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METABOLISM OF THE PHYTOALEXINS MEDICARPIN AND MAACKIAIN BY *FUSARIUM SOLANI*

TIMOTHY P. DENNY and HANS D. VANETTEN

Department of Plant Pathology, Cornell University, Ithaca, NY 14853, U.S.A.

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Key Word Index—*Fusarium solani*; *Nectria haematococca*; fungi; phytoalexin metabolism; pterocarpan; oxygenation; isoflavanone; 1a-hydroxydienone; 6a-hydroxypterocarpan.

Abstract—Non-inhibitory concentrations of the pterocarpan phytoalexin medicarpin were completely metabolized by isolates of *Fusarium solani* f. sp. *pisi*, f. sp. *cucurbitae*, f. sp. *phaseoli* and two other *F. solani* isolates genetically related to f. sp. *pisi* during 24 hr of growth in liquid medium. The major metabolic products accumulated without significant further degradation. Medicarpin was modified at one of three adjacent carbon atoms to form either an isoflavanone derivative, a 1a-hydroxydienone derivative or 6a-hydroxymedicarpin. Whereas each isolate degraded medicarpin to one or more metabolites, the isolates varied as to which metabolite they produced. Maackiain, another pterocarpan phytoalexin, was also metabolized by all the isolates to products analogous to those formed from medicarpin. The ability to metabolize medicarpin and maackiain was not always associated with the ability to metabolize pisatin and phaseollin, two other pterocarpan phytoalexins that were degraded by several of the isolates. Tolerance of medicarpin and maackiain was similarly not always associated with tolerance to pisatin.

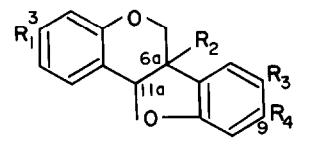
INTRODUCTION

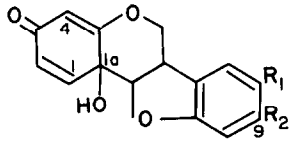
The ability of some fungal pathogens to degrade phytoalexins to less inhibitory compounds may be part of a tolerance mechanism that enables such pathogens to survive in infected plant tissues containing high concentrations of phytoalexins. Although phytoalexin degradation is not always required for pathogenicity [1–3], a recent genetic analysis demonstrated the association of virulence on pea with the ability of pathogenic isolates of *Nectria haematococca* to detoxify (and to tolerate) the pea phytoalexin pisatin [4]. Although basic information on how micro-organisms degrade phytoalexins is necessary for an evaluation of how important the ability to degrade phytoalexins is for pathogenicity, such studies are still limited in number.

Barz *et al.* showed that 15 strains of *Fusarium* (13 species and 3 formae speciales) degraded a variety of polyphenols [5], but that only some of these strains degraded the pterocarpan phytoalexins pisatin and medicarpin [6, 7]. Three formae speciales of the common plant pathogen *Fusarium solani*—*pisi*, *phaseoli* and *cucurbitae*—were not examined in the above studies. The formae speciales (f. sp.) nomenclature denotes known pathogenic specialization: *pisi*, *phaseoli*, and *cucurbitae* imply pathogenicity on *Pisum sativum* L. (pea), *Phaseolus vulgaris* L. (French bean) and cucurbits respectively. Earlier studies reported that, during *in vitro* culture, *F. solani* f. sp. *pisi* and f. sp. *phaseoli* degrade pisatin (**3**) and phaseollin (**7**), the major phytoalexins from their respective host plants, to less toxic derivatives (**4a** and **7a**) [8–10]. Cucurbits do not produce pterocarpanoid phytoalexins and *F. solani* f. sp. *cucurbitae* has not been observed to degrade either pisatin or phaseollin in culture [11].

Some *F. solani* isolates also have a sexual stage, identified as *Nectria haematococca* Berk. and Br. [12], and the ability to interbreed provides a way other than by pathogenicity to establish biological relationships. Within *N. haematococca* there are seven mating populations (MP) that are intra-fertile but not inter-fertile. *Fusarium solani* f. sp. *pisi* and f. sp. *cucurbitae* belong to MP VI and MP I respectively [13, 14], but the sexual stage of *F. solani* f. sp. *phaseoli* is still unknown. The f. sp. and MP designations are not always synonomous [14]; many members of MP VI, including two isolates used in this study, are not pathogenic on pea. Within mating populations, therefore, there is the potential for combining genetics with biochemistry in studying the enzymology of phytoalexin metabolism and its relationship to pathogenicity.

