Isoneorautenol and Other Pterocarpan Phytoalexins from *Calopogonium mucunoides*

John L. Ingham

Phytochemical Unit, Department of Botany, University of Reading, P. O. Box 221, Reading RG6 2AS, England

and

Satoshi Tahara

Department of Agricultural Chemistry, Faculty of Agriculture, Hokkaido University, Kita-ku, Sapporo 060, Japan

Z. Naturforsch. **40c,** 482–489 (1985); received March 16, 1985

Leguminosae, *Calopogonium,* Isoflavonoids, (−)−Pterocarpans, Phytoalexins

Following inoculation with the fungus *Helminthosporium carbonum,* excised leaflets of the tropical papilionate legume *Calopogonium mucunoides* have been found to produce isoneorautenol, a new dimethylpyrano-substituted isoflavonoid (pterocarpan) phytoalexin. This compound accumulates together with demethylmedicarpin, neodunol, tuberosin, sophorapterocarpan A, and a sixth highly antifungal isoflavonoid (calopocarpin) characterised as 3,9-dihydroxy-2-(3,3-dimethylallyl)pterocarpan. Chromatographic and spectroscopic examination of 'homoedudiol' (a pterocarpan from *Neorautanenia edulis* roots previously considered to possess the structure now assigned to calopocarpin) has shown that in reality this substance is identical with sophorapterocarpan A. The systematic relationship of *Calopogonium* to other genera within the legume tribe Phaseoleae is briefly discussed.

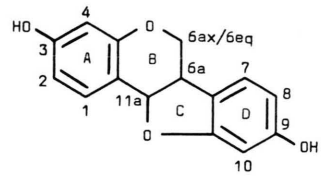
Introduction

A considerable number of phenolic isoflavonoid phytoalexins [1] have now been obtained from the fungus-treated tissues of tropical species belonging to the subfamily Papilionoideae of the Leguminosae [1–4]. Many of these compounds are pterocarpans which characteristically possess a tetracyclic ring system with oxygenation at C-3 and C-9 on rings A and D respectively (see structures **1**–**9**). Apart from pterocarpans, however, other types of isoflavonoid, including various isoflavones and isoflavanones [1, 2], are also known to accumulate as phytoalexins in some tropical Leguminosae. Our continuing search for new phytoalexin structures recently centred on *Calopogonium mucunoides* Desv. (Leguminosae-Papilionoideae; tribe Phaseoleae), a creeping, or occasionally twining, hairy perennial widely distributed throughout the tropics. In parts of South East Asia and Australia (Queensland), *C. mucunoides* has proved to be of some agricultural value being grown as a cover crop (*e.g.* in young rubber plantations) and as a source of green manure [5]. Although previous studies have revealed that the seeds of *C. mucunoides* are rich in isoflavones [6, 7], there are no reports on the isolation of constitutive or induced isoflavonoids from the herbaceous tissues of this legume.

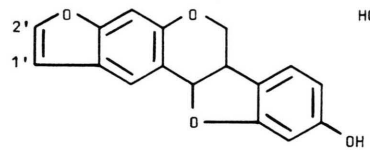
In the present study we have investigated the ability of excised *C. mucunoides* leaflets to produce isoflavonoid phytoalexins following treatment with a spore suspension of the fungus, *Helminthosporium carbonum* Ullstrup. Six fungitoxic, laevorotatory pterocarpans (**1**–**6**) have been found to accumulate in leaf diffusates, and one of these (isoneorautenol, **3**) is reported for the first time. In addition, 3,9-dihydroxy-2-(3,3-dimethylallyl)pterocarpan (**5**) is also produced as a *Calopogonium* phytoalexin, but this compound differs from a pterocarpan (named 'homoedudiol' and characterised as **5**) earlier obtained from the root bark of *Neorautanenia edulis* C. A. Sm. (Leguminosae-Papilionoideae; tribe Phaseoleae) [8], a comparatively close botanic relative of *C. mucunoides*. Re-examination of the *Neorautanenia* compound has revealed it to be an isomer of **5** with the structure now attributed to sophorapterocarpan A [3,9-dihydroxy-8-(3,3-dimethylallyl)pterocarpan, **4**].

Reprint requests to Dr. J. L. Ingham.

