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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10 We thank Dr Ruth Desqueyroux-Faundez, Museum d'Histoire Naturelle, Genève, Switzerland, for sponges classification and The Organization of the American States for financial support.

0014-4754/85/010034-02$1.50 + 0.20/0

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

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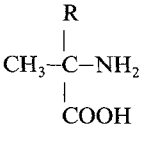
Summary. Enzymatic hydrolysis of several racemic N-phenylacetyl-α-methyl-α-amino acids containing an additional aliphatic, aromatic or polar substitutent on the chiral carbon atom, has been studied by using benzylpenicillinacylase from *Escherichia coli* A.T.C.C.9637. Both the rate of hydrolysis and 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-

Compounds examined and results of the hydrolysis experiments

Compound	M.p. of the starting racemic N-PA-α-methyl-α-amino-acid (°C)	Hydrolysis rate[a]	$[\alpha]_D$ of the amino acid released by the enzyme[b]	Obtained by HCl hydrolysis of the recovered N-PA-derivative	Literature (L or D isomer)	Optical purity of the isolated α-methyl amino acid (%)
1 α-Methylphenylglycine	181–182	4.5	+ 74.9° (c = 2)[c]		+ 86.3° (L)[19]	87
2 α-Methylglutamic acid	139–140	3.6	+ 9.0° (c = 5)[d]		+ 12.1° (L)[20]	74
3 α-Methylaspartic acid	161–162	3.6	+ 35.0° (c = 2)		− 52.9° (D)[21]	66
4 α-Methylserine	127–128	13.0	+ 4.0° (c = 2)		+ 6.3° (L)[22]	63
5 α-Methylvaline	217–218	0.8		+ 2.3° (c = 2.2)	− 3.9°[e] (L)[5]	59
6 α-Methyl-α-amino-butyric acid (isovaline)	198–200	16.7		− 0.6° (c = 10)	+ 11.1° (L)[23]	5
7 α-Methylleucine	182–183	7.6		− 2.5° (c = 5)	+ 34.0° (L)[f]	7
1a Phenylglycine		450	+112.0° (c = 1)	−113.0° (c = 1)	+113.0° (L)[24]	
2a Glutamic acid		360	+ 31.0° (c = 1)[g]	− 31.7° (c = 1)[g]	+ 31.5° (L)[23]	
3a Aspartic acid		360	+ 24.5° (c = 1)[g]	− 25.0° (c = 1)[g]	+ 24.9° (L)[23]	
4a Serine		270	− 7.5° (c = 1.2)	+ 7.8° (c = 1.5)	− 7.8° (L)[23]	
5a Valine		70	+ 6.0° (c = 2)	− 6.2° (c = 2)	+ 5.6° (L)[23]	
6a α-Aminobutyric acid		1400	+ 7.8° (c = 2)	− 8.0° (c = 2)	+ 7.9° (L)[23]	
7a Leucine		800	+ 15.5° (c = 1)[g]	− 15.7° (c = 1)[g]	+ 15.6° (L)[23]	

See text for experimental details. All [α]D were determined in water at 20 °C unless otherwise specified. All the products obtained by chemical methods or by enzymatic hydrolysis showed the expected elemental composition and spectroscopic data. [a]Expressed as micromoles of substrate hydrolyzed per h per mg of enzyme. 4.0 and 0.6 mg of BPA per mmol of substrate were used for compounds **1–7** and **1a–7a**, respectively. [b]Optical rotations are referred to the amino acids obtained after one crystallization from H_2O-EtOH. [c]Solvent 1N HCl. [d]Solvent 6N HCl. [e]The [α]D reported in ref. 5 is referred to L-α-methylvaline obtained by enzymatic resolution with CPA. Since this value appears to be rather low, and no other optical data are available in the literature, a chemical resolution of this substrate was performed in the course of the present work. Fractional crystallization (85% ethanol) of the brucine salt of (±)-N-formyl-α-methylvaline gave optically pure L-α-methylvaline: [α]D−4.0 (c = 6, water), in accordance with the enzymatic resolution. [f]This value was obtained in the course of the present work as reported in the text. [g]Solvent 5N HCl.

