The Structures of Alboleersin and Luteoleersin; the Identity of Luteoleersin with Cochlioquinone A

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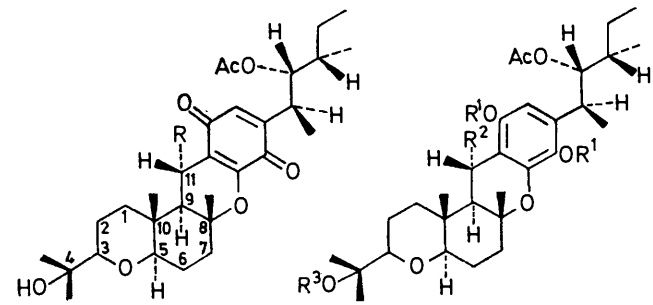
Structural studies of alboleersin and luteoleersin, two metabolites of the plant pathogen fungus *Helminthosporium leersii*, have established that luteoleersin is identical with cochlioquinone A, a metabolite of *Cochliobolus miyabeanus*, and that alboleersin is the corresponding hydroquinone. A number of derivatives characterizing these metabolites are described.

THE fungus *Helminthosporium leersii* Atkinson is widely distributed in America and has been found as a parasite on the leaf blades of *Leersia virginica* (*Homolocenchrus virginicus*).[1] In 1938 Ashley and Raistrick[1] isolated two related metabolites from cultures of *H. leersii*, which they named luteoleersin and alboleersin. They reported that luteoleersin behaved as a quinone or semiquinone and suggested that alboleersin was the corresponding phenol. A limited number of chemical transformations and attempted reactions were also reported.

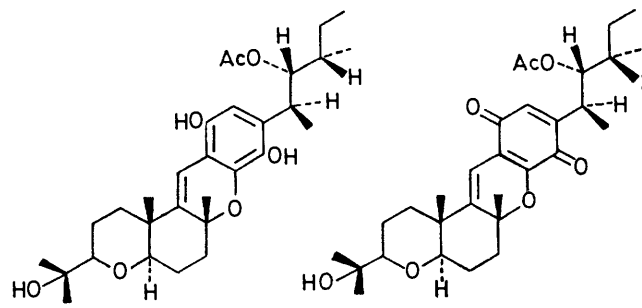
Shortly after we had commenced structural investigations on these original samples it became apparent that luteoleersin was identical with cochlioquinone A (Ia), $C_{30}H_{44}O_8$, a metabolite of *Cochliobolus miyabeanus*.[2] The structure of cochlioquinone A has recently been determined from chemical, spectroscopic, and *X*-ray crystallographic evidence.[2] We propose that the use of the names alboleersin and luteoleersin be discontinued.

Raistrick[1] assigned the formulae $C_{26}H_{40}O_7$ and $C_{26}H_{38}O_7$ to the hydroquinone and quinone, respectively, on the basis of elemental analysis. The mass spectrum of the quinone (Ia) shows a molecular ion at *m/e* 532 and mass measurement established the formula $C_{30}H_{44}O_8$. The hydroquinone (IIa) showed a very weak molecular ion at *m/e* 534, but a much stronger ion at *m/e* 516 ($M^+ - H_2O$) suitable for mass measurement; the formula of this ion is $C_{30}H_{44}O_7$, and therefore that of the hydroquinone (IIa) is $C_{30}H_{46}O_8$. These formulae agree well with the elemental analyses. The i.r. spectrum of the hydroquinone (IIa) shows acetate and hydroxy-bands, and that of the quinone (Ia) shows acetate, *p*-quinone, and hydroxy-absorption. The u.v. spectrum of compounds (Ia) and (IIa) show maxima typical of a *p*-benzoquinone and a hydroquinone, respectively. As compound (IIa) forms a tetra-acetate (IIc) (see later) and the two remaining oxygen functions are chemically inert (presumably ethereal), and since there is no spectroscopic evidence for any further unsaturation, the molecule is presumably tetracyclic. The n.m.r. spectrum of compound (Ia) shows a signal at τ 3·54 (1H, s) for the single olefinic proton, and a double doublet at τ 5·05