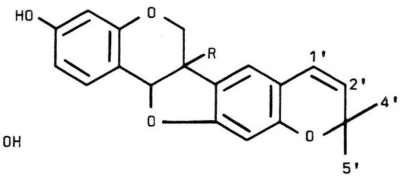
Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/85/0700–0482 $ 01.30/0



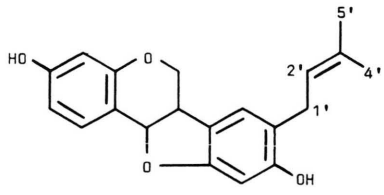
1: Demethylmedicarpin



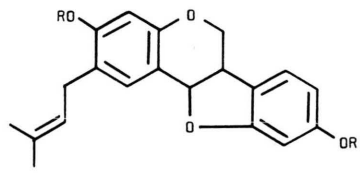
2: Neodunol



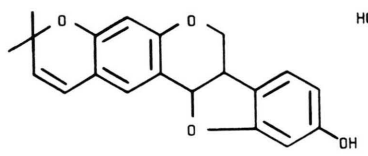
3: R = H, Isoneorautenol
6: R = OH, Tuberosin



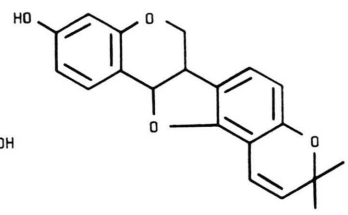
4: Sophorapterocarpan A
(= 'Homoedudiol')



5: R = H, Calopocarpin
9: R = CH_3



7: Neorautenol



8: Phaseollin

Results and Discussion

Ethyl acetate extracts of 48 h diffusates from control (H_2O-treated) or *H. carbonum*-inoculated leaflets of *C. mucunoides* were reduced to dryness under reduced pressure (40 °C), and the residue was then chromatographed (Si gel TLC) in $CHCl_3$–MeOH (20:1). No antifungal material of any kind was associated with the control diffusate extract. In contrast, diffusates from the fungus-inoculated leaflets contained several phenolic (diazotised *p*-nitroaniline–positive) substances, all of which inhibited the growth of *Cladosporium herbarum* Fr. on TLC plates [9, 10]. Particularly prominent antifungal bands occurred at approx. R_F 0.78, 0.61 and 0.43, with less well-defined inhibition zones being evident at approx. R_F 0.41 and 0.34. The antifungal material was eluted (MeOH) from corresponding zones on unsprayed chromatograms and then purified by TLC as described in the Experimental section to yield six compounds including demethylmedicarpin (3,9-dihydroxypterocarpan, **1**), the furano-pterocarpan neodunol (**2**), sophorapterocarpan A (**4**), and the di-

Table I. ^{1}H NMR chemical shifts of demethylmedicarpin (**1**), neodunol (**2**), neorautenol (**7**), and the *Calopogonium* phytoalexins isoneorautenol (**3**) and tuberosin (**6**)[a].

Proton	Demethylmedicarpin[b] (**1**)	Isoneorautenol (**3**)	Tuberosin (**6**)	Neorautenol[c] (**7**)	Neodunol[c] (**2**)
H-1	7.32d J = 8.3	7.32d J = 8.1	7.31d J = 8.3	7.15s	7.78s
H-2	6.56dd J = 8.3 & 2.4	6.55dd J = 8.1 & 2.3	6.55dd J = 8.3 & 2.4	–	–
H-4	6.36d J = 2.4	6.36d J = 2.3	6.32d J = 2.4	6.25s	7.05brs
H-7	7.13d J = 8.0	7.01s	7.07s	7.14d J = 7.9	7.18d J = 8.1
H-8	6.37dd J = 8.1 & 2.2	–	–	6.38dd J = 7.9 & 2.2	6.39dd J = 8.1 & 2.2
H-10	6.29d J = 2.2	6.19s	6.16s	6.29d J = 2.2	6.30d J = 2.2
H-6eq	4.24m	4.27m	4.14d J = *ca.* 13	4.27m	4.32m
H-6ax	3.56m (2H)	3.59m (2H)	4.04d J = *ca.* 13	3.57m (2H)	3.69m (2H)
H-6a			–		
H-11a	5.46d J = 5.9	5.50brd J = 5.6	5.28s	5.47brd J = 5.9	5.71brd J = 5.9
H-1′	–	6.33d J = 10.0	6.37d J = 9.8	6.42d J = 9.8	6.88dd J = 2.2 & 1.0
H-2′	–	5.53d J = 10.0	5.56d J = 9.8	5.66d J = 9.8	7.77d J = 2.2
H-4′	–	1.37s (2 × 3H)	1.38s (3H)	1.40s (3H)	–
H-5′	–		1.35s (3H)	1.39s (3H)	–