leased amino acid. The incubation was continued until the amount of the released amino acid accounted for 50% hydrolysis of the starting racemic substrate. Oxalic acid (2 mmoles) was added to the reaction mixture and the N-PA-derivative, together with phenylacetic acid, extracted with ethyl acetate. The aqueous phase was filtered and evaporated under vacuum; crystallization of the residue afforded the amino acid released by the enzyme. The organic layer was evaporated to dryness and the residue subjected to acid hydrolysis by refluxing for 24 h in 2N HCl. Phenylacetic acid was separated by washing the hydrochloric solution with ether. Evaporation of the aqueous phase and treatment of the residue with Amberlite IR-4B gave the amino acid.

The stereochemical preference of BPA in the case of the compounds **1–4** has been deduced from a direct comparison of the sign of the optical rotation of the amino acids released by the enzyme with that reported in the literature for L and D isomers. In the case of compounds **5**, **6** and **7** the enantiomeric excess was very low and the purification of the enzyme-released amino acids could be achieved only by crystallization; this procedure, however, gave almost racemic products. Optical rotations were then determined on the amino acids obtained by HCl-hydrolysis of the recovered N-PA-derivatives; purification of these latter compounds could in fact be easily performed by column chromatography on silica gel.

Results and discussion. The results reported in the table for compound **1–7** show that, as is found with natural α-amino acids[8,17], BPA splits the L-isomers more rapidly. However, as can be seen by comparing the hydrolytic data for compounds **1–7** with those of compounds **1a–7a**, the replacement of the α-hydrogen with a methyl group greatly affects both the stereoselectivity and the rate of hydrolysis. With the significant exception of the N-PA-derivative of α-methylserine, whose corresponding α-hydrogen analog is not a good substrate for BPA (see table), the rate of enzymatic hydrolysis is lowered by a factor of about 10^2 by the introduction of the methyl group on the α-carbon atom. This effect is analogous to, although more pronounced than, that recently found during the resolution of some unacylated α-methyl amino esters by chymotrypsin[6].

Data on optical purity reported in the table clearly indicate the strong dependence of the stereoselectivity upon the nature of the R substituent. When R is an aliphatic chain not containing polar groups, the stereoselectivity decreases rapidly as the steric hindrance is removed from the chiral center. Thus the N-PA-derivatives of the amino acids **6** and **7**, with an unsubstituted β-carbon atom, show the lowest degree of stereoselectivity (5 and 7% respectively), branching at the β-carbon, as in compound **5**, enhances the optical purity to about 60%. When a polar group is present on the R substituent, as in compounds **2**, **3** and **4**, the degree of asymmetric hydrolysis ranges from 74 to 63% and seems not to be significantly affected by the position of the polar group. It is worth noting that only when a phenyl group is located on the chiral carbon atom does the extent of selectivity become comparable with that found in the case of the natural amino acids **1a–7a**. The considerable although often unpredictable influence of the phenyl group on the steric course of the reactions has already been reported[9,18]; the present result is then not surprising, and emphasizes that both steric and electronic factors are important in determining the stereoselectivity of the hydrolysis.

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0014-4754/85/010035-03$1.50 + 0.20/0

Prostaglandin (15S)-PGA_2 derivatives in the gorgonian *Plexaura homomalla* (Esper), forma *kükenthali* Moser[1]

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Summary. The lipid fraction, 17%, of air-dried *Plexaura homomalla* forma *kükenthali,* collected in the Caribbean at Puerto Rico, yielded 50% of its weight as the mammalian prostaglandin (15S)-PGA_2 methyl ester. The freeze-dried gorgonian yielded (15S)-PGA_2 largely as the acetate of the methyl ester. (15S)-PGA_2 was also obtained from material collected at St. Croix and at South Caicos. Field observations indicate that *Plexaura kükenthali* may be a species separate from *Plexaura homomalla* and that it is abundant on some shallow water reefs in the Caribbean. Prostaglandins could not be detected in the lipid fraction of eggs isolated from *Plexaura homomalla* (Esper) forma *homomalla.*
Key words. Gorgonia; *Plexaura homomalla;* prostaglandins.

