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ALKALOIDS, COUMARINS, TRITERPENES AND A FLAVANONE FROM THE ROOT OF *ZANTHOXYLUM DIPETALUM**

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Key Word Index—*Zanthoxylum dipetalum*; Rutaceae; alkaloids; canthin-6-one; chelerythrine; nitidine; tembetarine; coumarins; avicennol; xanthoxyletin; triterpenes; lupeol; sitosterol; flavanones; hesperidin; chemotaxonomy.

Abstract—The root bark of *Zanthoxylum dipetalum* contained the alkaloids canthin-6-one, chelerythrine, nitidine and tembetarine, the pyranocoumarins avicennol and xanthoxyletin, the triterpene lupeol and the flavanoid hesperidin. The MS fragmentation pattern for avicennol is discussed and a tentative structure is proposed for a third coumarin, designated ZD/1. The root wood of the type species and the stem bark of the γ variety contained most of the above compounds plus sitosterol and, in the root wood only, magnoflorine. The chemotaxonomic implications of these findings are briefly discussed.

INTRODUCTION

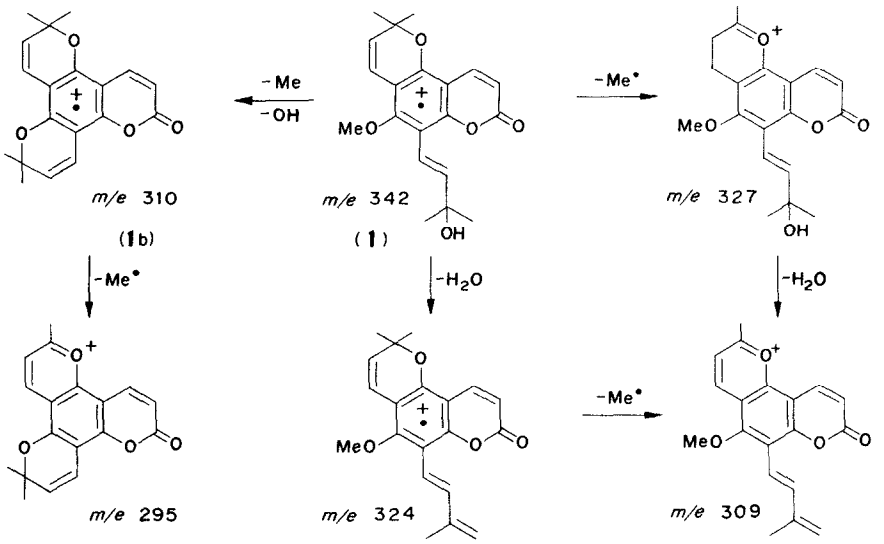
The large pan-tropical genus *Zanthoxylum* L (including *Fagara* L) (fam. Rutaceae) is represented in the Hawaiian Islands by six endemic species most of which have been sub-divided into several varieties [1]. *Zanthoxylum dipetalum* H. Mann (syn. *Fagara dipetala* Engl.), is a small to medium sized tree, distinguished from other Hawaiian species by a reduction in the number of petals to two due to the coalescence of two contiguous petals; by the occurrence of a pair of reduced basal leaflets adherent to the lowest pair of normal leaflets and by the mode of inflorescence [2]. Several varieties have been recorded differing from the type species in that they retain the original tetramerous state [1–3].

As part of a continuing study on the secondary metabolites of *Zanthoxylum* and their possible chemotaxonomic significance [4] we have examined samples of the root of the type species and of the stem of *Z. dipetalum* var γ Hillebr. (*F. dipetala* v. *mannii* Sherf).

