Steroidal Glycosides from *Dregea sinensis* var. *corrugata* Screened by Liquid Chromatography−Electrospray Ionization Tandem Mass Spectrometry

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Received January 23, 2008

The MS fragmentation behavior of the C-21 steroidal glycosides from *Dregea sinensis* var. *corrugata* was investigated by positive and negative ion electrospray ionization MS using a multistage tandem mass spectrometer equipped with an ion trap analyzer. The mass fragmentation patterns of steroidal glycosides substituted with an orthoacetate group were summarized, and the fragmentation patterns were applied to the online structure identification of the steroidal glycosides in the extract. Eighteen new C-21 steroidal glycosides were identified by means of HPLC-HRESIMS and HPLC-DAD-ESIMS^n. Three new compounds (**1**, **4**, and **7**) were identified by HPLC-DAD-ESIMS^n, and their structures were elucidated by use of 1D and 2D NMR methods. The structures identified by MS are fully consistent with those elucidated by NMR data. The present study shows that HPLC-DAD-ESIMS^n can be used as an effective tool to rapidly identify compounds and guide the isolation of target compounds from crude plant extracts.

Dregea sinensis var. *corrugata* belongs to the family Asclepiadaceae and has been widely used as an antiepileptic and diuretic in China.[1] In previous papers, we have reported the isolation of a series of C-21 steroidal glycosides, including nine new steroidal glycosides substituted with orthoacetate groups, from the roots of the plant.[2,3] Since the structural complexity of this type of compound complicates their isolation by traditional chromatographic techniques, improved methods are needed to guide their purification.

HPLC-DAD-ESIMS^n has been extensively applied and plays an increasingly important role in the online analysis of natural products. This technique has also proven to be a convenient method to detect the compounds that are undetectable by other methods. It provides fragmentation patterns and the corresponding masses of fragmentation ions, which are of great importance in determining the structures of natural products.[4−17]

In the present paper, the fragmentation behavior of 17 steroidal glycosides (**S1**−**S17**)[2,3] was investigated by ESIMS^n in both positive and negative ion modes. The MS fragmentation pathways of different types of steroidal glycosides were studied and the patterns of the MS fragmentation summarized. HPLC-HRMS and HPLC-DAD-ESIMS^n experiments were then performed to identify the compounds in three fractions from *D. sinensis* var. *corrugata*. As a result, 30 compounds, including 18 new compounds, were identified. On the basis of the results of the online screening and identification by HPLC-HRMS and HPLC-DAD-ESIMS^n, three of the 18 compounds (**1**, **4**, and **7**) were further isolated and their structures were unambiguously elucidated by NMR spectroscopic data. The absolute configurations of the sugars were identified by use of chemical methods.

Table 1. Key ESIMS and ESIMS^n Fragment Ions for **S1**−**S17**

compounds	$[M + Na]^+$	MS^n fragment ions
S1	1090	967, 942, 924, 819, 801, 783, 775, 489, 471
S2	1089	967, 923, 601, 575, 471, 327
S3	1090	967, 942, 924, 923, 924, 906, 819, 801, 471, 327
S4	1115	967, 949, 947, 471, 329
S5	1234	1086, 963, 945, 615, 489, 471, 327
S6	981	965, 947, 601, 489, 471
S7	1113	965, 947, 905, 605, 489, 471
S8	1087	965, 947, 905, 601, 575, 471, 442, 329, 272
S9	1041	981, 919, 921, 859, 799, 471, 327
S10	1203	1143, 1081, 1021, 633, 489, 403
S11	1347	1287, 1227, 1225, 1165, 1105, 777, 633, 489, 403
S12	1365	1305, 1183, 1245, 1243, 1123, 1093, 795, 651, 633, 489
S13	1509	1449, 1387, 1327, 1267, 939, 795, 651, 489
S14	1041	981, 919, 921, 859, 799, 471, 327
S15	1203	1143, 1081, 1021, 633, 489, 403
S16	1347	1287, 1227, 1225, 1165, 1105, 633, 489, 403
S17	1365	1287, 1225, 1165, 1105, 1227, 777, 633, 489

Results and Discussion

MS Fragmentation Pathways of Compounds S1−S17. ESIMS^n in both negative and positive modes was performed on compounds **S1**−**S17**, which were isolated in our previous study.[2,3] These glycosides all possess a sugar moiety linked at C-3. The sugar moieties are composed of a linear rather than a branched saccharide chain. The sugar residues are the 6-deoxyhexoses (thevetose), 2,6-dideoxyhexoses (cymarose, oleandrose, and digitoxose), and glucose. The linkage modes of the sugars are all 1→4. In tandem mass experiments, fewer product ions were observed in the negative mode. The $[M + Na]^+$ and product ions were in sufficient abundance in the positive mode for MS^n analysis. Therefore, positive ESIMS^n was selected for all analyses. The $[M + Na]^+$ ion was observed in (+) ESIMS of compounds **S1**−**S17**. Acetic acid (0.03%) was added to the solvent to increase the intensity of the fragmentation ions.

On the basis of their aglycones, compounds **S1**−**S17** were classified into four types (type **A**, **B**, **C**, and **D**) (see Figure 1). The

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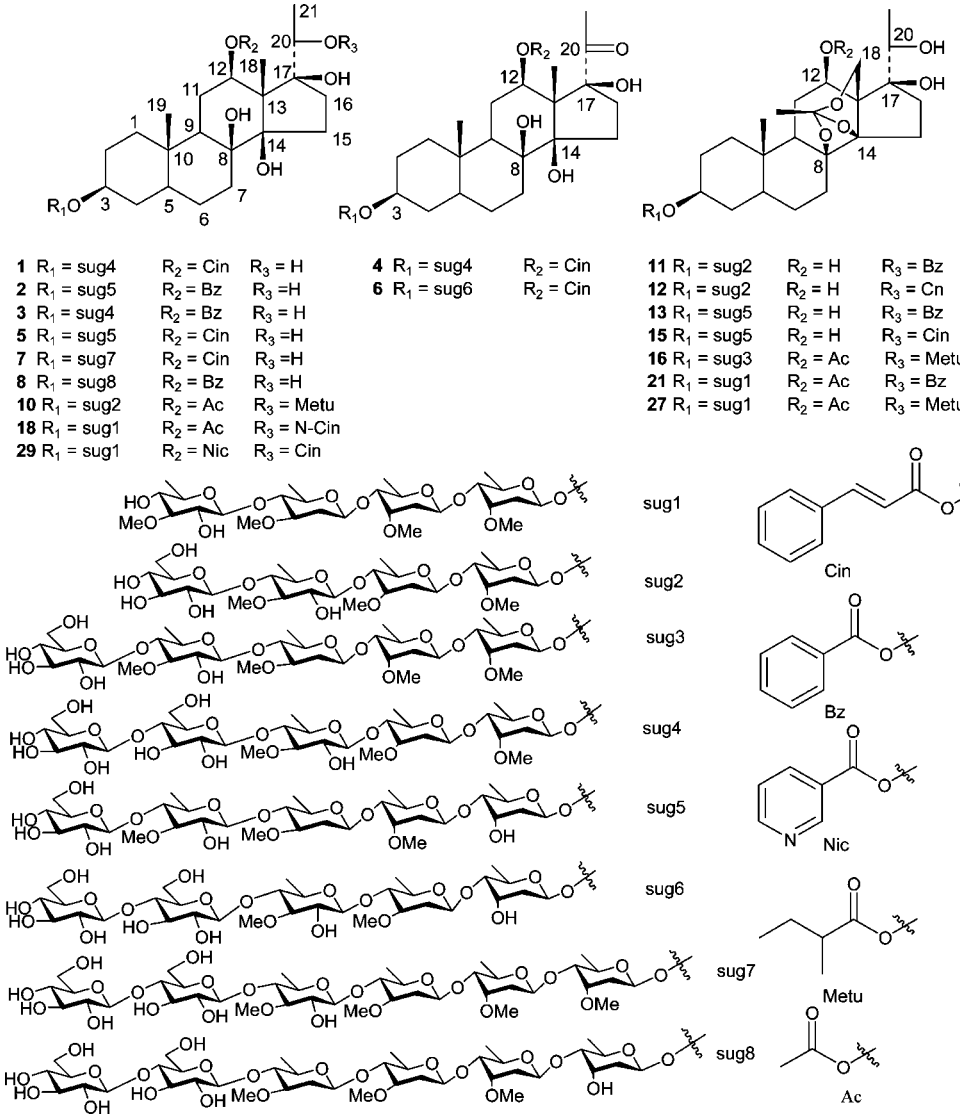
10.1021/np800048n CCC: $40.75
Published on Web 02/09/2009

