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Short Communications

A new sterol from the sponge *Haliclona chilensis* (Thiele)

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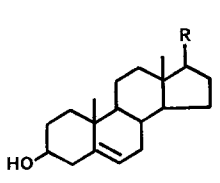
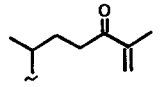
Summary. From the sponge *Haliclona chilensis* (Thiele) a minor keto-sterol was isolated and characterized as 24-keto-cholesta-5,25-dien-3β-ol by means of spectroscopic (^{1}H and ^{13}C-FT NMR, MS) methods.
Key words. Sponge; *Haliclona chilensis;* 24-keto-cholesta-5,25-dien-3β-ol; sterol.

Research on sterol components of marine invertebrates has shown that several species of lower organisms contain a variety of sterols with modified side chains[1,2]. Continuing with our investigations on sterols from aquatic organisms[3] we studied the sterol fraction of the sponge *Haliclona chilensis* (Thiele) which was collected at Chiloé, Chile, in March 1983 and frozen immediately. Work up was done as previously described[3]. As well as sterols that are already known[4], separated by chromatography on silver-nitrate-impregnated silica gel, the fraction contained a new sterol **1** which was purified as acetate by reverse phase HPLC (Whatman Partisil ODS-2) with RRT 0.35 (cholesterol acetate = 1 in abs. methanol) and was homogeneous by GC analysis (12 m × 0.02 mm fused silica capillary column coated with methylsilicone, Hewlett-Packard, 200–280°, 8/min) with RRT 1.19 (cholesterol acetate = 1). Its mass spectrum (398, M^+) displayed the typical pattern of Δ^5-3β-hydroxy sterols[5] (m/z 213, 231 and 253) and the base peak at m/z 271 suggested the presence of unsaturation in the side chain. Its IR spectrum presented a band of α,β-unsaturated ketone at 1680 cm^{-1} that should be located at the side chain. The ^{1}H-FT NMR spectrum (table) showed the presence of a terminal vinyl



1 R=



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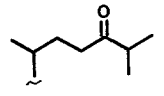

^{1}H-FT NMR (100 MHz, $CDCl_3$)		^{13}C-FT NMR (25.2 MHz, $CDCl_3$, TMS as int.st.)					
H	δ ppm (TMS)	C	δ ppm	C	δ ppm	C	δ ppm
3	4.62 (m)	1	36.95t	11	21.01t	21	18.54q
6	5.38 (m)	2	27.74t	12	39.67t	22	30.74t
18	0.68 (s)	3	73.85d	13	42.30s	23	34.36t
19	1.02 (s)	4	38.08t	14	56.59d	24	202.00s
21	0.94 (d, J = 6 Hz)	5	139.45s	15	24.23t	25	144.39s
26a*	5.76 (m)	6	122.41d	16	28.10t	26	123.95t
26b*	5.96 (m)	7	31.83t	17	55.81d	27	17.70q
27	1.87 (b.s.)	8	31.83d	18	11.85q	CH_3CO	21.37q
CH_3CO	2.04 (s)	9	49.95d	19	19.28q		
		10	36.54s	20	35.45d		

* Determined by double irradiation.

group conjugated to a carbonyl group and a methyl group attached to an olefinic carbon, in addition to the C-6 olefinic signal. Moreover, the ^{13}C-FT NMR spectrum (table) showed the typical signal of the carbonyl carbon atom (202.00 ppm) of a conjugated system and the corresponding signals for the double bond. Besides these, it exhibited chemical shifts in accordance with those of the Δ^5-3β-hydroxyl steroid nucleus[6]. Multiplicities were determined by the method of Le Cocq and Lallemand[7]. Another sterol present in trace amounts (HPLC RRT 0.40 in methanol, GC RRT 1.15) was characterized as **2** on the basis of the mass spectrum (400, M^+) and of the ^{1}H-FT NMR spectrum[8]. The 24-keto-cholesterol (**2**) appears to be a dietary constituent since it has been found in algae[8,9] and could be the direct precursor of the unsaturated analog **1**[10].

