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IRIDOIDS WITH ALGICIDAL PROPERTIES FROM *ALLAMANDA CATHARTICA*

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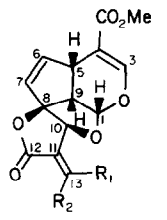
Key Word Index—*Allamanda cathartica*, Apocynaceae, iridoids, plumieride coumarate, plumieride coumarate glucoside

Abstract—Two new iridoid glycosides, plumieride coumarate and plumieride coumarate glucoside, have been isolated from *Allamanda cathartica* and detected in other *Allamanda* and *Plumeria* spp

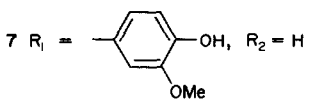
INTRODUCTION

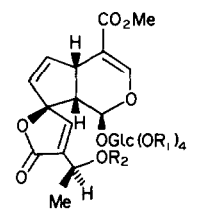
Over 200 iridoids have so far been found to occur in nature, the vast majority of them differing only in the degree and type of substitution in the basic cyclopentanopyran ring system A rather rare group of iridoids contains a spiro-lactone ring as an additional feature and comprises isoplumericin (**1**), plumericin (**2**) and plumieride (**3**), whose structures were established by Schmid *et al* [1–3], and allamandin (**4**), allamandicin (**5**) and allamdin (**6**), isolated by Kupchan *et al* [4] Apart from a single report of the isolation of **2** from *Nerium indicum* [5], all other work on these compounds has involved plants of the genera *Plumeria* [1–3, 6–9] or *Allamanda* [4, 10, 11] Recently, a further member of the group, oruwacin (**7**), was reported from *Morinda lucida* [12]

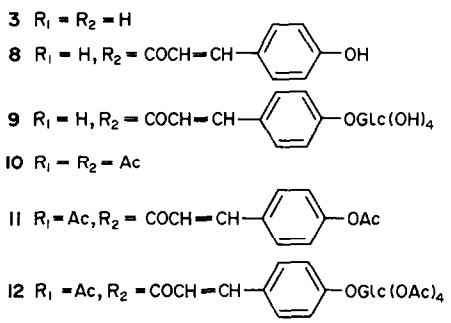
We have been interested for some time in the biological activity of **1** and **2** and initially at the possibility of exploiting their antifungal action against human and plant pathogens [11] However, following our discovery that both compounds are also strongly algicidal and barnicidal [13], and, therefore, offer promise for use as marine anti-fouling agents, we have looked more closely at alcoholic extracts of the plants from which they are derived [14] and detected a number of hitherto unreported iridoids, all of which appear to be structurally related to **1**, **2** or **3** We report here the isolation and characterization of the two most abundant compounds, also found to be algicidal, which we have named plumieride coumarate (**8**) and plumieride coumarate glucoside (**9**)



1 R_1 = H, R_2 = Me
2 R_1 = Me, R_2 = H

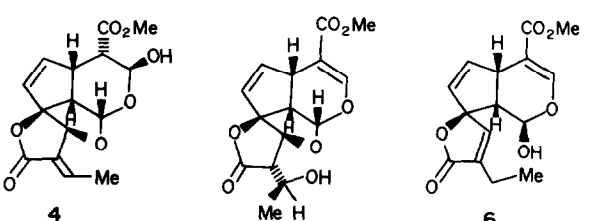






RESULTS AND DISCUSSION

Plumieride coumarate (**8**), $C_{30}H_{32}O_{14}$, was isolated from a methanolic extract of *Allamanda cathartica* L roots as an amorphous, buff-coloured solid which resisted all attempts at crystallization Its TLC characteristics suggested a close structural relationship to plumieride (**3**) and this view was supported by the IR spectrum which showed strong bands at 1749 (α,β-unsaturated lactone C = O) and 1697 (α,β-unsaturated ester C = O) cm^{-1} The presence of bands at 1604 and 1513 cm^{-1} suggested, in addition, an aromatic ring, possibly with a hydroxyl group attached (IR ν_{max} 1160 cm^{-1}), and this was confirmed by



the bathochromic shift of the UV absorption with alkali (λ_{max} 319 to 378 nm) Major fragment ions in the MS at m/z 164 $[HO-C_6H_4-CH=CH-CO_2H]^+ = [R-CO_2H]^+$, 147 $[R-CO]^+$ and 119 $[R]^+$ indicated the presence of a coumaroyl moiety

