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63. Preparation and Some Properties of Maleimido Acids and Maleoyl Derivatives of Peptides

by **Oskar Keller**[1]) and **Josef Rudinger**

Institut für Molekularbiologie und Biophysik,
Eidgenössische Technische Hochschule, CH–8049 Zürich

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Summary. N-Alkoxycarbonylmaleimides **3** have been prepared and used to convert amino acids to maleimido acids (**6–8**) in aqueous solution. The carboxyl group of maleimido acids can be activated for amide or peptide synthesis (*e.g.*, in the N-succinimidyl esters **10**); *t*-butyl-based protecting groups can be cleaved without damage to the maleimide moiety. Peptides carrying maleimide groups are accessible either from the maleimido acids (*e.g.*, **11b**, **15**) or by direct maleoylation (*e.g.*, **16b**). The maleoyl group can be cleaved off by successive mild alkaline and acid hydrolysis or by hydrazinolysis. The reactivity of maleimides toward thiol groups suggests the use of maleimido acids and maleoylpeptides for preparing a wide range of conjugates of biochemical interest.

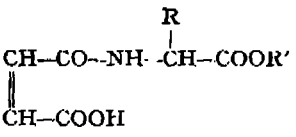
All attempts to prepare maleoylamino acids (maleimido acids) reported in the literature so far [2] [3] have failed.

Maleylamino acids (3-carboxyacryloylamino acids, **1**, R′= H) are readily accessible by reaction of the amino acids with maleic anhydride (see [2], [3] and references given there) but the cyclisation of the maleamic acid to the maleimide grouping (**1** → **2**, R′= H) has proved difficult in the presence of the additional free carboxyl group. *Helferich & Wesemann* [3] have cyclised the maleamic acid **1** (R = Me, R′= Et) derived from D,L-alanine ethyl ester to the maleimido ester

[1]) Part of the Doctoral Dissertation of *O. Keller* [1].

2 (R = Me, R′ = Et) with chloromethyl cyanide and triethylamine at about 90°, and converted (3-carboxyacryloyl)glycine (**1**, R = R′ = H) to maleoylglycine cyanomethyl ester (**2**, R = H, R′ = CH_2CN) under the same conditions but the preparation of the free maleimido acids from the esters has not been described. If the route to maleimido acids through the esters were to be used, carboxyl-protecting groups sensitive to alkaline hydrolysis or hydrogenation would evidently be unsuitable (see also below) but acid-labile protecting groups might be satisfactory. In preliminary studies it was indeed, found that maleimido acid *t*-butyl esters (**2**, R′ = Bu^t) can be prepared by the procedure of *Helferich & Wesemann* [3] and converted to the free maleimido acids by treatment with trifluoroacetic acid[2]). However, we felt that a mild procedure applicable directly to amino acids would be welcome.

Nefkens et al. [4] have developed a simple and elegant method for the preparation of phthalimido acids from amino acids in aqueous solution using N-carbethoxyphthalimide as the reagent. We have now been able to show that analogous reagents can also be prepared from maleimide and used for the preparation of maleimido acids.



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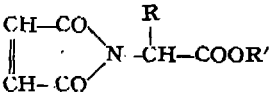

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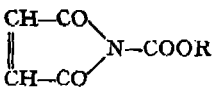
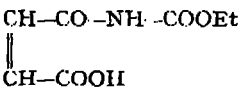


3a, R = Me **3c**, R = Bu^t
3b, R = Et **3d**, R = CH_2Ph
3e, R = $CH_2C_6H_4NO_2$-*p*



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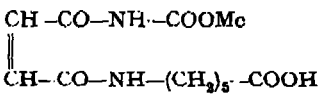

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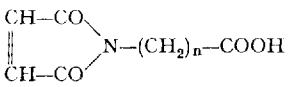
Several N-alkyloxycarbonylmaleimides (**3**, **a**–**e**) were prepared from maleimide by reaction with the appropriate alkyl chloroformates and N-methylmorpholine in ethyl acetate (Table 1). The methoxy- and ethoxycarbonyl derivatives (**3a**, **b**) proved to be reasonably soluble in water and were therefore used in all further work with aqueous solutions.

Reaction of N-ethoxycarbonylmaleimide with glycine, phenylalanine[3]), or 6-aminocaproic acid under the conditions used for the preparation of phthalimido acids [3] gave inhomogeneous materials from which pure products could be isolated only with difficulty and in low yield. We first suspected that the main contaminant might be N-ethoxycarbonylmaleamic acid (**4**), formed by hydrolysis of the reagent, but chromatographic comparison with an authentic sample of **4** showed it to be present only in traces. The course of the reaction of 6-aminocaproic acid with **3a**

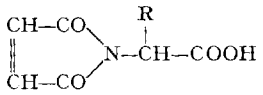
[2]) These unpublished experiments were carried out with Miss *Míla Cimrová* at the Institute of Organic Chemistry and Biochemistry of the Czechoslovak Academy of Science, Prague, in 1967–1968.

[3]) All chiral amino acids are of the L configuration. Abbreviations for amino acids and protecting groups follow current rules [5]; by analogy, 'Mal' is used to symbolise the maleoyl group. Other abbreviations: DCCI = dicyclohexylcarbodiimide, DCHA = dicyclohexylamine, diglyme = bis-2-methoxyethyl ether, DMF = N,N-dimethylformamide, DTNB = 5,5′-dithiobis-2-nitrobenzoic acid, HOBt = 1-hydroxybenzotriazole, NMM = N-methylmorpholine, tris = 2-amino-2-hydroxymethylpropane-1,3-diol.

