Studies on the Constituents of *Calliandra anomala* (KUNTH) MACBR. IV. Structure Analysis by HPLC Retention Time and FAB-MS Spectrum

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Three new triterpenoidal saponins were isolated from the branches of *Calliandra anomala* (KUNTH) MACBR. On elucidation of their structures, we noticed correlations between a series of structures and their HPLC retention time. By this behavior and the FAB-MS spectrum, the structures of three saponins (calliandra saponin M, N and O) were established.

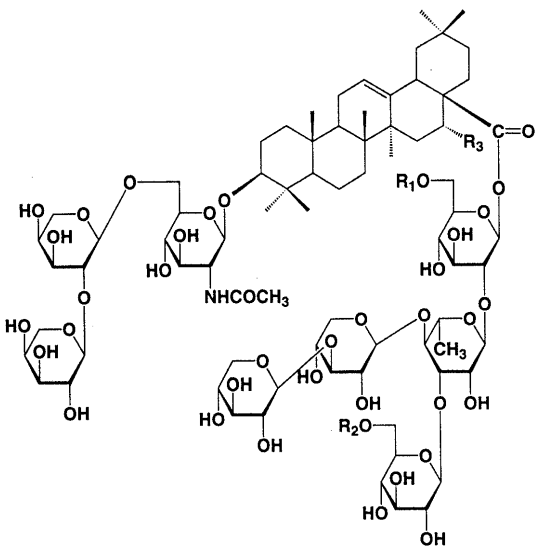
Key words *Calliandra anomala*; Leguminosae; calliandra saponin; HPLC; retention time; FAB-MS

Aqueous extracts of the branches of *Calliandra* (*C.*) *anomala* (KUNTH) MACBR. (Leguminosae) are used as an antimalarial and antifebrile agent in Mexico.[1] In our preceding papers,[2–4] we reported the structures of twelve triterpenoidal sponins, called calliandra saponins (A—L). The structures of these saponins have some similarities and their retention times (t_R) on reversed phase HPLC showed some properties that correlate with their structures. On further investigation, we isolated three components that are supposed to saponins. Using those behavior and the FAB-MS spectrum, we proposed structures for these saponins.

The *n*-butanol soluble fraction of the methanol extract was dissolved in methanol, and ether was added to give a precipitate. The ether precipitate was separated by Lobar RP-18 chromatography and subjected to repeated semi-preparative HPLC on an Asahipak ODP-50 reversed-phase column. We isolated all three saponins (**1**—**3**).

These calliandra saponins have a common formula: the aglycone is triterpenolic acid, the trisaccharide moiety has an *N*-acetyl glucosamine attached to the 3 position of the genin by an ether linkage and the pentasaccharide moiety has one or two monoterpene glycosides attached to the 28 position of the genin by an ester linkage. From the structures of a series of calliandra saponins, A—L (Chart 1), we found three pairs of structures. a) The sugar of the monoterpene glycoside moiety is D-xylose or D-quinovose (*e.g.* A and B), b) the aglycone is echinocystic acid or oleanolic acid (*e.g.* E and J), c) the outer glucose of the C-28 sugar moiety is not-acetylated or acetylated (*e.g.* E and F). The analytical HPLC of calliandra saponins A—L well showed a trend for the lower polarity structures to have a longer t_R (Table 1). Calculating the ratio of each pair of t_R values, we noticed that the ratio of the same combination was nearly equivalent. For example, with rule a), the ratio of calliandra saponin A to calliandra saponin B is 0.84, and calliandra saponin C to calliandra saponin D is 0.84, too. With the other rules, the t_R ratio indicated that the pairs of structures correlated with the HPLC t_R (Table 1) to a significant extent.

