Triterpene Esters from Australian *Acacia*

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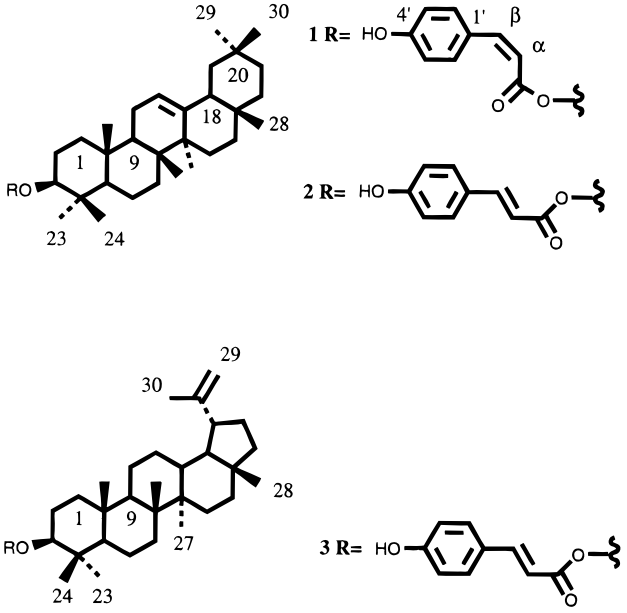
Three new compounds, the hydroxycinnamoyl esters of the triterpenes amyrin (**1**, **2**) and lupeol (**3**), were isolated from the leaves and twigs of *Acacia linarioides* and *Acacia trineura*. Their structures were determined by 1D and 2D NMR spectroscopy.

The Australian native flora is an abundant source of novel bioactive secondary metabolites. As part of our chemical investigation of Australian *Acacia*, we have studied *Acacia linarioides* and *Acacia trineura*. *A. linarioides* (Benth.) and *A. trineura* (F. Muell.) both belong to the family Leguminosae, which has been a rich source of secondary metabolites.[1] *Acacia linarioides* is a shrub up to 1.5 m long found almost exclusively in the Northen Territory,[2] while *A. trineura* is a shrub or tree growing up to 3 m high, found throughout the southern states of Australia (NSW, VIC, SA, WA).[3] We report here the isolation of the *cis*- and *trans*-*p*-hydroxycinnamoyl esters of amyrin and the *trans*-*p*-hydroxycinnamoyl ester of lupeol.

A. linarioides afforded the previously unreported *cis*- and *trans*-*p*-hydroxycinnamoyl esters **1** and **2**, respectively, of the triterpene amyrin, while the *trans*-*p*-hydroxycinnamoyl ester (**3**) of the triterpene lupeol was isolated from *A. trineura*. HRMS indicated that each of the isolated compounds had the molecular formula $C_{39}H_{56}O_3$. The ^{1}H NMR spectrum of **1, 2**, and **3** indicated a cinnamoyl unit, with **2** and **3** having a *trans* olefin group, at δ 7.62 and 6.32 (J H$_\alpha$−H$_\beta$ = 18.2 Hz) and δ 7.57 and 6.29 (J H$_\alpha$−H$_\beta$ = 16.2 Hz), respectively, while **1** had a *cis*-olefin group at δ 6.77 and 5.78 (J H$_\alpha$−H$_\beta$ = 13.2 Hz). The ^{13}C spectra of **1**, **2**, and **3** suggested the presence of ester groups at δ 166.8, 167.4, and 167.2, respectively, which was confirmed by the HMQC and HMBC spectra. Further examination of the HMQC and HMBC spectra confirmed the presence of the *p*-hydroxycinnamoyl ester group in **1**, **2**, and **3**.[4] On the basis of the molecular formula and the DEPT spectrum, it was highly probable that the *p*-hydroxycinnamoyl groups were esterified to a triterpene. The parent triterpene structures were identified as amyrin and lupeol[5,6] by a combination of HMQC, HMBC, and COSY spectra. Tables 1 and 2 summarize the ^{1}H and ^{13}C chemical shift assignments of **1**, **2**, and **3**, which are in close agreement with literature values for the parent triterpenes amyrin and lupeol.[5] Interestingly the three isolates are stable to the chromatographic conditions employed and exposure to $CDCl_3$, as there have been previous reports of *p*-hydroxycinnamoyl esters of triterpenes freely interconverting between the *cis* and *trans* isomers in less than 48 h.[4]



Experimental Section

General Experimental Procedures. Melting points were recorded on a UWS Nepean melting point apparatus Model No. SRTE 8806 and are uncorrected. Optical rotations were measured with a Atago Polax-D polarimeter. UV spectra were determined in spectroscopic grade $CHCl_3$ on a Shimadzu UV−visible recording spectrophotometer (UV-240). NMR spetra were obtained with a Varian Unity-plus 300 spectrometer [^{1}H (300 MHz) and ^{13}C (75 MHz)]. Chemical shift values were reported in δ (ppm). Mass spectral data was recorded on a VG Auto-Spec high-resolution mass spectrometer. Column chromatography was performed on columns of Si gel [Lab Supply Cat. No. 6031690] All extraction and HPLC solvents were distilled prior to use. HPLC was perfomed using a Waters 510 solvent delivery system and a Waters R401 refractive index detector. HPLC was carried out in normal phase using Econosil column (250 × 8mm).