(I) a; R = OH
b; R = H

(II) a; $R^1 = R^3 = H$, $R^2 = OH$
b; $R^3 = H$, $R^2 = OH$, $R^1 = Me$
c; $R^1 = R^3 = Ac$, $R^2 = OAc$
d; $R^1 = R^2 = R^3 = H$



(III) (IV)

(J 4·6 and 7·2 Hz) is assigned to a >CH–C*H*(OAc)–CH< system. The allylic proton at C-11 appears as a doublet at τ 5·15 (J 10·5 Hz) and is disposed *trans* diaxially to the

[1] J. N. Ashley and H. Raistrick, *Biochem. J.*, 1938, **32**, 449.
[2] J. R. Carruthers, S. Cerrini, W. Fedeli, C. G. Casinovi, C. Galeffi, A. M. Torracca Vaccaro, and A. Scala, *Chem. Comm.*, 1971, 164.

proton at C-9. The overlapping multiplets at τ 6·65—7 (3H) are due to the protons at C-3 and C-5 (each α to an ethereal oxygen atom) and the allylic proton of the side chain. At higher fields the following signals appear: τ 8·08 (OAc), 8·82 (4-Me_2), 8·89 (8-Me), 8·68 (10-Me), 8·85 and 9·11 (each d, J 6·5 Hz, secondary Me groups of side chain), and 9·12 (t, J 6·5 Hz, terminal Me).* In the hydroquinone (IIa) the olefinic proton signal is at higher field (τ 3·75).

The presence of the acetate group in (Ia) was verified by mild basic hydrolysis and characterization of the acetic acid formed. The hydroquinone (IIa) formed a dimethyl ether (IIb), $C_{32}H_{50}O_8$, on treatment with diazomethane and a tetra-acetate (IIc), $C_{38}H_{54}O_{12}$, on vigorous acetylation. The derivative (IIc) shows no OH absorption bands in the i.r. spectrum. The quinone (Ia) rapidly absorbs 1 mol. equiv. of hydrogen on catalytic hydrogenation, forming the hydroquinone (IIa), and then a slower reaction occurs as a further equiv. of hydrogen is absorbed. The product is the deoxyhydroquinone (IId), $C_{30}H_{46}O_7$, the benzylic alcohol having undergone hydrogenolysis. This hydroquinone (IId) is prone to aerial oxidation; the spectrum of the initially colourless product invariably shows a weak band at $\lambda_{max.}$ 390 nm (ε 100—200) presumably due to the quinone (Ib). Treatment of the hydroquinone (IIa) with strong acid yields a product (III), m.p. 174—175°, previously thought [1] to be an isomer of (IIa). Mass measurement of the molecular ion at *m/e* 516 established the formula as $C_{30}H_{44}O_7$, *i.e.* that of a dehydration product of (IIa), and there is no evidence of an ion at *m/e* 534 [*cf.* (IIa)]. The u.v. spectrum, $\lambda_{max.}$ 330 (ε 3500) and 289 nm (13,100), shows the extended chromophore in (III). The n.m.r. spectrum shows a new signal at τ 3·6 (1H, s) and there is no doublet at τ 5·15 (J 10·5 Hz) [*cf.* (Ia)]. The only other differences in the n.m.r. spectrum are minor changes in chemical shift, and we assign the chromen structure (III) to this product. The red product [1] obtained by oxidation of (III) with iron(III) chloride is therefore the quinone (IV). The i.r. spectra of the hydroquinones (IIa), (IId), and (III) show exceptionally low frequency carbonyl absorption at 1700 cm^{-1} for the acetate in the side chain. After oxidation, methylation, or acetylation of the phenolic hydroxy-groups [compounds (Ia), (IIb), and (IIc)] this absorption has moved to 1720 cm^{-1}. Possibly this arises from hydrogen bonding to the phenolic hydroxy-group in a nine-membered ring. Models show that after formation of the hydrogen bond the acetate grouping can come within the deshielding area of the aromatic ring; this could explain the unusually high chemical shift (τ 8·21) of the acetate in the n.m.r. spectrum of (IIa). An authentic sample of cochlioquinone A † showed i.r., u.v., n.m.r., and mass spectra identical with those of the quinone (Ia).

