1110. *The Structure of Lambertellin*

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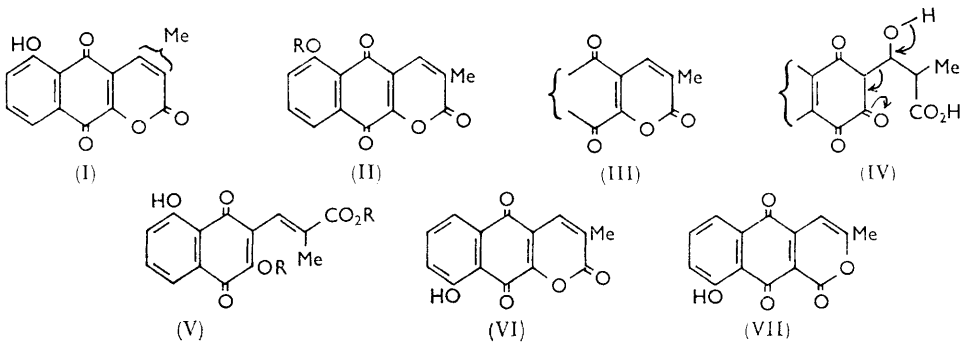
Lambertellin, a metabolite of *Lambertella corni-maris* and *L. hicoriae*, is shown to have the naphthaquinone structure (II; R = H).

Chloroform extracts of culture filtrates of the fungus *Lambertella corni maris* yield an antifungal orange-red pigment, m. p. 253—254°, $C_{14}H_8O_5$. In a lecture [1] Sproston has described the isolation of the same pigment * to which he gave the name lambertellin, from *Lambertella hicoriae* Whetzel and has suggested structure (I) for the compound. Our work supports this structure and enables us to locate the methyl group as in structure (II; R = H).

Several of its properties suggest that lambertellin possesses a naphthaquinone system with a hydroxyl group in the *peri* position. Its infrared spectrum shows a band at 1625 cm.$^{-1}$ characteristic of a hydrogen-bonded quinone group and its ultraviolet absorption ($\lambda_{max.}$ 290 and 430 mμ, $\lambda_{infl.}$ 284 mμ) resembles that of a *peri*-hydroxy-1,4-naphthaquinone.† Lambertellin forms a neutral monoacetate (II; R = Ac) ($\nu_{max.}$ 1774 cm.$^{-1}$) which no longer shows the band at 1625 cm.$^{-1}$ and which gives a long-wavelength maximum in the ultraviolet at 346 mμ.[2] Reductive acetylation of lambertellin gives a dihydro-triacetate which has the ultraviolet absorption spectrum of a naphthalene. In accord with the presence of a *peri*-hydroxyl group, lambertellin is acidic, dissolving in sodium carbonate to give a deep violet-red solution, but is not readily methylated with diazomethane.[3] It can, however, be methylated by the method used by Garden and Thomson [4] for *peri*-hydroxyl groups. The hydroxyl group gives no sharp band in the infrared due to hydrogen-bonding.

The presence of the hydroxynaphthaquinone moiety was confirmed by oxidation of lambertellin monoacetate with chromium trioxide in acetic acid–acetic anhydride followed by alkaline hydrolysis of the ether-soluble product to give 3-hydroxyphthalic acid.



(I) (II) (III) (IV) (V) (VI) (VII)

Because lambertellin is sparingly soluble in organic solvents its nuclear magnetic resonance (n.m.r.) spectrum was determined for a solution of its sodium salt in deuterium oxide. The spectrum shows a multiplet due to three aromatic protons, a one-proton quartet (J = 1·5 c./sec.) at τ 3·23, and a three-proton doublet (J = 1·5 c./sec.) at τ 7·29. The last two signals are characteristic of the system ·CH:CMe. Since the three aromatic

* The identity of the pigments was established by direct comparison of our compound with a sample of lambertellin kindly provided by Professor Sproston. We have never encountered the dimeric form of the pigment reported by Professor Sproston.[1]

† The presence of a 1,4- rather than a 1,2-naphthaquinone system was further indicated by our inability to prepare a quinoxaline derivative from lambertellin.

