Acacia concinna Saponins. II. Structures of Monoterpenoid Glycosides in the Alkaline Hydrolysate of the Saponin Fraction

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From the AcOEt-soluble fraction of the alkaline hydrolyzate of the highly polar saponin fraction of pods of *Acacia concinna*, two monoterpenes, (2*E*)-2,6-dimethyl-6-hydroxy-2,7-octadienoic acid (menthiafolic acid, 1) and (2*E*)-6-hydroxy-2-hydroxymethyl-6-methyl-2,7-octadienoic acid (4), and their glycosides, (6*R*)- and (6*S*)-menthiafolic acid-6-*O*-*β*-D-quinovoside (2a and 2b) and (6*R*)- and (6*S*)-menthiafolic acid-6-*O*-*β*-D-xyloside (3a and 3b), were isolated. A more polar fraction gave, after methylation with diazomethane, (6*R*)- and (6*S*)-(2*E*)-6-hydroxy-2-hydroxymethyl-6-methyl-2,7-octadienoic acid-6-*O*-*β*-D-quinovoside as their methyl esters (5a and 5b). Compounds 2a, 3a, 4, 5a, and 5b are new. The structures of the above compounds were determined mainly by the application of spectroscopic methods.

Key words *Acacia concinna*; monoterpene glycoside; menthiafolic acid; *β*-D-quinovoside; *β*-D-xyloside; ^{13}C-NMR

Saponins from pods of a leguminous plant, *Acacia* (*A.*) *concinna*, are a complex mixture of highly polar compounds, which, on alkaline hydrolysis, give prosapogenols and monoterpene-glycoside components. In a previous paper, we clarified structures of the prosapogenols, which were obtained from a butanol extract of the hydrolysate.[1] In this paper, we deal with the structures of the monoterpenoids, most of which were obtained from an ethyl acetate-soluble fraction of the hydrolysate.

Results and Discussion

Hydrolysis of a saponin fraction of pods of *A. concinna* with 0.5 N NaOH in MeOH at room temperature and fractionation of the product as described previously[1] gave AcOEt-soluble and BuOH-soluble fractions. The AcOEt-soluble fraction was separated by silica gel and reversed-phase silica gel column chromatography to yield four chromatographically pure compounds **1**—**4** from relatively mobile fractions. Although compounds **1** and **4** were single compounds, compounds **2** and **3** were each revealed to be a mixture of two compounds with closely related structures. Thus, they were separated by recycling HPLC on an octadecyl silica gel (ODS) column to yield compounds **2a** and **2b**, and **3a** and **3b**, respectively (Fig.

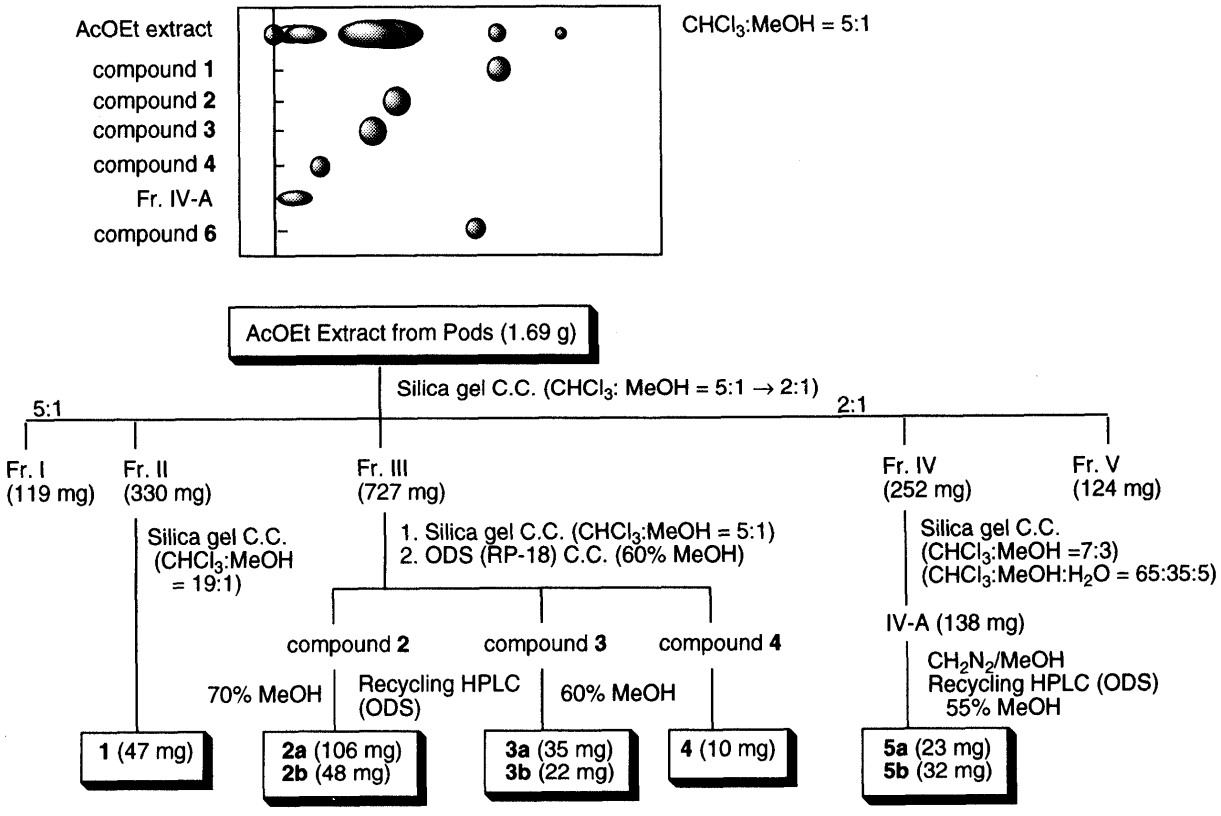

Fig. 1. Fractionation of AcOEt Extract from Alkaline Hydrolysate of *A. concinna* Saponin Fraction

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Table 1. ^{1}H-NMR of Compounds **1**, **2** and **3** (CD_3OD)

