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THE OCCURRENCE OF IRIDOIDS IN *PLUMERIA* AND *ALLAMANDA*

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Key Word Index—*Plumeria*, *Allamanda*, Apocynaceae, iridoids, isoplumericin, plumericin, plumieride, plumieride coumarate, plumieride coumarate glucoside

Abstract—As many as 210 samples of roots, stem, leaves and flowers from *Plumeria* and *Allamanda* spp have been examined for the presence of the iridoids isoplumericin, plumericin, plumieride, plumieride coumarate and plumieride coumarate glucoside, all of which, with the exception of plumieride, have algicidal properties Roots, and in particular the root bark, contained the highest concentrations of iridoids Isoplumericin and plumericin, the compounds with the strongest algicidal activity, were rarely found in aerial parts Age and size of roots are not guides to predicting the presence or absence of these two compounds, roots taken from stem cuttings of *P obtusa* after only 6 weeks growth contained significant amounts of them (0 6%, dry wt basis) and 6 0% total iridoids *Plumeria* samples were found to contain two further highly polar, but unidentified, iridoids One of these was often either the sole iridoid present or found together with plumieride only Another unidentified iridoid was detected in *Allamanda* spp *A neriifolia* was notable in containing the most complex mixture of iridoids

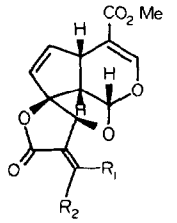
INTRODUCTION

Plants from the genera *Plumeria* and *Allamanda* are a source of the rather rare lactone-containing iridoids isoplumericin (**1**), plumericin (**2**), plumieride (**3**), plumieride coumarate (**4**) and plumieride coumarate glucoside (**5**) The isolation and structure elucidation of **4** and **5** have been reported by us in a previous paper [1] Examination of the literature on **1**–**3** shows that, hitherto, only a limited number of plant species have been investigated, and with the exception of **3** [2,3], no systematic study has been made on the occurrence of these compounds in different parts of the plant Following our discovery of the algicidal and barnicidal properties of **1** and **2**, and also **4** and **5** [4], we wished to identify a high-yielding source of these compounds We, therefore, formulated a programme of work aimed at screening as many *Plumeria* and *Allamanda* species as we could acquire, from as many parts of the world as possible We now present the results of this survey

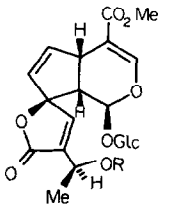
RESULTS AND DISCUSSION

Tables 1 and 2 summarize the results of analysing methanol extracts of various plant parts of some *Plumeria* and *Allamanda* spp Each extract was analysed by TLC under standard conditions using four different solvent systems (see Experimental) Compounds J and K, found in *Plumeria* spp only, and compound D, found only in *Allamanda*, are as yet unidentified but their TLC characteristics and colour reactions indicate that they are further iridoids of the isoplumericin–plumieride type (but not members of the allamdin group of iridoids isolated by Kupchan *et al* [5]) Compounds J and K are highly polar and appear to be glycosides of **3** and **4**, respectively Compound D is slightly less polar than **3** but probably still glycosidic in nature

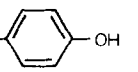
Several features of the distribution of iridoids in *Plumeria* and *Allamanda* are apparent from the Tables As far as the algicidal compounds **1**, **2**, **4** and **5** are concerned roots are by far the best source In *Plumeria*, **1** and **2** were absent from all aerial parts examined but were found in 9 out of 15 root samples * Compounds **4** and **5**, although

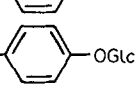


1 R_1 = H, R_2 = Me
2 R_1 = Me, R_2 = H



3 R = H

4 R = COCH=CH–  –OH

5 R = COCH=CH–  –OGlc

*It has been found that under certain conditions **1** and **2** interconvert to form an equilibrium mixture containing *ca* 85% **2** and it is probable that the proportions of each found in a plant extract reflect the precise conditions of extraction rather than their natural occurrence

