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THE ANTIFUNGAL ACTIVITY OF PTEROCARPANS TOWARDS *MONILINIA FRUCTICOLA*

DAWN R. PERRIN and I. A. M. CRUICKSHANK

Division of Plant Industry, C.S.I.R.O., Canberra, Australia

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Abstract—The antifungal activity towards *Monilinia fructicola* of some naturally occurring pterocarpans and a number of related compounds obtained by substitution, degradation and partial synthesis has been examined. Compounds that were effective as antifungal agents had ED_{50} values around 2×10^{-5} M. A structure-activity relationship is proposed.

INTRODUCTION

PTEROCARPANS are naturally occurring oxygen heterocyclics having the ring skeleton (I).[1] The first two members of this series, pterocarpin and homopterocarpin,[2] were characterized in 1940. Since 1961 a further twelve pterocarpans have been described.[3–10] With the exceptions of pisatin from *Pisum sativum* L.[4] and phaseollin from *Phaseolus vulgaris* L.[5] which develop following fungal infection, all the pterocarpans have been extracted from apparently healthy tissue removed from plants of the Papilionatae or Caesalpinioideae subfamilies of the family Leguminosae. Pisatin and phaseollin are known to be inhibitory to a wide range of phytopathogenic fungi.[11, 12] Antifungal activity has also been claimed for trifolirhizin from *Trifolium pratense* L.[8] Each of these three pterocarpans is considered to play a major role in the natural disease resistance of the plant from which it has been extracted. As pterocarpans have been isolated from economically important plants, ranging from agricultural crops to tropical timber trees, it seemed worthwhile to investigate other pterocarpans and related compounds for possible antifungal activity.

RESULTS AND DISCUSSION

Table 1 lists the compounds tested. Ten of the known pterocarpans are included together with a number of related compounds obtained by substitution, degradation or partial

[1] This nomenclature and numbering is based on the recommendation of the Editor, Chemical Society. *Chem. Comm.* **309** (1965). (Formerly known as coumaranochroman with numbering system as isoflavanoid derivative.)
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[11] I. A. M. CRUICKSHANK, *Australian J. Biol. Sci.* **15**, 147 (1962).
[12] I. A. M. CRUICKSHANK and D. R. PERRIN, unpublished results.

TABLE 1. GROWTH INHIBITION OF *Monilinia fructicola in vitro*

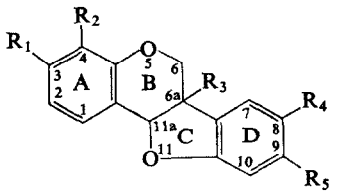
(Incubation period 96 hr, 20°)

Compound number	Compound	Structure		Growth inhibition	Source
Naturally occurring pterocarpans					
1.	(+)Maackiain	I,	$R_1=OH$, $R_2=R_3=H$, $R_4R_5=OCH_2O$	+++	*Sophora japonica* L.
2.	(±)Maackiain			+++	*S. japonica* L.
3.	(−)Maackiain			+++	*Trifolium pratense* L.
4.	(−)Maackiain			+++	*Sophora tomentosa* L.
5.	(−)Maackiain			+++	*S. tetraptera* J. Mill
6.	(−)Maackiain			+++	*S. subprostrata* Chun *et* T. C. Chen
7.	(−)Phaseollin	V		+++	*Phaseolus vulgaris* L.
8.	(+)Pisatin	I,	$R_1=OCH_3$, $R_2=H$, $R_3=OH$, $R_4R_5=OCH_2O$	++	*Pisum sativum* L.
9.	(−)Homopterocarpin	I,	$R_1=R_5=OCH_3$, $R_2=R_3=R_4=H$	+++	*Pterocarpus* sp.
10.	(−)Edulin[6]	IV		−	*Neoaurautanenia edulis* C. A. Sm.
11.	(+)Pterocarpin[9]	I,	$R_1=OCH_3$, $R_2=R_3=H$, $R_4R_5=OCH_2O$	±	*Sophora japonica* L.
12.	(±)Pterocarpin[9]			±	*S japonica* L.
13.	(−)Pterocarpin			−	*Pterocarpus* sp.
14.	Trifolirhizin[8]	I,	$R_1=O.C_6H_{11}O_5$, $R_2=R_3=H$, $R_4R_5=OCH_2O$	±	*Trifolium pratense* L.
15.	Trifolirhizin[9]			±	*Sophora subprostrata* Chun *et* T. C. Chen
16.	Sophojaponicin[9]	I,	$R_1=O.C_6H_{11}O_5$, $R_2=R_3=H$, $R_4R_5=OCH_2O$	−	*S. japonica* L.
Coumestans					
17.	Coumestrol	II,	$R_1=R_4=OH$, $R_2=R_3=H$	−	*Trifolium repens* L. cv. ladino
18.	Trifoliol	II,	$R_1=R_2=OH$, $R_3=H$, $R_4=OCH_3$		*T. repens* L. cv. ladino
19.	Medicagol	II,	$R_1=OH$, $R_2=H$, $R_3R_4=OCH_2O$	−	*Medicago sativa* L.
20.	4′-*O*-methyl coumestrol	II,	$R_1=OH$, $R_2=R_3=H$, $R_4=OCH_3$	−	
Acetates of *Swartzia* compounds					
21.	Acetoxymethoxypterocarpan	I,	$R_1=OCOCH_3$, $R_2=R_3=R_4=H$, $R_5=OCH_3$	++	*Swartzia madagascariensis* Desv.
22.	Acetoxymethylenedioxypterocarpan	I,	$R_1=OCOCH_3$, $R_2=R_3=H$, $R_4R_5=OCH_2O$	+	*S. madagascariensis* Desv.
23.	Acetoxydimethoxypterocarpan	I,	$R_1=OCOCH_3$, $R_3=R_4=H$, $R_2=_5R=OCH_3$	++	*S. madagascariensis* Desv.
Brazilin and related compounds					
24.	Brazilin	III,	R=H	−	"Soluble red woods" from *Caesalpinia* spp.

