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STRUCTURE OF FLAVENSOMYCINIC ACID

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FLAVENSOMYCIN is a new substance having antibiotic and anti-fungin properties, recently isolated by Giolitti and co-workers.[1,2]

The methanolysis of flavensomycin carried out in the presence of Dowex 50-X8 ion-exchange resin, gave a mixture of products from which a crystalline compound was isolated (pale-yellow needles, m.p.232-233°). This compound was assigned the empirical formula $C_{10}H_{11}NO_5$; owing to its acidic properties we called it "flavensomycinic acid" (I).

The flavensomycinic acid is an optically inactive strong acid, which gives negative ninhydrin, Ehrlich, triphenyl-tetrazolium chloride tests; it gives a red-violet colour with ferric chloride and the hydroxamic acid test[3] is positive under the conditions specific for esters and lactones.

The acid (I) contains a α-β unsaturated carbomethoxy group [I.R.: 1724 cm^{-1} (Nujol)]: by mild alkaline hydrolysis a crystalline dibasic acid (II) was obtained; II, called by us flavensomycinoic acid, by esterification with methanol in the presence of Dowex 50 resin reforms the acid (I). Methylglyoxylate (III) was obtained by ozone-oxidation of the sodium salt

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[1] R. Craveri and G. Giolitti, Nature, Lond. 179, 1307 (1957).

[2] R. Craveri, A. Lugli and G. Giolitti, Nuovi Ann. d'Igiene e Microb. IX (2), 185 (1958).

[3] A.I. Vogel, Practical Organic Chemistry (3rd Ed.) p.1063. (1956).

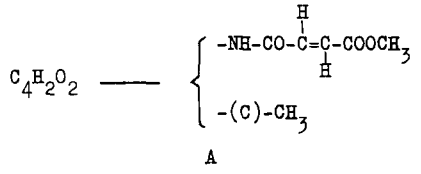
of I in water and by catalytic hydrogenation the acid (I) yields a dihydroflavensomycinic acid (IV) [I.R.: 1736 cm^{-1} (saturated ester)] for which both the hydroxamic acid and the ferric chloride tests were positive.

The I.R. spectrum of I has bands at 1295, 1172, 993, 763, 666 cm^{-1} typical of the fumaric acid derivatives[4]; the acid or alkaline hydrolysis of I and IV under drastic conditions yields respectively fumaric and succinic acid.

The nitrogen atom of I was ascribed to a secondary amidic group. The I.R. spectrum of I shows absorption maxima at 3200 cm^{-1} (-NH- stretching), at 1613 and 1563 cm^{-1} (secondary amide I and II band) which are shifted respectively to 3400, 1631, 1536 cm^{-1} in the spectrum of IV in chloroform.

The acid (I) reacts rapidly in the cold with chromic acid in acetic solution; from the reaction mixture was isolated the fumaramic acid methylester (V): this fact can only be explained if the amidic nitrogen of I is bound to a carbon atom which is easily oxidized to a carboxyl group.

The flavensomycinic acid (I) contains a methyl group bound to carbon; the above facts are consistent with the partial formulation of I as (A):



A

The two oxygen atoms of the $C_4H_2O_2$- moiety are ascribable to a β-dicarbonyl structure, which would account for the enolic and acidic properties of the flavensomycinic acid and of its dihydroderivative. The acids I and IV react with the usual carbonyl reagents; however no definite product could be identified from the last reaction owing to the small available amounts of flavensomycin.

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[4] W.L. Walton and R.B. Hughes, Analyt. Chem. 28, 1388 (1956).

The U.V. spectrum of the dihydroacid IV shows a maximum at 259 mμ; the absorption curve is very similar to those of the enolized "trans-fixed" β-diketones.[5] It follows from the above facts and considerations that the β-diketone group should belong to a cyclic system with four carbon atoms.

The characteristic properties of the 1,3-cyclobutanedione derivatives as described by Woodward[6] are in perfect agreement with those of I and its derivatives; the most remarkable property is their powerful acidity. By potentiometric titration it is possible to determine for the flavensomycinoic acid (II) the pK'_{a_1} 3.5 and pK'_{a_2} 4.7. The dihydroflavensomycinoic acid (VI) obtained in solution by catalytic hydrogenation of II in water had pK'_{a_1} 2.8 and pK'_{a_2} 3.6. The pK'_{a_1} 3.5 of II and pK'_{a_1} 2.8 of VI could be ascribed to the enol dissociation.

The strong acidity of I and IV is further showed by their spectroscopic behaviour in the U.V. The acid (I) has two absorption maxima in methanol at 330 mμ (log ε 3.92) and at 259 mμ (log ε 4.38); the former disappears in the spectrum of its dihydro-derivative, while the latter remains unchanged ($\lambda_{max}^{CH_3OH}$ 259 mμ; log ε 4.36). The U.V. spectrum of IV is substantially the same in alkaline solution; in acid solution the maximum shows a mild hypsochromic effect (λ_{max} 252 mμ), a lower molecular extinction coefficient (log ε 3.95) and a considerable shoulder appears at 259 mμ (log ε 3.92). It is clear that in diluted methanol solution the acid IV is completely dissociated and that the anion is present in a considerable amount even in acid solution.

