A NEW ANTIBIOTIC, HONDAMYCIN. III

CHEMICAL STUDIES ON HONDAMYCIN. I

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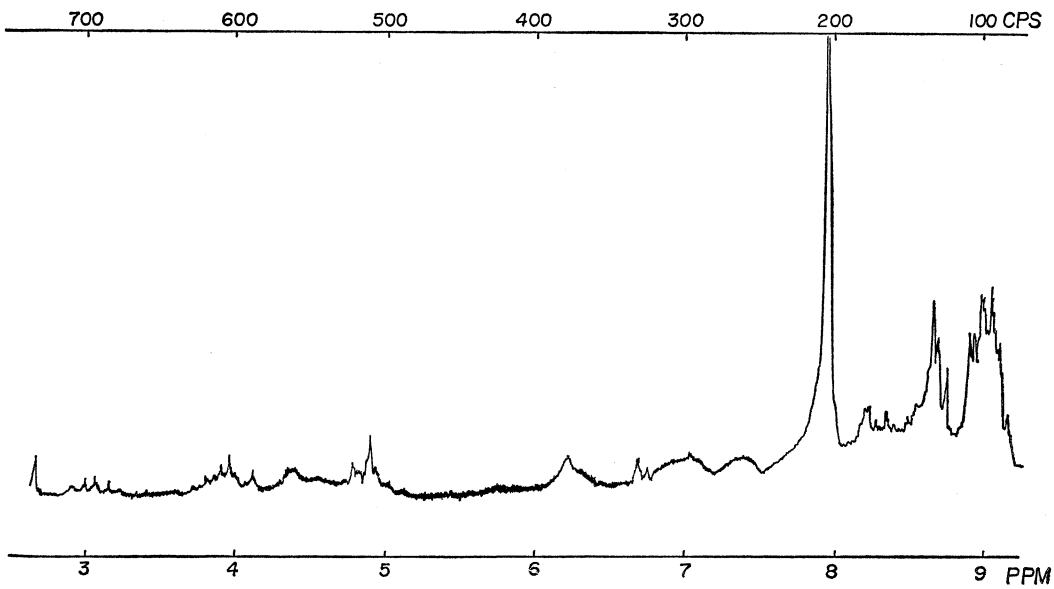
The oxidation and alkaline degradation of the antibiotic hondamycin ($C_{47}H_{78}O_{13}$) give a tentative identification of the various functional groups. Evidence for an α,β-unsaturated ester group was obtained from ultraviolet, infrared and nuclear magnetic resonance spectra. Catalytic hydrogenation resulted in the reduction of four double bonds.

Hondamycin is a crystalline antibiotic produced by *Streptomyces griseochromogenes* var. *albicus* and its isolation and characterization has been described in previous reports of this series[1,2]. The empirical formula $C_{47}H_{78}O_{13}$ was established by elemental analysis and the molecular weight determined by several methods including mass spectrometry.

Hondamycin was found to contain no methoxyl or acetoxyl groups, and no nitrogen, halogen or sulfur. C-methyl determination gave a minimum value of 10 such groups, and the presence of 8 hydroxyl groups and 3 carbonyl groups was indicated. Under routine conditions hondamycin triacetate yields crystalline needles, and the ion peak of its triacetate was found at m/e 958 (958.5957; calcd. for $M^+-H_2O=C_{53}H_{82}O_{15}$: 958.5653)[3].

Fig. 1. N. M. R. spectrum of hondamycin triacetate (in $CDCl_3$, 100 MC, TMS)



Absorption maxima in the ultraviolet spectrum occur at 225 and 232.5 mμ with inflections at 219, 240 and 270~295 mμ indicating a conjugated diene system and another chromophore.

Hondamycin was hydrogenated in ethanol with a platinum catalyst to a derivative (2), m. p. 74~76 (dec.). The uptake of hydrogen was 4.0 moles based on a molecular weight of 851 and microanalysis of octahydrohondamycin supported the molecular formula $C_{47}H_{86}O_{13}$. Its ultraviolet spectrum showed only weak end absorption (ε_{218} 560) indicating that octahydrohondamycin is a saturated compound. Hondamycin (potassium bromide disk) shows a strong band at 1700 cm^{-1} in the infrared which weakened on hydrogenation. A strong band at 1290 cm^{-1} disappeared with new bands at 1730, 1250, 1190 and 1170 cm^{-1} appearing at the same time. The nuclear magnetic resonance spectrum of hondamycin displayed a one-proton double doublet at τ=3.26 (J=10, 18 cps) and a one-proton doublet at τ=4.16 (J=18 cps). These observations are characteristic of *trans* olefinic protons conjugated with a lactone.

