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CHROMOMYCINONE, THE AGLYCONE OF CHROMOMYCIN A_3

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The structure of the chromophore chromomycinone was deduced as indicated in the following scheme.

Chromomycin A_3

↓ CH_3COOH, boil 2 hrs

Chromomycinone (CHR)

UV same as chromomycin A_3
∴ Chromophore still intact.

↓ i) Hydrogenation
ii) $NaBH_4$, then aerial oxidation in alkali.

Chromomyciquinone (CHQ)**

Chromomyciquinone (CHQ) $C_{21}H_{26}O_9$, m.p. 209-210°(dec.); with pyridine/acetic anhydride or a trace of conc. sulfuric acid/acetic anhydride it gave a hexaacetate, $C_{33}H_{38}O_{15}$,

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* On leave from Varian Associates, Palo Alto, California.

** After repeated trials, CHQ could be produced in two steps from CHR in a yield of 60% as shown in the Scheme. Hydrogenation with PtO_2/EtOH gave a hemiketal 10, which was then subjected to $NaBH_4$ reduction followed by aerial oxidation in alkali.

m.p. 183-185°, having a typical naphthoquinone UV spectrum, λ_{max}^{EtOH} 259 mμ(4.33*), 276 mμ(4.04), 355 mμ(3.56); whereas with zinc dust, sodium acetate and acetic anhydride it afforded a reductive octaacetate, $C_{37}H_{44}O_{17}$, m.p. 130°, having a naphthalenoid UV spectrum, λ_{max}^{EtOH} 235 mμ(4.80), 285 mμ(3.27). CHQ with acetone and a trace of conc. sulfuric acid yielded an acetonide, $C_{24}H_{30}O_9$, m.p. 130°, which could be converted into CHQ acetonide tetraacetate, $C_{32}H_{38}O_{13}$, m.p. 176° (FIG.1a and 1b). This acetonide tetraacetate was hydrolyzed by acetic acid to the diol tetraacetate, $C_{29}H_{34}O_{13}$, m.p. 206° (FIG.1c).

The structure of CHQ was deduced by examining the 100 mc spectra** of CHQ acetonide tetraacetate in $CDCl_3$ (FIG.1a) and in C_6H_6 (FIG.1b) and of CHQ diol tetraacetate in $CDCl_3$ (FIG.1c), together with UV and IR spectra and chemical evidence.

In FIG.1a the tall peak at 1.36 is due to the isopropylidene group protons while the two strong signals at 1.28 correspond to a secondary methyl group. The absorption due to the latter group appears as a single peak when proton (A) whose resonance occurs at 4.08 is irradiated. Conversely the multiplet (A) at 4.08 changes into a doublet when the secondary methyl signals are irradiated. Furthermore, the triplet at 3.70 arising from proton (B) also becomes a doublet when the resonance pattern around 5.15 is irradiated. The last two decoupled patterns due to protons (A) and (B) appear to be mirror images of each other, indicating that the two protons

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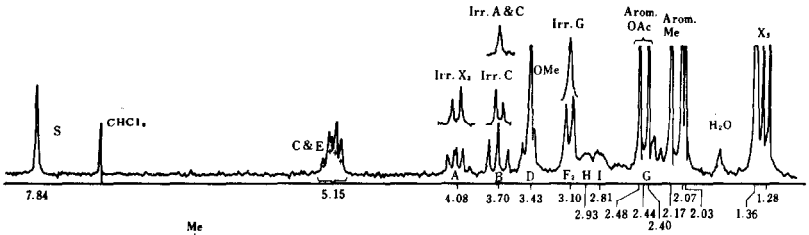
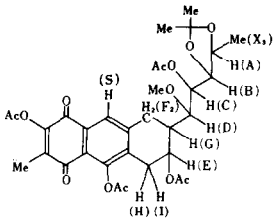
* Log ε values enclosed in parenthesis.

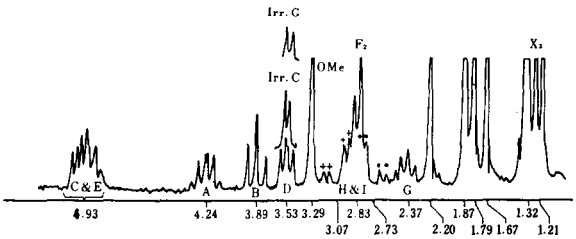
** The spectra where obtained on a Varian HR-100 spectrometer equipped to accomplish proton-proton spin-decoupling. TMS was used as internal reference, the chemical shifts being given in ppm.

FIG.1 NMR spectra, 100 mc.