No.	Compound **1**	Compound **2a**	Compound **2b**	Compound **3a**	Compound **3b**
H-3	6.77 (1H, tq, J=7.6, 1.5 Hz)	6.77 (1H, tq, J=7.8, 1.5 Hz)	6.85 (1H, br t, J=7.3 Hz)	6.76 (1H, br t, J=6.8 Hz)	6.73 (1H, br t, J=6.8 Hz)
H-4	2.22 (2H, m)	2.32 (2H, m)	2.37 (2H, m)	2.30 (2H, m)	2.24 (2H, m)
H-5	1.61 (2H, m)	1.73 (2H, t, J=8.3 Hz)	1.79 (2H, m)	1.71 (2H, m)	1.66 (2H, t, J=8.3 Hz)
H-7	5.91 (1H, dd, J=17.1, 10.7 Hz)	6.03 (1H, dd, J=17.8, 11.2 Hz)	6.02 (1H, dd, J=17.6, 11.2 Hz)	6.01 (1H, dd, J=17.6, 10.7 Hz)	5.90 (1H, dd, J=17.6, 11.2 Hz)
H-8a	5.05 (1H, dd, J=10.7, 1.5 Hz)	5.17 (1H, dd, J=11.2, 1.0 Hz)	5.30 (1H, dd, J=11.2, 1.0 Hz)	5.17 (1H, dd, J=10.7, 1.0 Hz)	5.18 (1H, br d, J=11.2 Hz)
H-8b	5.23 (1H, dd, J=17.1, 1.5 Hz)	5.23 (1H, dd, J=17.8, 1.0 Hz)	5.36 (1H, dd, J=17.6, 1.0 Hz)	5.23 (1H, dd, J=17.6, 1.0 Hz)	5.24 (1H, br d, J=17.6 Hz)
H-9	1.79 (3H, d, J=1.0 Hz)	1.80 (3H, d, J=1.5 Hz)	1.88 (3H, br s)	1.80 (3H, br s)	1.76 (3H, br s)
H-10	1.27 (3H, s)	1.33 (3H, s)	1.46 (3H, s)	1.33 (3H, s)	1.33 (3H, s)
		Qui	Qui	Xyl	Xyl
H-1′		4.31 (1H, d, J=7.8 Hz)	4.45 (1H, d, J=7.8 Hz)	4.28 (1H, d, J=7.3 Hz)	4.29 (1H, d, J=7.8 Hz)
H-2′		3.16 (1H, dd, J=9.3, 7.8 Hz)	3.25 (1H, dd, J=9.3, 7.8 Hz)	3.14 (1H, dd, J=9.3, 7.8 Hz)	3.12 (1H, dd, J=9.3, 7.8 Hz)
H-3′		3.27 (1H, t, J=9.3 Hz)	3.36 (1H, t, J=9.3 Hz)	3.28 (1H, t, J=9.0 Hz)	3.25 (1H, t, J=9.0 Hz)
H-4′		2.97 (1H, t, J=9.3 Hz)	3.07 (1H, t, J=9.0 Hz)	3.46 (1H, m)	3.42 (1H, m)
H-5a′		3.19 (1H, dq, J=9.3, 5.9 Hz)	3.31 (1H, dq, J=9.3, 6.4 Hz)	3.08 (1H, t, J=11.0 Hz)	3.09 (1H, t, J=10.8 Hz)
H-5e′				3.76 (1H, dd, J=11.5, 5.6 Hz)	3.75 (1H, dd, J=11.2, 5.4 Hz)
H-6′		1.22 (3H, d, J=5.9 Hz)	1.33 (3H, d, J=6.4 Hz)		

Table 2. ^{1}H-NMR of Compounds **4**, **5** and **6** (CD_3OD)

No.	Compound **4**	Compound **5a**	Compound **5b**	Compound **6**
H-3	6.92 (1H, t, J=7.8 Hz)	6.92 (1H, t, J=7.8 Hz)	6.91 (1H, br t, J=7.8 Hz)	6.92 (1H, t, J=7.3 Hz)
H-4	2.36 (2H, m)	2.46 (2H, m)	2.43 (2H, m)	2.36 (2H, m)
H-5	1.64 (2H, m)	1.76 (2H, dd, J=9.0, 7.1 Hz)	1.73 (2H, m)	1.67 (2H, m)
H-7	5.91 (1H, dd, J=17.6, 10.7 Hz)	6.03 (1H, dd, J=17.8, 11.0 Hz)	5.94 (1H, dd, J=17.8, 11.0 Hz)	5.80 (1H, dd, J=18.1, 10.7 Hz)
H-8c	5.05 (1H, dd, J=10.7, 1.5 Hz)	5.17 (1H, dd, J=11.0, 1.2 Hz)	5.21 (1H, dd, J=11.0, 1.2 Hz)	5.19 (1H, dd, J=10.7, 1.5 Hz)
H-8t	5.23 (1H, dd, J=17.6, 1.5 Hz)	5.23 (1H, dd, J=17.8, 1.2 Hz)	5.28 (1H, dd, J=17.8, 1.2 Hz)	5.16 (1H, dd, J=18.1, 1.5 Hz)
H-9	4.30 (2H, s)	4.31 (2H, s)	4.31 (2H, s)	4.30 (2H, s)
H-10	1.27 (3H, s)	1.34 (3H, s)	1.37 (3H, s)	1.27 (3H, s)
OMe		3.74 (3H, s)	3.74 (3H, s)	
		Qui	Qui	OBu
H-1′		4.31 (1H, d, J=7.8 Hz)	4.35 (1H, d, J=7.8 Hz)	3.31 (2H, m)
H-2′		3.15 (1H, dd, J=9.3, 7.8 Hz)	3.16 (1H, dd, J=9.3, 7.8 Hz)	1.49 (2H, m)
H-3′		3.27 (1H, t, J=9.3 Hz)	3.27 (1H, t, J=9.0 Hz)	1.38 (2H, m)
H-4′		2.97 (1H, t, J=9.3 Hz)	2.97 (1H, t, J=9.3 Hz)	0.92 (3H, t, J=7.3 Hz)
H-5′		3.19 (1H, m)	3.23 (1H, m)	
H-6′		1.22 (3H, d, J=6.3 Hz)	1.23 (3H, d, J=5.9 Hz)	

