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CONSTITUENTS OF THE STEM BARK FROM *BUTEA MONOSPERMA* (LEGUMINOSAE)

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Abstract : The anti–fungal compound isolated from the petroleum and ethyl acetate extracts of the stem bark from *Butea monosperma* was identified as (–)–3–hydroxy–9–methoxypterocarpan [(–)–medicarpin]. Both (–)–medicarpin and its acetate were active against *Cladosporium cladosporioides.* The petroleum extract also yielded lupenone, lupeol and sitosterol. Two isoflavones isolated from the ethyl acetate extract were found to be 5–methoxygenistein and prunetin.

1. Introduction

Butea monosperma (Lam.) Taub. (= *B. frondosa* Koenig ex Roxb.), Leguminosae, is a tree of moderate size found in Burma, India, Java and Sri Lanka. The stem and root barks, the gum exuding from the bark, and the flowers and seeds of the tree find medicinal use in Sri Lanka.[6] Previous chemical studies on *B. monosperma* include the isolation of proanthocyanidins from the bark and gum.[1,3] Phytochemical and pharmacological studies have been carried out on the seeds and flowers of this tree.[7,14] We now report the isolation and identification of a pterocarpan, two isoflavonoids, a steroid and two triterpenes from the stem bark.

2. Results and Discussion

The petroleum extract of the stem bark from *B. monosperma* was fractionated using medium pressure liquid chromatography (MPLC). A fraction which eluted with 40% MeOH in CH_2Cl_2 showed fungicidal activity.[1] The fungicidal compound 1 was isolated and purified as the acetate 2 after repeated MPLC and fractional precipitation of inactive material. A pure sample of 1 was obtained by mild alkaline hydrolysis of 2. High resolution mass data of 1 and 2 analysed for $C_{16}H_{14}O_4$ and $C_{18}H_{16}O_5$ respectively, indicating the presence of one OH group in 1 that can be acetylated with Ac_2O in pyridine. The UV spectral data of 1 were consistent with a pterocarpan skeleton.[10] The ^1H and ^{13}C–NMR spectral data of 1 and 2 (Tables 1 and 2) suggested that the aromatic rings of the pterocarpan nucleus contain only two substituents. The singlet at δ 3.76 ppm in the ^1H–NMR spectra of 1 and 2 was assignable to a methoxy group revealing the two substituents of 1 as OMe and OH groups respectively. Comparison with m.p., IR and ^1H–NMR data reported [4,11] established compounds 1 and 2 to be (–)–3–hydroxy–9–methoxypterocarpan [(–)– medicarpin] and (–)–3–acetoxy–9–methoxypterocarpan respectively. Analysis of the homonuclear ^1H–^1H

2D COSY spectrum of 2 and inspection of Dreiding models supported the previous conclusions regarding the steric structure of pterocarpan derivatives.[9]

Table 1 : ^{1}H NMR[a] of (−)–Medicarpin (1) and (−)–Medicarpin acetate (2)

1	2	Assignment
	2.78	$OCOCH_3$
3.57 (2H)	3.60 (2H)	6a, 6 α
3.76 (3H)	3.76 (3H)	OCH_3
4.21 (1H)	4.24 (1H)	6 β
5.47 (1H)	5.52 (1H)	11a
6.39–6.50 (4H)	6.45 (2H)	8, 10
	6.72 (1H)	4
	6.80 (1H)	2
7.18 (1H)	7.12 (1H)	7
7.34	7.53 (1H)	1

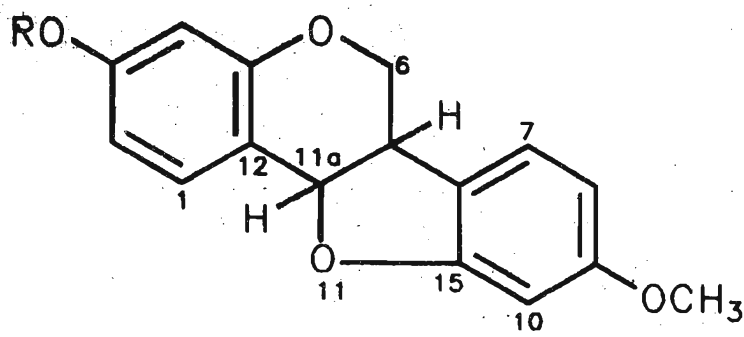
[a]Spectra run in $CDCl_3$ with TMS as internal standard.