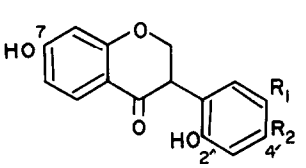
The major objective of this study was to examine the ability of selected members of *N. haematococca* MP VI to degrade the phytoalexins (−)-medicarpin (**1**) and (−)-maackiain (**2**). The degradation of these compounds is of interest because they are produced by chickpea (*Cicer arietinum* L.), a species that is a host for some members of *N. haematococca* MP VI [15, 16]. *Nectria haematococca* MP I and *F. solani* f. sp. *phaseoli* are not known to be pathogenic on chickpea and single isolates of each were included for comparison.

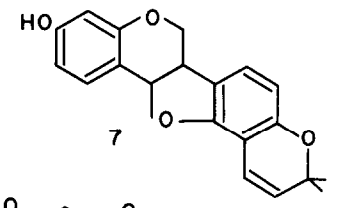




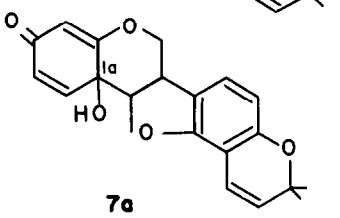
1 R_1 =OH; R_2 =R_3=H; R_4=OMe
2 R_1 =OH; R_2 =H; R_3=R_4=OCH_2O
3 R_1 =OMe; R_2=OH; R_3 = R_4=OCH_2O
4a R_1 =R_2=OH; R_3=H; R_4 = OMe
4b R_1 =R_2=OH; R_3=R_4 = OCH_2O

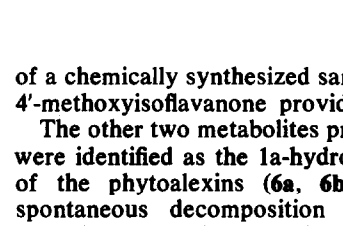
6a R_1=H; R_2=OMe
6b R_1=R_2= OCH_2O





5a R_1=H; R_2=OMe
5b R_1=R_2= OCH_2O





7a

RESULTS

Medicarpin and maackiain metabolites made by N. haematococca *MP VI*

Nectria haematococca MP VI isolate T-30 is pathogenic on both pea and chickpea, and when 24 hr old mycelium was washed and resuspended in medium with 12 μg/ml of either medicarpin or maackiain (a non-inhibitory concentration), it degraded the phytoalexins. Two major metabolites from each phytoalexin appeared simultaneously between 1 and 3 hr incubation and increased in concentration until *ca* 12 hr. The added phytoalexins completely disappeared between 12 and 24 hr, but concentrations of the metabolites decreased only slightly during this time. After 24 hr incubation and one TLC purification, an average 53% (range 40–75%, uncorrected for extraction efficiency) of the phytoalexins added were recovered as metabolites.

One of the two metabolites from each phytoalexin was identified as the isoflavanone derivative of that phytoalexin (**5a**, **5b**). The MWs of the metabolites were 16 mass units heavier than the parent phytoalexins, suggesting the addition of oxygen. The ease of fragmentation in electron impact mass spectrometry, loss of the 11a proton signal from the NMR spectrum [$(CD_3)_2CO$, 90 MHz, data not shown], large reversible bathochromic shifts in basic ethanol (58 nm) [17] and a positive Gibbs reaction for **5a** and not for **5b** [18] indicated an oxidative opening of the dihydrofuran ring to form the isoflavanones. This assignment was confirmed by cyclization of the maackiain derivative (**5b**) to 3-hydroxy-8,9-methylenedioxypterocarpan-6a-ene and the perfect match of the medicarpin derivative's (**5a**) IR spectrum with that of a chemically synthesized sample of 7,2'-dihydroxy-4'-methoxyisoflavanone provided by P. M. Dewick.