1). A more polar fraction (Fr. IV) was treated with CH_2N_2 in methanol and the product was separated by recycling HPLC to yield two monoterpene-glycosides as their methyl esters (**5a** and **5b**). Another monoterpenoid, compound **6**, was obtained from the BuOH extract of the hydrolysate.[1b]

Compound **1** was a colorless oil with the molecular formula $C_{10}H_{16}O_3$. Its ^{1}H-NMR spectrum in CD_3OD showed the presence of a quaternary methyl (δ 1.27, 3H, s), an olefinic methyl (δ 1.79, 3H, d, J=1.0 Hz), a terminal olefin (δ 5.05, 1H, dd, J=10.7, 1.5 Hz; 5.23, 1H, dd, J=17.1, 1.5 Hz; 5.91, 1H, dd, J=17.1, 10.7 Hz), and an olefinic proton (δ 6.77, tq, J=7.6, 1.5 Hz). These data, together with an IR absorption (1688 cm^{-1}), suggested that it is a monoterpenoid α,β-unsaturated carboxylic acid. It was concluded to be (2*E*)-2,6-dimethyl-6-hydroxy-2,7-octadienoic acid (menthiafolic acid)[2a] from the identity of its ^{13}C-NMR data with those reported.[2b] Since compound **1** showed a small but distinct optical rotation ($[\alpha]_D$ −3.0°), we concluded that it is a mixture of enantiomers with an excess of the (6*R*)-enantiomer ((6*R*):(6*S*)=*ca.* 1.4:1), since the reported $[\alpha]_D$ for the (6*S*)-enantiomer[3] is +19.3°.

Compounds **2a** and **2b** showed very similar spectral data. From the NMR data, they were suggested to be glycosides of compound **1** at the C-6 hydroxyl group, because large glycosylation shifts (+7.2 and +7.3 ppm) were observed at C-6 for **2a** and **2b**, respectively. The sugar moiety was identical in both compounds and was revealed to be β-quinovoside from the ^{1}H- and ^{13}C-NMR data (Tables 1 and 3). Compound **2b** was identified as (6*S*)-menthiafolic acid-6-*O*-β-D-quinovoside, because its spectral data were in good agreement with those reported in the literature.[4] Since the ^{13}C chemical shifts of **2a** and **2b** were almost identical in the sugar part and differences (δ_{2a}—δ_{2b}) were observed only for C-5

Table 3. ^{13}C-NMR Data in CD_3OD

No.	1	2a	2b	3a	3b	4	5a	5b	6
1	172.4	172.6	172.7	172.6	172.6	169.4	170.1	170.1	171.5
2	129.6	129.6	129.7	129.6	129.6	131.5	133.2	133.1	133.5
3	144.8	145.1[a]	144.8[a]	144.8[a]	144.78[a]	146.4	149.1	149.1	148.5
4	25.3	24.9	24.3	24.8	24.3	23.1	24.9	24.5	23.5
5	42.6	40.3	41.9	40.5	41.9	40.7	40.5	42.1	40.8
6	74.4	81.6	81.7	81.7	81.8	72.3	81.5	81.6	78.7
7	146.7	144.9[a]	144.9[a]	145.0[a]	144.81[a]	144.6	145.1	144.8	145.0
8	113.2	116.0	116.6	116.0	116.7	111.1	116.0	116.7	116.0
9	13.1	13.3	13.3	13.2	13.2	55.3	57.3	57.3	57.5
10	28.6	25.0	25.2	25.1	25.2	26.6	25.0	25.0	24.9
OMe							53.0	53.0	
		Qui	Qui	Xyl	Xyl		Qui	Qui	OBu
1′		99.9	100.1	100.8	100.9		99.9	100.1	63.7
2′		76.2	76.2	75.8	75.9		76.2	76.2	34.5
3′		78.7	78.8	78.8	78.8		78.7	78.7	21.3
4′		77.9	77.9	72.0	72.0		77.9	77.8	15.1
5′		73.7	73.9	67.4	67.4		73.7	73.7	
6′		19.1	19.1				19.1	19.1	

a) Interchangeable in each column.

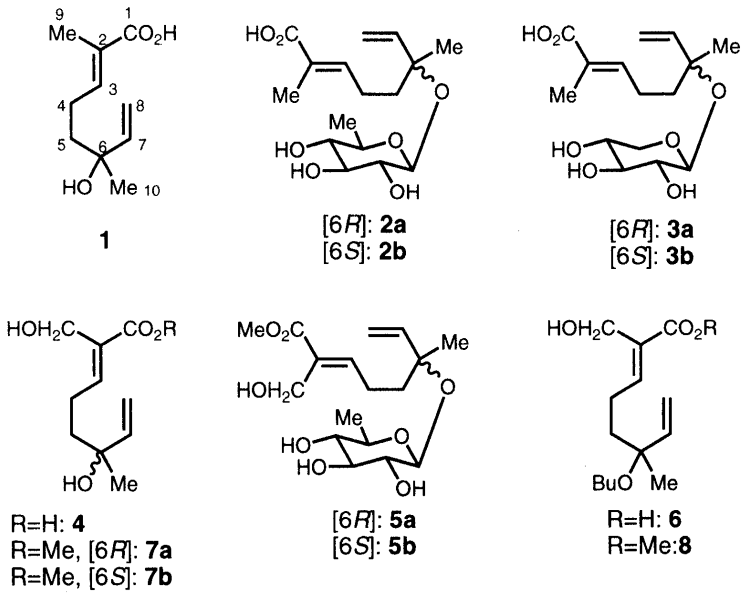

Chart 1

(−1.6 ppm), C-4 (+0.6 ppm) and C-8 (−0.6 ppm), compound **2a** was concluded to be the stereoisomer of **2b** at C-6, (6*R*)-menthiafolic acid-6-*O*-*β*-D-quinovoside.

