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A Novel Diterpene Glycoside from the Seeds of *Acacia farnesiana*

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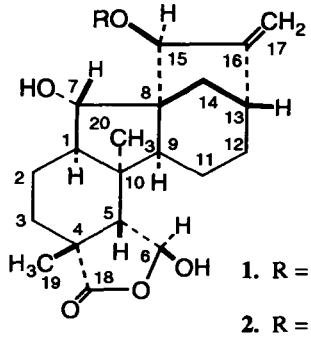
Abstract: Structure of farnesiaside (**1**), the β-D-glucoside of a diterpene bearing a novel skeleton [formally a 7(6→1)-*abeo*-*ent*-kaurene], has been deduced from spectral studies. Conformational change in the aglycone resulted in extensive changes in NMR spectra, explained through MM and MD studies. © 1997 Elsevier Science Ltd.

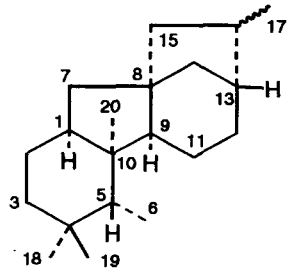
Acacia farnesiana Willd., a thorny bush or small tree occurring gregariously almost all over India, is used as folk remedy for different ailments.[1] Previous chemical investigations have demonstrated the presence of homoterpene lactone, flavone glycosides and polyphenolic compounds.[2,3] We now wish to report the isolation and characterization of a novel diterpene glycoside, designated farnesiaside (**1**), possessing a new carbon skeleton to which we recommend the name acafarnane.

The *n*-BuOH soluble fraction of the defatted *A. farnesiana* seeds on repeated chromatographic purification over silica gel gave compound **1**, as colourless needles, mp 110-112 °C (dec.), $[\alpha]^{25}_{D}$ -25.3° (*c* 0.19, MeOH), IR ν_{max}3388 (OH) and 1754 (5-ring lactone) cm^{-1}. The molecular formula $C_{26}H_{38}O_{10}$ was determined from its high resolution FABMS (*m/z* 533.2377 $[M+Na]^+$) as well as from its ^{13}C DEPT NMR spectra. Of the 26 carbons, 20 were assigned to the aglycone **2** and 6 to the carbohydrate unit, identified as glucose by enzymatic hydrolysis.[4] From its NMR spectra, the diterpene moiety of farnesiaside was found to contain an exocyclic double bond, two secondary hydroxyl groups and two tertiary methyl groups; it was linked to the sugar through an allylic carbon (HMBC correlation between anomeric proton/carbon, δ 5.11/106.9 ppm, and C-15 proton/carbon, δ 4.52/90.9 signals), orientation of the glucose unit being β (δ 5.11, d, *J*=7.6 Hz for the anomeric proton). From COSY, HOHAHA, HSQC (Table 1), and HMBC spectra, **1** was concluded to possess most of the structural elements of a kauranoid lactone framework depicted in bold lines in Fig. 1. However, the chemical



1. R = β-D-glucose

2. R = H



Acafarnane

shifts attributed to H-6 (δ 6.39) and C-6 (δ 100) were strikingly downfield. This, and the absence of COSY correlation between H-5, H-6 and H-7 signals necessitated that the C_6-C_7 bond be cleaved and C-6 carry a second oxy substituent. The presence of an additional methine carbon attached to C-7 (COSY), along with HMBC correlation noticed between H-7 and C-2, C-10 signals thereafter allowed us to assume the existence of a C_7-C_1 bond, leading to the novel acafarnane [7(6→1)*abeo-ent*-kaurane] skeleton. The orientation of the protons and the relative stereochemistry were established from a phase-sensitive NOESY. The methyl group at C-10 (supposed to be α from a biogenetic point of view for an *ent*-kaurane type skeleton) showed strong nOe interactions with H-1 and H-9, suggesting the *cis*-fusion of rings A and B, B and C. The β-orientation of H-5, H-19, and H-7 was suggested by the lack of nOes between H-20 and these protons. The *cis*-fusion of the rings A, B and C brought the protons at the β-face in close proximity, resulting in strong nOes between H-5 and H-2β, H-7, H-11β, H-12β, H-14β. A boat conformation for ring A was indicated from the nOes between H-1/H-3α, H-2β/H-5 and H-3α/H-20. The lack of nOes between H-11β/H-14β suggested the C-ring to have a chair-like form. Other supporting information was from the nOes observed between H-5/H-11β, H-5/H-12β, H-6/H-11β as well as H-12β/H-14β (weak). The lack of scalar coupling (but proximity in space as observed from NOESY) between H-5 and H-6 can be explained by the nearly 90° dihedral angle between them (91.3°, J_{cal} = 0.8 Hz from MM and MD calculations[5]).