Table 1 Occurrence of compounds 1–5 and J and K in *Plumeria* spp *

	No of samples				1/2				3				4				5				J				K			
	r	s	l	f	r	s	l	f	r	s	l	f	r	s	l	f	r	s	l	f	r	s	l	f	r	s	l	f
P rubra L †	7	9	13	7	2	0	0	0	7	8	7	3	7	1	2	1	7	4	2	2	2	4	11	6	0	0	3	3
P rubra (L) fma *acutifolia* (Poir) Woodson	2	8	9	3	2	0	0	0	2	7	2	0	2	1	1	0	2	1	0	0	1	7	7	1	0	0	0	0
P rubra (L) fma *bicolor* (R & P) Woodson	1	0	1	0	1	—	0	—	1	—	1	—	1	—	1	—	1	—	0	—	0	—	1	—	0	—	0	—
P rubra (L) fma *lutea* (R & P) Woodson	1	0	1	0	1	—	0	—	1	—	1	—	1	—	1	—	1	—	1	—	0	—	0	—	0	—	0	—
P rubra (L) fma *tricolor* (R & P) Woodson	1	1	1	0	1	0	0	—	1	0	1	—	1	0	1	—	1	0	0	—	0	0	1	—	0	0	0	—
P obtusa L ‡	1	6	7	5	1	0	0	0	0	5	4	2	1	5	4	1	0	5	2	1	0	5	1	2	0	0	0	0
P alba L	2	1	2	1	1	0	0	0	2	1	2	1	2	1	1	0	2	1	1	1	0	0	0	1	0	0	0	0
Plumeria sp	0	4	4	2	—	0	0	0	—	3	4	1	—	0	2	1	—	0	1	1	—	1	4	1	—	0	1	0
Total	15	29	38	18	9	0	0	0	14	24	22	7	15	8	13	3	14	11	7	5	3	17	25	11	0	0	4	3

*Expressed in terms of the number of samples in which the compounds were detected, traces have been regarded here as negative r, Roots, s, stems, l, leaves, f, flowers Nomenclature for identification of plant material has been made consistent by reference to Woodson [6]

†One sample of seed pods examined contained none of the compounds

‡One sample of seed pods contained 3–5 and J

Table 2 Occurrence of compounds 1–5 and D in *Allamanda* spp *

	No of samples				1/2				3				4				5				D			
	r	s	l	f	r	s	l	f	r	s	l	f	r	s	l	f	r	s	l	f	r	s	l	f
A cathartica L	6	10	11	9	6	1	2	0	6	10	11	8	6	2	1	5	6	3	2	5	0	1	5	3
A cathartica (L) var *grandiflora*	2	2	2	0	1	0	0	—	1	2	2	—	2	0	0	—	2	0	0	—	1	2	1	—
A cathartica (L) var *hendersonii*	1	2	2	1	1	0	0	0	1	2	2	1	1	1	0	0	1	1	1	0	1	1	1	0
A cathartica (L) var *nobilis*	2	3	3	1	2	0	0	0	2	3	3	1	2	1	1	0	2	1	1	0	1	1	1	0
A cathartica (L) var *williamsii*	0	1	1	1	—	0	0	0	—	1	1	1	—	0	0	0	—	0	0	1	—	0	1	0
A neriifolia Hook †	3	5	5	2	1	1	0	0	3	5	5	2	3	3	5	2	3	4	5	1	0	4	5	0
A violacea Gardn & Field	0	2	2	1	—	1	0	0	—	2	2	1	—	1	0	1	—	1	0	1	—	0	1	0
A blanchetii A DC	1	1	1	0	0	0	0	—	0	0	0	—	0	0	0	—	0	0	0	—	0	0	0	—
Total	15	26	27	15	11	3	2	0	13	25	26	14	14	8	7	8	14	10	9	8	3	9	15	3

*As for Table 1

†One sample of fruit examined contained 3 and 5

having a rather more general distribution within the plant, were still found in a higher proportion of root samples than aerial parts, all but one of the former containing both compounds As well as being present in a greater number of samples, 4 and 5 were also present in greater concentrations in the roots In *Allamanda*, the same general patterns are evident, though 1 and 2 were found in five samples of stems and leaves

The discovery of compounds J, K and D, and their distribution in the plant, merits their further investigation and identification In *Plumeria* samples, where only one iridoid was detected, it was either 3 (8 samples) or J (15 samples), while in those cases where only two iridoids were present it was usually 3 and J (17 out of 22 samples) The same pattern is evident in Table 3 which presents the results of analysing nine named varieties of *P rubra* collected at the same time and from the same location, J was detected in all but 2 of the 27 samples Here, flowers were the richest source of iridoids (roots were not available) and six samples contained compound K It seems likely that J, as 3, is an early product in the biosynthesis of the *Plumeria* iridoids Presumably, plants from *Allamanda* either lack the enzyme responsible for production of J or contain one that allows its degradation, if formed The place of D in the biosynthesis of iridoids in *Allamanda*, while less obvious, is equally intriguing