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Hydrolysis of N-phenylacetyl-*α*-methyl-α-amino acids by benzylpenicillinacylase

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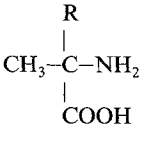
Summary. Enzymatic hydrolysis of several racemic N-phenylacetyl-α-methyl-α-amino acids containing an additional aliphatic, aromatic or polar substitutent on the chiral carbon atom, has been studied by using benzylpenicillinacylase from *Escherichia coli* A.T.C.C.9637. Both the rate of hydrolysis and the stereoselectivity were found to be considerably lower than in the case of natural α-amino acids. Steric and electronic factors in the side chains influencing the stereoselectivity are discussed.

Key words. Benzylpenicillinacylase; enzymatic hydrolysis; α-methyl-α-amino acids.

α-Alkyl-α-amino acids have recently received increasing attention, principally because of their activity as specific enzyme inhibitors[1,2] and because of the considerable restriction of the conformational freedom shown by derived peptides[3]. Although the stereospecific action of enzymes such as acylase I[4], carboxypeptidase A (CPA)[5] and chymotrypsin[6] has been used for the resolution of some racemic α-methyl-α-amino acids, a systematic examination of the factors which control the steric course of the enzymatic hydrolysis in these systems has not been undertaken.

We examined previously the enzymatic hydrolysis and the configurational correlations of the N-phenylacetyl derivatives (N-PA-derivatives) of several classes of amino compounds[7-9], including α-amino acids with α-hydrogen. Since the benzylpenicillinacylase (BPA) from *Escherichia coli* A.T.C.C.9637, used for such studies, showed a high degree of stereoselectivity together with a low degree of substrate specificity, it seemed interesting to investigate the hydrolytic action of BPA on a series of N-PA-derivatives of α-methyl-α-amino acids containing an aliphatic, aromatic or a polar side chain (R) on the chiral carbon atom.



1 R = C_6H_5 **4** R = CH_2OH **7** R = $CH_2CH(CH_3)_2$
2 R = $(CH_2)_2COOH$ **5** R = $CH(CH_3)_2$
3 R = CH_2COOH **6** R = CH_2CH_3

The amino acids examined have known absolute configurations with the exception of α-methylleucine (**7**). The absolute configuration of this latter compound was determined in the course of the present work by using commercially available CPA[5].

Material and methods. Compounds **1** and **2** were obtained by following the Strecker reaction starting from acetophenone[10] and ethyl 4-oxo-pentanoate[11] respectively; compound **4** was prepared by hydroxymethylation of alanine[12]; compounds **3**, **5** and **7** were synthesized by hydrolyzing the corresponding hydantoins obtained in turn from ethyl acetoacetate[13], 3-methyl-2-butanone[14,15] and 4-methyl-2-pentanone[16] respectively.

To define the absolute configuration of α-methylleucine (**7**), N-trifluoroacetyl-DL-α-methylleucine was prepared and incubated with CPA[5]. The L-α-methylleucine released by the enzyme was isolated from the aqueous solution: [α]D+34.0° (c = 3.0, water); hydrolysis with hydrochloric acid of the recovered N-trifluoroacetyl-D-α-methylleucine ([α]D−26.0° (c = 5.0, ethanol)) afforded the D-α-methylleucine: [α]D−34.0° (c = 3.0, water).

The N-PA-derivatives were prepared by treating the amino acids with phenylacetyl chloride in aqueous alkaline medium and were purified by crystallization. Hydrolysis experiments were accomplished as follows. N-PA-derivative (2 mmoles) was dissolved in water (60 ml) containing $CaCO_3$ (2 mmoles); a purified preparation of BPA[8] was added, and the mixture allowed to stand, under stirring, at 37 °C. The progress of the hydrolysis was followed by ninhydrin determination of re-