The other two metabolites produced by isolate T-30 were identified as the 1a-hydroxydienone derivatives of the phytoalexins (**6a**, **6b**). The unpredictable, spontaneous decomposition of these metabolites complicated their identification; reliable storage required that dry residues be kept in containers flooded with argon gas. An increase of 16 mass units for the parent ions of the metabolites again suggested the addition of oxygen to the phytoalexins. The IR spectrum showed the presence of a conjugated carbonyl group, but little could be easily concluded from the UV spectra or from the mass spectral fragments. However, proton NMR at 300 MHz produced nearly first order spectra of the phytoalexins and their derivatives. Comparison of the metabolites' spectra to each other and to those of the parent phytoalexins showed little change in the D-ring (C-7–C-10) substituents, the retention of four protons with the same relative positions in the aliphatic region (C-6, C-6a, C-11a) and the retention of all three protons in their ABX system in the A-ring (C-1, C-2 and C-4). Spin–spin decoupling during NMR of the maackiain derivative confirmed the assignment of the aliphatic protons. The NMR spectra suggested that the phytoalexins had been modified at a previously unprotonated position. Comparing the change in chemical shifts of the medicarpin and maackiain metabolites relative to their parent phytoalexins with those changes that occur when phaseollin is degraded to form 1a-hydroxyphaseollone by *F. solani* f. sp. *phaseoli* (Table 1) shows that medicarpin and maackiain were also modified by 1a-hydroxylation and dienone formation. Furthermore, like 1a-hydroxy-

Table 1. Change of chemical shifts (in ppm) relative to the chemical shifts of the parent phytoalexins for the analogous protons of three phytoalexin metabolites*

	Protons									
	Aromatic						Aliphatic			
Metabolites	1	2	4	7	8	10	6eq	6ax	6a	11a
1a-OH phaseollone†	0.71	0.44	0.92	0.06	0.02	—	−0.85	−0.72	−0.42	0.33
1a-OH-dienone from medicarpin‡	0.69	0.38	0.89	0.06	−0.02	0.26	−0.87	−0.74	−0.43	0.34
1a-OH-dienone from maackiain‡	0.76	0.44	0.86	0.08	—	0.19	−0.86	−0.67	−0.47	0.33

*A positive value represents an upfield shift in the metabolite's signal relative to the analogous signal of the parent compound. Changes for the protons that are unique to each pterocarpan were less than 0.28 ppm.

†Data are from 90 MHz spectra reported in ref. [10].

‡Data from spectra of medicarpin or maackiain made for this study at 300 MHz.

phaseollone [10], the medicarpin metabolite was reduced to the parent phytoalexin by zinc and acetic acid.

The average ratio of the isoflavanone to the 1a-hydroxydienone derivative of medicarpin and maackiain produced by *N. haematococca* MP VI isolate T-30 was 1:2, but the range was from 1:5 to 1:1. The isoflavanones were never the major constituents.

A third route of medicarpin and maackiain degradation by *N. haematococca* MP VI was found in isolate T-213. The metabolites produced were readily identified as the 6a-hydroxypterocarpan derivatives (**4a**, **4b**) from their UV spectra [19], dehydration by acid to form the pterocarp-6a-enes (identified by UV spectra and mass spectrometry of the medicarpin dehydration product, data not shown) and the NMR spectrum of the maackiain derivative [compare to the spin–spin decoupled spectrum of 1a - hydroxy - 8,9 - methylenedioxypterocarp - 1(2),4 - diene - 3 - one].

Medicarpin and maackiain metabolites made by N. haematococca *MP I and* F. solani *f. sp.* phaseoli

Nectria haematococca MP I isolate T-145 made the 6a-hydroxypterocarpan derivatives of medicarpin and maackiain. Unlike isolates of *N. haematococca* MP VI, however, isolate T-145 degraded the phytoalexins more completely when mycelium was resuspended in buffer rather than in GA medium before treatment.

Fusarium solani f. sp. *phaseoli* isolate T-232 made the 1a-hydroxydienone derivatives of medicarpin and maackiain without accumulating detectable amounts of the isoflavanones. Phytoalexin degradation was almost complete after only 7 hr incubation.