Table 3 Occurrence of compounds 1–5 and J and K in named varieties of *P rubra**

	1/2			3			4			5			J			K		
	s	l	f	s	l	f	s	l	f	s	l	f	s	l	f	s	l	f
June Bride	−	−	−	+	+	+	−	−	−	−	−	−	+	+	+	−	−	+
Little Beauty	−	−	−	+	+	+	−	−	+	−	−	+	+	−	+	−	−	−
Carmine Flush	−	−	−	+	+	+	−	−	−	−	−	+	+	+	+	−	−	+
Firefly	−	−	−	+	+	+	−	−	−	−	−	+	+	+	+	−	−	+
Pink Pearl	−	−	−	+	+	+	−	−	+	−	−	+	+	+	+	−	−	+
Flamingo	−	−	−	+	+	+	−	+	+	+	+	+	+	+	+	−	−	−
Celadine	−	−	−	+	+	+	−	−	−	+	−	+	+	+	+	−	−	+
Peace	−	−	−	+	+	+	−	+	+	−	+	+	+	−	+	−	−	−
Lady in Pink	−	−	−	+	+	+	−	−	−	+	+	+	+	+	+	−	−	+
Total	0	0	0	9	9	9	0	2	4	3	3	8	9	7	9	0	0	6

*Expressed in terms of the presence (+) or absence (−) of the compounds in one sample of each part of the plant, traces have been regarded as negatives s, Stem, l, leaves, f, flowers

Table 4 Distribution of compounds 1–5 in *A cathartica* roots*

	1/2	3	4	5
Bark	0 16 (1 1)	0 48 (3 2)	0 30 (2 0)	0 89 (5 9)
Inner part	0	0 07 (0 5)	0 08 (0 5)	0 20 (1 3)

*Expressed as wt (g) of compound isolated from 15 g dry wt each of bark and inner part, figures in parentheses are percentage yield

The number of samples, particularly root, available from each species was generally insufficient to draw definitive conclusions as to whether there exist genuine species differences in production of the algicidal compounds However, of seven *P rubra* root samples only two contained **1** and **2**, while seven out of eight root samples from six other species did contain them *P rubra* and *P rubra* fma *acutifolia* stem samples were also generally lacking in **4** and **5** while *P obtusa* stems usually contained them Of the *Allamanda* spp , roots of *A cathartica* var *hendersonii* and *A cathartica* var *nobilis* contained **1** and **2**, as well as **3**–**5**, while *A cathartica* var *grandiflora* and *A neriifolia* roots of the same age (1 year) and growing in the same place, under the same conditions, contained only **3**–**5** *A neriifolia* stood out as containing the most complex mixture of iridoids All five samples of leaves, for example, contained both **4** and **5** (in addition to **3**), in contrast to only 4 out of 22 leaf samples from seven other species containing **4** and/or **5** Each of the *A neriifolia* leaf samples also contained D and three of them contained yet another unidentified iridoid, designated F, not present in any other species Compound F is very close to **4** in mobility on TLC and shows the same red fluorescence as **4** and **5**, its structure, therefore, probably also contains a coumaric acid moiety

The distribution of the iridoids within the root, the richest source of them, was examined Roots of *A cathartica* up to *ca* 10 mm thickness separated readily into three parts bark, an adjacent thin woody layer and the hard core Compounds **1** and **2**, the most active of the algicidal compounds, were located almost entirely in the bark, traces were detected in the adjacent layer and none at all was found in the core In view of this, and the strong antifungal properties of **1** and **2** (against both plant and human pathogens) it is tempting to speculate that their function in the plant is to ward off attack by soil-borne fungi The differences in distribution of **4** and **5**, which have algicidal, but no antimicrobial, activity [4] are much less marked but in the same sense

Samples of older, thicker roots from both *Plumeria* and *Allamanda* were divided into bark and inner part and showed the same distribution pattern as above, with **1** and **2** confined to the bark Table 4 presents the quantitative data obtained by isolating chromatographically the individual iridoids from extracts of divided *A cathartica* roots As many as 85 % of the total iridoids were present in the bark

