Pergamon

Tetrahedron, Vol. 52, No. 4, pp. 1235-1238, 1996

0040-4020/96 $15.00 + 0.00

0040-4020(95)00978-7

New Stereoselective Beckmann-type Rearrangement Leading to Ring Contraction

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Abstract: In a new stereospecific reaction the known oxime TAN-1030A gave rise to a ring contraction to yield compound **2** closely related to the metabolite K-252a. The structure was elucidated by spectroscopic comparison with K-252a. The compound strongly inhibited protein kinase C with IC_{50} values of 0.18 μM. This reaction suggests that TAN-1030A is a biosynthetic precursor of K-252. The absolute stereochemistry of K-252a was assigned by comparison of CD spectra.

INTRODUCTION

Indolocarbazole alkaloids such as staurosporine [1], K-252a (**3**) [2] and TAN-1030A (**1**) [3,4] are secondary metabolites produced by various strains of the *Actinomycetes* family of bacteria. They are potent inhibitors of a key enzyme of the signal transduction pathway protein kinase C (PKC) and extensively investigated as potential drugs for the treatment of high blood pressure, inflammation and cancer. TAN-1030A (**1**) has originally been isolated from culture broths of *Streptomyces* sp. C-71799 as an activator of macrophage functions [3]. In our own laboratories this compound was isolated from extracts of a new isolate *Streptomyces longisporoflavus* R-19 producing staurosporine as the main metabolite and numerous by-products [5]. Chemical derivatization of the oxime **1** aimed at improving the potency and selectivity of the PKC-inhibitory activity led to the discovery of a surprising rearrangement product formed under Beckmann reaction conditions. Instead of the expected Beckmann fission product [6], we isolated a new compound closely related to the known PKC inhibitor K-252a (**3**), which has been isolated earlier by Japanese researchers [2] from a *Nocardiopsis* species. The new type of ring contracting reaction is highly stereoselective and provides new hints on the potential biosynthetic pathway for the formation of the metabolite K-252a.

RESULTS AND DISCUSSION

The rection proceeded by refluxation with H_2SO_4 in dioxane containing traces of water and yielded product **2** and some starting material.

Elemental composition and comparison of ^{1}H-NMR and ^{13}C-NMR spectra (DMSO-d_6; Table 1) with those of the known secondary metabolite K-252a [7] led to the proposal of structure **2** in a straightforward manner. The only notable difference between the data of the two compounds is the carbon shift at C-3' [8], where the new amino group is attached. Since no long range effects can be observed, the stereochemistry on center 3' is presumed to be identical.

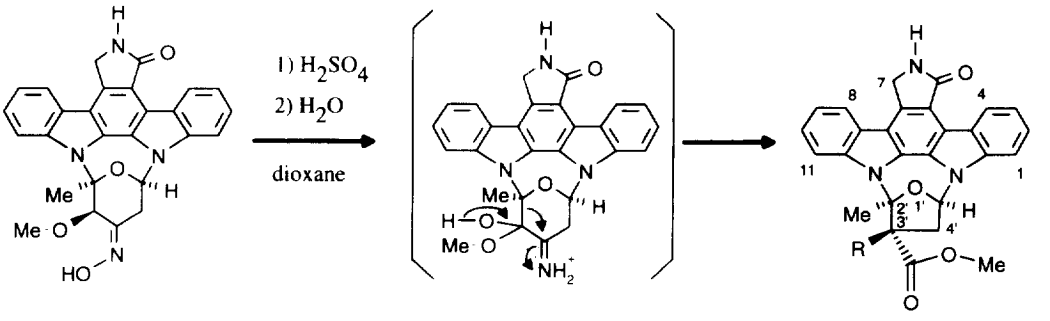
Table 1: ^{1}H- and ^{13}C-NMR Data of **2** and of K-252a (**3**) [7]