This assignment was confirmed by enzymatic hydrolysis of compound **2a**, which, on treatment with *β*-glucosidase, gave compound **1** and quinovose. The optical rotation of compound **1** thus obtained was $[\alpha]_D$ −18.0°, indicating that it is the (6*R*)-isomer. Quinovose in the hydrolysate was also confirmed to be the D-isomer from its $[\alpha]_D$ +24.7° (lit.[5] $[\alpha]_D$ +30° for D-quinovose). Thus, the structure of compound **2a** was confirmed to be (6*R*)-menthiafolic acid-6-*O*-*β*-D-quinovoside. This is the first report concerning the (6*R*)-isomer.

Compounds **3a** and **3b** also showed very similar spectral data to each other and were glycosides of compound **1** at the C-6 hydroxyl group (glycosylation shifts of C-6 are +7.3 ppm for **3a** and +7.4 ppm for **3b**). The sugar part in both compounds was revealed to be *β*-xyloside from the ^{1}H- and ^{13}C-NMR data (Tables 1 and 3). Compound **3b** was identified as (6*S*)-menthiafolic acid-6-*O*-*β*-D-xyloside by comparison of its spectral data with those reported in the literature.[6] Since the ^{13}C chemical shifts of the monoterpene parts in **3a** and **3b** were almost identical with those for **2a** and **2b**, respectively (Table 3), compound **3a** was concluded to be (6*R*)-menthiafolic acid-6-*O*-*β*-D-xyloside by analogy with the case of **2a**. Again, this is the first report of the (6*R*)-isomer.

Compound **4** was a colorless oil with a small optical rotation ($[\alpha]_D$ −3.4°), as in the case of **1**. Its ^{1}H-NMR spectrum (Table 2) was similar to that of compound **1**, except that it exhibited a hydroxymethyl signal at *δ* 4.30 (2H, s) instead of the olefinic methyl signal at *δ* 1.83 in **1**, suggesting its structure. The high-resolution MS ($[M-H]^-$ at *m/z* 199.0972, $C_{10}H_{15}O_4$) also supported the molecular formula. The stereochemistry of the $\Delta^{2,3}$ double bond was determined as *E* from the chemical shift of H-3

(δ 6.92), because the olefinic proton of the *Z*-isomer would appear at a higher field.[2b,7] Thus, the structure of compound **4** was concluded to be (2*E*)-6-hydroxy-2-hydroxymethyl-6-methyl-2,7-octadienoic acid. Although our paper describes the first characterization of this compound, it has been found in a saponin (GS-C) of a leguminous plant, *Gleditsia japonica*, as a monoterpene component,[8] whose reported ^{13}C-NMR data are in good agreement with those of **4** (Table 3). The very weak optical activity of compound **4** again suggested that it is a racemic compound with slight excess of the (6*R*)-enantiomer (see below).

Compounds **5a** and **5b** showed similar spectral data to each other, and were found to be glycosides of compound **4** methyl ester at the C-6 hydroxyl group. The sugar moiety was β-quinovoside in both compounds, based on the ^{1}H- and ^{13}C-NMR data (Tables 2 and 3).

The stereochemistry of **5a** and **5b** was proved as follows. Compounds **5a** and **5b** were separately hydrolyzed with β-glucosidase as described for **2a** to give the corresponding aglycones (**7a** and **7b**, respectively) and quinovose. Compound **7a** obtained from **5a** showed an optical rotation of $[\alpha]_D$ $-22.5°$, and **7b** from **5b** showed $[\alpha]_D$ $+18.7°$, indicating that they are the (6*R*)- and (6*S*)-isomers, respectively. The quinovose obtained from **5a** and **5b** had an optical rotation of $[\alpha]_D$ $+22.4°$ and $[\alpha]_D$ $+22.8°$, respectively, indicating the D-isomer in both cases. Thus, compounds **5a** and **5b** were determined to be methyl esters of (6*R*)- and (6*S*)-(2*E*)-6-hydroxy-2-hydroxymethyl-6-methyl-2,7-octadienoic acid-6-*O*-β-D-quinovoside, respectively. The original compounds must therefore be the corresponding acids.

Compound **6** was obtained from the BuOH-soluble fraction as described previously.[1] Its NMR data were very similar to those of compound **4**, except that **6** showed signals of an extra butoxy group [δ_C: 15.1, 21.3, 34.5, 63.7; δ_H: 0.92 (3H, t, J=7.3 Hz), 1.38 (2H, m), 1.49 (2H, m), 3.31 (2H, m)]. The parent ion in the MS ($[M-H]^-$: *m/z* 255) was 56 mass units (C_4H_8) larger than that of compound **4**. The position of the butoxy group was determined at C-6 from the down-field shift (+6.4 ppm) of the C-6 signal compared to that of **4**, and the configuration of the $\Delta^{2,3}$ double bond was deduced as *E* from the H-3 chemical shift (δ 6.92) as discussed above. On treatment with CH_2N_2 in MeOH, **6** gave a methyl ester (**8**). The ^{1}H-NMR spectrum of **8** in $CDCl_3$ showed a hydroxymethyl signal at δ 4.34 (2H, d, J=6.8 Hz), which coupled with a hydroxy signal at δ 2.57 (1H, t, J=6.8 Hz, disappeared on addition of D_2O). These data showed that the carboxyl and hydroxymethyl groups in **6** are free, supporting the above assignment. Thus, its structure was determined as (2*E*)-6-butoxy-2-hydroxymethyl-6-methyl-2,7-octadienoic acid. Since compound **6** was obtained from the BuOH extract and was not detected in the AcOEt-soluble fraction (by TLC comparison), we concluded that it is an artefact produced in the course of BuOH extraction. However, the small but distinct $[\alpha]_D$ of compound **6** ($-4.3°$) suggests that some asymmetric factor influences the formation process of this compound.