The ^1H NMR spectrum of **8** (Table 1) clearly showed one part of the AA'BB' pattern expected for a *p*-disubstituted aromatic ring (2H, δ 6 73, J_{ortho} = 8 5 Hz), the other being included in a composite lower field signal at δ 7 65–7 4 Also evident was part of an AB pattern attributable (spin-decoupling experiments) to the vinylic protons of a *trans* double bond (δ 6 33, J_{trans} = 16 Hz), the other part again lying within the region δ 7 65–7 4 The presence of analagous signals at higher field (δ 5 74, J_{cis} = 13 Hz and *ca* δ 6 70) suggested that **8** was a mixture of *trans* and *cis* isomers (*ca* 8 2, respectively, from relative signal intensities) This was verified by photochemical experiments irradiation of the NMR solution with UV light at 366 nm increased the intensity of the δ 5 74 signal at the expense of that at δ 6 33 The existence of two isomers also accounted for the observation of two peaks on GC of **8** (TMSi ether), which had appeared homogeneous on TLC

The spectral data demonstrated, therefore, that **8** was a *p*-coumaric acid derivative of **3** The stereochemistry at C-1, C-5 and C-9 was assumed to be the same as in **3**, this is in accord with the similar chemical shifts and coupling constants of the relevant protons That the site of esterification was at the hydroxyl group on C-13, rather than at one of the glucose hydroxyls, was shown by the

Table 1 ^1H NMR data of compounds **3** and **8–12***

	3†	**8**†	**9**†	**10**‡	**11**‡	**12**‡
H-1	5 17 *d* (4 0)	5 16 *d* (5 0)	5 25 *d* (5 0)	5 06 *d* (3 0)	5 09 *d* (2 5)	5 09 *d* (2 5)
H-3	7 48 *d* (1 5)	§	§	7 36 *d* (1 5)	7 38 *d* (1 0)	7 37 *d* (1 0)
H-5	‖	‖	‖	‖	‖	‖
H-6	6 39 *dd* (5 0, 2 5)	6 35 *dd* (5 0, 2 5)	6 40 *dd* (5 0, 2 0)	6 42 *dd* (5 5, 3 0)	6 42 *dd* (5 0, 3 0)	6 42 *dd* (5 5, 3 0)
H-7	5 55 *dd* (5 0, 2 0)	~ 5 55	~ 5 60	5 43 *dd* (5 5, 1 5)	5 45 *dd* (5 0, 1 5)	5 45 *dd* (5 5, 1 5)
H-9	2 82 *dd* (8 0, 4 0)	2 80 *dd* (7 0, 5 0)	2 81 *dd* (7 0, 5 0)	3 12 *dd* (8 5, 3 0)	3 13 *dd* (8 5, 2 5)	3 14 *dd* (8 5, 2 5)
H-10	7 25 *d* (1 0)	§	§	6 93 *d* (1 0)	6 99 *d* (1 0)	6 98 *d* (1 0)
H-13	4 36 *dq* (6 0, 1 0)	~ 5 55	~ 5 60	5 63 *dq* (7 0, 1 0)	5 77 *dq* (6 5, 1 0)	5 77 *dq* (6 5, 1 0)
H-14	1 28 *d* (6 0)	1 46 *d*, 1 42** *d* (6 5, 6 5)	1 50 *d*, 1 47** *d* (6 5, 6 5)	1 52 *d* (7 0)	1 60 *d* (6 5)	1 59 *d* (6 5)
CO_2Me	3 70 *s*	3 68 *s*	3 69 *s*	3 75 *s*	3 74 *s*	3 74 *s*
H-a	—	6 33 *d*, 5 74** *d* (16 0, 13 0)	6 50 *d*, 5 89** *d* (16 0, 13 0)	—	6 40 *d* (16 0)	6 35 *d* (16 0)
H-b	—	§, 6 70**	§, 6 96**	—	7 70 *d* (16 0)	7 67 *d* (16 0)
H-c	—	§	§	—	7 55 *d* (9 0)	7 49 *d* (9 0)
H-d	—	6 73 *d* (8 5)	7 03 *d* (8 5)	—	7 12 *d* (9 0)	6 98 *d* (9 0)
OAc	—	—	—	2 09, 2 07, 2 02, 1 99, 1 92	2 30, 2 05, 2 02 1 98, 1 91	2 15–1 90
H-1′	4 52 *d* (7 0)	4 53 *d* (7 0)	4 55 *d*, 4 90†† *d* (7 0, ~ 6 0)	¶	¶	¶
H-2′–H-4′	3 25–3 0	3 3–3 0	3 4–3 0‡‡	¶	¶	¶
H-5′	3 25–3 0	3 3–3 0	3 4–3 0‡‡	‖	‖	‖
H-6′	‖	‖	‖	4 31 *dd*, 4 07 *dd* (12 0, 4 0; 12 0, 3 0)	4 29 *dd*, 4 06 *dd* (12 0, 4 0, 12 0, 2 5)	4 45–3 95