[1] T. Sproston, "Aspects of Plant Phenolics Chemistry," Proc. Third Ann. Symp., Plant Phenolics Group of N. America, Toronto, 1963, p. 69; *Chem. Abs.*, 1964, **61**, 8560.
[2] Cf. A. K. Macbeth, J. R. Price, and F. L. Winzor, *J.*, 1935, 325.
[3] R. Kuhn and K. Wallenfels, *Ber.*, 1939, **72**, 1407.
[4] J. F. Garden and R. H. Thomson, *J.*, 1957, 2483.

protons are accounted for by the formation of 3-hydroxyphthalic acid, the quinone ring must be fully substituted.

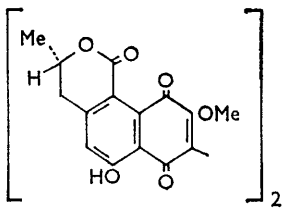
Allocation of the two remaining oxygen atoms to a lactone ring was suggested by titration experiments and by the presence of a band in the infrared at 1746 cm.$^{-1}$ which was absent from a barium salt prepared by precipitation from an alkaline solution of lambertellin. To meet the requirements of the molecular formula and the n.m.r. spectrum the lactone must include both the double bond of the allyl group and positions 2 and 3 of the 1,4-naphthaquinone nucleus and must therefore be six-membered. Of the four possible arrangements of the lactone ring, only structure (III) is consistent with the formation of propionaldehyde by alkaline hydrolysis of lambertellin. The propionaldehyde can arise by decarboxylation of the β-aldehydo-acid derived from the intermediate (IV) resulting from opening of the lactone ring and hydration of the double bond.

Consistent with the known properties of coumarins,[5] lambertellin is recovered unchanged from short treatment with dilute alkali whereas prolonged treatment in the presence of light gives a hydroxy-acid which is reconverted into lambertellin only on prolonged exposure to light in acid solution. The stable hydroxy-acid should have a coumaric (rather than coumarinic) acid structure and we therefore formulate it as the *trans*-acrylic acid (V; R = H). Methylation of the acid (V; R = H) with diazomethane gives an acidic dimethyl derivative, presumably (V; R = Me) since *peri*-hydroxyl groups are resistant to methylation.[3] Attempts to reduce the olefinic group in lambertellin, its acetate, or its leuco-acetate were unsuccessful.

On the evidence so far presented there are two possible structures, (II; R = H) and (VI), for lambertellin. We consider (II; R = H) to be correct for the following reason. The first non-volatile product of alkaline hydrolysis of structure (II; R = H) or (VI) would be 2,5- or 3,5-dihydroxynaphthaquinone respectively. Under the hydrolysis conditions used for lambertellin we find that 3,5-dihydroxy-1,4-naphthaquinone is stable but that 2,5-dihydroxy-1,4-naphthaquinone is degraded to a mixture of products. Neither dihydroxynaphthaquinone could be detected in thin-layer chromatographs of hydrolysates of lambertellin but the chromatographic pattern was the same, with two extra spots one of which is due to lambertellin itself, as that given by the hydrolysis products of 2,5-dihydroxy-1,4-naphthaquinone.

Sproston [1] suggested his structure (I) for lambertellin on the basis of spectroscopic evidence and because no isomer is detected on opening the lactone ring with alkali and immediately reclosing it with acid, an observation best explained by strong hydrogen-bonding between the 5-hydroxyl group and the carbonyl group at position 4. The only degradation products reported by Sproston are naphthalene, obtained by zinc-dust distillation, and acetaldehyde, obtained by oxidation with alkaline permanganate.

Biogenetically, the structure (II; R = H) suggested for lambertellin is somewhat unusual [at first we considered the alternative structure (VII), readily derivable from seven "acetate" units, to be more likely]. The branched-chain structure of lambertellin could, however, arise from a "polyketomethylene" precursor either by introduction of a one-carbon unit, with loss of a carbon atom elsewhere, or by direct degradation.



