Constituents of *Acacia cedilloi* and *Acacia gaumeri.* Revised Structure and Complete NMR Assignments of Resinone

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The rare lupene derivative named resinone has only been isolated before from *Fluorensia resinosa*. We now report the isolation of this compound from the bark of the new recently described *Acacia cedilloi* (Fabaceae), and the revision of its structure to 16β-hydroxylup-20(29)-en-3-one, based on NMR and MS spectral data. The detailed ^{1}H and ^{13}C NMR assignments of resinone and its acetate achieved by 1D and 2D NMR experiments (including DEPT, COSY, HMQC and HMBC) are reported. In addition, the study of *A. cedilloi* and *A. gaumeri* afforded the known related lupenes lupeol and lupenone, the acyclic squalene, the sterols β-sitosterol, stigmasta-7,22-dien-3β-ol (spinasterol) and stigmasta-5,22,25-trien-3β-ol (22-dehydroclerosterol) as well as α-tocopherol and β-carotene.

Introduction

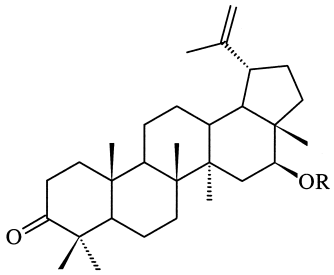
The family Fabaceae the third biggest family of Angiosperms with about 13,000 species, is the predominant family of the flora of the Yucatán Peninsula, where is represented by about 244 species. As part of our search for biologically active compounds of plants from the Yucatán Peninsula, we have undertaken the phytochemical study of *Acacia cedilloi* and *A. gaumeri* two endemic legume species which have not been chemically investigated before. *A. cedilloi* is a new recently described species which grows in the ecological reserve "El Eden" in the state of Quintana Roo (Rico-Arce, 1994) and *A. gaumeri*, an important melliferous plant which has also been documented as a medicinal species in the Maya traditional medicine, for the treatment of children's nycturia.

Results and Discussion

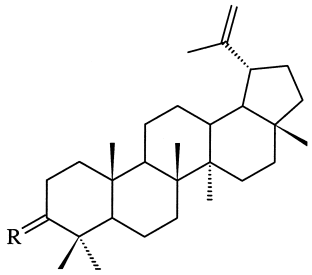
Column chromatography of the hexane and dichloromethane extracts of *A. cedilloi* afforded three triterpenes 16β-hydroxylup-20(29)-en-3-one identified as resinone (**1**), lupeol (**3**) and the acyclic squalene, in addition to common C_{12}-C_{20} hydrocarbons, palmitic and oleic acids together with their corresponding methyl esters. While the extracts of *A. gaumeri* afforded lupeol (**3**) and lupenone (**4**), in addition to sterols β-sitosterol, stigmasta-7,22-dien-3β-ol (spinasterol) and stigmasta-5,22,25-trien-3β-ol (22-dehydroclerosterol), α-tocopherol (vitamin E) and β-carotene.

The triterpene **1** was obtained from *A. cedilloi* as a crystalline compound, mp 171–172 °C, which showed low antimicrobial activity against *C. albicans*. The mass spectrum of **1** showed a molecular ion peak at *m/z* 440 corresponding to a molecular formula $C_{30}H_{48}O_2$ as confirmed the ^{13}C NMR and DEPT experiments which indicated the presence of 30 carbon atoms due to seven methyls, ten methylenes, six methines and seven non-protonated carbons. The ^{1}H and ^{13}C NMR and mass spectral data suggested the presence of a pentacyclic triterpene structure of the lupene type with hydroxyl and carbonyl groups according to the IR absorptions at 3340, 1704 and 1640, 880 cm^{-1}. The EI mass spectrum showed characteristic fragment ion peaks at m/z 205, 219, 234, which together with the presence of a signal at δ 218.1 for a carbonyl group in the ^{13}C NMR spectrum suggested the presence of the keto group at C-3, as in lupenone (**4**).

