β-LEPROSOL: ATTEMPTED ELUCIDATION OF STRUCTURE BY THE SYNTHESIS OF RESORCINOL DERIVATIVES

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An attempt has been made to elucidate the structure of β-leprosol by synthesising on biogentic grounds certain alkyl substituted resorcinol derivatives and comparing their UV spectra and colour reactions with that of the original compound.

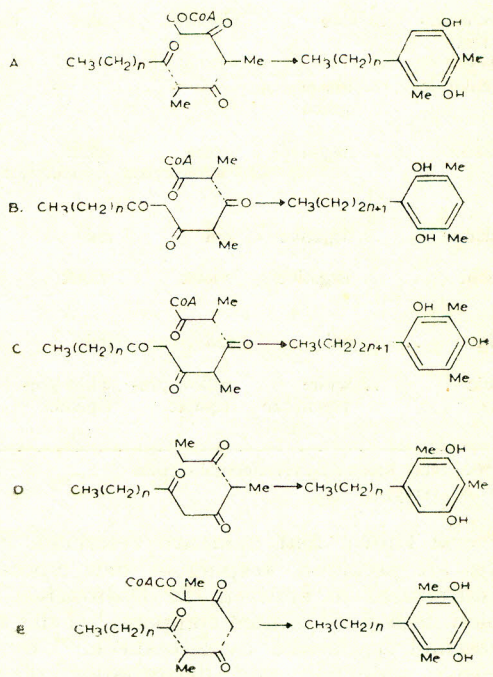
The biosynthesis of polyketides is known to involve malonyl co-enzyme-A[1] and occasionally methyl-malonyl coenzyme-A.[2] The macrolide antibiotics like methymycin, erthromycin are known to be built up wholly or partially in this way.[3] Lederer[4] has shown that propionate (i.e. methylmelonyl co-enzyme A) is involved in producing the terminal unit (carboxylic end) of some branched chain acids from *Mycobacteria*. Although 'acetate' is found to be frequently involved in the production of the aromatic rings,[5] so far 'propionate' has not been shown to take part. Macrocyclic lactone curvularin has been shown[6] to be derived solely from 'acetate' and the ease with which a transannular cylisation occurs to give a naphthol suggests an interesting structural and possibly biosynthetic link between the macrolide antibiotics such as methymycin and acetate derived phenols.

Woodward[7] has suggested that the macrolides (chiefly 'propionate' derived) may be produced basically because of inhibition of cyclisation reactions due to the extra methyl groups.

It seems therefore of considerable interest to discover whether any route to an aromatic compound involves 'propionate'. Possible compounds appeared to be the leprosols isolated from *Mycobacterium leprae*,[8] Unfortunately this organism cannot be readily grown and the substances are difficultly accessible. Hence it is rather difficult to carry out a tracer experiment. The preliminary investigations[9] suggested that these may be alkyl substituted resorcinol derivatives and also quoted detailed UV spectra and colour reactions. Butenandt and Stodola[9] concluded on the basis of colour tests and UV absorption spectra (λmax 287 mμ) that norleprosols are resorcinol derivatives. They also synthesised a number of possible models and quoted their UV and colour reactions.

It was therefore thought worthwhile to synthesise possible structures based on a possible biogenetic relation to the branched chain fatty acids

SCHEME I



of mycobacteria, in order to compare their spectra and colour reactions with those of natural compounds. Indications were that three alkyl substituents are present in a resorcinol ring.

There are a number of possible routes involving a straight chain fatty acid and two 'propionate' units (Scheme I, A,B,C). If ring closure could occur on a carbon carrying a methyl, then a varient of A is possible (Scheme I, D,E); as already noted this is unlikely.

Model compounds synthesised by Butenandt and Stodola did not carry a substituent between the hydroxyls. It was our first aim, therefore, to synthesise compounds of this type and to check with less substituted models that they do not in fact behave like nor-β-leprosol (Table 1).

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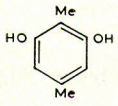
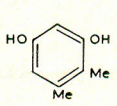
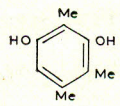
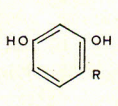
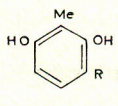
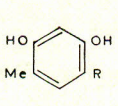
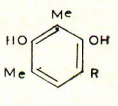
TABLE 1.