Based on the optical rotation of compound **1** and the yields of **2a**, **2b**, **3a** and **3b**, menthiafolic acid (**1**) in this plant is considered to exist as a mixture of the enantiomers with a slight excess of the (6*R*)-isomer. Although glycosides of menthiafolic acid have been isolated from several natural sources,[9] they are usually single stereoisomers in one plant source. To our knowledge, there is only one

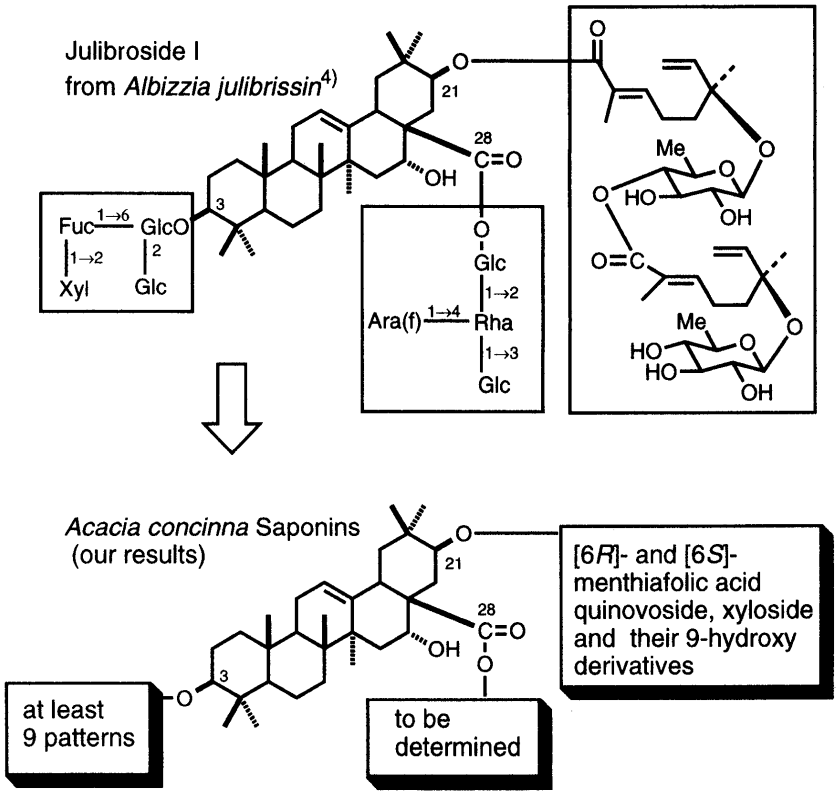

Fig. 2. Expected Structures of Genuine Saponins of *A. concinna*

report that both (6*R*)- and (6*S*)-menthiafolic acid and their glucosides coexist, *i.e.*, among the iridoid glucosides in *Jasmium hemsley*.[9b] In that case, the ratio of (6*R*)- and (6*S*)-isomers varied in the range of 47 : 53 to 18 : 82.

The BuOH-soluble fraction of the alkaline hydrolysate of *A. concinna* saponins gave nine prosapogenols, all of which were 3-*O*-glycosides of acacic acid lactone.[1] We have now identified eight monoterpenoid constituents, five of which were new compounds, in the AcOEt-soluble fraction. We postulate that the genuine saponins of *A. concinna* are constructed from these fragments, because closely related leguminous saponins, acasiasides A and B (from *A. auriculifolius*)[9e] and julibrosides I—III (from *Albizzia julibrissin*)[4] (Fig. 2), have been reported. All of them are 3-*O*-acacic acid derivatives carrying a monoterpene glycoside moiety at the C-21 hydroxy group together with a sugar moiety at the C-28 carboxyl group. In julibrosides, three patterns of C-3 sugars, and only (6*S*)-menthiafolic acid-6-*O*-quinovoside as the monoterpene glycoside moiety have been reported. Assuming that *A. concinna* saponins have similar structures, the combinations of nine patterns for the C-3 sugar moiety and six additional patterns in the monoterpenoid part would generate considerable complexity of the genuine saponins in this plant. The isolation and characterization of the genuine saponins are in progress.

Experimental

General Unless otherwise noted, the following procedures were adopted. Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. IR spectra were taken with a Shimadzu IR-460 spectrometer and the data are given in cm^{-1}. NMR spectra were measured on a JEOL GSX-500 (500 MHz for ^1H, 125 MHz for ^{13}C) spectrometer with tetramethylsilane as an internal standard and the chemical shifts are given in δ values. Mass spectra were taken with a JEOL JMS-SX102 spectrometer and major peaks are indicated as *m/z* (%). Optical rotation was recorded on a Horiba SEPA-300 polarimeter. Column chromatography was performed on LiChroprep® RP-18 (ODS, 40—63 μm, Merck) for reversed-phase and Micro Bead Silica Gel 4B (100—200 mesh, Fuji Silysia Chemical) for normal phase. Recycling HPLC was performed with a JAI LC-908 HPLC system (Japan Analytical Industry) on an Inertsil Prep-ODS column (20 × 250 mm, Gasukuro Kogyo). For thin-layer chromatography (TLC), Kieselgel 60 F_{254} and RP-18 $F_{254}S$ precoated plates (Merck) were used and the spots were developed by spraying 10% H_2SO_4 and heating the plates until coloration took place.

Isolation of Monoterpenoids from the Alkaline Hydrolysate The AcOEt-soluble fraction (1.67 g) obtained from the alkaline hydrolysate of the saponin fraction[1] was fractionated by silica gel column chromatography ($CHCl_3$: MeOH) into five fractions: Fr. I (5 : 1, 119 mg), Fr. II (5 : 1, 330 mg), Fr. III (5 : 1, 727 mg), Fr. IV (2 : 1, 252 mg), Fr. V (MeOH, 124 mg). Rechromatography of Fr. II on silica gel ($CHCl_3$: MeOH = 19 : 1) gave compound **1** (47 mg) along with a mixture of compounds **2** and **3** (93 mg). Repeated chromatography of Fr. III on silica gel ($CHCl_3$: MeOH = 5 : 1) and ODS (60% MeOH) afforded compounds **2** (**2a** + **2b**, 264 mg), **3** (**3a** + **3b**, 125 mg), and **4** (26 mg), each of which was subjected to recycling HPLC on an ODS column (70% MeOH for **2**, 60% MeOH for **3** and **4**) to separate **2a** (106 mg), **2b** (48 mg), **3a** (35 mg), **3b** (22 mg), and **4** (10 mg).