[a] Data (δ values) are for spectra determined in acetone-d_6 (TMS reference) at either 100 MHz (compounds **2**, **3**, **6** and **7**) or 270 MHz (compound **1**). Coupling constants (J) are given in Hz. All signals integrated for one proton except where indicated by data in parentheses.

[b] δ values for demethylmedicarpin (**1**) are taken from Woodward [13].

[c] Material extracted from the root bark of *N. edulis* [8] and kindly provided by Dr. G. J. H. Rall. Comparative data for neodunol derived from *C. mucunoides* are as follows: δ 7.78 (1H, s, H-1), 7.77 (1H, d, J = 2.2, H-2′), 7.18 (1H, d, J = 7.8, H-7), 7.05 (1H, br s, H-4), 6.88 (1H, dd, J = 2.2 & 1.0, H-1′), 6.39 (1H, dd, J = 7.8 & 2.2, H-8), 6.30 (1H, d, J = 2.2, H-10), 5.77 (1H, br d, J = 5.6, H-11a), 4.34 (1H, m, H-6eq), 3.67 (2H, m, H-6a/H-6ax).

methylpyrano-6a-hydroxypterocarpan tuberosin (**6**). Pterocarpans **1**, **2**, **4** and **6** were identified by UV, TLC (all four compounds) and ^{1}H NMR (**2** only) comparison with authentic samples. ^{1}H NMR data for **6** from *C. mucunoides* (Table I) were also entirely consistent with the proposed structure. Sophorapterocarpan A (**4**) has previously been found as a normal component of *Sophora franchetiana* Dunn

(Leguminosae-Papilionoideae; tribe Sophoreae) roots [11], but this is the first account of its accumulation as a phytoalexin in the Leguminosae. Compounds **1**, **2** and **6** are known to occur constitutively or as phytoalexins in a variety of papilionate legumes [1, 2], many of which belong, as does *C. mucunoides,* to the tribe Phaseoleae.

Of the two remaining *Calopogonium* phytoalexins, one (for which we suggest the name, isoneorautenol) was found to be a new dimethylpyrano-substituted pterocarpan (**3**; M^+ 322) isomeric with neorautenol (**7**) and phaseollin (**8**). In the ^1H NMR spectrum of **3** (Table I), signals attributable to the A-ring protons (H-1, -2 and -4) were in excellent agreement with those similarly given by the model pterocarpans, demethylmedicarpin (**1**) and tuberosin (**6**), an observation which permitted the dimethylpyrano group [defined by two olefinic doublets at δ 6.33 (H-1′) and δ 5.53 (H-2′) both with J = 10 Hz, and a 6H singlet at δ 1.37 (2 × $C\underline{H}_3$)] to be readily placed on ring D. Moreover, H-7 and H-10 of isoneorautenol appeared as uncoupled singlets (δ 7.01 and δ 6.19 respectively) and hence the side-structure must be attached linearly to ring D as in tuberosin (**6**) (*cf.* phaseollin (**8**) and its 6a-hydroxy derivative where the dimethylpyrano unit occupies an angular position and the two D-ring protons, H-7 and H-8, resonate as *ortho*-coupled doublets [12, 13]). Lastly, the H-11a signal of **3** was evident as a broad doublet (*cf.* corresponding data for the 6aH-pterocarpans **1**, **2**, **4**, **5** and **7** in Tables I and II) whilst H-6a, which was absent from the tuberosin spectrum (Table I), resonated together with H-6ax at approx. δ 3.60 (2H, multiplet). If oxygenation at C-9 is assumed, isoneorautenol (**3**) must therefore be the 6a-deoxy analogue of tuberosin (**6**).