Table 2 : ^{13}C NMR[a] of (−)–Medicarpin (1) and (−)–Medicarpin acetate (2)

Carbon atom	1	2	Multiplicity
1	132.22	131.78	(d)
2	106.54	110.73	(d)
3	157.08	151.67	(s)
4	109.86	115.31	(d)
6	66.57	66.52	(t)
6a	39.53	39.57	(d)
7	124.77	124.77	(d)
8	99.99	96.94	(d)
9	160.64[b]	160.59[b]	(s)
10	103.71	106.59	(d)
11	78.61	78.08	(d)
12	112.63	117.84[c]	(s)
13	156.64	156.25	(s)
14	119.16	118.77[c]	(s)
15	161.12[b]	161.22[b]	(s)
OCH_3	55.56	55.51	(q)
$COCH_3$	–	169.07	(s)
$COCH_3$	–	21.10	(q)

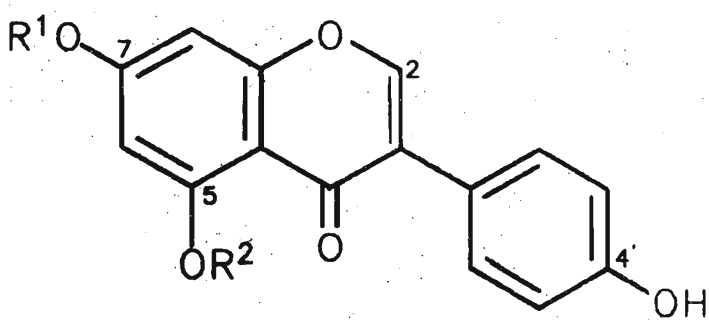
[a]Spectra run in $CDCl_3$ with TMS as internal standard ; Chemical shift values in δ scale ; 25.2 MHz.

[b,c]Assignment may be interchanged.



(1) (-)-Medicarpin, R=H

(2) (-)-Medicarpin acetate, R=$COCH_3$



(3) 5-Methoxygenistein, R^1=H, R^2=CH_3

(4) Prunetin, R^1=CH_3, R^2=H

Column chromatography of the petroleum extract gave three other compounds which were identified as lup–20(29)–en–3–one (lupenone), lup–20(29)–en–3β–ol (lupeol) and sitosterol by comparison with authentic samples.

The ethyl acetate extract of the stem bark was adsorbed on silica gel and extracted with CH_2Cl_2. Repeated column chromatography of the CH_2Cl_2 extract gave yellow crystalline compounds 3 and 4 in addition to more (–)medicarpin (1). Compounds 3 and 4 were found to be isoflavones having isomeric structures.

The molecular ion peak of 3 at *m/z* 284 was consistent with the formula $C_{16}H_{12}O_5$. The IR bands at 1675 and 1620 cm^{-1} together with the one proton singlet at δ 8.19 downfield from the signals of the aromatic protons at δ 6.53 (1H), 6.54 (1H), 6.92 (2H) and 7.42 (2H) indicated 3 to be an isoflavone with three substituents on the aromatic rings.[10] The mass fragments at *m/z* 166 and 118 due to *retro*–Diels–Alder cleavage, and the ^{1}H–NMR data suggested that 3 is 7,4'–dihydroxy–5–methoxy isoflavone (5–methoxygenistein).[3] Compound 4 also displayed the same molecular ion at *m/z* 284 and identical *retro*–Diels–Alder fragments at *m/z* 166 and 118 suggesting that 4 is the ring A isomer of 3. Accordingly, the acetate of 4 showed a D_2O exchangeable singlet at δ 12.76 due to the strongly chelated hydroxy group at C–5. Hence 4 was deduced to have the structure 5,4'–dihydroxy–7–methoxyisoflavone (prunetin).[2]

