Tetrahedron Vol. 46, No. 16, pp. 5603-5608, 1990
Printed in Great Britain

0040-4020/90 $3.00+.00
Pergamon Press plc

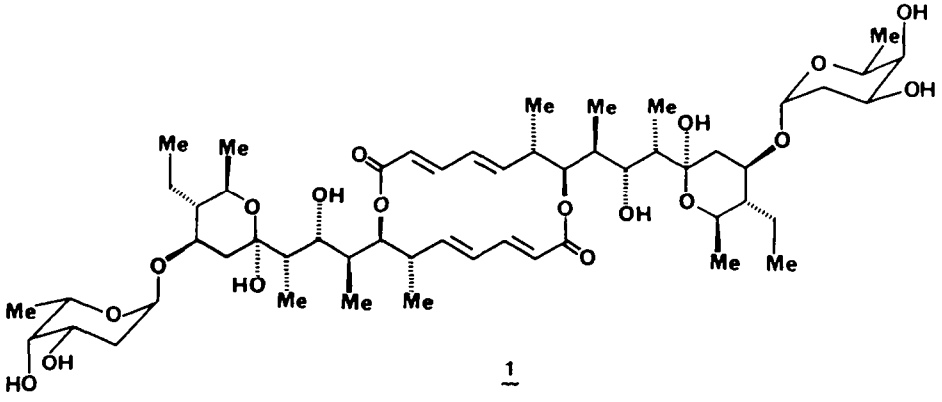
SECONDARY METABOLITES BY CHEMICAL SCREENING[1]-5. CYCLOADDITION REACTIONS OF ELAIOPHYLIN

Peter Hammann and Gerhard Kretzschmar*
Hoechst Aktiengesellschaft, Postfach 80 03 20, 6230 Frankfurt a. M.

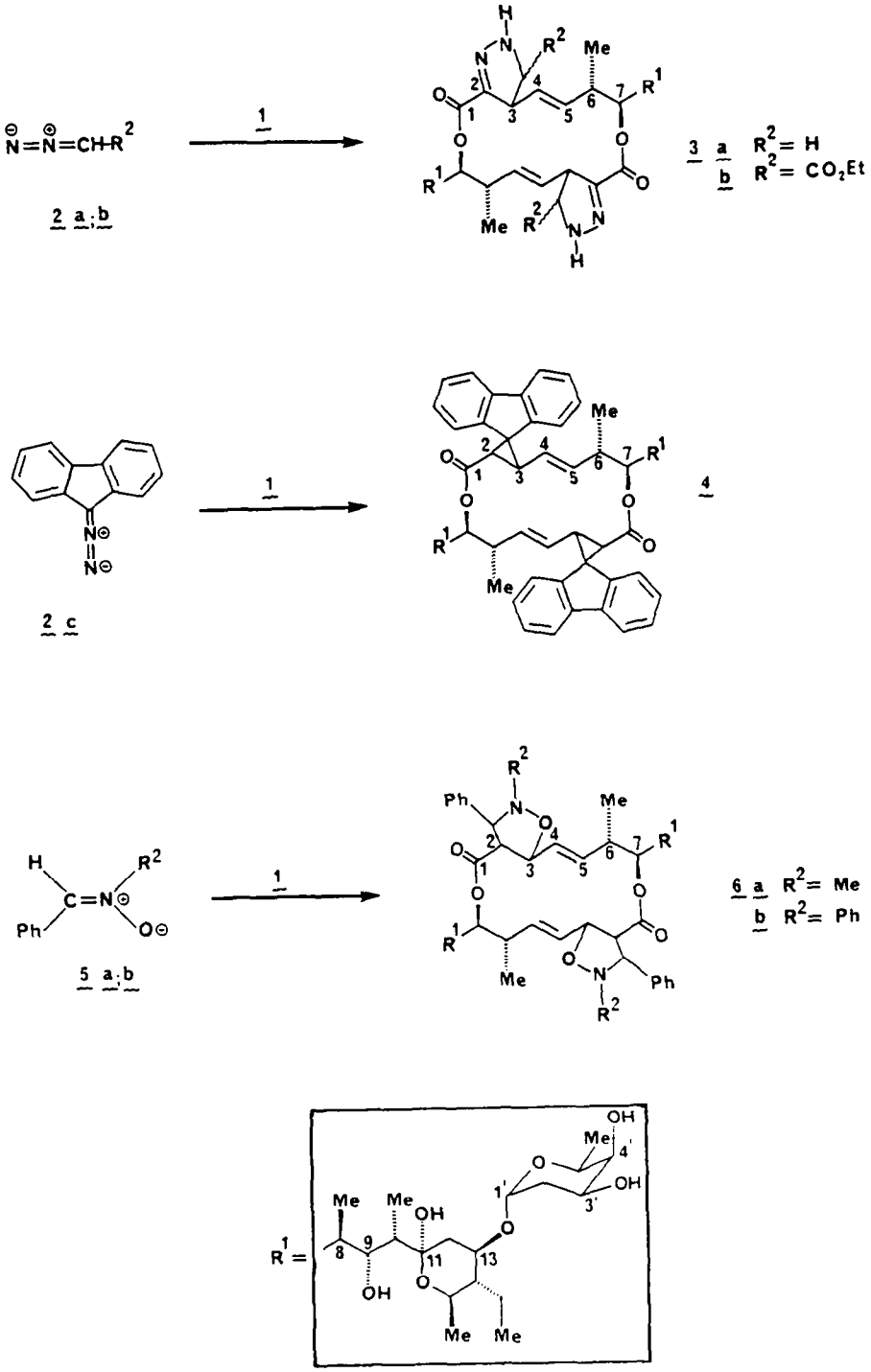
(*Received in Germany* 16 *March* 1990)

