Monatshefte für Chemie 123, 251 – 256 (1992)

Monatshefte für Chemie
Chemical Monthly
© Springer-Verlag 1992
Printed in Austria

Hydrogenation of Vermistatin

Bohumil Proksa[1,*], **Stanislava Uhrínová**[1], **Jana Adamcová**[2], and **Ján Fuska**[2]

[1] Institute of Chemistry, Slovak Academy of Sciences, CS-842 38 Bratislava, Czechoslovakia
[2] Department of Biochemical Technology, Faculty of Chemistry, Slovak Technical University, CS-812 37 Bratislava, Czechoslovakia

Summary. Hydrogenation of vermistatin, a metabolite of *Penicillium vermiculatum*, afforded *cis*- and *trans*-3-(2′-carboxy-4′,6′-dimethoxybenzyl)-6-propyltetrahydro-4*H*-pyran-4-one and 4,6-dimethoxy-3-(4′-propylcyclopentanon-2′-yl)phthalide. The latter compound was found to be the strongest inhibitor of RNA synthesis in P 388 leukemia cells of all compounds tested.

Keywords. Hydrogenation over Pd/C; Leukemia P 388; RNA synthesis inhibition; Vermistatine derivatives.

Hydrierung von Vermistatin

Zusammenfassung. Bei der Hydrierung von Vermistatin, einem neuen sekundären Metabolit aus *Penicillium vermiculatum*, wurden *cis*- und *trans* 3-(2-′Carboxy-4′,6′-dimethoxybenzyl)-6-propyltetrahydro-4-pyran-4-on und 4,6-dimethoxy-3-(4′-propylcyclopentanon-2′-yl)phthalid erhalten und spektroskopisch charakterisiert. Die letztgenannte Verbindung zeigte von allen getesteten Verbindungen die stärkste Hemmwirkung auf die RNA Synthese in P 388 Leukämiezellen.

Introduction

Vermistatin (**1**), a secondary metabolite of *Penicillium vermiculatum* [1] inhibits the RNA synthesis in EAC and P 388 leukemia cells [2]. The 4*H*-pyran-4-one moiety in the structure of compound **1** representing a masked tricarbonyl system might be responsible for the biological activity of vermistatin (**1**). In this paper considerable attention has been paid to the preparation of vermistatin derivatives with a modified supposed reactive center and to biological evaluation of the synthesized compounds.

Results and Discussion

Hydrogenation of vermistatin (**1**) over Pd/C in methanol and subsequent chromatographic purification of the mixture afforded compounds **2** – **4**. According to the molecular formula compound **2** is an isomer of octahydrovermistatin.

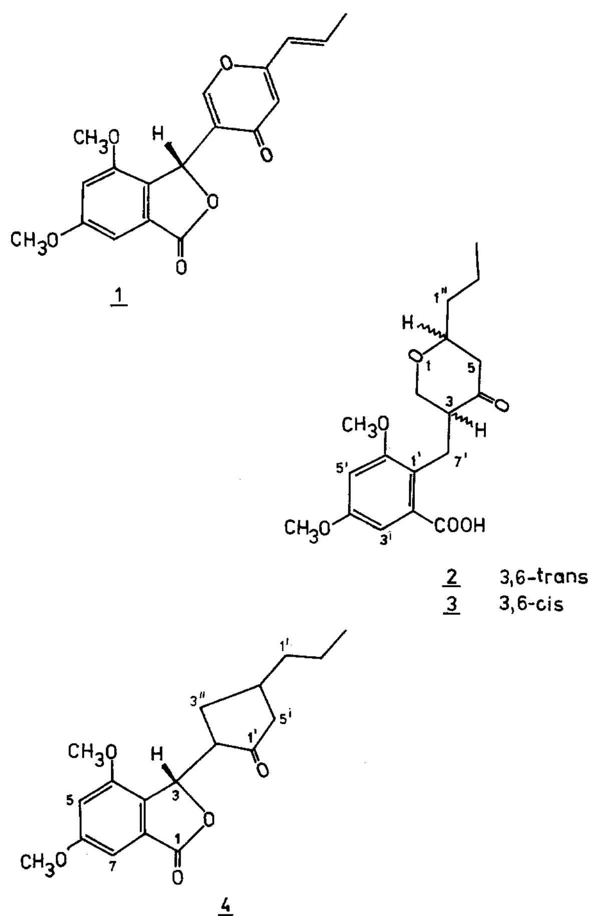

Table 1. ^{13}C-NMR data of compounds **2** and **3** (gd/ppm)

