Pergamon

Tetrahedron Letters 40 (1999) 4515–4518

TETRAHEDRON LETTERS

The Absolute Stereochemistry of the Ester Functions of Fumonisin B_1

Oliver E. Edwards*[1], Barbara A. Blackwell*[2], Alex B. Driega[1], Corrine Bensimon[3] and John W. ApSimon[1]

[1]Ottawa-Carleton Chemistry Institute, Carleton University, Ottawa, Ontario, Canada, K1S 5B6.

[2]Eastern Cereal and Oilseed Research Centre, Agriculture and Agrifood Canada, Ottawa, Ontario, Canada, K1A 0C6.

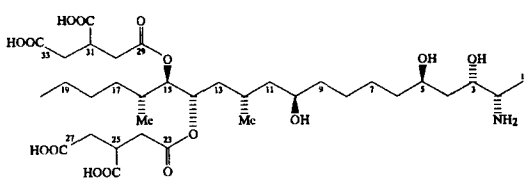
[3] MDS Nordion, 447 March Rd., Kanata, Ontario, Canada, K2K 1X8.

Received 24 March 1999; revised 22 April 1999; accepted 23 April 1999

Abstract: Synthesis of an optically active γ-lactone related to tricarballylic acid (TCA) and correlation of this to the same lactone derived from the two sidechain TCA esters at C-14 and C-15 of fumonisin B_1 has established that these esters have the *R* configuration. Crown copyright © 1999 Published by Elsevier Science Ltd. All rights reserved.

Keywords: fumonisins, TCA ester configuration, *R*(-) 3-(2'-hydroxylethyl)-(γ)-butyrolactone

In recent years, a variety of approaches by our own group[1,2] and others[3,4,5] has led to the assignment of the relative and absolute stereochemistry of the pentaoxyamine backbone of the mycotoxin FB_1 (**1**), using chemical derivatization methods. A very recent publication by Hartl and Humpf[6], using the circular dichroism exciton method, has confirmed the absolute configuration for the amino terminus of FB_1 to be 2*S*, 3*S* and 5*R* in agreement with our previous results. However, disagreement has arisen over the stereochemistry of the two tricarballylic acid (TCA) esters present at positions C-14 and C-15 in this and related molecules[7,8]. In this report we describe evidence that supports the *R* configuration in agreement with Shi, Peng and Kishi[9].



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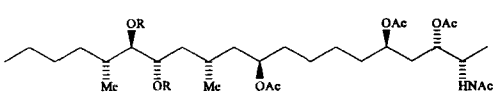

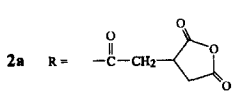

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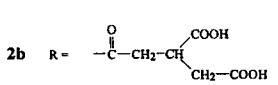

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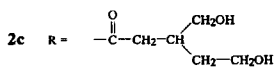
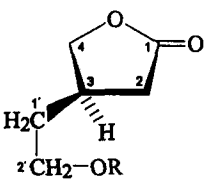


2c

Our approach started by stabilization of the asymmetric centres in the TCA units of naturally isolated FB_1 by borane reduction of the free carboxyl groups, as was also done by Shier et al[7]. In practice, this required solubilization of FB_1 in tetrahydrofuran (THF), which was accomplished by conversion to the N-acetyl triacetate bis-anhydride **2a**, (ν_{max} 3440, 1785, 1730 and 1675 cm^{-1}) using acetic anhydride. This was followed by partial hydrolysis in aqueous THF at room temperature to the acid **2b** (ν_{max} 1735, 1675, and 1635 cm^{-1}), a compound which is the triacetate of the naturally occurring Fumonisin A_1 (FA_1) first described by Bezuidenhout et al[12]. Reduction of **2b** using excess THF/BH_3 in THF gave the *N*-acetyl triacetyl tetraol **2c**

PII: S0040-4039(99)00824-2

(ν_{max} 3660, 3600, 1729, and 1620 cm^{-1}). **2c** is the same as compound **1c** of Shier et al[7]. Complete hydrolysis of **2c** using potassium hydroxide in aqueous methanol, followed by acidification, evaporation of the methanol, and then extraction with chloroform gave a mixture rich in hydroxy γ-lactone **3** (R=H), as determined by infrared (ν_{max} 1775). Benzoylation of this mixture and separation on SiO_2 using 1:1 ethyl acetate/hexane gave the benzoyloxy γ-lactone **3** (R=COC_6H_5, 74% overall yield). This showed ν_{max} 1774 and 1717 cm^{-1} (CH_2Cl_2) and $[\alpha]_D^{25}$ -13° (c 1.1, $CHCl_3$); EIMS, m/z 234 (M^+, 4), 122 (50), 112 (19), 105 (100), 84 (30), 77 (65), 51 (26), 40 (24); ^1H and ^{13}C NMR, see Table 1.



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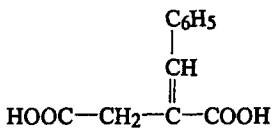

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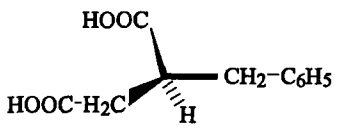

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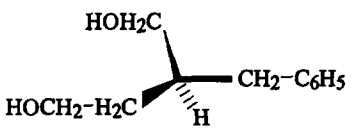

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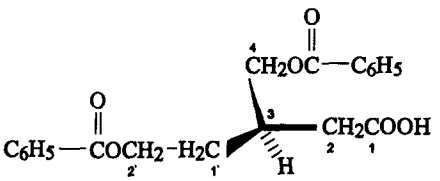

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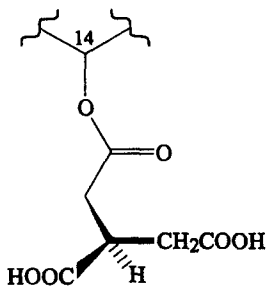
TABLE 1. Selected ^1H and ^{13}C Chemical Shift Assignments of Compounds **3** (R=COC_6H_5) and **7** in (in $CDCl_3$, ppm from TMS, J_{HH} in Hz).