RESULTS AND DISCUSSION

The major component of the petrol extract from the root bark of *Z. dipetalum* was the pyrano[2,3-*f*]coumarin, avicennol (**1**), which has been isolated previously only from the Asian species *Z. avicennae* (Lam.) DC [5,6]. Again it occurred in high concentration (in excess of 1%). Avicennol was identified by direct comparison of physical and spectral data with that previously reported and, in addition, a detailed study was made of the MS, for which accurate mass measurements of significant ions were obtained. The major fragmentation pattern can be rationalized as dehydration of the 3-hydroxy-3-methylbut-1-enyl side-chain and loss of a Me radical from the pyran ring to give a fragment at *m/e* 309. This is in agreement with previously published MS for pyranocoumarins [7]. A minor

* Part 5 in the series Chemosystematics in the Rutaceae. For Part 4 see Waterman, P. G. (1975) A Review of the Chemosystematics of the genus *Zanthoxylum* (Rutaceae) in South East Asia in, *Roles and Goals of Tropical Botanic Gardens* (Stone, B. C. ed.), University of Malaya, Kuala Lumpur.



Scheme 1.

pathway, giving fragments at *m/e* 310 and 295 may be explained by elimination of methanol with subsequent cyclization to the pyranocoumarin (**1b**) followed by loss of a Me radical (Scheme 1).

Four other compounds were isolated from the petrol extract by PLC; lupeol, canthin-6-one and two coumarins one of which was identified as xanthoxyletin. The second coumarin, designated ZD/1, gave UV and IR spectra typical of pyranocoumarins [7], the IR spectrum showing a marked similarity to that of avicennol. Accurate mass measurement indicated the empirical formula $C_{19}H_{18}O_4$ with a fragmentation pattern showing loss of only a Me radical. These data suggest ZD/1 may be a coumarin of novel type with a di-pyran ring system. Although no coumarins with such a ring system are known an analogous dihydro-dipyrano acridone alkaloid has recently been reported from *Atalantia monophylla* Correa (Rutaceae) [8]. If these tentative conclusions are correct the most likely structure for ZD/1 would seem to be (**1b**). Unfortunately this compound has proved difficult to purify and, as yet, very little material is available.

Investigation of the $CHCl_3$ and MeOH extracts yielded three quaternary alkaloids, the benzophenanthridines chelerythrine and nitidine and the 1-benzyltetrahydroisoquinoline tembetarine. Chelerythrine has previously been reported to be the major alkaloid of *Z. semi-articulatum* St. John & Hosaka [9], the only other Hawaiian *Zanthoxylum* species investigated to date. The MeOH extract also yielded large quantities of the flavanone glycoside hesperidin.

The root wood and the stem of the γ variety contained a similar range of compounds but nitidine was absent from the root wood and hesperidin and tembetarine were not present in the γ variety. Sitosterol and magnoflorine were detected; the former in both samples, the latter only in the root wood.

In view of the disparity between the plant parts examined little chemotaxonomic value can as yet be placed on these findings from the intraspecific viewpoint although the absence of hesperidin from the stem of the γ variety does seem unusual. At the generic level however, the occurrence of avicennol in *Z. dipetalum* (sect. Blackburnia) and *Z. avicennae* (sect. Paniculatae-Gerontogaeae) and of canthin-6-one in widespread and seemingly distantly related species [10] would appear to cast further doubt on Englerian classification of the *Zanthoxylum/Fagara* complex [4].

EXPERIMENTAL

UV spectra were recorded in EtOH and IR spectra in KCl. NMR (60 M Hz) spectra were recorded with TMS as internal standard. MS were determined at 70 eV. Mps (uncorr) were determined on a Kofler hot stage.

Plant material. Root bark and root wood of *Zanthoxylum dipetalum* H. Mann (Voucher: GS 8 at BISH, HLA, NBV) [11] were collected at the Pupukea-Paumalu Forest Reserve, Koolau Mountains, Oahu, Hawaii State. Stem bark of *Z. dipetalum* var. *γ* (Voucher: Herbst 1030 at BISH, US, HLA, NBV) [11] was collected at the junction of the Kokee and Halemanu roads, Kokee State Park, Kauai, Hawaii State.