C	**2**	**3**
2	71.6	71.5
3	51.3	52.4
4	209.6	210.3
5	48.5	45.5
6	79.0	78.0
1′	122.2	122.8
2′	131.5	130.5
3′	105.8	106.4
4′	158.6	158.7
5′	102.7	103.1
6′	158.9	158.7
7′	21.5	27.6
1″	38.6	38.1
2″	18.4	18.4
3″	14.0	14.0
COOH	172.2	172.1
OCH_3	55.5; 55.7	55.5 (2 ×)

Table 2. ^{1}H-NMR data of the tetrahydro-4*H*-pyran-4-one moiety of compounds **2** and **3** (δ/ppm)

H	**2**		**3**	
	δ/ppm	*J*/Hz	δ/ppm	*J*/Hz
2a	3.48 dd	11.3, 11.3	3.76 dd	11.7, 3.6
2e	4.03 dd	11.3, 6.5	4.10 dd	11.7, 2.3
3a	3.08 ddddd	11.3, 8.7, 6.5 4.5, 1.2	–	–
3e	–	–	2.61 ddddd	9.8, 5.0, 3.6 2.3, 1.3
5a	2.35 ddd	13.6, 11.4, 1.2	2.77 dd	14.4, 10.5
5e	2.41 dd	13.6, 2.3	2.34 ddd	14.4, 3.0, 1.3
6	3.62 m	10.5, 3.1	3.66 m	10.5, 3.1
7′a	3.33 dd	13.5, 4.5	3.48 dd	13.5, 9.8
7′b	2.86 dd	13.5, 8.7	3.27 dd	13.5, 5.0

The IR spectrum of **2** disclosed one carbonyl band at 1 705 cm^{-1} but lacked bands associated with the phthalide moiety and γ-pyrone carbonyls present in the original spectrum of vermistatin. The ^{13}C-NMR spectrum of compound **2** revealed signals of sp^2 carbons of substitued benzoic acid, one C = O carbon bound in a sixmembered ring and further ten sp^3 carbons (Table 1). In comparison with **1** substantial changes were observed in the phthalide moiety. Hydrogenolysis of this lactone was evidenced by signals of a benzyl carbon (δ 21.5 ppm), a downfield shift of the carboxyl carbon (δ 172.2 ppm vs. 170.0 in the spectrum of vermistatin and the absence of that of one sp^3 carbon bound to a heteroatom (δ 73.6 ppm in the vermistatin spectrum). The M^+ peak of compound **2** was not the base peak as observed in the spectrum of **1**, the most intese peaks corresponded to fragments triggered by the methylene bridge rupture.

According to these data compound **2** was assigned the structure of 3-(2′-carboxy-4′,6′-dimethoxybenzyl)-6-propyltetrahydro-4*H*-pyran-4-one. The MS of **3** was identical with that of compound **2**. The main differences in the ^{13}C-NMR of both compounds were observed in signals of the tetrahydro-4*H*-pyran-4-one protons and carbons (Table 1). Analysis of coupling constants (Table 2) together with an NOE experiment disclosed the difference in orientation of the C-3 substituents. The benzyl moiety in the structure **2** has an equatorial orientation contrary to that in structure **3** with an axial arrangement (Fig. 1). The propyl group attached to C-6 is in an equatorial position in both structures. The mutual orientation of tetrahydro-4*H*-pyran-4-one substituents is *trans* in compound **2** and *cis* in the isomer **3**. The thermodynamically less favourable *cis* isomer **3** isomerizes slowly into the *trans* isomer **2** in solution; about a 30% conversion was observed after 4 days.