*Run at 90 MHz with TMS as internal standard Shifts are in δ-values (ppm) Coupling constants are in Hz

†Run in DMSO-d_6

‡Run in $CDCl_3$

§Composite signal at δ 7 65–7 4 for **8** and 7 8–7 4 for **9**

‖Included in signals at *ca* δ 3 9–3 5, H-5 located at 3 94 for **3** in D_2O by spin decoupling

¶Included in signals at δ 5 35–4 75

**Shifts due to *trans* and *cis* isomers, respectively, confirmed by photochemical experiments

††Shifts due to H-1′ and H-1″, respectively (signals for H-2″–H-6″ included in those for H-2′, etc)

‡‡Partially obscured by H_2O

paramagnetic shift suffered by the H-13 proton in the NMR spectrum of **8** compared with **3** ($\Delta\delta$ 1 2) Irradiation at its suspected position (δ 5 55, coincident with H-7) collapsed the H-14 methyl doublet to a singlet, confirming its assignment Compound **8** is therefore 13-(*p*-coumaryl) plumieride or, simply, plumieride coumarate

Acid hydrolysis of **8** afforded *p*-coumaric acid and glucose, together with small amounts of three unidentified compounds, all of which were derived from the plumieride moiety (shown by a 'control' experiment in which **3** was hydrolysed under identical conditions) Acetylation of **8** provided a penta-acetate **11** whose ^{1}H NMR spectrum was entirely consistent with the deduced structure Compound **11** was obtained as the major product from the crude acetylated mixture by chromatography over Si gel and its NMR spectrum showed no evidence of a *cis* isomer being present

Compound **9** was isolated, again as an amorphous solid, from the more polar fractions of the methanol extract from which **8** was obtained Its IR spectrum was very similar to that of **8**, as was its UV spectrum in ethanol However, addition of alkali to the latter solution failed to change the spectrum, indicating the absence of a phenolic hydroxyl The ^{1}H NMR spectrum of **9** (Table 1) likewise resembled that of **8** and still contained signals appropriate for a *p*-disubstituted aromatic ring, suggesting, therefore, that the phenolic hydroxyl was now *O*-substituted *O*-Glycosylation seemed probable from the highly polar nature of **9** on TLC and examination of the NMR spectrum after deuterium exchange showed that a total of eight hydroxyl groups was present, with two anomeric protons resonating at δ 4 55 (cf δ 4 53 for **8**) and δ 4 90 Coupling constants of 7 ($J_{1',2}$) and 6 ($J_{1',2}$) Hz, respectively, indicated that both sugar units had diaxial protons on the anomeric and adjacent carbons Incubation of **9** with emulsin produced **8** and glucose, thereby identifying the additional sugar unit and confirming its β-configuration * Acid hydrolysis of **9** afforded glucose as the only sugar, together with *p*-coumaric acid and small amounts of the same three unidentified compounds as obtained from **8** and **3**, and since H-13 was still well downfield of its position in **3**, the site of esterification remains the same as in **8** Compound **9** is, therefore, 13-(*O*-glucosyl-*p*-coumaryl) plumieride or, simply, plumieride coumarate glucoside The existence of a *trans*–*cis* mixture (*ca* 7 3) was again evident from the NMR spectrum Acetylation of **9** provided an octa-acetate **12** whose spectral data were in complete agreement with the proposed structure

Both **8** and **9** have been found in species of *Allamanda* other than *A cathartica*, and also in *Plumeria* species, and these findings are reported separately [14]

EXPERIMENTAL

Mps are uncorr EIMS were run at 70 eV CC was performed using Si gel 60 and solvents as indicated below, fractions were monitored by TLC using procedures described elsewhere [14]