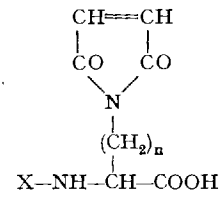
was then examined under a variety of conditions. When the reaction was carried out at room temperature in the presence of $NaHCO_3$ (2 mol) the pH of the solution dropped from about 8.3 to 7.0 during 30 sec, rose again to 8.5 during 20 min and then remained constant over 40 min. It was surmised that the first phase of the reaction was the formation of the imide-amide derivative **5** (*cf.* [4]), the appearance of the acidic N-acylcarbamate group being responsible for the decrease in pH and the formation of the maleimide **6c** and (neutral) methyl carbamate for the subsequent rise. When **3a** was allowed to react with 6-aminocaproic acid in NaOH in the absence of carbonate, the pH decreased from 11 to 6–7 but remained constant at this value; the product isolated under these conditions was characterised by elemental analysis and the NMR.-spectrum as the imide-amide **5**. Cyclisation of **5** to the maleimide **6c** at a useful rate was found to require the presence of carbonate and a pH about 8.5, suggesting a catalytic effect of hydrogen carbonate ion [4]. However, at pH 8.5 and above, hydrolysis of the maleimide **6c** to the maleamic acid **9** already takes place at an appreciable rate. We have so far been unable to find any set of conditions under which cyclisation of **5** to **6c** would proceed to completion



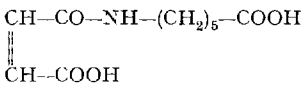
6a, n = 1
6b, n = 2
6c, n = 5



7a, R = CH_2Ph
7b, R = $CH_2CH(CH_3)_2$



8a, X = Boc, n = 3
8b, X = H, n = 3
8c, X = Z, n = 4



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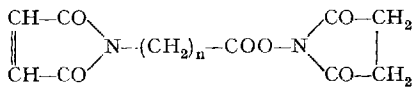
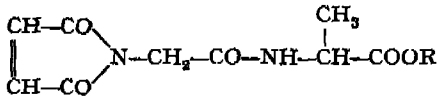


10a, n = 1
10b, n = 2
10c, n = 5

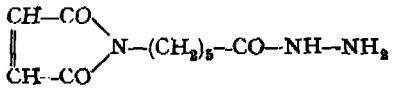
without some concomitant hydrolysis of **6c** to **9** (see Experimental Part). The conditions eventually adopted for the maleoylation of individual amino acids (aqueous $NaHCO_3$ initially at 0°, then at higher temperatures; *cf.* Table 2) were those found by preliminary TLC. studies to give the best yield of the maleimido acids. Clean separation from the maleamic acid by-products could be achieved by filtration through a column of silica gel.

By this procedure the maleoyl derivatives of glycine (**6a**), phenylalanine (**7a**), leucine (**7b**), N(α)-*t*-butyloxycarbonyl-ornithine (**8a**), N(α)-benzyloxycarbonyl-lysine (**8c**), β-alanine (**6b**), and 6-aminocaproic acid (**6c**) were obtained in yields of 60–70% (Table 2). The acids **7b**, **8a**, and **8c** were non-crystalline but afforded crystalline dicyclohexylamine salts.

The carboxyl groups of the maleimido acids can be rendered reactive by the usual procedures of peptide chemistry. Thus the maleimido acids **6a–c** were converted to the N-succinimidyl esters **10a–c** (Table 3) with N-hydroxysuccinimide and N,N-dicyclohexylcarbodiimide. The ester **10a** was coupled with alanine *t*-butyl ester to give maleoylglycylalanine *t*-butyl ester (**11a**); the same dipeptide derivative was also obtained directly from the acid **6a** and alanine *t*-butyl ester by coupling with dicyclohexylcarbodiimide. The *t*-butyl ester group of **11a** could be cleaved with trifluoroacetic acid, without damage to the maleimide grouping, to afford maleoylglycylalanine (**11b**). The resistance of the maleimide group to the standard conditions of acidolytic *t*-butyl cleavage was confirmed by the conversion of N(α)-*t*-butyloxycarbonyl-N(δ)-maleoyl-ornithine (**8a**) to N(δ)-maleoyl-ornithine hydrochloride (**8b** · HCl) with hydrogen chloride in ethyl acetate. On the other hand, treatment



11a, R = Bu^t
11b, R = H



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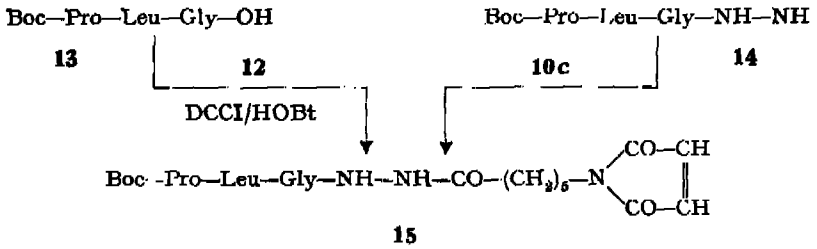
of the lysine derivative **8c** with hydrogen bromide under the standard conditions used for removal of N-benzyloxycarbonyl protecting groups gave a product containing covalently bound bromine and lacking the characteristic NMR. spectrum of the maleimido derivatives, presumably an N(δ)-bromosuccinoyl-lysine; and, as expected, catalytic hydrogenolysis of the protecting group of **8c** was attended by saturation of the double bond, forming a succinimide derivative (NMR.).

