Tetrahedron Letters No. 30, pp 3103 - 3106, 1972. Pergamon Press. Printed in Great Britain.

THE STRUCTURE OF THE ANTIBIOTIC K16. I. THE DIPEPTIDE SIDE CHAIN.

J.G. Batelaan, J.W.F.K. Barnick, J.L. van der Baan and F. Bickelhaupt

Scheikundig Laboratorium der Vrije Universiteit, Amsterdam-Z, The Netherlands

(Received in UK 8 June 1972; accepted for publication 22 June 1972)

From a strain of Streptomyces rimosus var. paromomycinus an antibiotic K16 was isolated which is of interest because of its antiprotozoic activity, especially against trypanosomes[1,2]. K16 was obtained as a white, nonvolatile, presumably microcristalline powder, insoluble in organic solvents, slightly soluble in water and readily soluble in aqueous acid or base. On heating in the solid state or by boiling in water for 5 minutes it yielded one mole CO_2 and one mole biologically inactive K16A.

Dried K16 and K16A were hygroscopic and contained variable amounts of water; the elemental analysis was therefore not easily reproducible and indicated the formulae $C_{13-14}H_{24-28}N_4O_{11-14}$ for K16 and $C_{12-13}H_{20-22}N_4O_{7-9}$ for K16A. Mass spectra could not be obtained without decomposition. The IR-spectrum (KBr) of freeze-dried K16 had broad bands at 3600-2500, 1730, 1670, 1600 and 1450 cm^{-1}; material which had been carefully precipitated at the isoelectric point (pH 2.7) showed instead of the shoulder at 1730 cm^{-1} a medium strong double peak at 1760 and 1740 cm^{-1}; K16A had no absorption between 1700 and 1800 cm^{-1}. UV-spectrum of K16: in H_2O λ_{max} 240 nm ($\varepsilon > 6600$), λ_{max} 277.5 nm ($\varepsilon > 7600$); in 5.4N HCl λ_{max} 274 nm ($\varepsilon > 6700$), λ_{min} 233 nm ($\varepsilon > 1800$). NMR--spectrum (60 MHz, D_2O): complex pattern at δ = 2.8-4.0 ppm. pK_a of K16 (H_2O): 0.8 (connected with change in UV-spectrum, spectrophotometrically), ca. 1.9, 4.2, ca. 7.8, ca. 9.2 (potentiometric titration); pK_a of K16A (H_2O): 0.65 (spectrophotometrically), 3.4, ca. 7.9, ca. 9.2. Optical rotation of K16: $[\alpha]_{577}^{20} = -64 \pm 4^{\circ}$ (c = 1, H_2O, $6.0 \leqslant pH \leqslant 9.5$, adjusted with NaOH). Both K16 and K16A gave a positive $FeCl_3$-test.

K16 consists of two parts, a chromophore[3] and a peptide side chain, which could be investigated separately. The structure of the latter will be dealt with in this communication.

After refluxing K16 in 6N HCl for 16 hrs. nearly one mole of each of the amino

acids L-serine (L-ser) and D,L-aspartic acid (D,L-asp) and one mol ammonia could be isolated. On treatment with 2,4-dinitrofluorobenzene (DNFB) in aqueous ethanol and $NaHCO_3$[4], K16 and K16A were converted to DNP_2-K16 and DNP_2-K16A, respectively. According to UV--spectra and titration, two dinitrophenyl groups had been introduced. Similarly, treatment of K16A with acetic anhydride or benzoylchloride gave the diacylated products Ac_2-K16A and Bz_2-K16A. Hydrolysis of DNP_2-K16A with 6N HCl yielded, besides fragments derived from the chromophore, asp and nearly one mole DNP-ser, which proves that ser is an N-terminal amino acid and asp is not.

After refluxing K16A in 0.2N HCl for 3 hrs. a mixture of amino acids could be separated by TLC; after dinitrophenylation DNP-ser, DNP-asp and a new compound DNP-X were isolated. The latter proved to be identical with a product obtained from ozonolysis ($NaHCO_3/H_2O$, 0°) of DNP_2-K16A[3]. On hydrolysis (6N HCl, 16 hrs.) DNP-X yielded DNP-ser and asp in equal amounts. DNP-X was proven to be a mixture of the diastereoisomers of L-DNP-ser-D,L-asp by its synthesis from L-DNP-ser and dimethyl D,L-aspartate with dicyclohexylcarbodiimide, followed by alkaline hydrolysis. The natural and the synthetic diacid had identical IR-spectra and R_f-values; the corresponding dimethylesters (m.p. 164-168°) were identical in mixed melting point, R_f-value, IR-, NMR-, and mass spectrum.

The site of attachment of the CO_2 which is readily eliminated from K16 was proven to be a carbon atom of the peptide side chain by decarboxylation of K16 in D_2O to K16A(D). In the NMR-spectrum of this compound a multiplett of one proton at δ 4.61 ppm (D_2O), present in ordinary K16A, was absent. Hydrolysis (6N HCl), followed by dinitrophenylation and esterification (CH_3OH/HCl) yielded (besides ordinary DNP-ser-OMe) DNP--asp-$(OMe)_2$ which according to the mass spectrum contained 80% deuterium in place of one proton; the NMR-spectrum proved the site of deuteration to be the α-carbon atom (see fig. 1).