TLC of the products of acid hydrolysis was performed using $CHCl_3$–MeOH (4 1), S1, and PrOH–EtOAc–H_2O (7 2 1), S2, plates were visualized by spraying with 50% H_2SO_4 and heating at 120° for a few min (iridoids) or 1% $FeCl_3$ in MeOH (coumaric acids) GC was carried out to determine the purity of iridoids isolated and the composition of mixtures of isomers using the following column and conditions 0 4 m × 4 mm glass packed with 1 5% OV-17, N_2 at 50 ml/min, FID, detector temp 320°, on-column injection Column temps, isothermal (R_t, min) 190° (1, 6 0, 2, 7 0), 240° (TMSi **3**, 8 3), 300° (TMSi **8** *cis*, 5 7, *trans*, 8 2) Trimethylsilylation of glycosides was carried out using HMDS–TMCS in pyridine **1** and **2** were found to undergo isomerization in pyridine to give an equilibrium mixture containing *ca* 15% **1** and 85% **2** Acetylations were carried out on a steam-bath (3 hr) using Ac_2O–pyridine

Isolation of iridoid fractions Mature roots of *Allamanda cathartica* were excavated in January 1977 from trees growing in the campus of the University of the Philippines, Quezon City, Manila, collection and identification of the plant material was made by Professor A Castillo of the Department of Pharmacy A portion (500 g) of the dried, ground roots was extracted twice by boiling with MeOH (4 l each) for 1 hr with stirring The combined extracts were concd and the residue chromatographed on Si gel (2 5 kg) Elution with petrol (5 l) and then mixtures of petrol–Et_2O (11 l), Et_2O (3 l), Et_2O–$CHCl_3$ (4 l), $CHCl_3$–MeOH (29 l) and MeOH (8 l) provided fractions which were monitored by TLC and bulked accordingly I (petrol–Et_2O, 2 3 to $CHCl_3$–MeOH, 95 5) contained **1** and **2**, II ($CHCl_3$–MeOH, 4 1) **8**, III ($CHCl_3$–MeOH, 4 1 to 3 2) **8** and **3**, IV ($CHCl_3$–MeOH, 3 2 to all MeOH) **3** and **9**

Isoplumericin (**1**) *and plumericin* (**2**) I was triturated with Et_2O to give crude **1** + **2** as a solid (4 2 g) Chromatography of part of this (1 0 g) on Si gel (40 g) and elution with petrol (200 ml) and mixtures of petrol–Et_2O (2 l) of increasing polarity gave, finally, from successive fractions with petrol–Et_2O (3 2), pure **1** (250 mg) as rectangular plates, mp 198–200°, lit mp 200 5–201 5° dec [3], a mixture of **1** and **2** (290 mg), and pure **2** (140 mg), mp 209–211°, lit mp 211 5–212 5° dec [3]

Plumeride coumarate (**8**) A portion of II (8 0 g) was re-chromatographed on Si gel (300 g) Elution with mixtures of $CHCl_3$ and MeOH yielded, from $CHCl_3$–MeOH (9 1), **8** (5 1 g) as a buff-coloured amorphous solid, homogeneous on TLC GC of TMSi **8** showed it to be a mixture of *ca* 20% *cis* and 80% *trans* isomers, $[\alpha]_D^{24}$ −60 0° (MeOH, *c* 0 9), MS, *m/z* (rel int) 273 $[M-OGlu-RCO_2H]^+$ (5), 169 $[C_8H_9O_4]^+$ (33), 164 $[RCO_2H]^+$ (100), 163 $[RCO_2]^+$ (31), 147 $[RCO]^+$ (41), 139 $[C_7H_7O_3]^+$ (12), 119 $[R]^+$ (30), UV λ_{max}^{EtOH} nm (log ε) 230 (4 34), 302 (sh), 318 (4 28), IR ν_{max}^{nujol} cm^{-1} 3380, 1749, 1697, 1634, 1604, 1513, 1160 (Found C, 55 85, H, 5 51 $C_{30}H_{32}O_{14}$ 1 5 H_2O requires C, 55 99, H, 5 48%)

