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A NEW CLASS OF NATURAL PRODUCT: HOMOLOGUES OF JUGLONE BEARING 4-HYDROXY-5-METHYLCOUMARIN-3-YL UNITS FROM *DIOSPYROS* SPECIES

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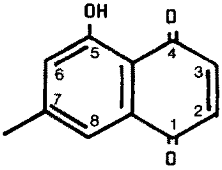
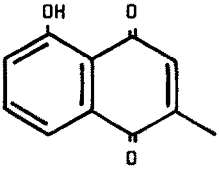
Summary 7-Methyljuglone bearing 4-hydroxy-5-methylcoumarin-3-yl units at C(2) and C(3) has been isolated from the bark of *Diospyros ismailii* Ng (Ebenaceae), and 2-methyljuglone (plumbagin) carrying the same coumarin-3-yl unit at C(3) has been isolated from *Diospyros canaliculata* De Wild; and the structures of both members of this new class of natural product confirmed by synthesis.

7-Methyljuglone (1a) and plumbagin (1b), and their oligomers are well-known metabolites of many species of *Diospyros*.[1] In this paper we report the occurrence of two members of a new class of natural product that consists of combinations of (1a) or (1b) with 4-hydroxy-5-methylcoumarin units: namely, ismailin (2a; other tautomers are possible) from *D. ismailii*, a tree endemic to West Malaysia,[2] and canaliculatin (4) from *D. canaliculata*, a small tree found throughout the forest zone of West and Central Africa.[3]

Ismailin was obtained as the major component of the methanol extract of the stem bark of *D. ismailii*, and isolated by preparative TLC on SiO_2 (solvent $CHCl_3$). It was sparingly soluble in most solvents, and crystallised from dimethylsulphoxide as the hemi-hydrate, m.p. (Kofler) 284-285° (decomp.). The high resolution mass-spectrum suggested ismailin to be $C_{31}H_{18}O_9$, and thus not an oligomer of (1a) or (1b). The red methanol solution became purple on addition of sodium hydroxide; in the visible region the λ_{max} were consistent with a juglone chromophore, and the molar extinction coefficients showed that the molecule contained one such unit (MeOH: neutral, λ_{max} 422 nm, log ε 3.76; + NaOH, λ_{max} 510 nm, log ε 3.83). Treatment with zinc and acetic acid gave a mixture of colourless products the major of which was found, by high-resolution mass spectrometry, to be a pentaacetate of a dihydro-ismailin (three acetate units for the reduced juglone part, plus two others).



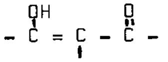
(1a)



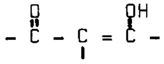
(1b)

High field ^1H n.m.r. (360 MHz, $CDCl_3$) showed three methyl groups (δ 2.42, 2.73, 2.74), of which the first was typical of 7-methyljuglone with protons on C(6) and C(8);[4] with doublets at δ 7.13 and 7.55 (1H each, ^4J 2 Hz) for H(6) and H(8), respectively, of 7-methyljuglone. Signals downfield of δ 12.0 (3H) indicated three hydroxyl groups, fitting the results of the reductive acetylation. Signals for the six remaining protons occurred in the range δ 7.09 to 7.55; their δ-values and splitting patterns showed that neither C(2) nor C(3) of the juglone unit carried hydrogen. These signals were not further analysed at this stage, and are discussed below.

Treatment of ismailin with methyl iodide and silver oxide gave a mixture of non-phenolic products which were partly resolved by preparative TLC on SiO_2 (solvent $CHCl_3$). The compounds that moved fastest (2b), and slowest (3), were shown by high resolution mass spectrometry to be isomeric trimethyl ethers of ismailin ($C_{34}H_{24}O_9$). The ^{13}C n.m.r. spectrum of (2b) showed 24 signals for 34 carbons. Those at δ_C 22.5 (2C) and 22.7 were due to the three methyl substituents, those at δ_C 56.7 and 61.7 (2C) to the three O-methyl groups, and the signals furthest downfield, at δ_C 183.8 and 181.9, to the carbons of the quinonoid carbonyl groups. The remaining signals showed 13 singlets; and five doublets to account for eight tertiary carbons. Atoms C(6) and C(8) of the juglone unit accounted for two doublets, leaving three signals of approximately twice their strength to account for the remaining six CH-units. The apparent simplicity of the spectrum clearly indicated that this trimethyl ether, and hence ismailin, contained a 7-methyljuglone moiety carrying two indistinguishable groups, one at C(2) and one at C(3). If the methyl ethers (2b) and (3) differ as a consequence of tautomerism:



$-\overset{\text{OH}}{\overset{|}{C}}=\underset{|}{C}-\overset{\text{O}}{\overset{\|}{C}}-$



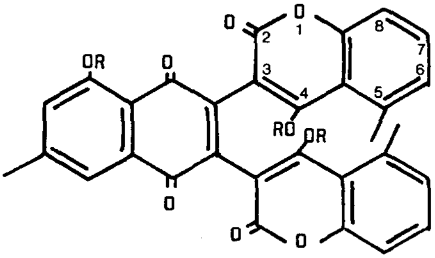
$-\overset{\text{O}}{\overset{\|}{C}}-\underset{|}{C}=\overset{\text{OH}}{\overset{|}{C}}-$

then a 4-hydroxycoumarin / 2-hydroxychromenone tautomerism would agree with the observed data; and Jurd[5] has recently reported that 4-hydroxycoumarin will add, through the 3-position, to C(2) and C(3) of 1,4-naphthoquinone.

Further interpretation of spectroscopic data supported this hypothesis. Thus the u.v. spectrum of (2b) showed λ_{max} 287 nm (log ε 4.41) typical for 5-methyl-4-oxycoumarin,[6] and the i.r. spectrum ν_{max} 1700 cm^{-1} typical of a coumarin carbonyl, while those of (3) showed λ_{max} 294 nm typical for 2-oxychromenone[6] and ν_{max} 1630 cm^{-1} for the chromone carbonyl. The C-methyl group for each unit of the coumarin/chromenone system must, therefore, be sited on the benzene ring. Analysis of the high-field ^1H NMR spectrum of ismailin showed that the δ 7.09 to 7.55 signals, referred from above, were now understandable: δ 7.09 (2H, dd, ^3J 8 Hz, ^4J 2 Hz), 7.19 (2H, dd, ^3J 8 Hz, ^4J 2 Hz), 7.55 (2H, dd, ^3J 8 Hz and 8 Hz). This pattern is consistent only with three adjacent protons, thus requiring placement of the methyl group in each system at either C(5) or C(8). The strongly deshielded positions of the methyl resonances (δ 2.73, 2.74) favoured placement at C(5), *peri* to the oxygen substituent at C(4), rather than at C(8) at which a resonance position of about δ 2.40 would be anticipated.[6]

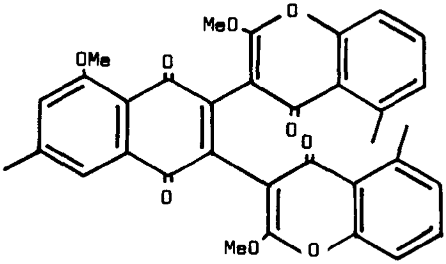
The implied structure (2a) for ismailin has been confirmed by synthesis. Reaction of 7-methyljuglone with two moles of 4-hydroxy-5-methylcoumarin, under the conditions used by Jurd,[5] gave synthetic ismailin, physically and spectroscopically identical with the natural product. Methylation of the synthetic material, under the conditions used for the natural product gave a mixture of the methyl ethers, including (2b) and (3).

Canaliculatin was obtained from the chloroform extract of the stem bark of *D. canaliculata*, and isolated by preparative TLC on SiO_2 (solvent toluene:ethyl acetate:acetic acid - 40:9:1) as a minor product. The high resolution mass spectrum suggested $C_{21}H_{14}O_6$. The spectral characteristics of canaliculatin showed marked similarities to those of ismailin.



