3-ALKANOYL-5-HYDROXYMETHYL TETRONIC ACID HOMOLOGUES: NEW INHIBITORS OF HIV-1 PROTEASE

II. STRUCTURE DETERMINATION

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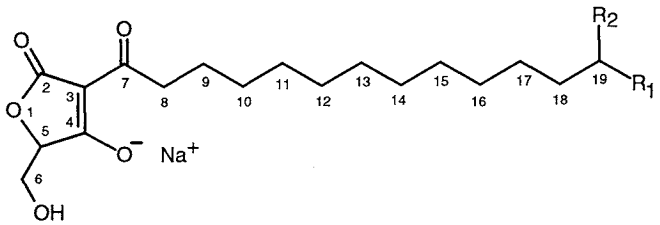
The structures of six new homologues of 3-alkanoyl-5-hydroxymethyl tetronic acid (**1**~**6**) were determined by spectroscopic methods.

Six new homologues of the 3-alkanoyl-5-hydroxymethyl tetronic acid (**1**~**6**) (Scheme 1) have been identified in extracts of the *Actinomycete* strain DSM 7357 as inhibitors of HIV-1 protease. In the preceding paper[1] we described the fermentation, isolation and biological properties of these secondary metabolites **1**~**6**. The length and substitution pattern of the alkanoyl side chain of the tetronic acids (**1**~**6**) differ from a recently described naturally occurring tetronic acid[2]. Agglomerins A~D[3] are tetronic acid derivatives with a different substitution of the butyrolactone ring, but exhibiting alkanoyl side chains of different length and branching. In this paper we will present the structure elucidation and physico-chemical properties of the tetronic acids **1**~**6**.

The FTIR spectrum of the tetronic acid **2**, prepared as a potassium bromide pellet, is shown in Fig. 1. The spectrum is representative of all tetronic acid homologues and shows bands at 3429 (OH and moisture), 2920, 2851 (alkyl chain), 1720 (lactone C=O), 1647 (ketone C=O), 1564 (C=C), 1462 (mainly CH), 1367 (CH_3), 1088, 1030 (C–O), 989, 779 and 721 cm^{-1}.

Scheme 1. Chemical structures of six new homologues of 3-alkanoyl-5-hydroxymethyl tetronic acid (**1**~**6**).



1 $R_1 = \overset{20}{C}H_3$, $R_2 = H$
2 $R_1 = \overset{20}{C}H_2\overset{21}{C}H_3$, $R_2 = H$
3 $R_1 = \overset{20}{C}H_3$, $R_2 = \overset{23}{C}H_3$
4 $R_1 = \overset{20}{C}H_2\overset{21}{C}H_2\overset{22}{C}H_3$, $R_2 = H$
5 $R_1 = \overset{20}{C}H(\overset{21/24}{C}H_3)_2$, $R_2 = H$
6 $R_1 = \overset{20}{C}H(\overset{24}{C}H_3)(\overset{21}{C}H_2\overset{22}{C}H_3)$, $R_2 = H$

Fig. 1. FTIR spectrum of tetronic acid **2**.

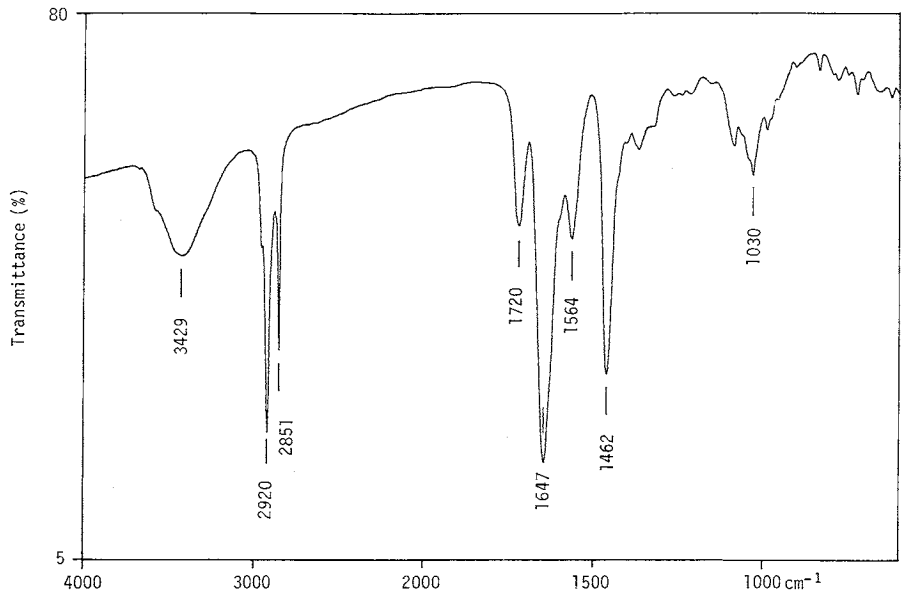


Table 1. ^{1}H NMR chemical shifts of tetronic acids (**1**~**6**).

