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STRUCTURE AND SYNTHESIS OF FK409, A NOVEL VASODILATOR ISOLATED FROM STREPTOMYCES AS A SEMI-ARTIFICIAL FERMENTATION PRODUCT

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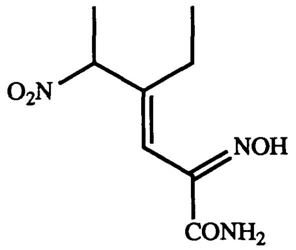
The structures of FK409 (1), a semi-artificial fermentation product and its precursor (2) have been established on the basis of chemical and spectroscopic evidence and confirmed by conversion of 2 to 1 *via* a synchronous nitrosation-nitration reaction.

KEYWORDS natural product; fermentation; Streptomyces griseosporeus; vasodilation activity; nitrosation reaction

In the course of our search for biologically active substances from microbial sources, we encountered an actinomycete, *Streptomyces griseosporeus* No. 16917 whose cultured broths in nutrient media containing nitrate produced, on acidification after fermentation, a substance (1) with vasodilation activity. This product (FK409) was considered to be formed *via* a synchronous occurrence of nitrosation and nitration of a precursor 2, which was later proved to exist in the cultured broths. The conversion of 2 to 1 seemed to proceed, under acidic conditions, with nitrite formed by microbial reduction of nitrate. The fermentation and isolation of the vasoactive compound 1 and its precursor 2 will be reported elsewhere.1) This paper describes the structural determination of 1 and 2, and the chemical conversion of 2 to 1.



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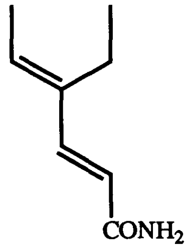
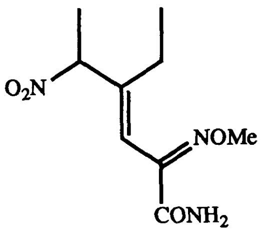


2

FK409 (1) was isolated as colorless prisms : $C_8H_{13}N_3O_4$ (FDMS, m/z 215 (M^+); Anal. Calcd for $C_8H_{13}N_3O_4$: C, 44.65; H, 6.09; N, 19.53. Found : C, 44.64; H, 5.92; N, 19.60); mp 140°C(dec); $[\alpha]_D$ 0°(c 1.0, MeOH); UV λmax (MeOH) 240nm (shoulder), 273 (ε 13,500); IR νmax(nujol) 3500, 3300-3200, 1660, 1600, 1560cm^{-1}. The ^{13}C and ^1H NMR spectra (CD_3OD and Me_2CO-d_6, respectively) revealed the presence of the following functional groups in 1: an ethyl (δ_C 12.9(q), 25.6(t); δ_H 1.02 (t, J=7Hz, 3H), 2.22(q, J=7Hz, 2H)), a 1-substituted ethyl group (>$CHCH_3$)(δ_C 18.4(q), 87.1(d); δ_H 1.72 (d, J=7Hz, 3H), 5.37 (q, J=7Hz, 1H)), a tri-substituted olefin (δ_C 119.5(d), 146.4(s)(or 149.8); δ_H 6.20(s, 1H)), and an amide group ($CONH_2$) (δ_C 168.1(s); δ_H 7.09(br s, 1H, exchangeable), 6.63(br s, 1H, exchangeable)). The existence of the amide function was corroborated by the IR (nujol) absorption at 1660cm^{-1}. These structural units accounted for seven of the eight carbons and 12 of the 13 hydrogens. The remaining one carbon and one proton, which resonated at δ_C 149.8(s) (or 146.4) and δ_H 11.17 (br s, 1H, exchangeable), respectively, were attributable to an oxime group. The existence of this function was supported by a positive color reaction of 1 to $FeCl_3/K_3Fe(CN)_6$ and by methylation of 1 with CH_2N_2 (MeOH), giving methoxime 3 (FDMS, m/z 229(M^+); δ_H 4.00 (s, 3H)).2)

The IR spectrum of 1 showed, in addition to the amide absorption (see above), a strong band at 1560cm^{-1}, suggesting the presence of a nitro group in 1. The low-field resonances of the methine carbon and proton (δ_C 87.1 and δ_H 5.37, respectively, as described above) suggested the attachment of the nitro group of this methine as a 1-nitroethyl unit. Ozonolysis of 1 (O_3/MeOH) followed by reductive workup (Me_2S) gave 2-nitropentan-3-one,$^{3)}$ which was identified with the sample prepared after the reported procedure.$^{4)}$ This chemical evidence thus established the location of the nitro group at C-5 and provided the partial structure A. The combination of this partial structure with the oxime and amide functions led to the full structure of 1. The double bond geometry was assigned to be E as shown in 1 on the basis of the observation that the olefinic proton (3-H) brought about NOEs between both 5-H and 6-Me (Fig.1). The structure 1 was thus proposed for FK409 except for the geometry of the oxime group.