Coupling of 6-maleimidocaproic acid (*e.g.*, as the succinimidyl ester **10c**) with *t*-butyl carbazate followed by acid treatment gave the hydrazide **12** (as the hydrochloride). This could be acylated with *t*-butyloxycarbonylprolyl-leucyl-glycine (**13**) to give the diacyl hydrazide **15**, which was also obtained from the tripeptide hydrazide **14** by acylation with **10**.



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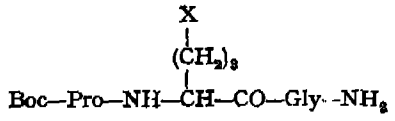
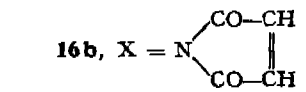
Maleoyl groups can also be introduced directly into peptides containing free amino groups under conditions similar to those used to prepare maleimido acids. The conversion of *t*-butyloxycarbonyl-prolyl-ornithyl-glycine amide (**16a**) [6] to the



16a, X = NH_2



16b, X =

maleoyl tripeptide **16b** with N-methoxycarbonyl-maleimide (**3a**) may serve as an example.

The rate of hydrolysis of the maleimide group in 6-maleimidocaproic acid was measured at three pH values (see Experimental Part); the second-order rate constant for hydrolysis by OH^- ion was calculated to be 22 l mol^{-1} s^{-1} (22°). The corresponding value for the alkaline hydrolysis of 6-phthalimidocaproic acid, determined polarographically [7], is 5.85 l mol^{-1} s^{-1} (25°). The maleimide grouping is thus appreciably more alkali sensitive than the phthalimide group. In a preparative experiment, 6-(3-carboxyacryloylamino)caproic acid ('6-maleylaminocaproic acid', **9**) was isolated as the product of alkaline hydrolysis in 94% yield.

It is well known that 3-carboxyacrylamides are hydrolysed to the free amines under mildly acidic conditions and this reaction has been exploited in protein chemistry, where the 'maleyl' (3-carboxyacryloyl) group is used for the reversible protection of amino groups [8]. As expected, the maleamic acid **9** gave 6-aminocaproic acid under these conditions.

The sequential hydrolysis at pH ~10 and at pH 2–3, without the isolation of the intermediate maleamic acid, would seem to offer a mild procedure for removing the maleoyl group (see [9] for a similar cleavage of phthaloyl groups). This conclusion was confirmed by the conversion of **6c** to 6-aminocaproic acid and **7a** to phenylalanine. The maleoyl group, like the phthaloyl group, can also be cleaved by hydrazine under mild conditions. It is evident that the maleoyl group meets the requirements for an amino-protecting group in peptide chemistry, sharing with the phthaloyl group the advantage that it replaces both hydrogen atoms of the amino group. While we do not mean to suggest its routine use in peptide synthesis, we believe that the maleoyl group may prove useful in specific situations.

A potentially more useful property of the maleimido acids and maleoylpeptides is their ability, as N-alkylmaleimides, to react rapidly and rather specifically with thiol groups.

This ability is illustrated by an experiment in which N(δ)-maleoylornithine was allowed to react with sodium 2-mercaptoethanesulfonate: Addition of the thiol to the maleimide group was essentially complete in less than 1 min at pH 7.2 (TLE.). The same reaction was also exploited to detect maleimide derivatives after TLC. and TLE.: After spraying with a solution of sodium 2-mercaptoethanesulfonate and the thiol reagent, DTNB, the maleimides gave rise to white (thiol-negative) spots on a yellow background.

Maleimide-containing reagents have been used to conjugate a variety of groups (labels, 'reporter' groups etc.; see, *e.g.*, [10]) to thiol-containing proteins, or for cross-linking such proteins [11]. Maleimide structures have also been built into drugs as potential 'anchor' groups for covalent attachment to target tissues [12]. The work described here opens up similar possibilities for peptide derivatives.

The maleimido acids **6a–c** and their carboxyl-activated derivatives such as **10a–c** particularly commend themselves as a (readily supplemented) set of variable-length 'adapters' useful for linking moieties containing amino groups on the one hand and thiols on the other. Potential combinations include hapten – carrier (to give an antigen); ligand – insoluble carrier (affinity sorbent); specific ligand – binding site (affinity labelling); label or reporter group – peptide or protein; or

suitably spaced groups within the same protein molecule or its subunits (topochemical probing; *cf.* [11]).

Obviously, derivatives such as the hydrazide **12** offer similar opportunities for linking carboxyl-containing with thiol-containing moieties (*cf.* **13** → **15**).

These possibilities are being actively explored. A first example has been provided by *Möschler & Schwyzer* [13] who have conjugated angiotensin **II** to a modified agarose by means of 6-maleimidocaproic acid (**6c**).

Experimental Part

General. For materials and general methods (determination of m.p. 's and optical rotations, basic procedures of thin-layer chromatography (TLC.) and electrophoresis (TLE.), elemental analyses) see [14]. Samples for elemental analysis were dried at 22° or 60° and 0.01 Torr for at least 24 h. Solutions in water-immiscible organic solvents were washed with satd. NaCl, dried over $MgSO_4$, and evaporated in a rotary evaporator at 25–40° and 12 Torr unless otherwise stated.

TLC. and TLE. Silica gel on glass was used for TLC. with the solvent systems (compositions by volume) A: MeOH/$CHCl_3$ 1:1; B: $CHCl_3$/AcOH 95:5; C: *n*-BuOH/AcOH/H_2O 4:1:1. For TLE., the electrolytes were D: pyridine/AcOH/H_2O 23:6:970 and E: 1M AcOH. In addition to standard detection methods (ninhydrin, *Reindel-Hoppe*, iodine vapour; *cf.* [14]) a new procedure (see below) was used to detect maleimides.