EXPERIMENTAL

M.p.s were taken on a Kofler hot-stage apparatus. I.r. spectra were recorded on a Unicam SP 200 spectrophotometer for solutions in chloroform or Nujol mulls. N.m.r. spectra were recorded for solutions in deuteriochloroform on a Varian HA-100 spectrometer. Mass spectra were taken at 70 eV on an A.E.I. MS9 spectrometer. Rotations were measured for solutions in chloroform on a Perkin-Elmer 141 polarimeter.

Cochlioquinone A (Ia).—The sample of luteoleersin (m.p. 126—135°) was repeatedly crystallized from ethyl acetate–light petroleum (b.p. 60—80°) and finally from light petroleum alone, yielding cochlioquinone A (Ia) as yellow needles, m.p. 132—136° (lit.,[1] 130—132°), $[\alpha]_D^{23}$ +158° (*c* 0·09), $\nu_{max.}$ 1720 (C=O) and 3350 cm^{-1} (OH), $\lambda_{max.}$ 390 (ε 900), 280 (11,000), and 245 nm (3300), τ 3·54 (1H, s), 5·05 (1H, dd, J 4·6 and 7·2 Hz), 5·15 (1H, d, J 10·5 Hz), 6·65—7 (3H, m), 8·08 (3H, s), 8·68 (3H, s), 8·82 (6H, s), 8·85 (3H, d, J 6·5 Hz), 8·98 (3H, s), 9·11 (3H, d, J 6·5 Hz), and 9·12 (3H, t, J 6·5 Hz), *m/e* 532 (M^+), 516, 514, 501, 499, 473, 456, 441 (100%), and 386 (Found: M^+ 532·2981. Calc. for $C_{30}H_{44}O_8$: M, 532·3036). The mother liquors from this purification were evaporated and the residue was repeatedly extracted with light petroleum (b.p. 60—80°). The insoluble residue crystallized from ethyl acetate–light petroleum as pale yellow needles, m.p. 200—206°. The mass spectrum indicated that this material was an isomer of cochlioquinone A but the small amount of material available prevented a structure determination (Found: M^+, 532·2986). The fragmentation pattern of this compound was different from that of cochlioquinone A and a mixed m.p. determination showed depression.

Cochliohydroquinone A (IIa).—Repeated crystallization of alboleersin from benzene yielded cochliohydroquinone A as *plates*, m.p. 210—212° (lit.,[1] 215°), $[\alpha]_D^{23}$ +98° (*c* 0·06), $\nu_{max.}$ 3400 (OH) and 1700 cm^{-1} (C=O), $\lambda_{max.}$ 295 nm (ε 3300), τ 3·75 (1H, s), 4·94 (1H, dd, J 4·2 and 8·5 Hz), 4·98 (1H, s), 8·2 (3H, s), 8·75 (3H, s), 8·82 (6H, s), 8·83 (3H, d, J 6·5 Hz), 8·95 (3H, s), 9·09 (3H, d, J 6·5 Hz), and 9·11 (3H, t, J 7·0 Hz), *m/e* 534 (M^+), 516, 501, 456, 441 (100%), and 387 (Found: $M^+ - H_2O$, 516·3036. $C_{30}H_{44}O_7$ requires 516·3087).