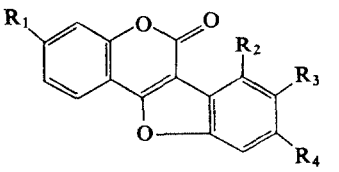
25.	Haematoxylin	III,	R=OH	−
26.	Tetramethylhaematoxylin			−
27.	Anhydrohaematoxylin			−
Compounds from substitution, degradation or chemical synthesis				
28.	(±)Pisatin[12]	I,	$R_1=OCH_3$, $R_2=H$, $R_3=OH$, $R_4R_5=OCH_2O$	++
29.	(±)Homopisatin[12]	I,	$R_1=R_5=OCH_3$, $R_2=R_4=H$, $R_3=OH$	+++
30.	(±)Dihydromaackiain(dihydroxy-methylenedioxyisoflavan)	VI,	$R_1=R_3=OH$, $R_2=R_4=H$	−
31.	(±)Maackiain acetate[9]	I,	$R_1=OCOCH_3$, $R_2=R_3=H$, $R_4R_5=OCH_2O$	++
32.	(±)Dihydromaackiain acetate[9]	VI,	$R_1=R_3=OCOCH_3$, $R_2=R_4=H$	−
33.	Dimethoxyisoflavan[12]	VI,	$R_1=R_3=OCH_3$, $R_2=R_4=H$	±
34.	Dihydroxydimethoxyisoflavan[12]	VI,	$R_1=R_3=OCH_3$, $R_2=R_4=OH$	±
35.	Sophojaponicin tetraacetate[9]			−
36.	Trifolirhizin tetraacetate[9]			−
37.	Anhydropisatin[4]			−
38.	Acetoxydimethoxyisoflaven[12]	VII		++
39.	Phenolic ether from pisatin[4]	VIII		++

Key: +++ represents ED_{50} less than 2×10^{-5} M; ++, ED_{50} 2 to 5×10^{-5} M; +, ED_{50} 5 to 10×10^{-5} M; ±, slight inhibitory effect at concentrations above 10^{-4} M; −, no inhibitory effect detected at concentrations up to 10^{-3} M.

synthesis. In three instances the naturally occurring phenol was unstable during purification, and it was necessary to use the corresponding acetate. As far as possible an attempt has been made to list the compounds in such a manner as to show the effect on antifungal activity of



(I)



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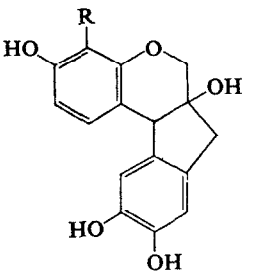

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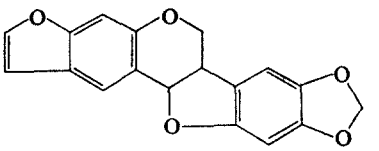

