

Avicennol: a New Pyranocoumarin from the Root-bark of *Zanthoxylum avicennae*, and its Conversion into Avicennin

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A new angular pyranocoumarin, avicennol (4) {6-(3-hydroxy-3-methyl-*trans*-but-1-enyl)-5-methoxy-2,2-dimethyl-2*H*-benzo[1,2-*b*:3,4-*b'*]dipyr-8-one}, has been isolated from the root-bark of *Zanthoxylum avicennae* (Rutaceae): the structural assignment is based on chemical and spectral evidence. A lanthanide shift reagent and the internal nuclear Overhauser effect have been used to differentiate between possible isomers. Avicennol has been converted into avicennin, previously reported from this and other species; thus the total structure of avicennin is determined. From this species avicennin may be an artefact.

PREVIOUS examinations of the root-bark of *Zanthoxylum avicennae* (Lam.) DC (family Rutaceae; sub-family Rutoideae) yielded the flavonoid glycosides hesperidin and diosmin,^{1a} the benzophenanthridine alkaloid avicine,^{1b} and a novel pyranocoumarin, avicennin.^{1c} The structure of avicennin was partially defined by Arthur and Ollis,² leaving three possible isomers (1)–(3). Avicennin has also been isolated from a second rutaceous species, *Eriostemon coccineus* C. A. Gard.³ We now report the isolation and structural elucidation of a novel coumarin, avicennol (4), from the root-bark of *Zanth-*

oxylum avicennae and confirm the structure of avicennin as (2) by its synthesis from avicennol.

The light petroleum extract of the ground root-bark gave, on cooling, a high yield (2·3%) of yellow crystalline material which proved to be a single compound (t.l.c.). U.v. and i.r. spectra indicated that the compound possessed a coumarin nucleus,⁴ probably having extended conjugation and with a non-phenolic hydroxy-group. Elemental analysis and accurate mass measurement gave the molecular formula C₂₀H₂₂O₅. The mass fragment-

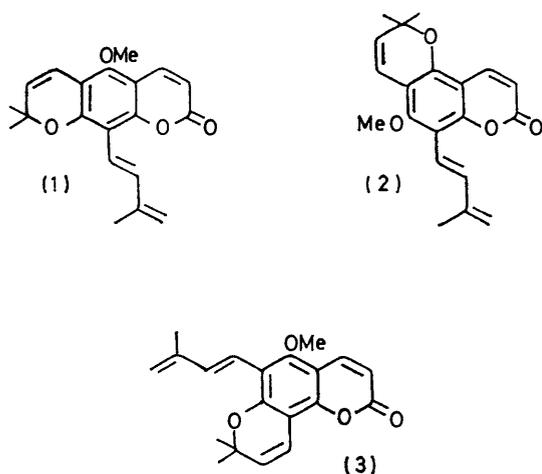
¹ (a) H. R. Arthur, W. L. Hui, and C. N. Ma, *J. Chem. Soc.*, 1956, 632; (b) H. R. Arthur, W. H. Hui, and Y. L. Ng, *ibid.*, 1959, 4007; (c) H. R. Arthur and C. M. Lee, *ibid.*, 1960, 4654.

² H. R. Arthur and W. D. Ollis, *J. Chem. Soc.*, 1963, 3910.

³ P. W. Chow, A. M. Duffield, and P. R. Jeffries, *Austral. J. Chem.*, 1966, **19**, 483.

⁴ B. E. Nielsen, *Dansk Tidsskr. Farm.*, 1970, **44**, 111.