Cochliohydroquinone A Dimethyl Ether (IIb).—A solution of cochliohydroquinone A (IIa) (50 mg) in chloroform was treated with an excess of ethereal diazomethane. Next day the solution was evaporated and the product purified by preparative t.l.c. (silica gel GF254). The major band was eluted from the silica with chloroform; the *ether* crystallized from light petroleum (b.p. 40—60°) as *plates*, m.p. 72—78°, $[\alpha]_D^{23}$ +130° (*c* 0·12), $\nu_{max.}$ 1725 (C=O) and 3500 cm^{-1} (OH), $\lambda_{max.}$ 289 nm (ε 15,000), *m/e* 562 (M^+) and 544 ($M^+ - H_2O$) (Found: $M^+ - H_2O$, 544·3402. $C_{32}H_{48}O_7$ requires 544·3400).

Cochliohydroquinone A Tetra-acetate (IIc).—The hydroquinone (IIa) was heated under reflux with acetic anhydride–sodium acetate for 6 h. After the usual work-up the tetra-acetate (IIc) precipitated from light petroleum (b.p. 40—60°) on cooling at −78° as *flakes*, m.p. 101—102°, $[\alpha]_D^{23}$ +135° (*c* 0·05), $\nu_{max.}$ 1765 and 1740 cm^{-1} (C=O), $\lambda_{max.}$ 305 (ε 3500) and 280 nm (10,000), *m/e* 702 (M^+) (Found: M^+, 702·3617. Calc. for $C_{38}H_{54}O_{12}$: 702·3615).

Deoxycochliohydroquinone A (IId).—A solution of the quinone (Ia) (20 mg) in ethanol (5 ml) was hydrogenated over palladium–charcoal. One mol. equiv. of hydrogen was rapidly absorbed (10 min) and a second equiv. was consumed during 6 h. Filtration, evaporation, and

* Numbering is non-systematic; see formula (I).

† We thank Professor Scala for this sample.

crystallization from cyclohexane yielded the deoxyhydroquinone (IId) as *prisms*, m.p. 155—159°, $[\alpha]_D^{23}$ +74° (*c* 0·05), $\nu_{max.}$ 1720 (C=O) and 3400 cm^{-1} (OH), $\lambda_{max.}$ 270 (ε 7800) and 390 nm (100—200 depending on age of sample), *m/e* 518, 458, and 388 (Found: M^+, 518·3231. $C_{30}H_{46}O_7$ requires 518·3242).

Basic Hydrolysis of Cochlioquinone A (Ia).—The quinone (Ia) (100 mg) was added to a solution of sodium hydroxide (1 g) in methanol–water (10 ml; 1 : 1) and left overnight. After evaporation of the methanol under reduced pressure the solution was acidified with 6N-sulphuric acid (50 ml), filtered, and steam distilled. A total of 200 ml of distillate was collected, titrated (phenolphthalein) with sodium hydroxide solution, and evaporated. The residue was converted into the *p*-bromophenacyl derivative in the usual way, and the product (20 mg, 40%) crystallized from light petroleum (b.p. 60—80°) as needles, m.p. 84—85°, identical (mixed m.p.) with an authentic sample of *p*-bromophenacyl acetate.

The Chromen (III).—The chromen (III) was prepared from the hydroquinone (IIa) as described by Raistrick [1] and crystallized from aqueous ethanol as plates, m.p. 174—175°, $[\alpha]_D^{23}$ +14° (*c* 0·05), $\nu_{max.}$ 1710 (C=O) and 3400 cm^{-1} (OH), $\lambda_{max.}$ 289 (ε 13,100) and 330 nm (3500), τ 3·6 (1H, s), 3·83 (1H, s), 4·9—5 (2H, m), 6·6—7 (4H, m), 8·21 (3H, s), 8·64 (3H, s), 8·88 (6H, s), 8·92 (3H, d, *J* 7 Hz), 8·98 (3H, s), and 9·15 (3H, t, *J* 6·5 Hz), *m/e* 516, 501, 456, 441, and 387 (Found: M^+, 516·3087. Calc. for $C_{30}H_{44}O_7$: 516·3086).

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