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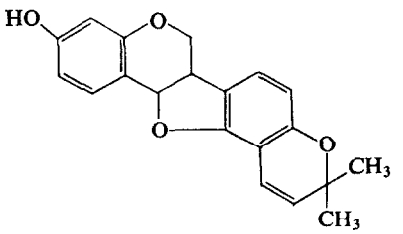

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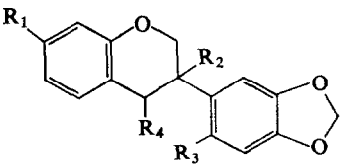

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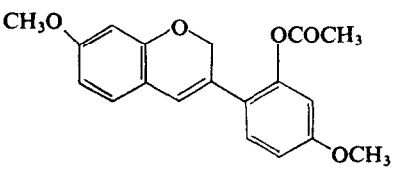

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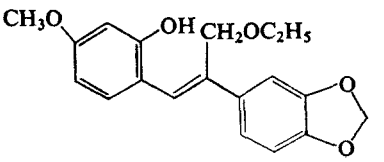

(VIII)

progressive changes in the substitution pattern. Several coumestans(II) have been included, as well as representatives of the brazilins(III).[13] Dehydrogenation across the 6a,11a bond

[13] R. ROBINSON, "Brazilin and haematoxylin", in *Chemistry of the Carbon Compounds* (edited by F. H. RODD), Ch. 9, Vol. IVB, Elsevier, Amsterdam (1959).

of pterocarpan gives rise to pterocarpen (the only available member of this class was anhydropisatin). Further replacement of the two hydrogens on position 6 by oxygen leads to the coumestans. The brazilins have many features in common with the pterocarpans, namely rings A and B as in the latter with a similar substitution pattern, and also the angular hydroxyl group as in pisatin and homopisatin.[14] However, the dihydrofuran ring C is replaced by a carbocyclic ring and the second aromatic ring D is displaced to a neighbouring position. All antifungal activity has been measured towards *Monilinia fructicola* (Wint.) Honey and is hereafter referred to as "antifungal activity".

The most active compounds tested were maackiain (syn. inermin, demethylpterocarpi n trifolirhizin aglycone) (1–6), phascollin (7), homopterocarpin (12) and homopisatin (29) In this discussion the numbers in parantheses refer to the compound numbers from Table 1. Maackiain was available in *dextro-*, *laevo-* and racemic forms isolated from three species of the genus *Sophora* and one species of the genus *Trifolium.* Acetylation of the hydroxyl group of maackiain produced a slight reduction in antifungal activity (cf. 1–6, 31). The presence of a sugar residue on the same position as in trifolirhizin and sophojaponicin markedly diminished the antifungal activity (cf. 1–6, 14–16).

The addition of a furano-group to ring A as in edulin (10)[6] resulted in complete loss of antifungal activity. All known pterocarpans have oxygen-containing groups on both carbons 3 and 9. Of those tested, the greatest antifungal activity was recorded for those pterocarpans with hydroxyl or methoxyl groups on carbon 3. The only exception is that of pterocarpin (11–13) for which testing was extremely difficult on account of inadequate solubility of the test compounds. The conversion of homopterocarpin to homopisatin by the introduction of the non-phenolic hydroxyl at the junction of the benzpyran-dihydrobenzfuran rings (position 6a), caused no change in antifungal activity (cf. 9, 29).

Changes in substitution such as methoxy-(9), methylenedioxy-(1–6, 8) and 2-methyl but-3-ene-2yl oxy-(7) on ring D had little effect on antifungal activity, but in each of these compounds the ether linkage at position 9 remains intact.

Opening of the dihydrofuran ring as in the catalytic hydrogenation of maackiain to give dihydroxymethylenedioxyisoflavan[9] resulted in loss of antifungal activity. A similar loss was observed for the corresponding acetates (compare 1–6, 31; 30, 32). Two dimethyl derivatives (33, 34) of the isoflavan showed only slight antifungal activity.

No antifungal activity was recorded for anhydropisatin (35) or for any of the coumestans (17–20), although the ring A and D substituents of the latter group are identical with those in some of the antifungal pterocarpans. Unlike anhydropisatin (37) from which they are derived, the isoflaven VII (38) and phenolic ether VIII (39) showed moderate antifungal activity.