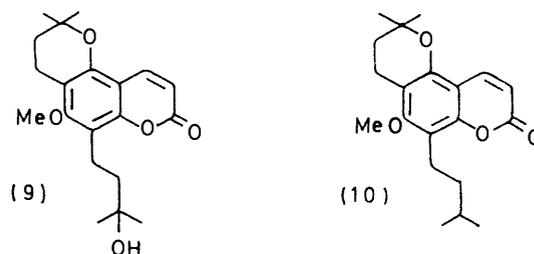
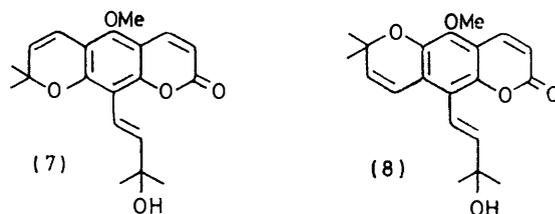
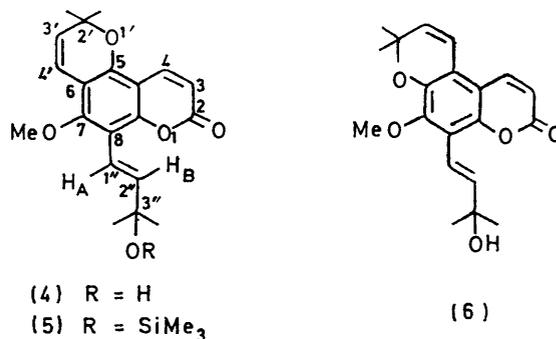
ation pattern was simple and suggested only loss of water and a methyl radical to give, as base peak, the ion $C_{19}H_{17}O_4$.



The n.m.r. spectrum defined all twenty-two protons. The occurrence of an AB quartet (J 10 Hz) with one half downfield at δ 8.06 confirmed the presence of the $\alpha\beta$ -unsaturated δ -lactone ring of the coumarin nucleus.^{4,5} A second AB quartet (J 10 Hz) and a signal for *gem*-methyl groups at δ 1.48 indicated the presence of the 2,2-dimethylchromen ring system encountered in pyranocoumarins.⁶ A three-proton singlet at δ 3.80 indicated the presence of a methoxy-substituent, leaving only hydroxy- and *trans*-olefinic (J 16 Hz) protons and two further *gem*-methyl groups unaccounted for. As one of the two remaining positions on the benzenoid ring of the pyranocoumarin must be filled by the methoxy-group these signals can only be attributed to a single unit C_5H_9O . This can be accounted for by the unusual 3-hydroxy-3-methyl-*trans*-but-1-enyl side-chain previously encountered only by Chan *et al.*⁷ in chromones from the rutaceous plant *Spathelia sorbifolia* L. Despite the helpful n.m.r. spectrum of avicennol there remained twelve possible isomers varying in the positioning of the substituents on the benzene ring.

A large contribution to the determination of the absolute structure of avicennol was made by using the lanthanide shift reagent tris-(7,7-dimethyl-1,1,1,2,2,3,3-heptafluoro-octane-4,6-dionato)europium(III) [Eu(fod)₃] according to a method described elsewhere.⁸ We have shown⁸ that Eu(fod)₃ complexes with the carbonyl oxygen atom of coumarins in such a way that an 8-substituent shows a large downfield shift. With avicennol itself results were complicated by preferred complexation at the side-chain hydroxy-group, so this group was masked by formation of the *O*-trimethylsilyl derivative (5). Complexation then proceeded as expected. The same approach as pre-

viously⁸ was used, and the best fit was obtained between observed and calculated shifts for values of ϕ (the C-O-Eu angle) = 152.5° and d (the O-Eu distance) = 2.75 \AA . This gave exactly the observed shift for the H-4 (0.31), relative to H-3* (1.00, by definition⁸) and reasonable values for all other shifts. We give in detail only the observed shifts for the side-chain olefinic protons



and those calculated with the side-chain in the four possible positions on the coumarin benzene ring (Table 1).

TABLE 1

Observed shifts for H_A and H_B of *O*-trimethylsilylavicennol (5) and their calculated values for attachment of the side-chain at positions 5, 6, 7, and 8 (relative of H-3 shift = 1.00)

	Observed	Calculated for			
		C-5	C-6	C-7	C-8
H _A	0.40	0.12	0.11	0.15	0.33
H _B	0.52	0.15	0.11	0.15	0.45

The calculations are based on average values for the two possible conformations of the side-chain double bond in

⁷ W. R. Chan, D. R. Taylor, and C. R. Willis, *J. Chem. Soc. (C)*, 1967, 2540.