The ether precipitate, the crude saponin fraction, contained many other compounds, and it was possible that these substances were triterpenoidal saponins and had



	R_1	R_2	R_3
A:	MA - *Xyl*	H	OH
B:	MA - *Qui*	H	OH
C:	MA - *Xyl* - MA - *Xyl*	H	OH
D:	MA - *Xyl* - MA - *Qui*	H	OH
E:	MA - *Xyl* - MA	H	OH
F:	MA - *Xyl* - MA	$COCH_3$	OH
G:	MA - *Qui* - MA	H	OH
H:	MA - *Qui* - MA	$COCH_3$	OH
I:	MA - *Xyl* - MA - *Qui* - MA	H	OH
J:	MA - *Xyl* - MA	H	H
K:	MA - *Xyl* - MA - *Qui* - MA	$COCH_3$	OH
L:	MA - *Xyl* - MA	$COCH_3$	H

MA : Monoterpene carboxylic acid
Qui : Quinovose
Xyl : Xylose

Chart 1

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structural similarities. Also, on acid hydrolysis with 2 N sulfuric acid, the ether precipitates gave echinocystic acid and oleanolic acid as aglycones, L-arabinose, D-glucose, L-rhamnose, D-xylose, and *N*-acetyl D-glucosamine as the component sugars, and (6*S*)-2-*trans*-2,6-dimethyl-6-hydroxy-2,7-octadienoic acid as the monoterpene carboxylic acid. However, repeated semi-preparative HPLC gave only a small amount of compound, so we have not been able to establish these structures based on 1D ^{13}C-NMR and 2D NMR. As another method for structural elucidation, we applied the relationship between the structures and the t_R ratio with FAB-MS data.

Table 1. Result of Calculating the Ratio of t_R in Each Rule

Rule	Name	t_R (min)	Ratio
a) D-Xylose or D-quinovose	A/B	11.8/14.1	0.84
	C/D	17.3/20.5	0.84
b) Echinocystic acid or	E/J	27.4/75.8	0.36
oleanolic acid	F/L	35.5/102.0	0.35
c) Not acetylated or acetylated	E/F	27.4/35.5	0.77
	G/H	36.7/48.3	0.76
	I/K	61.1/81.3	0.75
	J/L	75.8/102.0	0.74

Table 2. Result of Calculating the Ratio of t_R between Calliandra Saponin I and Compounds **1**—**3**

Rule	Name	t_R (min)	Ratio
a) D-Xylose or D-quinovose	**1**/I	46.0/61.1	0.75
	2/**3**	136.9/172.4	0.80
b) Echinocystic acid or	**1**/**2**	46.0/136.9	0.34
oleanolic acid	I/**3**	61.1/172.4	0.36

The FAB-MS spectrum of compound **1** gave an $[M+Na]^+$ ion peak at *m/z* 2460 and the fragments at *m/z* 962, 1521. Supposing compound **1** was one of the calliandra saponins, this FAB-MS data indicated that the aglycone of **1** was echinocystic acid and the C-28 sugar moiety was the same as calliandra saponin I ($[M+Na]^+$ 2474),[4] except for the monoterpene glycoside moiety, *i.e.* monoterpenexylose replaced monoterpenequinovose. The $[M+Na]^+$ ion peak at *m/z* 2460 was 14 fewer than that of calliandra saponin I, which was in agreement with the supposition that **1** was a xylose-type of calliandra saponin I. The peak at *m/z* 2444 $[M+Na]^+$, and the fragments at *m/z* 946, 1521 in the FAB-MS of compound **2** indicated that the aglycone was oleanolic acid and the C-28 sugar moiety was the same as **1**, *i.e.*, **2** was an oleanane-type of **1**. The FAB-MS spectrum of compound **3** showed the $[M+Na]^+$ ion peak at *m/z* 2458, which suggested that **3** was an oleanane-type of calliandra saponin I. Subjecting calliandra saponin I and the three new compounds to the t_R rules a) and b), we obtained the relationship illustrated in Chart 2. The analytical HPLC t_R was 46.0 min for **1**, 136.9 min for **2** and 172.4 min for **3**. We calculated the ratios of these t_R values according to the t_R rules a) and b) (Table 2). Comparing with Table 1, rule a) was only slightly different, where rule b) was almost equivalent. This result and the FAB-MS data supported the finding that compounds **1**, **2** and **3** could possibly be triterpenoidal saponins similar to calliandra saponins, and their structures may be as shown in Chart 2. So, we called compounds **1**, **2**, and **3**, calliandra saponins M, N, and O, respectively.