Reagents	Nor-β-leprosol							
M.p.	104–105°	161–162°	135–136°	148–149°	92–93°	89–90°	69–70°	94–95°
UV	287 mμ log ε	*281 mμ log ε	282 mμ log ε	281 mμ log ε	283 mμ log ε	279 mμ log ε	287 mμ log ε	283 mμ log ε
absorption	3.51	3.24	3.34	3.2	3.49	3.24	3.59	3.56
Phosphomolybdic acid $+NH_3$	blue	brown	blue	bluish violet	blue	greenish blue	blue	blue
Ferric chloride	bluish green	—	—	—	—	—	—	—
Fluorescein test	negative	green fluorescence	green fluorescence	green fluorescence	green fluorescence	light green fluorescence	bright green fluorescence	negative
Libermann test	negative	red	red	negative	blue	blue	negative	negative
Guareschi test	negative	violet	violet	very light pink	red	reddish violet	negative	negative
Vanillin test	red	red	red	red	red	red	red	light red
Mercuric nitrate	white precicitate	yellow precipitate	white precipitate	white precipitate	white precipitate	white precipitate	white precipitate	white precipitate

*For other peaks see experimental section
R=$C_{18}H_{37}$

Several known and unknown resorcinol derivatives are prepared, keeping in view especially the substituent in between the hydroxyls. The simplest dialkyl substituted compound of this type is β-orcinol synthesised by Kostanecki[10] by the successive reduction and diazotisation of 3,5-dinitro-*p*-xylene. The compound in our hands was however very conveniently prepared by refluxing orcinol dimethyl ether with butyl-lithium prepared in diethyl ether according to Gilman.[11] Subsequent addition of methyl iodide followed by a further continuous refluxing for some time yielded β-orcinol dimethyl ether, which was demethylated by boiling hydriodic acid to give β-orcinol. By reacting orcinol with zinc cyanide in presence of HCl, orcylic aldehyde was obtained[12] which on Clemmensen reduction yielded 4,5-dimethylresorcinol. By using exchange reaction with butyl-lithium described above, an extra methyl group was introduced on C_2 of 4,5-dimethylresorcinol dimethyl ether. Demethylation in the usual way gave 3,5-dihydroxy ψ-cumene. This is one of the representative compounds consisting of three alkyl substituents in resorcinol which could be biosynthesised by route A (Scheme I), described earlier, although the UV absorption spectrum and the colour reactions do not agree with nor-β-leprosol. (Table 1).

4-Octadecyl resorcinol was prepared by the Clemmensen reduction of stearoylresorcinol synthesised by refluxing resorcinol and stearic acid in trichloroethylene with a continuous flow of born trifluoride[13] Methylation of 4-octadecylresorcinol in acetone in presence of anhydrous potassium carbonate and dimethyl sulphate yielded 4-octadecylresorcinol dimethyl ether. Nuclear methylation by the usual exchange reaction with butyl-lithium gave 2-methyl-4-octadecyl-resorcinol dimethyl ether. This was demethylated by hydriodic acid to 2-methyl-4-octadecyl resorcinol. The NMR spectrum of 2-methyl-4-octadecyl-resorcinol dimethyl ether has a signal at 7.92τ corresponding to one aromatic methyl, peaks at 6.28τ and 6.38τ corresponding to two methoxyl groups. The two hydrogens in *ortho* relationship appear as doublets at 3.15τ and 3.56τ showing the expected large *ortho* coupling constant (~9 c/s). 2,4-Dihydroxy-5-octadecyl benzaldehyde was obtained by reacting octadecylresorcinol with

zinc cyanide in dry ether in presence of dry hydrogen chloride. Clemmensen reduction of this aldehyde gave 4-methyl-6-octadecylresorcinol Methylation with dimethyl sulphate in presence of anhydrous potassium carbonate in acetone yielded 4-methyl-6-octadecylresorcinol dimethyl ether. The NMR spectrum shows absorption at 7.85τ equivalent to one aromatic methyl, a signal at 6.18τ corresponding to two methoxyls and two singlets at 3.1τ and 3.55τ corresponding to two aromatic protons. By the usual exhange reaction with butyl-lithium followed by addition of methyl iodide, 2,4-dimethyl-6-octadecylresorcinol dimethyl ether was obtained. The NMR spectrum shows absorption of six protons at 7.85τ corresponding to two aromatic methyls, a signal at 6.4τ corresponds to two methoxyls and a singlet at 3.3τ showing the single aromatic proton. 2,4-Dimethyl-6-octadecylresorcinol was obtained by its demethylation. The signals from the octadecyl group have not been mentioned above, since we were interested only in the substitution of methyl groups in known octadecyl resorcinol. The UV absorption spectrum and the colour reactions are given in Table 1.