(VIII)

Growth of *L. corni-maris* in the presence of [^{14}C]formate gave a very low incorporation of radioactivity into lambertellin, rendering the first mechanism less likely. However, in view of the low yield of lambertellin in this experiment, the result is inconclusive; we propose to do no further work on this topic. The only other naturally-occurring naphthaquinone lactone to have been reported is xanthomegnin (VIII), whose structure is readily derivable from " acetate " units.[6]

Lambertellin inhibits the germination of fungal spores *in vitro* at concentrations in the

[5] F. Feigl and D. Goldstein, *J. Amer. Chem. Soc.*, 1955, **77**, 4162; W. Gruber, *Monatsh.*, 1944, **75**, 14.
[6] G. Just, W. C. Day, and F. Blank, *Canad. J. Chem.*, 1963, **41**, 74.

range 1—25 μg./ml., though some species are more resistant, and is weakly antibacterial, solutions containing 100 μg./ml. giving zones of inhibition of 14 mm. in cylinder plate assays against *B. subtilis*.

Experimental

Unless otherwise stated, infrared spectra were determined for Nujol mulls, ultraviolet spectra were measured for methanol solutions, and nuclear magnetic resonance spectra were determined for deuterochloroform solutions on a Varian A.60 spectrometer, with tetramethylsilane as internal standard. Hopkin and Williams's silica gel M.F.C. was used for column chromatography, Merck silica gel G was used for thin-layer chromatography and light petroleum had b. p. 60—80°.

Isolation of Lambertellin.—*Lambertella corni-maris* (C.B.S., Baarn; No. 1017 in our collection) was grown as surface culture at 25° on Czapek Dox medium with added dextrolact (5%) and yeast extract (0·1%). The fungus was harvested 28 days after inoculation and the culture filtrate (48 l.) was extracted at the natural pH (3·2) with chloroform (2 × 460 ml.) to give an orange-red gum (6·6 g.) which was chromatographed on silica gel. The appropriate fraction, eluted with benzene–chloroform (4 : 1), was crystallised from acetone to give orange-red needles (1·34 g.), m. p. 242—246°. Two crystallisations from acetone gave *lambertellin* as orange-red needles, m. p. 253—254° [Found: C, 65·3, 65·9; H, 3·1, 3·2%; Equiv. (lactone) 125; *M* (*X*-ray) 254 ± 3. $C_{14}H_8O_5$ requires C, 65·6; H, 3·15%; *M*, 256], $\lambda_{max.}$ 290 (ε 12,500) and 430 mμ (ε 4800), $\lambda_{infl.}$ 284 mμ (ε 12,100), $\nu_{max.}$ 1746s, 1665m, 1657m, 1625s, 1603w, and 1594w cm.$^{-1}$. Crystallisation from ethanol gave orange plates with the same physical properties as the orange-red needles. The nuclear magnetic resonance spectrum was determined for a solution of the sodium salt in deuterium oxide: τ (number of protons in parentheses) 1·74—2·58 (3, multiplet), 3·23 (1, quartet, J = 1·5 c./sec.) and 7·29 (3, doublet, J = 1·5 c./sec.).

The *acetyl compound* (II; R = Ac), prepared in pyridine with acetic anhydride at room temperature, formed yellow felted needles, m. p. 252—256°, from acetone–light petroleum (Found: C, 64·5; H, 3·4. $C_{16}H_{10}O_6$ requires C, 64·4; H, 3·4%), $\lambda_{max.}$ 234 (ε 13,200), 283 (ε 16,800), and 346 mμ (ε 3700), $\nu_{max.}$ 1774s, 1749s, 1677s, 1666s, 1639m, and 1591s cm.$^{-1}$, τ 1·76—2·71 (4, multiplet), 7·5 (3, singlet) and 7·7 (3, doublet, J = 1·5 c./sec.).

The *methyl ether* (II; R = Me), prepared by the method of Garden and Thomson,[4] formed yellow needles, m. p. 248—250°, from acetone–light petroleum (Found: C, 66·6; H, 3·9. $C_{15}H_{10}O_5$ requires C, 66·7; H, 3·7%), $\nu_{max.}$ 1748s, 1677s, 1667s, 1641m, and 1589s cm.$^{-1}$.