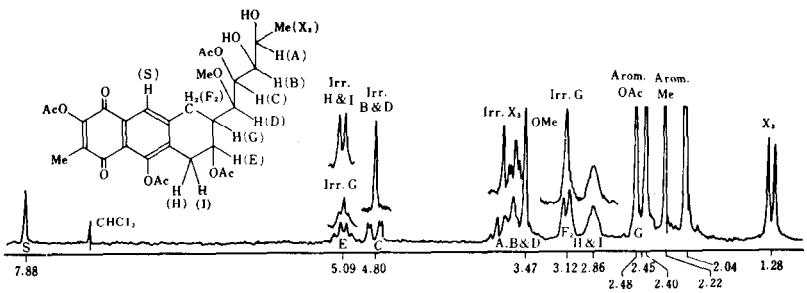




1a. chromomyciquinone acetonide tetraacetate, in $CDCl_3$.

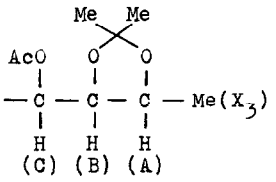


1b. chromomyciquinone acetonide tetraacetate in C_6H_6.



1c. chromomyciquinone diol tetraacetate in $CDCl_3$ shaken with D_2O.

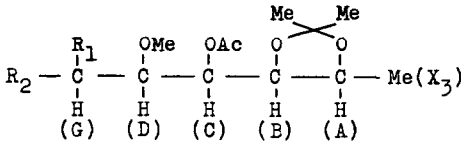
(A) and (B) are coupled to each other with a coupling constant of 7.8 cps. Since proton (B) appears as a triplet, its coupling constant to proton (C) (whose signal is obscured with that of another proton) must also be 7.8 cps. On the basis of the chemical shifts of the protons involved, the molecule is thought to contain the following moiety:



CHQ acetonide tetraacetate

The complicated resonance pattern around 5.15 (FIG.1a) which accounts for two protons is seen more clearly in the spectrum of CHQ diol acetate (FIG.1c). Conversely, the signals at 4.08 and 3.70 in FIG.1a are bunched together and overlap the resonance of still another proton in FIG.1c. Proton (C) appearing as a quartet at 4.80 (FIG.1c) is not only coupled to (B) but also to proton (D); the chemical shifts of protons (B) and (D) are very close together. The large coupling of 9 cps observed in the signal pattern of proton (C) is due to J_{BC} while the smaller one of 2.2 cps may be attributed to J_{CD}. The resonance due to proton (D) is scrambled with other signals in FIG.1a and 1c; however, in FIG.1b it is unmasked and is located at 3.53. As shown in FIG.1b, double resonance experiments indicate that proton (D) is coupled not only to proton (C) with a coupling constant of 5.5 cps but also to proton (G) (resonating at 2.37) with a coupling constant of 4.5 cps. The strong signals at 3.43, 3.29 and 3.47 in FIG.1a, b and c, respectively are due to a methoxyl group adjacent to proton (D). Therefore

from consideration of the chemical shifts of all the protons involved, the sample should contain the following moiety:



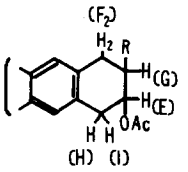
CHQ acetonide tetraacetate

where R_1 and R_2 = saturated carbon atoms.

It should be noted here that J_{BC} and J_{CD} are somewhat different in the two derivatives of CHQ. This may be due to either restricted rotation along the carbon-carbon bonds or electronegativity effects of the functions attached or both. This will be clarified while examining the stereochemistry of these compounds and will be reported later.

As shown in FIG.1a, the two peaks at 3.10, representing two protons, become a single absorption when the multiplet at 2.44 due to proton (G) is irradiated. This indicates that proton (G) is coupled to magnetically equivalent methylene group protons (F_2). FIG.1c indicates that proton (G) is coupled not only to protons (D) and (F_2) but also to proton (E), resonating at 5.09, since irradiation of proton (G) causes the multiplet at 5.09 to become a triplet. This experiment also indicates that proton (E) is further coupled to two other protons (H) and (I) with the same coupling constants, namely 2.5 cps. This can be confirmed by observing the resonance pattern for proton (E) while irradiating protons (H) and (I). The resonances for protons (G), (H) and (I) can be seen more clearly in FIG.1b. Protons (H) and (I) constitute an AB type quartet (J=12 cps) and the latter is further split (J=5 cps)

into an octet because of the adjacent proton (E). Examination of the chemical shifts and coupling patterns of the protons (E), (F_2), (G), (H) and (I) indicate that the following moiety is present in the sample.



