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THE STRUCTURE OF THE ANTIBIOTIC K16. II. CHROMOPHORE AND TOTAL STRUCTURE.

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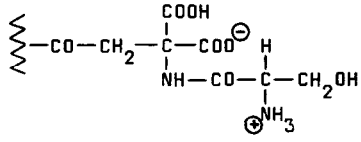
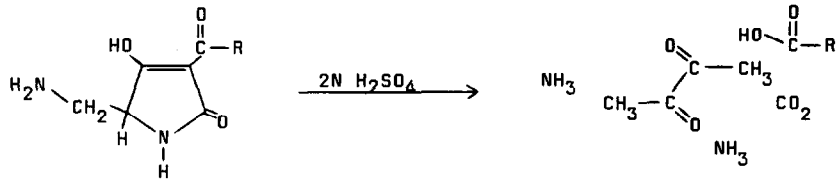
In the preceding paper we derived the partial structure (1) for the antibiotic K16.



(1)

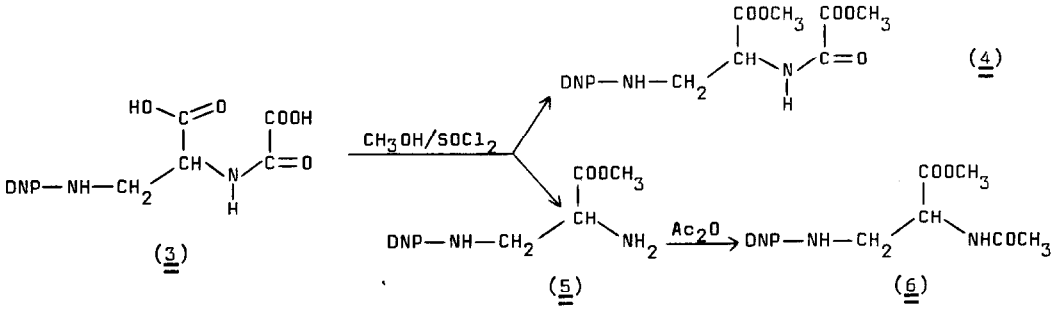
The determination of the structure of the other part of the molecule, the chromophore, was complicated by the fact that on mild acidic cleavage, which released the peptide side chain[1], the chromophoric rest, although detectable by TLC and UV-spectrum, could not be isolated in pure form due to ready decomposition.

The UV-spectra and the positive $FeCl_3$-test of K16 and K16A were compatible with an acyltetramic acid structure[2]; several acyltetramic acid derivatives have been found in nature[3,4]. An acyltetramic acid structure of K16 could also explain the cleavage of the side chain on mild acidic hydrolysis[1] in analogy to the behaviour of tenuazonic acid[4]. Support for this hypothesis came from the formation of a second mole of CO_2 on boiling K16 in 2N H_2SO_4, accompanied by ca. 15% biacetyl (identified as its 2,4-dinitrophenylhydrazon); this could be explained on the basis of the partial structure (2).

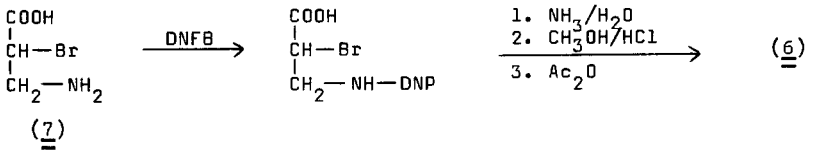


(2)

Structure (2) was confirmed by ozonolysis of DNP_2-K16A ($NaHCO_3/H_2O$, 0^o). By means of TLC two yellow compounds could be isolated: the side chain product DNP-X[1] and DNP-Y. DNP-Y was shown to be β-dinitrophenylamino-α-oxalylaminopropionic acid (3) as follows.