KUHN-ROTH oxidation of hondamycin gave acetic acid and propionic acid which

Fig. 2. Infrared spectrum of octahydrohondamycin (KBr)

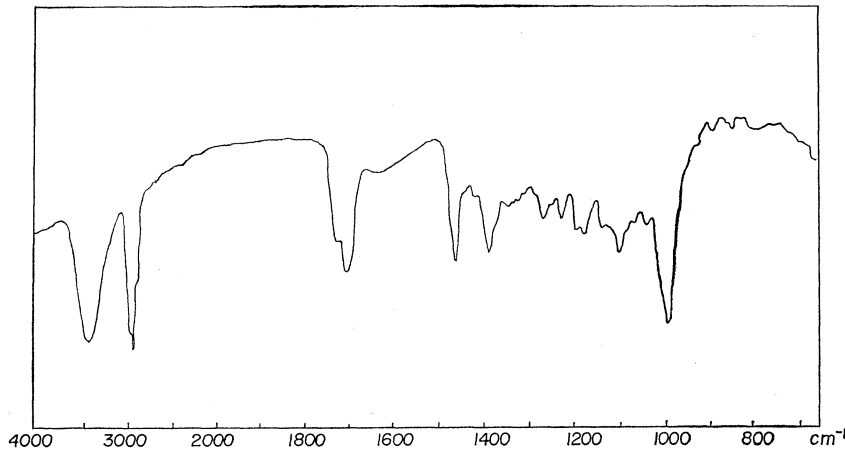
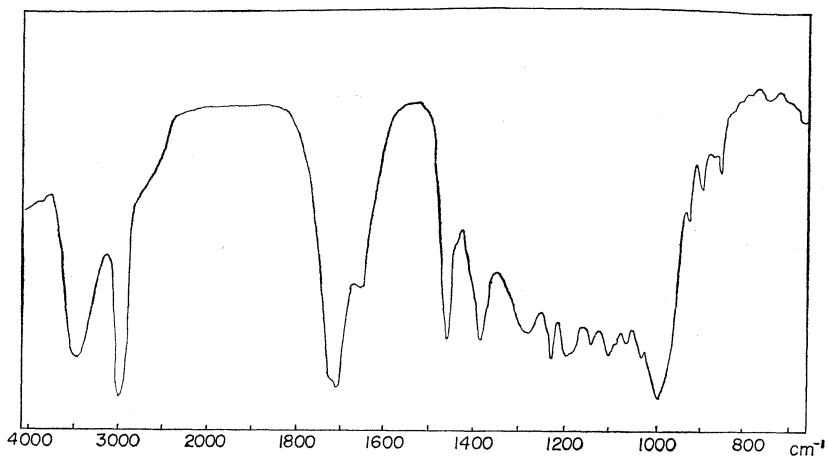


Fig. 3. Infrared spectrum of monocarboxylic acid (3)



were identified by paper and gas chromatography[4]. Alkaline degradation of hondamycin in methanol gave tiglic aldehyde, identified as its 2,4-dinitrophenylhydrazone.

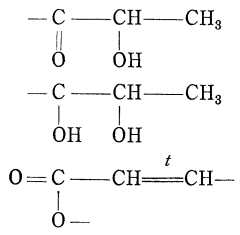
Hondamycin consumed two molar equivalents of periodate in neutral solution to give about two moles of acetaldehyde after 24 hours. The oxidation of hondamycin with sodium periodate gave an acid (3), m.p. 78~80, $C_{43}H_{68}O_{12}$, pKa 5.9, which showed an absorption maximum at 225 mμ and inflections at 220 and 240 mμ in the ultraviolet spectrum. These findings together with positive TOLLENS and FEHLING reactions suggest the presence of an alpha-ketol group in hondamycin.

The ultraviolet spectrum of acid (3) was nearly identical to the spectrum of hondamycin thus showing that no major change had taken place in the polyene system and that the chromophore in acid (3) is the same as in hondamycin. The neutral equivalent and molecular weight determinations of the acid (3) established that it was monocarboxylic.

Since the antibiotic failed to condense with 2,4-dinitrophenylhydrazine, it was assumed that the carbonyl group was sterically hindered. Evidence for the presence of a sugar in hondamycin was negative from the result of methanolysis.

Hondamycin belongs, in many of its properties, to a group of antibiotics such as oligomycin A[5], mycoticin[6], fungichromin[7]. These observations are in agreement with partial formula I in which the tiglic aldehyde is formed by a retroaldol condensation and dehydration.

3 *sec.* OH
ter. OH
4 C=C
C=O



I

Experimental

Hondamycin

A sample of the antibiotic which had been purified by chromatography and counter current distribution, was crystallized from methanol as hexagonal plates, m. p. 149~150 (dec.) $[\alpha]_D^{13.5}$ −48.1, λ_{max}^{MeOH} 225 (log ε 4.58), 232.5 (log ε 4.54), 240 (log ε 4.35), 270~295 mμ (log ε 1.97), ν_{max}^{KBr} 1700, 1650, 1290 cm^{-1}.