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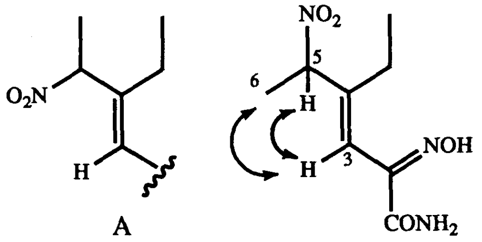

Fig.1. Partial Structure A and NOEs Observed in 1

The precursor 2 exhibited the following physical characteristics: colorless prisms; $C_8H_{13}NO$ (FDMS, m/z 139(M^+); Anal. Calcd for $C_8H_{13}NO$: C, 69.03; H, 9.41; N, 10.06. Found: C, 67.48; H, 9.12; N, 9.78); mp 57-8°C; IR νmax($CHCl_3$) 1670, 1620, 1590cm^{-1}. The absorption at λmax 260nm (ε 20,000) in the UV spectrum (MeOH) suggested the presence of a conjugated dienecarboxamide moiety, whose carbonyl bond in the IR spectrum ($CHCl_3$) was observed at 1670cm^{-1}. The ^1H NMR spectrum ($CDCl_3$) revealed the following functions: an ethyl (δ 1.00 (t, J=7Hz, 3H), 2.27(q, J=7Hz, 2H)), a methyl (δ 1.78(d, J=7Hz, 3H)) coupled to a tri-substituted olefin proton (δ 5.89 (q, J=7Hz, 1H)), and a di-substituted trans-olefin (δ 7.17 (d, J=16Hz, 1H), 5.83(d, J=16Hz, 1H)). Catalytic reduction of 2 (10% Pd-C/MeOH) gave γ-ethyl-n-capronamide,$^{5)}$ which was identical with the sample prepared by the known method.$^{6)}$