R = side chain as shown earlier.

The tall peak at 2.17 (FIG.1a), and 2.22 (FIG.1c), are assigned to an aromatic methyl group. This chemical shift establishes that the methyl group is located on the quinonoid ring, since attachment of the methyl group on the second aromatic ring would make its position peri to a carbonyl group, in which case its resonance would be observed at a much lower field*.

The somewhat broad signals at 7.84 and 7.88 in FIG.1a and c is attributed to the aromatic proton peri to a carbonyl group. This broad signal becomes quite sharp when protons (F_2) are irradiated, indicating that the aromatic proton (S) exhibits a small coupling to the methylene group protons (F_2). In FIG.1a the remaining four tall resonances can be attributed to the acetate group protons; namely, the signals at 2.03 and 2.07 to aliphatic acetates and those at 2.40 and 2.48 to aromatic acetates.

Careful oxidation of CHQ with hydrogen peroxide cleaved the quinonoid ring to afford a phenolic dicarboxylic acid, 1 $C_{18}H_{24}O_{10}$ (thiouronide, m.p.176°), which lacked the aromatic

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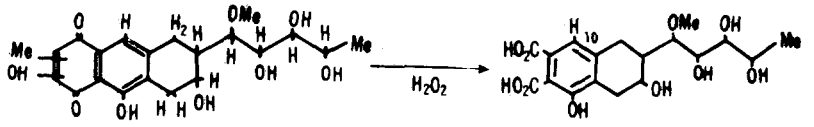
* Varian NMR Spectra Catalog, Vol.2, spectrum 650 (1963).

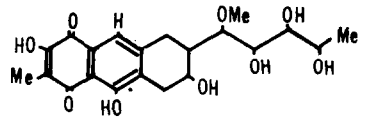
methyl NMR signal; this also indicated that the methyl group is located on the quinonoid ring.

Thus the structure for CHQ would be:

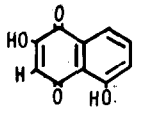


1

The exact location of the aromatic methyl on the quinonoid ring in CHQ was established by comparing its UV and IR spectra with those of appropriate model compounds, 2 and 3*. Thus the structure of CHQ is established as shown below:



$\nu_{cm^{-1}}^{KBr}$ 1650 and 1610



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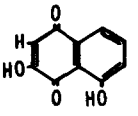
$\nu_{cm^{-1}}^{KBr}$ 1653 and 1618



3

$\nu_{cm^{-1}}^{KBr}$ 1625, broad

The preparation of pure crystalline chromomycinone proved to be troublesome. It was eventually accomplished by crystallisation of the ethyl acetate extract of crude CHR from acetic acid which yielded crystals containing two moles of acetic acid; heating in vacuo afforded solvent free material. Chromomycinone(CHR) $C_{21}H_{24}O_9$, m.p. 184-7°: Its UV spectrum,

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* In neutral media, the UV spectra of 2 and 3 are identical [M.Asano and J.Hase, J.Pharm.Soc. Japan, 63, 83 (1943)]; however, in 0.1N aq. NaOH they can be readily differentiated.

λ_{max}^{EtOH} 232 mμ(4.38), 282 mμ(4.60), 326 mμ(3.85), 340 mμ(3.85), 412 mμ(4.01), was superimposable on that of chromomycin A_3, and also very similar to that of 1-keto-8,9-dihydroxy-1,2,3,4-tetrahydroanthracene, λ_{max}^{EtOH} 267 mμ(4.67), 295 mμ(3.65), 304 mμ (3.55), 410 mμ(3.84). Zinc dust distillation of CHR yielded anthracene and 2-methylanthracene. Acetylation of CHR under various conditions gave a tetraacetate (5), a pentaacetate (6) and a hexaacetate (7), all three having different UV spectra. The IR spectrum of the hexaacetate indicated the absence of any hydroxyl band.

FIG.2 shows the 100 mc spectrum and spin-decoupling data for chromomycinone tetraacetate in $CDCl_3$. The chemical shifts are given in ppm.