Extraction of root bark of Z. dipetalum. Milled bark (55 g) was extracted in a Soxhlet separately and successively with petrol (bp 40–60°), $CHCl_3$ and MeOH. The extracts were conc under red pres. On standing a ppt formed in the petrol concentrate and it was collected by decanting the supernatant sol. The crystals were washed with *n*-hexane to yield avicennol (560 mg). The supernatant extract was further conc and an aliquot (50%) subjected to PLC on alumina (Woelm, neutral activity I) with *n*-hexane–EtOAc (4:1) as eluting solvent. Xanthoxyletin (R_f 0·3; 12 mg), lupeol (R_f 0·4; 15 mg) and an unidentified compound designated ZD/1 (R_f 0·5, 10 mg) were isolated. A second aliquot was treated by PLC on Si gel G with $CHCl_3$ as eluting solvent to give ZD/1 (R_f 0·5; 12 mg) and canthin-6-one (R_f 0·1; 8 mg). The $CHCl_3$ concentrate, on shaking with 1% aq HCl, gave a yellow ppt in the aq phase which was filtered off to yield chelerythrine chloride (91 mg). The acid extract was basified with NH_4OH and re-extracted with $CHCl_3$ to give canthin-6-one (15 mg).

On standing the MeOH concentrate deposited an amorphous buff ppt of hesperidin (1·5 g). The supernatant extract was purified by ion-exchange chromatography [12] and from the resulting EtOH extract yellow crystals of nitidine chloride (13 mg) were obtained. The EtOH extract was then evap to dryness and the alkaloid mixture dissolved in the minimum H_2O. A saturated soln of KI was added dropwise to ppt the alkaloids as the iodides from which, after repeated recrystallization from EtOH–EtOAc, tembetarine iodide (104 mg) was obtained.

Avicennol (**1**). Recrystallized from *n*-hexane–EtOAc (99:1) as yellow plates mp 124·5–125·5° (lit. [5] 124·5–125·5°). Found M^+ 342. 1477, $C_{20}H_{22}O_5$ requires *M* 342·1467. UV λ_{max} nm (log ϵ): 250 (4·50), 257 (4·60) and 301 (4·26 IR ν_{max} cm^{-1}: 3475 (OH), 1725 (C=O), 1585, 1140 and 825. NMR ($CDCl_3$): δ 1·48 (12H, *s*, 2′-Me_2 and 3″–Me_2), 2·15 (1H, *s*, D_2O exchange, OH), 3·80 (3H, *s*, OMe), 5·69 and 6·65 (2H, *ABq*, *J* 10 Hz, 3′-H and 4′-H), 6·80 and 6·92 (2H, *ABq*, *J* 16 Hz, 2″-H and 1″-H), 6·27 and 8·06 (2 H, *ABq*, *J* 10 Hz, 3-H and 4-H). MS *m/e* (rel. int.): 342 (23), 327 (100), 324 (52), 310 (16), 309 (66), 295 (6). On admixture with an authentic sample of avicennol from *Z. avicennae* there was no depression of mp.

Xanthoxyletin. Recrystallized from MeOH as colourless prisms mp 132–133° (lit. [13] 133°). UV λ_{max} nm: 223, 268·5 and 346. Identical in all respects (UV, IR, TLC, mmp) with an authentic sample of xanthoxyletin.

Compound ZD/1. Amorphous yellow powder from MeOH with no sharp mp. Found M^+ 310·1196. $C_{19}H_{18}O_4$ requires 310·1205 UV λ_{max} nm: 223, 243(*sh*), 250, 296, 306(*sh*) and 336. IR ν_{max} cm^{-1}: 1730 (C=O), 1610, 1360, 1135, 1025, 825, 740 and 705. MS *m/e* (rel. int.): 310 (26), 295 (100).