Proton	1	2	3	4	5	6
5	4.30 dd	4.30 dd	4.29 dd	4.30 br m	4.30 dd	4.33 dd
6	3.87 m,	3.87 m,	3.87 m,	3.87 m,	3.87 m,	3.88 m,
	3.73 m	3.72 m	3.72 m	3.72 m	3.72 m	3.75 m
8	2.73 m	2.72 m	2.72 m	2.72 m	2.73 m	2.74 m
9	1.56 m	1.56 m	1.55 m	1.56 m	1.55 m	1.58 m
10~18			1.30 m			
10~19	1.30 m				1.30 m	1.30 m
10~20		1.30 m				
10~21				1.30 m		
19			1.55 m			
20	0.89 t		0.88 d		1.53 m	1.57 m
21		0.90 t			0.88 d	1.30 m
22				0.90 t		0.87 t
23			0.88 d			
24					0.88 d	0.89 d

Chemical shifts given in ppm. Solvent CD_3OD.

The molecular weights of all tetronic acids (**1**~**6**) were confirmed by the corresponding base peaks *m/z* 385 $(M_{Na}+Na)^+$ for compound **1**, *m/z* 399 $(M_{Na}+Na)^+$ for compounds **2** and **3**, *m/z* 413 $(M_{Na}+Na)^+$ for compounds **4** and **5**, *m/z* 427 $(M_{Na}+Na)^+$ for compound **6** in the individual FAB-MS spectra. From these results it could be deduced that the tetronic acid homologues **1**~**6** are present as sodium salts. In the case of compound **2**, a high resolution mass spectrum was recorded confirming the molecular formula of $C_{20}H_{33}NaO_5$ for tetronic acid derivative **2**.

The shift assignments in the ^{1}H NMR spectra of all tetronic acids (**1**~**6**) are summarized in Table 1. The numbering of the carbon atoms of the different compounds **1**~**6** are given in Scheme 1. The only

proton of the five membered lactone ring at carbon atom C-5 shows a chemical shift of 4.3 ppm in compound **2** and couples with the two neighbouring protons of the hydroxymethyl side chain, forming a typical ABX system. The coupling constants between the proton at C-5 and the protons at C-6 have values of $J_{5,6}=2.7$ Hz and 5.2 Hz, respectively. The chemical shifts of the protons at the carbon atom C-6 are 3.87 ppm and 3.72 ppm in compound **2**. These protons show a geminal coupling pattern with a coupling constant of $J_{5,5}=12.3$ Hz. The shift of the C-8 protons at 2.72 ppm in the alkanoyl side chain are characteristic for protons neighbouring a carbonyl group. The remaining protons of the alkyl side chain have chemical shifts in the range of 0.8 ppm to 1.6 ppm and the number of protons can be calculated from the overall integral. The assignment of the signals in the ^{1}H NMR spectrum to single protons of the alkanoyl side chain is not possible except for the methyl groups at the end of the chain. In the case of the tetronic acids **1**, **2** and **4** the alkanoyl side chain is not branched and the signal with the lowest ppm-value corresponds to the methyl group at the end of the aliphatic side chain and forms a triplet coupling with protons of the adjacent methylene group. Compounds **3** and **5** show a doublet in their ^{1}H NMR spectra for the signal which is shifted most upfield. This signal can be assigned to two methyl groups at the end of the alkanoyl side chain which is coupled with the proton of the neighbouring methine groups at 1.55 ppm and at 1.53 ppm, respectively. The ^{1}H NMR spectrum of the tetronic acid **6** reveals that the alkanoyl side chain is branched, but the position of the branching could only be determined by the interpretation of the ^{13}C NMR spectrum of **6** (see below).

Table 2. ^{13}C NMR chemical shifts of tetronic acids **1**, **2** and **6**.

Carbon	**1**	**2**	**6**[a]
2	178.7 s	178.7 s	175.0 s
3	98.3 s	98.3 s	96.3 s
4	196.3 s	196.2 s	193.4 s
5	83.1 d	83.1 d	81.1 d
6	62.8 t	62.8 t	61.3 t
7	198.7 s	198.7 s	194.3 s
8	41.4 t	41.4 t	39.0 t
9	26.5 t	26.6 t	24.6 t[b]
10~17	30~31 t		
10~18		30~31 t	
10~17, 21			28.5~29.5 t
18	33.1 t		26.5 t[b]
19	23.7 t	33.1 t	36.0 t
20	14.4 q	23.8 t	33.7 d
21		14.4 t	
22			11.2 q
24			19.1 q

Chemical shifts given in ppm. Solvent CD_3OD except for **6**.
[a] Solvent: DMSO.
[b] Inter-convertible signals.