The ^{1}H NMR spectrum of **1** displayed signals for typical pentacyclic triterpenoid methyl groups at δ 0.81, 0.94, 1.01, 1.02, 1.07, and 1.08 (δ_C 11.7, 16.1, 15.9, 21.0, 26.6 and 15.8 respectively accord-



1 R = H
2 R = Ac



3 R = βOH, αH
4 R = O

Fig. 1.

ing with the HMQC experiment). The presence of a vinyl methyl broad singlet at δ 1.68 and two vinyl proton signals at δ 4.71 (brd, J = 2.5 Hz) and 4.60 (dq, J = 2.5, 1.5 Hz) with ^{13}C absorptions at δ 19.3, 149.9 and 109.8 are characteristic of the isopropenyl group of triterpenes of the lupene type. The ^{1}H NMR spectrum also showed a doublet of doublets at δ 3.61 (J = 11.0, 4.5 Hz) due to a hydroxymethine group (δ_C 76.9). The multiplicity and the coupling constant values indicated an axial orientation of the proton and limited the location of the hydroxyl group to C-7, C-15 or C-16. Positions C-7 and C-15 can be excluded by comparison of the ^{13}C NMR data of **1** with those of the known 7β-hydroxylup-20(29)-en-3-one (Anaya *et al.*, 1989), and lup-20(29)-en-3β,15α-diol (Tanaka *et al.*, 1993). On the other hand, comparison of the ^{13}C NMR data of compound **1** with those of lupenone (**4**) and those reported for lup-20(29)-en-3β,16β-diol were in good agreement with the presence of the keto group at C-3 and the hydroxyl group at C-16 (Wenkert *et al.*, 1978). The HMBC experiment confirmed the presence of the carbonyl group at C-3 and the hydroxyl group at C-16. Long-range couplings were observed between the carbonyl signal at δ 218.1 and the *gem*-dimethyl proton signals at δ 1.08 and 1.03 (C-23 and C-24). These two methyl signals were in turn coupled with a quaternary carbon at δ 47.3 and a methine group at δ 54.9 allowing the assignment of these signals to C-4 and C-5 respectively. Further coupling of the methine carbon at δ 54.9 (C-5) with the methyl signal at δ 0.93 indicated that this signal is due to the methyl group at C-10 (H-25). The long-range coupling between the methine signal at δ 76.9 and the methyl signal at δ 0.81 (H-28), indicated that the secondary alcohol must be at C-16. The β-orientation of the hydroxyl group was indicated by the coupling constants of the H-16 proton. Therefore the structure of compound **1**, was determined as 16β-hydroxylup-20(29)-ene-3-one. Acetylation of **1**, gave the corresponding acetate **2**. All spectral NMR data of acetate **2** were in good agreement and confirmed the proposed structure **1**.

A search of the literature indicated that structure **1** must correspond to resinone, a rare pentacyclic triterpene isolated from *Flourensia resinosa* (Asteraceae) (Rodriguez-Hahn and Rodriguez, 1972). The structure of resinone was established as the 12β-hydroxy derivative of lupenone (**4**) by chemical correlation with the oxidation product of thurberin (or calenduladiol) a lupene diol independently isolated from *Lemaireocereus thurberi* and *Calendula officinalis*, whose structure had been erroneously established as lupen-20(29)-3β,12β-diol (Jolad and Steelink, 1969; Kasprzyk and Pyrek, 1968). Later on, the identity of thurberine and calenduladiol, was recognized and the structure revised to lupen-20(29)-3β,16β-diol keeping the name calenduladiol (Kasprzyk *et al.*, 1970; Protiva *et al.*, 1977). Moreover, the revised structure of thurberin and calenduladiol corresponds with that of beyeriadiol, a compound isolated before from *Beyeria leschenaultii* (Baddeley *et al.*, 1964). Therefore, the structure of resinone must be revised to 16β-hydroxylup-20(29)-en-3-one (**1**) and the names thurberin and calenduladiol eliminated from the literature. This is the second report on the natural occurrence of compound **1**.