Detection of maleimides. Reagent *a*: 0.1% 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) in EtOH/tris-HCl buffer (pH 8.2), 1:1; reagent *b*: 2% Na 2-mercaptoethanesulfonate (Mesna®, *Organica UCB* S.A., Brussels, Belgium) in 80% aq. EtOH. The silicagel or cellulose-coated plate was sprayed with reagent *a* and then with reagent *b* until the background was bright yellow; maleimide derivatives appeared as white spots. The contrast could be increased by respraying with reagent *a*. Where an acid solvent or electrolyte had been used the yellow coloration was intensified by spraying with 10% Na_2CO_3 solution. The limit of detection was 10^{-8} mol of maleimide.

Spectra. ^{1}H-NMR. spectra were recorded at 60 MHz with a *Varian* T-60 instrument; chemical shifts are given in ppm from Me_4Si as internal standard. UV. spectra were measured on a *Pye-Unicam* SP 1800 spectrophotometer and are recorded as λ_{max} in nm, followed in brackets by ε (1000 cm^2/mol). Only selected NMR. and UV. spectra are described; for others, and for IR. spectra, see [1].

Measurements of pH and pH-stat rate measurements were made with an autotitrator assembly (*Radiometer*, Copenhagen, Denmark) consisting of Model TTT titrator, Model ABU 13 autoburette, and Titrigraph Type SBR 2c.

N-Alkoxycarbonylmaleimides and Derivatives. – *N-Alkoxymaleimides* (**3**). Maleimide (388 mg; 4 mmol) and NMM (404 mg; 4 mmol) in EtOAc (20 ml) were treated at 0–3° with the appropriate alkyl chloroformate (4 mmol). After 30 min the precipitate was filtered off, washed with EtOAc, filtrate and washings were washed, dried, and evaporated to dryness. The products (Table 1) were recrystallised from EtOA/*i*-Pr_2O unless otherwise stated. NMR. of **3a** (acetone-d_6): 3.93 (3 H, *s*); 7.0 (2 H, *s*).

N-Ethoxycarbonylmaleamic acid (**4**). **3b** (300 mg; 1.7 mmol) was stirred with $Na_2CO_3 \cdot 10H_2O$ (280 mg; 1.7 mmol) in water (10 ml) at 22° 2 h. The product was isolated from the acidified (pH 1.5) solution by extraction with EtOAc, evaporation, and recrystallisation from EtOAc/light petroleum; 300 mg (94%), m.p. 110°, Rf 0.36 (A), 0.03 (C). – NMR. (acetone-d_6): 9.76 (1 H, *s*, broad), 8.97 (1 H, *s*, broad), 7.0–6.0 (2 H, AB system), 4.20 (2 H, *q*, J = 7 Hz), 1.25 (3 H, *t*, J = 7 Hz).

$C_7H_9NO_5$ (187.1) Calc. C 44.92 H 4.85 N 7.48% Found C 44.86 H 4.87 N 7.46%

Maleimido Acids and Derivatives. – *N-(5-Carboxy-1-pentyl)-N'-ethoxycarbonylmaleamide* (**5**). 6-Aminocaproic acid (530 mg; 4 mmol) in water (5 ml) was brought to pH 11 with 1M NaOH and treated, at 0°, with **3b** (590 mg; 3.5 mmol). During 1 h the pH decreased to 6–7. Acidification to pH 1–2 with 1M H_2SO_4, extraction into EtOAc, washing, evaporation, and re-

Table 1. *N-Alkoxycarbonylmaleimides* (**3**)

Compound R	Yield, % M.p., °C	Rf (A) Rf (B)	Formula M.wt.	Calc./Found, % C	H	N
3a	64	0.67	$C_6H_5NO_4$	46.46	3.25	9.03
CH_3	61–63	0.45	155.1	46.29	3.29	8.94
3b	62	0.69	$C_7H_7NO_4$	49.71	4.17	8.28
CH_2CH_3	58–59	0.48	169.1	49.57	4.13	8.22
3c	58[a]	0.72	$C_9H_{11}NO_9$	54.82	5.62	7.10
$CH_2CH(CH_3)_2$	67–68	0.51	197.2	54.92	5.69	7.03
3d	57[b]	0.73	$C_{12}H_9NO_4$	62.34	3.92	6.06
$CH_2C_6H_5$	104	0.51	231.2	62.16	3.88	5.86
3e	58	0.73	$C_{12}H_8N_2O_6$	52.18	2.92	10.14
$CH_2C_6H_4NO_2$-*p*	149	0.42	276.2	52.17	2.85	10.02

[a] From *i*-Pr_2O. [b] From EtOAc/light petroleum.

crystallisation from EtOAc/light petroleum afforded 650 mg (62%) of **5**, m.p. 106°, Rf 0.46 (A), 0.03 (B). – NMR. (CD_3OD): 6.29 (2 H, *s*); 5.21 (2 H, *q*, J = 7 Hz); *ca.* 3.23 (under CHD_2OD signal; 2 H, pseudo-*t*); 2.29 (2 H, pseudo-*t*); 2.0–1.0 (9 H, *m*; at 1.27, 3 H, *d*, J = 7 Hz).