Chart 1



following MS fragmentation patterns of the steroidal glycosides were observed:

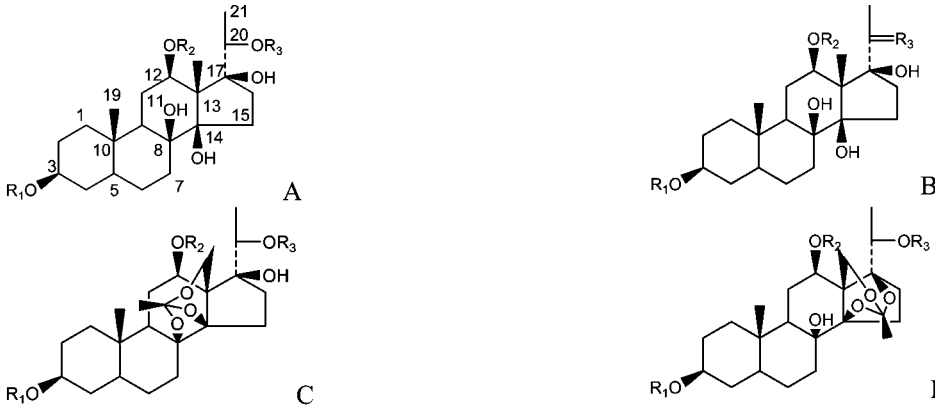
Type **A** (**S1**−**S6**): Ions resulting from loss of acetic acid (−60 Da), benzoic acid (−122 Da), cinnamic acid (−148 Da), nicotinic acid (−123 Da), and H_2O (−18 Da) were observed in the ESIMS^n spectra (Table 1 and Scheme 1). ESIMS^n spectra and proposed fragmentation pathways of **S1** are shown in Scheme 1a as an example.

Type **B** (**S7**, **S8**): C-20 of the **B**-type compounds was oxidized to carbonyl groups. An ion due to loss of acetaldehyde (−42 Da) was shown in the ESIMS^n. This was not observed for the other types. ESIMS^n spectra and proposed fragmentation pathways of **S8** are shown in Scheme 1b.

Types **C** (**S9**−**S14**) and **D** (**S15**−**S17**): Compounds of types **C** and **D** were substituted with orthoacetate groups at C-8, C-14, C-17 (type **C**) and at C-14, C-17, C-18 (type **D**). Besides the ions due to loss of acetic acid (−60 Da) and benzoic acid (−122 Da), another ion found in MS^2 and MS^3 spectra of compounds of types **C** and **D** was assigned as one due to loss of acetic acid (−60 Da) as a result of the rearrangement of the orthoacetate group. Because the orthoacetate group affords two degrees of unsaturation and the acetyl only one, the ions due to the loss of acetic acid can be considered as the diagnostic fragment ions useful for identifying the presence of an orthoacetate group. This can be done by calculating the degree of unsaturation of corresponding fragmentation ions. There were no distinguishable differences between the fragmentation ions of compounds of types **C** and **D**. The proposed fragmentation pathways of **S11** (type **C**) and **S16** (type **D**) are shown in Schemes 1c,d.

The fragmentation nomenclature of sugar units is based on the rules described by Costello.[18,19] The main fragmentation mode of the sugar chain residues was glycosidic cleavage. The B_n ions were key to identifying the sugar units. In the discussion of the fragmentation pathways of the sugar chains, the fragmentation pattern of **S11** was used as an example (Table 1). There were three major fragment ions in the ESIMS^n spectra of **S11** in positive ion mode: B_5, B_4, and B_3 at *m*/*z* 777, 633, and 489. The weak B_5 ion at *m*/*z* 777 represented the intact sugar moiety. The B_4 ion at *m*/*z* 633 was produced by cleavage of the inner cymarosyl unit from the B_5 ion. The outer cymarosyl unit of the B_4 ion was cleaved to generate the B_3 ion at *m*/*z* 489.

Identification of Steroidal Glycosides Present in Fractions I, II, and III from *D. sinensis* var. *corrugata*. HPLC-HRESIMS and HPLC-DAD-ESIMS^n experiments were performed on fractions **I** (Figure 2), **II** (Figure 3), and **III** (Figure 4). Similar fragmentation patterns to **S1**−**S17** were observed. HPLC-HRESIMS was performed in the positive ion ESI mode to afford their accurate masses and molecular formulas (Table 2). HPLC-DAD-ESIMS^n experiments provided their UV spectra, fragmentation patterns, and the corresponding masses of fragment ions (see Tables 3 and 4).



compounds	types	R_1	R_2	R_3
S1	A	thev-(1→4)-olean-(1→4)-cym	Cin	Nic
S2	A	thev-(1→4)-olean-(1→4)-digt-(1→4)-cym	Benz	H
S3	A	thev-(1→4)-olean-(1→4)-cym	Nic	Cin
S4	A	thev-(1→4)-olean-(1→4)-digt-(1→4)-cym	Cin	OH
S5	A	thev-(1→4)-olean-(1→4)-cym-(1→4)-cym	Cin	Nic
S6	A	thev-(1→4)-olean-(1→4)-cym	Ac	Mebu
S7	B	thev-(1→4)-olean-(1→4)-digt-(1→4)-cym	Cin	O
S8	B	thev-(1→4)-olean-(1→4)-digt-(1→4)-cym	Benz	O
S9	C	thev-(1→4)-olean-(1→4)-cym	Ac	Benz
S10	C	glc-(1→4)-thev-(1→4)-olean-(1→4)-cym	Ac	Benz
S11	C	glc-(1→4)-thev-(1→4)-olean-(1→4)-cym-(1→4)-cym	Ac	Benz
S12	C	glc-(1→4)-glc-(1→4)-thev-(1→4)-olean-(1→4)-cym	Ac	Benz
S13	C	glc-(1→4)-glc-(1→4)-thev-(1→4)-olean-(1→4)-cym-(1→4)-cym	Ac	Benz
S14	C	thev-(1→4)-olean-(1→4)-cym	Ac	Benz
S15	D	glc-(1→4)-thev-(1→4)-olean-(1→4)-cym	Ac	Benz
S16	D	glc-(1→4)-glc-(1→4)-thev-(1→4)-olean-(1→4)-cym	Ac	Benz
S17	D	glc-(1→4)-glc-(1→4)-thev-(1→4)-olean-(1→4)-cym	Ac	Benz

Figure 1. Structures of compounds **S1**−**S17**.