Reductive Acetylation of Lambertellin.—Pyridine (2 drops) was added to a suspension of finely powdered lambertellin (0·74 g.) and zinc dust (0·38 g.) in acetic anhydride (4 ml.) and the mixture was cooled until the reaction subsided. Further zinc dust (0·1 g.) was added and the mixture was heated on the water-bath until all the red solid had turned yellow. The mixture was poured on crushed ice and the solid collected, washed with water, dried, and washed with methanol. The pale yellow residue was dissolved in acetone, filtered free from zinc dust, and crystallised from acetone–light petroleum as pale yellow prisms (0·72 g.), m. p. 248—252°, which were dissolved in chloroform and filtered through Grade 1 neutral alumina. The recovered product was further crystallised from acetone–light petroleum to give the *triacetyl compound* as pale yellow needles, m. p. 250—256° [Found: C, 62·5; H, 4·2%; *M*, 418 (Rast). $C_{20}H_{16}O_8$ requires C, 62·7; H, 4·3%; *M*, 384], $\lambda_{max.}$ 230 (ε37,100), 269 (ε 36,100), 280 (ε 35,600), 314 (ε 14,200), and 328 mμ (ε 17,700), $\lambda_{infl.}$ 301 mμ (ε 8900), $\nu_{max.}$ 1775s, 1765s, 1739s, 1638m, and 1611m cm.$^{-1}$, τ 2·17—2·89 (4, multiplet), 7·47 (3, singlet), 7·50 (3, singlet), 7·58, (3, singlet), and 7·8 (3, doublet, J = 1·5 c./sec.).

Oxidation of Lambertellin Monoacetate.—The method was essentially that used by Astill and Roberts [7] for flaviolin. To a mixture of lambertellin monoacetate (II; R = Ac) (100 mg.), acetic anhydride (4·0 ml.) and acetic acid (2·0 ml.) were added 2·0 ml. of an oxidising reagent prepared by dissolving chromic oxide (2·5 g.) in water (2·0 ml.) and adding acetic acid (25 ml.). The mixture was heated at 85° for 45 min., further oxidising reagent (2·0 ml.) was added, and the heating continued for a further 25 min. The mixture was cooled, diluted with water, washed with chloroform and exhaustively extracted with ether to give a yellow crystalline mass (35 mg.). A solution of this solid (24 mg.) in 0·2N-sodium hydroxide (10 ml.) was heated for 1 hr. on the water-bath, cooled, and passed down a column of Amberlite IR-120 (H^+) resin. The filtrate was evaporated to dryness and the residual yellow solid was extracted with ether. The ether was evaporated off and an ethanolic solution of the product was treated with charcoal, filtered, and evaporated. The white residue was crystallised from ether–light petroleum to

[7] B. D. Astill and J. C. Roberts, *J.*, 1953, 3302.

give 3-hydroxyphthalic acid (14 mg.) as prisms, m. p. and mixed m. p. 160—161° (Found: C, 52·7 H, 3·5. Calc. for $C_8H_6O_5$: C, 52·7; H, 3·3%).

Hydrolysis of Lambertellin.—(*a*) A solution of lambertellin (100 mg.) in N-potassium hydroxide (10 ml.) was heated under reflux for 4 hr. during which time a stream of nitrogen passed first through the solution and then through a solution of 2,4-dinitrophenylhydrazine in 3N-hydrochloric acid. The yellow precipitate (31 mg.) was collected, washed with water, and crystallised from ethanol to give propionaldehyde 2,4-dinitrophenylhydrazone as orange needles, m. p. and mixed m. p. 155—156° (Found: C, 45·4; H, 4·4. Calc. for $C_9H_{10}N_4O_4$: C, 45·4; H, 4·2%). In a second experiment the gas stream passed through a cold trap and the contents of the trap were treated with a saturated aqueous solution of dimedone to give propionaldehyde dimethone as plates, m. p. and mixed m. p. 154—156°.

