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ANTHRAQUINONES AND ANTHRAQUINOLS FROM *MORINDA LUCIDA*

THE BIOGENETIC SIGNIFICANCE OF ORUWAL AND ORUWALOL

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Abstract—Two novel anthraquinols—oruwal and oruwalol; ten anthraquinones: damnacanthal, nor-damnacanthal, soranjidiol, alizarin-1-methyl ether, rubiadin, rubiadin-1-methyl ether, 2-methylanthraquinone, anthraquinone-2-aldehyde, 1-hydroxy-2-methylanthraquinone, 1-methoxy-2-methylanthraquinone; and hexacosanoic acid were obtained from the stem of *Morinda lucida*. The structure of oruwal was confirmed as 9,10-dimethoxyanthracene-2-aldehyde by synthesis, while oruwalol was characterized as 5- or 8-hydroxy-9, 10-dimethoxyanthracene-2-aldehyde from analytical and spectroscopic evidence. The biogenetic significance of the co-occurrence of the anthraquinones, oruwal and oruwalol is discussed.

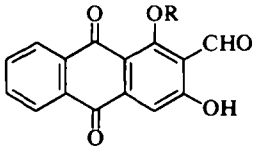
We reported in our preliminary communication[1] the isolation of four known anthraquinones: damnacanthal (1), alizarin-1-methyl ether (2) rubiadin-1-methyl ether (3) soranjidiol (4) and a novel anthraquinol pigment oruwal (5) from the light petroleum extract of the stem of *Morinda Lucida* Benth. (Rubiaceae). Six more known anthraquinones: 2-methylanthraquinone, 1-hydroxy-2-methyl-anthraquinone (7), 1-methoxy-2-methyl-anthraquinone (8), rubiadin (9), nor-damnacanthal (6), anthraquinone-2-aldehyde are characterised. Apart from soranjidiol and alizarin-1-methyl ether, the structures of the known anthraquinones are confirmed either by synthesis or by comparison with authentic specimens. In addition structure is assigned to a new compound m.p. 198–201°, which we name oruwalol.

Like many other plants, *Morinda lucida* is chemically very variable depending on the locality where it is found. Specimens were collected from two main sources: some in Ibadan—near Eleiyele and others in Akure about 150 miles north east of Ibadan. The specimens from both sources contain the same sort of anthraquinones, but no anthraquinol derivative was found in the samples from Akure. Indeed it was in a second attempt to confirm the presence of oruwal in another Ibadan tree that oruwalol was isolated along with oruwal and other anthraquinones.

The crude extracts from *Morinda lucida* were either subjected to successive chromatography on silica gel or fractionated using potassium bicarbonate, sodium carbonate, sodium hydroxide. Latterly the combination of both methods was used as will be reported in the experimental section. Light petroleum eluted an oil and an aliphatic acid M^+ 396 whose fragmentation pattern and other spectral and analytical data suggest it to be hexacosanoic acid. Light petroleum: ether (9:1) mixture eluted in succession oruwal, a white crystalline solid m.p. 162°, M^+ 426, and soranjidiol from either a silica gel or deactivated alumina column. The solid m.p. 162° M^+ 426, was not studied further, but its IR and NMR indicate it to be a steroid.

Oruwal, m.p. 157°, M^+ 266 was obtained from a highly fluorescent fraction after evaporation. The IR spectrum shows no band at *ca* 1580 cm^{-1} characteristic of anthraquinones while the UV absorption spectrum shows weak absorptions at higher wavelength (> 330 nm) as in anthracene. The NMR spectrum shows resonances attributed to two groups of aromatic methoxy protons (δ4·11 and 4·18 ppm), an aldehydic proton (δ10·2 ppm), and seven aromatic protons of which six resonate between δ7·5 and 8·47, while the seventh at 8·80 (J = 2Hz) suggests meta coupling and an adjacent deshielding nucleus. On the basis of these spectra data and analytical data, oruwal was regarded as a dimethoxyanthracene aldehyde, with the aldehydic function at C-2.

