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SHORT COMMUNICATION

SOME CONSTITUENTS OF *PITAVIA PUNCTATA**

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Abstract—Extracts from the stems and leaves of *Pitavia punctata* Mol. were examined. The neutral fraction yielded β-sitosterol, daucosterin, quercetin, avicularin, and the previously undescribed quercetin 3-rhamnosylarabinoside. Braylin was co-extracted with the basic constituents, dictamnine, skimmianine and γ-fagarine. Acid hydrolysis of the leaves yielded cyanidin and delphinidin.

INTRODUCTION

IN CONTINUATION of our study of the chemistry of Chilean flora[1] an investigation of the species *Pitavia punctata* Mol. has been made. The plant belongs to the *Rutaceae* members of which often contain alkaloids of the quinoline, furoquinoline and quinazoline types.[2] Initially the alkaloid content of the plant was examined. This was followed by isolation of the major neutral constituents.

RESULTS

Extraction of the dried leaves and stems of *P. punctata* Mol., collected in March 1968 at Rocoto near Concepcion, with benzene was followed by isolation of the acid soluble portion. Paper chromatography indicated only two alkaloids which were isolated and identified, by comparison with authentic samples, as dictamnine[3] (0·004%), based on dry plant weight, and skimmianine[4] (0·0005%). A further alkaloid, γ-fagarine,[5] was isolated in small quantities from the acid soluble ethanolic extract. These alkaloids are all examples of furoquinolines, which have only been isolated from other members of the *Rutaceae*.[2b]

Co-occurring with the alkaloid γ-fagarine was a neutral compound which showed IR absorption at 1725 and 1580 cm^{-1} and UV absorption characteristic of a coumarin.[6] Its

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mass spectrum indicated a molecular formula of $C_{15}H_{14}O_4$ and its NMR spectrum revealed the presence of one methoxyl group and a cyclic isopentenyl moiety. The structure was tentatively assigned as braylin.[7] In order to rule out isomeric structures the effect of solvent shifts on its NMR spectrum were studied using $CDCl_3$ and benzene as solvent.[8] The effects are presented in the table and substantiate the assignment. The assignment was confirmed by direct comparison with an authentic sample.

The neutral portion of the benzene extract afforded, by column chromatography, a hydrocarbon fraction, the major component being nonacosane. This was followed by β-sitosterol and, finally, daucosterin, identified IR spectral comparison with an authentic sample, and by conversion into the acetate.

The neutral, polar constituents of the leaves were extracted with methanol followed by re-extraction with ethyl acetate. Column chromatography afforded three components identified as the flavonol quercetin, its 3-arabinoside, avicularin,[9] and a new glycoside of quercetin identified as the 3-rhamnosylarabinoside. The structure of the latter compound was confirmed on the basis of its spectral properties. That the sugar residues were attached at one position only was indicated by the NMR spectrum of the derived nonacetate which showed four aromatic acetoxy groups and five aliphatic acetoxy moieties. Characteristic UV maxima shifts were obtained on the addition of aluminium chloride, typical for a 3-substituted flavonol.[10]

Hydrochloric acid catalysed hydrolysis of fresh leaves (dried) followed by paper chromatography afforded two anthocyanidins. These were eluted from the paper with ethanol for spectroscopic measurements and identified, by direct comparison, as delphinidin and cyanidin.

EXPERIMENTAL

M.ps were taken on a Kofler block and are uncorrected. The microanalyses were determined by Dr. A. Bernhardt, Mulheim, Germany. UV spectra were recorded with a Unicam SP 800 spectrophotometer for ethanolic solutions and the IR spectra using a Perkin–Elmer Model 457B spectrometer. NMR spectra were determined on a Varian HA 100 spectrometer with deuteriochloroform as solvent and tetramethylsilane as internal reference. Mass spectra were obtained with an A.E.I. MS9 double focussing instrument. The alumina and silica used were the activated grades from Merck. Merck silica gel GF_{254} was used for TLC. Light petroleum refers to the fraction of boiling range 60–80°

Extraction of Alkaloids

The powdered dry leaves and stems of *P. punctata* (7 kg) was exhaustively extracted with benzene, the solvent removed and the residue macerated with cold 0·5 N HCl. The acid soluble portion was adjusted to pH 9 with K_2CO_3 before extraction with EtOAC. The residue (6 g) showed only two alkaloids by paper chromatography,[11] using Whatman No. 1 paper with *n*-BuOH–HOAc–H_2O (4:1:5) and spraying with Dragendorff reagent. The mixture was then separated by column chromatography through alumina (grade II). Elution with 2:1 light petroleum–benzene afforded dictamnine (300 mg), m.p. 131–132° (from light petroleum), (lit.[3] m.p. 132°), $[\alpha]_D^{20}$ 0·00° (*c* 1, $CHCl_3$), ν_{max}^{Nujol} 2825, 1587, 1504 cm^{-1}, λ_{max} 236, 310 and 330 nm (ε 10,500, 15,621 and 14,328), τ 5·68 (3Hs, methoxyl), 3·00 (1Hd, *J* 2·9 Hz), 2·4 (1 Hd, *J* 2·9 Hz), 2·35–1·9 (4Hm), m.s. peaks at *m/e* 199 (M^+), 184 (M^+-CH_3), 156 (M^+-CH_3CO). The material was identified by direct comparison with an authentic sample.