Eight new compounds (**1**−**8**) and one known compound (**9**) were identified by analysis of their ESIMS^n ions from Fr. **I** (Table 3).

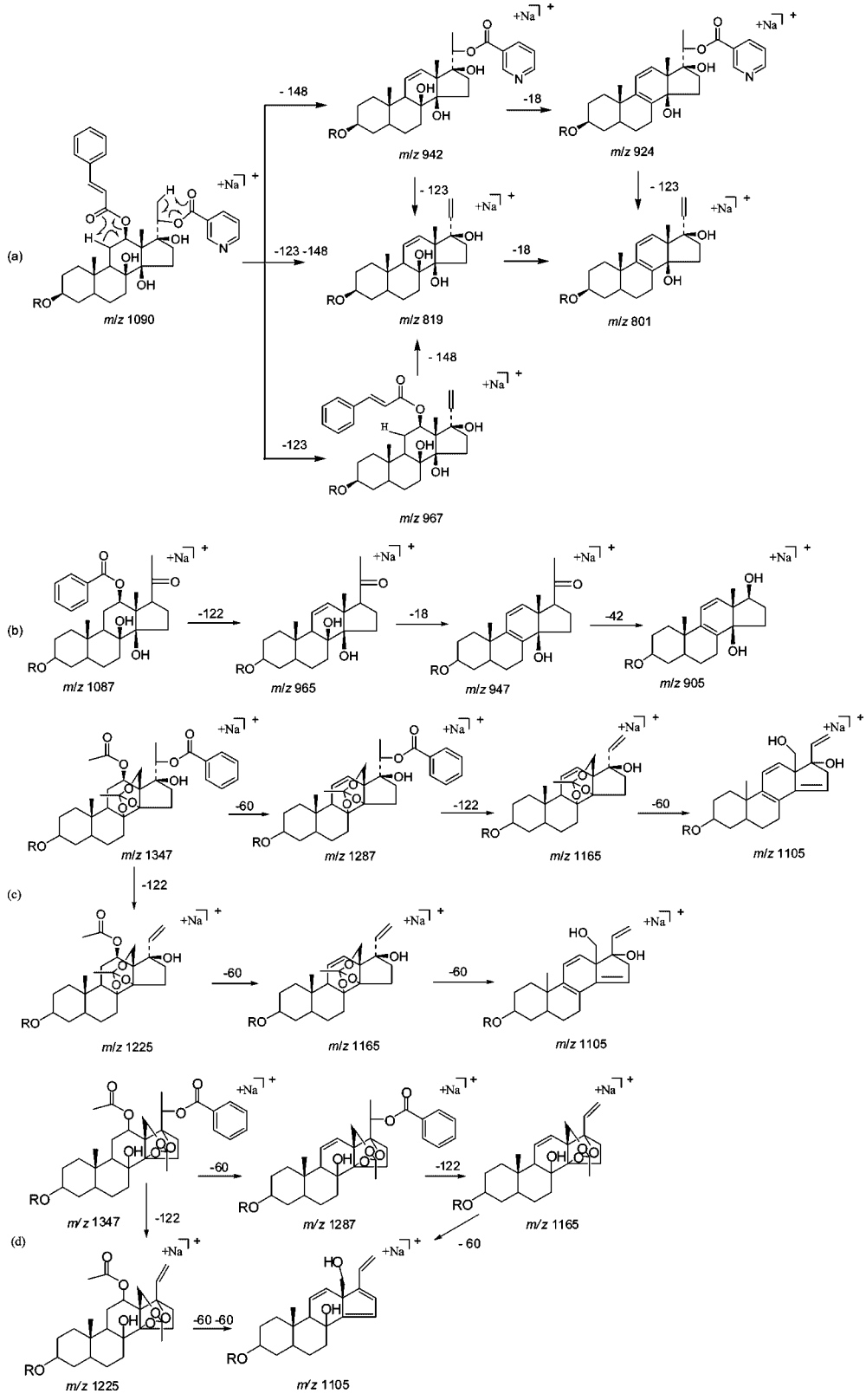
The HPLC-DAD-UV spectrum of **1** showed a UV absorption maximum at 280 nm, indicating the presence of an α,β-unsaturated carbonyl group. The molecular formula of **1** was determined as $C_{63}H_{98}O_{27}$ on the basis of HPLC-HRESIMS, which gave *m/z* 1309.6175 (calcd for $C_{63}H_{98}O_{27}Na$: 1309.6193). The ESIMS^n showed an $[M + Na]^+$ ion at 1309. The *m/z* 795 ion represented the fragment ion (B_5) bearing an intact sugar unit, which was the same ion as that in **S12**. The ions at *m/z* 651 and 507 corresponded to B_4 and B_3 ions, respectively. The Y_n ions at *m/z* 999 (Y_4), 837 (Y_3), 677 (Y_2), and 533 (Y_1) were, respectively, due to the loss of the outer glucosyl, inner glucosyl, one thevetosyl, and one oleandrosyl unit from the ion at *m/z* 1161. On the basis of the above ions and the cross-ring cleavage fragment ions $^{3,5}A_3$ at *m/z* 405 and A_4 at *m/z* 566, the sugar unit was proposed as *O*-glucopyranosyl-(1→4)-*O*-glucopyranosyl-(1→4)-*O*-thevetopyranosyl-(1→4)-*O*-oleandropyranosyl-(1→4)-*O*-cymaropyranoside. Thus, the structure of **1**, belonging to type **A**, was determined as 12-*O*-cinnamoyl dihydrosarcostin 3-*O*-glucopyranosyl-(1→4)-*O*-glucopyranosyl-(1→4)-*O*-thevetopyranosyl-(1→4)-*O*- oleandropyranosyl-(1→4)-*O*-cymaropyranoside (see Scheme 2).