Heating oruwal in sulphuric acid for 3 min gave a pale yellow product m.p. 185–187°, M^+ 236. The IR, ν_{max} (Nujol) 1690, 1660, 1590 and 710 cm^{-1}, indicated an anthraquinone with one aromatic ring unsubstituted.[4] Detailed study of the NMR which revealed the loss of the two OMe groups, the presence of one aldehyde proton, and seven aromatic protons allowed the unambiguous assignment of the transformation product as the known anthraquinone-2-aldehyde which was also obtained as a natural product from a later fraction, and whose identity has been confirmed by synthesis. Oruwal is there-



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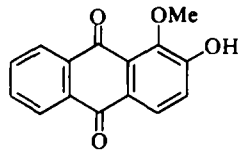

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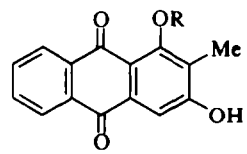

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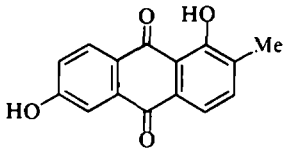

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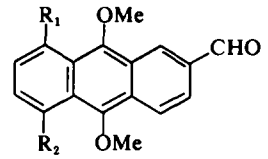
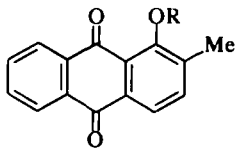
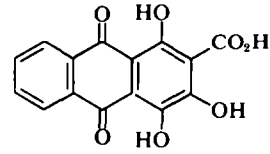


5: $R_1 = R_2 = H$
6a: $R_1 = H, R_2 = OH$
b: $R_1 = OH, R_2 = H$



7: R = H
8: R = Me



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fore 9,10-dimethoxyanthracene -2-aldehyde (**5**). The mass spectral fragmentation of oruwal with prominent ions at *m/e* 266, 251, 235, 223, 207, 193, 179, 165 and 151, due to cleavages of the OMe groups followed by expulsion of carbon monoxide, supports the assignment.

Final proof of the structure has been achieved by synthesis. Anthraquinone-2-aldehyde was reductively methylated in an inert atmosphere to give oruwal in very good yield.

The third compound oruwalol which was eluted by light petroleum: ether (3:1) as a faint fluorescent fraction was shown by elemental analysis and by its mass spectrum to have the formula $C_{17}H_{14}O_4$, which is one O atom more than oruwal. The IR spectrum does not have a band at *ca* 1580 cm^{-1} characteristic of anthraquinones, and apart from the OH peak at ν_{max} 3320 cm^{-1}, it is similar to that of oruwal. The UV absorption spectrum was also very similar to that of oruwal, but the λ_{max} at 284 nm of the most intense peak represents a small shift to longer wavelength due to the presence of an OH group. The difference in their fluorescence makes comparison of their absorbance unreliable. The most prominent peaks in the mass spectrum apart from base peak, M^+ 282, at *m/e* 267, 251, 223, 195, 167, and 139 due to the loss of Me radicals and carbon monoxide also suggest that oruwalol is a hydroxyoruwal. The NMR spectrum shows resonances attributed to two aromatic OMe resonances (δ4·08 and 4·18 ppm), an aldehydic proton (δ10·2) a phenolic -OH (δ9·60 ppm, disappeared with D_2O), and six aromatic protons of which five resonate between δ 6·9 and 8·37, while the sixth at δ 8·62 ppm (J = 2Hz), suggests meta coupling, and an adjacent deshielding nucleus. H-1 is probably present as in oruwal.