⁸ A. I. Gray, R. D. Waigh, and P. G. Waterman, *J.C.S. Chem. Comm.*, 1974, 632.

* In order to clarify the text, the non-systematic numbering system illustrated in formula (4) is used to refer to positions on the avicennol skeleton.

⁵ E. V. Lassak and J. T. Pinhey, *J. Chem. Soc. (C)*, 1967, 2000.

⁶ T. Tomimatsu, M. Hashimoto, T. Shingu, and K. Tori, *Chem. Comm.*, 1969, 168.

the plane of the benzene ring and therefore are only approximate, since the side-chain conformation is not known. However, it is clear that only a side-chain in the 8-position offers anything approaching a shift of sufficient magnitude. This observation reduces the possible structures for avicennol from twelve to four (4) and (6)—(8). Further distinction between structures on the basis of $\text{Eu}(\text{fod})_3$ -induced shifts is less certain, since the shifts for protons of substituents in the 5-, 6-, and 7-positions are smaller, and so, therefore, are the calculated differences amongst the four remaining isomers. However, the observed methoxy-shift (0.15) offers clear evidence in favour of structures (4) and (6) (calculated shift 0.15) rather than (7) and (8) (calculated shift 0.11).

The structure (4) for avicennol was confirmed by measurement of the intramolecular nuclear Overhauser effect (n.O.e.) involving the methoxy-group and the olefinic protons; this technique was used earlier⁶ for a similar pyranocoumarin. The observed n.O.e. is shown in Table 2. Structure (4) is the only one in which the

TABLE 2

Observed nuclear Overhauser effects for avicennol on saturation of the methoxy signal at δ 3.80
Increase in integrated intensities of signals of

H-3	H-4	H-3'	H-4'	H-1'' and -2''
Nil	Nil	Nil	9%	5%

methoxy-group is close to both H-4' and the side-chain olefinic protons, if structures which do not fit the shift reagent data are excluded.

It seemed possible that previous isolation^{1c,2} of avicennin (in small quantity) rather than avicennol (in bulk) from *Z. avicennae* may have been due to differences in extraction and purification techniques, which in the earlier work involved treatment with acid, base, and boiling chloroform. Treatment of pure avicennol with acid and base as described^{1c} gave no useful result, but phosgene quickly effected the dehydration, producing avicennin in high yield, with the same physical constants as already reported.^{1c,2} Thus avicennin has the structure (2).

Catalytic hydrogenation of avicennol afforded two products: the expected tetrahydroavicennol (9), m.p. 165—165.5°, and hexahydroavicennin (10), m.p. 81—82°, for which the data obtained were in agreement with those reported by Arthur and Ollis.²

N.m.r. shift experiments with avicennin and hexahydroavicennin gave results substantially similar to those with the trimethylsilyl ether of avicennol.

EXPERIMENTAL

M.p.s were determined with a Kofler hot-stage apparatus. U.v. spectra (solvent ethanol) were recorded with a Unicam SP 800A spectrophotometer, and i.r. spectra (for KCl discs) with a Perkin-Elmer 237 grating spectrophotometer. Optical rotations were measured with a Bellingham and Stanley Polarimeter. N.m.r. spectra were recorded for solutions in deuteriochloroform with a Perkin-Elmer R12 60

MHz instrument, with the exception of the n.O.e. experiment in which a Perkin-Elmer 90 MHz instrument was used. Mass spectra and accurate mass determinations were obtained with an A.E.I. MS 902 spectrometer.

Extraction of the Root-bark.—The dried, powdered root-bark (150 g) of *Zanthoxylum avicennae** was extracted (Soxhlet) with light petroleum (b.p. 40—60°; 3 l) for 10 h. On cooling, pale yellow crystals (3.55 g) were deposited from the extract. T.l.c. on alumina (Woelm; activity I), with chloroform-methanol (98 : 2) as eluant, showed a single spot (R_F 0.4) fluorescing orange under screened u.v. light (366 nm).