In addition to five aromatic protons, ^1H NMR analysis of the final *Calopogonium* phytoalexin, calopocarpin (**5**; M^+ 324: *Dimethyl ether,* **9**; M^+ 352) revealed signals at δ 1.73 and 1.74 (both s, each 3H, $C\underline{H}_3$), 5.36 (br t, 1H, olefinic hydrogen) and 3.30 (br d, 2H, methylene) characteristic of a 3,3-dimethylallyl sidechain (*cf.* data for **4** in Table II). The heterocyclic region of the spectrum closely resembled that of demethylmedicarpin (**1**) thereby confirming that, like isoneorautenol, compound **5** was a 6aH-pterocarpan. Three of the aromatic protons exhibited chemical shift values closely coincident with those reported for H-7, H-8 and H-10 (ring D) of **1** and neodunol (**2**) (Table II), and on this basis the sidechain was allocated to ring A. The two residual signals (δ 7.17 and δ 6.39) in the ^1H NMR spectrum of **3** appeared as 1H singlets and were readily attributable to H-1 and H-4 respectively. Since all naturally occurring pterocarpans are oxygenated at C-3 [2], it follows from the above data that the prenyl group is situated at C-2, and hence calopocarpin can be formulated as 3,9-dihydroxy-2-(3,3-dimethylallyl)pterocarpan (**5**). As well as occurring in *C. mucunoides,* calopocarpin has also been isolated as a minor phytoalexin from the *H. carbonum*-inoculated leaflets of two other papilionate legumes, namely *Pueraria phaseoloides* (Roxb.) Benth. (tribe Phaseoleae) and a species of *Alysicarpus* (tribe Desmodieae) [14]. A phytoalexin, termed PE-2, previously obtained from the etiolated stems of

Table II. ^1H NMR data for calopocarpin (**5**), authentic sophorapterocarpan A (**4**), and 'homoedudiol' (= **4**) from *N. edulis* root bark[a,b].

Proton	Calopocarpin (**5**)	Sophoraptero-carpan A (**4**)	'Homoedudiol' (= **4**)
H-1	7.17s	7.30d J = 8.3	7.30d J = 8.3
H-2	–	6.54dd J = 8.3 & 2.4	6.55dd J = 8.3 & 2.4
H-4	6.39s	6.35d J = 2.4	6.36d J = 2.4
H-7	7.13d J = 8.1	7.04s	7.03s
H-8	6.36dd J = 8.1 & 2.1	–	–
H-10	6.29d J = 2.1	6.32s	6.34s
H-6eq	4.22m	4.23m	4.15m
H-6ax, H-6a	3.51m (2H)	*ca.* 3.53m (2H)	3.52m (2H)
H-11a	5.44 br d J = 5.4	5.42 br d J = 5.6	5.41 br d J = 5.9
H-1′	3.30 br d J = 7.3	3.25 br d J = 7.3	3.25 br d J = 7.3
H-2′	5.36 br t J = 7.3	5.32 br t J = 7.3	5.33 br t J = 7.3
H-4′	1.73s (3H)	1.71s (3H)	1.70s (3H)
H-5′	1.74s (3H)	1.72s (3H)	1.72s (3H)

[a] δ values measured in acetone-d_6 at 100 MHz. Coupling constants (J) are in Hz. All signals are 1H except where indicated by 2H or 3H in parentheses.

[b] Comparative data for the aromatic ring protons of the model pterocarpan demethylmedicarpin (**1**) are: *A-ring,* δ 7.32 (d, J = 8.3, H-1), 6.56 (dd, J = 8.3 & 2.4, H-2) and 6.36 (d, J = 2.4, H-4); *D-ring,* δ 7.13 (d, J = 8.0, H-7), 6.37 (dd, J = 8.1 & 2.2, H-8) and 6.29 (d, J = 2.2, H-10) [13]. For other chemical shift values see Table I.

Pachyrrhizus erosus (L.) Urban (Leguminosae-Papilionoideae, tribe Phaseoleae) has also been provisionally assigned structure **5** [15]. This identification has now been confirmed by direct UV, MS and TLC comparison with the *Calopogonium* pterocarpan.