All the data suggest that oruwalol is a monohydroxyoruwal, whose OH function is not at C1. The 3-position is rejected on the ground that NMR shows no protons that would be equivalent to H-1 and H-4 in a 3-substituted oruwal. Furthermore the OH group cannot be on C-6 or C-7 because there was no other singlet or narrow doublet attributable to H-5 or H-8: careful study of the well spaced out signals between δ6·9 and 8·37 precludes the possibilities of other signals obscuring either an H-5 or H-8. Therefore oruwalol is either 5-hydroxy or 8-hydroxyoruwal (**6a** or **6b**). Two attempts at demethylating oruwalol by a method similar to that used for oruwal failed; in both cases there was some charring and work up of the reaction product did not yield any anthraquinone or anthracene derivative. Unfortunately, insufficient of the compound remained for other attempts at degradation.

We think that the isolation of oruwal and

oruwalol is of biogenetic interest. Anthraquinones are biogenetically derivable by the acetate–malonate pathway,[2-4] or by the shikimic acid-mevalonate pathway.[3-6] The isolated anthraquinones from *Morinda lucida* are structurally related to pseudopurpurin (**11**) which is known to be derived from shikimate and mevalonate precursors.[6] The co-occurrence of these anthraquinones with oruwal and oruwalol supports the idea that anthraquinones in Rubiaceae are formed by prenylation of a naphathol precursor: it is conceivable that oxidative cyclization of such a precursor will give rise to oruwal or oruwalol and subsequently their congeners.

EXPERIMENTAL

M.ps were taken with a Kofler hot-stage apparatus and are uncorrected. IR spectra were measured for Nujol mulls, and UV spectra are for solns in cyclohexane unless stated otherwise. NMR spectra were taken for solns in $CDCl_3$ at room temperature with a Varian A 56/60 instrument. Mass spectra were recorded with a Perkin-Elmer Hitachi RMU6E instrument. Light petroleum refers to the fraction of b.p. 60–80°, Silica gel refers to Merck or MN (Machery, Nagel and Co), Mesh 0·05mm–0·2mm.

Extraction. Morinda lucida stem was extracted with boiling light petroleum. Concentration gave a residue which was chromatographed over silica gel. Elution with light petroleum gave an oil and a crystalline compound m.p. 85–87°M^+ 396. The mass spectra fragmentation pattern, the presence of -COOH (IR, NMR, chemical test) suggest the solid is hexacosanoic acid. Elution with light-petroleum: ether (9:1) mixture gave a solid m.p. 271–273°M^+ 254, soranjidiol, whose structure was confirmed by comparison with recorded literature data; oruwal (**5**), crystallised from MeOH in yellow needles m.p. 157–158°M^+ 266, (Found: C, 76·9; H, 5·7, $C_{17}H_{14}O_3$ requires C, 76·7, H, 5·3%); ν_{max} 1695, 1680, 1620w and 695 cm^{-1}; λ_{max} 238sh. 243, 272sh, 277, 360, and 375 nm. (log ϵ 4·41, 4·43, 4·76, 4·81, 3·63 and 3·75); Addition of a drop of NaOH to a methanolic soln produced no change as to be expected but 2 drops of HCl gave new spectrum bands λ_{max} 253, 262, 354, 375 nm. A solid M^+ 426, m.p. 162° was also eluted with this mixture. Further elution with light petroleum: ether (3:1) mixture gave **12**, as pale yellow crystals from ether: light petroleum (1:1) m.p. 198–201°M^+ 282 (Found: C. 72·5, H, 5·25; $C_{17}H_{14}O_4$ requires: C, 72·3; H, 5·0 %) ν_{max} 3320 (OH) 1685(m) 1675(s), 1610w cm^{-1}; λ_{max} 233, 238, 245, 253, 284, 362 and 382 nm. [log ϵ 4·63, 4·65, 4·67, 4·70, 4·87, 4·43 and 4·46.] The methanolic soln absorbs at λ_{max} 255, 287, 344 and 383 nm. Addition of one drop NaOH gave a broad absorption λ_{max} 256 sh, 281, 392 nm, which narrowed considerably on adding 2 drops HCl to λ_{max} 247, 267, 355, 372 nm.