Avicennol (4).—Recrystallised from n-hexane-ethyl acetate (99 : 1) *avicennol* gave yellow plates, m.p. 124.5—125.5°, $[\alpha]_D^{21}$ 0° (*c* 1.00 in CHCl_3) (Found: C, 70.35; H, 6.65%; M^+ , 342.1463. $\text{C}_{20}\text{H}_{22}\text{O}_5$ requires C, 70.2; H, 6.45%; M , 342.1467), λ_{max} 250 (log ϵ 4.51), 257 (4.61), and 301 nm (4.28), ν_{max} 3480 (OH), 2960, 1725 (CO), 1585, 1255, and 1140 cm^{-1} , δ 1.48 (12H, s, 2'-Me₂, 3''-Me₂), 2.58 (1H, s, exchanged with D₂O, OH), 3.80 (3H, s, OMe), 6.65 and 5.69 (2H, ABq, *J* 10 Hz, H-4' and -3'), 6.95 and 6.81 (2H, ABq, *J* 16 Hz, H-1'' and -2''), and 6.27 and 8.06 (2H, ABq, *J* 10 Hz, H-3 and -4), *m/e* 342 (M^+ , 3%), 327 ($\text{C}_{20}\text{H}_{20}\text{O}_4$, 78), 309 ($\text{C}_{19}\text{H}_{17}\text{O}_4$, 100), 277 (14), 263 (6), 253 (5), 251 (8), and 162 (3).

3''-O-Trimethylsilylavicennol (5).—Avicennol (120 mg) was dissolved in benzene (sodium-dried) and an excess of *NO*-bis(trimethylsilyl)acetamide was added. The reaction was followed by t.l.c. on alumina with the system already described. After 4 h, the mixture was evaporated to dryness under reduced pressure, and the resulting yellow *solid* recrystallised from methanol; m.p. 100—101° (Found: M^+ , 414.1840. $\text{C}_{23}\text{H}_{30}\text{O}_5\text{Si}$ requires M , 414.1862), λ_{max} 251 (log ϵ 4.93), 257 (4.94), and 301 nm (4.62), ν_{max} 2950, 1730, 1585, 1140, 1050, and 840 cm^{-1} (Si-C), δ 0.18 (9H, s, SiMe₃), 1.48 (12H, s, 2'-Me₂, 3''-Me₂), 3.80 (3H, s, OMe), 6.65 and 5.69 (2H, ABq, *J* 10 Hz, H-4', H-3'), 6.95 and 6.81 (2H, ABq, *J* 16 Hz, H-1'', H-2''), and 6.27, 8.06 (2H, ABq, *J* 10 Hz, H-3 and -4), *m/e* 414 (M^+ , 24%), 399 ($\text{C}_{22}\text{H}_{27}\text{O}_5\text{Si}$, 100), 371 (12), 279 (21), and 180 (60).

Catalytic Hydrogenation of Avicennol.—Avicennol (200 mg) in absolute ethanol (100 ml) was hydrogenated for 10 h at room temperature and atmospheric pressure over Adams catalyst (20 mg). The mixture was then filtered; t.l.c. on alumina (as already described) indicated two compounds, R_F 0.4 (A) and 0.7 (B), both showing a blue fluorescence in screened u.v. light (366 nm). Separation was achieved by preparative t.l.c. on neutral alumina (Woelm activity I) with chloroform-ethanol (98 : 2) as eluant. The products were extracted from the bands with chloroform.

