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DIPETALOLACTONE: A NOVEL PYRANOCOUMARIN FROM THE ROOT BARK OF *ZANTHOXYLUM DIPETALUM*

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Key Word Index—*Zanthoxylum dipetalum*; Rutaceae; pyranocoumarins; dipetalolactone; tetrahydrodipetalolactone; dipetaline; synthesis.

Abstract—A novel dipyranocoumarin, dipetalolactone {2-oxo-6,6,10,10-tetramethylbenzo[1,2-b:3,4-b':5,6-b"]tripyran}, has been isolated from the root bark of *Zanthoxylum dipetalum* and its structure proven by the synthesis of tetrahydrodipetalolactone. A second new pyranocoumarin, dipetaline, has been assigned the tentative structure of 6-(3,3-dimethylallyl)-5-methoxy-2,2-dimethyl-2H-benzo[1,2-b:3,4-b']dipyran-8-one on the basis of PMR analysis using the lanthanide shift reagent $Eu(fod)_3$.

INTRODUCTION

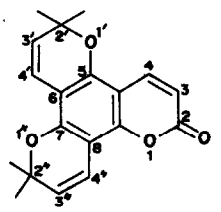
In a previous publication [1] we reported the isolation of the alkaloids canthin-6-one, chelerythrine, nitidine, tembetarine and magnoflorine, the pyranocoumarins avicennol and xanthoxyletin, the triterpenes lupeol and sitosterol, and the flavanone hesperidin from the Hawaiian tree *Zanthoxylum dipetalum* H. Mann (Rutaceae). A third pyranocoumarin (designated ZD/1), isolated only in small amounts and in an impure state, was tentatively assigned a unique dipyranocoumarin nucleus, largely on the basis of MS data.

We now report the complete structure elucidation of this novel coumarin, to which we give the trivial name dipetalolactone (**1**), and the synthesis of its tetrahydro derivative. In addition it has been shown that the contaminant preventing the crystallisation of dipetalolactone from ZD/1 is another pyranocoumarin. This compound, which we have called dipetaline, is tentatively identified as the angular pyrano[2,3-f]coumarin (**8**) which is closely related to avicennol, the major coumarin of this species.

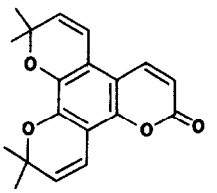
RESULTS AND DISCUSSION

The petrol extract of the root bark of *Z. dipetalum*, after deposition of avicennol, was subjected to repeated PLC over alumina to give 65 mg of a yellow oil designated ZD/1 [1]. Although exhibiting only a single spot on TLC this material was shown (by PMR) to be a mixture of two coumarins in the approximate ratio of 4:1. Repeated recrystallisation of the oil from absolute EtOH gave dipetalolactone (46 mg) mp 119–120°. The MS, UV and IR, reported previously [1], were all typical of pyranocoumarins [2]. Accurate mass measurement gave a molecular ion M^+ 310·1206 ($C_{19}H_{18}O_4$) and a base peak at *m/e* 295 for loss of Me$^\cdot$. Further loss of Me$^\cdot$ from the ion at *m/e* 295 gave a doubly charged fragment at *m/e* 140.

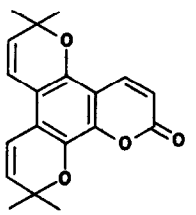
The PMR spectrum readily resolved all eighteen protons of dipetalolactone. An AB quartet (*J* 10 Hz) δ 6·19 (1H) and 8·02 (1H) confirmed the presence of the α,β-unsaturated lactone ring of the coumarin nucleus [2]. The presence of two of the 2,2-dimethylchromene ring systems encountered in pyranocoumarins [3,4] was indicated by the occurrence of a singlet for *gem*-methyl groups at δ 1·48 (12H) and two AB quartets (both *J* 10 Hz) centered at δ 6·88 (1H), 5·65 (1H) and δ 6·71 (1H), 5·62 (1H). This easily rationalised PMR spectrum confirmed that the major component of the oil ZD/1 had the previously suggested [1] dipyranocoumarin structure but failed to distinguish between the four possible isomers (**1–4**).



Dipetalolactone (1)



(2)



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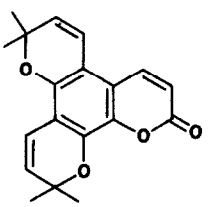

(4)

Of the possible isomeric structures (**1**) was considered the most likely in view of the following. Firstly, almost all naturally occurring coumarins are oxygenated in the

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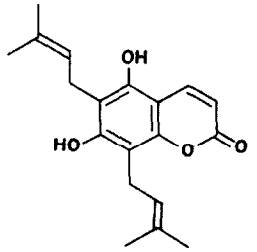
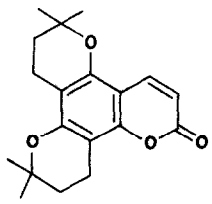
7-position* [5], thereby requiring that the C-4″ attachment be in the 8- position, as in structures (**1**) and (**2**). Secondly, avicennol and xanthoxyletin, the two coumarins already reported from this species, both exhibit a 5,7-oxygenation pattern [cf. (**1**)].