In 1969, Weinheimer and Spraggins[2] reported the isolation of derivatives of the 'non-mammalian' prostaglandin, 15-epi PGA_2 or (15R)-PGA_2, from the gorgonian *Plexaura homomalla* (Esper) forma *homomalla*[3] collected off the Florida Keys. A few years later derivatives of the prostaglandins identical with those derived from mammalian sources, (15S)-PGA_2 and PGE_2, were obtained from *Plexaura homomalla* collected at Grand Cayman[4].

Bayer[5] has raised the question whether the collection in which (15R)-PGA_2 was found might have been a mixture of the typical *homomalla* and the *kükenthali* forms of the gorgonian, and suggested that the *kükenthali* form should be investigated for its prostaglandins. Our first observations at Puerto Rico and St. Croix indicated that the *kükenthali* form is very abundant on some shallow water reefs, that it occurs side by side with the 'true' *Plexaura homomalla,* and that the living gorgonians differ in a number of ways. It is likely that *Plexaura kükenthali* is a species separate from *Plexaura homomalla.* Preliminary surveys, in 1974, indicated the *kükenthali* form to be rich in prostaglandins, established in 1978 as derivatives of the mammalian (15R)-PGA_2.

Kung and Ciereszko[6] found eggs in the gorgonian *Pseudopterogorgia americana* (Gmelin) to be very rich in lipids in which the saturated wax cetyl palmitate made up some 70% of the total lipid. In contrast, the eggs obtained from *Plexaura homomalla* forma *homomalla* were found to contain much unsaturated lipid leading us to suspect the presence of prostaglandins. We therefore analyzed the egg lipids of *homomalla* for PGA_2, with negative results.

Materials and methods. Specimens of *Plexaura homomalla* forma *kükenthali* were collected in the Caribbean Sea, on San Cristobal Reef off La Parguera, Puerto Rico, at Tague Bay, St. Croix, US Virgin Islands, and at South Caicos, in December 1976 and January 1978. Specimens collected at Puerto Rico were deposited in the collection of the National Museum of Natural History, Smithsonian Institution, Washington, D.C., as vouchers. The live *Plexaura kükenthali* had appearance and texture clearly different from those of live *P. homomalla.* Some specimens were frozen fresh and freeze-dried shortly after collection, before they were discolored. Others were hung on a line to drip dry. Drying was completed with a hot air blower. The South Caicos sample was preserved in alcohol. Air and freeze dried samples were ground and extracted with methanol-chloroform (1:1) or with n-hexane in a Soxhlet extrator. Lipid content of the dried cortex approached 17%. The lipid extracts were analyzed by chromatographic and spectrophotometric methods.

Eggs were obtained, at Puerto Rico, in December 1976, from freshly collected *P. homomalla* by placing them for an hour in fresh water and then transferring them to sea water. Exposure to fresh water softened the cortex of the gorgonian so that the cortex could be stripped off the skeleton easily, releasing eggs which floated. Eggs were removed from the surface of the sea water by skimming with a fine sieve and washing with sea water. The eggs were preserved in ethanol and later extracted with ethanol, ether and chloroform to yield an unsaturated lipid extract. No prostaglandin could be detected in the lipid extract by chromatographic analysis[7].

Results and discussion. The dark brown lipid extract of air-dried, *P. kükenthali* showed on thin-layer chromatography[7] a strong UV absorbing spot, R_f 0.61, like that of authentic (15S)-PGA_2 methyl ester. On gas chromatography (6 ft. 1% OV-17 at 220°) the largest peak eluted had a retention time of 5.1 m, corresponding to that of (15S)-PGA_2 methyl ester. The UV spectrum in 95% EtOH showed strong absorption around 218 nm which shifted to 278 nm on addition of 45% KOH to the