It has already been claimed [8] that a pterocarpan (named 'homoedudiol') with structure **5** occurs in the root bark of *Neorautanenia edulis*. In fact, we have found that the *Calopogonium*- and *Neorautanenia*-derived pterocarpans can easily be separated on Si gel thin-layer plates developed in $CHCl_3$–MeOH, 20:1 (*Calopogonium* material, R_F 0.61; *Neorautanenia* material, R_F 0.54). However, the latter pterocarpan co-chromatographed with authentic sophorapterocarpan A (**4**) [11] in a variety of solvent systems, and a ^{1}H NMR comparison (Table II) finally confirmed that the two compounds were identical. The structure of *Neorautanenia* 'homoedudiol' must therefore be revised to **4**.

Although Brink and his co-workers reported [8] that 'homoedudiol' from *N. edulis* could be converted into neorautenol (**7**) and vice versa, such a transformation is clearly impossible. Indeed, the difference, with respect to A/D-ring substitution, between 'homoedudiol' and other *Neorautanenia* pterocarpans (**2** and **7**) isolated at the same time is immediately evident from the ^{1}H NMR data published by Brink *et al.* [8]. Thus, in $CDCl_3$ the D-ring protons of neodunol (**2**) and neorautenol (**7**) were found to resonate respectively at δ 7.09/7.11 (H-7), 6.36/6.39 (H-8) and 6.40/6.40 (H-10) whereas corresponding chemical shift values reported for 'homoedudiol' were δ 7.41 ('H-7'; Δ δ approx. 0.31), 6.57 ('H-8'; Δ δ approx. 0.19) and 6.45 ('H-10'; Δ δ 0.05) in the same solvent. It is worth noting that the last three δ values are actually in close accord with data reported by Woodward [13] for H-1 (δ 7.39), H-2 (δ 6.56) and H-4 (δ 6.42) of demethylmedicarpin (**1**; $CDCl_3$ spectrum), thereby providing indirect proof that ring A of 'homoedudiol' carries only an OH group at C-3. In view of the structure revision required for 'homoedudiol', we have also re-determined the ^{1}H NMR spectra of neodunol (**2**) and neorautenol (**7**) from *N. edulis* (Table I) and have confirmed that both compounds are substituted as originally described by Brink *et al.* [8].

As shown in Table III, diffusates from the *H. carbonum*-inoculated leaflets of *C. mucunoides* contained large quantities of tuberosin and smaller, but still substantial, amounts of neodunol, calopocarpin and isoneorautenol. In contrast, demethylmedicarpin and sophorapterocarpan A were always minor diffusate components, never exceeding concentrations of 5 and 10 μg/ml respectively. Demethylmedicarpin is presumably the immediate biosynthetic precursor of both calopocarpin and sophorapterocarpan A, with the latter compound affording isoneorautenol by cyclisation of the 3,3-dimethylallyl group to the OH substituent at C-9. Neodunol might be similarly formed from calopocarpin by sidechain cyclisation coupled with the loss of three carbon atoms as is thought to occur in furanocoumarin biosynthesis [17]. The biosynthetic origin of tuberosin is less certain. It could conceivably arise by direct C-6a hydroxylation of isoneorautenol or, since prenylation appears to be a late step in pterocarpan biosynthesis [18], precursors such as glycinol (3,6a,9-trihydroxypterocarpan) and its 8-prenylated analogue may be involved. However, no evidence has yet been obtained to suggest that such compounds accumulate in *C. mucunoides* diffusates.

As all six *Calopogonium* phytoalexins were found to be laevorotatory, they therefore possess either the 6a*R*; 11a*R* (compounds **1**–**5**) or 6a*S*; 11a*S* (compound **6**) absolute configuration [1]. Until now, tuberosin (**6**) has only been obtained as its (+)-

Table III. Typical levels (μg/ml) attained by phytoalexins **1**–**6** in *C. mucunoides* leaf diffusates 48 h after treatment with droplets of an *H. carbonum* spore suspension[a].

Phytoalexin	Diffusate Concentration[b] [μg/ml]
Demethylmedicarpin (**1**)	4
Neodunol (**2**)	18
Isoneorautenol (**3**)	30
Sophorapterocarpan A (**4**) (= 'homoedudiol')	7
Calopocarpin (**5**)	42
Tuberosin (**6**)	68

[a] Compounds **1**–**6** were not produced by excised leaflets treated with droplets of deionised H_2O.

