Phytochemistry, Vol. 30, No. 11, pp. 3631–3634, 1991
Printed in Great Britain.

0031 9422/91 $3.00 + 0.00

CO-OCCURRENCE OF HYDROXYLATED LUPANINES AND THEIR CORRESPONDING ANGELATE ESTERS IN *PEARSONIA* SPECIES

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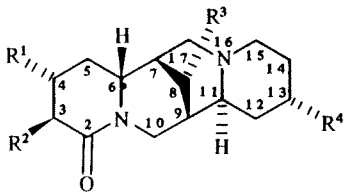
(*Received* 26 *March* 1991)

Key Word Index—*Pearsonia*; Leguminosae; 13α-hydroxylupanine; 3β,13α-dihydroxylupanine; 8α,13α-dihydroxylupanine; 3β,8α,13α-trihydroxylupanine; lebeckianine; angelate esters.

Abstract—Hydroxylated lupanines were found to co-occur with their corresponding angelate esters in specimens of different species of *Pearsonia*. The structures of two new compounds, 8α,13α-dihydroxylupanine and 3β,8α,13α-trihydroxylupanine, are reported. The comparative ^{13}C NMR data of all hydroxylated compounds and their angelate esters are reported.

INTRODUCTION

In recent years, the use of alkaloids in studies of the biochemical systematics of higher order plants has developed as a new field in research [1–3]. In the tribe Crotalarieae (family Fabaceae, subfamily Papilionoideae), alkaloids proved to be significant characters at the generic level [3]. The genus *Pearsonia* has an interesting combination of lupanine type alkaloids, including several esters of hydroxylated lupanines [4, 5]. Being lupanine esters of angelic acid, the products lupanine-13α-angelate (**1**), cajanifoline (**2**), sessilifoline (**3**), cryptanthine (**4**) and pearsonine (**5**) are of significance for the generic status of *Pearsonia* in the tribe Crotalarieae. The only other report of esters in the tribe Crotalarieae is from the genus *Rothia* [6] which has angelic, tiglic, 2-methylbutyric and vanillic acid esters of 13α-hydroxylupanine. The presence of only angelate esters, combined with the absence of tiglate and other esters, appears to be a unique character for the genus *Pearsonia*. These interesting results prompted us to carry out a detailed investigation of *Pearsonia* to determine possible variations in alkaloidal content resulting from provenance, seasonal differences and different morphological parts.



	R¹	R²	R³	R⁴
1	H	H	H	OAng
2	H	OH	H	OAng
3	OAng	OH	H	H
4	H	H	OH	OAng
5	H	OH	OH	OAng
6	H	OH	H	H
7	H	H	H	OH
8	OH	OH	H	H
9	H	OH	H	OH
10	H	OH	OH	OH
11	H	H	OH	OH

Studies of alkaloids in the genera *Calpurnia* [7–9], *Cadia* [9, 10] *Lupinus* [11], *Rothia* [6] and *Virgilia* [12], indicated the co-occurrence of alkaloidal esters and their corresponding hydroxylated compounds as a feature in most cases. It was, therefore, envisaged that *Pearsonia* may have a similar combination of esters and hydroxylated lupanines. GC analysis of extracts of different plant parts of several specimens of *P. cajanifolia* subsp. *cajanifolia* and subsp. *cryptantha* and *P. sessilifolia* subsp. *marginata* revealed the presence of several hitherto undetected minor compounds with GC retention times in the expected region of hydroxylated lupanines (see Table 1 for GC parameters and retention times). The GC-MS analysis of these samples proved that the product with R_t 24.27 min (*m/z* 264) was an isomer of 3β-hydroxylupanine (**6**) which was subsequently identified as 13α-hydroxylupanine (**7**), an alkaloid which is widely distributed in the Fabaceae. Compounds with R_ts of 25.75 and 26.50 min (*m/z* 280) were both structural isomers of lebeckianine (**8**) R_t 23.53 min, *m/z* 280) which was generally detected in *P. sessilifolia*, whereas the product with R_t 27.87 min showed a $[M]^+$ *m/z* 296, indicating that it is a trihydroxylupanine derivative.