Selectivity of pterocarpan metabolism

All five isolates were compared for their ability to degrade medicarpin, maackiain, pisatin and phaseollin and the results (Table 2) revealed a selectivity in their metabolism of these phytoalexins. Unlike *N. haematococca* MP VI isolate T-30, MP VI isolate 126–80 did not accumulate detectable amounts of isoflavanones during production of the 1a-hydroxydienone derivatives of medicarpin and maackiain. In this respect, MP VI isolate 126–80 was like *F. solani* f. sp. *phaseoli* isolate T-232. In contrast to isolate T-232, though, MP VI isolates 126-80 and T-30 apparently lacked the ability to degrade phaseollin. With the conditions used, no major phytoalexin metabolites other than those listed in Table 2 accumulated in the cultures. Tests were not extensive enough, however, to conclude that other conditions would not alter the observed selectivity.

An earlier study [4] demonstrated that *N. haematococca* MP VI isolates T-213 and 126-80, in addition to being incapable of demethylating pisatin, have a decreased tolerance of pisatin when growing on agar medium amended with high concentrations of this phytoalexin. Further tests of these isolates revealed a selectivity in their tolerance on pisatin, medicarpin and maackiain (Table 3). Tolerance correlated with metabolic ability (Table 2); hyphal growth was significantly inhibited only by the phytoalexin that an isolate was unable to metabolize.

DISCUSSION

Although the isoflavanones (**5a** and **5b**) have been found as natural products [20, 21] and can serve as biosynthetic precursors of the phytoalexins [22, 23], we believe this is the first example of a fungus degrading pterocarpans via ring opening to the isoflavanones. All other reported ring openings, including that by another isolate of *N. haematococca* MP VI, occurred via reduction to form isoflavans [24–27]. The isolation of the 1a-hydroxydienone derivatives of medicarpin and maackiain has also not been reported previously.

Other research has demonstrated that medicarpin, and probably maackiain, are almost always detoxified when modified by the three routes described in this study [1, 16, 28–30]. Detoxification is one mechanism by which fungi might tolerate phytoalexins [27], and the data in Table 3 support this hypothesis.

Table 2. Primary metabolites produced by *N. haematococca* and *F. solani* isolates during 24 hr incubation with non-inhibitory concentrations of four phytoalexins

	Medicarpin and maackiain*			Pisatin*	Phaseollin†
	iso-flavanone	1a-OH-dienone	6a-OH	DMDP‡	1a-OH-dienone
N. haematococca					
MP VI T-30	+	+	−	+§	−
MP VI T-213	−	−	+	−	NT¶
MP VI 126-80	−	+	−	−	−
MP I T-145	−	−	+	−	−
F. solani					
T-232	−	+	−	±‖	+

*Phytoalexin concentration 12 μg/ml.

†Phytoalexin concentration 5 or 12 μg/ml.

‡DMDP is 3,6a-dihydroxy-8,9-methylenedioxypterocarpan.

§Best metabolism occurred when mycelium was in buffer rather than in GA medium, and when DMSO was used as the carrier solvent instead of ethanol.

‖Degradation was quite slow and only small amounts of DMDP were produced during the first 24 hr under all culture conditions tested.

¶NT = Not tested.

Table 3. Percentage inhibition of radial mycelial growth for three *N. haematococca* MP VI isolates treated with the phytoalexins pisatin, medicarpin and maackiain*

Isolate	Pisatin	Medicarpin	Maackiain
T-30	8	5	8
T-213	57	12	8
126-80	62	12	6

*Growth was linear ($r = 0.999$) over the 6–12 days of the assay. Rate of growth was measured from calculated slopes of the lines. Controls grew *ca* 3.4 mm/day. Phytoalexin concentrations were: pisatin = 165 μg/ml (0.5 mM); medicarpin = 180 μg/ml (0.65 mM); maackiain = 160 μg/ml (0.5 mM).

However, phytoalexin tolerance apparently requires more than just the ability to detoxify phytoalexins, since *N. haematococca* MP I isolate T-145 which is able to detoxify medicarpin and maackiain is still sensitive to these compounds [16].

The hydroxylations of medicarpin and maackiain by the three isolates of *N. haematococca* MP VI occurred on a cluster of three adjacent carbon atoms: C-1a, C-11a and C-6a. It could be that all three reactions are catalysed by closely related enzymes, with the observed selectivity being the result of minor differences in alignment during formation of the enzyme substrate complexes. Mono-oxygenase enzymes, which can cause the direct insertion of oxygen into C–H bonds [31], could catalyse all of the observed reactions. Conversion of simple phenols to dienone derivatives is a well known mono-oxygenase catalysed reaction [31] and recent work has shown that it is a mono-oxygenase enzyme in *F. solani* f. sp. *phaseoli* isolate T-232 that catalyses 1a-hydroxylation of phaseollin [32]. The 1a-hydroxylation of medicarpin and maackiain by *N. haematococca* MP VI isolates is probably similarly catalysed by a mono-oxygenase, albeit one with an apparently different substrate specificity.