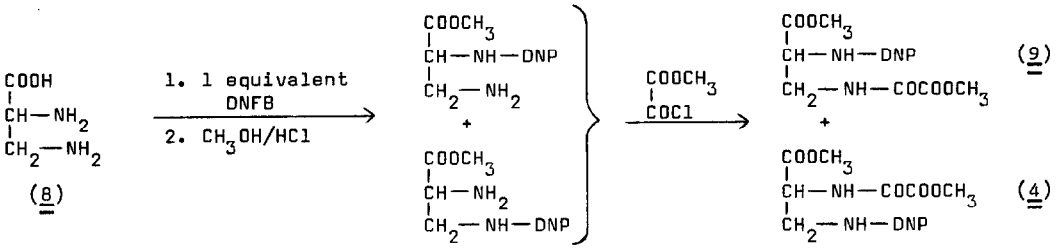


Treatment of (3) with $CH_3OH/SOCl_2$ (1 hr. reflux[5]) yielded a neutral ester (4) and a basic ester (5); (5) was acetylated to give (6), m.p. 181-183°. The structure of (6) followed from the spectral data - especially the mass spectrum[6] - and from independent synthesis from β-amino-α-bromopropionic acid[7] (7):



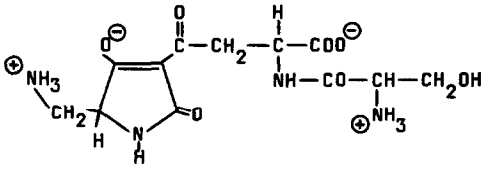
Natural and synthetic (6) were identical in their spectral properties and R_f-values; however, synthetic (6) had m.p. 158.5-161° (m.m.p. 158-175°). The reason for this difference in melting points, which might be configurational in origin, has not yet been established.

Similarly, the structure of (4) was proven by a nonspecific synthesis from α,β-diaminopropionic acid (8):



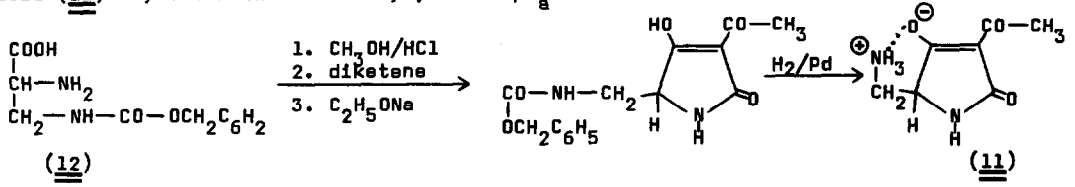
(4) (m.p. 133-135°) and (9) (m.p. 137.5-139.5°) were separated by TLC. Synthetic (4) was identical with the natural product according to IR- and mass spectrum[6] [m/e 370, $M^{\cdot+}$; m/e 196, DNP-$NHCH_2^+$; m/e 175, $(M - DNP\text{-}NHCH_2 + H)^{\cdot+}$]; (9) had a characteristically different fragmentation pattern [m/e 370, $M^{\cdot+}$; m/e 255, $(M - CH_2NHCOCOOCH_3 + H)^{\cdot+}$; m/e 254, $(M - CH_2NHCOCOOCH_3)^+$; m/e 116, $CH_2NHCOCOOCH_3^+$; m/e 115 $(CH_2NHCOCOOCH_3 - H)^{\cdot+}$].

On the basis of this evidence, structure (10) could be proposed for K16A (at the isoelectric point). This would imply the molecular formula $C_{12}H_{18}N_4O_7$. As earlier elemental analyses did not exclude a formula with one more carbon atom, a series of careful combustions by different methods were performed. In good agreement they lead to a carbon-nitrogen ratio C : N = 2.98 : 1 as compared to C_{12} : N_4 = 3 : 1 and C_{13} : N_4 = 3.25 : 1. The averaged formula derived from these combustions was $C_{11.89}H_{19.60}N_{4.00}O_{7.14}$.