The six compounds isolated from the stem bark of *B. monosperma* and the acetate derivative of medicarpin were tested for antifungal activity against *Cladosporium cladosporioides* using the TLC–bioassay method.[8] Medicarpin (1) and its acetate (2) inhibited the growth of the fungus. The fungicidal activity of 1 was found to be greater than that of Benlate, a standard fungicide.[1] Lupenone, lupeol, sitosterol and the two isoflavones were inactive against *C. cladosporioides*.

3. Experimental

Melting points were determined on a Kofler hot–stage apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer Model 241 polarimeter at 25°. UV spectra were recorded on a Varian DMS90 spectrophotometer and IR spectra on a Shimadzu Model IR–408. ^{1}H–NMR (400 MHz) and ^{13}C–NMR spectra were recorded at the Department of Organic Chemistry, University of Stockholm, using tetramethylsilane as the internal standard. Mass spectra were recorded at the Research School of Chemistry, The Australian National University. For column chromatography Silica gel 60, 70–230 mesh and for preparative TLC (PTLC) Silica gel 60, $PF_{254+366}$, (both Merck), were used. Petrol refers to the fraction boiling at 60–80° and hexane to *n*–hexane.

Extraction and isolation of compounds. Dried, powdered stem bark (4.5 Kg) was extracted successively with petrol and EtOAc in a soxhlet. Evaporation of the petrol extract gave a brown solid (26.3 g), a part of which (15 g) was chromatographed on silica gel using mixtures of petrol, CH_2Cl_2 and MeOH. The fraction which eluted with petrol–CH_2Cl_2 (4:6) was separated by PTLC (petrol–CH_2Cl_2, 1:1) to give a white solid (158 mg) which was recrystallised from MeOH and identified as *lupenone,* m. p. 168–169°C, $[\alpha]_D$ +61.4° [lit. (15) m.p. 170°C, $[\alpha]_D$ + 63.5°]. Elution with CH_2Cl_2 gave a fraction which on purification by PTLC and recrystallisation from MeOH–CH_2Cl_2 gave *lupeol* (209 mg), m.p. 213–215°C, $[\alpha]_D$ + 24.6° [lit. (12) m.p. 206–208°C, $[\alpha]_D$ + 25.2°]. Elution with CH_2Cl_2–MeOH (8:2) gave a fraction from which *sitosterol*, m.p. 137–139°, [lit.(5) m.p. 136–137°C] separated. The mother liquor available after the separation of sitosterol and the fraction which eluted with CH_2Cl_2–MeOH (6:4) were combined and re–chromatographed on silica gel. Elution with petrol–EtOAc (19:1) gave a brown semi–solid which was acetylated and purified by PTLC (petrol–CH_2Cl_2, 4:6) as described below. The resulting acetate 2 was deacetylated using 5% KOH in MeOH. PTLC with MeOH–CH_2Cl_2 (1:49) and recrystallisation with CH_2Cl_2–hexane gave *(–)–medicarpin* 1 (74 mg), m.p. 127–129°C, $[\alpha]_D$–230° [lit. (4) m.p. 127.5–128.5°C, $[\alpha]_D$–234°]; UV $\lambda_{max.