Compound (A) was recrystallised from methanol to give *tetrahydroavicennol* (9) as prisms, m.p. 165—165.5° (Found: M^+ , 346.1779. $\text{C}_{20}\text{H}_{26}\text{O}_5$ requires M , 346.1780), λ_{max} 213 (log ϵ 4.42), 258 (3.84), and 316 nm (4.09), ν_{max} 3420 (OH), 2940, 1710 (CO), 1600, and 1115 cm^{-1} , δ 1.35 (6H, s, Me₂), 1.39 (6H, s, Me₂), 1.83 (4H, m, 2''- and 3'-H₂), 1.96 (1H, s, exchanged with D₂O, OH), 2.83 (4H, m, 1''- and 4'-H₂), 3.90 (3H, s, OMe), and 6.27 and 8.12 (2H, ABq, *J* 10 Hz, H-3 and -4), *m/e* 346 (M^+ , 21%), 331 (6), 329 (15), 328 (66), 313 (12), 273 ($\text{C}_{16}\text{H}_{17}\text{O}_4$, 100), and 217 (51).

Compound (B) was recrystallised from light petroleum (b.p. 40—60) to give hexahydroavicennin (10) as white

* Voucher Specimen No. STRATHCLYDE 30 has been deposited at the Herbarium of the Royal Botanic Garden, Edinburgh.

prisms, m.p. 81—82° (lit.,^{1c} 81—82°) (Found: M^+ , 330·1824. Calc. for $C_{20}H_{26}O_4$: M , 330·1831), λ_{\max} . 217, 257, and 315 nm, ν_{\max} . 2920, 1725 (CO), 1600, and 1120 cm^{-1} , m/e 330 (M^+ , 91%), 275 (29), 274 (35), 273 ($C_{16}H_{17}O_4$, 100), and 217 (23), n.m.r. spectrum identical with that previously recorded.²

Avicennin (2).—Avicennol (100 mg) was dissolved in benzene (sodium-dried) and a 12·5% solution (0·25 ml) of phosgene in toluene added. After 3 h the excess of phosgene was degraded by addition of absolute ethanol (1 ml). The mixture was evaporated to dryness to yield a solid which was recrystallised from methanol to give avicennin as yellow needles, m.p. 138—140° (lit.,³ m.p. 136·5—138·5°) (Found: M^+ , 324·1359. $C_{20}H_{20}O_4$ requires M , 324·1361), λ_{\max} . 270, 278, and 301 nm, ν_{\max} . 2940, 1730 (CO), 1585, and 1140 cm^{-1} , n.m.r. spectrum identical with that previously recorded.²

N.m.r. Shift Experiments.—All experiments were carried out with CDCl_3 as solvent, tetramethylsilane as internal standard, and $\text{Eu}(\text{fod})_3$ as shift reagent. The weighed sample (60—80 mg) was dissolved (0·3—0·5 ml solvent) and the spectrum recorded. The shift reagent was then added in weighed quantities of *ca.* 10 mg, at least four spectra

being recorded for each compound, each with a larger quantity of shift reagent. Graphs were then plotted of shift (Hz) *versus* weight of $\text{Eu}(\text{fod})_3$ added to find shifts for all protons, which were then expressed ⁸ relative to that of H-3 (= 1·00). The results were as follows: avicennol (4) H-3 (1·00), H-4 (0·40), H-2'' (2·56), H-1'' (2·08), MeO (0·40), H-4' (0·26), H-3' (0·14), 2'-Me₂ (0·14), 3''-Me₂ (1·59), OH (8·57); 3''-O-trimethylsilylavicennol (5) H-3 (1·00), H-4 (0·31), H-2'' (0·52), H-1'' (0·40), MeO (0·15), H-4' (0·13), H-3' (0·08), 2'-Me₂ (0·08), 3''-Me₂ (0·13); avicennin (2) H-3 (1·00), H-4 (0·29), H-2'' (0·38), H-1'' (0·41), MeO (0·13), H-4' (0·12), H-3' (0·08), 2'-Me₂ (0·08), 3''-Me (0·09), 4''-H₂ (0·03, 0·09); hexahydroavicennin (10) H-3 (1·00), H-4 (0·30), 2''-H₂ (0·24), 1''-H₂ (0·36), MeO (0·13), 4'-H₂ (0·13), 3'-H₂ (0·11), 2'-Me₂ (0·09), 3''-Me₂ (0·11).

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