A significant contribution to the assignment of structure (**1**) to dipetalolactone was made by PMR studies using the lanthanide shift reagent Eu(fod)$_3$ (tris-(7,7-dimethyl-1,1,1,2,2,3,3-heptafluoro-octane-4,6-dionato)europium) according to a method described elsewhere [6]. Complexation proceeded at the carbonyl group as expected with the observed shift of the 4-H (relative to 3H = 1·00) as before [7]. However other shifts, particularly that of the putative C-8 pyran substituent, were less than the calculated values. This may be explained by a preferred out-of-plane conformation for the lactone ring due to interaction of the 4″-H with the electron clouds of the lactone oxygen and thereby resulting in the formation of a non-coplanar complex [8]. Despite the variation of shift values obtained from those observed previously [6,7] the results obtained still gave a 'best-fit' for, and could only be assigned to, structure (**1**).

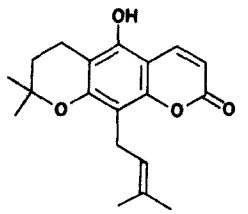
In order to confirm the structure of dipetalolactone its tetrahydro derivative (**6**) was synthesised. The nuclear prenylation of 5,7-dihydroxy-coumarin, in which one of the major products was 5,7-dihydroxy-6,8-di-(3,3-dimethylallyl)-coumarin (**5**), has been described [9]. Acid-catalysed cyclisation of an *ortho*-(3,3-dimethylallyl)-hydroxycoumarin (osthenol) to the corresponding dihydropyranocoumarin (dihydroseselin) has also been reported [10]. These synthetic steps provide a convenient method for the preparation of 2-oxo-6,6,10,10-tetramethyl-7,8,11,12-tetrahydro-benzo[1,2-b:3,4-b′:5,6-b″]tripyran (**6**), although the alternative cyclisation to the linear dihydropyranocoumarin dihydrotrachyphyllin (**7**) might also occur. Refluxing (**5**) with formic acid and subsequent PLC gave (**6**) mp 154–155° as the major product with minor amounts of a second compound, possibly (**7**).



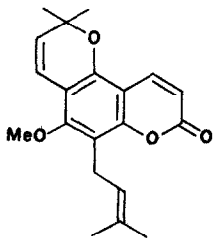
(**5**)



(**6**)



(**7**)



Dipetaline (**8**)

Hydrogenation of dipetalolactone over Adam's catalyst yielded colourless plates, mp 154–155°, undepressed on admixture with (**6**). Tetrahydrodipetalolactone and (**6**) were found to be identical in all respects (UV, IR, PMR, MS) thereby confirming the structure of dipetalolactone as (**1**). A lanthanide shift experiment with tetrahydrodipetalolactone gave essentially the same results as dipetalolactone.

The minor coumarin, dipetaline, which remained in a 1:1 mixture with dipetalolactone in the supernatant after the deposition of the latter, was not further purified. MS of the mixture indicated a molecular formula for dipetaline of $C_{20}H_{22}O_4$ with a major ion at *m/e* 311 for the loss of Me$^{\cdot}$. PMR of the mixture permitted all twenty-two protons of dipetaline to be distinguished from those of dipetalolactone. An AB quartet (*J* 10 Hz) at δ 6·30 (1H) and 8·10 (1H) demonstrated the presence of an α,β-unsaturated lactone ring. A second AB quartet (*J* 10 Hz) at δ 5·71 (1H) and 6·64 (1H) together with a singlet for *gem*-methyl groups at δ 1·48 (6H) was again indicative of the 2,2-dimethylchromene ring system of pyranocoumarins. The presence of a methoxy substituent was shown by a singlet at δ 3·85 (3H) whilst broad singlets at δ 1·72 (3H) and 1·87 (3H) together with multiplets at δ 3·50 (2H) and 5·33 (1H) could be accounted for by a 3,3-dimethylallyl unit. Therefore dipetaline is a pyranocoumarin with the two remaining positions on the benzenoid nucleus filled by methoxy and 3,3-dimethylallyl substituents, respectively.