Abstract: The title compound **1** reacts by cycloaddition with diazoalkanes **2a** and **2b** to pyrazolines **3a** and **3b** and with nitrones **5a** and **5b** to isoxazolidines **6a** and **6b**. Reaction of **1** with diazofluorene yields the cyclopropane adduct **4**. The reaction proceeds regio- and stereoselectively in high yields preserving the symmetry of the macrodiolide system.

Elaiophylin (**1**) was originally isolated from cultures of Streptomyces melanosporus[2] and is now available in quantities by fermentation of other streptomyces species.[3] The macrolide **1** has a 16-membered unsaturated lactone ring like leucanicidin,[4] the bafilomycins[5] and the avermectins[6] and belongs to the small family of macrolides together with boromycin, conglobatin and the aplasmomycins.[7] Recently the structure of **1** has been definitely established by X-ray crystallography[8] and two independant groups reported the total synthesis of **1**.[9] Although this compound has stimulated widespread interest due to its attractive C_2-symmetry and biological activity,[10] little is known about the chemistry of this highly base and acid sensitive molecule itself,[10] and except hydrogenation[11] nothing has been reported about the reactivity of the dienelactone system.



1



Scheme 1

In the course of our investigations in the field of biologically important macrolides[1] we observed that joining elaiophylin (**1**) with reactive 1,3-dipole reagents like diazoalkanes **2** or nitrones **5** gives rise to the cycloaddition products **3**, **4** and **6**, respectively, in high yields (scheme 1). These reactions proceed in a regio- and stereoselective manner and all other functionalities present in **1** remain unchanged, thus no protective group manipulations are required. The 1,3-dipoles **2** and **5** exclusively attack the directly conjugated 2,3-double bonds and only a single stereoisomer with respect to the new carbon-carbon and carbon-oxygen bonds formed could be detected in each case, although their absolute configuration has not yet been assigned. ^{13}C-NMR spectra of products **3**, **4** and product **6**[12] show only a single set of signals for half of all carbon atoms indicating that the high symmetry (C_2) of the starting molecule has been preserved in the adducts. The experimental procedures are fairly simple and can be easily performed on multigram scales.

Diazomethane (**2a**) smoothly reacts at room temperature within 1 - 2 hours while the addition of diazoester **2b** is completed within 24 hours at reflux temperature in tetrahydrofuran. The preparation procedures for compound **6** are strictly analogous with nitrones **5** requiring reflux temperatures in toluene for 2 - 5 hours. Diazofluorene (**2c**) adds quantitatively to **1** within 1.5 hours at 77° C.