Further elution of the column with 2.1 benzene–light petroleum afforded another alkaloid, skimmianine (35 mg), m.p. 178–179° (lit.[4] m.p. 176°), ν_{max}^{KBr} 3000, 2840, 1618, 1582 and 1493 cm^{-1}, λ_{max} 249, 318 and 332 nm (ε 83,050, 8863, 8303), τ 6·0 (3Hs, methoxyl), 5·95 (3 Hs, methoxyl), 3·05, 2·50 (2H, AB doublets, *J* 2 7 Hz),

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2·80, 2·50 (2H, AB doublets, J 9·5 Hz), m.s. peaks at m/e 259 (M^+), 244 (M^+-15). The NMR was identical to that of an authentic specimen.

The benzene insoluble plant material was further extracted with EtOH, the solvent removed *in vacuo* and the residue extracted with 0·5 N HCl before adjusting the pH of the aqueous layer to pH 9 with K_2CO_3. Extraction of the alkaline solution with $CHCl_3$ and evaporation of solvent afforded a residue (2·5 g) which showed a small quantity of one alkaloid by paper chromatography. Separation by chromatography through alumina (grade II) gave, with 3:2 light petroleum–benzene, braylin (400 mg) as pale yellow needles, m.p. 150° (from light petroleum) (lit.[7] m.p. 150°), ν_{max}^{KBr} 2841, 1725 (CO), 1580, 1504 (conjugated C=C) cm^{-1}, λ_{max} 226, 258, 304, 353 nm (ϵ 34,250, 13,050, 8600, 12,200), τ 2·38, 2·28, 3·10, 3·55, 3·70 and 6·18 (see Table 1), m.s. peaks at m/e 258 (M^+). (Found: C, 69·62; H, 5·46. Calc. for $C_{15}H_{14}O_4$ C, 69·75; H, 5·45%). The sample was identical by direct comparison with an authentic sample.

TABLE 1. SOLVENT INDUCED CHEMICAL SHIFTS FOR BRAYLIN

Group	Observed shift*	Calculated
Dimethyl	+0·27	—
Methoxy	+0·48	+0·5 to +0·7†
5-H	+0·54	+0·53 to +0·57
3-H	+0·32	+0·15 to +0·29
4-H	+0·80	+0·76 to +0·79
3′-H	+0·52	+0·50†
4′-H	+0·08	+0·10†

* Carried out on a HA100 instrument using 10% w/v solutions. Dilution showed no significant change in the chemical shifts; Shifts as $\Delta\tau = \tau_{C_6D_6} - \tau_{CDCl_3}$.

† Estimated by comparison with model compounds.[8]

Further elution of the column, with benzene, gave γ-fagarine (10 mg), m.p. 142° (lit.[5] m.p. 139°), ν_{max}^{KBr} 3175, 2874, 1460, 1381, 1304, 1238, 1095 and 1053 cm^{-1}, λ_{max} 238, 332, 370 nm (ϵ 57,300, 7600, 7800), τ 6·01 (3Hs, methoxyl), 5·65 (3Hs, methoxyl) 3·01, 2·44 (2H, AB quartet, J 2·7 Hz), 3·05 (1 Hm), 2·7 (1 Hm), 2·2 (1 Hm), m.s. peaks at m/e 229 (M^+), 214 (M^+-CH_3), 200, 199, 184. The sample was identical to authentic γ-fagarine by TLC and paper chromatography as above.

Neutral constituents. The acid insoluble portion of the initial benzene extract (360 g) was studied. A portion (10 g) was chromatographed through alumina (grade I) eluting with solvent of increasing polarity which afforded the following compounds.

n-*Nonacosane.* 100 mg eluted with light petroleum, m.p. 58–60° (from MeOH), ν_{max}^{Film} 2933, 2874, 1471, 737, 720 cm^{-1}, m.s. peak at m/e 408, corresponding to $C_{29}H_{60}$. Small peaks (< 10%) also occurred at 422, 394, 380, due to small quantities of homologous hydrocarbons, as well as peaks characteristic of fragmentation of the hydrocarbon.

β-Sitosterol. 150 mg eluted with 1:1 benzene–light petroleum, m.p. 133–135° (from EtOH) (lit.[12] m.p. 139–140°), $[\alpha]_D^{20}$ −20·9° (c 0·5, $CHCl_3$), ν_{max}^{KBr} 3425, 2940, 1450, 1370, 1050, 840, 800 cm^{-1}, λ_{max} 206 nm (ϵ 3250). A portion was acetylated to give the monoacetate, m.p. 118–120° (from EtOH). Both the free alcohol and its acetate were identical by co-chromatography and mixed m.p. with authentic specimens.