Plant Material. Leaves and twigs of *A. linarioides* (accesion no. D0068217) and *A. trineura* (accesion no. 842766) were collected from the Kakadu National Park Northern Territory and the Mount Annan Botanic Gardens NSW, respectively.

Extraction and Isolation. Milled *A. linarioides* leaves and twigs (700 g dry wt) were extracted exhaustively with dichloromethane. The solvent was evaporated under reduced pressure to afford a residue of 23.6

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Table 1. ^{1}H and ^{13}C Chemical Shifts for Compounds **1** and **2**[a]

position	^{13}C (**1**)	HMQC (**1**)	^{13}C (**2**)	HMQC (**2**)
1	38.2 *t*	1.05 (*m*), 1.65 (*m*)	38.2 *t*	1.13 (*m*), 1.68 (*m*)
2	23.5 *t*	1.70 (*m*), 1.90 (*m*)	23.8 *t*	1.72 (*m*), 1.92 (*m*)
3	81.1 *d*	4.62 (*m*)	81.1 *d*	4.70 (*m*)
4	36.8 *s*		38.6 *s*	
5	55.3 *d*	0.81 (*m*)	55.6 *d*	0.91 (*m*)
6	18.2 *t*	1.45 (*m*), 1.54 (*m*)	18.5 *t*	1.45 (*m*), 1.56 (*m*)
7	32.54 *t*	1.38 (*m*), 1.55 (*m*)	32.9 *t*	1.36 (*m*), 1.55 (*m*)
8	39.8 *s*		40.1 *s*	
9	47.5 *d*	1.54 (*m*)	47.8 *d*	1.6 (*m*)
10	37.7 *s*		37.4 *s*	
11	23.5 *t*	1.70 (*m*), 1.90 (*m*)	23.8 *t*	1.87 (*m*), 1.92 (*m*)
12	121.6 *d*	5.16 (*m*)	121.9 *d*	5.22 (*m*)
13	145.2 *s*		145.5 *s*	
14	41.7 *s*		42.0 *s*	
15	26.1 *t*	0.98 (*m*), 1.15 (*m*)	26.4 *t*	0.98 (*m*), 1.16 (*m*)
16	26.9 *t*	0.80 (*m*), 2.00 (*m*)	27.2 *t*	0.82 (*m*), 2.01 (*m*)
17	32.46 *s*		32.7 *s*	
18	47.2 *d*	1.94 (*m*)	47.5 *d*	1.97 (*m*)
19	46.8 *d*	1.66 (*m*)	47.1 *d*	1.70 (*m*)
20	31.1 *s*		31.3 *s*	
21	34.7 *t*	1.01 (*m*), 1.37 (*m*)	34.1 *t*	1.12 (*m*), 1.35 (*m*)
22	37.1 *t*	1.24 (*m*), 1.43 (*m*)	37.4 *t*	1.24 (*m*), 1.44 (*m*)
23	28.0 *q*	0.83 (*s*)	28.6 *q*	0.86 (*s*)
24	15.5 *q*	0.96 (*s*)	15.8 *q*	1.01 (*s*)
25	16.7 *q*	0.84 (*s*)	17.1 *q*	0.96 (*s*)
26	16.8 *q*	0.94 (*s*)	17.1 *q*	1.0 (*s*)
27	25.6 *q*	1.14 (*s*)	26.1 *q*	1.17 (*s*)
28	28.4 *q*	0.83 (*s*)	28.4 *q*	0.86 (*s*)
29	33.3 *q*	0.88 (*s*)	33.6 *q*	0.90 (*s*)
30	23.7 *q*	0.88 (*s*)	23.9 *q*	0.89 (*s*)
1′	127.3 *s*		127.8 *s*	
2′, 6′	132.2 *d*	7.59 (*d*, *J* = 8.8)	130.1 *d*	7.45 (*d*, *J* = 8.0)
3′, 5′	115.1 *d*	6.76 (*d*, *J* =8.8)	116.1 *d*	6.85 (*d*, *J* = 8.0)
4′	156.8 *s*		157.7 *s*	
β	143.5 *d*	6.77 (*d*, *J* = 13.2)	144.1 *d*	7.62 (*d*, *J* = 18.2)
α	117.5 *d*	5.78 (*d*, *J* = 13.2)	116.7 *d*	6.32 (*d*, *J* = 18.2)
CO2	166.8 s		167.4 *s*	

[a] Values in parentheses are coupling constants in hertz (solvent $CDCl_3$, 300 MHz).