The mass spectrum of compound **4** displays a weak M^+ peak (*m*/*z* 318) accompanied by intense peaks at *m*/*z* 194 and 124. The IR spectrum revealed bands of a phthalide lactone (1 760 cm^{-1}) and

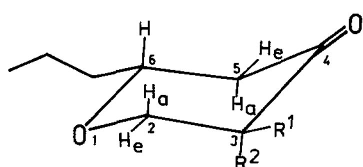

Fig. 1. Arrangement of substituents in the tetrahydropyranone moiety of compounds **2** and **3**. **2**: $R^1 = C_{10}H_{11}O_4$, $R^2 = H$; **3**: $R^1 = H$, $R^2 = C_{10}H_{11}O_4$

Table 3. NMR data of compound **4**

Position	^{13}C	^{1}H	
	δ/ppm	δ/ppm	*J*/Hz
1	170.3	–	–
3	77.3	5.94 d	2.4
3 a	128.8	–	–
4	154.7	–	–
5	104.9	6.67d	2.0
6	162.7	–	–
7	98.6	6.91 d	2.0
7 a	129.5	–	–
1′	215.9	–	–
2′	51.0	3.11 dddd	12.2, 8.2, 2.4, 1.0
3′	28.1	1.62 dddd[a]	12.3, 6.1, 8.2, 2.1
		1.03 ddd[b]	12.3, 12.2, 11.4
4′	34.2	2.04 m	12.2, 11.4, 6.7, 6.1
5′	45.3	2.50 dddd[a]	17.8, 6.7, 2.1, 1.0
		1.88 dd[b]	17.8, 12.2
1″	37.8	1.2 – 1.5 m	
2″	20.9	1.2 – 1.5 m	
3″	14.1	0.86 t	7.0
OCH_3 (2 ×)	55.8	3.86 s	

[a] 3′a; 5′a
[b] 3′b; 5′b

one alicyclic ketone (1 730 cm^{-1}). The ^{13}C-NMR spectrum confirmed the presence of a dimethoxyphthalide grouping attached to a propylcyclopentanone group [3].

These data indicate a contraction of the γ-pyrone ring during vermistatin hydrogenation. Arrangement of substituents in the cyclopentanone ring of compound **4** and the relative configuration were deduced from its ^{1}H-NMR spectrum (Table 3). It is more complex than that of compounds **2** and **3** due to the absence of a heteroatom in the alicyclic moiety. The long-range coupling constants $^{4}J_{H,H}$ extracted from the coupling patterns of protons H-5′a with H-2′ and H-3′b, together with the vicinal coupling constants indicate a *quasi* equatorial orientation of both cyclopentanone substituents in the structure of compound **4** (Fig. 2). These facts led us to ascribe the structure of 4,6-dimethoxy-3-(4′-propylcyclopentanon-2′-yl)phthalide to compound **4**. The compounds **1** – **4** were tested as inhibitors of ^{14}C-uridine incorporation into P 388 leukemia cells [4]. Vermistatin (**1**) caused a 18% inhibition of incorporation of the labelled RNA synthesis precursor in a 150 μg/

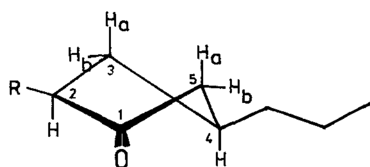

Fig. 2. Spatial arrangement of the cyclopentanone ring substituents in structure **4**; $R = C_{10}H_9O_4$

ml concentration; tetrahydropyranones **2** and **3** did not show any inhibibition, but an enhancement of incorporation (+20 and +32%, respectively). The most significant inhibitor in this test was the phthalide **4**, which lowered the uridine incorporation to 68% at 50 μg/ml concentration. These data indicate the phthalide moiety to be essential for the cytoxic activity of vermistatin (**1**).