(10)

The pK_a-values[1] of K16A can now be assigned as follows: 0.65, -OH (chromophore); 3.4, -COOH (asp); 7.9, $>CH-\overset{\oplus}{N}H_3$ (ser); 9.2, $-CH_2\overset{\oplus}{N}H_3$. Of these, only the first one seems to be unusual, as acyltetramic acids normally have pK_a's of about 3-3.5 in water[3,4]. However, the model compound (11), obtained from β-N-benzyloxycarbonyldiaminopropionic acid (12)[8] by the method of Lacey[9], had a pK_a of 1.93.

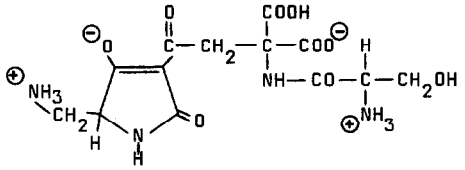


(12) (11)

We therefore assume that the proximity of the positive amoniomethylgroup (and possibly specific hydrogen bonding) has a pronounced effect on the acid strength of the enolic hydroxyl group. In agreement with this assumption, the hydroxyl group of DNP_2-K16A has a normal pK_a value (ca. 3.4 in H_2O).

A point of initial concern was the 220 MHz NMR-spectrum[10] of K16A in D_2O, as it was too complex for the simple proposed structure (10). However, the spectrum was strongly pH-dependent, and by comparison of the spectra at pH 8.5 and at pH 11.5 [pH 11.5: δ 2.88, d(J = 4.3), 2H, $-CH_2-\overset{\oplus}{N}D_3$; δ 2.99 and 3.02, 2 x d of d(J = 15.2 and 4), 1H, $-CO-CH_AH_B-$; δ 3.36-3.53, m, 2H [at pH 8.5 resolved to: δ 3.37 and 3.39, 2 x d of d(J = 15.2 and 9), 1H, $-CO-CH_AH_B-$; δ 3.52 and 3.53, 2 x t(J = 5), $>CH-CH_2OD$]; δ 3.63 and 3.68, 2 x d(J = 5), 2H, $>CH-CH_2OD$; δ 3.76, t(J = 4.3), 1H, $>CH-CH_2\overset{+}{N}D_3$; δ 4.54 and 4.55, 2 x d of d(J = 9 and 4), 1H, >CH- of asp] it could be completely assigned[11] on the assumption that K16A is a mixture of about equal amounts of two diastereoisomers (D,L-asp on

hydrolysis!), which have slightly different chemical shifts for the six non-exchangeable protons of the peptide side chain. The NMR-spectrum of compound (11) [D_2O, pH 12: δ 2.89, d(J = 4.2), 2H, $-CH_2-\overset{\oplus}{N}D_3$; δ 3.77, t(J = 4.2), 1H, $>CH-CH_2\overset{\oplus}{N}D_3$] furnishes an excellent model for the three protons of the chromophore of K16A.



(13)

We can therefore assign structure (13) to K16. Two problems, namely the configuration at the asymmetric carbon atom of the chromophore, and the bands at 1760 and 1740 cm^{-1} in the IR-spectrum of (microcrystalline?) K16[1] (reversible ring formation of one of the carboxyl groups in the solid state?) remain at present unsolved. The interesting problem of the biosynthesis of the unique aminomalonic acid unit[1] and of the tetramic acid chromophore[12] is under investigation.

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2 We thank Drs. C. Vos and coworkers from Gist-Brocades N.V., Delft, for the communication of this and other preliminary results on K16 and for valuable discussions.

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6 We thank Mr.E.A.I.M. Evers for the measurement and interpretation of the mass spectra.

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10 We thank Drs. D.E.J. Verwiel, Central Laboratory, TNO, Delft for the measurement of the 220 MHz NMR-spectra and for discussions; we acknowledge financial support for these measurements from the Netherlands Organization for Advancement of Pure Research (ZWO) through the Netherlands Foundation for Chemical Research (SON).

11 We thank Miss Dr. N.H. Velthorst for a computer simulation of the NMR-spectrum of K16A.

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