In an attempt to determine the substitution pattern of dipetaline it was examined using Eu(fod)$_3$. Addition of Eu(fod)$_3$ to the mixture allowed, in the light of previously obtained shift values for the protons of dipetalolactone, the determination of the shift for the protons of dipetaline (relative to 3H = 1·00). In this case 'best-fit' was obtained between observed and calculated shifts for values of ϕ (C–O–Eu bond angle) of 152·5° and *d* (O–Eu distance) of 2·75Å, in agreement with our previous findings [6,7]. The large observed shifts for the allylic protons (Table 1) indicated the placement of the 3,3-dimethylallyl unit in the 8-position. The observed methoxy shift (0·15) suggested its placement in the 7-position (calculated shift 0·15) rather than the 5-position (calculated shift 0·11). Furthermore observed and calculated shifts for the pyran ring methyl protons offer marginally better fits with pyran ring attachment through the oxygen at C-5 rather than that at C-7 and therefore also support the angular structure (**8**) for dipetaline. Circumstantial evidence in favour of this assignment for dipetaline is

Table 1. Observed and calculated shift values for dipetaline with Eu(fod)$_3$ (relative to 3H = 1·00)

(a) for the allylic protons:

	observed	calculated for attachment at			
		C-5	C-6	C-7	C-8
CH_2*	0·35	0·15	0·12	0·15	0·32
CH*	0·54	0·17	0·12	0·16	0·48

(b) for the methoxy and pyran ring methyl groups:

	observed	calculated for attachment to oxygen at	
		C-5	C-7
OMe	0·15	0·11	0·15
Me_2	0·08	0·08	0·09

* In order to clarify the text, the non-systematic numbering system illustrated in formula (**1**) is used to refer to positions on the dipetalolactone skeleton.

* The calculated values are the average of the two extreme conformations of the side-chain in the plane of the coumarin benzene ring with the allylic protons *trans*-orientated.

provided in its obvious relationship to avicennol, the major coumarin of *Z. dipetalum*.

The presence of several derivatives of 5,7-dihydroxy-6,8-(3,3-dimethylallyl)coumarin in *Z. dipetalum* is of systematic interest. A similar range of 5,7-dihydroxy-6,8(3,3-dimethylallyl)-2-methylchromones, including the dipyranoid compound spatheliabischromene, occur in the small aberrant genus *Spathelia* L. [11]. The co-occurrence of this unusual type of substitution might be considered to offer further chemical support to that already available [12] in favour of the retention of *Spathelia* in the Rutaceae and against its transfer to the Simaroubaceae. Other dipyranoid substituents have been reported among the acridone alkaloids of the rutaceous genus *Atalantia* Correa [13] and among the chromones of *Cneorum tricoccum* L. [14] of the closely allied family Cneoraceae.

EXPERIMENTAL

UV spectra were recorded in EtOH and IR spectra in KCl. PMR (60 MHz) were recorded in $CDCl_3$ with TMS as internal standard. MS were recorded on an AEI MS902 spectrometer at 70 eV. Mp's (uncorr) were determined on a Kofler hot stage. PLC were carried out on 1 mm thick layers eluting with $CHCl_3$.

Plant material. Root bark of *Zanthoxylum dipetalum* H. Mann (Voucher: GS 8 at BISH, HLA and NBV [15]) was collected at the Pupukea-Paumalu Forest Reserve, Koolau Mountains, Oahu Hawaii.

Extraction and isolation. Milled bark (55 g) was extracted in a Soxhlet with petrol (bp 40–60°) and the extract conc under red. pres. On standing a ppt. of avicennol (560 mg) was obtained [1]. An aliquot of the supernatant (50%) was subjected to PLC on alumina (Woelm, neutral, activity I) with *n*-hexane–EtOAc as eluting solvent. Xanthoxyletin and lupeol were isolated and identified as previously described [1]. A third yellow band, designated ZD/1 (R_f 0·5, 10 mg), was eluted and on careful re-PLC of adjacent alumina bands a further 15 mg of this material was obtained. The remainder of the petrol extract was similarly treated by PLC on Si gel G, eluting with $CHCl_3$, to give further ZD/1 (R_f 0·5, 45 mg) together with canthin-6-one. Repeated PLC of the combined ZD/1 fractions over alumina gave 65 mg, as a yellow oil, which was shown, by PMR ($CDCl_3$), to be a mixture of two coumarins.

Dipetalolactone (**1**). Recrystallisation of the mixture ZD/1 from absolute EtOH gave, after several days, yellow plates, mp 119–120°. Found M^+ 310·1206, $C_{19}H_{18}O_4$ requires 310·1205. UV λ_{max} nm (log ϵ): 222(4·17), 244*sh*(4·37), 250(4·47), 294*sh*(4·37), 297(4·38), 307*sh*(4·28), 344(4·04); absence of bathochromic shift on addition of NaOH solution. IR ν_{max} cm^{-1}: 2990, 1730(C=O), 1610, 1360, 1135, 1025, 825, 740, 705. MS *m/e*: 310(35%), 295(100), 140(M^{2+} ion). PMR δ: 1·48 (12H, *s*, 2′-Me_2, 2″-Me_2), 5·62, 6·71 (2H, AB*q*, *J* 10 Hz, 3′-H, 4′-H†), 5·65, 6·88 (2H, AB*q*, *J* 10 Hz, 3″-H, 4″-H†), 6·19, 8·02 (2H, AB*q*, *J* 10 Hz, 3-H, 4-H).