The HPLC-HRESIMS analysis of **4** gave an $[M + Na]^+$ ion at *m/z* 1307.5951 (calcd for $C_{63}H_{96}O_{27}Na$: 1307.6037), which suggested the molecular formula of **4** as $C_{63}H_{96}O_{27}$. The loss of 42 Da from the ion at *m/z* 1141 produced the ion at *m/z* 1099, suggesting that **4** belongs to type **B**. According to the B_n ions at *m/z* 795 (B_5) and 651 (B_4), the Y_n ions at *m/z* 997, 835, and 675, and the cross-ring cleavage fragment ion $^{3,5}A_4$ at *m/z* 565, the sugar unit was assigned as glucopyranosyl-(1→4)-*O*-glucopyranosyl-(1→4)-*O*- thevetopyranosyl-(1→4)-*O*-oleandropyranosyl-(1→4)-*O*-cymaropyranoside. Therefore, the structure of **4** was proposed as 12-*O*-cinnamoyltayloron 3-*O*-glucopyranosyl-(1→4)-*O*-glucopyranosyl-(1→4)-*O*-thevetopyranosyl-(1→4)-*O*-oleandropyranosyl-(1→4)-*O*-cymaropyranoside.

The same experimental procedures were further employed to screen the steroidal glycosides present in Fr. **II** and **III** and led to the identification of 10 new compounds (**10**, **11**, **12**, **13**, **15**, **16**, **18**, **21**, **27**, and **29**). Eleven compounds (**14**, **17**, **19**, **20**, **22**, **23**, **24**, **25**, **26**, **28**, and **30**) were also identified as the known compounds **S17**, **S10**, **S15**, **S14**, **S1**, **S2**, **S3**, **S8**, **S4**, **S7**, and **S5**, respectively (Table 3, Figures 3 and 4).

Compound **11** is used for the discussion of the MS data. According to the HPLC-HRESIMS ion at *m/z* 1161.5687 $[M + Na]^+$, the molecular formula of **11** was determined as $C_{57}H_{86}O_{23}Na$. Ions at *m/z* 1161 $[M + Na]^+$, 1039 $[1161 - 122]^+$, 979 $[1039 - 60]^+$, and 949 $[979 - 30]^+$ were observed in the ESIMS^n spectra. The ions at *m/z* 1039 and 979 were, respectively, due to the loss of one benzoic acid unit from the ion at *m/z* 1161 and one acetic acid moiety from the ion at *m/z* 1039. The loss of the acetic acid moiety was due to the cleavage of an orthoacetate group. According to the B_4 ion at *m/z* 633, the B_3 ion at *m/z* 489, Y_3 ion at *m/z* 877, Y_2 ion at *m/z* 717, Y_1 ion at *m/z* 571, and the cross-ring cleavage fragment ions $^{3,5}A_3$ at *m/z* 403 and A_4 at *m/z* 547, the sugar unit was identified as glucopyranosyl-(1→4)-*O*-thevetopyranosyl-(1→4)-*O*-oleandropyranosyl-(1→4)-*O*-cymaropyranosyl. Thus, there are two possible structures for **11** depending on the positions at which the orthoacetic oxygen atoms are attached in the aglycone: 12-*O*-benzoyl-8,14,17-orthoacetate-dihydrosarcostin 3-*O*-glucopyranosyl-(1→4)-*O*- thevetopyranosyl-(1→4)-*O*-oleandropyranosyl-(1→4)-*O*-

Scheme 1. (a) Proposed Fragmentation Pathways of **S1** Type **A**; (b) Proposed Fragmentation Pathways of **S8** Type **B**; (c) Proposed Fragmentation Pathways of **S11** Type **C**; (d) Proposed Fragmentation Pathways of **S16** Type **D**



cymaropyranoside or 12-*O*-benzoyl-14,17,18-orthoacetate-dihydrosarcostin 3-*O*-glucopyranosyl-(1→4)-*O*-thevetopyranosyl-(1→4)-*O*-oleandropyranosyl-(1→4)-*O*-cymaropyranoside (see Scheme 3).

Structural Elucidation of Compounds 1, 4, and 7 by NMR Spectroscopy. Three compounds (**1**, **4**, and **7**) which were abundant in Fr. **I** were isolated. Compounds **1**, **4**, and **7** all showed positive Liebermann−Buchard and Keller−Kiliani reactions, suggesting that they were steroidal glycosides with 2-deoxysugar moieties. The IR spectra of **1**, **4**, and **7** all showed the absorption bands for hydroxy, carbonyl, and phenyl groups. Compounds **1**, **4**, and **7** were hydrolyzed, and the constituent sugars were confirmed to be D-type deoxysugars by comparison of their TLC and HPLC profiles with authentic sugar samples.[20]

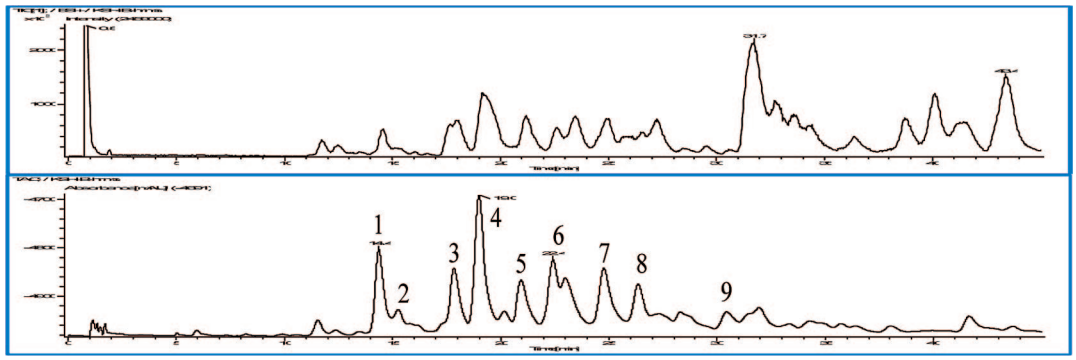


Figure 2. Liquid chromatogram and total ion chromatogram of Fr. **I** at 280 nm.

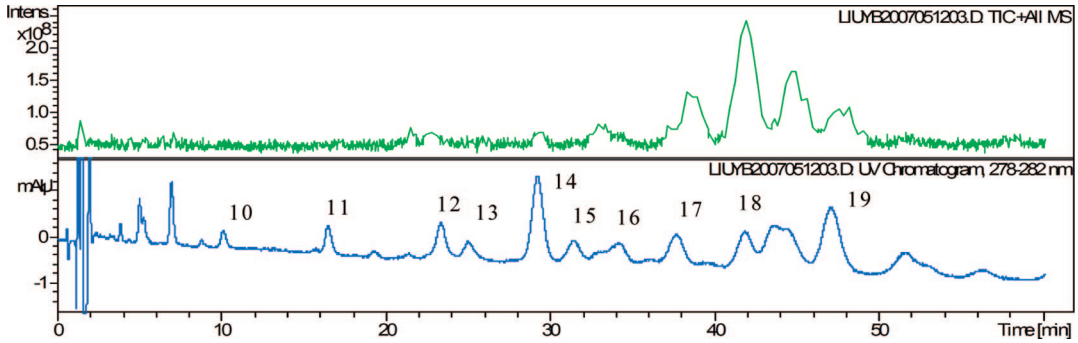


Figure 3. Liquid chromatogram and total ion chromatogram of Fr. **II** at 280 nm.