Daucosterin. 120 mg eluted with EtOH, m.p. 270–275° (from EtOH), (lit.[13] m.p. 272–275°), ν_{max}^{KBr} 3350, 2930, 1445, 1360 and 1050 cm^{-1}, identical to that of an authentic sample. Acetylation afforded a tetraacetate, m.p. 167–168° (from EtOAc) (lit. m.p. 167–168°), ν_{max}^{KBr} 1758, 1626, 1460 and 1380 cm^{-1}. Found: C, 69·11; H, 9·09. Calc. for $C_{43}H_{68}O_{10}$, C, 69·31; H, 9·20%.

Acid hydrolysis of the glycoside with 0·2 N HCl under reflux for 6 hr afforded β-sitosterol. The aqueous solution was examined by paper chromatography and glucose was detected as the only sugar present.

Polar neutral constituents. Fresh dry leaves and stems of *P. punctata* (500 g) were extracted with MeOH in a Soxhlet apparatus for 24 hr. The extract was evaporated to dryness and the residue dissolved in hot H_2O. The aqueous phase was extracted with EtOAc in a liquid–liquid extractor and the solution dried (Na_2SO_4). After evaporation of solvent the residue (3 g) was chromatographed through silica gel using solvent of increasing polarity. The following compounds were eluted.

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Quercetin. 40 mg eluted with EtOAc, m.p. 313–314° from H_2O, λ_{max}^{EtOH} 256, 270 (inf), 374 nm, $\lambda_{max}^{EtOH+AlCl_3}$ 267, 432 nm, $\Delta\lambda^{AlCl_3}$ 59 nm[10]; λ_{max}^{MeOH} 255, 373 nm, $\lambda_{max}^{MeOH+AlCl_3}$ 272, 455 nm, $\Delta\lambda^{AlCl_3}$ 82 nm. The sample was identical, by direct comparison (mixed m.p. and co-chromatography) with authentic quercetin.

Avicularin. Quercetin-3α-L-arabofuranoside (60 mg), eluted with EtOAc, m.p. 225–230° (from aq. EtOH) (lit.[9] m.p. 222–223°), λ_{max}^{EtOH} 258, 267, 360 nm, $\lambda_{max}^{EtOH+AlCl_3}$ 269, 402 nm, $\Delta\lambda_{max}^{AlCl_3}$ 42 nm, indicating attachment of the sugar at position 3; λ_{max}^{MeOH} 256, 358 nm, $\lambda_{max}^{MeOH+AlCl_3}$ 434 nm. This material was converted into its heptaacetate with Ac_2O in pyridine, m.p. 138–140° (from EtOH), λ_{max} 254, 305 nm, τ 8·02, 7·98 7·96 (3 aliphatic acetates), 7·74 (9H, 3 aromatic acetates), 7·62 (3H, aromatic acetate), 6·5 (1Hm, —CHO—), 5·0–4·5 (4Hm, —CH—OAc), 3·26 (1Hm), 2·78 (2Hm), 2·12 (2Hm), as required for the arabinose in the furanose form. Acidic hydrolysis under standard conditions afforded quercetin and arabinose only.

Quercetin-3-rhamnosylarabinoside. 100 mg eluted with EtOAc–EtOH, m p. 168–170° (from aq. EtOH), λ_{max}^{EtOH} 256, 265, 354 nm, $\lambda_{max}^{EtOH+AlCl_3}$ 271, 402 nm, $\Delta\lambda_{max}^{AlCl_3}$ 48 nm; λ_{max}^{MeOH} 256, 352 nm, $\lambda_{max}^{MeOH+AlCl_3}$ 432 nm, $\Delta\lambda_{max}^{AlCl_3}$ 77 nm.

The material was acetylated to give a colourless *nonaacetate*, m.p. 84° (from EtOH), λ_{max} 260, 298 nm (ε 28,100, 13,500), τ 8·08 (12Hbs, 4 aliphatic acetates), 7·96 (3Hs, aliphatic acetate) 7·76, 7·44, 7·73, 7·64 (4 aromatic acetates); the aromatic region between 3·5–2·0, was almost identical to that in avicularin acetate. (Found: C, 55·86; H, 4·88. $C_{44}H_{46}O_{24}$ requires C, 55·26; H, 4·86%). Acidic hydrolysis of the glycoside, under standard conditions, afforded quercetin, arabinose and rhamnose only. Partial hydrolysis could not be controlled to give avicularin, only starting material and the products being observed.

Leuco-anthocyanidins. Fresh, dry leaves (20 g) were extracted with hot 2 N HCl for 40 min at 100°. The acid extract was examined for anthocyanidins by the usual methods[14] and the hydrolysate separated by paper chromatography (Whatman No. 1), using the Forestal solvent (HOAc–conc. HCl–H_2O, 3:1:3). Two anthocyanidins were detected. These were eluted from the paper by 95% EtOH for spectral measurements.

Cyanidin. R_f in BAW, 0·69, in Forestal, 0 47, in formic acid 0·22, $\lambda_{max}^{EtOH+HCl}$ 548 nm.[15]

Delphinidin. R_f in BAW, 0 43, in Forestal 0·30, in formic acid 0·08, $\lambda_{max}^{EtOH+HCl}$ 558 nm.

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