The ^{13}C NMR spectra of the 3-alkanoyl-5-hydroxymethyl tetronic acids **1**, **2** and **6** were recorded at 100 MHz and the data of these spectra are shown in Table 2. The even or odd number of attached protons at each carbon atom was determined by DEPT spectra. In the ^{13}C NMR spectrum of the compound **2** the signals were assigned in the following order: 198.7 ppm to the ketone carbonyl, C-7, 196.2 ppm to the enolized carbonyl, C-4, 178.7 ppm to the lactone carbonyl, C-2, and 98.3 ppm to the *sp*-hybridized, carbon, C-3, by analogy to the interpretation of the ^{13}C NMR spectrum of malonomicin[4]. By low power selective decoupling experiments the mentioned attributions of the three carbonyls C-7, C-4 and C-2 could unequivocally be proven: C-7 couples with protons at C-8 and C-9, C-4 couples with protons at C-5 and C-6 and C-2 with protons at C-5. The signals at 83.1 ppm appears in the region of the chemical shifts of carbon atoms that are close to heteroatoms and it shows in addition an odd number of attached protons in the DEPT spectrum. Therefore this signal was assigned to the carbon atom, C-5, in the tetronic acid **2**. The hydroxymethyl carbon C-6 was attributed to the signal at 62.8 ppm showing two attached protons as could be deduced from the DEPT spectrum of **2** at this position. The signals at 41.4 ppm and at 26.6 ppm in the ^{13}C NMR spectrum of **2** correspond to the methylene group C-8 in α-position to the ketone carbonyl

and the methylene group C-9. For compound **2**, the methyl group and the two adjacent methylene groups at the end of the alkanoyl side chain can be assigned to the signal at 14.4 ppm, 23.8 ppm and 33.1 ppm. The additional signals between 30 and 31 ppm in the ^{13}C NMR spectrum of the tetronic acid **2** correspond all to the carbon atoms of the methylene groups in the alkanoyl side chain of the molecule.

The interpretation of the ^{13}C NMR spectra of the compounds **1** and **6** is essentially the same. In the case of the tetronic acid **6** the position of the additional methyl group C-24 could be established by comparison of the chemical shifts and DEPT signals of compound **6** with the corresponding data from 3-methylnonane[5]. The signals at 11.2 ppm and 19.1 ppm were assigned to the methyl groups C-22 and C-24, respectively. The corresponding values from 3-methylnonane are 11.5 ppm and 19.4 ppm. The methine group C-20 and the adjacent methylene group C-19 correspond to the signals at 33.7 ppm and 36.0 ppm, which again can be compared with the reported values of 34.8 ppm and 37.0 ppm.

The structures of the six new homologues of 3-alkanoyl-5-hydroxymethyl tetronic acid (**1**~**6**) were established by analysis of the IR-, FAB-MS, ^1H NMR- and ^{13}C NMR-spectra.

Experimental

The following instruments were used in this study: Bruker spectrometer IFS 48, ZAB-HF mass spectrometer (FISONS Instruments, Mainz-Kastel), NMR spectrometer Varian VXR-400 S.

Spectroscopic Data of **1**

Lyophilized white powder. IR ν_{max} (KBr) cm^{-1} 3396, 2922, 2853, 1720, 1645, 1570, 1462, 1373, 1248, 1092, 1045, 953, 723; FAB-MS *m/z* 385 $(M_{Na}+Na)^+$; ^1H NMR (400 MHz, CD_3OD): see Table 1; ^{13}C NMR (100 MHz, CD_3OD): see Table 2.

Data of **2**

Lyophilized white powder. IR ν_{max} (KBr) cm^{-1} 3429, 2920, 2851, 1720, 1647, 1564, 1462, 1367, 1088, 1030, 989, 721; FAB-MS *m/z* 399 $(M_{Na}+Na)^+$; HRFAB-MS *m/z* 399.2121 ($C_{20}H_{33}Na_2O_5$, δ_m 0.2 mmu); ^1H NMR (400 MHz, CD_3OD): see Table 1; ^{13}C NMR (100 MHz, CD_3OD): see Table 2.

Data of **3**

Lyophilized white powder. IR ν_{max} (KBr) cm^{-1} 3404, 2924, 2853, 1720, 1645, 1572, 1464, 1367, 1248, 1094, 1040, 953, 723; FAB-MS *m/z* 399 $(M_{Na}+Na)^+$; ^1H NMR (400 MHz, CD_3OD): see Table 1.

Data of **4**

Lyophilized white powder. IR ν_{max} (KBr) cm^{-1} 3418, 2920, 2851, 1720, 1645, 1568, 1466, 1369, 1325, 1252, 1092, 1030, 984, 723; FAB-MS *m/z* 413 $(M_{Na}+Na)^+$; ^1H NMR (400 MHz, CD_3OD): see Table 1.

Data of **5**

Lyophilized white powder. IR ν_{max} (KBr) cm^{-1} 3389, 2922, 2853, 1720, 1645, 1570, 1466, 1367, 1329, 1259, 1096, 1036, 953, 721; FAB-MS *m/z* 413 $(M_{Na}+Na)^+$; ^1H NMR (400 MHz, CD_3OD): see Table 1.

Data of **6**

Lyophilized white powder. IR ν_{max} (KBr) cm^{-1} 3273, 2957, 2918, 2851, 1724, 1639, 1570, 1470, 1375, 1329, 1258, 1096, 1047, 855, 795, 719; FAB-MS *m/z* 427 $(M_{Na}+Na)^+$; ^1H NMR (400 MHz, CD_3OD): see Table 1; ^{13}C NMR (100 MHz, DMSO): see Table 2.

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