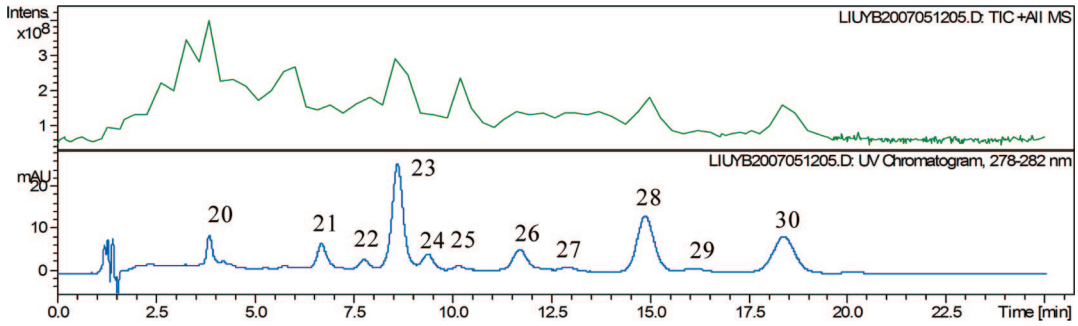


Figure 4. Liquid chromatogram and total ion chromatogram of Fr. **III** at 280 nm.

The NMR data for the aglycone moiety of compound **1** were identical to those reported for compound **S4**.[2] The ^{13}C NMR signals at δ_C 166.9 (cin-1), 119.9 (cin-2), 145.2 (cin-3), 135.0 (cin-4), 128.6 (cin-5/9), 129.2 (cin-6/8), and 130.5 (cin-7) suggest the presence of a cinnamoyl group. The C-12 position of the cinnamoyl group was based on the long-range correlation between the carbonyl of the cinnamoyl group (δ_C 166.9) and H-12 (δ_H 5.28) in the HMBC spectrum. The aglycone of compound **1** was thus identified as 12-*O*-cinnamoyldihydrosarcostin.

Resonances due to five anomeric protons [δ_H 5.30 (1H, d, J = 9.5 Hz), 5.18 (1H, d, J = 8.0 Hz), 5.07 (1H, d, J = 8.0 Hz), 4.86 (1H, d, J = 8.0 Hz), and 4.68 (1H, d, J = 9.5 Hz] were observed. The splitting patterns of anomeric proton indicated that **1** possessed five sugar units with β-linkages. On the basis of ^1H, ^{13}C, $^1H-^1H$ COSY, HMQC, and HMBC NMR spectroscopic data, five sugar units of **1** were determined to be two β-glucopyranoses, one β-thevetopyranose, one β-oleandropyranose, and one β- cymaropyranose. The linkage and sequences of the five sugar units in **1** were ascertained by the HMBC spectrum, which showed distinct correlations for δ_H 5.18 (H-1 of terminal β-glucopyranosyl) to δ_C 81.6 (C-4 of inner β-glucopyranosyl), δ_H 5.07 (H-1 of inner β-glucopyranosyl) to δ_C 83.5 (C-4 of β- thevetopyranosyl), δ_H 4.86 (H-1 of β-thevetopyranosyl) to δ_H 79.3 (C-4 of β-oleandropyranosyl), δ_H 4.68 (H-1 of β-oleandropyranosyl) to δ_H 83.6 (C-4 of β-cymaropyranosyl), and δ_H 5.30 (H-1 of β- cymaropyranosyl) to δ_H 76.2 (C-3 of aglycon). The D-absolute configuration of the sugars was confirmed by HPLC. Thus, the structure of **1** was determined as 12-*O*-cinnamoyl-dihydrosarcostin 3-*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-thevetopyranosyl-(1→4)-*O*-β-D-oleandropyranosyl-(1→4)-*O*-β-D-cymaropyranoside (see Table 4 for ^{13}C NMR data).

The NMR data analysis showed that the structure of **4** was similar to **1**. Comparison of the ^{13}C NMR data of the aglycone moiety of **4** with that of **1** showed that the signals for C-17, C-20, and C-21 were shifted downfield by 3.4, 139.4, and 8.3 ppm, respectively. This suggested that C-20 was oxidized to a carbonyl group.[2] Therefore, the structure of **4** was identified as 12-*O*-cinnamoyltayloron 3-*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-thevetopyranosyl-(1→4)-*O*-β-D-oleandropyranosyl-(1→4)-*O*-β-D-cymaropyranoside, which was further confirmed by 2D NMR experiments (see Table 4 for ^{13}C NMR data).

Table 2. Accurate Masses and Molecular Formulas of Compounds **1**−**30** by HPLC-HRMS in Positive Ion ESI Mode

fraction	compounds	ESIMS $[M + Na]^+$	molecular formula	HRESIMS
Fr. **I**	**1**	1309	$C_{63}H_{98}O_{27}Na$	1309.6175
	2	1251	$C_{61}H_{96}O_{25}Na$	1251.6172
	3	1267	$C_{61}H_{96}O_{25}Na$	1267.6088
	4	1307	$C_{63}H_{96}O_{27}Na$	1307.5951
	5	1277	$C_{63}H_{98}O_{25}Na$	1277.6279
	6	1293	$C_{63}H_{98}O_{26}Na$	1293.6245
	7	1453	$C_{70}H_{110}O_{30}Na$	1453.6827
	8	1411	$C_{67}H_{104}O_{30}Na$	1411.6919
	9	1365	$C_{65}H_{98}O_{29}Na$	1365.6112
Fr. **II**	**10**	1206	$C_{58}H_{89}NO_{24}Na$	1206.5708
	11	1161	$C_{57}H_{86}O_{23}Na$	1161.5687
	12	1187	$C_{59}H_{88}O_{23}Na$	1187.5609
	13	1291	$C_{63}H_{96}O_{26}Na$	1291.6071
	14	1365	$C_{65}H_{98}O_{29}Na$	1365.6803
	15	1317	$C_{65}H_{98}O_{26}Na$	1317.6244
	16	1287	$C_{62}H_{104}O_{26}Na$	1287.6709
	17	1203	$C_{60}H_{92}O_{23}Na$	1203.5569
	18	1203	$C_{60}H_{92}O_{23}Na$	1203.5548
	19	1313	$C_{63}H_{102}O_{27}Na$	1313.6515
Fr. **III**	**20**	1041	$C_{57}H_{86}O_{20}K$	1057.47929
	21	1185	$C_{60}H_{90}O_{22}Na$	1185.6163
	22	1090	$C_{57}H_{82}O_{18}N$	1068.5504
	23	1089	$C_{55}H_{86}O_{20}Na$	1089.5649
	24	1090	$C_{57}H_{82}O_{18}N$	1068.5581
	25	1087	$C_{57}H_{88}O_{20}Na$	1087.5434
	26	1115	$C_{64}H_{94}O_{21}N$	1115.5971
	27	1165	$C_{58}H_{94}O_{22}Na$	1165.5609
	28	1113	$C_{49}H_{82}O_{18}Na$	1113.5613
	29	1234	$C_{64}H_{99}NO_{21}$	1212.6324
	30	1234	$C_{64}H_{99}NO_{21}$	1212.6294