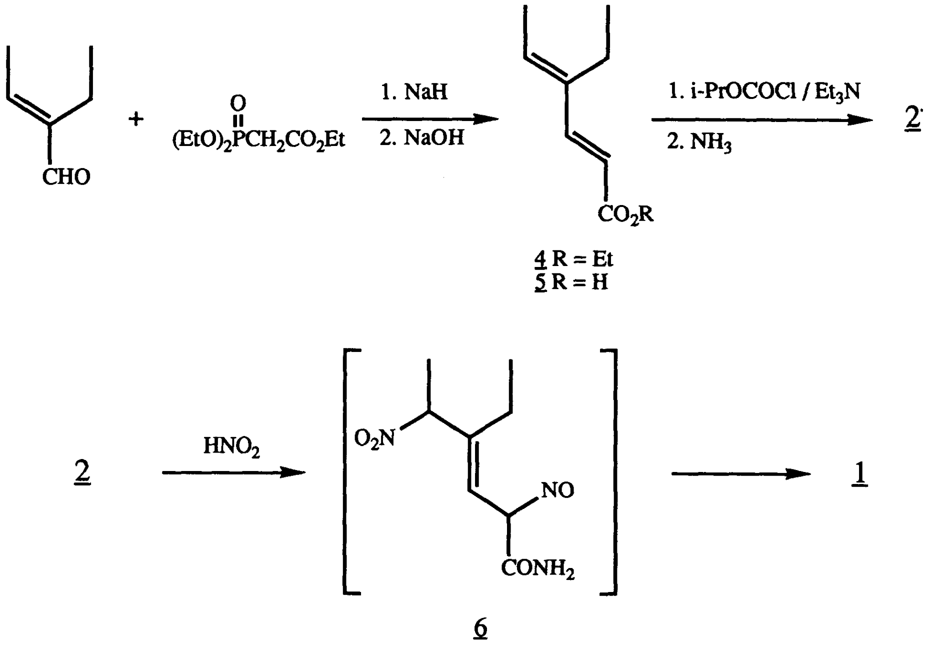

Chart 1

The structure of the precursor was thus defined as 2. The structure was further confirmed by a synthesis from (E)-2-ethyl-2-butenal and triethyl phosphonoacetate by the Horner-Emmons reaction (NaH/benzene, r.t.) to 4 (86%),7) followed by alkaline hydrolysis (1N NaOH/MeOH, r.t.) to 5 (97%)7) and ammonolysis *via* activation (1. i-PrOCOCl/Et$_3$N/CH$_2$Cl$_2$, -20°C; 2. NH$_3$ gas, -20°C; 89%).

Chemical conversion of 2 to 1 was carried out according to the usual method using sodium nitrite under acidic conditions. Thus, sodium nitrite (excess) was slowly added to a solution of 2 in 10% aq MeOH at pH 3.0 (HCl) at 0°C. After 15 min at room temperature, the product was extracted (AcOEt) and purified by recrystallization (MeOH) to yield pure compound 1 (41%), which was identical in all respects with the sample isolated from the fermentation broth.

The reaction might be accounted for by electrophilic 1,4-nitroso-nitro addition of dinitrogen trioxide formed in situ from nitrous acid,8) the nitroso group in the intermediate (8) being isomerized to the oxime form in the isolated product 1.

The present study disclosed the structure of FK409 as being 1 expect for the oxime geometry9) and provided ample evidence that FK409 is a semi-artificial fermentation product. As far as we are aware, FK409 is the first example of this structural type and its activity as a vasodilator is of special interest.10) Moreover, the synthesis of FK409 involves the attractive feature of using a novel 1,4-nitrosation-nitration reaction, which can be used to synthesyze analogous nitro-oxime and/or nitro-nitroso compounds.

REFERENCES AND NOTES

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2) ^{1}H NMR data of 3 (CDCl$_3$) : δ 6.56 (br s, 1H), 6.08 (s, 1H), 5.50 (br s, 1H), 5.17 (q, J=7Hz, 1H), 4.00 (s, 3H), 2.12 (q, J=7Hz, 2H), 1.74 (d, J=7Hz, 3H), 1.02 (t, J=7Hz, 3H).

3) IR (CHCl$_3$) 1730, 1560, 1460cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 5.30 (q, J=6Hz, 1H), 2.62 (q, J=7Hz, 2H), 1.70 (d, J=6Hz, 3H), 1.07 (t, J=7Hz, 3H).

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5) mp 82-3°C ; IR (CDCl$_3$) 1670, 1590, 1460cm^{-1}; ^{1}H NMR (CD$_3$OD) δ 2.22 (t, J=5Hz, 2H), 0.87 (t, J=6Hz, 6H).

6) E. Rothstein, W.G. Schofield, *J. Chem. Soc.*, 1965, 4566.

7) Physical data of the intermediates. 4 : bp 120°C (20mmHg); IR (CDCl$_3$) 1690, 1620cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.20 (d, J=16Hz, 1H), 6.07-5.67 (m, 2H), 4.20 (q, J=7Hz, 2H), 2.26 (q, J=7Hz, 2H), 1.78 (d, J=7Hz, 3H), 1.27 (t, J=7Hz, 3H), 0.97 (t, J=7Hz, 3H); MS, m/z 168(M$^+$). 5 : mp 79-81°C; IR (CHCl$_3$) 1680, 1620cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 11.20 (brs, 1H), 7.33 (d, J=16Hz, 1H), 6.00 (q, J=7Hz, 1H), 5.80 (d, J=16Hz, 1H), 2.29 (q, J=7Hz, 2H), 1.83 (d, J=7Hz, 3H), 1.00 (t, J=7Hz, 3H); MS, m/z 140 (M$^+$).

8) See e.g. a) J.H. Ridd, *Quart. Rev.* 15, 418 (1961) ; b) J.R. Park, D.L.H. Williams, *J. Chem. Soc. Perkin Trans. II.*, 1976, 828.

9) The oxime geometry of 1 was determined to be E by X-ray crystal analysis. Details will be reported in due course.

10) Some preliminary data for the activity will be described in ref 1a. Detailed pharmacological data will be reported in due course.

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