Carbon	**2** [a]			**3**			**2** [a]		**3**
1	8.03*	d		7.9	d	8	108.5	d	109.0
2	7.47	t		7.49	br t		124.9	d	125.4
3	7.27*	t		7.29	br t		118.9	d	119.4
4	9.23	d		9.24	d	8	125.2	d	125.6
4a							122.1	s	122.6
4b							115.2	s	115.8
4c							119.1	s	119.5
5							171.3	s	171.7
6	8.29	s br		8.64	br s				
7	4.98	≈d	17	5.04/5.00		17	45.0	t	45.4
7a							132.4	s	132.9
7b							114.2	s	114.6
7c							123.8	s	124.1
8	8.06*	d		8.05	d	8	120.8	d	121.2
9	7.35*	t		7.36	br t		120.0	d	120.4
10	7.47	t		7.49	br t		124.6	d	125.0
11	7.83	d		7.95	d	8	114.5	d	114.7
11a							139.5	s	139.8
12a							128.2	s	128.3
12b							123.7	s	123.9
13a							136.3	s	136.8
2'							100.3	s	99.3
2'-**CH_3**	2.15	s		2.16	s		22.5	q	22.8
3'							71.8	s	84.9
3'-**CO**							172.9	s	172.8
3'-$COOCH_3$	3.92	s		3.94	s		52.4	q	52.6
3'-**NH_2**	2.10	s br							
4'a	2.04	dd	14/5	2.04	dd	14/5	42.5	t	42.5
4'b	3.36	dd	14/8	3.41	dd	14/7			
5'	7.07	dd		7.15	dd	7/5	84.5	d	85.0

a: 100/400 MHz, DMSO-d_6, ^{3}J(H-H) in Hz

As compound **1** and staurosporine are produced by the same microorganism simultanously, it should have the same absolute stereochemistry as the one of staurosporine which was recently established [9]. Comparison of the CD-spectra of **2** and **3** (Figure 1) prove that both compounds have the same absolute configuration. Thus K-252a must have also the configuration of TAN-1030A (**1**) at the bridgehead positions 2' and 6' which remain unaffected by the rearrangement. The absolute configuration is therefore inverse to the commonly used formula.

There is no obvious explanation for the observed reaction course, since it does not lead to the usual Beckmann rearrangement or fission product [10]. A hypothetical mechanism is proposed in Scheme 1. Loss of hydrogen 3' of the protonated oxime gives an enamine. Protonation of the leaving hydroxy group by different reagents [11] and addition of water to the double bond leads to (**i**) which upon protonation rearranges to the final product **2**, whereby the migrating 1'-2'-bond is antiperiplanar to the π-electrons of the leaving group. This could explain the stereoselectivity: As no major peaks were observed in the HPLC chromatogram of the crude product except for **1** and **2**, the selectivity of the reaction has to be at least 10:1.



Scheme 1: Chemical structure of TAN-1030A, **2**, K-252a and hypothetical mechanism of the rearrangement reaction

This reaction is proposed to represent a key step in the biosynthesis of K-252a (**3**). This hypothesis is supported by the fact that both TAN-1030A and K-252a are co-produced in small amounts by our strain *Streptomyces longisporoflavus* R-19 which produces staurosporine as the main product [5,12]. It is quite conceivable that **1** constitutes the branching point of the biosynthetic pathway for the formation of the two metabolites.

Compound **2** inhibits porcine PKC [13] much more effectively (IC_{50} = 0.18 μM) than TAN-1030A (IC_{50} = 1.2 μM) and in a similar range compared to K-252a (IC_{50} = 0.22 μM). Although weaker inhibitors of PKC than staurosporine, K-252a and K-252b attracted considerable interest as they are able to selectively potentiate neurotrophin-3 actions and might be therapeutically useful in a variety of neuropathologic conditions [14].