$C_{13}H_{20}N_2O_6$ (300.2) Calc. C 51.99 H 6.71 N 9.33% Found C 52.09 H 6.84 N 9.25%

Cyclisation of **5** *to 6-maleimidocaproic acid* (**6c**). **5** (45 mg; 0.15 mmol) with $NaHCO_3$ (25 mg; 0.3 mmol) in water (15 ml) at 22° was kept at pH 8.7 with 0.1M NaOH (pH-stat). Samples (0.4 ml) taken at 5, 10, 20, 30, and 60 min were acidified with 1M H_2SO_4 (0.1 ml), extracted with EtOAc (0.2 ml), and 20 µl of each extract analysed by TLC. in solvent A. Amounts were estimated visually from fluorescence quenching (254 nm). All of **5** had disappeared after 30 min, but **8** began to appear at 20 min; the highest concentration of **6c** was observed after 30 min. Similar experiments were carried out at pH values from 7 to 10 and at 0 and 22°.

Table 2. *Maleimido acids* (**6**–**8**)

Compound	Proc./time[a] Yield, %	M.p., °C Solvent[b]	$[\alpha]_D$, deg. *c*, solvent	Rf (A) Rf (B)	Formula M. wt.	Calc./Found, % C	H	N
6a Mal=Gly	Aa/45	105–106	–	0.40	$C_6H_5NO_4$	46.46	3.25	9.03
	70	Et_2O/PE		0.12	155.1	46.32	3.22	9.01
6b Mal=βAla	Aa/45	106–107	–	0.48	$C_7H_7NO_4$	49.71	4.17	8.28
	60	EtOAc/PE		0.26	169.1	49.58	4.25	8.17
6c Mal=εNle	Aa/30	89–90	–	0.64	$C_{10}H_{13}NO_4$	56.87	6.20	6.63
	70	Me_2CO/*i*-Pr_2O		0.43	211.2	56.72	6.26	6.59
7a Mal=Phe	Bd/60	168–169	−124	0.45	$C_{13}H_{11}NO_4$	63.67	4.52	5.71
	57	H_2O	5, MeOH	0.22	245.2	63.84	4.48	5.61
7b Mal=Leu	Ab/60	193–194	−12.2	0.61	$C_{22}H_{36}N_2O_4$	67.32	9.24	7.14
DCHA salt	60	*i*-PrOH/Me_2CO	1, MeOH	0.28	392.5	67.03	9.11	6.90
8a Boc-Orn(=Mal)	Ba/50	141–142	+13.7	0.63	$C_{26}H_{43}N_3O_6$	63.26	8.78	8.51
DCHA salt	70	MeOH/*i*-Pr_2O	1, MeOH	0.19	493.6	63.12	9.10	8.14
8c Z-Lys(=Mal)	Ba/45	140–142	+11.7	0.64	$C_{30}H_{43}N_3O_6$	66.52	8.00	7.76
DCHA salt	70	Me_2CO/*i*-Pr_2O	4, $CHCl_3$	0.21	541.7	66.52	8.18	7.54

[a] Reaction time (min) at room temperature or 40°.
[b] For recrystallisation; yields refer to recrystallised product.

Attempts to convert **5** to **6c** in nonaqueous media in the presence of bases, bifunctional catalysts such as imidazole or 8-hydroxyquinoline, or triethylamine carbonate were unsuccessful.

Maleimido acids **6–8**. The amino acid (5 mmol) in satd. $NaHCO_3$ solution (25 ml) was treated, at 0° under vigorous stirring, with finely ground **3a** (775 mg; 5 mmol). After 10 min the solution was diluted with (A) water (100 ml) or (B) dioxan or tetrahydrofuran (50 ml) and stirred at (a) room temperature or (b) 40° for 30–40 min, brought to pH 6–7 with conc. H_2SO_4, evaporated to *ca.* 30 ml, acidified to pH 1–2 with 1M H_2SO_4, and extracted with EtOAc. The washed and dried extracts were evaporated, the residue in $CHCl_3$ with 5% AcOH (5–10 ml) was passed through a column of silica gel (20 g) and eluted with the same solvent. The eluate was evaporated, finally at 1 Torr, freed of residual AcOH by evaporation with water, and recrystallised (Table 2). DCHA salts were prepared with 1.1 mol-eq. DCHA in Et_2O (**7b**) or acetone/*i*-Pr_2O (**8a, c**). – NMR. of **6a** (acetone-d_6): 10.32 (1 H, *s*); 6.95 (2 H, *s*); 4.25 (2 H, *s*).

Maleimido acid N-succinimidyl esters (**10**). The maleimido acid (1–5 mmol) as a *ca.* 0.2M solution in the appropriate solvent (Table 3) was treated at 0° with N-hydroxysuccinimide (1.1 mol-eq.) and DCCI (1.1 mol-eq.). After 1 h more at 0° and 3 h at room temperature, treatment with a few drops of AcOH, and 1 h more at 0–3° the solution was filtered and evaporated to dryness. The product was dissolved in the minimal amount of boiling *i*-PrOH and allowed to crystallise at 0° overnight (Table 3). – NMR. of **10a** (CD_3CN): 6.89 (2 H, *s*); 4.61 (2 H, *s*); 2.78 (4 H, *s*).