Although general antibiotic properties[15] have been ascribed to brazilin and haematoxylin and their derivatives (24–27), they showed no antifungal activity towards *M. fructicola.*

An examination of molecular models of the pterocarpan and pterocarpen or coumestan ring-skeletons suggested that the differences in biological activity might be steric in origin. Recent stereochemical studies on several members of the pterocarpan class indicate that the B/C ring junction is likely to be the unstrained *cis* form in all known pterocarpans.[16, 17] From molecular models (Dreiding, Courtauld and Catalin) it appears that the two aromatic rings

[14] C. W. L. BEVAN, A. J. BIRCH, B. MOORE and S. K. MUKERJEE, *J. Chem. Soc.* 5991 (1964).
[15] A. SANCHEZ-MARROQUIN, L. GARCIA and M. MENDEZ, *Rev. Latinam. Microbiol.* **1**, 225 (1958), cited in *Chem. Abstr.* **54**, 24982f (1960).
[16] S. ITO, Y. FUJISE and A. MORI, *Chem. Comm.* 595 (1965).
[17] H. SUGINOME, *Bull. Chem. Soc. Japan* **39**, 1544 (1966).

are almost perpendicular to each other for all pterocarpans. However, for anhydropisatin and the coumestans, dehydration or dehydrogenation has completely altered the shape of the molecule, and atomic models strongly suggest that the pterocarpens and coumestans are planar (Fig. 1).

Consideration of the above results has led us to suggest that the antifungal activity of the pterocarpans may be associated with two factors, namely (*a*) the aromatic rings A and D do not lie in the same plane; (*b*) small oxygen-containing substituents are present on the periphery of the molecule, especially at positions 3 and 9. To illustrate (*a*) and (*b*) the naturally occurring compounds, maackiain, phaseollin, pisatin and homopterocarpin and several of the corresponding acetates, as well as the synthetic compounds (±)pisatin and (±)homopisatin show moderate to high antifungal activity (1–6, 7, 8, 9, 21–23, 31) whereas edulin, anhydropisatin and the coumestans (10, 17–20, 37) were inactive. Slight activity only was found in the glycosides (14–16).

In the above antifungal compounds, substituent groups at position 3 include hydroxyl, methoxyl and acetoxyl and in each case the substituent is free to rotate about the ether linkage. In edulin (10) the aromatic character of the furan ring would greatly diminish any bonding by the furan oxygen to the receptor surface. For the glycosides it is not possible to decide whether the reduced antifungal activity is simply a consequence of the bulkiness of the sugar moiety, or whether the marked hydrophilic properties of the compound are responsible. Substitution changes on ring D have less effect. Activity is observed not only in systems where the ether oxygen is free to rotate as in homopterocarpin, but also in molecules such as maackiain and phaseollin where the oxygen at position 9 is held rigidly in a ring system, planar with the dihydrobenzfuran portion of the molecule (cf. 1–6, 7, 9).

The two factors given above which appear to be necessary for antifungal activity possibly reflect the needs of a biological receptor surface. The absence of any fungus-inhibiting activity in the planar molecules is consistent with this suggestion. The apparently anomalous activities of the two compounds VII and VIII obtained by hydrogenolytic fission of the rings in anhydropisatin would be explained if ring D was not in the same plane as rings A and B. Some rotation of the single bond joining the two ring systems is to be expected, particularly in view of the bulkiness of the acetoxyl and ethoxymethyl groups respectively. U.v. absorption spectra are consistent with loss of planarity. A hypsochromic shift of the long wavelength band, and a diminution of absorption intensity, are found when the anhydropisatin molecule undergoes fission of either the furan or the pyran ring to yield the compounds VII and VIII (Table 2). A comparable shift is observed when *trans*-stilbene (which is planar) is methylated in positions which cause the molecule to become nonplanar.[18]

TABLE 2. ABSORPTION MAXIMA (LONG WAVELENGTH BAND)

Compound	λ_{max}, nm in EtOH	log ϵ
Anhydropisatin	339, 358	4·58, 4·60
VII	319	4 39
VIII	321	4 03
trans-Stilbene	295, 310	4·49, 4 48
2,4,6-Trimethylstilbene	280	4·28

[18] R. N. BEALE and E. M. F. ROE, *J. Am. Chem. Soc.* **74**, 2302 (1952)

Isoflavanoid compounds with a 4-phenyl substitution and ring C carbocyclic as in the brazilin series, also have a very different shape from that of the pterocarpans.