Discussion

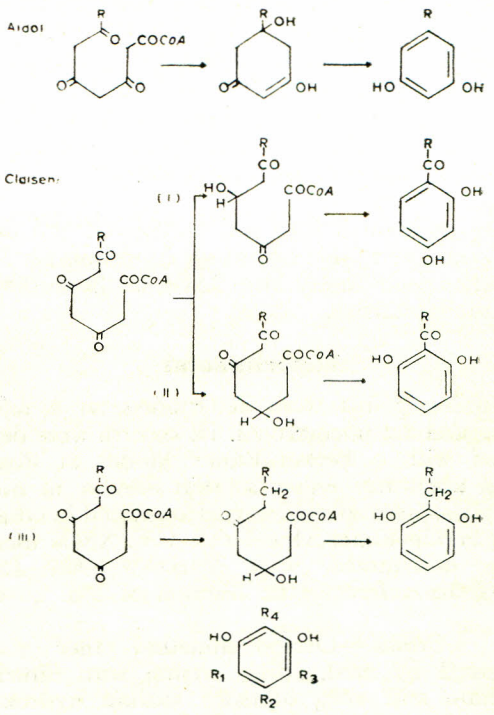
Neglecting the somewhat dubious ferric test, the colour test alone compare with nor-β-leprosol in cases of compound I ($R_{1,4}$= Me, R_2=H, R_3=$C_{18}H_{37}$). The large number of negative tests with nor-β-leprosol is an indication of high substitution and particularly the absence of free *para* position. The UV spectra of these two compounds (λmax 285 mμ, log ε 3.47; and λmax. 283 mμ log ε 3.56) can be compared with λmax. 287 mμ, log ε 3.51 for nor-β-leprosol. Since small differences are vital, it is not clear, however, what differences exist between methyl and larger groups in their effects on the spectra. The m.p. of 2,4-βdimethyl-6-octadecylresorcinol which may be a higher homologue of nor-β-leprosol is 94–95°, whereas that of nor-β-leprosol is 104–105°.

On biogenetic grounds formula I ($R_{1,3}$=Me, R_2=H, R_4=alkyl) is also possible which is in some respect more satisfactory. The ring closure which generates the resorcinol ring results in loss of an oxygen. This is automatic in the aldol type closure but requires reduction of a carbonyl in a Claisen type closure.

Scheme 2 would show a few possibilities of aldol and Claisen type of ring closure.

The aldol ring can therefore readily give compounds with a saturated side chain since appropriate reactivity is available in the ring. The Claisen route must involve reduction before aromatisation, i.e. before ring closure, since this would generate the keto from of the phloroglucinol immediately. Thus by route (i) the carbonyl in the side chain is necessary for ring closure to occur. Later reduction of the carbonyl seems unlikely in view of the common occurrence of acyl rather than alkyl phloroglucinols and resorcinols in nature.

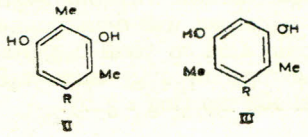
Scheme II



I

The Claisen route (iii) is therefore the more likely one. This would however generate a symmetrical compound which seems unlikely in view of the statement[9] that one hydroxyl methylates much more readily than the other although no experimental evidence is given.

We are therefore led back to the aldol route and putting in two methyl groups we obtain as possibilities either II or III, of which the latter is unlikely on two grounds; that it is also symmetrical, and that the aldol ring closure would have to occur on —CHMe rather than —CH_2.



II III

The drawback to formula II is that it contains a free *para* position; however, it might not give colour tests because of the two *ortho* substituents, one being very large. In this case R would obviously contain an odd number of carbon atoms. A model compound of methyl analogue (3,5-dihydroxy-Ψ-cumene) has been synthesised which however has a low UV absorption spectrum and indicates signs of *para*-reactivity.

The problem is thus still unresolved and there seems no alternative but to apply techniques such as mass spectrometry and NMR to the authentic natural substances.

Experimental

Melting points were determined on a Kofler block and are uncorrected. IR spectra were determined with a Perkin Elmer Model 21 double beam apparatus either as thin film or in nujol. UV absorption spectra were determined in ethanol on a Perkin-Elmer Model 137 UV. NMR spectra were determined with Varian A-60. Light petroleum refers to the fraction of b.p. 40–60°.