Lupeol. Recrystallized from Me_2CO as white needles mp 217–218° (lit. [14] 215–216°). Identical in all respects (IR, TLC, mmp) with an authentic sample of lupeol.

Canthin-6-one. Recrystallized from MeOH as pale cream needles mp 162–163° (lit. [8] 162–163°). Found M^+ 220·0613, $C_{14}H_8N_2O$ requires *M* 220·0637. UV λ_{max} nm: 227, 250, 259, 268, 299, 346, 362 and 379. Identical in all respects (UV, IR, TLC, mmp) with an authentic sample of canthin-6-one.

Chelerythrine chloride. Recrystallized from EtOH–1N HCl as yellow needles mp 200–202° (lit. [15] 202–203°). UV λ_{max} nm: 271, 279(*sh*), 318, 339(*sh*) and 394. Identical in all respects (UV, IR, TLC, mmp, conversion to dihydrochelerythrine) with an authentic sample of chelerythrine chloride.

Nitidine chloride. Recrystallized from EtOH as yellow needles mp 240° decomp. (lit. [16] 240° decomp.). UV λ_{max} nm: 231, 272, 281, 301, 329 and 385. Identical in all respects (UV, IR, TLC, mmp, conversion to dihydronitidine) with an authentic sample of nitidine chloride.

Hesperidin. Recrystallized from MeOH as buff coloured needles mp 257° decomp. (lit. [17] 255–260° decomp.). UV λ_{max} nm: 283 and 329; λ_{max} $AlCl_3$ nm: 306 and 380 [18]. A positive reaction was obtained with the Mg/HCl test for flavanones [18] and the material was identical in all respects (UV, IR, TLC, mmp) with an authentic sample of hesperidin.

Tembetarine iodide. Recrystallized from EtOH–EtOAc as white plates mp 155–160°. Found M^+ −1 343·1774, $C_{20}H_{25}NO_4$ requires M −1 343·1783. $[\alpha]_D^{21}$ + 91° (*c* 0·32 EtOH). UV λ_{max} nm: 286; λ_{max}^{NaOH} nm: 296. IR ν_{max} cm^{-1}: 3350 (OH), 1620, 1520, 1270, and 1240. MS *m/e* (rel. int.): 343 (7), 192 (100), 177 (18), 137 (2). *Tembetarine chloride.* Deposited from the purified EtOH extract before evaporation and addition of KI and recrystallized from EtOH–EtOAc (15:3) as white prisms mp 236° (lit. [19] 237°). Identical spectral data (UV, IR, MS) was obtained to that recorded for tembetarine iodide.

Extraction of root wood of Z. dipetalum. Milled root wood (80 g) was extracted as previously described for the root bark. Examination of the petrol extract on TLC (alumina, solvent $CHCl_3$) indicated the presence of traces of avicennol, xanthoxyletin, canthin-6-one, lupeol, sitosterol and compound ZC/1. Examination of the $CHCl_3$ extract (alumina, $CHCl_3$–MeOH, 49:1) showed further traces of canthin-6-one and of chelerythrine chloride whilst the MeOH extract (Cellulose, 0·1 N HCl) contained tembetarine and a further quaternary alkaloid, probably magnoflorine. *Hesperidin* (375 mg) was isolated from the MeOH extract and identified as previously described.

Extraction of the stem bark of Z. dipetalum *var.* Milled bark (150 g) was extracted in the manner previously described. The petrol concentrate was subjected to column chromatography on alumina (Woelm, activity IV). Elution with *n*-hexane gave *lupeol* (20 mg), *n*-hexane–EtOAc (9:1) gave *xanthoxyletin* (24 mg) and *n*-hexane–EtOAc (4:1) gave *sitosterol* (30 mg). All were identified by direct comparison with authentic material. Trace quantities of canthin-6-one and avicennol were detected by TLC (systems as before). Similarly traces of chelerythrine and canthin-6-one were detected in the $CHCl_3$ extract.

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