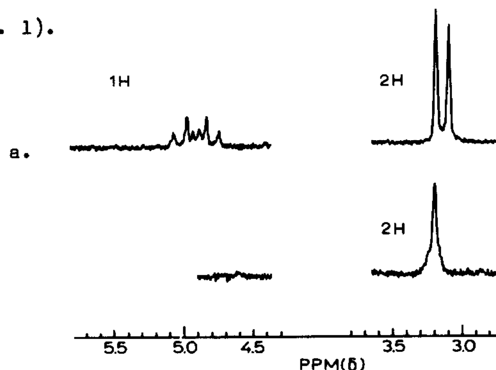


Fig. 1

NMR-spectrum(D_2O) of DNP-asp-$(OMe)_2$

a. authentic sample

b. obtained from K16 after decarboxylation in D_2O

This leaves two possible structures for the peptide side chain of K16 (Fig. 2): one with the labile carboxyl group bound to the α-carbon atom (malonic acid derivative,(1)) or to the β-carbon atom (β-keto acid,(2)). Both structures can explain the formation of D,L-asp on hydrolysis, (1) being achiral and both (1) and (2) decarboxylating via an achiral, enol-type transition state.

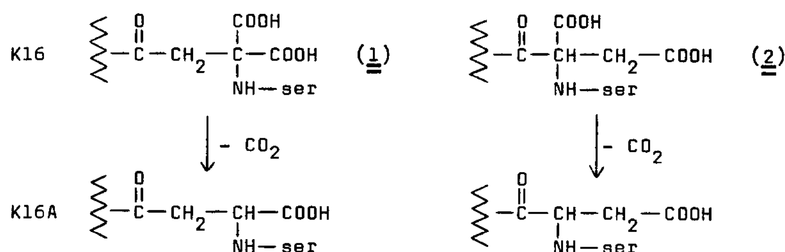
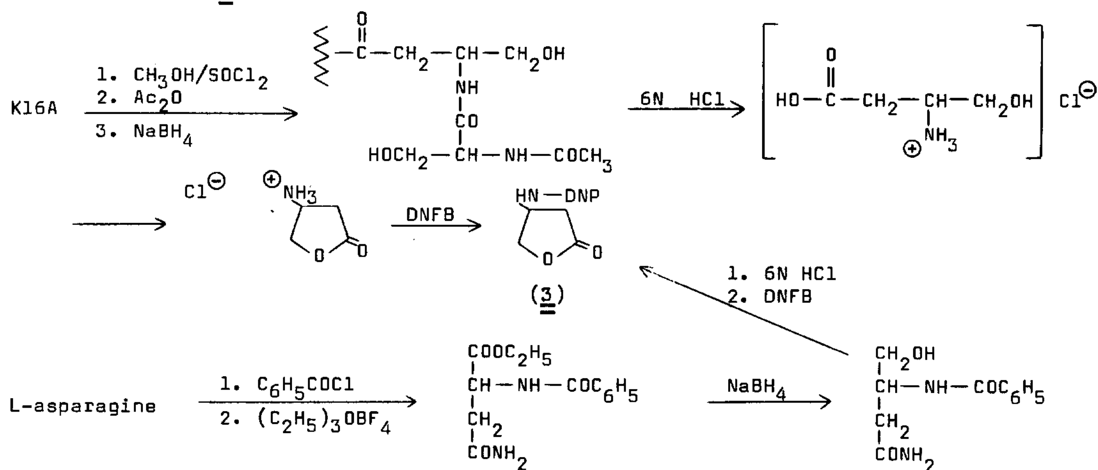


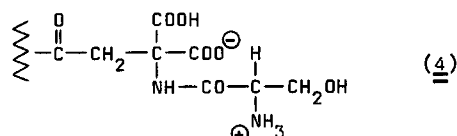
Fig. 2 Two possible structures for the side chain of K16 and K16A

The pK_a (ca. 1.9) of K16 is in better agreement with structure (1) than with structure (2). A distinction was made in favour of structure (1) by the very mild reduction of peptide esters to the corresponding alcohols with excess sodium borohydride[5]. For this purpose K16A was esterified ($CH_3OH/SOCl_2$)[6], acetylated (excess acetic anhydride in $NaHCO_3/H_2O$, 0°) and, after adjustment to pH 10, treated with a large excess of sodium borohydride (the UV-spectrum remained unchanged). Hydrolysis (6N HCl) and dinitrophenylation yielded only a trace of DNP-asp and 24% β-dinitrophenylaminobutyrolactone (3). Without prior esterification of K16A this reaction sequence gave only DNP-asp and no (3). The structure of (3) was proven by independent synthesis:



The (racemic) natural and the (optically active) synthetic (**3**) were completely identical in their spectral properties; the only difference was found in the melting points (188-189° and 196-200°, respectively). Racemic (**3**) (prepared in an analogous but nonspecific way by reduction of dimethyl D,L-aspartate and separated from the α-isomer) was identical with natural (**3**) also in melting point and mixed melting point.

The evidence presented above proves that K16 posesses an L-seryl-α-carboxy-β-aspartyl side chain (**4**). To our knowledge this is the first time that a derivative of an α-aminomalonic acid has been found in nature, although there has been considerable speculation on the possible occurrence of α-aminomalonic acid itself[7]. It is significant that the biological activity is lost[2] on decarboxylation.



(**4**)

References:

1 Koninklijke Nederlandsche Gist- en Spiritusfabriek N.V., Neth. Appl. 67 01356, 27 January 1967.

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

3 J.G. Batelaan, J.W.F.K. Barnick, J.L. van der Baan and F. Bickelhaupt, Tetrahedron Letters, 1972, accompanying paper.

4 K.R. Rao and H.A. Sober, *J. Amer. Chem. Soc.* **76**, 1328 (1954).

5 O. Yonemitsu, T. Hamada and Y. Kanaoka, *Chem. Pharm. Bull.* **17**, 2075 (1969).

6 M. Brenner and W. Huber, *Helv. Chim. Acta* **36**, 1109 (1953).

7 J.W. Thanassi, *Biochemistry* **9**, 525 (1970).