Fraction IV was chromatographed on silica gel with $CHCl_3$: MeOH = 7 : 3 and then $CHCl_3$: MeOH : H_2O = 65 : 35 : 5 to give Fr. IV-A (138 mg). This fraction was treated with an ethereal solution of diazomethane in MeOH and the products were separated by silica gel column chromatography ($CHCl_3$: MeOH = 6 : 1) and recycling HPLC (ODS, 55% MeOH) to give compounds **5a** (23 mg) and **5b** (32 mg). Examination of the faster-moving fractions than **5a** on the ODS column indicated the presence of a xyloside of compound **4** (as a methyl ester), but this could not be isolated because the amount was too small.

Isolation of compound **6** from the BuOH extract was described in a previous paper (compound **11** in ref. 1).[1]

Compound 1 [(2*E*)-2,6-Dimethyl-6-hydroxy-2,7-octadienoic Acid, Menthiafolic Acid] Colorless oil, $[\alpha]_D^{30}$ −3.2° (*c* = 1.0, $CHCl_3$). FAB-MS (neg.): 183 (M − H, 100), 168 (49). IR ($CHCl_3$): 1688. ^1H-NMR ($CDCl_3$): 1.32 (3H, s, H-10), 1.66 (2H, m, H-5), 1.83 (3H, d, *J* = 1.4 Hz, H-9), 2.25 (2H, m, H-4), 5.10 (1H, dd, *J* = 10.7, 1.3 Hz, H-8a), 5.24 (1H, dd, *J* = 17.5, 1.3 Hz, H-8b), 5.91 (1H, dd, *J* = 17.5, 10.7 Hz, H-7), 6.89 (1H, tq, *J* = 7.8, 1.5 Hz, H-3). ^{13}C-NMR ($CDCl_3$): 12.0 (C-9), 23.7 (C-4), 28.1 (C-10), 40.5 (C-5), 73.1 (C-6), 112.3 (C-8), 127.1 (C-2), 144.5, 144.7 (C-3, 7), 172.8 (C-1). ^1H-NMR (CD_3OD): see Table 1. ^{13}C-NMR (CD_3OD): see Table 3.

Compound 2a [(6*R*)-Menthiafolic Acid 6-*O*-β-D-Quinovoside] Colorless crystalline solid, mp 101.5—103 °C, $[\alpha]_D^{25}$ −13.6° (*c* = 0.4, MeOH). FAB-MS (neg.): 329 (M − H, 100). IR (KBr): 1692. HR-FAB-MS (neg.) M − H (*m/z*): 329.1627. Calcd for $C_{16}H_{25}O_7$: 329.1600. ^1H-NMR (pyridine-d_5): 1.49 (3H, s, H-10), 1.61 (3H, d, *J* = 5.9 Hz, H-6′), 1.91 (2H, t, *J* = 8.3 Hz, H-5), 2.08 (3H, br s, H-9), 2.58 (2H, m, H-4), 3.71 (overlapped, H-4′, H-5′), 4.00 (1H, dd, *J* = 8.8, 7.8 Hz, H-2′), 4.12 (1H, t, *J* = 8.8 Hz, H-3′), 4.85 (1H, d, *J* = 7.8 Hz, H-1′), 5.20 (1H, dd, *J* = 10.7, 1.0 Hz, H-8a), 5.35 (1H, dd, *J* = 17.6, 1.0 Hz, H-8b), 6.34 (1H, dd, *J* = 17.6, 10.7 Hz, H-7), 7.22 (1H, br t, *J* = 7.8 Hz, H-3). ^{13}C-NMR (pyridine-d_5): 12.9 (C-9), 18.8 (C-6′), 23.6 (C-4), 24.7 (C-10), 38.8 (C-5), 72.7 (C-5′), 75.4 (C-2′), 76.9 (C-4′), 78.4 (C-3′), 79.5 (C-6), 99.2 (C-1′), 114.1 (C-8), 129.0 (C-2), 142.4 (C-3), 144.4 (C-7), 170.6 (C-1). ^1H-NMR (CD_3OD): see Table 1. ^{13}C-NMR (CD_3OD): see Table 3.

Enzymatic Hydrolysis of Compound 2a Compound **2a** (10 mg) was hydrolyzed with β-glucosidase (from almonds, 7.0 units/mg, Sigma, G-0395, 19.6 mg) in 0.1 M acetate buffer (pH 5.0, 1 ml) at room temperature for 2.5 d. The mixture was passed through a SEP-PAC® C_{18} cartridge (Waters Associates) and the cartridge was washed with water (15 ml) and then eluted with EtOH (5 ml). The EtOH eluate was concentrated to dryness and the residue was purified by silica gel column chromatography ($CHCl_3$: MeOH = 5 : 1) to give (6*R*)-**1** (5.1 mg), $[\alpha]_D^{27}$ −18.0° (*c* = 0.17, MeOH) (*cf.* (6*S*)-**1**, $[\alpha]_D$ +19.3° (*c* = 0.15, $CHCl_3$)).[3] The ^1H-NMR spectrum of this material was identical with that of compound **1**. The water washings were concentrated to dryness and the residue was chromatographed on silica gel ($CHCl_3$: MeOH = 2 : 1) to afford D-quinovose (3.6 mg), $[\alpha]_D^{26}$ +24.6° (*c* = 0.12, H_2O). The NMR data of this material were identical with those of an authentic sample.