[b] The concentrations of neodunol (**2**), sophorapterocarpan A (**4**) and tuberosin (**6**) were calculated from spectrophotometric measurements using the following previously published extinction coefficients: **2**, ε = 21880 at 246 nm [8], **4**, ε = 9120 at 290 nm [11] and **6**, ε = 6607 at 286 nm [16]. Demethylmedicarpin (**1**), isoneorautenol (**3**), and calopocarpin (**5**) were quantified using ε for medicarpin (3-hydroxy-9-methoxypterocarpan, ε = 7762 at 287 nm [22]), tuberosin and sophorapterocarpan A respectively.

isomer from tubers of *Pueraria tuberosa* DC. [16], and from excised leaflets of *P. thunbergiana* (Sieb. & Zucc.) Benth. [= *P. lobata* (Willd.) Ohwi] where it functions as a phytoalexin [2].

At present, *Calopogonium* is placed within subtribe Diocleinae of the tribe Phaseoleae, but this is largely an arrangement of convenience and the natural allies of this peculiar legume genus remain to be established [19]. One reason for undertaking the present study, therefore, was to determine if the leaf phytoalexin response of *C. mucunoides* could provide any information on the systematic relationship of *Calopogonium* to other taxa inside or outside the Phaseoleae. Interestingly, *C. mucunoides* has been found to produce a number of 'complex' pterocarpan phytoalexins (**2** and **4**–**6**) which are known to occur either constitutively or as phytoalexins [1, 2, 14] in only a very limited number of legume genera, those most relevant to *Calopogonium* being *Pachyrrhizus* (which has compounds **2** and **5** in common with *Calopogonium*), *Neorautanenia* (compounds **2** and **4**) and *Pueraria* (compounds **5** and **6**). Like *Calopogonium,* the tropical genus *Pachyrrhizus* is placed in subtribe Diocleinae [19], but its close chemical similarity to *Neorautanenia* (now regarded as a peripheral genus of the subtribe Phaseolinae*) has already been discussed elsewhere [15]. Lastly, whilst *Pueraria* is currently associated with subtribe Glycininae, it contains species (*e.g.* the tuberosin sources *P. tuberosa* and *P. thunbergiana* = *P. lobata* [1, 2, 16]) which appear to have links with the subtribe Diocleinae [19]. Surveys of phytoalexin formation in numerous genera belonging to the Phaseoleae and neighbouring tribes [14] have generally failed to reveal compounds **2** and **4**–**6** although traces of **5** accumulate in an *Alysicarpus* species (tribe Desmodieae) [14], and as mentioned earlier the roots of *Sophora franchetiana* (tribe Sophoreae) contain **4** as a normal constituent [11]. Despite the occurrence of **4** and **5** in *Sophora* and *Alysicarpus* respectively, the available evidence suggests that in terms of phytoalexin chemistry, *C. mucunoides* most closely resembles species of *Pachyrrhizus, Neorautanenia* and *Pueraria,* and it is amongst these three latter genera that the nearest taxonomic relatives of *Calopogonium* may eventually be found to reside.

* Erroneously referred to as subtribe Glycininae in ref. [15].

Experimental

Plant and fungus material

Between 10 and 15 plants of *Calopogonium mucunoides* Desv. were grown in John Innes No. 1 compost from seeds supplied by Dr. E. F. Henzell, The Cunningham Laboratory, St. Lucia, Queensland, Australia. The plants were kept in a warm (approx. 20 °C) glasshouse under natural daylight, and received soil-applied liquid fertilizer (Maxicrop) at 4–5 week intervals. Sufficient leaflets to provide batches of diffusate in excess of 20 ml were collected periodically, starting when the plants were 8–10 weeks old. Details of fungus (*Helminthosporium carbonum* Ullstrup and *Cladosporium herbarum* Fr.) culture conditions are given in ref. [10].

