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GALBONOLIDES A AND B - TWO NEW NON-GLYCOSIDIC ANTIFUNGAL MACROLIDES FROM STREPTOMYCES GALBUS

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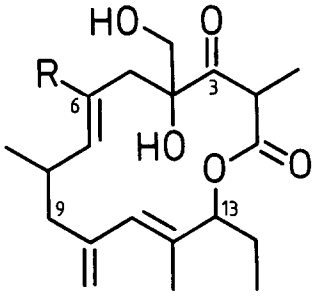
Summary - The title compounds 1 and 2 have been isolated and their structures established; 1 and 2 represent new non-glycosidic 14-membered macrolides with significant antifungal activity.

In the course of a screening program for antimicrobial compounds, we discovered two new neutral 14-membered macrolide antibiotics with antifungal activity, designated galbonolide A (1) and B (2), respectively. The producing microorganism was isolated from soil (1) and classified as Streptomyces galbus ssp. eurythermus (TÜ 2253) (2).



Galbonolide A : 1 (R = OCH_3)

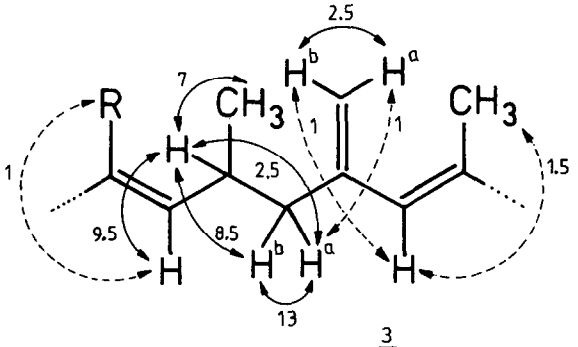
Galbonolide B : 2 (R = CH_3)

The mycelium was extracted with methanol and the solvent removed in vacuo. In order to avoid decomposition the galbonolides had to be separated and isolated by multiple stage column chromatography at 2°C on the following systems: Sephadex-LH 20 (methanol), Sephadex-LH 20 (methanol/petroleum ether), Fractogel TSK HW40(S) (methanol); the separation procedures were monitored by testing the antibiotic activity.

Crystallization (petroleum ether; -20°C) yields colourless needles; 1: m.p. 68°C, $[\alpha]_D^{20}$ -231° (c=0.5, acetone); 2: m.p. 109-112°C, $[\alpha]_D^{20}$ -237° (c=0.4, acetone).

In solution at 20°C the galbonolides are unstable, particularly in the presence of base or acid; even the presence of silica causes rapid decomposition. The electron-impact MS exhibits the highest mass ion at m/z 348.1936 for 1, corresponding to $C_{20}H_{28}O_5$ (calcd 348.1937); however, DCI MS of 1 shows the MH^+ ion at m/z 381 and indicates $C_{21}H_{32}O_6$ to be the elemental composition. M^+ for 2 appears in the EI MS at m/z 364.2250 (= $C_{21}H_{32}O_5$; calcd 364.2250), and this is corroborated by DCI MS.

^1H NMR and ^{13}C NMR studies reveal partial structures 3 (1: R = OCH_3; 2: R = CH_3), 4 and 5 besides an additional $-CH_2-$ and a -CO- group. This accounts for all 21 carbon atoms present in 1 and 2, respectively (see Table).



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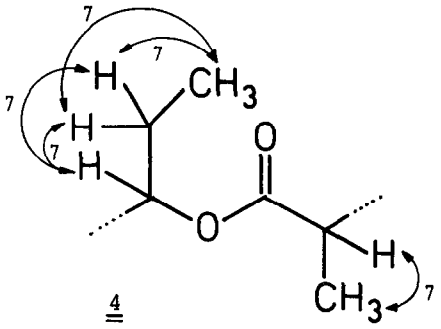

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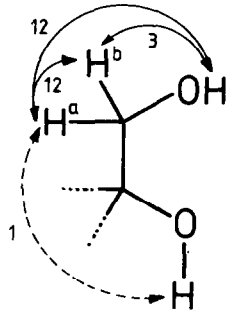
The correlations originally were deduced from $^1H\{^1H\}$- and $^{13}C\{^1H\}$ NMR decoupling experiments on 2; they are fully verified for 1 by $^{13}C,^1H$ shift correlated 2-D NMR (via $^1J_{C,H}$). Additional $^{13}C,^1H$ shift correlated 2-D NMR (via $^{13}C,^1H$ long-range couplings) of 1 allows the connection of the partial structures as shown in formula 6.