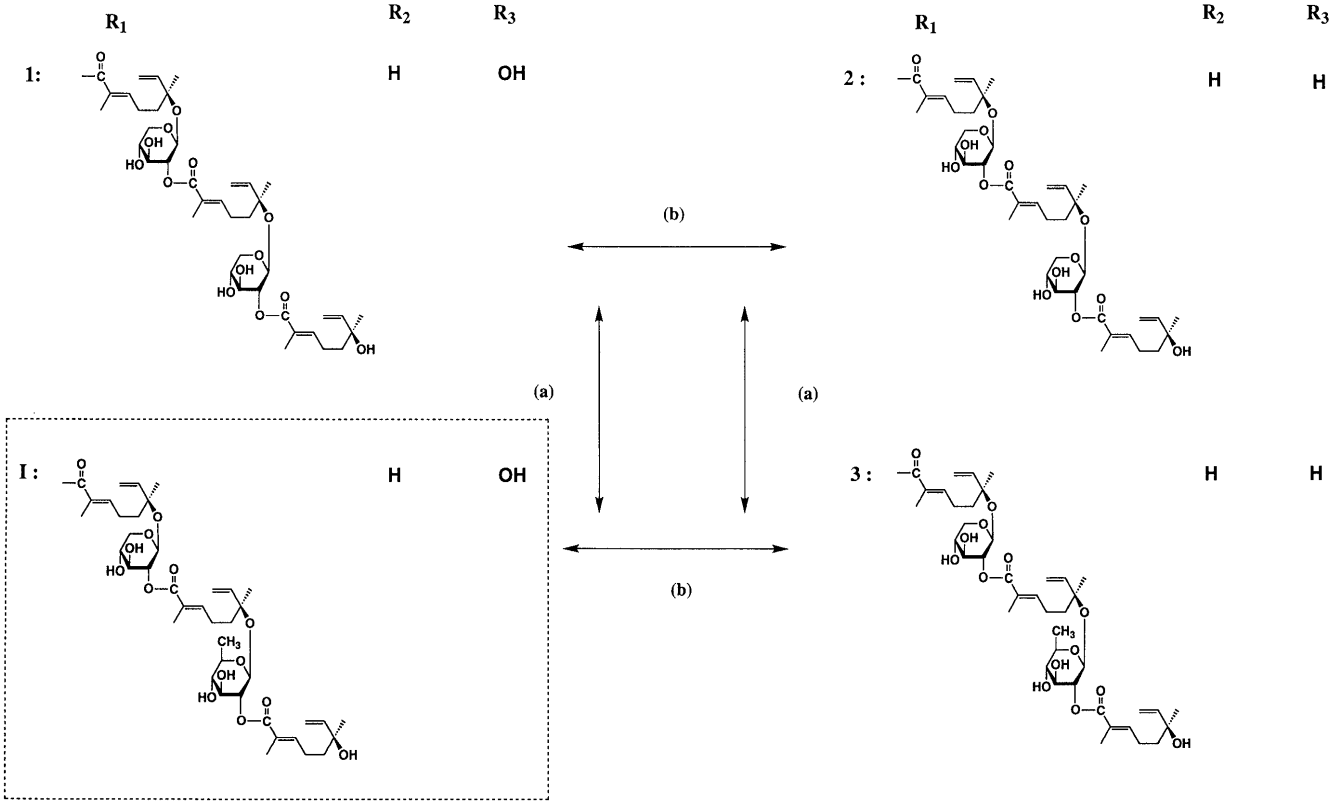

Chart 2

Experimental

The instruments used for obtaining physical data and the experimental conditions for chromatography were the same as described in our previous paper.[3)]