RESULTS AND DISCUSSION

13α-Hydroxylupanine (**7**) was isolated in ample quantities from seeds of *P. cajanifolia* subsp. *cajanifolia*. The product was authenticated by comparing its ^{13}C NMR spectrum with reported data [13]. It proved to be the major seed alkaloid of both subspecies of *P. cajanifolia*

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Table 1. Retention times of lupanine type alkaloids from *Pearsonia*

Compound	No.	R_t (min)*
Lupanine		20.45
3β-Hydroxylupanine	6	21.95
Lebeckianine	8	23.43
13α-Hydroxylupanine	7	24.27
3β,13α-Dihydroxylupanine	9	25.75
8α,13α-Dihydroxylupanine	11	26.49
3β,8α,13α-Trihydroxylupanine	10	27.87
Lupanine-13α-angelate	1	28.82
Cajanifoline	2	30.30
Cryptanthine	4	30.65
Sessilifoline	3	30.85
Pearsonine	5	32.10

*GC parameters. Detector: PND. Column: fused silica capillary 30 m × 0.25 mm i.d. DB-1 0.25 μm thickness. Carrier gas: N_2 @ 4 ml min^{-1}. Temp: inlet 230°, PND 300°. Temp. programme: 150° to 320° at 6° min^{-1}, 15 min isothermal. Split ratio: 30:1, injection volume: 1 μl.

and was also present in the leaves of most specimens. GC-analysis indicated that whenever lupanine-13α-angelate (**1**) was present, the alcohol (**7**) occurred complementary to the ester. The other alcoholic derivative commonly found in *P. cajanifolia* was the one with R_t 25.75 min. Co-occurrence of cajanifoline (**2**) with this compound led to the assumption that it had to be the corresponding diol 3β,13α-dihydroxylupanine [3*S*-(3β,7β,7aα,9α,14β,14aβ)-dodecahydro-2,9-dihydroxy-7,14-methano-4*H*,6*H*-dipyrido-[1,2-*a*:1′2′-*e*][1,5]diazocin-4-one] (**9**). Alkaline hydrolysis of **2** gave **9** which was co-injected (GC) with specimens containing the unknown product. This gave the evidence of the unknown being the diol **9**. It was then isolated in small quantities from leaves of *P. cajanifolia* subsp. *cajanifolia*. The ^{13}C NMR data were virtually identical to those reported earlier for the product (**9**) isolated from *Cytisus scoparius* [13]. A comparison with the ^{13}C NMR spectrum of cajanifoline (**2**) revealed that the chemical shifts assigned to C-4 and C-5 should be exchanged (for corrected data see Table 2). Differences in the chemical shifts of C-11 to C-15 compared to the chemical shifts for the corresponding carbon atoms in **2**, was in accordance with the de-esterification of C-13. Analysis of other specimens of *Pearsonia* indicated the co-occurrence of the two esters (**1** and **2**) and their corresponding alcohols (**7** and **9**) as a general feature in *P. cajanifolia*.

The leaves of a specimen of *P. cajanifolia* subsp. *cryptantha* gave surprising results. The GC of the extract indicated that pearsonine (**5**) was essentially the only alkaloid present. On the basis of the co-occurrence of esters and alcohols in other specimens, a trace of a product at R_t 27.87 min was assumed to be 3β,8α,13α-trihydroxylupanine (**10**). The quantity, however, was too small to attempt isolation of the product by large scale extraction. In order to verify the identity of this product, **5** was subjected to alkaline hydrolysis to afford a product of high polarity on TLC. The ^{1}H NMR spectrum of this compound was quite similar to that of **5** apart from the H-13 which shifted upfield from δ5.10 to 3.60 and the absence of signals of the angeloyl moiety. In the ^{13}C NMR spectrum, C-13 shifted to δ63.7 compared to 66.6 in **5**. The chemical shifts for C-13 in **7** and **9** were of the same magnitude (Table 2). Other carbon resonances affected by the de-esterification were C-12 (δ38.9 to 42.0), C-14 (δ29.7 to 32.7) and to a lesser extent C-15 (δ48.6 to 49.6). The mass spectrum showed a strong $[M]^+$ (*m/z* 296, 100%) and the consecutive loss of two molecules of water. Co-injection of this synthetic triol with the extract proved that it was identical to the natural product at R_t 27.87 min. This new product, 3β,8α,13α-trihydroxylupanine [3*S*-(3β,7β,7aα,9α,14β,14aβ)]-dodecahydro-3,9-dihydroxy-7,14-α-hydroxymethano-2*H*,6*H*-dipyrido [1,2-*a*: 1′,2′-*e*][1,5]diazocin-4-one (**10**) was subsequently found in all specimens of *Pearsonia* which contained pearsonine (**5**).