6-Maleimidocaproic acid hydrazide hydrochloride (**12**. HCl). The succinimidyl ester **10c** (450 mg; 1.46 mmol) and *t*-butyl carbazate (200 mg; 1.52 mmol) in MeCN (1 ml) were stirred at 22° 6 h, diluted with EtOAc (50 ml), washed with satd. $NaHCO_3$, 0.1M HCl and satd. NaCl solution, dried, and evaporated. The residual oil [Rf 0.66 (A), 0.32 (B), 0.31 (C)] was treated with 2M HCl in dioxan

Table 3. *Maleimido acid N-succinimidyl esters* (**10**)

Compound	Solvent[a]) Solvent[b])	Yield, % M.p.	Rf (A) Rf (B)	Formula M.wt.	Calc./Found, % C	H	N
9a	diglyme	183–184	0.36	$C_{10}H_9N_2O_6$	47.36	3.20	11.11
Mal=Gly-ONSu	*i*-PrOH	82	0.31	252.2	47.71	3.23	11.03
9b	diglyme/DMF	85	0.70	$C_{10}H_{10}N_2O_6$	49.63	3.79	10.52
Mal=βAla-ONSu	diglyme/*i*-PrOH	160–163	0.38	266.2	49.61	3.88	10.30
9c	EtOAc	87	0.73	$C_{14}H_{16}N_2O_6$	54.54	5.23	9.09
Mal=εNle-ONSu	$CHCl_3$/*i*-Pr_2O[c])	62–65[d])	0.43	308.3	53.90	5.26	8.85

[a]) Reaction medium. [b]) Recrystallisation. [c]) At –25°. [d]) Dec.

(5 ml) at 22° 40 min, the hydrochloride was precipitated with *i*-Pr_2O and dried over NaOH at 15 Torr; 260 mg (69%), Rf 0.55 (A), 0.05 (C). A sample recrystallised from MeOH/*i*-PrOH and dried at 60°/0.01 Torr 48 h (m.p. 138–145°, dec.) had evidently lost some HCl on drying.

$C_{10}H_{15}N_3O_3 \cdot 0.75$ HCl	Calc.	C 47.55	H 6.29	N 16.64	Cl 10.53%
(252.6)	Found	,, 47.77	,, 6.35	,, 16.46	,, 10.29%

N(δ)-Maleoylornithine hydrochloride (**8b**. HCl). The oily Boc derivative **8c** (1.0 g), obtained from the DCHA salt by treatment with 1M H_2SO_4, extraction with EtOAc, and evaporation, was treated with 4M HCl in EtOAC (10 ml) at 22° 50 min. The product was precipitated with *i*-Pr_2O (50 ml), collected after 30 min at 4°, and dried over NaOH; 620 mg. From the filtrate, 50 mg more were recovered by evaporation and treatment with *i*-Pr_2O; total yield 84%, m.p. 165–170° (EtOAc/*i*-Pr_2O), $[\alpha]_D = +6.6°$ ($c = 1.8$, H_2O), m_{ArH} 0.31 (D), 0.29 (E). – NMR. (D_2O): 6.86 (2 H, *s*); 4.1 (1 H, *t*, $J = 6$ Hz); 3.56 (2 H, *t*, $J = 6$ Hz); 1.87 (4 H, *m*). – UV. (DMF): 267 (620), 293 (610).

$C_9H_{12}N_2O_4 \cdot$ HCl $\cdot H_2O$	Calc.	C 40.53	H 5.66	N 10.50	Cl 13.29%
(266.7)	Found	,, 40.07	,, 5.72	,, 10.24	,, 13.10%

Reaction of Z-Lys(= Mal) with HBr. The acid **8c** (760 mg), obtained from the DCHA salt as above, was treated with 30% HBr in AcOH at 22° 40 min. The solution was evaporated with repeated additions of AcOH, the residue taken into MeOH (5 ml) and neutralised with pyridine. The product was collected after 5 h at 4°, washed with MeOH and dried over P_2O_5; 420 mg; m.p. 152–154°. – NMR. (1M DCl/D_2O, from Na 2,2,3,3-tetradeuterio-3-trimethylsilylpropionate): *ca.* 5.0 (1 H, partly under HOD peak); 4.11 (1 H, *t*, J = 6 Hz); 3.57 (2 H, *t*, J = 7 Hz); 3.6–2.8 (2 H, *m*, *ABX* system); 2.2–1.2 (6 H, *m*).

$C_{10}H_{15}BrN_2O_4$ (307.1) Calc. C 39.11 H 4.92 N 9.12% Found C 39.12 H 4.89 N 8.97%

Qualitative test for halogen positive, for ionisable halogen negative.

Maleoyl Derivatives of Peptides. – *Mal=Gly-Ala-OBu^t* (**11a**). a) The ditosylamine salt of Ala-OBu^t (470 mg; 1 mmol), **6a** (155 mg; 1 mmol), NMM (0.11 ml; 1 mmol), and DCCI (220 mg; 1.07 mmol) in diglyme (2 ml) were stirred at 22° 2 h. The precipitate was filtered off, washed with diglyme (10 ml), the combined filtrates were taken to dryness, and the residue in ether (40 ml) was washed at 0° with satd. $NaHCO_3$ solution, 0.05M H_2SO_4 and satd. NaCl solution. After evaporation, dissolution of the residue (320 mg) in acetone (2 ml), filtration after 12 h at 4°, and evaporation, the product was precipitated from $CHCl_3$ with *i*-Pr_2O and light petroleum; 220 mg (78%), m.p. 117–118°, Rf 0.70 (A), 0.68 (C), $[\alpha]_D$ = –58.6° (c = 1, MeOH). – NMR. ($CDCl_3$) confirmed the presence of the maleimide (6.8, 2 H, *s*) and *t*-butyl (1.47, 9 H, *s*) groups.