}$ (log. ϵ) : 214 (3.71), 230 (3.61), 280 (3.38), 286 (3.43) nm; IR $\nu_{max.}$: 3350, 1620, 1580, 1495, 1290 and 1210 cm^{-1} ; ^1H–NMR ($CDCl_3$) : δ 7.34 (1H, H–1), 7.18 (1H, H–7), 6.39–6.50 (4H, H–2, H–4, H–8, H–10), 5.47 (1H, H–11a), 4.21 (1H, H–6β), 3.76 (3H, OCH_3), 3.57 (2H, H–6*a*, H–6α) ; EI–MS *m/z* (rel. int. %) 270 (100, M^+), 269 (45), 268 (14), 267 (14), 256 (12), 255 (41), 253 (8), 161 (7) and 148 (13). (Found M^+ 270.0892 ; Calc. for $C_{16}H_{14}O_4$, 270.0892). A part of the EtOAc extract (37 g) was dissolved in hot EtOAc and adsorbed on silica gel (mesh 35–70) and re–extracted in a soxhlet with CH_2Cl_2. The CH_2Cl_2 extract (10 g) was chromatographed on silica gel. Elution with CH_2Cl_2–MeOH (7:3) gave a fraction which on condentration yielded a yellow precipitate. The filtrate gave (–)–medicarpin on purification, while the yellow precipitate on PTLC with $CHCl_3$ and recrystallisation from acetone–MeOH gave orange crystals (23 mg) of compound 3 m.p. 301–303°C decomp., which was identified as *5–methoxygenistein* [lit. (3) m.p. 305°C decomp.; IR $\nu_{max.}$ 3400, 1675 and 1620 cm^{-1} ; ^1H–NMR (d_6–acetone) : 3.92(3H, s, OCH_3), 6.53 (1H, d, J 2.0 Hz), 6.54 (1H, d, J 2.0 Hz), 6.92 (2H, d, J 8.5 Hz), 7.42 (2H, d, J 8.4 Hz), 8.19 (1H, s, C_2–H), 8.44 (1H, s, D_2O exchangeable) ; EI–MS *m/z* (rel. int %) 284 (100, M^+) 166 (40), 118 (16). Elution with CH_2Cl_2–MeOH (4:6) gave a fraction from which a yellow solid (12.3 mg) separated after silica gel chromatography. The yellow solid was identified as *prunetin*,4 m.p. 239–242°C, [lit. (2) m.p. 242°C] ; IR $\nu_{max.}$ 3400, 1660 and 1610 cm^{-1} ; ^1H–NMR of acetate (d_6–acetone), 2.30 (3H, s, $OCOCH_3$), 3.90 (3H, s, OCH_3), 6.40 (2H, s), 7.20 (2H, d, J 8.2 Hz), 7.57 (2H, d, J 8.8 Hz), 7.92 (1H, s, C_2–H), 12.76, s, C_5–OH) ; MS *m/z* (rel. int. %), 284 (100, $[M]^+$), 166 (22) and 118 (11).

Acetylation of 1. Compound 1 was acetylated with acetic anhydride in pyridine at room temperature for 1 h. The usual work up followed by PTLC separation gave a solid which recrystallised from CH_2Cl_2–hexane to yield *(–)–3–acetoxy–7–methoxypterocarpan* 2 as white crystals, m.p. 124–125°C, $[\alpha]_D$ –221° [lit.(11) m.p. 122–123°C, $[\alpha]_D$ –182°] ; (Found M^+ 312.0985, Calc. for $C_{18}H_{16}O_5$ 312.0998 ; UV λ_{max}. 207 (4.17), 225 (3.71), 280 (3.52) and 285 (3.54) nm ; IR ν_{max}. 1740, 1620, 1590, 1500, 1235, 1145, 950 and 895 cm^{-1} ; ^1H–NMR ($CDCl_3$) : δ 7.53 (1H, H–1), 7.12 (1H, H–7), 6.80 (1H, H–2), 6.72 (1H, H–4), 6.45 (2H, H–8, H–10), 5.52 (1H, H–11a), 4.24 (1H, H–6β), 3.76 (3H, OCH_3), 3.60 (2H, H–6a, H–6α), 2.78($OCOCH_3$) ; EI–MS *m/z* (rel. int. %) 312 (40) $[M]^+$, 271 (17), 270 (100), 269 (38), 255 (20) and 148 (9).

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