These findings could presumably be extrapolated to assume that species with sessilifoline (**3**) and cryptanthine (**4**) would also contain lebeckianine (3β,4α-dihydroxylupanine) (**8**) and 8α,13α-dihydroxylupanine (**11**). A small quantity of lebeckianine [2*R*-(2α,3β,7β,7aα,14β,14aβ)-dodecahydro-2,3-dihydroxy-7,14-methano-4*H*,6*H*-dipyrido[1,2-*a*:1′,2′-*e*][1,5]diazocin-4-one] (**10**) was isolated from seeds of *P. sessilifolia* subsp. *marginata*. In order to obtain sufficient quantities of **11** for structural analysis and co-injection with extracts, cryptanthine (**4**) was hydrolysed under basic conditions to afford the diol (**11**) quantitatively. The ^{1}H and ^{13}C NMR spectra of this compound were virtually identical to those of cryptanthine (**4**), apart from the expected changes in the resonance frequencies of H-13, C-13, C-12, C-14, C-15 (similar to differences in NMR spectral properties of the other esters and their related alcohols) and the disappearance of signals belonging to the angeloyl moiety; C-13 shifted from δ66.7 in **4** to δ63.4 in this product. Both C-12 and C-14 experienced slight deshielding and shifted downfield. A comparison of the ^{13}C NMR data of the pairs **1** and **7**, **2** and **9**, **4** and **11** and **5** and **10** (Table 2) showed that the differences in chemical shifts for C-11 to C-15 between two molecules of a pair, were virtually the same for all the pairs. For **3** and **8**, significant differences were only recorded for C-3 and C-4. In the mass spectrum of compound (**11**), the $[M]^+$ (*m/z* 280) lost a molecule of water followed by the loss of a hydroxyl group, confirming the presence of two carbinol groups. Analytical GC and GC-MS of an extract of *P. sessilifolia* subsp. *marginata* indicated the presence of significant quantities of both cryptanthine (**4**) and sessilifoline (**3**) as well as lebeckianine (**10**) and an unknown diol with R_t 26.50 min. Co-injection of the synthetic diol (**11**) and all extracts containing the unknown with R_t 26.50 min, proved that it was **11**. This is the first report of the new product, 8α,13α-dihydroxylupanine [9*S*-(7β,7aα,9α,14β,14aβ)]-dodecahydro-9-hydroxy-7,14-α-hydroxymethano-2*H*,6*H*-dipyrido- [1,2-*a*:1′,2′-*e*][1,5]diazo-cin-4-one (**11**). The absence of lupanine-13α-angelate (**1**), cajanifoline (**2**), pearsonine (**5**) and their corresponding alcohol derivatives **7**, **9** and **10** in the extract of *P. sessilifolia* subsp. *marginata*, confirmed our findings on the co-occurrence of esters and their corresponding alcohols. Complementary to this, inspection of the GC traces of extracts of both subspecies of *P. cajanifolia* revealed the absence of sessilifoline (**3**) and lebeckianine (**10**).