Anal. Calcd. for $C_{47}H_{78}O_{13}$: C 66.31, H 9.24, O 24.45, 10×C-CH_3 17.67
Found : C 66.38, H 9.25, O 24.37, C-CH_3 17.3

Acetylation of hondamycin

A solution of 100 mg of hondamycin in 20 ml of acetic anhydride and 5 ml of pyridine was allowed to stand for 24 hours at room temperature and then concentrated to 10 ml *in vacuo*. After 100 ml of ice-water was added, the reaction mixture was allowed to stand for 24 hours at room temperature and filtered. The precipitate was washed with water and dried *in vacuo*. Crystallization from hexane-acetone gave crystalline needles, m. p. 120 (dec.) $[\alpha]_D^{13.5}$ −90, ν_{max}^{KBr} 1740, 1715, 1240 cm^{-1}.

Anal. Calcd. for $C_{53}H_{84}O_{16}$: C 65.16, H 8.61, O 26.23
Found : C 65.41, H 8.19, O 26.40
m/e 958.5957 ($M-H_2O=C_{53}H_{82}O_{15}$: 958.5653)

Octahydrohondamycin

A solution of 729 mg of hondamycin was hydrogenated with 60 mg of palladium black catalyst in 50 ml of absolute ethanol for 18 hours at room temperature. The hydrogenated product was crystallized from acetone-hexane, m. p. 74~76 (dec.), $[\alpha]_D$ −70.2, ν_{max}^{KBr} 1730, 1700, 1170, 1190, 1250 cm^{-1}, λ_{max}^{MeOH} 285 mμ (log ε 1.38). These data indicate the presence of a carbonyl group and the absence of any additional double bonds.

Anal. Calcd. for $C_{47}H_{86}O_{13}$: C 65.70, H 10.09, O 24.21
Found : C 66.26, H 9.88, O 23.86

Substantially the same results were obtained when platinum oxide was substituted for the palladium catalyst.

KUHN-ROTH oxidation of hondamycin

Twenty mg of hondamycin was oxidized following the modified procedure of GARBERS, SCHMID and KARRER[4]. The paper chromatogram showed spots corresponding to acetic acid and propionic acid.

Hondamycin (103 mg) was oxidized with 5 ml of chromium trioxide-sulfuric acid mixture and 20 ml of water following the method of ENSTRE and KARRER[8]. The distillate (70 ml) was collected and extracted with ether. The ether extract was dried over sodium sulfate and treated with diazomethane. The ether was slowly distilled and the remaining methyl esters were subjected to gas chromatography at 100°C using a column of polyethylene glycol 200. Results showed the presence of the methyl esters of acetic and propionic acids. Authentic samples of the methyl esters were used as standards.

Quantitative periodate oxidation of hondamycin

A sample of hondamycin, 250 mg, was dissolved in 150 ml of ethanol-water mixture (2 : 1) and to it was added 100 ml of 0.02 M potassium metaperiodate solution. Aliquots (25 ml) were removed for titration by the thiosulfate method at intervals from 3 hours to 24 hours. The values ranged between 1.80 and 2.11 molar equivalents of periodate consumed and slow uptake of periodate was observed.

Periodate oxidation of hondamycin

Hondamycin, 1.411 g, was dissolved in 350 ml of ethanol and 400 ml of 0.02 M sodium metaperiodate solution was added. The reaction mixture was allowed to stand at room temperature for 4 days. After removal of ethanol under diminished pressure, the remaining solution was adjusted to pH 9.5 and filtered. The colorless clarified aqueous solution was acidified with hydrochloric acid and the remaining precipitate was filtered and washed with water. By repeating this treatment several times, 1.233 g of monocarboxylic acid was obtained as a colorless crystalline powder, m. p. 78~80 (dec.), λ_{max}^{MeOH} 225 (log ε 4.48), 220 (log ε 4.46, inf.), 230 (log ε 4.44, inf.), pKa 5.9 (86.6 % of methanol aqueous solution), titration eq. 791, M. W. 821 (thermoelect.).

Anal. Calcd. for $C_{43}H_{68}O_{12}$: C 66.44, H 8.82, O 24.74
Found : C 65.76, H 9.02, O 25.22

Isolation of tiglic aldehyde from hondamycin

Hondamycin (312 mg) was dissolved in 5 ml of methanol and 3 ml of 1 N sodium hydroxide was added. After heating on a water bath (90~95°C) for 6 hours, the reaction mixture was steam-distilled into a solution of 2,4-dinitrophenylhydrazine in aqueous hydrochloric acid. The mixture was allowed to stand overnight and the precipitate was collected, dried (73 mg) and chromatographed on a silica gel column. Elution with benzene gave tiglic aldehyde, m. p. 219.5. A mixed melting point with authentic material was undepressed. The infrared spectra of the two samples were identical.

Acetaldehyde and propionic aldehyde 2, 4-dinitrophenylhydrazone were also identified in the other fractions.

Acknowledgements

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