Thus the observed reactivity scale is in perfect agreement with FMO theory predictions[13] for dipole-HOMO controlled cycloadditions. As expected, 1,3-dipoles with lower HOMO energies than -9 eV like nitrile oxides or azides do not react with 1. Interestingly the open chain half of elaiophylin (seco-acid[14]) resists to any of these cycloaddition reagents even at elevated temperatures (110°C). These results can be explained by consideration of the X-ray crystallographic data[8] of **1** which highlight the nonplanarity of both the trans-olefinic double bonds in **1** and established rotations of between 15° and 18° around these bonds. This reveals the observed regioselectivity in favour of the more electron-deficient 2,3-double bonds of the macrolide and suggests that the reactivity of elaiophylin may in part be due to some steric release in the transition state of the cycloaddition. In contrast, open chain conjugated dienoic esters with planar olefinic bonds like methyl butadiene-trans-carboxylate prefer - at least with diazomethane - cycloaddition to the remote 3,4-double bond[15].

The biological activity of the cycloaddition products is currently under investigation.[16]

Tab.1: ^{13}C NMR data of the cycloadducts[a]

Carb.No	3a	3b	4	6a	6b
1	164.0	162.7	169.8	172.0	171.0
2	142.1	140.8	38.9	64.0	63.5
3	45.8	49.3	39.4	80.3	81.4
4	135.6	137.3	126.0	137.7	141.2
5	128.5	128.2	138.0	131.4	122.7
6	38.5	40.6	41.2	40.4	40.8
7	76.1	77.1	76.7	76.7	76.3
8	38.0	37.5	37.1	37.8	37.1
9	70.7	70.3	70.4	70.5	71.3
10	42.7	42.8	43.5	42.9	43.3
11	99.9	100.0	99.8	100.4	100.3
12	39.1	39.4	38.9	39.6	39.3
13	70.0	69.9	69.8	69.8	69.7
14	49.1	49.1	49.0	49.3	49.1
15	67.6	67.5	67.6	67.6	67.6
16	19.7	19.7	19.5	20.0	19.4
17	16.8	16.7	16.3	17.2	17.2
18	9.2	8.9	9.3	9.3	9.7
19	7.4	7.1	7.1	7.4	7.4
20	19.7	19.6	19.7	19.8	19.8
21	9.2	9.2	9.3	8.8	9.3
1'	94.2	94.1	94.1	94.2	94.0
2'	34.2	34.1	34.2	34.2	34.1
3'	66.4	66.3	66.5	66.4	66.4
4'	72.0	71.9	72.1	72.0	72.0
5'	66.8	67.1	66.8	67.4	67.9
6'	17.7	17.5	17.7	17.7	17.7
N-C[b]	56.2	69.4	44.3	74.6	78.4
NH-CH$_3$				42.6	
COEt		162.3			
CH$_2$		60.2			
CH$_3$		14.4			
spiro-C			44.3		
arom.C[c]			143.7	139.1	150.2
			143.5	129.1	142.2
			141.5	129.0	129.6
			140.8	128.6	129.1
			127.7		129.0
			127.5		128.6
			126.9		128.1
			126.0		116.2
			123.9		
			123.0		
			120.3		
			120.6		

a: Recorded on a Bruker NMR spectrometer AM 300, 75.5 MHz in pyridin
b: C-atom in the five membered ring system
c: the aromatic carbons are not assigned

Acknowledgement: We like to thank the Bundesministerium für Forschung und Technologie for financial support and Dr. H. Kluge for valuable NMR discussions.

EXPERIMENTAL

Melting points were determined with a Kofler hot stage apparatus and are uncorrected. NMR spectra were recorded on a Bruker AM 300. Chemical shifts are expressed in ppm with TMS as internal standard. IR spectra on a Perkin-Elmer 197 spectrometer. The FAB MS were taken by a MS 50 Kratos Analytical with 3-nitrobenzyl alcohol as matrix. Thin layer chromatography was carried out on silica gel plates (Merck F_{254}).