It was evident from our results that the presence of lupanine esters in *Pearsonia* generally indicated the occurrence of their corresponding alcohols, albeit in small

Table 2. Comparison of ^{13}C chemical shifts of esters and hydroxylated lupanines from *Pearsonia*

C	**1**	**7***	$\Delta\delta$	**2**†	**9**	$\Delta\delta$	**4**	**11**	$\Delta\delta$	**5**†	**10**	$\Delta\delta$	**3**	**8**‡	$\Delta\delta$
2	170.9 *s*	170.9		173.8	173.8		171.0	171.5		173.7	174.0		171.5	171.5	
3	32.9 *t*	32.9 *t*		67.7 *d*	68.0 *d*		32.7 *t*	32.9 *t*		67.9 *d*	68.0 *d*		70.9 *d*	73.8 *d*	+2.9
4	19.6 *t*	19.6 *t*		24.3 *t*	24.4 *t*		19.7 *t*	19.8 *t*		24.6 *t*	24.7 *t*		70.3 *d*	68.4 *d*	−1.9
5	26.5 *t*	26.6		26.1	26.2		27.6	27.7		27.5	27.5		26.3	26.6	+0.3
6	60.6 *d*	60.8		61.3	61.6		59.3	59.5		60.4	60.5		57.8	58.2	+0.4
7	34.0 *d*	34.2		33.6	33.9		41.0	41.3		40.9	40.9		34.0	34.3	
8	27.4 *t*	27.3 *t*		27.2 *t*	27.2 *t*		72.9 *d*	73.1 *d*		72.7 *d*	72.7 *d*		32.5 *t*	33.0 *t*	
9	32.2 *d*	31.6		32.2	32.2		39.2	39.3		39.2	39.2		31.7	32.0	
10	46.6 *t*	46.8		47.8	47.8		45.1	45.4		46.3	46.3		48.3	48.3	
11	57.9 *d*	57.0	−0.9	57.9	57.0	−0.9	57.6	56.7	−0.9	58.1	57.0	−1.1	64.1	64.0	
12	36.4 *t*	39.9	+3.5	35.8	39.8	+4.0	38.9	42.0	+3.1	38.9	42.0	+3.1	30.2	31.7	
13	67.7 *d*	64.0 *d*	−3.7	67.9 *d*	64.6 *d*	−3.3	66.5 *d*	63.4 *d*	−3.1	66.6 *d*	63.7 *d*	−2.9	24.4 *t*	24.8 *t*	
14	28.8 *t*	32.4	+3.6	28.3	31.6	+3.3	29.6	32.1	+3.5	29.7	32.7	+3.0	24.3	24.5	
15	49.8 *t*	49.2	−0.6	49.7	49.0	−0.7	49.3	48.5	−0.8	49.6	48.6	−1.0	55.4	55.3	
17	52.0 *t*	52.4		51.7	52.3		52.1	52.3		52.4	52.4		52.1	52.6	
1′	167.4 *s*	—		167.3 *s*	—		167.1 *s*	—		167.2 *s*	—		167.8 *s*	—	
2′	128.2 *s*	—		128.1 *s*	—		127.9 *s*	—		128.0 *s*	—		127.8 *s*		
3′	137.6 *d*	—		137.6 *d*	—		137.7 *d*	—		137.7 *d*	—		139.2 *d*	—	
4′	16.0 *q*	—		15.9 *q*	—		15.8 *q*	—		16.0 *q*	—		15.5 *q*	—	
5′	20.8 *q*	—		20.7 *q*	—		20.6 *q*	—		20.8 *q*	—		20.1 *q*	—	

*Data from ref. [13].
†Data corrected from those presented in ref. [4].
‡Data from ref. [15].

amounts. The opposite, however, was not always true. Hydroxylated lupanines were found in the absence of their corresponding esters in several specimens of *Pearsonia*. The diversity and functionalization of lupanine type alkaloids were determined by the morphological parts, developmental stages of plants as well as provenance. Seeds of *P. cajanifolia*, for example, produced large quantities of the alcohols **7** and **9**–**11** with the esters only present as traces or absent in some specimens. Similarly, seeds of *P. sessilifolia* subsp. *marginata* contained the alcohols **8** and **11** whilst the expected esters **3** and **4** were absent from the extracts. An investigation of the seasonal variation of alkaloids showed that plants in the sprouting stage tended to produce esters as their major constituents. In the fruiting stage, the dominant alkaloids were generally hydroxylated lupanines. Plants of the same species from different localities produced alkaloidal patterns of such a variety that specimens often did not seem to be related on the basis of their alkaloidal contents. A full account of the study of alkaloids in the genus *Pearsonia* will be published elsewhere [14].