There is no experimental evidence that mono-oxygenases are active in the other modifications of medicarpin and maackiain. However, demethylation of aromatic ethers is normally catalysed by mono-oxygenases [31], and an aromatic ether bond is cleaved during the transformation of medicarpin and maackiain to their isoflavanones. This reaction might occur via an oxygenation at C-11a to form an unstable hemiketal intermediate analogous to the hemiacetal generated during demethylation of aromatic ethers. Hydroxylation at C-6a could also be catalysed directly by a mono-oxygenase, although oxidation to an alkene and subsequent hydration is another possible pathway. An oxidation–hydration process seems unlikely since no alkenes were isolated even though the most probable oxidation products of medicarpin and maackiain, the conjugated 6a,11a-ene derivatives, are easily detected at low concentrations.

One degradative reaction of *N. haematococca* MP VI isolates that is known to be catalysed by a mono-oxygenase type enzyme is the demethylation of pisatin [33]. The similarity of isoflavanone production to the demethylation of aromatic ethers was noted above. However, even though *N. haematococca* MP VI isolates T-213 and 126–80 degraded medicarpin and maackiain, both are incapable of demethylating pisatin [4]. These results would seem to rule out the possibility that the hydroxylations of medicarpin and maackiain, and the demethylation of pisatin are catalysed by the same mono-oxygenase enzyme system(s).

The observation that *N. haematococca* MP I isolate T-145 and *F. solani* f. sp. *phaseoli* isolate T-232 could degrade medicarpin and maackiain illustrates that the ability to degrade pterocarpan phytoalexins is not limited to pathogens of plants that produce such phytoalexins. Similar stitutions have been reported previously [1, 28, 30]. Nevertheless, degradation of medicarpin and maackiain may still be required by *N. haematococca* MP VI for pathogenicity of chickpea. A survey of *N. haematococca* isolates has found no additional maackiain metabolites, but has detected some isolates that apparently cannot degrade maackiain (Lucy, M. C. and VanEtten, H. D., unpublished results). The combination of this information with the ability to manipulate *N. haematococca* MP VI genetically offers an excellent opportunity to evaluate whether the degradative enzymes are important for pathogenicity on chickpea, as well as resolving the number of enzymes that are involved in producing the derivatives of medicarpin and maackiain.

EXPERIMENTAL

Biological. Fungal isolates were maintained on V-8 slants as described elsewhere [16]. The isolates used were: *N. haematococca* Berk. and Br. mating population (MP) VI field isolates T-30 (ATCC 42973) and T-213 and the ascospore isolate 126-80 [4]; *F. solani* f. sp. *phaseoli* isolate T-232 [32]; and *F. solani* f. sp. *cucurbitae* isolate T-145 [16]. *Nectria haematococca* MP VI isolates T-213 and 126-80 are not pathogenic on pea [4], and thus cannot be referred to as *F. solani* f. sp. *pisi.* Published procedures were used to assess the antifungal activities of the phytoalexins [34].

Phytoalexins. The phytoalexins were isolated from plant sources and purified as described elsewhere [8, 16, 35].

Phytoalexin metabolism. The procedure for metabolism is described in detail elsewhere [16]. Briefly, the method was as follows. Spores washed from 1–2 week old V-8 slants were resuspended in glucose–asparagine (GA) liquid medium and incubated for *ca* 24 hr at 27°. After collection on a cloth filter (pore size *ca* 50 μm) the mycelium was washed with 50 mM $NaPO_4$ buffer, resuspended in either GA medium or in buffer and treated with phytoalexins dissolved in EtOH (2% final EtOH concn). After another 24 hr incubation metabolites were recovered by extracting the culture with $CHCl_3$ (2–3 × 2 vol.) and taking the organic phase to TLC (Si gel, Analtech GF) in chambers fully satd with one of the following solvent systems: (A) toluene–EtOAc (1:1); (B) $CHCl_3$–MeOH (10:1); (C) $CHCl_3$–MeOH (25:1). Medicarpin and maackiain have identical mobilities in each system and these were: R_f 0.65, 0.65 and 0.50 in solvents A, B and C respectively.