The known lupeol (**3**) and lupenone (**4**), were identified by comparison of their NMR and MS spectral data. ^{1}H and ^{13}C NMR assignments based on 1D and 2D experiments, including DEPT,

Table I. ^{1}H and ^{13}C NMR spectral data for 16β-hydroxylup-20(29)-en-3-one (**1**) (500 MHz, 125 MHz) and its acetate (**2**) (400 MHz, 100 MHz), in $CDCl_3$.

	δ_C (**1**)	δ_H (**1**)	HMBC (**1**)	δ_C (**2**)	δ_H (**2**)	HMBC (**2**)
1a	39.6 t	1.38 m*	H-25	39.6	1.39	H-2a, 2b, 25
1b		1.90 ddd (13.0, 7.5, 4.5)			1.89	
2a	34.1 t	2.40 ddd (16.0, 8.5, 4.5)		34.1	2.42	H-1b
2b		2.49 ddd (16.0, 9.5, 7.5)			2.48	
3	218.1 s	– – –	H-23, 24, 2b	218.1	– – –	H-1, 2, 23, 24
4	47.3 s	– – –	H-23, 24	47.3	– – –	H-23, 24
5	54.9 d	1.30 m*	H-23, 24, 25	54.7	1.32	H-23, 24, 25
6a, b	19.6 t	1.48 m* (2H)		19.6	1.48	
7a, b	33.5 t	1.43 m* (2H)	H-26	33.5	1.42	H-26
8	40.9 s	– – –	H-26, 27	40.9	– – –	H-26, 27
9	49.4 d	1.36 m*	H-25, 26	49.3	1.37	H-25, 26
10	36.8 s	– – –	H-25	36.8	– – –	H-1b, 2a, 25
11a	21.4 t	1.26 m*		21.4	1.25	
11b		1.42 m*			1.45	
12a	24.8 t	1.04 m*		24.7	1.06	
12b		1.70 brd (15.0)			1.70	
13	37.4 d	1.64 m*	H-27	37.5	1.69	H-27
14	44.1 s	– – –	H-26, 27	44.2	– – –	H-26, 27
15a	36.8 t	1.30 m*,	H-27	33.5	1.32	H-16, 27
15b		1.57 t (12.5)			1.63	
16	76.9 d	3.62 dd (11.0, 4.5)	H-15, 28	79.0	4.87	H-15a, 15b, 28
17	48.6 s	– – –	H-28	47.3	– – –	H-16
18	47.7 d	1.40 m*	H-28	47.7	1.51	H-16, 28
19	47.6 d	2.50 td (11.5, 5.5)	H-30, 29a, 29b	47.5	2.50	H-29b, 30
20	149.9 s	– – –	H-30	149.8	– – –	H-19, 30
21a	29.9 t	1.40 ddd (12.0, 8.5, 1.5)		29.7	1.52	H-19
21b		1.98 m			1.95	
22a	37.7 t	1.28 m*	H-28	37.6	1.33	H-16, 28
22b		1.65 m*			1.69	
23	26.6 q	1.07 s	H-24	26.7	1.06	H-24
24	21.0 q	1.02 s	H-23	21.0	1.02	H-23
25	16.1 q	0.94 s		16.0	0.95	
26	15.8 q	1.08 s		15.8	1.07	
27	15.9 q	1.01 s	H-15	15.9	1.06	
28	11.7 q	0.81 s	H-18, 22	12.7	0.86	H-16
29a	109.9 t	4.60 dq (2.0, 1.5)	H-30	110.0	4.60	H-19, 30
29b		4.71 brd (2.5)			4.71	
30	19.3 q	1.68 brs	H-29a, 29b	19.2	1.68	H-29a, 29b
AcO–				21.4	2.03	
				170.8		H-16

* Assignments and chemical shifts of overlapped ^{1}H multiplets are based on COSY NOESY and HMQC experiments. a,b refers to relative configuration of protons.