(*b*) A solution of lambertellin (275 mg.) in N-potassium hydroxide (10 ml.) was heated under reflux for 1 hr. in an atmosphere of nitrogen. The solution was acidified and extracted with ethyl acetate and the product was chromatographed on silica gel. Fractions eluted with benzene–chloroform (9 : 1) gave lambertellin (25 mg.), m. p. 250—254°. The remaining fractions gave no pure product. Samples were examined by thin-layer chromatography on silica gel in di-isopropyl ether–formic acid (9 : 1), using as standards 2,5- and 3,5-dihydroxy-1,4-naphthaquinone and the products of alkali treatment of these dihydroxy-naphthaquinones. Neither quinone was detected in any of the fractions. 3,5-Dihydroxy-1,4-naphthaquinone was unaffected by treatment with alkali but the 2,5-isomer was hydrolysed to a mixture of products having R_F 0·66 (faint), 0·57, 0·40, 0·19, and 0·06. Compounds with these R_F values were prominent in the column fractions and, in addition, three other prominent compounds had R_F values of 0·79, 0·53, and 0·60 (lambertellin).

(*c*) Solutions of lambertellin (4 mg.) and 2,5-dihydroxy-1,4-naphthaquinone (4 mg.), each in 2N-potassium hydroxide (0·5 ml.) were heated in sealed tubes at 100° for 4 hr. The involatile products were isolated, chromatographed on thin-layer silica gel plates in methylene chloride–ethanol–formic acid (93 : 6 : 5) and detected by spraying with chromic acid and heating at 120°. The major products, R_F 0·40, 0·36, 0·28, 0·15, and 0·04, were common to both compounds and in addition the hydrolysate of lambertellin contained products with R_F values of 0·56 and 0·74 (lambertellin).

trans-α-*Methyl*-β-(2,5-*dihydroxy*-1,4-*naphthaquinon*-3-*yl*)*acrylic Acid* (V; R = H).—A solution of lambertellin (100 mg.) in 0·2 N-sodium hydroxide (20 ml.) was exposed to daylight at room temperature for seven days. The solution was acidified with dilute hydrochloric acid, extracted with ethyl acetate, and the extract was shaken with saturated sodium hydrogen carbonate. The bicarbonate solution was acidified with dilute hydrochloric acid and extracted with ethyl acetate to give an orange-red solid. The solid was washed with chloroform and the residue (7 mg.) was crystallised from methanol to give the *acrylic acid* (V; R = H) as orange-red needles, m. p. 210—217° (Found: C, 61·0; H, 3·7. $C_{14}H_{10}O_6$ requires C, 61·3; H, 3·7%), $\lambda_{max.}$ 232 (ε 12,500), 270 (ε 14,500), and 415 mμ (ε 4300), $\nu_{max.}$ 3130br, 2600br, 1702s, 1653sh, 1646s, 1591m, and 1578s cm.$^{-1}$.

The *dimethyl compound* (V; R = Me), prepared with ethereal diazomethane, formed orange needles, m. p. 148—150°, from acetone–light petroleum (Found: C, 63·7; H, 4·6. $C_{16}H_{14}O_6$ requires C, 63·5; H, 4·7%), $\nu_{max.}$ 1722 cm.$^{-1}$, τ −1·78 (1, singlet), 2·26—2·86 (4, multiplet), 5·89 (3, singlet), 6·17 (3, singlet), and 8·18 (3, doublet, J = 1·5 c./sec.).

Isomerisation of the Acid (V; R = H).—A solution of the acid (V; R = H) (10 mg.) in methanol (5 ml.) containing concentrated hydrochloric acid (4 drops) was exposed to daylight at room temperature for 16 days, whereupon lambertellin (2 mg.) separated as red plates, m. p. and mixed m. p. 249—254°.

Isolation of [^{14}C]*Lambertellin.*—*L. corni-maris* was grown as described previously on 2 l. of culture medium. When antifungal activity, as shown by the suppression of germination of spores of *Botrytis allii*, was detected in the culture medium, sodium [^{14}C]formate (250 μc) was added. The fungus was harvested after 28 days' growth and lambertellin (6·5 mg.; $1·67 \times 10^{-3}$ μc; 0·0007% incorporation, m. p. 247—253°, was isolated.

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