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OBSCUROLIDES, A NOVEL CLASS OF PHOSPHODIESTERASE INHIBITORS FROM STREPTOMYCES

II. MINOR COMPONENTS BELONGING TO THE OBSCUROLIDE B TO D SERIES

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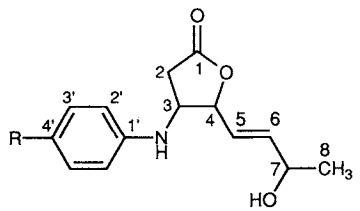
We previously[2] reported the isolation, structural elucidation and biological properties of new phosphodiesterase inhibitors, the obscurolides A_1 to A_4 (**1** to **4**) from the cultures of *Streptomyces viridochromogenes* (strain Tü 2580). A careful search for minor components by chemical screening methods[3,4] revealed eight further members of the obscurolide-complex[2] (Scheme 1). They were assigned to the B, C and D series signifying their differences in the polyketide moiety (C-1 to C-8), whereas the subscripts indicated the oxidation level of the functional group at C-4'. In this paper the isolation, physico-chemical and biological characterization of these new compounds are described. All obscurolides were isolated as diastereomeric mixtures at C-7 (A and C series) and C-5 (B series), respectively. Only in the cases of **5**/**6** and **9**/**10** could the isomers be separated.

The raw product of a 10-liter fermentation[2] was purified by flash chromatography on silica gel (column 40 × 5 cm, $CHCl_3$-MeOH, 9:1) to give 1.9 g of the obscurolide-complex, which consisted mainly of **2** and **3**. The minor components were isolated directly from the complex by preparative

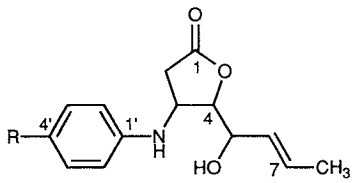
Scheme 1. Structures of the obscurolides belonging to the A to D series.

A series



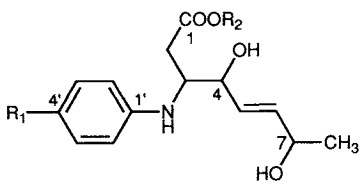
1 (A_1) R = COOH
2 (A_2) R = CHO
3 (A_3) R = CH_2OH
4 (A_4) R = CH_2OCH_3

B series



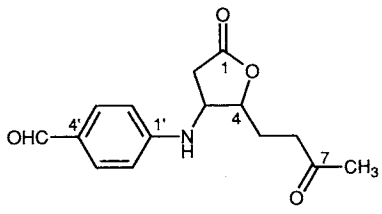
5 ($B_{2\alpha}$) R = CHO
6 ($B_{2\beta}$) R = CHO
7 (B_3) R = CH_2OH
8 (B_4) R = CH_2OCH_3

C series



9 ($C_{2\alpha}$) R_1 = CHO R_2 = H
10 ($C_{2\beta}$) R_1 = CHO R_2 = H
11 R_1 = CHO R_2 = CH_3

D series



12 (D_2)

† See ref 1).

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Table 1. Physico-chemical properties of the obscurolides $B_{2\alpha}$ to D_2 (**5** to **12**).

Compound	Rf-values[a]	Molecular formula	HREI-MS (M^+) found	Calcd for the given formula	IR (KBr cm^{-1})
$B_{2\alpha}$ (**5**)	I: 0.69 II: 0.30	$C_{15}H_{17}NO_4$	275.1157	275.1152	3350, 1760, 1660
$B_{2\beta}$ (**6**)	I: 0.78 II: 0.36	$C_{15}H_{17}NO_4$	275.1154	275.1152	3340, 1770, 1655
B_3 (**7**)	I: 0.38 II: 0.15	$C_{15}H_{19}NO_4$	277.1314	277.1308	3470, 1770, 1705
B_4 (**8**)	I: 0.60 II: 0.45	$C_{16}H_{21}NO_4$	291.1471	291.1464	3380, 1770, 1710
$C_{2\alpha}$ (**9**)	I: 0.10 II: 0.04	$C_{15}H_{19}NO_5$	[b]	293.1257	3400, 1655, 1595
$C_{2\beta}$ (**10**)	I: 0.10 II: 0.03	$C_{15}H_{19}NO_5$	[b]	293.1257	3400, 1655, 1600
11	I: 0.37 II: 0.07	$C_{16}H_{21}NO_5$	307.1419	307.1413	3470, 1720, 1665
D_2 (**12**)	I: 0.51 II: 0.21	$C_{15}H_{17}NO_4$	275.1149	275.1152	3450, 1770, 1705

[a] TLC silica gel, I: $CHCl_3$-MeOH (9:1), II: EtOAc-petroleum ether (1:1).
[b] No EI-MS was obtainable.