	3		7	
Position	^1H	^{13}C	^1H	^{13}C
1	-	176.4	-	188.7
2	2.28 (dd, 16.2, 7.5)	34.4	2.56 (m)	36.3
	2.68 (m)			
3	2.71 (m)	33.5	2.56 (m)	32.3
4	4.00 (dd, 9.1, 7.4)	72.9	4.35 (dd, 5.3, 11.1)	66.6
	4.47 (dd, 9.1, 7.6)		4.43 (m)	
1'	1.95 (ddd, 12.8, 6.6, 3.9)	32.1	1.98 (ddd, 13.2, 7.4, 3.7)	30.4
2'	4.36 (m)	62.8	4.44 (m)	62.3
C=O	-	166.3	-	166.4, 166.5
C_6H_5:				
C-1"	-	129.8	-	130.0, 130.1
C-ortho	8.00 (d, 7.7)	129.5	8.00 (d, 6.8)	129.4, 129.5
C-meta	7.43 (dd, 7.7, 7.5)	128.5	7.39 (dd, 7.6, 6.8)	128.4. 128.5
C-para	7.55 (d, 7.5)	133.2	7.52 (d, 7.6)	133.0, 133.1

An authentic sample of optically active **3** (R=COC_6H_5) was prepared from *E*-phenylitaconic acid **4**. Asymmetric reduction[13] of **4** gave *S*(-) 2-benzylsuccinic acid **5** ($[\alpha]_D^{25}$ -27° (c 1.5, EtOAc)). The absolute stereochemistry assigned to this[13,14] was confirmed by X-ray crystallography using the Bijvoet method[15]. Conversion of **5** to the diol **6** using borane-THF, then benzoylation and ruthenium tetraoxide oxidation[16] gave the dibenzoyloxy acid **7** ($[\alpha]_D^{25}$ +4° (c 7.0, $CHCl_3$), ^1H and ^{13}C NMR, see Table 1). Alkaline hydrolysis followed by acidification gave the *R*(-) hydroxy γ-lactone **3** (R=H); $[\alpha]_D^{25}$ -5° (c 3.8, $CHCl_3$); ν_{max} 3600, 3470, and 1770 cm^{-1}. Finally, benzoylation converted this to *R*(-) **3** (R=COC_6H_5) with $[\alpha]_D^{25}$ -13° (c 2.3 in $CHCl_3$), identical by MS, IR and both ^{13}C and ^1H NMR to the product from FB_1.



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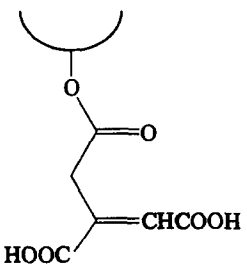

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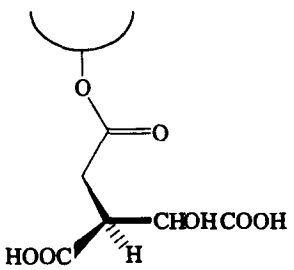

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It follows that both the TCA units in FB_1 have the *R* configuration illustrated in **8**. Thus, our conclusions agree with those of Kishi and coworkers[9] rather than those of Shier et al[7]. Our detailed NMR assignments for the carbons and hydrogens of the TCA units in FB_1 (as well as FB_2 and FB_3)[2,17] agree well with those in the literature[12], making it improbable that two optical isomers of FB_1 have been isolated. It is evident both from our work and that of Boyle and Kishi[8], that only one configuration exists for the TCA units at both the C-14 and C-15 positions in the backbone for all the fumonisins isolated to date. Moreover, it is interesting to observe that no fumonisin has yet been isolated with TCA units at any other position on the backbone, implying a biosynthetic preference for those sites. Comparison of the present results with previous biosynthetic studies[2,18] raise an intriguing question with respect to the biosynthetic origin of these TCA units. Studies using ^{13}C-enriched glutamate have shown that the secondary carboxyl functions (C-28 and C-34) are derived from C-5 of *L*-glutamic acid, while studies with ^{13}C-enriched acetate have shown that the unesterified four carbon unit of the TCA unit (C-25, 26, 27, 28 and C-31, 32, 33, 34) is formed before the addition of a third acetate unit (leading to C-23, 24 and C-29, 30). The specific incorporation of glutamic acid shows that the TCA units are derived from the Kreb's acid cycle. These results can be explained by three possible mechanisms: a) simple chiral esterification using TCA itself, b) esterification with *cis*-aconitate as in **9**, followed by chiral reduction of the double bonds or c) esterification with the chiral intermediate 2*R*-3*S* isocitrate to give **10**, followed by deoxygenation at C-24 and C-30. In the latter case, the *R* configuration of the TCA units would arise, consistent with the results presented here. However, this route invokes a rather rare deoxygenation step.

Acknowledgments: The authors would like to thank The National Research Council of Ottawa for the use of their x-ray equipment, J. Nikiforuk for the NMR spectra, and the Ottawa Carleton Mass Spectrometry Centre for the mass spectra. This work constitutes publication no. 991395 of Eastern Cereal and Oilseed Research Centre.

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