Compound 2b [(6*S*)-Menthiafolic Acid 6-*O*-β-D-Quinovoside] Colorless syrup, $[\alpha]_D^{25}$ −28.3° (*c* = 0.43, MeOH) (lit. $[\alpha]_D$ −16.8° (*c* = 0.32, MeOH)).[4] FAB-MS (neg.): 329 (M − H, 100). IR (KBr): 1688. HR-FAB-MS (neg.) M − H (*m/z*): 329.1597. Calcd for $C_{16}H_{25}O_7$: 329.1600. ^1H-NMR (pyridine-d_5): 1.56 (3H, s, H-10), 1.61 (3H, d, *J* = 5.4 Hz, H-6′), 1.82 (2H, m, H-5), 2.02 (3H, br s, H-9), 2.49 (2H, m, H-4), 3.71 (overlapped, H-4′, H-5′), 4.00 (1H, dd, *J* = 8.8, 7.8 Hz, H-2′), 4.11 (1H, t, *J* = 8.8 Hz. H-3′), 4.89 (1H, d, *J* = 7.8 Hz, H-1′), 5.23 (1H, dd, *J* = 10.7, 1.5 Hz, H-8a), 5.44 (1H, dd, *J* = 17.6, 1.5 Hz, H-8b), 6.23 (1H, dd, *J* = 17.6, 10.7 Hz, H-7), 7.16 (1H, br t, *J* = 6.8 Hz, H-3). ^{13}C-NMR (pyridine-d_5): 12.8 (C-9), 18.9 (C-6′), 23.8, 23.9 (C-4, C-10), 40.6 (C-5), 72.6 (C-5′), 75.6 (C-2′), 76.9 (C-4′), 78.4 (C-3′), 79.6 (C-6), 99.4 (C-1′), 114.8 (C-8), 129.1 (C-2), 142.2 (C-3), 144.2 (C-7), 170.7 (C-1). ^1H-NMR (CD_3OD): see Table 1. ^{13}C-NMR (CD_3OD): see Table 3.

Compound 3a [(6*R*)-Menthiafolic Acid 6-*O*-β-D-Xyloside] Colorless glassy solid, mp 111—113 °C, $[\alpha]_D^{25}$ −5.5° (*c* = 1.0, MeOH). FAB-MS (neg.): 315 (M − H, 100). IR (KBr): 1687. HR-FAB-MS (neg.) M − H (*m/z*): 315.1454. Calcd for $C_{15}H_{23}O_7$: 315.1444. ^1H-NMR (pyridine-d_5): 1.49 (3H, s, H-10), 1.89 (2H, m, H-5), 2.07 (3H, br s, H-9), 2.55 (2H, m, H-4), 3.64 (1H, t, *J* = 10.7 Hz, H-5a′), 3.98 (1H, t, *J* = 8.3 Hz, H-2′), 4.13 (1H, t, *J* = 8.8 Hz, H-3′), 4.21 (1H, m, H-4′), 4.27 (1H, dd, *J* = 10.7, 5.4 Hz, H-5e′), 4.83 (1H, d, *J* = 7.8 Hz, H-1′), 5.20 (1H, dd, *J* = 10.7, 1.0 Hz, H-8a), 5.33 (1H, dd, *J* = 17.6, 1.0 Hz, H-8b), 6.32 (1H, dd, *J* = 17.6, 10.7 Hz, H-7), 7.20 (1H, br t, *J* = 7.8 Hz, H-3). ^{13}C-NMR (pyridine-d_5): 12.8 (C-9), 23.7 (C-4), 24.5 (C-10), 39.1 (C-5), 67.0 (C-5′), 71.1 (C-4′), 75.1 (C-2′), 78.6 (C-3′), 79.6 (C-6), 100.1 (C-1′), 114.2 (C-8), 129.1 (C-2), 142.2 (C-3), 144.2 (C-7), 170.6 (C-1). ^1H-NMR (CD_3OD): see Table 1. ^{13}C-NMR (CD_3OD): see Table 3.

Compound 3b [(6*S*)-Menthiafolic Acid 6-*O*-β-D-Xyloside] Colorless syrup, $[\alpha]_D^{25}$ −25.6° (*c* = 0.73, MeOH). FAB-MS (neg.): 315 (M − H, 100). IR (KBr): 1687. HR-FAB-MS (neg.) M − H (*m/z*): 315.1447. Calcd for $C_{15}H_{23}O_7$: 315.1444. ^1H-NMR (pyridine-d_5): 1.56 (3H, s, H-10), 1.82 (2H, m, H-5), 2.02 (3H, br s, H-9), 2.48 (2H, m, H-4), 3.65 (1H, dd, *J* = 11.2, 9.8 Hz, H-5a′), 3.99 (1H, dd, *J* = 8.8, 7.8 Hz, H-2′), 4.13 (1H,

t, J=8.8 Hz, H-3′), 4.21 (1H, ddd, J=9.8, 8.8, 5.4 Hz, H-4′), 4.29 (1H, dd, J=11.2, 5.4 Hz, H-5e′), 4.87 (1H, d, J=7.8 Hz, H-1′), 5.23 (1H, dd, J=10.7, 1.3 Hz, H-8a), 5.42 (1H, dd, J=17.6, 1.3 Hz, H-8b), 6.23 (1H, dd, J=17.6, 10.7 Hz, H-7), 7.15 (1H, br t, J=7.3 Hz, H-3). ^{13}C-NMR (pyridine-d_5): 12.7 (C-9), 23.8×2 (C-4, C-10), 40.6 (C-5), 66.9 (C-5′), 71.1 (C-4′), 75.2 (C-2′), 78.6 (C-3′), 79.6 (C-6), 100.3 (C-1′), 114.9 (C-8), 129.1 (C-2), 142.1 (C-3), 144.0 (C-7), 170.6 (C-1). ^{1}H-NMR (CD_3OD): see Table 1. ^{13}C-NMR (CD_3OD): see Table 3.

Compound 4 [(2*E*)-6-Hydroxy-2-hydroxymethyl-6-methyl-2,7-octadienoic Acid] Colorless oil, $[\alpha]_D^{28}$ −3.4° (c=0.33, MeOH). FAB-MS (neg.): 199 (M−H, 100). IR ($CHCl_3$): HR-FAB-MS (neg.) M−H (m/z): 199.0972. Calcd for $C_{10}H_{15}O_4$, 199.0970. ^{1}H-NMR (CD_3OD): see Table 2. ^{13}C-NMR (CD_3OD): see Table 3.