The NMR data of compound **7** suggested that it possessed the same aglycone unit as that in **1**. The ^{1}H NMR, ^{13}C NMR, TLC, and HPLC profiles of the sugar moieties of **7** indicated the presence of six sugar units: two β-D-cymaropyranosyl, one β-D-oleandropyranosyl, one β-D-thevetopyranosyl, and two glucopyranosyl moieties. The positions, linkage, and sequences of the six sugar units in **7** were ascertained by ^{1}H, ^{13}C, and 2D NMR spectroscopy. The structure of **7** was established as 12-*O*- cinnamoyldihydrosarcostin 3-*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-thevetopyranosyl-(1→4)-*O*-β-D-oleandropyranosyl-(1→4)-*O*-β-D-cymaropyranosyl-(1→4)-*O*-β-D-cymaropyranoside (see Table 4 for ^{13}C NMR data).

In conclusion, the present HPLC-DAD-ESIMS^n method is a valuable and effective tool for the online identification of steroidal glycosides in complex plant extracts without further purification. It provides much structural information to screen this kind of compound rapidly and results in reduction of labor costs.

Experimental Section

General Experimental Procedures. Melting points were measured on an XT-4 micromelting point apparatus and were uncorrected. Optical rotations were determined on a Perkin-Elmer 241 automatic digital polarimeter. UV spectra were obtained on a Shimadzu UV-260 spectrometer. IR spectra were recorded on a Nicolet-Impact 400 IR spectrometer using KBr discs. 1D and 2D NMR experiments were performed on an Inova 500 FT-NMR spectrometer. TMS was used as internal standard. ESIMS was performed on an Agilent 1100 Series LC/MSD Trap mass spectrometer. HRESIMS was measured on a Bruker FTMS APEXIII 7.0T mass spectrometer.

Planta Materials. The stems of *D. sinensis* var. *corrugata* were collected in November 2004 from Guangxi Province, China, and were identified by Lin Ma, Department of Chemistry of Natural Products, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College. A voucher specimen (No. 20040618) was deposited in the herbarium of the Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College.

Extraction and Isolation. The dried bark was chopped and extracted with 95% EtOH. The EtOH extract was suspended in H_2O and subsequently partitioned with EtOAc and *n*-BuOH. The *n*-BuOH-soluble part was loaded on a Daion HP-20 and eluted with H_2O/EtOH (70:30, 50:50, 30:70, 10:90). The 70% EtOH fraction was then subjected to a Sephadex LH-20 column to yield Fr. **I**. A portion of the EtOAc extract was subjected to Si gel column chromatography and eluted with $CHCl_3$/MeOH (100:1, 50:1, 30:1, 20:1, 10:1, 5:1, 1:1, 0:100, v/v) to yield eight fractions (Frs. **A**−**H**). Fr. **C** was applied to a Sephadex LH-20 column and eluted with MeOH to obtain Fr. **II**. Fraction **E** was loaded on an RP-18 Si gel column and eluted with MeOH/H_2O (60:40 → 100:1, v/v) to afford Fr. **III**.

ESIMS^n Analysis of Compounds S1−**S17.** The positive and negative ion ESIMS^n experiments were measured on an Agilent 1100 Series LC/MSD Trap mass spectrometer. The ESI conditions were as follows: drying temperature, 325 °C; drying gas, 6.0 L/min; nebulizer, 15 psi; skimmer, 40 V; injection rate, 5 μL/min. The concentration of each compound was 1 mg/mL.

High-Resolution Mass Measurements. HRMS were measured on an Agilent 1100 Series LC/MSD TOF mass spectrometer. The positive ion ESI conditions were as follows: gas temperature, 300 °C; drying gas, 8 L/min; nubulizer, 20 psi. The TOF conditions were as follows: fragmentor, 200 V; skimmer, 60 V; OCTRFV, 200 V. The mobile phase and concentration for each fraction were the same as that used in HPLC-MS^n analysis. HPLC separation was carried out on an Agilent XDB-C18 column (2.1 × 150 mm, 5 μm) using a mobile phase of MeCN and H_2O at wavelength 280 nm (flow rate, 1 mL/min; temperature, 25 °C). Fractions **I**, **II**, and **III** were chromatographed using 40:60, 35:65, and 45:55 MeCN/H_2O, respectively.

Table 3. Key ESIMS^n Fragment Ions for 18 New Compounds in Frs. **I**, **II**, and **III**

	ESIMS $[M + Na]^+$	$[M - acid + Na]^+$ or $[M - acid - nH_2O + Na]^+$	Y_5	Y_4	Y_3	Y_2	Y_1	B_6	B_5	B_4	B_3	$^{3,5}A_3$	$^{3,5}A_4$
1	1309	1161, 1143		999	837	677	533		795	651	507	405	565
2	1251	1129, 1111, 1085		967	807				763	633	489	403	547
3	1267	1145, 1129, 1101		983	821	677			779	635	491	405	565
4	1307	1159, 1141, 1099		997	835	675	531		795	651	507	405	565
5	1277	1129, 1111, 1085		967	807	663	519		763	633	489	403	547
6	1293	1145, 1127, 1101		983	821	661			779	635	491	405	565
7	1453	1305	1143	981	821	677	533	967	795	651	507	405	565
8	1411	1289, 1271, 1127	1127	965	821	677	533	923	779	635	491	405	565
10	1206	1057, 997, 1039, 979			835	675	531			633	489	403	547
11	1161	1039, 979, 949			877	717	571			633	489	403	547
12	1187	1039, 979, 949			877	717	571			633	489	403	547
13	1291	1169, 1109		1007	847	703	559		763	633	489	403	547
15	1317	1169, 1079, 1109		1007	847	703	559		763	633	489	403	507
16	1287	1185, 1227, 1125		963	803	659	515		777	633	489	403	547
19	1313	1211		989	889	745			763	633	489	403	547
21	1185	1125, 1065, 1003			861	741				615	471	385	
27	1165	1105, 1045, 1003			861	741				615	471	385	
29	1234	1086, 963			803	659				615	471	385	

Table 4. ^{13}C NMR Data of **1**, **4**, and **7** from Fr. **I** in Pyridine-d_5 (125 MHz)