Table 2. ^{1}H and ^{13}C Chemical Shifts for Compound **3**

position	^{13}C	HMQC
1	38.5 *t*	1.00 (*m*), 1.66 (*m*)
2	23.9 *t*	1.59 (*m*), 1.67 (*m*)
3	80.9 *d*	4.56 (*m*)
4	38.1 *s*	
5	55.5 *d*	0.81 (*m*)
6	18.3 *t*	0.74 (*m*), 1.38 (*m*)
7	34.3 *t*	1.38 (*m*), 1.42 (*m*)
8	40.9 *s*	
9	50.4 *d*	1.25 (*m*)
10	37.2 *s*	
11	21.0 *t*	1.28 (*m*), 1.40 (*m*)
12	25.2 *t*	1.08 (*m*), 1.66 (*m*)
13	38.1 *d*	1.35 (*m*)
14	42.9 *s*	
15	27.5 *t*	0.85 (*m*), 0.90 (*m*)
16	35.6 *t*	1.32 (*m*), 1.40 (*m*)
17	43.0 *s*	
18	48.3 *d*	1.39 (*m*)
19	48.0 *d*	2.36 (*m*)
20	151.0 *s*	
21	29.9 *t*	1.23 (*m*), 1.30 (*m*)
22	40.0 *t*	1.15 (*m*), 1.36 (*m*)
23	28.0 *q*	0.94 (*s*)
24	16.0 *q*	1.03 (*s*)
25	16.7 *q*	0.87 (*s*)
26	16.2 *q*	0.94 (*s*)
27	14.6 *q*	0.90 (*s*)
28	18.0 *q*	0.76 (*s*)
29	109.4 *t*	4.56 (*m*), 4.69 (*m*)
30	19.3 *q*	1.67 (*s*)
1′	127.6 *s*	
2′, 6′	129.9 *d*	7.42 (*d*, *J* = 8.7)
3′, 5′	115.8 *d*	6.82 (*d*, *J* = 8.7)
4′	157.4 *s*	
β	143.8 *d*	7.57 (*d*, *J* = 16.2)
α	116.5 *d*	6.29 (*d*, *J* = 16.2)
CO2	167.2 *s*	

[a] Values in parentheses are coupling constants in hertz (solvent $CDCl_3$, 300 MHz).

g, which was subjected to flash chromatography using gradient elution with ethyl acetate and petroleum ether to afford six fractions. Fractions 4 and 5 were combined and subjected to isocratic flash column chromatography (7% ethyl acetate and 93% petroleum ether). Further purification was carried out by normal phase HPLC (250 × 10 mm, Si 10 μm column, 20% ethyl acetate, and 80% petroleum ether eluent) affording 14 mg (0.002% dry wt) of **1** and 29 mg (0.004% dry wt) of **2**.

Milled *A. trineura* milled leaves and twigs (543 g dry wt) were extracted exhaustively with MeOH, and the extract was initially subjected to flash column chromatography with gradient elution as mentioned above. Fraction 2 was further purified by isocratic column chromatography (20% ethyl acetate and 80% petroleum ether, and then 5% ethyl acetate and 95% petroleum ether). Exhaustive normal phase HPLC (250 × 10 mm, Si 10 μm column, 25% ethyl acetate and 75% petroleum ether eluent) afforded 2 mg (0.0004% dry wt) of **3.**

***cis*-Hydroxycinnamoyl ester of amyrin (1):** pale yellow amorphous solid; mp 75−77 °C; IR 3400, 2926, 2856, 1700, 1601, 1166, 830, 756 cm^{-1}; UV ($CHCl_3$) λ_{max} 293 nm (ϵ 9600); $[\alpha]^{25}_D$ +218.9° (*c* 0.004 in 20 mL of C_6H_6); HREIMS *m*/*z* $[M]^+$ calcd for $C_{39}H_{56}O_3$ 572.4229, found 572.4221; ^{1}H-NMR and ^{13}C-NMR ($CDCl_3$, 300 MHz) (see Table 1).

***trans*-Hydroxycinnamoyl ester of amyrin (2):** white solid; mp 104−107 °C; IR 3424, 2948, 1705, 1635, 1604, 830, 756 cm^{-1}; UV ($CHCl_3$) λ_{max} 290 nm (ϵ 12 000); $[\alpha]^{25}_D$ +160.0° (*c* 0.005 in 20 mL of C_6H_6); HREIMS *m*/*z* $[M]^+$ calcd for $C_{39}H_{56}O_3$ 572.4229, found 572.4221; ^{1}H-NMR and ^{13}C-NMR ($CDCl_3$, 300 MHz) (see Table 1).

***trans*-Hydroxycinnamoyl ester of lupeol (3):** pale yellow solid; mp 82−85 °C; IR 3427, 1705, 1639 cm^{-1}; UV ($CHCl_3$) λ_{max} 293 nm (ϵ 8500); $[\alpha]^{25}_D$ +240.0° (*c* 0.005 in 20 mL of $CHCl_3$); HREIMS *m*/*z* $[M]^+$ calcd for $C_{39}H_{56}O_3$ 572.4229, found 572.4221; ^{1}H-NMR and ^{13}C-NMR ($CDCl_3$, 300 MHz) (see Table 2).

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