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Chemical Studies of Coumermycin A_1, a New Antibiotic

Hiroshi KAWAGUCHI*

A new antibiotic, coumermycin A_1, was isolated from fermentation broth of *Streptomyces rishiriensis* nov. sp. which was isolated from the soil sample collected in Rishiri Island, Hokkaido, Japan.

Coumermycin A_1 is a colorless acidic substance having a molecular formula of $C_{55}H_{59}N_5O_{20}$ and its monosodium salt crystallizes as colorless needles melting at 245°C with decomposition. The ultraviolet spectrum of coumermycin A_1 has two absorption maxima at 280 mμ and 330 mμ, and the latter absorption maximum shows a characteristic hypsochromic shift of novobiocin type in going from acid to alkaline solution. Coumermycin A_1 is optically active : $[\alpha]_D^{25}=-141°$.

Coumermycine A_1 exhibits antimicrobial activity both in vitro and in vivo against a variety of microorganisms including Grams-postive, Grams-negative and acid-fast bacteria. It is especially active against staphylococci, the activity being about 30 times more potent than novobiocin. It shows, however, reduced activity against a laboratory strain of staphylococcus which was made resistant to novobiocin, suggesting an existence of cross-resistance between the two drugs.

The further investigation was undertaken to elucidate the chemical structure of coumermycin A_1. Titration of coumermycin A_1 free acid gave a neutral equivalent of 550–570 with a pK'a value of 6.35 in aqueous 75 % dimethylformamide (DMF). An osmometric molecular weight of the acid was approximately 1100 without showing change on dilution of the sample. These observations suggested coumermycin A_1 to be a dibasic acid having two functions of the same acidity. The antibiotic gives positive Fehling and Molisch reactions and decolorizes bromine in acetic acid. Coumermycin A_1 was stable to catalytic hydrogenation. Methylation with diazomethane introduced two methyl groups to give neutral methyl coumermycin A_1 which was devoid of biological activity.

Acid methanolysis of coumermycin A_1(I) yielded two moles of an optically active, neutral compound II ($C_{15}H_{23}NO_6$), which appeared to be a methyl glycoside, and one mole of an optically inactive acidic substance III ($C_{27}H_{21}N_3O_{10}$), which was designated coumermic acid. The glycoside, II, was hydrolyzed by dilute sulfuric acid to an acylated sugar IV ($C_{14}H_{21}NO_6$), designated coumerose.

Upon treatment with barium hydroxide, II was split into two fractions, a methyl

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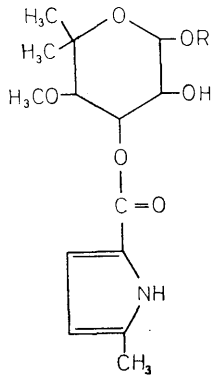
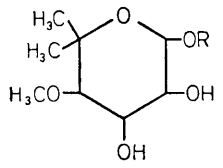
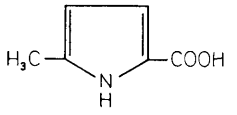
glycoside(V), $C_9H_{18}O_5$, and a barium salt of nitrogen-containing acid(VI). The methyl glycoside, V, and its hydrolyzed product (XIII), $C_8H_{16}O_5$, were identified with methyl novioside and noviose, respectively. The barium salt (VI) was decomposed with dilute sulfuric acid to the free acid, VII, which analyzed as $C_6H_7NO_2$ containing one C-methyl group. It showed UV absorption maximum at 273 mμ and gave positive Ehrlich reaction for pyrrole. The IR spectrum and pK'a determination indicated that the acid function of VII was carboxyl. These data suggested VII to be a methylpyrrole having a carboxyl radical at the α-position, since the β-acids should have the UV absorption maximum at shorter wave length. Of the three isomers for methylpyrrole-α-carboxylic acid, the melting point of 5-methypyrrole-2-carboxylic acid in literatures was in best agreement with that of VII. Therefore, 5-methyl-pyrrole-2-carboxylic acid was synthesized, and its indentity with VII was proved by. IR spectra and mixed melting point determination. Since IV was a neutral substance, VII should estrify either the 2- or 3-position of XIII. Compound IV consumed one.mole of periodate. For the compound to possess an α-glycol group, the methylpyrrole group must be substituted at the 3-position of noviose. Thus, coumerose (IV) is 3-O-(5'-methyl-2'-pyrroyl)-4-O-methyl-5, 5-dimethyl-L-lyxose, or more simply, 3-(5'-methyl-2'-pyrroyl)noviose.



II : R=CH_3
IV : coumerose, R=H



V : R=CH_3
XIII : noviose, R=H

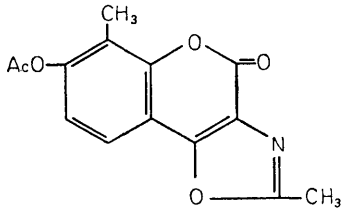


VII

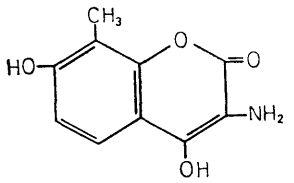
The aglycone, III, which was designated coumermic acid, was fairly stable to acid hydrolysis. A successful cleavage of III was performed using hot acetic anhydride in pyridine to yield three main degradation products : a monoacetyl dibasic acid VIII ($C_{19}H_{16}N_2O_6$), a diacetyl neutral substance IX($C_{14}H_{11}NO_5$), and an acidic substance X$(C_7H_5NO_3)_n$, of very poor solubility.