(a) β-*Orcinol*.—Orcinol dimethyl ether (3.0g), prepared by methylating orcinol with dimethyl sulphate and 10% aqueous sodium hydroxide, was refluxed with butyl-lithium (~1.5 g) in ether for 4 hr. After the addition of methyl iodide refluxing was continued for further 2 hr. The reaction mixture was cooled and water was added cautiously. The ether layer was separated and the aqueous layer was twice extracted with ether (500 ml). The combined ether-soluble extract (2.67 g) was refluxed with an excess of hydroiodic acid for 1½ hr. The ether extract from this reaction mixture yielded β-orcinol (76 mg, 2.3%) on fractional recrystallisation from benzene–light petroleum. m.p. 161–62° (lit. 163)[14] λmax. 281, 276, 272 mμ (log ε 3.24, 3.25, 3.26).

(b) 4,5-*Dimethylresorcinol*.—Orcylic aldehyde (8.0 g) prepared by following the method of Adam and Levine[12] was converted by a Clemmensen reduction to 4,5-dimethyl resorcinol (4.12 g, 76%) m.p. 135–136° (lit 136–137[14] λmax 282 mμ (log ε 3.34).

(c) 2,4,5-*Trimethylresorcinol*.—4,5-Dimethyl-resorcinol dimethyl ether (3.0 g) prepared by methylating 4,5-dimethyl resorcinol with dimethyl sulphate and 10% sodium hydroxide solution, was subjected to nuclear methylation by usual exchange reaction with butyl-lithium. The product thus obtained was demethylated by boiling hydroiodic acid to yield 2,4,5-trimethyl resorcinol (384 mg; 14.2%) m.p. 148–149° (lit. 156°)[15] λmax. 281 mμ (log ε 3.2).

(d) *Stearoylresorcinol*.—A mixture of recrystallised, thoroughly dried resorcinol (20 g) and stearic acid (40 g) suspended in trichloroethylene (350 ml) was refluxed on a heating mantle for 3 hr with a continuous flow of boron trifluoride. The colourless suspension immediately turned red and a homogeneous solution was obtained. Water was then added to the cold reaction mixture and the ethylene trichloride layer was separated. The water layer was extracted with ether and the combined extracts dried (Na_2SO_4). The residue left after removal of the solvent was crystallised from petroleum–ether (60–80°C) (45.7 g, 86.3%). Recrystallisation from 95% ethanol and subsequently from petroleum ether (60–80°C) gave colourless crystals of stearoylresorcinol m.p. 98–99° (lit. 99°)[117] λmax 314, 277, 231 mμ (log ε 3.88, 4.068, 3.94), νmax. (in nujol) 1640, 1600 cm^{-1}.

(e) *Octadecylresorcinol*.— Stearoylresorcinol (20 g) was refluxed for 16 hr with zinc amalgam (250 g) in ethanol (50 ml) and 4N hydrochloric acid (120 ml). On cooling, a colourless precipitate was obtained which was extracted with ether. The crystalline residue obtained after the removal of the solvent was recrystallised from a mixture of benzene and light petroleum giving octadecylresorcinol (16.85 g, 87.7%) m.p. 92–93° (lit. 92–93°), λ283 mμ (log ε 3.49), νmax, (in nujol) 3450, 3275, 1610, cm-1.

(f) *Octadecylresorcinol dimethyl ether*.—Octadecylresorcinol (4.2 g) was refluxed in acetone (100 ml) with anhydrous potassium carbonate (20 g) and dimethyl sulphate (12.0 ml) for 8½ hr. Most of the acetone was removed under vacuum. On adding water a colourless crystalline material precipitated out which was recrystallised from moist acetone yielding octadecylresorcinol dimethyl ether (3.2 g, 71.1%) m.p. 51–52°. (Found: C, 79.6; H, 11.4; $C_{26}H_{46}O_2$ requires C, 80.0; H, 11.7%), λmax 279, 285 mμ (log ε 3.512, 3.455), ν max (in nujol) 1618, 1595 cm^{-1}.