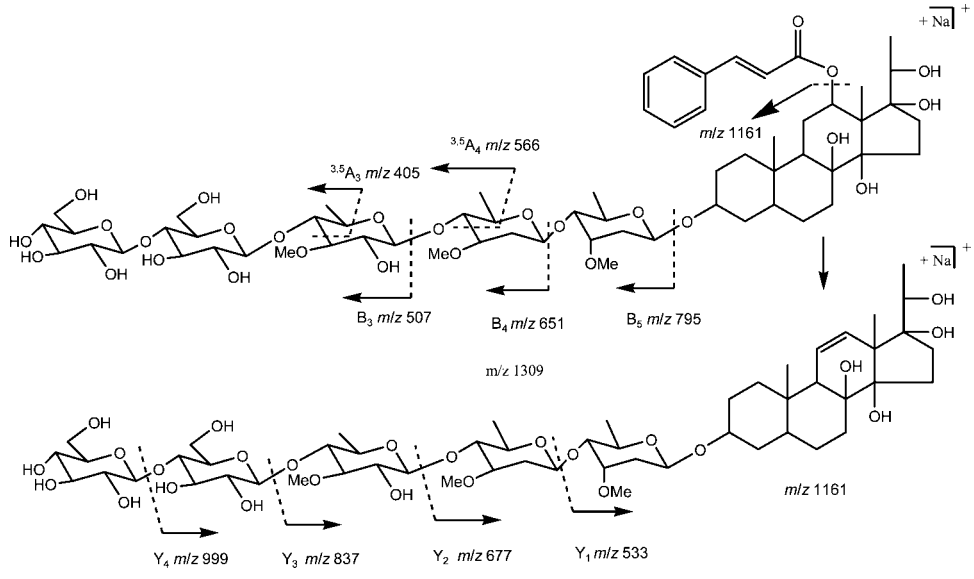
aglycone		**1**			**4**			**7**	
1	38.1	Cym-1	95.9	38.0	Cym-1	95.9	38.0	Cym-1	95.9
2	32.9	2	37.6	33.3	2	37.6	32.9	2	37.6
3	76.9	3	77.9	76.9	3	77.9	76.9	3	77.8
4	37.3	4	83.6	37.3	4	83.6	37.3	4	83.5
5	45.3	5	68.9	45.2	5	68.9	45.3	5	68.9
6	25.2	6	18.7	25.1	6	18.7	25.2	6	18.7
7	34.5	3-OMe	58.8	34.5	3-OMe	58.8	34.5	3-OMe	58.8
8	75.9	Ole-1	101.9	76.1	Ole-1	101.9	75.9	Cym-1′	100.4
9	46.9	2	37.3	47.2	2	37.3	46.9	2′	37.3
10	36.5	3	79.3	36.5	3	79.2	36.5	3′	78.1
11	29.6	4	83.3	29.6	4	83.2	29.6	4′	83.2
12	75.0	5	71.9	75.0	5	71.9	75.0	5′	69.1
13	58.5	6	18.7	58.5	6	18.7	58.8	6′	18.7
14	88.4	3-OMe	57.4	89.3	3-OMe	57.4	88.4	3′-OMe	58.9
15	33.8	The-1	104.0	33.6	The-1	104.0	33.8	Ole-1	101.9
16	34.5	2	75.4	34.4	2	75.4	34.5	2	37.0
17	88.9	3	83.5	92.3	3	83.5	88.9	3	79.2
18	12.3	4	86.4	11.2	4	86.4	12.2	4	83.1
19	13.0	5	71.6	13.0	5	71.5	13.0	5	72.0
20	70.8	6	18.8	210.2	6	18.7	70.8	6	18.7
21	19.4	3-OMe	60.6	27.7	3-OMe	60.6	19.4	3-OMe	57.3
		Glc-1	105.0		Glc-1	105.0		The-1	104.0
		2	74.8		2	74.1		2	75.4
		3	76.2		3	76.9		3	83.5
	Cin at C-12	4	81.6	Cin at C-12	4	81.6	Cinat C-12	4	86.4
1	166.9	5	78.3	166.9	5	78.2	166.9	5	71.6
2	119.7	6	62.5	119.7	6	62.5	119.7	6	18.5
3	145.2	Glc-1′	104.7	145.2	Glc-1′	104.7	145.1	3-OMe	60.6
4	135.0	2′	75.3	135.0	2′	74.8	135.0	Glc-1	105.0
5	128.6	3′	76.7	128.6	3′	76.6	128.6	2	74.8
6	129.2	4′	71.6	129.2	4′	71.9	129.2	3	76.3
7	130.5	5′	78.5	130.5	5′	78.5	130.5	4	81.7
8	129.2	6′	62.5	129.2	6′	62.5	129.2	5	78.3
9	128.6			128.6			128.6	6	62.5
								Glc-1′	104.7
								2′	75.3
								3′	76.6
								4′	72.0
								5′	78.5
								6′	62.5

HPLC-DAD-ESIMS^n. For the online HPLC-MS^n analyses, an Agilent 1100 Series liquid chromatography system was utilized, coupled with an ion trap mass spectrometer. The positive ion ESIMS^n experiments were conducted using the same conditions as those for the pure compounds described above. HPLC separation was carried out on an Agilent XDB-C18 column (2.1 × 150 mm, 5 μm) (0.3 mL/min). The mobile phase and concentration for each fraction were the same as those used in HPLC/HRMS analyses.

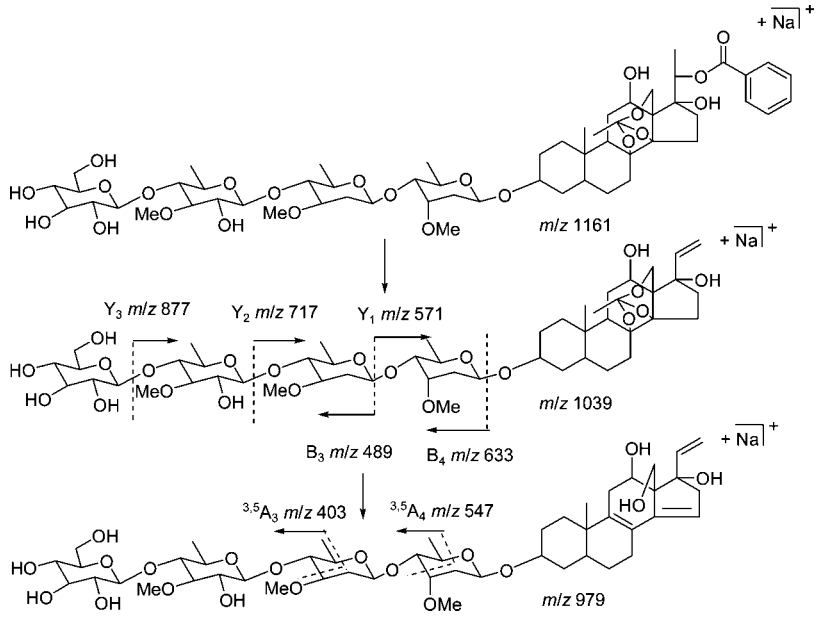
Isolation of 1, 4, and 7 from Fr I. Fr. **I** (500 mg) was subjected to preparative HPLC on a RP-18 column (250 mm × 20 mm, wavelength 280 nm, flow rate 5 mL/min) and eluted with MeCN/H_2O (40:60, v/v) to give **1** (52 mg, t_R = 17.5 min), **4** (72 mg, t_R = 21.2 min), and **7** (49 mg, t_R = 25.2 min).