Isolation and purification of phytoalexins **1**–**6**

Excised *C. mucunoides* leaflets were inoculated on their lower surface with droplets of a spore suspension of *H. carbonum* [20], and after 48 h incubation [21] the pale yellow, frequently cloudy, diffusate (normally between 25 and 30 ml) was collected by suction and extracted (×3) with equal volumes of EtOAc. Control leaflets were treated with deionised H_2O on two separate occasions, and each time yielded diffusate samples that were clear and completely colourless. The components in extracts of diffusates from *H. carbonum*-treated leaflets were initially separated by Si gel TLC (Merck precoated, glass-backed plates; F-254; layer thickness, 0.25 mm) in $CHCl_3$–MeOH (CM, 20:1) to afford neodunol, **2** + isoneorautenol, **3** (R_F 0.78), calopocarpin, **5** (R_F 0.61), sophorapterocarpan A, **4** (= 'homoedudiol') (R_F 0.54), tuberosin, **6** (R_F 0.43) and demethylmedicarpin, **1** (R_F 0.34). After elution from the Si gel with MeOH, pterocarpans **2** and **3** were resolved by TLC in *n*-pentane–Et_2O–glacial HOAc (PEA, 75:25:6; **2** = R_F 0.59 and **3** = R_F 0.67). The PEA solvent system was also used to purify calopocarpin, **5** (R_F 0.41) and sophorapterocarpan A, **4** (R_F 0.39) prior to UV, MS and ^{1}H NMR analysis. Demethylmedicarpin (**1**) from the CM plates was subsequently chromatographed in PEA (75:25:6, ×3) followed by elution and further TLC in benzene–MeOH (9:1, R_F 0.19). Purification of tuberosin (**6**) was by Si gel TLC in Et_2O–*n*-hexane–MeOH (75:25:2, R_F 0.52). Pterocarpans **1**–**6** were absent from EtOAc extracts of the control diffusate.

Physico-chemical data for phytoalexins **1**–**6**

Optical rotation measurements in MeOH at 589 nm established that all six pterocarpans were laevorotatory, but precise [α] values were not determined. ^{1}H NMR data for four of the *Calopogonium* phytoalexins are given in Tables I (compounds **2**, **3** and **6**) and II (compound **5**).

Demethylmedicarpin (**1**) *and neodunol* (**2**)

Colour with diazotised *p*-nitroaniline reagent, orange. UV data as lit. [15, 21]. ^{1}H NMR data for *Phaseolus*-derived **1** are given in Table I.

Isoneorautenol (**3**)

Colour with diazotised *p*-nitroaniline reagent, yellow (*cf.* neorautenol **7**, orange). UV: λ max, nm (rel. int.): MeOH, 210 (100%), 281 (20%), 287 (20%), 314 (18%), 326sh (16%); + NaOH, 210, 253sh, 293, 314, 325sh. [UV maxima recorded for authentic neorautenol (**7**) were as follows: MeOH, 208sh (68%), 228 (100%), 277sh (22%), 286 (25%), 295sh (20%), 308 (15%), 320 (14%); + NaOH, 214, 274sh, 288sh, 307, 321sh]. MS (rel. int.) $[M]^+$ 322 (37%), *m/z* 321 (5%), 320 (6%), 308 (21%), 307 (100%; loss of CH_3 from the dimethylpyran side-structure). The mass spectrum of **3** was almost identical with that of neorautenol which gave $[M]^+$ 322 (34%), *m/z* 321 (4%), 308 (37%), 307 (100%). On Si gel thin-layer plates developed in $CHCl_3$–MeOH (20:1), neorautenol (R_F 0.78) ran slightly ahead of isoneorautenol (R_F 0.74).

Sophorapterocarpan A (**4**) (= *'homoedudiol'*)

Colour with diazotised *p*-nitroaniline reagent, orange to orange-yellow. UV: λ max, nm (rel. int.): MeOH, 208 (100%), 230sh (43%), 282sh (26%), 289 (30%); + NaOH, 211, 249, 298. ^{1}H NMR data for *Sophora*-derived **4** are given in Table II.