Spectroscopy. IR spectra were made on 10–25 mM solns in $CHCl_3$ using matched 0.5 mm light path salt cells. Electron impact mass spectrometry used a heated direct inlet probe (70 eV) whereas chemical ionization mass spectrometry used CH_4 carrier gas (70 eV). All ^1H NMR spectra were obtained from $CDCl_3$ solns at 300 MHz using Fourier transformation. Peaks were assigned relative to the δ 7.24 signal of the 1% residual $CHCl_3$.

7,2'-Dihydroxy-4'-methoxyisoflavanone (**5a**). TLC: R_f 0.50, 0.31, 0.20 (solvents A, B, C respectively); diazotized *p*-nitoaniline, yellow–orange; Gibbs reagent, blue. UV λ_{max} (EtOH) nm: 228 sh, 278, 312; $\lambda_{max}^{EtOH+NaOH}$ nm: 300 sh, 336 (acid reversible), UV $\lambda_{max}^{EtOH+HCl}$ no change. CIMS *m/z* (rel. int.): 287 $[M+1]^+$ (100); EIMS *m/z* (rel. int.): 286 $[M]^+$ (30), 150 (100), 137 (73), 121 (12), 107 (10). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580 (OH), 3250 *br* (OH), 1655 w (conj. C=O), 1608 and 1583 (aromatic ring).

7,2' - Dihydroxy - 4',5' - methylenedioxyisoflavanone (**5b**). TLC: R_f same as 7,2'-dihydroxy-4'-methoxyisoflavanone; diazotized *p*-nitroaniline, pale yellow; Gibbs reagent, no reaction. UV λ_{max}^{EtOH} nm: 232, 278, 307; UV $\lambda_{max}^{EtOH+NaOH}$ nm: 247, 336 (acid reversible); UV $\lambda_{max}^{EtOH+HCl}$ no change. EIMS *m/z* (rel. int.): 300 $[M]^+$ (22), 190 (14), 165 (9), 164 (80), 163 (32), 162 (11), 137 (100), 133 (24). IR like 7,2'-dihydroxy-4'-methoxyisoflavanone. 3 - Hydroxy - 8,9 - methylenedioxypterocarp - 6a - ene was produced by refluxing in acidic MeOH [22] (UV λ_{max}^{EtOH} nm: 339, 357; it co-chromatographed with a standard prepared by dehydrating demethylpisatin [8]).

1a - *Hydroxy* - 9 - *methoxy* - *pterocarp* - 1(2),4 - *diene* - 3 - *one* (**6a**). TLC: R_f 0.27, 0.49, 0.26 (solvents A, B, C respectively); diazotized *p*-nitroaniline, pale tan; Gibbs reagent, no reaction. UV λ_{max}^{EtOH} nm (*ca* log ϵ): 229 (4.10), 287 (3.83), 290 sh (3.83), 304 (3.71); UV $\lambda_{max}^{EtOH+NaOH}$ nm: 286 (immediate decomposition); UV $\lambda_{max}^{EtOH+HCl}$ nm: 284 (decomposition during 1 hr at room temp.). CIMS *m/z* (rel. int.): 287 $[M+1]^+$ (61), 271 $[M-Me]^+$ (24), 163 (18), 162 (21), 161 (100); EIMS *m/z* (rel. int.): 286 $[M]^+$ (27), 204 (18), 175 (13), 162 (54), 161 (100), 149 (10), 148 (17), 147 (12), 133 (18). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580 (OH), 3260 *br* (OH), 1665 *s* (conj. C=O), 1627 and 1602 (aromatic ring). ^1H NMR almost first order: δ 7.05 (1H, *dd*, J = 8.09, 0.74 Hz, C-7); 6.68 (1H, *d*, J = 9.56 Hz, C-1); 6.46 (1H, *dd*, J = 8.09, 2.21 Hz, C-8); 6.27 (1H, *d*, J = 2.21 Hz, C-10); 6.15 (1H, *dd*, J = 9.56, 1.84 Hz, C-2); 5.51 (1H, *d*, J = 1.84 Hz, C-4); 5.14 (1H, *d*, J = 10.295 Hz, C-11a); 5.095 (1H, *dd*, $J \sim$ 10.7, 3.3 Hz, C-6eq); 4.34 (1H, *dd*, $J \sim$ 10.1, 0.7 Hz, C-6ax); 3.94 (1H, *br dd*, $J \sim$ 10.1, 3.1 Hz, C-6a); 3.71 (3H, *s*, Me). Reduction: a portion (300 μg) was treated with 0.5 ml HOAc buffered with 5% w/v NaOAc, and 1 mg Zn powder. The mixture was stirred 10 hr at *ca* 18° and then adjusted to pH 5.5 with 10% w/v Na_2CO_3 after removing the Zn powder. The soln was extracted with CH_2Cl_2 (2 × 10 vols) and the evaporated extracts purified by TLC (solvent A). Medicarpin was recovered in greater that 70% yield. Conditions suitable for 1a-hydroxyphaseollone reduction (non-buffered HOAc) gave medicarpin in less than 20% yield; at normal room temp. no medicarpin was recovered and the starting material was destroyed.