(g) 2-*Methyl*-4-*octadecylresorcinol*. — Octadecylresorcinol dimethylether (3.0 g) was refluxed with butyl-lithium for 4½ hr under an atmosphere of nitrogen and was left to stand overnight. An excess of methyl iodide was added and refluxing continued for a further 3 hr. After cooling water was added cautously. The ether layer was separated and the aqueous protion extracted with ether (500 ml). The combined ether extracts were dried (Na_2SO_4) and the solvent was removed. The yellow oil thus obtained was chromatographed on alumina (grade 'H'). Elution with light petroleum gave colourless crystals of 2-methyl-4-octadecylresorcinol dimethylether (2.98 g, 95.5%). Recrystallisation from acetone gave a pure

sample, m.p. 38–39° (Found: C, 80.85; H, 12.0. $C_{27}H_{48}O_2$ requires C, 80.1; H, 11.8%),λmax. 282, 277,273 mμ (log ε 3.178, 3.199, 3.185).

2 - Methyl - 4 - octadecylresorcinol dimethylether (250 mg) was boiled with an excess of hydriodic acid at 160–180° for 2½ hr. Cooling and addition of water gave a sticky solid which was extracted with ether. The dark yellow ethereal extract was decolourised by shaking with powdered sodium sulphite. Filtration and evaporation of the solvent gave a white solid which was chromatographed on Florex. Elution with a mixture of light petroleum and ether (1:1) gave 2-methyl-4-octadecylresorcinol which was recrystallised from light petroleum giving colourless crystals (74.2 mg., 32.1%) m.p. 89–90°,λmax 279 mμ (log ε 3.244), ν max. (in nujol) 3470, 3200, 1605 cm-1.

(h) 2,4 - *Dihydroxy* - 5 - *octadecylbenzaldehyde*.—Octadecylresorcinol (5.0 g) was subjected to Gatterman reaction in the presence of anhydrous zinc cyanide and dry hydrogen chloride. Ether was decanted from the reaction mixture and the aldimine hydrochloride was decomposed by adding water. The crystalline solid thus obtained, was recrystallised from dilute ethanol giving 2,4-dihydroxy-5-octadecylbenzaldehyde (5.1 g, 96.2%) m.p. 119–120°. (Found: C, 76.65; H, 10.5 $C_{25}H_{42}O_3$ requires C, 76.9; H, 10.7%), λmax. 326, 283,237 mμ (log ε 3.87, 4.21,4.146), νmax (in nujol) 1610, 1580 cm^{-1}.

(i) 4 - *Methyl* - 6 - *octadecylresorcinol*.—2,4 - Dihydroxy-5-octadecylbenzaldehyde (5.0 g) was subjected to Clemmensen reduction. The crude product was purified through a column of Florex using petroleum ether-ether (1:1) as eluent. Crystallisation from light petrol gave 4-methyl-6-octadecyl-resorcinol as colourless needles (3.25 g., 67.4%) m.p. 69–70°, λmax 287 mμ (log ε 3.59) νmax 1618,1590 cm^1.

(j) 2,4-*Dimethyl*-6-*octadecylresorcinol*.—Methyl-6-octadecyl resorcinol (3.0 g) was methylated using dimethyl sulphate and anhydrous potassium carbonate in acetone. The dimethyl ether (2.78 g, 86%) was recrystallised from moist acetone giving colourless crystals, m.p. 49–50°. (Found: C, 79.75; H, 11.35. $C_{27}H_{48}O_2$ requires C, 80.1; H, 11.8%),λmax 285 mμ (log ε 3.63).

The above dimethyl ether (2.4 g) was refluxed with butyl-lithium in ether and subsequently with methyl iodide in an atmosphere of nitrogen. The reaction product was worked up as usual. A colourless oil (2.38 g, 95.2%) was obtained which crystallised on standing. Recrystallisation from moist acetone gave 2,4-dimethyl-6-octadecylresorcinol dimethyl ether, m.p. 34–35°. (Found: C, 81.0; H, 12.1 $C_{28}H_{50}O_2$ requires C, 80.38; H, 11.96%), λmax 278, 275, 270 mμ (log ε 2.962, 2.936, 2.893).

2,4-Dimethyl-6-octadecylresorcinol dimethyl ether (467 mg.) was boiled with an excess of hydriodic acid. The crude product was purified by passing through a column of florex. Recrystallisation from light petroleum afforded 2,4-dimethyl-6-octadecylresorcinol, (178 mg, 41.8%) m.p. 94–95°, λ max. 283 mμ (log ε 3.56), λ max (in nujol) 3350, 1608 cm^{-1}.

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