Determination of the Absolute Configurations of the Sugars in Compounds 1, 4, and 7. A solution of l-(−)-MBA (α-methylbenzy-

Scheme 2. Structure and Proposed Fragmentation Pathways of **1**



Scheme 3. Possible Structure and Proposed Fragmentation Pathways of **11**



lamine) (20 mg) and $NaBH_3CN$ (sodium cyanoborohydride) (4 mg) in 0.5 mL of MeOH was added to a solution of an authentic sugar sample (20 mg) in 0.5 mL of H_2O. The mixture was allowed to stand overnight, acidified to pH 3−4 by HOAc, and evaporated to dryness. The resultant oily material was acetylated with Ac_2O/Py (1:1) (2 mL) at 100 °C for 1 h in a sealed tube. After codistillation of the Ac_2O with toluene, H_2O (2 mL) was added to the residue and the mixture was extracted with $CHCl_3$ (1 mL). The $CHCl_3$ layer was evaporated to give an oily residue and subjected to HPLC (Inertsil sil-100A column, 250 mm × 4.6 mm, 5 μm; solvent system, CH_2Cl_2/MeOH (65:1); flow rate, 1.0 mL/min; detection at 230 nm). The retention times of the derivatives of authentic sugar samples were as follows: D-cymaropyranose 5.627 min, D-digitoxopyranose 11.012 min, D-oleandropyranose 6.388 min, and D-thevetopyranose 7.588 min. To each solution of **1**, **4**, and **7** in MeOH (5 mL) was added 0.05 M H_2SO_4 (5 mL), and the solution was kept at 50 °C for 1 h. The solution was then neutralized with aqueous saturated $Ba(OH)_2$, and the precipitate was removed by filtration. The filtrate was partitioned with $CHCl_3$ to give the aglycone, and the aqueous phase was retained to study the sugar components by HPLC. The aqueous portions obtained from the acid hydrolysis of compounds **1**, **4**, and **7** were treated in the same way as the authentic samples, and the retention times were compared with those of the authentic samples. The types and absolute configurations of the sugars were determined by HPLC, which showed different behaviors of acyclic diastereoisomeric 1-deoxy-1-(*N*-acetyl-α-methylbenzylamino)alditol acetates derived from different monosaccharides. This is consistent with the reported procedure.[20]

1: white powder; $[\alpha]_D^{25}$ +39.3 (*c* 0.46, CH_3OH); IR (KBr) ν_{max} 3402, 2932, 1703, 1634, 1162, 1065 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) δ_H 8.13 (1H, d, *J* = 16.0 Hz, H-3 of Cin), 6.92 (1H, d, *J* = 16.0 Hz, H-2 of Cin), 5.30 (1H, d, *J* = 9.5 Hz, H-1 of D-Cym), 5.18 (1H, d, *J* = 8.0 Hz, H-1 of outer D-Glc), 5.07 (1H, d, *J* = 8.0 Hz, H-1 of inner D-Glc), 4.86 (1H, d, *J* = 8.0 Hz, H-1 of D-Thev), 4.68 (1H, d, *J* = 9.5 Hz, H-1 of D-Olean), 3.89 (3H, s, OMe of D-Thev), 3.59 (3H, s, OMe of D-Cym), 3.49 (3H, s, OMe of D-Olean), 2.13 (3H, s, H-18), 1.16 (3H, s, H-19), 1.32 (3H, d, *J* = 5.0 Hz, H-21); ^{13}C NMR (pyridine- d_5, 125 MHz) see Table 4; ESIMS *m/z* 1309 $[M + Na]^+$; HRESIMS *m/z* 1309.6193 $[M + Na]^+$ (calcd for 1309.6175).

4: white powder; $[\alpha]_D^{25}$ +17.4 (*c* 0.46, CH_3OH); IR (KBr) ν_{max} 3416, 2930, 1707, 1636, 1162 cm^{-1}; ESIMS *m/z* 1307 $[M + Na]^+$; HRESIMS *m/z* 1307.6027 $[M + Na]^+$ (calcd for 1307.6037); ^1H NMR (pyridine-d_5, 500 MHz) δ_H 7.79 (1H, d, *J* = 16.0 Hz, H-3 of Cin), 6.81 (1H, d, *J* = 16.0 Hz, H-2 of Cin), 5.29 (1H, d, *J* = 8.5 Hz, H-1 of D-Cym), 5.17 (1H, d, *J* = 7.5 Hz, H-1 of outer D-Glc), 5.07 (1H, d, *J* = 7.5 Hz, H-1 of inner D-Glc), 4.85 (1H, d, *J* = 7.5 Hz, H-1 of D-Thev), 4.68 (1H, d, *J* = 9.0 Hz, H-1 of D- Olean), 3.88 (3H, s, OMe of D-Thev), 3.58 (3H, s, OMe of D-Cym), 3.49 (3H, s, OMe of D-Olean), 2.12 (3H, s, H-18), 1.18 (3H, s, H-19), 2.49 (3H, s, H-21); ^{13}C NMR (pyridine-d_5, 125 MHz) see Table 4; ESIMS *m/z* 1309 $[M + Na]^+$; HRESIMS *m/z* 1309.6193 $[M + Na]^+$ (calcd for 1309.6175).

7: white powder; $[\alpha]_D^{25}$ +11.3 (*c* 0.35, CH_3OH); IR (KBr) ν_{max} 3400, 2930, 1702, 1634, 1160 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) δ_H 8.13 (1H, d, *J* = 16.0 Hz, H-3 of Cin), 6.92 (1H, d, *J* = 16.0 Hz, H-2 of Cin), 5.31 (1H, d, *J* = 10.0 Hz, H-1 of outer D-Cym), 5.17 (1H, d, *J* = 8.0 Hz, H-1 of outer D-Glc), 5.11 (1H, d, *J* = 10.0 Hz, H-1 of inner D-Cym), 5.07 (1H, d, *J* = 10.0 Hz, H-1 of inner D-Glc), 4.86 (1H, d, *J* = 8.5 Hz, H-1 of D-Thev), 4.66 (1H, d, *J* = 9.5 Hz, H-1 of D-Olean), 3.88 (3H, s, OMe of D-Thev), 3.59 (3H, s, OMe of outer D-Cym), 3.55 (3H, s, OMe of inner D-Cym), 3.49 (3H, s, OMe of D-Olean), 2.12 (3H, s, H-18), 1.18 (3H, s, H-19), 1.32 (3H, d, *J* = 5.5 Hz, H-21); ^{13}C NMR (pyridine-d_5, 125 MHz) see Table 4; ESIMS *m/z* 1453 $[M + Na]^+$; HRESIMS *m/z*: 1453.6846 $[M + Na]^+$ (calcd for 1453.6827).

Acknowledgment. This project was supported by the National Natural Science Fund for Distinguished Young Scholars (No. 30625040) and National Key Basic R&D (973) Project (No. 2004CB518906). We are grateful to the Department of Natural Products Chemistry of the Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, for performing ESIMS^n, HPLC- DAD-ESIMS^n, and HPLC-HRMS experiments.

Supporting Information Available: ESIMS^n and 1D and 2D NMR spectra of compounds **1**, **4**, and **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP800048N