By methylation with diazomethane the dihydroflavensomycinic acid gives

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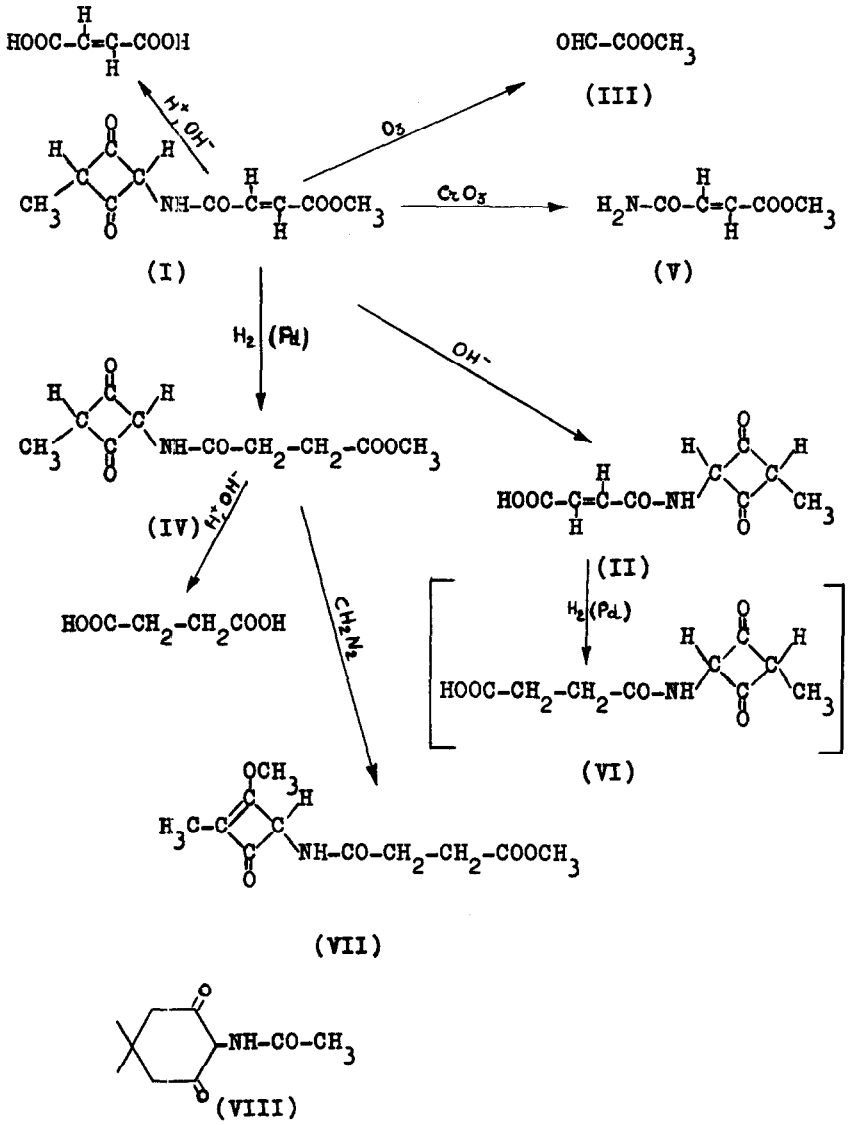
[5] R.B. Woodward and E.R. Blout, J.Amer.Chem.Soc. 65, 562 (1943); E.R. Blout, V.W. Eager and D.C. Silverman, Ibid. 68, 566 (1946); B. Eistert and W. Reiss, Chem.Ber. 87, 108 (1954).

[6] R.B. Woodward and G. Small, Jr., J.Amer.Chem.Soc. 72, 1297 (1950).



a neutral methylether (VII) [white needles, m.p.117-118°. I.R.: 1739 cm^{-1} (saturated ester), 1692 cm^{-1} (α-β unsaturated ketone), 1258 cm^{-1} (vinylic

ether[7])] which has negative reaction with ferric chloride and is very readily hydrolysed to the dihydroacid (IV).

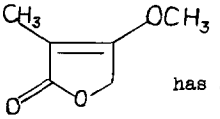
The U.V. spectrum of VII shows an absorption maximum at 249 mμ[8] with a hypsochromic effect of 10 mμ ($\lambda^{\beta\text{-diket. in alk. media}}_{max}$ — $\lambda^{enolether}_{max}$) with respect to the anion of IV; this shift is of the same order of magnitude as that observed for 1,3-cyclobutandiones.