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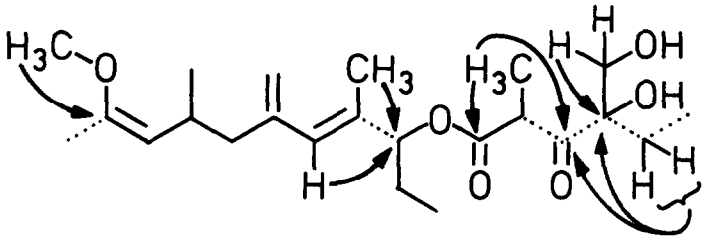

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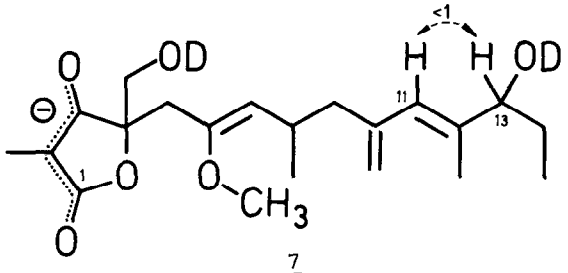
Position	δ_C	1	2	δ_H		1	2
Partial structure 3:							
6-R	Q	56.4	18.8	6-R		3.15 (s)	1.61 (d)
C-6	S	149.1	128.7				
C-7	D	122.7	137.0	7-H		4.83 (d)	5.32 (dq)
C-8	D	30.7	33.2	8-H	ddqd	2.94	2.63
8-$\underline{C}H_3$	Q	20.8	19.5	8-$C\underline{H}_3$	d	0.92	0.85
C-9	T	46.4	45.8	9-H^a	ddd	2.40	2.26
				9-H^b	dd	2.05	2.06
C-10	S	144.2	144.2				
10=$\underline{C}H_2$	T	116.8	116.5	10-$C\underline{H}^a$	dd	5.03	5.03
				10-$C\underline{H}^b$	dd	4.90	4.89
C-11	D	129.1	128.1	11-H	dq	5.98	5.98
C-12	S	135.1	135.1				
12-$\underline{C}H_3$	Q	15.4	15.8	12-$C\underline{H}_3$	d	1.73	1.73
Partial structure 4:							
14-$\underline{C}H_3$	Q	9.9	9.9	14-CH_3	t	0.78	0.81
C-14	T	26.2	26.4	14-H_2	m	1.5	1.5
C-13	D	81.2	80.8	13-H	dd	5.00	4.94
C-1	S	168.9	168.7				
C-2	D	51.0	50.0	2-H	q	3.71	3.79
2-$\underline{C}H_3$	Q	14.4	15.2	2-$C\underline{H}_3$	d	1.42	1.45
Partial structure 5:							
-CH_2OH	T	67.7	68.1	H^a	ddd	3.70	3.57
				H^b	dd	3.44	3.33
				OH	dd	1.97	1.82
C-OH	S	82.8	84.4	OH	d	3.60	3.52
additional structural groups:							
C=O	S	208.4	209.0				
CH_2	T	33.9	41.7	H^a	d	2.64	2.80
				H^b	d	2.41	1.97

Table : NMR data for structural elements (δ[ppm] in C_6D_6).

The reaction with base is in agreement with the β-ketolactone moiety present in 1 and 2: on treatment of 1 with K_2CO_3/CD_3OD lactone 7 is produced; this conversion is accompanied on the ^1H NMR by loss of the signal of H-2 and collapse of the original doublet of the adjacent CH_3-group to a singlet. Furthermore, in 7 a new long-range coupling becomes observable connecting 13-H (δ = 3.88 ppm) with 11-H.



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The β-ketolactone system also well accounts for the UV absorption [λ_{max} = 230 nm (lg ε = 3.9), shifting to about 260 nm (lg ε = 4.0) on addition of NaOH]. The configurations at the double bonds are deduced from the ^{13}C NMR data: the high-field positions of the 12-CH_3 signals give evidence for the (E)-configuration at the 12,13 double bond (3). From the same reason, the (E)-configuration of the 6,7 double bond of 2 is demonstrated by the low-field position of the C-5 signal. In 1, the same stereochemical feature is corroborated by a positive $^1H\{^1H\}$ NOE effect between 5-H and 7-H.

The galbonolides differ from the known non-glycosidic 14-membered macrolides by the structural feature of a conjugated exomethylene group, a non-conjugated methyl enol ether (in case of 1) and by an extremely strong antifungal activity (MIC about 10^{-12} mol/6 mm Ø; disc diffusion assay).

Very recently, a Japanese patent application claims a structure corresponding to 1 (or a diastereomer of 1) for a metabolite (P59B1) isolated from a Micromonospora genus (4).

Literature

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