Methylation of Compound 4 Compound **4** (5 mg) in MeOH (1 ml) was treated with an excess amount of ethereal CH_2N_2 and the product was purified by chromatography to give a methyl ester (**7**) as a colorless oil (3 mg). ^{1}H-NMR ($CDCl_3$): 1.31 (3H, s, H-10), 1.68 (2H, m, H-5), 2.35 (2H, m, H-4), 2.59 (1H, br s, OH), 3.77 (3H, s, OMe), 4.34 (2H, br s, H-9), 5.10 (1H, dd, J=10.9, 1.0 Hz, H-8c), 5.24 (1H, dd, J=17.5, 1.0 Hz, H-8t), 5.90 (1H, dd, J=17.5, 10.9 Hz, H-7), 6.91 (1H, t, J=7.9 Hz, H-3). ^{13}C-NMR ($CDCl_3$): 23.3 (C-4), 28.3 (C-10), 40.7 (C-5), 51.9 (OMe), 57.2 (C-9), 73.1 (C-6), 112.4 (C-8), 130.7 (C-2), 144.3 (C-3), 146.1 (C-7), 168.0 (C-1).

Compound 5a [Methyl (6*R*)-(2*E*)-6-Hydroxy-2-hydroxymethyl-6-methyl-2,7-octadienoate 6-*O*-β-D-Quinovoside] Colorless syrup, $[\alpha]_D^{23}$ −9.4° (c=0.34, MeOH). FAB-MS (pos.): 383 (M+Na, 59). HR-FAB-MS (pos.) M+H (m/z): 361.1846. Calcd for $C_{17}H_{29}O_8$: 361.1862. ^{1}H-NMR (CD_3OD): see Table 2. ^{13}C-NMR (CD_3OD): see Table 3.

Enzymatic Hydrolysis of Compound 5a Compound **5a** (10 mg) was hydrolyzed with β-glucosidase as described for **2a** to give compound **7a** (3.8 mg) of $[\alpha]_D^{22}$ −22.5° (c=0.13, MeOH) and D-quinovose (3.3 mg), $[\alpha]_D^{22}$ +22.4° (c=0.11, H_2O), whose structures were supported by the ^{1}H-NMR spectra.

Compound 5b [Methyl (6*S*)-(2*E*)-6-Hydroxy-2-hydroxymethyl-6-methyl-2,7-octadienoate 6-*O*-β-D-Quinovoside] Colorless syrup, $[\alpha]_D^{23}$ −26.1° (c=0.71, MeOH). FAB-MS (pos.): 383 (M+Na, 100). HR-FAB-MS (pos.) M+H (m/z): 361.1874. Calcd for $C_{17}H_{29}O_8$: 361.1862. ^{1}H-NMR (CD_3OD): see Table 2. ^{13}C-NMR (CD_3OD): see Table 3.

Enzymatic Hydrolysis of Compound 5b Compound **5b** (9 mg) was hydrolyzed with β-glucosidase as described for **2a** to give compound **7b** (3.8 mg) of $[\alpha]_D^{22}$ +18.7° (c=0.13, MeOH) and D-quinovose (3.6 mg), $[\alpha]_D^{22}$ +22.8° (c=0.12, H_2O), whose structures were confirmed by ^{1}H-NMR.

Compound 6 [(2*E*)-6-Butoxy-2-hydroxymethyl-6-methyl-2,7-octadienoic Acid] Colorless oil, $[\alpha]_D^{24}$ −4.3° (c=0.33, $CHCl_3$). FAB-MS (neg.): 255 (M−H, 100). IR ($CHCl_3$): 1703. ^{1}H-NMR ($CDCl_3$): 0.91 (3H, t, J=7.3 Hz), 1.26 (3H, s), 1.36 (2H, m), 1.49 (2H, m), 1.66 (2H, t, J=8.1 Hz), 2.35 (2H, m), 3.27 (2H, m), 4.34 (2H, br s), 5.14 (1H, br d, J=17.6 Hz), 5.17 (1H, br d, J=10.7 Hz), 5.77 (1H, dd, J=17.6, 10.7 Hz), 7.03 (1H, t, J=7.8 Hz). ^{13}C-NMR ($CDCl_3$): 14.0 (C-4′), 19.5 (C-3′), 22.2 (C-4), 23.2 (C-10), 32.5 (C-2′), 38.9 (C-5), 56.9 (C-9), 62.0 (C-1′), 76.5 (C-6), 114.7 (C-8), 130.3 (C-2), 142.9 (C-7), 148.5 (C-3), 172.2 (C-1). ^{1}H-NMR (CD_3OD): see Table 2. ^{13}C-NMR (CD_3OD): see Table 3.

Methylation of Compound 6 Compound **6** (10 mg) in MeOH was treated with ethereal CH_2N_2 to give a methyl ester (**8**) as a colorless oil (8 mg). ^{1}H-NMR ($CDCl_3$): 0.91 (3H, t, J=7.6 Hz, H-4′), 1.25 (3H, s, H-10), 1.35 (2H, m, H-3′), 1.49 (2H, m, H-2′), 1.65 (2H, m, H-5), 2.32 (2H, m, H-4), 2.57 (1H, t, J=6.8 Hz, OH), 3.26 (2H, m, H-1′), 3.77 (3H, s, OMe), 4.34 (2H, d, J=6.8 Hz, H-9), 5.13 (1H, dd, J=17.6, 1.5 Hz, H-8), 5.16 (1H, dd, J=10.7, 1.5 Hz, H-8), 5.76 (1H, dd, J=17.6, 10.7 Hz, H-7), 6.91 (1H, t, J=8.1 Hz, H-3). ^{13}C-NMR ($CDCl_3$): 14.0 (C-4′), 19.4 (C-3′), 22.2, 22.9 (C-4 or 10), 32.5 (C-2′), 39.0 (C-5), 51.8 (OMe), 57.2 (C-9), 61.9 (C-1′), 76.4 (C-6), 114.6 (C-8), 130.6 (C-2), 142.9 (C-7), 146.3 (C-3), 168.0 (C-1).

References and Notes

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