Acid hydrolysis of compound IX gave 3-amino-4, 7-dihydroxy-8-methyl-coumarin (XII, $C_{10}H_9NO_4$) which was obtained from novobiocin by similar degradation procedures. The UV spectrum of IX showed a characteristic fine structure with maxima at 286, 310 and 323 mμ, and the IR spectrum showed complete absence of OH and

NH bands. These observations suggested a possible identity of IX with 2,6-dimethyl-7-acetoxy-4H-(1)benzopyrano-(3, 4-d)oxazol-4-one, and the direct comparison was made with the authentic sample to confirm the identity.



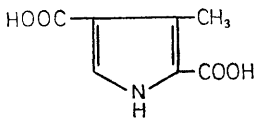
IX



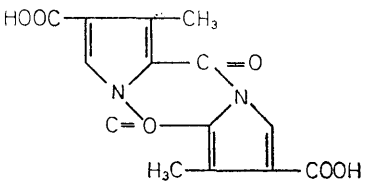
XII

The acidic substance of low solubility, X, resisted acid hydrolysis, but on treatment with refluxing sodium hydroxide solution, a nitrogen-containing acid, XI, with a formula of $C_7H_7NO_4$ was obtained. Group analysis of XI indicated the presence of one C-methyl group and it gave a positive Ehrlich reaction for pyrrole nucleus. The IR spectrum of XI showed the absorptions attributable to NH and carboxyl groups, and the potentiometric titration indicated that XI was a dibasic acid showing two pK'a values of 7.40 and 9.50 in 75 % DMF solution. Methylation with diazomethane converted XI into the dimethyl ester, $C_9H_{11}NO_4$(XV), melting at 125–127°C. These findings suggested XI was a methylpyrrole-dicarboxylic acid.

From the interpretation of NMR spectrum of XV, the methyl group at the β-position and two carboxyls at α- and β-positions of the pyrrole nucleus seemed most probable for the structure of XI. Of the two isomeric β-methylpyrrole-α, β-dicarboxylic acids, XI was identified with 3-methylpyrrole-2,4-dicarboxylic acid. Heating of XI with acetic anhydride in pyridine gave X. Therefore, compound X was assumed to be a secondary degradation product arising from XI during the acetolysis of coumermic acid. From the IR spectrum (lack of NH band) and the NMR spectrum (presence of ring proton) along with the elementary analysis, the structure of X is supposed to be of a pyrocoll type.



XI

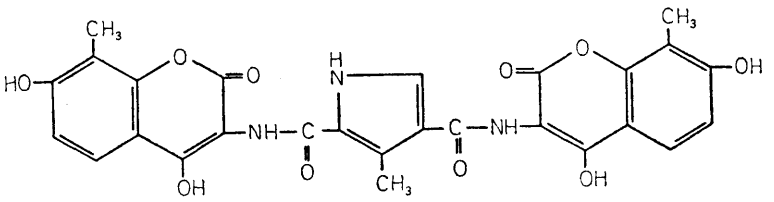


X

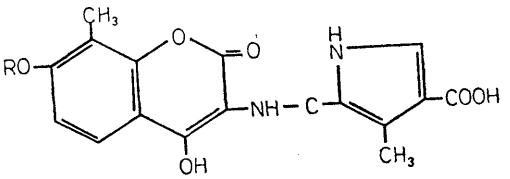
Another degradation product, VIII, was obtained as colorless needles and analyzed for $C_{19}H_{16}N_2O_8$ with one acetyl group. Titration of VIII showed two acidic functions with pK'a values of 5.45 and 8.70 in 75 % DMF solution. Acid hydrolysis of VII gave a des-acetyl compound, $C_{17}H_{14}N_2O_7$ (XIV), which was designated coumeroic acid. Further acetolysis of VIII yielded compounds IX and X. Thus, it appeared

that both coumermic acid (III) and coumeroic acid (XIV) should be constructed by the same constituents, the coumarin derivative (XII) and the pyrrole-dicarboxylic acid (XI).

The structural relationship between III and XIV was elucidated by analyzing the NMR spectra of both compounds that coumermic acid consists of two moles of 3-amino-4,7-dihydroxy-8-methylcoumarin (XII) and one mole of 3-methylpyrrole-2,4-dicarboxylic acid (IX), while coumeroic acid contains one mole each of XI and O-acetyl XII. This conclusion was also supported by the titration data of III and VIII: the pK'a value of III (6.75 in 75 % DMF) is in the range of enolic OH of the β-diketone type, while VIII shows two pK'a values (5.45 and 8.70), one for enolized β-diketone and the other for carboxyl. Thus, one of two carboxyl radicals of XI is free in VIII, the site of the free carboxyl being determined by synthesis to be at the β-position of pyrrole nucleus.



Coumermic acid (III)



Coumeroic acid (XIV) : R=H
O-acetyl coumeroic acid (VIII) : R=$COCH_3$

Since coumermycin A_1 yields two moles of methyl coumeroside and one mole of coumermic acid by acid methanolysis, two coumerose were undoubtedly connected to coumermic acid at the phenolic hydroxyl groups of the coumarins through glycosidic linkages. Therefore, the following structure was given to coumermycin A_1.