.4 min[(*S*)-(-)-N-1-phenylethyl-(*S*)-2-methylbutyramide [*cf.* (*S*)-(-)-N-1-phenylethyl-(*R*)-2-methylbutyramide, R_t: 4.5 min].

Fr. 4 (11 mg) was further purified by HPLC [Kusano CIG Si gel, 100 mm × 22 mm; *n*-hexane-EtOAc (6:1)] to yield colourless needles (4 mg), mp 68-70 , [α]$_D$-13.8 (*c*=0.4, CHCl$_3$). ^{1}H NMR (CDCl$_3$, 400 MHz): δ 1.25 (3H, *d*, $J=7.0$ Hz, H$_3$-4), 1.30 (3H, *d*, $J=7.0$ Hz, H$_3$-5), 2.62 (1H, *dq*, $J=7.0$, 7.0 Hz, H-2), 3.97 (1H, *dq*, $J=7.0$, 6.5 Hz, H-3), 5.33, 5.43 (each 1H, *d*, $J=16.5$ Hz, C*H*$_2$CO-Ph), 7.63, 7.79 (each 2H, *ddd*, $J=8.5$, 2.0, 2.0 Hz, *arom.* H). These data were identical with those of an authentic sample of *p*-bromophenacyl (2*R*, 3*R*)-nilate.

Isolation of glycosidic acids **9**, **10**, **11** *and* **12**.

The glycosidic acid fraction (280 mg) was subjected to prep HPLC on Inertsil ODS (GL Sciences, 250 mm × 20 mm, 80% MeOH) to give fr. 5 (57 mg), fr. 6 (81 mg), fr. 7 (98 mg) and fr. 8 (43 mg). Fr. 5 was chromatographed over Cosmosil 75 C$_{18}$-OPN (75% MeOH) to give **9** (39 mg). Fractions 6 (30 mg), 7 (35 mg) and 8 (38 mg) were each subjected to HPLC [Chemcosorb Si-5, CHCl$_3$-MeOH-H$_2$O (6:4:0.8)] to afford **10** (12 mg), **11** (11 mg) and **12** (15 mg), respectively.

9. White powder, mp 128–131 , [α]$_D$-66.2 (*c*=2.55, MeOH). Negative ion FAB MS *m/z*: 859, 713, 576, 405, 243. Found: C, 53.09; H, 7.87. C$_{38}$H$_{68}$O$_{21}$ requires: C, 53.01; H, 7.96; ^{1}H NMR (600 MHz, pyridine-d_5) and ^{13}C NMR (150 MHz, pyridine-d_5): Table 2.

10. White powder, mp 119–123 , [α]$_D$-81.3 (*c*=1.09, MeOH). Negative FAB MS *m/z*: 829, 683, 537, 357, 243. Found: C, 53.41; H, 8.13. C$_{37}$H$_{66}$O$_{20}$ requires: C, 53.48; H, 8.01; ^{1}H NMR (600 MHz, pyridine-d_5) and ^{13}C NMR (150 MHz, pyridine-d_5): Table 2.

11. White powder, mp 118–122 , [α]$_D$-77.0 (*c*=0.89, MeOH). Negative FAB MS *m/z*: 843, 697, 551, 389, 243. Found: C, 54.07; H, 8.15. $C_{38}H_{68}O_{20}$ requires: C, 54.01; H, 8.11; ^{1}H NMR (600 MHz, pyridine-d_5) and ^{13}C NMR (150 MHz, pyridine-d_5): Table 2.

12. White powder, mp 121–124°, $[\alpha]_D$-63.7° ($c = 1.21$, MeOH). Negative FAB MS *m/z*: 887, 741, 595, 433, 271. Found: C, 54.11; H, 8.21. $C_{40}H_{72}O_{21}$ requires: C, 54.04; H, 8.16; ^{1}H NMR (600 MHz, pyridine-d_5) and ^{13}C NMR (150 MHz, pyridine-d_5): Table 2.

Acidic hydrolysis of **9**, **10**, **11** *and* **12**

Compounds **9** (11 mg), **10** (9 mg), **11** (8 mg) and **12** (9 mg) were separately dissolved in 1 M H_2SO_4 (2 ml) and heated at 95° for 1 hr. The reaction mixture was diluted with H_2O (2 ml) and extracted with Et_2O (2 ml × 3). The Et_2O layer was washed with H_2O and dried over Na_2SO_4 and then treated with CH_2N_2 in Et_2O. The mixture was evaporated to give colourless needles (3 mg from **9**, 2 mg each from **10**, **11** and **12**). The products was examined by GC (conditions as for **6a**), R_t: (min): 4.81 (methyl convolvulinolate) for **9**, **10** and **11**, and 8.37 (methyl jalapinolate) for **12**. The absolute configurations at C-11 of methyl convolvulinolate and methyl jalapinolate were both determined to be *S* by Mosher's method [7] as for **6** and **7**, respectively.

Each aq layer was neutralized with 3% KOH and desalted by chromatography over LH-20 (MeOH) to afford a sugar mixture as a syrup. They were analyzed by GC according to Hara et al. as for **8** described above, R_t: (min): 19.13 (L-rhamnose) and 27.02 (D-glucose) from **9**; 17.27 (D-xylose), 19.18 (L-rhamnose) and 26.98 (D-glucose) from **10**; 18.37 (D-quinovose), 19.18 (L-rhamnose) and 27.01 (D-glucose) from **11**; 19.17 (L-rhamnose) and 27.01 (D-glucose) from **12**.

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REFERENCES

1. Jiang Su New Medicinal College, in *Dictionary of Chinese Crude Drugs*, Scientific Technologic Publisher, Shanghai, 1977, p. 2006.
2. Miyahara, K., Du, X-M., Watanabe, M., Sugiura, C., Yahara, S. and Nohara, T., *Chem. Pharm. Bull.*, 1996, **44**, 481.
3. Wagner, H., in *Medicine and Natural Sciences, Chemistry in Botanical Classification*, ed. by Bendz, G., Santesson, J., Academic Press, New York and London, 1974, pp. 235–240.
4. Mayer, W., *Justus Liebigs Ann. Chem.*, 1855, **95**, 129.
5. Helmchen, G., Volter, H. and Schule, W., *Tetrahedron Lett.*, **1977**, 1417.
6. Ono, M., Noda, N., Kawasaki, T. and Miyahara, K., *Chem. Pharm. Bull.*, 1990, **38**, 1892.
7. Ono, M., Yamada, F., Noda, N., Kawasaki, T. and Miyahara, K., *Chem. Pharm. Bull.*, 1993, **41**, 1023.
8. Hara, S., Okabe, H. and Mihashi, K., *Chem. Pharm. Bull.*, 1987, **35**, 501.
9. Tanaka, O., *Yakugaku Zassshi*, 1985, **105**, 323.