EXPERIMENTAL

Mps: uncorr. IR were recorded as thin films of $CHCl_3$ solns. Optical rotations were measured for a pathlength of 1 cm in the solvents stated for each sample. ^1H and ^{13}C NMR spectra were recorded at 200 and 50 MHz, respectively, in $CDCl_3$ using the $CHCl_3$ signal (δ7.24) as ref. TLC was performed on Merck 60 F_{254} silica gel plates using $CHCl_3$–C_6H_{12}–$HNEt_2$ (4:5:1). Chromatograms were visualized after drying by spraying with iodoplatinate soln. Details of the extraction and purification of alkaloids are reported elsewhere [5]. Species and voucher specimen details are presented in ref. [14].

3β,13α-Dihydroxylupanine (**9**). Cajanifoline (**2**) (100 mg) was stirred in 0.2 M NaOH containing 5% EtOH (15 ml) at 100° for 1 hr. Extraction of the product from the aq. soln with $CHCl_3$ (3 × 15 ml) gave the diol (**9**) (64 mg). The spectral properties of this compound were identical to those of **9** isolated from *P. c.* subsp. *cajanifolia*. $[\alpha]_D^{22}$ −11° ($CHCl_3$; *c* 1.2). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3280 br (OH), 1625 (lactam C=O). ^1H NMR (200 MHz, $CDCl_3$) (D_2O added): δ4.27 (1H, *dd*, $J_{8eq,10eq} = J_{9,10eq} = 2.33$ and $J_{10ax,10eq} = 13.30$ Hz, H-10eq), 4.06 (1H, *quin*, $J_{12,13} = J_{13,14} = 2.72$ Hz, H-13), 3.99 (1H, *dd*, $J_{3,4ax} = 11.13$ and $J_{3,4eq} = 6.01$ Hz, H-3), 3.32 (1H, $J_{5ax,6} = 9.48$, $J_{5eq,6} = 6.05$ and $J_{6,7} = 1.0$ Hz, H-6), 2.86 (1H, *dd*, $J_{7,17eq} = 10.99$ and $J_{17ax,17eq} = 12.94$ Hz, H-17_{eq}), 2.62 (1H, *dd*, $J_{9,10ax} = 2.31$ and $J_{10ax,10eq} = 13.30$ Hz, H-10_{ax}), 2.52 (1H, *ddd*, $J_{14ax,15eq} = 2.08$, $J_{14eq,15eq} = 4.87$ and $J_{15ax,15eq} = 12.00$ Hz, H-15_{eq}), 2.34 (1H, *dt*, $J_{14ax,15ax} = J_{15ax,15eq} = 12.00$ and $J_{14eq,15ax} = 3.01$ Hz, H-15ax), 2.25–1.55 (11H, *m*, 2 × H-4, 2 × H-5, H-7, H-8eq, H-9, H-11, 2 × H-12, 2 × H-14 and H-17_{ax}), 1.25 (1H. *dt*, $J_{7,8ax} = J_{8ax,9} = 2.23$ and $J_{8ax,8eq} = 9.80$ Hz, H-8_{ax}). EIMS (probe) 70 eV (rel. int.): 280 $[M]^+$ (100), 263 $[M-OH]^+$ (34), 262 $[M-H_2O]^+$ (38), 261 $[M-H_2O-H]^+$ (27), 245 (11), 181 (9), 165 (43), 152 (95), 134 (40), 122 (15), 108 (25), 95 (26), 82 (25), 69 (33), 55 (49).