Extraction and Isolation This was performed as described in a previous report.[3)] The material was collected in Morelos, Mexico, in 1987 and voucher specimens were deposited in the Jardin de Etno-botanico, Instituto Nacional de Antropologia e Historia. The material was identified as *C. anomala* (KUNTH) MACBR. by Dr. Guillermo Suarez Ortega (Botanical Garden Director, Jardin de Etnobotanica, Morelos, Mexico). The methanol extract was concentrated under reduced pressure and the residue (80.3 g) was suspended in water. The suspension was extracted with *n*-butanol and then the *n*-butanol soluble fraction was concentrated *in vacuo* to give a residue (33.4 g). This residue was dissolved in methanol (20 ml), and ether (1 l) was added to give a precipitate (10.7 g). The ether precipitate (1.8 g) of the methanol extract was chromatographed on Lobar RP-18 with 35% acetonitrile solution as mobile phase to give a crude saponin fraction. The repeated semi-preparative HPLC of the crude saponin fraction separately on an Asahipak ODP-50 column (10 × 250 mm) with 35% acetonitrile solution yielded calliandra saponins M (**1**) (2.1 mg), N (**2**) (1.2 mg), and O (**3**) (0.8 mg). HPLC conditions: column, Asahipak ODP-50 (4.6 i.d. × 250 mm); flow rate, 0.5 ml/min; detection, 221 nm; eluent, CH_3CN–THF–H_2O (33 : 1 : 66).

Retention time (min): calliandra saponin A, 11.8; B, 14.1; C, 17.3; D, 20.5; E, 27.4; F, 35.5; G, 36.7; H, 48.3; I, 61.1; J, 75.8; K, 81.3; L, 102.0; M, 46.0; N, 136.9; O, 172.4.

Calliandra Saponin M (1) $[\alpha]_D^{22}$ −19.1° (c = 0.002, MeOH). ^{1}H-NMR (pyridine-d_5: D_2O = 9 : 1) aglycone moiety δ: 0.95 (3H, s, H_3-25), 1.00 (6H, s, H_3-24, H_3-29), 1.09 (3H, s, H_3-26), 1.12 (3H, s, H_3-30), 1.28 (3H, s, H_3-23), 1.80 (3H, s, H_3-27), 1.66 (3H, d, J = 6.1 Hz, Rham, Me-6), 2.20 (3H, s, $NHCOCH_3$); monoterpene glycoside moiety δ: 1.50, 1.51 (6H, s, H_3-10, H_3-10″), 1.53 (3H, s, H_3-10′) , 1.92 (3H, s, H_3-9), 1.97 (3H, s, H_3-9″), 1.98 (3H, s, H_3-9′).

Calliandra Saponin N (2) $[\alpha]_D^{22}$ −28.0° (c = 0.002, MeOH). ^{1}H-NMR (pyridine-d_5: D_2O = 9 : 1) aglycone moiety δ: 0.88 (3H, s, H_3-29), 0.89 (3H, s, H_3-25), 0.93 (3H, s, H_3-30), 0.95 (3H, s, H_3-24), 1.01 (3H, s, H_3-26), 1.25 (3H, s, H_3-23), 1.33 (3H, s, H_3-27), 1.67 (3H, d, J = 6.1 Hz, Rham, Me-6), 2.21 (3H, s, $NHCOCH_3$); monoterpene glycoside moiety δ: 1.51 (6H, s, H_3-10, H_3-10″), 1.53 (3H, s, H_3-10′), 1.93 (3H, s, H_3-9), 1.99 (6H, s, H_3-9′, H_3-9″).

Calliandra Saponin O (3) $[\alpha]_D^{22}$ −26.0° (c = 0.004, MeOH). ^{1}H-NMR (pyridine-d_5: D_2O = 9 : 1) aglycone moiety δ: 0.88 (3H, s, H_3-29), 0.89 (3H, s, H_3-25), 0.93 (3H, s, H_3-30), 0.95 (3H, s, H_3-24), 1.01 (3H, s, H_3-26), 1.25 (3H, s, H_3-23), 1.35 (3H, s, H_3-27), 1.67 (3H, d, J = 5.8 Hz, Rham, Me-6), 2.21 (3H, s, $NHCOCH_3$); monoterpene glycoside moiety δ: 1.50, 1.51 (6H, s, H_3-10, H_3-10″), 1.53 (3H, s, H_3-10′), 1.58 (3H, s, J = 5.2 Hz, Qui, Me-6), 1.93 (3H, s, H_3-9), 1.98 (6H, s, H_3-9′, H_3-9″).

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