Table 2. ^1H NMR signals in acetone-d_6 (200 MHz, δ in ppm relative to internal TMS) of the obscurolides $B_{2\alpha}$ to D_2 (**5** to **12**).

Proton	$B_{2\alpha}$ (**5**)	$B_{2\beta}$ (**6**)	B_3 (**7**)[a]	B_4 (**8**)[b]	$C_{2\alpha}$ (**9**)	$C_{2\beta}$ (**10**)	**11**[c]	D_2 (**12**)
2-Ha	2.44 dd	2.39 dd	2.35 dd	2.38 dd	2.40 dd	2.40 dd	2.62 dd	2.48 dd
2-Hb	3.13 dd	3.17 dd	3.06 dd	3.10 dd	2.55 dd	2.58 dd	2.76 dd	3.10 dd
3-H	4.43 m	4.42 m	4.32 m	4.38 m	4.24 m	4.21 m	4.38 m	4.06 m
3-NH	6.48 br d	6.49 br d	5.39 br d	5.51 br d	6.62 br d	6.60 br d	6.12 br d	6.46 br d
4-H	4.43 m	4.42 m	4.32 m	4.38 m	4.05 m	4.04 m	4.08 m	4.42 dt
5-H	4.43 m	4.42 m	4.32 m	4.38 m	5.79 m	5.80 m	5.77 m	1.85 ddt[d]
5-OH	4.56 d	4.75 d	2.93 br	2.90 br	3.03 br[e]	3.00 br[e]	4.52 br	2.21 m[f]
6-H	5.62 dd	5.64 dd	5.61 dd	5.66 dd	5.79 m	5.80 m	5.96 m	2.72 dd[g]
7-H	5.92 dq	5.93 dq	5.80 dq	5.85 dq	4.24 m	4.21 m	4.38 m	—
8-CH_3	1.69* d	1.74* d	1.69 d	1.70 d	1.18 d	1.13 d	1.27* d	2.19 s
2′-H/6′-H	6.83 d	6.81 d	6.66 d	6.73 d	6.76 d	6.75 d	6.73 d	6.63 d
3′-H/5′-H	7.70 d	7.69 d	7.14 d	7.17 d	7.63 d	7.62 d	7.74 d	7.76 d
7′-H	9.73 s	9.74 s	4.47 s[h]	4.31 s[h]	9.73 s	9.73 s	9.77 s	9.79 s

[a] 7′-OH: $\delta = 3.03$ br s.
[b] 8′-CH_3: $\delta = 3.29$ q.
[c] OCH_3: $\delta = 3.70$ q.
[d] 5-Ha.
[e] 7-OH.
[f] 5-Hb.
[g] 6-H_2.
[h] 7′-CH_2.
* Split signal, main peak.

reversed phase HPLC (Nucleosil 100 C-18, 10 μm, H_2O-MeOH, 70:30 to 0:100) in amounts of 9 mg (**5**), 7 mg (**6**), 9 mg (**10**), 8 mg (**11**) and 5 mg (**12**) and from fractions, which were obtained from the complex after flash chromatography on silica gel (column 40 × 5 cm, ethyl acetate-petroleum ether, 1:1) and subsequent chromatography on Sephadex LH-20 with methanol yielding 25 mg of **7**, 18 mg of **8** and 16 mg of **9**. All obscurolides were easily detectable due to their UV-absorption at 254 nm and their yellow color on TLC plates after staining with EHRLICH's reagent. Some physico-chemical properties of the new obscurolides are given in Table 1, whereas the ^1H and ^{13}C NMR data are shown

Table 3. ^{13}C NMR signals (50.3 MHz, δ in ppm relative to internal TMS) of the obscurolides $B_{2\alpha}$ to D_2 (**5** to **12**) in acetone-d_6, multiplicity assignments by attached proton test (APT).