Age and size of roots were not useful criteria in predicting the presence of **1** and **2**, and seasonal, climatic and edaphic factors presumably affect their production Very young plants, however, do produce the active compounds, and yields from the roots of *P obtusa* stem cuttings after 10 weeks growth are given in Table 5

Table 5 Yields of compounds 1–5 in young *P obtusa* roots*

1/2	3	4	5
06	20	10	24

*Roots taken from stem cuttings grown for 6 weeks in a mist propagator and for 4 weeks hydroponically Yields estimated by TLC using standard solutions and expressed as a percentage on dry wt basis (means of results from four plants)

Compounds **1**–**5** were present in similar amounts when the roots were first analysed at 6 weeks

Plumericin (**2**) has been reported to occur in *Nerium indicum* roots [7] Two samples of *N indicum* roots from different sources and samples of *N oleander* leaves and stem examined by us were devoid of any iridoids

EXPERIMENTAL

General analytical procedure Plant material was separated into its component parts on receipt and dried at 60° overnight The dried, ground material (2 g) was extracted with MeOH in a Soxhlet apparatus for 8 hr and the extract concd and made up to 50 ml Each extract (5 μl) was analysed by TLC using Si gel 60 and the following solvents (with R_f values as indicated) C_6H_6–EtOAc (4 1) (0 39 **1**, 0 35 **2**), $CHCl_3$–MeOH (4 1) (0 38 D, 0 32 **4**, 0 24 **3**, 0 06 **5**), $CHCl_3$–MeOH (7 3) (0 52 **4**/D, 0 45 F, 0 42 **3**, 0 20 **5**), PrOH–EtOAc–H_2O (7 2 1) (0 67 **4**, 0 60 **3**, 0 54 **5**, 0 47 J, 0 37 K) Plates were visualized by spraying with 50% H_2SO_4 and heating at *ca* 120° for a few min All iridoids appeared yellow in visible light, **1**–**3** and D and J had a 'whitish' fluorescence under UV light at 366 nm and **4**, **5**, F and K a 'reddish' fluorescence

Quantitative analysis (*Table* 4) Mature dried roots of *A cathartica* collected at the University of the Philippines were separated into bark and inner part and a portion of each (15 0 g) was extracted successively with $CHCl_3$ and MeOH in a Soxhlet apparatus for 14 hr The $CHCl_3$ extract (1 7 g) of the bark was chromatographed on Si gel (80 g) and eluted with mixtures of petrol, Et_2O, $CHCl_3$ and MeOH of increasing polarity Petrol–Et_2O (3 2) gave a fraction containing **1** and **2**, which on trituration with Et_2O afforded pure **1** and **2** (160 mg), $CHCl_3$–MeOH (3 2) contained **3** and **4** which, together with the MeOH extract (2 8 g) of the bark, was chromatographed on Si gel (150 g) deactivated with H_2O (15 ml) Elution with mixtures of $CHCl_3$ and MeOH of increasing polarity gave several fractions $CHCl_3$–MeOH (9 1) afforded pure **4** (150 mg), $CHCl_3$–MeOH (4 1) afforded **5** (480 mg) and a mixture containing **3** and **4**, which on partition between H_2O and EtOAc yielded **3** (180 mg) and **4** (150 mg), respectively, $CHCl_3$–MeOH (3 2) afforded a mixture containing **3** and **5** which was purified by further CC to give **3** (300 mg) and **5** (410 mg) The combined $CHCl_3$ (810 mg) and MeOH (920 mg) extracts of the inner part of the roots, which contained no **1** or **2**, were chromatographed on Si gel (75 g) deactivated with H_2O (7 5 ml) Elution with mixtures of $CHCl_3$ and MeOH gave several fractions which were purified as before to yield, finally, **3** (70 mg), **4** (80 mg) and **5** (200 mg) Cubic crystals (70 mg) recovered from the later fractions were identified as sucrose (TLC, IR)

Quantitative analysis (*Table* 5) A number of different standard solns of **1**–**4** in MeOH (equivalent to 0 1–3 0% in the plant on a dry wt basis) were applied alongside extracts on TLC plates and spots in the latter were matched in size and intensity of fluorescence with those of the standard solns At lowest concns, 0 1% standard was weakly but definitely discernible and distinguishable from 0 2%

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