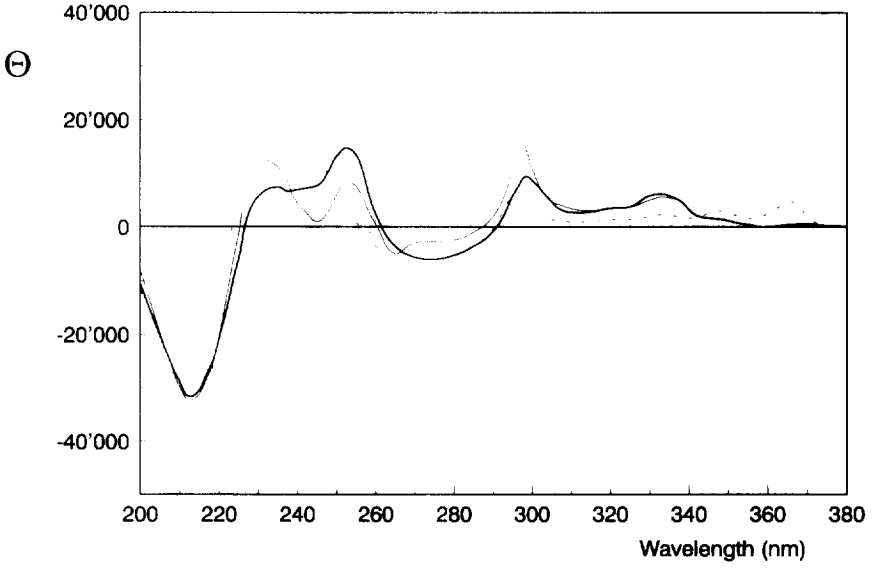


Figure 1: CD-Spectra of TAN-1030A (·····), K-252a (——) and of **2** (——) in EtOH

EXPERIMENTAL

A mixture of TAN-1030A (**1**, 250 mg) and sulphuric acid (56 µl; 96%) in 20 ml dioxane (99.5 %) was refluxed for 40 min. After a short period a brownish solid, the hydrogen sulfate salt of **2** according to IR, started to precipitate. The mixture was dissolved in MeOH and CH_2Cl_2, extracted with Na_2CO_3, dried and the solvent was removed *in vacuo* to give 240 mg of crude organic extract. The pure product was obtained by column chromatography on silica gel (LiChroprep Si 60, 15-25 µm; column volume 130 ml; solvent system CH_2Cl_2, H_2O saturated; fractions from 200 to 600 ml) to give 130 mg of **2**.

The physical data of **2**, $C_{27}H_{22}N_4O_4 \cdot 0.7\ CH_2Cl_2$ are as follows: **mp** = 283-290°C (cryst. from MeOH/CH_2Cl_2); **EI-MS:** m/z 466.1646 (M^+, δM = 0.5 mmu, 5%), 406 (2), 353 (2), 311 (5), 111 (7), 97 (13), 84 (23), 82 (16), 71 (44), 69 (18), 57 (76), 55 (33), 44 (100), 43 (62); **IR** (KBr): ν [cm^{-1}] = 3420, 3300, 3040, 1730 (s), 1680 (s), 1630, 1590, 1460, 1430, 1390, 1370, 1350, 1305, 1275, 1230, 1210, 1160, 1145, 1060, 1030, 1020, 770, 760, 730, 620; **Anal:** Calcd for $C_{27}H_{22}O_7 \cdot 0.7\ CH_2Cl_2$: C 63.49, H 4.49, N 10.70, Cl 9.08. Found: C 63.46, H 4.72, N 10.78, Cl 9.12.

ACKNOWLEDGMENTS

We are indebted to Jean Metzger for skilled technical assistance. Thanks are also due to Dr. Yang Cai for the isolation of TAN-1030A and K-252a, to Dr. Hermann Fuhrer for the NMR spectra, to Dr. Giorgio Caravatti for investigation of the REACCS database and to Dr. Thomas Meyer for providing the data on the biological activity. We would like to acknowledge helpful discussions of the reaction mechanism with Prof. A. Eschenmoser and Dr. R. Heckendorn.

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(*Received in Germany* 9 *October* 1995; *accepted* 31 *October* 1995)