6- and 12a-Hydroxylation of 6-Methylpretetramid

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Summary Oxygenation of 6-methylpretetramid (I) in Me_2N·CHO–0·1N-KOH, or Me_2SO–saturated-$Mg(OAc)_2$, resulted in specific hydroxylation at the 6- and 12a-positions.

THERE is good evidence in support of the suggestion that 6-methylpretetramid (I) is a biosynthetic precursor of tetracycline (III) and that enzyme-catalysed hydroxylation processes are involved in this transformation.[1] In previous publications,[2] we have described the conversion of 6-methylpretetramid into the quinone (II) by oxygenation in aqueous 0·1N-KOH. We now report that, if the solvent is changed to Me_2N·CHO–0·1N-KOH (1 : 1 to 10 : 1) or to saturated aqueous $Mg(OAc)_2$–Me_2SO (1 : 99), oxygenation of 6-methylpretetramid takes another course. There is a specific uptake of two molecular equivalents of oxygen to give the product (IV) in virtually quantitative yield. This reaction is evidently of interest in relation to the possibility of synthesising tetracyclines from pretetramids.

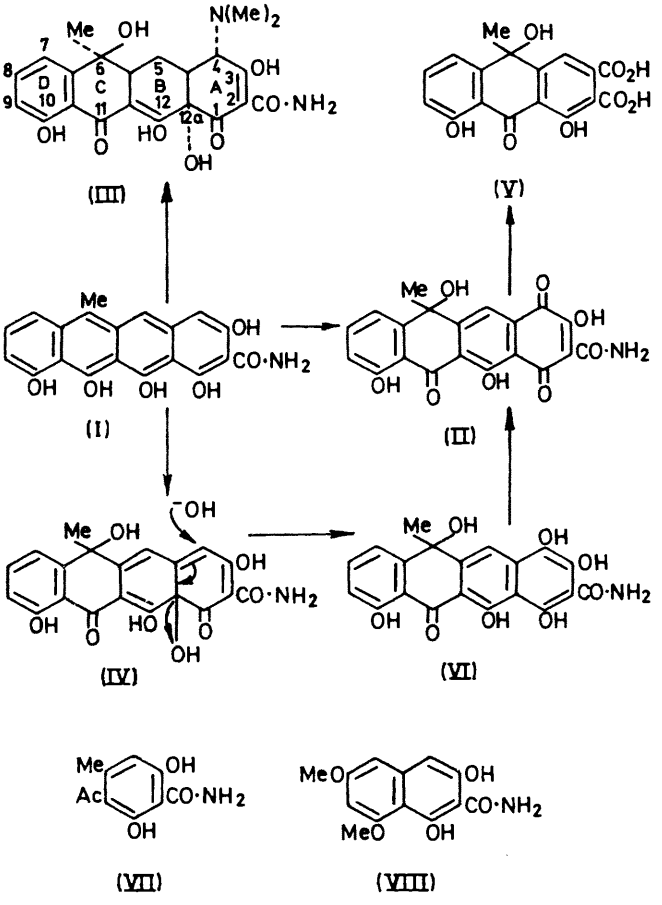
The molecular structure (IV) of the oxygenation product has been established by means of spectroscopic evidence and degradation. The formula $C_{20}H_{15}NO_8$ was defined by high resolution mass spectrometry and analysis by combustion. Evidence of the relevant metastable ions in the mass spectrum and accurate mass determinations confirmed each stage of the fragmentation sequence:

$$\begin{array}{ccc} C_{20}H_{15}NO_8 & \xrightarrow{-H_2O} & C_{20}H_{13}NO_7 \\ (m/e\ 397) & & (m/e\ 379) \\ & & \downarrow -NH_3 \\ C_{19}H_{10}O_6 & \xleftarrow{-CO} & C_{20}H_{10}O_7 \\ (m/e\ 334) & & (m/e\ 362) \end{array}$$

The preparation of tri-acetyl and hexasilyl derivatives also served to establish the molecular formula.

When the compound was refluxed with hydriodic acid in phenol for 1·5 minutes it was converted back in almost quantitative yield into 6-methylpretetramid (I). This indicated that the carbon skeletons of both compounds were the same. Treatment with alkaline hydrogen peroxide at 60° for 2 h gave the acid (V), a known product of oxidation of the quinone (II).[2,3] The formation of this acid from both the new compound and this quinone suggested that one might be converted into the other under the reaction conditions. This was confirmed. Treatment of the new compound (IV) in air with 0·1N-KOH at 60° gave a good yield of the quinone. However, it was established that the new compound was not an intermediate in the conversion of 6-methylpretetramid into the quinone (II) by alkaline oxygenation at room temperature. Under these conditions it was recovered unchanged from the reaction mixture. We suggest that the quinone is formed through the hydroquinone (VI) by hydroxyl-ion attack of the dienone (IV) at the 4-position. This resembles the conversion of 4-hydroxy-4-phenylcyclohexa-2,5-dien-1-one into 1,2-dihydroxy-4-phenylbenzene in alkali.[4]

The ^1H n.m.r. spectrum of the new compound and its tri-acetyl and hexasilyl derivatives gave important information concerning the molecular structure. The signals for the 6-methyl group in these three compounds τ 8·46 [$(CD_3)_2SO$]; 8·24 [$(CD_3)_2SO$] 8·2, 8·29 (3*H*, in CCl_4), respectively, established that this function was not attached to an aromatic ring c.[5] It followed that the 6-hydroxy-group, as in the anthrone (V), was a substituent in the new compound. With this, only one of the oxygen atoms remained unaccounted. A detailed study of the signals in the



aromatic region of the ^1H n.m.r. spectrum confirmed that there were three protons in adjacent positions with chemical shifts [τ 3·07, 2·35, 2·69 for (IV), $(CD_3)_2SO$] and coupling constants ($J_{7,8} = J_{8,9} = 8$ Hz) as for the 7,8,9 positions of tetracyclines. Moreover, the effects of NaOD and of *O*-silylation on the chemical shifts of these protons were very similar for the new compound and the tetracyclines. Evidently ring D of the new compound had not been further substituted. The ^1H n.m.r. spectrum included two singlets [τ 2·72, 3·35; $(CD_3)_2SO$] which we have assigned to protons at the 4- and 5-positions. Each signal integrated for one proton and the chemical shift was virtually unaffected by the addition of NaOD; this indicated that these

protons in the new compound were not attached to phenolic rings. An examination of the ^1H n.m.r. spectra of the model compounds (VII) and (VIII) established that there were as expected for these cases, substantial changes (25, 13 Hz) in the chemical shifts of the protons at positions 4, 5, respectively, as a result of addition of sodium deuteroxide. This has led, unequivocally, to the formulation of the compound as (IV). Structures with hydroxyl substituents at alternative positions, or with peroxide functions, could not account for the properties of the new oxygenation product. Also the structure (IV) and ring A tautomers, alone accounted in a satisfactory way for the band at 442 nm in the u.v. absorption spectrum. Similar calculations based on the addition of values for known chromophores, and on the use of Woodward's rules, gave results in good agreement with observed values in the case of tetracycline and 4a,12a-anhydrotetracycline.

(*Received, June 23rd*, 1970; *Com.* 994.)

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