SOME STRUCTURAL FEATURES OF BORRELIDIN, AN ANTI-VIRAL ANTIBIOTIC

By Drs. K. ANDERTON and R. W. RICKARDS

Department of Chemistry, University of Manchester

THE isolation from fermentation media of *Streptomyces* C2989 of material, λ_{max} 258 mμ, with high activity *in vitro* against *Corynebacteria* and viruses, has been described by Lumb *et al.*[1]. Purification of this substance by fractionation with alkali and chromatography on silica gel afforded a crystalline antibiotic $C_{28}H_{43}NO_6$, m.p. 146°–148°, $[\alpha]_D^{21}$ −28·8° (in ethanol), λ_{max} 258 mμ (log ε 4·54, in ethanol). These properties agree with those recorded for borrelidin, m.p. 145°–146°, $[\alpha]_D^{27}$ −28° (in ethanol), λ_{max} 256 mμ ($E_{1\,cm}^{1\%}$ 550, in isopropanol), an acid with anti-borrelia activity[2] isolated by Berger, Jampolsky and Goldberg[3] from *S. rochei* and suggested to have the formula $C_{28}H_{43}NO_6$. Diazomethylation of our acid gave a methyl ester, m.p. 155·5°–156·5°, which was identical (mixed m.p. and infra-red spectrum) with authentic borrelidin methyl ester[3], m.p. 153°–154°. Acetylation of our ester gave a methyl ester diacetate, m.p. 190°–192°; the corresponding borrelidin derivative[3] has m.p. 190°.

The previously suggested formula, $C_{28}H_{43}NO_6$, for borrelidin itself was confirmed by the present micro-analytical data, and by high-resolution measurement of the mass-to-charge ratios of the molecular ions in the mass spectra of borrelidin methyl ester (found, on ^{12}C scale: 503·3236 ± 0·002; $C_{29}H_{45}NO_6^+$ requires 503·3247) and its diacetate (found, on ^{12}C scale: 587·3444 ± 0·004; $C_{33}H_{49}NO_8^+$ requires 587·3458). We thank Dr. M. Barber, Associated Electrical Industries, Ltd., for these spectra, which were obtained with an MS9 double-focusing spectrometer.
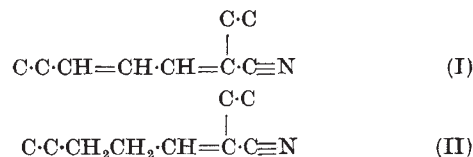
Borrelidin has no carbonyl activity, and the oxygen functions clearly comprise a carboxylic acid, a lactone or ester, and two hydroxyl groups. Thus borrelidin methyl ester lacks the dominant carboxyl absorption (ν_{max} in 'Nujol' 3,500–2,500 and 1,730–1,700 cm^{-1}) present in the infra-red spectrum of the parent compound, and shows two hydroxyl peaks (ν_{max} in 'Nujol' 3,500 and 3,400 cm^{-1}) and two saturated ester-type maxima (ν_{max} 1,738 and 1,720 cm^{-1}). Conversion into the methyl ester diacetate removes the hydroxylic absorption, while the four ester-type carbonyls are now unresolved (ν_{max} in 'Nujol' 1,735–1,725 cm^{-1}).

The lactone or ester function of borrelidin terminates on a secondary carbon atom (as in $-CO_2CH<$), giving rise to a multiplet (1H) centred at τ 5·1 in the proton magnetic resonance (p.m.r.) spectrum of borrelidin methyl ester. P.m.r. spectra were recorded at 60 Mc/sec for deuterochloroform solutions containing tetramethylsilane as internal reference, except where otherwise stated. Further broad absorption (2H) between τ 5·8 and the methoxyl singlet (3H) at τ 6·3 is due to protons attached to hydroxyl-bearing carbon, and undergoes a paramagnetic shift to τ 4·7–5·2 on mild acetylation, indicative[4] of secondary alcohol systems. Confirmation of the secondary nature of both hydroxyl functions in borrelidin was obtained from the p.m.r. spectrum of the methyl ester in dimethyl sulphoxide[5]. In this solvent, the strongly hydrogen-bonded hydroxylic protons appeared as doublets at τ 4·65 and 5·68 ($J = 3\cdot8$ and 4·5 c/s respectively), which were rapidly removed by deuterium exchange.

The ultra-violet absorption of borrelidin (λ_{max} 258 mμ, log ε 4·54) is unchanged by the addition of aqueous sodium hydroxide, by methylation alone (λ_{max} 258 mμ, log ε 4·50), or by methylation and acetylation (λ_{max} 259 mμ, log ε 4·55), indicating that the carboxyl and both hydroxyl functions are isolated from the chromophore. Hydrogenation of borrelidin methyl ester over palladium-charcoal

rapidly afforded a dihydro derivative, λ_{max} 214 mμ (log ε 4·09), whereas after extended reduction tetrahydroborrelidin methyl ester, showing end absorption only, was obtained. These compounds represent successive stages in the reduction of a conjugated diene-nitrile system. Thus *trans, trans*-sorbonitrile[6] ($Me\cdot CH=CH\cdot CH=CH\cdot C\equiv N$) has λ_{max} 254 mμ (log ε 4·48), *trans*-α-methylcrotononitrile[7] ($Me\cdot CH=CMe\cdot C\equiv N$) has λ_{max} 208 mμ (log ε 4·02), while saturated nitriles[8] are transparent in the near ultra-violet. In agreement, the sharp absorption due to conjugated $C\equiv N$ in the 2,220–2,170 cm^{-1} region of infra-red spectra of borrelidin and its simple esters is retained in the dihydro methyl ester (ν_{max} in CCl_4 2,195 cm^{-1}), but shifts to 2,240 cm^{-1} (in CCl_4), corresponding[9] to saturated $C\equiv N$, in the tetrahydro-derivative. Tetrahydroborrelidin methyl ester also lacks the olefinic absorption between 1,645 and 1,630 cm^{-1} which is present in the less saturated derivatives. Alkaline hydrolysis of borrelidin yields ammonia, as expected.

The p.m.r. spectrum of borrelidin methyl ester shows three protons attached to the conjugated olefinic system (multiplets, τ 3·0–3·9), whereas the dihydro ester has only one (quartet, τ 3·51). In conjunction with the formation of glyoxal on oxononolysis of borrelidin, together with the absence of allylic–methyl p.m.r. absorption, this evidence necessitates that the dienenitrile chromophore must be substituted as in (I), with system (II) as the dihydro chromophore.

$$C\cdot C$$
$$C\cdot C\cdot CH=CH\cdot CH=\overset{|}{C}\cdot C\equiv N \qquad (I)$$

$$C\cdot C$$
$$C\cdot C\cdot CH_2CH_2\cdot CH=\overset{|}{C}\cdot C\equiv N \qquad (II)$$

Hydrogenation past the tetrahydro stage over a platinum catalyst resulted only in reduction of the nitrile group itself. There is thus no evidence for additional olefinic bonds in borrelidin, and from the formula $C_{28}H_{43}NO_6$ the antibiotic must then be bicyclic. One ring must be carbocyclic, while the fragmentation patterns observed in mass spectra of borrelidin derivatives indicate the presence of a lactone ring, rather than a simple ester function. Growth of *Streptomyces* C2989 on a medium containing sodium [2-^{14}C] propionate afforded labelled borrelidin (0·2 per cent tracer incorporation) with little randomization of isotope. Thus borrelidin is probably an antibiotic of the macrolide type[10], although possessing structural features which are unique among this group.

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