Pyrazoline adducts 3a and 3b

1.02 g (1 mmol) elaiophylin (**1**) is dissolved in 50 ml of methanol and 1 ml water, and 200 ml of an ether solution containing about 10 equivalents of the relevant diazoalkane **2a** or **2b** are added. After reaction at room temp. for about 2 h with **2a** or refluxing the reaction mixture with **2b** in 50 ml tetrahydrofuran for 25 h, the solution is concentrated, and the residue is recrystallized with diisopropyl ether to **3a** or chromatographed on silica gel with dichloromethane/methanol (40 : 1) for **3b**.

3a: Yield: 987 mg (89%); m.p. 156 - 158° C; $[\alpha]_D^{20}$ -24° (c = 1, CH_3OH); Analysis for $C_{56}H_{92}N_4O_{18}$ (1109.3): calcd C, 60.6; H, 8.4; found C, 61; H, 8.5; FAB MS ($M+Na^+$) m/e = 1131; ^1H NMR (400 MHz, pyridine-d_5): δ = 2.65(m, 1H, 6-H), 2.8(d, d, 1H, J = 12 Hz, J = 4.5 Hz, 12-Heq), 3.58(d, d,, J = 10.5 Hz, J = 3.8 Hz), 3.78(pseudo , J = 10.5 Hz, J = 1 Hz, a-H), 3.95(m, 1H, 3-H), 4.1(pseudo s, 1H, 4'-H, 4.2(m, 2H, 5'-H, 15-H), 4.45(m, 2H, 3'-H, 13-H), 4.6(pseudo d, 1H, J = 9.8 Hz, 9-H), 5.4(pseudo d, J = 3.7 Hz, 1'-H), 5.9(pseudo d, J = 11 Hz, 7-H), 5.8(m, 2H, 4-H, 5-H).

3b: Yield: 1.22 g (98%); $[\alpha]_D^{20}$ -104° (c = 1, CH_3OH); Analysis for $C_{62}H_{100}N_4O_{22}$ (1252.49) ; calcd C, 59.4; H 8.0; found C, 59.0; H, 7.8; FAB MS ($M+Na^+$) m/e = 1275; ^1H NMR (400 MHz, pyridine-d_5): δ = 2.5(m, 1H, 6-H), 2.8(pseudo d, 1H, 12-Heq), 4.1(pseudo s, 1H, 4'-H), 4.3(m, 4H), 4.5(m, 5H), 4.8(pseudo d, 1H), 5.4-6.0(m, 4H).

Cyclopropane adduct 4

6.0 g (5.85 mmol) elaiophylin (**1**) and 4.5 g (23.4 mmol) diazofluorene (**2c**) are stirred under reflux for 1.5h in 100 ml of ethyl acetate. After the evaporation of the solvent the residue is dissolved in dichloromethane and filtered through a plug of silica gel to recover excess reagent of **2c**. The product is eluted with ethyl acetate and crystallizes from diisopropyl ether. **4**: Yield 7.91 g (100%); m.p. 196°C; $[\alpha]_D^{20}$ -27° (c = 1, CH_3OH); Analysis for $C_{80}H_{104}O_{18}$ (1353.7) : calcd C, 71.0; H, 7.7; found C, 71.1; H, 7.8; FAB MS ($M+Na^+$) m/e = 1375; ^1H NMR (400 MHz, pyridine-d_5): δ = 2.4-2.6(m, 3H, 12-Heq, 6-H, 2'-Hax), 3.7(m, 2H, 2-H, 3-H), 4.05(m, 3H, 9-H, 4'-H, 15-H), 4.2(m, 1H, 5'-H), 4.4(m, 1H, 13-H), 4.52(m, 1H, 3'-H), 5.35(pseudo s, 1H, 1'-H), 5.8(m, 2H, 7-H, 5-H), 6.3(m, 1H, 1'-H), 7.05-8.0(m, 8H, aromatic protons and solvent pyridine)

Isoxazolidine adducts 6a and 6b

1.02 g (1 mmol) elaiophylin (**1**) and 5 mmol of the relevant nitrone **5a** or **5b** in 20 ml toluene are stirred under reflux for 3h. The reaction mixture is concentrated, and the product is separated from excess reagent by column chromatography on silica gel with dichoromethane/ methanol

(20 : 1) and are further purified by crystallization from diisopro ether.