The column was thereafter eluted with MeOH. The eluate was concentrated and the residue taken in cold chloroform and filtered. The insoluble material was dissolved in refluxing chloroform and adsorbed on silica gel. The preparation was introduced dry to a silica gel column. Ether eluted damnacanthal while ether: MeOH (9:1) eluted nor-damnacanthal from the column. The filtrate was extrated successively with $KHCO_3$ (2M), Na_2CO_3 (1M), and NaOH(1M), acidified and reextracted with chloroform to give portions A, B, and C respectively. Portion D was the chloroform soln left after the base treatments. All the four portions were separately chromatographed on silica gel columns.

Chromatography of portion A. Light petroleum: ether (3:1) eluted rubiadin, the mixture (2:1) eluted rubiadin-1-methyl ether (identical with authentic samples), while ether eluted alizarin-1-methyl ether m.p. 181–183°, M^+ 254 whose structure was deduced by analysis of the spectra, and comparison of its data with those in literature, and confirmed by demethylation to give alizarin.

Chromatography of portion B. Light petroleum: ether (3:1) eluted rubiadin and rubiadin-1-methyl ether; damnacanthal came down the column with ether.

Chromatography of portion C. Light petroleum: ether (2:1) eluted 2-methyl-anthraquinone whose structure was confirmed by synthesis. Damnacanthal was also eluted with ether.

Chromatography of portion D. Light petroleum: ether (9:1) eluted 1-hydroxy-2-methylanthraquinone while 1-methoxy-2-methylanthraquinone was eluted by the mixture (3:1), structures of both compounds were confirmed by comparison with authentic specimens.* More 2-methyl-anthraquinone was eluted by the mixture (3:1) while the mixed solvents (2:1) eluted anthraquinone-2-aldehyde, whose structure was confirmed by synthesis. Damnacanthal was eluted with ether.

Although there is quantitative difference in the amounts of compounds isolated from different plants, the last specimen investigated gives a fair picture of the yields to be expected. 10 kg fresh stem gave damnacanthal 2·4 g; nor-damnacanthal, 40g; alizarin-1-methyl ether, 90mg; soranjidiol, 260mg; anthraquinonone-2-aldehyde 180mg; 2-methylanthraquinone, 80mg; 1-hydroxy-2-methyl-anthraquinone, 45mg; 1-methoxy-2-methylanthraquinone, 30mg; rubiadin, 220mg; rubiadin-1-methyl ether, 250mg; oruwal 94mg, and oruwalol 23mg.

Demethylation of oruwal. Oruwal (25mg.) in conc H_2SO_4 (2·5ml) was heated at 150–160°. The mixture turned deep blue after 30 sec and then reddish brown within 3 min. Water (excess) was added to the cooled soln, and evaporation of the chloroform extract gave a pale yellow solid m.p. 182–185°, M^+ 236, (m.p. 185–187° on recrystallization) identical with anthraquinone-2-aldehyde.

Synthesis of oruwal. Anthraquinone-2-aldehyde (0·6g) was suspended in MeOH (8ml) and water (2ml) on a hot water bath. With N_2 gas passing through the mixture, saturated aqueous soln of sodium dithionite was added. The mixture turned red and the soln was refluxed for 10 min. Then a mixture of 2N NaOH (20ml) and $MeSO_4$ (5ml) was added cautiously. The red soln turned blue and after about 1 min turned red, and there was some evolution of foul odour (SO_2!). After 5 min the mixture turned yellow with a brown ppt at the bottom; refluxing was continued for a further 15 min; cooled and filtered to give crude oruwal (0·62g) m.p. 150–154°. Dissolution in chloroform, filtering and careful crystallisation afforded pure oruwal, which recrystallised from MeOH m.p. 157° and mixed m.p. 156–158° with a natural sample.

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