β-Leprosol: the Identification of a Trialkylresorcinol from Bacterial Lipids

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The surviving sample of β-leprosol, obtained in 1936 from lipids of *Mycobacferium leprae*, is identified as a mixture of the monomethyl ethers of 5-n-heptadecyl- and 5-n-pentadecyl-4,6-dimethylresorcinol.

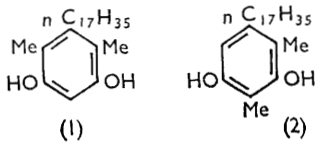
OVER thirty years ago, Crowder, Stodola, and Anderson obtained α- and β-leprosol from the lipids of *Mycobacterium leprae*, describing them as isomeric monomethyl ethers of alkylresorcinols, $[C_{19}H_{42}][C_6(OH)OMe]$.[1] Subsequently Butenandt and Stodola prepared a 4-alkyl-, a 5-alkyl-, a 4,6-dialkyl-, and 4,5,6-trimethyl-resorcinol

[1] J. A. Crowder, F. H. Stodola, and R. J. Anderson, *J. Biol. Chem.*, 1936, **114**, 431.

as models, and from u.v. data and colour reactions they concluded that nor-β-leprosol was a 4,5,6-trialkylresorcinol.[2] However, Asano and Iguti, presuming that the alkyl substituents were n-heptadecyl and dimethyl, synthesized several such trialkylresorcinols with the remaining aromatic hydrogen at each of the three possible positions (2, 4, and 5), and showed that neither the colour reactions nor the u.v. spectra justified any decision concerning the substitution pattern. Nor did the m.p. of any of their products correspond closely enough to the published data to permit identifications; the closest correspondence was between 5-heptadecyl-4,6-dimethylresorcinol, m.p. 97—98° (dimethyl ether, m.p. 62—63°) and nor-β-leprosol, m.p. 104—105° (methyl β-leprosol, m.p. 68—69°).[3] The leprosols seem to have eluded subsequent attention, and we were pleased when Dr. Stodola, having apprised us of the problem, also entrusted us with the remnant of the original isolates, *viz.* a few mg of β-leprosol and its demethylation product, nor-β-leprosol; no α-leprosol samples had survived.

The mass spectrum showed β-leprosol to be a mixture of homologues, principally $C_{26}H_{46}O_2$ and $C_{24}H_{42}O_2$ in the ratio 1·4 : 1; similarly the nor-β-leprosol sample comprised $C_{25}H_{44}O_2$ and $C_{23}H_{40}O_2$ in the ratio 1 : 5. There was no significant proportion of other homologues or of dehydro- (alkenyl) analogues, and in what follows we shall speak as if only the higher homologue were present in each case. The n.m.r. spectrum of β-leprosol shows one aromatic CH, one aromatic OMe, and two aromatic CMe groups; the compound is therefore a methyl ether of n-heptadecyldimethylresorcinol, as postulated by Asano and Iguti.[3]

In the mass spectrum of nor-β-leprosol a large peak for $C_9H_{12}O_2$ corresponds to loss of $C_{16}H_{32}$ from the heptadecyl side-chain, and one for $C_8H_9O_2$ to a further loss of CH_3. Similar peaks occur in the spectra of β-leprosol and methyl β-leprosol, with metastable peaks for both steps in every case. Compared with these losses of $C_{16}H_{32}$, the peaks corresponding to loss of $C_{16}H_{33}$ in each case were relatively small. Now in long-alkyl phenols, loss of the side-chain as an alkene (by β-cleavage with hydrogen transfer to the nucleus) predominates over direct β-cleavage when the long alkyl group is *meta* to the oxygen, whereas direct β-cleavage (with loss of alkyl) predominates when the oxygen is *para* and a quinomethide ion can be formed.[4] We find this generalisation is equally true for alkylresorcinols; thus direct cleavage predominates for 4-octadecylresorcinol but β-cleavage with hydrogen transfer predominates for cardol (5-pentadecylresorcinol) and for '4-methylcardol' and '6-methylcardol' (2-methyl-5-pentadecyl- and 4-methyl-5-pentadecyl-resorcinol respectively). We therefore assign the heptadecyl group of β-leprosol to the 5-position, so that nor-β-leprosol must be either (1) or (2).