The moiety which is present in CHQ and its derivatives is also present in CHR tetraacetate, as shown by spin-decoupling experiments (FIG.2). The secondary methyl signal is at 1.34, while the absorption due to protons (A) and (B) is located at 5.48 and 5.29, respectively. However, in this spectrum the

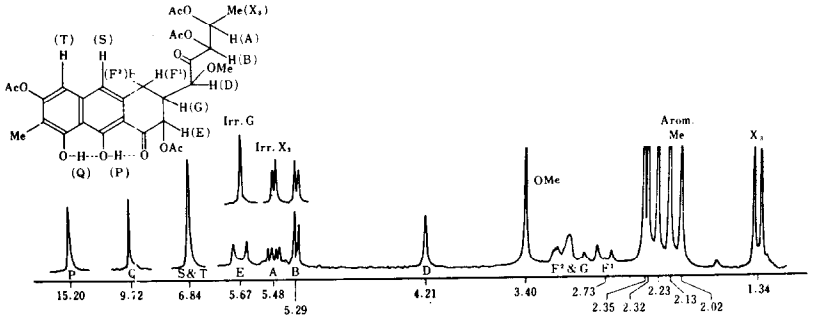
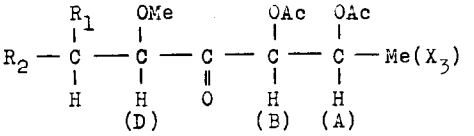


FIG.2 NMR spectrum of chromomycinone tetraacetate, 100 mc, in $CDCl_3$.

resonance pattern corresponding to proton (B) (unlike that of the CHQ derivatives) is only a doublet, suggesting that proton (C) is missing. The signal at 4.21 is attributed to proton (D). The absorption pattern for this proton shows only a slight coupling to the adjacent proton (G). This clearly indicates that proton (C) is not present in the molecule. Furthermore, the chemical shift of 4.21 for proton (D) is at a much lower field than for the same proton in CHQ derivatives. This fact along with the absence of proton (C) strongly suggests the carbon atom on which proton (C) was located has been replaced by an electronegative function such as a carbonyl group. IR and UV data showed the presence of a saturated carbonyl group in CHR, $\nu_{C=O}^{nujol}$ 1727 cm^{-1}; sodium borohydride reduction afforded dihydro-CHR which had practically the same UV spectrum but lacked this carbonyl absorption. Thus the side chain in the sample of CHR tetraacetate would be



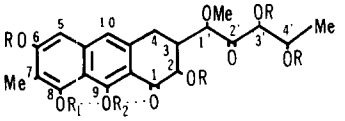
R_1 and R_2 = saturated carbon atoms.

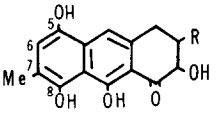
The signals at 5.67 are assigned to proton (E). These peaks at 5.67 become a single absorption when irradiated at the frequency of proton (G). It may be recalled that this proton (E) became a triplet under similar conditions in the sample of CHQ diol acetate. This would indicate that protons (H) and (I) are absent in the CHR tetraacetate. Furthermore the chemical shift of 5.62 for the proton (L) is at a much lower field than in the earlier sample, indicating that another electronegative function such as carbonyl has been introduced in place of the

methylene group. This was determined while examining the two downfield peaks at 9.27 and 15.20 which were assigned to aromatic hydroxyls peri to a carbonyl group. These two peaks disappeared upon addition of D_2SO_4 to the sample tube. These results were confirmed by examining the behaviour of corresponding peaks in the sample of 1-keto-8,9-dihydroxy-1,2,3,4- tetrahydroanthracene.

The resonance at 6.84 is assigned to the two aromatic protons (S) and (T) which are not coupled to each other. The rest of the tall peaks at 2.02, 2.23, 2.32, 2.35, 2.13 and 3.40 were attributed to four acetate groups, and aromatic methyl and methoxyl protons respectively.

Thus the structure of CHR may be written as 4 or 8 :



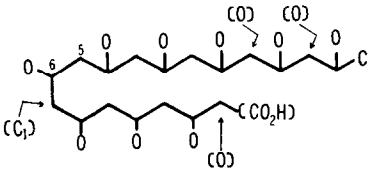


8

4 R = R_1 = R_2 = H, 5 R = Ac, R_1 = R_2 = H,

6 R = R_1 = Ac, R_2 = H, 7 R = R_1 = R_2 = Ac,

As mentioned earlier chromomyciquinone was obtained from chromomycinone. This can only occur if CHR has structure (4). Since, if structure (8) were correct oxidation to a quinone would occur without introduction of an additional hydroxyl



9

group, whereas in fact the formation of chromomyciquinone skeletons under various conditions is always accompanied with the introduction of an additional hydroxyl group. Furthermore, its biogenesis from the acetogenin 9 would require the introduction (C_5) and removal (C_6) of additional oxygen functions. Thus the structure of the aglycone chromomycinone is 4 and chromomyciquinone is formed by reduction and hydrogenolysis of the C_1 oxygen (leading to hemiketal 10) and subsequent reduction and oxidation.