Carbon	$B_{2\alpha}$ (**5**)	$B_{2\beta}$ (**6**)	B_3 (**7**)	B_4 (**8**)[a]	$C_{2\alpha}$ (**9**)	$C_{2\beta}$ (**10**)	**11**[b]	D_2 (**12**)
1	175.7 s	175.5 s	176.3 s	176.2 s	179.9 s	180.0 s	172.8 s	174.8 s
2	36.5 t*	36.7 t*	36.8 t*	36.7 t*	39.7 t	40.4 t	35.3 t*	35.8 t
3	51.7 d*	50.1 d*	52.2 d*	52.1 d*	56.4 d	56.8 d	55.2 d	54.7 d
4	88.4 d	88.3 d	88.7 d	88.6 d	74.4 d	75.2 d	73.1 d*	84.8 d
5	73.2 d*	72.7 d*	73.4 d*	73.3 d	130.4 d	131.2 d	129.2 d*	28.3 t
6	129.1 d	128.9 d	128.7 d	128.7 d	137.2 d	138.0 d	137.6 d*	39.3 t
7	130.9 d	130.6 d	131.2 d	131.2 d	68.8 d	69.7 d	67.8 d*	206.5 s
8	17.9 q	18.0 q	17.9 q	17.9 q	23.4 q	24.0 q	23.9 q*	30.5 q
1′	153.3 s	153.0 s	147.0 s	148.8 s	155.8 s	156.4 s	154.2 s	153.3 s
2′/6′	113.2 d	113.2 d	113.9 d	113.8 d	113.5 d	114.0 d	113.2 d	113.2 d
3′/5′	132.7 d	132.6 d	129.1 d	130.2 d	133.6 d	134.2 d	132.5 d	132.6 d
4′	128.0 s	127.9 s	132.3 s	128.3 s	126.5 s	127.1 s	127.2 s	128.1 s
7′	190.2 d	190.2 d	64.7 t*	75.0 t	192.1 d	192.7 d	189.9 d	190.2 d

[a] OCH_3: $\delta = 57.5$ q.
[b] OCH_3: $\delta = 51.7$ q.
* Split signal, main peak.

in Table 2 and Table 3, respectively.

The molecular formula $C_{15}H_{17}NO_4$ of the obscurolides $B_{2\alpha}$ (**5**) and $B_{2\beta}$ (**6**) was identical with that of **2**, and the physico-chemical properties apart from the specific rotation ($[\alpha]_D^{20} = +65.9°$ for **5** and $+23.4°$ for **6**) were very similar. In the ^1H NMR spectra a downfield shift of 8-CH_3 from δ 1.22 (**2**) to δ 1.69 (**5**) and δ 1.74 (**6**) revealed the location of the double bond between C-6 and C-7. Accordingly 5-H showed an upfield shift from δ 5.95 (**2**) to δ 4.43/4.42 (**5**/**6**) demonstrating the shifting of the secondary hydroxy group from C-7 to C-5. Thus **5** and **6** (Scheme 1) differ in the stereochemistry at C-5 and for the substituents at C-3/C-4 we assume *trans* configuration as shown for an obscurolide A_2 derivative[2]. The related obscurolides B_3 (**7**) and B_4 (**8**) exhibited the same rearrangement of the allylic alcohol in the side chain as **5** and **6**. They were isomers of obscurolide A_3 (**3**) and A_4 (**4**), respectively, showing the typical signals for a hydroxymethylene group (**7**, δ_H, 4.47, δ_C 64.7) and its corresponding methyl ether (**8**, δ_H 3.29/4.31, δ_C 57.5/75.0) attached to the benzene ring at C-4′.

The C series of the obscurolides combines those compounds, in which the γ-lactone was opened to the γ-hydroxycarboxylic acid (**9**/**10**) and its methyl ester (**11**), while the structure of the side chain follows the A series. Characteristic for all these compounds was the smaller distance of the ^1H NMR signals of the diastereotopic methylene protons 2-Ha and 2-Hb (*e.g.* δ 2.40 and 2.55 for **9**), which were fixed no longer in a γ-lactone ring. All open chain obscurolides had an aldehyde group in 4′-position, **9** and **10** were diastereomers regarding C-7.

Table 4. Effect of the obscurolides $B_{2\alpha}$ to D_2 (**5** to **12**) on calcium/calmodulin-dependent phosphodiesterase from bovine brain.

Obscurrolide	IC_{50} (mM)
$B_{2\alpha}$ (**5**)	2.5
$B_{2\beta}$ (**6**)	>15
B_3 (**7**)	1.5
B_4 (**8**)	0.4
$C_{2\alpha}$ (**9**)	10
$C_{2\beta}$ (**10**)	12
11	0.8
D_2 (**12**)	15

The only representive of the D series, obscurolide D_2 (**12**), showed a saturated side chain, in which starting from **2** the hydroxy group at C-7 has been oxidized to the ketone (δ_C 206.5).

The minor components of the obscurolide complex were tested as inhibitors of the calcium/calmodulin-dependent cAMP phosphodiesterase from bovine brain (Table 4)[2]. Although no definitive structure-activity relationship could be made with this limited data, it seems likely that the biological activity increases with the degree of reduction of the substituent at C-4′. The influence of the stereochemistry at C-5 becomes apparent if one compares the IC_{50} values of the diastereomers **5** and **6**. Although some of the minor components of the obscurolide complex are twenty-fold more active than the main components **2** and **3**, additional improvement was performed through enzymatic bromination, which will be the subject of a following

paper[5]. In analogy to the obscurolides of the A series the obscurolides of the B to D series revealed no growth inhibiting potency against bacteria, yeast and fungi.

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