The behaviour of IV to reduction is also typical for 1,3-cyclobutandione structure. In fact IV, unlike larger cyclodiones,[9] is not hydrogenated in the presence of catalyst and is not reduced by $NaBH_4$ while the acyclic β-diketones are.[10]

In agreement with the behaviour of cyclo β-diketones,[11] IV is oxidized rapidly by sodium periodate in neutral solution at 0°: oxalic and succinamic acids are obtained through a mechanism similar to that reported by Wolfrom and Huebner (loc.cit.).

Besides, by treatment with an excess of bromine in CCl_4, IV gives a monobromoderivative, which reduces triphenyltetrazolium chloride in the cold as common to α-halogenoketones.

By strong alkaline hydrolysis the β-diketone system of IV is cleaved. The mixture of cleavage products, unlike IV, reduces triphenyltetrazolium chloride and by oxidation with H_2O_2 in alkaline solution gives succinic and propionic acids.



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[7] The tetrinic acid methyl ether has a band at 1259 cm^{-1} (unpublished observation from our own laboratories).

[8] The 1,1-diphenyl-2-ethoxy-3-methylcyclobut-2-ene-4-one has a maximum at 248 mμ [J.Nieuwenhuis and J.F. Arens, Rec.Trav.Chim. 77, 1153 (1958)]. The 1,3-dipentyl-2-ethoxycyclobut-en-4-one has a maximum at 245 mμ [J. Nieuwenhuis and J.F. Arens, Rec.Trav.Chim. 77, 761 (1958)].

[9] J.P. Wibaut and H.P.L. Gitsels, Rec.Trav.Chim. 60, 577 (1941).

[10] J. Dale, J.Chem.Soc. 910 (1961).

[11] M.L. Wolfrom and J.M. Bobbitt, J.Amer.Chem.Soc. 78, 2489 (1956): C.F. Huebner, S.R. Ames and E.C. Bubl, Ibid. 68, 1621 (1946).

Further information on the structure of flavensomycinic acid is obtained from comparison of the I.R. spectra of I and IV with that of 2-acetylamino-dimedone (VIII).[12] The spectrum in Nujol of the latter compound shows a very broad band at 2632 cm^{-1} (chelated OH) while it has no absorption in the region 1700 cm^{-1} (the keto form of dimedone absorbs at 1702 cm^{-1} [13]). The band of amidic -C=O of VIII masks nearly completely the band indicative of the "conjugated chelate" type of enolization which would fall in the same region. The spectrum of IV, very similar to that of VIII, has at 2632 cm^{-1} the very broad band of chelated OH, while it does not have any absorption in the regions corresponding to the keto and the α-β unsaturated form (this latter should fall at 1692 cm^{-1}, as shown by the spectrum of VII). It follows that the dihydroflavensomycinic acid (IV) in the solid state is in the "enol conjugated chelate" form, unlike the dimethylcyclobutandione.[14] The spectrum of I in Nujol has a broad absorption at 2632 cm^{-1} and a band of medium intensity at 1692 cm^{-1}, which can be probably ascribed to the α-β unsaturated keton group. Besides, a band is present at 917 cm^{-1} which, according to Reid (loc.cit.), is typical skeletal vibration of cyclobutanes.

The NMR spectrum of the enolether VII[15] shows that it contains fifteen protons: one in N-H (elongated signal at 460 cps), three in $-COOCH_3$ (signal at 219 cps, three in H_3C-O-C=C- (signal at 242 cps), three in H_3C-C=C- (signal at 158 cps) and five protons in the neighbourhood of 158 cps ascribable to the system A_2B_2 of the -N-CO-CH_2-CH_2-CO- group and to the residual

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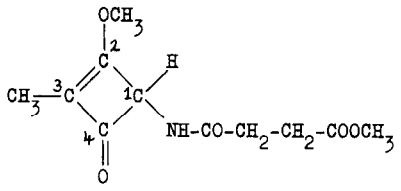
[12] Unpublished from our own laboratories.

[13] R.S. Rasmussen, D.D. Tunnicliff and R.R. Brattain, J.Amer.Chem.Soc. 71, 1068 (1949).

[14] E.B. Reid and S.J. Groszos, J.Amer.Chem.Soc. 75, 1655 (1953).

[15] We are greatly indebted to Dr. A. Melera for the measurement of the NMR spectra. Measurements were carried out on a Varian 4302, 60 megacycles spectrophotomer with electronic integrator, in the Research Laboratory of the Varian A.G., Zürich. Sample was dissolved in $CDCl_3$ and TMS was used as internal reference.

proton bound to the cyclobutane ring. This spectrum proves that the enolic double bond is in 2-3 (3-4), as in (B)



B

Acknowledgement - We are greatly indebted to Prof. G. Giolitti for providing the flavensomycin and to Dr. G. Ricca for discussions about the I.R. spectra.