$C_{13}H_{18}N_2O_5$ (282.3) Calc. C 55.31 H 6.43 N 9.92% Found C 55.26 H 6.28 N 9.68%

b) The ditosylamine salt of Ala-OBu^t (510 mg; 1.08 mmol), NMM (0.12 ml; 1.1 mmol), and the ester **10a** (225 mg; 0.9 mmol) in diglyme (6 ml) were stirred at 22° 2 h. Since the reaction was incomplete (TLC.) the suspension was diluted with MeCN (4 ml), stirred for 1 h more, and filtered. The residue was washed with diglyme (10 ml) and the combined filtrates were worked up as in a). The crude product (220 mg) was recrystallised from $CHCl_3$/light petroleum; 180 mg (71%), m.p. 116–118°, $[\alpha]_D$ = –58.1° (c = 1, MeOH), identical on TLC. with the product from a).

Mal = Gly-Ala (**11b**). **11a** (110 mg) was stirred with CF_3COOH (3 ml) at 22° 3 h. After evaporation (0.1 Torr) the residue was dried over NaOH and crystallised from MeOH/*i*-Pr_2O; 80 mg (91%), m.p. 155–157°, $[\alpha]_D$ = –34.0° (c = 1, MeOH), Rf 0.35 (A). – NMR. confirmed the absence of the *t*-butyl group.

$C_9H_{10}N_2O_5$ (226.2) Calc. C 47.79 H 4.46 N 12.39% Found C 47.71 H 4.57 N 12.07%

Boc-Pro-Orn(= Mal)-Gly-NH$_2$ (**16b**). Boc-Pro-Orn-Gly-NH_2 (**16a**) (385 mg; 1 mmol) in satd. $NaHCO_3$ solution (5 ml) was treated, at 0° under stirring, with **3a** (310 mg; 2 mmol). After 10 min at 0° the solution was diluted with water (25 ml), stirred at 22° for 15 min more, and extracted with EtOAc/*n*-BuOH 4:1 (3 × 30 ml). The extracts were washed, dried, evaporated to *ca.* 5 ml and diluted with Et_2O; 290 mg (63%), m.p. 209–210° (dec.), $[\alpha]_D$ = –68.2° (c = 1, CF_3CH_2OH), Rf 0.64 (A), 0.02 (B). The NMR. (Me_2SO-d_6) showed the characteristic peak of the maleimide protons at 6.98 (2 H, *s*).

$C_{21}H_{33}N_5O_7$ (467.5) Calc. C 53.95 H 7.11 N 14.98% Found C 53.94 H 6.81 N 14.84%

Boc-Pro-Leu-Gly-NH-NH(Mal=εNle) (**15**). a) Boc-Pro-Leu-Gly-N_2H_3 [15] (200 mg; 0.5 mmol) and **10c** (155 mg; 0.5 mmol) were stirred in DMF (1.5 ml) at 22° 20 h. The residue after evaporation (0.1 Torr) in EtOAc (50 ml) was washed with satd. $NaHCO_3$ solution, 0.1M $KHSO_4$/0.2M K_2SO_4 [16], and satd. NaCl solution and after evaporation to 3 ml precipitated with *i*-Pr_2O; 190 mg (64%), m.p. 89–93° (sintering from 79°), $[\alpha]_D$ = –45.1° (c = 1, EtOH), Rf 0.78 (A), 0.65 (C). Elemental analysis indicated the presence of about $^1/_2$ mol H_2O and $^1/_3$ mol *i*-Pr_2O even after drying at 40°/0.01 Torr 24 h.

$C_{28}H_{44}N_6O_8 \cdot 0.5\ H_2O \cdot 0.33\ C_6H_{14}O$	Calc.	C 56.67	H 7.77	N 13.22 H_2O 1.41%
(635.8)	Found	,, 56.83	,, 7.65	,, 13.31 ,, 1.10%

b) Boc-Pro-Leu-Gly-OH [15] (100 mg; 0.26 mmol), the hydrazide hydrochloride **12**. HCl (68 mg; 0.26 mmol), NMM (29 µl; 0.26 mmol), HOBt (40 mg; 0.3 mmol) and DCCI (60 mg; 0.3 mmol) in DMF (2 ml) were stirred at 0° 1 h and at 22° 20 h, treated with a few drops of AcOH, stirred at 22° 2 h, and kept at 4° 2 h. The filtrate was worked up as in a) and the product precipitated from *i*-PrOH with *i*-Pr_2O; 80 mg (52%), m.p. 90–94° (sintering from 64°), $[\alpha]_D$ = –44.3° (c = 1, EtOH), identical with the sample from a) by TLC.

Reactions of Maleimide Groups. – *Hydrolytic cleavage* – a) *Alkaline hydrolysis*: *6-(3-Carboxyacryloylamino)caproic acid* (**9**). **6c** (170 mg) was stirred with 5% Na_2CO_3 solution (4 ml) at 22° 1 h. Acidification to pH 1 with 1M H_2SO_4 precipitated 135 mg of **9**, extraction of the filtrate with EtOAc and evaporation afforded 20 mg more; total yield 84%, m.p. 164–166°, Rf 0.23 (A), 0.02 (B). – NMR. (pyridine-d_5): 13.9 (2 H, *s*); 10.0 (1 H, *s*, br.); 6.58 (2 H, *AB* system); 3.8–3.1 (2 H, *m*); 2.6–2.2 (2 H, *m*, pseudo-*t*); 2.0–1.2 (6 H, *m*).