8α,13α-Dihydroxylupanine (**11**). Cryptanthine (**4**) (84 mg) was dissolved in 0.2 M NaOH containing 5% EtOH (15 ml) and stirred for 8 hr at 65°. After cooling the soln to ambient, it was extracted with $CHCl_3$ (3 × 30 ml). The extract was evapd to afford the pure diol (**11**) (61 mg). $[\alpha]_D^{22}$ +29° ($CHCl_3$; *c* 2.4). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3260 br (OH), 1625 (lactam (C=O). ^1H NMR (200 MHz, $CDCl_3$): δ5.52 (2H, *br s*, 2 × OH), 4.46 (1H, *dd*, $J_{9,10eq} = 3.01$ and $J_{10ax,10eq} = 13.62$ Hz, H-10_{eq}), 3.99 (1H, *quin*, $J_{12,13} = J_{13,14} = 2.71$ Hz, H-13), 3.57 (1H, *t*, $J_{7,8} = J_{8,9} = 2.90$ Hz, H-8), 3.24 (1H, *ddd*, $J_{5ax,6} = 11.26$, $J_{5eq,6} = 4.95$ and $J_{6,7} = 1.46$ Hz, H-6), 2.89 (1H, *dd*, $J_{7,17eq} = 10.27$ and $J_{17ax,17eq} = 11.34$ Hz, H-17_{eq}), 2.51 (1H, *dt*, $J_{14ax,15eq} = J_{14eq,15eq} = 3.48$ and $J_{15ax,15eq} = 11.25$ Hz, H-15_{eq}), 2.44 (1H, *dd*, $J_{9,10ax} = 2.62$ and $J_{10ax,10eq} = 13.62$ Hz, H-10_{ax}), 2.39–1.70 (15H, *m*, 2 × H-3, 2 × H-4, 2 × H-5, H-7, H-9, H-11, 2 × H-12, 2 × H-14, H-15ax and H-17ax). EIMS (probe) 70 eV *m/z* (rel. int.): 280 $[M]^+$ (70), 262 $[M-H_2O]^+$ (100), 247 (10), 235 (11), 223 (10), 208 (14), 207 (13), 182 (13), 181 (10), 168 (49), 165 (30), 164 (32), 150 (62), 126 (41), 113 (18), 112 (39), 98 (50), 82 (59), 72 (27), 69 (41), 56 (62), 55 (78).

3β,8α,13α-Trihydroxylupanine (**10**). Pearsonine (**5**) (54 mg) was dissolved in 0.2 M NaOH containing 5% EtOH (15 ml) and stirred at 60° for 8 hr. Extraction of the product from the aq. soln with $CHCl_3$ (3 × 15 ml) gave pure **10** (12 mg). Co-TLC, co-inj. (GC and GC-MS) proved that the acquired triol (**10**) was identical to that present in *P. cajanifolia*. $[\alpha]_D^{22}$ +2° ($CHCl_3$; *c* 2.2). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300 br (OH), 1620 (lactam C=O). ^1H NMR (200 MHz, $CDCl_3$) (D_2O added): δ4.31 (1H, *dd*, $J_{9,10eq} = 2.93$ and $J_{10ax,10eq} = 13.67$ Hz, H-10_{eq}), 4.04 (1H, *quin*, $J_{12,13} = J_{13,14} = 3.18$ Hz, H-13), 4.01 (1H, *dd*, $J_{3,4ax} = 11.90$ and $J_{3,4eq} = 5.37$ Hz, H-3), 3.60 (1H, *t*, $J_{7,8} = J_{8,9} = 2.81$ Hz, H-8), 3.31 (1H, *m*, H-6), 2.96 (1H, *dd*, $J_{7,17eq} = 10.44$ and $J_{17ax,17eq} = 11.29$ Hz, H-17_{eq}), 2.64–2.51 (2H, *m*, H-10_{ax} and H-15_{eq}), 2.37–2.08 (4H, *m*, H-7, H-5_{eq}, H-15_{ax}, H-17_{ax}), 1.94–1.52 (9H, *m*, 2 × H-4, H-5_{ax}, H-9, H-11, 2 × H-12 and 2 × H-14). EIMS (probe) 70 eV (rel. int.): 296 $[M]^+$ (100), 279 $[M-OH]^+$ (23), 278 $[M-H_2O]^+$ (23), 277 (16), 261 (7), 251 (6), 239 (5), 225 (4), 181 (9), 168 (19), 166 (16), 164 (15), 150 (30), 126 (15), 114 (14), 96 (16), 82 (17), 69 (23), 57 (28), 55 (31), 46 (28).

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