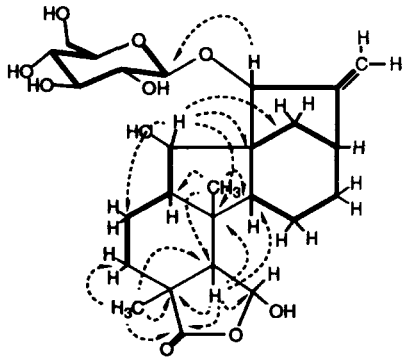


Fig. 1. Connectivities established by COSY, HOHAHA, HSQC (bold lines), and HMBC (arrows).

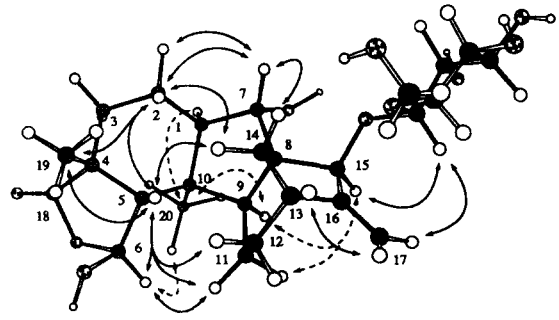


Fig. 2. Relative stereochemistry of farnesiaside (**1**) based on NOESY (the structure was optimized from MM and MD simulations).

Though attempted acid hydrolysis of **1** caused extensive decomposition of the aglycone, treatment with β-glucosidase[4] afforded the genuine aglycone farnesin (**2**) quantitatively. Surprisingly, the ^1H and ^{13}C spectra of **2** showed great difference from that of **1** and the assignments can not be made by simple comparison only as in other cases. On the basis of COSY, HSQC, HMBC and phase-sensitive NOESY, the protons and carbons of the aglycone were positively assigned (Table 1) and all these data supported the established structure of farnesiaside (**1**). A plausible explanation for the significant displacement in some of the chemical shifts was that the attachment of a glucose at C-15 has changed the conformation of the aglycone, which had a highly flexible five-membered B-ring close to where the glucose was attached.

In order to shed light on the seemingly perplexing phenomenon, molecular mechanics and dynamics study were carried out on farnesiaside (**1**) and its aglycone (**2**), respectively. The resulting energy-minimized conformers fitted quite well with the information from 2D-NOESY. For **1**, the MM and MD simulations gave a boat A-ring, and a chair-like C-ring, as indicated from the nOe study (Fig. 2). The five-membered B-ring took an envelope form with C-8 above the plane defined by C-1,7,9,10. In the case of **2**, the energy-minimized conformer showed the C-ring in a boat conformation, which is supported by the nOe observed between H-11β/H-14β. Very interestingly, the cyclopentane B-ring existed in an envelope form with C-1 below the plane, different from that in **1**. For the aglycone, the hydroxyl at C-7 is in a position (six-membered ring) to form hydrogen bond with the free hydroxyl at C-15, which is not available in **1**. The phase-sensitive NOESY of **2** showed that there was close contact between the hydrogens belonging to the hydroxyls at C-7 and C-15. Further evidence for the existence of the hydrogen bond was the doublet splitting pattern (δ7.31, 3.7 Hz) observed for the hydrogen of C_7-OH (Table 1). Apart from the difference in the conformations of B and C-rings as discussed above, conformational discrepancy between **1** and **2** is also reflected in the following trans-space nOe relationships. For **2**, nOes were observed between H-7 and H-14α/β, H-6 and H-11α/β, while for **1**, only nOes between H-7 and H-14α, H-6 and H-11β were observed.

It is well known that there is no preferred conformation for the cyclopentane framework itself and substituted cyclopentane will take up conformations such that the interactions of the substituents with the framework and with each other are minimized. Along with the change in the conformation, the torsion angles, the bond angles and the exocyclic angles change as well. In our case, the change in the conformation of the cyclopentane B-ring can be gauged from the change of the vicinal coupling constants (or the appearance of the signals) in ^{1}H NMR (H-1, H-7, H-9 as well as other protons in the molecule). The above evidence indicated that the absence of the bulky glucose of farnesiaside (**1**) in **2** allowed the formation of a hydrogen bond between 7-OH and 15-OH changing the conformation of the aglycone. Proton and carbon chemical shifts are very sensitive to their stereo surroundings, and therefore, the change in the conformation would result in significant displacement of these.