$C_{10}H_{15}NO_5$ (229.2) Calc. C 52.40 H 6.59 N 6.11% Found C 52.03 H 6.69 N 5.90%

b) *Acid cleavage of the maleyl group.* The maleamic acid **9** (130 mg) was suspended in 1M AcOH (5 ml), dissolved by addition of dioxan, and kept at 40° 20 h. Evaporation and crystallisation from H_2O/*i*-PrOH gave 70 mg (94%) of 6-aminocaproic acid, Rf 0.32 (C), m_{Arg} 0.54 (D).

c) *Cleavage by successive hydrolysis with alkali and acid.* Maleoylphenylalanine (**7a**) (120 mg; 0.49 mmol) stirred in Na_2CO_3/$NaHCO_3$ buffer (pH 10; 5 ml) for 3 h at 22° gave a clear solution which was treated with AcOH (20 ml; final pH 2.5), stirred 40 h more and passed through a column (6 ml) of Dowex 50W (H^+). Elution with 5% pyridine, evaporation, and crystallisation from H_2O/*i*-PrOH gave 53 mg (65%) of a product identical by TLC. [Rf 0.36 (C)] and optical rotation ($[\alpha]_D = -31.9°$, $c = 2.3$, H_2O) with authentic L-phenylalanine. By the same procedure, 6-aminocaproic acid [Rf 0.32 (C); m_{Arg} 0.54 (D)] was obtained in 70% yield from **6c**.

Hydrazinolysis. The reaction of **6c** (20 mg; 0.1 mmol) with $N_2H_4 \cdot H_2O$ (10 mg; 0.2 mmol) in 2.5% $NaHCO_3$ solution (2 ml) at 40° was followed by TLE. After 1 h all the **6c** had disappeared and 6-aminocaproic acid [m_{Arg} 0.54 (D)] was the only material (in addition to hydrazine) detected by the *Reindel-Hoppe* reagent.

Reaction of **8b** *with 2-mercaptoethanesulfonate* (W. Fischli). Solution *a*: 10 mM **8b**. HCl in tris buffer (pH 7.2); solution *b*: 10 mM Na 2-mercaptoethanesulfonate in water; solution *c*: 10 mM 1,2-diiodoethane (freshly crystallised) in EtOH. Solutions *a* (1 ml) and *b* (2 ml) were mixed at room temperature, 0.1-ml samples taken at 1-min intervals were immediately treated with solution *c* (0.2 ml) and analysed by TLE. (D; detection with ninhydrin). In a control experiment the oxidation of the thiol with diiodoethane was found to be complete within seconds. After 1 min, practically all **8b** had reacted (only a trace of neutral material was found by TLC. and this did not diminish during 15 min).

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64. Neue Umlagerungsreaktionen des Trichothecangerüsts

Verrucarine und Roridine, 31. Mitteilung [1]

von **Beat Müller** und **Christoph Tamm**

Institut für Organische Chemie der Universität Basel

(24. XII. 74)

Summary. Treatment of the apotrichothecane derivative **4** with H_2SO_4 in dioxan gave the acetal **6** and with H_2SO_4 in acetone the ketal **9**. Whereas the oxidation of **4** with Ag_2CO_3 yielded the hydroxy aldehyde **7**, the reaction with CrO_3 or MnO_2 led to the α,β-unsaturated ketone **8**. Upon treatment of **8** with base the cyclic keto ether **11** was obtained due to 1,4-addition. Acetylation of the latter compound gave a mixture consisting of the enolacetate **13** and the acetylketone **14**. The oxim **15** of ketone **14** was transformed to the nitrile **16** and not the *Beckmann* fragmentation product **18**. For the identification of the C(11) hydrogen atom in biosynthetic studies the triol **22** was oxidized to the keto aldehyde **26** which, upon treatment with methanolic K_2CO_3, gave the spirolactol **30** and the cyclic acetal **29** as second product when the reaction was carried out in dilute solution. The spirolactol **30** was oxidized to the spirolactone **31**. The corresponding **19** possessing the intact 12,13-epoxy group underwent rearrangement to the apotrichothecane derivatives **20** and **21** under the same conditions. Oxidation of the triol **22** with MnO_2 or CrO_3 gave a mixture of the acetal **23** and the keto acid **24**. – The mechanisms of the rearrangements observed are discussed.

1. Einleitung. – Im Zusammenhang mit Untersuchungen über die Biosynthese des Verrucarols (**1**) [1] [2] haben wir einige neue Umwandlungsprodukte hergestellt und sind auf unerwartete Umlagerungen des Trichothecangerüsts gestossen, über die wir im folgenden berichten.

Bei der Behandlung von Di-O-acetyl-verrucarol (**2**) mit H_2SO_4 in Dioxan – eine Reaktion, die zum Apotrichothecangerüst [3] führt – erhielten wir neben dem bekannten umgelagerten Di-O-acetyltetrol **4** [4] eine Verbindung, die anstelle der beiden freien HO-Gruppen eine Acetalgruppierung enthielt: sie besitzt die Strukturformel **6**, denn im IR.-Spektrum waren entsprechende Banden bei 1730, 1680, 1370, 1230, 1125 und 1050 cm^{-1} und im ^1H-NMR.-Spektrum (vgl. Tab.) ein Dublett bei 1,32 ppm (3H) der CH_3-Gruppe und bei 4,80 ppm ein Quartett für das Methinproton sichtbar.

Durch Hydrolyse mit methanolischer K_2CO_3 oder KOH liessen sich die Acetylgruppen abspalten, wodurch das Acetaldiol **5** entstand, das im IR.-Spektrum keine Carbonylschwingungen sondern nur assoziierte HO-Gruppen zeigte. Die Acetalgruppierung war durch entsprechende Signale im ^1H-NMR.-Spektrum erkennbar. Im Massenspektrum trat die Basisspitze bei *m/e* 163 auf, das Molekel-Ion bei *m/e* 310.