6a: Yield: 868.7 mg (67%); m.p. 148 - 150°C $[\alpha]_D^{20}$ -61.8° (c = CH_3OH); Analysis for $C_{70}H_{106}N_2O_{20}$ (1296.6) : calcd C, 64.8; H, 8.3; fo C, 65.0; H,8.1; FAB MS ($M+Na^+$) m/e = 1318; ^1H NMR (400 MHz, pyridine-d δ = 2.6(s, 3H, N-CH_3), 2.85(d, d, J = 12 Hz, J = 4.5 Hz, 1H, 12-He 3.6(m, 1H, 2-H), 4.1(m, 2H, 4'-H, CH-C_6H_5), 4.3(m, 2H, 5'-H, 15-H), 4.5 3H, 3'-H, 9-H, 13-H), 5.4(m, 2H, 1'-H, 3-H), 5.75(m, 2H, 5-H, 7-H), 6.1 1H, 4-H), 7.45(m, 2H, b-H,c-H), 7.82(m, 1H, a-H).

6b: Yield: 1.18 g (83%); $[\alpha]_D^{20}$ -91° (c = 1; CH_3OH; Analysis $C_{80}H_{110}N_2O_{20}$ (1419.75) : calcd C, 67.6; H, 7.8; found C, 67.3; H, 7.7; MS ($M+Na^+$) m/e = 1414; ^1H NMR (400 MHz, pyridine-d_5): δ = 2.8(m, 12-Heq), 3.7(m, 1H,2-H), 4.1(m, 1H, 4'-H), 4.3(m, 2H, 5-H, 15-H), 4.5 3H, 3'-H, 9-H, 13-H), 5.4(m, 5H, 1'-H, J = 4.5 Hz, 7-H, CH-C_6H_5).

REFERENCES AND NOTES

1. Part 4 of this series: C. Giani, S. Grabley, P. Hammann, H. Kluge, J. Wink, P. Kricke and A. Zeeck, J. Antibiotics, submitted **1990**.
2. F. M. Arcamone, C. Bertazzoli, M. Ghione and T. Scotti, J. Microbiol **1959**, 7, 207.
3. Part 2 of this series: S. Grabley, P. Hammann, W. Raether, J. Wink a A. Zeeck, J. Antibiotics, submitted, **1989**.
4. A. Isogai, S. Sakuda, S. Matsumatu, M. Ogura, K. Furihata, H. Seto a A. Suzuki, Agric. Biol. Chem., **1984**, 48, 1379.
5. M. Deeg, H.-P. Hagenmaier and A. Kretzschmer, J. Antibiotics, **1987**, 90, 320.
6. M. H. Fisher, Spec. Publ.-R. Soc. Chem., **1985**, 53, 53.
7. For a review see: S. Omura, Ed. Macrolide Antibiotics, Chemistry, Biology and Practice, Academic Press, New York, **1984**.
8. S. V. Ley, D. Neuhaus and D. J. Williams, Tetrahedron Lett., **1982**, 23, 1207.
9. K. Toshima, K. Tatsuta and M. Kinpshita, Tetrahedron Lett., **1986**, 27, 4741.0; D. Seebach, H. F. Chow, R. F. W. Jackson, M. A. Sutter, S. Thaisrivongs and J. Zimmermann, Liebigs Ann. Chem. **1986**, 1281.
10. T. Wakamatsu, S. Yamada and H.Nakamura, Heterocycles, **1987**, 25, 43; R. F. W. Jackson, M. A. Sutter and D. Seebach, Liebigs Ann. Chem., **1985**, 2313.
11. H. Kaiser and W. Keller-Schierlein, Helv. Chim. Acta, **1981**, 64, 407.
12. Further NMR data and assignments: P. Hammann, G. Kretzschmar and H. Kluge, Magn. Reson. Chem., in preparation.
13. I. Fleming, Frontier Orbitals in Organic Chemical Reactions, J. Wile New York, **1976**.
14. Part 6 of this series: P. Hammann and G. Kretzschmar, Tetrahedron, submitted, **1990**.
15. R. Huisgen, A. Ohta and J. Geitner, Chem. Pharm. Bull., **1975**, 23, 2735.
16. Only compound **6a** expresses a good antibacterial activity, all other elaiophylin derivatives exhibit no significant activities against gi positive bacteria.