1a - *Hydroxy* - 8,9 - *methylenedioxy* - *pterocarp* - 1(2),4 - *diene* - 3 - *one* (**6b**). TLC: R_f and colour reactions same as 1a - hydroxy - 9 - methoxy - pterocarp - 1(2),4 - diene - 3 - one. UV λ_{max}^{EtOH} nm (*ca* log ϵ): 236 (4.04), 309 (4.00); UV $\lambda_{max}^{EtOH+NaOH}$ nm: 305 (immediate decomposition); UV $\lambda_{max}^{EtOH+HCl}$ nm: 300 (decomposition during 1 hr at room temp.) EIMS *m/z* (rel. int.): 300 $[M]^+$ (32), 176 (64), 175 (100), 163 (25), 162 (58), 161 (25), 151 (11), 138 (29), 133 (12). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580 (OH), 3260 *br* (OH), 1669 *s* (conj. C=O), 1610 (aromatic ring). ^1H NMR almost first order: δ 6.66 (1H, *d*, J = 9.93 Hz, C-1), 6.61 (1H, *s*, C-7), 6.23 (1H, *s*, C-10), 6.155 (1H, *dd*, J = 9.93, 1.84 Hz, C-2), 5.88 (2H, pair of *d*, J = 1.10 and 1.47 Hz, OCH_2O), 5.54 (1H, *d*, J = 1.84 Hz, C-4), 5.125 (1H, *d*, J = 9.93 Hz, C-11a), 5.06 (1H, *dd*, J = 11.03, 3.31 Hz, C-6eq), 4.295 (1H, *d*, J = 11.03 Hz, C-6ax), 3.92 (1H, *br dd*, $J \sim$ 10.3, 3.3 Hz, C-6a). ^1H NMR spin–spin decoupled (irradiate δ 3.92 = C-6a): δ and J for C-1, C-2, C-4, C-7, C-10 and OCH_2O unchanged; δ 5.13 (1H, *s*, C-11a), 5.065 (1H, *dd*, J = 11.03, 1.47 Hz, C-6eq); 4.30 (1H, *d*, J = 11.03 Hz, C-6ax).

6a-Hydroxymaackiain (**4b**). TLC: R_f same as the isoflavanones (**5a**, **5b**); diazotized *p*-nitroaniline, yellow. UV λ_{max}^{EtOH} nm: 281 sh, 287, 309; UV $\lambda_{max}^{EtOH+HCl}$ nm: 290 sh, 340,

357 (after 1.5 hr at room temp.). ^{1}H NMR: δ 7.33 (1H, *d*, J = 8.46 Hz, C-1); 6.78 (1H, *s*, C-7); 6.55 (1H, *dd*, J = 8.46, 2.57 Hz, C-2); 6.39 (1H, *d*, J = 2.57 Hz, C-4); 6.38 (1H, *s*, C-10); 5.91 (2H, pair of *d*, J = 1.47 Hz, OCH_2O); 5.25 (1H, *s*, C-11a); 4.16 (1H, *dd*, J = 11.40, 0.74 Hz, C-6eq); 3.98 (1H, *d*, J = 11.40 Hz, C-6ax).

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