In this study we were fortunate in having available three pterocarpans present in enantiomorphic forms. It is common for one isomer of an enantiomorphic pair to possess all or almost all of the biological activity. From Table 1, however, both forms of maackiain and pisatin have comparable antifungal activity. Dreiding atomic models show that rings A, C

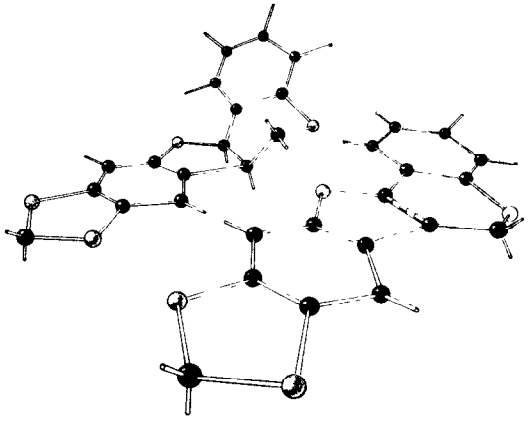

FIG. 1. MOLECULAR MODELS (BASED ON DREIDING STEREOMODELS) ILLUSTRATING DIFFERENCES IN CONFORMATION BETWEEN METHYLENEDIOXYPTEROCARPAN (UPPER) AND METHYLENEDIOXYPTEROCARPEN (LOWER).

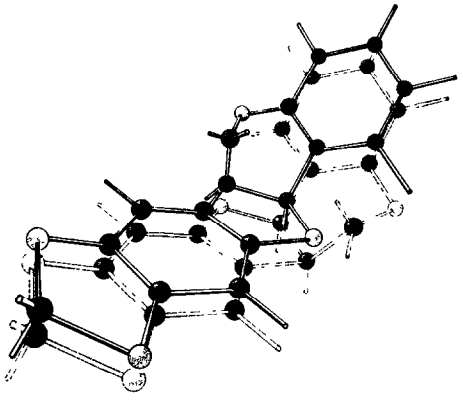

FIG. 2. MOLECULAR MODELS (BASED ON DREIDING STEREOMODELS) SHOWING THAT (+)METHYLENEDIOXYPTEROCARPAN IS SUPERIMPOSABLE ON (−)METHYLENEDIOXYPTEROCARPAN.

and D of the pairs of enantiomorphs are superimposable, but rings B lie in different positions relative to the plane of ring A (Fig. 2). This suggests that the active site has sufficient width to accommodate the two possibilities and that bonding to the receptor through the pyran and dihydrofuran rings is not important.

Although this study has been limited to the activity of the test compounds towards mycelial growth of *M. fructicola*, the results could well indicate a simple structure-activity relationship, based primarily on the stereochemical relationships within the pterocarpan molecule, the main feature being the partially staggered benzpyran ring system is steeply

angled to the planar benzfuran portion. Such a molecule could slip into a curved bioreceptor site, where ether linkages, especially at positions 3 and 9, could be essential for bonding of the chemical compound to the fungal receptor.

It is intended to extend the present studies to other natural compounds and synthetic molecules which would be expected to have similar stereochemical relationships as the pterocarpans.

EXPERIMENTAL

A study was made of solvents suitable for use as carriers to improve solubility of the compounds under test without appreciably affecting mycelial growth. The following alternatives were acceptable (the values denoting maximum concentrations are given in parentheses)—acetone (4 per cent), $CHCl_3$ (1 per cent), dimethylformamide (0·5 per cent), dimethysulphoxide (1 per cent) and ethanol (2–3 per cent).

With the exception of pterocarpin (11–12), satisfactory solubilization was achieved for all the compounds tested. For the pterocarpin isomers, the assay was done in 1 per cent $CHCl_3$, but at this concentration in agar, some precipitation of pterocarpin occurred.

Antifungal activity was assessed by the radial growth assay procedure already described.[11] Compounds in the appropriate solvent were aseptically added to a glucose, phosphate, yeast extract agar medium (pH 5·5). Agar plates (5 cm dia.) were inoculated centrally with mycelial disks of *Monilinia fructicola* and incubated at 20°. Radial growth of fungal colonies was measured after 96 hr. Assays were carried out in triplicate over a five-point dilution series. Each compound showing antifungal activity was tested in at least two separate tests. Growth of *M. fructicola* (expressed as a percentage of control) was plotted against concentration of the test compound and the median effective dose (ED_{50}) was read off the resulting dosage–response curve.

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