Tetrahydrodipetalolactone (**6**). Hydrogenation of dipetalolactone (30 mg) over Adam's catalyst (20 mg) in EtOH (absolute), hydrogen uptake occurring over 4 hr, gave, on filtration and recrystallisation from MeOH, tetrahydrodipetalolactone (25 mg) as colourless plates mp 154–155°. Found M^+ 314·1514, $C_{19}H_{22}O_4$ requires 314·1518. UV λ_{max} nm (log ϵ): 211(4·55), 225*sh*(4·13), 253(3·80), 262(3·85), 337·5(4·16). IR ν_{max} cm^{-1}: 3000, 2950, 1730(C=O), 1620, 1380, 1165, 825, 820. MS *m/e*: 314 (80%), 259(100), 258(56), 243(23), 203(76). PMR δ: 1·37 (12H, *s*, 2′-Me_2, 2″-Me_2), 1·80, 2·64 (4H, 2 x *tr*, *J* 7 Hz, 3′-CH_2, 4′-CH_2†), 1·83, 2·85 (4H, 2 x *tr*, *J* 7 Hz, 3″-CH_2, 4″-CH_2), 6·16, 8·10 (2H, AB*q*, *J* 10 Hz, 3-H, 4-H).

† Assignments of pyran ring signals in the PMR spectra of (**1**) and (**6**) were made on the basis of observed shifts with $Eu(fod)_3$.

Synthesis of 2-oxo-6,6,10,10-tetramethyl-7,8,11,12-tetrahydrobenzo[1,2-b: 3,4-b′:5,6-b″]tripyran (**6**). *Preparation of 5,7-dihydroxycoumarin.* Perkin condensation [16] of phloroglucinaldehyde with Ac_2O in the presence of anhydrous NaOAc gave 5,7-diacetoxycoumarin, recrystallised from EtOH as prisms mp 140° (lit. [16] 140°). The previously reported [16] alkaline hydrolysis of 5,7-diacetoxycoumarin resulted in an intense red solution from which no 5,7-dihydroxycoumarin could be recovered. Acid hydrolysis of 5,7-diacetoxycoumarin was achieved by heating a conc ethanolic solution in 50% HCl for 4 hr at 100°C. Evaporation of the EtOH gave 5,7-dihydroxycoumarin, recrystallised from dil AcOH as needles (83% yield) mp 283–285° (lit. [16] 285–286°).

Preparation of 5,7-dihydroxy-6,8-di-(3,3-dimethylallyl)coumarin (**5**). Reaction of 5,7-dihydroxycoumarin with 2-methylbut-3-en-2-ol by the method of Mahey *et al.* [9] afforded an oil having identical characteristics (UV, IR, PMR) to those recorded for 5,7-dihydroxy-6,8-di-(3,3-dimethylallyl)coumarin [9].

Preparation of 2-oxo-6,6,10,10-tetramethyl-7,8,11,12-tetrahydrobenzo[1,2-b:3,4-b′:5,6-b″]tripyran (**6**). 5,7-Dihydroxy-6,8-di-(3,3-dimethylallyl)coumarin (60 mg) was heated on a water bath with formic acid for 4 hr. The reaction mixture was diluted with H_2O and extracted with CH_2Cl_2. Concn of the CH_2Cl_2 extract followed by PLC over alumina, eluting with $CHCl_3$ (blue fluorescent band, R_f 0·63), gave, on recrystallisation from MeOH, colourless plates (25 mg) mp 154–155° identical in all respects (UV, IR, PMR, MS, TLC, mmp) with tetrahydrodipetalolactone.

Dipetaline (**8**). The supernatant EtOH solution after deposition of dipetalolactone was subjected to MS and PMR analysis. The following data were obtained after comparison with spectra of pure dipetalolactone. Found M^+ 326·1513, $C_{20}H_{22}O_4$ requires 326·1518. MS *m/e*: 326, 311. PMR δ: 1·48 (6H, *s*, pyran-Me_2), 1·72 (3H, *s*, allylic Me), 1·87 (3H, *s*, allylic Me), 3·50 (2H, *br. d.*, allylic CH_2), 3·85 (3H, *s*, OMe), 5·33 (1H, *br. tr.*, allylic CH), 5·71, 6·64 (2H, AB*q*, *J* 10 Hz, pyran CH:CH), 6·30, 8·10 (2H, AB*q*, *J* 10 Hz, 3-H, 4-H).

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