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STRUCTURE OF A NEW CLASS OF C-NUCLEOSIDE ANTIBIOTIC, SHOWDOMYCIN

Yuzo Nakagawa, Hideo Kanō, Yoshiko Tsukuda and Hirozo Koyama

Shionogi Research Laboratory, Shionogi & Co., Ltd., Fukushima-ku, Osaka, Japan

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Showdomycin is a broad spectrum antibiotic, first isolated from Streptomyces showdoensis by Nishimura and his coworkers, in 1964 (1). The antibiotic has been found to exhibit definite activity against Ehrlich ascites tumor in vivo and against cultured HeLa cells (2). The structure has now been elucidated as a new class of C-nucleoside.

Showdomycin, m.p. 160–161°, has a molecular formula, $C_9H_{11}NO_6$ (1) and specific rotation of $[\alpha]_D^{22.5}$ +49.9° (c = 1, H_2O) (1). It is soluble in water and other polar solvents, and insoluble in nonpolar solvents. The ultraviolet spectrum of showdomycin has a main absorption maximum at 222 mμ (log ε 4.1) in 95% ethanol and the characteristic infrared absorption bands are assignable as follows: 3465, 3403, 3234, 3164, 2929 cm^{-1} (OH and/or NH); 1775, 1725, 1705, 1645 cm^{-1} (C=O, C=C). The ultraviolet spectrum and the carbonyl region in the infrared spectrum closely resemble those of citraconimide. Moreover its pKa value (9.60 ± 0.01) also resembles that of citraconimide (10.21 ± 0.01). The detection of 0.75 molar NH_3 by treatment of showdomycin with 30% KOH solution, again strongly suggests the compound possesses a maleimide-type imide group.

Acetylation of showdomycin with a mixture of acetic anhydride and phosphoric acid produced a triacetate (2), $C_{15}H_{17}NO_9$, m.p. 115–116°, and the reaction with acetone–$CuSO_4$ yielded an acetonide (3), $C_{12}H_{15}NO_6$, m.p. 140.5–141°. Both the acetate and the acetonide have the same absorption spectra as those of showdomycin, and reproduced showdomycin by treatment with dil. hydrochloric acid. The formation of these derivatives shows that showdomycin contains at least three hydroxyl groups and two of them have a cis configuration if they are vicinal. Catalytic hydrogenation of both the triacetate and the acetonide gave the corresponding dihydro derivatives: (4) $C_{15}H_{19}NO_9$, oil, and (6) $C_{12}H_{17}NO_6$, m.p. 173–174°, respectively.

Periodic acid oxidation of showdomycin did not afford formic acid, any volatile ketone or aldehyde, although one mole of periodic acid was consumed.

The above experimental results suggest that the structure of showdomycin consists of a maleimide portion and a furanose group bearing two cis vicinal hydroxyl groups. Both the sugar and the base moieties would be linked with C-C bond, since showdomycin resists hydrolysis with conc. hydrochloric acid.

In the n.m.r. spectrum of the triacetyl showdomycin (2) in deuterochloroform, a broad proton signal at 8.0 ppm assignable to an imide NH, and a triplet vinyl one proton signal at 6.6 ppm were observed. On addition of deuterium oxide the signal at 8.0 ppm disappeared and the one at 6.6 ppm changed to a sharp doublet signal, which shows NH and -CH= group are located as these protons are coupled to each other. Comparison of the n.m.r. spectrum of showdomycin in DMSO-d_6/CH_3COOD with that of a C-nucleoside antibiotic, formycin (3), and the analysis of the former using decoupling method, gave further informations: seven protons (H_a, H_b, H_c, H_d, $2H_e$ and an olefinic proton H_f) are all assigned as shown in FIG. 4. The H_a proton signal appears at 4.63 ppm as a quartet and couples with H_b and H_f with coupling constant 3.0 and 1.5 cps, respectively. The appearance of H_a at relatively higher field in comparison with that of adenosine (at 6.15 ppm) gives further support for a C-nucleoside structure. Lemiux and Lineback (4) have pointed out that in a furanose ring the coupling constant for vicinal cis protons theoretically requires a value larger than 3.5 cps and that for trans protons can vary down to 0 cps. The observed H_a-H_b coupling constant 3.0 cps is rather favouring to H_a-H_b trans configuration, and this was confirmed by the following n.m.r. study of the cycloshowdomycin derivative.