Experimental Part

Melting points were determined on a Kofler micro hot-stage the IR spectra were recorded with a Perkin Elmer, model 983 spectrometer. The EI-MS were measured with a Jeol JMS 100 D apparatus at 70 eV and 300 μA. The ^{1}H- and ^{13}C-NMR spectra of $CDCl_3$ solutions were run with a Bruker AM-300 instrument operating at 300 and 75 MHz, respectively (δ relative to *TMS*). The 2D-quantum filtered H,H-COSY spectra [5, 6] were recorded under following parameters: spectral width 1 150 Hz, acquisition time 0.9 s, 2 K data points in F_2, relaxation delay 1 s; 16 scans (two dummy) in each of 256 FIDs were zero filled to 512 data points to yield the final digitization of 0.25 Hz per point. Quadrature detection in t_1 was accomplished by the time-proportional phase-increment method [6]. The HETCOR spectra were acquired with ^{1}H decoupling in the F_1 domain [7, 8] with following parameters: relaxation delay 2 s, acquisition time 0.14 s; 128 scans (2 dummy); spectral width in F_1 was ±560 Hz; 128 increments were acquired and zero filled to 256 in F_1. The spectral width in F_2 was 7 698 Hz. The 1D-NOE difference experiment [9, 10] parameters were: acquisition time 4 s, number of scans 80, time of presaturation of individual multiplet 6 s. Silufol UV_{254} in S-1: chloroform-benzene-methanol (10 : 1 : 1) and visualization at 254 nm were used in analytical TLC; Kieselgel 60 PF_{254} was employed for preparative TLC.

Vermistatin Hydrogenation

Vermistatin (**1**, 250 mg) dissolved in methanol (100 ml) was hydrogenated over 10% Pd/C (10 mg) at an ambient temperature for 4 h. The catalyst was filtered off, the filtrate was concentrated, the rest was purified by preparative TLC in S-1. The individual zones were eluted with chloroform methanol (1 : 1), the solvents were evaporated and the residue was crystallized from diethylether − *n*-heptane to give pure compounds **2** (120 mg), **3** (45 mg) and **4** (36 mg).

trans-3-(2′-Carboxy-4′,6′-dimethoxybenzyl)-6-propyltetrahydro-4H-pyran-4-one (**2**)

M.p. 214−215°C, R_F=0.24. For $C_{18}H_{24}O_6$ (336.4) calc. 64.27, H 7.19; found C 64.22, H 7.24. IR ($CHCl_3$) 3 004, 2 962, 2 935 (C-H), 1 705 (C=O), 1 604, 1 517 (aromatics) cm^{-1}. MS: *m/z*=336(M^+, 12), 318(7), 288(5), 273(6), 221(7), 196(24), 195(100), 182(28), 154(14), 141(9). NMR: see Table 1 and 2.

cis-3-(2′-Carboxy-4′,6′-dimethoxybenzyl)-6-propyltetrahydro-4H-pyran-4-one (**3**)

M.p. 212−213°C, R_F=0.20. For $C_{18}H_{24}O_6$ (336.4) calc. C 64.27, H 7.19; found C 64.19, H 7.12. IR ($CHCl_3$) 3 000, 2 954, 2 930 (C-H), 1 705 (C=O), 1 606, 1 517 (aromatics) cm^{-1}. NMR: see Tables 1 and 2.

4,6-Dimethoxy-3-(4′-propylcyclopentanon-2′-yl)phthalide (**4**)

M.p. 162−164°C, R_F=0.38. For $C_{18}H_{22}O_5$ (318.4) calc C 67.91, H 6.96; found C 67.85, H 6.88. IR ($CHCl_3$): 3 000, 2 958, 2 938 (C-H), 1 760, 1 730 (C=O), 1 610, 1 525 (aromatics). MS *m/z*: 318(M^+, 3), 276(1), 274(1), 194(100), 124(40), NMR: see Table 3.

References

[1] Fuska J., Uhrín D., Proksa B., Votický Z., Ruppeldt J. (1986) J. Antibiot. **39**: 1605
[2] Fuska J., Fusková A., Nemec P. (1979) Biológia (Bratislava) **34**: 735
[3] Stothers J., Tan C. T. (1974) Can. J. Chem. **52**: 308
[4] Fuska J., Miko M., Nemec P., Drobnica L. (1971) Neoplasma **18**: 631
[5] Wagner G., Ernst R. R., Wüthrich K. (1983) Biochem. Biophys. Res. Commun. **117**: 479
[6] Marion D., Wüthrich K. (1983) Biochem. Biophys. Res. Commun. **113**: 967
[7] Bax A. (1983) J. Magn. Reson. **53**: 517
[8] Wilde J. A., Bolton P. H. (1984) J. Magn. Reson. **59**: 343
[9] Neuhaus D. (1983) J. Magn. Reson. **53**: 109
[10] Kövér K. E. (1984) .J. Magn. Reson. **59**: 485

Received May 23, 1991. Accepted June 17, 1991