(1) (2)

In the n.m.r. spectrum of β-leprosol, the two aromatic methyl signals are resolved (τ 7·86 and 7·89 in $CDCl_3$). However, in three derivatives with equivalent oxygen substituents, *viz.* methyl β-leprosol, nor-β-leprosol, and nor-β-leprosol diacetate, the aromatic methyls give only one sharp 6-proton singlet in $CDCl_3$, and the OMe and COMe groups of the dimethyl ether and the diacetate similarly give sharp 6-proton singlets. For this to occur in every case, absolute symmetry must be invoked, rather than a mere coincidence of chemical shifts, indicating structure (1) for nor-β-leprosol. This was confirmed by comparing the n.m.r. spectra of methyl β-leprosol in $CDCl_3$ and in C_6D_6; despite the quite large shifts (CMe, τ 7·87 to 7·63; OMe, τ 6·20 to 6·54) both 6-proton singlets remained unresolved. In the dimethyl ether of the alternative structure (2), the CMe at C-2 would be expected to show a much larger shift than that at C-4.[5]

As already noted, compound (1) was actually synthesized as a nor-leprosol analogue by Asano and Iguti.[3] The recorded differences of m.p. are presumably due to differing proportions of homologues, and perhaps alkenyl analogues, in the natural product and the synthetic material (the latter being ultimately derived from a natural, and probably heterogeneous, fatty acid). We ourselves also synthesized (1), by a route essentially similar, obtaining a 4 : 1 mixture of the 5-heptadecyl and 5-nonadecyl compounds with m.p. 102—105° (dimethyl ether, m.p. 68—70; diacetate, 48—50°); these m.p. data actually correspond quite adequately with the literature data for nor-β-leprosol and its derivatives. More significantly, the mass spectra, n.m.r. spectra, and g.l.c. retention times of the heptadecyl components of the naturally derived and synthetic materials were identical.

β-Leprosol is therefore the monomethyl ether of (1), *i.e.* a mixture of 3-n-heptadecyl-5-methoxy-2,4-dimethylphenol and 5-methoxy-2,4-dimethyl-3-n-pentadecylphenol. The side-chains suggest that β-leprosol arises by polyketide extension of palmitate and stearate, but the *C*-methylation pattern of the ring is unexpected; a comparison with the orcinol homologues from higher plants, and particularly with the series from *Anacardium* (cashew nut-shell) [6] is of interest.

EXPERIMENTAL

Mass spectra, with high resolution mass measurement of all significant ions, were measured with an A.E.I. MS9

[2] A. Butenandt and F. H. Stodola, *Annalen*, 1939, **539**, 40.
[3] M. Asano and S. Iguti, *J. Pharm. Soc. Japan*, 1951, **71**, 1218, 1223 (*Chem. Abs.*, **46**, 6095g).
[4] J. L. Occolowitz, *Analyt. Chem.*, 1964, **36**, 2177.
[5] R. G. Wilson, J. H. Bowie, and D. H. Williams, *Tetrahedron*, 1968, **24**, 1407; H. M. Fales and K. S. Warren, *J. Org. Chem.*, 1967, **32**, 501.
[6] J. H. P. Tyman, *Chem. Comm.*, 1967, 982; J. H. P. Tyman and L. J. Morris, *J. Chromatog.*, 1967, **27**, 287.

instrument; all molecular formulae quoted are based on high resolution mass measurements correct to within +3 p.p.m. N.m.r. spectra were measured with a Varian HA-100 instrument with tetramethylsilane as reference, for solutions in deuteriochloroform except where noted. Samples of cardol, 4-methylcardol, and 6-methylcardol [6] were given by Dr. Tyman (Brunel University).