(2a) R = H

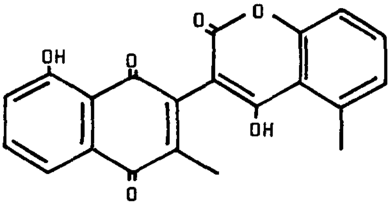
(2b) R = Me



(3)

Thus, the yellow methanol solution gave λ_{max} 285 nm (4-oxycoumarin) and 410 nm (juglone derivative); and turned purple on addition of sodium hydroxide. The ^{1}H n.m.r. spectrum (90 MHz, $CDCl_3$ + 10% dimethylsulphoxide-$\underline{d}_6$) gave signals at δ 2.73 (3H, s), 7.07 (1H, dd), 7.17 (1H, dd) and 7.42 (1H, dd) in close agreement with those of the 3-substituted 4-hydroxycoumarin units of ismailin. However, the signals for the juglone-derived unit did not agree with those previously noted for 7-methyljuglone but showed a singlet at δ 2.08 (3H) for a methyl substituent, and aromatic signals at δ 7.23 (1H, dd, ^{3}J 8 Hz and 8 Hz), 7.62 (1H, dd, ^{3}J 8 Hz, ^{4}J 1 Hz) and 7.64 (1H, dd, ^{3}J 8 Hz, ^{4}J 1 Hz); data consistent with that obtained for plumbagin (1b) which is the major extractive of D. canaliculata. The absence of a signal for H(3) of plumbagin suggested that the link was from C(3) of the naphthoquinone to C(3) of the coumarin, in a manner analogous to the linkage in ismailin.

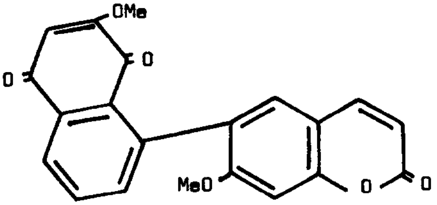
The implied structure (4) for canaliculatin was confirmed by its synthesis from plumbagin and 4-hydroxy-5-methylcoumarin using the method of Jurd.[5] The synthetic canaliculatin was



(4)

identical physically and spectroscopically with the natural product, with m.p. (Kofler) in excess of 300°. One characteristic difference between ismailin and canaliculatin was that the latter only exhibited one hydrogen-bonded hydroxyl signal in the ^{1}H n.m.r. spectrum whereas the former had signals for three such protons. It is suggested that this is due to the two hydroxyl groups of the ismailin coumarin units being able to hydrogen bond to the alternate coumarin unit whereas in canaliculatin this is not possible.

Ismailin and canaliculatin represent a type of naphthoquinone derivative not previously isolated from a natural source. The closest similarity is with the naphthocoumarin, naphthoherniarin (5), from *Ruta graveolens*[7] (Rutaceae) but from a biogenetic standpoint the coumarin unit of naphthoherniarin is derived from cinnamic acid and phenylalanine whilst in ismailin and canaliculatin it is, like the juglone-derivatives, formed from the polymalonate route. A full account of the characterisation of ismailin and canaliculatin (u.v., i.r., mass spectrometry, ^{1}H and ^{13}C n.m.r.) will be given in the full publication.[8]



(5)

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References

1. R. H. Thomson, *Naturally Occurring Quinones* (2nd. Edn.). Academic Press, London, (1972).
2. F. S. P. Ng, *Tree Flora of Malaya*, Vol. 3, p. 56. Longman, Kuala Lumpur, (1978). A voucher specimen has been deposited at the Herbarium of the Forestry Research Institute, Kepong, Malaysia.
3. F. White, *Bull. Jard. Bot. Nat. Belg.*, *48*, 245 (1978). A voucher specimen, GC-46658 has been deposited at the Herbarium of the University of Ghana.
4. T. J. Lillie and O. C. Musgrave, *J. Chem. Soc. Perkin Trans. I*, 355 (1977).
5. L. Jurd, *Aust. J. Chem.*, 33, 1603 (1980).
6. J. I. Okogun, U. V. Enyenihi and D. E. U. Ekong, *Tetrahedron*, *34*, 1221 (1978).
7. Z. Rosza, I. Mester, J. Reisch and K. Szendrei, *Planta Med.*, *39*, 219 (1980).
8. J. A. D. Jeffreys, Muhamad bin Zakaria, P. G. Waterman and S. Zhong, *J. Chem. Soc. Perkin Trans. I*, - to be submitted.

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