Calopocarpin (**5**)

Colour with diazotised *p*-nitroaniline reagent, orange. UV: λ max, nm (rel. int.): MeOH, 211 (100%), 232sh (47%), 284sh (31%), 288 (34%), 293sh (32%); + NaOH, 211, 249, 300. MS (rel. int.): $[M]^+$ 324 (100%), *m/z* 323 (11%), 322 (12%), 307 (12%), 270 (13%), 269 (77%; $M^+ - 55$), 268 (28%; $M^+ - 56$). The above data resembled those previously recorded for a minor phytoalexin, designated PE–2, isolated from *Pachyrrhizus erosus* [15]. *Dimethyl ether* (**9**) (CH_2N_2). R_F, UV and MS data as given for the dimethyl ether of *Pachyrrhizus* phytoalexin PE–2 [15].

Tuberosin (**6**)

Colour with diazotised *p*-nitroaniline reagent, yellow. UV: λ max, nm (rel. int.): MeOH, 210sh (80%), 224 (100%), 281 (27%), 287 (27.5%), 312 (24%), 318sh (23%), 324sh (21%); + NaOH, 215, 254sh, 293, 312, 319sh, 325sh; + conc. HCl (*d.* 1.16, 3 drops per ml cuvette volume; recorded after 10 min at room temp.), numerous peaks and shoulders between 260 and 325 nm, plus diagnostic maxima at 349 and 365 nm due to formation of the anhydro derivative.

Acknowledgements

We thank Mr. R. W. Butters for mass spectral measurements and Miss Y. Hatakeyama for determining the ^{1}H NMR spectra. Samples of neodunol, 'homoedudiol' and neorautenol from *Neorautanenia edulis* were kindly supplied by Dr. G. J. H. Rall (Dept. of Chemistry, University of the Orange Free State, Bloemfontein, South Africa). We are also grateful to Dr. Y. Shirataki (Faculty of Pharmaceutical Sciences, Josai University, Saitama, Japan) for a generous gift of sophorapterocarpan A.

[1] J. L. Ingham, Phytoalexins (J. A. Bailey and J. W. Mansfield, eds.), p. 21, Blackie, Glasgow 1982.
[2] J. L. Ingham, Fortschr. Chem. Org. NatStoffe **43,** 1 (1983).
[3] J. L. Ingham and S. Tahara, Z. Naturforsch. **38c,** 899 (1983).
[4] J. L. Ingham and K. R. Markham, Z. Naturforsch. **39c,** 13 (1984).
[5] J. W. Purseglove, Tropical Crops, Dicotyledons. **Vol. 1,** Longmans, London 1968.
[6] C. Vilain, Bull. Soc. R. Sci. Liège **44,** 306 (1975).
[7] C. Vilain, Bull. Soc. R. Sci. Liège **45,** 468 (1976).
[8] A. J. Brink, G. J. H. Rall, and J. P. Englebrecht, Phytochemistry **13,** 1581 (1974).
[9] A. L. Homans and A. Fuchs, J. Chromatogr. **51,** 327 (1970).
[10] J. L. Ingham, Phytopathol. Z. **87,** 353 (1976).
[11] M. Komatsu, I. Yokoe, and Y. Shirataki, Chem. Pharm. Bull. Tokyo **29,** 532 (1981).
[12] D. R. Perrin, C. P. Whittle, and T. J. Batterham, Tetrahedron Lett. **1972,** 1673.
[13] M. D. Woodward, Phytochemistry **19,** 921 (1980).
[14] J. L. Ingham, unpublished data.
[15] J. L. Ingham, Z. Naturforsch. **34c,** 683 (1979).
[16] B. S. Joshi and V. N. Kamat, J. Chem. Soc. Perkin Trans. I **1973,** 907.
[17] R. D. H. Murray, Fortschr. Chem. Org. NatStoffe **35,** 199 (1978).
[18] S. Banks and P. M. Dewick, Phytochemistry **22,** 2729 (1983).
[19] J. A. Lackey, Advances in Legume Systematics (R. M. Polhill and P. H. Raven, eds.), p. 301, Her Majesty's Stationary Office, London 1981.
[20] J. L. Ingham, Advances in Legume Systematics (R. M. Polhill and P. H. Raven, eds.), p. 599, Her Majesty's Stationary Office, London 1981.
[21] J. L. Ingham, Phytochemistry **15,** 1489 (1976).
[22] S. H. Harper, A. D. Kemp, and W. G. E. Underwood, Chem. & Ind. **1965,** 562.