Biogenetically, it is presumed that farnesiaside originated from a 6,7-dihydroxy-*ent*-kaurene derivative *via* cleavage of the B-ring between C-6 and C-7, rotation around C_9-C_{10} and formation of a bond between C-1 and C-7 (Fig. 3). To our knowledge, farnesin is the first example of a diterpenoid with such carbon skeleton.

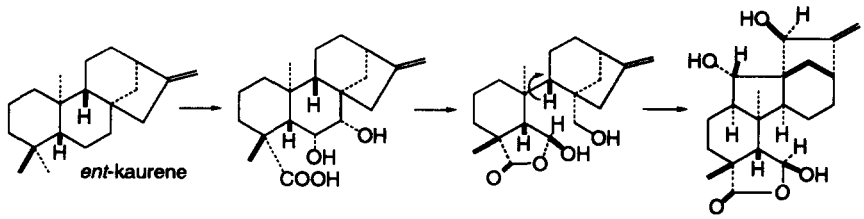


Fig. 3. The possible biosynthetic pathway for farnesin from *ent*-kaurene

Table 1. ^{13}C and ^{1}H-NMR Data of Farnesiaside (**1**) and Farnesin (**2**)†

	Farnesiaside (**1**)		Farnesin (**2**)	
	^{13}C	^{1}H	^{13}C	^{1}H
1	58.4 d	2.08 ddd (12.5, 6.1, 2.4)	52.6	1.94*
2	24.5 t	1.83* α, 1.40 (β) m	18.8	1.71* (α), 1.92* (β)
3	30.7 t	1.78* (2H)	27.6	1.75* (α), 2.27 (β) m
4	42.1 s	—	41.1	—
5	53.7 d	2.77 s	51.3	2.43 s
6	100.0 d	6.39 s	99.7	6.10 s
7	81.3 d	4.19*	77.6	4.50 dd (10.1, 2.4)
8	59.2 s	—	51.9	—
9	53.3 d	2.28 dd (7.9, 2.0)	55.3	1.47*
10	43.2 s	—	40.2	—
11	17.8 t	1.76* α, 1.90 (β) m	17.5	1.62 (α) *, 1.83* (β)
12	32.5 t	1.60 (α) m, 2.20 (β) m	32.9	1.43* (α), 2.11 m (β)
13	41.1 d	2.81 m	37.9	2.72 t (6.1)
14	34.1 t	2.12 (α) dd (11.3, 5.0) 1.71 (β) d (11.3)	31.8	2.57 (α) dd (11.3, 5.3) 1.52 (β) d (11.3)
15	90.9 d	4.52 s	82.0	4.58 s
16	155.4 s	—	161.8	—
17	113.2 t	5.29 s, 5.95 s	110.3	5.19 s, 5.39 s
18	183.5 s	—	182.4	—
19	28.7 q	1.77 s	30.1	1.74 s
20	29.5 q	1.24 s	24.4	1.08 s
Glc-1	106.9 d	5.11 d (7.6 Hz)		
Glc-2	75.6 d	3.98 t (8.2)		
Glc-3	78.9 d	4.18*		
Glc-4	71.8 d	4.18*		
Glc-5	78.5 d	3.95 m		
Glc-6	62.9 t	4.50 dd (12.0, 2.4) 4.33 dd (12.0, 5.5)		

†Recorded in pyridine-d_5 on a JOEL α-500 (500 MHz) spectrometer. The ^{1}H and ^{13}C assignments were based upon COSY, HOHAHA, DEPT, HSQC, HMBC and phase-sensitive NOESY.
*Overlapped by other signals.

References and Notes:

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4. A solution of farnesiaside (17 mg) in acetate buffer (pH 5.0, 1 ml) was treated with β-glucosidase (Oriental yeast, from almond, *ca* 30 mg) at 37 °C for 15 h. AcOEt extraction afforded farnesin (**2**, 8 mg). The sugar was identified to be D-glucose by co-HPLC analysis with authentic sample (t_R=10.1 min., Asahipak NH2P-50, 4.6 x 250 mm, 75% CH_3CN-H_2O, 0.8 ml/min, RI) and specific rotatory power measurement $[\alpha]^{25}_D$ +47.5° (*c* 0.04, H_2O, measured 24 h after dissolution in water).
5. MM and MD simulations were performed using the MacroModel and BatchMin V5.0 molecular modeling programs.
6. Farnesin (**2**): mp. 265-267 °C; $[\alpha]^{23}_D$ -23.1 ° (*c* 0.7, MeOH); IR (KBr) $\nu_{max}cm^{-1}$: 3374, 3264, 1749; EI-MS (rel. int.): m/z 348 $[M]^+$ (0.8%) 330 $[M-H_2O]^+$(8.3), 312 $[M-2H_2O]^+$ (15.2), 91 (100).

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