On treatment of showdomycin acetonide (3) with 10% potassium carbonate solution at room temperature a Michael type addition of the primary alcohol to the double bond proceeded to yield cycloshowdomycin acetonide, $C_{12}H_{15}NO_6$, m.p. 213.5°, which lacks the ultraviolet absorption at 222 mμ. This isomeric acetonide can be readily methylated with dimethyl sulfate to give N-methyl cycloshowdomycin acetonide (7), $C_{13}H_{17}NO_6$, m.p. 171-172°, which after hydrolysis to N-methyl cycloshowdomycin, $C_{10}H_{13}NO_6$, m.p. 175-176°, with dil. hydrochloric acid, was acetylated to give diacetyl N-methyl cycloshowdomycin (8), $C_{14}H_{17}NO_8$, m.p. 212-215°. The n.m.r. spectrum of the diacetate (8) could be well assigned as shown in FIG. 5. The observed coupling constant of nearly 0 cps between H_a and H_b gives a definite evidence for the trans H_a-H_b configuration, in fact a Dreiding molecular model for this compound shows that the angle between those protons is almost 90°. The formation of cycloshowdomycin indicates the maleimide and methylene alcohol group should be located in the same side to the furanose ring, and therefore the structure

FIG. 1. UV-spectra of showdomycin and citraconimide

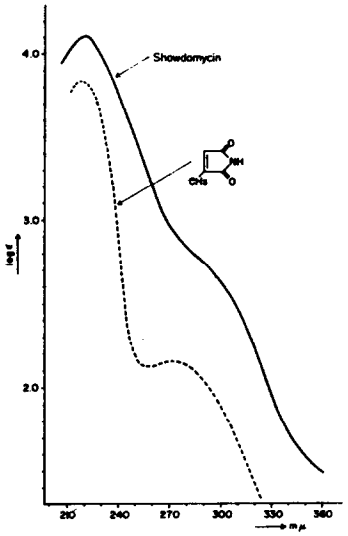
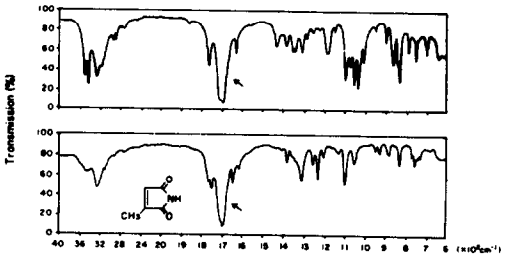
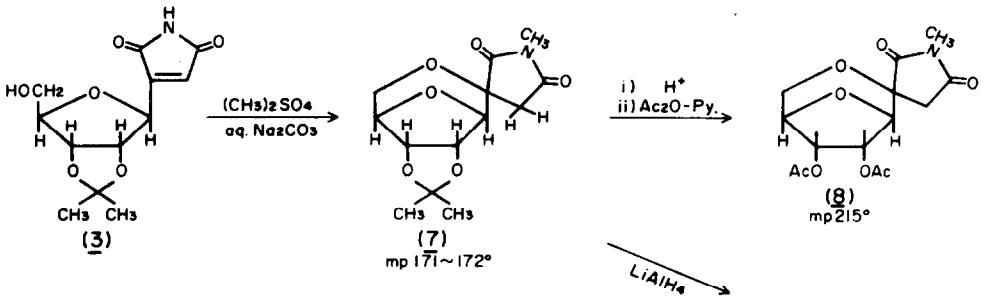


FIG. 2. IR-spectra of showdomycin and citraconimide

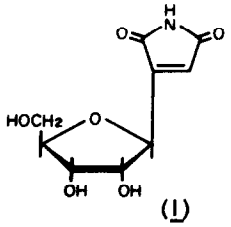




(3)

(7)
mp 171~172°

(8)
mp 215°



(1)
Showdomycin

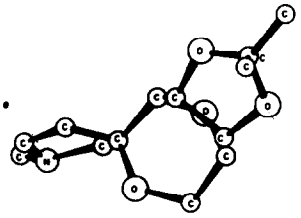
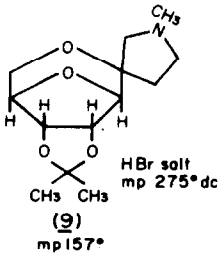


FIG. 3. The molecular structure of 9.



(9)
mp 157°

FIG. 4. N.m.r. spectrum of showdomycin in DMSO-d_6/AcOD at 100 Mcps.