COSY, NOESY, HMQC and HMBC are in good agreement with the recently reported data (Burns *et al.*, 2000).

Experimental

General

Melting points were determined on a Fischer Jones type apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ solutions on a Jasco DIP 360 polarimeter. IR spectra were recorded on KBr disks on a Nicolet Magna Fourier transform IR spectrometer 550. EIMS were obtained on a Hewlett-Packard 5892 mass spectrometer using a Hewlett-Packard 5970 series II gas chromatograph as injection system. NMR spectra were recorded on a Varian Unity Plus 500 and Bruker Advance 400 spectrometers in $CDCl_3$ solutions with TMS as internal standard, chemical shifts are recorded in δ values.

Plant material

Acacia cedilloi (L) Rico was collected at the "El Eden" ecological reserve located 21° 3′ N, 87° 11′ W of Cancún Q. R. (México) in July 1996. *A. gaumeri* Blake, was collected at the 8 km on the road Kanasin-Acancéh in the state of Yucatán (México) in January 1999. Voucher specimens have been deposited at the herbarium Alfredo Barrera Marin, Universidad Autónoma de Yucatán, Mérida, Yuc., México.

Extraction and isolation

Dried and ground bark (664 g) and leaves (380 g) of *A. cedilloi* were extracted separately at room temperature by percolation with hexane and CH_2Cl_2 to give 2.7 g and 26.0 g of residue respectively. The bark (400 g) and leaves (450 g) of *A. gaumeri* were extracted in the same way giving 9.0 g and 10.0 g of residue, respectively. Column chromatography of the bark residue of *A. cedilloi*, on silica gel using hexane and hexane-CH_2Cl_2 mixtures of increasing polarity. GC-MS of the low polar fractions indicated the presence of squalene, mixtures of common $C_{12}-C_{20}$ hydrocarbons, palmitic and oleic acid together with their corresponding methyl esters. Fractions eluted with hexane-CH_2Cl_2 7:3 v/v afforded the crystalline 16β-hydroxylup-20(29)-en-3-one (**1**). From the chromatography of the leaf residue, lupeol (**3**) and β-sitosterol were obtained from fractions eluted with hexane-EtOAc 9:1 v/v and hexane-EtOAc 7:3 respectively. The CH_2Cl_2 extract of the bark of *A. gaumeri* was fractionated by column chromatography on silica gel using mixtures of hexane-EtOAc (9:1, 8:2, 6:4 v/v). Lupenone (**4**) and lupeol (**3**) were obtained from fractions eluted with hexane-EtOAc 9:1. Fractions eluted with hexane-EtOAc 8:2 gave a sterol identified as stigmasta-7,22-diene-3β-ol (spinasterol). Column chromatography over silica gel of the leaf extract using mixtures of hexane-EtOAc afforded α-tocopherol (vitamin E), β-carotene in the low polar fractions and stigmasta-5,22,25-triene-3β-ol (22-dehydroclerosterol) in fractions eluted with hexane-EtOAc 8:2.

*16β-hydroxylup-20(29)-en-3-one (**1**)*

Colorless crystals, mp 171–172°, $[\alpha]_D^{25}$ + 43.8° (*c* 1.6 mg/ml, $CHCl_3$); IR (KBr) ν_{max} 3341, 3072, 1704, 805 cm^{-1}; EIMS m/z 440 [M]+ (100), 422 (69), 407 (28), 358 (53), 313 (22), 234 (17), 219 (19), 205 (51), 121 (35), 108 (71), 95 (52), 81 (59), 68 (83), 55 (68), 43 (49); ^1H and ^{13}C NMR (see Table I).

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