Penta-acetylplumeride coumarate (**11**) Chromatography of the acetylation product of **8** (1 5 g) on Si gel and elution with Et_2O afforded pure **11** (1 1 g) as a white solid, $[\alpha]_D^{23}$–103 3° ($CHCl_3$, *c* 0 9), MS, *m/z* (rel int) 479 $[M-OGluOAc_4]^+$ (2), 331 $[GluOAc_4]^+$ (36), 273 (9), 206 $[R(OAc)CO_2H]^+$ (2), 189 $[R(OAc)CO]^+$ (3), 169 (64), 164 (25), 163 (4), 147 (19), 139 (7), 119 (4) (Found C, 57 66, H, 5 17 $C_{40}H_{42}O_{19}$ O 5H_2O requires C, 57 49, H, 5 19%)

Acid hydrolysis of **8** Carried out by heating **8** (100 mg) in 1 N H_2SO_4 (5 ml) under reflux for 2 hr Dark insoluble material was filtered off and the filtrate extracted with EtOAc (3 × 5 ml) Evaporation of the solvent gave a residue which was shown by comparison with authentic compounds to contain *p*-coumaric acid, small amounts of unchanged **8** and lesser amounts of an unidentified compound, iridoid in nature† (R_f 0 61, S1) The aq soln was neutralized with Amberlite IR 45 (OH form) and concd

*The β-glucose attached to the plumieride moiety was resistant to enzymic cleavage, in accord with the observation of Schmid *et al* [1]

†Possibly the aglycone of **3**

TLC and GC showed it contained glucose, together with **3** and small amounts of two other unidentified iridoid compounds* (R_fs 0.53 and 0.43, S2)

Acid hydrolysis of **3** Carried out using the same conditions as above. The EtOAc extract contained, as sole product, the same unidentified compound (R_f 0.61, S1) as obtained from **8**. The aq soln contained glucose, unchanged **3** and, again, the same unidentified compounds (R_fs 0.53 and 0.43, S2) as obtained from **8**.

Plumieride (**3**). A portion of III (10.0 g) was partitioned between H_2O (400 ml) and EtOAc (3 × 300 ml). The EtOAc extract contained mainly **8** and was chromatographed to yield 3.8 g **8**. The aq phase was evaporated and the resulting solid crystallized (MeOH–Et_2O) to give pure **3** (1.8 g), mp 225–226°, $[\alpha]_D^{24}$ −112.2° (H_2O, *c* 0.9). Lit. mp 224–225°, $[\alpha]_D^{16}$ −114° (H_2O, *c* 0.54) [1].

Plumieride coumarate glucoside (**9**). A portion of IV (30.0 g) was rechromatographed on Si gel (1.0 kg) deactivated with H_2O (100 ml). Elution with mixtures of $CHCl_3$ and MeOH of increasing polarity yielded, from $CHCl_3$–MeOH (4:1), **9** (6.2 g) as a buff-coloured amorphous solid, homogeneous on TLC, $[\alpha]_D^{25}$ −64.4° (MeOH, *c* 0.9), UV λ_{max}^{EtOH} nm (log ε) 226 (4.46), 300 (sh), 309 (4.36), IR ν_{max}^{nujol} cm^{-1} 3350, 1746, 1695, 1630, 1600, 1511 (Found: C, 52.15; H, 5.60. $C_{36}H_{42}O_{19}$ 3 H_2O requires C, 51.92; H, 5.81%).

Octa-acetylplumieride coumarate glucoside (**12**). Chromatography of the acetylation product of **9** (1.6 g) on Si gel and elution with Et_2O afforded pure **12** (1.1 g) as a white solid, $[\alpha]_D^{23}$ −88.9° ($CHCl_3$, *c* 0.9), MS, *m/z* (rel. int.): 753 $[M - GluOAc_4 - OMe + H]^+$ (1), 331 (29), 273 (6), 169 (71), 164 (5), 147 (3), 139 (7). (Found: C, 55.70; H, 5.31. $C_{52}H_{58}O_{27}$ 0.5 H_2O requires C, 55.57; H, 5.29%).

Enzymic hydrolysis of **9**. Carried out by incubating **9** (25 mg) in 0.1 M citrate buffer (pH 5.0, 25 ml) with β-glucosidase (25 mg) at 30° for 6 hr. TLC examination of the soln identified **8** and glucose as the only products.

Acid hydrolysis of **9**. Carried out using the same conditions as for **8** except that the period of heating was 3 hr. The number and distribution of products from the reaction were precisely the same as those obtained from **8**, there being slightly less *p*-coumaric acid and rather more glucose produced.

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*One of which is probably plumieride acid, the other may be the corresponding aglycone.

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