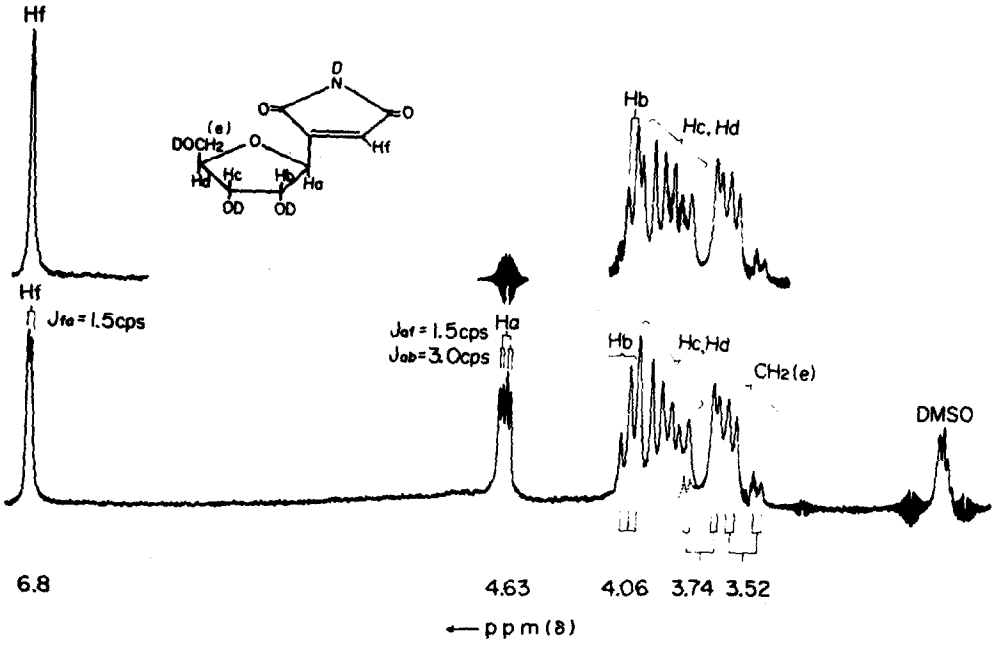
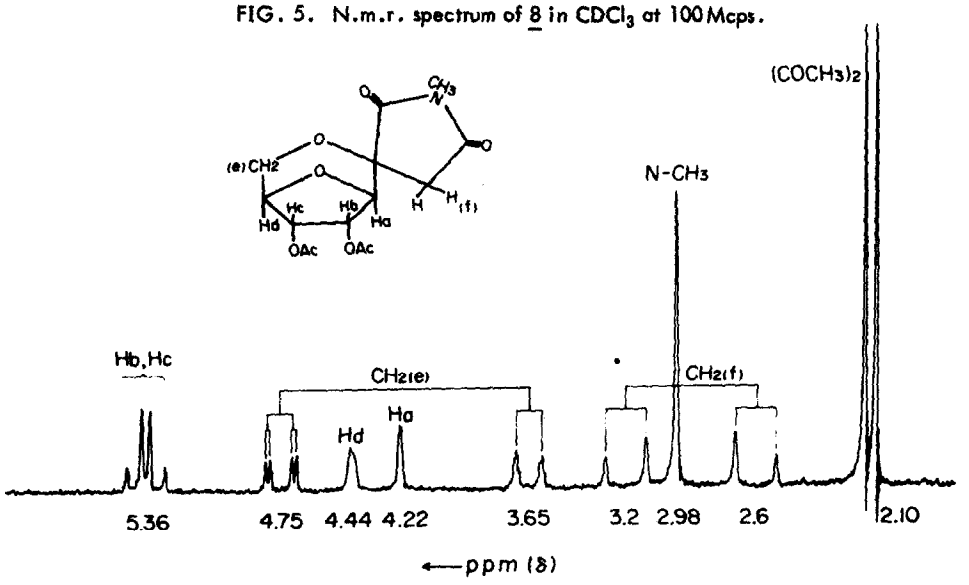


FIG. 5. N.m.r. spectrum of 8 in $CDCl_3$ at 100 Mcps.



of showdomycin has been assigned to 2-(β-D-ribofuranosyl)maleimide or its mirror image.

To confirm the structure, an X-ray structure analysis of the N-methyl bisdeoxo cycloshowdomycin acetonide (9) hydrobromide,* which was derived from N-methyl cycloshowdomycin acetonide by reduction with lithium aluminum hydride,** was carried out and the absolute configuration shown in FIG. 3 was obtained.***

From the structural relationship between this derivative and showdomycin, the structure of showdomycin has been established as 2-(β-D-ribofuranosyl)maleimide (1). To our knowledge, this is the third C-nucleoside obtained from natural source; firstly pseudouridine (5) and secondly formycin antibiotics (6).

After we finished this work, a report on the structure of showdomycin has been presented by R. K. Robins and his coworkers (7), in which they also have reached to the same conclusion by chemical and spectral investigations.

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* Some attempts to obtain a more simple crystalline derivative containing a heavy atom and suitable for the analysis were unsuccessful.

** Reduction with lithium aluminum deuteride afforded bisdeoxo N-methylcycloshowdomycin acetonide-d_4.

*** The details was read by one of us, Tsukuda at the 20th Annual Meeting of the Chemical Society of Japan, March 1967, and will be published in the Chemical Communication.