Synthesis of Nor-β-leprosol.—The intermediates are those of the earlier route,[3] but individual steps have been improved. By Wenkert's procedure,[7] 3,5-dimethoxybenzyl alcohol gave 3,5-dimethoxy-n-heptadecylbenzene (70%), which with dimethylformamide–phosphoryl chloride in *o*-dichlorobenzene [8] gave quantitative a 1 : 4 mixture, resolved by chromatography in light petroleum–benzene–diethyl ether on alumina (H), of 4-n-heptadecyl-2,6-dimethoxybenzaldehyde and the required 2-n-heptadecyl-4,6-dimethoxybenzaldehyde, m.p. 54—55° (lit.,[3] 56—57°). This was converted into the semicarbazone, m.p. 108—109°, which with an excess of potassium t-butoxide in boiling toluene [9] gave 1-n-heptadecyl-3,5-dimethoxy-2-methylbenzene, m.p. 51—53° (lit.,[3] 46—47°). By repeating the formylation and reduction steps, this gave 2-n-heptadecyl-4,6-dimethoxy-3-methylbenzaldehyde (98%), m.p. 67—70° (lit.,[3] 63—64°), and 1-n-heptadecyl-3,5-dimethoxy-2,6-dimethylbenzene (methyl β-leprosol) (75%), m.p. 68—70° (lit.,[1] 68—69°; lit.,[3] 62—63°). Demethylation of the latter (pyridine–HCl; yield 97%) gave 5-heptadecyl-4,6-dimethylresorcinol (nor-β-leprosol), m.p. 102—105° (lit.,[1] 104—105° lit.,[3] 97—98°); diacetate, m.p. 48—50° (lit.,[1] 48°).

Mass Spectra.—*Naturally derived nor-β-leprosol: m/e* 376 (M^+, $C_{25}H_{44}O_2$) (20%), 348 (M^+, $C_{23}H_{40}O_2$) (100), 152 [$C_9H_{12}O_2$; M – RCH:CH_2 (*m** 61·5 and 66·4)] (77), 151 ($C_9H_{11}O_2$) (32), and 137 (152 – 15, *m** 123).

Natural β-leprosol: m/e 390 (M^+, $C_{26}H_{46}O_2$) (39%), 362 (M^+, $C_{24}H_{42}O_2$) (28), 166 [$C_{10}H_{14}O_2$; M – RCH:CH_2 (*m** 70·5 and 76·0)] (100), 165 ($C_{10}H_{13}O_2$) (34), and 151 (166 – 15, *m** 137·5) (18).

Side-chain cleavage of long-alkyl resorcinols. Approximate peak height ratios for β-cleavage with hydrogen transfer to direct β-cleavage were: for 4-n-octadecylresorcinol 0·09 : 1; for cardol 5 : 1; for 4-methylcardol 3·7 : 1; for 6-methylcardol 3·6 : 1; for nor-β-leprosol 2·4 : 1; and for β-leprosol 3·0 : 1.

N.m.r. Spectra.—*β-Leprosol:* τ 3·73 (s, Ar–H), 5·22br (s, ArO–H), 6·25 (3H,s, ArO–Me), 7·86 (3H,s, Ar–Me), and 7·89 (3H,s, Ar–Me).

Nor-β-leprosol: τ 3·81 (s, Ar–H) and 7·87 (6H,s, Ar–Me).

Methyl β-leprosol: τ 3·64 (s, Ar–H), 6·20 (6H, s, Ar–OMe), 7·87 (6H, s, Ar–Me); in C_6D_6, τ 3·77 (s), 6·54 (6H, s), and 7·63 (6H, s).

Nor-β-leprosol diacetate: τ 3·36 (s, Ar–H), 7·72 (6H, s, ArO–Ac), and 7·92 (6H, s, Ar–Me).

Gas Chromatography.—On a column (3 ft.) packed with 3% SE-30 on DMCS-Chromosorb W at 220° in nitrogen, relative retention times were: 1-n-heptadecyl-3,5-dimethoxy-2,6-dimethylbenzene (synthetic methyl β-leprosol) 1·00, n-nonadecyl component in the foregoing 1·87, and naturally derived methyl β-leprosol 1·00 and 0·54 (n-pentacyl component). Similarly, at 220° relative retention times were: 1-n-heptadecyl-3,5-dimethoxybenzene 1·00, 1-n-heptadecyl-3,5-dimethoxy-4-methylbenzene 1·15, and 1-n-heptadecyl-3,5-dimethoxy-2-methylbenzene 1·33.

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